Molecular Phylogenetics and Evolution xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

# Fast radiation of the subfamily Lacertinae (Reptilia: Lacertidae): History or methodical artefact?

Mihaela Pavlicev [a,b,*], Werner Mayer [b]

[a] Department of Anatomy and Neurobiology, Washington University, 660 S Euclid Ave., Box 8108, St. Louis, 63110 MO, USA
[b] Natural History Museum Vienna, Laboratory for Molecular Systematics, Burgring 7, A-1010 Vienna, Austria

ARTICLE INFO

*Article history:*
Received 22 October 2008
Revised 12 April 2009
Accepted 30 April 2009
Available online xxxx

*Keywords:*
Lacertinae
Speciation
Radiation
Miocene
*Atlantolacerta andreanskyi*
*Algyroides*

ABSTRACT

Lacertinae is one of the three lacertid lizard subfamilies with a geographical distribution confined to the Palaearctic. Several past attempts to reconstruct its phylogeny resulted in unresolved bush-like topologies. We address the question of whether the lack of resolution is due to insufficient data or whether this lack reflects a rapid succession of speciation events. We analyzed four partial and one complete gene sequences from mitochondrial and nuclear genomes, totalling roughly 3600 bp. We included 29 species representing all 19 genera suggested in recent revision of Lacertinae [Arnold, E.N., Arribas, O., Carranza, S., 2007. Systematics of the palaearctic and oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae), with descriptions of eight new genera. Zootaxa 1430, 1–86]. The resulting phylogeny, first, corroborates monophyly at the genus level for the suggested genera, as well as the finding that *Atlantolacerta andreanskyi*, until recently part of Lacertinae, belongs to the subfamily Eremiadinae. Second, we find that increasing the sequence length and combining multiple nuclear and mitochondrial sequences did not resolve the polytomy, suggesting that the inferred topology indicates a multiple cladogenesis within a short geological period, rather than a methodical artefact. Divergence time estimates, based on previous estimates of several node ages, range from 13.9 to 14.9 million years for the radiation event, however with very broad confidence interval. To associate the radiation with a narrower geological time we consider palaeogeographic and palaeoclimatic data, assuming that the Lacertinae probably evolved in Central Europe and W Asia after the collision of Africa and Eurasia. We suggest that this radiation may date to the late Langhian (ca. 14–13.5 million years) when geological events caused abrupt changes in regional water–land distribution and climate, offering a window of distinct conditions.



## 1. Introduction

The family Lacertidae is composed of three well supported monophyletic groups (Harris et al., 1998; Mayer and Pavlicev, 2007; Arnold et al., 2007) that until recently were considered subfamilies: Lacertinae, Eremiainae and Gallotiinae. Arnold et al. (2007) in their recent work adjusted the name Eremiainae into the grammatically correct form Eremiadinae. In the same publication the rank was downscaled for both the Lacertinae and the Eremiadinae from subfamily to tribus, yielding Lacertini and Eremiadini within a subfamily Lacertinae. We consider this latter change to be a rather unnecessary cladistic complication and treat both groups in this paper further as subfamilies, focusing here on Lacertinae (i.e., Lacertini).

The Lacertinae as defined above are distributed from eastern Asia to the Atlantic Ocean. The subfamily's approximately 100 species have been classified in the recent thorough revision (Arnold et al., 2007) into19 genera. One of the most notable taxonomic changes of this revision was transferring *Lacerta andreanskyi* from the subfamily Lacertinae (or tribus Lacertini, after Arnold et al., 2007) into a new genus *Atlantolacerta* within Eremiadinae.

In the recent years, the predominantly molecular characterisation enabled recognition of the monophyletic units within Lacertinae. Among other taxonomic changes, this characterisation also led to the successive separation of single distinct units (as genera) from the originally highly polyphyletic genus *Lacerta* (*Teira*, *Timon*, *Zootoca*: Mayer and Bischoff, 1996; *Iberolacerta*, *Darevskia*: Arribas, 1997), but leaving the remaining group still heterogeneous. The recent study and taxonomical reordering by Arnold et al. (2007) includes the most complete set of taxa so far. Apart from the thorough morphological study, the molecular phylogenetic part of the above study is based on the analysis of relatively short mitochondrial sequences when all taxa are considered (291 bp *cyt b*, 329 bp 12S rRNA). Mitochondrial sequences have been used

* Corresponding author. Present address: CEES, Department of Biology, University of Oslo, P.O. Box 1066 Blindern, Oslo, Norway. Fax: +47 22 85 40 01.
*E-mail addresses:* mihaela.pavlicev@bio.uio.no, pavlicev@pcg.wustl.edu (M. Pavlicev), werner.mayer@nhm-wien.ac.at (W. Mayer).

1055-7903/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2009.04.020

before to infer phylogenetic relationships in Lacertinae; for example Fu (2000) used a long (>4000 bp) mitochondrial sequence, but on a less complete set of taxa. Nevertheless, in contrast to the subfamily Eremiadinae that is well resolved even by the less variable nuclear markers (Mayer and Pavlicev, 2007), so far none of the analyses yielded a resolved phylogenetic inference of the subfamily Lacertinae. In general, this lack of resolution can be either due to the markers which were insufficient or inappropriate to reflect the respective cladogenesis (soft polytomy), or due to a multiple cladogenesis within a short time (hard polytomy). The resolution of a phylogeny depends on the variation in the marker sequences between the taxa studied. Too much as well as too little variation can obscure the results. Highly variable markers, like those from mitochondrial DNA, can lead to saturation when distances between taxa are high and the probability that the same position has mutated multiple times increases. This situation compresses the branch length relative to true divergence, as new mutations are not noticeable when they take place repeatedly on the already mutated position or even reverse to the ancestral state. The opposite is trivial: too low variation in markers, as can be the case in nuclear markers with a low rate of sequence change, or in a short sequence, precludes the resolution of phylogeny. Changing the marker or increasing the sequence length can improve the amount of variation between the taxa and the power to distinguish them; the effect of length being simply due to the longer sequences having increased probability to include variable sites. The resolution therefore is affected by the length of the sequence in the way every statistical representation is affected by the size of the sample. Combining the information from multiple sequences, both mitochondrial and nuclear, from the same taxa should surpass this problem as (i) it is unlikely that all mitochondrial sequences consistently show too much, and all nuclear sequences too little diversification, and (ii) the increase in the total sequence length increases the probability to detect sufficient differences, if the lack of diversification should be a problem (e.g., Gontcharov et al., 2004).

In this paper, we study multiple mitochondrial and nuclear DNA sequences with a total length of approximately 3600 bp, encompassing representatives of all presumptive groups of the subfamily Lacertinae. We asked whether this extended data from different markers can resolve the apparent bush-like radiation of Lacertinae or whether the topology mirrors an actual multiple cladogenesis. We aim furthermore to associate the estimates of divergence age based on our inferred topology and previous node age estimates to the recent palaeogeographic and palaeoclimatic knowledge.

