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Molecular Phylogenetics, tRNA Evolution, and Historical Biogeography in Anguid Lizards and Related Taxonomic Families

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Phylogenetic relationships among lizards of the families Anguidae, Anniellidae, Xenosauridae, and Shinisauridae are investigated using 2001 aligned bases of mitochondrial DNA sequence from the genes encoding ND1 (subunit one of NADH dehydrogenase), $tRNA^{Ile}$, $tRNA^{Gln}$, $tRNA^{Met}$, ND2, $tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, $tRNA^{Tyr}$, and COI (subunit I of cytochrome *c* oxidase), plus the origin for light-strand replication (O_L) between the $tRNA^{Asn}$ and the $tRNA^{Cys}$ genes. The aligned sequences contain 1013 phylogenetically informative characters. A well-resolved phylogenetic hypothesis is obtained. Because monophyly of the family Xenosauridae (*Shinisaurus* and *Xenosaurus*) is statistically rejected, we recommend placing *Shinisaurus* in a separate family, the Shinisauridae. The family Anniellidae and the anguid subfamilies Gerrhonotinae and Anguinae each form monophyletic groups receiving statistical support. The Diploglossinae*, which appears monophyletic, is retained as a *metataxon* (denoted with an asterisk) because its monophyly is statistically neither supported nor rejected. The family Anguidae appears monophyletic in analyses of the DNA sequence data, and statistical support for its monophyly is provided by reanalysis of previously published allozymic data. Anguid lizards appear to have had a northern origin in Laurasia. Taxa currently located on Gondwanan plates arrived there by dispersal from the north in two separate events, one from the West Indies to South America and another from a Laurasian plate to Morocco. Because basal anguine lineages are located in western Eurasia and Morocco, formation of the Atlantic Ocean (late Eocene) is implicated in the separation of the Anguinae from its North American sister taxon, the Gerrhonotinae. Subsequent dispersal of anguine lizards to East Asia and North America appears to have followed the Oligocene drying of the Turgai Sea. The alternative hypothesis, that anguine lizards originated in North America and dispersed to Asia via the Bering land bridge with subsequent colonization of Europe and Morocco, requires a phylogenetic tree seven steps longer than the most parsimonious hypothesis. North African, European, and West Asian anguines were isolated from others by the rapid uplift of Tibet in the late Oligocene to Miocene. Phylogenetic analysis of evolutionary changes in the gene encoding $tRNA^{Cys}$ suggests gradual reduction of dihydrouridine (D) stems by successive deletion of bases in some lineages. This evolutionary pattern contrasts with the one observed for parallel elimination of the D-stem in mitochondrial tRNAs of eight other reptile groups, in which replication slippage produces direct repeats. An unusual, enlarged TψC (T) stem is inferred for $tRNA^{Cys}$ in most species. © 1999 Academic Press

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Anguid lizards, found predominately in the northern hemisphere, are an exciting group for a molecular phylogenetic study of biogeographic fragmentation between North America and Eurasia. The anguimorph family Anguidae contains three subfamilies. The subfamily Gerrhonotinae occurs strictly in North America and Central America. The subfamily Diploglossinae ranges from Mexico and the West Indies to South America. The subfamily Anguinae, comprising the genera *Anguis* and *Ophisaurus*, is the only anguid subfamily that occurs in the Old World. *Anguis* is restricted to Europe, whereas *Ophisaurus* is found in eastern North America, eastern Asia, western Asia, and adjacent Europe and Morocco.

Some taxonomists consider the anguimorph lizard family Anniellidae a fourth subfamily of the Anguidae (see Gauthier, 1982). It comprises two species, *Anniella geronimensis* and *A. pulchra*, from the west coast of North America. Three major hypotheses have been

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considered for the phylogenetic position of the Anniellidae relative to the Anguidae: (1) *Anniella* is the sister group to all anguid taxa (Good, 1987), (2) *Anniella* is the sister group to the Anguinae (Gauthier, 1982), and (3) *Anniella* is the sister taxon to *Anguis* of Europe (Keqin and Norell, 1998).

The anguimorph family Xenosauridae occurs in the New World (*Xenosaurus*) and Old World (*Shinisaurus*). Some authors consider *Shinisaurus* a separate monotypic family, the Shinisauridae (see Zhao and Adler, 1993). No previous molecular study has examined the relationships of these taxa.

A major question within the Anguinae is the phylogenetic position of *Anguis fragilis, Ophisaurus apodus,* and *O. koellikeri,* which occur between two extremely large barriers to faunal distributions, the Atlantic Ocean and the Tibetan Plateau. Current taxonomy implies at least two separate origins of anguid lizards in this region. Mitochondrial DNA sequences are reported for these taxa as well as for the East Asian *O. harti* and the North American *O. attenuatus* and *O. ventralis.*

We examine all genera within the Gerrhonotinae except *Coloptychon,* which is known from only three specimens. *Abronia oaxacae, Barisia imbricata, Gerrhonotus liocephalus,* and *Mesaspis moreleti* represent the four tropical genera in our analysis. Five species of *Elgaria,* primarily from the temperate part of North America, are examined. *Elgaria coerulea,* sampled from coastal California, is the most northern species of the Gerrhonotinae. Two other species are included from California, *E. multicarinata* collected from the east side of the Sierra Nevada and *E. panamintina* from an adjacent population in the Inyo Mountains. *Elgaria kingii,* sampled from Arizona, occurs along the west coast of Mexico in the Sierra Madre Occidental opposite Baja California where *E. paucicarinata* was obtained from the Sierra de La Laguna. This choice of species allows an examination of taxa from both sides of the Gulf of California, a region of rifting between tectonic plates.

All genera of the neotropical subfamily Diploglossinae are examined. *Ophiodes striatus* represents the only endemic anguid genus in South America. *Celestus enneagrammus* from Mexico and *Diploglossus bilobatus* from Costa Rica represent mainland North American diploglossines, and *Diploglossus pleei, Sauresia agasepsoides,* and *Wetmorena haetiana* represent West Indian taxa.

Our sampling includes a comprehensive representation of species in the Anniellidae (*Anniella geronimensis* and *A. pulchra*) and genera of the Xenosauridae (*Shinisaurus crocodilurus* from China and *Xenosaurus grandis* from Mexico).

Heloderma suspectum and *Varanus griseus,* New World and Old World representatives of the Varanoidea, serve as outgroups to root the tree. Previous phylogenetic analyses of morphological data (Estes *et al.,* 1988; Macey *et al.,* 1997a; Schwenk, 1988) cannot determine whether the Varanoidea or the Xenosauridae is closer to the anguid and anniellid clade; this question, however, is not a focus of this study.

Phylogenetic relationships are examined using 2001 aligned positions (1013 informative) of mitochondrial DNA sequence. The region sequenced extends from the protein-coding gene, ND1 (subunit one of NADH dehydrogenase), through the genes encoding $tRNA^{Ile}$, $tRNA^{Gln}$, $tRNA^{Met}$, ND2, $tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, and $tRNA^{Tyr}$ to the protein-coding gene COI (subunit I of cytochrome *c* oxidase), and includes the replication origin for the light strand (O_L) between the $tRNA^{Asn}$ and the $tRNA^{Cys}$ genes.

Previously published allozymic data (Good, 1987, 1988) are reanalyzed and compared with the results obtained from the new DNA sequence data to provide a comprehensive assessment of relationships among the taxa investigated.

The mitochondrial genomic region sequenced demonstrates several unusual characteristics among squamate reptiles (Kumazawa and Nishida, 1995; Kumazawa *et al.,* 1996; Macey *et al.,* 1997a,b,c; Seutin *et al.,* 1994). Within anguimorph lizards, gene sequences encoding $tRNA^{Cys}$ lack a dihydrouridine (D) stem and instead contain a D-arm replacement loop in *Varanus* (Macey *et al.,* 1997b). A model involving replication slippage has been proposed for the formation of D-arm replacement loops in mitochondrial tRNAs (Macey *et al.,* 1997b). Under this model direct repeats are expected and the size of the D-arm replacement loop should be less than 12 bases, the minimum number of bases normally found between the amino acid-acceptor (AA) and the anticodon (AC) stems when a D-stem is present (Macey *et al.,* 1997b). Alternatively, gradual relaxation of pairing among bases in the D-stem would not produce either repeats or deletion of bases. Gradual deletion of bases or base pairs would not produce repeats but could produce length variation that may result in less than 12 bases between the AA- and AC stems. Under a model of gradual deletion of bases or base pairs, a tRNA that has a single base pairing in the D-stem could result, as has been observed in the $tRNA^{Asn}$ gene of the frog, *Xenopus laevis* (Dirheimer *et al.,* 1995; Roe *et al.,* 1985). Mitochondrial tRNAs that have a single base pairing in the D-stem have a tertiary structure distinct from both standard tRNAs and tRNAs in which no pairings are observed between the AA- and AC-stems (Steinberg *et al.,* 1994). A phylogenetic analysis of secondary structure for $tRNA^{Cys}$ within the Anguimorpha serves to test the hypothesis that D-arm replacement loops are formed by replication slippage versus gradual relaxation or deletion of bases within the D-stem.

MATERIALS AND METHODS

Specimen Information

Museum numbers and localities for voucher specimens from which DNA was extracted, and GenBank accession numbers are presented below. Acronyms are CAS for California Academy of Sciences, San Francisco; MVZ for Museum of Vertebrate Zoology, University of California at Berkeley; USNM for United States National Museum, Washington, DC; UTA-R for University of Texas at Arlington; and ZISP for Zoological Institute, St. Petersburg, Russia. Acronyms followed by a dash RM or TP represent field numbers of the first or sixth author, respectively, for uncatalogued specimens being deposited in the Museum of Vertebrate Zoology. The acronym followed by a dash SBH represents a field number of S. Blair Hedges for an uncatalogued specimen being deposited in the United States National Museum. The three previously reported sequences have been extended by 303 bases to include 101 additional amino acid positions of the ND1 gene, and the GenBank accessions have been updated accordingly.

Heloderma suspectum: no voucher, AF085603, probably Arizona. *Varanus griseus:* ZISP 19576, U71334 (Macey *et al.*, 1997a), east side of Nephtezavodsk which is 30 km WNW of Deynau (39° 15′N 63° 11′E), Chardjou Region, Turkmenistan. *Shinisaurus crocodilurus:* MVZ 204291, AF085604, China. *Xenosaurus grandis:* MVZ 137789, U71333 (Macey *et al.*, 1997a), slopes behind Casa de Miguel Ceron, Cuatlapan, Veracruz, Mexico. *Anniella geronimensis:* MVZ 134196, AF085605, beach, 3.5 miles W of Colonia Guerrero, Baja California Norte, Mexico. *Anniella pulchra:* MVZ-TP24334, AF085606, SW 1/4 Sec. 23, T. 2 N., R. 2 E., sand dune on N side of railroad tracks, 0.2 miles SE Jct of Hwy 4 and Big Break Road, Oakley, Contra Costa Co., California. *Celestus enneagrammus:* MVZ 191045, AF085607, Elev. 2125 m, La Joya, Veracruz, Mexico. *Diploglossus bilobatus:* MVZ 207334, AF085608, 3.3 km E ranch headquarters at Moravia on road to indian reservation, Prov. Cartago, Costa Rica. *Diploglossus pleei:* MVZ-TP24475, AF085609, Bosque de Guajataca, Vereda Salomé, approx. 7 km airline SW Quebradillas (18° 24.5′ N 66° 57.8′ W), Puerto Rico. *Ophiodes striatus:* MVZ 191047, AF085610, Edo. São Paulo, Brazil. *Sauresia agasepsoides:* USNM-SBH194829, AF085611, Bucan Detwi (17° 44.0′ N 71° 30.3′ W), Pedernales, Dominican Republic. *Wetmorena haetiana:* USNM 328858, AF085612, 15.3 km S, 6.7 km E (by road) Cabral, Barahona, Dominican Republic. *Barisia imbricata:* MVZ 191048, AF085613, 3070 m, Mex. Hwy 190, Mexico, Mexico. *Gerrhonotus liocephalus:* UTA-R-12225, AF085614, 2377 m, El Tejocote, Oaxaca, Mexico. *Abronia oaxacae:* MVZ 144197, AF085615, Cerro San Felipe, 20 km NNE of Oaxaca (by Hwy 175) to La Cumbre then 4 km NW (by dirt road), Oaxaca, Mexico. *Mesaspis moreleti:* MVZ 143472, AF085616, Elev. 9550 ft., 4.5 km by Road E of Todos Santos, Depto. Huehuetenango, Guatemala. *Elgaria coerulea:* MVZ-TP24365, AF085617, San Pablo Ridge, Wildcat Canyon Road at Inspiration Point, Contra Costa Co., California. *E. kingii:* MVZ-RM1192, AF085618, 10.2 miles NE of Tanque Verde Road on Catalina Hwy (Mt. Lemmon Rd.), Pima Co., Arizona. *E. paucicarinata:* MVZ 191079, AF085619, La Laguna, Sierra de La Laguna, Baja California Sur, Mexico. *E. multicarinata:* MVZ 227733, AF085620, Elev. 5700 ft., NE 1/4 Sec. 16, T. 13 S., R. 34 E., south fork of Oak Creek, 5.0 miles west (airline) of Independence, Inyo Co., California. *E. panamintina:* MVZ 227761, U82692 (Macey *et al.*, 1997c), Elev. 2030 m, 10.1 miles E of Big Pine on Hwy 168, Inyo Co., California. *Ophisaurus koellikeri:* MVZ 178120, AF085621, 10.1 km S of Kenitra (34° 16′ N 6° 36′ W) on P-29A, Kenitra, Morocco. *Anguis fragilis:* MVZ 219518, AF085622, 2 km SE of Babukal, also 53 km ENE of Dagomys (43° 40′ N 39° 38′ E) on road, Krasnodarsky Territory, Russia. *Ophisaurus apodus:* CAS 182911, AF085623, Tersko-Kumskaya Nizmennast, 3 km WNW of Voskresenskaya, which is approx. 25 km NNW of Gudermes (43° 21′ N 46° 06′ E), Schelkovskaya District, Chechenia Autonomous Republic, Russia. *O. harti:* MVZ 224111, AF085624, Elev. 900–1100 m, Tam Dao (21° 27′ N 105° 37′ E), Vihn Yen District, Vihn Thu Province, Vietnam. *O. attenuatus:* MVZ-RM10468, AF085625, 2.4 miles south of Weldon Springs at I-40 on Hwy 94, St. Charles Co., Missouri. *O. ventralis:* MVZ 137541, AF085626, Surf City, Pender Co., North Carolina.

