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DNA phylogeny of *Lacerta* (*Iberolacerta*) and other lacertine lizards (Reptilia: Lacertidae): did competition cause long-term mountain restriction?

Abstract West European Rock lizards, *Lacerta* (*Iberolacerta*) have small widely separated ranges in highland areas. Mitochondrial and nuclear DNA sequences corroborate the monophyly of the group and show it is not closely related to any of the other Rock lizards with which it was formerly placed in *Archaeolacerta*, an assemblage for which there is no evidence of clade status. *L.* (*Iberolacerta*) consists of four main units: *L.* (*I.*) *horvathi* of NW Croatia and neighbouring regions; the Pyrenees species, *L.* (*I.*) *bonnali, L.* (*I.*) *aranica* and *L.* (*I.*) *aurelioi; L.* (*I.*) *cyreni* of the Iberian Sistema Central, with distinctive populations in the Sierras de Béjar, Gredos and Guadarrama; and *L.* (*I.*) *monticola* of the Serra da Estrela of Central Portugal and NW Spain, this unit also contains *L.* (*I.*) *cyreni martinezricai* of La Peña de Francia, W. Spain and a distinctive population in the Montañas de Sanabria. *L.* (*Iberolacerta*) has persisted in some mountain ranges for at least 4.2 ± 1.4 Ma and may have been restricted to mountains by competition from Wall lizards (*Podarcis*). Its clade status shows it has lost range extensively and has produced few external branches since its initial fragmentation. In contrast, *Podarcis* diversified about the time *L.* (*Iberolacerta*) fragmented, producing a series of widespread lineages that have persisted until the present time. The mainly European subfamily Lacertinae, to which both *L.* (*Iberolacerta*) and *Podarcis* belong, diversified rapidly 13–9 Ma ago, probably largely replacing other lacertid lizards of earlier origin. As another round of replacement started at approximately 9 Ma ago with the spread of *Podarcis*, this may be a recurrent phenomenon in the evolution of some lizard communities.

Key words mitochondrial DNA, nuclear DNA, cytb, 12S rRNA, c-*mos*, evolution, phylogeography, mountain restriction, competition

Introduction

West European Rock lizards, *Lacerta* (*Iberolacerta*), are an assemblage of lacertine lacertids that is almost entirely confined to small widely separated mountain areas (Fig. 1) and has been the subject of considerable recent taxonomic revision (Arribas, 1996, 1999*b*, 2000*a*, 2001). We investigate their evolutionary relationships here using DNA sequences, and then employ the phylogenies produced and molecular clocks to test the following hypotheses: (1) Rock lizards have persisted in and around highland areas for very long periods; (2) *L.* (*Iberolacerta*) and most other European Rock lizards were restricted to mountains by the spread of Wall lizards (*Podarcis*) (Arnold, 1981). The general history of lacertines is also considered.

(a) Systematics of *Iberolacerta*

At present, the following nine species and subspecies are assigned to *Lacerta* (*Iberolacerta*) (Arribas, 2002*a*,*b*,*c*; Pérez-Mellado, 2002; Pérez-Mellado *et al.*, 1993). *L.* (*I.*) *monticola* comprises *L.* (*I.*) *m. monticola* restricted to the Serra da Estrela of Portugal (locality 4 in Fig. 1) and *L.* (*I.*) *m. cantabrica* distributed across a much wider area in northwest Spain including the Cantabrian and León Mountains (localities 2, 5–7 in Fig. 1), the Galaico-Duriense massif, and the northern coast of Galicia (locality 3 in Fig. 1). Some populations in this last region are found at exceptionally low altitudes, living almost at sea level in deep fluvial gorges (Galán, 1999). *L.* (*I.*) *cyreni* occurs in the Spanish Sistema Central where three subspecies have been described: *L.* (*I.*) *cyreni cyreni* for the Sierra de Guadarrama (locality 9 in Fig. 1), *L.* (*I.*) *cyreni castiliana* for the Sierra de Béjar and Sierra de Gredos (localities 8 and 10 in Fig. 1)

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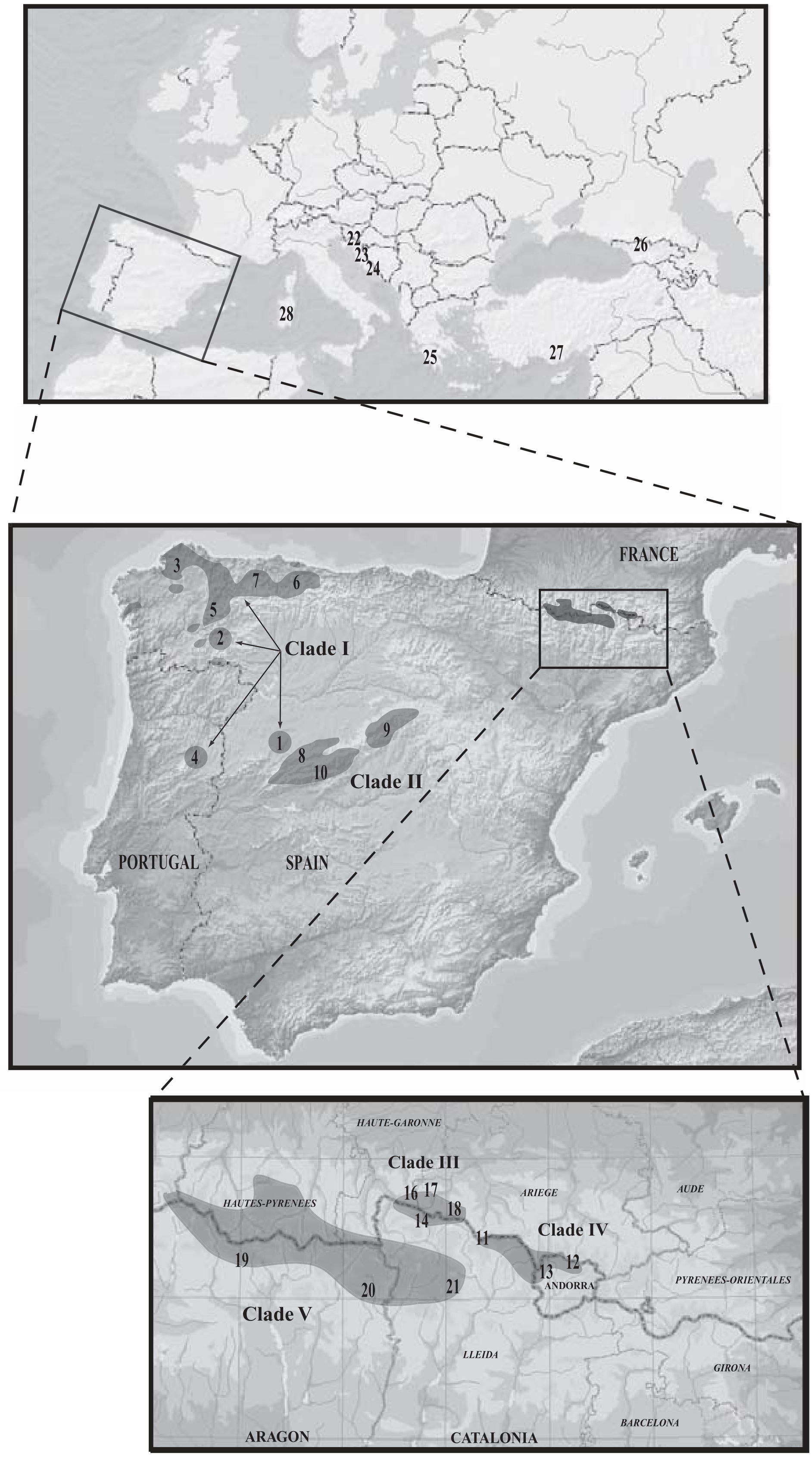

Figure 1 Map of Europe and the Iberian Peninsula showing localities of Rock lizard (*Archaeolacerta*) samples used in the present study including those of the Western Rock lizards, *L.* (*Iberolacerta*); see Table 1 and Fig. 3 for further details.

and *L.* (*I.*) *cyreni martinezricai* which is restricted to the Peña de Francia (locality 1 in Fig. 1). Three further species of *L.* (*Iberolacerta*) have been described in the Pyrenean region: *L.* (*I.*) *bonnali* in the Central Pyrenees, extending from El Portalé in the west (close to locality 19 in Fig. 1) to La Bonaigua in the east (close to locality 21 in Fig. 1); *L.* (*I.*) *aranica* restricted to the Maubèrme Massif, between the valleys of Aran, Spain (locality 14 in Fig. 1) and Ariège, France (localities 16–18 in Fig. 1); and *L.* (*I.*) *aurelioi* in the massifs of Montroig (locality 11 in Fig. 1), Pica d'Estats, Coma Pedrosa (locality 13 in Fig. 1) and Sorteny (locality 12 in Fig. 1). The last member of *Iberolacerta*, *L.* (*I.*) *horvathi*, is found 1100 km further east, inhabiting several mountain areas in north and west Croatia (locality 22 in Fig. 1), Slovenia and small adjoining regions of north-east Italy and Austria. Recent records also exist from the border region of southern Germany and Austria (Capula, 1990).

In the past, all Iberian *L.* (*Iberolacerta*) populations were assigned to *Lacerta monticola* (Mertens & Wermuth, 1960; Arnold, 1973; Arnold & Burton, 1978; Böhme, 1984; Arnold, 1989). Species status was later given to some populations by the following authors: *L* (*I.*) *bonnali* – (Arribas, 1993*a*; Perez-Mellado *et al*., 1993); *L.(I.) aranica* – (Mayer & Arribas, 1996); *L. cyreni* – (Arribas, 1996); and a new species, *L.* (*I.*) *aurelioi* (Arribas, 1994), was described from the eastern Pyrenees. The validity of *Lacerta* (*Iberolacerta*) has been argued on morphological and karyological grounds (Arribas, 1999a). A mitochondrial DNA study covering many lacertid taxa confirmed the affinity of the three *L.* (*Iberolacerta*) species that were included, namely *L.* (*I.*) *monticola, L.* (*I.*) *bonnali* and *L.* (*I.*) *horvathi* (Harris *et al*., 1998).

(b) Relationships to other *lacertine* Rock lizards

The species now assigned to *L.* (*Iberolacerta*) and other lacertine lacertids generally known as Rock lizards, were formerly referred to *Archaeolacerta* Mertens, 1921, a subgenus of *Lacerta* that has been used formally or informally in many publications, by for instance (by Lanza *et al*., 1977; Arnold, 1989; Arribas, 1993*b*; Mayer & Benyr, 1994). The content of this group has varied over time but, besides the members of *L.* (*Iberolacerta*), has included the following taxa (Arnold, 1973): *Lacerta bedriagae, L. oxycephala, L. mosorensis, L. graeca*, the *L. danfordi* group (*L. danfordi, L. anatolica* and *L. oertzeni*), *L. kulzeri*, and most of those now placed in *Lacerta* (*Darevskia*) (Arribas, 1999*a*). Recently, Arribas (1999*a*) has restricted *Archaeolacerta* to the first three species. The distinctive features of *Archaeolacerta* in its broad sense include dorsoventral compression of the head and body, a range of cranial features involving reduction in ossification, smooth often flat dorsal body scales, a reduced smooth-edged collar beneath the neck, rectangular belly scales with little posterior overlap and toes that are laterally compressed and often kinked in the vertical plane (Arnold, 1973, 1989, 1998*a*); other features tend to be primitive among the Lacertidae. One of the reasons why the boundaries of *Archaeolacerta* have been unstable is that the morphological characteristics of the group are variable in their degree of development and also occur, to varying extents, in some other lacertine species including *Lacerta cappadocica*, *L.* (*Teira*) *perspicillata* and some populations of *Podarcis hispanica* s. lat. These features are functionally associated with living on rocky surfaces and the use of crevices in them as refuges, conferring advantage in this situation. As there is also evidence they are labile and easily evolved (Arnold, 1973, 1989, 1998*a*,*b*), it is possible the characters of *Archaeolacerta* were acquired more than once and that the group has multiple origins.

