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Toward the Phylogeny of the Family Lacertidae: Implications from Mitochondrial DNA 12S and 16S Gene Sequences (Reptilia: Squamata)

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A phylogeny of the family Lacertidae was derived from mtDNA gene sequence data. Seventeen species, representing 16 currently recognized genera and subgenera, were included in the analysis. A total of 954 bp was obtained and aligned from 12S and 16S partial gene sequences. A preferred tree was selected based on weighted parsimony and functional ingroup and outgroup analyses. Decay analysis, bootstrapping, and permutation tail probability were used to evaluate support for the recovered nodes. The genus *Gallotia* was resolved as the basal taxon and the sister group of all remaining lacertids. *Takydromus* branched off next. All African lacertids grouped together and formed a monophyletic clade with the Eurasian genera *Eremias* and *Ophisops*. The remaining Eurasian lacertids sequentially branched off near the base of the tree in a "comb-like" fashion. The basal position of *Gallotia* and the monophyly of African lacertids are consistent with previous hypotheses. The European-origin hypothesis of lacertids is favored, and the distribution of lacertids in Africa is likely a Miocene dispersal event. Most of the extant European lacertids probably arose after the Eocene. The classification of the family needs to be revised.

INTRODUCTION

Lizards of the family Lacertidae are widely distributed throughout most of Eurasia and all of Africa. The range of the family extends eastward as far as Malaysia and Indonesia, but never reaches New Guinea and Australia. They are absent from Madagascar as well. Presently, approximately 259 species of lacertid lizards have been assigned to 24 genera (Bischoff, 1990, 1991a,b,c, 1992a,b). A wide range of investigations have been carried out, including comparative morphology, karyology, immunocytochemistry, ecology, population dynamics, behavior, and parasitology. The lacertids are among the most studied vertebrates, especially in Europe (Valakos *et al.*, 1993). In terms of phylogenetics, recent hypotheses have been derived from morphological data (Arnold, 1973, 1983, 1989a,b, 1991) and biochemical data (Mayer and Tiedemann, 1982; Mayer and Lutz, 1989, 1990; Mayer and Benyr, 1994; Murphy *et al.*, 1996; Fu *et al.*, 1997).

In spite of this wealth of knowledge, the phylogenetic relationships of the genera remain unclear. Arnold (1989a, p. 210) stated: "[t]he Lacertidae are one of the few large or medium-sized families of lizards not to have been subjected to some form of overall phylogenetic analysis in recent years." Although this statement no longer holds, only two treatments of phylogeny exist at the family level. Arnold (1989a) pursued an overall phylogeny of the family using morphological data. His proposed phylogeny is a largely unresolved tree, especially at the base. Although a fully resolved tree was presented, most of its nodes were considered tentative. Two problems were probably responsible for the inability of Arnold's morphological data to fully resolve a phylogeny. First, an insufficient number of informative characters left many nodes ambiguous. My reevaluation of Arnold's original data set using PAUP (version 3.1.1; Swofford, 1993) resulted in 19,400 equally most parsimonious trees (MPTs) with a CI of 0.326. The basal part of the strict consensus tree is a "bush." Second, conflict in the data resulted in a lack of confidence. In addition, Arnold used a problematic method of data analysis, i.e., compatibility analysis (Swofford *et al.*, 1996), and erroneous assumptions (e.g., common equals primitive). More recently Mayer and Benyr (1994) presented another phylogeny of the family. Unfortunately, the albumin immunological (MC'F) method they used is a questionable method for inferring phylogeny. The method groups taxa on the basis of similarity (Maxson and Maxson, 1990) and not on the basis of shared derived character states, which violates the taxa grouping rule of phylogenetics (Wiley *et al.*, 1991; Murphy and Doyle, in press).

The use of DNA sequence data for inferring phylogeny has exploded in the past decade. Among the many advantages of DNA sequence data, the enormous gene pools of organisms supply an almost unlimited number

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of characters for phylogenetic reconstruction. More importantly, these characters have a tremendous scope of variation, ranging from the most conservative to the most variable (Miyamoto and Cracraft, 1991; Hillis *et al.*, 1996a). Because of these attributes, they allow for the examination of different and diverse evolutionary questions.

Considering the contradiction between the poor estimation of the genealogical relationships and the wealth of knowledge about the other aspects of its biology, a robust phylogeny of the family Lacertidae is highly desirable. I conducted a molecular study of the phylogeny of the family Lacertidae using mtDNA sequence data, the results of which are reported herein.

MATERIALS AND METHODS

Species Examined

Seventeen species were selected to represent the main lineages of the family Lacertidae (Table 1). Sixteen currently recognized genera and subgenera were included. The genus *Lacerta* has long been acknowledged as a non-monophyletic group (Arnold, 1973; Böhme and Corti, 1993; Mayer and Benyr, 1994), and thus five species were used to represent five generally accepted natural groups. The ranges of the selected species covered most of the distribution of the family from the Canary Islands to Vietnam and from South Africa to northern Russia. The family Teiidae was selected as the primary outgroup based on Estes *et al.* (1988). Two species, *Ameiva ameiva* and *Cnemidophorus tigris maximus*, were used in the analysis (Table 1).

TABLE 1

Species Examined in This Study

Species	Localities	Voucher no.
Algyroides fitzingeri	Italy	ROM 24642
Ameiva ameiva	Guyana	ROM 20530
Ameiva auberi	GenBank	
Cnemidophorus tigris maximus	Mexico, Baja California	ROM RWM647
Eremias velox	Russia, Daghestan	ROM 23498
Gallotia galloti	Spain, Canary Islands	Unavailable
Heliobolus spekii	Kenya, Kajiado District	CAS 198923
Lacerta (Archaeolacerta) bedriagae	Italy	ROM 24640
Lacerta (Timon) lepida	GenBank	
Lacerta media (s.str.)	Armenia, Abovyan	ROM 24267
Lacerta saxicola	Russia, Dombay	ROM 24392
Lacerta (Zootoca) vivipara	Russia, St. Petersburg	ROM 24750
Latastia longicaudata	Kenya, Kajiado District	CAS 198982
Meroles ctenodactylus	South Africa, Cape Province	LSUMZ H-13110
Nucras tessellata	South Africa, Richtersveld National Park	LSUMZ H-13111
Ophisops elegans	Armenia, Chosrov	ROM 23506
Pedioplanis namaquensis	South Africa, Richtersveld National Park	LSUMZ H-13109
Podarcis sicula	Italy	ROM 24637
Podarcis (Teira) dugesii	GenBank	
Takydromus sp.	Vietnam, Sapa	ROM 26345

Genes Selected

Two ribosomal RNA genes, 12S and 16S, from the mitochondrial genome were selected to reconstruct the phylogeny. Ribosomal RNAs are functionally important in protein synthesis, which makes them relatively resistant to evolutionary change (Mindell and Honeycutt, 1990). This trait may help overcome the problem of ecological adaptation common to many morphological attributes. According to Mindell and Honeycutt (1990), the 12S and 16S genes are potentially informative for divergence as far back as 300 million years ago (MYA), and they seem best suited for divergence of about 150 MYA or less. The divergence of lacertids is thought to be within this range (Estes 1983a).

Amplification and Sequencing Protocols

Standard phenol-extraction methods were used to extract DNA from tail muscle or liver tissues. Laboratory protocols follow Palumbi (1996) and Hillis *et al.* (1996a). Polymerase chain reaction (PCR) was used for amplifying the DNA sample; parameters and settings follow the manufacturer's recommendations and Palumbi (1996). Sequencing used Autoload Solid Phase Sequencing Kits (Pharmacia) and an ALF automated sequencer (Pharmacia). Protocols follow manufacturer's recommendations with minor modifications. Two 12S gene primers (12S-1 5′ caa act ggg att aga tac ccc act at 3′, 12S-2 5′ agg gtg acg ggc ggt gtg t 3′; Kocher *et al.*, 1989) and two 16S gene primers (16S-1M 5′ ccg act gtt tac caa aaa cat 3′, modified from Palumbi 1996; 16S-2 5′ ccg gat ccc cgg ccg gtc tgt tga act cag atc acg 3′, Palumbi, 1996) were used for PCR and sequencing. All sequences were completed from both directions.

Clustal W (version 1.6, Thompson *et al.*, 1994) was used for sequence alignment. The aligned sequences were subsequently edited in ESEE (version 3, Cabot and Beckenbach, 1989). Minor modifications were made by eye to correct the computer-aligned sequences.

Phylogenetic Analysis

The two genes were initially analyzed separately, because different genes may experience different evolutionary pathways. Further, the corroboration from independent data sets provides strong evidence for the

reliability of phylogenetic trees (Hillis, 1987; Miyamoto and Fitch, 1995). Second, a combined data analysis was conducted. Tree length distribution skewness (g_1 statistics; Huelsenbeck, 1991; Hillis and Huelsenbeck, 1992) and permutation tail probability (PTP; Archie, 1989; Faith and Cranston, 1991) were used for assessing character covariance in the data sets.

The maximum parsimony criterion was used for inferring phylogeny. Each base site was treated as a nonadditive (=unordered) character. The initial analysis was conducted with equal weights to all characters. Subsequently, transversion weighting was applied (Hillis *et al.*, 1994).

Because DNA sequence data are highly homoplastic, it is advisable to examine the suboptimal trees as well (Swofford, 1991; Cracraft and Helm-Bychowski, 1991). Therefore, decay analysis (Bremer, 1988) was used to assess the most parsimonious tree from the initial analysis. Based on the results of this assessment, functional ingroup and outgroup analyses (FIG/FOG; Watrous and Wheeler, 1981; Fu and Murphy, 1997) were used for further testing of the recovered relationships.

Two statistical methods, bootstrap proportions (BSPs; Felsenstein, 1985) and PTP, were also applied. However, the BSP may not be a valid method for assessing nodal confidence (Kluge and Wolf, 1993; Trueman, 1993; Sanderson, 1995; Murphy and Doyle, in press). An extended "conditional PTP" (Faith and Cranston, 1991) was used to evaluate the recovered nodes. By using one representative from each clade to form a subset and subsequently applying the PTP test to the subset, the confidence limit of the relationships among the clades can be examined. The method has been proven to be powerful for DNA sequence data (Fu and Murphy, in review).

The computer programs PAUP (version 3.1.1; Swofford, 1993), MacClade (version 3.04; Maddison and Maddison, 1992), and Random Cladistics (version 40.3, Siddall, 1997), which interacts with Hennig86 (version 1.5; Farris, 1988), were used for the analysis.

