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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

Joana Mendes [a,b], D. James Harris [a], Salvador Carranza [b], Daniele Salvi [a,*]

[a] CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal
[b] Institute of Evolutionay Biology (CSIC-Universitat Pompeu Fabra), Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain

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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*, *pdc*, *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.

1. Introduction

The squamate reptile family Lacertidae is a clade of small-bodied lizards distributed in the Palaearctic 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

* Corresponding author.
E-mail address: danielesalvi.bio@gmail.com (D. Salvi).

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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*, *Psammodromus 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.,

Table 1
Sample codes, species, sampling locality and sequences' GenBank accession numbers for the 88 samples used in this study.

Code	Species	Locality	*12S*	*nd4*	*acm4*	*βfib*	*mc1r*	*pdc*	*reln*
330	*Algyroides fitzingeri*	Cagliari, Sardinia, Italy	KX080559	KX081002	KX080921	KX080640	KX080711	KX080793	KX080858
701	*Algyroides fitzingeri*	Restonica, Corsica, France	KX080560	KX081003	KX080922	KX080641	KX080712	KX080794	KX080859
4029	*Algyroides fitzingeri*	Mt. Albo, Sardinia, Italy	KX080561	KX081004	KX080923	KX080642	KX080713	KX080795	KX080860
1768	*Algyroides marchi*	La Hueta's waterfall, Spain	KX080563	KX081006	KX080925	KX080644	KX080715	KX080797	KX080862
1859	*Algyroides marchi*	El Toril, Spain	KX080564	KX081007	KX080926	KX080645	KX080716	KX080798	KX080863
1889	*Algyroides marchi*	Puente de las Herrerías, Spain	KX080562	KX081005	KX080924	KX080643	KX080714	KX080796	KX080861
458b	*Algyroides moreoticus*	Kalivia, Greece	KX080555	KX081000	KX080917	KX080637	KX080707		KX080855
4324	*Algyroides moreoticus*	Roitika Patras, Greece	KX080558		KX080920		KX080710	KX080792	KX080857
4325	*Algyroides moreoticus*	Kalavryta, Greece	KX080556		KX080918	KX080638	KX080708	KX080790	
4332	*Algyroides moreoticus*	Zarouchla, Greece	KX080557	KX081001	KX080919	KX080639	KX080709	KX080791	KX080856
416	*Algyroides nigropunctatus*	Metsovo, Greece	KX080552	KX080997	KX080914	KX080634	KX080704	KX080787	
3237	*Algyroides nigropunctatus*	Vitsa, Greece	KX080550	KX080995	KX080913		KX080702	KX080786	
3246	*Algyroides nigropunctatus*	Voidomatis, Greece	KX080551	KX080996		KX080633	KX080703		
15438	*Algyroides nigropunctatus*	Vanganel, Slovenia	KX080553	KX080998	KX080915	KX080635	KX080705	KX080788	
15441	*Algyroides nigropunctatus*	Vanganel, Slovenia	KX080554	KX080999	KX080916	KX080636	KX080706	KX080789	
S10390	*Anatololacerta danfordi*	Çamıyayla, Turkey	KX080617	KX081055	KX080981	KX080690			
12033	*Apathya cappadocica*	Göksun, Turkey	KX080619	KX081057	KX080983		KX080772	KX080843	KX080900
S10388	*Apathya cappadocica*	Eastern Turkey	KX080618	KX081056	KX080982	KX080912	KX080771	KX080842	KX080899
RE1	*Archaeolacerta bedriagae*	Restonica, Corsica, France	KX080585	KX081026	KX080947	KX080659	KX080737	KX080814	
RE2	*Archaeolacerta bedriagae*	Restonica, Corsica, France	KX080586	KX081027	KX080948	KX080660	KX080738	KX080815	KX080880
5015	*Atlantolacerta andreanskyi*	Tizin Tichka, Morocco	JX462057.1	JX462200.1	JX461988.1	KX080693	JX461804.1	JX461634.1	
5058	*Atlantolacerta andreanskyi*	Tizin Tichka, Morocco	JX462054.1	JX462196.1	JX462000.1	KX080694	JX461816.1	JX461644.1	
S10353	*Dalmatolacerta oxycephala*	Bosnia and Herzegovina	KX080610	KX081049	KX080973	KX080684	KX080763	KX080836	
S10354	*Dalmatolacerta oxycephala*	Bosnia and Herzegovina	KX080609	KX081048	KX080972	KX080683	KX080762	KX080835	
7802	*Darevskia derjugini*	Abastumani, Georgia	KX080583		KX080945	KX080657	KX080735	KX080813	KX080878
7803	*Darevskia derjugini*	Abastumani, Georgia	KX080584		KX080946	KX080658	KX080736		KX080879
4985	*Darevskia raddei*	Pia, Georgia	KX080582	KX081025	KX080944	KX080656	KX080734	KX080812	
10126	*Darevskia raddei*	Ganzasar, Nagorno-Karabakh Republic	KX080581	KX081024	KX080943	KX080655	KX080733	KX080811	KX080877
3	*Dinarolacerta montenegrina*	Đebeza, Prokletije Mountains, Montenegro		KX081012	KX080930	KX080909	KX080721	KX080803	KX080868
18	*Dinarolacerta montenegrina*	Đebeza, Prokletije Mountains, Montenegro	KX080566	KX081009	KX080927	KX080646	KX080718	KX080800	KX080865
19	*Dinarolacerta montenegrina*	Đebeza, Prokletije Mountains, Montenegro	KX080567	KX081010	KX080928	KX080907	KX080719	KX080801	KX080866
20	*Dinarolacerta montenegrina*	Đebeza, Prokletije Mountains, Montenegro	KX080565	KX081008		KX080906	KX080717	KX080799	KX080864
22	*Dinarolacerta montenegrina*	Đebeza, Prokletije Mountains, Montenegro	KX080568	KX081011	KX080929	KX080908	KX080720	KX080802	KX080867
9	*Dinarolacerta mosorensis*	Međuvršje, Montenegro	KX080570	KX081014	KX080932	KX080647	KX080723	KX080804	KX080870
13	*Dinarolacerta mosorensis*	Međuvršje, Montenegro	KX080571		KX080933	KX080648	KX080724	KX080805	
15	*Dinarolacerta mosorensis*	Virak, Montenegro	KX080569	KX081013	KX080931		KX080722		KX080869
AM1	*Dinarolacerta mosorensis*	Lovćen Mountains, Montenegro	KX080572	KX081015	KX080934	KX080649			
1244	*Gallotia atlantica*	Nazaret, Lanzarote, Spain	KX080625	KX081062	KX080988	KX080695	KX080778	KX080847	KX080902
1341	*Gallotia atlantica*	Yaiza, Lanzarote, Spain	KX080626	KX081063	KX080989	KX080696	KX080779	KX080848	
1350	*Gallotia stehlini*	San Andrés, Gran Canaria, Spain	KX080627	KX081064	KX080990	KX080697	KX080780	KX080849	
1412	*Gallotia stehlini*	Aldea Blanca, Gran Canaria, Spain	KX080628	KX081065	KX080991	KX080698	KX080781	KX080850	KX080903
456b	*Hellenolacerta graeca*	Agia Kyriaki, Greece	KX080612		KX080975	KX080686	KX080765	KX080838	KX080896
456c	*Hellenolacerta graeca*	Agia Kyriaki, Greece	KX080613		KX080976	KX080687	KX080766	KX080839	KX080897
S10387	*Hellenolacerta graeca*	Greece	KX080611	KX081050	KX080974	KX080685	KX080764	KX080837	KX080895
62	*Iberolacerta cyreni*	Navacerrada, Spain	KX080578	KX081021	KX080940	KX080651	KX080730	KX080809	KX080874
MON1	*Iberolacerta cyreni*	Rascafría, Spain	KX080577	KX081020	KX080939	KX080652	KX080729		KX080873
4282	*Iberolacerta monticola*	Sosas de Laciana, Spain	KX080579	KX081022	KX080941	KX080653	KX080731		KX080875
4283	*Iberolacerta monticola*	Sosas de Laciana, Spain	KX080580	KX081023	KX080942	KX080654	KX080732	KX080810	KX080876
5143	*Iranolacerta brandtii*	Esfahan, Iran	KX080623	KX081060	KX080986		KX080776		
5146	*Iranolacerta brandtii*	Ardabil, Iran	KX080624	KX081061	KX080987		KX080777	KX080846	KX080901
S10397	*Lacerta agilis*	Studland, United Kingdom	KX080600	KX081039	KX080962	KX080673	KX080752	KX080829	KX080888
S10401	*Lacerta agilis*	Alp, Spain	KX080601	KX081040	KX080963	KX080674	KX080753	KX080830	

Table 1 (*continued*)

