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# Toward the phylogeny of the family Lacertidae—Why 4708 base pairs of mtDNA sequences cannot draw the picture

JINZHONG FU*

*Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, 100 Queen's Park, Toronto, Canada M5S 2C6*

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A phylogeny of the family Lacertidae was derived from DNA sequences of six mitochondrial genes. Only a few nodes were confidently resolved using maximum parsimony, although the data yielded a total of 1664 phylogenetically informative characters. The lacertids grouped into two subfamilies, the Gallotiinae which includes genera *Gallotia* and *Psammodromus*, and the Lacertinae which includes the remaining lacertids. The Lacertinae split into two additional groups. The African group included all African and Arabian lacertids and two Eurasian genera, *Eremias* and *Ophisops*; the remaining Eurasian lacertids were included in the Eurasian group. Most of the relationships within the African and Eurasian groups cannot be confidently resolved. A permutation tail probability test suggested that there is very little character covariance in the data to support these unresolved relationships. A recent explosive speciation hypothesis was invoked to explain the lack of structure of the data. The common ancestor of the Eurasian group, as well as the ancestor of the African group, experienced simultaneous, or almost simultaneous, multiple speciation events, which left none or very few characters fixed on the internodes. The phylogenetic reconstruction at the family level will be very difficult, if not impossible. Future phylogenetic research should focus on lower levels.



ADDITIONAL KEY WORDS:—Lacertids – Lacertinae – Gallotiinae – African group – Eurasian group – explosive speciation hypothesis – permutation.

CONTENTS



* Present address: Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada. E-mail: jinzhongfu@netscape.net



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## INTRODUCTION

In spite of several attempts, the phylogeny of the family Lacertidae remains controversial and largely unresolved. Lack of a solid phylogeny has underscored many comparative studies of the family. The strong contrast between the wealth of knowledge about many aspects of lacertid biology (e.g. ecology, behaviour, parasitology) and the poor understanding of their genealogical relationships has made the overall phylogeny of the family very desirable. Arnold (1989) pursued a phylogeny of the family using morphological data. Although a fully resolved tree was presented, many of its nodes were considered as tentative. Mayer and Benyr (1994) presented another phylogeny of the family using albumin immunological (MC′F) data. The analysis also left a number of unresolved nodes and the results were highly contradictory to those of Arnold's (1989).

Two recent works using DNA sequence data shed more light on the relationships of the family Lacertidae (Fu, 1998; Harris *et al.*, 1998a). However, the small size of the data set, 954 base pair (bp) by Fu (1998) and 1049 bp by Harris *et al.* (1998a), limited the resolution. DNA sequence data have many advantages in phylogenetic construction. Among them are the almost unlimited number of characters and the tremendous scope of variation ranging from the most conservative to the most variable (Miyamoto & Cracraft, 1991; Hillis *et al.*, 1996). These make DNA sequence data one of the most promising ways of pursuing the phylogeny of lacertids.

The sole objective of this study is to resolve a phylogeny of the main lineages of the family Lacertidae. These lineages were defined based on the current understanding of their genealogical relationships and classification (Arnold, 1973, 1989; Mayer & Benyr, 1994; Bischoff, 1996; Fu, 1998). Using species representatives of the lineages and mtDNA sequence data, a variety of phylogenetic methods were employed in an attempt to answer this long-standing question.

## MATERIAL AND METHODS

Thirty-one species, representing 18 of 24 currently recognized genera (Bischoff, 1996), were used to reconstruct a phylogeny of the main lineages of the family Lacertidae. The genus *Lacerta* has long been acknowledged as a non-monophyletic group (e.g. Arnold, 1973, 1989; Böhme, 1984; Mayer & Benyr, 1994), therefore, 10 species were used to represent five generally accepted natural groups. Multiple representatives were also used for *Adolfus*, *Gallotia*, *Meroles*, and *Podarcis*. The families Teiidae and Gymnophthalmidae were selected as the primary outgroups based on a phylogeny of lizard families (Estes *et al.*, 1988). Three species, *Ameiva ameiva*, *Cnemidophorus tigris maximus* (Teiidae) and *Neusticurus* sp. (Gymnophthalmidae) were examined in this study. Voucher specimens and locality data are listed in Appendix 1.

Four mitochondrial genes, 12S, 16S, cytochrome *b* (cyt-*b*) and COI, were selected

to reconstruct the phylogeny. Two tRNA genes, $tRNA^{Val}$ and $tRNA^{Thr}$, which are adjacent to the four major genes, were also sequenced. Ribosomal RNAs (12S and 16S) are functionally important in protein synthesis, which makes them relatively resistant to evolutionary change, and the 12S and 16S genes seem best suited for divergence of about 150 Mya or less (Mindell & Honeycutt, 1990). The divergence of lacertids is thought to be within this range (Estes, 1983). Partial sequences of 12S and 16S genes used in this study have been published (Fu, 1998). Cyt-*b* has been used for a wide range of phylogenetic construction up to 80 MYr (Irwin *et al.*, 1991) or older (Meyer & Wilson, 1990). COI is a less frequently used gene in phylogenetic reconstruction. Zardoya and Meyer (1996) evaluated the performance of mitochondrial protein-coding genes in resolving relationships among vertebrates, and found COI, cyt-*b* and ND2 to be the best.