(c) Long-term persistence of mountain taxa

Lacerta (*Iberolacerta*) is just one instance where mountains have endemic species, for many other animals and plant taxa are largely or wholly confined to highland areas. Sometimes whole clades are involved. For example *Asaccus* geckos are rooted in the Hajar Mountains of eastern Arabia and have five species there that vary in body size, other morphological features and ecology (Arnold & Gardner, 1994). Such endemism and radiation suggest that the taxa concerned may have been in highland areas for considerable periods. Molecular clocks provide a means of assessing whether this is actually so.

(d) How mountains get their endemic taxa

There are a number of potential mechanisms that could have produced montane restriction in animals like lizards that are incapable of aerial dispersal (Arnold, 2004). These are not necessarily exclusive and include the following.

1. Mountains that were glaciated during the Pleistocene period are likely to have gained their present faunas recently. With climatic amelioration, cold-tolerant forms in the lowlands may have moved into the cooler mountains after the ice melted.
2. Forms already adapted to mesic mountain-type conditions may have invaded a massif across a temporary bridge of suitable habitat.
3. Lineages may have simply been converted into montane forms by uplift of their original range during the process of mountain building.
4. Mountain taxa may have been derived from the surrounding lowlands by being restricted and perhaps also displaced upwards by competition from ecologically similar taxa.

These different possible mechanisms may generate the following indicators.

1. *Climatic amelioration*. Populations in neighbouring mountains show little differentiation and similar dates of origin that match the time of climatic amelioration.
2. *Bridges*. Populations in the massif concerned have close relatives in another mesic area including a sister taxon and more distant ones; there may be geological or palaeoclimatic evidence of a habitat bridge that would have enabled spread from the source area to the massif.
3. *Uplift*. The endemic montane taxa originated at or before the beginning of mountain building.
4. *Competition*. Possible indicators include: (a) an ecologically similar potential competitor in the surrounding areas; (b) evidence of present competitive interaction between the restricted form and that restricting it, such as precise

replacement at contact areas, and expansion of the restricted taxon when the other is experimentally removed (as has been demonstrated in North American *Plethodon* salamanders – Hairston, 1987); (c) indications of better adaptation of the supposed competitor, for example possession of more derived (apomorphic) features, more versatile reproductive strategies, aggressive behaviour upon other species, etc.; (d) evidence that the restricted form has lost range, such as a disjunct distribution, with the restricted form confined to isolated mountains and the supposed competitor occupying the intervening lowlands; (e) any close relatives of the restricted form outside the range of the competitor may remain in the lowlands and retain a relatively large continuous range; (f) the competing form may arise later than the restricted one, or at least only make contact with it some time after its origin; (g) limitation of the restricted form to mountains is correlated on phylogenies with spread and persistence of the competing form; (h) absence of indicators characterising other kinds of origin of mountain endemics.

(e) Possible competitive restriction of *Lacerta* (*Iberolacerta*) and other Rock lizards

A case has been made (Arnold, 1981, 2004) that Rock lizards were initially restricted to their present largely montane distribution in Europe as a result of competition from Wall lizards (*Podarcis*), rather than by other possible mechanisms. The evidence, admittedly circumstantial, is as follows (letters refer to possible indicators of this kind of restriction listed in the previous section): (a) populations of Rock lizards are usually surrounded by those of Wall lizards which have similar body sizes and general appearance and are ecologically comparable in being heliothermic rock climbing lizards that often forage actively and eat similar mainly arthropod food (Arnold, 1987); (b) in some cases Rock and Wall lizards are in close contact and replace each other over very short distances (for instance, *Podarcis bocagei* and *L.* (*I.*) *monticola cantabrica* in Galicia – Galán, 1999; *Podarcis hispanica* and *L.* (*I.*) *monticola* in the Peña de Francia, Serra da Estrela and Sierra de Gredos – Lizana *et al.* 1988; *Podarcis muralis* and *Lacerta (I) monticola cantabrica* in Spain – Braña, 1983; Arnold, 1987; *Podarcis muralis* and the Pyrenean mountain lizards (*L.* (*I.*) *bonnali*, *L.* (*I.*) *aranica* and *L.* (*I.*) *aurelioi*) – Arribas (2000*a*, *b*, *c*) and *P. muralis* and *L.* (*I*) *horvathi* in Slovenia – Arnold, 1987); (c) Wall lizards have many stable derived morphological features (Arnold, 1973), which might possibly confer competitive superiority and are not found in Rock lizards; (d) in contrast to Wall lizards, Western Rock lizards, *L.* (*Iberolacerta*), have fragmented ranges, something that is true of European Rock lizards as a whole; (e) Rock lizards that are not sympatric with Wall lizards often have larger ranges, sometimes at generally lower attitudes; (f) the often low levels of morphological differentiation between species of Wall lizards and their compact continuous joint range suggest the group may have expanded only relatively recently; (g) the fact that populations of Rock lizards in neighbouring massifs often tend to be morphologically well differentiated makes very recent primary occupation of mountains after climatic amelioration following the last glaciations unlikely; there is no clear evidence that populations of Rock lizards have reached their present mountain ranges across previous habitat bridges.

Phylogenies with even a rough time dimension in the form of molecular clocks would allow the hypothesis of competitive restriction to be tested. It would be possible to see if the Rock lizards are really a clade and test the validity of supposed subclades within it such as *L.* (*Iberolacerta*), so that fragmentation in these units (d) could be confirmed or rejected. It may also be possible to see if Wall lizards arose and expanded at or after the time that Rock lizards originated (f) and whether restriction of Rock lizards actually coincided with spread of Wall lizards (d). Molecular clocks may also reject the possibility that the Rock lizards in particular massifs originated when these were uplifted, or that they reached their montane ranges very recently, as a result of climatic amelioration.

(f) History of the Lacertinae

The Lacertinae comprise about 80 species found in Europe and surrounding mesic areas. Some aspects of their history have recently been discussed elsewhere (Arnold, 2004). Studies involving relatively few lacertine species (Fu, 1998; Harris *et al.*, 1998; Fu, 2000) suggest that the group diversified rapidly. Inclusion of a much greater range of taxa in a phylogenetic analysis would enable this preliminary interpretation to be properly tested and a molecular clock would provide some indication of when diversification occurred.

Here we use a total of 678 bp of mitochondrial DNA gene fragments (up to 303 bp of cytochrome *b* and 375 bp of 12S rRNA) and 335 bp of the c-*mos* nuclear gene to explore the systematics and history of *Lacerta* (*Iberolacerta*), the possible competitive restriction and persistence of it and other Rock lizards, and the history of the Lacertine lacertids.

Materials and methods

Samples, DNA extraction and amplification

To test the monophyly of *Lacerta* (*Iberolacerta*) and explore its relationships to other Rock lizards, a total of 130 individuals of the subfamily Lacertinae were used in this study and 12 individuals of the Gallotiinae employed as outgroups. The Lacertinae comprise representatives of *Podarcis*, *Algyroides* and all the recognised subgenera of *Lacerta*, and of 10 species of *Lacerta* not included in these units. All species and subspecies of *L.* (*Iberolacerta*) described to date (Arribas, 1996, 1999*b*, 2000*a*, 2001) are included as well as most *Podarcis*, to see if the expansion of this group correlates with the restriction of *L.* (*Iberolacerta*). Specimen data are given in Table 1 and selected localities shown in Fig. 1. DNA extraction, PCR amplification and sequencing of the PCR products followed procedures described elsewhere (Carranza *et al.*, 2000). Primers used in both amplification and sequencing were 12Sa and 12Sb (Kocher *et al.*, 1989) for the 12S rRNA gene, cytochrome *b*1 and cytochrome *b*2 (Kocher *et al.*, 1989) for the cytochrome *b* (cytb) gene, and G73 and G74 (Saint *et al.*, 1998) for the nuclear c-*mos* gene.

		ACCESSION NUMBERS
TAXA	**LOCALITY**	**Cyt _b_ / 12SrRNA / C-_mos_**
Psammodromus algirus-1	S. of Tizi Chika, High Atlas (Morocco)	AF080309 / AF080308
*_Psammodromus algirus_-2		AY151835 / AY151914 / AY151998
*_Gallotia stehlini_	Gran Canaria (Canary Islands)	AY151838 / AY151917 / AY152001
*_Gallotia atlantica atlantica_	Fuerteventura (Canary Islands)	AY151836 / AY151915 / AY151999
*_Gallotia atlantica majoratae_	Lanzarote (Canary Islands)	AY151837 / AY151916 / AY152000
*_Gallotia intermedia_	Tenerife (Canary Islands)	AY151844 / AY151923 / AY152007
*_Gallotia simonyi machadoi_	El Hierro (Canary Islands)	AF101219 / AY151924 / AY152008
*_Gallotia caesaris gomerae_	La Gomera (Canary Islands)	AY151842 / AY151921 / AY152005
*_Gallotia caesaris caesaris_	El Hierro (Canary Islands)	AY151843 / AY151922 / AY152006
*_Gallotia galloti palmae_	La Palma (Canary Islands)	AY151841 / AY151920 / AY152004
*_Gallotia galloti eisentrauti_	N. Tenerife (Canary Islands)	AY151839 / AY151918 / AY152002
*_Gallotia galloti galloti_	S. Tenerife (Canary Islands)	AY151840 / AY151919 / AY152003
*_L._ (_Iberolacerta_) _cyreni martinezricai_-1	Peña de Francia (Spain) [1]	AY151897 / AY151977
*_L._ (_Iberolacerta_) _cyreni martinezricai_-2	Peña de Francia (Spain) [1]	AY151895 / AY151975 / AY152009
*_L._ (_Iberolacerta_) _monticola cantabrica_-1	Montañas de Sanabria (Spain) [2]	AY151863 / AY151943 / AY152010
*_L._ (_Iberolacerta_) _monticola cantabrica_-2	Rio Eume (Spain) [3]	AY151865 / AY151945 / AY152011
*_L._ (_Iberolacerta_) _monticola cantabrica_-3	Rio Eume (Spain) [3]	AY151866 / AY151946
*_L._ (_Iberolacerta_) _monticola cantabrica_-4	Rio Eume (Spain) [3]	AY151868 / AY151948
*_L._ (_Iberolacerta_) _monticola monticola_-1	Serra da Estrela (Portugal) [4]	AY151870 / AY151950 / AY152012
*_L._ (_Iberolacerta_) _monticola monticola_-2	Serra da Estrela (Portugal) [4]	AY151871 / AY151951
*_L._ (_Iberolacerta_) _monticola monticola_-3	Serra da Estrela (Portugal) [4]	AY151872 / AY151952
*_L._ (_Iberolacerta_) _monticola cantabrica_-6	Sierra de Caurel (Spain) [5]	AY151857 / AY151937 / AY152013
*_L._ (_Iberolacerta_) _monticola cantabrica_-7	Sierra de Caurel (Spain) [5]	AY151858 / AY151938 / AY152014
*_L._ (_Iberolacerta_) _monticola cantabrica_-8	Sierra de Caurel (Spain) [5]	AY151859 / AY151939
*_L._ (_Iberolacerta_) _monticola cantabrica_-9	Sierra de Caurel (Spain) [5]	AY151860 / AY151940 / AY152015
*_L._ (_Iberolacerta_) _monticola cantabrica_-11	Somiedo (Spain) [7]	AY151864 / AY151944 / AY152016
*_L._ (_Iberolacerta_) _monticola cantabrica_-12	Somiedo (Spain) [7]	AY151856 / AY151936
*_L._ (_Iberolacerta_) _monticola cantabrica_-13	Somiedo (Spain) [7]	AY151855 / AY151935
*_L._ (_Iberolacerta_) _monticola cantabrica_-10	Puerto de Vegerada (Spain) [6]	AY151869 / AY151949 / AY152017
*_L._ (_Iberolacerta_) _monticola cantabrica_-14	Puerto de Vegerada (Spain) [6]	AY151861 / AY151941 / AY152018
*_L._ (_Iberolacerta_) _monticola cantabrica_-15	Puerto de Vegerada (Spain) [6]	AY151862 / AY151942
*_L._ (_Iberolacerta_) _horvathi_	NW Croatia [22]	AY256648 / AY256653 / AY256658
*_L._ (_Iberolacerta_) _cyreni castiliana_-4	Sierra de Bejar (Spain) [8]	AY151851 / AY151931
*_L._ (_Iberolacerta_) _cyreni castiliana_-5	Sierra de Bejar (Spain) [8]	AY151850 / AY151930
*_L._ (_Iberolacerta_) _cyreni castiliana_-7	Sierra de Bejar (Spain) [8]	AY151849 / AY151929 / AY152019
*_L._ (_Iberolacerta_) _cyreni cyreni_-8	Navacerrada (Spain) [9]	AY151846 / AY151926 / AY152020
*_L._ (_Iberolacerta_) _cyreni cyreni_-9	Navacerrada (Spain) [9]	AY151845 / AY151925 / AY152021
*_L._ (_Iberolacerta_) _cyreni cyreni_-10	Navacerrada (Spain) [9]	AY151847 / AY151927
*_L._ (_Iberolacerta_) _cyreni castiliana_-11	Sierra de Gredos (Spain) [10]	AY151854 / AY151934
*_L._ (_Iberolacerta_) _cyreni castiliana_-12	Sierra de Gredos (Spain) [10]	AY151852 / AY151932 / AY152022
*_L._ (_Iberolacerta_) _cyreni castiliana_-13	Sierra de Gredos (Spain) [10]	AY151853 / AY151933
*_L._ (_Iberolacerta_) _aurelioi_-1	Montroig (Spain) [11]	AY151883 / AY151963 / AY152023
*_L._ (_Iberolacerta_) _aurelioi_-2	Sorteny (Andorra) [12]	AY151882 / AY151962 / AY152024
*_L._ (_Iberolacerta_) _aurelioi_-3	Circ de Comapedrosa (Spain) [13]	AY151880 / AY151960 / AY152025
*_L._ (_Iberolacerta_) _aurelioi_-4	Circ de Comapedrosa (Spain) [13]	AY151881 / AY151961
*_L._ (_Iberolacerta_) _aranica_-1	Coll de Barrados (Spain) [14]	AY151879 / AY151959 / AY152026
*_L._ (_Iberolacerta_) _aranica_-3	Serre de Ventaillou (France) [16]	AY151876 / AY151956 / AY152028
*_L._ (_Iberolacerta_) _aranica_-4	Combre de Muntanyole (France) [17]	AY151875 / AY151955
*_L._ (_Iberolacerta_) _aranica_-5	Combre de Muntanyole (France) [17]	AY151874 / AY151954 / AY152029
*_L._ (_Iberolacerta_) _aranica_-6	Muntanyes de Barlongere (France) [18]	AY151873 / AY151953 / AY152030
_L_ (_Iberolacerta_) _bonnali_-2	Ordesa (Spain) [19]	AF080291 / AF080290