## 2. Materials and methods

To infer phylogenetic relationships we used multiple sections of both nuclear and mitochondrial DNA, comprising in total approximately 3600 bp (1600 bp nuclear: *c-mos* and *rag1*, 2000 bp mitochondrial: 12S rRNA, 16S rRNA, *cyt b*). The exact lengths of gene sequences are listed in Table 1. All sequences were analyzed in a total of 33 species (Table 2). For 12 of the 19 genera (after Arnold et al., 2007) we included one representative species in the analysis (six of them are monospecific); the remaining seven genera were represented by two or more species (Table 3). *Gallotia galloti, Eremias arguta*, and *Ophisops elegans* were included as outgroups in the first part of the study, establishing the phylogenetic position of *Atlantolacerta andreanskyi*. In the second part *Atlantolacerta andreanskyi* was also included in outgroup.

It is important to note here that a genus with respect to formal nomenclature is defined by its *species typica*. In order to infer the phylogeny at the genus level it is therefore important to represent genera by their corresponding *species typicae*. In this study, we were able to follow this principle in all genera except *Darevskia* (spec. typ. *saxicola*). The sequences of all gene fragments were derived from the same individual for each taxon, to avoid combining phylogenetic signal from different sources (i.e., possibly divergent populations).

### 2.1. DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from frozen or alcohol-preserved soft tissues following a standard phenol–chloroform procedure (Sambrook et al., 1989). Amplifications of all PCR fragments were performed in 25 μl reaction mixtures containing PCR buffer with 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM of each PCR primer, and 0.5 U of *Taq* polymerase (Amersham Biosciences, UK). Reaction conditions comprised an initial denaturation step of 2 min at 94 °C, 35 cycles of 10 s at 95 °C, 15 s at annealing temperature, 50 s at 72 °C, and a final extension step of 7 min at 72 °C. Negative and positive PCR controls were included in all PCR amplifications. Sequences and annealing temperatures of primers used in the study are given in Table 4.

We sequenced PCR products directly and as cloned PCR fragments. The important advantage of the first method is that it can reveal polymorphic sites within individuals. These polymorphisms can be due to heterozygosity as well as due to diverged multiple copies (functional and non-functional, i.e., pseudogenes). Potential paralogous copies (Pavlicev and Mayer, 2006) could therefore be identified and excluded from the dataset of the present study.

For direct sequencing, we repeated PCR amplifications using the preamplified segment as a template. The nested primers used for reamplifications, as well as sequencing primers are listed in Table 4. Gel-purified (QIAquick® Gel extraction kit, Qiagen, Venlo, The Netherlands) amplified PCR fragments were cloned using the TA vector (TOPO TA Cloning Kit, Invitrogen, Carlsbad, CA, USA). Sequencing was performed by MWG Biotech (Ebersberg, Germany), and AGOWA (Berlin, Germany).

### 2.2. Sequence analysis

The sequences were combined, aligned and edited manually in the programme BioEdit (Version 7.0.1; Hall, 1999). In the coding genes (*cyt b*, *rag1*, and *c-mos*), we found a single 3 bp in-frame deletion in *cyt b* of *Parvilacerta fraasii*. This deletion has been confirmed by independently sequencing four individuals from two localities. This and further indels of non-coding genes were treated in the analysis as missing characters (see below for testing the effect of this model). Sequence segments in rRNA genes with ambiguous alignment were excluded from the analysis (see Online Supplementary material for detail). Separate trees were calculated for each of the five partial gene sequences and for different concatenated datasets. Two concatenated datasets will be addressed in more detail here: one of them consisting of all sequences, and the second consisting of nuclear sequences only (*rag1* and *c-mos*).

**Table 1**
The length of the sequence segments and the corresponding evolutionary models used in the analyses, as determined by the ModelTest. The values in parentheses are the approximative lengths of the sequences before deletion of the ambiguous, unalignable parts.

| Sequence | Length (bp) | Evolutionary model |
|---|---|---|
| *cyt b* (complete sequence) | 1143 | GTR+I+G |
| 12S rRNA (segment) | 411 (450) | GTR+I+G |
| 16S rRNA (segment) | 451 (500) | TrN+I+G |
| *c-mos* (segment) | 581 | TVMef+G |
| *rag 1* (segment) | 1012 | TIM+I+G |

GTR, general time reversible model; TrN, Tamura–Nei model; TVMef, transversion equal frequencies model; TIM, transversion model; I, a proportion of invariable sites; G, gamma distributed substitution rate across sites).

Please cite this article in press as: Pavlicev, M., Mayer, W. Fast radiation of the subfamily Lacertinae (Reptilia: Lacertidae): History or methodical artefact?. Mol. Phylogenet. Evol. (2009), doi:10.1016/j.ympev.2009.04.020

**Table 2**
List of analyzed specimens, their geographical origin and the GenBank accession numbers for all partial gene sequences. All DNA samples are stored at the Natural History Museum Vienna (NMW), Austria.

| Species | Geographical origin | GenBank | | | | |
|---|---|---|---|---|---|---|
| | | *c-mos* | *rag 1* | *cyt b* | *12S rRNA* | *16S rRNA* |
| *Algyroides fitzingeri* | Italy: Sardinia | GQ142147 | GQ142157 | GQ142134 | GQ142081 | GQ142104 |
| *Algyroides marchi* | Southeastern Spain | GQ142146 | GQ142156 | GQ142133 | GQ142080 | GQ142103 |
| *Algyroides moreoticus* | Greece: Korinthia, Feneos | EF632254 | EF632209 | GQ142131 | GQ142079 | GQ142102 |
| *Algyroides nigropunctatus* | Greece: Preveza, Parga | EF632255 | EF632210 | GQ142132 | AY942787 | AF942795 |
| *Anatololacerta anatolica* | Greece: Samos Island | GQ142150 | GQ142160 | GQ142138 | GQ142086 | GQ142109 |
| *Anatololacerta danfordi* | Turkey: Icel, Camliyayla | DQ461743 | EF632224 | DQ461765 | GQ142084 | GQ142107 |
| *Anatololacerta oertzeni* | Greece: Rhodos Island | GQ142149 | GQ142159 | GQ142137 | GQ142085 | GQ142108 |
| *Apathya cappadocica* | Turkey: Kayseri, Mt. Ercyas | EF632268 | EF632223 | GQ142127 | GQ142076 | GQ142099 |
| *Archaeolacerta bedriagae* | France: Corsica | EF632256 | EF632211 | GQ142126 | AF440599 | AF440614 |
| *Atlantolacerta andreanskyi* | Marocco: Jebel Toupkal | GQ142144 | GQ142154 | GQ142117 | GQ142070 | GQ142093 |
| *Dalmatolacerta oxycephala* | Croatia: Hvar Island | EF632271 | EF632228 | GQ142129 | AF440601 | AF440616 |
| *Darevskia valentini* | Armenia: Rasdan | EF632257 | EF632212 | GQ142123 | GQ142075 | GQ142098 |
| *Dinarolacerta mosorensis* | Montenegro: Durmitor Mts. | EF632270 | EF632227 | GQ142130 | AF440600 | AF440615 |
| *Dinarolacerta montenegrina* | Albania: Shkoder | GQ142153 | GQ142163 | GQ142141 | GQ142078 | GQ142101 |
| *Eremias arguta* | Ukraine | EF632258 | EF632213 | GQ142115 | AY035827 | AF035837 |
| *Gallotia galloti* | Spain: Tenerife Island | EF632260 | EF632215 | GQ142114 | GQ142068 | GQ142091 |
| *Hellenolacerta graeca* | Greece: Lakonia, Monemvasia | EF632269 | EF632225 | GQ142128 | GQ142077 | GQ142100 |
| *Iberolacerta horvathi* | Austria: Carinthia, Rattendorf | EF632264 | EF632219 | GQ142125 | AJ238186 | AF149943 |
| *Iberolacerta monticola* | Portugal: Sierra Estrela | EF632265 | EF632220 | GQ142124 | AF440589 | AF440604 |
| *Iranolacerta brandtii* | Iran: Kuh-e-Sahand | GQ142152 | GQ142162 | GQ142140 | GQ142088 | GQ142111 |
| *Lacerta agilis* | Austria: Lower Austria, Weitra | EF632267 | EF632222 | GQ142118 | AF149947 | AF149963 |
| *Ophisops elegans* | Greece: Evros, Gianuli | EF632278 | EF632235 | GQ142116 | GQ142069 | GQ142092 |
| *Parvilacerta parva* | Turkey: Malatya | EF632279 | EF632236 | GQ142135 | GQ142082 | GQ142105 |
| *Parvilacerta fraasii* | Leban: Mt. Lebanon | GQ142148 | GQ142158 | GQ142136 | GQ142083 | GQ142106 |
| *Phoenicolacerta troodica* | Cyprus: Pafos | DQ461715 | EF632226 | DQ461746 | AJ238181 | AF149939 |
| *Phoenicolacerta kulzeri* | Leban: Jebel Barouk | GQ142151 | GQ142161 | GQ142139 | GQ142087 | GQ142110 |
| *Podarcis muralis* | Austria: Lower Austria, Gumpoldskirchen | EF632282 | EF632239 | AY185096 | AY190305 | AF190306 |
| *Teira dugesii* | Portugal: Madeira Island | EF632289 | EF632246 | GQ142121 | GQ142073 | GQ142096 |
| *Timon lepidus* | Spain: Alicante | EF632290 | EF632247 | GQ142119 | GQ142071 | GQ142094 |
| *Scelarcis perspicillata* | Spain: Menorca Island | GQ142145 | GQ142155 | GQ142122 | GQ142074 | GQ142097 |
| *Takydromus sexlineatus* | Unknown (animal trade) | EF632288 | EF632245 | GQ142143 | GQ142090 | GQ142113 |
| *Takydromus amurensis* | Russia: Amur region | EF632287 | EF632244 | GQ142142 | GQ142089 | GQ142112 |
| *Zootoca vivipara* | Austria: Lower Austria, Schneeberg | EF632292 | EF632249 | GQ142120 | GQ142072 | GQ142095 |