Laboratory Protocols

Genomic DNA was extracted from liver using the Qiagen QIAamp tissue kit. Amplification of genomic DNA featured a denaturation at 94°C for 35 s, annealing at 50°C for 35 s, and extension at 70°C for 150 s with 4 s added to the extension per cycle, for 30 cycles. Negative controls were run for all amplifications. Amplified products were purified on 2.5% Nusieve GTG agarose gels and reamplified under similar conditions. Reamplified double-stranded products were purified on 2.5% acrylamide gels (Maniatis *et al.*, 1982). Template DNA was eluted from acrylamide passively over 3 days with Maniatis elution buffer (Maniatis *et al.*, 1982). Cycle-sequencing reactions were run using the Promega fmol DNA-sequencing system with a denaturation at 95°C for 35 s, annealing at 45–60°C for 35 s, and extension at 70°C for 1 min for 30 cycles. Sequencing reactions were run on Long Ranger sequencing gels for 5–12 h at 38–40°C.

Amplifications from genomic DNA used different primer combinations (Table 1): (1) L3002-H4419b, (2) L4160-H4980, (3) L4437-H5934, (4) L3878-H4980, (5) L3881-H5934, (6) L4221-H5934, and (7) L4437-H6564. Both strands were sequenced using the primers in Table 1. Primer numbers refer to the 3′ end on the

TABLE 1

Primers Used in This Study

Human position[a]	Gene	Sequence[b]	Reference
L3002	16S	5′-TACGACCTCGATGTTGGATCAGG-3′	Macey *et al.*, 1997a
L3428	ND1	5′-CGAAAAGGCCCAAACATTGTAGG-3′	This study
L3878	ND1	5′-GCCCCATTTGACCTCACAGAAGG-3′	Macey *et al.*, 1998b
L3881	ND1	5′-TTTGACCTAACAGAAGGAGA-3′	Macey *et al.*, 1997a
L4160	ND1	5′-CGATTCCGATATGACCARCT-3′	Kumazawa and Nishida, 1993
L4178	ND1	5′-CAACTAATACACCTACTATGAAA-3′	Macey *et al.*, 1997a
L4221	$tRNA^{Ile}$	5′-AAGGATTACTTTGATAGAGT-3′	Macey *et al.*, 1997a
H4419a	$tRNA^{Met}$	5′-GGTATGAGCCCAATTGCTT-3′	Macey *et al.*, 1997a
H4419b	$tRNA^{Met}$	5′-GGTATGAGCCCGATAGCTT-3′	Macey *et al.*, 1997a
L4437	$tRNA^{Met}$	5′-AAGCTTTCGGGCCCATACC-3′	Macey *et al.*, 1997a
L4645	ND2	5′-ACAGAAGCCGCAACAAAATA-3′	Macey *et al.*, 1997a
L4882	ND2	5′-TGACAAAAACTAGCCCC-3′	Schulte *et al.*, 1998
H4980	ND2	5′-ATTTTTCGTAGTTGGGTTTGRTT-3′	Macey *et al.*, 1997a
L5002	ND2	5′-AACCAAACCCAACTACGAAAAAT-3′	Macey *et al.*, 1997a
H5540	$tRNA^{Trp}$	5′-TTTAGGGCTTTGAAGGC-3′	Macey *et al.*, 1997a
L5556a	$tRNA^{Trp}$	5′-AAGAGCCTTCAAAGCCCTAAG-3′	Macey *et al.*, 1997a
L5556b	$tRNA^{Trp}$	5′-GCCTTCAAAGCCCTAAA-3′	Macey *et al.*, 1997a
L5617	$tRNA^{Ala}$	5′-AAAGTGTCTGAGTTGCATTCAG-3′	Macey *et al.*, 1997a
L5638	$tRNA^{Ala}$	5′-CTGAATGCAACTCAGACACTTT-3′	Macey *et al.*, 1997a
H5692	$tRNA^{Asn}$	5′-TTGGGTGTTTAGCTGTTAA-3′	Macey *et al.*, 1997a
H5934a	COI	5′-AGRGTGCCAATGTCTTTGTGRTT-3′	Macey *et al.*, 1997a
H5937a	COI	5′-GTGCCAATGTCTTTGTG-3′	Macey *et al.*, 1997a
H5937b	COI	5′-AGGGTTCCGATATCTTTRTG-3′	This study
H6564	COI	5′-GGGTCTCCTCCTCCAGCTGGGTC-3′	Macey *et al.*, 1998a

[a] Primers are designated by their 3′ ends which correspond to the position in the human mitochondrial genome (Anderson *et al.*, 1981) by convention. H and L designate heavy-strand and light-strand primers, respectively.

[b] Positions with mixed bases are labeled with the standard one-letter code: R = G or A.

human mitochondrial genome (Anderson *et al.*, 1981), where L and H correspond to light and heavy strands, respectively.

Phylogenetic Analysis

DNA sequences were aligned manually. Protein-coding sequences were translated to amino acids using MacClade (Maddison and Maddison, 1992) for confirmation of alignment. Transfer RNA secondary structure was determined manually using the criteria of Kumazawa and Nishida (1993) to ensure proper alignment (Macey and Verma, 1997). Positions of ambiguous alignment were excluded from phylogenetic analysis (see Results).

Phylogenetic trees were estimated using PAUP* beta version 4.0b1 (Swofford, 1998) with 100 heuristic searches featuring random addition of sequences. Bootstrap resampling was used to assess support for individual nodes with 1000 bootstrap replicates using 100 heuristic searches with random addition of sequences per replicate. Decay indices (="branch support" of Bremer, 1994) were calculated for all internal branches of the tree using two methods. First, 100 heuristic searches with random addition of sequences, which retained suboptimal trees, were run for nodes with decay indices of 1 to 15. For nodes with decay indices above 15, a phylogenetic topology containing the single node in question was constructed using MacClade (Maddison and Maddison, 1992) and analyzed as a constraint in PAUP* beta version 4.0b1 (Swofford, 1998) with 100 heuristic searches featuring random addition of sequences. In these searches, trees that did not contain the imposed constraint were retained. All searches conducted on allozymic data were exhaustive.

Wilcoxon signed-ranks tests (Felsenstein, 1985; Templeton, 1983) were used to examine statistical significance of the shortest tree relative to alternative hypotheses. This test asks whether the most parsimonious tree is significantly shorter than an alternative or whether their differences in length can be attributed to chance alone (Larson, 1998). Wilcoxon signed-ranks tests were conducted both as one- and two-tailed tests. Felsenstein (1985) showed that one-tailed probabilities are close to the exact probabilities for this test but not always conservative, whereas the two-tailed test is always conservative. Tests were conducted using PAUP* beta version 4.0b1 (Swofford, 1998), which incorporates a correction for tied ranks.

Alternative phylogenetic hypotheses were tested using the most parsimonious phylogenetic topologies compatible with them. To find the most parsimonious tree(s) compatible with a particular phylogenetic hypothesis, phylogenetic topologies were constructed us-

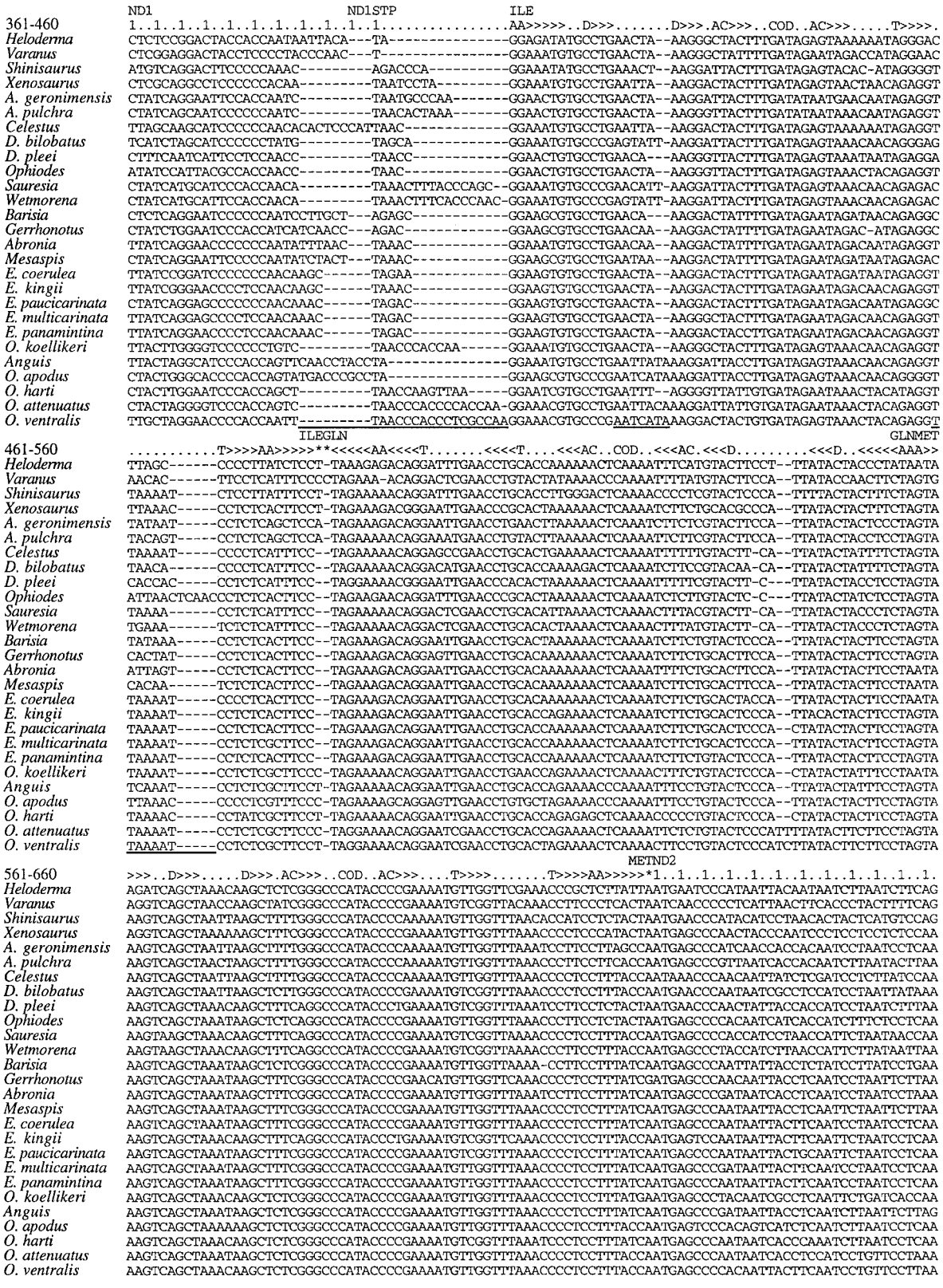

FIG. 1. Length-variable regions among the 2101 aligned mitochondrial DNA sequences as used in the phylogenetic analysis. Eleven regions totaling 100 positions are excluded from the analysis and are underlined. Positions 1–360 and 661–1600 from the ND1 and ND2 genes, respectively, are not shown because this region had no length variation except for a single codon deletion in *Heloderma* at positions 211–213. Sequences are presented as light-strand sequence and tRNA secondary structure is designated above the sequence. Stems are indicated by arrows in the direction encoded: AA, amino acid-acceptor stem; D, dihydrouridine stem; AC, anticodon stem; and T, TψC stem. The tRNA anticodons are designated COD. Asterisks indicate the unpaired 3′ tRNA position 73. Periods indicate bases located outside stem regions; 1 depicts the first codon position of protein-coding sequences. STP indicates stop codons.