RESULTS

Seventeen specimens, each representing a species, were sequenced for 12S, and sequences for another three species, *Ameiva auberi*, *Lacerta lepida*, and *Podarcis dugesii*, were obtained from GenBank. A total of 411 bp were resolved and aligned (Fig. 1). Pairwise comparisons showed that the site variability between ingroup and outgroup members ranged from 27 to 32%. The variability among ingroup members ranged from 6 to 23%. Hillis and Dixon (1991) suggested that sites with ambiguous alignment should be excluded from the analysis, because the homology cannot be confidently assumed. Consequently, 41 sites were excluded from the analysis. The pairwise difference became 25 to 33% between ingroup and outgroup members and 5 to 21% among ingroup members.

Seventeen specimens were sequenced for 16S. A total of 543 bp were resolved and aligned (Fig. 1). Pairwise comparisons showed that the site variability between ingroup and outgroup members ranged from 24 to 27%. The variability among ingroup members ranged from 8 to 21%. Ninety-one sites were excluded from the analysis due to ambiguous alignment. Subsequently, the pairwise difference became 17 to 20% between ingroup and outgroup members and 5 to 15% among ingroup members. Electronic files of the aligned sequences are available upon request.

Invariant sites and those with a single variable taxon (autapomorphic change) were excluded from the phylogenetic analysis, because they contribute no information to taxon grouping. One hundred forty-six potentially phylogenetically informative characters were obtained from the 12S gene, and 124 from the 16S gene.

Both 12S and 16S data resulted in significant PTP values of 0.001 (999 replicates). Data from the 12S gene resulted in a g_1 value of -1.95, and -1.53 for the 16S gene (1000 replicates). Both measurements indicated that there is significant character covariance in the data sets (Faith and Cranston, 1991; Hillis and Hulsenbeck, 1992; but see Murphy and Doyle, in press).

Eleven MPTs were found in the analysis of the 12S data, with 514 steps, CI of 0.472, and RI of 0.479. However, the strict consensus tree only resolved five nodes (Fig. 2A), indicating inconsistent and insufficient data. Three MPTs resulted from analysis of the 16S data, with steps of 378, CI of 0.487, and RI of 0.464. Better resolution was achieved from the 16S data set (Fig. 2B). As is typical for DNA sequence data, the low CI and RI values implied that the data are highly homoplastic. The results from these two genes are largely compatible except for the slightly different placements of *Gallotia*. Because both the 12S and 16S genes are rRNA coding genes, and both are located in the mitochondrial genome, they are less likely to have different evolutionary pathways in this case. Thus, a combined data approach was appropriate (Doyle, 1992; Huelsenbeck *et al.*, 1996). The larger data set should reinforce the signal presented in the small data sets and better overwhelm the "noise." One MPT was resolved from the combined data, with 919 steps, CI of 0.464, and RI of 0.442 (Fig. 3A), and with resolution significantly improved.

Gallotia was resolved as the sister group to all other lacertids. *Takydromus* was the next most basal taxon. The other lacertids were divided into two monophyletic groups: the African clade, including all African lacertids plus Eurasian *Ophisops* and *Eremias*, and the

Eurasian clade. While *Eremias* was resolved at the base of the African clade, *Ophisops* occurred as the sister group of (*Haliobolus, Latastia*) (Fig. 3A).

Five suboptimal trees were found at 920 steps (1 step longer than MPT). A strict consensus tree (including the MPT) resolved five nodes, including the nodes defining the basal position of *Gallotia* and *Takydromus,* and the African clade. Keeping all trees equal to or shorter than 921 steps resulted in 36 trees. Although the strict consensus did not resolve any nodes, the majority rule consensus tree indicated that 94% of the 36 trees supported the basal placements of *Gallotia* and *Takydromus,* as well as the monophyly of the African clade. As a preliminary conclusion, the decay analysis supports the basal position of *Gallotia* and *Takydromus,* and the monophyly of the African clade, but the monophyly of the Eurasian clade is not supported.

Inversely weighting the transversions (two times) over transitions, reflecting the ratio of observed changes (Williams and Fitch, 1989), resulted in one tree (Fig. 3B). While *Gallotia* and *Takydromus* kept their basal positions and the African clade was retained, the Eurasian clade collapsed. The members of the Eurasian clade formed a comb-like shape at the base of the tree, and *Eremias* and *Ophisops* formed a sister group relationship at the base of the African clade. This tree required 924 steps on the unweighted data. Ignoring transitions all resulted in 105 trees. The strict consensus resolved the basal position of *Gallotia,* and the relationships among the African clade members remained as in the above weighted tree.

Examination of the branch lengths showed that a fairly long branch connected the ingroup to the primary outgroup. This indicated that the primary outgroup was probably too distantly related to the ingroup. To reduce the potential effects of long branch attraction, the FIG/FOG analysis was conducted to evaluate the stability of the recovered nodes. The well-supported basal position of *Gallotia* and *Takydromus* facilitated their use as the FOG. Consequently, a progressive functional outgroup approach (Fu and Murphy, 1997) was conducted. First, *Gallotia* was used as the FOG and the three primary outgroup members were excluded from the analysis. One tree was resolved. The placement of *P. (Teira) dugesii* and *Podarcis sicula* were altered compared to the initial analysis. Other relationships remained the same. Mapping the tree on the original data set resulted in 920 steps. Next, *Takydromus* was used as the FOG and *Gallotia* was excluded from the analysis. One tree was resolved. The topology was quite different from the initial analysis, but very similar to the results from the weighted parsimony. The African clade was upheld, and the Eurasian lacertids were placed basely, forming a comb-like tree. This tree requires 921 changes to explain the original data (Fig. 4A).

DISCUSSION

The Preferred Phylogeny

Hillis *et al.* (1994) argued that weighted parsimony may be more accurate at representing the evolutionary history for molecular data. In addition, the FIG/FOG analysis may be more appropriate for DNA sequence data. Reanalyzing the amphibian family phylogeny derived from 12S and 16S gene sequence data (Hay *et al.,* 1995) indicated that a distantly related outgroup could misroot the tree and/or resulted in less reliable trees (Fu and Lathrop, in review). The tree topologies that resulted from the weighted parsimony and FIG/FOG analyses were largely the same in this study, with only a few different placements of the terminal taxa. Therefore, the tree which resulted from using *Takydromus* as FOG was selected as the preferred phylogeny (Fig. 4A). This tree requires 921 steps, 2 steps longer than the MPT and 2 steps shorter than the weighted tree. Examining the suboptimal trees, there are 36 trees with steps equal to or less than 921. The results of PTP nodal evaluation and decay analysis were mapped onto the preferred tree. BSPs greater than 0.20 were also mapped on the tree.

Four nodes of the preferred tree were supported by the PTP analysis, which indicates that these relationships are the result of significantly covaried data and that the pattern presented in the data cannot be derived by chance alone (Faith and Cranston, 1991). Beside these four, an additional node was supported by decay analysis. The position of *Gallotia* was supported by all trials, as was the position of *Takydromus.* The monophyly of African lacertids along with *Eremias* and *Ophisops* was also well supported by decay analysis, FIG/FOG analysis, and transversion weighting. The sister group relationships of *Algyroides* with the *Lacerta saxicola* complex and *Haliobolus* with *Latastia* were also well supported. Because relationships among Eurasian lacertids were very unstable, and differed from one another among different trials, they are not likely to be a monophyletic group. They are placed at the basal region of the preferred tree as a paraphyletic group. The topology of the Eurasian lacertids reflects the results of weighted analyses and part of the FIG/FOG analysis. However, the relationships should be regarded as tentative.

The positions of *Eremias* and *Ophisops* within the African clade are very interesting. *Eremias* was located at the base of the clade on all trials. However, *Ophisops* was grouped with (*Haliobolus, Latastia*) in the initial analysis. They also grouped together under the weighting and the FIG/FOG analyses. The sister group relationship of (*Eremias, Ophisops*) was established from morphology (Arnold, 1989a) and immunology (Mayer and Benyr, 1994). Subsequently, a topology-constrained search was conducted, forcing *Eremias* and *Ophisops* to

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|12S
                                                                  50                                                                 100
Aamei GCCTGGGAGT TAACCACGAT AGGCCGA--- CACAAT--CT ATCCGCCAGA GAATTACGGG CGAAAG-CCT AAAACTCAAA AGACTTGACG GTGTCCCAAC CCTGCCTAGA
Aaube ..T.A.A... .........C .A...CT.AT A....C..T. G......... .G........ T......... .......... .......... .........T T..A......
Ctigr ..T.A.A... ....A....C .AA..AC..T A.......T. G......... A......... T....A.... .......... .....T...-. ...A......
Afitz ..TAA.CCC. A...ATT... TA...T-.TT AG...G.A.. T......... ...C...AA. T....AA.T. G......... G......... .........CA T.G.......
Evelo ..TAA.CCC. ....ATT..C ..CA.A-.CC T...C..G.. C......... ...C...AA. T....AA.T. .......... G......G.. .........TA T.GA......
Ggall ..TAA.CCA. A...ATT... ..TTAA-.TA T...TA.... T......... ...C...AA. T....AA.T. .......... G......G.. .........CA T.GA......
Hspek ..TAA.CCA. A...ATT... ..C.AA-.AC T..T.AG..C .......... ...C...AA. TA...AA.T. .......... G..T...G.. .........CA T.AAA.....
Lbedr ..TAAACCC. A...ATT... ..CTTA-.TA T....C.... T......... ...C...AA. T....AA.T. G......... G......... .........TA T.G.......
Llepi ..TAA.CCC. A...ATT... TA.TTA-.TA T....C.A.. C......... ...C...AA. T....AA.T. G......... G......... ..A.....TA T.GA......
Lmedi ..TAA.CCC. A...ATT... TA...A-.TA T....C..T. C......... ...C...AA. T....AA.T. .......... G......... .........TA T.GA......
Lsaxi ..TAA.CCC. ....ATT... -A.TAT-.TA T......... T......... ...C...AA. T....AA.T. G......... G......... .........TA ..GA......
Lvivi ..TAA.CCC. A...ATT... TAAT.A-.TA T....C.A.. C......... ...C...AA. T-...AA.T. G......... G......... .........TA T.GA......
Llong ..TAA.CCA. .T..ACT... ..TTAA-.AC T...TAT... .......... ...C...AA. T....AA.T. .......... G......G.. .........TA T.A.A.....
Mcten ..TA..CCC. A...AT...A ..CT.A-.TC .....-.G.. T......... ...C...AA. T....AA.T. .......... G......... .........TA ..GA......
Ntaes ..TAA.TC.. A...ATT... G.C?AT-.AC T....A.G.C T......... .......AA. TA...AA.T. G......... G......G.. .........A T.AA......
Oeleg ..TAA.CCC. A...ATT... ..CTAA-.CA T..T.C.G.. T......... .......AA. .....TG.T. G......... G......G.. ..A......A T.GA......
Pnama ..TAAACC.. A...ATT... ..C.TC-.AA T...CACG.. T......... ...C...AA. T....AG.T. .......... G......G.. ..A.....CA T.GC......
Pduge ..TAA.CCA. ....ATT..A ..CTAT-.CA T......G.. T......... ...C...AA. T....AA.T. .......... G......... .........TA T.GA......
Psicu ..TAA.CCC. G...ATT..A ..TTAC-.TA T..TT..A.. T......... ...C....A. T....AA.T. .......... G......... .........TA T.G.......
Takyd ..T.A.TTT. A...ATT..G CAATAT.ATC T...CCCATG C......... ...C...AAA T....AATT. .......... G......... .........TA T.GA......