Table 1 Details of material and sequences used in the present study. Numbers after taxa refer to Fig. 2, those after localities to Fig. 1. All specimens specifically sequenced for this work have been marked with an asterisk. All the rest of the sequences used have been downloaded from Genbank and are mainly from Harris _et al._ (1998, 1999, 2002) and Fu (2000).

		ACCESSION NUMBERS		
TAXA	**LOCALITY**	**Cyt _b_**	**12SrRNA**	**C-_mos_**
*_L. (Iberolacerta) bonnali_-3	Ordesa (Spain) [19]	AY151890	AY151970	AY152032
*_L. (Iberolacerta) bonnali_-4	Possets (Spain) [20]	AY151894	AY151974	AY152033
*_L. (Iberolacerta) bonnali_-5	Possets (Spain) [20]	AY151892	AY151972	
*_L. (Iberolacerta) bonnali_-6	Possets (Spain) [20]	AY151893	AY151973	
*_L. (Iberolacerta) bonnali_-7	Port de Rus (Spain) [21]	AY151889	AY151969	AY152035
*_L. (Iberolacerta) bonnali_-8	Port de Rus (Spain) [21]	AY151888	AY151968	
*_L. (Iberolacerta) bonnali_-9	Port de Rus (Spain) [21]	AY151887	AY151967	
*_Lacerta mosorensis_-1	Southern Croatia etc. [23]	AY151902	AY151982	
*_Lacerta mosorensis_-2	Southern Croatia etc. [23]	AY151903	AY151983	
*_Lacerta mosorensis_-3	Southern Croatia etc. [23]	AY151904	AY151980	
*_Lacerta mosorensis_-4	Southern Croatia etc. [23]	AY151905	AY151985	AY151995
*_Lacerta mosorensis_-5	Southern Croatia etc. [23]	AY151901	AY151981	
*_Lacerta mosorensis_-6	Southern Croatia etc. [23]	AY151900	AY151984	
Lacerta bedriagae bedriagae-1	Foret d'Ospidale (Corsica)	AF080326	AF080325	
*_Lacerta bedriagae bedriagae_-2	Corsica (France)	AY256649	AY256654	
*_Lacerta bedriagae bedriagae_-3	Corsica (France)	AY256650	AY256655	
*_Lacerta oxycephala_-1	Bosnia [24]	AY256651	AY256656	AY256659
*_Lacerta oxycephala_-2	Bosnia [24]	AY256652	AY256657	AY256660
Lacerta kulzeri		AF112295	AF112294	AF148712
Lacerta danfordi danfordi	Bolkar Mountains (Turkey) [27]	AF080323	AF080322	
Algyroides marchi	Sierra de Cazorla (Spain)	AF080307	AF080306	
Lacerta brandtii	Kuh Rang (Iran)	AF080320	AF080319	
Lacerta graeca	Feneus Mati (Greece) [25]	AF080272	AF080271	
Lacerta cappadocica	Eastern Turkey (Turkey)	AF080329	AF080328	
Lacerta laevis	Mount Scopus, Jerusalem (Israel)	AF080332	AF080331	
*_Lacerta (Zootoca) vivipara_-1	Andorra	AY151913	AY151993	
Lacerta (Zootoca) vivipara-2	Surrey (UK)	AF080335	AF080334	
Lacerta (Teira) andreanszkyi	Oukaimeden, High Atlas (Morocco)	AF206537	AF206603	AF211203
*_Lacerta (Teira) perspicillata_-1	Taza (Morocco)	AY151898	AY151978	
Lacerta(Teira) perspicillata-2	Oukaimeden, High Atlas (Morocco)	AF080304	AF080303	
Lacerta (Teira) dugesii -1	San Miguel, Azores (Portugal)	AF080314	AF080313	
Lacerta (Teira) dugesii -2				AF315398
Lacerta (Timon) pater	Ouarzazate (Morocco)	AF080294	AF080293	
Lacerta (Timon) lepida-1	Spain	AY151899	AY151979	AY151994
Lacerta (Timon) lepida-2	Badajoz (Spain)	Z48049	Z48050	
Lacerta (Timon) princeps	SE Turkey (Turkey)	AF080383	AF080382	
Lacerta (Darevskia) chlorogaster	Near Tangerud (Azerbaijan)	AF080285	AF080284	
Lacerta (Darevskia) saxicola brauneri	Western Caucasus (Russia) [26]	AF080282	AF080281	
Lacerta (Lacerta) agilis	Roermond (Netherlands)	AF080299	AF080298	
Lacerta (Lacerta) media	Arailer Mountains (Armenia)	U88603	AF206590	
Lacerta (Lacerta) bilineata	France			AF211204
Lacerta (Parvilacerta) fraasii	Sammim Mountains (Lebanon)	AF080317	AF080316	
*_Podarcis muralis_-1	Andorra	AY151908	AY151988	
*_Podarcis muralis_-2	Andorra	AY151909	AY151989	
*_Podarcis muralis_-3	Somiedo, Asturias (Spain)	AY151912	AY151992	
*_Podarcis muralis_-4	Navacerrada, Madrid (Spain)	AY151910	AY151990	
*_Podarcis muralis_-5	Navacerrada, Madrid (Spain)	AY151911	AY151991	
Podarcis muralis-6		AF133455	AF133454	
Podarcis muralis-7	Near Cannes (France)	AF080278	AF080277	
Podarcis muralis-8	Benasque (Spain)	AF206572	AF206600	
Podarcis peloponnesiaca	Peloponnese (Greece)	AF133452	AF133451	
Podarcis taurica	Russia	AF080280	AF080279	

Table 1 _Continued..._

Podarcis milensis	Milos Island (Greece)	AF133450 / AF133449
Podarcis gaigeae	Skyros Island (Greece)	AF133445 / AF133444
Podarcis filfolensis	St. Pauls Bay (Malta)	AF133443 / AF133442
Podarcis tiliguerta	Sardinia (Italy)	AF133457 / AF133456
Podarcis lilfordi	Balearic Islands (Spain)	AF052639 / AF133447
Podarcis pityusensis	Balearic Islands (Spain)	AF052640 / AF133453
Podarcis atrata-1	Columbretes Islands (Spain)	AF052636 / AF133439
Podarcis carbonelli-1	Serra da Estrela (Portugal)	AF372079 / AF469418
Podarcis bocagei-1	Vairao (Portugal)	AF372087 / AF469421
Podarcis bocagei-2		/ AF315399
Podarcis hispanica-1	Medinaceli (Spain)	AF469436 / AF469435
*_Podarcis hispanica_-2	Andorra	AY134703 / AY134738 / AY151996
Podarcis hispanica-3	Barcelona (Spain)	AF469432 / AF469431
Podarcis hispanica-4	Leiria (Portugal)	AF469458 / AF469457
Podarcis hispanica-5	Portalegra (Portugal)	AF372086 / AF469456
Podarcis hispanica-6	Madrid (Spain)	AF469460 / AF469459
Podarcis hispanica-7	Montesinho (Portugal)	AF469449 / AF469448
Podarcis hispanica-8	Sierra de Gredos (Spain)	AY134704 / AY134739
*_Podarcis hispanica_-9	Peña de Francia (Spain)	AY151906 / AY151986 / AY151997
*_Podarcis hispanica_-10	Peña de Francia (Spain)	AY151907 / AY151987
Podarcis hispanica-11	Granada (Spain)	AF469428 / AF469427
Podarcis hispanica-12	Cuenca (Spain)	AF469430 / AF469429
Podarcis hispanica-13		/ AF148702
Podarcis hispanica vaucheri-1	Ain Draham (Tunisia)	AY134700 / AY134735
Podarcis hispanica vaucheri-2	S. of Ain Draham (Tunisia)	AY134698 / AY134733
Podarcis hispanica vaucheri-3	10Km S. of Tabarca (Tunisia)	AY134699 / AY134734
Podarcis hispanica vaucheri-4	Mairena del Aljarace (Spain)	AY134684 / AY134719
Podarcis hispanica vaucheri-5	Sevilla (Spain)	AY134685 / AY134720
Podarcis hispanica vaucheri-6	Taza (Morocco)	AY134693 / AY134728
Podarcis hispanica vaucheri-7	15Km S.W. of Zinat (Morocco)	AY134689 / AY134724
Podarcis hispanica vaucheri-8	Bab-Berred (Morocco)	AY134690 / AY134725
Podarcis hispanica vaucheri-9	Bab-Berred (Morocco)	AY134691 / AY134726
Podarcis hispanica vaucheri-10	Azrou (Morocco)	AY134702 / AY134737
Podarcis hispanica vaucheri-11	N. of Oukaimeden (Morocco)	AY134683 / AY134718
Podarcis hispanica vaucheri-12	15Km S.W. of Zinat (Morocco)	AY134688 / AY134723
Podarcis hispanica vaucheri-13	8Km S.W. of Zinat (Morocco)	AY134687 / AY134722
Podarcis hispanica vaucheri-14	Jebel Musa (Morocco)	AY134701 / AY134736
Podarcis hispanica vaucheri-15	8Km S.W. of Zinat (Morocco)	AY134686 / AY134721
Podarcis hispanica vaucheri-16	El-Had (Morocco)	AY134694 / AY134729

Table 1 *Concluded.*

Phylogenetic analyses

Three data sets were used in the phylogenetic analyses. Data set I included 138 specimens listed in Table 1 and 589 bp of aligned mtDNA sequence data (291 bp of cytochrome *b* (cytb) and 298 bp of 12S rRNA). Data set II included a total of 1013 bp of mitochondrial (303 bp of cytb and 375 bp of 12S rRNA) and nuclear (335 bp of c-*mos*) DNA for at least one representative of every single population of *Iberolacerta* listed in Table 1, 6 representatives of the subfamily Lacertinae and 11 Gallotinae. Data set III included 335 bp of the nuclear c-*mos* gene for all 42 lacertids included in data set II plus seven new sequences downloaded from GenBank. In all data sets, DNA sequences were equal in length.