**Table 3**
The number of species per genus included in the analyses, and in parentheses the total number of species in the respective genus (taxonomy after Arnold et al., 2007).

| Genus | No. of species |
|---|---|
| *Algyroides* | 4 (4) |
| *Anatololacerta* | 3 (3) |
| *Apathya* | 1 (2) |
| *Archaeolacerta* | 1 (1) |
| *Dalmatolacerta* | 1 (1) |
| *Darevskia* | 1 (18 + 7*) |
| *Dinarolacerta* | 2 (2) |
| *Hellenolacerta* | 1 (1) |
| *Iberolacerta* | 2 (8) |
| *Iranolacerta* | 1 (2) |
| *Lacerta* | 1 (8) |
| *Parvilacerta* | 2 (2) |
| *Phoenicolacerta* | 2 (4) |
| *Podarcis* | 1 (17) |
| *Scelarcis* | 1 (1) |
| *Teira* | 1 (1) |
| *Timon* | 1 (4) |
| *Zootoca* | 1 (1) |
| *Takydromus* | 2 (18) |
| *Gallotia* | 1 (7–8) |
| *Eremias* | 1 (~30) |
| *Ophisops* | 1 (7) |
| *Atlantolacerta* | 1 (1) |

* The parthenogenetic species in genus *Darevskia*.

The combinability of the sequences was tested with a partition homogeneity test (PAUP Version 4.0b10; Swofford, 2000). The results of the pair-wise tests revealed no significant conflicts between pairs of fragments (*P*-values are listed in Table 5).

We applied multiple inference algorithms. Bayesian inference (MrBayes, version 3.1; Huelsenbeck and Ronquist, 2001) was applied in all analyses. We used ModelTest (version 3.7; Posada and Crandall, 1998) to estimate the optimal evolutionary models and priors for single gene fragments. In the analyses of the concatenated dataset we assigned the separate evolutionary models to the individual genes (partitions). The evolutionary models are listed in Table 2 for separate sequences. Four Mio generations were run in all Bayesian analyses except the one on a total dataset, with a sampling frequency of 100. All taxa except *Gallotia galloti* were included in these analyses and the results compared across single-gene trees and the concatenated dataset trees. For the analysis of the total concatenated dataset including *cyt b*, 10 Mio generations were run to reach convergence. Due to apparent destabilization when adding *cyt b* to the dataset, we compared the trees inferred from the concatenated dataset including and excluding the *cyt b* sequence. A majority consensus tree was built with the final 50% of the sampled trees.

For the total dataset, we also inferred the phylogeny using the Maximum likelihood (ML), Maximum parsimony (MP) as well as Neighbor Joining (NJ) algorithms. MP analysis was conducted using the heuristic search mode with 100 repeats, randomised input orders of taxa, and tree bisection–reconnection (TBR) branch-swapping with all codon positions weighted equally. Nonparametric bootstrapping (100 pseudoreplicates, 10 addition-sequence replicates) was used to assess the stability of internal branches in the trees in MP and ML algorithms, while 1000 bootstrap replicates were used in NJ algorithm.

Please cite this article in press as: Pavlicev, M., Mayer, W. Fast radiation of the subfamily Lacertinae (Reptilia: Lacertidae): History or methodical artefact?. Mol. Phylogenet. Evol. (2009), doi:10.1016/j.ympev.2009.04.020

