NAT. CROAT. | VOL. 8 | No 3 | 161–180 | ZAGREB | September 30, 1999

ISSN 1330-0520
UDK 598.112:577.22(592)

original scientific paper / izvorni znanstveni rad

MOLECULAR SYSTEMATICS AND EVOLUTION OF LACERTID LIZARDS

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Harris, D. J.: Molecular systematics and evolution of lacertid lizards. Nat. Croat., Vol. 8, No. 3., 161–180, 1999, Zagreb.

In recent years there has been an exponential increase in the amount of published DNA sequences, as it has become one of the most popular sources of data for reconstructing phylogenies. A number of studies have attempted to resolve the relationships of groups of lacertids using mitochondrial DNA sequence data, in particular *Podarcis* and *Gallotia* (e.g. THORPE *et al.*, 1994; 1996; GONZALEZ *et al.*, 1996; FU *et al.*, 1997; HARRIS, 1997; HARRIS *et al.*, 1998a; 1999; CASTILLA *et al.*, 1999a,b). Reassessment of these data suggest some alternative phylogenies to those previously published, and also highlight possible problems.

Two studies have attempted to unravel the phylogeny of the whole family using mitochondrial DNA sequences (FU, 1998; HARRIS *et al.*, 1998b). By combining these essentially congruent data sets, and reanalysing levels of support for the phylogenies produced, a more complete estimate of relationships across the family is produced. This is then compared to alternative sources of evidence, primarily morphological (ARNOLD, 1989), biochemical (MAYER & BENYR, 1994) and karyological (OLMO *et al.*, 1993). Reasons for discrepancies are discussed, as are the relative advantages and disadvantages of the different sources of phylogenetic inference.

Key words: phylogeny, Lacertidae, *Podarcis, Gallotia, Algyroides.*

Harris, D. J.: Molekularna sistematika i evolucija lacertidnih guštera. Nat. Croat., Vol. 8, No. 3., 161–180, 1999, Zagreb.

Posljednjih godina objavljuje se sve veći broj DNA sekvencija, što je postalo jednim od najpopularnijih izvora podataka pri rekonstrukciji filogenije. Više radova pokušalo je riješiti odnose skupina lacertida koristeći sekvence mitohondrijske DNA, posebno *Podarcis* i *Gallotia* (npr. THORPE *et al.*, 1994; 1996; GONZALEZ *et al.*, 1996; FU *et al.*, 1997; HARRIS, 1997; HARRIS *et al.*, 1998a; 1999; CASTILLA *et al.*, 1999a,b). Ponovno proučavanje tih podataka sugerira neke alternativne filogenije u odnosu na one koje su prethodno objavljene, a također i naglašava moguće probleme.

Dva rada pokušala su objasniti filogeniju cijele porodice koristeći sekvencije mitohondrijske DNA (FU, 1998; HARRIS *et al.*, 1998b). Kombinacijom tih zapravo sukladnih podataka, i ponovnom analizom stupnjeva potpore proizašlih filogenija, stvorena je potpunija procjena odnosa u porodici. Ona je tada uspoređena s alternativnim izvorima podataka, prvenstveno morfološkim (ARNOLD, 1989), biokemijskim (MAYER & BENYR, 1994) i kariološkim (OLMO *et al.*, 1993). Raspravlja se o razlozima razilaženja, kao što su relativne prednosti i nedostaci različitih izvora filogenetičkih zaključaka.

Ključne riječi: filogenija, Lacertidae, *Podarcis, Gallotia, Algyroides.*

INTRODUCTION

Attempts to unravel evolutionary history, or phylogenies, date back at least as far as Darwin. Modern systematic classifications attempt to reflect phylogeny (MAYR, 1953), and since systematics underpins comparative biology (SIEBERT, 1992) only by understanding phylogenetics can we answer many biological questions within such diverse fields as ecology, ethology and physiology (ARNOLD, 1993).

Phylogenetic studies require characters that accurately reflect evolutionary history (AVISE, 1994). In this respect there has been a general trend towards assuming that molecular characters have clear advantages over morphological ones (eg. HEDGES & MAXSON, 1996). With DNA sequences the data set is effectively limitless. Many genes are homologous across a range of taxa, allowing homologous characters to be identified irregardless of gross morphology. So with DNA sequencing becoming technically easier and cheaper, it is rapidly becoming the main source of inference for new systematic studies.

However these studies still have many of the problems that any classical morphological assessment of phylogeny has – are the characters truely homologous? Is the sampling adequate? How well supported is the estimated of phylogeny? Are alternative phylogenies reasonable too? These questions can be addressed by examining some of the alternative phylogenies suggested for groups of lacertids, especially where multiple data sets from difference sources are available. In this study three genera of lacertids from the Mediterranean region have been examined – *Gallotia*, *Algyroides* and *Podarcis*. Then alternative phylogenies for the whole family are compared, in particular the largest data sets of morphological (ARNOLD, 1989), biochemical (MAYR & BENYR, 1994), karyological (OLMO *et al.*, 1993) and mitochondrial DNA (HARRIS *et al.*, 1998b; FU, 1998) characters.

1. *Gallotia*

Of the family Lacertidae, only *Gallotia* is found on the Canary Islands, where it is endemic (ARNOLD, 1973). The two largest extant species, *Gallotia simonyi* and *G. stehlini* are sister taxa based on estimates of phylogeny using morphological characters (ARNOLD, 1989), whereas albumin immunological data associates *G. galloti* and *G. simonyi* (MAYR & BISCHOFF, 1991). An array of data sets using mitochondrial DNA (mtDNA) sequence data have also been published (MCGREGOR, 1992; BLACK, 1994; THORPE *et al.*, 1994; GONZALEZ *et al.*, 1996; RANDO *et al.*, 1997). The most recent of these (RANDO *et al.*, 1997) claim that the mtDNA sequence data indicates that *G. simonyi* and *G. galloti* are sister taxa. However they did not include all the available DNA sequence data in their analysis, nor did they discuss alternative possible phylogenies.