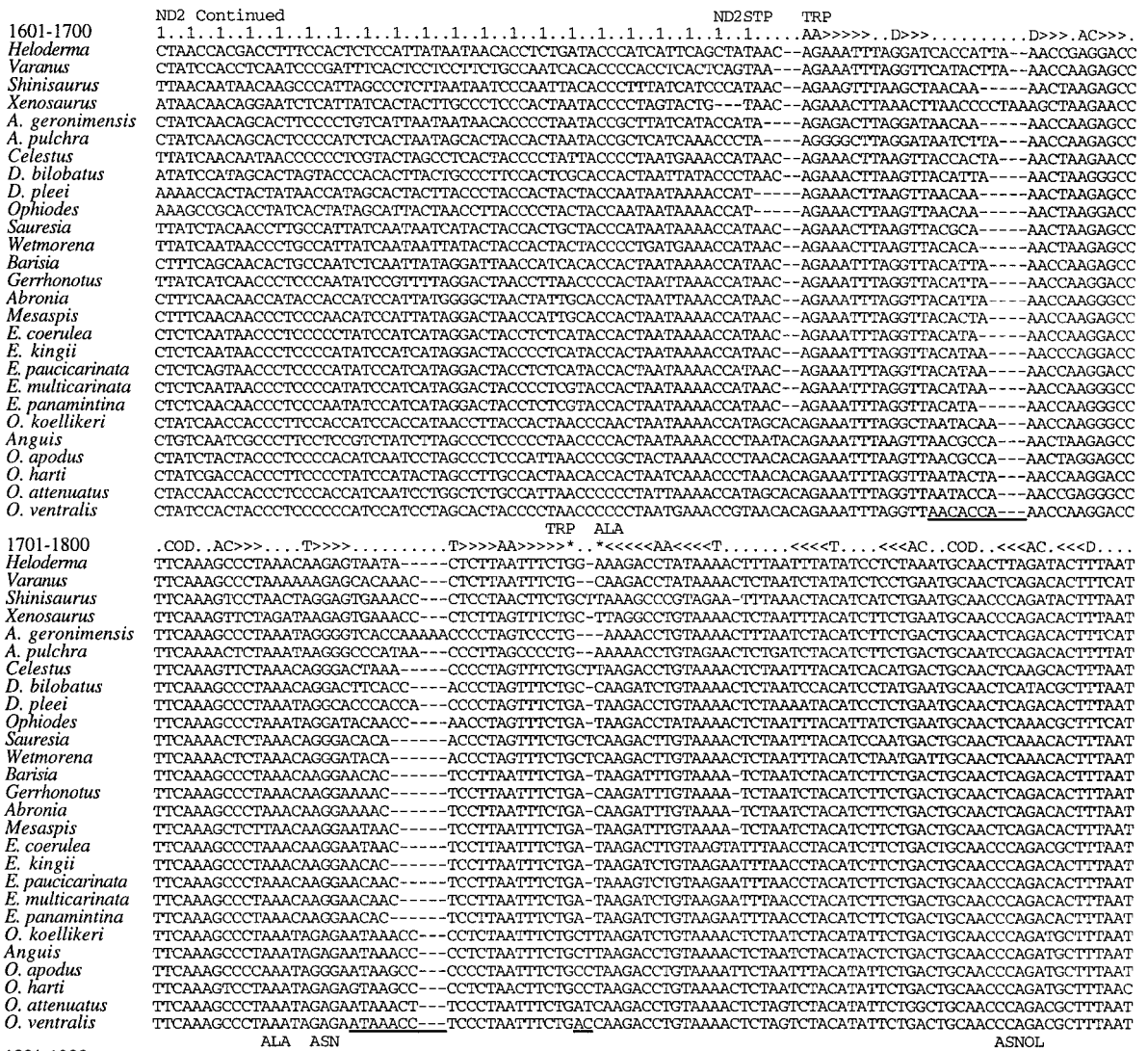

FIG. 1—*Continued*

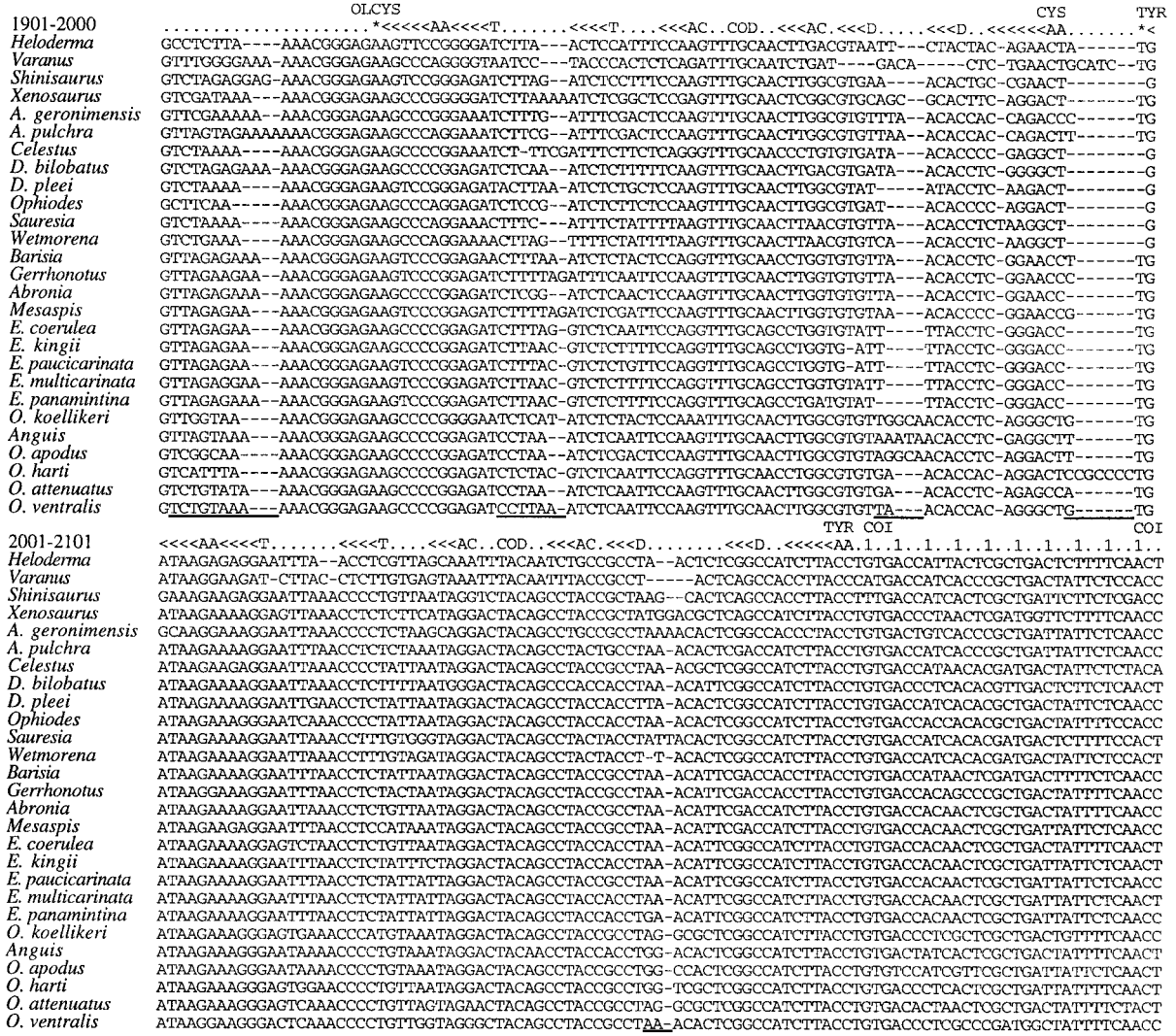

FIG. 1—*Continued*

ing MacClade (Maddison and Maddison, 1992) and analyzed as constraints using PAUP* beta version 4.0b1 (Swofford, 1998) with 100 heuristic searches with random addition of sequences.

Cladistic Analyses of Allozymic Data

Previously reported allozymic data of Good (1987, 1988) were coded in two ways for cladistic analysis. Although presence–absence coding of alleles has received considerable criticism for a lack of independence of alleles and the possibility of no allele being reconstructed for an ancestral node, it remains the method that provides the greatest amount of resolution. Alternatively, combinations of alleles for a particular locus may be coded as discrete character states (Buth, 1984). If step matrices are used to connect character states, a greater amount of information can be retained (Mabee and Humphries, 1993). In our analysis, step matrices were constructed on the basis of gains and losses of alleles. For example, a fixed difference between two alleles was counted as two steps, one allele lost and another gained. If a two-allele polymorphism in one population shares one allele with another monomorphic population, a single gain or loss was counted as one step. Additional polymorphisms were counted in the same manner.

In the allozymic data for the Gerrhonotinae (Good, 1987), no outgroup was completely scored. Instead, alleles found in *Abronia*, *Barisia*, and *Mesaspis* that were present in *Elgaria* were recorded. When no allele was reported for a particular locus in the outgroup, we inferred that at least one unique allele was present.

Phylogenetic analysis of tRNA stem regions used MacClade (Maddison and Maddison, 1992). The number of stem pairings was ordered in these analyses.

RESULTS

Sequences ranging in size from 2034 to 2061 bases of mitochondrial DNA for 27 taxa of anguimorph lizards are presented as 2101 aligned positions in Fig. 1.

Authentic Mitochondrial DNA

Several observations suggest that the DNA sequences analyzed here are from the mitochondrial genome and are not nuclear-integrated copies of mitochondrial genes (see Zhang and Hewitt, 1996). Protein-coding genes do not have premature stop codons, suggesting that these sequences represent functional copies that encode a protein. Transfer-RNA genes appear to code for tRNAs with stable secondary structures, indicating functional genes. The presence of strand bias further supports our conclusion that the 27 DNA sequences reported here are from the mitochondrial genome. The sequences reported here show strong strand bias against guanine on the light strand (G = 11–14%, A = 30–36%, T = 22–29%, and C = 25–35%), which is characteristic of the mitochondrial genome but not the nuclear genome. See Macey *et al.* (1997a,c, 1998a) for similar strand bias across most squamate-reptile families for the same region of the mitochondrial genome.

Assessment of Homology and Sequence Alignment

Sequences reported correspond to positions 3874 to 5936 on the human mitochondrial genome (Anderson *et al.*, 1981). This sequence contains the genes encoding ND1 (subunit one of NADH dehydrogenase), $tRNA^{Ile}$, $tRNA^{Gln}$, $tRNA^{Met}$, ND2, $tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, $tRNA^{Tyr}$, and COI (subunit I of cytochrome *c* oxidase), plus the O_L between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes (Fig. 1). Except for the last couple of codon positions in the ND1 and ND2 genes and a single deletion of a codon position in the ND1 gene of *Heloderma*, no length variation is found in protein-coding genes, making alignment straightforward. Among protein-coding sequences, only the last few amino acid positions encoding ND1, the stop codon, and noncoding sequences between the ND1 and $tRNA^{Ile}$ genes are excluded from phylogenetic analyses (positions 382–407) because of considerable length variation. Gaps are placed in the *Heloderma* sequence in positions 211–213 corresponding to codon position 71 of the ND1 gene fragment included in this study.

Among tRNA genes, a few loop regions are unalignable as are some noncoding sequences between genes. Phylogenetic analyses do not include regions encoding the dihydrouridine (D) and TψC (T) loops of the $tRNA^{Ile}$ (positions 421–427, 460–471), $tRNA^{Trp}$ (positions 1680–1689, 1721–1730), and $tRNA^{Cys}$ (positions 1973–1977, 1935–1941) genes. Part of the region encoding the D-loop of the $tRNA^{Tyr}$ gene (positions 2050–2052) also is excluded.

The $tRNA^{Cys}$ gene can be particularly problematic to align for phylogenetic analyses because gene sequences that do not encode a D-stem can have stem realignment in the AA- and T-stems (Macey *et al.*, 1997b). The previously published sequence for *Varanus griseus* (Macey *et al.*, 1997a,b) appears to be homologous to other sequences analyzed. Most of the bases from the D-arm replacement loop are placed in the excluded D-loop region in the alignment. In addition, the $tRNA^{Cys}$ gene sequences from *Elgaria kingii* and *E. paucicarinata* appear to have a T base deleted from the region encoding the D-stem and a gap is placed at position 1970. In the $tRNA^{Cys}$ gene, the T-stem in some taxa may be extended beyond the normal five pairs. The phylogenetic analysis includes only the five paired positions normally observed. *Sauresia* has an unusual $tRNA^{Cys}$ in which a T has been inserted in the region encoding the AA-stem, forcing three bases between the AA- and D-stems in the encoded tRNA. A gap is placed in all other taxa at position 1985.

In the $tRNA^{Gln}$ gene a deletion of an A from the AA-stem at position 486 appears to have occurred in *Varanus*. This deletion has resulted in a realigned T-stem. In the phylogenetic analysis a gap is placed in the *Varanus* sequence at position 492, and the *Varanus* sequence is aligned to the secondary structure observed in the other taxa.

Sequences between the $tRNA^{Trp}$ and the $tRNA^{Ala}$ genes and between the $tRNA^{Cys}$ and the $tRNA^{Tyr}$ genes (positions 1744–1745 and 1992–1998, respectively) are not used in the phylogenetic analyses. The loop region of the replication origin for the light strand is mostly unalignable and therefore not used (positions 1902–1912).

Among the 2101 aligned positions only 100 sites, constituting less than 5% of the aligned sequences, are excluded from the phylogenetic analyses.

Variation of Stems in $tRNA^{Cys}$

Tremendous variation in stem lengths occurs among the 27 $tRNA^{Cys}$ gene sequences reported here (Fig. 2) and in Macey *et al.* (1997b). Both the D- and T-stems show variation for the number of base pairings in stem regions that deviate from the typical four pairs in D-stems and five pairs in T-stems.

In *Varanus*, $tRNA^{Cys}$ is known to lack a D-stem and instead contains a D-arm replacement loop (Macey *et al.*, 1997b). This structure has been postulated to result from slipped-strand mispairing of noncontiguous repeats during replication (Fig. 2; Macey *et al.*, 1997b). The number of pairings in the D-stem varies among other taxa between one and six. *Ophisaurus koellikeri* has an unusually large six-base D-stem that contains two extra pairs. Three other taxa, *Xenosaurus* and the two *Anniella* species, have five-base D-stems, hence containing one extra pair. Most taxa (*Heloderma*, *Sau-*

