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# Phylogeny of Xantusiid Lizards: Concern for Data and Analysis

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**Phylogenetic analyses of new DNA sequence data from the mitochondrial 16S rRNA gene in xantusiid lizards support the intergeneric relationships obtained previously (S.B. Hedges, R.L. Bezy, and L.B. Maxson, 1991, *Mol. Biol. Evol.* 8:767–780) with data from the 12S rRNA and cytochrome b (cyt b) genes. The total data set now includes 1028 alignable sites, 471 of which are variable and informative for the distance analyses and 281 of which are informative for the parsimony analyses. Crother and Presch (1993), *Mol. Phylogenet. Evol.* 1:289–294) claim that their reanalyses of our 12S rRNA and cyt b sequence data do not support a robust phylogeny for xantusiid lizards. However, that conclusion is not supported by their own analyses of the combined data from those two genes, which result in the same phylogenetic tree of xantusiid genera that we obtained in the original study with the same method (maximum parsimony). This result was unchanged when Crother and Presch eliminated sites containing insertions/deletions and ambiguities, and when transversions were weighted. The less robust result for the separate cyt b analyses, probably due to the smaller size of the data set, was already noted (Hedges *et al.*, 1991). We believe that the best estimate of relationships, in this case, is obtained by combining the sequence data from these tightly linked mitochondrial genes. We also refute the criticisms by Crother and Presch of the neighbor-joining method. To correct for a higher rate of transitions in mitochondrial sequence data, they weight transversions more heavily (5×) than transitions. We present theoretical criticisms of this weighting method and advocate the use of scaled corrections available with distance methods. Crother and Presch also claim that a robust phylogeny of xantusiid lizards is not obtained when some morphological data (13 informative characters) are combined with the molecular data in a single analysis. However, there are serious problems with their morphological data and methods of analysis. We reevaluate the three pivotal morphological characters in their alternative phylogeny for xantusiid genera and demonstrate that none of the three provides unambiguous support for their alternative arrangement. Crother and Presch implement a new approach whereby the entire morphological data set is weighted equally to the molecular data set in a combined analysis, thus resulting in a very inflated weight assigned to each character in the small morphological data set. Using this rationale, each of the 13 informative morphological characters would receive a greater than million-fold weight if combined with the sequence data for the entire genome of these lizards. We do not advocate such an approach.** 

## INTRODUCTION

In a recent study, we presented DNA sequence data from portions of the mitochondrial 12S ribosomal RNA (rRNA) and cytochrome (cyt) b genes that supported a robust phylogeny for representatives of the lizard family Xantusiidae (Hedges *et al.*, 1991). One of the results obtained was that *Lepidophyma* and *Xantusia* are sister groups. This was supported by high bootstrap *P*-values (>95%) in both neighbor-joining and parsimony analyses for the 12S rRNA and combined data sets. In contrast, an earlier morphological analysis (Crother *et al.*, 1986) found that *Cricosaura* and *Lepidophyma* are sister groups. That analysis was based largely on morphological data extracted from the work of Savage (1955, 1963). Although we briefly discussed the Crother *et al.* study, we chose not to examine it critically because we considered it to be preliminary due to the small number of characters employed: 13 informative for parsimony, with only two supporting the *Lepidophyma* + *Cricosaura* clade.

Crother and Presch (1993) have combined our DNA sequences from the 12S rRNA and cyt b genes with the morphological data and claim that a robust xantusiid phylogeny is not yet available. We disagree, and will here examine the pivotal morphological characters in their phylogeny and will demonstrate that these traits do not provide unambiguous support to the clades they recognize. We will also discuss our objections to their methods of analysis, including their weighting of each morphological character 40 times greater than each nucleotide character (site). Finally, we will present new DNA sequence data from the mt 16S rRNA gene that support the same intergeneric relationships of



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xantusiid lizards as we obtained with 12S rRNA and cyt b DNA sequences.

## MATERIALS AND METHODS

The DNA samples used in this study were identical to those used in Hedges *et al.* (1991). The methods of DNA amplification and sequencing were similar to those outlined in Hedges *et al.* (1991, 1992), with the following differences: (1) the polymerase chain reaction protocol (Perkin–Elmer Model 9600 Thermal Cycler) was 94°C (15 s), 50°C (15 s), and 72°C (45 s), for 30 cycles (double-stranded DNA) and 94°C (15 s), 60°C (15 s), and 72°C (45 s), for 35 cycles (single-stranded DNA), with no mineral oil overlay; (2) single-stranded DNA was purified with 30,000 MW filters; and (3) the ampliTaq polymerase enzyme (Perkin–Elmer) was utilized for DNA sequencing following manufacturer's recommendations.

The aligned DNA sequences were analyzed with several methods: the programs NJBOOT2 and TREEVIEW (Koichiro Tamura, Pennsylvania State University) for neighbor-joining analyses; ME-TREE (Andrey Rzhetsky, Pennsylvania State University) for minimum evolution analyses; and PAUP 3.0s (David Swofford, Illinois Natural History Survey) for maximum parsimony. The Exhaustive Search algorithm was used with PAUP. Nucleotide sites containing ambiguities or deletions were omitted in the distance analyses, and were treated as missing data in the parsimony analyses. All applications of the bootstrap method (Felsenstein, 1985) involved 2000 replications in order to examine statistical significance at the 95% level (Hedges, 1992).

