

HOW LIZARDS TURN INTO SNAKES: A PHYLOGENETIC ANALYSIS OF BODY-FORM EVOLUTION IN ANGUID LIZARDS

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Abstract.—One of the most striking morphological transformations in vertebrate evolution is the transition from a lizardlike body form to an elongate, limbless (snakelike) body form. Despite its dramatic nature, this transition has occurred repeatedly among closely related species (especially in squamate reptiles), making it an excellent system for studying macroevolutionary transformations in body plan. In this paper, we examine the evolution of body form in the lizard family Anguinae, a clade in which multiple independent losses of limbs have occurred. We combine a molecular phylogeny for 27 species, our morphometric data, and phylogenetic comparative methods to provide the first statistical phylogenetic tests of several long-standing hypotheses for the evolution of snakelike body form. Our results confirm the hypothesized relationships between body elongation and limb reduction and between limb reduction and digit reduction. However, we find no support for the hypothesized sequence going from body elongation to limb reduction to digit loss, and we show that a burrowing lifestyle is not a necessary correlate of limb loss. We also show that similar degrees of overall body elongation are achieved in two different ways in anguines, that these different modes of elongation are associated with different habitat preferences, and that this dichotomy in body plan and ecology is widespread in limb-reduced squamates. Finally, a recent developmental study has proposed that the transition from lizardlike to snakelike body form involves changes in the expression domains of midbody Hox genes, changes that would link elongation and limb loss and might cause sudden transformations in body form. Our results reject this developmental model and suggest that this transition involves gradual changes occurring over relatively long time scales.

Key words.—Comparative methods, development, macroevolution, morphology, phylogeny, reptiles.

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The evolutionary history of animals is marked by many radical transformations in body plan (Raff 1996). These transformations are often associated with the origin of higher taxa, such as arthropods, echinoderms, tetrapods, whales, turtles, and snakes (Carroll 1997). A fundamental question in evolutionary biology is whether the mechanisms and rates of morphological change associated with the origin of higher taxa are actually different from those that typically occur within populations and among closely related species (e.g., Stanley 1979; Charlesworth et al. 1982; Raff 1996; Carroll 1997). Unfortunately, understanding these macroevolutionary transformations is generally difficult because they occurred long ago and were unique. The antiquity of these changes makes it difficult to extrapolate the results of present-day ecological, functional, and developmental comparisons. Their uniqueness makes robust statistical analyses difficult.

Because these problems limit macroevolutionary studies in many groups of organisms, the evolution of an elongate, limbless body plan (“snakelike” or “serpentine” hereafter) in squamate reptiles is an excellent model system for understanding major changes in body form. The transition from lizardlike to snakelike body form is common in squamates (including dozens of seemingly independent lineages such as amphisbaenians, snakes, and limbless members of seven families of lizards; Greer 1991; Pough et al. 1998), which facilitates statistical comparisons. Furthermore, this transition occurs among species that are relatively recently diverged, with fully limbed and fully limbless lizard species sometimes occurring within the same genus (e.g., the skink genera *Brachymeles*, *Chalcides*, *Lerista*, *Scelotes*; Lande 1978), thus facilitating ecological, functional, and developmental comparisons.

In many ways, the transition from lizardlike to snakelike body form in squamates has been both well and poorly studied. The transition is well studied in that numerous authors have addressed the evolution of snakelike squamates, but is poorly studied in that none have used phylogeny-based comparative methods. Several authors have noted a relationship between increase in vertebral number and decreases in limb size and number of digits or phalanges (e.g., Camp 1923; Stokeley 1947; Presch 1975; Greer 1987; Caputo et al. 1995). Gans (1975) discussed morphological, functional, and ecological correlates of limb loss in tetrapods, noting that body elongation, reduced body size, and undulatory locomotion seem to be associated consistently with limb reduction. Lande (1978) provided an excellent review and analysis of evolutionary rates, developmental mechanisms, morphological correlates, and quantitative genetic models of limb loss. Lee (1998) showed that repeated evolution of elongate, limb-reduced burrowing forms (and the characters associated with this ecomorph) might mislead phylogenetic analyses of squamates based on morphological data. Cohn and Tickle (1999) studied the developmental basis of limb loss and body elongation in the origin of snakes and suggested that anterior expansion of domains of expression of midbody Hox genes may simultaneously account for both of these changes. A number of authors have recently discussed (and disputed) the loss of limbs and the possible reappearance of well-developed hind limbs among the primitive lineages of snakes (e.g., Caldwell and Lee 1997; Zaher and Rieppel 1999; Greene and Cundall 2000a,b; Lee et al. 2000; Tchernov et al. 2000).

Despite these numerous studies of limb loss in squamates, many long-standing and fundamental hypotheses have not been tested rigorously. Previous authors have hypothesized

relationships between body elongation and reduction in limb size and between reduction in limb size and loss of digits (Gans 1975; Presch 1975; Lande 1978; Caputo et al. 1995; Greer et al. 1998; Lee 1998), between limb reduction and reduction in overall body size (e.g., Rieppel 1988; Griffith 1990; Lee 1998), and between the evolution of snakelike body form and a burrowing lifestyle (e.g., Gans 1975; Rieppel 1988; Caputo et al. 1995; Lee 1998). These hypotheses generally have been formulated by comparing species with different degrees of limb reduction and (implicitly) assuming that the phylogeny tracks a morphocline of increasing limb reduction and that different species represent different stages in a process that is largely uniform among species (e.g., Gans 1975; Lande 1978; Caputo et al. 1995; Greer et al. 1998). Rigorously testing these hypotheses requires statistical methods that incorporate phylogeny (e.g., Felsenstein 1985a). Unfortunately, most phylogenetic hypotheses within and between squamate families with limb-reduced forms have been based on morphological characters, and these phylogenetic analyses potentially have been compromised by character nonindependence, given that a suite of traits may be associated with limb reduction, cranial modifications for burrowing, and miniaturization (Lee 1998). A statistical, phylogenetic analysis of the morphological and ecological correlates of limb loss has never been published.

Anguid lizards provide an invaluable opportunity for studying the transition from lizardlike to snakelike body form. The Anguidae consists of 102 species and 15 genera, found in North, Central, and South America, the West Indies, Europe, Asia, and North Africa (Pough et al. 1998). The current taxonomy of anguid lizards suggest that there has been repeated evolution of serpentiform body plan, with elongate, limb-reduced species occurring in three of the four subfamilies. A phylogeny for 23 species of the family based on mitochondrial DNA (mtDNA) sequences has been published recently (Macey et al. 1999), which includes representatives of all of the limb-reduced genera. MtDNA sequences provide a set of characters that should be unaffected by changes associated with the evolution of body form. Although sequence data have their own problems in phylogeny reconstruction (see review in Hillis and Wiens 2000), the phylogeny estimated by Macey et al. (1999) is largely congruent with previous analyses based on morphology and allozymes (e.g., Gauthier 1982; Good 1987a,b; but see Gao and Norell 1998). Finally, the Anguidae have an extensive fossil record (e.g., Gauthier 1982; Gao and Norell 1998), which, along with molecular and biogeographical data, allows some evaluation of the time frame over which changes in body form have occurred.

In this paper, we use anguid lizards as a case study for understanding the evolution of body form in snakelike vertebrates. We combine our morphological data, the molecular data of Macey et al. (1999), and statistical phylogenetic methods (i.e., Felsenstein 1985a) to address the following questions: Is body elongation correlated with limb reduction (i.e., decrease in relative limb length)? Is limb reduction correlated with digital reduction (i.e., decrease in the number of digits)? Is the evolution of serpentiform morphology associated with reduced body diameter (i.e., miniaturization)? We also qualitatively address the following questions: Does body elon-

gation have the same morphological basis in different clades, or are different mechanisms involved (Gans 1975)? Is there a sequence in the evolution of snakelike body form involving body elongation, limb reduction, and digit loss (Gans 1975; Lande 1978)? Is there an association between burrowing and limb reduction (Lee 1998)? Is there morphological evidence that elongation and limb loss are linked through expansion of domains of Hox gene expression (Cohn and Tickle 1999)? Over what time scales might limb loss occur?

MATERIALS AND METHODS

Morphometric Data

Morphometric data were obtained from preserved museum specimens for the same species sampled by Macey et al. (1999). The analysis was restricted to these species so that molecular phylogenetic information and branch lengths would be available for all species. Taxon sampling included representatives of 14 of the 15 extant genera of anguids. The only genus not included (*Coloptychon*) is very similar in body form to other members of Gerrhonotinae (Good 1988a). Our sampling of taxa should incorporate all putative transitions between lizardlike and snakelike body form in anguids. Furthermore, simulations (Ackerly 2000) show that the phylogenetic comparative method that we use in this study (independent contrasts) can be robust to incomplete taxon sampling.

Specimens examined are listed in Appendix 1. An average of 8.7 individuals per species was examined (range 1–20). Only presumed adult specimens with unregenerated tails were included to avoid biasing the results with ontogenetic shape changes (Lande 1978) or the reduced length of regenerated tails. In some cases, this reduced sample sizes—particularly for species that are poorly represented in collections. Data from conspecific individuals from different populations were pooled for all species. Little intraspecific variation in body form was observed, especially relative to the dramatic differences among species. Anguids are difficult to sex externally, and dissections to determine sex are problematic for rare species. We therefore did not distinguish the sex of sampled specimens. Anguid species show little or no variation in shape between sexes (some males may have wider heads in some species of gerrhonotines and anguines; Fitch 1981) and only minor sexual size dimorphism (male body size is within 10% of female body size in species of *Anniella*, *Elgaria*, *Ophisaurus*, *Sauresia* and *Wetmorena* and within 25% in species of *Anguis* and *Diploglossus*; Fitch 1981; Schwartz and Henderson 1991). Interspecific differences in head width should have little impact on this analysis, because the increases in head width observed in some males appear to be largely posterior to the orbits (J. J. Wiens, pers. obs.).