Standard phenol–chloroform methods were used to extract DNA from tail muscle or liver tissues. Laboratory protocols follow Palumbi (1996). Polymerase Chain Reaction (PCR) with *taq* DNA polymerase (Boehringer Mannheim) was used to amplify the DNA sample; double stranded DNA was sequenced directly using $^{33}P$ labelled terminator cycle sequencing method (Amersham). Protocols follow manufacturer's recommendations with minor modifications. Primers used for PCR and sequencing are listed in Appendix 2. Both heavy and light strands were sequenced for most regions.

The alignment of RNA genes was accomplished by computer program ClustalW with the following parameters: Gap opening penalty = 5.00; gap extension penalty = 0.05 (version 1.6, Thompson *et al.*, 1994). Minor modifications of the computer output alignments were made by eye. Sites with ambiguous alignment were excluded from the phylogenetic analysis, because the homology cannot be assumed confidently (Hillis and Dixon, 1991).

Phylogenetic analyses were conducted using computer programs PAUP* (version 4.0b1; Swofford, 1998) and MacClade (version 3.04; Maddison & Maddison, 1992). Initially, the four major genes were analysed independently. Different genes may evolve at different rates and are best suitable to resolve nodes at different levels (Hillis, 1987). Further, the corroboration from independent data sets provides strong evidence for the reliability of phylogenetic trees (Miyamoto & Fitch, 1995). In the case where the well-supported elements of the tree topologies were not conflicting, combined data analyses were conducted.

For assessing character covariance in the data sets, permutation tail probability (PTP; Faith & Cranston, 1991), and homoplasy excess ratio (HER; Archie, 1989; Fu & Murphy, 1999) were used. The maximum parsimony criterion was used for inferring phylogeny. Each nucleotide site was treated as a non-additive (= unordered) character. Alignment gaps were treated as missing data. The initial analyses were conducted with equal weights to all characters.

Decay analysis (Bremer, 1988), bootstrap (Felsenstein, 1985) and Templeton's test (Templeton, 1983) were conducted. The bootstrap proportion (BSP), in conjunction with decay index (DI), was used to evaluate nodal support. However, caution should be exercised not to take the BSP as an absolute measurement for confidence estimation (e.g. Kluge & Wolf, 1993; but see Sanderson, 1995). All BSPs were calculated from 100 replicates. Templeton's test was applied to test whether an alternative topology is significantly different from another.

Based on the results of the nodal support assessment, functional ingroup and outgroup analysis (FIG/FOG; Watrous & Wheeler, 1981) was used for further

examination of the recovered nodes. Several recent observations indicated that use of a distantly related outgroups could misroot the resulting trees, therefore present wrong tree topologies (e.g. Lee *et al.*, 1997).

## RESULTS

### *The independent analyses*

Number of species, length of each fragment sequenced, number of base pairs analysed are summarized in Table 1. All sequences are deposited in GenBank (accession numbers U88603, U88611, AF206528-616). The sequence of *Lacerta (Zootoca) vivipara* was obtained from GenBank (accession number U69834). No insertions/deletions were found in cyt-*b* and COI genes.

Uninformative characters were excluded from the phylogenetic analysis. All four major genes yielded substantial phylogenetically informative characters (Table 1). PTP indicated the presence of significant character covariance in the data sets (Table 1; Faith & Cranston, 1991). Given that the data have significant cladistic structure, the phylogenetic analyses were performed.

One most parsimonious tree (MPT) was found from the COI gene data (Table 1, Fig. 1A). Eight nodes received high BSP values (i.e. greater than 0.70; Hillis & Bull, 1993, and references hereafter) and they were exactly the same eight nodes which received the highest DIs (greater than 4). The BSPs and DIs were highly congruent on the COI data, as well as other genes. Five genera (groups) with multiple representatives, *Gallotia*, *Timon*, *Lacerta* (*s.s.*), *Podarcis*, and *Meroles* were confirmed to be monophyletic. However, the monophyly of *Adolfus* and *Archaeolacerta* was questioned. The monophyly of the subfamily Gallotiinae that includes genera *Gallotia* and *Psammodromus*, as well as the subfamily Lacertinae that includes the remaining lacertids, was also well-supported.

One MPT was found from the cyt-*b* data (Table 1, tree not shown). The clade of *Gallotia* and *Psammodromus* obtained the highest DI (over 8) and BSP value, which is the only BSP greater than 0.70. The subfamily Lacertinae was also present on the tree, although one African genus (*Acanthodactylus*) fell out at the very base of the tree instead of being grouped with the other Lacertinae members.