Three distance measures were used to correct for multiple hits: Jukes-Cantor (JC; Jukes and Cantor, 1969), Kimura 2-parameter (K) for transition/transversion bias (Kimura, 1980), and the Tamura 3-parameter (T) for transition/transversion bias and base compositional bias (Tamura, 1992). Some statistics were calculated with the program MEGA (Sudhir Kumar, Koichiro Tamura, and Masatoshi Nei; Pennsylvania State University).

For the morphological comparisons, the following specimens were examined: *Cricosaura typica,* LACM 3777; USNM 138485-88, 138490, 138497-503; *Lepidophyma flavimaculatum,* LACM 131092, 131094, 131096, 131101, 131103, 131107-11; *L. gaigeae,* LACM 106770, 106807-09, 109153, 127169, 131143, 136770, UCM 49849-50, UTEP 13866-67; *L. mayae,* JAC 16923; *L. micropholis,* LACM 136369-70; *L. pajapanensis,* LACM 135510, 137605, UAZ 28765, 28804, 28808-11; *L. smithii,* LACM 136359, 136677; *L. sylvaticum,* LACM 136366-67; *L. tuxtlae,* LACM 136352, 136355; *Xantusia bolsonae,* LACM 55957-64, 136791-92, 136794; *X. henshawi,* LACM 100716, 100723, 100725, 100737, 100739, 100746, 100756, 100767-68, 1006716, 106755, 127158; *X. riversiana,* LACM 108652, 108655-56, 108670, 108677, 108681, 108688, 108690, 108695, 108705, 127131; *X. vigilis,* LACM 127152, 136255, 136285, 136292, 136298, 136303, 136305-06, 136311, 136320, 136322.

## RESULTS

### *Morphology*

The morphological data that Crother and Presch (1993) combine with our molecular data are those extracted by Crother *et al.* (1986) for xantusiid genera from the work of Savage (1955, 1963). Crother *et al.* (1986) list 30 characters (10 from squamation, 20 from osteology) with 25 consisting of two states and five involving three-state linear transformation series based on morphoclines (Savage, 1955). There is disagreement on how many characters in their morphological data set can be considered to be informative under the conditions of parsimony. We count a maximum of 13, whereas they variably claim 18 (Crother *et al.,* 1986:39) or 17 (Crother and Presch, 1993). We judge only 9 characters (numbers 3, 5, 6, 11, 12, 13, 14, 16, and 28) to be informative by the criterion of having two or more states that are present in two or more OTUs, and four additional characters (2, 26, 27, 29) might be considered informative due to their assumption of a linearly ordered transformation series (which may be inappropriate; Houser and Presch, 1991).

We cannot evaluate or reproduce their rooting methods, as they state only that they constructed hypothetical ancestral states "on a character by character basis" by "outgroup comparisons with other lizard groups." No information is supplied as to which outgroups were examined and what character states were observed (Crother *et al.,* 1986:38–39). They were unable to assign a state to the hypothetical ancestor for 12 of the 30 characters, due to extensive variation in the outgroup.

In their most parsimonious tree based on the morphological data of Savage (1955, 1963) and the karyological tree of Bezy (1972), the *Lepidophyma* + *Cricosaura* node is supported by two allegedly unambiguous characters and the *Xantusia* + *"Klauberina"* (*X. riversiana*) node is supported by only one. Each of these nodes is supported by one additional character which they consider ambiguous due to unresolved conditions in the outgroup. A full critique of the morphological analysis of Crother *et al.* (1986) is reserved for a subsequent paper, but we here reevaluate the three "unambiguous" characters supporting the nodes in their parsimony tree to document what we consider to be serious problems with the morphological information they combine with the molecular data.

The *Xantusia* + *"Klauberina"* (*X. riversiana*) node is supported by the allegedly unique feature of the presence of "enlarged gulars," whereas *Cricosaura,*

*Lepidophyma,* and the hypothetical ancestor are coded as having "gulars same size as pregulars" (*Crother et al.,* 1986: their Table 1, Fig. 4, and Appendix). In *Lepidophyma,* the gulars, posterior pregulars, and anterior pregulars (sensu, Savage, 1963:8–9) form a continuous series of uniformly small granular scales (Savage, 1955:157, 1963:27; R.L. Bezy, personal observation). In *Xantusia* (inclusive of *X. riversiana*) the gulars are enlarged relative to both the posterior and anterior pregulars (Savage 1955:115, 126, 163:18, 21; R.L. Bezy, pers. obs.). In *Cricosaura typica,* the gulars and anterior pregulars consist of rectangular plates that are enlarged relative to the posterior pregulars (or to the gulars and pregulars of *Lepidophyma* and the pregulars of *Xantusia*) (Savage, 1955:105, 1963:14, 1964:540, Fig. 2; R.L. Bezy, pers. obs.). Savage (1955:87) states that "the gulars are enlarged in *Cricosaura, Klauberina,* and *Xantusia,*" and this is confirmed by our observations. In addition, *Cricosaura* has the unique feature of enlarged anterior pregulars. Thus, the presence of enlarged gulars is not an unambiguous shared-derived character uniting the members of *Xantusia* (inclusive of *X. riversiana*).