DNA sequences were aligned by hand using the alignment editor BIOEDIT v. 5.0.9 (Hall, 1999) and taking into account the published secondary structure (Hickson *et al.*, 1996). Alignment gaps were inserted to resolve length differences between sequences, and positions that could not be unambiguously aligned were excluded. Cytb sequences were translated into amino acids prior to analysis and did not show any stop codons, suggesting that all were functional. Three different methods of phylogenetic analysis were employed: maximum-likelihood (ML), Bayesian analysis and maximum parsimony (MP). MODELTEST (Posada & Crandall, 1998) was used to select the most appropriate model of sequence evolution for the ML and Bayesian analyses, under the Akaike Information Criterion. For data sets I and II this was the General Time Reversible (GTR) model, taking into account the shape of the Gamma distribution (G) and the number of invariable sites (I), while for data set III it was the GTR model.

Both ML and MP analyses were performed in PAUP* 4.0b10 (Swofford, 1998). For data sets II and III they included heuristic searches involving tree bisection and reconnection (TBR) branch swapping with 100 random stepwise additions of taxa. Because of the large size of data set I, the search strategy used avoided unnecessary swapping involving replicates that do not locate one of the "islands" containing optimal trees (Giribet & Wheeler, 1999). This strategy involved setting the maxtrees command in PAUP* to 10 000, followed by a heuristic search with TBR branch swapping in which not more than 10 trees of length ≥ 1 were stored, and then inactivating this constraint and swapping on all stored trees to completion. In all MP analyses, gaps were included as a fifth state. In order to correct for the observed transitions (ts) : transversions (tv) ratio, in the MP analyses of data sets I and II, transversions were given the same weight as transitions and four times that weight in different analyses; for data set III, the same weight and two times that weight were used. Nodal support for all MP trees and for the ML tree of data set III was assessed using bootstrap analysis (Felsenstein, 1985) involving 1000 pseudoreplications. Bayesian phylogenetic analyses were performed with MRBAYES v. 2.01 (Huelsenbeck & Ronquist, 2001) using the GTR+I+G model of sequence evolution (see above) with parameters estimated as part of the analysis and four incrementally heated Markov chains with the default heating values. All analyses started with randomly generated trees and ran for 2.5×10^6 generations, with sampling at intervals of 100 generations that produced 25 000 sampled trees. To ensure that the analyses were not trapped on local optima, all data sets were run three times independently, each run beginning with a different starting tree. The log-likelihood values of the 25 000 trees in each analysis were plotted against the generation time. All the trees produced prior to reaching stationarity were discarded, making sure that burn-in samples were not retained. Although stationarity was reached very rapidly (data not shown), only the last 5000 trees in each of the three independent analyses were used to estimate separate 50% majority rule consensus trees for these. The frequency of any particular clade, among the individual trees contributing to the consensus tree, represents the posterior probability of that clade (Huelsenbeck & Ronquist, 2001); only values above 95% were regarded as indicating that clades were significantly supported.

The incongruence length difference (ILD) test (Mickevich & Farris, 1981; Farris *et al*., 1994) was used to check for incongruence between all three genes in data set III. In this test, 10 000 heuristic searches were made and invariable characters were removed before starting the analysis (Cunningham, 1997).

Where appropriate, topological constraints were generated with MRBAYES (Huelsenbeck & Ronquist, 2001) for data set I and with MacClade v. 4.0 (Maddison & Maddison, 1992) for data set II, and compared with our optimal topologies using the Shimodaira-Hasegawa (SH) (Shimodaira & Hasegawa, 1999) test implemented in PAUP * 4.0b10 (Swofford, 1998) and employing RELL bootstrap with 1000 replicates.

Molecular clock considerations

To establish approximate dates for some of the nodes resulting from the analysis of data sets I and II, two different methods were employed. For data set II, the likelihood ratio test (Huelsenbeck & Crandall, 1997) was first used to assess the statistical significance of the difference between the log likelihood of the trees calculated with and without molecular clock assumptions. If the difference between both ML trees were not significant, it would indicate that the gene fragment used to infer the phylogeny was evolving in a clocklike manner and genetic distances between taxa could be used to infer approximate dates. To consider any bias produced by the use of different evolutionary models when calculating distance matrices to subsequently infer evolutionary dates, we used two different models of sequence evolution. The GTR+I+G, selected by MODELTEST as the most appropriate model for data set II, and the Kimura 2-parameters model, used in previous work to infer evolutionary dates from distance matrices in reptiles (Carranza *et al*., 2000, 2001; Paulo *et al*., 2001; Carranza *et al*., 2002; Carranza & Arnold, 2003; Maca-Meyer *et al*., 2003).

Divergence times on trees derived from data sets I and II were also estimated using the Nonparametric Rate Smoothing (NPRS) method implemented in the r8s program (Sanderson, 1997). The source code was compiled and run on a PC under Linux. To avoid the problem of finding only local optima the searches were started at three initial time guesses (num_time-guess = 3). We checked the local stability of the solutions for each guess by perturbing them and restarting the search three times (num_restarts = 3; perturb_factor = 0.05). Given that the NPRS method for estimating divergence times depends on both topology and branch lengths, age ranges were calculated for each node based on four different branch length optimization methods (GTR+I+G, Kimura 2-parameters, ACCTRAN and DELTRAN).

The age of El Hierro island in the Canaries islands, which is estimated as 1 Myr (Guillou *et al*., 1996), was used for calibration. This was on the assumption that the resident *Gallotia caesaris caesaris* colonised this island, soon after its formation, from neighbouring La Gomera, where *G. c. gomerensis* occurs. These taxa are suitable for use in calibration as they are reciprocally monophyletic sister species with low intraspecific variability (Maca-Meyer *et al*., 2003). Apart from the assumption that El Hierro was colonised rapidly, factors that could affect clock calibrations include stochastic variation at low levels of sequence divergence and the possibility of extinct or unsampled lineages (Emerson *et al*., 2000*a*,*b*; Emerson, 2002), although there is no evidence for any of these occurring in *Gallotia* (González *et al*., 1997; Barahona *et al*., 2000; Maca-Meyer *et al*., 2003). For the c-*mos* data set III, no dates could be inferred because there is no difference between *G. c. caesaris* and *G. c. gomerensis* in the gene fragment used.

Results

(a) Analysis of data set I – mitochondrial genes for Lacertinae and Gallotiinae

Monophyly of the Western Rock lizards, *L*. (*Iberolacerta*) and of Rock lizards (*Archaeolacerta* s. lat) in general was investigated using 589 bp of mitochondrial sequence, 273 being

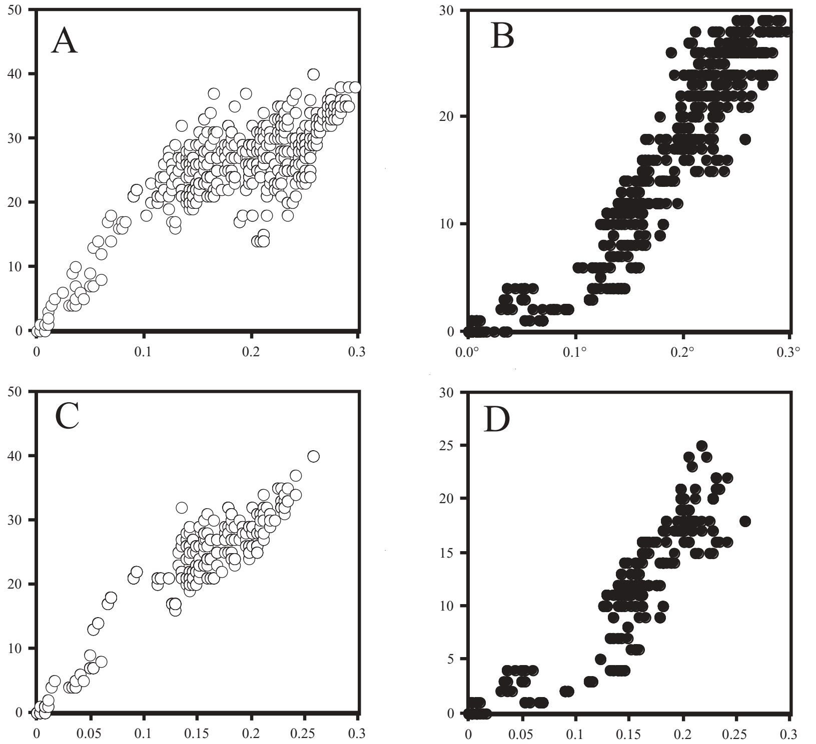

Figure 2 Saturation curves produced when observed number of transitions and transversions within the Lacertinae are plotted against uncorrected genetic distances for the cytochrome b 3rd codon positions. (A) Data set I cytb 3rd codon transitions; (B) Data set I cytb 3rd codon transversions; (C) Data set II cytb 3rd codon transitions; and (D) Data set II cytb 3rd codon transversions.

variable and 247 parsimony-informative. Before the phylogenetic analysis, separate saturation tests were carried out, in which the observed number of ts and tv was plotted against the uncorrected genetic distances. This was done independently for the 12S rRNA and for all three codon positions of cytb. No saturation was apparent, even for the most variable cytb 3rd codon ts (see Fig. 2), so all positions were included in the phylogenetic analyses.

A phylogenetic tree for the Lacertinae and Gallotiinae is shown in Fig. 3. The three independent Bayesian analyses differed only slightly in their overall topology for the Lacertinae, so only the tree with the highest likelihood value is illustrated (1st Bayesian replicate; see Table 2). MP trees were also slightly different from the Bayesian topology in Fig. 3, usually showing less resolution at the base of the tree. Relevant data for the MP analyses with data set I, II and III are shown in Table 3. In all phylogenetic analyses, relationships within the Gallotiinae were identical and very similar to those found in many other studies of the group (e.g. González *et al.*, 1997; Maca-Meyer *et al.*, 2003), indicating that sequences in data set I are sufficient to recover phylogenetic relationships providing there was sufficient time between branching points. Within the Lacertinae, the monophyly of *L.* (*Iberolacerta*) is supported in all analyses by relatively high bootstrap and high Bayesian posterior probability values. But this is not true of *Archaeolacerta* in its broad sense or in the narrow sense of Arribas (1999*a*). To test this conclusion further, constraint analyses were carried out. In these, a tree in which species assigned to *Archaeolacerta* were constrained to monophyly was compared with the topology shown in Fig. 3. The results clearly reject both concepts of *Archaeolacerta* as a monophyletic unit (Table 2). In contrast, Wall lizards (*Podarcis*) are a well supported clade and their internal relationships are congruent with previous analyses (Harris & Arnold, 1999; Harris *et al.*, 2002; Harris & Sa-Sousa, 2002). The *Podarcis hispanica* group of southwest Europe and northwest Africa has up to six monophyletic units in the Iberian Peninsula that can be regarded as separate species, although their taxonomy is not fully resolved (Harris *et al.*, 2002; Harris & Sa-Sousa, 2002).

The tree also indicates some other included species assigned to subgenera of *Lacerta* are closely related to each other. This is true of *Lacerta* (*Lacerta*) *agilis* and *L.* (*L.*) *media*, and for *L.* (*Darevskia*) *saxicola* and *L.* (*D.*) *chlorogaster*. While *L.* (*Timon*) *lepida* and *L.* (*T.*) *pater* appear closely related to each other on the tree, *L.* (*T.*) *princeps* is not placed with them, but a constraint analysis does not reject this association (Table 2). Similarly, while *L.* (*Teira*) *dugesii* and *L.* (*T.*) *perspicillata* are clearly closely related on the tree, *L. andreanszkyi*, which is also sometimes placed in *L.* (*Teira*), is not associated with them but again the supposed relationships of these three forms does not fail a constraint test.