**Table 4**
Primer sequences and the corresponding annealing temperatures (for amplification primers).

| Name | Sequence | *T* (°C) | Gene | Purpose | Taxa |
|---|---|---|---|---|---|
| Hcmos3 | 5′-ggt gat ggc aaa tga gta gat-3′ | 55 | *c-mos* | PCR(1) | All |
| L-1zmos | 5′-cta gct tgg tgt tct ata gac tgg-3′ | 55 | *c-mos* | PCR(1)/PCR(2) | All |
| Hcmos1 | 5′-gca aat gag tag atg tct gcc-3′ | 56 | *c-mos* | PCR(2) | All |
| CMS-77L | 5′-cta cgt acc atg gag cta c-3′ | | *c-mos* | Sequencing | All |
| CMS-482H | 5′-ttg gga aca tcc aaa gtc tc-3′ | | *c-mos* | Sequencing | All |
| RAG-R1 | 5′-aaa atc tgc ctt cct gtt att g-3′ | 52 | *rag1* | PCR(1) | All |
| RAG-fo | 5′-gaa aag ggc tac atc ctg g-3′ | 52 | *rag1* | PCR(1)/PCR(2) | All |
| RAG-re | 5′-cca gtt att gct ttt aca gtt c-3′ | 52 | *rag1* | PCR(2) | All |
| RGS-380L | 5′-ctc agt acc aag atc ctt gc-3′ | | *rag1* | Sequencing | All |
| RGS-587H | 5′-agc caa act gtt gag gat ac-3′ | | *rag1* | Sequencing | All |
| L01091 | 5′-aaa ctg gga tta gat acc cca cta t-3′ | 50/56 | *12S* | PCR(1)/PCR(2) | All |
| H01557 | 5′-gta cac ttt acc ttg tta cga ctt-3′ | 50/56 | *12S* | PCR(1)/PCR(2) | All |
| HE1478 | 5′-agg gtg acg ggc ggt gtg t-3′ | | *12S* | Sequencing | All |
| LE1318 | 5′-acg tca ggt caa ggt gta gc-3′ | | *12S* | Sequencing | All |
| LE2190 | 5′-gta ggc ctc aaa gca gcc ac-3′ | 50/56 | *16S* | PCR(1)/PCR(2) | All |
| H03056 | 5′-ccg gtc tga act cag atc acg-3′ | 50/56 | *16S* | PCR(1)/PCR(2) | All |
| LE2493 | 5′-cca act gtt tac caa aaa cat ag-3′ | | *16S* | Sequencing | All |
| LgluLK | 5′-aac cgc tgt tgt ctt caa cta-3′ | 50 | *cyt b* | PCR(1) | *Zootoca vivipara* |
| NTheH | 5′-ggt tta caa gac cag tgc ttt-3′ | 50/55 | *cyt b* | PCR(1)/PCR(2) | All except *Dalmatolacerta oxycephala* |
| Sicnt-L | 5′-ttt gga tcc ctg tta ggc ctc tgt t-3′ | 55 | *cyt b* | PCR(2) | *P. muralis* |
| MURNUM | 5′-agg cac ctc tat agt tca cc-3′ | | *cyt b* | Sequencing | *P. muralis* |
| HPod | 5′-ggt gga atg gga ttt tgt ctg-3′ | | *cyt b* | Sequencing | *P. muralis* |
| NGluL2 | 5′-gct tga tat gaa aaa cca tcg ttg-3′ | 50/52 | *cyt b* | PCR(1)/PCR(2)/sequencing | *Zootoca vivipara* |
| H15888 | 5′-gct ttg gag ttt aag cta cta gg-3′ | 52 | *cyt b* | PCR(2) | *Zootoca vivipara* |
| LPod | 5′-cag aca aaa ttc cat tcc acc-3′ | | *cyt b* | Sequencing | *Zootoca vivipara* |
| HProLK | 5′-agt ttt ggg ggc tag tga tgg-3′ | 50/55 | *cyt b* | PCR(1)/PCR(2) | *Dalmatolacerta oxycephala* |
| LE14841 | 5′-cca tca aac att tca gct tga tga aa-3′ | 55 | *cyt b* | PCR(2) | *Dalmatolacerta oxycephala* |
| OX14700L | 5′-agg tac ctc tat aat tca cc-3′ | | *cyt b* | Sequencing | *Dalmatolacerta oxycephala* |
| OX14850H | 5′-ggg tct cct agt agg tta gg-3′ | | *cyt b* | Sequencing | *Dalmatolacerta oxycephala* |

**Table 5**
The *p*-values of the pair-wise tests of compatibility of single fragments. Partition homogeneity test was used (PAUP Version 4.0b10; Swofford 2002). The null hypothesis of homogeneity could not be rejected in any case.

| | *cyt b* | *c-mos* | *rag1* | *16s* |
|---|---|---|---|---|
| *12s* | 0.91 | 1.00 | 0.53 | 0.40 |
| *16s* | 1.00 | 1.00 | 0.35 | |
| *rag1* | 1.00 | 0.56 | | |
| *c-mos* | 1.00 | | | |

### 2.3. Model testing and molecular clock

We used Bayes factors to test three hypotheses: the presence of the effect of treating indels as missing data, the specific aspect of topology, and the equal evolutionary rates among branches. Bayes factor test is analogous to other likelihood ratio tests (e.g., Kishino–Hasegawa topology tests, Kishino and Hasegawa, 1989), with an advantage that the values need not be corrected for the different number of parameters used (Goodman, 1999; Kass and Raftery, 1995; Nylander et al., 2004). The resulting harmonic means (*HM*) of the marginal likelihood values across the post burn-in trees are compared between two alternative models $M_1$ and $M_2$ by the forming of Bayes factor $B_{10} = \frac{HM_1}{HM_2}$. Twice the logarithm of $B_{10}$ ($= 2 * \Delta \log_{10}(HM_i)$) is used to decide the relative fit of the models. $B_{10} < 1$ ($= 2 * \Delta \log_{10}(HM_i) < 0$) yields evidence for $M_2$. As $B_{10}$ increases over 1, the evidence for the $M_1$ increases, however only values of $B_{10} > 3$ ($= 2 * \Delta \log_{10}(HM_i) > 1$) yield significant support for the $M_1$ and only values $B_{10} > 100$ ($= 2 * \Delta \log_{10}(HM_i) > 4$) are considered decisive support for the $M_1$ (Goodman, 1999; Kass and Raftery, 1995; Nylander et al., 2004).

We first tested the bias introduced by treating the indels as missing characters. To this end we applied a binary model in Bayesian inference coding indels as a variable, to include information from missing characters into the inference. We inferred the tree with this setting (we restricted this test to the tree based on all sequences and the species-dataset without *G. galloti*) and compared the fit and topology of this tree with the tree resulting from the inference treating the indels as missing values. Including the information about deletion did not increase the fit and did not change the topology. Second, we used Bayes factor test to test the topology. For this purpose we constrained the topology in Bayesian inference with specific hypothesis (see below) and compared the fit of the resulting inference to the fit of the unconstrained topology.

Finally, the Bayes factor was used to test the applicability of molecular clock to the data set. In this test, we compared the likelihoods of the non-clock tree and the tree constrained with equal rates on all branches. As the constraint of equal rates did not significantly affect the fit, we estimated divergence times by using the nonparametric rate smoothing method (NPRS) with Powell algorithm, as implemented in r8s (ver. 1.70; Sanderson, 2004). In absence of objective calibration points (e.g., fossil data), we utilised previous estimates for nodes within Lacertinae, derived on the basis of mitochondrial sequences that were included also in our dataset. Where possible, these previously published estimates of node ages were introduced as constraints (with minimum and maximum ages), taking their expected deviations into account when calibrating the calculation. The estimates include the age of the split of *Iberolacerta* at 8.1 Mio years before present (Mya) (±2.3 Mya; Carranza et al., 2004), and the split of *Takydromus* species (Lin et al., 2002) at 10 Mya. We also utilised the previous estimate (Mayer and Pavlicev, 2007, and the references therein) of the separation of the two groups at the next deeper node, Eremiadinae and Lacertinae, assumed (Mayer and Benyr, 1994) to be caused by the colonisation of Africa in the time of first Neogenic contact of Africa and Eurasia 17–19 Mya (Rögl and Steininger, 1983). The standard deviations of the estimates have been generated by bootstrapping the data matrices (PHYLIP, ver. 3.6, Felsenstein, 2005) and assessing the distribution of estimated divergence times by r8s.

