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The phylogeny of the family Lacertidae (Reptilia) based on nuclear DNA sequences: Convergent adaptations to arid habitats within the subfamily Eremiainae

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Abstract

The family Lacertidae encompasses more than 250 species distributed in the Palearctis, Ethiopis and Orientalis. Lacertids have been subjected in the past to several morphological and molecular studies to establish their phylogeny. However, the problems of convergent adaptation in morphology and of excessively variable molecular markers have hampered the establishment of well supported deeper phylogenetic relationships. Particularly the adaptations to xeric environments have often been used to establish a scenario for the origin and radiation of major lineages within lacertids. Here we present a molecular phylogenetic study based on two nuclear marker genes and representatives of 37 lacertid genera and distinct species groups (as in the case of the collective genus *Lacerta*). Roughly 1600 bp of the nuclear *rag1* and *c-mos* genes were sequenced and analyzed. While the results provide good support to the hitherto suggested main subfamilies of Gallotiinae (*Gallotia* and *Psammodromus*), Eremiainae and Lacertinae [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], they also suggest unexpected relationships. In particular, the oriental genus *Takydromus*, previously considered the sister-group to the three subfamilies, is nested within Lacertinae. Moreover, the genera within the Eremiainae are further divided into two groups, roughly corresponding to their respective geographical distributions in the Ethiopian and the Saharo-Eurasian ranges. The results support an independent origin of adaptations to xeric conditions in different subfamilies. The relationships within the subfamily Lacertinae could not be resolved with the markers used. The species groups of the collective genus *Lacerta* show a bush-like topology in the inferred Bayesian tree, suggesting rapid radiation. The composition of the subfamilies Eremiainae and Lacertinae as well as their phylogeography are discussed.

Keywords: Lacertidae; Eremiainae; Lacertinae; Phylogeography; Adaptation

1. Introduction

Boulenger's systematics of the family Lacertidae (1920, 1921) based on external morphological traits persisted nearly unchanged until the early 70s despite several apparent deficiencies arising from the use of plesiomorphic characters. Arnold (1973) separated the genera *Gallotia* and *Podarcis* from *Lacerta* on the basis of osteological traits. Szczerbak (1975) further ascribed generic status to Boulenger's 'sections' of *Eremias*. A concise phylogenetic study of the family Lacertidae (Arnold, 1989) was founded on numerous, predominantly morphological characters. It revealed two groups: a 'primitive Palearctic and Oriental assemblage' and an 'advanced Saharo-Eurasian and Ethiopian clade'. First examinations of molecular characters (albumin-immunological studies by Lutz and Mayer, 1984, 1985) indicated greater divergence of the genera *Gallotia* and *Psammodromus* from all other studied lacertids. Mayer and Benyr (1995) applied the same method with an extended data set including almost all lacertid genera.

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They confirmed the distinct position of *Gallotia* and *Psammodromus* and accordingly established a subfamily Gallotiinae encompassing these two genera and contrasting them to the subfamily Lacertinae, which comprised all remaining taxa. Furthermore, this study implied paraphyly of Arnold's (1989) 'advanced Saharo-Eurasian and Ethiopian clade', with part of the Saharo-Eurasian genera being closely related to the European taxa. Harris et al. (1998) and Fu (2000) presented sequence data of several mitochondrial (mt) genes (partial sequences of *12SrRNA*, *16SrRNA* and *cytochrome b*) for various lacertid taxa. However, these data alone were not able to resolve the phylogenetic relationships within the family Lacertidae. In the study of Harris et al. (1998) a combination of the morphological data originally compiled by Arnold (1989) with mtDNA sequence data led to a phylogenetic hypothesis generally congruent with the results of the morphological analyses. Consequently the Lacertinae were confined to the group corresponding roughly to the 'primitive Palearctic and Oriental assemblage' as described by Arnold (1989). Furthermore, a newly established subfamily Eremiainae was assigned to the group matching Arnold's (1989) 'advanced Saharo-Eurasian and Ethiopian clade'.

In all previous studies the position of the genus *Takydromus* remained unresolved. Due to the hemipenal structure that is characteristic for this genus, Arnold (1986) considered it a possible sister-group to all other Lacertidae. The later analysis of numerous morphological features (Arnold, 1989) placed it within the 'primitive Palearctic and Oriental assemblage' close to *Zootoca*. Albumin-immunological data indicated either a 3-way split with the 'Eurasian' and 'African' groups or within the 'African' group (Mayer and Benyr, 1995). The results obtained from mtDNA sequences (Fu, 2000) were contradictory depending on the outgroup and the genes used for the analysis.

Within the subfamily Lacertinae (sensu Harris et al., 1998; Arnold, 2004) three to eight genera are recognized today, depending on the authors. The reason is that some authors favour a successive splitting of the obviously paraphyletic genus *Lacerta* (e.g., Bischoff and Mayer, 1996) whereas others prefer to uphold an extensive genus *Lacerta* and to treat some species groups at the subgeneric level until precise relationships are established, to avoid instability in the nomenclature (e.g., Harris et al., 1998).

