



Evaluating the phylogenetic signal limit from mitogenomes, slow evolving nuclear genes, and the concatenation approach. New insights into the Lacertini radiation using fast evolving nuclear genes and species trees



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ABSTRACT

Estimating the phylogeny of lacertid lizards, and particularly the tribe Lacertini has been challenging, possibly due to the fast radiation of this group resulting in a hard polytomy. However this is still an open question, as concatenated data primarily from mitochondrial markers have been used so far whereas in a recent phylogeny based on a compilation of these data within a squamate supermatrix the basal polytomy seems to be resolved.

In this study, we estimate phylogenetic relationships between all Lacertini genera using for the first time DNA sequences from five fast evolving nuclear genes (*acm4*, *mc1r*, *pdg*, *βfib* and *reln*) and two mitochondrial genes (*nd4* and *12S*). We generated a total of 529 sequences from 88 species and used Maximum Likelihood and Bayesian Inference methods based on concatenated multilocus dataset as well as a coalescent-based species tree approach with the aim of (i) shedding light on the basal relationships of Lacertini (ii) assessing the monophyly of genera which were previously questioned, and (iii) discussing differences between estimates from this and previous studies based on different markers, and phylogenetic methods.

Results uncovered (i) a new phylogenetic clade formed by the monotypic genera *Archaeolacerta*, *Zootoca*, *Teira* and *Scelarcis*; and (ii) support for the monophyly of the *Algyroides* clade, with two sister species pairs represented by western (*A. marchi* and *A. fitzingeri*) and eastern (*A. nigropunctatus* and *A. moreoticus*) lineages. In both cases the members of these groups show peculiar morphology and very different geographical distributions, suggesting that they are relictual groups that were once diverse and widespread. They probably originated about 11–13 million years ago during early events of speciation in the tribe, and the split between their members is estimated to be only slightly older. This scenario may explain why mitochondrial markers (possibly saturated at higher divergence levels) or slower nuclear markers used in previous studies (likely lacking enough phylogenetic signal) failed to recover these relationships.

Finally, the phylogenetic position of most remaining genera was unresolved, corroborating the hypothesis of a hard polytomy in the Lacertini phylogeny due to a fast radiation. This is in agreement with all previous studies but in sharp contrast with a recent squamate megaphylogeny. We show that the supermatrix approach may provide high support for incorrect nodes that are not supported either by original sequence data or by new data from this study. This finding suggests caution when using megaphylogenies to integrate inter-generic relationships in comparative ecological and evolutionary studies.

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1. Introduction

The squamate reptile family Lacertidae is a clade of small-bodied lizards distributed in the Palearctic and Africa. It comprises two sub-families, the Gallotiinae (2 genera, 13 species) and the Lacertinae (41 genera, 308 species), with the latter divided in two

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tribes, Eremiadini (22 genera, 184 species) and Lacertini (19 genera, 124 species) (Arnold et al., 2007; Uetz and Hošek, 2015). As the most common lizard family in Europe, lacertids have been widely used as model species to answer questions on ecology and evolutionary biology, such as testing hypotheses on functional ecology (e.g. Vanhooydonck and Van Damme, 1999; Herrel et al., 2008; Baeckens et al., 2015), natural selection (e.g. Salvi et al., 2009; Heulin et al., 2011) or biogeography (e.g. Harris et al., 2002; Carranza et al., 2004; Poulakakis et al., 2005; Salvi et al., 2013). All such diverse assessments require an understanding of the evolutionary history of the group, so that comparisons can be drawn within a phylogenetic framework.

Over the last decades several morphological, bio-chemical and molecular studies have been conducted in order to infer the phylogeny of Lacertidae (Harris et al., 1998; Fu, 1998, 2000; Arnold et al., 2007; Mayer and Pavlicev, 2007; Hipsley et al., 2009; Pavlicev and Mayer, 2009; Cox et al., 2010). While the phylogenetic relationships within Gallotiinae and Eremiadini are relatively well known (e.g. Mayer and Pavlicev, 2007; Cox et al., 2010), the phylogeny of the tribe Lacertini is still mainly unresolved, with conflicting hypotheses and little corroboration between studies, particularly in the internal nodes. Indeed, although a few relationships within the tribe have been estimated with confidence and consistently across the previous studies, such as the case of the sister taxa relationships between the monotypic genera *Scelarcis* and *Teira* or between the genera of green lizards *Lacerta* and *Timon*, the phylogenetic position of the majority of taxa remains unknown. Moreover, the monophyly of the genus *Algyroides* was recently questioned (Pavlicev and Mayer, 2009). Since the lack of phylogenetic resolution shown by early studies may be due to insufficient data, Mayer and Pavlicev (2007) and Pavlicev and Mayer (2009) performed phylogenetic analyses including nuclear sequence data and an increasing taxon sampling. Their results yielded no improvements in the basal resolution of the phylogenetic tree and therefore discarded the hypothesis of a soft polytomy due to a methodological artefact. However, a possible alternative explanation for the lack of improvements in this last phylogenetic assessments may be that the nuclear data used in these two later studies consisted in two extremely slow-evolving genes (*c-mos* and *rag1*), possibly holding low information content to recover speciation nodes within Lacertini. On the other hand, a recent study from Pyron et al. (2013) with a wide focus on relationships between 4161 Squamata taxa, appears to have successfully solved the internal branching within Lacertini recovering high statistical support from internal to tip nodes. In this study, the authors used mainly the same two slow evolving nuclear markers employed by Mayer and Pavlicev and mitochondrial information from previous studies, and applied a non-parametric Shimodaira–Hasegawa-Like implementation of the approximate likelihood-ratio test (SHLaLRT) (Anisimova and Gascuel, 2006). Consequently, the current state of knowledge on Lacertini evolutionary history has two contrasting phylogenetic hypotheses drawn from concatenated dataset using mostly the same DNA sequences from mitochondrial and slow evolving nuclear markers.