                                                                 150                                                                200
Aamei GGAGCCTGTT TCATAATCGA TAACCCCCGC TCAACCCAAC CTTCCCTCGA A-CAT--CTC AGCCTATATA CCGCCGTCTT ACTTCTAGCT TACCTTCTGA AAGAAATACA
Aaube ......... ......... ......... ..C..... ..CTT..... .AT....T.. .......... ...AA...C. T.-.T.C... .....T... ....C.A...
Ctigr ......... ......... .........A .......G.. ...T...T.. .AT..A.... .......... ........C. T--.TAC... ......T.?? ??..T.....
Afitz .........C CT........ ..CT..... ..C..... .CCAA..A.C .TGTG..-.. .......... ........GA T----C...C ....C.G... .G.TCCA...
Evelo .........C CT........ ..CT..... ..T...T... ..CAA..A.C .C.CCA.-A. .......... ........GA -----C..TC ....CCA... GG.CTCA...
Ggall .........C CT........ ..CT..... ..T...TC.. .AG.A..A.C .C..A.CT.. .......... ........GA -----C.... ....CC.... GG.TTTA...
Hspek .........C CT........ ..C.....T ..C..... ..AAA..A.C .TA--..T.. .......... ........GA -----C...C ....CCA... GG.TTT..T.
Lbedr .........C CTG....... ..C.....T ..C..... ...AA..A.T .A-.A.TA.. .......... ........GA -----C..TC ....C.A... .G.CCCA...
Llepi .........C CT........ ..C...T... .TC....C.. .C.AA..A.C .CACATTA.. .......... ........GA T----C...- ....C.A... .G.TCTA.T.
Lmedi .........C CT........ ..CT..A..T ..T...T... .C.AA..A.C .CA.-.TAA. .......... ........GA -----C...C ....C.A... .G.TCTA...
Lsaxi .........C CT........ ..CT..... ..C..... .CCAA..A.C .TTCC..-.. .......... ........GA T----C...C ....C.A... ...TCCA.T.
Lvivi .........C CT........ .........T ..C....C.. .C.AA..A.C .TAC-..--. ...T..... ........GA -----C...C ....CCT... GG.TT.A...
Llong .........C CT........ ..CT..... ..C..... ...AA..A.C .TA--..TC. .......... ........GA -----C...C ....CCA... GG..CCA.T.
Mcten .........C CT........ ..C..... ......TG.. ...AG..A.C CATCC..--. .......... ........GA -----C..TC ....CCA... GG.CTCA...
Ntaes .........C CT........ ..C..... ..C..... ...AG..A.C CAAG...--. .......... ........AA -----C..TC ....CCA... GG.CCCA...
Oeleg .........C CT........ ..C.....T ..C..... ..C.A..A.T .CTT-..-.. .......... ........GA T----C...C ....C.G... GG.TCTA.T.
Pnama .........C CT........ ..C.....T ..C..... .CCAGT.A.C CC..C..--. .......... ........GA -----C..TC ....CCA... GG.C.CA...
Pduge .........C CT........ .GCT.....T ..C....G.. .CCAG..A.C .AT.A.AA.. .......... ........GA -----C-.TC ....C.A... .G.CCCA...
Psicu .........C CT........ ..C.....T ..C..... .CCAA..A.C .A-.A.CA.. .......... ........GA -----C..T. ....C.A... .G.TCCA.T.
Takyd .........C CT........ .G.A..... ..C...TT.. ...AA..A.C .CTTA..-C. .......... ........CC -----C...C ....CCA... .G.TTTA...
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FIG. 1. The aligned sequences of 411 bp of mitochondrial 12S rRNA gene for 17 lacertids and 3 teiids and 543 bp of mitochondrial 16S rRNA gene for 15 lacertids and 2 teiids. Dashes correspond to gaps and question marks are missing data. The underlined sites are excluded from the phylogenetic analysis. Aamei, *Ameiva ameiva;* Aaube, *Ameiva auberi;* Ctigr, *Cnemidophorus tigris maximus;* Afitz, *Algyroides fitzinger;* Evelo, *Eremias velox;* Ggall, *Gallotia gallotia;* Hspek, *Heliobolus spekii;* Lbedr, *Lacerta bedriagae;* Llepi, *Lacerta lepida;* Lmedi, *Lacerta media;* Lsaxi, *Lacerta saxicola;* Lvivi, *Lacerta vivipara;* Long, *Latastia longicaudata;* Mcten, *Meroles ctenodactylus;* Ntaes, *Nucras taessellata;* Oeleg, *Ophisops elegans;* Pnama, *Pedioplanis namaquensis;* Pduge, *Podarcis dugesii;* Psicu, *Podarcis sicula;* Takyd, *Takydromus.*

be sister groups. One tree was resolved and it was identical to the result of the FIG/FOG analysis (the preferred tree). Except for the sister group relationship of *Haliobolus* and *Latastia,* other relationships in the African clade were unstable among the trials, and nodal confidence could not be obtained. A phylogenetic framework (Lanyon, 1993) that reflects the confidence elements of the resolved phylogeny was generated (Fig. 4B). The association of *Eremias* and *Ophisops* represented not only the DNA data, but also other sources as well. The nodes that are polychotomies represent uncertainties, not multiple simultaneous speciation events. The following discussion is based on the phylogenetic framework.

Comparison of Hypotheses

The two previous assessments of phylogenetic hypotheses of lacertids were made by Arnold (1989a; hereafter referred to as ENA) and Mayer and Benyr (1994; hereafter referred to as MB). When only the taxa in common with my study were depicted on the tree, ENA's tree required 768 steps for the DNA data, which was 48 steps longer than my preferred tree. MB's tree required 632 steps, which was 22 steps longer than my preferred tree. Although there were some relationships common to all three hypotheses, my phylogeny differed from the other two in several aspects.

The basal position of *Gallotia* was recognized by MB and my DNA data, but not by ENA. On ENA's tree, the placement of *Gallotia* and its relatives was unresolved. ENA tentatively associated *Gallotia* and its relatives with [*Algyroides, Archaeolacerta, L. saxicola* complex, *Podarcis* and *P. (Teira) dugesii*]. This relationship was not depicted on my DNA sequence tree. ENA also failed to find a placement for *Takydromus,* although it was grouped tentatively with *Lacerta (Zootoca) vivipara.* MB did not include *Takydromus* in their analysis. The basal placement of *Gallotia* and *Takydromus* is well supported by the DNA sequence data.

Both ENA and MB recognized the monophyly of African lacertids, which is also supported by the DNA

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                                         250                                                          300
Aamei GTAAGCACAA TAGTTCC--C A--ACTAGAA AGTCAGGTCA AGGTGTAGCT TATGAGAAGG AGAAAATGGG CTACATTTTC TGTCACAGAA CATCCACGGA AAATATTCTG
Aaube ......C... .....T.AA. .......A.. .......... .......... .....AG... .......... .......... .A........ ....T..... ..G.......
Ctigr ......CT.. .....TATTT .......A.. .A........ .......... ....GAG... .......... .......... .A...T...T A.C--...A. ..G.......
Afitz ...GA..... C..CC...TT C..G...AT. C......... .........A A...TTGG.. .AG.G..T.. .........T .A.A.T.A.. A..--...A. ...C.C.A..
Evelo ...GA..... ...CC.A.A. C..G...AC. C......... .........A C...CTG... .AG.G..TT. .........T .ACATT.A.. A.C--...A. .TGC.ACA..
Ggall .......... ....C--.CT .CC....AC. C......... .......... A...T.CT.. CAG.G..T.. .........T .A.TTT.A.. A.C--...A. .TGCTA.A..
Hspek ...GA..... ....GTATAT .TT....AC. C......... .........A C...TTT... .AG.G..T.. .........T AA.TTT.A.. A..--..... .TGCCCAA..
Lbedr ...GA..T.. ...CC...C. CCCG...AC. C......... .........A A...TT.... .AG.G..T.. .........T .AAA.T.A.. A.C--...A. ..GC.C.A..
Llepi ...GA..... ...CC.A.A. C..G-..AT. C......... .........A A...TT.G.. .AG.G..T.. .........T .A.G.T.A.. A.C--...A. .-G....A..
Lmedi ...GA..... ...CC.A.A. C..G....T. C......... .........A A...CT.G.. .AG.G..T.. .........T .A.G.T.A.. A.C--...A. ..G....A..
Lsaxi ...GA..... ...CC.T.AT C..G...AT. C......... .........A A...TTGT.. .AG.G..T.. .........T .A.T.T.A.. A..--...A. .....C.A..
Lvivi ...GA..... .T.C..A.A. C..GT..AT. C......... .........A C...TT.G.. .AGCG..T.. .........T .A..TT.A.. G..--...A. .GGCCC.A..
Llong ...G...T.. ....A-ATAA .TT....A.. C......... .......... A...TT.... .AG.G..T.. .........T AAAATT.A.. ..C--..... .TGC.C.A..
Mcten ...GA..... C..CC...T. CC.G...AC. C......... .........A ....CT.... .AG.G..T.. .........T .AATTT.A.. A.C.-...A. .TGC.GCA..
Ntaes ...GAT.T.. ...CA...C. CCTG...AT. C......... .........C C...CT.... .AG.G..T.. .........T ...A...A.. A..TT...A. TTGC.C.A..
Oeleg ...GA..... ...CCTA.CT C..G....T. C......... .......... A...TAG... .AG.G..T.. .........T .A.TCT.A.. A..--..... .T...ACA..
Pnama ...GA..T.. A..CAA..AA CC.G...AT. C......... .........A A..ACTGG.. .AG.G..T.. .........T .TATTT.A.. A..TA...A. TTGC.C.A..
Pduge ...GAT.... ...CC...C. CCC....AT. C.A....... .........A A...CTGG.. .AG.G..T.. .........T .A.G.T.A.. A..--...A. .TGC.C.A..
Psicu ...GA..... ...CC.T.AA C..G...AT. C......... .........A A...CTGG.. .AG.G..T.. .........T .A.A.T.A.. A.C--...A. TTG..CCA..
Takyd ...GA..... ...CC--.CT C......AT. C......... .........A G...TT.... .AG.G..T.. .........T .AAA.T.A.. A.C--...A. ..G.T..G..