Code	Species	Locality	*12S*	*nd4*	*acm4*	*βfib*	*mc1r*	*pdc*	*reln*
15306	*Lacerta bileneata*	Bosco Magnano, Italy	KX080602	KX081041	KX080964	KX080675	KX080754		
15307	*Lacerta bileneata*	Pantana, Italy	KX080603	KX081042	KX080965	KX080676	KX080755		KX080889
15308	*Lacerta bileneata*	Abruzzo, Italy	KX080604	KX081043	KX080966	KX080677	KX080756		KX080890
1912	*Lacerta schreiberi*	Garganta de las Lancha, Spain	KX080598	KX081037	KX080960	KX080671	KX080750	KX080827	KX080887
3866	*Lacerta schreiberi*	Tanes, Spain	KX080599	KX081038	KX080961	KX080672	KX080751	KX080828	
445	*Lacerta trilineata*	Agios Vasilios, Greece		KX081044	KX080968	KX080679	KX080758	KX080831	
446	*Lacerta trilineata*	Agios Vasilios, Greece	KX080606	KX081045	KX080969	KX080680	KX080759	KX080832	KX080892
447	*Lacerta trilineata*	Dorio, Greece	KX080607	KX081046	KX080970	KX080681	KX080760	KX080833	KX080893
451	*Lacerta trilineata*	Koutsouroumpas, Greece	KX080608	KX081047	KX080971	KX080682	KX080761	KX080834	KX080894
S10399	*Lacerta trilineata*	Golbasi, Turkey	KX080605		KX080967	KX080678	KX080757		KX080891
S10398	*Parvilacerta parva*	Çorum, Turkey	KX080616	KX081054	KX080980	KX080911	KX080770	KX080841	
JamJB	*Phoenicolacerta kulzeri*	Barouk, Jordan	KX080622	KX081059	KX080985	KX080692	KX080775	KX080845	
Petra	*Phoenicolacerta kulzeri*	Petra, Jordan	KX080621		KX080984	KX080691	KX080774		
S10389	*Phoenicolacerta kulzeri*	Ainata, Lebanon	KX080620	KX081058			KX080773	KX080844	
509	*Podarcis muralis*	Florence, Italy	KX080575	KX081018	KX080937	KX080650	KX080727	KX080807	KX080872
5937	*Podarcis muralis*	Sierra delle Ciavole, Italy	KX080576	KX081019	KX080938		KX080728	KX080808	
771	*Podarcis sicula*	Vulcano Island, Sicily, Italy	KX080573	KX081016	KX080935		KX080725		KX080871
9103	*Podarcis sicula*	Pizzo, Italy	KX080574	KX081017	KX080936	KX080701	KX080726	KX080806	
2347	*Psammodromus algirus*	Iminifri, Morocco	KX080631	KX081068		KX080699	KX080784	KX080853	
2356	*Psammodromus algirus*	Azrou, Morocco	KX080632	KX081069	KX080994	KX080700	KX080785	KX080854	
1723	*Psammodromus hispanicus*	Jaén, Spain	KX080629	KX081066	KX080992		KX080782	KX080851	KX080904
1850	*Psammodromus hispanicus*	Jaén, Spain	KX080630	KX081067	KX080993		KX080783	KX080852	KX080905
139	*Scelarcis perspicillata*	Sidi Yahya Ousaad, Morocco	KX080591	KX081031	KX080953	KX080664	KX080743	KX080820	
3456	*Scelarcis perspicillata*	Taza, Morocco	KX080592		KX080954	KX080665	KX080744	KX080821	
S9282	*Takydromus sexlineatus*	Gangaw, Myanmar	KX080614	KX081051	KX080977	KX080688	KX080767		KX080897
S9294	*Takydromus sexlineatus*	Nat Ma Taung National Park, Myanmar	KX080615	KX081052	KX080978	KX080689	KX080768		KX080898
S10392	*Takydromus smaragdinus*	Okinawa, Japan		KX081053	KX080979		KX080769	KX080840	
5222	*Teira dugesii*	Santa Maria, Azores, Portugal	KX080595	KX081034	KX080957	KX080668	KX080747	KX080824	
5223	*Teira dugesii*	Santa Maria, Azores, Portugal	KX080596	KX081035	KX080958	KX080669	KX080748	KX080825	
5224	*Teira dugesii*	Santa Maria, Azores, Portugal	KX080597	KX081036	KX080959	KX080670	KX080749	KX080826	
4012	*Timon lepidus*	Vairão, Portugal	KX080589		KX080951	KX080662	KX080741	KX080818	KX080883
4846	*Timon lepidus*	Burgos, Spain	KX080590	KX081030	KX080952	KX080663	KX080742	KX080819	KX080884
14	*Timon tangitanus*	Morocco	KX080587	KX081028	KX080949	KX080661	KX080739	KX080816	KX080881
27	*Timon tangitanus*	Cirque de Jafar, Morocco	KX080588	KX081029	KX080950	KX080910	KX080740	KX080817	KX080882
15305	*Zootoca vivipara*	Russia	KX080594	KX081033	KX080956	KX080667	KX080746	KX080823	KX080886
zoo	*Zootoca vivipara*	Baikal Lake, Russia	KX080593	KX081032	KX080955	KX080666	KX080745	KX080822	KX080885

1989). For a reduced number of samples for which the saline extraction failed we used the Qiagen DNeasy® Blood & Tissue extraction kit, following the manufacture's protocol.

Fragments from the mitochondrial DNA genes NADH Dehydrogenase 4 plus the flanking tRNAs Histidine and Serine (*nd4*), and of the ribosomal 12SrRNA gene (*12S*), and the nuclear genes Acetylcholinergic Receptor M4 (*acm4*), Melanocortin 1 Receptor (*mc1r*), Phosducin (*pdc*), intron 7 of *β*-fibrinogen (*βfib*) and intron 61 of Reelin (*reln*) were amplified through standard Polymerase Chain Reaction (PCR). We selected these mitochondrial and nuclear markers because they have been shown to be highly variable in previous studies on Lacertinae (e.g. Pinho et al., 2008; Salvi et al., 2010, 2014; Barata et al., 2012). Primers and PCR protocols used for the amplification of the molecular markers are reported in Table 2. Purification and sequencing of PCR products were carried out by a commercial sequencing company (Macrogen Europe: www.macrogen.com), using the same primers employed for amplification.

2.3. Phylogenetic analyses

Sequences were aligned using the MUSCLE algorithm (Edgar, 2004) in Geneious (Biomatters Ltd.) with default settings. Ambiguous and poorly aligned positions were removed by Gblocks v.0.91b using default settings (Castresana, 2000).

Haplotype reconstruction for nuclear gene fragments was performed in PHASE v. 2.1 (Stephens et al., 2001; Stephens and Scheet, 2005). Input files were created in SEQPHASE (Flot, 2010; available at http://seqphase.mpg.de/seqphase/). Haplotypes defined from heterozygous insertion–deletions were manually phased and were incorporated as known phases to improve haplotype determination following Flot et al. (2006). Phase was run three times to assure consistency, with a phase probability threshold of 0.7 and the remaining settings by default.

Recombination detection was performed in RDP v.3.44 (Martin et al., 2010) using five different algorithms, RDP (Martin and Rybicki, 2000), GENECONV (Padidam et al., 1999), MaxChi (Smith, 1992), BootScan (Martin et al., 2005) and SiScan (Gibbs et al., 2000) with default options and applying the auto-masking tool to remove the outgroup and very divergent or very similar sequences, in order to increase statistical power (Martin et al., 2010).

Phylogenetic relationships among the Lacertidae species were inferred by Maximum Likelihood (ML), Bayesian Inference (BI) and the Bayesian species tree approach based on the multi-locus coalescent. For the ML and BI analyses, unphased sequence data

Table 2
Primers and PCR protocols used for the amplification of the molecular markers used in this study.

Gene	Primer	Sequence (5′–3′)	Source	PCR conditions (°C (s) × number of circles)
nd4	ND4 Leu	CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC CAT TAC TTT TAC TTG GAA TTT GCA CCA	Arévalo et al. (1994)	94(180), [94(30), 50(30), 72(60) × 35], 72(600)
12S	12Sa 12Sb	CTG GGA TTA GAT ACC CCA CTA T GAG GGT GAC GGG GCG GTG TGT	Kocher et al. (1989)	94(180), [94(30), 50(30), 72(45) × 35], 72(600)
acm4	TgF TgR	CAA GCCTGA GAG CAA RAA GG ACY TGA CTC CTG GCA ATG CT	Gamble et al. (2008)	92(180), [92(30), 62↓0.5(30), 72(45) × 20], [92(30), 50(30), 72(45) × 15], 72(600)
βfib	BF8 BfibR	CAC CAC CGT CTT CTT TGG AAC ACT G CAG GGA GAG CTA CTT TTG ATT AGA C	Pinho et al. (2008)	92(180), [92(30), 62↓0.5(30), 72(60) × 20], [92(30), 50(30), 72(60) × 15], 72(600)
mc1r	MC1R-F MC1R-R	GGC NGC CAT YGT CAA GAA CCG GAA CC CTC CGR AAG GCR TAG ATG ATG GGG TCC AC	Pinho et al. (2009)	92(180), [92(30), 62↓0.5(30), 72(60) × 25], [92(30), 50(30), 72(60) × 15], 72(600)
pdc	PHOF2 PHOR1	AGA TGA GCA TGC AGG AGT ATG A TCC ACA TCC ACA GCA AAA AAC TCC T	Bauer et al. (2007)	92(180), [92(30), 58(30), 72(60) × 35], 72(600)
reln	62F 63R	GAG TMA CTG AAA TAA ACT GGG AAA C GCC ATG TAA TYC CAT TAT TTA CAC TG	Pinho et al. (2009)	92(180), [92(30), 57(30), 72(60) × 35], 72(600)

were concatenated in three different matrices: mitochondrial DNA (mtDNA), nuclear DNA (nucDNA) and mitochondrial-nuclear DNA (mt-nucDNA) data. Within each matrix the data was partitioned by gene fragment (seven mt-nucDNA partitions).