All previous Lacertini phylogenies were based on the analysis of concatenated sequences from multiple genes. Such concatenation approach can prove problematic due to discordances between gene histories and the true evolutionary relationships among species, or in other words, between the gene trees and the species tree. While several processes can account for the discrepancy between gene trees and species trees (Maddison, 1997), recent studies demonstrate that the common approach of concatenating sequences from multiple genes can result in a well-supported but incorrect tree (Kubatko and Degnan, 2007). Bias caused by the concatenation approach can be produced, for instance, by the overuse of genetically linked and more variable mitochondrial genes, which regularly

drives the tree, hiding the information of less variable, usually nuclear, genes. Another major, yet frequently unconsidered, challenge is allele selection in the concatenation process. This substantially influences the phylogenetic results, as heterozygous alleles may have gene tree coalescences deeper than their species divergence, causing gene tree variations according to the chosen allele (Weisrock et al., 2012). Moreover, incongruence across gene tree topologies is an issue of concatenation: if topologies are not significantly different, species trees can be estimated through a concatenation approach. On the other hand, theoretical work has shown that the coalescent process can produce substantial variation in single-gene histories. When single-gene trees are significantly different and incongruent, as it seems the case for Lacertini, the concatenation approach leads to statistically inconsistent estimation of phylogenies (Degnan and Rosenberg, 2006; Kubatko and Degnan, 2007; McVay and Carstens, 2013). In all these cases, bootstraps can provide strong support for an incorrect phylogeny (Kubatko and Degnan, 2007). New methodologies of species tree estimation based on multilocus data from multiple individuals per species allow the reconciliation of a set of gene trees embedded in a shared species phylogeny. Thus, the species tree methods offer a promising tool to assess the reliability of previous phylogenies based on mainly mitochondrial dataset and to dissect the very different phylogenetic estimates of Lacertini based on the concatenation approach.

In this study we generate a comprehensive DNA sequence dataset for Lacertini, including all the tribe's genera, by sequencing multiple specimens per species, with additional taxa relative to previous studies, and including, for the first time, five fast evolving nuclear molecular markers to complement mitochondrial sequence data. In addition to the common approach of concatenating sequences from multiple genes, we implement a species tree approach to infer the phylogeny of Lacertini. Our main aim is to explore whether the addition of DNA sequences from fast-evolving nuclear genes, combined with a multi-species coalescent approach can resolve or improve the inference of basal relationships of the tribe Lacertini, as well as provide more resolution on the relationships between genera and support for genera monophyly. We also compare the species tree with trees derived from the concatenation approach based on mitochondrial and nuclear genes from this study and previous ones. By doing this, we investigate the phylogenetic resolution of mitochondrial and nuclear markers, as well as comparing the phylogenetic inferences made by different phylogenetic methods.

2. Material and methods

2.1. Sampling

A total of 78 specimens from all the 19 genera of Lacertini were employed in the phylogenetic analyses. We used an average of two specimens per species, with a minimum of one and a maximum of five specimens. Ten additional samples, two for each of the species *Gallotia atlantica*, *G. stehlini*, *Psammotromus algirus* and *P. hispanicus* from the sub-family Gallotiinae, and *Atlantolacerta andreanskyi* from the tribe Eremiadini were used as outgroups following previous studies (e.g. Arnold et al., 2007; Harris et al., 1998). All samples were obtained from the collections of Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto (CIBIO-InBIO) and the Institute of Evolutionary Biology – CSIC-UPF (IBE). Information regarding the sample codes, species, sampling locality and GenBank accession numbers is given in Table 1.

2.2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from alcohol-preserved tail muscle following standard high-salt protocols (Sambrook et al.,

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