The evolutionary rate and consequent variability of a particular molecular marker determine the levels of phylogenetic diversification that can be addressed by the comparative analysis of that marker. In animals, mt sequences evolve rapidly relative to the nuclear (nc) genome. When applied to infer phylogenetic relationships at deeper levels, such as families, they are prone to homoplasy and thus inappropriate, as demonstrated in a study based on about 4700 bp of the mt genome (Fu, 2000). Therefore nc sequences are often used to resolve relationships at these levels. Partial sequences of the nc *c-mos* gene were employed first by Harris et al. (1999, 2001) to resolve squamate relationships. Townsend et al. (2004) used a combination of mt and nc DNA to address the phylogeny of squamate reptiles. Carranza et al. (2004) used *c-mos* along with mtDNA sequences to investigate relationships among lacertine lizards.

In this study we applied partial sequences of two nc genes, the *recombination activating gene* (*rag1*) and *c-mos* gene to investigate phylogenetic relationships among representatives of most lacertid genera (except *Australolacerta* and *Gastropholis*) as well as among numerous presumptive species groups within *Lacerta*.

2. Materials and methods

A 581 bp section of the nc *c-mos* gene and 1012 bp of the nc *rag1* gene of 44 representatives of 31 lacertid genera and of one species of the family Gerrhosauridae (Table 1) were sequenced and analyzed. Most genera are represented in our study by a single species only. In cases where more than one congeneric species (apart from the collective genus *Lacerta*) was analyzed, monophyly of genera was highly supported in all calculations of phylogenetic inference.

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 units 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 2.

We sequenced PCR products directly and as cloned PCR fragments. The important advantage of the first method is that it can reveal polymorphic sites. These can be due to heterozygosity as well as to diverged multiple copies (functional and non-functional, i.e., pseudogenes). Although *rag1* and *c-mos* have both been considered single copy genes, paralogous copies of *c-mos* were detected in *Lacerta laevis* (Pavlicev and Mayer, 2006). The obvious pseudogene sequences were excluded from the data set of the present study. We found no evidence of pseudogenes in other species.

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 2. 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,

Table 1
List of analyzed specimens, their geographical origin and the GenBank accession numbers for both partial gene sequences (*c-mos* and *rag1*)

Species	Geographical origin	GenBank: *c-mos*	GenBank: *rag1*
Acanthodactylus boskianus	Egypt[a]	EF632251	EF632206
Acanthodactylus scutellatus	Egypt: Abu Simbel	EF632252	EF632207
Adolfus jacksoni	Rwanda: Rukiva	EF632253	EF632208
Algyroides moreoticus	Greece: Korinthia, Feneos	EF632254	EF632209
Algyroides nigropunctatus	Greece: Preveza, Parga	EF632255	EF632210
Archaeolacerta bedriagae	France: Corsica	EF632256	EF632211
Darevskia valentini	Armenia: Rasdan	EF632257	EF632212
Eremias arguta	Ukraine	EF632258	EF632213
Eremias pleskei	Armenia: Ararat region	EF632259	EF632214
Gallotia galloti	Spain: Tenerife Island	EF632260	EF632215
Gerrhosaurus nigrolineatus	Namibia: Windhoek	EF632250	EF632205
Heliobolus lugubris	Namibia: Haruchas	EF632261	EF632216
Heliobolus spekii	Tanzania: Lake Natron	EF632262	EF632217
Holaspis laevis	Tanzania: Usambara Mts.	EF632263	EF632218
Iberolacerta horvathi	Austria: Carinthia, Rattendorf	EF632264	EF632219
Iberolacerta monticola	Portugal: Sierra Estrela	EF632265	EF632220
Ichnotropis squamulosa	Mozambique[a]	EF632266	EF632221
Lacerta agilis	Austria: Lower Austria, Weitra	EF632267	EF632222
Lacerta cappadocica	Turkey: Kayseri, Mt. Ercyas	EF632268	EF632223
Lacerta danfordi	Turkey: Icel, Camliyayla	DQ461743	EF632224
Lacerta graeca	Greece: Lakonia, Monemvasia	EF632269	EF632225
Lacerta laevis	Cyprus: Pafos	DQ461715	EF632226
Lacerta mosorensis	Montenegro: Durmitor Mts.	EF632270	EF632227
Lacerta oxycephala	Croatia: Hvar Island	EF632271	EF632228
Latastia longicaudata	Eritrea: Nakfa	EF632272	EF632229
Meroles suborbitalis	Namibia: Rosh Pinah	EF632273	EF632230
Mesalina guttulata	Tunesia: Tamerza	EF632274	EF632231
Mesalina rubropunctata	Egypt: Hurghada	EF632275	EF632232
Nucras lalandii	South Africa: Stellenbosch	EF632276	EF632233
Omanosaura jayakari	United Arab Emirates: Fujayrah	EF632277	EF632234
Ophisops elegans	Greece: Evros, Gianuli	EF632278	EF632235
Parvilacerta parva	Turkey: Malatya	EF632279	EF632236
Pedioplanis undata	Namibia: Nauchas	EF632280	EF632237
Philochortus spinalis	Eritrea: Ghinda	EF632281	EF632238
Podarcis muralis	Austria: Lower Austria, Gumpoldskirchen	EF632282	EF632239
Poromera fordii	Cameroon: Mt. Nlonako	EF632283	EF632240
Psammodromus algirus	Spain: Lerida	EF632284	EF632241
Psammodromus hispanicus	Spain: Barcelona	EF632285	EF632242
Pseuderemias smithi	Kenya: Lake Turkana	EF632286	EF632243
Takydromus amurensis	Russia: Amur region	EF632287	EF632244
Takydromus sexlineatus	Indonesia[a]	EF632288	EF632245
Teira dugesii	Portugal: Madeira Island	EF632289	EF632246
Timon lepidus	Spain: Alicante	EF632290	EF632247
Tropidosaura gularis	South Africa: SW-Cape	EF632291	EF632248
Zootoca vivipara	Austria: Lower Austria, Schneeberg	EF632292	EF632249