Please cite this article in press as: Pavlicev, M., Mayer, W. Fast radiation of the subfamily Lacertinae (Reptilia: Lacertidae): History or methodical artefact?. Mol. Phylogenet. Evol. (2009), doi:10.1016/j.ympev.2009.04.020

## 3. Results

*Gallotia galloti*, *Eremias arguta*, and *Ophisops elegans* were used as outgroups in the first step of this study. The relatively distant phylogenetic position of *Gallotia* enabled us to test the phylogenetic position of *Atlantolacerta andreanskyi* relative to the remaining taxa. The results support the position of *A. andreanskyi* outside of the Lacertinae, as suggested by Arnold et al. (2007). The total tree including *G. galloti* is shown in Online Supplementary material. In further detailed analysis we excluded the distant species *G. galloti* in attempt to gain resolution within Lacertinae, and retained *A. andreanskyi*, *Eremias arguta* and *Ophisops elegans* as outgroups. However the deeper nodes nevertheless could not be resolved.

The test of bias due to treating indels as missing characters revealed no significant bias (harmonic mean of the marginal likelihoods for the inference in which indels were coded as variable: $HM = -31214.2$; harmonic mean for the run where indels were missing characters: $HM = -31210.4$; $2 * \Delta \log(HM) = 1.1e-4$; $B_{10} \sim 1$).

We inferred separate gene trees for each of the five analyzed gene segments (not shown), as well as two trees based on concatenated datasets: the one including all five studied sequences and the second with only nuclear sequences. The two concatenated datasets were analyzed with all taxa as well as with the reduced set of taxa, i.e., excluding *G. galloti*, but the report to follow on the relationships within Lacertinae refers to the inference excluding *G. galloti*. It is also noteworthy, that adding the *cyt b* sequence to the data caused the apparent instability, as reflected in the increased duration of Bayesian inference to reach convergence, however the topology and branch support was not significantly affected when comparing trees derived by test-wise inclusion and exclusion of *cyt b* sequence (not shown). We present the inferred phylogenies based on the two concatenated datasets in comparison in Fig. 1. The topologies of the two trees differ, however the main lineages to be discussed below are well supported in both (Fig. 1). Furthermore, the support values of different algorithms (given in parentheses) correspond for these lineages. Fig. 1 shows all the nodes with posterior probabilities (PP) greater than 0.6, however we discuss only the nodes with PP > 0.95. We also provide the support values for the Neighbor Joining (NJ), the Maximum Likelihood (ML) and the Maximum Parsimony (MP) algorithms that were run for the total dataset only. The results from different algorithms are generally in accord with the Bayesian support. The differences between the two combined trees in Fig. 1 are mostly restricted to the nodes that have low support or to the differences in degree of resolution. For example, *Takydromus* clusters with Lacertinae in all trees, but branches off basally in the total tree.

In Table 6, we summarise the evidence with respect to the main findings of the study in parallel for the gene trees derived on only mitochondrial, only nuclear and combined sequence data (with and without *cyt b*). The single-gene trees are not shown separately here (except for the information on *cyt b* in Table 6). The overall evidence is in support of multiple equivalent lineages branching off quasi-simultaneously. The genera included in this study with multiple species are monophyletic in all trees: *Phoenicolacerta*, *Iberolacerta*, *Parvilacerta*, and *Anatololacerta*. Further, clustering of *Scelarcis* and *Teira* is well supported in all trees. Note that both genera consist only of a single species each (Table 3). All the trees based on dataset without *cyt b* support the association of *Parvilacerta* with *Anatololacerta* (Table 6). On the contrary, we do not find support for the clustering of *Darevskia valentini* with *Iranolacerta* suggested by Arnold et al. (2007). The evidence is also weak for the distinct groupings of the following potential sister-taxa: *Archaeolacerta bedriagae* and *Darevskia valentini*, *Timon lepidus* and *Apathya cappadocica*, as well as *Lacerta agilis* and *A. cappadocica*. The genus *Algyroides* is the only seemingly paraphyletic genus in this inference. In all trees, *Dinarolacerta* branches off within the *Algyroides* clade. This finding seems surprising; therefore we tested this particular topological aspect by a Bayes factor test, comparing the fit of the unconstrained topology against the fit of the topology constrained with *Algyroides* as a monophyletic group. The resulting *HM* of the marginal likelihood values across the post burn-in trees were similar (unconstrained: $HM_1 = -31210.4$ and constrained topology: $HM_2 = -31216.7$; $2 * \Delta \log(HM) = 1.8e-4$) for the dataset including all gene sequences. We also performed analogous test for the inference based on the nuclear genes alone (*rag1* and *c-mos*). The result was equivalent (unconstrained: $HM_1 = -6006.1$; constrained $HM_2 = -6009.6$; $2 * \Delta \log(HM) = 5.1e-4$), therefore we conclude that the resulting paraphyly does not have sufficient statistical support, in spite of it appearing repeatedly in all inferred trees.

## 4. Discussion

Several conclusions at the level of genera are well supported in this study. The position of *A. andreanskyi* outside of Lacertinae (Arnold et al., 2007) is clearly corroborated in our study in all single gene datasets, as well as in the combined datasets when *Gallotia* is included (Table 6; also supplementary material). Furthermore, the monophyly of all but one polytypic genera suggested by Arnold et al. (2007) is well supported in our study, in cases where more than one species was included in our dataset. *Takydromus* clusters with Lacertinae in all trees, but branches off basally in the total tree, indicating possible divergence of this eastern palaearctic genus from the rest before the divergence of the western palaearctic Lacertinae.

The only potentially paraphyletic genus is *Algyroides*, as *Dinarolacerta* (*D. mosorensis* and *D. montenegrina*) consistently clusters with *Algyroides* in all trees. In spite of this association being present repeatedly, the difference in fit between the tree constrained with monophyly and the unconstrained tree is not statistically significant ($B_{10} \sim 1$), thus the support for the paraphyly of *Algyroides* is not sufficient given the present data. Nevertheless, in the light of morphological differences between *Algyroides* and *Dinarolacerta* even an indication of the very close relationship is surprising and should be examined closer. The morphological characters considered synapomorphies of *Algyroides* (e.g., large dorsal scales with micro ornamentation; Arnold et al., 2007) are practically absent in *Dinarolacerta*. If *Dinarolacerta* were a subclade within *Algyroides*, these characteristics would therefore have been lost secondarily, for example as an adaptation to narrow crevices, i.e., a special niche within the rock habitats. A perhaps useful hint for the morphological study, could be that *Dinarolacerta* always appears in the same position and splits *Algyroides* into two groups reflecting the distributions in the western (*A. fitzingeri* + *A. marchi*), respectively, more eastern part of the Mediterranean region (*A. moreoticus* + *A. nigropunctatus*). These relationships within the genus *Algyroides* have been found before based on morphology and molecular data (Harris et al., 1999b).