                                                                                      12S|
                        350                                                 400        411
Aamei AAATAAAATA TAAGAAGGCG GATTTAGCAG TAAGTTAAAT CAGAGACTTT AACTAAAACA AGCTCTGGGA CATGTACACA ?
Aaube ...C...... C.CA...... .......T.. ..G--C...C T....-TG.. G.....C.-- -.......-. .......??? .
Ctigr .......T.. C.C....-T. A...-..... ..G--.T... TT???????? ?????????? ?????????? ?????????? .
Afitz ...CCG--.G C.T.....T. A......T.. ...AA..... A......... .TT.T..TAG C......... .GC....... C
Evelo ...CCC--.G C.T.....T- A......T.. ....C.T... G..G..-... .CT.-..CA. C...-..... .GC--....? .
Ggall ...A.C--AG C.T....... A......T.. ...AAC.G.C A.....G.C. GCT.T..CA. C......... .GC....... C
Hspek .....C--A. C.TA....T. A......... ...AA....C A...CTA... .TT.T..CAT T......... .GC.C...A. .
Lbedr ...CCC--.G C.T.....T. A......T.. ...AA..G.. A....TG.C. .TT.T..CA. C......... .GC....... C
Llepi ...C..--.. C.T.....T. A......T.. ...AA..G.. A.....G.C. .TT.T..CA. C......... TGC....??? .
Lmedi ...CTG--.. C.T.....T. A......T.. ...AA..G.. A....TGCC. .TT.T..CA. C......... .GC....... C
Lsaxi ...C.G--.. ..T.....T. A......T.. ...AA...CC A..G.TGC.. .TT.T..CA. C......... .CG....... .
Lvivi ..CC..--.G C.T.....GT A......T.. ...AG.-G.. A..G.TT--C GCT.T..CA. CCGC...... .CG-.????? .
Llong ....CC--.G C.T.....T. A......... ...AAA.G.. A....TG.C. TTT....C.C T......... .GC.C..... C
Mcten ...C.C--.. C.TA....T. A......... ...AC..G.C A.....G.C. .TT.T..C.T C......... .GC.C..... C
Ntaes .....G--.. C.T.....GT A......... ...AA.GC.A A..GTG.CA. TTT.G..CAT T...-..... .GGCC...AC A
Oeleg ...CCT--.T ..T.....T. A......... ...AA..G-. A.....AC.. .TT.T..CAG C......... TGC....... C
Pnama ...CC.--.G C.T.....G. A......T.. ...AA..T.A AG.GTCTA.. TTT....C.C C.-....... TGC.C...?? .
Pduge ...C.C--.G C.T....... A......T.. ...AA..... A....TA... .TT.T..CA. C......... TGC....??? .
Psicu ...A.G--.. C.T.....T. A....T.T.. ...AAC.G.. A....TG.C. GTT.T..CA. C......... .GC....... .
Takyd ...A..--A. C.T.....T. A......T.. ...AA....C T....TG... .TT.T..C.G C......... .GC....... C

|16S
                                                         50                                                     100
Aamei TTTAGCCCAA CAAGTATTAA AGGTGATGCC TGCCCAGTGA ----T-TCTT C-AACGGCCG CGGGATCCTA CACCGTGCAA AGGTAGCATA ATCAATTGTC ATCTAAATAA
Ctigr .......A.. .......... ....A..... .......... .....A.T.. T......... .......... .......... .......... ....C..... ..........
Afitz ....------ .......... ....ACC... .......... ....CTCT.. TA........ ...T...... AC.-...... .......... ....CC.... CC.......G
Evelo ........?? .......... ....CCC-.. .......... ....CCC..C -A........ ...T...... AC.-...... .......... ....C..... CC.......G
Ggall C......TC. .......... ....CCC... .......... .....A.T.. TA........ ...T...... AC.-...... .......... ....C..... CC.......G
Hspek ?????????? ?????????? ?????????? ?????????? ....?????? ???....... ...T...... AC.-...... .......... ....C..... TC.......G
Lbedr .......TT. .......... ....CCC... .......... ....CTCT.. -A........ ...T...... AC.-...... .......... ....C..... CC.......G
Lmedi ......T.T. .......... ....CCC... .......... ....CTCT.. -A........ ...T...... AC.-...... .......... ....C..... CC......CG
Lsaxi .......TC. .......... ....CTC... .......... ....CTCT.. -A........ ...T...... AC.-...... .......... ....C..... CCT......G
Lvivi ......TT-. .......... ....CTC... .......... ....CGCT.. -A........ ...T...... AC.-...... .......... .......... TCT......G
Llong ?????????? ?????????? ?????????? ?????????? ....?????? ??........ ...T...... AC.-...... .......... ....C..... -C.......G
Mcten ......-T.. .......... ....-TCA.. .......... ....CT.T.. -A........ ...T...... AC.-...... .......... ....C..... CC.......G
Ntaes ......TT.. .......... ....CTC... .......... ....CCC... -A........ ...T...... AC.-...... .......... ....C..... CC.......G
Oeleg .......AC. .......... ....CT.... .......... ACCACTCT.. TA........ ...T...... AC.-...... .......... ....C..... TC.......G
Pnama .......TG. .......... ....CCCA.. .......... ....CTCT.. -A........ ...T...... AC.-...... .......... ....C..... CC.......G
Psicu ?......T-. .......... ....CCC... .......... ....CTCT.. -A........ ...T...... AC.-...... .......... ....C..... CC.......G
Takyd ........T. .......... ....CCC... .......... ....CTCT.. .A........ ...T...... AC.-...... .......... ....C..... TCT......G

                                              150                                                     200
Aamei TGACTAGTAT GAATGGCCCA ATGAGGACTT TCCTGTCTCT TGTA-ACCAA TCAATGAAAC TGATCTTTCA GTTCAAAAGC TGAAATACTC ACACAAGACG AAAAGACCCT
Ctigr .......... .........T .......... C......... .......... .......... ...C.CCC.. .......G.. ..G....AAT T......... ..........
Afitz G......A.. .......TA. .........A AA........ ..C.GG.TGG C........T ....T..C.. ..A....... ..G....AAA ...T...... .G........
Evelo G......A.. ...C...TA. .........A AA........ ..C.G-AA.G C......... .......C.. ..C....... .......T.A T..T...... .G........
Ggall G......... ...C...TA. ........C. .A........ ..CTG-...G .......... ......CC.. ..C....... ..G....ACT C..T.....A ..........
Hspek A......A.. .......TA. ........AA AA........ .AC.C-T... CT.......T .......... ..A....... .......AA. C..T...... .G........
Lbedr G......A.. ...C...TA. .........A CT........ CACGGG-T.. C........T .A.....CT. .......... .AG....AAT ...T...... .G........
Lmedi G......A.. .......TA. .........A AA........ .AC.GG.T.G C........T .......C.. ..A....... ..G.....A. C..T...... .G........
Lsaxi G....G.A.. .......TA. .........A AA........ .A.GGGTTG- .........T ....T..C.. ..A....... ..G....AG. ...T...... .G........
Lvivi A......A.. .......TA. .........A AT........ .ACGG..... C........T .A........ ..A....... .......AAA ...T...... .G........
Llong G......... ...C...TA. .......... AA.......C ..A.T-TTG. C......... .......... ..C....... .......TAA T..T...... .G........
Mcten G......A.. ...C..TTA. ......G.C. CA........ .AC.T-TTG. C....A..C. .......... ..C....... .......AA. G..T...... .G........
Ntaes G......A.. ...C...TAC .........A A......... ..C.T-T... .........T ......C... ..C....... ...G...AC. C..T...... .G........
Oeleg A......A.. ...C...TAC .........A AA.......C .A.GAGGATC C......... .......... ..C....... ......TT.A ....T..... .G........
Pnama G......... ...C..T.A. .......... AA........ .AC.T-TT.. C....A.... .......... ...A...... .......AAA ...T...... .G........
Psicu G....G.A.. .......TA. ........AA AA........ .A.GGC.T.G C........T .....C.C.. ..A....... ..G....AA. ...T...... .G........
Takyd A......A.. ...A...TA. ......G... A......... .AC.G-.TGG .........T ...CT.CC.. ..C....G.. ..GG..TT.T ...T...... .G........
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FIG. 1—*Continued*

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                                  250                                                                 300
Aamei GTGGAACTTT -AAGTGCTAA TC-AACA--C ATGA--CACA AACACTTTTA GTTGGGGCAA CTTTGGAACA AAACAGAACT TCCAA----T -ACAAAG--A CCACCTCCAA
Ctigr .........C C......... ..C..T.A.. .......... .C........ .......... .......... .....A.... .......... T..CGG.AT. .T..T.TA..
Afitz .....G...- C..AAC.G.. CTC.TT.AA. -ATGTTTCTT CGG.T..... ........G. ...C...GA. C..A.A...C .....AACA- TGACCTACCC A.TTTGT...
Evelo .....G...- T..AAC..-T G.C.CT.AA. .ATGG..C.C TTGGT..... ........G. ...C...G.. T..A.A.C.C ...G-.A.AA A..T.TATT. AG.T.CA..C
Ggall .....G.... ...AAC.A.. A.C.C.TACA ..TGTC...- TTGGT..... ........G. ...C...G.. ..GACA...C ...G.AACAA TG--G.CCTC A.CATAT...
Hspek .....G...- TT.AAT.AGC AAC.CT-.AT .ATGCT...- CTG.T...C. ........G. .......GT. T..A.A-C.C .....CACAA ...T..CATT AT----TT..
Lbedr .....G...- C..AAC.AG. C-C.C..GA. .-TGCT.CTC -GG.T..... ........G. ...C...G.. T..A.A.C.C .....A.TA- T.TT---..- --TTTGTT..
Lmedi .....G...- C..AAC.A.. C.ATC--AGT .-TGTC.CT- TGGTT..... ........G. ...C....T. C....A.... .....AATA- AGATT.ATCC A-----TT..
Lsaxi .....G...- C..AAC.A.G .TC.T..AC. .ATGTT.CTT -GG.T..... ........G. .......GT. C..A.A.C.C .....AACA- TGTT..ACC. AT.AAATT..
Lvivi .....G...- C..AAC.A.. CAC.C.CTG. .-TGCT.CTC -.GGT..... ........G. ...C...GT. C..T.A...C .....AAT.- .-.TT--..T -T.T..T...
Llong .....G...A .T.AAC.C.G C.C.CT.AAA TGCCTC.C.- TGGGT....G ........G. .C.C...GA. C..A.A...C ...G.C.CAA GG--..CTTT ATT---....
Mcten .....G...C AT.AACTA.. ..C.TATAA. C.A.TGG... TTAGT..... ........G. .......G.. C..T.A.C.C .........C T..GG.ACTC ----T.T.T.
Ntaes .....G...A TT.AACTA.G C.C.C.GA.. .-TGAA.G.C T-AGT...C. ........G. ...C...G.. C..A.A.C.C ....-.A.A. T.ATCCAACC AG.TTAA..C
Oeleg .....G...C C..AAC...T G.C..T.CCT -ATGGGTT.T .CGGT..... ........G. ...C...GA. C..A.A...C ...C.ACCAG G.---.ATCC T.CTGATT..
Pnama .....G...- .C.AAC.A.. C.C.GTTC.. CATGGAGCGC --AGT..... ........G. ...C...G.. C..A.A.C.C ...G-.ACAA A.TT.TATT. AG...AA..C
Psicu .....G...- C..AAC.A.. A.C.CT.AA. -ATGCT.T.T TGG.T..... ........G. .......G.. ...A.A.C.C .....AAA.- .-TTT..TTT -T.TTAT...
Takyd .....G...- T..AAC.A.. A...TT.A.. -ATGTC...T -.AGT..... ........G. ...C...G.. T..A.A.C.C .....AACAC T..CCTACTT TGCAAAAT.C