All DNA samples are stored at the Natural History Museum Vienna (NMW), Austria.
[a] Samples provided from the animal trade.

USA). All sequencing was performed by MWG Biotech (Ebersberg, Germany).

2.2. Sequence analysis

The sequences were combined, aligned and edited manually in the programme BioEdit (version 7.0.1; Hall, 1999). No insertions/deletions (indels) were found in the alignment of the Lacertid dataset, but there were two indels (21 bp and 3 bp long) in the *c-mos* sequence of the *Gerrhosaurus nigrolineatus*, a taxon used in the preliminary analysis as an outgroup to explore the suitability of the Gallotiinae as a more closely related outgroup (see the next section). The indels were treated in the analysis as missing characters. Separate trees were calculated for each of the two partial gene sequences, *c-mos* and *rag1*, as well as for the combined data set, consisting of both sequences, and the results were compared. Bayesian inference (BI) (MrBayes, version 3.1; Huelsenbeck and Ronquist, 2001) was used for all three data sets, the combined data set was furthermore analyzed by Maximum Parsimony analysis (MP) using PAUP (version 4.0b10; Swofford, 2000).

The combinability of the *c-mos* and *rag1* sequence data was tested with a partition homogeneity test (PAUP; ver-

Table 2
Primer sequences and the corresponding annealing temperatures (for amplification primers)

Name	Source	Sequence	T (°C)	Gene	Purpose
Hcmos3	This study	5′-ggt gat ggc aaa tga gta gat-3′	55	*c-mos*	PCR (1)[a]
L-1zmos	Whiting et al., 2003[b]	5′-cta gct tgg tgt tct ata gac tgg-3′	55	*c-mos*	PCR (1)/PCR (2)[c]
Hcmos1	This study	5′-gca aat gag tag atg tct gcc-3′	56	*c-mos*	PCR (2)
CMS-77L	This study	5′-cta cgt acc atg gag cta c-3′		*c-mos*	sequencing
CMS-482H	This study	5′-ttg gga aca tcc aaa gtc tc-3′		*c-mos*	sequencing
RAG-R1	This study	5′-aaa atc tgc ctt cct gtt att g-3′	52	*rag1*	PCR (1)
RAG-fo	This study	5′-gaa aag ggc tac atc ctg g-3′	52	*rag1*	PCR (1)/PCR (2)
RAG-re	This study	5′-cca gtt att gct ttt aca gtt c-3′	52	*rag1*	PCR (2)
RGS-380L	This study	5′-ctc agt acc aag atc ctt gc-3′		*rag1*	sequencing
RGS-587H	This study	5′-agc caa act gtt gag gat ac-3′		*rag1*	sequencing

[a] PCR (1) refers to the initial PCR.
[b] The referred primer was modified for this study.
[c] PCR (2) refers to the reamplification (in the case of consequent direct sequencing, see text).

