

Phylogenetic Placement of the Pygmy Alligator Lizard Based on Mitochondrial DNA

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ABSTRACT.—Morphological studies have proven inconsistent in establishing the phylogenetic placement and taxonomic assignment of *Elgaria parva*. Originally classified as *Gerrhonotus parvus* Knight and Scudday, this taxon was reassigned to *Elgaria* based on morphology. To investigate its phylogenetic affinities, we generated mitochondrial DNA sequence data and conducted phylogenetic analyses together with published sequences for a broad taxonomic sampling of anguid lizards. We conducted parsimony, likelihood, and Bayesian analyses of the data. Our results indicate that *E. parva* forms a clade with other *Gerrhonotus* rather than *Elgaria*. Furthermore, *Elgaria* and *Gerrhonotus* are not sister taxa. Based on our new molecular evidence, we suggest that *E. parva* be classified as *Gerrhonotus parvus* as originally described.

Knight and Scudday (1985) described a new species of alligator lizard, *Gerrhonotus parvus*, based on two specimens from Galeana, Nuevo León, México. Characters that distinguish this taxon from other species of *Gerrhonotus* are small adult size, smooth dorsal scales, nasals in contact with rostral, second primary temporal in contact with fifth medial supraocular, suboculars separated from lower primary temporal by an upper labial, and wide pale crossbands on the tail. Knight and Scudday suggested that *G. parvus* and *Gerrhonotus lugoi* are sister species. *Gerrhonotus lugoi* is another small species with smooth dorsal scales but differs from *G. parvus* in some characters. Knight and Scudday (1985) noted that there was debate over generic taxonomy among gerrhonotines and classified the new species as *G. parvus* without commenting on its possible placement within other genera, other than to note that *G. parvus* closely resembles neither *Gerrhonotus liocephalus* nor *Barisia imbricata*. Smith (1986) allocated *G. parvus* to *Elgaria* (emending the epithet to *parva*) based on several characters including nasal-rostral contact, anterior internasal absence, contact of the supranasals, lack of postnasals, cantholoreal presence, and oviparity. Good (1988) agreed with Smith (1986) and pointed out that the first three characters, all related to loss of the anterior internasal and thereby not independent, are derived and unique to *Elgaria*. Notably, Knight and Scudday found *G. parvus* to be superficially almost indistinguishable from *G. lugoi*, which also occurs in the Sierra Madre Oriental about 300 km north of Galeana. However, Good (1988:69) suggested that *G. lugoi* is sister to *G. liocephalus* by stating, "It can in many respects be thought of as a miniaturized *G. liocephalus*." Apparently these authors based their generic allocation of *G. parvus* on somewhat different sets of characters. *Gerrhonotus parvus* has been classified as *E. parva* since Good (1988). To avoid confusion, we will refer to the taxon of interest as *G. parvus*.

In a study of relationships among anguid genera, Macey et al. (1999) provided a large molecular dataset. They used 2001 aligned bases of several mitochondrial protein and transfer RNA genes. They sampled 27 species from 17 putative genera, including representatives of *Abronia*, *Barisia*, *Elgaria*, *Gerrhonotus*, and *Mesaspis*. Their parsimony analysis found strong support for the monophyly of *Elgaria* and found *Gerrhonotus* to be more closely related to a clade composed of *Abronia*, *Barisia*, and *Mesaspis* than to *Elgaria*. *Gerrhonotus parvus* was not included in the dataset of Macey et al. (1999), but their study greatly facilitated reconsideration of the phylogenetic position of *G. parvus* based on DNA sequence data, independent of morphological characters that may be plesiomorphic or convergent.