                  350                                                                 400
Aamei -CAAACCAAG GCCCCACGGC CAACGTTTGC CTCTTATACT GATC-CAGT- ACTA-CTGAT CAAAGAAACA AGCTACCCCA GGGGATAACAG CGCCATCCCC TCTTAGAGTC
Ctigr AT...A.T.. .TT.....A. ....C...TA A..CGCC..C ..C......T .......... A......... .......... .......... .......... ..........
Afitz GATC.A..TA C.AA.A-.AA .T..A.GA.A .C.AGCA... A--.C.TAAT GT..T....C ...T...C.. ..T....... .......... .......... .TC.......
Evelo .ATC.AAGTA A.-----.-- --.AC..-.A .C.AGTAGT. -T.TA-CCAA ....T....C .T.T...C.. ..T....... .......... ...T...... .TC.......
Ggall GGCC.A..C. C.AA.ATAA. ...AAC...A .C.AG.CCT. A---.---AT TTC-.-...C ...T...C.. ..T....... .......... .......TT. .T........
Hspek GATT.A..CA T.AA.T.TTA .T-TACC..A .C.AATA.T. T..GC.TTAA .T..TT.... ...T.G.C.. ..T....... .......... ...T...... CTC.......
Lbedr GATT.A..CA T.AA.A-TAA .T.TA.CA.A .C.AGCA.T. TTA.T..AAT .T.GT..... ...T...C.. ..T....... .......... .......... ..CC......
Lmedi GGTT.A..CA C.AA.A-TCT TC..A..-.A .C.AGCA... A.CT.----. TT.GT....C ...T...C.. ..T....... .......... ...A...T.. .T.......T
Lsaxi GA.C.A.... C.AA.A---A .T..AAAA.A .C.AGCA.TC A-A.C.C-AT GT.GT....C ...T...C.. ..T....... .......... ...T...... .TC.......
Lvivi GATT.A...A C.AA.A.--- .TTTA..A.A .C.AGCA.T- --GTAT.-AC .T.GT....C ..CT...C.. ..T....... .......... .......... ..C.......
Llong AAC..GATTA A.AA.TCT.. -...TC...A .C.AGTG..C A---.---AC ..C.A..... ...C...C.. ..T....... .......... ........T. .T........
Mcten GAT.TA...A T.AA.AC.-- .TG.CC.A.A .C.AGTAGT. ---TAATTAG ....T....C .T.T...C.. ..T....... .......... ...T....T. .TC.......
Ntaes .ATCGA.--- A.-----.-- --T.C..-.A .C.AGTAGT. -T.TAACCCT C...C..... ...T...... ..T....... .......... .......... .TCA......
Oeleg GATC.A..CA T.TA.A-TAG .TTTAC...A .C.AGCAG.- --A.C.CC.T T..GT..... ...G...C.. ..T....... .......... ...T....T. .T........
Pnama .GTC.A..TA TTT---..-- --..AC.-.A .C.GATA-TC -TC..-CT.A GA..TTC... ...T...C.. ..T....... .......... .......... .TC.......
Psicu GACT.A..TA T.AA.A.TAA .TTTTA.A.A .C.AGCA.T- --ATTTTTAT .T.GT..... ...T...C.. ..T....... .......... ........T. .TC.......
Takyd .AC.TATTTA A.TA.G.T-- --..A..A.A .C.AG.G..C AT.TA..ACA GT.CT....C T..T...C.. ..T....... .......... .......... .TCA......