Measurements were taken mostly with vernier and dial calipers (to the nearest 0.1 mm), but a meter stick (to the nearest 1 mm) was used to measure very large individuals. The following data were obtained from each specimen: snout-vent length (SVL), from the tip of the snout to the posterior end of the cloaca; tail length (TL), from the posterior end of the cloaca to the posterior tip of the outstretched tail; head width (HW), the width of the head at the level of the posterior corner of the eye; head length (HL), from the tip of the snout

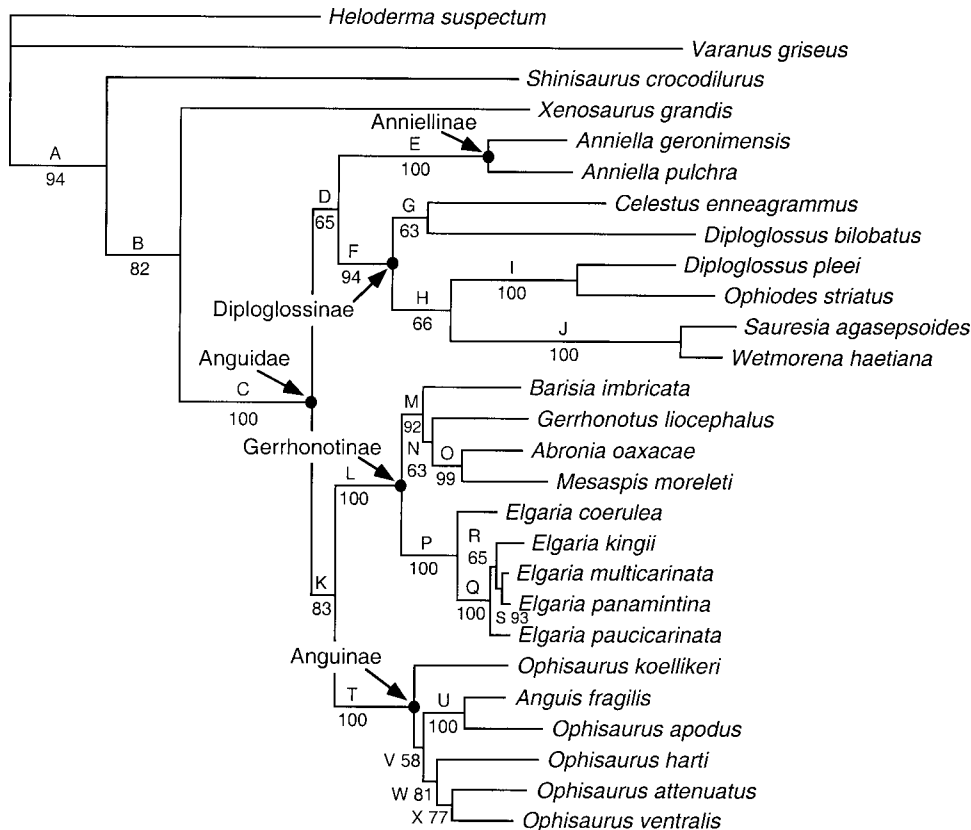


FIG. 1. Phylogeny of anguid lizards (and relatives) based on maximum-likelihood analysis of mitochondrial DNA sequence data. Numbers associated with branches are bootstrap proportions (bootstrap values < 50% not shown). Branch lengths are drawn proportional to lengths estimated using maximum likelihood. Estimated lengths for each internal branch (labeled with capital letters on figures) and terminal branch are given below: A, 0.152; B, 0.116; C, 0.209; D, 0.037; E, 0.236; F, 0.088; G, 0.054; H, 0.090; I, 0.199; J, 0.360; K, 0.035; L, 0.104; M, 0.032; N, 0.015; O, 0.046; P, 0.087; Q, 0.052; R, 0.010; S, 0.090; T, 0.126; U, 0.062; V, 0.015; W, 0.021; X, 0.024; *Heloderma suspectum*, 0.486; *Varanus griseus*, 1.053; *Shinisaurus crocodilurus*, 0.644; *Xenosaurus grandis*, 0.548; *Anniella geronimensis*, 0.122; *Anniella pulchra*, 0.131; *Celestus enneagrammus*, 0.277; *Diploglossus bilobatus*, 0.416; *Diploglossus pleei*, 0.151; *Ophiodes striatus*, 0.213; *Sauresia agasepsoides*, 0.086; *Wetmorena haetiana*, 0.065; *Barisia imbricata*, 0.154; *Gerrhonotus liocephalus*, 0.150; *Abronia oaxacae*, 0.097; *Mesaspis moreleti*, 0.134; *Elgaria coerulea*, 0.062; *Elgaria kingii*, 0.044; *Elgaria multicarinata*, 0.011; *Elgaria panamintina*, 0.013; *Elgaria paucicarinata*, 0.032; *Ophisaurus koellikeri*, 0.144; *Anguis fragilis*, 0.065; *Ophisaurus apodus*, 0.124; *Ophisaurus harti*, 0.158; *Ophisaurus attenuatus*, 0.117; *Ophisaurus ventralis*, 0.096.

to the posterior corner of the eye; forelimb length (FLL), from the posterior insertion of the fully extended forelimb to the tip of the claw of the longest (usually the third) finger; hind limb length (HLL), from the posterior insertion of the fully extended hind limb to the tip of the claw of the longest (usually the fourth) toe; the number of fingers on the manus; and the number of toes on the pes. Note that functionless, internal limb remnants may be present in some species that we consider limbless based on external data. In some species of anguids, the hind limb is reduced to a clawless, attenuate stump or flap; these taxa arbitrarily were considered to have a single digit because the precise morphology of the foot (i.e., presence of metatarsals or phalanges) was difficult to determine. Digitizing specimens for use of morphometric landmark methods was impractical for this study because of the manner in which the specimens were preserved.

Phylogenetic Trees

Two trees were used to examine the evolution of body form in anguids. First, we used a phylogeny based on a re-

analysis of the mtDNA dataset of Macey et al. (1999) using maximum likelihood (Figs. 1, 2). This dataset consists of 2001 aligned base pairs (1013 parsimony-informative characters) from the mitochondrial ND1, ND2, and COI genes and several adjacent transfer RNAs. We used the alignment provided by these authors, which incorporates information on the secondary structure of the tRNAs. Macey et al. (1999) analyzed their data using only equally weighted (i.e., unweighted) parsimony. Equally weighted parsimony for DNA sequence data implicitly assumes (among other things) that all types of substitutions are equally likely and that all sites evolve at the same rate; these assumptions are probably violated in mtDNA datasets for protein-coding genes because of higher rates of transitions and of changes at third codon positions. Furthermore, use of parsimony to estimate branch lengths for use in comparative studies is problematic because parsimony may underestimate the length of long branches (Swofford et al. 1996). To obtain potentially more accurate estimates of phylogeny and branch lengths, we reanalyzed these data using maximum likelihood.

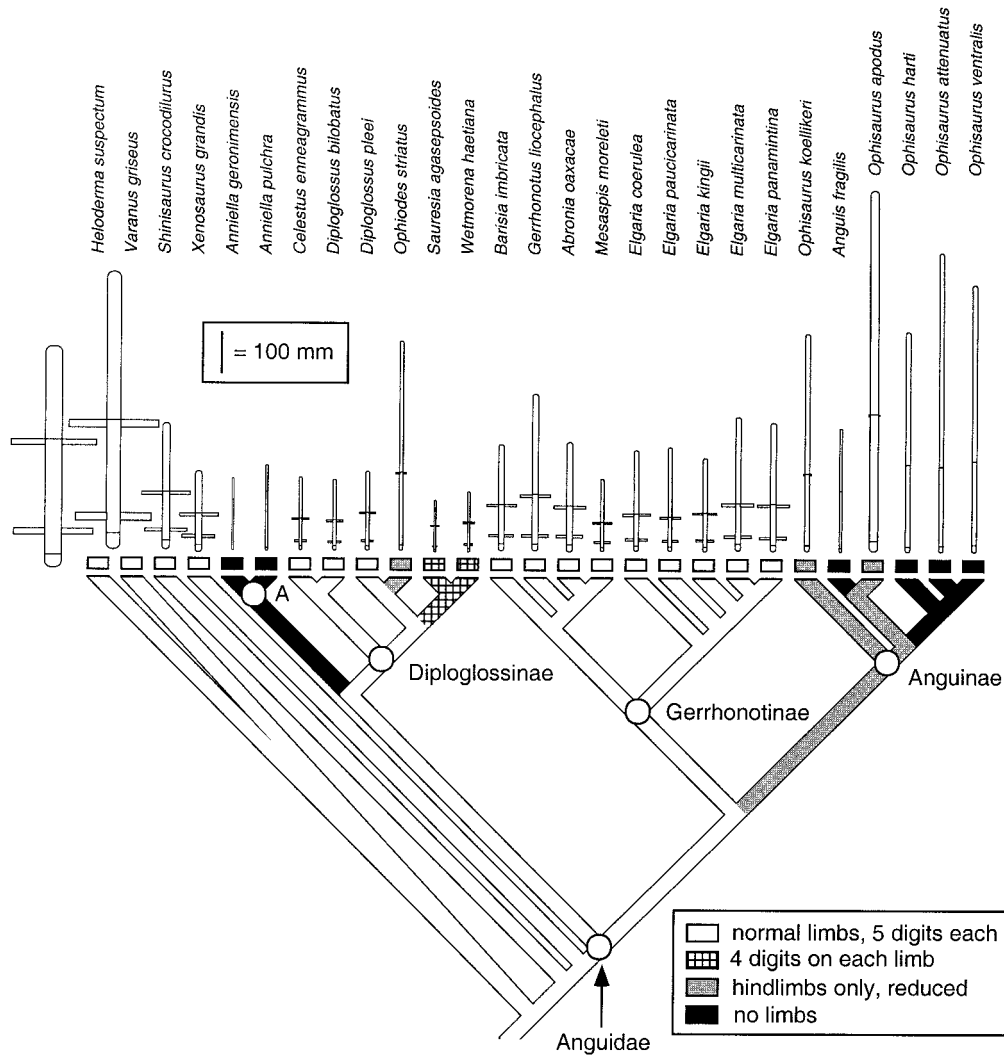


FIG. 2. Evolution of body form in anguid lizards and their relatives, mapped onto a phylogeny based on maximum-likelihood analysis of mitochondrial DNA sequence data (Fig. 1). The reconstruction of limb loss is one of several equally parsimonious optimizations; the one illustrated was selected because it does not require reacquisition of limbs or digits. The mean head length, head width, snout-vent length, tail length, and limb lengths of each species are illustrated diagrammatically. A, Anniellinae.