MATERIALS AND METHODS

Sampling.—We used the aligned sequences of Macey et al. (1999; GenBank accession numbers AF085603–AF085626). Additionally, specimens of *Elgaria* and other anguid lizards were collected from several localities in México (Appendix 1). Details of their acquisition and identification are described in detail elsewhere (Bryson et al., 2003). All specimens were deposited at Universidad Autónoma de Nuevo León (UANL). DNA was extracted from tissue preserved in ethanol using the Qiagen DNeasy Tissue Kit following the instructions provided by the manufacturer. Using primers described in Macey et al. (1999), we amplified portions of homologous data (Appendix 1) for one *Abronia graminea*, one *Barisia levicollis*, one *Elgaria kingii*, two *Gerrhonotus infernalis*, one *G. liocephalus*, and three *G. parvus* (Appendix 1). Most data overlapped portions of mitochondrial tRNA Methionine, NADH dehydrogenase 2, tRNA Tryptophan, tRNA Alanine, tRNA Asparagine, light strand replication origin, tRNA Cysteine, tRNA Tyrosine, and a short portion of cytochrome c oxidase I. For one *Abronia* (E-6) we were able to sequence a portion of NADH dehydrogenase 1, tRNA Isoleucine and tRNA Glutamine. PCR was conducted in a volume of 25 µl with 1 µM of each

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primer, 0.75 mM dNTPs, 2.5 μ l of PCR buffer from Roche and 0.125 μ l Taq from Roche. Successful PCRs were cleaned with Qiagen QIAquick PCR Purification Kit. DNA was sequenced on either an ABI 377 or 3730 with standard protocols with BigDye® Terminator Cycle Sequencing Kits and aligned by eye with Sequencher software version 4.2.2 (Gene Codes Corp., Ann Arbor, MI, 2000).

Phylogenetic Analyses.—We conducted equally weighted parsimony searches in PAUP* (D. L. Swofford, Vers. 4. Sinauer Associates, Sunderland, MA, 2003). To explore tree space, we used 500 random additions of taxa. We ran searches with fewer outgroups, but because there was little difference in topology or clade support from the full dataset, we did not focus on those results. To provide a measure of confidence for the inferred clades, we performed 2500 bootstrap replicates with unweighted heuristic searches. Our overall search strategy was simpler than that of Macey et al. (1999), but our topology did not differ from theirs. To address whether the data could support monophyly of *Elgaria* including *G. parvus*, we constrained parsimony searches to find trees compatible with monophyly of all the *Elgaria* plus *G. parvus* sequences and ran Kishino-Hasegawa (Kishino and Hasegawa, 1989), Templeton (1983), and winning-sites (Prager and Wilson, 1988) tests in PAUP*.

We used ModelTest (Posada and Crandall, 1998) with PAUP* to test for an appropriate model of nucleotide substitution for our data for likelihood analysis (i.e., sufficiently complex but with the fewest parameters of competing models). Using a hierarchical likelihood-ratio test, ModelTest identified TVM + I + Γ as the most appropriate. This model stipulates one rate for transitions, four separate rates of transversions, unequal base frequencies, a gamma-distributed pattern of rate heterogeneity, and a proportion of invariant sites. We used PAUP* for phylogenetic analysis under the likelihood criterion using the model and parameters estimated by ModelTest. We used a heuristic search on the likelihood criterion and factory default values for the search. We also constrained a search for monophyly of *Elgaria*, including *G. parvus*, and ran Shimodaira-Hasegawa (Shimodaira and Hasegawa, 1999) test with full optimization and Kishino-Hasegawa (Kishino and Hasegawa, 1989) test with normal approximation between the unconstrained and constrained trees, as implemented in PAUP*.

We used MrBayes version 3.0b4 (Huelsenbeck and Ronquist, 2001) for a Bayesian analysis of our sequences. Because model choice is limited to 1, 2, or 6 model-substitution types, we elected to use 6, with a gamma-distributed pattern of substitution, and a proportion of invariant sites to best match our likelihood analysis. We started with uniform priors and ran four chains for 500,000 generations, sampling every 100 generations. We did this four times to verify that we adequately searched tree space. Our burnin (i.e., trees discarded in early part of the optimization procedure) at 100,000 generations was selected based on visual observation of plots of generations versus negative log likelihoods. We generated consensus trees of every 100th tree for the postburnin trees, or 4000 trees. Because of their similarity, we only present the consensus from the first run. Consensus trees all had the same topology and only differed by a few posterior probability points.