Four MPTs derived from the 12S gene data (Table 1; trees not shown). The ambiguities were the relationships among the three species in *Podarcis* and the placement of *Lacerta (Teira) andreanszkyi*. Five nodes obtained BSPs over 0.70 and they are among the six nodes that received the highest DIs (greater than 4). Four groups with multiple representatives, *Lacerta (s.s.)*, *Lacerta* (*Timon*), *Meroles*, and *Podarcis* were resolved as monophyletic and received strong support from the data. The association of *Gallotia* and *Psammodromus* was also well-supported. The node receiving high DI but not BSP is the sister group relationship of *Latastia* and *Heliobolus*. However, in contrast to the COI and cyt-*b* data, the Gallotiinae clade was placed in the middle of the tree instead of at the base. All members of the African clade, previously identified by Arnold (1989), Fu (1998) and Harris *et al.* (Eremiainae; 1998a), were grouped together. The 12S gene data placed *Takydromus* at the base of the tree. The other Eurasian lacertids formed a pectinate pattern and were located at the base of the tree next to *Takydromus*.

TABLE 1. Summary of the results. POG = primary outgroup, bp = base pair, PIC = phylogenetically informative character, MPT = most parsimonious tree, TL = tree length, CI = consistency index, RI = retention index, TS = transition, TV = transversion, PTP = permutation tail probability, HER = homoplasy excess ratio. All PTPs and HERs were calculated form 1000 replicates without randomizing the POG. Numbers in parentheses are PICs from 1st & 2nd codon positions, and 3rd codon positions

| Gene | bp aligned | bp analysed | No. PICs | No. taxa | No. MPTs | TL | CI | RI | TS/TV | PTP | HER |
|---|---|---|---|---|---|---|---|---|---|---|---|
| COI | 1080 | 1080 | 434 (87–347 | 33 | 1 | 3665 | 0.222 | 0.297 | 1.83 | 0.001 | 0.073 |
| cyt-*b* | 1045 | 1045 | 492 (163–329) | 25 | 1 | 3319 | 0.279 | 0.275 | 1.42 | 0.001 | 0.046 |
| 12S | 948 | 765 | 285 | 33 | 4 | 1409 | 0.347 | 0.415 | 1.70 | 0.001 | 0.167 |
| 16S | 1510 | 1116 | 442 | 33 | 2 | 2298 | 0.326 | 0.425 | 1.72 | 0.001 | 0.180 |
| tRNAs | 125 | 81 | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| Combined | 4708 | 4087 | 1664 | 29 | 1 | 10378 | 0.286 | 0.299 | 1.70 | 0.001 | 0.080 |
| (No POGs) | 4708 | 4087 | 1495 | 26 | 1 | 9210 | 0.287 | 0.275 | 1.67 | n/a | n/a |