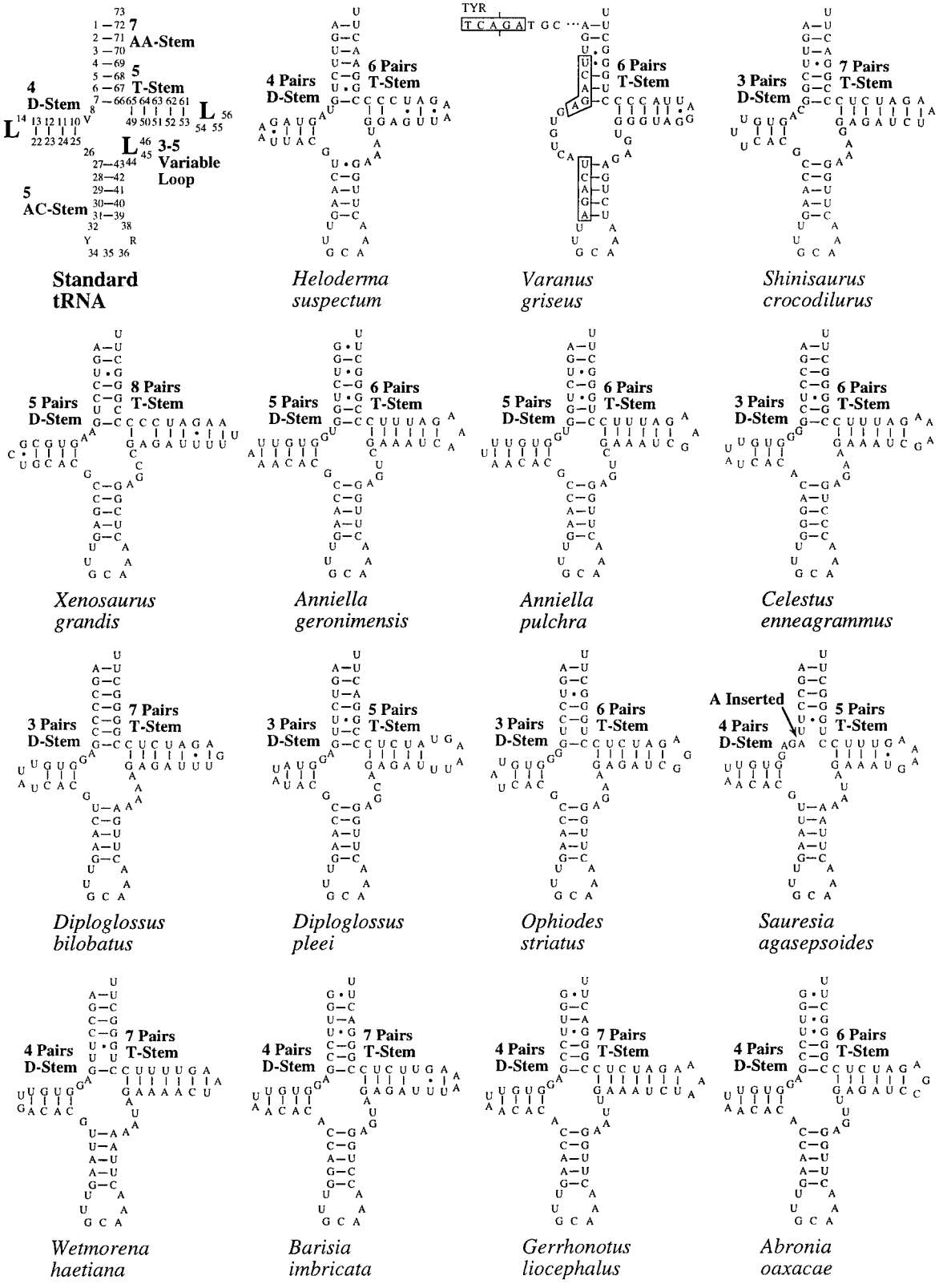

FIG. 2. Potential secondary structures derived from 27 $tRNA^{Cys}$ gene sequences presented in Fig. 1. A standard tRNA with a four-base D-stem and a five-base T-stem is depicted first, where R = G or A, Y = C or T, and V = G, C, or A (after Kumazawa and Nishida, 1993). L indicates the three loop regions where length variation is standardly observed. *Varanus* lacks a D-stem and instead contains a D-arm replacement loop. Bases boxed represent three potential noncontiguous repeats postulated to have resulted from slipped-strand mispairing during replication (Macey *et al.*, 1997b). *Sauresia* has an unusual $tRNA^{Cys}$ in which an A has been inserted in the AA-stem, forcing three bases between the AA- and D-stems instead of the two bases normally observed. Note the tremendous variation in sizes of both D- and T-stems. The position where an A was deleted destroying the D-stems in *Elgaria kingii* and *E. paucicarinata* is indicated.

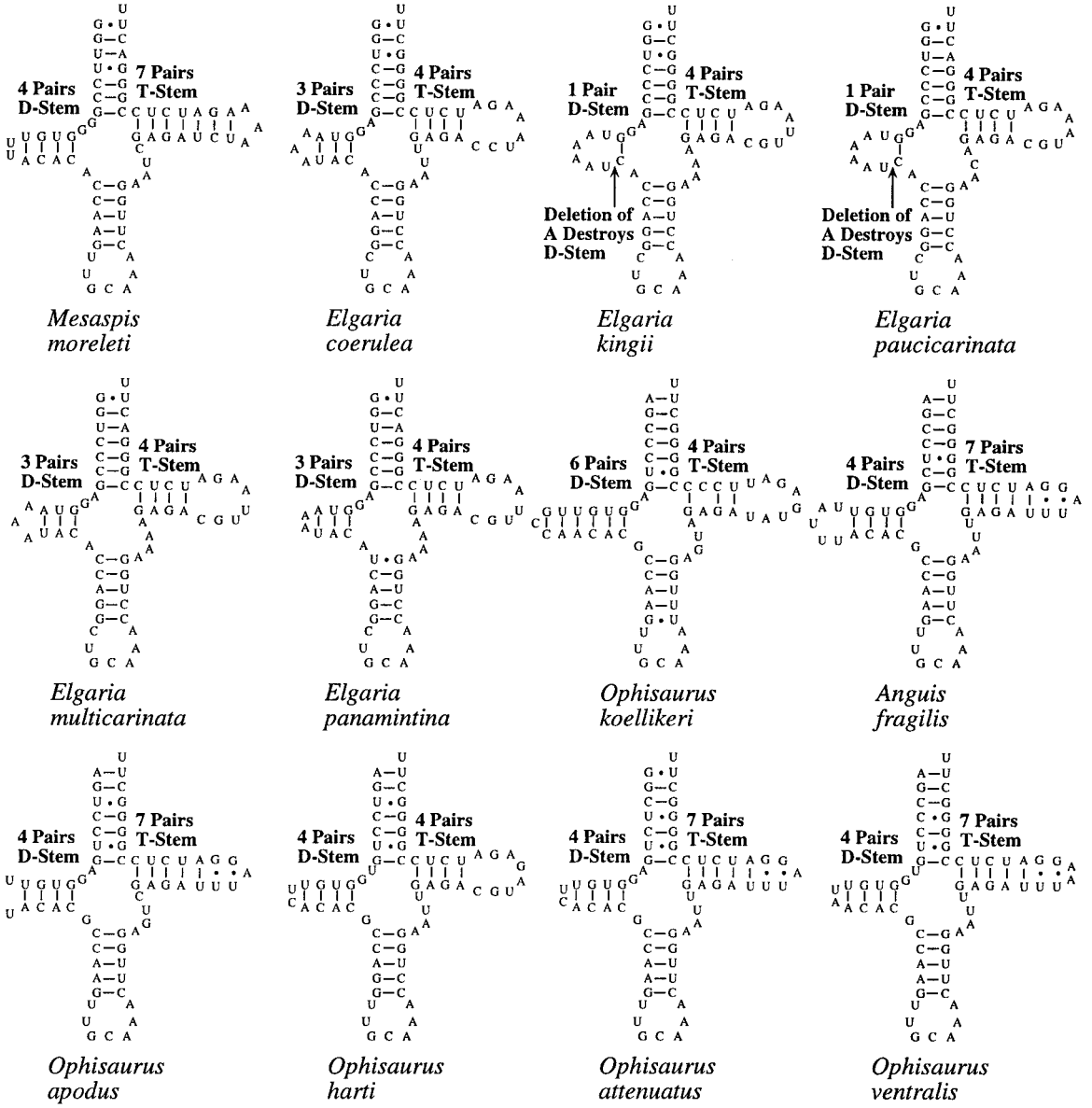

FIG. 2—*Continued*

resia, Wetmorena, Barisia, Gerrhonotus, Abronia, Mesaspis, Anguis, and all *Ophisaurus* species except *O. koellikeri*) have the normal four base pairings in the D-stem. Eight taxa (*Shinisaurus, Celestus, Diploglossus bilobatus, D. pleei, Ophiodes, Elgaria coerulea, E. multicarinata,* and *E. panamintina*) have a reduction of one pair to produce D-stems composed of three base pairings. Interestingly, *D. pleei* and *E. panamintina* have only two bases in the D-loop, which is less than the minimal three bases required to restore through base substitutions a fourth D-stem base pairing while retaining at least one D-loop base.

Only *Elgaria kingii* and *E. paucicarinata* are observed to have a single base pairing in the D-stem. These taxa have only six bases in the D-loop, which is less than the minimum of seven bases required to restore through base substitutions three additional base pairings in the D-stem while retaining at least one D-loop base. From comparison with the other $tRNA^{Cys}$ gene sequences, it appears that a T encoding an A in the tRNA is deleted, destroying the D-stem. In addition, no direct repeats or noncontiguous repeats are observed; such repeats are implicated, however, in the formation of D-arm replacement loops among eight other lepidosaurian taxa (Macey *et al.*, 1997b).

Striking variation is observed also in the number of base pairings among T-stems in $tRNA^{Cys}$ gene sequences (Fig. 2). Only two taxa, *Diploglossus pleei* and *Sauresia,* have the typical five base pairings in the T-stem. Instead, a surprising situation occurs in which

T-stems are either reduced by one base pairing to produce four pairs, or lengthened to as many as eight pairs. The five *Elgaria* species, *Ophisaurus koellikeri,* and *O. harti* have T-stems that have lost a single pairing, producing a T-stem with four base pairings. *Heloderma, Varanus, Anniella, Celestus, Ophiodes,* and *Abronia* all show an additional pair, producing an increase in size of the T-stem to six base pairings. *Shinisaurus, Diploglossus bilobatus, Wetmorena, Barisia, Gerrhonotus, Mesaspis, Anguis, Ophisaurus apodus, O. attenuatus,* and *O. ventralis* all show two extra base pairings to produce T-stems seven pairs in length, and *Xenosaurus* has three extra pairs to produce eight base pairs in the T-stem. Note that with the exception of *Heloderma, Varanus,* and *Sauresia,* all taxa that contain less than seven base pairings in the T-stem contain T-loops large enough to produce additional pairs through base substitutions that would result in enlarged T-stems of seven base pairs.

Genic Variation

Different levels of DNA substitutional variation are observed among the 3 protein-coding genes, 8 tRNA-coding genes, and four noncoding regions (Table 2). All 11 genes contain phylogenetically informative characters. The 8 tRNA genes each have phylogenetically informative sites in stem and nonstem regions. Each of the 3 protein-coding genes contains phylogenetic information in first, second, and third codon positions. Most of the variation and phylogenetically informative sites are from protein-coding regions. Only 23% of variable and 21% of phylogenetically informative sites are from tRNA genes and noncoding regions. Of the 802 phylogenetically informative characters from protein-coding regions, 448 are from third positions of codons. Third-position sites account for slightly less than half of the phylogenetically informative sites in the total data set. Only 150 phylogenetically informative sites occur in regions encoding stems of tRNAs, suggesting that compensatory substitutions do not compromise the phylogenetic analysis.

Phylogenetic Relationships

Two trees of equal length are produced from the parsimony analysis of the 2001 aligned DNA sequences containing 1013 phylogenetically informative base positions (Fig. 3, Table 2). Phylogenetic relationships are well resolved for most nodes of the tree. All ingroup taxa are grouped to the exclusion of the Varanoidea (the New World *Heloderma* and the Old World *Varanus*) with good support (bootstrap 82%, decay index 12). The Old World *Shinisaurus* and the New World *Xenosaurus,* often grouped as the Xenosauridae, appear not to form a monophyletic group. Instead, *Shinisaurus* is excluded from a monophyletic group containing *Xenosaurus,* the Anniellidae, and the Anguidae (bootstrap 91%, decay index 18). A monophyletic grouping of *Anniella* and the Anguidae receives considerable support (bootstrap 98%, decay index 29). A monophyletic *Anniella* (bootstrap 100%, decay index 51) forms the sister taxon to the Anguidae, which appears monophyletic but with weak support (decay index 3).

Within the Anguidae, the Diploglossinae appears monophyletic with weak support (decay index 4) and forms the sister taxon to a clade composed of the Gerrhonotinae and Anguinae (bootstrap 80%, decay index 6). Monophyly of both the Gerrhonotinae (bootstrap 100%, decay index 22) and Anguinae (bootstrap 100%, decay index 40) receives strong support.

In the Diploglossinae, *Celestus* and *Diploglossus bilobatus* from mainland Mexico and Central America form a monophyletic group with good support (bootstrap 93%, decay index 13). A second clade comprising West Indian and South American taxa, *Diploglossus pleei, Ophiodes, Sauresia,* and *Wetmorena* (bootstrap 82%, decay index 9) can be recognized in the Diploglossinae. Two well-supported groups are observed within this clade: (1) the Puerto Rican *D. pleei* and South American *Ophiodes* (bootstrap 100%, decay index 47), and (2) the Hispaniolan *Sauresia* and *Wetmorena* (bootstrap 100%, decay index 102).

Two clades are recognized in the Gerrhonotinae, one containing the largely Neotropical *Barisia, Gerrhonotus, Abronia,* and *Mesaspis* (bootstrap 69%, decay index 4), and the other composed of the five species from the more temperate North American genus *Elgaria* (bootstrap 100%, decay index 39). In the tropical group, only the sister-taxon relationship of *Abronia* and *Mesaspis* is recovered with strong support (bootstrap 99%, decay index 17). Among the five species of *Elgaria,* all branches are well supported. *Elgaria coerulea,* the most northern gerrhonotine, is the sister taxon to a group comprising the remaining species (bootstrap 100%, decay index 29). The more eastern species, *E. kingii,* is the sister taxon to a monophyletic group of *E. paucicarinata, E. multicarinata,* and *E. panamintina* (bootstrap 94%, decay index 9). *Elgaria multicarinata* and *E. panamintina* from California form a monophyletic group (bootstrap 98%, decay index 8).

In the Anguinae, most relationships are not well supported. *Ophisaurus koellikeri* from Morocco forms the sister taxon to the remaining species (decay index 1). A monophyletic grouping of the western Eurasian *Ophisaurus apodus* and *Anguis fragilis* is well supported (bootstrap 100%, decay index 24) and forms the sister taxon to a weakly supported clade composed of the East Asian *O. harti* and the North American *O. attenuatus* and *O. ventralis* (decay index 1). North American *Ophisaurus* appear monophyletic with moderate support (bootstrap 77%, decay index 7).