All phylogenetic analyses were performed using PAUP*, version 4.0b4a (Swofford 2000). We used parsimony and neighbor joining to generate an initial set of trees, compared the likelihoods of these trees under different models, tested the fit of the different models to that tree with the overall highest likelihood (using the procedure outlined by Huelssenbeck and Crandall 1997), and then used the best-fitting model to estimate the phylogeny using maximum likelihood. For neighbor-joining analyses we used four models of substitution: (1) Jukes-Cantor, assuming equal rates of change for transitions and transversions and equal base frequencies (JC; Jukes and Cantor 1969); (2) Kimura two-parameter, assuming different rates of change for transitions and transversions and equal base frequencies (K2P; Kimura 1980); (3) Hasegawa-Kishino-Yano, assuming different rates for transitions and transversions and unequal base frequencies (HKY85; Hasegawa et al. 1985); and (4) general time re-

versible, assuming a different rate for all six classes of substitutions (GTR; Yang 1994a).

Trees from the parsimony and distance analyses were compared using 16 models of DNA sequence evolution in a likelihood framework. These models were combinations of the four substitution models listed above with four approaches for dealing with different rates of change among sites: (1) assuming equal rates of change among all sites; (2) assuming that some sites are invariant and that all variable sites evolve at the same rate (I; Hasegawa et al. 1985); (3) assuming that all sites follow a gamma distribution (Γ ; Yang 1994b; using four rate categories); and (4) assuming that some sites are invariant and that variable sites follow a gamma distribution (I + Γ ; Gu et al. 1995). Specific parameters for each combination of model and tree were estimated using likelihood.

The equally weighted parsimony analyses yielded two shortest trees (as found by Macey et al. 1999). The neighbor-

joining analyses yielded a topology that differed slightly from the parsimony trees (involving the placement of Anniellinae) and was consistent across the different distance measures. Comparison of the different models across these three trees revealed that one of the trees from the parsimony analyses had the highest likelihood for most models. Models of increasing complexity were tested to determine if adding parameters to the model significantly increased the fit between the model and data; if the difference between models was not significant, the less complex model (fewer parameters) was chosen. Because this model-fitting test can potentially be biased by the sequence in which parameters are added (e.g., Cunningham et al. 1998), two different sequences of parameter addition were tested: one in which among-site rate heterogeneity was added to the most complex substitution model, the other in which rate heterogeneity was added to the least complex model. Statistical significance of differences in likelihoods of the models was evaluated using the likelihood-ratio test statistic, $-2\log\Lambda$, the difference between the negative log likelihoods for the two models, multiplied by two. This statistic should approximate a chi-square distribution with the degrees of freedom equal to the difference in the number of parameters between the two models (Yang et al. 1995; but see Whelan and Goldman 1999). A sequential Bonferroni correction was used to adjust significance levels for multiple statistical tests (Rice 1989).

The best-fitting model was then used in a heuristic search to find the overall best likelihood topology using tree-bisection-reconnection branch swapping and 10 random addition sequence replicates. Because of the large number of taxa, model parameters were difficult to estimate during the search and were estimated from the best-fitting parsimony tree instead. Support for the likelihood tree was evaluated using nonparametric bootstrapping (Felsenstein 1985b), with 100 pseudoreplicates and one addition sequence replicate per bootstrap pseudoreplicate. Because of the time-intensive nature of the bootstrap searches, neighbor-joining was used to obtain an initial tree for maximum-likelihood optimization and the subtree-pruning-grafting method was used for branch swapping.

The second tree used (Fig. 3) was based largely on a composite of earlier morphological and allozyme studies (referred to hereafter as the "traditional tree"). The traditional tree differs from the equally weighted parsimony trees of Macey et al. (1999) only in the following respects: (1) the monophyly of the Xenosauridae was assumed, following previous morphological analyses (e.g., Estes et al. 1988; Gao and Norell 1998); (2) relationships within the Gerrhonotinae were based on congruent phylogenies from osteology (Good 1987a) and external morphology (Good 1988a), with *Gerrhonotus* basal, *Elgaria* the next up the tree, and *Barisia* the sister taxon of *Mesaspis* + *Abronia*; and (3) relationships within *Elgaria* were based on external morphology (Good 1988a) and allozymes (Good 1988b). The unusual placement of *E. multicarinata* with *E. panamintina* by the mtDNA data (Macey et al. 1999) may be the result of a lateral transfer event, given that the allozymes and morphology concur that *E. panamintina* is closely related to *E. kingii* (T. Reeder, pers. comm.). Relationships among anguid subfamilies based on morphology are unclear. Gauthier (1982) found weak support for

grouping Gerrhonotinae and Diploglossinae (with the relationships between that clade, Anguinae, and Anniellidae unresolved), whereas Gao and Norell (1998) grouped Anguinae and Anniellidae as sister taxa (but left relationships among that clade, Gerrhonotinae, and Diploglossinae, unresolved). The allozyme data of Good (1987b) strongly support Anguinae and Gerrhonotinae as sister taxa, with Diploglossinae as the sister group to these two subfamilies (based on the reanalysis by Macey et al. 1999). We have used the subfamilial relationships suggested by allozymes (also congruent with parsimony analysis of the mtDNA) in this tree, especially given the possibility that the morphology-based analyses are misled by convergence and character nonindependence in the snakelike forms. There have been no explicit phylogenetic hypotheses within Anguinae and Diploglossinae, apart from the mtDNA analysis of Macey et al. (1999), so the mtDNA phylogeny was used for relationships within these subfamilies. We did not attempt a combined analysis of the morphology, allozymes, and mtDNA because most of the morphological data used in phylogenetic analyses of anguids (particularly outside of Gerrhonotinae) have never been reported explicitly.

Character Mapping

Major changes in body form and digit number initially were examined by mapping characters onto the two phylogenies (although these reconstructions were merely examined qualitatively and were not used in any statistical analyses). Morphometric variables were reconstructed using squared-change parsimony (Huey and Bennett 1987; Maddison 1991) as implemented in MacClade (Maddison and Maddison 1992) and using linear generalized least squares (GLS; Martins and Hansen 1997) with COMPARE (Martins 1999a). Ancestral reconstructions using squared-change parsimony are equivalent to maximum-likelihood estimates when equal branch lengths are assumed (Schluter et al. 1997) and reconstructions using GLS-linear are equivalent to maximum-likelihood estimates with unequal branch lengths (Martins 1999b) or to estimates based on independent contrasts (Garland et al. 1999). We reconstructed ancestral trait values using squared-change parsimony on the traditional phylogeny (for which branch lengths are unknown and were assumed to be equal) and the mtDNA phylogeny (assuming equal branch lengths). We used linear GLS to reconstruct ancestral trait values using the phylogeny and branch lengths estimated from maximum-likelihood analysis of the mtDNA data (see above). Digit numbers for the manus and pes were treated as separate, ordered, multistate characters and were reconstructed using linear parsimony with MacClade (Maddison and Maddison 1992).

Statistical Analyses of Morphometric Data

Multivariate and bivariate statistical analyses were performed on morphological variables that were adjusted for the phylogenetic relatedness of taxa using Felsenstein's (1985a) independent contrasts method. Independent contrasts is a widely used phylogenetic comparative method (e.g., Losos 1990; Harvey and Pagel 1991; Westneat 1995; Martins 1996; Garland et al. 1999) for continuous variables that assumes a