For this study I concentrated on the partial cytochrome *b* data sets, for which the largest number of individuals had been sequenced. I combined the data from the previous studies, and using PAUP*4.0.d63 (SWOFFORD, 1998), I calculated an uncorrected neighbour-joining (NJ) tree, with *Psammodromus hispanicus* as the outgroup (Fig. 1). Support was estimated using the bootstrap technique (1000 replicates – FEL-

SENSTEIN, 1985). A maximum parsimony (MP) analysis produced 192 equally parsimonious trees of length 177 (CI = 0.6328, RI = 0.8434). The 50% bootstrap consensus

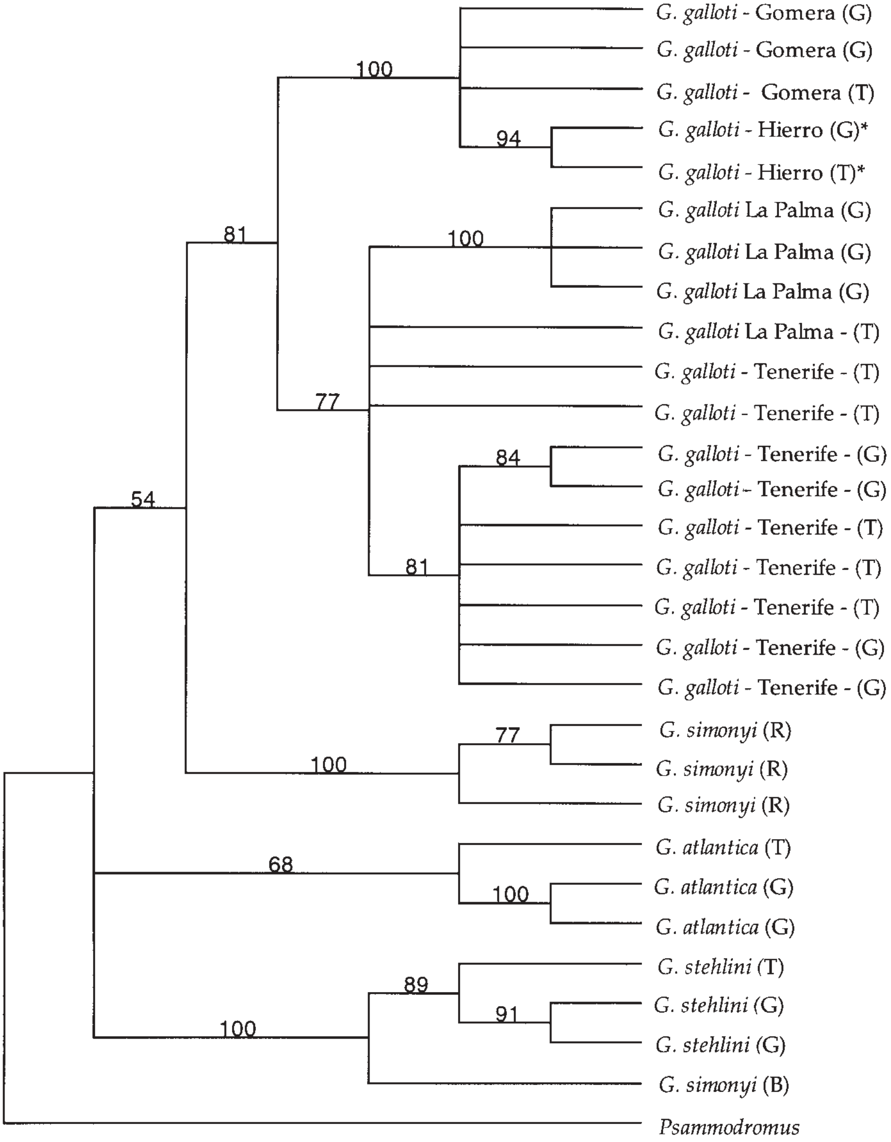

Fig. 1. 50% bootstrap consensus tree derived from a NJ analysis using partial cytochrome *b*. (G) represent data from GONZALEZ *et al.*, 1996, (R) RANDO *et al.*, 1997, (T) THORPE *et al.*, 1994; 1996, (B) BLACK, 1994. A 50% bootstrap consensus tree derived from MP was identical except that the node grouping *G. galloti* and *G. simonyi* was unsupported. **G. galloti caesaris* was given specific rank by LOPEZ-JURADO *et al.* (1996).

of this analysis was identical to the tree in Fig. 1, except that the *G. galloti/G. simonyi* node was not supported.

This analysis of the cytochrome *b* data supports the monophyly of *G. galloti*, *G. atlantica* and *G. stehlini* (81%, 68% and 89% bootstraps respectively), but *G. simonyi* appeared paraphyletic, with the individuals sequenced by RANDO *et al.* (1997) being sister group to *G. galloti* (54% bootstrap), while the individuals sequenced by BLACK (1994) where strongly associated with *G. stehlini* (100% bootstrap). The most likely explanation for this discrepency is a mistake by one or other group. It is also worth noting that support for the relationships between species, other than that of one *G. »simonyi«* and *G. stehlini*, is weak – no species pair are associated on the 50% bootstrap consensus tree derived from the MP analysis. Also *G. galloti caesaris*, endemic to Hierro, has recently been suggested to value species rank (LOPEZ-JURADO *et al.*, 1996). Using the estimate of phylogeny given here, and from that based on the partial 12S DNA sequence data (not shown), if we regard *G.caesaris* as a species different from *G. galloti*, it is evident to include *G. g. gomerensis* (from Gomera) in *G. caesaris.*

This reanalysis of the cytochome *b* sequence data set for *Gallotia* raises three important points. Firstly contamination is a problem for DNA characters, and phylogenetic hypotheses based on DNA sequence data that are at odds with well accepted phylogenies based on other data sets (eg morphological characters) should be thoroughly checked for this possibility. Secondly combining data sets is very important – simulations suggest that adding taxa improves phylogenetic accuracy much more quickly than adding additional characters (GRAYBEAL, 1998). The data from THORPE *et al.* (1994) and BLACK (1994) was available long before the studies of GONZALEZ *et al.* (1996) and RANDO *et al.* (1997), and should have been included in these later analyses. Finally there is still not a well supported phylogeny available for *Gallotia*, irregardless of how often this is suggested.

2. *Algyroides*

Algyroides is a clade of four species of lacertids with largely disjunct ranges in the Mediterranean region (Fig. 2). The most recent analysis of relationships within *Algyroides* combined a matrix of previously published morphological characters (ARNOLD, 1973; 1989a; 1989b; submitted), and comparing this with an estimate of phylogeny based on two partial mtDNA gene regions, 12S rRNA and 16S rRNA (HARRIS *et al.*, 1999). MP, NJ and maximum likelihood (ML) analyses all resulted in a single tree topology, and most nodes were supported by high bootstrap values (Fig. 3).