sion 4.0b10; Swofford, 2000). The test revealed no significant conflict between fragments ($P = 0.36$). Additionally, we partitioned the concatenated data set and assigned the individual evolutionary models to the separate genes. We used ModelTest (version 3.7; Posada and Crandall, 1998) to estimate the optimal evolutionary models to be used. BI was applied in all analyses. For the *rag1* and the concatenated sequence, the preferred model was TrN+I+G (Tamura and Nei, 1993), with unequal rates of nucleotide substitutions and a proportion of invariable sites. For *c-mos*, the proposed model was somewhat simpler, K80+G. These models were used for partitioned and separate analysis. The following settings were implemented in BI of the *rag-1* and the concatenated sequence: unequal rates of substitutions (nst = 6) and gamma-shaped rate variation with a proportion of invariable sites (rates = invgamma). Apart from suggesting the frame settings of a model (the above settings), ModelTest also estimates specific prior values (stationary frequencies, substitution rates, proportion of invariable sites and the shape parameter of gamma distribution). We ran both the analysis constrained with fixed prior values, as estimated by ModelTest, as well as the default, less constrained analysis with only the above settings. Both analyses resulted in same topologies, with only three of all posterior-probabilities differing at the third decimal place. Thus the analysis chosen for the further work was the unconstrained analysis. Five Mio generations were run in all BI analyses with a sampling frequency of 100. A majority consensus tree was built with the final 5000 sampled trees. The topology of the tree inferred by BI from the combined data set was subsequently compared to the tree obtained by applying an alternative algorithm for phylogenetic inference, Maximum Parsimony (MP), to this dataset. MP analysis was conducted using the heuristic search mode with 100 repeats, randomized input orders of taxa, and tree bisection–reconnection (TBR) branch-swapping with all codon positions weighted equally. Non-parametric bootstrapping (100 pseudoreplicates, 10 addition-sequence replicates) was used to assess the stability of internal branches in the trees.

2.3. Hypothesis testing

We tested two explicit hypotheses: the goodness-of-fit of the resulting topology in comparison to the previously proposed hypothesis (Arnold, 1989, 2004: p. 23), and the equal rates of substitutions in all lineages. To compare the topologies we applied the Bayes factors (Kass and Raftery, 1995; Nylander et al., 2004), comparing the logarithmically transformed harmonic mean of the likelihood values of the unconstrained analysis to that derived from the a-priori constrained analysis based on the specific hypothesis. This method is analogous to likelihood ratio tests (e.g., Kishino-Hasegawa test; Kishino and Hasegawa, 1989) to compare the goodness-of-fit of two models, but does not require a correction for the differences in the number of parameters in the compared models. The specific topology used to constrain the BI was the topology of the clade Eremiainae, following the pectinate tree in Arnold (1989, 2004). To test the second hypothesis, the homogeneity of evolutionary rates, we performed the relative rate test implemented in PHYLTEST 2.0 (Kumar, 1996).

3. Results

Gerrhosaurus nigrolineatus was used as an outgroup to test the position of the Gallotiinae (*Gallotia* and *Psammodromus*) relative to the remaining species under study. According to the MP and BI trees (not shown) the Gallotiinae form the sister taxon to the rest of the lacertids. We then used the Gallotiinae as the outgroup in the remaining analyses to reduce the effect of homoplasy, as generally introduced by a distantly related outgroup.

The group Gallotiinae is not discussed here further, although, apart from being an outgroup, it is also the third subfamily constituting the lacertid family. When we refer to the two main clades, we mean Eremiainae and Lacertinae. The *p*-distances between pairs of taxa range from 1.4% (23 substitutions between *Archaeolacerta bedriagae* and *Zootoca vivipara*) to 9.4% (150 substitutions between *Psammodromus hispanicus* and *Heliobolus spekii*).

As already mentioned, in cases where more than one congeneric species (apart from the collective genus *Lacerta*) was analyzed, monophyly of genera was highly supported in all calculations of phylogenetic inference. A single exception is the genus *Iberolacerta* of the clade Lacertinae (clade A in Fig. 1), which is variably supported in different calculations. However, branch support is generally low in this clade. MP and BI yielded similar topologies. The MP analysis of the combined data (1593 characters, 968 constant, 381 parsimony-informative) resulted in 1593 most parsimonious trees (length = 836, CI = 0.628, RI = 0.614, RC 0,385). Fig. 1 presents the BI tree, with the bootstrap values obtained in the MP analysis presented as a second value below the posterior-probability values. The MP algorithm yielded low resolution within Eremiainae (clade B), clearly supporting one clade (equivalent to B_1, see below) but leaving the rest largely unresolved (bootstrap value below 50%). The partitioned analysis resulted in identical topology and node support (not shown). Separate calculation of phylogenetic inference from both partial gene sequences by BI yielded topologies comparable to the total-sequence BI phylogeny, unsurprisingly with lower support values (not shown). The differences between the phylogenetic trees inferred separately from the *c-mos* and the *rag1* data were basically limited to differences in branch support, *c-mos* data providing less support than *rag1*. This, however, may be due to the shorter length of the *c-mos* sequence (581 bp versus 1012 bp in *rag1*). Thus, all trees are essentially in accord in that they all support the main findings of this study and in the following we refer to a single tree, resulting from the analysis of the combined data set (Fig. 1).