                                                                                                                                16S|
                  450                                                                 500                                       543
Aamei CCAATCGACG AGCGGGGCTT ACAGACTTCG ATGTTGGATA AGGGCACCAC AAAAAGTGCA GCAG-TGTTA AAGGTTTGTT TGTT-AACAA ATTAATGA-- CTA
Ctigr .A..... ......-... ..-...C... .........C ...---..C. ...C..... ....C..... ..... ....C..... T....GT-.C ...
Afitz ..T.....A ..-....T.. ..-...C... .........C ...A..T.C. ..-TG..... ..C.CCA... ......C... ....C...G. -....--.GT ...
Evelo .AT.....A ..-..... ..-...C... .........C ...AG...CT ..-T..... A.C.C.A... ......C... ....C...G. -....--.AT ...
Ggall ..T.....A ..-AA..T.. ..-...C... .........C ...A....C. ..-T..... ..C.C.A... ......C... ....C...G. -....-C.GT ...
Hspek ..T.....A G.-..... ..-...C... .........C .A.A....CT ..-T..... ....C.A... ......C... ....C...G. -....--TGT ...
Lbedr .AT.....A ..-....T.. ..-...C... .........C ...A....C. ..-TG..... ..C.CCA... ......C... ....C...?? ?????????? ???
Lmedi .AT.....A ..-....T.. ..-...C... .....A...C ...A....C. ..-TG..... ..C.CCA... ......C... ....C...G. -....-C.GT ...
Lsaxi ..T.....- ..-....T.. ..-...C... .........C ...A....C. ..-TG..... ..C.CCA... ......C... ....C...G. -....--.GT ...
Lvivi .AT.....A ..-....T.. ..-...C... .........C ...A....C. ..-TG..... ..C.CCA... ....G.C... ....C...G. -.....C.GT ...
Llong .AT.....A ..-A..... ..-...C... .........C ...A....C. ..-T..... ....C.A... ......C... ....C...G. -....--TGT ...
Mcten ..T.....A ..-A...T.. ..-...C... .........C ...A....CT ..-T..... ..C.C.A... ......C... ....C...G. -....--.GT ...
Ntaes ..C.....A ..-....T.. ..-...C... .........C ...A....C. ..-T..... T.C.C.A... .C....C... ....C...G. -....--.GT ...
Oeleg ..T.....A ..-A..... ..-...C-.. .----...-C ..-A...??? ??-??????? ?????????? ?????????? ?????????? ?????????? ???
Pnama ..T.....A ..-....T.. ..-...C... .........C ...A....C. ..-T..... ..C.C.A... ......C... ....C...G. -....--.GT ...
Psicu .TT.....A ..-....T.. ..-...C... .........C ...A....C. ..-T..... ..C.C.A... ......C... ....C...G. -....--.GT ...
Takyd .TT.....A ..-....T.. ..-...C... .........C ...A....C. ..-T..... ..C.C.A... .......... ....C..... -....--.GT .C.
```

FIG. 1—*Continued*

sequence data. However, MB did not include the Eurasian genera *Eremias* and *Ophisops* in the clade. The association of African lacertids with *Eremias* and *Ophisops* is represented on ENA's tree, and the DNA sequence data strongly support this association (by PTP and decay analysis). The difference is that ENA places *Eremias* and *Ophisops* at the top of the clade, while the DNA data place them at the base of the clade.

Neither ENA nor MB acknowledge the sister group relationship of *Algyroides* and the *L. saxicola* complex. The relationship was shown on the DNA sequence tree and is well supported. MB found a sister group relationship of *Latastia* and *Haliobolus,* which was also shown on the DNA tree.

The monophyly of Eurasian lacertids (except *Gallotia, Eremias,* and *Ophisops*), which appeared on the initial analysis, was not supported by either ENA or MB. The relationships among the European lacertids, as well as their relationships with other lacertids, are uncertain. This uncertainty is clearly revealed by the largely unresolved tree from morphology and poorly supported nodes from DNA sequence data.

Taxonomic Inferences

Mayer and Benyr (1994) proposed a two-subfamily classification with the genera *Gallotia* and *Psammodromus* forming the subfamily Gallotiinae, and the rest forming the subfamily Lacertinae. The DNA sequence phylogeny supports this classification and the monophyly of the subfamily Lacertinae. However, one question arises from this classification. If *Gallotia* (with *Psammodromus*) is recognized as a subfamily, then the taxonomic position of *Takydromus* needs to be revised. Should *Takydromus* (including *Platyplacopus*) be recognized as another subfamily? More work needs to be done to answer this question.

It has long been recognized that the genus *Lacerta* is non-monophyletic (Arnold, 1973; Böhme and Corti,

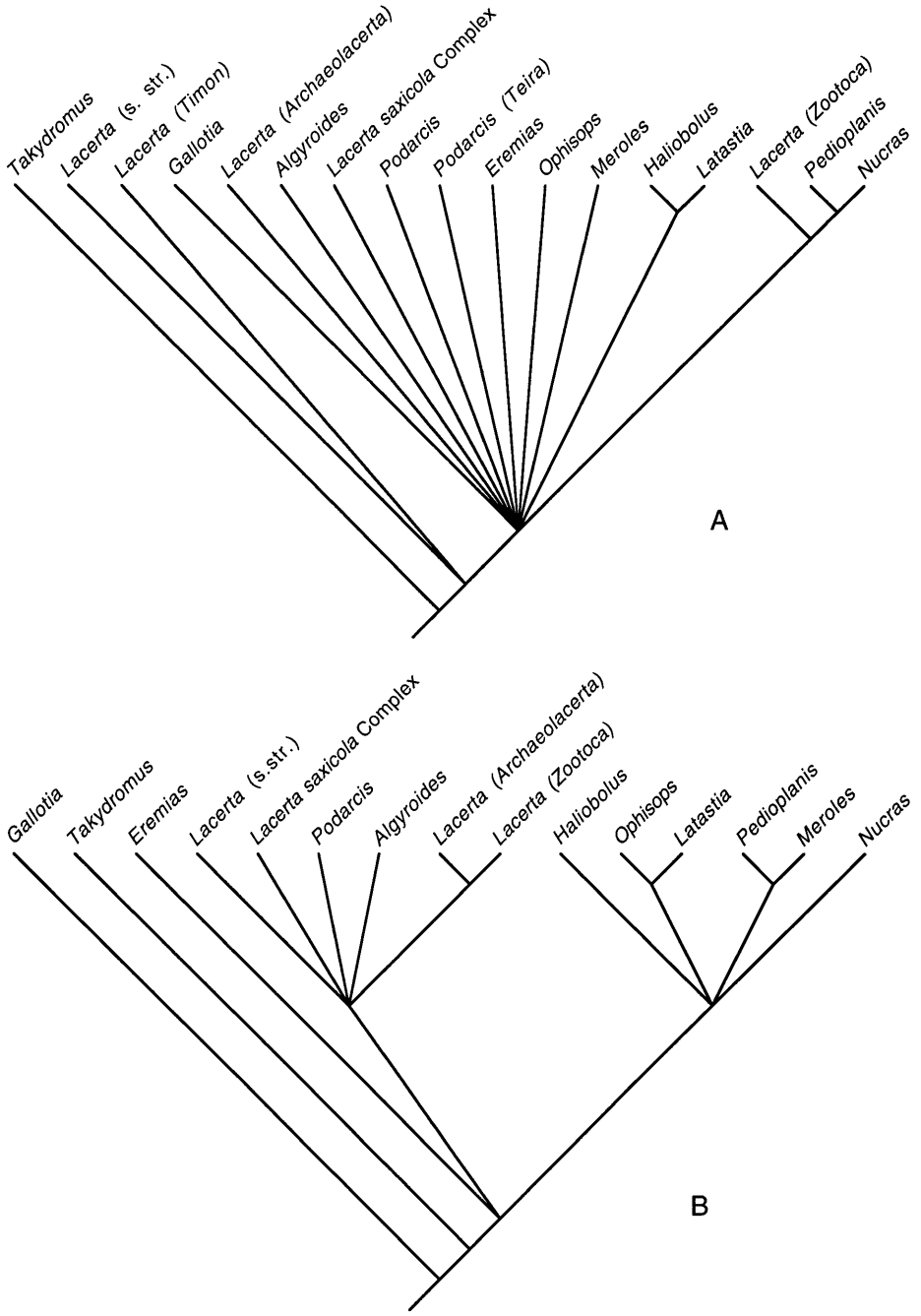

FIG. 2. (A) The strict consensus tree resulting from the phylogenetic analysis of 12S gene sequence data. (B) The strict consensus tree resulting from the phylogenetic analysis of 16S gene sequence data.

1993). A taxonomic revision of this genus is required. Most of the current subgenera should be recognized as genera. The subgenus *Archaeolacerta* is non-monophyletic as well. The European *Archaeolacerta* and Caucasian *Archaeolacerta* (*Lacerta saxiola* complex) are not sister groups, as clearly shown on the DNA sequence tree (Fig. 4). The Caucasian *Archaeolacerta* are more closely related to *Algyroides*. This sister group relationship is well supported by the DNA sequence data. The European *Archaeolacerta* should be regarded as a genus, as should the *L. saxicola* complex.

Historical Biogeography

Modern biogeographic analysis has been dominated by two major theories: dispersal and vicariance theory. Although vicariance theory has been proven to be the major trend in many groups (e.g., amphibian families; Savage, 1973; Duellman and Trueb, 1986), the distribu-

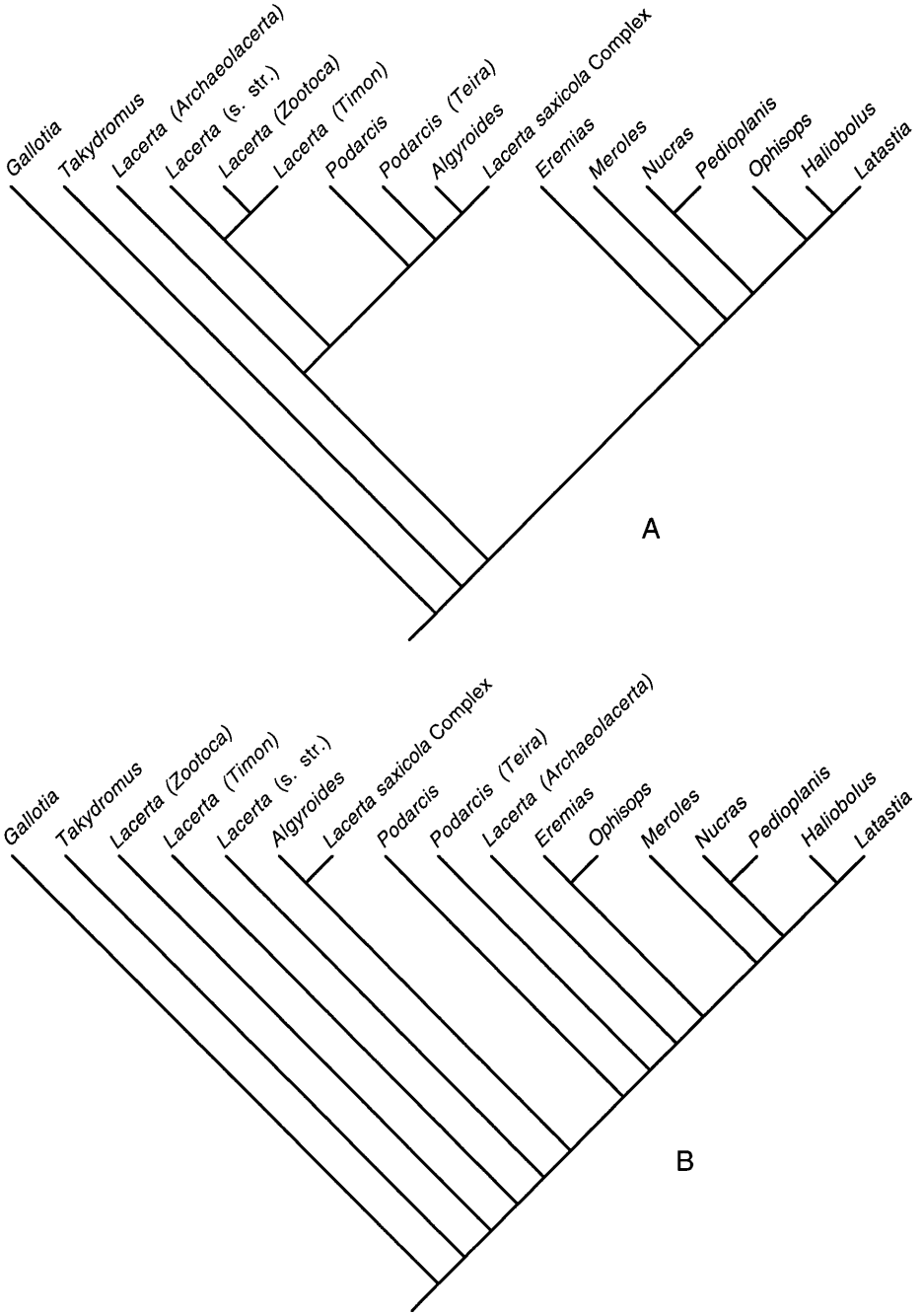

FIG. 3. (A) The single most parsimonious tree from combined 12S and 16S gene sequence data. All characters are equally weighted. (B) The single most parsimonious tree from the weighted combined data. Transversion changes were weighted two times over transitional changes.

tion pattern of lacertids is probably explained best by dispersal. Presently, 17 of 24 lacertid genera occur in Africa, 6 genera are found in Europe and adjacent central and western Asia (including the paraphyletic genus *Lacerta*), and 1 genus, *Takydromus*, is distributed in East Asia. Fossil records are comparatively rare. Most of them are from the Cenozoic of Europe, although a single record is known from the Miocene of Morocco (Estes, 1983b).

Two hypotheses have been proposed to explain the origin of lacertids: the ancestor of lacertids originated from Europe or from Africa (Estes, 1983a). The DNA sequence phylogeny supports the European-origin hypothesis. All of the basal forms are Eurasian in distribution and most are currently distributed in Europe. *Gallotia* occurs only in the Canary Islands, but its hypothetical sister group, *Psammodromus*, is found in the Iberian Peninsula, which is one of the oldest former

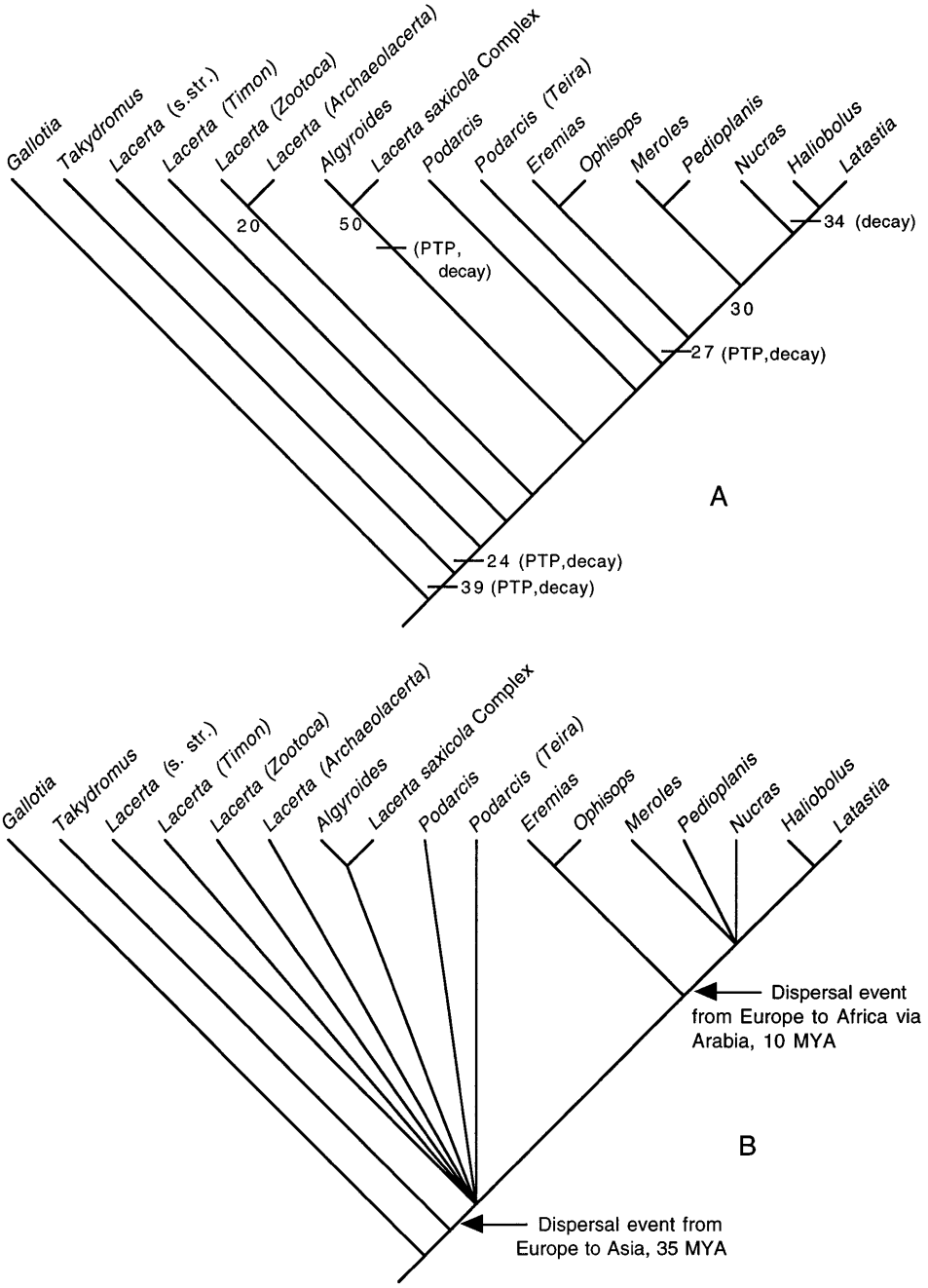

FIG. 4. (A) The preferred phylogenetic hypothesis. The tree was obtained from a functional ingroup/functional outgroup analysis. Numbers below nodes are the bootstrap values over 0.20. Nodes supported by PTP and decay analyses are marked by PTP and/or decay. (B) The phylogenetic framework. Only the confidence elements were used for discussion. Two hypothetical dispersal events were mapped on the framework.

islands of Europe. The African clade, which is located at the top of the tree, can best be explained as a dispersal event.