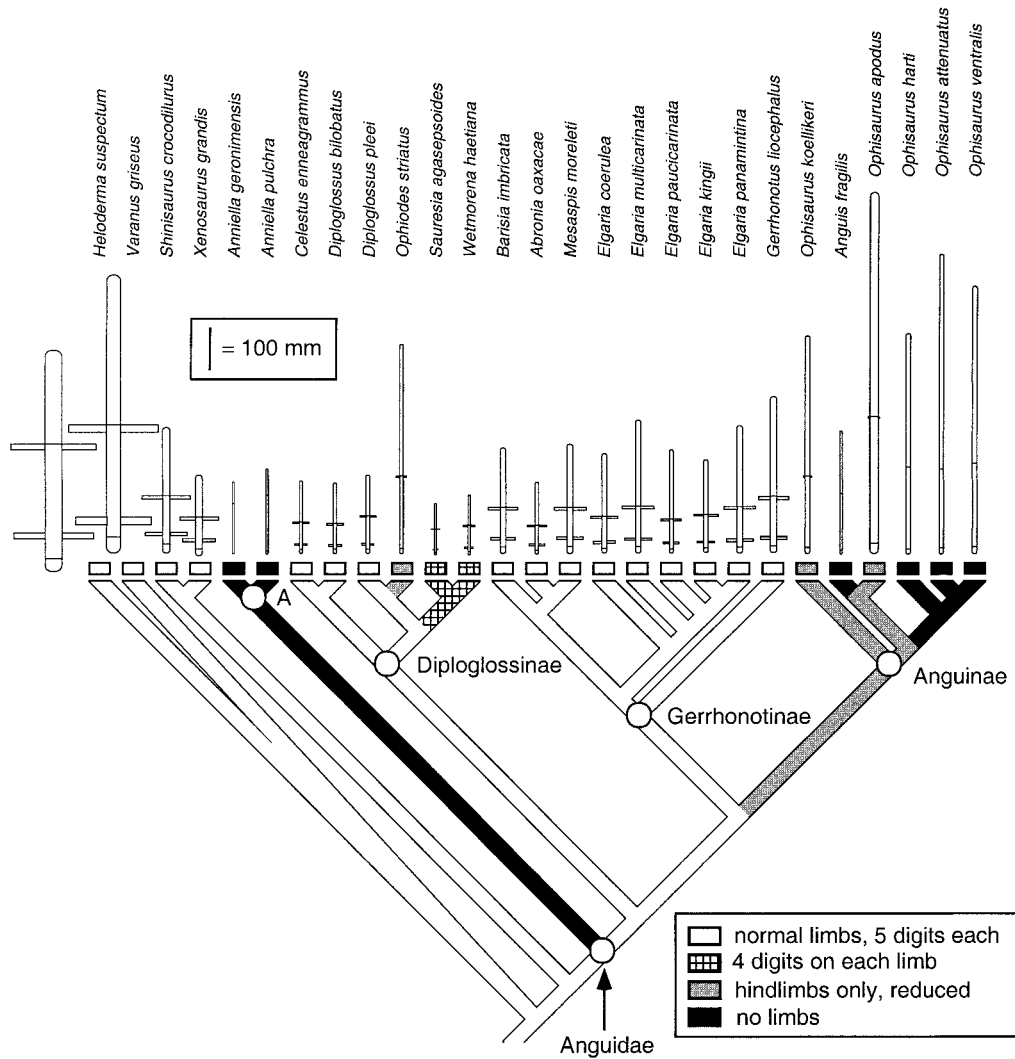


FIG. 3. Evolution of body form in anguid lizards and their relatives, mapped onto the traditional phylogeny based on morphology, allozymes, and mitochondrial DNA sequences. See Figure 2 for explanation of trait mapping, symbols, and abbreviations.

Brownian motion model of evolution. Three combinations of trees and branch lengths were used: (1) the maximum-likelihood tree estimated from our analyses of the mtDNA data of Macey et al. (1999), with branch lengths estimated using likelihood; (2) the maximum-likelihood tree with branch lengths assumed to be equal; and (3) the tree based on the composite allozyme, morphology, and mtDNA phylogenies, with all branch lengths assumed to be equal (estimating branch lengths would be difficult because of the multiple, nonoverlapping datasets). To verify that the independent branch lengths were adequately standardized by their estimated branch lengths (for the mtDNA data), the absolute values of each independent contrast for each node were regressed on their standard deviations (the square root of the sum of the branch lengths for that contrast), following Garland et al. (1992). No significant relationships were found, and the contrasts were therefore considered to be adequately standardized. Independent contrasts for each branch and character were obtained using the Contrasts package in PHYLIP, version 3.57c (Felsenstein 1995).

To quantify and assess overall changes in body form, we performed a series of multivariate analyses, using principal components analysis (PCA). We first generated a correlation matrix derived from linear regression analyses of each pair of morphological variables (using independent contrasts of natural log-transformed variables), with the regression model forced through the origin (following Clobert et al. 1998). The correlation matrix was then subjected to PCA using SYSTAT, version 5.2.1 (Wilkinson 1992). Analyses were performed both with all eight variables (including digit numbers for the hand and foot) and with the six morphometric variables alone. We used the broken-stick method to determine how many PCs to interpret (following Jackson 1993; Legendre and Legendre 1998). PCA was performed for all three sets of trees and branch lengths. Because many of the measurements involved structures that are lost in some taxa (e.g., limbs, digits), and because the log of zero is undefined, the value one was added to each measurement prior to log transformation.

We also performed a series of bivariate analyses designed

to explicitly test the hypotheses of previous authors. Previous authors have divided body and limb measurements by head length to obtain indices of elongation and relative limb size (Lande 1978; Caputo et al. 1995); the relative length of the body and limbs vary dramatically in lizards, whereas the size and shape of the skull are relatively conservative (Stokely 1947). Because many anguids lack a distinct neck or ear openings, the distance from the snout to the posterior corner of the eye was used as an index of skull length (HL), again following Lande (1978). The shape of this anteriormost part of the skull and the relative position of the eye seem to be extremely conservative in anguids, despite extensive change in other parts of the body (Figs. 2, 3; J. J. Wiens, pers. obs.). Numerous studies suggest that elongation in squamates proceeds primarily through increases in vertebral number (e.g., Camp 1923; Stokeley 1947; Presch 1975; Greer 1987; Caputo et al. 1995) and is independent of HL. HL is tightly correlated with HW in anguids (using independent contrasts, $R > 0.800$ and $P < 0.0001$, for all trees, branch lengths, and data transformations). This observation suggests the conservativeness of head shape in anguids and, because HW is related to body width (and elongation can be thought of as an increase in body length relative to width), supports the use of HL to obtain indices of shape.

Indices of elongation and relative limb size were based on residuals from linear regression of independent contrasts of HL (independent variable) versus contrasts of other measurements (dependent variables: SVL, TL, FLL, HLL). Residuals were calculated by making contrasts of HL positive (and switching signs of all other contrasts accordingly if HL contrasts were negative), and forcing the regression model through the origin (Garland et al. 1992). Overall body elongation in lizards may be achieved by lengthening the trunk, the tail, or both (e.g., Gans 1975). We used three measures of elongation: SVL, TL, and total length (TOTAL = SVL + TL).

The following bivariate analyses were performed. First, the relationship between the three measures of body elongation and the relative size of the fore and hind limbs was evaluated (i.e., residuals from regressing contrasts of HL versus contrasts of SVL, TL, TOTAL, FLL, HLL). Second, the relationship between the relative size of the limbs and independent contrasts of digit number was examined. Finally, the relationships between contrasts of HW and indices of body elongation, limb reduction, and digit reduction were tested. HW was used as a proxy for body diameter (i.e., body size independent of body elongation) because there are no clear landmarks for determining body width in limbless species. These analyses were performed on all three combinations of trees and branch lengths, and using both the raw and log-transformed morphometric data. The relationship between each pair of variables was examined using least-squares regression (using the Statview software package, Abacus Concepts, Berkeley, CA), forcing the model through the origin (Felsenstein 1985a; Garland et al. 1992), with significance levels based on regression coefficients. A sequential Bonferroni correction was used to adjust significance levels (Rice 1989). This correction was applied to a given set of independent contrast analyses (i.e., for one tree and set of

branch lengths), rather than to all analyses performed in the study.

Ecology

Data on general habitat preferences for the species in this study were compiled from the literature and observations of these species in nature by J. J. Wiens. The relationship between limb loss (absence of forelimbs and/or hind limbs; dependent variable) and fossoriality (burrowing; independent variable) was examined using Maddison's (1990) concentrated changes test, implemented in MacClade (Maddison and Maddison 1992). The test was performed on both the mtDNA and traditional phylogenies. Burrowing was defined as underground locomotion associated with digging by the lizard (e.g., sand swimming and the construction and use of tunnels by the lizard) and is distinguished from being merely cryptic (e.g., hiding under rocks or logs) or use of burrows made by other species. Detailed data on habitat preferences are not available for most species, but we consider a generalized characterization to be sufficient for our purposes. Diet might also potentially influence the evolution body form, but anguid species for which data are available feed mostly on invertebrates, and there seems to be little interspecific differentiation in diet (see references in Table 3). However, larger species of anguid (e.g., *E. multicaudata*, *Ophisaurus apodus*) may sometimes feed on small vertebrates (Arnold and Burton 1978; Stebbins 1985).

Skeletal Morphology

Previous authors have suggested that elongation in serpentiform squamates is associated with an increase in the number of presacral vertebrae (e.g., Camp 1923; Stokeley 1947; Presch 1975; Greer 1987; Caputo et al. 1995). Furthermore, the model of Cohn and Tickle (1999) implicitly assumes that species that are elongate and lack forelimbs should lack the associated limb girdles, because they postulate that elongation and forelimb loss are associated with anterior expansion of expression of midbody Hox genes. Data on the number of vertebrae and presence and absence of limb girdles were obtained from museum specimens prepared as skeletons using dermestid beetles. Skeletons were available from a representative of a fully limbed clade (*Elgaria coerulea*) and one or more representatives of each of the three clades of elongate, limb-reduced anguids (*Anniella pulchra*, *Ophiodes striatus*, *Anguis fragilis*, *Ophisaurus ventralis*).

RESULTS

Phylogeny

The most parameter-rich of the likelihood models of DNA sequence evolution that was examined, GTR + I + Γ , was found to have the best fit to the data of Macey et al. (1999), regardless of the order in which parameters were added (results not shown). This observation suggests that the simple assumptions of a parsimony analysis with equal weighting provide a very poor fit to these data. However, the optimal tree from the maximum-likelihood analysis using the GTR + I + Γ model (Fig. 1) was similar to the trees based on equally weighted parsimony (Macey et al. 1999). The main

difference is that in the parsimony tree, *Anniella* is the sister taxon of Anguinae, whereas in the likelihood tree, *Anniella* is nested inside Anguinae as the sister taxon of Diploglossinae. Because of the conflicting support for the monophyly of Anguinae exclusive of *Anniella*, we consider *Anniella* to be a subfamily of Anguinae (following Pough et al. 1998) rather than a separate family (contra Macey et al. 1999), a classification that is consistent with both phylogenies. The only other difference between the parsimony and likelihood trees is in the placement of *E. kingii* and *E. paucicarinata*; the positions of these two species are reversed in the parsimony and likelihood trees. Not all parts of the mtDNA tree based on maximum likelihood are strongly supported based on bootstrapping. Nevertheless, the three elongate, limb-reduced clades (Anniellinae, Anguinae, and *Ophiodes*) are each strongly supported as monophyletic (bootstrap = 100%; but *Ophiodes* is represented by a single species) and are separated from each other by one or more well-supported branches (bootstrap = 83–100%). Thus, the presence of three independent origins of snakelike body form in anguins is well supported by the molecular data (Fig. 1).