Phylogenetic analysis in all cases revealed two main clades, named A and B in Fig. 1. They correspond in general to the subfamilies Lacertinae (A) and Eremiainae (B), respectively, as suggested by Harris et al. (1998), using the analyses of both, the mtDNA sequences (Harris et al., 1998) and the morphological traits (Arnold, 1989).

The Lacertinae (clade A) show an unresolved, bush-like topology, where the successive splits of the genera and subgeneric units of *Lacerta* s.l. cannot be inferred. The Eremiainae (clade B) reveals two well supported clades that correspond to almost strictly Ethiopian (i.e., African south of the Saharan desert) genera (clade B_1) and to predominantly Saharo-Eurasian genera (clade B_2).

The variation in the sequences is relatively high in both clades, therefore the presence of polytomies is an indication of multiple splitting events within relatively short time periods, rather than a sign of the low variability of the molecular marker.

We tested the topology resulting from unconstrained analysis against the topology proposed by Arnold (1989), using the Bayes factors. The comparison of log likelihood values of the total species sample (using harmonic means after reaching stationary phase) of the unconstrained analysis (−9654.73) and constrained analysis (−9999.38) revealed significantly better fit of the data with the unconstrained model ($2\log(B_{10}) = 689.3$ [≫150]). When the clade B was considered separately, using *Lacerta agilis* as outgroup, the topology test yielded analogous result (log likelihood values −6727.49 for unconstrained and −7057.6 for constrained analysis; $2\log(B_{10}) = 660.22$ [≫150]).

Branch lengths in the tree (Fig. 1) suggest substantial differences in evolutionary rates that make it difficult to apply a molecular clock. The existence of uniform molecular clock implying the homogeneity of evolutionary rates was tested with relative rate test (PHYLTEST, Kumar, 1996). The test indicated that constancy of mutation rate between clades A and B is rejected at the 5% level ($Z = 5.44531$). Consequently the homogeneity of the rates cannot be assumed and a molecular clock was not applied.

4. Discussion

Most genera of the subfamily Lacertinae as well as species within genus *Lacerta* form a clade with little internal structure. This low resolution does not allow implications about the evolutionary history of the subfamily. The separation of *Lacerta agilis* (*Lacerta* s. str.) and *Zootoca* from the remaining taxa (Gallotiinae excluded), as suggested by the study of albumins (Mayer and Benyr, 1995), was not supported in our study. In contrast, both genera clearly are members of the largely unresolved clade A, the Lacertinae. *Algyroides*, *Podarcis* and *Takydromus* have long been accepted as genera. Even if *Archaeolacerta* (sensu Mayer and Arribas, 2003; comprising exclusively *A. bedriagae*), *Darevskia*, *Iberolacerta*, *Parvilacerta*, *Teira*, *Timon* as well as *Zootoca* (the groups often treated as subgenera of *Lacerta*) are regarded as genera, monophyly of the remaining *Lacerta* group, containing altogether seven lineages, is uncertain. Interestingly, the results also suggest close relationships among two pairs of taxa usually not considered close relatives. Well supported sister-group relationships occur between *Lacerta danfordi* and the genus *Parvilacerta*, and between *L. mosorensis* and *Algyroides*. The grouping of *Lacerta danfordi* with *Parvilacerta* is also indicated by the study of Carranza et al. (2004) based on mt sequences. While the sister-group relationship of the first pair is supported by all algorithms used, the BI analysis of *c-mos* sequences alone does not support the close relationship of the *L. mosorensis* and *Algyroides*. Therefore, these presumptive closer relationships are being further tested and will be presented elsewhere.

Perhaps one of the most surprising results of our study is the position of the oriental genus *Takydromus* within Lacertinae. This genus consists of about 18 species, inhabiting a large range in eastern Asia. In its geographical distribution, *Takydromus* is for the most part separated from the rest of the lacertid genera. The lineage *Takydromus* formed the sister-group to both main clades in the preferred phylogeny of Harris et al. (1998). Arnold (2004) mentioned several morphological similarities of *Takydromus* with different species of the Eremiainae but considered its position unclear.