Approximate dates for some diversification events within the Lacertinae were inferred using the NPRS method. They are shown in Fig. 3 and indicate this subfamily underwent rapid splitting during the mid-late Miocene period about 13–9 Ma ago. Data set I suggests diversification in *Podarcis* and *Iberolacerta* occurred at similar times about 9–8 Ma ago, in the Late Miocene. Speciation within the Iberian *P. hispanica*

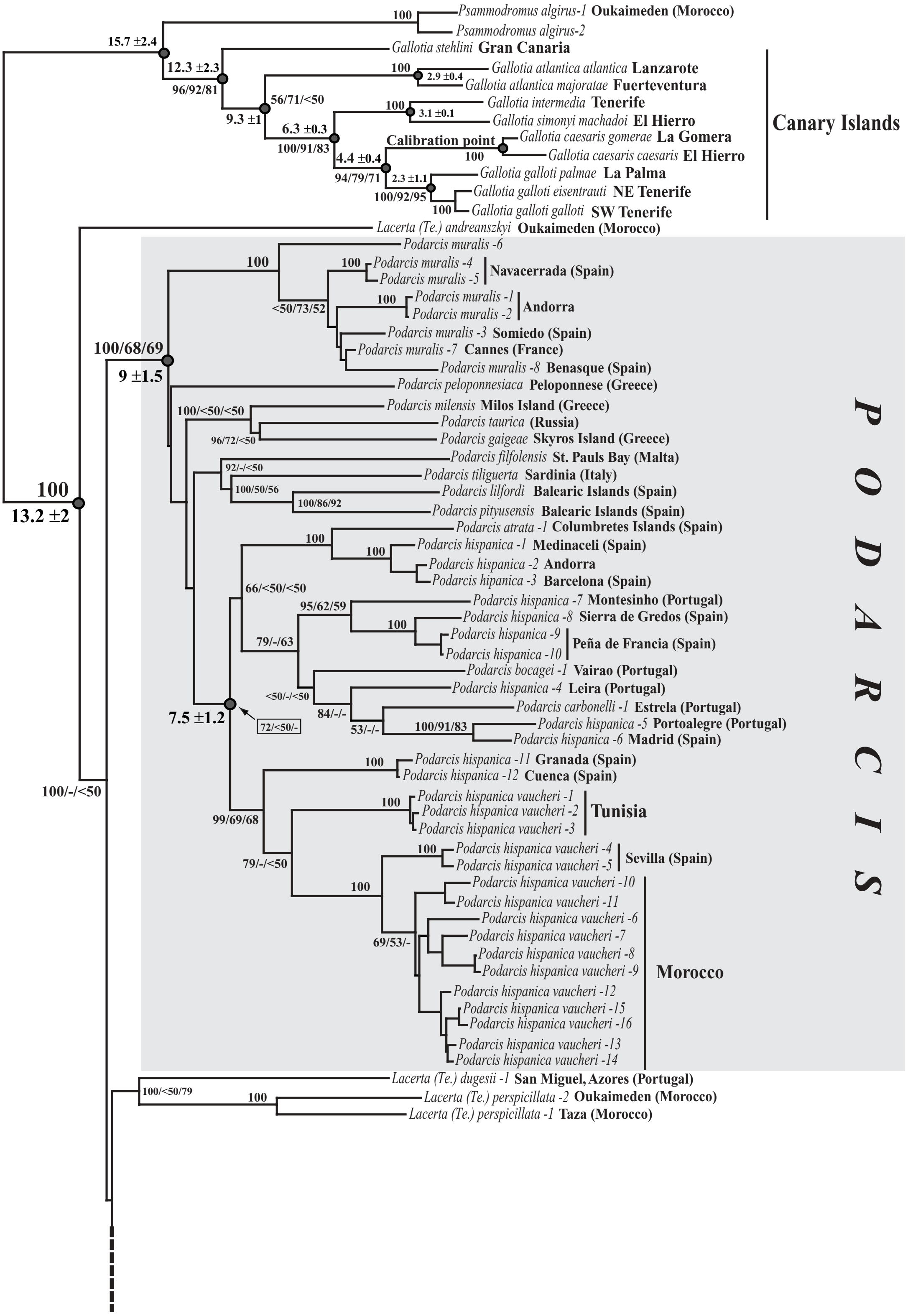

Figure 3 Bayesian tree for Lacertinae and Gallotiinae inferred from data set I (mtDNA sequence). Bootstrap support and posterior probability values are shown at the corresponding nodes: Left, posterior probability values derived by Bayesian analysis (1st replicate); Middle, bootstrap support derived by MP (ts = tv); Right, bootstrap support derived by MP (ts = 1; tv = 4). When difference between the bootstrap and posterior probability values was < 5% only the average value is shown. The '<' symbol is used to show that the bootstrap/posterior probability support for that node is lower than 50% and the '-' symbol indicates that a particular node is never recovered when using this method. Estimated mean ages and standard deviations are given for selected nodes marked by filled circles, including those in *Gallotia*, the first bifurcation within the Lacertinae, and those within *Podarcis* as a whole, including the *P. hispanica* group, and *L.* (*Iberolacerta*). Ages have been calculated using the NPRS method implemented in r8s (see Material and Methods). Italic numbers after taxon names refer to different individual lizards, details of which can be found in Table 1; numbers in square brackets refer to localities shown in Fig. 1.

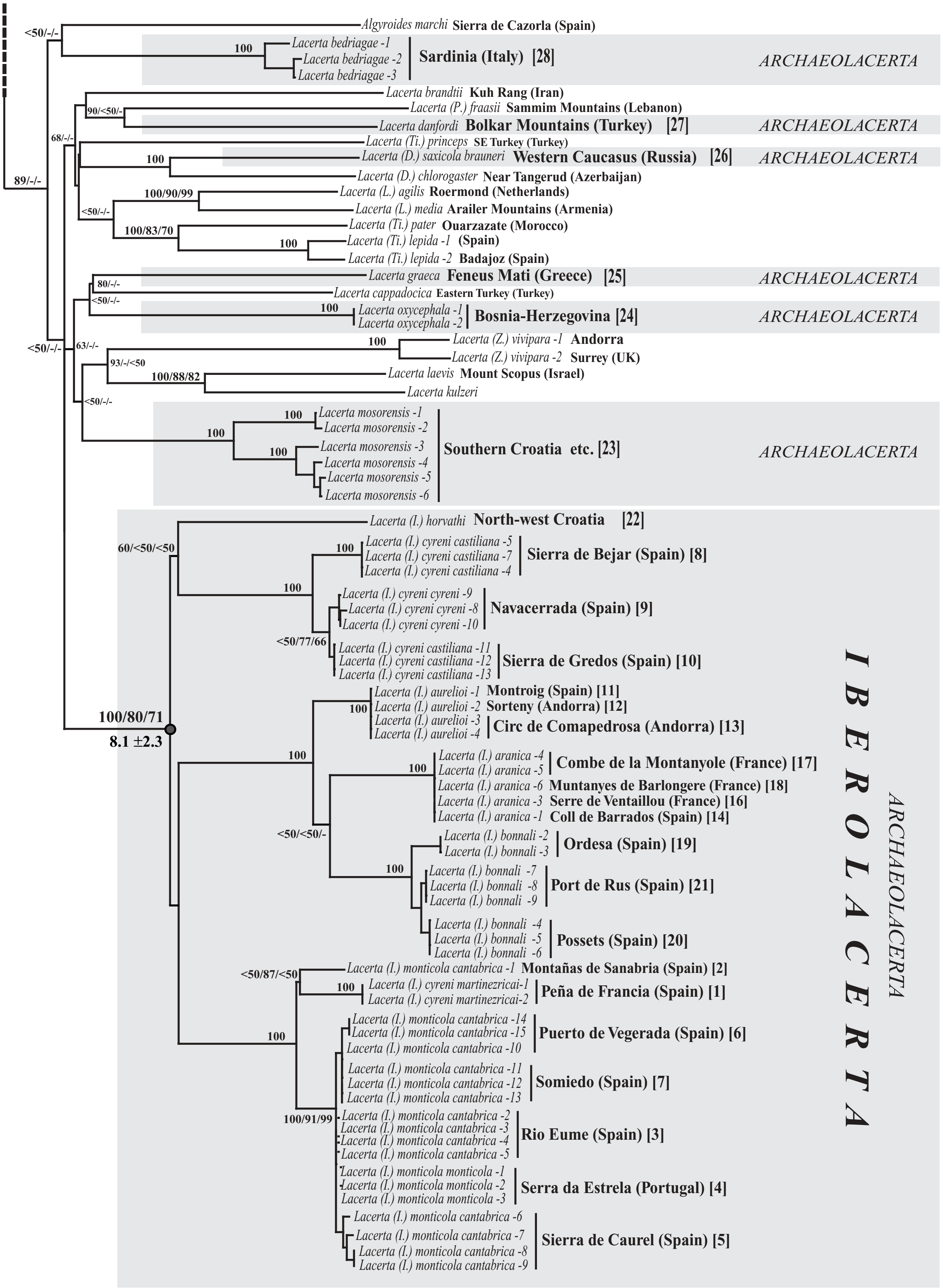

Figure 3 Continued

Tree	−Log likelihood	Δ−Log likelihood	SH *P*
Unconstrained Bayesian tree (Fig. 3, 1st replicate)	9764.09120	(best)	
Unconstrained Bayesian tree (2nd replicate)	9772.05133	7.96012	0.874
Unconstrained Bayesian tree (3rd replicate)	9768.09065	3.99945	0.950
Constrained (*Archaeolacerta s.* l. monophyletic) (1st replicate)	9883.51612	119.42492	0.000*
Constrained (*Archaeolacerta s.* l. monophyletic) (2nd replicate)	9858.60184	94.51063	0.000*
Constrained (*Archaeolacerta s.* l. monophyletic) (3rd replicate)	9892.23583	128.14463	0.000*
Constrained (*Archaeolacerta s.* nov. monophyletic) (1st replicate)	9861.94101	97.84981	0.000*
Constrained (*Archaeolacerta s.* nov. monophyletic) (2nd replicate)	9825.49580	61.40459	0.029*
Constrained (*Archaeolacerta s.* nov. monophyletic) (3rd replicate)	9822.90827	58.81707	0.028*
Constrained (*Teira*. monophyletic) (1st replicate)	9790.67139	26.58019	0.392
Constrained (*Teira*. monophyletic) (2nd replicate)	9793.64288	29.55168	0.318
Constrained (*Teira*. monophyletic) (3rd replicate)	9816.86243	52.77123	0.086
Constrained (*Timon* monophyletic) (1st replicate)	9778.94335	14.85215	0.680
Constrained (*Timon* monophyletic) (2nd replicate)	9802.85191	38.76070	0.182
Constrained (*Timon* monophyletic) (3rd replicate)	9775.33382	11.24262	0.792

Table 2 Statistical support for alternative hypotheses of relationships of selected Lacertinae.(SH, Shimodaira–Hasegawa test; *indicates $P < 0.05$ and suggests that the constrained and unconstrained solutions are significantly different).
Archaeolacerta sens. lat. includes: *L. bedriagae, L. danfordi, L. saxicola, L. graeca, L. oxycephala, L. mosorensis, L.(I.) horvathi, L.(I.) aranica, L.(I.) bonnali, L.(I.) aurelioi, L.(I.) monticola* and *L.(I.) cyreni*. *Archaeolacerta sensu novo* Arribas 1999 includes: *L. bedriagae, L. mosorensis* and *L. oxycephala*. *Teira* includes: *L. dugesii, L. perspicillata* and perhaps *L. andreanskyi*. *Timon* includes: *L. pater, L. lepida* and *L. princeps*.