Evolutionary Changes in Body Form

Data on size and body form of each species are represented graphically on the mtDNA and traditional phylogenies (Figs. 2, 3) and are listed explicitly in Appendix 2. All phylogenies (traditional and mtDNA), branch lengths (equal and mtDNA), and reconstruction methods (squared-changes parsimony and GLS-linear) gave very similar results for character mapping analyses (i.e., reconstructing changes in body proportions), the results of which are described qualitatively below. In *Anniella*, there has been loss of fore and hind limbs and extreme elongation of the total length via elongation of SVL without elongation of the tail (we use SVL synonymously with trunk length herein; even though SVL also includes the head, HL is relatively conservative and elongation of SVL seems to occur primarily through increases in the number of trunk vertebrae; see Skeletal Morphology). Within diploglossines (*Diploglossus*, *Celestus*, *Ophiodes*, *Sauresia*, and *Wetmorena*), the forelimbs are lost entirely in *Ophiodes* and the hind limbs are reduced to small flaps. Limb loss in *Ophiodes* is accompanied by elongation of the total body length, which is the result of elongation of both the trunk and tail. The full complement of digits is present in the species of *Celestus* and *Diploglossus*, but one digit is absent from both the pectoral and pelvic limbs in *Sauresia* and *Wetmorena*. However, the exact phylogenetic placement of the loss of these digits depends somewhat on the optimization of the character and the phylogeny used. Although one possible optimization on both trees is for this loss to have occurred only in the common ancestor of *Sauresia* and *Wetmorena* (the optimization that we consider most likely), other equally parsimonious optimizations would involve loss of these digits in the common ancestor of Anniellinae and Diploglossinae, and reacquisition of the fifth digit in species of *Celestus* and *Diploglossus*. One equally parsimonious optimization suggests that the loss of the fifth digit occurred in the common ancestor of Anguinae and that it has been regained in Gerrhonotinae and some species of diploglossines. These opti-

mizations assume that gains and losses of digits are equally likely, however, and it seems that losses are much more likely than gains, given the many seemingly independent losses of digits and limbs that have occurred in squamates (Greer 1991). Furthermore, including additional species of *Diploglossus* and *Celestus* could also change this optimization to favor loss of digits (i.e., adding a species to either branch H or J in Fig. 1). *Sauresia* has a relatively elongate body, which seems to have arisen through some elongation of the trunk and slight elongation of the tail. Despite lacking a digit on the manus and pes, *Wetmorena haetiana* is not especially elongate, and has similar body proportions to *Diploglossus pleei*, a species with a full complement of digits (e.g., the range of individual specimen values for each species overlap for measures of body elongation and relative limb length). Both *Sauresia* and *Wetmorena* are small (i.e., small HL and HW) relative to other diploglossines, suggesting a possible role for miniaturization in the loss of digits in this clade.

No loss of limbs or digits occurs in Gerrhonotinae (*Abronia*, *Barisia*, *Elgaria*, *Gerrhonotus*) and body proportions are relatively conservative in this clade. The forelimbs are lost entirely in the common ancestor of Anguinae (*Ophisaurus* and *Anguis*), and the hind limb is reduced to a small stump. The hind limb stump is present in both *Ophisaurus koellikeri* and *O. apodus*. Given the phylogeny and equal weighting of gains and losses, this distribution requires either two losses of hind limbs in anguines (which we consider the most likely scenario) or the loss of hind limbs in anguines above *O. koellikeri* and their reacquisition in *O. apodus*. All anguines have extremely elongate bodies; this occurs through elongation of the trunk and elongation of the tail. Despite this elongation, the ratio of trunk to tail length in anguines is relatively similar to that of fully limbed species, such as gerrhonotines. Within anguines, *Anguis fragilis* has evolved a relatively elongate trunk, relatively short tail, and smaller overall body size (relative to other anguines), and its sister species, *O. apodus*, has evolved relatively large body size while maintaining similar body proportions. *Ophisaurus attenuatus* has evolved an even more elongate tail, without increasing relative trunk length.

In summary, both phylogenies show the independent evolution of limb-reduced and elongate body form in three different clades of anguins (anniellines, *Ophiodes*, and anguines). These three clades show similar levels of overall body elongation, but this is achieved in two very different ways. In *Anniella*, elongation occurs primarily through elongation of the trunk (SVL). In *Ophiodes* and anguines, elongation occurs in both the trunk and tail. Digit reduction is also present in *Sauresia* and *Wetmorena*. The similarity in body proportions between *W. haetiana* and *D. pleei* suggests that there is not necessarily a sequence of evolutionary change from body elongation to limb reduction to digit reduction; in at least one taxon, there is digit reduction without a significant increase in body elongation or limb reduction relative to a closely related species with a complete set of digits.

Correlated Character Evolution

Multivariate analyses (PCA) gave very similar results for all three sets of trees and branch lengths, and we describe

TABLE 1. Results of principal component analysis of independent contrasts of log-transformed morphological variables, both including and excluding digit numbers. Variable loadings, eigenvalues, and percentage of total variance explained are given for each principal component (PC). Only the first four PCs are shown, and only the first two are considered interpretable (using the broken-stick method). Independent contrasts are based on the tree and branch lengths estimated from the maximum-likelihood analysis of the mitochondrial DNA sequence data (Fig. 1).

	All variables			
	PC1	PC2	PC3	PC4
Snout-vent length	0.683	0.574	-0.419	-0.279
Tail length	0.617	0.641	-0.278	0.349
Head width	0.833	0.428	0.334	-0.090
Head length	0.799	0.547	0.310	-0.014
Forelimb length	0.775	-0.535	-0.018	0.139
Hind limb length	0.823	-0.416	0.386	0.114
Fingers	0.742	-0.515	-0.522	0.044
Toes	0.773	-0.572	0.020	-0.223
Eigenvalue	4.607	2.275	0.882	0.292
% variance	57.593	28.439	11.023	3.651
	Morphometric variables only			
	PC1	PC2	PC3	PC4
Snout-vent length	0.791	-0.499	0.262	0.222
Tail length	0.781	-0.465	0.112	-0.402
Head width	0.959	0.006	-0.203	0.168
Head length	0.969	-0.114	-0.205	0.044
Forelimb length	0.543	0.728	0.415	0.017
Hind limb length	0.645	0.724	-0.196	-0.116
Eigenvalue	3.805	1.533	0.375	0.255
% variance	63.417	25.545	6.246	4.252

only the results using the tree and branch lengths estimated using maximum likelihood for the mtDNA data (Table 1). With all eight variables included, the first PC explains 57.6% of the variation in the data. All variables show strong positive loadings on this component, which suggests that it reflects overall size (the loadings of digit numbers on this PC imply a relationship between reduced body size and digit reduction). The second PC explains 28.4% of the variance, and shows relatively strong positive loadings for SVL, TL, HL, and HW and strong negative loadings for limb lengths and numbers of fingers and toes. We interpret this PC as indicating an association between limb reduction and digit reduction, which is contrasted with body size. Additional PCs are considered uninterpretable (i.e., the third PC explains only 11.0% of the variance). When only the six morphometric variables are included, the first PC explains 63.4% of the variation and again shows strong positive loadings for all variables (presumably reflecting size). The second PC explains 25.6% of the variance and shows strong positive loadings for limb lengths and strong negative loadings for SVL and TL, suggesting a relationship between limb reduction and body elongation. Subsequent PCs were not interpretable (i.e., the third PC explains only 6.3% of the variance). In summary, the multivariate analyses support the relationships between limb reduction and digit reduction and limb reduction and body elongation suggested by previous authors. PCA also implies a relationship between miniaturization and digit reduction but not between miniaturization and elongation.

Relationships among variables are generally very similar

TABLE 2. Relationships between pairs of morphological variables based on regression analyses of independent contrasts. The variables rSVL (snout-vent), rFLL (forelimb), rHLL (hindlimb), rTOTAL (snout-vent and tail), and rTAIL (tail) are residuals from regression of independent contrasts of each these variables with contrasts for head length. HW denotes head width. Results shown used the topology and branch lengths estimated from maximum-likelihood analysis of the DNA sequence data (Fig. 1), with log-transformed morphometric data. Alternate topologies, branch lengths, and data transformations give very similar results. Results that are considered significant ($\alpha = 0.05$) after a sequential Bonferroni correction (Rice 1989) are marked with an asterisk.

Independent variable	Dependent variable	R	P
rSVL	rFLL	0.778	<0.0001*
rSVL	rHLL	0.723	<0.0001*
rTOTAL	rFLL	0.826	<0.0001*
rTOTAL	rHLL	0.668	<0.0001*
rTAIL	rFLL	0.671	0.0001*
rTAIL	rHLL	0.480	0.0112
rFLL	fingers	0.935	<0.0001*
rHLL	toes	0.868	<0.0001*
HW	rSVL	0.043	0.8299
HW	rTOTAL	0.117	0.5598
HW	rTAIL	0.140	0.4860
HW	fingers	0.185	0.3552
HW	toes	0.405	0.0362
HW	rFLL	0.105	0.6023
HW	rHLL	0.089	0.6602

across all bivariate analyses, regardless of phylogeny, branch lengths, and data transformation. A sample of the results, based on the tree and branch lengths estimated from maximum-likelihood analysis of the mtDNA data, is shown in Table 2 and Figure 4. In all analyses, there is a significant relationship between trunk elongation and limb reduction and between total elongation and limb reduction, and a weaker relationship between tail elongation and limb reduction (not significant for some comparisons with hind limb length). Reduction in limb size is consistently associated with loss of digits. The relationship between HW (a proxy for diameter or size) and elongation, digit reduction, and limb reduction is generally weak (a significant relationship was found only between HW and toe number and only on the traditional tree using log-transformed data). In summary, both PCA and bivariate analyses support the hypothesized correlations between elongation and limb reduction and between limb reduction and digit reduction. However, there is no consistent relationship between miniaturization and the evolution of limb reduction, digit loss, or elongate body form.