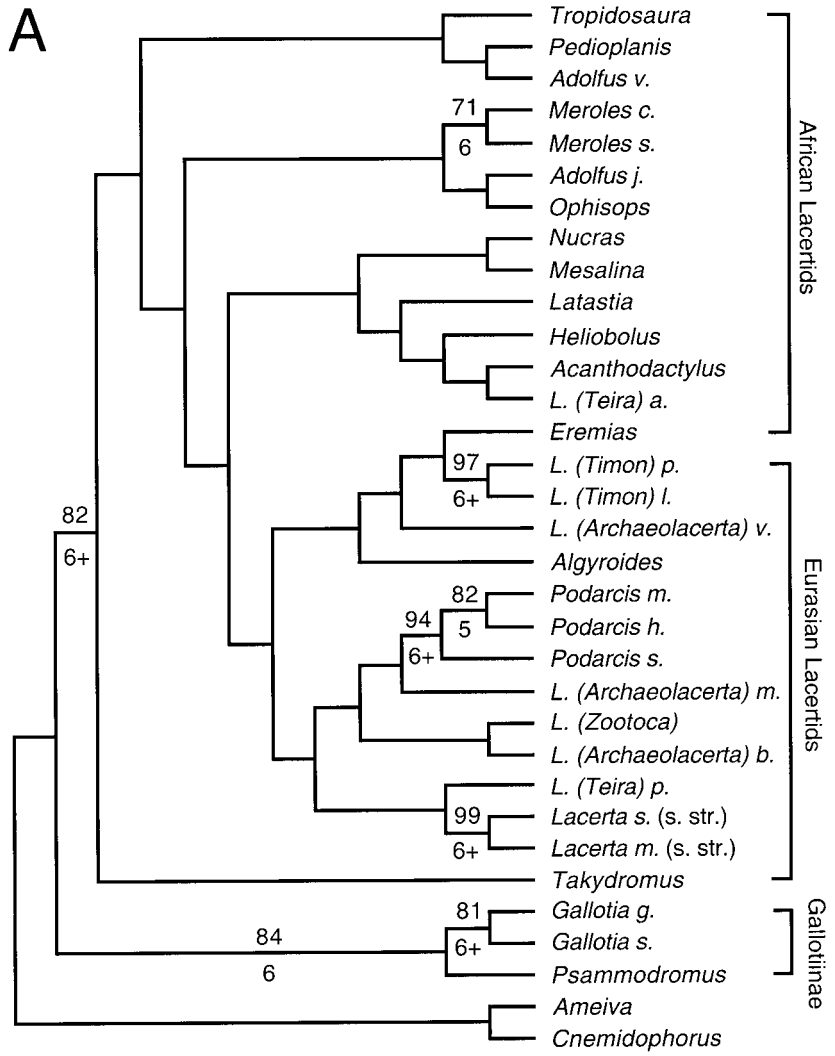


Figure 1. The most parsimonious trees (MPTs) from the independent analyses. The numbers above the lines are bootstrap proportions greater than 0.50. The numbers below the lines are decay indices greater than four. Taxa name abbreviations: *Adolfus j.* = *Adolfus jacksoni*; *Adolfus v.* = *Adolfus vaureselli*; *Gallotia g.* = *Gallotia galloti*; *Gallotia s.* = *allotia stehlini*; *L.* (*Archaeolacerta*) *b.* = *Lacerta bedriagae*; *L.* (*Archaeolacerta*) *m.* = *Lacerta monticola*; *L.* (*Archaeolacerta*) *v.* = *Lacerta valentini*; *Lacerta m.* (*s.s.*) = *Lacerta media*; *Lacerta s.* (*s.s.*) = *Lacerta schreiberi*; *Meroles c.* = *Meroles ctenodactylus*; *L.* (*Teira*) *a.* = *Lacerta andreanszkyi*; *L.* (*Teira*) *p.* = *Lacerta perspicillata*; *L.* (*Timon*) *l.* = *Lacerta lepida*; *L.* (*Timon*) *p.* = *Lacerta pater*; *L.* (*Zootoca*) = *L. vivipara*; *Meroles s.* = *Meroles suborbitalis*; *Podarcis h.* = *Podarcis hispanica*; *Podarcis m.* = *Podarcis muralis*; *Podarcis s.* = *Podarcis sicula*. A, the single MPT from the COI gene data. B (facing page), the strict consensus tree from the 16S gene data.

Two MPTs were obtained from the 16S data (Table 1, Fig. 1B). Seven nodes received BSP values greater than 0.70, and they were exactly the same seven nodes which received the highest DIs (great than 4). Similar to the results of 12S gene, the monophyly of *Lacerta (s.s.)*, *Lacerta* (*Timon*), *Meroles* and *Podarcis* was well-supported.

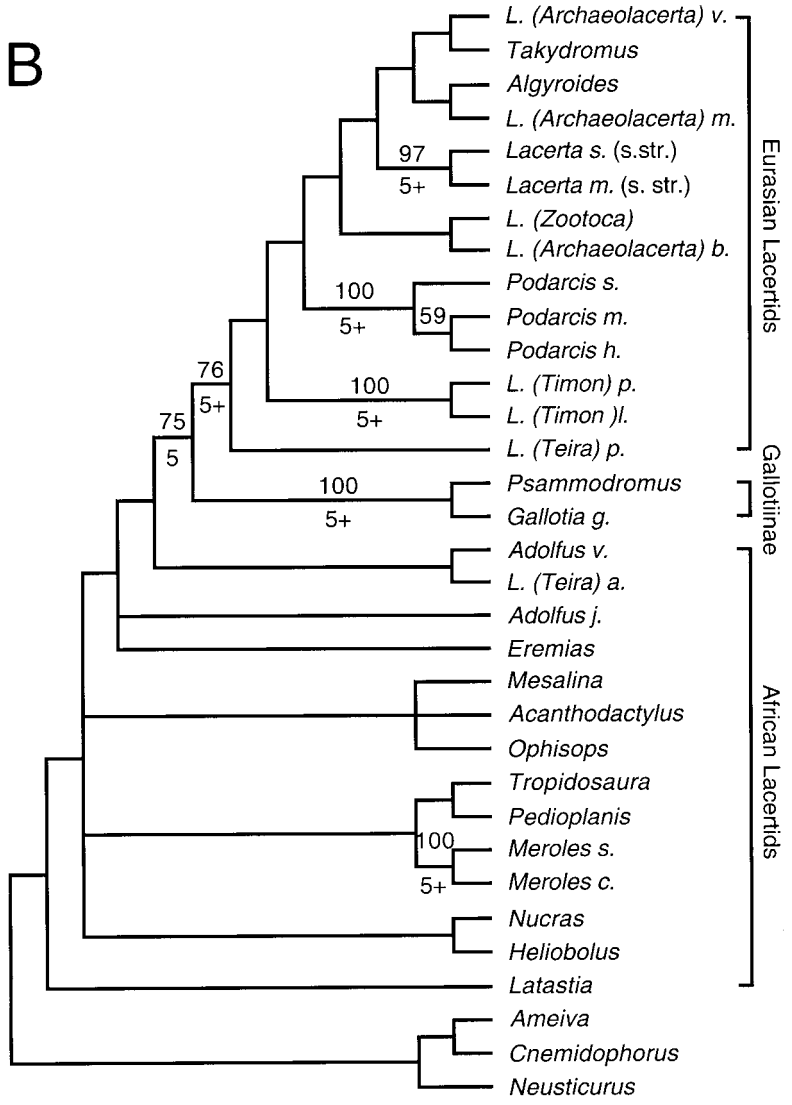


Figure 1B. For caption see facing page.

Again, the Gallotiinae was placed in the middle of the tree instead of at the base. In contrast to the 12S data, the Eurasian lacertids were grouped together at the top of the tree. The African lacertids formed a pectinate pattern and were located at the base of the tree.