Four characters – increase in number of presacral vertebrae in females, development of dorsal scales on flanks, dorsal and lateral scales lanceolate and more overlapping, and increase in number of ventral scales – are not congruent with the estimate of phylogeny. These features occur in both *A. moreoticus* and *A. fitzingeri*, and could either result from a single origin on the main lineage of the genus followed by reversal in the ancestor of *A. marchi*, or equally parsimoniously they could have developed independently in the two species. The later hypothesis is prefered for

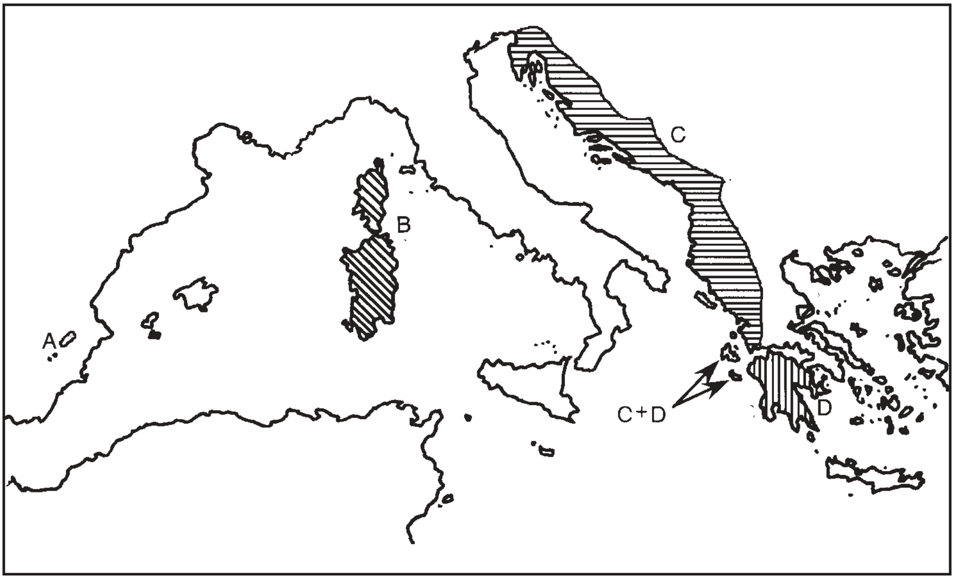

Fig. 2. Distribution of *Algyroides* species showing their largely allopatric and distinct ranges. A: *marchi*, B: *fitzingeri*, C: *nigropunctatus*, D: *moreoticus*. From Harris *et al.*, 1999.

three reasons. Firstly the features appear to evolve easily, arising independently several times within the Lacertidae. Secondly the features occur concurrently on the taxa with occupation of habitats in which they are likely to be advantageous, indicating the probability of selection for these features. Finally there are no residual signs in *A. marchi* that the lineage leading to it ever possessed these features.

The prediction that some of the morphological characters analysed are associated with niche occupation may also explain the superficial similarity of some tropical African lizards, now placed in the genus *Adolfus* that were long assigned to *Algyroides* (Boulenger, 1920; Loveridge, 1957). The morphological parallels between *Algyroides fitzingeri* and *Algyroides moreoticus* may be associated with greater occupation of litter and brushwood. In these often spiky habitats large flank scales, and increased overlap of scales provides protection (Arnold, 1973), and a higher number of presacral vertebrae could promote flexibility. *Aldofus alleni*, which has resemblances to both these species, utilizes similar vegetation matrixes.

Two alternative possible phylogenies for *Algyroides* are suggested on the basis of albumin evolution assessed by immunological means (Mayer & Lutz, 1990). One is that relationships are: *A. fitzingeri* (*A. marchi* (*A. nigropunctatus*, *A. moreoticus*)). The alternative is that *Alyroides marchi* is more closely related to a clade comprising *Podarcis* and *Lacerta graeca*, making *Algyroides* paraphyletic. Harris *et al.* (1999) assessed the likelihood of these alternative relationships, using the Likelihood Ratio Tests (LRT) of Kishino & Hasegawa (1989). Compared to the ML tree based on the mtDNA data set, both alternative topologies were significantly less likely. Since topologies produced from mtDNA and morphological data are similar, it seems more

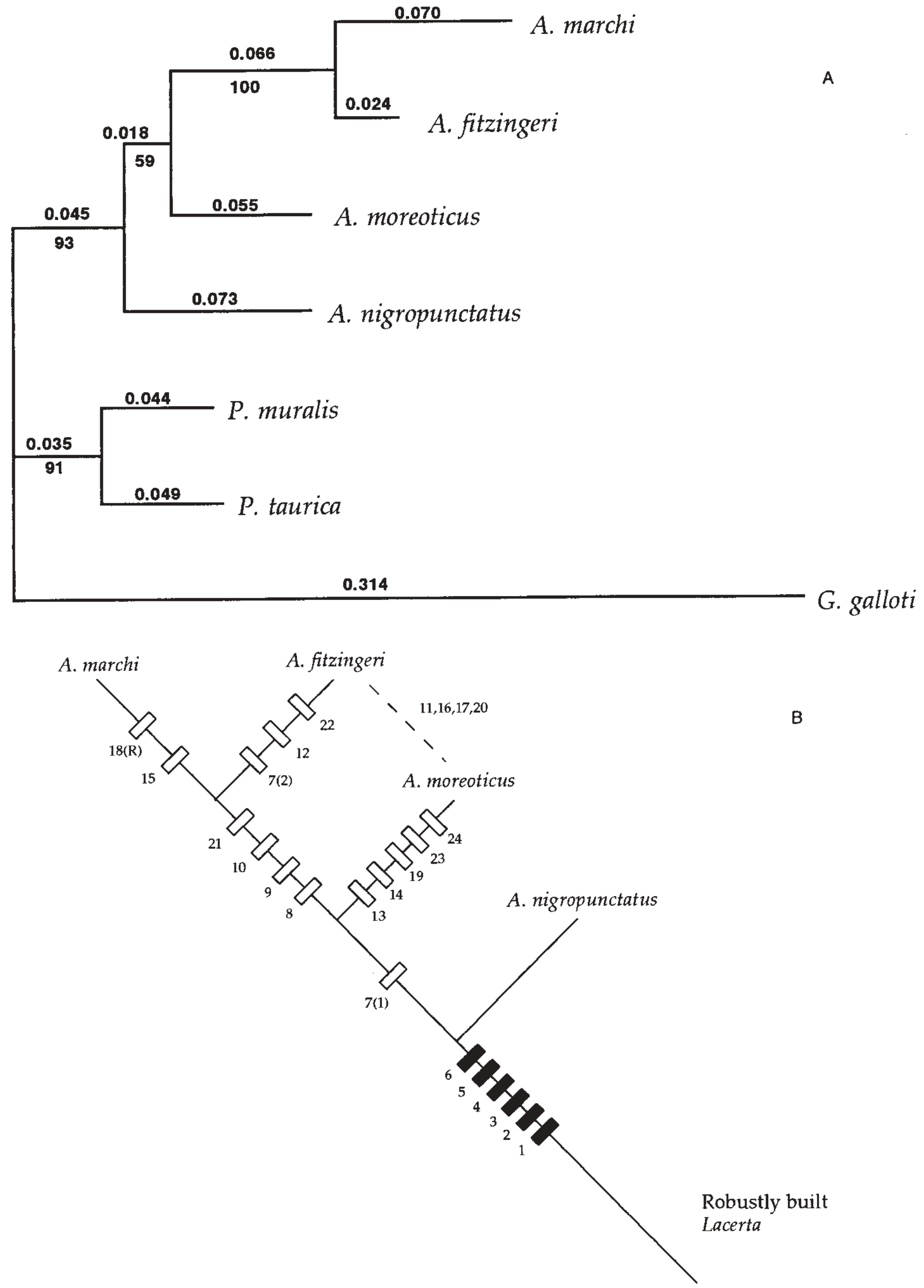

Fig. 3. A: ML tree based on combined 12S and 16S mtDNA sequences. The MP tree is identical, and support from this tree is indicated by bootstraps below nodes. B: Phylogeny of *Algyroides* showing principle changes in morphology. See HARRIS *et al.*, (1999) for details.