ML analyses were performed in RAxML GUI v.1.1.3 (Silvestro and Michalak, 2012), a graphical front-end for RaxML v.7.4.2 (Stamatakis, 2006). ML searches included 10 random addition replicates and 1000 nonparametric bootstrap replicates, applying the general time-reversible model with gamma model of rate heterogeneity (GTRGAMMA) for each of the three concatenated datasets.

BI analyses were performed in BEAST v.1.8.0 (Drummond et al., 2012) for each concatenated dataset. The best model of nucleotide substitution for each gene among 40 different models was assessed in jModelTest v.2.1.3 (Posada, 2008) under the corrected Akaike Information Criterion (AICc) (Table 3). We built the input file with evolutionary models, tree priors and Markov Chain Monte Carlo (MCMC) options using the BEAUTi utility included in the BEAST package. Models and prior specifications applied were as follows (otherwise by default): we implemented the K80 model in BEAST by specifying the HKY model with "base frequencies" set to "All Equal"; the tree model of all gene partitions was linked, while nucleotide substitution and clock models were unlinked; Relaxed Uncorrelated Lognormal Clock set for all genes, Yule process of speciation as tree prior, random starting tree, alpha Uniform (0, 10), ucld.mean Uniform, and operator kappa (2.0). The use of the Yule process of speciation prior requires only one sequence per species, whereas our concatenated alignments contain multiple samples per species. Therefore, to investigate the sensitivity of our estimates to the choice of tree prior, we performed an additional run for each dataset, applying the same settings as above but using only one representative sequence for each species. BEAST was run three times, with 100 million generations, sampling every 10,000 generations. We used Tracer v 1.5 (Rambaut and Drummond, 2007) to check the runs for convergence (burn-in = 10%) and to ensure that all effective sample sizes parameters (ESS) were higher than 200, as recommended in the manual. Runs were combined with LogCombiner and afterwards TreeAnnotator (both included in the BEAST package) was used to summarize the trees in a consensus tree representing the posterior distribution.

The species tree was inferred using the *BEAST extension of the BEAST software. *BEAST co-estimates a species tree along with the gene trees and effective population sizes of the species in a single Bayesian Markov Chain Monte Carlo analysis. For this analysis we used the phased alignments of the nuclear genes and their relative models of nucleotide evolution calculated in jModelTest, under the AICc (Table 3). Nucleotide substitution, clock and tree models were unlinked, with the exception of the tree model of the mitochondrial genes *12S* and *nd4* because these genes are genetically linked. The remaining settings were the same as in the BEAST analysis of the concatenated mt-nucDNA data. We used the available estimated rate of evolution of *12S* of lacertid lizards (Carranza and Arnold, 2012) to estimate cladogenetic events within Lacertini. Mean substitution rates and their standard errors for the same *12S* gene regions used in the present study were extracted from a fully-calibrated phylogeny (nine calibration points) including the lacertid lizard Canary Islands radiation of *Gallotia* sp. (Cox et al., 2010) and the Balearic islands *Podarcis pityusensis* and *P. lilfordi* (Brown et al., 2008). For a full account on the specific calibration points and methods used to infer the substitution rate of Lacertid lizard used in the present study please see Carranza and Arnold (2012). Absolute divergence times were estimated in *BEAST by setting a normal distribution prior for the ucld.mean parameter of the *12S* gene fragment with the following parameters: initial: 0.00553, mean: 0.00553, stdev: 0.00128. *BEAST was run five times with 400 million generations, sampling every 40,000 generations. Runs were performed in the CIPRES Science Gateway V. 3.3 (Miller et al., 2010, at http://www.phylo.org/). Convergence and ESS of the runs were verified in Tracer v 1.5. Runs were combined with LogCombiner and the maximum clade credibility tree was calculated in TreeAnnotator. All trees were visualized in FIGTREE v1.4 (available at http://tree.bio.ed.ac.uk/software/figtree/).

Table 3
Number of sequences for each gene, length of the gene fragments, models of sequence evolution for unphased and phased data as selected by jModelTest according to the AICc and number of variable positions calculated in MEGA 5 for the dataset with and without outgroup.

Gene	No. seq.	Length (bp)	Model unphased data	Model phased data	Variable positions Ingroup	Variable positions with outgroup
12S	85	362	GTR + I + G		125	136
nd4	77	726	TrN + I + G		417	433
acm4	84	379	HKY + I + G	K80 + G	73	90
β-fib	76	327	HKY	JC	143	175
mc1r	86	615	HKY + I + G	HKY + I + G	125	138
pdc	71	444	K80 + I + G	K80 + G	99	113
reln	51	681	HKY + G	HKY + G	265	318

2.4. Topology tests

In order to compare our phylogenetic hypothesis with previous phylogenies, we performed topological tests between our ML tree based on the concatenated mt-nucDNA dataset and ML trees

Table 4
Age, in million years (Mya), and node 95% highest posterior density (HPD) intervals for the major supported nodes in the species tree. Letters represent the nodes in the species tree (Fig. 4).

Node	Split/clade	Age (Mya)	Height 95% HPD (Mya)
a	Lacertinae – Gallotinae	33.22	11.77–51.28
b	Lacertini – Eremiadini	17.85	10.68–25.7
c	Lacertini	15.03	9.42–21.58
d	*Algyroides – Dinarolacerta*	11.29	6.98–16.48
e	*A. fitzingeri – A. marchi*	8.59	4.95–12.65
f	*Dinarolacerta* genus	2.9	1.35–4.76
g	*Podarcis* genus	6.85	3.79–10.42
h	*Iberolacerta* genus	4.32	2.09–6.85
i	*Archaeolacerta, Zootoca, Teira, Scelarcis*	12.85	7.95–18.66
j	*Teira – Scelarcis*	5.81	3.17–9.02
k	*Lacerta* genus	7.88	4.74–11.65
l	*Timon* genus	3.51	1.58–5.76
m	*Takydromus* genus	8.45	4.75–12.75
n	*Darevskia – Iranolacerta*	10.25	5.93–15.34
o	*Darevskia* genus	3.18	1.35–5.27
p	*Parvilacerta – Anatololacerta*	10.91	6.15–16.08
q	*Gallotia – Psammodromus*	14.92	8.92–22.38
r	*Gallotia* genus	10.8	5.74–16.55
s	*Psammodromus* genus	11.67	6.56–17.64

obtained by previous studies. First, we inspected supported nodes recovered in previous studies that conflicted with our results and then we enforced these nodes in our tree topology. In order to assess the relative contribution on topological comparisons of nodes with different levels of support we generated three constrained topologies enforcing all nodes obtained in previous studies with bootstrap support values equal or higher than (i) 95 or (ii) 90 or (iii) 85. The trees with topological constrains were generated in Mesquite version 3.03 (Maddison and Maddison, 2015). Constrained clades are presented in Table 5. Per-site log likelihood values were estimated in RAxMLGUI v.1.1.3. The constrained trees were compared with our best ML tree using the Shimodaira–Hasegawa (SH) and the approximately unbiased (AU) tests (Shimodaira and Hasegawa, 1999; Shimodaira, 2002, respectively), as implemented in CONSEL (Shimodaira and Hasegawa, 2001) to determine if any of the alternatives could be rejected at the 0.05 level.

Table 5
Results of topological tests using three sets of constrains based on relationships recovered in Pyron et al. (2013) with a node support ⩾85 or ⩾90 or ⩾95. The enforced relationships and *p*-value results of Shimodaira–Hasegawa (SH) and Approximately Unbiased (AU) tests are reported.