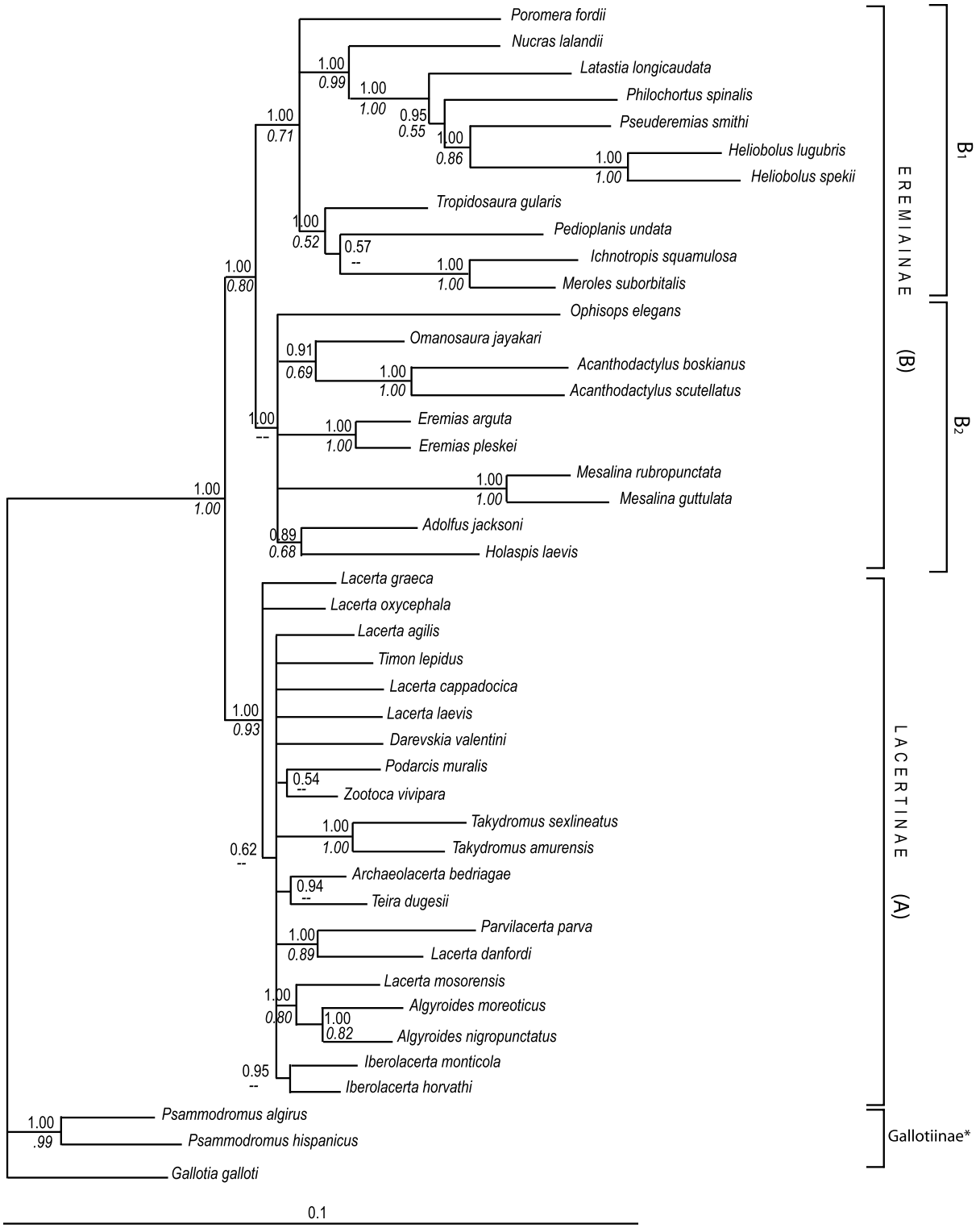

Fig. 1. Phylogenetic tree of the family Lacertidae based on the combined partial *c-mos* and *rag1* nuclear gene sequences and inferred by BI. Posterior-probability values are above the nodes, below in italics are bootstrap values >50% from MP analysis. *Galotiinae are used as the outgroup.

According to our results, it is nested within the Lacertinae (clade A, Fig. 1) and its affiliation to the subfamily Lacertinae is strongly supported, as is the monophyly of both studied species. The origination of this genus from the European clade (corresponding to Lacertinae) was suggested also by *16SrRNA* analyses (Fu, 2000), but has so far been treated as uncertain.

In contrast to the relative homogeneity of distances within the Lacertinae (clade A), the Eremiainae (clade B) are well structured and composed of two subclades,

marked as B_1 and B_2 in Fig. 1. The two main subclades correspond roughly to the geographic distribution of the species included in these clades. The general split of the African and (Saharo-) Eurasian lines was revealed by the study of albumins as well (Mayer and Benyr, 1995), although the affiliation of the genera *Acanthodactylus* and *Adolfus* to the former lineage as indicated by albumins is not supported by the present data. According to the nc sequence data, these two groups cluster clearly with Saharo-Eurasian species (clade B_2).