	MP (ts = tv)	MP (ts = 1; tv = 2)	MP (ts = 1; tv = 4)
Data set I	5846 trees; length: 2192	–	31 trees; length: 3759
	CI: 0.195; RI: 0.763	–	CI: 0.230; RI: 0.812
Data set II	6 trees; Length: 847	–	8 trees; Length: 1603
	CI: 0.469; RI: 0.829		CI:0.498; RI: 0.865
Data set III	5 trees; Length: 52	5 trees; Length: 72	–
	CI: 0.942; RI: 0.991	CI: 0.931; RI: 0.990	

Table 3 Data for the different MP analyses. CI = Consistency Index; RI = Retention Index. All values have been calculated excluding uninformative positions.

assemblage started approximately 7.5 ± 1.2 Ma ago, and therefore was also very close in time to diversification within *Iberolacerta*. The inference of these dates was based on homologous sequence and identical methods of analysis for independent clades within the Lacertinae. Consequently, even if there is error in the determination of the absolute age of the diversifying clades, the determination of relative ages should be similarly biased, and therefore directly comparable.

(b) Analysis of data set II – mitochondrial and nuclear genes for *L.* (*Iberolacerta*)

This analysis, which was based on more mtDNA sequence than in data set I plus a fragment of the c-*mos* nuclear gene, further explored the relationships of West European Rock lizards. Of the total 1013 bp, 315 were variable and 269 parsimony-informative. As in data set I, not even the cytb third codon ts appear to be saturated (see Fig. 2), so all sites were included in the analysis. An ILD test showed that all three genes were congruent with each other (ILD, $P > 0.80$) and were consequently combined in a total evidence analysis. Results are shown in Fig. 4 and statistics for the different analyses given in Table 3. All ML, MP and Bayesian trees have almost identical topologies, which are only slightly different from those obtained from data set I (see Fig. 3). *L.* (*Iberolacerta*) *horvathi* separates first and then the Pyrenean assemblage (clades III–V in Fig. 4), leaving all the other Iberian populations as a monophyletic group (clades I+II in Fig. 4). The three basal nodes involved (nodes J, K and N in Fig. 4) are recovered in all analyses, but have very low bootstrap and posterior probability values. This lack of clear basal resolution in *Iberolacerta*, despite 1013 bp of sequence from three different genes being used, suggests that the speciation events involved occurred over a short time. Speciation within the Pyrenean assemblage (clades III–V) was probably also very swift. The three species form a trichotomy in all MP strict consensus trees, while in the ML (GTR + I + G) tree (Fig. 4) and in the Bayesian analyses, *L.* (*I.*) *bonnali* separates first leaving *L.* (*I.*) *aurelioi* and *L.* (*I.*) *aranica* as a monophyletic group but with very low support. Constraint analyses, in which alternative hypotheses of relationships within the Pyrenean group were compared with the

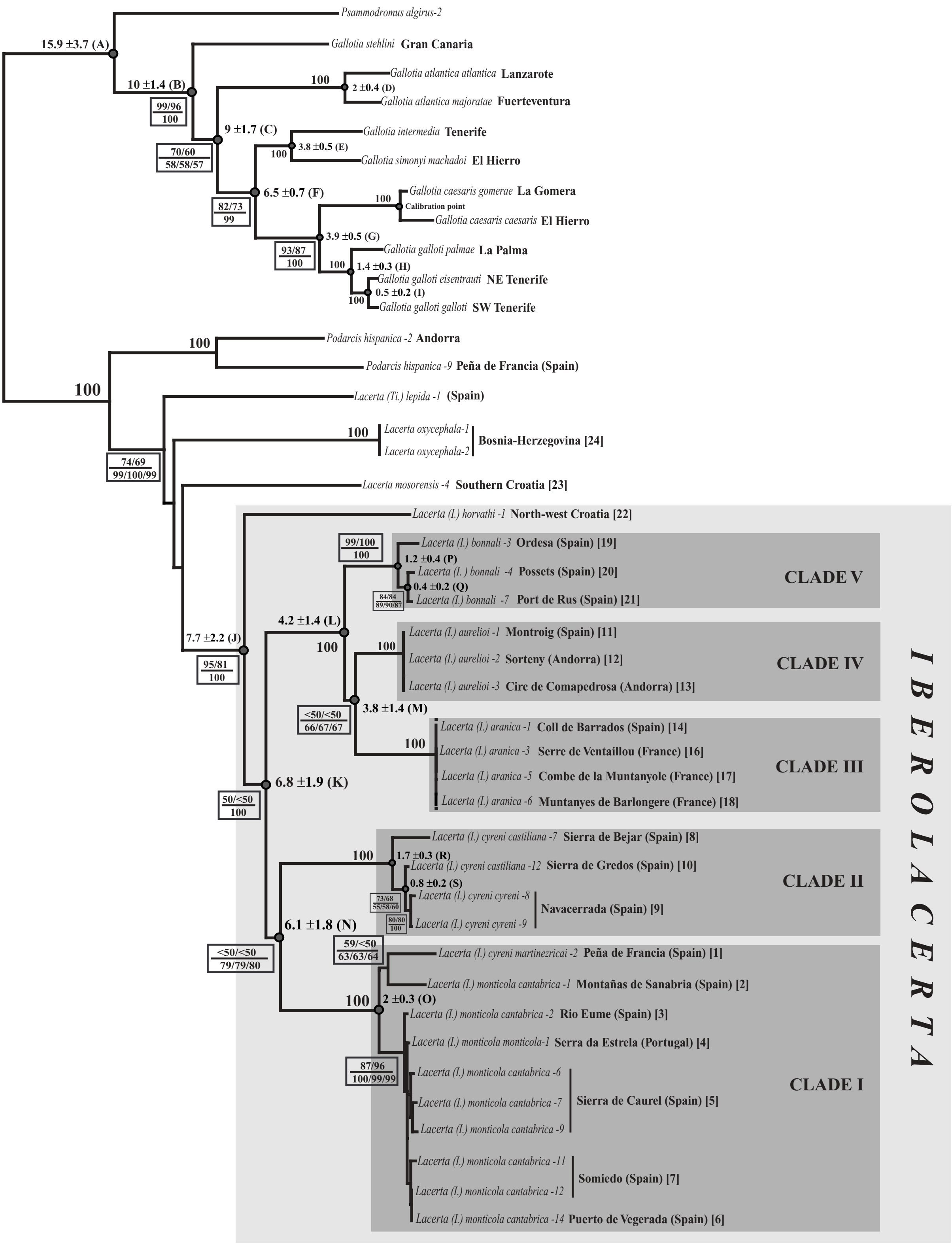

Figure 4 ML tree for *L.* (*Iberolacerta*) (Log likelihood –5456.67436, GTR+I+G model of sequence evolution) inferred from data set II which includes mtDNA and nuclear c-mos sequences. Bootstrap support and Bayesian posterior probabilities for particular nodes are shown in the boxes with the figures indicating the percentage support for different analyses: Upper left, bootstrap support derived from MP (ts = tv); Upper right, bootstrap support derived by MP (ts = 1; tv = 4); Lower left, posterior probability values derived from Bayesian analysis (1st replicate); Lower middle, posterior probability values derived from Bayesian analysis (2nd replicate); Lower right, posterior probability values derived from Bayesian analysis (3rd replicate). Estimated ages are given for some bifurcations, which are marked by filled circles and followed by a capital letter which links them to Table 6. When difference between the four support values or between all three Bayesian posterior probabilities is <5%, only the average value is shown. The '<' symbol is used to show that the bootstrap/posterior probability value for that node is lower than 50% and the '-' symbol indicates that a particular node is never recovered when using this method. Italic numbers after taxon names refer to different individual lizards, details of which can be found in Table 1; numbers in square brackets refer to localities shown in Fig. 1.

Tree	**−Log likelihood**	**Δ-Log likelihood**	**SH *P***
Unconstrained ML tree (Fig. 3)	5456.67436	(best)	
Constrained (*L* .(*I* .) *bonnali* sister to *L* .(*I* .) *aranica*)	5458.43425	1.75988	0.601
Constrained (*L* .(*I* .) *bonnali* sister to *L* .(*I* .) *aurelioi*)	5458.06716	1.39280	0.655
Constrained (*L* .(*I* .) *cyreni martinezricai* belongs to Clade II)	5491.55480	34.88044	0.002*

Table 4 *Statistical support for alternative hypotheses of Iberolacerta relationships.* (SH, Shimodaira–Hasegawa test; *indicates $P < 0.05$ and suggests that the constrained and unconstrained trees are significantly different)

	CLADE I	**CLADE II**	**CLADE III**	**CLADE IV**	**CLADE V**	***I. horvathi***	***Internal variability***
CLADE I	–						0.8 /2 / 0.46
CLADE II	6.1 / 16.3 / 3.5	–					0.7 /2.1 / 0.2
CLADE III	7.2 / 18.6 / 4.4	6.9 / 16.5 / 5.3	–				0
CLADE IV	6.4 / 14.5 / 5	6.2 / 12.7 / 6.1	3 / 7.4 / 2.2	–			0
CLADE V	6.7 / 17 / 4.3	6.7 / 16.1 / 5.1	3.7 / 9.8 / 2.4	2.5 / 6.2 / 1.8	–		0.6 /1.1 / 0.7
L.(I.) *horvathi*	7.4 / 16.7 / 5.2	7.5 / 16 / 6.1	8.6 / 21 / 6	7.4 / 17 / 5.6	7.7 / 18.5 / 5	–	–

	Béjar [8]	**Gredos [10]**	**Navacerrada [9]**	**Navacerrada [9]**
***L.(I.). cyreni castiliana* Sierra de Béjar [8]**	–			
***L.(I.) cyreni castiliana* Sierra de Gredos [10]**	1.1 / 3 / 0.5	–		
***L.(I.) cyreni cyreni* –8 Navacerrada [9]**	1.4 / 4 / 0.5	0.3 / 1/ 0	–	
***L.(I.) cyreni cyreni* –9 Navacerrada [9]**	1.3 / 3.7 / 0.5	0.2 / 0.7 / 0	0.09 / 0.3 / 0	–

	Peña de Francia [1]	**Sanabria [2]**	**[3–7]**
***L.(I.) cyreni martinezricai* Peña de Francia [1]**	–		
***L.(I.) monticola cantabrica* Montañas de Sanabria [2]**	1.9 / 5.1 / 0.8	–	
***L.(I.) monticola* [3-7]**	1.9 / 5.5 / 0.7	1.4 / 3.7 / 0/0.9	–

	Ordesa [19]	**Posets [20]**	**Port de Rus [21]**
***L.(I.) bonnali* Ordesa [19]**	–		
***L.(I.) bonnali* Possets [20]**	0.8 / 1.3 / 1	–	
***L.(I.) bonnali* Port de Rus [21]**	0.8 / 1.7 / 0.8	0.2 / 0.3 / 0.3	–

Table 5 Kimura 2-parameter genetic distances between all main clades within *Iberolacerta* (see Fig. 4) and between populations within *L.(I.) cyreni, L.(I.) monticola* and *L.(I.) bonnali.* Numbers between square brackets after population names refer to population codes in Table 1 and Figs 1, 3–5. The three different values from left to right correspond to % genetic distances calculated taking into account all three genes together (cytb + 12S rRNA + c-mos), cytb only and 12S rRNA only.

pattern of relationships shown in Fig. 4, indicates that the alternatives are not significantly less well supported (Table 4). Genetic distances between clades III–V are relatively high (6.2–9.8% genetic divergence for the cytb gene; see Table 5), in agreement with their species status. Despite samples being selected to cover the maximum geographical range of each of the three Pyrenean species, genetic variability within clades III and IV is 0% (see Table 5), each clade having only a single haplotype. In contrast, *L.* (*I.*) *bonnali* has a mean internal genetic variability of 1.1% for the cytb gene and each of the three populations sampled possesses a different haplotype. Within *L.* (*I.*) *bonnali*, the Ordesa population separates first, while the other two populations (Possets and Port de Rus; see Fig. 1), which are geographically close to each other, form a relatively well-supported monophyletic group.