However, the genus *Xantusia* (inclusive of *X. riversiana*) is united by a morphological feature not used by Crother *et al.* (1986). Members of the genus have vertically elliptical pupils, whereas all other living xantusiids have round pupils (Bezy, 1972:17, pers. obs.; Regal, 1968:85–86; Savage, 1963: Figs. 9, 12, 18, 22). Given the membership of the Xantusiidae in the Scincomorpha (Estes, 1983; Estes *et al.,* 1988; Presch, 1988), the presence of virtually elliptical pupils in *Xantusia* can be considered to be a derived condition within the family.

The *Cricosaura* + *Lepidophyma* node is alleged to be supported by the presence of caudal scales that are heterogeneous (rather than homogeneous) in size (Crother *et al.,* 1986: their Table 1, Fig. 4, and Appendix). This character combines several features of the caudal scales of xantusiids, none of which represent unambiguous evidence for the existence of this clade. The dorsal caudal scales are heterogeneous in size in all xantusiids with the possible exception of *Xantusia riversiana.* In *Lepidophyma,* each caudal segment consists of a ring of enlarged keeled scales (whorls) followed by one to five smaller annuli (interwhorls; Bezy, 1984, 1989; Bezy and Camarillo, 1992; Savage, 1955:157, 1963: Fig. 23; Smith, 1973). The dorsal interwhorls in *L. flavimaculatum* are relatively small (middorsal length averages 43% of preceding whorl; $n$ = 10; Bezy, 1989: Fig. 11) compared to those in *L. gaigeae* (76%, $n$ = 10; Bezy, 1984: Fig. 10). In *Cricosaura* (Fig. 1; Savage, 1955:105, 1963:14, 1964: Fig. 4) and *Xantusia* (Fig. 1; contra Savage, 1955:126, 1963:21), the whorls are followed dorsally by two slightly smaller interwhorls (*Cricosaura,* middorsal length 86% of the preceding whorl, $n$ = 13; *X. henshawi,* 65%, $n$ = 10; *X. bolsonae,* 76%, $n$ = 9; *X. vigilis,* 78%, $n$ = 10; *X. riversiana,* 91%, $n$ = 10). Thus, if the dorsal whorls and interwhorls are considered to be heterogeneous in size in *Cricosaura* and *Lepidophyma* (inclusive of *L. gaigeae*), they are also heterogeneous in *Xantusia* (with the possible exception of *X. riversiana*) and do not constitute an unambiguous shared-derived character of *Cricosaura* + *Lepidophyma.*

One aspect of caudal scale heterogeneity that is present in *Cricosaura* and comes closer to resembling the condition in *Lepidophyma* is the presence, on the basal third of the tail, of a single row of enlarged scales across the midventer for each pair of dorsal interwhorls (Savage, 1964: Fig. 4; R.L. Bezy, pers. obs.). This condition is similar to that found in most species of *Lepidophyma* which have three or four smaller dorsal interwhorls, only two of which are complete (and slightly enlarged) across the venter (Bezy, 1984, 1989; Bezy and Camarillo, 1992; Smith, 1973). However, in *L. gaigeae* the interwhorls are usually complete and unenlarged ventrally on at least the basal portion of the tail (Bezy and Camarillo, 1992; Smith, 1973:112). Thus, none of the three components of caudal scale heterogeneity (the presence of dorsal whorls and interwhorls differing in size, of ventrally incomplete interwhorls, and of ventrally enlarged interwhorls) can be considered an unambiguous shared-derived character of *Cricosaura* + *Lepidophyma.*

The other character found by Crother *et al.* (1986) to unite *Cricosaura* and *Lepidophyma* is the absence of a parietal foramen (coded as present in *Xantusia* including *X. riversiana*). In *X. bolsonae, X. henshawi,* and *X. vigilis,* the parietal foramen is relatively large (e.g., Savage, 1963: Fig. 6), but in *X. riversiana* it is small (e.g., Savage, 1963: Fig. 10) or absent (Savage, 1955:115; 1963:18; R.L. Bezy, pers. obs.). In *Cricosaura,* the parietal foramen is closed (Savage, 1963:17; R.L. Bezy, pers. obs.). In *Lepidophyma,* the parietal foramen in usually closed (Fig. 1; Savage, 1955:157, 1963:30), but is sometimes open (Fig. 1; R.L. Bezy, pers. obs.). In *L. smithii* (the *L. flavomaculatum* of Savage, 1955:42) the foramen is not visible dorsally, being thickly roofed over by the parietals (Fig. 1). In most other species of *Lepidophyma,* the parietal foramen is clearly visible dorsally, even though it is thinly roofed over by the parietal bones in larger individuals. For example, in eight *L. pajapanensis* examined, the parietal foramen was found to be dorsally visible in all, open in four (Fig. 1; SVL 42, 57, 62, and 74 mm), and thinly roofed over by the parietal bones in four (SVL 62, 72, 72, and 73 mm). The absence of the foramen in some *X. riversiana* (which involves co-ossification of osteoderms with the parietals) appears not to be homologous with the absence in *Cricosaura* and larger individuals of *Lepidophyma* (which is due exclusively to closure of the parietals). Nevertheless, the character is sufficiently variable in *Lepidophyma* that we con-