A morphology-based phylogeny of the Eremiainae produces pectinate tree, with xeric-adapted taxa representing a monophyletic group and more mesic species branching off from the more basal nodes (Arnold, 1989, 2004). Our results support several sister-relationships within Eremiainae that were implied by the study of morphological characters (e.g., *Holaspis* and *Adolfus*; the clade consisting of genera *Nucras, Latastia, Philochortus, Pseuderemias* and *Heliobolus; Meroles* and *Ichnotropis*). However, the previously suggested central notion of monophyletic trend from mesic to xeric species is not supported. We plotted a rough mesic-to-xeric assignment of the genera (according to Arnold, 2004) represented by the chosen species, onto our phylogenetic tree (Fig. 2). We also added the nodal support to inform about the reliability of the evidence. The assignment of even rough ecomorph categories is somewhat difficult due to the differences between micro- and macrohabitat, as well as due to the diversity of the species-specific habitats within the single genera. To account for this problem, we listed all ecomorphs categories represented in the genus. As can be seen in Fig. 2, according to the nc markers the mesic genera *Omanosaura*, *Adolfus*, *Holaspis*, *Tropidosaura* and *Poromera*, considered outgroups to the derived Eremiainae by Arnold (2004), show differential affinities to the two subclades of Eremiainae. *Poromera* and *Tropidosaura* cluster with the Ethiopian genera of Eremiainae (B_1), whereas *Omanosaura*, *Adolfus* and *Holaspis* are members of the Saharo-Eurasian subclade (B_2). The presence of both extreme ecomorphs, the clearly xeric and the clearly mesic, within well supported sister-subclades conflicts with the hypothesis of a unique monophyletic trend towards xeric taxa. Thus, in spite of the difficulties with the assignment of ecomorphs the diagram in Fig. 2 indicates that if the primitive condition was mesic (as implied by outgroup comparison), the xeric forms originated more than once independently in the evolution of the Eremiainae.

The Ethiopian subclade (B_1) includes three groups forming a trichotomy: a group distributed in southern Africa (including *Tropidosaura*, *Pedioplanis*, *Ichnotropis* and *Meroles*), another group with a more East African distribution (*Nucras* and allies), and the West African forest genus *Poromera*. The clustering of the predominantly East African genera *Latastia*, *Philochortus*, *Pseuderemias* and *Heliobolus* is congruent with the grouping based on derived morphological and behavioural traits as well as geographical coherence (Arnold, 2004). All of these genera inhabit increasingly arid regions of north-eastern Africa (Fig. 2). The genus *Nucras* with a more southern geographical distribution splits from the basal node of this clade. It was considered by Arnold (1989) the sister taxon to the other Eremiaine genera. Its position in our tree generally supported a similar hypothesis of mesic-to-xeric transition, but this change occurred within the East African subgroup of the Ethiopian subclade (B_1).

A closer relationship between *Ichnotropis* and *Meroles* was proposed already by the mtDNA study of Harris et al. (1998). In our study, both genera group also with *Pedioplanis* and the mesic *Tropidosaura*. These four genera are confined to southern Africa.

As noted above, the three mesic-adapted genera *Omanosaura*, *Adolfus* and *Holaspis* belong to the Saharo-Eurasian subclade (subclade B_2, Figs. 1 and 2). With respect to their geographical distribution, equatorial African *Adolfus* and *Holaspis* deviate from this otherwise Saharo-Eurasian clade. The four Palearctic genera *Acanthodactylus*, *Eremias*, *Mesalina* and *Ophisops* have also been proposed to constitute a clade based on morphological characters (Arnold, 2004). However, their proposed close relationship to the South African *Pedioplanis* (here B_1) is not supported in this study.

From a morphological, behavioral (Arnold, 1989) and probably also physiological point of view there are numerous genera of Eremiainae with apparently similar adaptations to xeric habitats that have been interpreted as synapomorphies. However, considering the molecular phylogeny, multiple parallel adaptations to xeric habitats are postulated. These may have been promoted by the formation of dry habitats as a result of the gradual cooling and drying of Africa, especially of the southern part, beginning in the Middle Miocene (Bobe, 2006; Jacobs, 2004). The basal radiation of the Lacertinae, estimated by Carranza et al. (2004) to have taken place 13 million years ago, probably occurred in the west-Palearctic. This is upheld by the fact that representatives of almost all lineages can still be found there today. Only *Takydromus* has reached eastern Asia and has undergone extensive cladogenesis there.

Mayer and Benyr (1995) proposed a colonization of Africa by lacertids 17–19 million years ago, immediately after the first Neogenic contact between Eurasia and Africa (Rögl and Steininger, 1983). According to our results this geological event might have influenced either the whole Eremiainae (clade B) or the Ethiopian subclade only (B_1). Arnold (2004), in agreement with the former possibility, suggested a secondary recolonization of southwest Asia from Africa by the 'Saharo-Sindian' group (corresponding to the clade B_2) across a land connection between the Horn of Africa and Arabia, existing until the early Pliocene (ca. 5 million years ago; Whybrow, 1984). Although a reasonable calibration of our tree is not possible (see above), it implies that the separation of the 'Saharo-Eurasian' lineage (B_2) and its first radiation occurred approximately simultaneously with the beginning of the radiation of the clade Lacertinae