Clade II of *L.* (*Iberolacerta*) contains *L.* (*I.*) *cyreni cyreni* and *L.* (*I.*) *cyreni castiliana* but not *L.* (*I.*) *cyreni martinezricai*, which unexpectedly forms part of the *L.* (*I.*) *monticola* group (clade I). When a tree, in which *L.* (*I.*) *cyreni martinezricai* is constrained to be most closely related to other *L.* (*I.*) *cyreni* populations, is compared with that shown in Fig. 4, it is significantly less well supported (Table 4). Relationships

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
GTR+I+G genetic distances	21.3	11.6	12.3	2.2	4.1	7.9	4.7	1.5	0.5	11.5	9.9	3.8	3.4	8.7	2	1.1	0.2	1.6	0.3
K2P genetic distances	12.4	8.3	8.5	2	3.5	6.2	4	1.4	0.5	8.8	7.5	3.5	3.2	6.8	1.9	1	0.2	1.6	0.3
r8s (GTR+I+G)	14.7	9	7.5	1.4	3.4	5.7	3.3	1	0.3	7.8	7.7	7	6.8	7.5	2.7	1.6	0.5	1.7	0.8
r8s (K2P)	13.8	9.8	8.6	2	4.7	7	4.2	2.1	0.7	6	5.5	3.5	3.4	5	1.8	1	0.4	1.2	0.5
r8s (ACCTRAN)	20	12	9.6	2.6	3.5	6.3	3.6	1.3	0.3	7	6	4.1	3.6	5	1.9	1.9	0.8	1.5	0.8
r8s (DELTRAN)	13.5	9.6	7.8	1.9	3.9	6.3	4	1.5	0.6	5.2	4.6	3.3	2.7	4	1.8	0.8	0.3	1	0.5
Mean	15.9	10	9	2	3.8	6.5	3.9	1.4	0.5	7.7	6.8	4.2	3.8	6.1	2	1.2	0.4	1.7	0.8
Standard deviation	3.7	1.4	1.7	0.4	0.5	0.7	0.5	0.3	0.2	2.24	1.9	1.4	1.4	1.8	0.3	0.4	0.2	0.3	0.2

Table 6 Ages for the 19 speciation events shown in Fig. 4 calculated using six different approaches. Values are in million years. Letters refer to marked nodes in Fig. 4

within *L.* (*I.*) *cyreni* (clade II; see Fig. 4) indicate that *L.* (*I.*) *cyreni castiliana* from the Sierra de Gredos (locality 10, Fig. 1) is more closely related to the geographically distant *L.* (*I.*) *cyreni cyreni* from Navacerrada (locality 9, Fig. 1) than to *L.* (*I.*) *cyreni castiliana* from the nearby Sierra de Bejar (locality 8, Fig. 1), although both bootstrap support and posterior probability values are low.

Within clade I, *L.* (*I.*) *cyreni martinezricai* is most closely related to *L.* (*I.*) *m. monticola* from Montañas de Sanabria but with very low support. This unit has a 4.6% genetic divergence in the cytb gene from all other populations of *L.* (*I.*) *monticola*, and genetic variability within it is very low (0.45% for the cytb gene), even though the samples occur over a large geographical area (see Fig. 1).

In order to estimate times of cladogenetic events on the *L.* (*Iberolacerta*) phylogenetic tree produced from data set II, a maximum likelihood ratio test was performed comparing the likelihood value of the ML tree from Fig. 4 (–5456.67436) with the log likelihood of the same tree with clock-like branch lengths (–5483.99400). The likelihood ratio test statistic showed that there was no significant difference between the two trees ($-2\log\Delta = 54.63928$ which approximates to an $X^2{}_{40}$ distribution under the null hypothesis; $P < 0.05$) and therefore the sequences could be used to estimate approximate dates. Calibrations based on the genus *Gallotia* from the Canary Islands (see p. 15) were carried out using two different models of sequence evolution (GTR+I+G and Kimura 2-parameters; see p. 14). The results were identical for both evolutionary models and indicated that all three genes together (cytb, 12S rRNA and c-*mos*) were evolving at a rate of 0.9% per million years (Ma), the combined cytb and 12S rRNA at 1.35% per Ma, the cytb alone at 2.3% per Ma, and the 12S rRNA alone at 0.5% per Ma. In our analyses, we only used the rate based on all three genes for which clock-like behaviour had been tested. Calibrations were also carried out applying the NPRS method (Sanderson, 1997) to the ML tree shown in Fig. 4 with branch lengths calculated using four different methods (GTR+I+G, Kimura 2-parameters, ACCTRAN and DELTRAN). The various dates for the most relevant nodes in Fig. 4 (marked as A-S) calculated using all six different approaches are shown in Table 6. As expected, dates for nodes situated at the base of the tree (A and J) have higher standard deviations, the effects of using different evolutionary models being more apparent here. Dates calculated using the same evolutionary model (GTR+I+G or Kimura 2-parameters) but different methods (either inferred directly from the distance matrix or using the NPRS method) were generally very similar (see Table 6). Despite being based on more mitochondrial sequence and an extra nuclear gene, dates for speciation events within the Gallotiinae and the origin of *Iberolacerta* in the present analysis are very similar to these shown in Fig. 3. Moreover, dates within the Canary lacertid lizards of the genus *Gallotia* are congruent with island ages and with previous published work (Maca-Meyer *et al.*, 2004).

Diversification within *Iberolacerta* started at approximately 7.7 ± 2.2 Ma ago. There was a second bout of speciation within *Iberolacerta* around 4.2 ± 1.4 Ma ago, when the Pyrenean assemblage split into three units (clades III, IV and V). Both genetic distance and NPRS methods show that *L.* (*I.*) *cyreni martinezricai* and *L.* (*I.*) *monticola cantabrica* from the Montañas de Sanabria (locality 2 in Fig. 1) separated from all the rest of populations of *L.* (*I.*) *monticola* in clade I at approximately 2 ± 0.3 Ma ago, during the Late Pliocene or the beginning of the Pleistocene. Further divergence within *L.* (*I.*) *cyreni* (clade II) and *L.* (*I.*) *bonnali* (clade V) occurred during the Pleistocene between 1.7 ± 0.3 and 0.4 ± 0.2 Ma ago (see Fig. 4).

(c) Analysis of data set III – Nuclear c-*mos* gene for *Iberolacerta* and the Gallotiinae

Variability in the gene fragment c-*mos* is low; only 69 bp out of 335 bp were variable and only 48 bp parsimony-informative. Because of this and also because c-*mos* is a nuclear gene, it was analysed independently. The c-*mos* tree presented in Fig. 5 shows that, within the Lacertinae, relationships are congruent with the total evidence analysis presented in Fig. 4 in that *L.* (*I.*) *horvathi* is the first taxon separating within *Iberolacerta* and the Pyrenean assemblage is sister to a monophyletic group formed by clades I and II, which are, in turn, reciprocally monophyletic. Moreover, *L.* (*I.*) *cyreni martinezricai* is sister to a genetically uniform unit made up of all *L.* (*I.*) *monticola* samples, including *L.* (*I.*) *m. cantabrica* from Montañas de Sanabria (see Fig. 3). All three Pyrenean species of *Iberolacerta* are genetically identical in the c-*mos* fragment sequenced

Figure 5 ML tree for *L.* (*Iberolacerta*) (Log likelihood – 938.10919, GTR model of sequence evolution) inferred from data set 3 (c-*mos* sequence). Bootstrap support and posterior probabilities for particular nodes are shown in the boxes with the figures indicating the percentage support for different analyses: Upper left, bootstrap support derived from ML (GTR); Upper middle, bootstrap support derived from MP (ts = tv); Upper right, bootstrap support derived from MP (ts = 1, tv = 2); Lower left, posterior probability values derived from Bayesian analysis (1st replicate); Lower middle, posterior probability values derived from Bayesian analysis (2nd replicate); Lower right, posterior probability values derived from Bayesian analysis (3rd replicate). When difference between the four bootstrap support values or between all three Bayesian posterior probabilities was < 5%, only the average value is shown. The '<' symbol is used to show that the bootstrap/posterior probability value for that node is lower than 50%. Italic numbers after taxon names refer to different individuals, details of which can be found in Table 1; numbers in square brackets refer to localities shown in Fig. 1.

and there is also no variability within clade II. In general, bootstrap and posterior probability values are very low (see Fig. 5). Nonetheless, the fact that the nuclear c-*mos* gene fragment supports several of the relationships produced by mitochondrial DNA alone increases confidence in these.

Discussion

(a) Relationships and taxonomy of West European Rock lizards, *Lacerta* (*Iberolacerta*)

Inclusion of all taxa of *L.* (*Iberolacerta*) in a phylogenetic analysis with a very wide range of other Lacertinae (data set I) provides strong support for its clade status, and *L.* (*Iberolacerta*) should consequently continue to be recognised as a formal unit. We follow (Harris & Carretero, 2003), in treating it as a subgenus of *Lacerta*, to avoid confusing change in species and subspecies names.

Relationships within *Iberolacerta* are largely resolved by DNA sequences, although there is some conflict with other data sources. In the DNA analysis presented here, The Pyrenean *Lacerta* (*I.*) *aurelioi* and *L* (*I.*) *aranica* form a clade, with *L* (*I.*) *bonnali* sister to it (Figs 3 and 4). Although bootstrap support and posterior probability values are both very low, these relationships are consistently recovered in our analyses, but other kinds of data give different results. Gross morphology suggests *L* (*I.*) *bonnali* and *L* (*I.*) *aranica* are most closely related (Arribas, 1993*b*, 2000*b*), while karyological (Odierna *et al*., 1996) and allozyme data (Mayer & Arribas, 1996) support a clade made up of *L* (*I.*) *bonnali* and *L* (*I.*) *aurelioi*. As noted earlier, a constraint analysis does not give precedence to any of these hypotheses (Table 4), so it is probable that division of the Pyrenean clade into three main units occurred rapidly.

In general, the nomenclature currently used within *L.* (*Iberolacerta*) is appropriate, but phylogenetic and constraint analyses indicate *L.* (*I*) *cyreni martinezricai* of the Peña de Francia should be removed from *L.* (*I.*) *cyreni*. An evaluation of the taxonomic status of the Peña de Francia Rock lizard will be published elsewhere (work in progress). Taxonomic change may also be eventually appropriate within *L.* (*I.*) *cyreni*. At present the easternmost populations in the Sierra de Guadarrama are named *L.* (*I.*) *cyreni cyreni*, while those from the adjoining Sierra de Gredos and Sierra de Bejar are called *L.* (*I.*) *cyreni castiliana*. But phylogenetic analysis suggests Sierra de Gredos animals are more closely related to those in the Sierra de Guadarrama than to ones in the Sierra de Bejar, although with low bootstrap support and posterior probability values. The validity of the subspecies *L.* (*I.*) *cyreni* consequently needs further investigation.

(b) Long-term persistence of Rock lizards in mountains

If a clade has two or more lineages in a restricted area, it is most parsimonious to assume that it has been in the region concerned since at least the time the lineages first diverged. The case increases in strength with the number of lineages involved. On this basis, clades III–V of *L.* (*Iberolacerta*) may have been in the Pyrenees mountains at least since these units diverged at 4.2 ± 1.4 Ma ago. The fact that they have adjacent but allopatric ranges suggests they may never have spread very far beyond their present distributions. In the rest of Iberia, phylogenetic relationships and approximate dates indicate the Estrela, Sanabria and Peña de Francia populations of *L.* (*I.*) *monticola* have been in the general montane area they inhabit for at least 2 ± 0.3 Ma and the Gredos, Guadarrama and Bejar populations of *L.* (*I.*) *cyreni* in central Iberia for at least 1.7 ± 0.3 Ma. No divergence date can be estimated within *L.* (*I.*) *horvathi*, as only a single population was included in the phylogenetic analyses. The three main Iberian clades of *L.* (*Iberolacerta*) and *L.* (*I*) *horvathi* may even have been in their present montane distributions since they separated in the basal polychotomy of *L.* (*Iberolacerta*), between 7.7 ± 2.2 and 8.1 ± 2.3 Ma ago, especially as mountains such as the Pyrenees, were already in place at this time (Dercourt *et al*., 1986). This would be the simplest interpretation of the tree, although the possibility that the basal speciation was not immediately accompanied by confinement of the main units of *L.* (*Iberolacerta*) to their present highland ranges cannot be entirely ruled out. However, there is no evidence that lineages of *L.* (*Iberolacerta*) once extended well outside their present range, in the form of relict populations in areas with suitable climatic conditions, such as isolated massifs. Isolates of this kind are common, for example in cold-adapted taxa that spread widely during the ice ages. Also, the basal branches of the three main Iberian units of *L.* (*Iberolacerta*) are relatively long and 'bald', before they diversify within particular montane areas, being without external branches. This may be because no speciation events occurred, which would be in agreement with a continuously small range since their divergence, or external basal branches may have existed but have been pruned by extinction. Either possibility would fit with long-term persistence only in small montane areas and lack of success in any long-term colonisation beyond these.