RESULTS

Not all of the primer pairs from Macey et al. (1999) yielded PCR products. Thus, only 260–1369 bases were produced per sample (Appendix 1), whereas Macey et al. (1999) analyzed ~2000 bases. However, as seen below, congeners clustered consistently with those in Macey et al. (1999). Base composition was similar between the two datasets.

Parsimony.—Unweighted parsimony searches resulted in two equally parsimonious trees of length 6128 (Fig. 1). Analysis of the sequence data from Macey et al. (1999) recovered nearly identical relationships to those found by Macey et al. (1999; using PAUP* vers. 4.0b1); the sole difference being that *G. liocephalus* was more closely related to *Abronia oaxacae* and *Mesaspis moreletti* in our analysis rather than forming a trichotomy with that clade and *Barisia imbricata* in Macey et al. (1999). When we included our samples, the inferred relationships among the existing taxa did not change. Our new sample of *B. levicollis* placed sister to *B. imbricata*. Our new *G. liocephalus* placed sister to the published sequence of this same taxon. Our *G. infernalis* placed sister to the two *G. liocephalus*. Our *A. graminea* placed sister to the published *A. oaxacae*. Our *E. kingii* placed sister to the published *E. kingii*. Our data suggest a sister relationship between *G. parvus* and *G. liocephalus* + *G. infernalis*. We found 82% support for a clade including *Abronia*, *Barisia*, *G. infernalis*, *G. liocephalus*, *G. parvus*, and *Mesaspis* and 100% bootstrap support for a clade with all of the *Elgaria*. The Kishino-Hasegawa, Templeton, and winning sites tests did not reject parsimony trees constrained to monophyly of *Elgaria* plus *G. parvus*. The Kishino-Hasegawa test failed to reject with $P = 0.4252$. The Templeton test failed to reject with $P = 0.4235$, and the winning-sites test failed to reject with $P = 0.6350$.

Likelihood Analysis.—MODELTEST, in conjunction with PAUP*, selected the TVM + I + Γ model with parameters as follows: frequency of A = 0.4055, C = 0.3245, G = 0.0745, T = 0.1956; [A \leftrightarrow C] = 0.4514, [A \leftrightarrow G = C \leftrightarrow T] = 3.9762, [A \leftrightarrow T] = 0.6409, [C \leftrightarrow G] = 0.3027, [G \leftrightarrow T] = 1.0000; proportion of invariable sites (I) = 0.1983; gamma distribution shape parameter = 0.5739. The maximum likelihood search resulted in one tree of $-\log$ -likelihood score 27184.73943 (Fig. 2). Most of the topology of the tree matched that of the parsimony tree. This tree supports the monophyly of *Elgaria* (minus *G. parvus*) and the placement of *G. parvus* sister to *G. liocephalus* and *G. infernalis*. The constrained search led to a tree different in $-\log$ -likelihood by 17.24. The Shimodaira-Hasegawa test had a P -value of 0.031, suggesting significant difference between constrained and unconstrained topologies. The Kishino-Hasegawa test had stronger support of difference with a P -value of 0.000.

Bayesian Analyses.—The four Bayesian searches provided very similar results. Posterior probabilities appeared to stabilize rapidly, settling well before 200,000 generations. The topology did not change between runs and Bayes posterior probabilities changed only by a few points. We only discuss the first analysis. The topology was the same as found in the maximum likelihood search (Fig. 3). Bayes posterior probabilities for branches ranged from 87–100. The clade including *Abronia*, *Barisia*, *G. infernalis*, *G.*