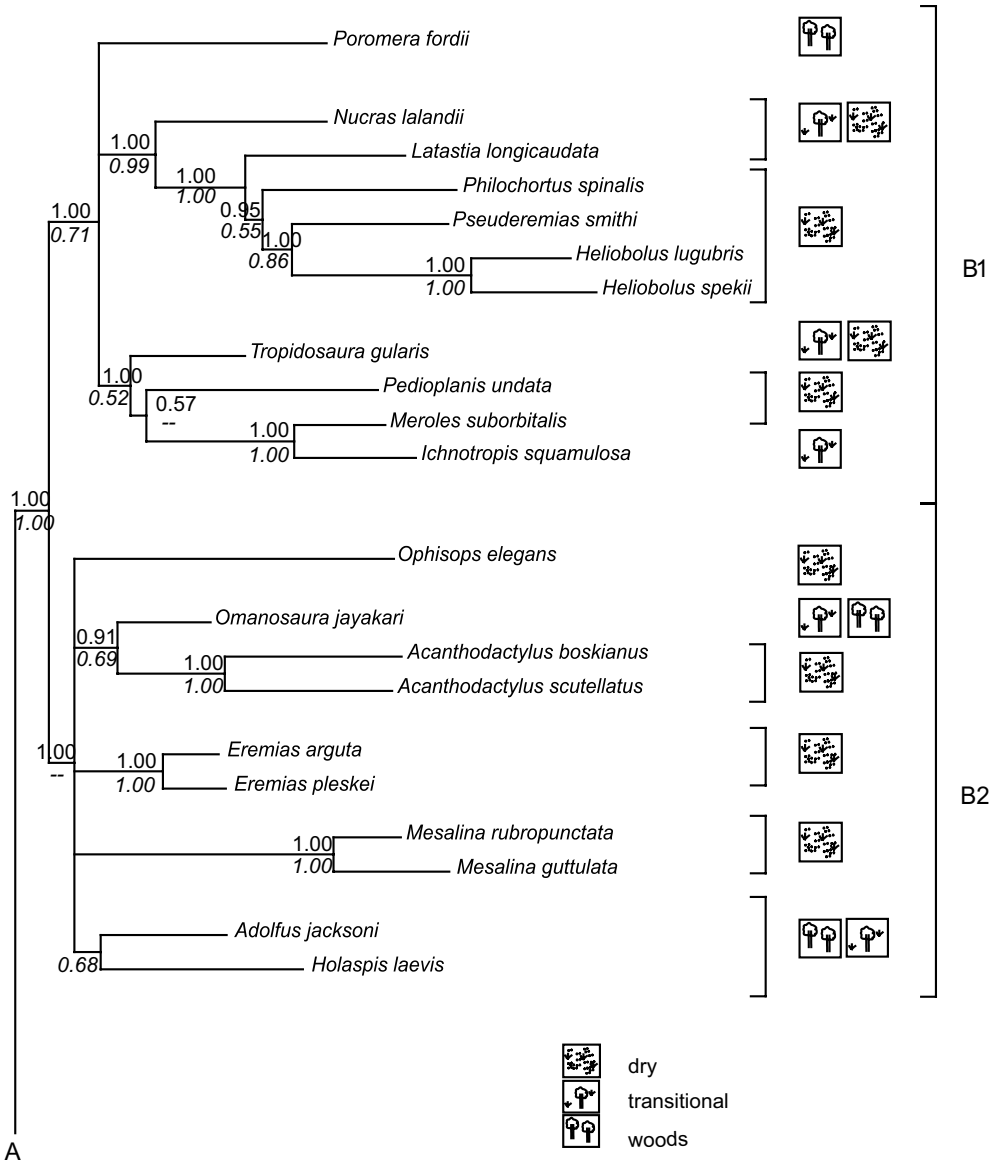

Fig. 2. Clade B of the phylogenetic tree of Lacertidae with the rough assignment of the mesic–xeric ecomorphs categories to genera (according to Arnold, 1989, 2004), represented here by the single species. Note that both clearly xeric and clearly mesophilic ecomorphs occur in clades B_1 and B_2.

(13 million years ago, according to Carranza et al., 2004).

The second feasible scenario would be a separation of the two lineages of the Eremiainae in the Near East and a following independent colonization of Africa by (i) the ancestor of the Ethiopian clade (only B_1) and (ii) the lineage leading to the central African group (*Adolfus* and allies). According to this scenario, the lineage leading to the extant genera *Eremias*, *Mesalina*, *Acanthodactylus* and *Omanosaura* (B_2) would never have left the Palearctic region more than marginally.

Assuming that the estimations of lacertid colonization of Africa (17 million years ago) and the separation of the clades B_1 and B_2 (13 million years ago) are correct, current knowledge of this issue favours the former scenario, under which Africa was colonized by Eremiainae before the split of clades B_1 and B_2, and an ancestor of the B_2 lineage recolonized the Palearctic secondarily.

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