Ecological Correlates

Most species of anguids are terrestrial (active on the surface of the ground), although *Abronina* are arboreal and *Anniella* are burrowing sand swimmers (Table 3). Previous authors have suggested a relationship between limb loss and burrowing (e.g., Gans 1975; Rieppel 1988; Caputo et al. 1995; Lee 1998). Limb loss has occurred three times in anguids (*Anniella*, *Ophiodes*, *Ophisaurus* + *Anguis*). *Anniella* are burrowers, but as far as is known, species of *Ophiodes*, *Ophisaurus*, and *Anguis* are not. Use of Maddison's concentrated-changes test shows no significant relationship between

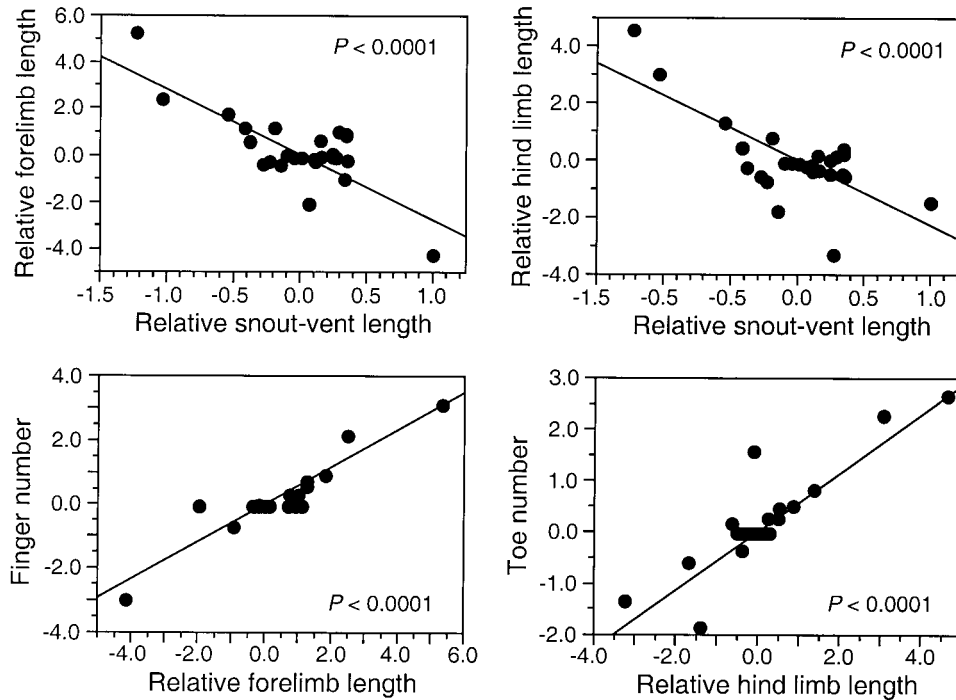


FIG. 4. Plots from least-squares linear regression analyses (forced through the origin) of selected pairs of morphological variables for anguid lizards, showing relationships between limb reduction and trunk elongation and between digit loss and limb reduction. Relative snout-vent length, forelimb length, and hind limb length are based on residuals from regression of independent contrasts of snout-vent length, forelimb length, and hind limb length with contrasts of head length. Finger and toe number also represent contrasts. All variables were natural-log transformed prior to calculating contrasts. Contrasts are based on the tree and branch lengths estimated using likelihood analysis of the mitochondrial DNA sequence data (Fig. 1). See Table 2 for further results.

TABLE 3. Ecological data for anguid species included in the analysis. Species described as cryptic are found under rocks, logs, and other surface cover. "Both" indicates that forelimbs and hind limbs are present. Data were unavailable for *Celestus enneagrammus* and *Ophisaurus koellikeri*.

Species	Limbs	Habitat	Source
Anniellinae			
<i>Anniella pulchra</i>	none	burrower in sand	Stebbins (1985)
<i>Anniella geronimensis</i>	none	burrower in sand	Stebbins (1985)
Diploglossinae			
<i>Diploglossus pleei</i>	both	terrestrial, cryptic	Schwartz and Henderson (1991)
<i>Ophiodes striatus</i>	hind only	terrestrial, grass-swimmer	Cei (1993); R. Espinoza and F. Lobo (pers. comm.)
<i>Sauresia agasepsoides</i>	both	terrestrial, cryptic	Schwartz and Henderson (1991)
<i>Wetmorena haetiana</i>	both	terrestrial cryptic	Schwartz and Henderson (1991)
Gerrhonotinae			
<i>Elgaria coerulea</i>	both	terrestrial, cryptic	Stebbins (1985)
<i>Elgaria multicarinata</i>	both	terrestrial, cryptic	Stebbins (1985); J. J. Wiens (pers. obs.)
<i>Elgaria kingii</i>	both	terrestrial, cryptic	Stebbins (1985); J. J. Wiens (pers. obs.)
<i>Elgaria panamintina</i>	both	terrestrial, cryptic	Stebbins (1985)
<i>Elgaria paucicarinata</i>	both	terrestrial, cryptic	Stebbins (1985)
<i>Abronia oaxacae</i>	both	arboreal	Campbell and Frost (1993)
<i>Barisia imbricata</i>	both	terrestrial	J. J. Wiens (pers. obs.)
<i>Mesaspis moreleti</i>	both	terrestrial	Alvarez del Toro (1982)
<i>Gerrhonotus liocephalus</i>	both	terrestrial, arboreal	Alvarez del Toro (1982)
Anguinae			
<i>Anguis fragilis</i>	none	terrestrial, cryptic	Arnold and Burton (1978)
<i>Ophisaurus apodus</i>	hind only	terrestrial	Arnold and Burton (1978)
<i>Ophisaurus attenuatus</i>	none	terrestrial, cryptic	Fitch (1989); Mitchell (1994); Palmer and Braswell (1996)
<i>Ophisaurus ventralis</i>	none	terrestrial, cryptic	Mitchell (1994); Palmer and Braswell (1996)

TABLE 4. Osteological data for anguid lizards (CM, Carnegie Museum of Natural History; AMNH, American Museum of Natural History).

Species	Presacral vertebrae	Pectoral girdle	Pelvic girdle	Source
<i>Elgaria coerulea</i>	30	present	present	CM 50843
<i>Ophiodes striatus</i>	66	present	present	AMNH 140844; Stokely (1947)
<i>Anniella pulchra</i>	73	present	present	Stokely (1947)
<i>Anguis fragilis</i>	64–65	present	present	AMNH 73056; Stokely (1947)
<i>Ophisaurus ventralis</i>	56–57	present	present	AMNH R140828, CM 112304

burrowing and limb loss in anguids ($P > 0.200$ on both trees). By using MacClade to manipulate the probabilities obtained from this test, we found that if there were three independent acquisitions of burrowing behavior associated with each of the three lineages of limb-reduced anguids, the test would be powerful enough to yield a significant result in this group ($P = 0.02$).

Skeletal Morphology

Osteological data from our observations and the literature (Table 4) suggest that elongation of the trunk is achieved through an increase in the number of presacral vertebrae in all three clades, as predicted by previous authors (Camp 1923; Stokely 1947; Presch 1975; Greer 1987). At least some elements of the pectoral and pelvic girdles are present in all of the limbless forms, although they are extremely reduced in *Anniella pulchra* (Stokely 1947). The presence of the pectoral girdle in the elongate, limbless forms suggests that elongation and loss of the forelimbs are not simply the result of anterior expansion of more posterior domains of Hox gene expression, as suggested by Cohn and Tickle (1999). Expansion of Hox gene domains would explain the complete absence of all limb girdle elements, but not the absence of the limbs alone.

DISCUSSION

In this study, we combine morphometrics, molecular phylogenetics, and phylogenetic comparative methods to provide the first statistical phylogenetic tests of the long-standing hypotheses of correlation between body elongation, limb reduction, and digit loss in the evolution of snakelike vertebrates. Our results support the hypotheses that limb reduction is correlated with body elongation and that digit loss is correlated with limb reduction. Surprisingly, our results do not support the hypothetical sequence of body elongation to limb reduction to digit loss, nor do they support the idea that limb loss is necessarily associated with miniaturization or a burrowing lifestyle. Our analyses show that elongate body form results from two different morphological mechanisms (trunk elongation vs. trunk and tail elongation), which are associated with two very different habitats (subterranean vs. surface-dwelling), and that these ecomorphs have evolved repeatedly among snakelike squamates. Our results also reject the developmental model of Cohn and Tickle (1999), who proposed that body elongation and limb loss are linked through changes in Hox gene expression patterns and that this transformation in body form occurs suddenly rather than gradually.

The correlations between body elongation and limb reduction and between limb reduction and digit reduction may

have very different explanations. Gans (1975) proposed that the correlation between body elongation and limb reduction has a functional explanation, in that lateral undulation is purportedly a more energy-efficient means of locomotion than quadrupedal locomotion. Lateral undulation is common in limbless squamates and requires an elongate body but does not require functional limbs, and limbs may be an impediment to movement in the burrows and dense grass favored by snakelike lizards (Gans 1975). However, Walton et al. (1990) showed that lateral undulation might have a similar energetic cost to limbed locomotion, based on physiological comparisons of a relatively derived snake (*Coluber constrictor*) and a group of skinks. Remarkably, no studies have compared the energetic efficiencies of limbed and limbless locomotion among closely related species, nor have any compared the locomotor performances of closely related limbed and limbless species (e.g., speed, endurance, maneuverability). The general question of what drives the repeated evolution of elongate, limb-reduced body form in squamates remains unanswered.