Node support level	Enforced relationships	SH	AU
95	1. (*Takydromus*, *Zootoca*) 2. (*Dalmatolacerta*, *Hellenolacerta*)	0.018	0.002
90	1. (*Takydromus*, *Zootoca*) 2. (*Dalmatolacerta*, *Hellenolacerta*) 3. ((*Timon*, *Lacerta*) (*Podarcis* (*Teira*, *Scelarcis*))) 4. (*Archaeolacerta*, *Apathya*) 5. (*Algyroides marchi*, *A. fitzingeri*) *Dinarolacerta*)	3e−004	6e−008
85	1. (*Takydromus*, *Zootoca*) 2. (*Dalmatolacerta*, *Hellenolacerta*) 3. ((*Timon*, *Lacerta*) (*Podarcis* (*Teira*, *Scelarcis*))) 4. (*Archaeolacerta*, *Apathya*) 5. (*Algyroides marchi*, *A. fitzingeri*) *Dinarolacerta*) 6. (*Dalmatolacerta*, *Hellenolacerta*, *Archaeolacerta*, *Apathya*, *Iberolacerta*, *Parvilacerta*, *Anatololacerta*, *Algyroides*, *Iranolacerta*, *Darevskia*)	3e−004	8e−006

3. Results

A total of 530 sequences were obtained and used in the phylogenetic analyses, among which 520 sequences were newly generated for this study and 10 sequences of the species *Atlantolacerta andreanskyi* were retrieved from GenBank. The percentage of missing data was 3% for *12S*, 12.5% for *nd4*, 4.5% for *acm4*, 15% for *βfib*, 2% for *mc1r*, 19% for *pdc* and 42% for *reln*. The percentage of missing nucleotides is very low in the genes *12S*, *nd4* and *reln*, with 1–3 samples having 0.05% of the total length of the alignment missing, and higher for the genes *acm4*, *bfib* and *mc1r*, with an average of 10% of the length missing in a maximum of 6 specimens. The number of sequences, multiple sequence alignments length, models of sequence evolution and number of variable positions are reported for each gene in Table 3. Multiple sequences alignments of protein coding genes (*nd4*, *acm4*, *mc1r* and *pdc*) did not require gap positions and their translation into amino acid sequences contained no stop codons. Alignments of both the intronic regions *βfib* and *reln* showed high sequence length polymorphisms. The recombination tests applied in RDP did not find statistically significant evidence for recombination in any of the nuclear genes. All sequences were deposited in GenBank (Table 1).

3.1. Phylogenetic relationships within Lacertidae

Phylogenetic results from the concatenation analyses and species tree present three consistent traits: (i) in the Bayesian trees based on the concatenated datasets and in the species tree, in which the outgroup is not enforced, the subfamilies Gallotiinae and Lacertinae are reciprocally monophyletic sister taxa (Figs. 1–4); (ii) the Lacertini tribe presents a basal polytomy pattern with a lack of support for basal nodes in all trees; (iii) all genera are monophyletic with high support (Bayesian Posterior Probabilities (BPP) ⩾ 95; Bootstrap Support (BS) ⩾ 70), except *Algyroides*, whose monophyly is recovered in all the trees but with low node support, except in the BI tree based on the concatenated mtDNA topology where *Algyroides* is paraphyletic relative to *Dinarolacerta* (Fig. 1, in orange). Supported sister genera relationships recovered by all analyses with high support include *Scelarcis perspicillata* and *Teira dugesii* (Figs. 1–4, in green[1]; BPP > 0.98, BS = 100). The sister genus relationships between *Darevskia* and *Iranolacerta* is recovered in all the trees and is statistically well supported (Figs. 1, 3 and 4, in pink; BPP > 0.94, BS > 83) except in the tree based on the concatenated nucDNA (Fig. 2). *Algyroides* and *Dinarolacerta* are also recovered as well supported sister taxa in the tree based on the concatenated mt-nucDNA and the species tree analyses (Figs. 3 and 4, in orange; BPP > 0.97, BS > 98), are recovered as sister taxa but not supported in the nucDNA tree (Fig. 2) and *Dinarolacerta* is nested within *Algyroides* in the BI tree based on the concatenated mtDNA (Fig. 1). Some clade relationships are recovered when using only one type of molecular markers: when using mtDNA data we recovered the relationships between *Anatololacerta* and *Parvilacerta* in the BI tree based on the concatenated dataset and species tree (Figs. 1, 3 and 4, in brown; BPP > 0.96) and the green lizard genera *Lacerta* and *Timon* in the trees based on the concatenated datasets (Figs. 1 and 3, in blue; BPP > 0.98, BS > 74); when using the nucDNA data we recovered a well-supported clade containing the taxa *Scelarcis perspicillata*, *Teira dugesii*, *Archaeolacerta bedriagae* and *Zootoca vivipara* (Figs. 2–4, in green; BPP = 1, BS > 87). The position of all the other genera, *Podarcis*, *Hellenolacerta*, *Phoenicolacerta*, *Takydromus*, *Iberolacerta*, *Dalmatolacerta* and *Apathya* is neither resolved nor consistent across the trees.

[1] For interpretation of color in Figs. 1–4, the reader is referred to the web version of this article.

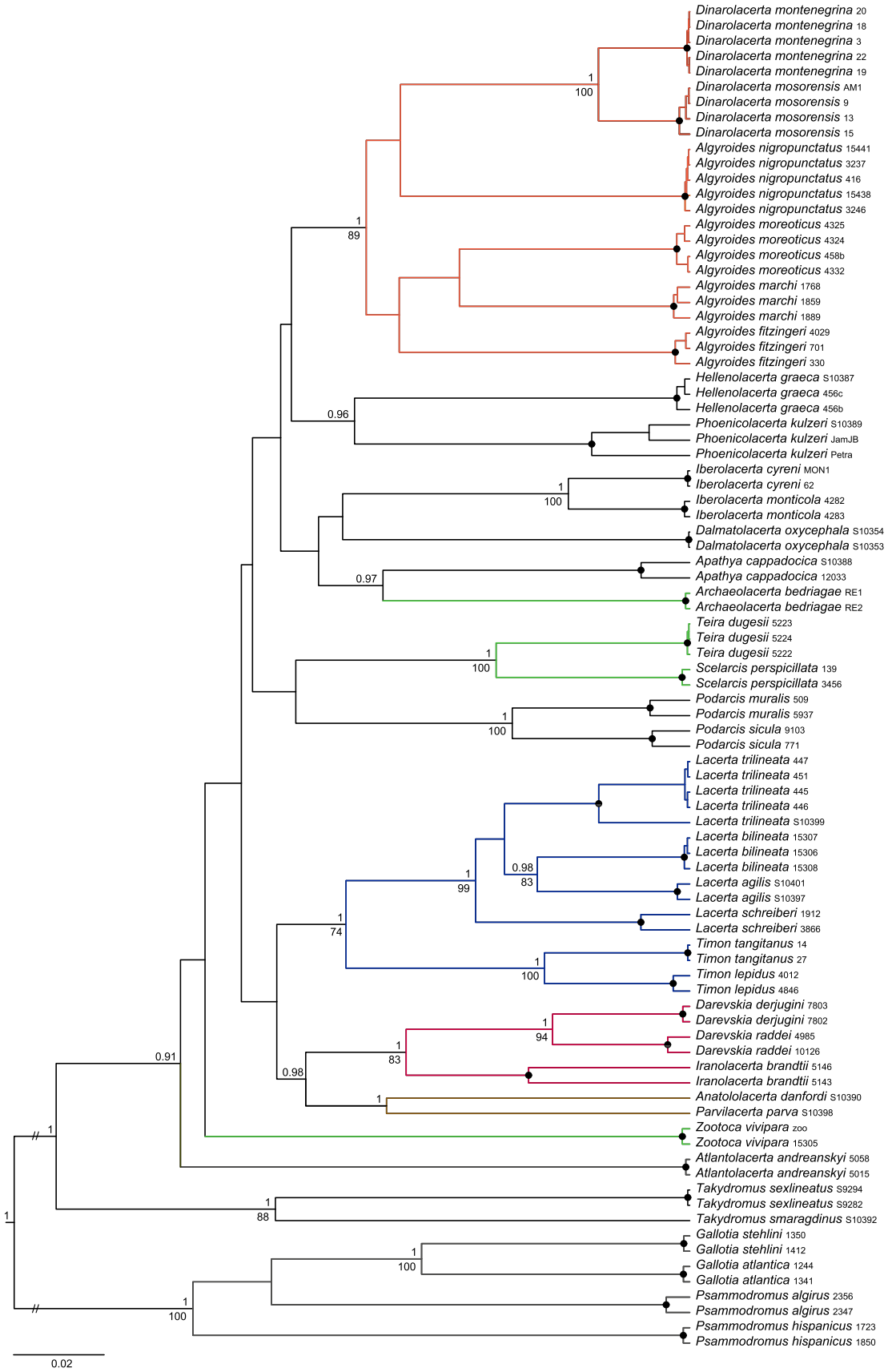

Fig. 1. Phylogenetic relationships of Lacertini based on Bayesian analyses of concatenated mitochondrial DNA sequences (*12S* and *nd4*). Bayesian posterior probabilities (BPP) ⩾ 0.90 are reported above nodes; bootstrap values from Maximum-Likelihood analyses (BS) ⩾ 90 are reported below nodes. Within species, black dots represent BPP of 1 and BS of 100 in both BI and ML analyses; grey part of the dots represent BPP of 0.9 ⩾ 0.99 and BS of 70 ⩾ 99, respectively.