Phylogenetic relationships among the Anguidae, Anniellidae, and Xenosauridae resolved from reanalysis of allozymic data (Good, 1987, 1988) are largely the same whether analyzed with allelic combinations as charac-

TABLE 2

Distribution of Phylogenetically Informative and Variable Positions

	ND1 Codon positions			$tRNA^{Ile}$ [a]		$tRNA^{Gln}$		$tRNA^{Met}$		ND2 Codon positions		
	1st	2nd	3rd	Stem	Non-stem	Stem	Non-stem	Stem	Non-stem	1st	2nd	3rd
Informative sites	44	22	118	19	6	18	10	13	6	188	96	321
Variable sites	59	34	125	25	7	28	16	19	9	236	150	340

	Noncoding[b] region 1	$tRNA^{Trp}$ [a]		$tRNA^{Ala}$		Noncoding[b] region 2	$tRNA^{Asn}$	
		Stem	Non-stem	Stem	Non-stem		Stem	Non-stem
Informative sites	1	23	2	19	8	1	13	7
Variable sites	2	28	5	30	12	1	16	10

	Noncoding[b] region 3	$tRNA^{Cys}$ [a]		$tRNA^{Tyr}$ [c]		Noncoding[b] region 4	COI Codon positions		
		Stem	Non-stem	Stem	Non-stem		1st	2nd	3rd
Informative sites	—	26	7	19	13	—	3	1	9
Variable sites	2	30	8	29	15	1	5	2	9

	Protein-coding codon positions			tRNA coding			
Total	1st	2nd	3rd	Stem	Non-stem	Noncoding regions	All aligned sequence
Informative sites	235	119	448	150	59	2	1013
Variable sites	300	186	474	205	82	6	1253

[a] Not including D- and T-loops which were excluded from the analyses.
[b] Noncoding region 1 includes the ND2 stop codon and sequences between the ND2 and the $tRNA^{Trp}$ genes. Noncoding region 2 is between the $tRNA^{Ala}$ and the $tRNA^{Asn}$ genes. Noncoding region 3 is between the $tRNA^{Asn}$ gene and the O_L. Noncoding region 4 is between the $tRNA^{Tyr}$ and the COI genes.
[c] Not including part of the D-loop, which was excluded from the analyses.

ter states using step matrices or presence/absence coding of alleles (Fig. 4). In the survey of higher-level taxa (Good, 1987), a topology is acquired that is completely concordant with the DNA sequence data (Figs. 4A and 4B). When the data are coded using allelic combinations as character states and analyzed with step matrices, only two loci provide phylogenetic information. Monophyly of the Anguidae (*Celestus*, *Elgaria*, and *Ophisaurus*) is supported with a bootstrap value of 79% and a decay index of 2, and monophyly of a group composed of the Gerrhonotinae (*Elgaria*) and Anguinae (*Ophisaurus*) is supported by a bootstrap value of 72% and a decay index of 1. The same phylogenetic relationships result from presence/absence coding of individual alleles. The 20 informative alleles in the latter analysis provide considerably better support. Monophyly of the Anguidae is supported with a bootstrap value of 99% and a decay index of 9, and monophyly of a group containing the Gerrhonotinae and Anguinae is supported by a bootstrap value of 96% and a decay index of 3.

The phylogenetic relationships between the five *Elgaria* species resolved from reanalysis of allozymic data (Good, 1988) are in conflict with the result of the DNA analysis (Figs. 4C and 4D). When the data are coded using allelic combinations as character states and analyzed with step matrices, five loci provide phylogenetic information. This analysis produces two equally most parsimonious trees in which the phylogenetic relationships of *E. coerulea* and *E. multicarinata* remain unresolved. Monophyly of a group containing *E. paucicarinata*, *E. kingii*, and *E. panamintina* is weakly supported (bootstrap 66%, decay index 1). Monophyly of a group containing *E. kingii* and *E. panamintina* receives better support (bootstrap 80%, decay index 2). A similar phylogenetic tree is resolved from presence/absence coding of individual alleles. The 23 informative alleles in the latter analysis provide considerably better support. In this analysis, *E. coerulea* is excluded from a monophyletic group composed of *E. multicarinata*, *E. paucicarinata*, *E. kingii*, and *E. panamintina* (bootstrap 84%, decay index 3). Monophyly of a group containing *E. paucicarinata*, *E. kingii*, and *E. panamintina* is not well supported (bootstrap 69%, decay index 1) but the grouping of *E. kingii* and *E. panamintina* receives good support (bootstrap 98%, decay index 6). Disagreement between analyses of the DNA sequence data and allozymic data for *Elgaria* species

occurs regarding the relative positions of *E. kingii, E. multicarinata, E. panamintina,* and *E. paucicarinata.*

The phylogenetic results provide an area cladogram for the Holarctic region. To confirm these results and to test support for the origins of clades found in separate historical regions, the Wilcoxon signed-ranks test (Felsenstein, 1985; Templeton, 1983) is applied (Table 3).

(1). Current taxonomy places *Shinisaurus* and *Xenosaurus* in a single family, the Xenosauridae. When the two shortest trees overall (A1–2 in Appendix 1) showing a nonmonophyletic Xenosauridae are compared to the shortest alternative trees (B1–4 in Appendix 1) showing a monophyletic Xenosauridae, this alternative is rejected in favor of the overall shortest trees by either the one-tailed or the two-tailed test (test 1 in Table 3). This result suggests that the Xenosauridae is not monophyletic.

(2). The two overall shortest trees from analysis of the DNA sequence data (Fig. 3) show that the Anniellidae, Anguidae, Diploglossinae, Gerrhonotinae, and Anguinae each form monophyletic groups. In addition, the overall shortest tree from analysis of allozymic data also shows monophyly of the Anguidae. When the two overall shortest trees (A1–2 in Appendix 1) from analysis of the DNA sequence data, which show a monophyletic Anniellidae, are compared to the shortest alternative trees (C1–2 in Appendix 1) having a nonmonophyletic Anniellidae, this alternative is rejected in favor of the overall shortest trees by the two-tailed test (test 2 in Table 3). Because the decay index on the branch leading to a monophyletic Anguidae is only 3 from analysis of the DNA sequence data, this branch cannot receive statistical support from the Wilcoxon signed-ranks test which requires at least 4 unopposed characters to be significant (Felsenstein, 1985).

When the overall shortest allozymic tree (Fig. 4B, allele presence/absence coded), which shows a monophy-

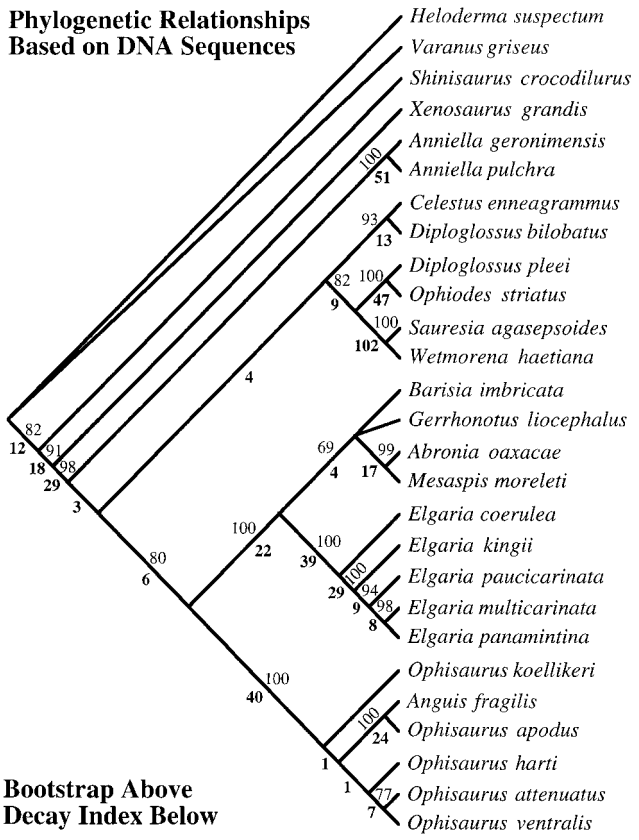

FIG. 3. Phylogenetic relationships based on DNA sequences. Strict consensus of two equally most parsimonious trees produced from analysis of the 2001 aligned (1013 phylogenetically informative) positions. The tree has a length of 5452 steps and a consistency index of 0.394. Bootstrap values are presented above branches and decay indices below branches.

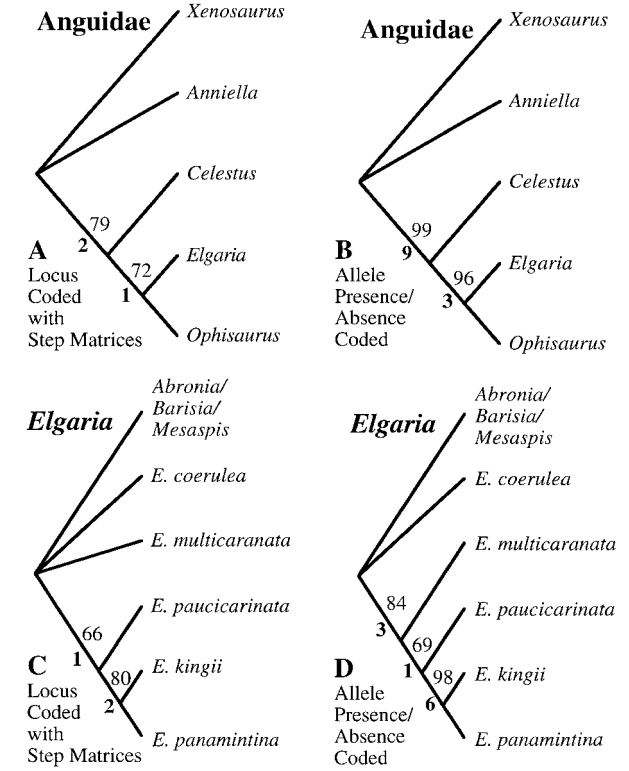

FIG. 4. Phylogenetic trees from analyses of allozymic data from the literature (Good, 1987, 1988). Bootstrap values are presented above branches and decay indices below branches. (A) The most parsimonious tree from our analysis of Good's (1987) data using allelic combinations as character states and analyzed with step matrices. Two of the 22 loci are phylogenetically informative. The tree has a length of 91 steps and a consistency index of 0.100. (B) The most parsimonious tree from our analysis of Good's (1987) data coded by the presence or absence of the 72 (20 informative) individual alleles. The tree has a length of 76 steps and a consistency index of 0.921. (C) Strict consensus tree of two equally most parsimonious trees from our analysis of Good's (1988) data using allelic combinations as character states and analyzed with step matrices. Five of the 18 loci are phylogenetically informative. The tree has a length of 88 steps and a consistency index of 0.955. (D) The most parsimonious tree from our analysis of Good's (1988) data coded by the presence or absence of the 67 (23 informative) individual alleles. The tree has a length of 75 steps and a consistency index of 0.867.

TABLE 3

Results of Wilcoxon Signed-Ranks Tests

Alternative hypotheses tested	Trees[a]	*N*[b]	Z[c]	P[d]
1. Monophyly of Xenosauridae	A1 vs B1	173	1.79	0.0370*
	A1 vs B2	149	1.97	0.0244**
	A1 vs B3	93	2.59	0.0048**
	A1 vs B4	119	2.14	0.0164**
	A2 vs B1	148	2.00	0.0230**
	A2 vs B2	173	1.84	0.0330*
	A2 vs B3	115	2.27	0.0115**
	A2 vs B4	93	2.59	0.0048**
2. Nonmonophyly of Anniellidae	A1 vs C1	188	3.63	0.0002**
	A1 vs C2	207	3.49	0.0003**
	A2 vs C1	159	3.97	0.0001**
	A2 vs C2	180	3.71	0.0001**
3. Nonmonophyly of Anguidae[e]	Fig. 4*B* vs D1	13	2.50	0.0063**
4. Nonmonophyly of Diploglossinae	A1 vs E1	125	0.35	0.3619
	A2 vs E1	93	0.41	0.3416
5. Nonmonophyly of Gerrhonotinae	A1 vs F1	72	2.59	0.0067**
	A1 vs F2	68	2.67	0.0055**
	A2 vs F1	36	3.67	0.0001**
	A2 vs F2	36	3.67	0.0001**
6. Nonmonophyly of Anguinae	A1 vs G1	187	2.82	0.0043**
	A1 vs G2	188	2.83	0.0054**
	A1 vs G3	193	2.73	0.0031**
	A2 vs G1	156	3.09	0.0010**
	A2 vs G2	159	3.04	0.0012**
	A2 vs G3	169	3.00	0.0014**
7. *Anniella* as the sister taxon to the Anguinae	A1 vs H1	119	1.38	0.0846
	A2 vs H1	89	1.59	0.0559
8. *Anniella* and *Anguis* form a monophyletic group	A1 vs I1	228	6.01	0.0001**
	A1 vs I2	204	6.29	0.0001**
	A2 vs I1	204	6.36	0.0001**
	A2 vs I2	235	5.90	0.0001**
9. *Ophiodes* and *Ophisaurus koellikeri* form a monophyletic group	A1 vs J1	289	8.79	0.0001**
	A2 vs J1	308	8.62	0.0001**
10. *Diploglossus pleei, Sauresia,* and *Wetmorena* form a monophyletic group	A1 vs K1	81	5.67	0.0001**
	A1 vs K2	114	4.60	0.0001**
	A2 vs K1	111	4.84	0.0001**
	A2 vs K2	81	5.67	0.0001**
11. *Ophisaurus koellikeri, Anguis fragilis* and *Ophisaurus apodus* form a monophyletic group	A1 vs L1	86	0.74	0.2291
	A1 vs L2	62	0.87	0.1927
	A1 vs L3	31	1.26	0.1044
	A2 vs L1	57	0.93	0.1769
	A2 vs L2	31	1.26	0.1044
	A2 vs L3	61	0.90	0.1851
12. *Anguis* and *Ophisaurus apodus* are not sister taxa	A1 vs M1	80	2.68	0.0037**
	A2 vs M1	112	2.21	0.0135**
13. *Elgaria kingii* and *E. panamintina* form a monophyletic sister group to *E. paucicarinata*	A1 vs N1	36	4.33	0.0001**
	A2 vs N1	68	3.15	0.0001**
14. *Elgaria multicarinata*[e] and *E. panamintina* form a monophyletic sister group to *E. paucicarinata*	Fig. 4*D* vs O1	11	2.71	0.0036**

[a] See Appendix 1 for phylogenetic topologies used in tests.
[b] Number of characters differing in minimum numbers of changes on paired topologies.
[c] Normal approximation for Wilcoxon signed-ranks test.
[d] Asterisk indicate a significant difference between the overall shortest tree and an alternative tree. One asterisk denotes significance using the one-tailed probability only and two asterisks denote significance using the two-tailed probability for the Wilcoxon signed-ranks test. One-tailed probabilities are shown and two-tailed probabilities are double these values. A significant result means that the alternative hypothesis as stated can be rejected.
[e] Tests using allozymic data; all other tests are done on DNA sequence data.