TABLE 1

**Pairwise Transitions (Ts) and Transversions (Tv)**

| | | | Total | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ts | Tv | TC | TA | TG | CA | CG | AG |
| Ameiva | vs | Gallus | 145 | 147 | 86 | 42 | 15 | 75 | 15 | 59 |
| Cricosaura | vs | Gallus | 132 | 143 | 79 | 46 | 15 | 61 | 21 | 53 |
| Cricosaura | vs | Ameiva | 126 | 134 | 70 | 52 | 7 | 61 | 14 | 56 |
| Lepidophyma | vs | Gallus | 124 | 149 | 72 | 40 | 12 | 73 | 24 | 52 |
| Lepidophyma | vs | Ameiva | 153 | 150 | 97 | 38 | 16 | 77 | 19 | 56 |
| Lepidophyma | vs | Cricosaura | 124 | 126 | 78 | 40 | 10 | 61 | 15 | 46 |
| Xbolsonae | vs | Gallus | 138 | 143 | 82 | 40 | 13 | 68 | 22 | 56 |
| Xbolsonae | vs | Ameiva | 131 | 138 | 75 | 45 | 9 | 66 | 18 | 56 |
| Xbolsonae | vs | Cricosaura | 119 | 113 | 72 | 53 | 4 | 48 | 8 | 47 |
| Xbolsonae | vs | Lepidophyma | 125 | 94 | 75 | 31 | 5 | 46 | 12 | 50 |
| Xhenshawi | vs | Gallus | 145 | 144 | 87 | 41 | 10 | 75 | 18 | 58 |
| Xhenshawi | vs | Ameiva | 136 | 134 | 83 | 52 | 11 | 60 | 11 | 53 |
| Xhenshawi | vs | Cricosaura | 111 | 118 | 68 | 58 | 7 | 46 | 7 | 43 |
| Xhenshawi | vs | Lepidophyma | 128 | 95 | 75 | 33 | 5 | 47 | 10 | 53 |
| Xhenshawi | vs | Xbolsonae | 95 | 40 | 65 | 12 | 3 | 22 | 3 | 30 |
| Xvigilis | vs | Gallus | 137 | 137 | 82 | 39 | 10 | 74 | 14 | 55 |
| Xvigilis | vs | Ameiva | 128 | 130 | 81 | 42 | 12 | 63 | 13 | 47 |
| Xvigilis | vs | Cricosaura | 117 | 117 | 78 | 52 | 7 | 51 | 7 | 39 |
| Xvigilis | vs | Lepidophyma | 122 | 91 | 73 | 30 | 5 | 44 | 12 | 49 |
| Xvigilis | vs | Xbolsonae | 84 | 28 | 62 | 11 | 3 | 7 | 7 | 22 |
| Xvigilis | vs | Xhenshawi | 88 | 33 | 63 | 10 | 2 | 20 | 1 | 25 |
| Xriversiana | vs | Gallus | 143 | 137 | 89 | 42 | 9 | 69 | 17 | 54 |
| Xriversiana | vs | Ameiva | 125 | 133 | 82 | 49 | 10 | 60 | 14 | 43 |
| Xriversiana | vs | Cricosaura | 115 | 112 | 72 | 50 | 6 | 50 | 6 | 43 |
| Xriversiana | vs | Lepidophyma | 124 | 86 | 80 | 33 | 3 | 39 | 11 | 44 |
| Xriversiana | vs | Xbolsonae | 79 | 29 | 58 | 9 | 3 | 11 | 6 | 21 |
| Xriversiana | vs | Xhenshawi | 83 | 34 | 56 | 11 | 2 | 21 | 0 | 27 |
| Xriversiana | vs | Xvigilis | 52 | 17 | 39 | 7 | 3 | 7 | 0 | 13 |