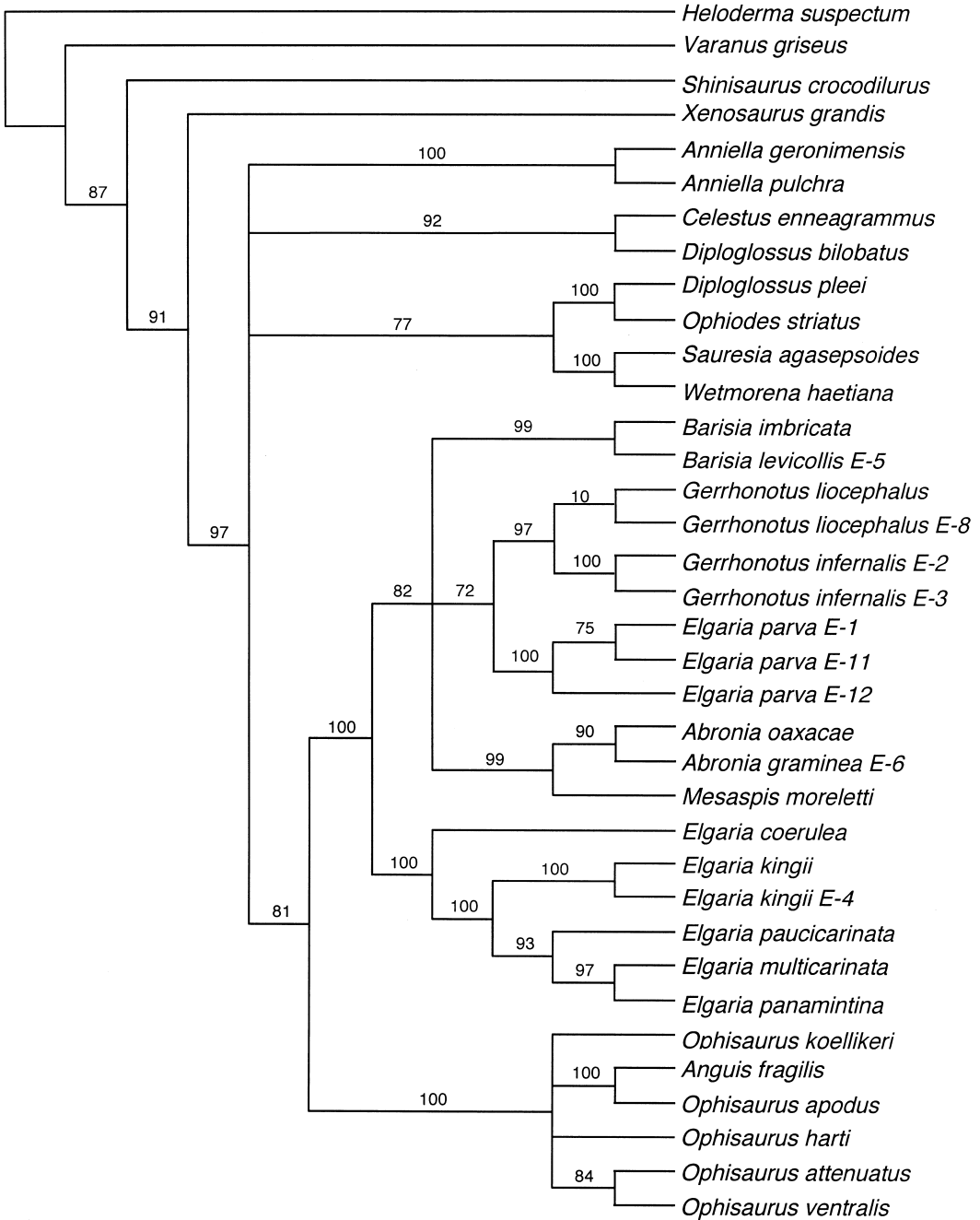


FIG. 1. Parsimony analysis. Consensus of 2500 bootstrap replicates with heuristic search. Values on branches indicate bootstrap support greater than 50%. Notations by species in this and Figures 2 and 3 refer to reference numbers in Appendix 1. Taxa without reference numbers are from Macey et al. (1999). Bold lines highlight the position of *Gerrhonotus parvus*.

liocephalus, and *G. parvus* had support of only 91%, but the *G. infernalis* + *G. liocephalus* and *G. parvus* clade had 100% support. Most relationships within the *Elgaria* clade were well supported (97–100%).