TABLE 4

Pairwise Comparisons of DNA Sequences among the Anguidae and Related Taxa[a]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1. *Heloderma*	—	30.3%	29.5%	28.8%	29.3%	29.6%	30.3%	30.9%	29.5%	28.9%	29.5%	30.1%	28.0%	27.4%	27.2%	27.8%	28.0%	27.7%	28.1%	27.8%	27.4%	28.2%	28.8%	29.5%	28.2%	29.3%	28.6%
2. *Varanus*	597	—	31.3%	31.0%	31.8%	30.8%	32.4%	32.8%	31.1%	31.9%	31.7%	31.0%	31.3%	31.9%	30.6%	31.5%	30.8%	30.6%	30.7%	30.7%	30.5%	31.4%	31.4%	30.5%	30.7%	30.6%	30.2%
3. *Shinisaurus*	584	618	—	29.6%	27.0%	27.9%	27.1%	28.4%	27.7%	28.1%	28.2%	28.2%	27.1%	27.8%	27.3%	27.4%	27.3%	27.5%	27.4%	27.2%	27.1%	27.8%	28.0%	28.9%	28.5%	29.2%	27.8%
4. *Xenosaurus*	571	611	586	—	27.1%	25.2%	26.6%	27.4%	26.0%	25.5%	27.4%	26.9%	25.8%	25.4%	24.9%	25.7%	25.3%	25.5%	25.7%	25.9%	25.6%	26.1%	26.4%	26.6%	26.1%	26.2%	25.5%
5. *A. geronimensis*	580	627	536	537	—	12.2%	21.3%	24.4%	23.0%	22.7%	23.3%	22.7%	20.4%	20.5%	20.5%	20.8%	20.0%	20.8%	20.5%	20.5%	20.1%	21.3%	20.9%	22.1%	21.1%	21.7%	20.2%
6. *A. pulchra*	588	608	553	500	243	—	21.4%	23.4%	23.1%	22.8%	23.7%	22.7%	21.3%	20.4%	20.2%	20.5%	20.4%	21.3%	21.1%	21.1%	21.2%	21.4%	20.7%	21.9%	21.0%	21.2%	20.4%
7. *Celestus*	600	639	537	527	422	425	—	21.7%	21.5%	21.9%	22.8%	22.7%	22.0%	21.8%	22.0%	22.8%	21.6%	21.6%	21.6%	21.0%	21.1%	22.8%	22.4%	23.8%	23.6%	23.4%	22.1%
8. *D. bilobatus*	613	647	564	543	483	464	431	—	23.1%	23.5%	25.3%	24.3%	23.8%	22.3%	22.5%	23.1%	23.2%	23.5%	23.8%	23.5%	23.3%	23.2%	23.3%	24.4%	24.2%	22.8%	23.5%
9. *D. pleei*	584	613	548	514	455	458	427	457	—	14.4%	22.3%	20.9%	22.7%	22.5%	21.8%	23.3%	22.4%	22.5%	22.7%	22.5%	22.3%	22.8%	22.5%	22.9%	23.0%	23.2%	22.3%
10. *Ophiodes*	571	628	557	506	451	452	434	466	286	—	22.3%	21.7%	22.8%	22.4%	22.2%	23.6%	21.9%	22.1%	22.4%	22.2%	22.0%	23.4%	22.5%	22.7%	22.1%	23.2%	20.9%
11. *Sauresia*	585	625	560	543	463	471	453	502	443	442	—	9.1%	23.6%	24.2%	24.0%	24.3%	23.6%	24.0%	24.7%	24.1%	24.2%	24.4%	24.4%	24.0%	24.2%	23.9%	22.5%
12. *Wetmorena*	597	611	559	533	450	451	452	483	414	430	181	—	22.3%	23.2%	22.3%	22.7%	22.9%	23.1%	23.3%	23.5%	23.3%	23.9%	23.1%	23.5%	23.5%	23.5%	21.7%
13. *Barisia*	556	617	537	513	405	423	437	472	449	451	468	442	—	13.6%	13.7%	15.6%	14.0%	14.4%	14.4%	14.3%	13.8%	18.6%	18.6%	20.0%	20.0%	18.3%	17.9%
14. *Gerrhonotus*	544	630	551	505	407	405	432	442	445	443	481	460	271	—	13.0%	14.3%	14.2%	14.6%	15.1%	15.3%	14.8%	19.4%	19.4%	20.7%	19.9%	18.6%	18.6%
15. *Abronia*	540	604	542	495	407	402	437	446	433	441	476	442	273	258	—	11.8%	13.8%	15.5%	15.1%	14.7%	14.7%	18.0%	17.8%	19.7%	19.6%	17.6%	17.7%
16. *Mesaspis*	552	622	544	511	412	407	453	459	462	467	482	450	310	285	234	—	15.3%	15.7%	15.4%	15.5%	15.1%	20.0%	19.6%	21.3%	20.7%	19.4%	19.1%
17. *E. coerulea*	556	609	541	503	398	405	430	461	444	435	469	455	279	283	275	304	—	8.6%	8.8%	8.4%	8.5%	18.3%	17.2%	19.1%	18.7%	18.0%	17.3%
18. *E. kingii*	549	605	546	507	412	424	429	467	446	439	477	459	286	290	308	312	171	—	5.9%	4.8%	4.8%	19.1%	18.4%	19.8%	18.9%	19.2%	17.8%
19. *E. paucicarinata*	557	607	543	510	407	419	430	473	450	444	491	462	287	299	300	307	175	117	—	4.4%	4.5%	18.9%	18.9%	20.3%	19.0%	19.3%	18.0%
20. *E. multicarinata*	551	606	540	515	407	420	418	466	446	441	479	467	285	304	293	309	167	95	87	—	2.0%	18.3%	18.0%	19.8%	18.8%	18.6%	17.9%
21. *E. panamintina*	544	603	538	509	400	421	420	462	442	436	481	463	274	295	292	300	170	96	90	39	—	18.3%	18.5%	19.8%	19.1%	18.6%	18.1%
22. *O. koellikeri*	559	620	552	518	423	426	454	461	453	464	484	475	370	386	357	397	364	379	376	364	365	—	13.5%	15.8%	14.7%	14.4%	13.6%
23. *Anguis*	572	620	556	524	415	412	446	462	447	447	485	459	369	385	354	390	342	365	375	358	369	269	—	11.7%	14.8%	14.7%	12.8%
24. *O. apodus*	585	603	573	528	439	436	473	485	455	451	477	467	398	411	392	424	380	393	404	393	393	315	233	—	15.6%	16.1%	14.1%
25. *O. harti*	560	606	566	519	420	418	469	480	457	439	481	468	397	395	389	411	372	376	377	375	379	292	295	311	—	14.0%	13.3%
26. *O. attenuatus*	582	605	579	521	431	421	466	453	460	461	476	467	363	370	350	385	359	382	384	370	370	286	293	320	278	—	11.6%
27. *O. ventralis*	567	597	552	506	401	406	439	466	442	415	447	432	355	370	351	380	345	354	357	356	360	271	255	281	265	231	—

[a] Percentage sequence divergence is shown above the diagonal and number of base substitutions between sequences is shown below the diagonal. Taxa are abbreviated with *A.* representing *Anniella*, *D.* representing *Diploglossus*, *E.* representing *Elgaria*, and *O.* representing *Ophisaurus*.

letic Anguidae, is compared to the shortest alternative tree (D1 in Appendix 1) having a nonmonophyletic Anguidae, this alternative is rejected using the allele presence/absence coded data (Good, 1987) in favor of the overall shortest tree by the two-tailed test (test 2 in Table 3).

When the two overall shortest trees (A1–2 in Appendix 1) from analysis of the DNA sequence data, which show a monophyletic Diploglossinae, are compared to the shortest alternative tree (E1 in Appendix 1) showing a nonmonophyletic Diploglossinae, this alternative cannot be rejected in favor of the overall shortest trees (test 4 in Table 3). When the two overall shortest trees (A1–2 in Appendix 1) from analysis of the DNA sequence data, which show a monophyletic Gerrhonotinae, are compared to the shortest alternative trees (F1–2 in Appendix 1) showing a nonmonophyletic Gerrhonotinae, this alternative is rejected in favor of the overall shortest trees by the two-tailed test (test 5 in Table 3). When the two overall shortest trees (A1–2 in Appendix 1) from analysis of the DNA sequence data, which show a monophyletic Anguinae, are compared to the shortest alternative trees (G1–3 in Appendix 1) showing a nonmonophyletic Anguinae, this alternative is rejected in favor of the overall shortest trees by the two-tailed test (test 6 in Table 3). Hence, the Anniellidae, Anguidae, Gerrhonotinae, and Anguinae each form monophyletic groups with statistical support, but statistical support is lacking for monophyly of the Diploglossinae.

(3). The phylogenetic position of *Anniella* is a point of disagreement. When the two overall shortest trees (A1–2 in Appendix 1), which show *Anniella* as the sister taxon to the Anguidae, are compared to the shortest alternative tree (H1 in Appendix 1) showing *Anniella* as the sister taxon to the Anguinae, this alternative cannot be rejected in favor of the overall shortest trees, but it is close to significance using the one-tailed test (test 7 in Table 3). When the two overall shortest trees (A1–2 in Appendix 1), which show *Anniella* as the sister taxon to the Anguidae, are compared to the shortest alternative trees (I1–2 in Appendix 1) showing the hypothesis of Keqin and Norell (1998) that *Anniella* is the sister taxon to *Anguis*, this alternative is rejected in favor of the overall shortest trees using the two-tailed test (test 8 in Table 3). *Anniella* is unlikely to represent the sister taxon to either Anguinae or *Anguis*.

(4). Only *Ophisaurus koellikeri* from Morocco and *Ophiodes* from South America are endemic to Gondwanan continents. When the two overall shortest trees (A1–2 in Appendix 1), in which *O. koellikeri* and *Ophiodes* do not form a monophyletic group, are compared to the shortest alternative tree (J1 in Appendix 1) showing them as sister taxa, this alternative is rejected in favor of the overall shortest trees using the two-tailed test (test 9 in Table 3). Taxa inhabiting Gondwanan plates therefore do not share a common Gondwanan origin.

(5). West Indian taxa appear not to form a monophyletic group. When the two overall shortest trees (A1–2 in Appendix 1), in which West Indian taxa (*Diploglossus pleei*, *Sauresia*, and *Wetmorena*) do not form a monophyletic group, are compared to the shortest alternative trees (K1–2 in Appendix 1) showing a monophyletic grouping of these taxa, this alternative is rejected in favor of the overall shortest trees using the two-tailed test (test 10 in Table 3). These results indicate that the South American genus *Ophiodes* is derived from the West Indies.

(6). The three Old World taxa that occur between the Atlantic Ocean and the Tibetan Plateau, *Ophisaurus koellikeri*, *Anguis fragilis*, and *O. apodus*, are found not to form a monophyletic group. When the overall shortest trees (A1–2 in Appendix 1), in which *Ophisaurus koellikeri*, *Anguis fragilis*, and *O. apodus* do not form a monophyletic group, are compared to the shortest alternative trees (L1–3 in Appendix 1) showing these species as a monophyletic group, this alternative costs seven steps but cannot be rejected in favor of the overall shortest trees (test 11 in Table 3). The most parsimonious trees suggest that the history of anguine lizards in western Eurasia and Morocco is older than anguine history in North America, contrary to the alternative hypothesis grouping taxa from western Eurasia and Morocco as closest relatives. *Anguis fragilis* and *O. apodus* form a monophyletic group. When the two overall shortest trees (A1–2 in Appendix 1), which group *A. fragilis* and *O. apodus* as sister taxa, are compared to the shortest alternative tree (M1 in Appendix 1) in which these species are not sister taxa, this alternative is rejected in favor of the overall shortest trees using the two-tailed test (test 12 in Table 3). As currently recognized, *Ophisaurus* is not monophyletic.

(7). The only point of disagreement between the analyses of the DNA sequence data and allozymic data is the relative placement of *Elgaria kingii*, *E. multicarinata*, *E. panamintina*, and *E. paucicarinata*. When the overall shortest DNA trees (A1–2 in Appendix 1), which show *E. multicarinata* and *E. panamintina* as a monophyletic sister group to *E. paucicarinata*, are compared to the shortest alternative tree (N1 in Appendix 1) showing *E. kingii* and *E. panamintina* as a monophyletic sister group to *E. paucicarinata*, this alternative is rejected by the DNA sequence data in favor of the overall shortest trees using the two-tailed test (test 13 in Table 3). When the shortest allozymic tree (Fig. 4D, allele presence/absence coded) showing *E. kingii* and *E. panamintina* as a monophyletic sister group to *E. paucicarinata* is compared to the shortest alternative tree (O1 in Appendix 1) showing *E. multicarinata* and *E. panamintina* as a monophyletic sister group to *E. paucicarinata*, this alternative is rejected by the allele presence/absence coded data (Good, 1988) in favor of

the overall shortest tree using the two-tailed test (test 14 in Table 3). The DNA sequence and allozymic data are in conflict with regard to the relative grouping of the species *Elgaria kingii, E. multicarinata, E. panamintina,* and *E. paucicarinata.* Two explanations for this discordance can be given. First, it is possible that the mitochondrial genome has undergone lineage sorting (Pamilo and Nei, 1988) and is misleading phylogenetically. Alternatively, the allozymic data of Good (1988) may be misleading because of small sample size; the sample size for *E. paucicarinata* is four individuals, *E. kingii* is two individuals, and for *E. panamintina* is only one individual. These sample sizes are not adequate for evaluating occurrence of alleles in a population or a species. Further work is needed to confirm our results inferred from mitochondrial DNA sequences, but we suggest that this hypothesis of phylogeny is the more reliable estimate.

DISCUSSION

Phylogeny and Biogeography of Extant Forms and the Fossil Record

Anguid lizards are inferred to have originated in the northern hemisphere. Our data considered in light of biogeographic and paleontological evidence clearly reject a Gondwanan origin for the Anguidae. No anguid fossils are known from tectonic regions of Gondwanan origin. Only two taxa occur exclusively on separate Gondwanan continents, *Ophiodes* in South America and *Ophisaurus koellikeri* in Morocco (Fig. 5). A sister group relationship between these taxa is statistically rejected, indicating that they do not share a common Gondwanan origin. The molecular phylogenetic analysis places these taxa in different clades of Laurasian origin in the northern hemisphere. *Ophiodes* is nested within West Indian diploglossines and monophyly of West Indian diploglossines is statistically rejected, indicating that *Ophiodes* descends from a lineage that originated in the West Indies and subsequently moved to South America. *Ophisaurus koellikeri* appears to be the sister taxon to the remaining members of the Anguinae. All other anguines occur in Europe, West Asia, East Asia, or North America, which consist primarily of Laurasian plates. Because other anguids, anniellids, and *Xenosaurus* occur in North America, the Anguinae is nested within a clade of northern forms of Laurasian origin (Fig. 5).