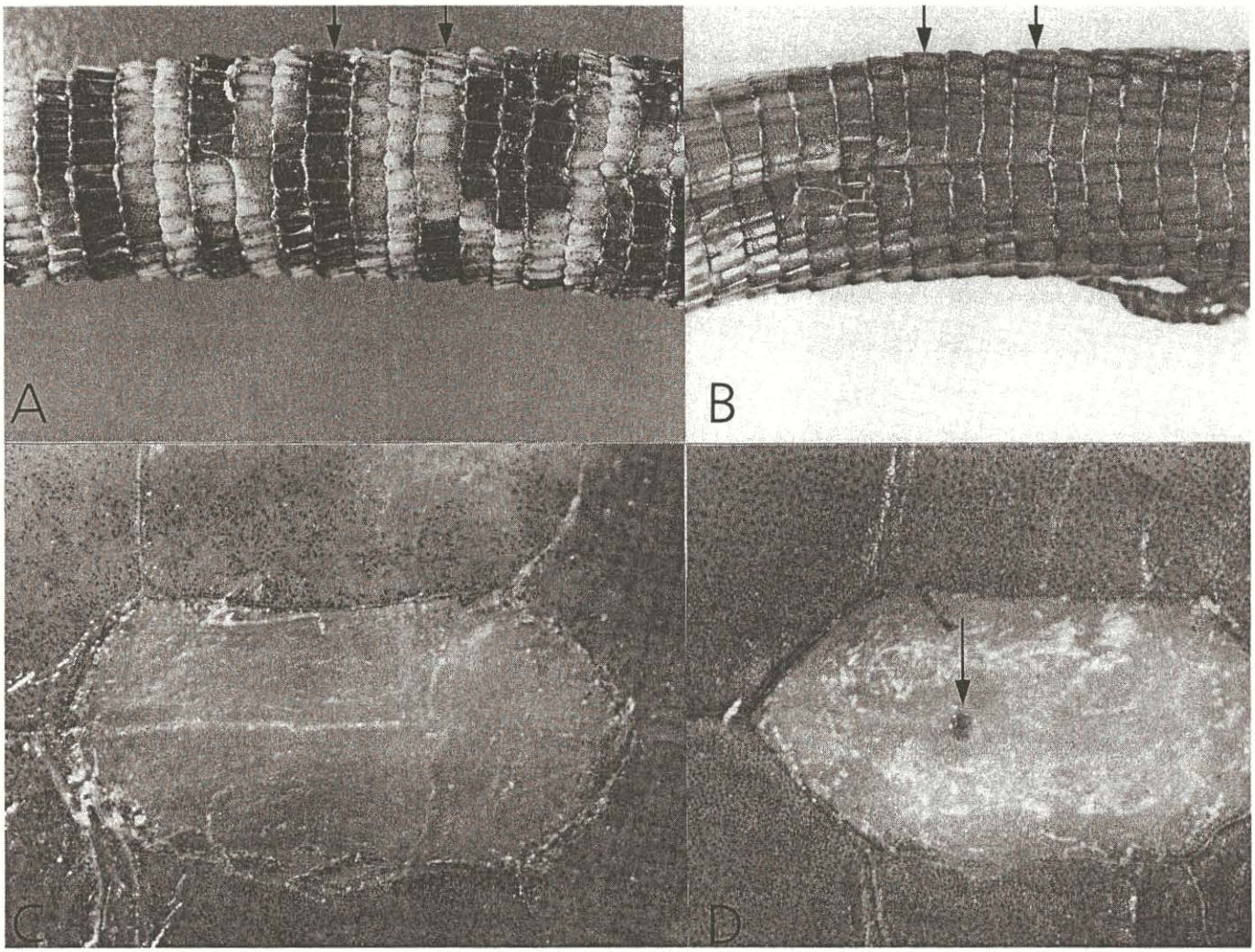


**FIG. 1.** Reevaluation of the two pivotal morphological characters used by Crother *et al.* (1986) and Crother and Presch (1993) to infer a sister-group relationship between *Cricosaura* and *Lepidophyma*. Dorsal view of the basal portion of the tail of (A) *Xantusia henshawi* (LACM 100716) and (B) *Cricosaura typica* (USNM 13850). Arrows indicate two of the slightly enlarged whorls separated by two smaller interwhorls; Crother *et al.* coded the caudal scales of *C. typica* (which have very slightly enlarged dorsal whorls) as "heterogeneous in size," whereas they code those of *X. henshawi* (which have more clearly enlarged whorls) as "homogeneous in size." Dorsal view of the head of (C) *Lepidophyma smithii* (LACM 136677) and (D) *L. pajapanensis* (UAZ 28804) with interparietal scale removed to expose parietal bones. Arrow indicates the presence of an open parietal foramen. Crother *et al.* coded the parietal foramen as absent in *Lepidophyma*.

clude it does not clearly support the existence of a *Lepidophyma* + *Cricosaura* clade.

*DNA Sequences*

Aligned sequences from all three mitochondrial genes are presented in Fig. 2. As in the original study (Hedges *et al.*, 1991), the teiid lizard *Ameiva auberi* was treated as the outgroup in the phylogenetic analyses. The published sequence of the chicken, *Gallus gallus* (Desjardins and Morais, 1990), was included for the purpose of having a second, more distant, outgroup.

There are two regions in the 16S rRNA sequence, indicated in Fig. 3, that are too variable to align with confidence and these were omitted from the analyses, as was the long insertion present in the chicken sequence. The complete data set consists of 1028 alignable sites, of which 471 are variable and "informative" for the distance analyses (excluding sites with deletions and ambiguities) and 281 are "informative" for the parsimony analyses. The 16S rRNA region consists of 318 alignable sites, of which 132 are variable and "informative" for the distance analyses (excluding sites with deletions and ambiguities) and 81 are "informative" for the parsimony analyses.