likely that the relationships based on immunological evidence are incorrect. The immunological method used groups taxa on the basis of similarity, not shared derived characters, something which is fundamentally problematic. Further with immunological data it is not possible to gauge support for internal branches, which tests like bootstrapping allow for DNA sequence data sets. Alternative topologies cannot be compared either in a statistical framework, something that LRT allow for DNA sequences. This is a severe limitation for this and similar data sets, where other estimates of phylogeny may well be no less likely given the data, but cannot be assessed.

3. *Podarcis*

Wall lizards, *Podarcis*, comprise 17 currently recognized species, all of which occur in the Mediterranean region where they are the dominant reptile group. Because the species are morphologically very similar, yet individually variable, the systematics of the group is unstable. This means that the extensive ecological and behavioural studies carried out on *Podarcis* lack a resolved phylogeny which would give these investigations a valuable historical context (eg BAUWENS, 1995; BAUWENS & DIAZ-URIARTE, 1997).

Morphological characters support *Podarcis* as a clade (ARNOLD, 1973; 1989), and suggest the closest relatives are *L. andreanszkyi* and then the sister species *L. perspicillata* and *L. dugesii*. These three have sometimes been placed in a separate genus, *Teira* Gray 1838 (MAYER & BISCHOFF, 1996). Within *Podarcis* various immunological and protein electrophoretic studies have been made, but these usually involve only a few species, and results often conflict (LANZA & CEI, 1977; MAYER & TIEDEMANN, 1982; LUTZ & MAYER, 1985; CAPULA, 1994; 1996; 1997a; 1997b).

Partial sequence data from the 12S rRNA mitochondrial gene of 562 aligned base pairs (bp) for seven species of *Podarcis*, and *Lacerta viridis* have recently been used in a phylogenetic analysis of the Italian *Podarcis* (OLIVEIRO *et al.*, 1998). Bootstrap support gives evidence to the clade status of *Podarcis*, and to close relationships between *P. raffonei* and *P. muralis*, and between *P. wagleriana*, *P. melisellensis* and *P. filfolensis*. However the *P. filfolensis* sequence has only a 1bp deletion that distinguishes it from the *P. melisellensis* sequences. Given that this region of the 12S gene often varies a lot more than this within species (eg present data – variation within *P. sicula*, and also in *Gallotia* – THORPE *et al.*, 1994) the validity of this sequence of *P. filfolensis* may be questionable. Further sequences of different *P. filfolensis* individuals for this particular region of the 12S gene would be extremely useful in confirming this result. This data set has since been extended (OLIVEIRO *et al.*, 1998).

Another recent study (HARRIS & ARNOLD, 1999) examined variation within another portion of the 12S gene for all currently recognized species of *Podarcis*, except *P. erhardii*, *P. raffonei* and *P. wagleriana*, and all three members of »*Teira*«. *Lacerta chlorogaster*, *L. bonnali* and *L. monticola* where used as outgroups. A portion of the cytochrome *b* gene was sequenced for the same species, except that *L. andreanskyi* and *P. melisellensis* failed to amplify with these primers. 12S sequences were aligned against other published lacertid 12S sequences (HARRIS *et al.*, 1998a), and 12 sites within 2 loop regions (between Helices 36/38 and 42/42' as given by HICKSON *et al.*, 1996) were omitted from the analysis as they could not be unambiguously aligned. NJ, MP and ML analyses were used to estimate phylogenies (Fig. 4).

This data set also corroborates the monophyly of *Podarcis* (96% support in the NJ analysis). The data suggests that *L. andreanskyi* is probably the sister group to *Podarcis*, as does the morphology, and that *L. perspicillata* and *L. dugesii* are sister taxa. This would make »*Teira*« paraphyletic, and therefore not a useful way of subsetting