Weighting schemes were employed which used only transversion substitutions for RNA encoding genes and only first and second codon position substitutions for protein encoding genes. Hillis *et al.* (1994) suggested that giving high weight to transversion change, as well as first and second codon position changes, would more accurately reflect the genealogical relationships, especially in the cases of deep divergence. However, in this case, character weighting largely reduced the resolution of the trees, especially at the base (trees were not shown).

### *The combined data analysis and the tree root*

Although each data set resulted in an almost fully resolved tree, the small number of well-supported nodes and low HERs indicated the small amount of cladistic structure in the data (Table 1, Fig. 1A, B). The topologies from the independent analyses were largely different from each other, notably the tree root. However, none of the well-supported nodes (i.e. BSP greater than 0.70 and high DIs) were in conflict. Subsequently, a combined data analysis was conducted. Several redundant representatives were omitted from the data set because cyt-*b* data were not available and the monophyly of the five genera (groups) with multiple representatives was confirmed by the analyses of individual genes.

The combined data analysis found one MPT (Table 1; Fig. 2A). Both subfamilies Gallotiinae and Lacertinae formed well-supported clades. In the Lacertinae clade, *Takydromus* located at the base and the remaining Eurasian lacertid group members grouped together, as well as all African lacertid group members.

Where should the tree be rooted? Several options are possible (Fig. 1A, B). However, the only well-supported option, in terms of BSP values and decay indices, was the solution from COI data, which placed the root between the Gallotiinae and the Lacertinae. The combined data analysis also strongly supported this solution, which may represent the signal from the COI gene. Mayer and Benyr (1994) and Harris *et al.* (1998a) also reached this conclusion.

Accepting this rooting inferred that the cyt-*b*, 12S and 16S genes misrooted their trees. Forcing the cyt-*b* data with a COI-like root resulted in MPTs 5 steps longer than the unconstrained topology. Similar constraints on the 12S and 16S data resulted in MPTs with an increase of 7 and 5 steps, respectively. The constrained and unconstrained topologies were almost equally parsimonious. Templeton's test revealed no significant difference between them ($P = 0.4011$–$0.5078$). Evidently, the characters supporting the erroneous root might have resulted from homoplastic changes. This is another example that outgroups may misroot the tree when DNA sequence data are used to reconstruct the phylogeny (e.g. Lee *et al.*, 1997). Because the primary outgroup misrooted the cyt-*b*, 12S and 16S tree, it could possibly have an adverse effect on the tree topology as well. To avoid this problem, a FIG/FOG analysis was conducted.

### *FIG/FOG and PTP analyses*

With the establishment of tree root, the Gallotiinae formed an ideal functional outgroup for the Lacertinae. Subsequently, the relationships among Lacertinae were further examined using FIG/FOG analyses and excluding the primary outgroups.

One MPT resulted from the combined data (Fig. 2B; Table 1). Both the African and Eurasian clades were resolved, and with significant support in terms of BSP, although the latter did not receive a high DI. Comparing the results of the analysis with the primary outgroup, the relationships within the African clade were the same, but the relationships within the Eurasian clade were slightly different. A notable difference was the location of *Takydromus*—it was grouped into the Eurasian group without the primary outgroups.

One interesting aspect of the MPT was its extremely long terminal branches and short internodes. The 24 terminal branches had an average of 207.4 unambiguous