Our original paper (Hedges *et al.*, 1991) reported neighbor-joining and maximum-parsimony analyses of sequences from the two genes (12S rRNA and cyt b) separately and then in combined analyses. Following along those same lines, we report here analyses of our new data from the 16S RNA gene separately, and then

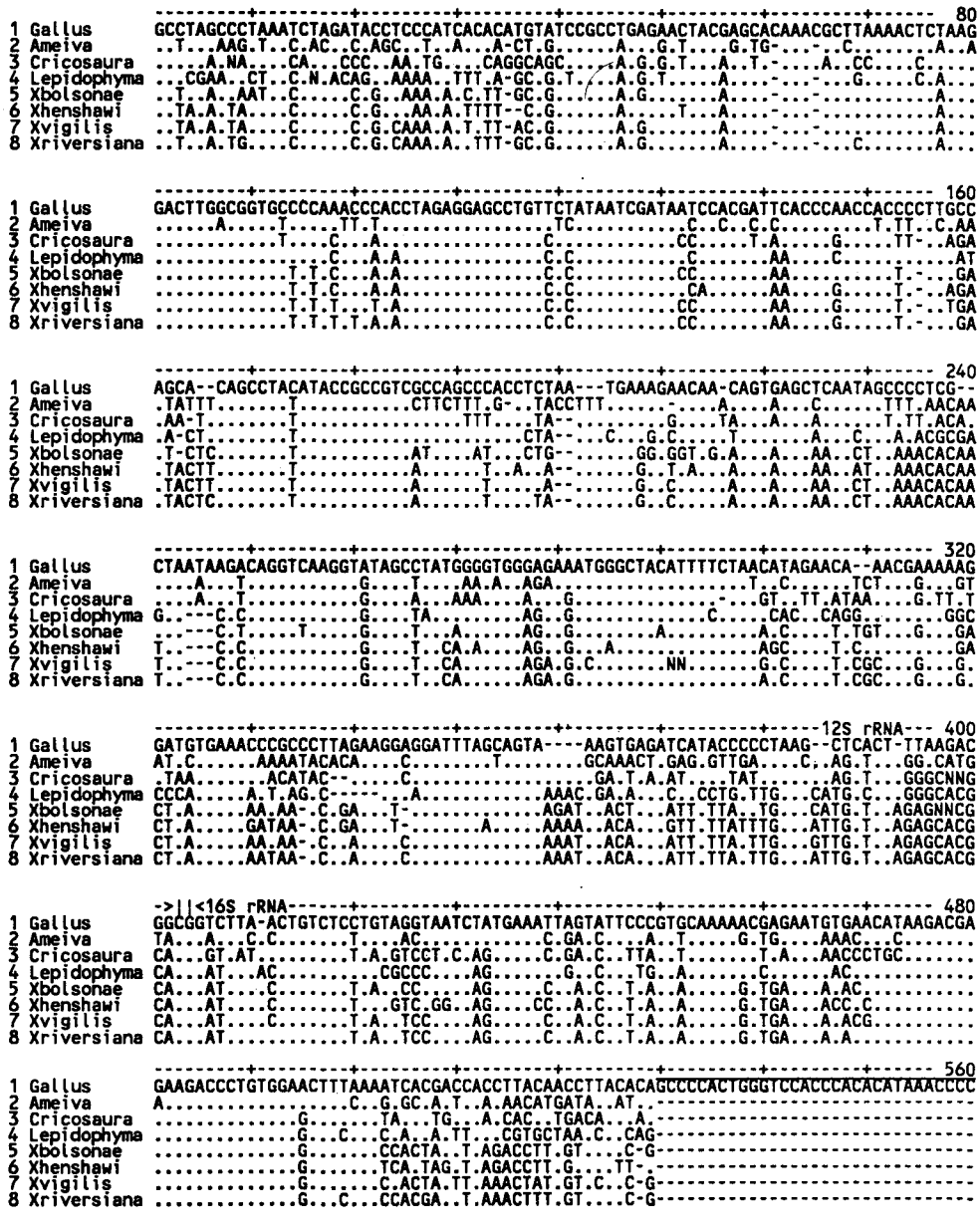


**FIG. 2.** Mitochondrial DNA sequences of portions of the 12S rRNA (sites 1–403), 16S rRNA (404–797), and cytochrome b (798–1104) genes in the chicken (*Gallus gallus;* Desjardins and Morais, 1990; corresponding to sites 1754–2139, 3354–3741, and 14991–15297, respectively), a teiid lizard (*Ameiva auberi*), and six species of xantusiid lizards. A solid dot denotes identity with the first sequence; a dash denotes a gap, and an N denotes an ambiguity. Highly variable regions where alignment is uncertain were not used in the analyses and are indicated by a solid line above the alignment. The 12S rRNA and cyt b lizard sequences are from Hedges *et al.* (1991) and the 16S rRNA lizard sequences are new to this study.