Apart from the older radiation of 17 lineages, our study reveals several clades resulting from later branching. These events gave rise to the following taxa, currently treated as genera: *Dinarolacerta* (discussed above), *Scelarcis and Teira*, *Parvilacerta* and *Anatololacerta* (Arnold et al., 2007). In the particular suggested taxonomy, two genera consist of a single species each: *Teira dugesii* and *Scelarcis perspicillata*. These species were previously unified in a subgenus *Teira* of the genus *Podarcis* by Richter (1980) on the basis of morphological features. Later, Mayer and Bischoff (1996) elevated this subgenus to genus level. Our analyses corroborate that *T. dugesii* and *S. perspicillata* are sister taxa. The current generic level

Please cite this article in press as: Pavlicev, M., Mayer, W. Fast radiation of the subfamily Lacertinae (Reptilia: Lacertidae): History or methodical artefact?. Mol. Phylogenet. Evol. (2009), doi:10.1016/j.ympev.2009.04.020

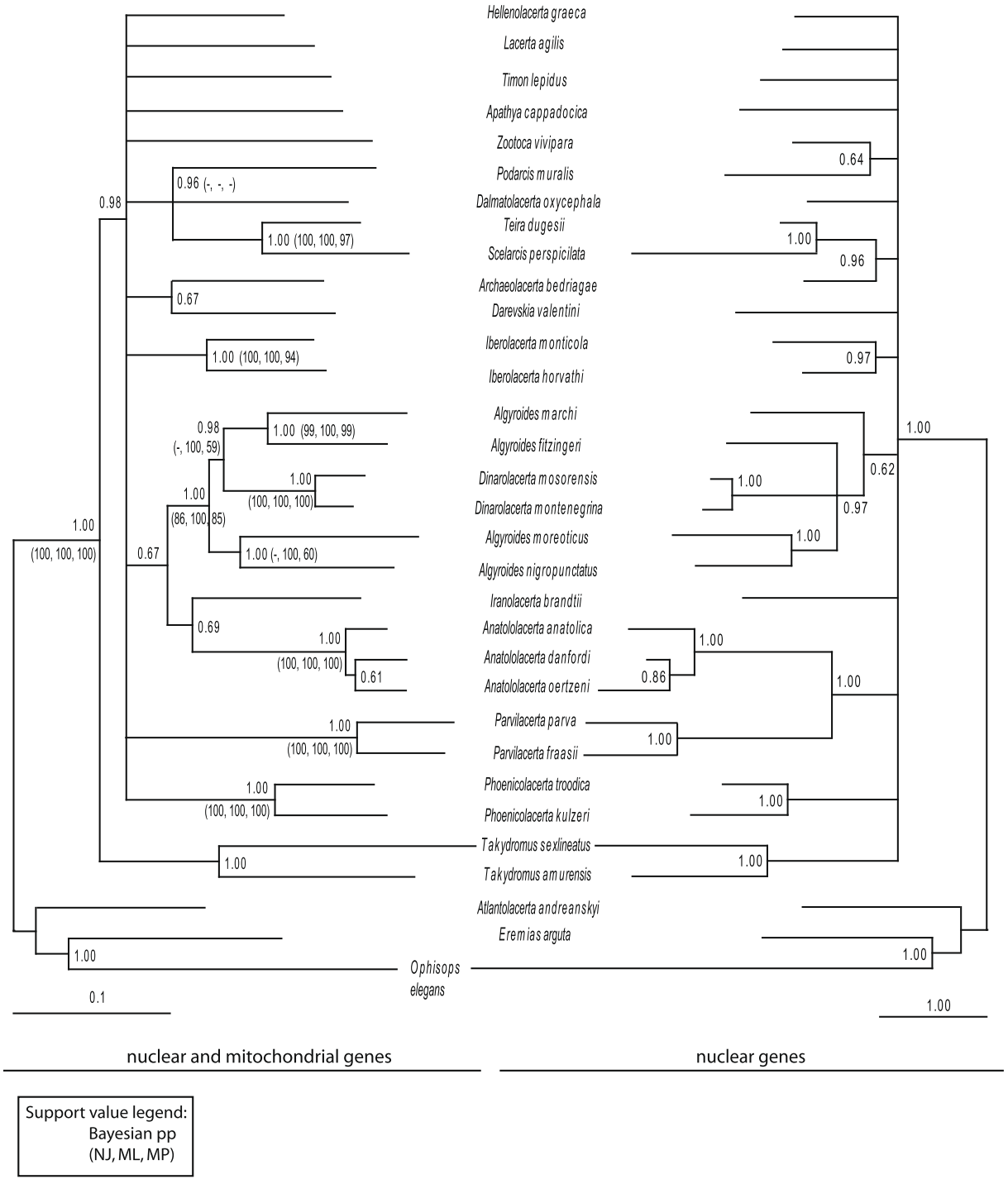


**Fig. 1.** Phylogenetic trees inferred on the basis of partial sequences of all five genes: *cyt b*, *12S rRNA*, *16S rRNA*, *rag1*, and *c-mos*. The tree on the left side is inferred by including all gene sequences and the one on the right based only on nuclear genes. The support values are reported for Bayesian inference (posterior probabilities; MrBayes, version 3.1; Huelsenbeck and Ronquist, 2001) NJ [1000 replicates], ML and MP (bootstrap values for 100 replicates) trees for the tree based on all five genes and only for Bayesian inference for the nuclear tree. In Bayesian method the major consensus tree was inferred from the 50% of the sampled trees (see Section 2 for detail). We collapsed the branches with Bayesian posterior probability of <0.6. The total length of this dataset is 3598 bp. The trees were inferred after *G. galloti* has been excluded from dataset (for the tree including *G. galloti*, see Online Supplementary material).

is based on arbitrary assignment of the taxonomic level of a genus to a certain genetic or morphological distance between solely two species. Although both these species show some diversification below the species level (island-forms in *T. dugesii*, Brehm et al., 2003; mitochondrial lineages but non-corresponding morphological diversification in *S. perspicillata*, Perera et al., 2007) they are closely related and do consist of single species each, hence taxonomy could be simplified by re-uniting them in the same genus (*Teira*).

Two overarching questions are of interest here: (1) the cladistic pattern of Lacertinae evolution and (2) when and why the presumptive basal radiation could have occurred. The main result of our study is the support for the hypothesis that the members of the subfamily Lacertinae group into at least 17 equivalent branches not only in fast evolving mitochondrial genes but also when considering only the comparatively slowly evolving nuclear genes. Therefore, saturation of substitutions in the mitochondrial se-

Please cite this article in press as: Pavlicev, M., Mayer, W. Fast radiation of the subfamily Lacertinae (Reptilia: Lacertidae): History or methodical artefact?. Mol. Phylogenet. Evol. (2009), doi:10.1016/j.ympev.2009.04.020

**Table 6**
Comparisons among the separate gene trees regarding the bayesian posterior probabilities for the specific nodes, reflecting sister-taxa relationships. Separate trees are based on nuclear, mitochondrial, and full datasets, respectively, including and excluding *cyt b*.

| | Gene sequence (s) | | | | |
|---|---|---|---|---|---|
| | *rag1/c-mos* | *12s/16s* | *cyt b* | All | All excl. *cyt b* |
| *A. andreanskyi*–Eremiadinae[a] | 1.00 | Basal | .99 | 1.00 | 1.00 |
| *Parvilacerta* | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| *Anatololacerta* | 1.00 | 1.00 | .95 | 1.00 | 1.00 |
| *Phoenicolacerta* | 1.00 | 1.00 | .97 | 1.00 | 1.00 |
| *Iberolacerta* | .97 | 1.00 | .97 | 1.00 | .97 |
| *Teira-Scelarcis* | 1.00 | 1.00 | .96 | 1.00 | 1.00 |
| *Parvilacerta–Anatololacerta* | 1.00 | .94 | //[b] | //[b] | 1.00 |
| *Phoenicolacerta–Lacerta* | //[b] | .73 | //[b] | //[b] | //[b] |
| *Algyroides–Dinarolacerta* | .97 | 1.00 | .99 | 1.00 | .97 |
| *Teira–A. bedriagae* | .96 | //[b] | //[b] | //[b] | .96 |
| *D. valentini–A. bedriagae* | //[b] | //[b] | .69 | .67 | //[b] |
| *D. valentini–I. brandtii* | //[b] | .91 | //[b] | //[b] | //[b] |
| *T. lepidus–A. cappadocica* | //[b] | 1.00 | //[b] | //[b] | //[b] |
| *L. agilis–A. cappadocica* | //[b] | //[b] | .55 | //[b] | //[b] |

"Basal" denotes the position of *A. andreanskyi* with respect to Eremiadini representatives, whether it is a sister-taxa relationship (posterior probability is given) or unresolved basal polytomy. Support for some of the nodes is reported due to previous suggestions in the literature, but is not discussed in text due to lack of sufficient evidence.