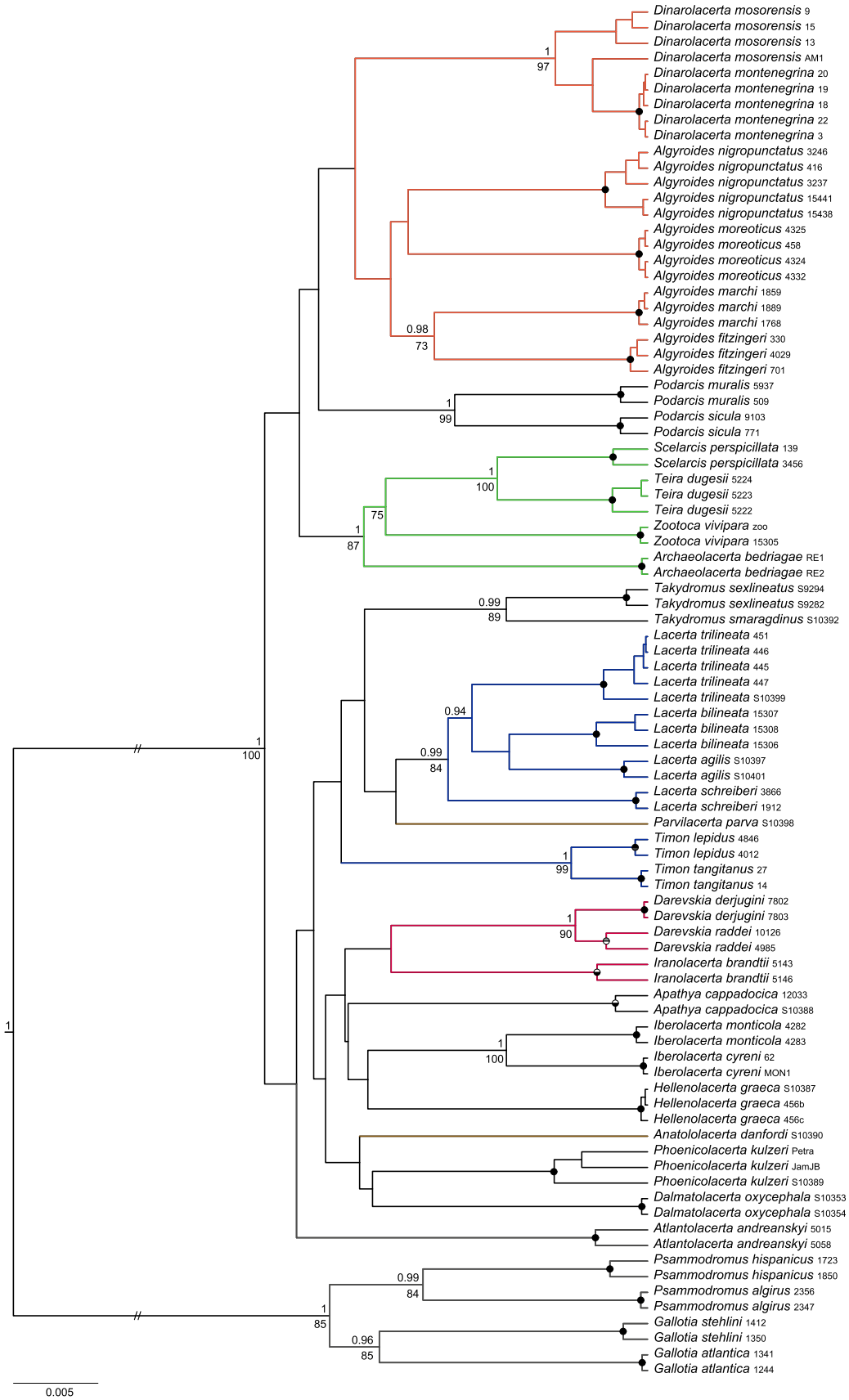

Fig. 2. Phylogenetic relationships of Lacertini based on Bayesian analyses of concatenated nuclear DNA sequences (*acm4*, *βfib*, *mc1r*, *pdc* and *reln*). Bayesian posterior probabilities ⩾0.90 are reported above nodes; bootstrap values from Maximum-Likelihood analyses ⩾0.90 are reported below nodes. Within species, black dots represent BPP of 1 and BS of 100 in both BI and ML analyses; grey part of the dots represent BPP of 0.9 ⩾ 0.99 and BS of 70 ⩾ 99, respectively and white part represents no support.

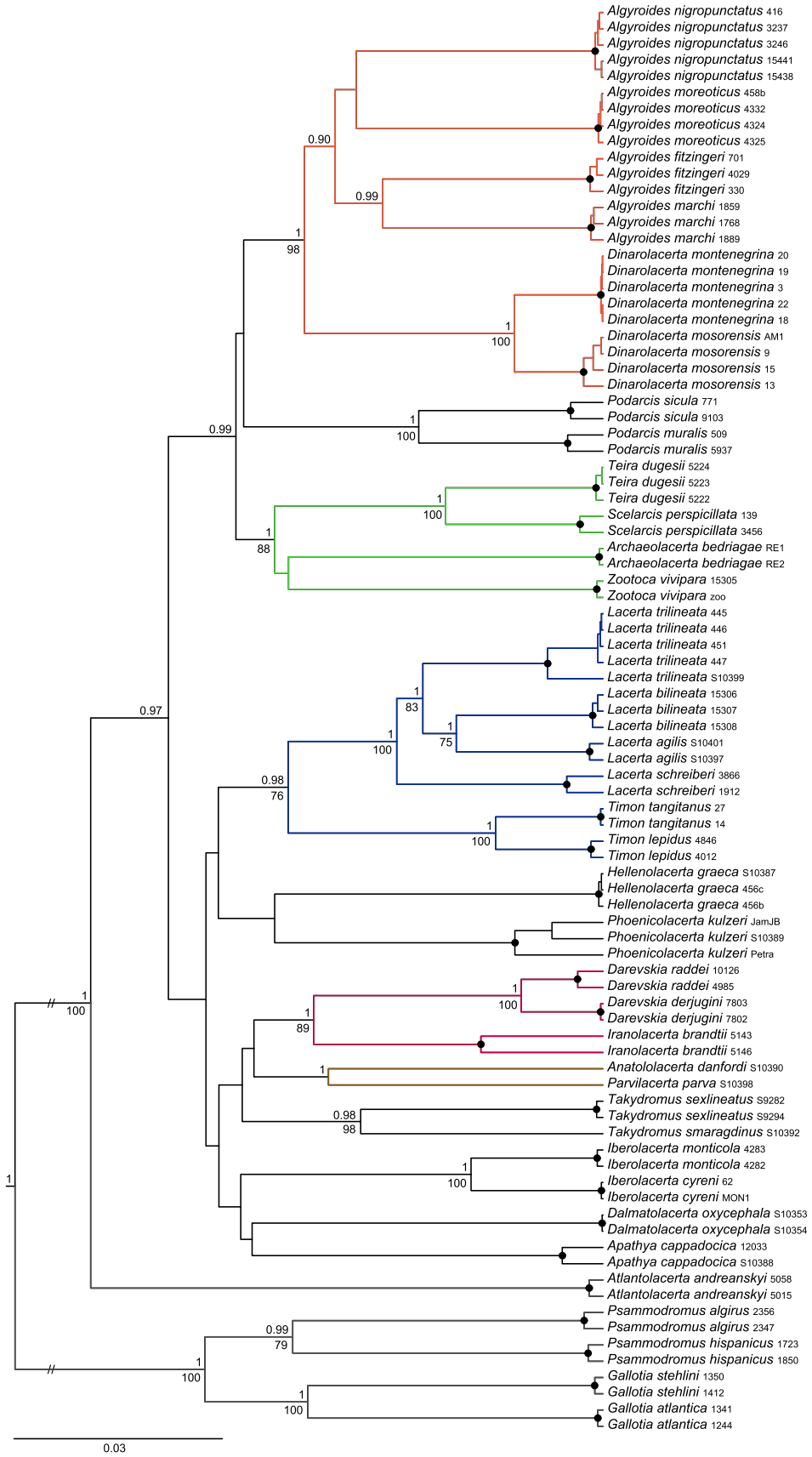

Fig. 3. Phylogenetic relationships of Lacertini based on concatenated mitochondrial (*12S* and *nd4*) and nuclear (*acm4*, *βfib*, *mc1r*, *pdc* and *reln*) DNA sequences. Bayesian posterior probabilities ⩾0.90 are reported above nodes; bootstrap values from Maximum-Likelihood analyses ⩾0.90 are reported below nodes. Within species, black dots represent BPP of 1 and BS of 100 in both BI and ML analyses.