Given that lacertids originated from Europe, the dispersal event of *Takydromus* from Europe to eastern Asia was likely to be a Tertiary event. During the Cretaceous, most of Europe was submerged and extensive habitats for lizard groups were not present (Estes, 1983a). Therefore, the diversity of early lacertids was likely limited, and the distribution of lacertids was restricted to Europe. Asia had been temporarily united with Europe twice during the Cretaceous. However, the dispersal of *Takydromus* probably did not occur during either of those two connections. Otherwise, as a large land mass with optimal subtropical climate, Asia would be rich with lacertid diversity, which is contrary to the

present situation. The total absence of a fossil record in Asia makes mass extinction events seem unlikely. The Turgai Sea persistently separated Europe and Asia until the end of the Eocene (about 35 MYA). Since then, the land connection between Europe and Asia has continued to the present. However, shortly after the connection, the uplift of the Tibetan plateau built another barrier between eastern Asia and the rest of the continent. The ancestor of *Takydromus* must have reached its present range during that short period between the establishment of the land connection and the Tibetan uplift.

If the above hypothesis is true, then the origins of most extant groups were likely to be late Tertiary events. Furthermore, the dispersal event of the African clade was even later, not, as Estes (1983a) suggested, a Cretaceous event. Since the late Miocene (about 8–10 MYA), Africa had a restricted link with Eurasia via Arabia. The common ancestor of African lacertids probably dispersed to Africa after the connection. The climate has also changed dramatically since the Miocene. In particular, southwestern Asia and northern Africa have become progressively more arid. The common ancestor of African lacertids and (*Eremias, Ophisops*) may have adapted to a xeric habitat during that time, penetrating the arid region of Africa southward and westward. The Eurasian remnants evolved into *Eremias* and *Ophisops* and dispersed further northward to central Asia. The African invader radiated to form the present African clade. Some groups adapted secondarily to mesic habitat. The only Indian lacertids, genus *Cabrita* (included into *Ophisops* by Bischoff, 1991c), probably also reached the Indian subcontinent through southwestern Asia. The affinity of *Cabrita* and *Ophisops* was recognized as early as Boulenger (1921).

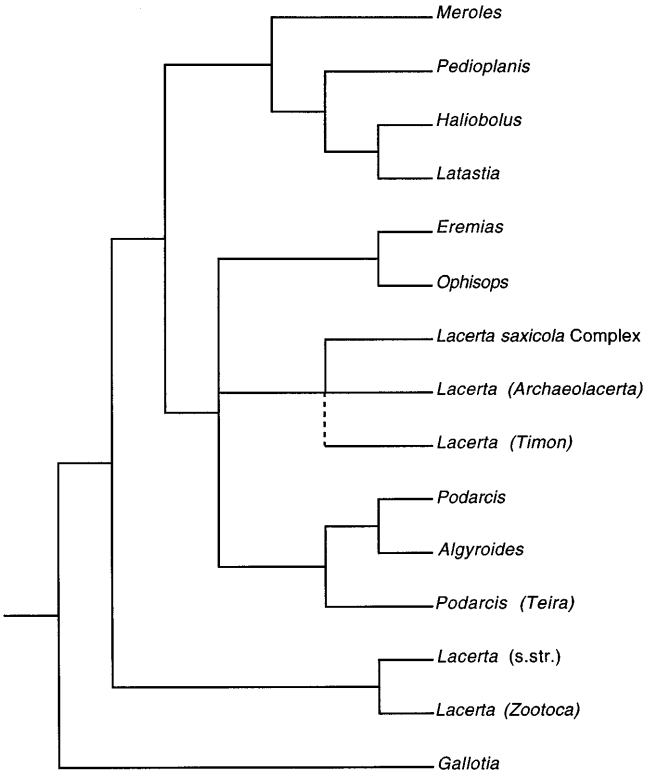

FIG. 6. Mayer and Benyr's tree (1994). Only the taxa in common with this study are depicted on the tree. Dashed line indicates uncertainty.

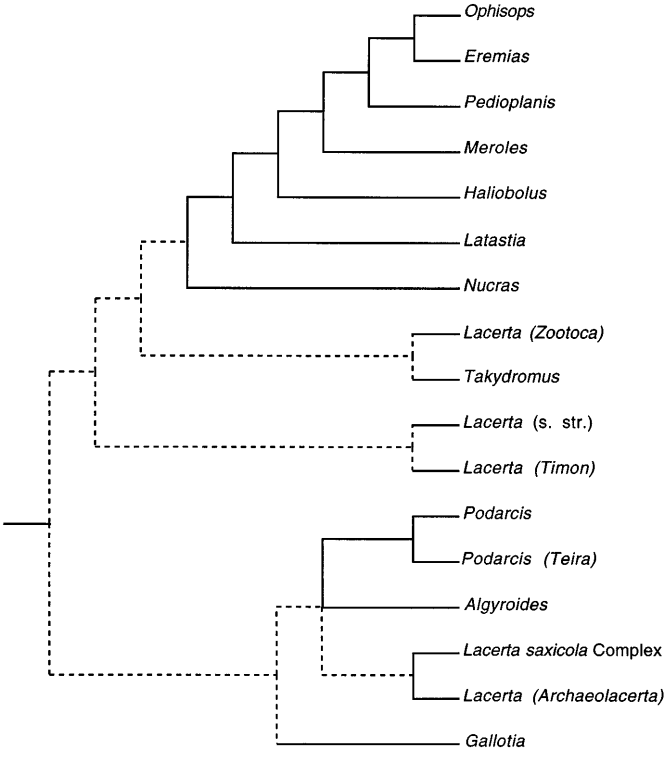

FIG. 5. Arnold's tree (1989a). Only the taxa in common with this study are depicted on the tree. Dashed lines indicate uncertainty.

Epilogue

A convincing phylogeny of the family Lacertidae is still far from realized. The data presented here confidently resolved only six nodes. More data are needed to generate a robust phylogeny. The explosive speciation events in a short time period leave very few characters fixed on many nodes. This may partially explain why the European lacertids are so resistant to phylogenetic reconstruction. At present, the morphological approach does not seem very helpful at the family level. DNA sequencing may prove more useful. The partial sequences of the 12S and 16S genes are informative in resolving some relationships. More data from these genes as well as others expressing different divergence levels are desirable to confidently resolve the more basal or terminal nodes and eventually achieve a robust phylogeny.

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REFERENCES

Archie, J. W. (1989). A randomization test for phylogenetic information in systematic data. *Syst. Zool.* **38:** 239–252.

Arnold, E. N. (1973). Relationships of the Palaearctic lizards assigned to the genera *Lacerta, Algyroides* and *Psammodromus* (Reptilia: Lacertidae). *Bull. Br. Mus. Nat. Hist. (Zool.)* ***25:*** *289–366.*

Arnold, E. N. (1983). Osteology, genitalia and the relationships of *Acanthodactylus* (Reptilia: Lacertidae). *Bull. Br. Mus. Nat. Hist. (Zool.)* **44:** 291–339.

Arnold, E. N. (1989a). Towards the phylogeny and biogeography of the Lacertidae: Relationships within an Old-World family of lizards derived from morphology. *Bull. Br. Mus. Nat. Hist. (Zool.)* **55:** 209–257.

Arnold, E. N. (1989b). Systematics and adaptive radiation of equatorial African lizards assigned to the genera *Adolfus, Bedriagaia, Gastropholis, Holaspis* and *Lacerta* (Reptilia, Lacertidae). *J. Nat. Hist.* **23:** 525–555.

Arnold, E. N. (1991). Relationships and evolution of the South African lacertids assigned to *Aporosaura, Meroles* and *Pedioplanis* (Reptilia: Lacertidae). *J. Nat. Hist.* **25:** 783–807.

Bischoff, W. (1990). Übersicht der Arten und Unterarten der Familie Lacertidae 1. Die Gattungen *Acanthodactylus, Adolfus, Algyroides* und *Australolacerta. Eidechse* **1:** 18–23.

Bischoff, W. (1991a). Übersicht der Arten und Unterarten der Familie Lacertidae 2. Die Gattungen *Eremias, Gallotia, Gastropholis, Heliobolus, Holaspis* und *Ichnotropis. Eidechse* **2:** 14–21.

Bischoff, W. (1991b). Übersicht der Arten und Unterarten der Familie Lacertidae 3. Die Gattung *Lacerta. Eidechse* **3:** 5–16.

Bischoff, W. (1991c). Übersicht der Arten und Unterarten der Familie Lacertidae 4. Die Gattungen *Latastia, Meroles, Mesalina, Nucras, Ophisops, Pedioplanis* und *Philochortus. Eidechse* **4:** 17–25.

Bischoff, W. (1992a). Übersicht der Arten und Unterarten der Familie Lacertidae 5. Die Gattung *Podarcis. Eidechse* **5,** 6–20.

Bischoff, W. (1992b). Übersicht der Arten und Unterarten der Familie Lacertidae 6. Die Gattungen *Poromera, Psammodromus, Pseuderemias, Takydromus* und *Tropidosaura. Eidechse* **6:** 13–17.

Böhme, W., and Corti, C. (1993). Zoogeography of the lacertid lizards of the western Mediteranean basin. *In* "Lacertidae of the Mediteranean Region, A Biological Approach" (E. D. Valakos, W. Böhme, V. Pérez-Mellado, and P. Maregou, Eds.), pp. 17–33, Hellenic Zool. Soc., Athens, Greece.

Boulenger, G. A. (1921). "Monograph of the Lacertidae," Vol. II, Trustees of the British Museum (Natural History), London.

Bremer, K. (1988). The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42:** 795–803.

Cabot, E. L., and Beckenbach, A. T. (1989). Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. *Comp. Appl. Biol.* **5:** 233–234.

Cracraft, J., and Helm-Bychowski, K. (1991). Parsimony and phylogenetic inference using DNA sequences: Some methodological strategies. *In* "Phylogenetic Analysis of DNA Sequences" (M. M. Miyamoto and J. Cracraft, Eds.), pp. 184–220, Oxford Univ. Press, New York.

Doyle, J. J. (1992). Gene tree and species tree: molecular systematics as one-character taxonomy. *Syst. Bot.* **17:** 144–163.

Duellman, W. E., and Trueb, L. (1986). Biology of Amphibians. John Hopkins Univ. Press, Baltimore, Maryland.

Estes, R. (1983a). The fossil record and early distribution of lizards. *In* "Advances in Herpetology and Evolutionary Biology" (A. Rhodin and K. Miyata, Eds.), pp. 365–398, Museum of Comparative Zoology, Cambridge, Massachusetts.

Estes, R. (1983b). "Encylcopedia of Paleoherpetology," Part 10A, Fischer Verlag, Stuttgart, New York.