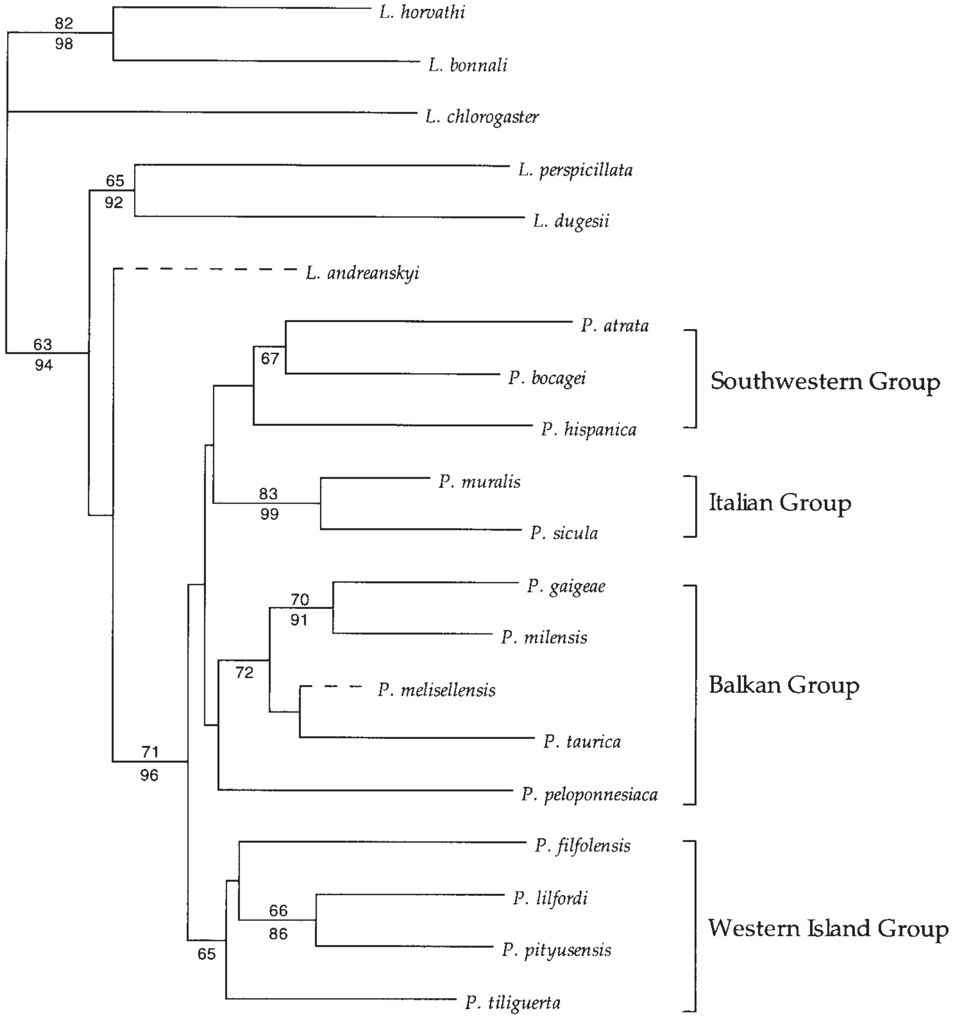

Fig. 4. NJ tree for *Podarcis* and »*Teira*« species based on a different region of 12S. Bootstraps above nodes are from a MP analysis, below from the NJ analysis. *Teira* may be paraphyletic. From HARRIS & ARNOLD, 1999.

the already paraphyletic genus *Lacerta*. The data also indicates that the species of *Podarcis* can be separated into four main groups that are geographically coherent: a Western Island group (*P. filfolensis, P. lilfordi, P. pityusensis* and *P. tiliguerta*), a Balkan group (*P. gaigae, P. milensis, P. melilensis* and *P. taurica*), an Italian group (*P. muralis* and *P. sicula*) and a South Western group (*P. atrata, P. bocagei* and *P. hispanica*). The data from the alternative region of 12S suggests strongly that *P. wagleriana* would be a member of the Balkan group. *P. peloponnesiaca* may also be a part of this group, and so then would be *P. erhardii*, which shares a unique hemipenial character with *P. peloponesiaca* (ARNOLD, 1973). These two are also associated by protein electrophoresis (LUTZ & MAYER, 1985).

Podarcis muralis has a very extensive distribution in Southern, Western and Central Europe, but it exhibits most of its morphological diversity in Italy, and its genetic variability in Italy is also much higher than in Spain or Austria (CAPULA, 1997b). This suggests that *P. muralis* may have arisen in Italy and spread to other areas quite recently. Distributions also suggest that *Podarcis* has largely replaced small members of *Lacerta* and *Algyroides* in Southern Europe (ARNOLD, 1973; 1981). From the estimation of phylogeny for *Podarcis* derived from mtDNA sequence data, it appears that similar replacement may have also occurred within *Podarcis*, with clades comprising one or more species having disjunct ranges with the intervening areas occupied by other forms. Although the new mtDNA sequence data has gone some way to providing an adequate phylogeny for *Podarcis*, there is clearly plenty of scope for further data for other gene regions to be used to help resolve some of the ambiguous nodes, and to support those from the separate studies. Many of the relationships suggested should still be taken as only the best estimate so far, and not the definative phylogeny.

4. Phylogeny of the family Lacertidae

Lacertids are some of the most studied vertebrates, especially in Europe where they make up about three quarters of the lizard fauna. They have been used to assess the role of biological process in phylogenetics (ARNOLD, 1996), in assessing correlated evolution (BAUWENS *et al.*, 1995; 1997) and in estimating colonization patterns across islands (THORPE *et al.*, 1994; 1996). However relationships within the family remain largely unresolved. This has led BAUWENS *et al.* (1995) to analyse their data twice using different estimates of phylogeny, while other ecological studies (eg BRANA, 1996) have incorporated phylogenies not supported by any single data set. There is therefore a clear need for further work on the phylogenetics of the group.

Previous hypotheses of relationships

The Lacertidae are undoubtedly a monophyletic group, with ESTES *et al.* (1989) defining ten morphological synapomorphies. Firmly established within the Scincomorpha Camp, 1923 the families closest relatives are thought to be the Teiioidea (CAMP, 1923; ESTES *et al.*, 1989). ARNOLD (1989a) attempted to produce a general phylogeny of the Lacertidae using 112 binary morphological characters applied to most known species. The 44 taxonomic units used were suggested on the basis of morphology and biogeography to be monophyletic, and as such have been widely accepted. The data suggested that the family could be split into two parts: a paraphyletic Palaearctic and Oriental group of primitive forms, and a monophyletic assemblage of Ethiopian and advanced Saharo-Eurasian taxa (ESE group) arising from it. This data set has recently been reanalysed (HARRIS *et al.*, 1998b), and shows strongly supported (using the bootstrap technique) nodes for many relationships within the ESE group (Fig. 5). A subset of this data was included in a study examining sensitivity of phylogenies to taxon sampling (POE, 1998) because it gave »a fully resolved, single most parsimonious tree«. This suggests that FU's (1998) evaluation of the same data, that »insufficient numbers of informative characters left many nodes unresolved... the basal part of the strict consensus is a bush« is misleading. There are a great number of well supported relationships suggested by this data set.

As well as the morphological data there is a large amount of karyological data available for the primitive lacertids. This includes using sex chromosome data (ODIERNA *et al.*, 1992), C banding (OLMO *et al.*, 1986), G banding (ODIERNA *et al.*,