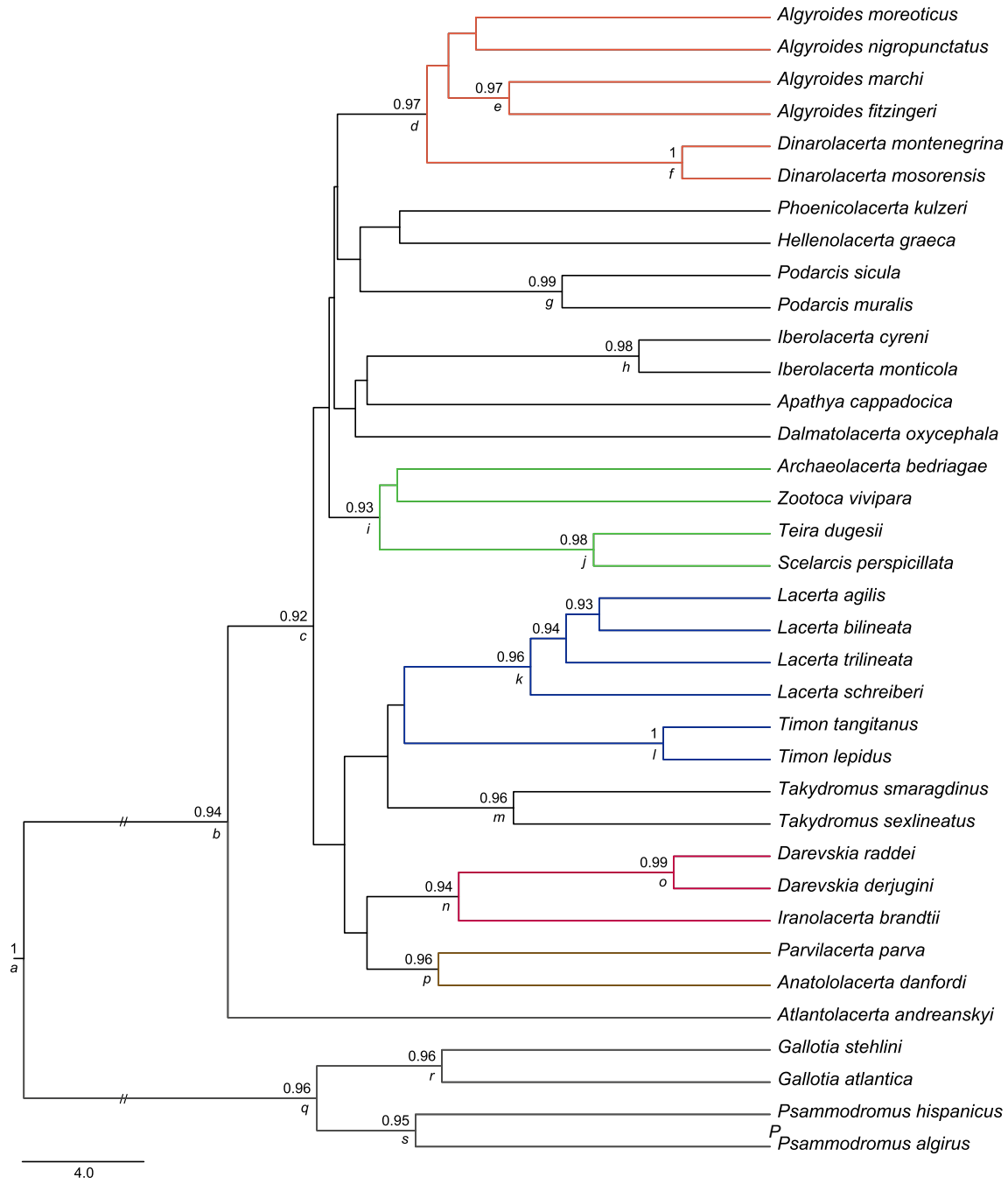

Fig. 4. Species tree of Lacertini inferred from mitochondrial (*12S* and *nd4*) and nuclear (*acm4*, *βfib*, *mc1r*, *pdc* and *reln*) DNA sequences using the multispecies coalescent model in *BEAST software. The posterior probabilities ⩾0.90 are shown above nodes. Node ages and 95% highest posterior density intervals (HPD) values for supported nodes (indicated by the letters a–s) are presented in Table 4.

3.2. Comparison between mitochondrial and nuclear trees, ML and BI trees, and the species tree

Overall we found higher Bayesian Posterior Probabilities (BPP) support than the Bootstrap Support (BS) when comparing BI and ML trees, irrespective of the dataset used. When comparing results obtained with different phylogenetic methods (BI *vs.* ML approach) or different datasets (mitochondrial *vs.* nuclear data), we found that the position and relationships of some taxa are more sensitive to the markers than to the method used, with some inconsistencies between the results based on mtDNA *vs.* nucDNA data. The Eremiadini species *Atlantolacerta andreanskyi* is sister taxon to the Lacertini tribe in all the analyses including mtDNA + nucDNA data (Figs. 3 and 4) but not in the trees based on the concatenated nucDNA, where it is positioned within Lacertini (Figs. 2 and S2) or in the BI tree based on the concatenated mtDNA, where it is sister taxon to all Lacertini with the exception of *Takydromus* (Fig. 1). The genus *Takydromus* is nested within the Lacertini tribe in all the trees, except in the BI tree based on the concatenated mtDNA where it is sister taxon to all the other Lacertini included in the analyses + *Atlantolacerta andreanskyi* (Fig. 1; BPP = 0.91). *Archaeolacerta* clustered in a group with *Zootoca*, *Scelarcis* and *Teira* in all the trees containing nucDNA data (Figs. 2–4, in green), in the tree based on the concatenated mtDNA it is either unresolved (ML tree, Fig. S1) or sister taxon to *Apathya* (BI tree, Fig. 1; BPP: 0.97). A relationship between *Hellenolacerta* and *Phoenicolacerta*

is supported only in the BI tree based on the concatenated mtDNA (Fig. 1; BPP: 0.96). Regarding *Algyroides*, the monophyly of the genus and sister taxa relationships between the species *A. marchi* and *A. fitzingeri*, and *A. nigropunctatus* and *A. moreoticus* are recovered in all the trees including nucDNA data. The clade formed by *A. marchi* and *A. fitzingeri* received high statistical support (Figs. 2–4, in orange; BPP > 0.97, BS = 73). In the trees based on mtDNA data only, the genus is either monophyletic (ML trees; Fig. S1) or paraphyletic (BI; Fig. 1) and a closer relationship between *A. moreoticus* and *A. marchi* is recovered with low statistical support (BPP = 0.85, BS = 59).

3.3. Molecular dating

Molecular dating results are shown in Table 4, along with the 95% highest probability density (HPD) intervals. The divergence between Gallotiinae and Lacertinae is estimated at around 30 million years ago (Mya) (HPD: 11.77–51.28; node a), with divergence between *Gallotia* and *Psammodromus* about 15 Mya (HPD: 8.92–22.38; node q). Divergence between the tribes Lacertini and Eremiadini is estimated at around 17 Mya (HPD: 10.68–25.7; node b). Within Lacertini, the majority of basal splits are placed in a short time span of about 2.5 million years during the Middle Miocene (15–12.5 Mya). The time to most recent common ancestors (TMRCAs) of the Lacertini genera are estimated in the late Miocene (11–5 Mya; Fig. 4, nodes d, f–p).

3.4. Topology tests

Supported nodes in ML trees recovered by previous studies were consistent with our results (Arnold et al., 2007; Fu, 2000, 1998; Harris et al., 1998; Hipsley et al., 2009; Mayer and Pavlicev, 2007; Pavlicev and Mayer, 2009), except the ML tree by Pyron et al. (2013) that shows 6 supported nodes (SHLaLRT values $\geqslant 85$) that are not recovered in our ML tree. All the topological hypothesis constrained according to the three levels of support (support $\geqslant 85$: six nodes; support $\geqslant 90$: five nodes; and support $\geqslant 95$: two nodes), were rejected by the SH and AU tests. Results are presented in Table 5.

4. Discussion

The addition of faster evolving nuclear molecular markers and the use of multi-locus coalescent approaches to infer the phylogeny of Lacertini enabled the detection of new relationships between genera and provided insights into previously open questions concerning genera monophyly and the rapid radiation of the tribe.

4.1. Corroborations and advances in the Lacertini phylogeny

Taxonomy of Lacertidae as described by Arnold et al. (2007) is consistent with our study, with the subfamily Gallotiinae (*Gallotia* and *Psammodromus*) being sister taxon to the subfamily Lacertinae. Within the latter, the Eremiadini tribe, here represented by *Atlantolacerta*, is sister taxon to Lacertini. In the trees based on the concatenated nucDNA *Atlantolacerta* is placed within Lacertini (Figs. 2 and S2). This result may be caused either by the lack of a proper taxon sampling within Eremiadini or by the inadequacy of nuclear molecular data. A short time span between the split of the two Lacertinae tribes and the onset of radiations within each tribe could be out of the scope of the nuclear genes used, but relationships corroborate the taxonomy when using the mitochondrial markers either alone or in combination with nuclear data (Figs. 1, 3 and 4).

A completely new and very interesting phylogenetic relationship was detected in all the trees containing nuclear data between the genera *Archaeolacerta*, *Zootoca* and the sister taxa *Teira* and *Scelarcis* that formed a clade. The position of these taxa has been highly unstable across all the previous phylogenetic studies. *Archaeolacerta*, for instance, has been placed with *Algyroides* (Harris et al., 1998; Carranza et al., 2004), *Darevskia* (Pavlicev and Mayer, 2009), *Zootoca* (Fu, 2000; Hipsley et al., 2009), *Scelarcis* (Salvi et al., 2011) and *Apathya* (Pyron et al., 2013), often with low statistical support. In our results, the clade (*Archaeolacerta*, *Zootoca*, *Teira* and *Scelarcis*) is highly supported, although the position of *Archaeolacerta* and *Zootoca* is unresolved. The geographic distribution of these four genera is allopatric: *Archaeolacerta* is endemic to Corsica and Sardinia, which were separated from the Iberian plate around 30–27 Mya, although land connections with Europe and North Africa existed in the Messinian Salinity Crisis at 5.96–5.33 Mya (Duggen et al., 2003); *Zootoca* has the widest distribution of all lacertids, covering most of Eurasia north of the Mediterranean peninsulas; *Teira* has the westernmost distribution in the Madeira archipelago; and *Scelarcis* is endemic to northwest Africa. In addition to unrelated geographic distribution, these genera are also morphologically very different, all presenting unique morphological characters within Lacertini and many features found only in a minority of other Lacertini (Arnold et al., 2007). Therefore, the peculiar morphology of the members of this group, which is today represented by four effectively monotypic genera whose geographical distribution show little commonality, indicate that it is a relictual group that was once diverse and widespread. An ancient relationship between the members of this group during early speciation events in the tribe may explain why only the faster evolving nuclear markers used in this study provided enough phylogenetic signal for this relationship. The fact that this group had never been recovered in previous phylogenetic studies could be the result of different non-exclusive scenarios: (i) the extinction of original lineages from this ancestral group; (ii) the possible loss of signal in the mtDNA due to saturation at deep nodes; and (iii) the use of slow nuclear markers in previous studies, which might have missed the discrimination between this old split and others slightly older. This finding emphasises the importance of using fast nuclear molecular markers in the phylogenetic inference of fast radiations as they may shed light on some basal polytomies when the clustering of internal nodes occur in a short time span, such as in the case of Lacertini.