DISCUSSION

Our results are consistent with those of Macey et al. (1999) in that original relationships were supported. Wiens and Slingluff (2001) also analyzed the Macey et

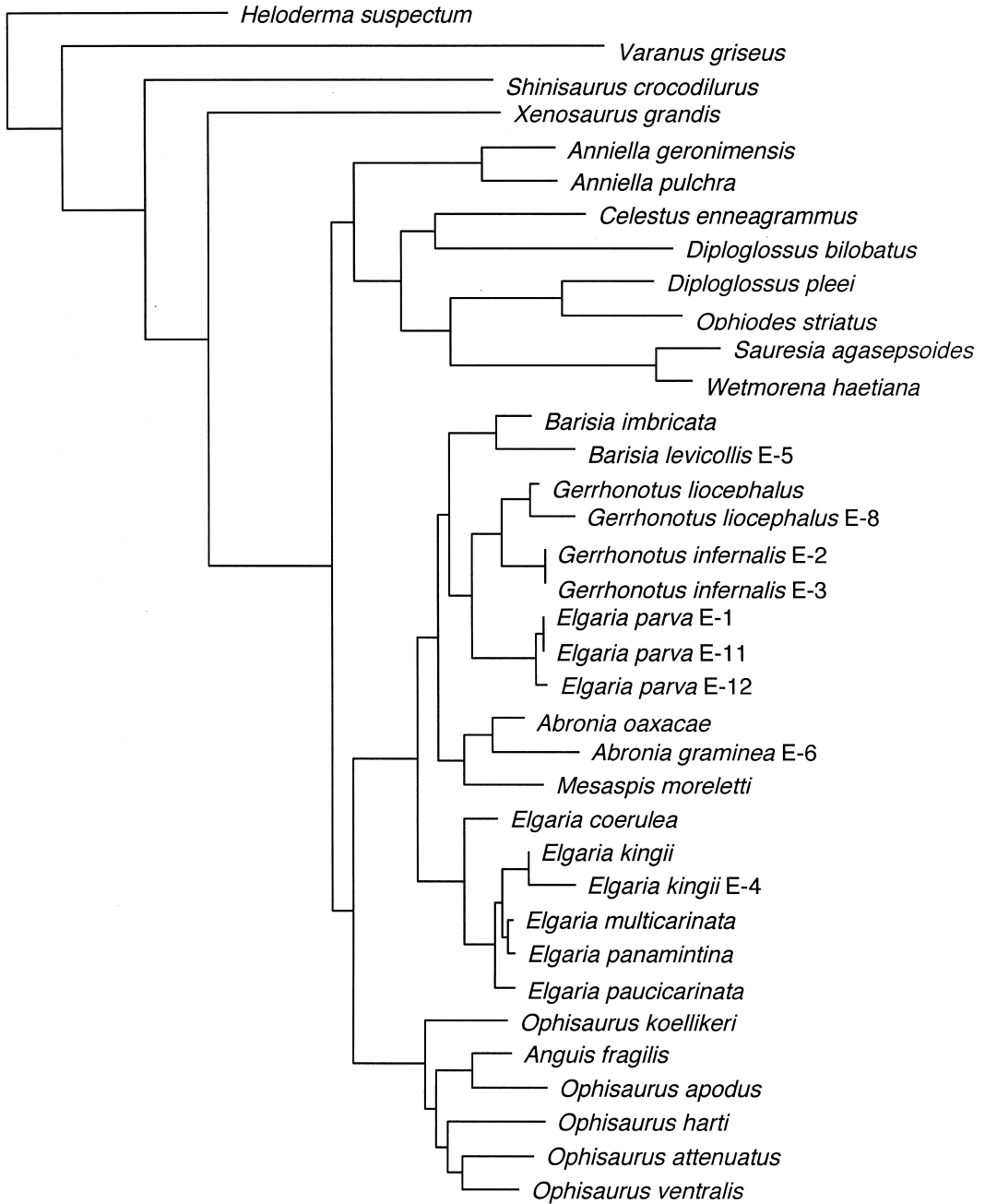


FIG. 2. Likelihood analysis. Results of a maximum likelihood heuristic search with TVM + I + Γ model of substitution. The search resulted in one tree of $-\log$ -likelihood score 27184.73943.

al. (1999) dataset with maximum likelihood and found a topology similar to ours.

Our findings suggest that the morphological characters used to ally *G. parvus* with *Elgaria* should be reevaluated. The small adult size of *G. parvus* relative to other species in either *Elgaria* or *Gerrhonotus* suggests

that characterizing its morphology may be confounded by heterochrony. *Gerrhonotus parvus* may have been allied with *Elgaria* based on its developmental trajectory, rather than homology (J. McGuire, pers. comm.). Richmond and Reeder (2002), with examples from scincid lizards, emphasized the necessity of a phyloge-