In contrast to the possibly functional relationship between body elongation and limb reduction, the relationship between limb reduction and digit loss may have a developmental explanation. Alberch and Gale (1983, 1985) showed that in amphibians, the number of digits in the adult limb is related to the number of cells in the developing limb bud. Similar experiments in lizards also suggest that the number of cells in the embryonic limb bud determines digit number (Raynaud 1985). The number of cells in the developing limb bud may be reflected in the size of the adult limb, and selection on the size of the adult limb may lead to loss of digits as a developmental by-product. In support of this hypothesis, there is a consistent relationship (based on regressions of independent contrasts) between reduction in absolute limb size and loss of digits for anguids ($P < 0.01$ for all combinations of limbs, trees, branch lengths, and data transformations; results not shown). This relationship may explain the loss of digits in the *Sauresia-Wetmorena* clade, which has comparatively little reduction in relative limb size. Lande (1978) suggested that absolute limb size was not important in digit reduction (e.g., *Tyrannosaurus rex* has only two digits in the forelimb), but we hypothesize that the threshold of limb size for determining digit loss may simply vary among clades.

Previous authors have hypothesized that there is a sequence in the evolution of snakelike body form (e.g., Gans 1975; Lande 1978), starting with body elongation, followed by reduction in relative limb size, which is then followed by digit loss. Our results suggest instead that these variables all change together, more or less simultaneously. We found sig-

nificant correlations between these variables using independent contrasts, which indicates that they change on the same branch of the phylogeny, rather than changes in one variable consistently preceding another phylogenetically. We also found one digit-reduced species (*W. haetiana*) in which the level of body elongation and relative limb-size reduction was very similar to a closely related species (*D. pleei*), which had a full complement of digits. Thus, digit loss need not be preceded by extensive body elongation and limb reduction (although the case of *Wetmorena* may be exceptional because of overall miniaturization in body size).

Previous authors also have suggested relationships between the evolution of snakelike body form and miniaturization and a burrowing lifestyle (e.g., Gans 1975; Rieppel 1988; Caputo et al. 1995; Lee 1998). Both of these correlations are unsupported in anguids, largely for the same reason; whereas the limbless *Anniella* is a small, burrowing form (as predicted), the other two limbless clades (*Ophiodes* and the anguines) are nonburrowers and are of moderate (*Ophiodes*) or variable (anguines) size.

These different habitats are associated with two different kinds of elongation in anguids, both of which result in a superficially similar snakelike body form. In the burrowing *Anniella*, the trunk is elongate, but the ratio of TL to HL is similar to that seen in nonelongate anguid species. This results in a morphology with an elongate trunk and a tail that is roughly half the length of the trunk (unlike most anguids, in which the tail is usually equal to or longer than the SVL). In the surface-dwelling *Ophiodes* and anguines, both the trunk and tail are elongated (relative to HL and relative to the lengths in other species), and the ratio of trunk length to TL is similar to that seen in nonelongate anguids (with the tail ranging from roughly equal to about twice the SVL).

Our results suggest that there are two ecomorphs of snake-like lizards that evolved independently many times and that differ in body proportions, size, and habitat use: small, short-tailed burrowers (e.g., *Anniella*) and large, long-tailed, grass-swimmers (e.g., *Ophiodes* and the anguines). This dichotomy in body form and habitat in snakelike lizards was first suggested by Camp (1923; p. 417), and seems to be extremely widespread. Based on data from our observations (J. J. Wiens, unpubl. data) and the literature, elongate trunks and relatively short tails are found in amphisbaenians (which are all burrowers; Pough et al. 1998), snakes (the primitive lineages of which are burrowers; Pough et al. 1998), and many groups of burrowing skinks (e.g., acontiines, feyliniines, some scincines; Klemmer 1975; Branch 1988; Caputo et al. 1995). In contrast, the cordylids (e.g., *Chamaesaura*) and gerrhosaurids (e.g., *Tetradactylus*) with reduced limbs and elongate trunks and tails are surface-dwelling grass swimmers (Branch 1988; J. J. Wiens unpubl. data). Pygopodids are all elongate and limb reduced, and the group contains both long-tailed surface dwellers and short-tailed burrowers (based on information in Cogger 2000). This dichotomy may even exist within a single genus (*Chalcides*; Caputo et al. 1995). Although this association between morphology and habitat use seems widespread and strong, it must be verified across squamates with phylogenetic comparative methods and extensive taxon sampling.

The reasons for this dichotomy are unclear, but we suggest

three preliminary (and not mutually exclusive) hypotheses. First, there may be advantages in locomotor performance for burrowers to be short-tailed and grass-swimmers to be long-tailed. Elongate tails seem to have evolved repeatedly in grass-swimming lizards, including those that have limbs (e.g., lacertid genus *Takydromus*; grass-bush ecomorph of Caribbean *Anolis*; Losos 1992). Second, increased allocation of length to the tail may be favored in surface-dwelling lizards because of higher predation pressure in these habitats than underground, and because damage to the tail may be less critical than damage to the trunk (most of the tail contains no vital organs). Many surface-dwelling squamates have long tails that are modified for easy breakage and regeneration (Pough et al. 1998), including the long-tailed, snakelike anguids (e.g., *Ophioides*, *Ophisaurus*). Caudal autotomy seems to be less common among burrowing snakelike species (e.g., *Anniella*). Third, small body size in burrowing species may place constraints on female body shape (leading to correlated change in both sexes). Miniaturization is widespread in burrowing squamates (Rieppel 1988; Lee 1998), and the only burrowing species of anguids (*Anniella*) are small. Increased allocation to trunk length may be selected for in miniaturized species to maintain an adequate volume of the abdominal cavity for developing embryos, because reduction of abdominal cavity volume should be constrained by selection against decreased size of offspring and/or clutches (Griffith 1990). In potential support of this hypothesis within anguids, we found that the nonburrowing but miniaturized *Anguis fragilis* has increased the relative length of the trunk and decreased the relative length of the tail. Furthermore, an increase in relative trunk length has evolved in three of the four smallest genera of anguids (*Anguis*, *Anniella*, and *Sauresia*).

Our data for anguids also have implications for a recent study on the role of Hox genes in the evolution of the snake-like body form. Cohn and Tickle (1999) compared Hox gene expression patterns in developing python and chicken embryos and suggested that loss of forelimbs and trunk elongation in snakes are linked through a common developmental mechanism: the expansion of midbody Hox gene expression domains anteriorly along the body axis. These authors suggested that "such higher order genetic changes could have resulted in sudden anatomical transformations, rather than gradual changes, during snake evolution" (Cohn and Tickle 1999, p. 478) and that the evolution of limblessness in other squamate lineages (specifically mentioning anguids) may have involved similar developmental mechanisms. In anguids, there is also loss of forelimbs and elongation of the trunk in three independent lineages (Anguinae, Anniellinae, and *Ophiodes*). However, in all three lineages, elements of the pectoral girdle are retained, despite the absence of forelimbs. Anterior expansion of more posterior Hox gene expression domains is only consistent with the absence of all forelimb and pectoral girdle elements. Thus, the presence of the pectoral girdle in these elongate species indicates that forelimbs are not lost in anguids because this region of the body has acquired a more posterior identity and that there is not a common developmental mechanism linking elongation and limb loss. Instead, the presence of reduced pectoral girdles and the existence of species with intermediate levels of digit loss, limb reduction, and body elongation (i.e., *Saure-*

sia), suggest that transition from lizard to snakelike body form is gradual and does not involve sudden anatomical transformations. In fact, species with intermediate degrees of limb loss and body elongation are extremely common in some groups of lizards, such as scincids and gymnophthalmids (Lande 1978). Unless the evolution of the serpentiform body plan in snakes involves fundamentally different developmental mechanisms from those seen in anguids and other squamates, we suggest that the model of Cohn and Tickle (1999) needs to be revised.

Paleontological, biogeographical, and molecular data for anguid lizards suggest that the transition from lizard to snake-like body form may be quite slow rather than sudden. None of the three limbless lineages of anguids appear to be less than 20 million years old. The anniellines are known from fossils in Wyoming that are 49–54 million years old (Gauthier 1982). Anguines are found in both North America and Europe (which became separated roughly 50 million years ago), and the oldest fossil anguines have been estimated to be 40–50 million years old (Gauthier 1982). No fossils of *Ophiodes* have been recorded. Estimating the age of this lineage using molecular data, given a rate of 0.65–0.69% uncorrected sequence divergence between sister taxa per million years (Macey et al. 1999), suggests that *Ophiodes* is at least 21–22 million years old. However, this date may be an underestimate of the age of *Ophiodes* because of saturation or an overestimate if there has been a large increase in rates within diploglossines, and the use of molecular data to date lineages is controversial (Hillis et al. 1996). Furthermore, the antiquity of these clades does not rule out the possibility of rapid limb loss and body elongation either early or late in the history of these lineages, and there is too little postcranial fossil material for anguids to adequately address this issue. Nevertheless, the expected pattern, given rapid evolution of body form (i.e., two recently diverged sister taxa, one snakelike and the other not), does not occur in any of the three serpentiform lineages of anguids. We support the idea that the origin of higher taxa (such as snakes) may proceed gradually through the same processes that occur within populations and among closely related species (Lande 1978), without invoking sudden changes or special genetic or developmental mechanisms.