Estes, R., de Queiroz, K., and Gauthier, J. (1988). Phylogenetic relationships within squamata. *In* "Phylogenetic Relationships of the Lizard Families" (R. Estes and G. K. Pregill, Eds.), pp. 119–281, Stanford Univ. Press, Stanford, California.

Faith, D. P., and Cranston, P. S. (1991). Could a cladogram this short have arisen by chance alone? On permutation tests for cladistic structure. *Cladistics* **7:** 1–28.

Farris, J. S. (1988). "Hennig86 Reference," version 1.5, distributed by the author, Port Jefferson Station, New York.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39:** 783–791.

Fu, J., Murphy, R. W., and Darevsky, I. S. Toward the phylogeny of Caucasian rock lizards: implication from mitochondrial DNA gene sequences (Reptilia: Lacertidae). *Zool. J. Linn. Soc.*, **121:**463–477.

Fu, J., and Murphy, R. W. (1997). Phylogeny of Chinese *Orelalax* and the use of functional outgroups to select among multiple equally parsimonious trees. *Asiatic Herpetol. Res.* **7:** 38–43.

Hay, J. M., Ruvinsky, I., Hedges, S. B., and Maxson, L. R. (1995). Phylogenetic relationships of amphibian families inferred from DNA sequences of mitochondrial 12S and 16S ribosomal RNA genes. *Mol. Biol. Evol.* **12:** 928–937.

Hillis, D. M. (1987). Molecular versus morphological approaches to Systematics. *Annu. Rev. Ecol. Syst.* **18:** 23–42.

Hillis, D. M., and Dixon, M. T. (1991). Ribosomal DNA: Molecular evolution and phylogenetic inference. *Q. Rev. Biol.* **66:** 411–453.

Hillis, D. M., and Huelsenbeck, J. P. (1992). Signal, noise and reliability in molecular phylogenetic analysis. *J. Hered.* **83:** 189–195.

Hillis, D. M., Huelsenbeck, J. P., and Cunningham, C. W. (1994). Application and accuracy of molecular phylogenies. *Science* **264:** 671–677.

Hillis, D. M., Mable, B. K., and Moritz, C. (1996a). Application of molecular Systematics. *In* "Molecular Systematics" (D. M. Hillis, C. Moritz, and B. K. Mable, Eds.), 2nd ed., pp. 515–543, Sinauer, Sunderland, Massachusetts.

Hillis, D. M., Mable, B. K., Larson, A., Davis, S. K., and Zimmer, E. A. (1996b). Nucleic acids IV: Sequencing and Cloning. *In* "Molecular Systematics" (D. M. Hillis, C. Moritz, and B. K. Mable, Eds.), 2nd ed., pp. 321–383, Sinauer, Sunderland, Massachusetts.

Huelsenbeck, J. P. (1991). Tree-length distribution skewness: an indicator of phylogenetic information. *Syst. Zool.* **40:** 257–270.

Huelsenbeck, J. P., Bull, J. J., and Cunningham, C. W. (1996). Combining data in phylogenetic analysis. *Tree* **11:** 152–158.

Kluge, A. G., and Wolf, A. J. (1993). Cladistics: What's in a word? *Cladistics* **9:** 183–199.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Paabo, S., Villablance, F. X., and Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* **86:** 6196–6200.

Lanyon, S. M. (1993). Phylogenetic frameworks: Towards a firmer foundation for the comparative approach. *Biol. J. Linn. Soc.* **49:** 45–61.

Maddison, W. P., and Maddison, D. R. (1992). "MacClade: Analysis of Phylogeny and Character Evolution," version 3.04, pp. 1–398, Sinauer, Sunderland, Massachusetts.

Mayer, W., and Benyr, G. (1994). Albumin-Evolution und Phylogenese in der Familie Lacertidae. *Ann. Naturhist. Mus. Wien* **96B:** 621–648.

Mayer, W., and Lutz, D. (1989). Chemosystematische untersuchungen zur phylogenese der sammelgattung *Lacerta* (Reptilia: Sauria: Lacertidae). *Z. Zool. Syst. Evol.* **27:** 338–349.

Mayer, W., and Lutz, D. (1990). Albumin-immunologische Untersuchungen zur Phylogenese der Gattung *Algyroides* und ihrer systematischen Position gegenüber der Sammelgattung *Lacerta* (Reptilia: *Zool. Anz.* **224:** 99–105.

Mayer, W., and Tiedemann, F. (1982). Chemotaxonomical investigation in the collective genus *Lacerta* (Lacertidae, Sauria) by means of protein electrophoresis. *Amphibia–Reptilia* **2:** 349–355.

Maxson, J. R., and Maxson, R. D. (1990). Proteins II: Immunological techniques. *In* "Molecular Systematics" (D. M. Hillis and C. Moritz, Eds.), pp. 127–155, Sinauer, Sunderland, Massachusetts.

Mindell, D. P., and Honeycutt, R. L. (1990). Ribosomal RNA in vertebrates: Evolution and phylogenetic application. *Annu. Rev. Ecol. Syst.* **21:** 541–566.

Miyamoto, D. D., and Cracraft, J. (1991). Phylogenetic inference, DNA sequence analysis, and the future of molecular systematics. *In* "Phylogenetic Analysis and DNA sequences" (M. M. Miyamoto and J. Cracraft, Eds.), pp. 3–17, Oxford Univ. Press, New York.

Miyamoto, D. D., and Fitch, W. M. (1995). Testing species phylogenies and phylogenetic methods with congruence. *Syst. Biol.* **44:** 64–76.

Murphy, R. W., Darevsky, I. S., Macculloch, R. D., Fu, J., and Kupriyanova, L. A. (1996). Evolution of the bisexual species of caucasian rock lizards: A phylogenetic evaluation of allozyme data. *Russian J. Herpetol.* **3:** 18–31.

Murphy, R. W., and Doyle, K. D. (in press). Phylophenetics: Frequencies and polymorphic characters in genealogical estimation. *Syst. Biol.*

Palumbi, S. R. (1996). Nucleic acids II: The polymerase chain reaction. *In* "Molecular Systematics" (D. M. Hillis, C. Mortiz, and B. K. Mable, Eds.), 2nd ed., pp. 205–247, Sinauer, Sunderland, Massachusetts.

Sanderson, M. J. (1995). Objections to bootstrapping phylogenies: A critique. *Syst. Biol.* **44:** 299–320.

Savage, J. M. (1973). The geographic distribution of frogs: patterns and predictions. *In* "Evolutionary Biology of the Anurans: Comptemporary Resuarch on Major Problems" (J. L. Vial, Ed.), pp. 352–445, Univ. Missouri Press, Columbia.

Siddall, M. S. (1997). "RandomCladistics," version 4.0.3, Univ. of Toronto, Department of Zoology.

Swofford, D. L. (1991). When are phylogeny estimates from molecular and morphological data incongruence? *In* "Phylogenetic Analysis of DNA Sequences" (M. M. Miyamoto, and J. Cracraft, Eds.), pp. 295–333, Oxford Univ. Press, New York.

Swofford, D. L. (1993). "PAUP: Phylogenetic Analysis Using Parsimony," version 3.1.1, computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Swofford, D. L., Olsen, G. J., Waddell, P. J., and Hillis, D. M. (1996). Phylogenetic inference. *In* "Molecular Systematics" (D. M. Hillis, C. Moritz, and B. K. Mable, Eds.), 2nd ed., pp. 407–514, Sinauer, Sunderland, Massachusetts.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence wighting, potition-specific gap penelties and weight matrix choice. *Nucleic Acids Res.* **22:** 4673–4680.

Trueman, J. W. H. (1993). Randomization confounded: A response to Carpenter. *Cladistics* **9:** 101–109.

Valakos, E. Böhme, W., Pérez-Mellado, V., and Maregou, P. (1993). "Lacertidae of the Miditeranean Region, A Biological Approach." pp. 1–281, Hellenic Zool. Soc., Athens, Greece.

Watrous, L. E., and Wheeler, Q. D. (1981). The outgroup comparison method of character analysis. *Syst. Zool.* **30:** 1–11.

Wiley, E. O., Siegel-Causey, D., Brooks, D. R., and Funk, V. A. (1991). "The Compleat Cladist, a Primer of Phylogenetic Procedures," Museum of Natural History, the University of Kansas, Lawrence, Kansas.

Williams, P. L., and Fitch, W. M. (1989). Finding the minimal change in a given tree. *In* "The Hierarchy of Life" (B. Fernholm, K. Bremer, and H. Jörnvall, Eds.), pp. 453–470, Elsevier, Amsterdam.