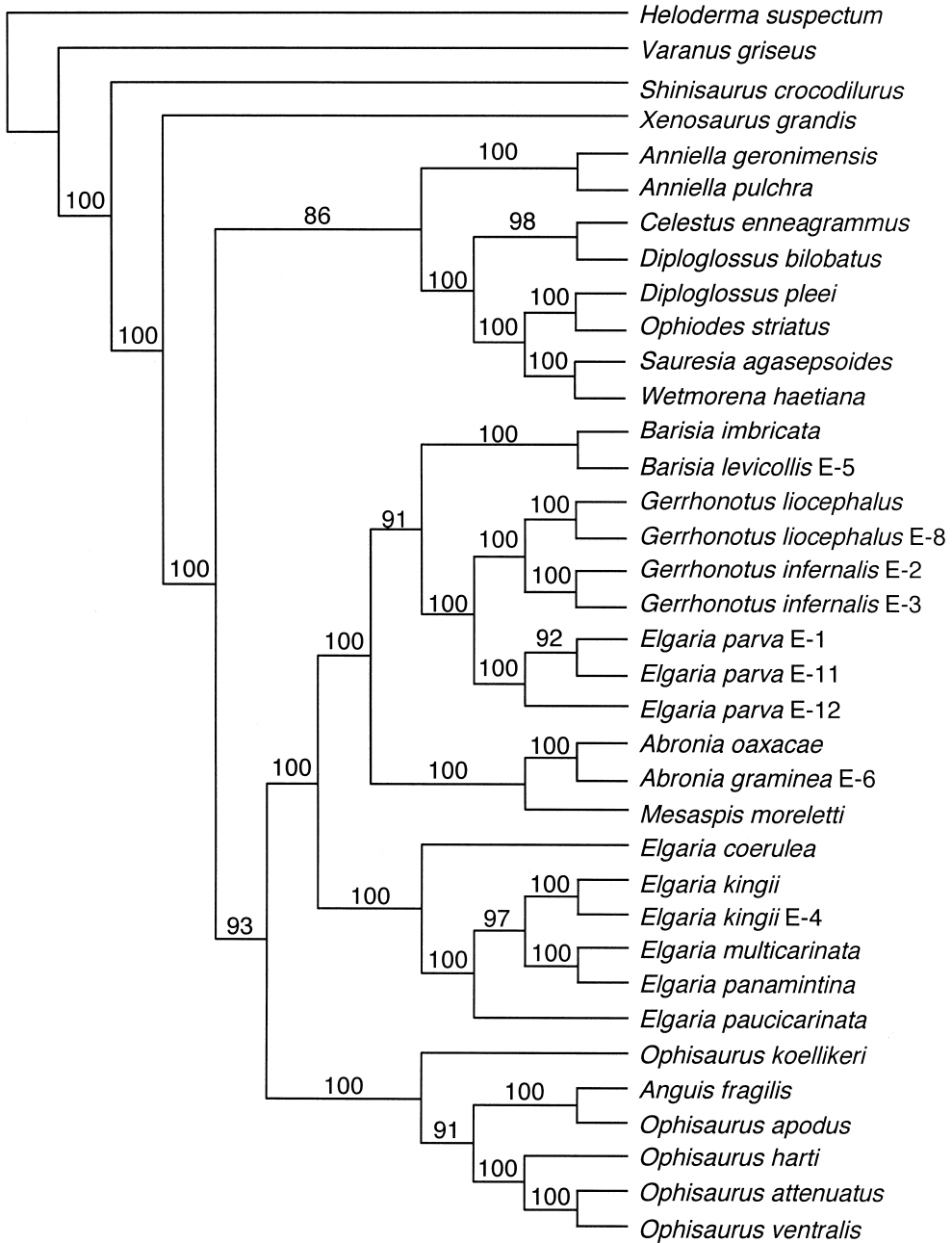


FIG. 3. Bayesian analysis. Values on branches are Bayes posterior probabilities computed from every 100th tree from last 400,000 of 500,000 generations.

netic approach to uncovering patterns of heterochrony. Future work should investigate the phylogenetic position of other small-bodied species in this group.

Our results indicate that *G. parvus* should be maintained within *Gerrhonotus* as originally described. Our mtDNA data indicate that the morphological characters used to assign *G. parvus* to *Elgaria*, a well-supported clade based on molecular data for other

species, fail to correctly classify *G. parvus*. Our tests of alternative topologies were ambiguous in that the parsimony alternative was not rejected, but the likelihood alternative was. However, we feel it is noteworthy that our three methods of analysis (parsimony, maximum likelihood, Bayesian) all suggested *G. parvus* should be aligned with *Gerrhonotus*, not with *Elgaria*.

We are presently investigating the phylogenetic position of *G. lugoi*. Determining its relationships to other anguoid lizards should be interesting because of the complexity of its characters states. It is also a relatively small anguoid lizard. Knight and Scudday (1985) found this species to be superficially similar to *G. parvus*, and