Although the evidence suggests at least some *L.* (*Iberolacerta*) persisted in or at least around montane areas for periods of millions of years, there must have been at least some minor range shift. This is because the mountains concerned were much colder during the Pleistocene ice ages and some at least were glaciated at high altitudes. Such climatic changes would have made it impossible for at least Pyrenean *L.* (*Iberolacerta*) to inhabit their present ranges, which lie mainly on the southern side of the mountains between 1700 m and 3000 m, but spread over the watershed on to the upper northern slopes. In the Pyrenees, the glacial maximum was reached approximately 50 000–45 000 years ago (Jalut *et al*., 1992; Montserrat-Martí 1992) but, while glaciers were abundant at high altitudes, the lower southern slopes of the mountains were undoubtedly warmer (Andrieu *et al*., 1988) and Pyrenean *L.* (*Iberolacerta*) may have survived by relatively small distributional shifts to lower altitudes on these. Analogous shifts must have occurred elsewhere in the range of *L.* (*Iberolacerta*), but any populations in Galicia and the Cantabrian Mountains may have been exterminated. Present populations in this area are *L.* (*I.*) *monticola* and are genetically very similar with each other and those in the Serra da Estrela of central Portugal, which suggests they may have arrived very recently, presumably after the end of the Würm glacial period. A northward extension from central Portugal is in agreement with the climatic amelioration that has occurred and is supported by the greatest diversity in *L.* (*I.*) *monticola* being to the south, where populations from the Peña de Francia, the Montañas de Sanabria and the Serra da Estrela (localities 1, 2 and 4 in Fig. 4) are all genetically different.

Why have West European Rock lizards often managed to persist for so long in the small mountain areas where they occur? Mountains may simply provide the conditions to which the lizards are now adapted and are also capable of preserving them over long periods through episodes of climatic change. This is because organisms adapted to particular habitats can maintain their climatic requirements by shifting upwards in warm periods and downwards in cool ones. Another possible cause is that patterns of intermittent gene flow which could reduce adaptation to local conditions may differ between forms confined to highland areas and ones that have invaded from the lowlands, to the advantage of the former (Arnold, 1981).

(c) Did *Podarcis* restrict *L.* (*Iberolacerta*) and other European Rock lizards?

Phylogenetic analysis based on DNA sequence and incorporating a molecular clock provides support for this hypothesis of competitive restriction. It is clear that *L.* (*Iberolacerta*) really is a clade and, as lizards are terrestrial animals, this means there must once have been continuity between its populations, although not necessarily simultaneously. *L.* (*Iberolacerta*) must therefore have undergone range restriction. The idea, based on their uniform morphology and compact continuous range, that Wall lizards (*Podarcis*) are a young group that has expanded to restrict West European Rock lizards quite recently, must be discounted, as the clade is old, perhaps separating from other Lacertines as long as 13–9 Ma ago and beginning to diversify at about 9 ± 1.5 Ma ago. It may nonetheless have restricted *L.* (*Iberolacerta*) around this period, as diversification of *Podarcis* took place around the time that *Iberolacerta* seems to have fragmented, including diversification of the *P. hispanica* clade, which occurred around 7.5 ± 1.2 Ma ago (see Fig. 3).

In contrast to *L.* (*Iberolacerta*), *Podarcis* diversified into many branches, many speciation events occurring after its initial polychotomy. In the *P. hispanica* clade this is especially true of the monophyletic groups in the northeast and west Iberian Peninsula that are sympatric with *Iberolacerta*. They cover large areas and show considerable diversification within these. The bare basal branches seen in *L.* (*Iberolacerta*) are absent, indicating the *Podarcis* clades have been successful in producing many early lineages that have persisted to the present day. The contrasts between *L.* (*Iberolacerta*) and *Podarcis* over a long period suggest they may have interacted through this time. Combined with observational evidence for possible competition between extant *L.* (*Iberolacerta*) and *Podarcis* (Arribas, 1996; Galán, 1999), the similar dates of diversification and the difference in phylogenetic pattern and range, all provide circumstantial evidence of competitive restriction.

If *Podarcis* has restricted the European species of *L.* (*Iberolacerta*) to mainly montane areas for very long periods, what is its competitive advantage? As stated, the genus does have a combination of derived morphological features not found in *L.* (*Iberolacerta*) and other Rock lizards, but how they might confer advantage is not obvious. Possibly, *Podarcis* was simply better adapted for warmer-drier situations and they do have higher voluntary maximum body temperatures in the field than *L.* (*Iberolacerta*) and other European Rock lizards (Arnold, 1987). However, it is always possible that *L.* (*Iberolacerta*) only adapted to relatively cool conditions as competitive restriction to mountains occurred, and that the competitive advantage of *Podarcis* is based on some other factor. At present, reconstructing the original preferred microclimates of *L.* (*Iberolacerta*) and other European Rock lizards is not possible on the basis of phylogeny, as detailed relationships of these taxa to other lacertines are uncertain and preferred microclimates are very varied in these.

Although the present study shows that other European Rock lizards are not closely related to *L.* (*Iberolacerta*), it is possible that they too have long been restricted to their small and often montane ranges by Wall lizards. For instance, the Mosor Rock lizard (*Lacerta mosorensis*), which has a restricted mountain distribution in south Croatia, southwest Bosnia and Montenegro (Yugoslavia), possesses a phylogeny with deep internal bifurcations and a bare basal stem (see Fig. 3), suggesting that it may have been restricted to this area for a long time.

Persistence of relatively primitive forms in mountain situations and presence of related and often more apomorphous relatives in the surrounding areas is a common phenomenon outside the European Lacertinae. For example, it occurs in *Pristurus* geckos in the mountains of Oman, Yemen and Somalia (Arnold, 1993); *Quedenfeldtia* geckos in Morocco (Arnold, 1990) and *Lacerta kulzeri* in the Levant.

(d) History of the Lacertinae

Phylogenetic analysis of data set I, which contains a wide and representative range of lacertine species, corroborates and extends previous studies involving fewer taxa (Fu, 1998, 2000; Harris *et al*., 1998) that indicate the subfamily underwent rapid diversification. As noted, the molecular clock estimates presented here indicates this happened about 13–9 Ma ago in the mid-late Miocene. This contrasts with a date in the early to mid-Miocene suggested on the basis of albumin immunology and protein electrophoresis (Lutz *et al*., 1986). It is probable that initial diversification involved relatively fast geographical spread combined with division into a number of taxonomic units that have remained largely or entirely allopatric. They are all assigned to *Lacerta* s. l. and include: *L.* (*Zootoca*) *vivipara*, which is widely distributed but does not reach the Mediterranean area; *L.* (*Teira*) *andreanszkyi* of Morroco; *L.* (*T.*) *perspicillata* of northwest Africa and *L.* (*T.*) *dugesii* which has colonised the oceanic island of Madeira; *L.* (*Iberolacerta*) including *L.* (*I.*) *monticola*, *L.* (*I.*) *bonnali*, *L.* (*I.*) *aranica* and *L.* (*I.*) *aurelioi* of the Iberian Peninsula and *L.* (*I.*) *horvathi* of Slovenia and northwest Croatia; *L. bedriagae* of Corsica and Sardinia; *L. oxycephala* of southwest Croatia, Montenegro etc.; *L. mosorensis* of southwest Croatia, Montenegro etc.; *L. graeca* of southern Greece; the *L. danfordi* group including *L. anatolica* and *L. oertzeni* of west and southwest Asiatic Turkey; *L. cappadocica* of east Asiatic Turkey, north Iraq etc.; *L.* (*Parvilacerta*) *parva* and *L.* (*P.*) *fraasi* of central Turkey and Lebanon respectively; *L. laevis* and *L. kulzeri* of Syria, Jordan and adjoining regions; *L. brandtii* of NW Iran and adjoining Turkey; and finally *L.* (*Darevskia*) in the Caucasus, northern Turkey, western Iran and the Kopet Dagh with one species reaching the eastern Balkan area of Europe. In slightly less than half the cases, speciation has occurred but nearly always to a modest degree producing only 2–4 extant species. The only exception is *Lacerta* (*Darevskia*), once generally known as the *Lacerta saxicola* group, which has many species including parthenogenetic ones. Where there has been such speciation, the products also tend to be largely or wholly allopatric and, in the case of *L.* (*Iberolacerta*), are strongly disjunct. Most of the units listed above tend to be at least primitively rock-dwellers and climbers to varying degrees, the main exceptions being the largely terrestrial *L.* (*Z.*) *vivipara* and *L.* (*P.*) *parva* + *L.* (*P.*) *fraasii*. Some species of *L.* (*Darevskia*) have become ground-dwelling.

Three or four clades in the Lacertinae depart from the widespread pattern just described, involving allopatry usually followed by low levels of speciation, although these clades too may have originated as small-scale geographical isolates. These units now have wide ranges, often more species, and are sympatric with at least four of the units of *Lacerta* s. l.

listed above and sometimes many more. Of the widespread clades, the Green lizard group, *Lacerta* (*Lacerta*), has about seven species and occurs widely over virtually the whole area occupied by the units of *Lacerta s. lat.* with restricted distributions, except for northern Europe and some mainly western Mediterranean islands. Sympatry between *L.* (*Lacerta*) and other *Lacerta s. lat.* may be permitted by the much bigger adult body size and prey size in this group and the tendency for its species to be associated with vegetation, making ecological coexistence possible. *Lacerta* (*Timon*), which may form a clade with *L.* (*Lacerta*) has five or so species and presumably achieved sympatry in a similar way as its members are extremely large-bodied.

Another clade to have dispersed widely is *Algyroides*, a specialised group of four small-bodied species with large dorsal scales that occupy relatively cool, often partly shaded habitats and occur from the West Balkan region to southeast Spain. The distinctive ecology of *Algyroides* again may permit it to coexist with other small lacertines. It has undergone fragmentation and its species now have small mainly disjunct ranges. The final widespread clade is made up of the 20 or so species of Wall lizards (*Podarcis*) occurring in Europe and northwest Africa. Unlike the others, it is ecologically similar to many species of *Lacerta s. lat* within its range and, as we have seen, has probably competed with them, displacing them into small largely montane habitats.

It is possible that replacement, or at least substantial displacement, of once quite widespread clades by others may be a recurrent phenomenon in community evolution. The rapid diversification of the Lacertinae at 13–9 Ma ago may represent a similar case to the spread of Wall lizards (*Podarcis*) at 9–8 Ma ago. Although the fossil record indicates Lacertidae have been in Europe since at least the Palaeocene period over 50 Ma ago (Estes, 1983), there is little trace within the range of the Lacertinae of groups of earlier origin. The only exception is *Psammodromus*, largely confined to southwest Europe and northwest Africa, and a member of the subfamily Gallotiinae, which originated earlier than the Lacertinae (Fu, 1998; Harris *et al.*, 1998). This suggests the Lacertinae may have replaced other members of the family in the way *Podarcis* has apparently largely supplanted various other groups within this subfamily. Such wholesale replacement may be a repeated pattern in community evolution (Arnold, 2004) and has also been reported in the fossil history of mammals (Kemp, 1998). However, the case of European Rock lizards including *L.* (*Iberolacerta*) indicates that mountains can enable remnants of previous lizard radiations to survive and so conserve faunal diversity.

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