The close relationship between the genera *Teira* and *Scelarcis* found in all our trees (Figs. 1–4) is consistent with previous studies. *Scelarcis* was once included in the genus *Teira* (Mayer and Bischoff, 1996), and it has been argued by Pavlicev and Mayer (2009) that they should be reunited again under the genus *Teira*. However, these genera exhibit unique morphological features and, considering the high intraspecific differentiation found within *Teira dugesii* and *Scelarcis perspicillata*, they may represent reciprocally monophyletic species complexes (Brehm et al., 2003; Perera et al., 2007). Moreover, from a taxonomic point of view the repetitive actions of splitting and lumping these two genera may produce taxonomic instability rather than simplifying it, and therefore we suggest to keep the taxonomy proposed by Arnold et al. (2007).

Concerning the monophyly of the Lacertini genera, a previous study by Pavlicev and Mayer (2009) raised the possibility that *Algyroides* could be paraphyletic, as in their results, the monophyletic *Dinarolacerta* clade is nested within the *Algyroides* clade. Our results confirm that these two genera are closely related and form a clade (Figs. 1–4). Moreover, the nucDNA data used in this study support the monophyly of *Algyroides,* which is recovered in all the trees based on nuclear data (Figs. 2–4, Fig. S3; but also in the ML mtDNA tree, see Fig. S1), whereas the paraphyly of this genus is recovered only in the BI mtDNA tree (Fig. 1). Since overall we

have no support for paraphyly from molecular data, and considering that the four *Algyroides* species share unique morphological characters that distinguish them from all other lacertids (Arnold, 1973; Arnold et al., 2007) it is highly probable that this genus is monophyletic. Intrageneric relationships of *Algyroides* consist of two sister species pairs, the western clade of *A. marchi* and *A. fitzingeri* from southeast Spain and Corsica–Sardinia, and the eastern clade of *A. nigropunctatus* and *A. moreoticus* from the Balkan Peninsula and Peloponnese. The split between these two clades is represented by very long branches in all the trees, with a short internode between them and the clade including *Dinarolacerta* species. This pattern suggests a scenario where the split between the two lineages including two extant pairs of *Algyroides* sister taxa occurred soon after the cladogenesis between *Algyroides* and *Dinarolacerta*, likely followed by extensive extinction within *Algyroides* lineages which are today represented by four species with a relictual distribution. This would explain the well supported relationships between sister taxa within *Algyroides* and *Dinarolacerta* and the blurred relationships between these genera especially when using mitochondrial (Harris et al., 1999; this study) and slow evolving nuclear markers (Pavlicev and Mayer, 2009; Pyron et al., 2013).

Several sister taxa relationships recovered by our results were previously described, such as the case of the green lizards *Lacerta* and *Timon* (Arnold, 1973; Harris et al., 1998; Fu, 2000; Carranza et al., 2004; Arnold et al., 2007; Pyron et al., 2013). The species from these two genera are morphologically different from all other Lacertini, sharing a significantly bigger size and numerous non-molecular features that do not usually appear in the small body-sized lizards from the rest of the tribe (Arnold et al., 2007).

The sister taxa relationships recovered between the genera *Anatololacerta* and *Parvilacerta* and between *Darevskia* and *Iranolacerta* have already been described before (Harris et al., 1998; Carranza et al., 2004; Arnold et al., 2007; Mayer and Pavlicev, 2007; Hipsley et al., 2009; Pavlicev and Mayer, 2009; Pyron et al., 2013). Species in each of these genera pair occupy the same geographic regions, the former species occur in Anatolia and Middle-East, the latter in the Caucasus and Middle-East, suggesting that they diverged from a common ancestor living somewhere near their shared geographical area.

4.2. Phylogenetic hypotheses on the evolutionary history of the Lacertini

The phylogenetic position of all other genera of the tribe (*Apathya*, *Dalmatolacerta*, *Iberolacerta*, *Hellenolacerta*, *Podarcis*, *Phoenicolacerta* and *Takydromus*) is not resolved in this study, despite the addition of information from fast evolving nuclear DNA and the application of coalescent-based phylogenetic methods. Therefore, our results support the hypothesis of a hard polytomy within the evolutionary tree of Lacertini (Pavlicev and Mayer, 2009). The basal polytomy observed in Lacertini would be indicative of a fast radiation, which, according to our molecular dating estimates, place the internal node divergence in a relatively short time span of about 2.5 million years in the Middle Miocene (from 15 to 12.5 Mya). A similar age for the radiation event has been described before by Pavlicev and Mayer (2009). The fast radiation hypothesis agrees with most of the previous molecular studies (Harris et al., 1998; Fu, 1998, 2000; Arnold et al., 2007; Mayer and Pavlicev, 2007) and was further corroborated by Pavlicev and Mayer (2009), but is in sharp contrast with the results from the supermatrix approach applied by Pyron et al. (2013), where the internal branching of the Lacertini (sub)tree is almost completely statistically supported. Results of topological tests comparing our ML tree with that of Pyron et al. (2013) indicate that differences between these trees are statistically significant even for relationships very highly supported in the latter study. While differences between our results and Pyron et al. (2013) can be explained by the use of different molecular data and phylogenetic methods, this cannot explain the differences between Pyron et al. (2013) and the previous studies. Indeed, Pyron et al. (2013) used mostly the data generated in previous studies and implemented the same concatenation approach. On the other hand, since Pyron and colleagues were focused on the species-level relationships between squamate reptiles rather than on Lacertini, they used a very large-scale taxon sampling including 4161 species of lizards and snakes and a non-squamate outgroup taxa, *Sphenodon punctatus*. Estimating a tree of this size required high-speed approximations of tree topology searches, substitution models parameter estimates, as well as to assess node support for which they relied on a non-parametric SHLaLRT approach,since bootstrap analysis was computationally intractable. Such a large-scale taxonomic focus also required the use of a large squamate sequence alignment and a non-squamate outgroup which are certainly appropriate to infer and root relationships between the main squamate lineages but maybe not optimal to assess relationships within Lacertini. These considerations suggest that the supermatrix approach may provide high support for relationships within tip-clades which are actually not supported and inconsistent with those phylogenetic studies, with a narrower taxonomic focus, from where the data used in the supermatrix originated.

Finally, while providing additional support that inferring basal relationships within Lacertini is challenging, this study also highlights how adding a few fast evolving nuclear markers helps to shed some light on many ancient relationships within the tribe. In this context, the application of Next Generation Sequencing approaches makes it possible to generate information from thousands loci across the whole genome at a reasonable cost (McCormack et al., 2013), thus representing a promising research direction to further investigate early cladogenesis within Lacertini.

5. Conclusion

This study corroborates the difficulties in the recovery of the evolutionary history of Lacertini lizards, with strong evidence that these difficulties reflect a fast radiation event. Implementing nuclear data in the analyses allowed the recovery of novel phylogenetic relationships that solved some basal polytomies in previous studies, as well as support for the monophyly of *Algyroides*, and an overall increase in the node statistical support. Adding many informative nuclear DNA markers to the phylogenetic analyses proved more helpful to the recovery of the evolutionary history of Lacertini than applying different phylogenetic methods. This exemplifies the benefits of the use of fast evolving nuclear DNA to enhance recovery of ancient relationships in groups that experienced fast radiation and extensive extinctions within old lineages.

New data from fast evolving nuclear markers and the multi-species coalescent approach, implemented for the first time in this study, allowed comparisons to be made between two contrasting phylogenetic hypotheses for Lacertini drawn from previous studies focusing on Lacertidae or on a large-scale phylogeny of squamate reptiles. Through topological comparison of supported relationships, we found that the taxon-wide concatenated supermatrix approach provided high support for nodes that are not supported either by analyses of the original sequences data or by new data from this study. These findings have far reaching implication for comparative studies relying on megaphylogenies from supermatrices (Roquet et al., 2013). Indeed, while new large-scale phylogenies built compiling molecular data from previous studies in a supermatrix may be a valuable resource for comparative macroecological and macroevolutionary studies with a focus on wide taxonomic groups or on higher-level relationships, caution is needed when using megaphylogenies as a guide for integrating tip-clades – such as inter-generic – relationships into ecological

studies. In the case of lacertids, relying on the phylogenetic estimates produced in the original studies in which the data were generated may be a better choice, especially when these studies are based on a comprehensive taxon and marker sampling. It remains to be investigated if this is a generality or if the case of Lacertini is a rare example in which the megaphylogeny approach appears to fail.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.04.016.