The opening of the Atlantic Ocean in the late Eocene [50 million years before present (MYBP)] may have produced the divergence between the Anguinae and the Gerrhonotinae, which are sister taxa. The location of the most basal anguine lineages in Morocco and western Eurasia supports this explanation. Miocene climatic changes and montane uplifting in North America may have separated the two major clades of gerrhonotines, one primarily tropical and the other primarily temperate.

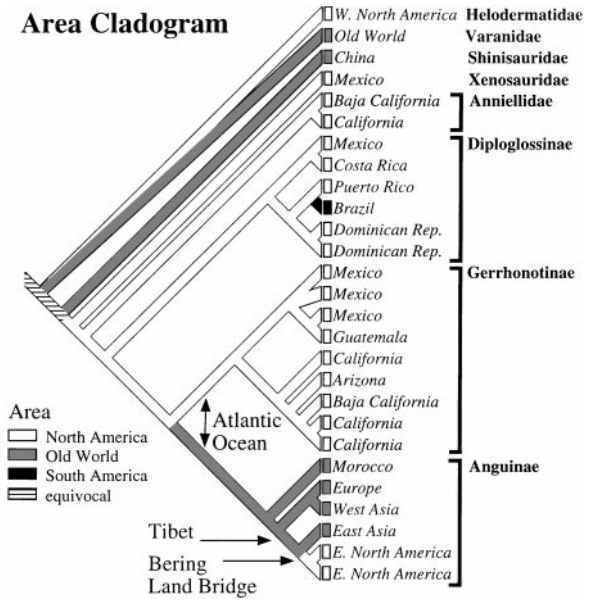

FIG. 5. Area cladogram for anguid lizards and related taxa. Taxonomy is shown to the right. Note deep divergences in North America with a dispersal event from the West Indies to South America and a nested position for Old World anguines. The shortest estimate of phylogeny suggests that the formation of the Atlantic Ocean separated the Gerrhonotinae and Anguinae. Following the Oligocene drying of the Turgai Sea, dispersal of anguine lizards from Europe to East Asia and across the Bering land bridge to North America was possible, but would have been interrupted rapidly by the uplift of the Tibetan Plateau. It costs seven steps on our phylogenetic estimate to construct a topology compatible with anguine taxa originating in North America and crossing the Bering land bridge to East Asia, with continued dispersal to Europe following the Oligocene drying of the Turgai Sea.

Phylogenetic relationships (Fig. 3) are surprisingly well resolved for branches ranging from the late Cretaceous (95–75 MYBP) to the Pleistocene (1.5 MYBP) and provide insights for interpreting biogeographic and paleontological data. Two groups of fossil lizards occurring in Europe have been referred to the Anguidae. The extinct Glyptosaurinae dates to the late Cretaceous (95–75 MYBP; Gauthier, 1982), and its phylogenetic position is not well understood. Fossils in Europe from two later periods may be related to extant forms within the Anguinae. The earliest fossils from the middle Eocene (40–50 MYBP) are either grouped with the modern Old World anguines (Meszoely and Haubold, 1975) or considered the sister lineage to all modern anguines (Gauthier, 1982). If these fossils are grouped with a modern Old World anguine lineage (Fig. 3), they place modern anguine lineages in Europe during the Eocene prior to the Oligocene drying of the Turgai Sea.

Our phylogenetic tree and interpretation of historical events suggest rapid separation of the western Eurasian anguine lineages from eastern Eurasian and North American anguines by the Tibetan uplift during

the Oligocene–Miocene. Within the Anguinae, fossils that are assigned to the *Anguis–Ophisaurus apodus* clade appear in Europe first in the late Oligocene (25–30 MYBP; Gauthier, 1982). This date coincides with the connection of Europe with Asia–America following drying of the Turgai Sea (Briggs, 1987). At this time a continuous land connection was available from Europe through Asia to North America. At this same time (30 MYBP), the first phase of uplifting of the Tibetan Plateau was coming to a close with the plateau reaching an average elevation of 3000 m (Dewey *et al.*, 1989). The second phase of uplifting maintained this elevation of 3000 m until 10 MYBP when faulting and uplifting of the Tibetan Plateau exceeded erosion (Shackleton and Chang, 1988), resulting in a third phase of uplifting to produce an average elevation of 5000 m (Dewey *et al.*, 1989). By the late Oligocene to early Miocene, taxa in Europe and western Asia probably were isolated from taxa in eastern Asia and North America after sharing a brief connection immediately following the drying of the Turgai Sea.

The ancestral anguine lineage may have entered Europe from North America in the Eocene prior to formation of the North Atlantic and then expanded its distribution southward to North Africa and eastward into West Asia. The drying of the Turgai Sea in the Oligocene could have permitted taxa to migrate to eastern Asia and then back to North America via the Bering land bridge. The Oligocene-to-Miocene uplifting of Tibet would have formed a barrier to migration between eastern and western Eurasia shortly after the drying of the Turgai Sea. This scenario predicts that North African, European, and West Asian anguines would not necessarily form a monophyletic group but that North American anguines should be a monophyletic group. This scenario is compatible with both our phylogenetic hypothesis and the hypothesis that Eocene European fossils of the Anguinae are affiliated with either the modern North African lineage or the European and West Asian lineage (Meszoely and Haubold, 1975). The European fossil anguines of the late Oligocene–Miocene are assigned to the *Anguis–Ophisaurus apodus* clade (Gauthier, 1982). The first fossil appearance of *Ophisaurus* in North America occurs in the late Miocene of Saskatchewan (Holman, 1970). This observation is consistent with a post-Oligocene arrival of *Ophisaurus* in North America by dispersal from the Bering land bridge across Canada to its current distribution in southeastern North America.

In an alternative scenario, the Anguinae arose in North America and spread to eastern Asia via the Bering land bridge prior to the Oligocene drying of the Turgai Sea. When the Turgai Sea was dry, western Eurasia would have been invaded and quickly blocked to the east by the uplifting of Tibet (Oligocene to Miocene). This scenario predicts that North American anguines would not necessarily form a monophyletic group but that North African, European, and West Asian anguines would. Phylogenetic predictions of this second scenario are not compatible with our most parsimonious tree. This second scenario also requires that Eocene European fossil anguines are phylogenetically outside a group containing Oligocene–Miocene European fossil anguines and all extant anguines from North Africa, Europe, and West Asia. A tree showing the North African and western Eurasian anguines forming a monophyletic group as predicted by the second scenario was not statistically rejected but was costly (seven extra steps required). Both hypotheses suggest that the drying of the Turgai Sea and the formation of Tibet were instrumental in shaping current biogeographic patterns.

DNA Sequence Divergence and the Fossil Record

Rate of molecular evolution for the mitochondrial DNA region sequenced here has been estimated for agamid lizards, bufonid frogs, and fishes (Bermingham *et al.*, 1997; Macey *et al.*, 1998a,b) as 0.65–0.69% change per lineage per million years. If this rate is approximately correct for anguid, anniellid, and xenosaurid lizards, then taxa sampled here are extraordinarily old. Relatively few divergences are under 10 million years (between *Anniella* species; between *Sauresia* and *Wetmorena;* between *Abronia* and *Mesaspis;* among *Elgaria* species; between *Anguis* and *Ophisaurus apodus;* and between *Ophisaurus attenuatus* and *O. ventralis;* Table 4). Of these taxa, only divergences among *Elgaria* species are less than 7 million years. After 10 million years, mitochondrial DNA is expected to saturate (Moritz *et al.*, 1987); hence, a linear relationship of nucleotide substitutions and time is not anticipated.

The branching event separating *Ophisaurus apodus* and *Anguis* appears to be approximately 9 MYBP, which may be an underestimate if some substitutional saturation has occurred. The fossil record for these taxa is difficult to interpret because small *Ophisaurus apodus*-like specimens can be confused with *Anguis*-like specimens (Gauthier, 1982). Note that the divergence between these taxa and *Ophisaurus koellikeri* of Morocco is greater than 10 MYBP.

The fossil record is consistent with our interpretation of very old divergences among the major lineages (Gauthier, 1982). Fossils of New and Old World xenosaurids and anguids are known from the late Cretaceous (95–75 MYBP). The taxa Anniellidae, Anguinae, Diploglossinae, and Gerrhonotinae all are known from at least the early Eocene (50–55 MYBP).

Within *Elgaria,* the molecular data estimate the divergence between the northern *E. coerulea* and the remaining taxa at 6.6 MYBP. The Gulf of California formed 5–6 MYBP (reviewed in Murphy, 1983) and could have separated *E. kingii* from the clade of western species (*E. paucicarinata, E. multicarinata,* and *E. panamintina*); the DNA sequences estimate 4.0 MYBP,

which is slightly less than expected. Alternatively, the formation of the Mojave Desert could have separated *E. kingii* from the western clade. Continued aridization of the Baja California peninsula (3.4 MYBP) could have separated *E. paucicarinata* from *E. multicarinata* and *E. panamintina*, and a Pleistocene (1.5 MYBP) divergence for *E. multicarinata* and *E. panamintina* may have occurred across the Owens Valley of California. This result is consistent with our molecular calibration and with current hypotheses of Pliocene drying of western North America that continued into the Pleistocene (Axelrod, 1979).

Taxonomic Recommendations

Two considerations should be addressed when making taxonomic changes to preserve monophyly. First, is the evidence for nonmonophyly of currently recognized groups statistically robust and second, how disruptive is the proposed taxonomic change?

Among higher taxa, the overall most parsimonious trees from analysis of DNA sequence data depict as monophyletic groups the Anniellidae, Anguidae, Diploglossinae, Gerrhonotinae, and Anguinae but not the Xenosauridae (*Xenosaurus* and *Shinisaurus*). The lizard family Xenosauridae as currently recognized contains two subfamilies, the Shinisaurinae and Xenosaurinae. Because monophyly of the Xenosauridae (*Shinisaurus* and *Xenosaurus*) is statistically rejected, we propose to recognize as separate lizard families the Shinisauridae (genus *Shinisaurus*) and Xenosauridae (genus *Xenosaurus*). This taxonomic change affects a single species, *Shinisaurus crocodilurus*, and therefore is not considered disruptive.

The Anniellidae, Gerrhonotinae, and Anguinae each receive statistical support as monophyletic groups from analysis of DNA sequence data. Recognition of the lizard family Anniellidae has been a topic of debate (Gauthier, 1982; Good, 1987; Keqin and Norell, 1998). The phylogenetic analyses of DNA sequences and allozymic data place the Anniellidae, which includes only two species, as the sister taxon to the Anguidae, and monophyly of the Anguidae receives statistical support from analysis of allozymic data. The Anniellidae appears not to be the sister taxon to either *Anguis* or the anguines as previously proposed (Gauthier, 1982; Keqin and Norell, 1998). Because the family Anniellidae has been recognized for a long time and is currently used in popular field guides as well as the scientific literature, placing this taxon in the Anguidae would be disruptive. Hence, we recommend continued recognition of the Anniellidae.

Statistical support was not obtained for monophyly of the Diploglossinae*, which therefore is retained as a *metataxon* (Estes *et al.*, 1988; Gauthier *et al.*, 1988) denoted with an asterisk, indicating that monophyly is neither statistically supported nor rejected (Schulte *et al.*, 1998). The genus *Diploglossus* is not monophyletic but a more detailed sampling is needed before stable taxonomic changes can be made.

Within the Anguinae, *Ophisaurus* is not monophyletic, and statistical support is obtained for the grouping of *Ophisaurus apodus* with *Anguis fragilis* rather than with the other species of *Ophisaurus*. Because old generic names exist, *Hyalosaurus* for *O. koellikeri* and *Pseudopus* for *O. apodus*, two options are presented. One option would be to change *O. koellikeri* from *Ophisaurus* to *Hyalosaurus* and *O. apodus* from *Ophisaurus* to *Pseudopus*. If these changes were made, the remaining *Ophisaurus* would still be considered a *metataxon* because monophyly of this group is supported only by a decay index of 1 and statistically is neither supported nor rejected. This change would not be disruptive unless other *Ophisaurus* species are found not to form a monophyletic group, thereby requiring more taxonomic changes. Alternatively, all taxa in the Anguinae could be referred to the genus *Anguis*, which would provide a long-lasting stable taxonomy. Because few species are involved, we favor recognition of a single genus, *Anguis*.

Evolution of tRNA^{Cys}

Tremendous variation occurs among species in potential stem sizes of both D- and T-stem regions of DNA sequences encoding tRNA^{Cys} (Fig. 2). Taxa basal on our phylogeny (*Heloderma*, *Varanus*, and *Shinisaurus*) have zero, three or four base pairings in the D-stem (Fig. 6). *Xenosaurus* and *Anniella* demonstrate enlarged stems of five base pairings. All other taxa except two groups have the normal four base pairings or a slightly reduced stem of three base pairings. An enlarged stem of six pairings occurs in *Ophisaurus koellikeri*, and variation occurs in *Elgaria* for either three base pairings or one base pairing. Because *E. kingii* and *E. paucicarinata* have a single D-stem pair and *E. coerulea*, *E. multicarinata*, and *E. panamintina* have three base pairings in the D-stem, an equivocal reconstruction is presented in Fig. 6. Two interpretations are possible: (1) two base pairings were lost to produce a stem of one base pairing in the ancestor of *E. kingii*, *E. paucicarinata*, *E. multicarinata*, and *E. panamintina* and then two base pairings were regained in the ancestor of *E. multicarinata* and *E. panamintina* to produce three base pairings or (2) two base pairings were lost independently in *E. kingii* and *E. paucicarinata*. Parallel deletion of a single base that destroys two base pairings in the D-stems of both *E. kingii* and *E. paucicarinata* (Figs. 1 and 2) seems more likely than reinsertion of a base in exactly the same place following its deletion, thereby favoring the second hypothesis.

Most species have enlarged T-stems (Fig. 2). The basal condition appears to be a stem size of six to eight base pairings instead of the normal five base pairings (Fig. 6). In the Anniellidae and Anguidae the ancestral condition is an enlarged stem of six base pairings.