Good (1988) suggested a relationship with *G. liocephalus*. Given the sharp incongruence of morphological and molecular results shown here for *G. parvus*, molecular data for *E. lugoi* may also provide surprising results.

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APPENDIX 1. Specimens used in this study for original data. UANL = Universidad Autónoma de Nuevo León. Nexus file available upon request from CJC.

Sample	Taxon	UANL number	Collecting locality	# Bases analyzed	GenBank #
E-6	<i>Abronia graminea</i>	6064	México: Veracruz: Puerto del Aire	1369	AY742917, AY742923, AY742929 AY742922
E-5	<i>Barisia levicollis</i>	5702	México: Chihuahua: Sierra del Nido	553	AY742922
E-4	<i>Elgaria kingii</i>	5700	México: Chihuahua: Sierra del Nido	909	AY742921, AY742928
E-2	<i>Gerrhonotus infernalis</i>	5841	México: Nuevo León: Carretera Santiago- Laguna de Sánchez	810	AY742919, AY742927
E-3	<i>G. infernalis</i>	5788	México: Nuevo León: Parque Ecológico Chipinque	538	AY742920
E-8	<i>G. liocephalus</i>	uncataloged	México: Guerrero, east of Omilteme	860	AY742924, AY742930
E-1	<i>G. parvus</i>	5844	México: Nuevo León: Cañon de San Isidro	900	AY742918, AY742926
E-11	<i>G. parvus</i>	6221	México: San Isidro, Nuevo Leon	260	AY742916
E-12	<i>G. parvus</i>	6220	México: Los Rayones, Nuevo Leon	788	AY742925, AY742931