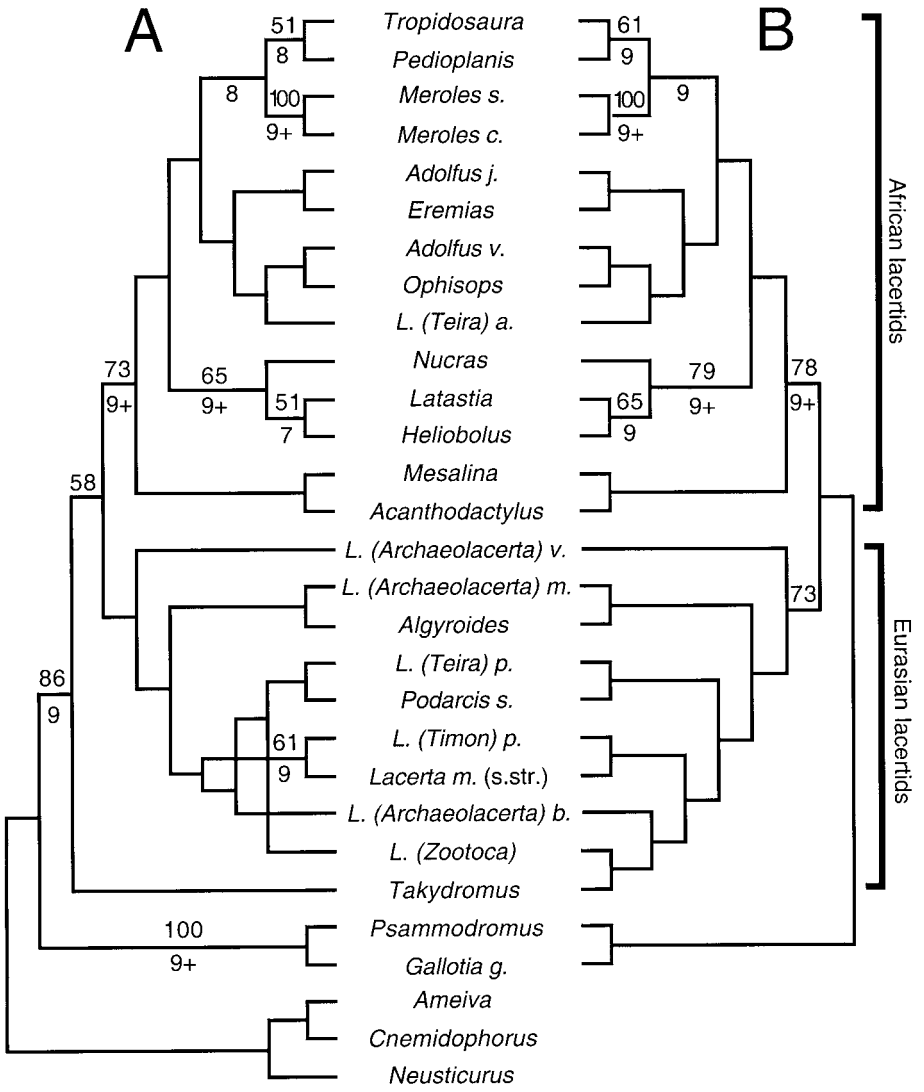


Figure 2. The most parsimonious trees (MPTs) from the combined data. The numbers above the lines are bootstrap proportions greater than 0.50. The numbers below the lines are decay indices greater than six. Taxon name abbreviations refer to Figure 1. A, the single MPT with the primary outgroups. B, the single MPT without the primary outgroups.

changes with a standard deviation of 59.3. The two internodes defining the African and Eurasian clades both had 50 changes. The 22 internodes within the African and Eurasian clades had an average of 38.5 changes with a standard deviation of 14.7. The longest internode was the node defining Lacertinae, which had 133 unambiguous changes.

Another striking feature of the trees resulting from the combined data analysis was that too few nodes were well supported. Only four nodes received BSP values over 0.70, and none of them was in the Eurasian clade. Furthermore, the relationships

within the Eurasian group and the African group have little in common among trees resulting from each gene and combined data (other than the monophyly of the groups with multiple representatives). This poor nodal support and incongruence were unlikely due to insufficient data, because of the large number of phylogenetically informative characters (Table 1). Rather, it is probably because of deficient structure in the data. To evaluate the structure, a PTP analysis was applied.

The combined data set was partitioned into two data sets. One consisted of only African clade members, and the other of Eurasian clade members (taxa components refer to Fig. 2).

No significant cladistic structure was detected from the subset composed of Eurasian group (PTP = 0.057, HER = 0.010), although 875 phylogenetically informative characters were present. Significant structure was detected in the African group subset (PTP = 0.001, HER = 0.040), with 1261 phylogenetically informative characters. However, a single well-supported node could make an otherwise random data set yield a significant PTP (Fu & Murphy, 1999). Two well-supported nodes were observed on the tree. These nodes may carry a large portion of the structure in the data. To test this, one of the two taxa from the clade with the highest BSP (100), *Meroles ctenodactylus*, was deleted from the data set. This deletion resulted in the loss of the structure at this node. The remaining data set was subjected to PTP test again and a significant PTP was maintained (PTP = 0.001, HER = 0.019), with 1241 phylogenetically informative characters. However, HER was reduced by half. When two of the three taxa from the clade with the second highest BSP (79), *Latastia* and *Heliobolus*, were deleted from the data, an insignificant PTP resulted (PTP = 0.116, HER = 0.007), indicating there is no significant structure remaining in the data, although there were 1111 phylogenetically informative characters.

## DISCUSSION

### *The preferred phylogeny of family Lacertidae*

The Family Lacertidae is clearly divided into two subfamilies: the Gallotiinae, which includes *Gallotia* and *Psammodromus* and the Lacertinae, which includes all remaining lacertids. The Lacertinae is further divided into two groups, the Eurasian group and African group. Within the two groups, the monophyly of *Gallotia*, *Lacerta (s.s.)*, *Lacerta* (*Timon*), *Meroles*, *Podarcis* are well-supported by COI, 12S and 16S gene data. The close relationship of genera *Nucras*, *Latastia* and *Heliobolus* is also well-supported. Although other relationships are also fully resolved, the PTP examination indicated that they are based on random data. Therefore, those relationships cannot be confidently considered as the genealogy of the organisms.

Previous studies also acknowledged the major clades on the preferred phylogeny. The Gallotiinae clade was recognized by Arnold (1989), Mayer and Benyr (1994), and Harris *et al.* (1998a). However, the Lacertinae clade was only accepted by Mayer and Benyr (1994), and Harris *et al.* (1998a). Furthermore, all three previous studies recognized the African lacertid clade, although the composition of the clade differs slightly, and two of them (Mayer & Benyr, 1994; Harris *et al.*, 1998a) also accepted the Eurasian lacertid clade. Interestingly, all three studies realized the difficulty in reconstructing the phylogeny among Eurasian lacertids. This uncertainty is clearly

revealed by the largely unresolved tree from morphology and poorly supported nodes from the DNA sequence data.