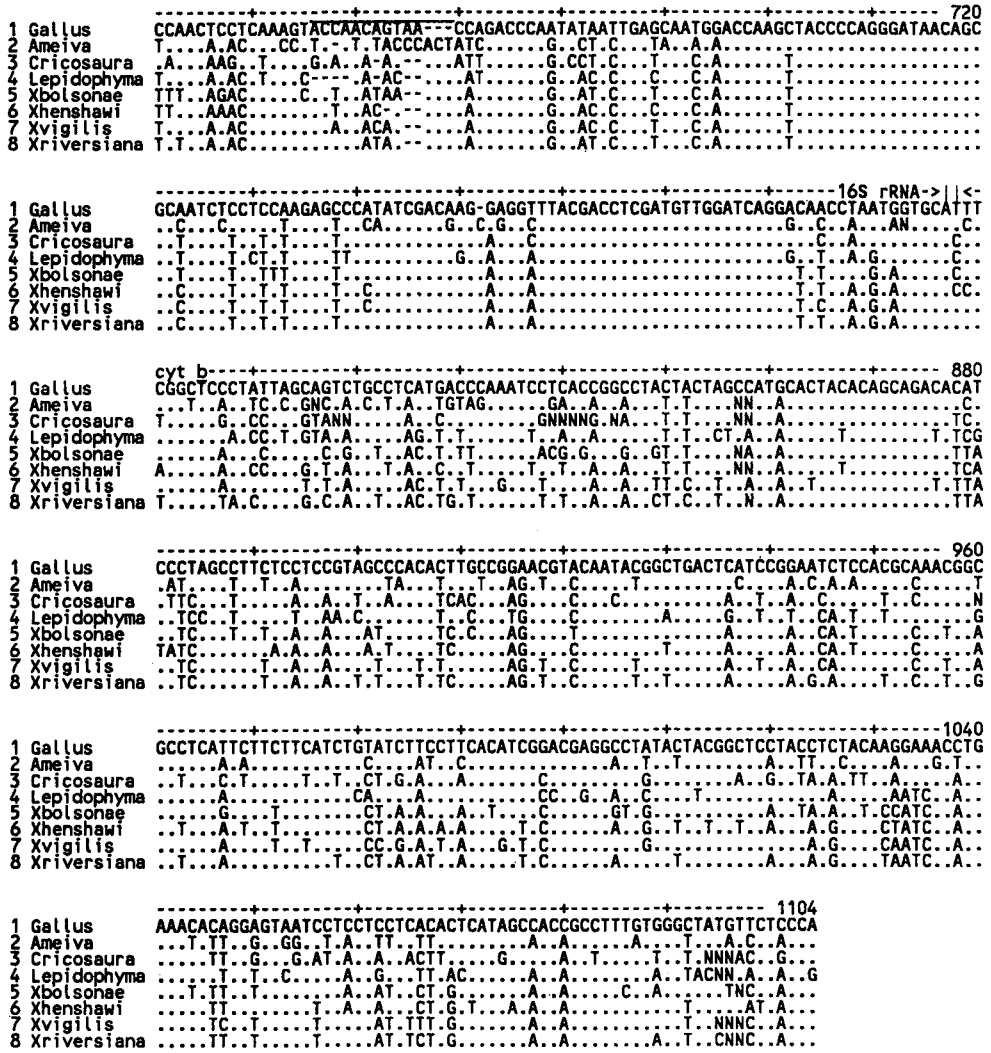

FIG. 2—*Continued*

analyses combining DNA sequence data from all three genes. Although we believe that the best estimate of phylogeny based on molecular data is obtained by combining DNA sequences from multiple genes, it is also useful to compare phylogenies derived from different genes for the purpose of appraising congruency (Swofford, 1991). However, this is less important for mitochondrial genes (than for nuclear genes) because they are tightly linked and inherited as a single unit.

The average base composition in the complete data set (Fig. 2) varies, with a slightly higher proportion of adenine and a slightly lower proportion of guanine: A (32.3%), C (25.5%), G (18.1%), T (24.1%). Substitution frequencies also vary, with a transition bias evident between closely related species, and a roughly equal ratio of transitions to transversions in comparisons involving *Gallus* (Table 1). This same pattern was found in our earlier study (Hedges *et al.*, 1991) and is typical of mitochondrial DNA data (Brown *et al.*, 1982).

### *16s rRNA Analyses*

All analyses of the new sequence data from the 16S rRNA gene support the intergeneric relationships found by Hedges *et al.* (1991), i.e., a sister-group relationship between *Lepidophyma* and *Xantusia* (Fig. 3). The addition of the second outgroup, *Gallus,* did not affect this conclusion. Due to the relatively small number of sites in the 16S rRNA data set, bootstrap values for the nodes are not high.