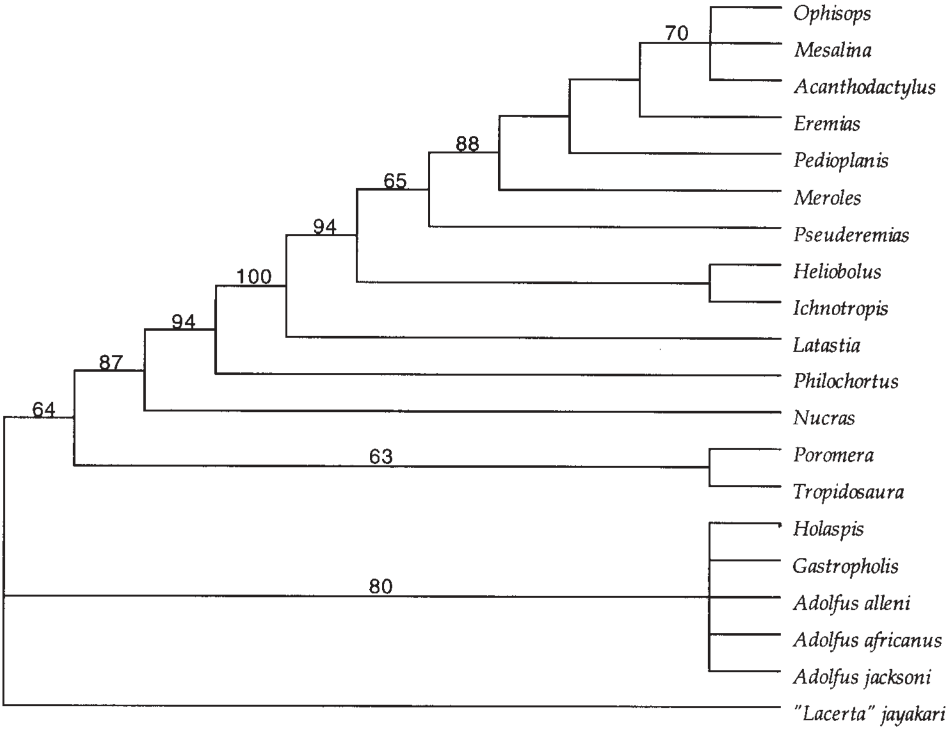

Fig. 5. 50% bootstrap consensus tree of relationships within the ESE clade based on the morphological data of ARNOLD (1989). From HARRIS *et al.*, 1998b. Many of the relationships are extremely well supported.

1993) and localisation of the Nucleolar Organising Region (NOR – ODIERNA *et al.*, 1987). Gross chromosomal features have had little value in assessing relationships, although some characteristics are informative, for example evidence for the monophyly of *Gallotia* (40 chromosomes in a diploid cell compared to the typical 38 – CANO *et al.*, 1984; LOPEZ-JURADO *et al.*, 1986). The localisation and amount of C banded heterochromatin has also not been very phylogenetically informative (OLMO *et al.*, 1986). G banding patterns may in the future be useful (ODIERNA *et al.*, 1993), but at present the limited samples examined makes assessment difficult. NOR positions have been shown to be the same in species within *Podarcis*, *Gallotia* and *Lacerta* parts I and II but different between them, thus supporting their raise to distinct genera (ODIERNA *et al.*, 1987).

Both albumin evolution (LUTZ & MAYER, 1985; MAYER, 1990; MAYER & BENYR, 1994) and protein electrophoresis (LUTZ *et al.*, 1986) have been used in an attempt to produce phylogenies based on molecular characters. All these studies, as well as morphological data, suggest that *Gallotia* and *Psammodromus* are closely related. However evidence from albumin evolution and protein electrophoresis suggests that *Gallotia* and *Psammodromus* are also very distinct from all other lacertids, to the point that they should be assigned to a subfamily *Gallotinae* separate from the subfamily *Lacertinae* containing all other lacertids. In most respects the phylogenies based on these data sets differ from each other and from morphology, making a consensus viewpoint almost impossible.

Until recently, attempts to investigate relationships among lacertids using DNA sequence data had been limited to investigations within *Gallotia* (eg THORPE *et al.*, 1994; 1996). Evolution of centromeric satellite DNA had also been used, but only in a small subset of species (CAPRIGLIONE *et al.*, 1991). However this has changed dramatically in the last year, with a study using DNA taxoprints (GRECHKO *et al.*, 1998), and a number of new studies using DNA sequence (HARRIS, 1997, HARRIS *et al.*, 1998a,b, in press; CASTILLA *et al.*, 1999a,b; FU *et al.*, 1997; FU, 1998). Two of these (HARRIS, 1997; FU, 1998) examine a number of taxa using partial 12S and 16S rRNA mitochondrial gene sequences, but infer different phylogenies. Since they use the same gene regions, the two data sets can be advantageously combined, and reanalysed. This is carried out in this study for the first time.

Combined mtDNA analysis

Sequences for 12S, 16S and cytochrome *b* (HARRIS *et al.*, 1998b), and the 12S and 16S sequences of FU (1998) were combined into a single data matrix. Some minor adjustments to the alignments were necessary, and were made by eye. The alignment used is available on request from the author. The slightly longer regions reported by FU (1998) where shortened so that sequences of the same length were compared. A number of previously unpublished sequences (all 3 partial gene regions for *L. kulzeri* and *O. elegans*, and the cytochrome *b* region of *Takydromus septentrionalis*) were also included. The cytochrome *b* sequence for *L. media* (FU *et al.*, 1997) was combined with the 12S and 16S data (FU, 1998), so all three gene regions could be included for this species. The resulting alignments had 1055 sites. Unalignable sites, and the third postions of the cytochrome b sequences, which have been shown to be saturated at this phylogenetic level for lacertids (HARRIS, 1997) where excluded. This left 842 characters, of which 348 were parsimoniously informative. The previously unpublished sequences have been submitted to Genbank.

An uncorrected NJ analysis was performed using PAUP* (SWOFFORD, 1998), and levels of supported were estimated using the bootstrap technique (1000 replicates – Fig. 6). A MP analysis was also carried out (10 replicate heuristic search), and resulted in 11 most parsimonious trees (2565 steps). Bootstrap values were calculated for this analysis also (Fig. 7).

These estimates of phylogeny are very similar to that produced by HARRIS *et al.*, (submitted), but differ from the phylogeny prefered by FU (1998). The most parsimonious tree shown by FU (1998) was more similar to the tree shown here, with an ESE clade (as morphological characters indicate), a »*Lacerta* and its allies« clade (*Lacerta*, *Podarcis* and *Algyroides*), and with *Gallotia* and *Psammodromus* basal. FU (1998) suggested that *Takydromus* is the sister group to all lacertids except *Gallotia* (and presumably *Psammodromus*, which was not included in this study), but he included only one species and the bootstrap support is extremely low (24). However FU disregards the most parsimonious tree in favour of a prefered hypothesis, where the »*Lacerta* and its allies« clade is paraphyletic. The combined analysis presented here suggests that his MP tree is perhaps the better estimate of phylogeny.

The combined mtDNA tree shows a number of supported clades within the Lacertidae, whose monophyly is again supported (100% – all bootstrap values given are from the NJ tree). *Gallotia* and *Psammodromus* are sister groups (100%), and basal to all other lacertids (79%). The armatured clade is very weakly supported (28%), but corroborated by strong morphological evidence. *Takydromus* is basal to the »*Lacerta* and its allies« group (51%), which is a clade (59%). Almost all the taxonomic units suggested by ARNOLD (1989) are supported as clades. A number of relationships in particular are worth noting:

a) *L. jayakari* and *L. cyanura*

LUTZ *et al.* (1985) using immunological and protein electrophoretic data suggested that the subgenus *Omanosaura* should be established containing these two species. Other morphological analyses have questioned this association (LEPTIEN & BÖHME, 1994). The mtDNA strongly suggests that the two species are a clade (89%), and part of the ESE group unlike any other *Lacerta*. The taxonomic value of *Omanosaura* is therefore upheld.

b) *L. lepida* and *L. agilis* group

The green lizards, *Lacerta agilis* group and *Lacerta lepida* group, come out as a clade. This agrees with the strong morphological evidence for this relationship, but is contrary to immunological evidence (MAYER & BENYR, 1994). On the immunological evidence RYKENA (1996) did not consider the *L. lepida* group when carrying out hybridization experiments within the green lizards. This is a further example of the need for an accepted phylogeny for the lacertids.