The genes used in this study are high complimentary to each other. For example, the COI data strongly support the monophyly of the two subfamilies, while the 12S and 16S genes contributed much to the monophyly of the Eurasian and African groups. The FIG/FOG analysis revealed an interesting outcome in that *Takydromus* moved from in the Eurasian group to the base of Lacertinae clade when the primary outgroup were included (Fig. 2). This could be a long branch attraction phenomenon, i.e. the primary outgroup, which have a long branch, attracted *Takydromus*, which also has a long branch. This aspect deserves further examination.

### *Hypothetical recent explosive speciation*

Why do 4708 bps of the mtDNA sequence fail to resolve a well-supported tree? It is not because the genes are overly conservative. The combined data yielded 1664 phylogenetically informative characters for 29 taxa (Table 1). The numbers of informative characters for the relationships among the Eurasian lacertids and African lacertids are also large. Those data confidently resolved several terminal nodes (e.g. the monophyly of five genera/subgenera). It is not because the genes are overly variable either. If so, the data would have resolved the terminal nodes and not the basal nodes. The mtDNA sequence data collected in this study successfully recovered the deep divergence of lacertids with confidence, but failed to decipher more recent divergence. This failure is due to lack of structure in the data, as the PTP analysis indicated. Futhermore, the lack of structure likely has arisen from recent explosive speciation events. This is suggested by the short internodes of the resulting tree.

Lutz *et al.* (1986) suggested that the ancestor of western European lacertids might have undergone rapid multiple divergence since the early Miocene. This is probably also true for African lacertids. After the separation of the ancestor of African lacertids from the ancestor of Eurasian lacertids, the subsequent speciation rates suddenly accelerated. The acceleration in Africa may have been correlated to the change of climate. Since the late Miocene, northern Africa has become progressively more arid (Duellman & Trueb, 1986). The divergence of African lacertids was possibly associated with this change in climate, which led to rapid multiple speciation events. This is evidenced by the fact that the greatest divergence of African lacertids is associated with adaptations to arid habitats.

The explosive speciation hypothesis can also account for the lack of resolution of the morphological study by Arnold (1989). In the explosive speciation scenario, the speciation events were so close to each other in time that none or only a few characters were fixed on the internodes, which makes the phylogenetic reconstruction difficult. Harris *et al.*'s (1998a) data also showed this trend. On their resulting trees from the DNA data, only two generic level associations (*Gallotia* with *Psammodromus*; *Ichnotropis* with *Meroles*) were resolved at the 50% BSP level. Although the small size of the data set may be the major reason, the explosive speciation is certainly responsible, at least partially, for the poor resolution. This hypothesis can be tested by collecting more data. If the tree becomes better resolved by increasing the size of the data set, then the explosive hypotheses would be falsified.

*Future direction of research on this topic*

If the explosive speciation hypothesis is true, collecting more data would be unlikely to improve the resolution of the phylogeny. Phylogenetic reconstruction at the family level may prove very difficult, if not impossible. Although the large picture is difficult, the close associations of a few genera (or other natural groups) are possible (e.g. the association of *Nucras*, *Latastia* and *Heliobolus* from this study). Future phylogenetic studies should focus on lower levels, such as genera or subgenera. Most of the currently recognized genera probably are monophyletic. When working on the phylogeny of a genus (or other assumed monophyletic groups), a large number of possibly closely related outgroup taxa should be used. This would test the monophyly of the ingroup while searching for the phylogeny of the ingroup members. For DNA sequence data, international databases, such as GenBank, can supply the data for a broad choice of outgroups. A few recent studies showed that phylogenetic construction is more fruitful at lower levels (e.g. Thorpe *et al.*, 1994; Fu *et al.*, 1997; Harris *et al.*, 1998b).

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## APPENDIX 1

### *Specimens examined in this study*

Abbreviations: CAS California Academy of Sciences, San Francisco; LSUMZ Museum of Natural Science, Louisiana State University; MNCN Museo Nacional de Ciencias Naturales, Madrid; MVZ Museum of Vertebrate Zoology, University of California at Berkeley; ROM Royal Ontario Museum, Toronto.