[a] The first row is based on trees including *G. galloti*, further rows are based on inferences calculated excluding distant outgroups (see text).

[b] Missing sister-taxa relationship.

quences can hardly be responsible for this result. In contrast, we assume that the polytomy reflects a multiple cladogenesis in a geologically short time. The second question, the dating of this event, is problematic mainly due to two reasons: the lack of reliable calibration points (i.e., the lack of appropriate fossil records), and the potential saturation of mtDNA substitutions that hamper the use of available mtDNA molecular clocks. Additionally, the applicability of molecular clock depends on homogeneity of evolutionary rates among branches. Previous studies using *c-mos* and mitochondrial genes (*cyt b*, 12S rRNA, 16S rRNA) at higher taxonomic levels have often found high evolutionary rate heterogeneity that precluded the application of molecular clock at the particular level (Harris et al. 1998, 1999a; Mayer and Pavlicev, 2007). At the studied level the Bayes factor test on the combined sequence indicated no significant effect on fit when constrained to equal rates on all branches, given the same topology ($HM_1 = -31262.5$ for the constrained uniform rates *vs.* $HM_2 = -31210.4$ for unconstrained rates, $2 * \Delta \log(HM) = 0.00145$; $B_{10} \sim 1$). However, applying molecular clock to estimate the age of the polytomy yields variable time estimates depending on the calibration points included in the calculation thus it should be treated with caution. Using the tree that includes *Gallotia galloti* and constraining the clock with estimates of the divergence of the two *Iberolacerta* species, the divergence of Eremiadinae and Lacertinae at 17 Mya and an estimated divergence of the two *Takydromus* species (10 Mya), yields 14.7 Mya (however with standard deviation [SD] = 7.45!). Using the tree without *Gallotia galloti* yields estimates of 13.9 Mya (SD = 3.00). Using the divergence of Eremiadinae and Lacertinae as the interval between 17 and19 Mya instead of fixed point yields an estimate of 14.9 Mya (SD = 2.73) for the divergence of Lacertinae.

Obviously, the estimates derived here depend on many assumptions, such as the accuracy of previous estimates (again depending on the assumptions that were made, including possibly different topology), and such as the assumption that the mtDNA saturation does not affect the rates at the deeper nodes. The standard deviations of the estimated divergence times are very high and thus estimates, considering their 95% confidence interval (i.e., ±1.96 * SD), yield little information. One existing estimate for this event has placed it into the mid-late Miocene (13–9 Mya; Carranza et al., 2004), however note that this estimate was based on a different tree topology of the Lacertinae which included *A. andreanskyi*, and again itself based on previous estimates of divergence times for *Gallotia*. Mayer and Benyr (1994) suggested the radiation to have taken place in the early Miocene based on the albumin immunological data.

Because the present study based on molecular data allows only broad estimate of the age of polytomy, limited with previous assumptions, we consider independent information from the recent palaeoclimatological and palaeogeographical data. The one assumption used here is that the separation at the next deeper node of the two subfamilies, Eremiadinae and Lacertinae, was associated with the time of the first Neogenic contact of Africa and Eurasia 17 to 19 Mio years before present (Rögl and Steininger, 1983) and the subsequent colonisation of Africa (Mayer and Benyr, 1994; Mayer and Pavlicev, 2007; and the references therein). Adopting this estimate, it follows that the main radiation of Lacertinae must have happened later, therefore somewhat after 17 Mya. The second assumption is that it took place in what is today south-eastern Europe and western Asia, where the diversity is still highest at present. We particularly look for a distinct period of ecological fragmentation potentially causing bigger population to diversify. The relevant ecological factors, on which data is available, are the distribution of land and water bodies due to changes in water levels, and the local climate, depending both on global climate and local geography. Both can significantly affect the dynamics of distribution of the land-bound ectothermic group (Böhme, 2003).

Indeed there exists congruent evidence of a relatively stable period, followed by distinct fragmentation of land and local diversification of climate. Palaeogeographic studies, specifically those of the S. European region during the Miocene (e.g., Rögl and Steininger, 1983; Rögl, 1999) suggest repeated redistributions of land, as the tectonic movements caused the two large water bodies, the Parathetys and the Mediterranean Sea to constantly change in size. However, the apparent pulsing of water bodies was interrupted for a somewhat extended period of time, when the African continent including Arabia collided with Eurasia approximately 19–17 Mya (Rögl and Steininger, 1983), causing continentalization of Middle Europe. The changes of water level started again with the water level rise, reinforcing the fragmentation in Langhian (16–13.5 Mya), and the drop in the Serravallian etc. (13.6–11.6 Mya; Rögl, 1999).

Furthermore, dramatic climate shifts paralleled geographical changes. The Langhian again is distinct in its relatively high temperature and humidity (Bruch et al., 2007), peaking slightly earlier, at about 18–16 Mya ("Miocene climatic optimum"; Böhme, 2003). Nevertheless, at the end of the Langhian period, about 13.9 Mya, a significant worldwide cooling began (Holburn et al., 2005).

Please cite this article in press as: Pavlicev, M., Mayer, W. Fast radiation of the subfamily Lacertinae (Reptilia: Lacertidae): History or methodical artefact?. Mol. Phylogenet. Evol. (2009), doi:10.1016/j.ympev.2009.04.020

Additionally, in the western Palaearctic the general elevation of land and thus the reduction of Parathetys, and local mountain-building (Carpathians), affected the climate on a local scale. The temperature decrease was accompanied by local spatial climate differentiation, presumably enhancing the ecological niche differentiation. Finally, the Middle Miocene Disruption is suggested to have taken place concurrently, dated at 14.8–14.5 Mya (Miller and Fairbanks, 1983; Allmon and Bottjer, 2001). This period is often associated with a wave of extinctions at least in the mammalian fauna; however its extent, importance, and causes are under discussion. Theoretically it could have been important for Lacertinae lizards in reducing predatory and/or competition pressure.

We suggest that this window of continentalization, followed by land fragmentation may coincide with the estimated time of multiple cladogenesis in Lacertinae. The fragmentation may have caused radiation on a large scale, as multiple populations may have become isolated relatively simultaneously from a big population, and speciation could have followed in allopatry. To what extent, and if at all, any of these events were crucial for the Lacertinae radiation is certainly a speculation. However, the geographical and climatic events described consistently date in a relatively narrow time window of the Langhian, 16–13.5 Mya, probably towards the end of this period (14–13.5 Mya). This time window differs only slightly from previous estimates by means of an mtDNA molecular clock (Carranza et al., 2004).