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- 37, 33812, 33818, 33825–26, 33830, 33837. *Barisia imbricata* ($n = 12$) CAS 95923–24, 135686, 169644–45, 169775. UTA 5949, 5974, R4182, R4194, R4459, R17931. *Celestus enneagrammus* ($n = 6$) UTA 8546, 8549, 8551, 10278, R30339, R32725. *Diploglossus bilobatus* ($n = 9$) KU 34296–97, 40988, 67427, 67430, 67432–33, 104093, 113658. *Diploglossus pleei* ($n = 2$) CAS 200731. USNM 221102. *Elgaria coerulea* ($n = 9$) CM 20310, 22645, 76225, 88714, 94672, 94709, 94752, 94688, 94723. *Elgaria kingii* ($n = 11$) CAS 12713, 34963, 48843, 48845. CM 19359, 33506, 37519, 51555, 52428, 59544, 69852. *Elgaria multicarinata* ($n = 11$) AMNH 595, 9047, 97215, 77343. CM 24893, 29083, 73814, S5517. USNM 106017, 196340, 297237. *Elgaria panamintina* ($n = 3$) MVZ 75918, 134111, 150326. *Elgaria paucicarinata* ($n = 8$) CAS 46723, 46728, 119123, 190915. MVZ 11770, 45367–68, 50078. *Gerrhonotus liocephalus* ($n = 10$) AMNH 100723, 100726, 102729, 102732–33, 106730–31, 106733, 106745, 106747. *Mesaspis moreleti* ($n = 15$) CM 41864, S8118–19. UTA 6426, 6564, R19694–95, R19731, R19736, R19740, R33183, R33201, R33651, R33656, R40106. *Ophiodes striatus* ($n = 3$) CM 55419, 68405. USNM 98609. *Ophisaurus apodus* ($n = 15$) AMNH 2244, 2409, 19899, 28652, 38229–30, 75318. CM 90197, S4861. USNM 7363, 14322, 58674, 149410, 154487–88. *Ophisaurus attenuatus* ($n = 13$) CM 9987, 25885, 32046, 32048, 32050, 32052, 58700, 61922, 92143, P1679, S4307, S7000, S8651. *Ophisaurus harti* ($n = 12$) AMNH 20981, 33538, 34950, 34952–55, 34957, 34960, 34963, 34966. USNM 60575. *Ophisaurus koellikeri* ($n = 4$) AMNH 84174, 99159, 139561. MVZ 178119. *Ophisaurus ventralis* ($n = 11$) AMNH 1922, 10435, 63779, 111142, 112934, 120179. CM 16807, 23876, 27796, 27812, 56922. *Sauresia agasepsoides* ($n = 3$) KU 227574–75. USNM 259974. *Wetmorena haetiana* ($n = 14$) KU 228269, 228279, 228282, 228298, 228308, 228314, 228324, 228426–27, 228431, 228434, 228439–40, 228446.
- Helodermatidae. *Heloderma suspectum* ($n = 5$) CM 19338, 21318, 25220, 142372, S6907.
- Xenosauridae. *Shinisaurus crocodilurus* ($n = 4$) AMNH 129138, 118981, 118982. CM 115318. *Xenosaurus grandis* ($n = 5$) AMNH 100837–40. CM 90203.
- Varanidae. *Varanus griseus* ($n = 3$) AMNH 88361. CM 33538, 65845.

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APPENDIX 1

Specimens Examined

Institutional abbreviations are explained in the Acknowledgments.

Anguillidae. *Abronia oaxacae* ($n = 1$) AMNH 98003. *Anguis fragilis* ($n = 13$) CM 28200, 28641, 28956–57, 55425, 58202, 60289, 60885, 60887, S4303–05, S6653. *Anniella geronimensis* ($n = 12$) MVZ 78805–06, 117305, 142015, 142017–18, 142023–24, 197492–95. *Anniella pulchra* ($n = 20$) CM 14463, 22092, 24894, 26027, 39263–65. MVZ 11223–24, 11227, 11229, 11230, 11236–

APPENDIX 2
 Raw morphometric data for anguillid lizards and relatives. All measurements are in millimeters. The mean and standard deviation are given.

Species	n	Variables									
		SVL	TL	HW	HL	FLL	HLL	Fingers	Toes		
<i>Heloderma suspectum</i>	5	300.3 ± 18.64	124.3 ± 14.56	39.6 ± 1.56	29.3 ± 1.56	75.2 ± 5.36	82.1 ± 2.72	5 ± 0	5 ± 0		
<i>Varanus griseus</i>	3	303.5 ± 12.58	355.3 ± 22.40	32.9 ± 4.51	37.1 ± 1.44	73.9 ± 6.85	90.4 ± 4.42	5 ± 0	5 ± 0		
<i>Shinisaurus crocodilurus</i>	4	135.5 ± 15.57	162.0 ± 19.27	18.2 ± 2.08	18.5 ± 1.95	40.7 ± 4.48	48.9 ± 3.22	5 ± 0	5 ± 0		
<i>Xenosaurus grandis</i>	5	100.5 ± 10.56	90.3 ± 12.38	16.3 ± 2.21	14.9 ± 1.53	30.7 ± 3.00	37.8 ± 3.03	5 ± 0	5 ± 0		
Anguillidae											
<i>Anniella geronimensis</i>	12	118.5 ± 9.79	51.9 ± 4.75	3.9 ± 0.39	4.3 ± 0.46	0.0 ± 0.00	0.0 ± 0.00	0 ± 0	0 ± 0		
<i>Anniella pulchra</i>	20	137.9 ± 11.18	62.9 ± 13.79	4.6 ± 0.40	5.0 ± 0.52	0.0 ± 0.00	0.0 ± 0.00	0 ± 0	0 ± 0		
<i>Celestes emeagrammus</i>	6	72.6 ± 11.49	99.2 ± 6.72	7.1 ± 0.68	7.3 ± 0.64	11.0 ± 1.72	16.1 ± 1.19	5 ± 0	5 ± 0		
<i>Diploglossus bilobatus</i>	9	70.3 ± 11.79	97.0 ± 13.37	7.9 ± 1.56	7.5 ± 1.39	10.7 ± 2.32	16.2 ± 3.52	5 ± 0	5 ± 0		
<i>Diploglossus pleii</i>	2	87.2 ± 12.66	97.6 ± 9.69	8.4 ± 2.12	7.8 ± 0.78	9.6 ± 0.07	15.3 ± 1.84	5 ± 0	5 ± 0		
<i>Ophiodon striatus</i>	3	184.2 ± 19.79	311.2 ± 19.66	8.2 ± 0.81	9.7 ± 0.95	0.0 ± 0.00	8.0 ± 2.50	0 ± 0	1 ± 0		
<i>Sauresia agasepsoides</i>	3	60.0 ± 8.422	61.0 ± 7.38	4.3 ± 0.26	4.0 ± 0.21	3.4 ± 0.38	6.8 ± 0.55	4 ± 0	4 ± 0		
<i>Wetmorena haetiana</i>	14	68.7 ± 9.61	72.0 ± 14.24	6.4 ± 0.79	6.2 ± 0.75	6.9 ± 1.36	11.1 ± 2.34	4 ± 0	4 ± 0		
<i>Barisia imbricata</i>	12	110.3 ± 20.93	139.4 ± 26.07	14.4 ± 3.14	13.8 ± 2.48	22.0 ± 4.03	28.0 ± 4.44	5 ± 0	5 ± 0		
<i>Gerrhonotus liocephalus</i>	10	131.7 ± 15.62	238.8 ± 44.97	14.8 ± 2.56	15.4 ± 2.20	24.0 ± 4.16	28.4 ± 3.63	5 ± 0	5 ± 0		
<i>Abronia oaxacae</i>	1	107.2 ± 0.00	150.8 ± 0.00	14.7 ± 0.00	13.8 ± 0.00	24.2 ± 0.00	29.9 ± 0.00	5 ± 0	5 ± 0		
<i>Mesaspis moreleti</i>	15	66.8 ± 9.41	103.8 ± 18.95	8.2 ± 1.18	8.2 ± 1.14	15.1 ± 2.96	19.0 ± 3.41	5 ± 0	5 ± 0		
<i>Elgaria coerulea</i>	9	86.5 ± 10.95	149.3 ± 33.84	10.9 ± 1.50	11.4 ± 1.55	19.8 ± 3.08	27.3 ± 4.24	5 ± 0	5 ± 0		
<i>Elgaria kingii</i>	11	79.6 ± 7.85	165.1 ± 13.44	8.6 ± 1.01	9.3 ± 1.10	14.2 ± 1.34	19.1 ± 1.36	5 ± 0	5 ± 0		
<i>Elgaria paucicarinata</i>	8	87.6 ± 11.22	132.5 ± 33.91	10.2 ± 1.18	11.0 ± 1.20	16.2 ± 2.59	23.3 ± 4.06	5 ± 0	5 ± 0		
<i>Elgaria multicarinata</i>	11	110.4 ± 19.35	204.5 ± 40.70	12.4 ± 2.42	13.5 ± 2.39	25.5 ± 5.02	32.0 ± 5.38	5 ± 0	5 ± 0		
<i>Ophisaurus koellikeri</i>	3	109.9 ± 7.78	192.7 ± 17.06	12.5 ± 1.42	13.2 ± 1.49	24.6 ± 0.50	31.1 ± 0.83	5 ± 0	5 ± 0		
<i>Ophisaurus koellikeri</i>	4	183.3 ± 80.70	333.0 ± 94.51	11.1 ± 6.21	12.0 ± 6.02	0.0 ± 0.00	4.4 ± 1.97	0 ± 0	1 ± 0		
<i>Anguis fragilis</i>	13	142.3 ± 24.24	148.1 ± 37.04	6.3 ± 1.04	6.7 ± 1.04	0.0 ± 0.00	0.0 ± 0.00	0 ± 0	0 ± 0		
<i>Ophisaurus apodus</i>	15	321.2 ± 37.81	534.2 ± 51.34	19.8 ± 2.65	21.0 ± 2.41	0.0 ± 0.00	2.8 ± 0.56	0 ± 0	1 ± 0		
<i>Ophisaurus harti</i>	12	205.2 ± 26.76	317.5 ± 53.07	11.5 ± 1.73	11.0 ± 1.61	0.0 ± 0.00	0.0 ± 0.00	0 ± 0	0 ± 0		
<i>Ophisaurus attenuatus</i>	13	200.2 ± 46.89	508.7 ± 140.27	10.4 ± 2.56	13.2 ± 2.66	0.0 ± 0.00	0.0 ± 0.00	0 ± 0	0 ± 0		
<i>Ophisaurus ventralis</i>	11	212.7 ± 27.87	419.6 ± 85.96	11.2 ± 1.72	13.8 ± 1.98	0.0 ± 0.00	0.0 ± 0.00	0 ± 0	0 ± 0		