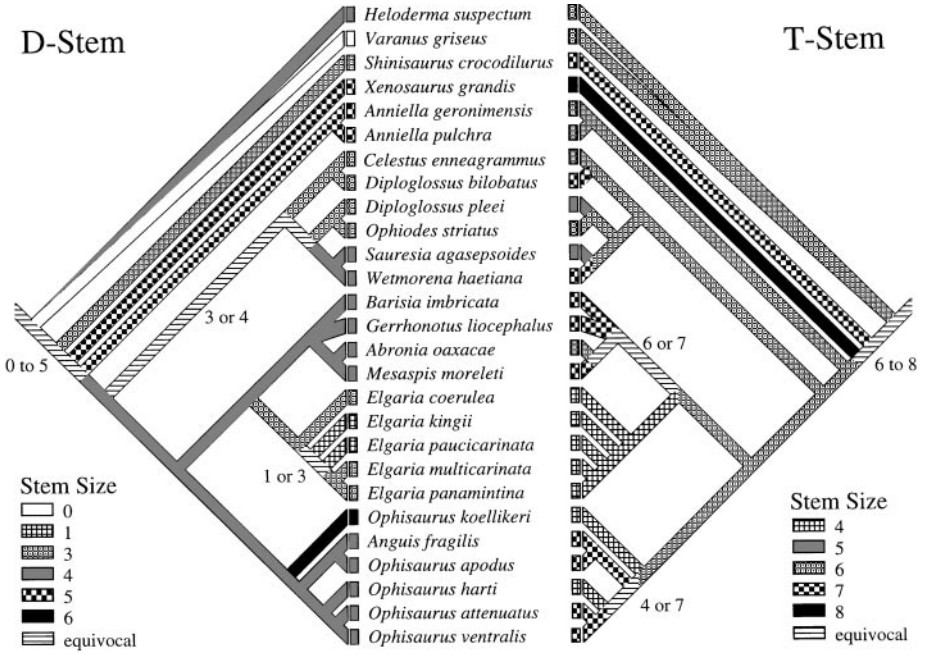

FIG. 6. Evolution of stem regions in $tRNA^{Cys}$ as inferred from DNA sequences derived from anguid lizards and related taxa. Stem size in number of pairs is mapped on the shortest estimate of phylogeny. Light patterns represent few or no pairings and black represents a large number of pairings.

Among diploglossines, two independent losses are observed to produce normal five-base stems, and two independent gains are observed to produce enlarged seven-base stems. All tropical gerrhonotines have enlarged stems of six or seven base pairings whereas all *Elgaria* species have reduced stems of four base pairings. The same situation is observed among the Anguinae; species exhibit either enlarged stems of seven pairings or reduced stems of four pairings.

The loss in *Elgaria* of base pairings in D-stems of $tRNA^{Cys}$ differs from the eight independent losses previously observed among lepidosaurian reptiles (Macey *et al.*, 1997b). In *Elgaria*, a single base pairing is observed in the D-stem of $tRNA^{Cys}$ whereas no such base pairings remained in the eight other independent losses. Transfer RNAs with single base pairs in the D-stem are thought to form a tertiary structure different from tRNAs with D-arm replacement loops (Steinberg *et al.*, 1994). Because the lineage ancestral to *Elgaria* is associated with D-stem size reduction from four to three base pairings, a gradual process of deletion is implicated in the formation of the unusual $tRNA^{Cys}$ observed in *E. kingii* and *E. paucicarinata*. In addition, no repeats that would indicate slippage events during replication (Macey *et al.*, 1997b) are observed.

The large T-stems are unique. Mitochondrial tRNAs have been shown to lose stem regions, but this is the first observation of massive stem increase. In addition, after the T-stem increased in size, decreases in size are observed to occur in parallel. Interestingly, return to a smaller T-stem is associated with reduction of the D-stem in the *Elgaria* species. Size changes in the two stems could be related, but a more detailed sampling of taxa should be used before any major conclusions are made about possible correlated evolution of these stems in $tRNA^{Cys}$ among anguid, anniellid, xenosaurid, and shinisaurid lizards.

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APPENDIX 1

Alternative hypotheses used in Wilcoxon signed-ranks tests (Felsenstein, 1985; Templeton, 1983). Lengths of trees and consistency indices (CI) (Swofford, 1998) are given in parentheses. Numbers refer to the following taxa: (1) *Heloderma suspectum*, (2) *Varanus griseus*, (3) *Shinisaurus crocodilurus*, (4) *Xenosaurus grandis*, (5) *Anniella geronimensis*, (6) *Anniella pul-*

chra, (7) *Celestus enneagrammus*, (8) *Diploglossus bilobatus*, (9) *Diploglossus pleei*, (10) *Ophiodes striatus*, (11) *Sauresia agasepsoides*, (12) *Wetmorena haetiana*, (13) *Barisia imbricata*, (14) *Gerrhonotus liocephalus*, (15) *Abronia oaxacae*, (16) *Mesaspis moreleti*, (17) *Elgaria coerulea*, (18) *Elgaria kingii*, (19) *Elgaria paucicarinata*, (20) *Elgaria multicarinata*, (21) *Elgaria panamintina*, (22) *Ophisaurus koellikeri*, (23) *Anguis fragilis*, (24) *Ophisaurus apodus*, (25) *Ophisaurus harti*, (26) *Ophisaurus attenuatus*, and (27) *Ophisaurus ventralis*.

The two overall most parsimonious trees using the DNA sequence data (length 5452 steps and CI of 0.394): A1. (1, (2, (3, (4, ((5, 6), (((7, 8), ((9, 10), (11, 12))), ((((13, 14), (15, 16)), (17, (18, (19, (20, 21)))))), (22, ((23, 24), (25, (26, 27)))))))))). A2. (1, (2, (3, (4, ((5, 6), (((7, 8), ((9, 10), (11, 12))), (((13, (14, (15, 16))), (17, (18, (19, (20, 21))))), (22, ((23, 24), (25, (26, 27)))))))))).

The most parsimonious trees derived by constraining *Shinisaurus* and *Xenosaurus* to form a monophyletic group using the DNA sequence data (length of 5477 steps and a CI of 0.392): B1. (1, (2, ((3, 4), ((5, 6), (((7, 8), ((9, 10), (11, 12))), (((13, (14, (15, 16))), (17, (18, (19, (20, 21))))), (((22, 25), (23, 24)), (26, 27)))))))). B2. (1, (2, ((3, 4), ((5, 6), (((7, 8), ((9, 10), (11, 12))), ((((13, 14), (15, 16)), (17, (18, (19, (20, 21))))), (((22, 25), (23, 24)), (26, 27)))))))). B3. (1, (2, ((3, 4), ((5, 6), (((7, 8), ((9, 10), (11, 12))), ((((13, 14), (15, 16)), (17, (18, (19, (20, 21))))), (22, ((23, 24), (25, (26, 27)))))))))). B4. (1, (2, ((3, 4), ((5, 6), (((7, 8), ((9, 10), (11, 12))), (((13, (14, (15, 16))), (17, (18, (19, (20, 21))))), (22, ((23, 24), (25, (26, 27)))))))))).

The most parsimonious trees derived by constraining *Anniella* not to form a monophyletic group using the DNA sequence data (length of 5503 steps and a CI of 0.390): C1. (1, (2, (3, (4, ((5, ((7, 8), (((9, 10), (11, 12)), (((13, (14, (15, 16))), (17, (18, (19, (20, 21))))), (((22, 25), (23, 24)), (26, 27)))))), 6))))). C2. (1, (2, (3, (4, ((5, (6, (7, 8))), (((9, 10), (11, 12)), (((13, (14, (15, 16))), (17, (18, (19, (20, 21))))), (((22, 25), (23, 24)), (26, 27)))))))))).

The most parsimonious tree derived by constraining the Anguidae not to form a monophyletic group using the allozymic data of Good (1987; length of 85 steps and a CI of 0.824): D1. (*Xenosaurus*, ((*Anniella*, (*Ophisaurus*, *Elgaria*)), *Celestus*)).

The most parsimonious tree derived by constraining the Diploglossinae not to form a monophyletic group using the DNA sequence data (length of 5456 steps and a CI of 0.394): E1. (1, (2, (3, (4, ((5, 6), ((7, 8), (((9, 10), (11, 12)), (((13, (14, (15, 16))), (17, (18, (19, (20, 21))))), (((22, 25), (23, 24)), (26, 27)))))))))).

The most parsimonious trees derived by constraining the Gerrhonotinae not to form a monophyletic group using the DNA sequence data (length of 5474 steps and a CI of 0.392): F1. (1, (2, (3, (4, ((5, 6), (((7, 8), ((9, 10), (11, 12))), (((13, (14, (15, 16))), (22, ((23, 24), (25, (26, 27))))), (17, (18, (19, (20, 21)))))))))))). F2. (1, (2, (3, (4, ((5, 6), (((7, 8), ((9, 10), (11, 12))), ((13, (14, (15, 16))), ((17, (18, (19, (20, 21))))), (22, ((23, 24), (25, (26, 27)))))))))))).

The most parsimonious trees derived by constraining the Anguinae not to form a monophyletic group using the DNA sequence data (length of 5492 steps and a CI of 0.391): G1. (1, (2, (3, (4, ((((((5, 6), (7, 8)), ((9, 10), (11, 12))), ((13, (14, (15, 16))), (17, (18, (19, (20, 21)))))))), ((22, 25), (26, 27))), (23, 24)))))). G2. (1, (2, (3, (4, (((5, 6), (7, 8)), (((9, 10), (11, 12)), ((((13, (14, (15, 16))), (17, (18, (19, (20, 21))))))), ((22, 25), (26, 27))), (23, 24)))))))). G3. (1, (2, (3, (4, ((((((5, 6), (7, 8)), ((9, 10), (11, 12))), ((13, (14, (15, 16))), (17, (18, (19, (20, 21)))))))), ((22, (23, 24)), (26, 27))), 25))))).

The most parsimonious tree derived by constraining *Anniella* to be the sister taxon to the Anguinae using the DNA sequence data (length of 5467 steps and a CI of 0.393): H1. (1, (2, (3, (4, (((((5, 6), (22, ((23, 24), (25, (26, 27))))))), ((13, (14, (15, 16))), (17, (18, (19, (20, 21)))))))), ((9, 10), (11, 12))), (7, 8)))))).

The most parsimonious trees derived by constraining *Anniella* and *Anguis* to form a monophyletic group using the DNA sequence data (length of 5551 steps and a CI of 0.387): I1. (1, (2, (3, (4, ((((((5, 6), 23), 24), ((22, 25), (26, 27))), ((7, 8), ((9, 10), (11, 12)))), ((13, (14, (15, 16))), (17, (18, (19, (20, 21))))))))))). I2. (1, (2, (3, (4, ((((((5, 6), 23), 24), ((22, 25), (26, 27))), ((7, 8), ((9, 10), (11, 12)))), (((13, 14), (15, 16)), (17, (18, (19, (20,21))))))))))).

The most parsimonious tree derived by constraining *Ophiodes* and *Ophisaurus koellikeri* to form a monophyletic group using the DNA sequence data (length of 5616 steps and a CI of 0.382): J1. (1, (2, (3, (4, (((((5, 6), (7, 8)), (9, (11, 12))), ((10, 22), ((23, 24), (25, (26, 27))))), (((13, 14), (15, 16)), (17, (18, (19, (20, 21))))))))))).

The most parsimonious trees derived by constraining West Indian taxa, *Diploglossus pleei*, *Sauresia*, and *Wetmorena* to form a monophyletic group using the DNA sequence data (length of 5503 steps and a CI of 0.390): K1. (1, (2, (3, (4, ((5, 6), (((7, 8), ((9, (11, 12)), 10)), ((((13, 14), (15, 16)), (17, (18, (19, (20, 21))))), (22, ((23, 24), (25, (26, 27)))))))))). K2. (1, (2, (3, (4, ((5, 6), (((7, 8), ((9, (11, 12))), 10)), (((13, (14, (15, 16))), (17, (18, (19, (20, 21))))), (22, ((23, 24), (25, (26, 27)))))))))).

The most parsimonious trees derived by constraining *Ophisaurus koellikeri*, *Anguis*, and *O. apodus* to form a monophyletic group using the DNA sequence data (length of 5459 steps and a CI of 0.393): L1. (1, (2, (3, (4, ((5, 6), (((7, 8), ((9, 10), (11, 12))), (((13, (14, (15, 16))), (17, (18, (19, (20, 21))))), (((22, (23, 24)), 25), (26, 27)))))))). L2. (1, (2, (3, (4, ((5, 6), (((7, 8), ((9, 10), (11, 12))), (((13, (14, (15, 16))), (17, (18, (19, (20, 21))))), ((22, (23, 24)), (25, (26, 27))))))))))). L3. (1, (2, (3, (4, ((5, 6), (((7, 8), ((9, 10), (11, 12))), ((((13, 14), (15, 16)), (17, (18, (19, (20, 21))))), ((22, (23, 24)), (25, (26, 27)))))))))).

The most parsimonious tree derived by constraining *Anguis* and *Ophisaurus apodus* not to form a monophyletic group using the DNA sequence data (length of 5476 steps and a CI of 0.392): M1. (1, (2, (3, (4, ((5, 6), (((7, 8), ((9, 10), (11, 12))), ((((13, 14), (15, 16)), (17, (18, (19, (20, 21))))), (((22, (25, (26, 27))), 23), 24)))))))).

The most parsimonious tree derived by constraining *Elgaria kingii* and *E. panamintina* to form a monophyletic sister group to *E. paucicarinata* using the DNA sequence data (length of 5478 steps and a CI of 0.392): N1. (1, (2, (3, (4, ((5, 6), (((7, 8), ((9, 10), (11, 12))), ((((13, 14), (15, 16)), (17, (((18, 21), 19), 20))), (22, ((23, 24), (25, (26, 27))))))))))).

The most parsimonious tree derived by constraining *Elgaria multicarinata* and *E. panamintina* to form a monophyletic sister group to *E. paucicarinata* using the allozymic data of Good (1988; length of 84 steps and a CI of 0.774): O1. (*Abronia/Barisia/Mesaspis*, (*Elgaria coerulea*, (((*Elgaria multicarinata*, *Elgaria panamintina*), *Elgaria paucicarinata*), *Elgaria kingii*)))).

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