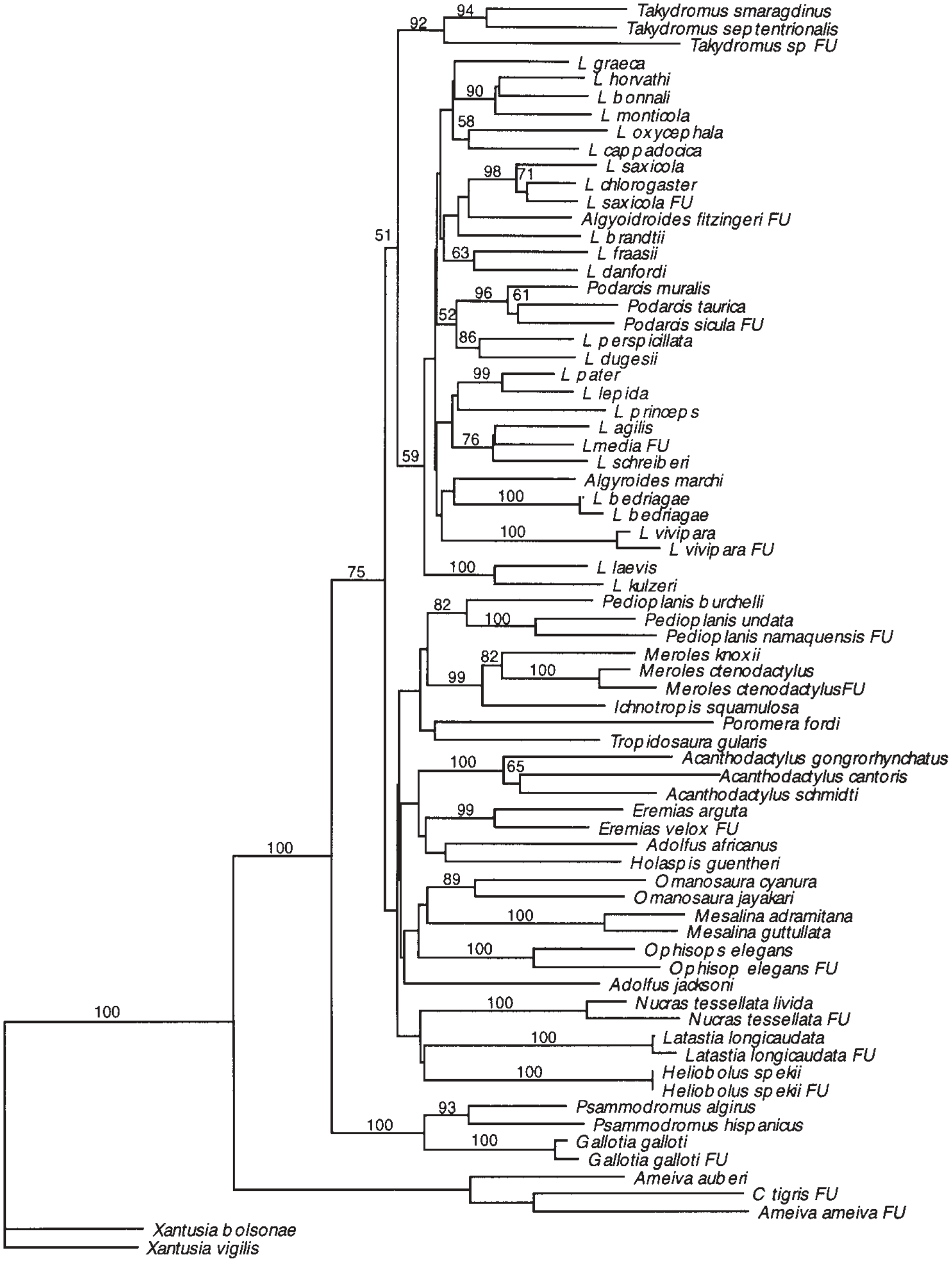

Fig. 6. Uncorrected NJ analysis of the combined available 12S, 16S and cytochrome *b* data for species of Lacertidae (data from HARRIS, 1997; FU, 1998 and new for this analysis). Bootstrap support (1000 replicates) is given above nodes. The tree was rooted using two xantusiids and three teiids. See text for details.