In conclusion, the present study provides support for a quasi-simultaneous radiation of Lacertines based on the divergence of nuclear and mitochondrial sequences and parallel palaeogeographic information. We show that the resolution of the phylogeny based on nuclear genes alone is not improved significantly when including even long mitochondrial sequences. The study corroborates most of the recently suggested monophyletic lineages. From the morphological viewpoint the most surprising result is a close association of *Dinarolacerta* with *Algyroides*, however the evidence is not sufficient to support paraphyly of *Algyroides*. Further data, both molecular and morphological, are necessary to clarify the importance of this association.

## Acknowledgments

We thank O. Arribas (Barcelona, Spain), W. Bischoff (Bonn, Germany), S. Carranza (Barcelona, Spain), J. Harris (Vairao, Portugal), J. Schmidtler (München, Germany), and B. Trapp (Wuppertal, Germany) for sharing samples. F. Steininger (Frankfurt, Germany) kindly provided help with the palaeogeographical references. Further we thank Simone Latkolik and Barbara Däubl for help in the laboratory, and E. Haring, L. Kruckenhauser, W. Pinsker (Vienna, Austria), and L. Mielke (St. Louis, USA) for valuable comments on previous drafts of the paper. We also particularly acknowledge very useful suggestions of two reviewers. M.P. thanks J. Cheverud (St. Louis, USA) for technical support.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.04.020.

## References

Allmon, W.D., Bottjer, D.J., 2001. Evolutionary Paleoecology: The Ecological Context of Macroevolutionary Change. Columbia University Press, New York.

Arnold, E.N., Arribas, O., Carranza, S., 2007. Systematics of the palaearctic and oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae), with descriptions of eight new genera. Zootaxa 1430, 1–86.

Arribas, O., 1997. Morfología, filogenia y bibliografía de las lagartijas de alta montana de los Pirineos. Ph.D. Thesis, Universitat Autonoma de Barcelona.

Böhme, M., 2003. The Miocene climatic optimum: evidence from ectothermic vertebrates of Central Europe. Palaeogeogr. Palaeoclimatol. Palaeoecol. 195, 389–401.

Brehm, A., Jesus, J., Spinola, H., Alves, C., Vicente, L., Harris, D.J., 2003. Phylogeography of the Madeiran endemic lizard *Lacerta dugesii* inferred from mtDNA sequences. Mol. Phyl. Evol. 26, 222–230.

Bruch, A.A., Uhl, D., Mosbrugger, V., 2007. Miocene climate in Europe – Patterns and evolution. A first synthesis of NECLIME. Palaeogeogr. Palaeoclimatol. Palaeoecol. 253, 1–7.

Carranza, S., Arnold, E.N., Amat, F., 2004. DNA phylogeny of Lacerta (*Iberolacerta*) and other lacertine lizards (Reptilia: Lacertidae): did competition cause long-term mountain restriction? Syst. Biodiversity 2, 57–77.

Felsenstein, J., 2005. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the Author. Department of Genome Sciences. University of Washington, Seattle.

Gontcharov, A.A., Marin, B., Melkonian, M., 2004. Are combined analyses better than single gene phylogenies? A case study using SSU rDNA and rbcL sequence comparisons in the Zygnematophyceae (Streptophyta). Mol. Biol. Evol. 21, 612–624.

Goodman, S.N., 1999. Toward evidence-based medical statistics. 2. The Bayes factor. Ann. Int. Med. 130, 1005–1013.

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

Harris, D.J., Arnold, E.N., Thomas, R.H., 1998. Relationships of lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. Proc. R. Soc. Lond. B 265, 1939–1948.

Harris, D.J., Sinclair, E.A., Mercader, N.L., Marshall, J.C., Crandall, K.A., 1999a. Squamate relationships based on c-mos nuclear DNA sequences. Herpetol. J. 9, 147–151.

Harris, D.J., Arnold, E.N., Thomas, R.H., 1999b. A phylogeny of the European lizard genus Algyroides (Reptilia: Lacertidae) based on DNA sequences, with comments on the evolution of the group. J. Zool. Lond. 249, 49–60.

Holburn, A., Kuhnt, W., Schulz, M., Erlenkeuser, H., 2005. Impacts of orbital forcing and atmospheric carbon dioxide on Miocene ice-sheet expansion. Nature 438, 483–487.

Huelsenbeck, J.P., Ronquist, F., 2001. Mr. Bayes: Bayesian inference of phylogenetic trees. Bioinformatics (Oxford, England) 17, 754–755.

Kass, R.E., Raftery, A.E., 1995. Bayes factors. J. Am. Stat. Assoc. 90, 773–795.

Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence. J. Mol. Evol. 29, 170–179.

Lin, S.-M., Chen, C.A., Lue, K.-Y., 2002. Molecular phylogeny and biogeography of the grass lizard genus *Takydromus* (Reptilia: Lacertidae) of East Asia. Mol. Phylogen. Evol. 22, 276–288.

Mayer, W., Benyr, G., 1994. Albumin-Evolution und Phylogenese in der Familie Lacertidae (Reptilia: Sauria). Ann. Naturhist. Mus. Wien 96B, 621–648.

Mayer, W., Bischoff, W., 1996. Beiträge zur taxonomischen Revision der Gattung Lacerta (Reptilia: Lacertidae). Teil I: *Zootoca*, *Omanosaura*, *Timon* und *Teira* als eigenstaendige Gattungen. Salamandra 32, 163–170.

Mayer, W., Pavlicev, M., 2007. The phylogeny of the family Lacertidae (Reptilia) based on nuclear DNA sequences: convergent adaptations to arid habitats within the subfamily Eremiainae. Mol. Phylogen. Evol. 44, 1155–1163.

Miller, K.J., Fairbanks, R.G., 1983. Evidence for Oligocene–middle Miocene abyssal circulation changes in the western North Atlantic. Nature 306, 250–253.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J.L., 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53, 47–67.

Pavlicev, M., Mayer, W., 2006. Multiple copies of coding as well as pseudogene c-mos sequence exist in three lacertid species. J. Exp. Zool. B 306, 539–550.

Perera, A., Vasconcelos, R., Harris, J.D., Brown, R.P., Carretero, M.A., Pérez-Mellado, V., 2007. Complex patterns of morphological and mtDNA variation in *Lacerta perspicillata* (Reptilia; Lacertidae). Biol. J. Linn. Soc. 90, 479–490.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics (Oxford, England) 14, 817–818.

Richter, K., 1980. *Lacerta dugesii* Milne-Edwards, 1829 und *Lacerta perspicillata* Duméril et Bibron, 1839 gehören zur Gattung *Podarcis* Wagler, Subgenus *Teira* Gray, 1838. Zool. Abh. Staatl. Mus. Tierkde. Dresden 36, 1–9.

Rögl, F., 1999. Mediterranean and Parathetys. Facts and hypotheses of an Oligocene to Miocene palaeogeography (short overview). Geol. Carpathica 50, 339–349.

Rögl, F., Steininger, F.F., 1983. Vom Zerfall der Tethys zu Mediterran und Paratethys. Die neogene Palaeogeographie und Palinspatik des zirkummediterranen Raumes. Ann. Naturhist. Mus. Wien 85, 135–163.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory Press.

Sanderson, M.J., 2004. R8s, Inferring absolute rates of molecular evolution and divergence times. Version 1, 70.

Swofford, D.L., 2000. PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Sinauer Associates, Sunderland, Massachusetts.