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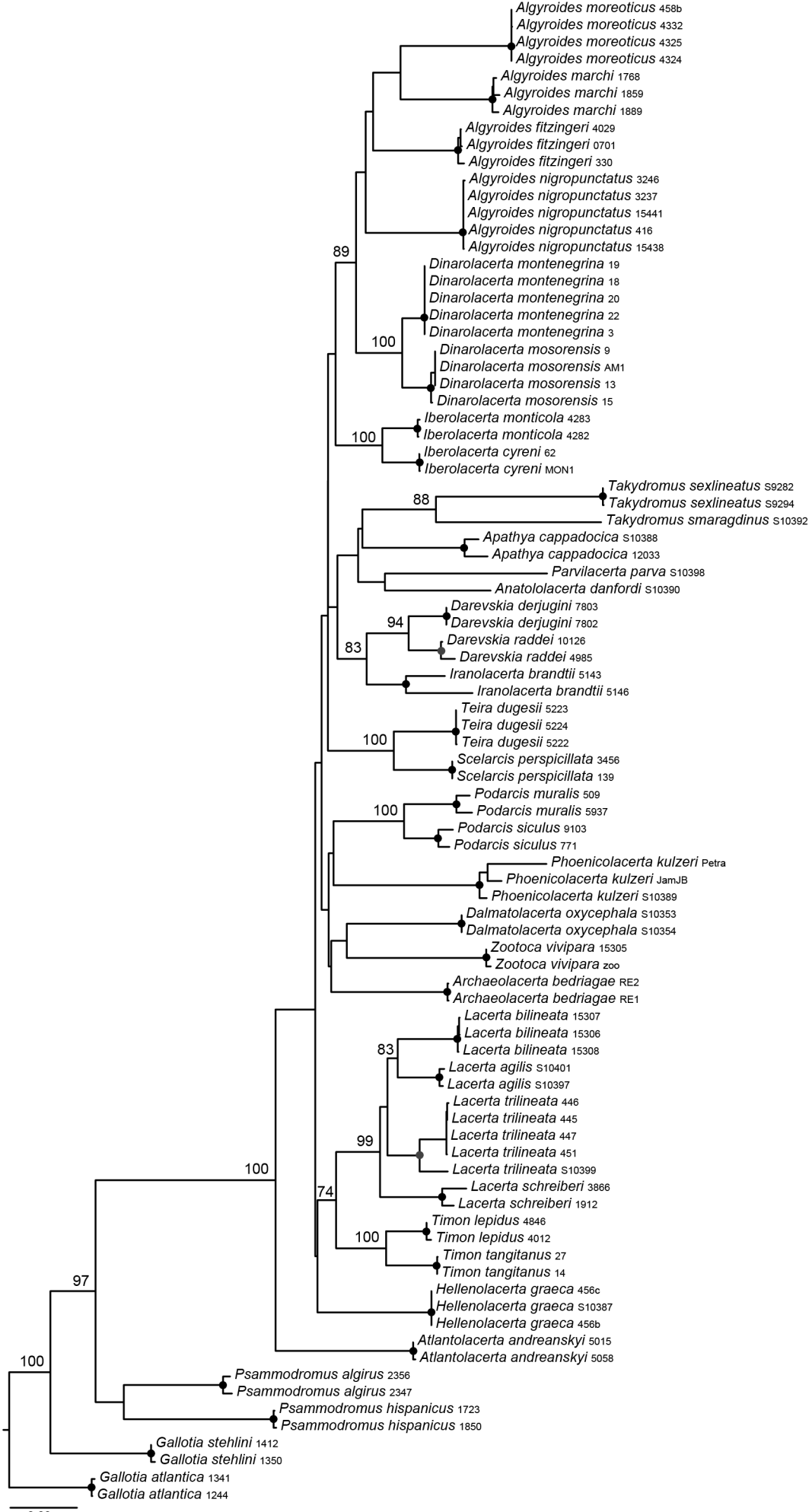

Fig. S1. Phylogenetic relationships of Lacertini based on Maximum Likelihood analyses of concatenated mitochondrial DNA sequences (*12S* and *nd4*). Bootstrap values (BS) ≥70 are shown above branches. Within species, black dots represent BS = 100; grey dots represent BS of 70 ≥ 99.

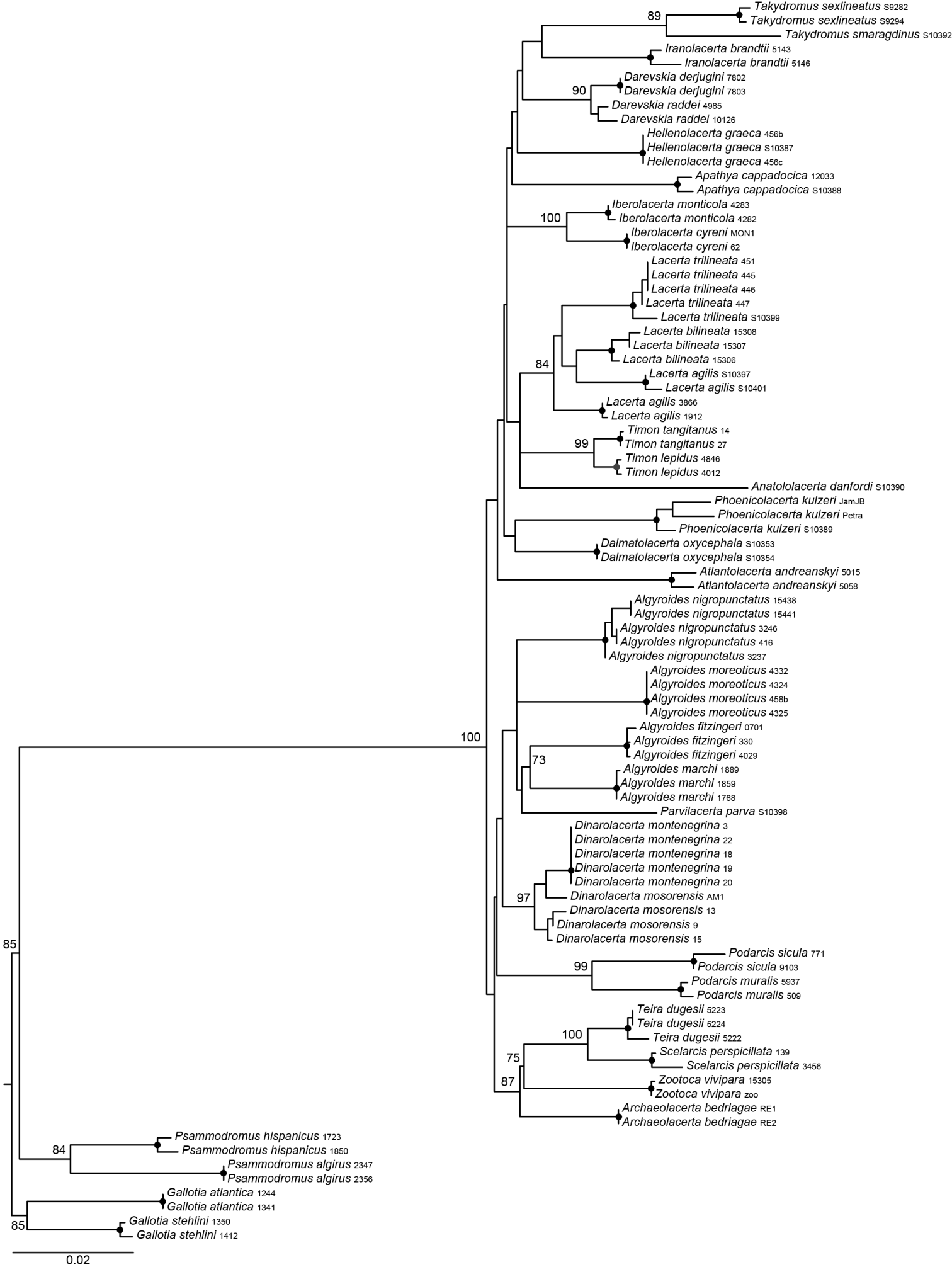

Fig. S2. Phylogenetic relationships of Lacertini based on Maximum Likelihood analyses of concatenated nuclear DNA sequences (*acm4*, *βfib*, *mc1r*, *pdc* and *reln*). Bootstrap values ≥ 70 are shown above branches. Within species, black dots represent BS = 100; grey dots represent BS of 70 ≥ 99.

Fig. S3. Phylogenetic relationships of Lacertini based on Maximum Likelihood analyses of concatenated mitochondrial (*12S* and *nd4*) and nuclear DNA sequences (*acm4*, *βfib*, *mc1r*, *pdc* and *reln*). Bootstrap values ≥ 70 are shown above branches. Within species, black dots represent BS = 100.