*Acanthodactylus erythrurus*, Spain: Cádiz: Punta Paloma, MNCN11931; *Adolfus vauereselli*, CAS201617, Uganda: Kabale Dist.: Bwindi Impenetrable National Park; *Adolfus jacksoni*, Uganda: Kabale Dist.: Bwindi Impenetrable National Park, CAS201605; *Algyroides fitzingeri*, Italy: Sardinia, ROM 24642; *Ameiva ameiva*, Guyana: Tukeit, ROM 20530; *Cnemidophorus tigris maximus*, Mexico: Baja California, ROM RWM647; *Eremias velox*, Russia: Daghestan, ROM 23498; *Gallotia galloti*, Spain: Canary Is., no voucher number available; *Gallotia stehlini*, Spain: Canary Is., no voucher number available; *Heliobolus spekii*, Kenya: Rift Valley Prov.: Kajiado Dist., CAS 198923; *Lacerta* (*Archaeolacerta*) *bedriagae*, Italy: Sardinia, ROM 24640; *Lacerta* (*Archaeolacerta*) *monticola*, Spain: Avila: Sierra de Gredos, MNCN13831; *Lacerta* (*Teira*) *andreanszkyi*, Morocco: Marrakech: Oukaïmedèn, MVZ178213; *Lacerta* (*Teira*) *perspicillata*, Morocco: Rabat-Salé: Rabat, MVZ186202; *Lacerta* (*Timon*) *lepida*, Spain: Cádiz: Benaup de Sidonia, MVZ186068; *Lacerta* (*Timon*) *pater*, Morocco: Âïn-Leuh, MVZ178286; *Lacerta media* (*s.s.*), Armenia: Abovyan, ROM 24267; *Lacerta schreiberi* (*s.s.*), Spain: Avila: Cuevas del Valle, MNCN13904; *Lacerta (Archaeolacerta) valentini*, Armenia: Sevan, ROM 23861; *Lacerta* (*Zootoca*) *vivipara*, Russia: St. Petersburg, ROM 24750; *Latastia longicaudata*, Kenya: Rift Valley Prov.: Kajiado Dist., CAS 198982; *Meroles ctenodactylus*, South Africa: Cape Prov.: 37.1 km S Alexander Bay, LSUMZ H-13110; *Meroles suborbitalis*, Namibia: East Spitzkoppe, no voucher number available; *Mesalina brevirostris*, no location data and voucher number available; *Neusticurus* sp., Guyana, ROM 22892; *Nucras tessellata*, South Africa: Cape Prov.: Richtersveld National Forest, LSUMZ H-13111; *Ophisops elegans*, Armenia: Chosrov, ROM 23506; *Pedioplanis namaquensis*, South Africa: Cape Prov.: Richtersveld National Park, LSUMZ H-13109; *Podarcis sicula*, Italy: Tuscany, ROM 24637; *Podarcis muralis*, Spain: Huesca: Baños de Benasque, MNCN23640; *Podarcis hispanica*, Morocco: Tétouan: Asilah, MVZ186232; *Psammodromus algirus*, Morocco: Tanger: Cap Spartel, MVZ178376; *Takydromussexlineatus*, Vietnam: Sapa, ROM 26345; *Tropidosaura gularis*, South Africa: East Cape: Montague Pass, no voucher number available.

## APPENDIX 2

*Primers used for PCR and sequencing in this study*

Primers are designed by their 3′ ends, which correspond to the position in the human mitochondrial genome (Anderson *et al.*, 1981). H and L designate heavy- and light-strand primers, respectively. L1091, H1478, L14841, and H15149 are from Kocher *et al.* (1989); H3060 is from Palumbi (1996); L6586 and H7086 are from Wüster *et al.* (1995); H15488 and H15915 are from Haddrath (pers. comm.). H1497 is complementary of H1478; L2510 is modified from Palumbi (1996); L15153 is complementary of H15149.

12S gene primers: L717 5′ TAC ACA TGC AAG TAT CCG CAC ACC AGT G 3′; L1091 5′ CAA ACT GGG ATT AGA TAC CCC ACT AT 3′; H1195 5′ ATC GAT TAT AGA ACA GGC TCC TCT A 3′; H1478 5″ AGG GTG ACG GGC GGT GTG T 3′; H1497 5′ ACA CAC CGC CCG TCA CCC TC 3′;

16S gene primers: L1921 5′ CCC GAA ACC AAA CGA GCA A 3′; H1990 5′ CCA GCT ATC ACC AAG TTC GGT AGG CTT TTC 3′; L2510 5′ CCG ACT GTT TAC CAA AAA CAT 3′; H2568 5′ CTA CCT TTG CAC GGT TAG GAT ACC GCG GC 3′; H3060 5′ CCG GAT CCC CGG CCG GTC TGA ACT CAG ATC ACG 3′;

COI gene primers: L6106 5′ GCC CAT GCA TTC GTA ATA ATT TTC TT 3′; L6208 5′ TTC CCG CGA ATA AAT AAC ATA AGC TT 3′; L6586 5′ GAA TTC CCT GCA GGA GGA GGA GAC CC 3′; H7086 5′ GAA TTC CCA GAG ATT AGA GGG AAT CAG TG 3′; H7319 5′ACT TCT CGT TTA GCT GCG AAG GCT TCT CA 3′;

cyt-*b* gene primers: L14841 5′ CCA TCC AAC ATC TCA GCA TGA TGA AA 3′; H15149 5′ GCC CCT CAG AAT GAT ATT TGT CCT CA 3′; L15153 5′ TGA GGA CAA ATA TCC TTC TGA GG 3′; H15488 5′ TTG CTG GGG TGA AGT TTT CTG GGT C 3′; L15369 5′ CAT GAA ACT GGA TCA AAC AAC CC 3′; H15915 5′ GTC TTC AGT TTT TGG TTT ACA AGA C 3′ (locating on $tRNA^{Thr}$).