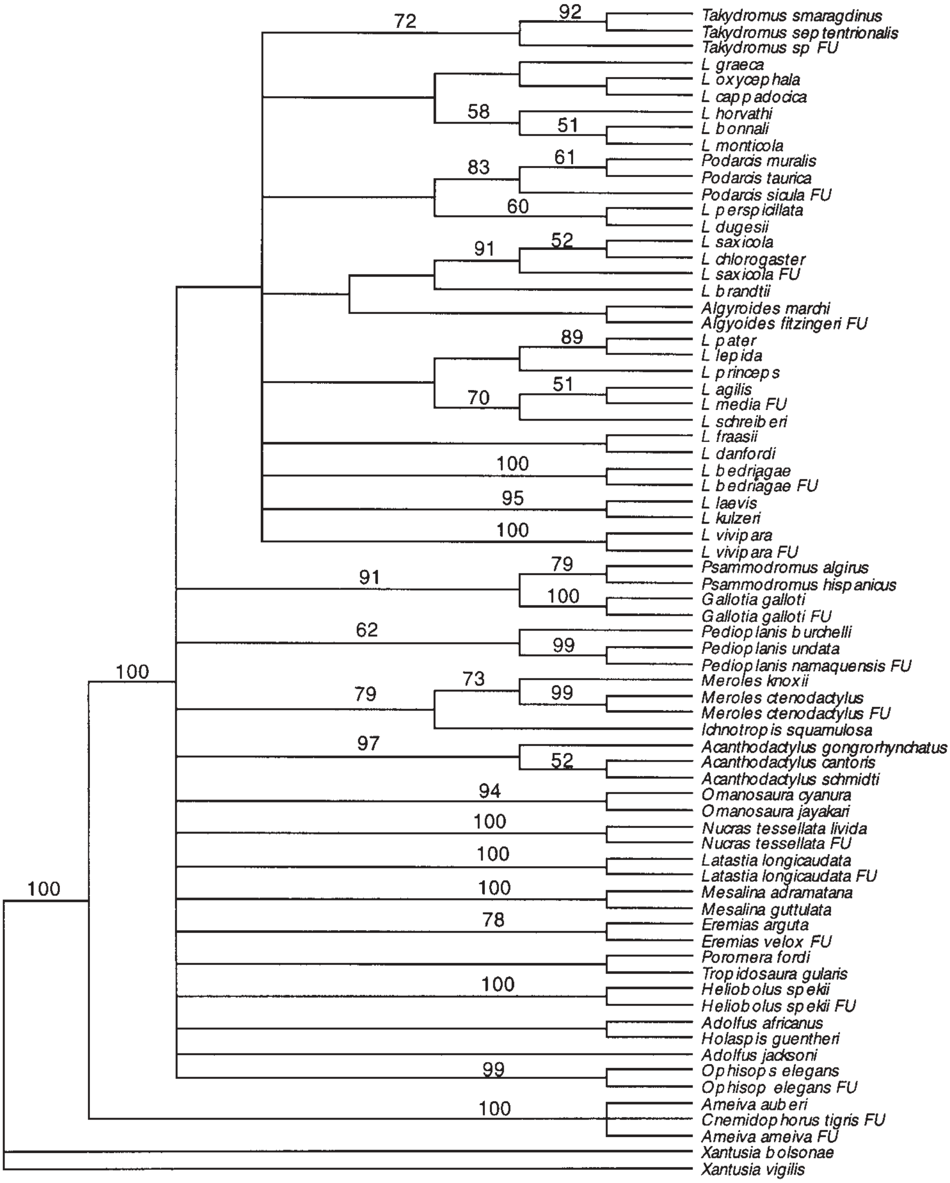

Fig. 7. Strict consensus of MP trees for the same data as Fig. 6. See text for details.

c) Relationships of *L. parva* and *L. brandtii*

Morphological characters support a *L. parva* group (including *L. fraasii*) association with *L. brandtii*, and more weakly with *Gallotia* and *Psammodromus* (Arnold, 1989). As already noted *Gallotia* and *Psammodromus* in fact appear to be the most basal lacertid lineage. The DNA evidence weakly supports a relationship between *L. brandtii* and the Rock lizards *L. saxicola* and *L. chlorogaster*, while *L. fraasii* is associated with *L. danfordi* (63% support). A possible association of *L. brandtii* with the Rock lizards group has been suggested previously (BÖHME, 1993), although this was based on a single hemipenial character and was not suggested as being decisive. However this relationship also makes sense biogeographically, with both *L. saxicola* and *L. chlorogaster* being in sympatry with *L. brandtii*, and other morphological similarities between these species have been previously noted (LANTZ & CYRÉN, 1939). *L. fraasii* and the *L. danfordi* group also have overlapping geographical ranges, and without strong contradictory evidence can be considered as sister groups.

d) Relationships of Rock Lizards

Several Rock lizards were included in this study; *L. graeca, L. oxycephala, L. horvathi, L. bonnali, L. monticola, L. saxicola, L. chlorogaster* and *L. bedriagae*. They have sometimes been placed in a subgenus, *Archaeolacerta* Mertens 1921, although most recent studies have regarded this as probably paraphyletic (ARNOLD, 1989A; MAYER & BENYR, 1994). Much of their morphological similarity appears to be functionally related to the problems of surviving in rocky habitats, and with using crevices as refuges (ARNOLD, 1973). ARNOLD (1989a) separated *L. oxycephala* and *L. graeca* from the others, and split the remaining species on a biogeographical basis with a more northern and western group (including *L. horvathi, L. bonnali* and *L. bedriagae* from this study) and a *L. saxicola* complex centered in the Caucasus (*L. saxicola* and *L. chlorogaster*). The DNA data strongly supports the *L. saxicola* group (98% support), and a *L. horvathi* relationship with *L. monticola* and *L. bonnali* (90% support) relationships of the other Rock lizards are less well supported. *L. bedriagae* is indicated as being the sister species of *Algyroides marchi* from the DNA data, but without strong support. Interestingly the two individuals of *Lacerta saxicola* included in this study do not come out as a clade, with one individual more closely related to *L. chlorogaster* (71%). GRECHKO *et al.* (1998) using DNA taxoprint did not find that *Lacerta saxicola* was a clade either, so it seems likely that it is infact a species group.

e) *Algyroides*

Surprisingly in the NJ analysis the two included species of *Algyroides* do not form a clade. This might be due to the fact that there is no cytochrome *b* data available for *A. fitzingeri*. The two species are associated in the strict consensus of the MP trees. This shows clearly how short branches (such as the ones separating these species in the NJ analysis), should always be treated extremely cautiously, unless they are corroborated by independent evidence.

f) E.S.E clade

Support for relationships based on sequence data within the advanced E.S.E. clade was far more limited than the support derived from morphological characters. Although the taxonomic units used were well supported, only one other relationship had more than 50% bootstrap support – the sister group relationship of *Ichnotropis* and *Meroles*. This is slightly different from the relationships indicated by morphological characters.

CONCLUSIONS

The phylogeny of the family Lacertidae, although still unresolved in part, is beginning to take shape. Formal names have recently been proposed for some supragenic groupings (HARRIS *et al.*, 1998b). *Gallotia* and *Psammodromus* seem to be sister group to all other lacertids, and deserve the status of subfamily, Gallotiinae Cano *et al*, 1984. Since the armatured E.S.E group is supported by both morphology and, weakly, by the mtDNA data sets, Eremiainae Shcherbak, 1975 can be applied to this clade. Lacertinae Linnaeus, 1758 is then available for *Lacerta* and its allies *Podarcis* and *Algyroides*. The exact taxonomic position of *Takydromus* within the family remains uncertain.

This study, as well as trying to advance our understanding of lacertid systematics, has aimed to highlight a number of points. Firstly the problem of contamination in previous studies and the lack of a resolved phylogeny for the genus *Gallotia*. Secondly the advantages of including both morphological and molecular characters in phylogenetic studies. It is surprising that extremely poorly supported molecular studies can be used to overturn well resolved and supported estimates of phylogeny based on morphological data sets. The high bootstrap support for relationships within Eremiainae based on morphological data, shows just how effective such characters can be in phylogeny reconstruction. The difficulties in resolving relationships within *Podarcis*, and the possible paraphyly of »*Teira*« has also been demonstrated. Finally the value of combining data sets, especially compatible mtDNA sequences has been shown, both in the case of *Gallotia*, and more extensively for the whole of the Lacertidae.

ACKNOWLEDGMENTS

This work was primarily funded by a Natural History Museum PhD studentship, and also by the Zoology Department of the Natural History Museum, London. Additional work was carried out in the Zoology Dept., Brigham Young University, Utah, USA. Thanks to E. N. Arnold who gave a great deal of assistance during my PhD studentship. W. Mayer and an anonymous reviewer gave extensive and constructive comments on an earlier draft of this manuscript. Many thanks to the enormous number of herpetologists who donated samples, which made the project possible. I hope this helps to answer some of their questions.

Received September 20, 1998

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