The monophyly of the genus *Xantusia* is supported in all of the analyses (Figs. 3A–3F), and with a relatively high degree of statistical confidence in the distance analyses (93–99%). Relationships among the four species of *Xantusia* are not well resolved in any of the 16S rRNA analyses apparently due to the relatively small number of sites in the data set. In all analyses where *Gallus* was included as the outgroup (Figs. 3B, 3D, and 3F), the monophyly of the family Xantusii-

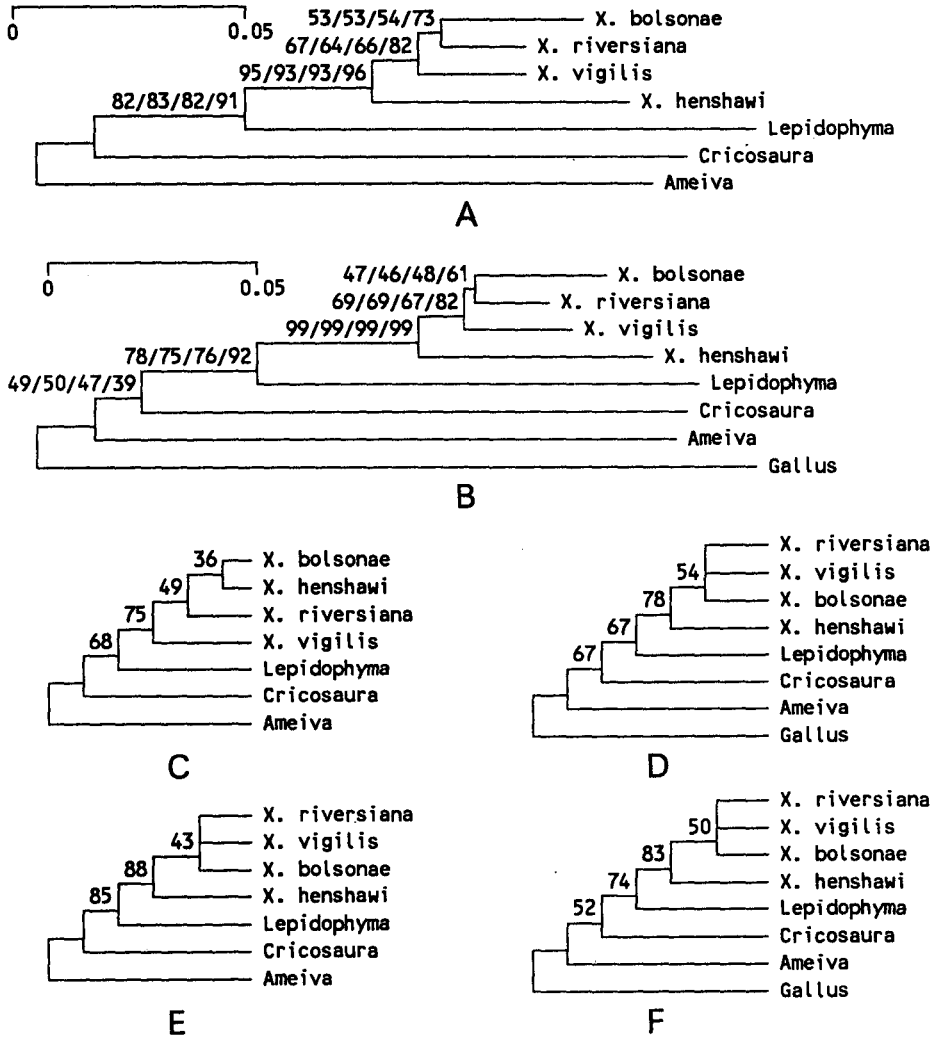


FIG. 3. Phylogenetic trees of xantusiid relationships obtained by analysis of the 16S rRNA data (318 sites). All bootstrap values shown were obtained with 2000 replications. (A, B) Minimum evolution trees (Kimura 2-parameter distance) obtained by rooting with *Ameiva* and *Gallus*, respectively. For each node, there are three bootstrap *P*-values representing three separate analyses (JC, K, and T distances) followed by the branch-length significance value. (C–F) Maximum parsimony trees obtained by rooting with *Ameiva* (C, E) and *Gallus* (D, F), and by using either no weighting scheme (C, D), or by weighting transversion five times transitions (E, F). Unresolved nodes indicate a consensus among multiple most-parsimonious trees.

dae was supported, although the confidence values were not high. Tree lengths, consistency indexes, and consistency indexes excluding "uninformative" sites for parsimony (respectively) for the two unweighted maximum parsimony analyses are: 193, 0.77, 0.63 (Fig. 3C) and 253, 0.73, 0.63 (Fig. 3D).

*Combined Analyses*

All analyses of the 1028-site data set combining sequences from all three genes support our original finding of a sister-group relationship between *Lepidophyma* and *Xantusia* (Fig. 4). With *Ameiva* as the outgroup, this result is statistically highly significant (98–100%) regardless of method of analysis, distance correction, or of weighting transversions (in the case of PAUP). When the second outgroup (*Gallus*) is added (Figs 4B, 4D, and 4F), the bootstrap *P*-values of the nodes decrease slightly (93–99%).

The monophyly of the genus *Xantusia* is supported in all of the analyses (Fig. 4) and with a very high degree of statistical confidence (99–100%). Within the genus *Xantusia*, a sister-group relationship between *X. vigilis* and *X. riversiana* is supported in all combined analyses, and this result is statistically significant (97–99%) both in distance analyses and in the unweighted parsimony analyses (Figs. 4A–4D). The sister group to this pair of species is not strongly resolved in any of the analyses, although the distance analyses support *X. bolsonae* (79–84%). In all analyses where *Gallus* was included as the outgroup (Figs. 4B, 4D,

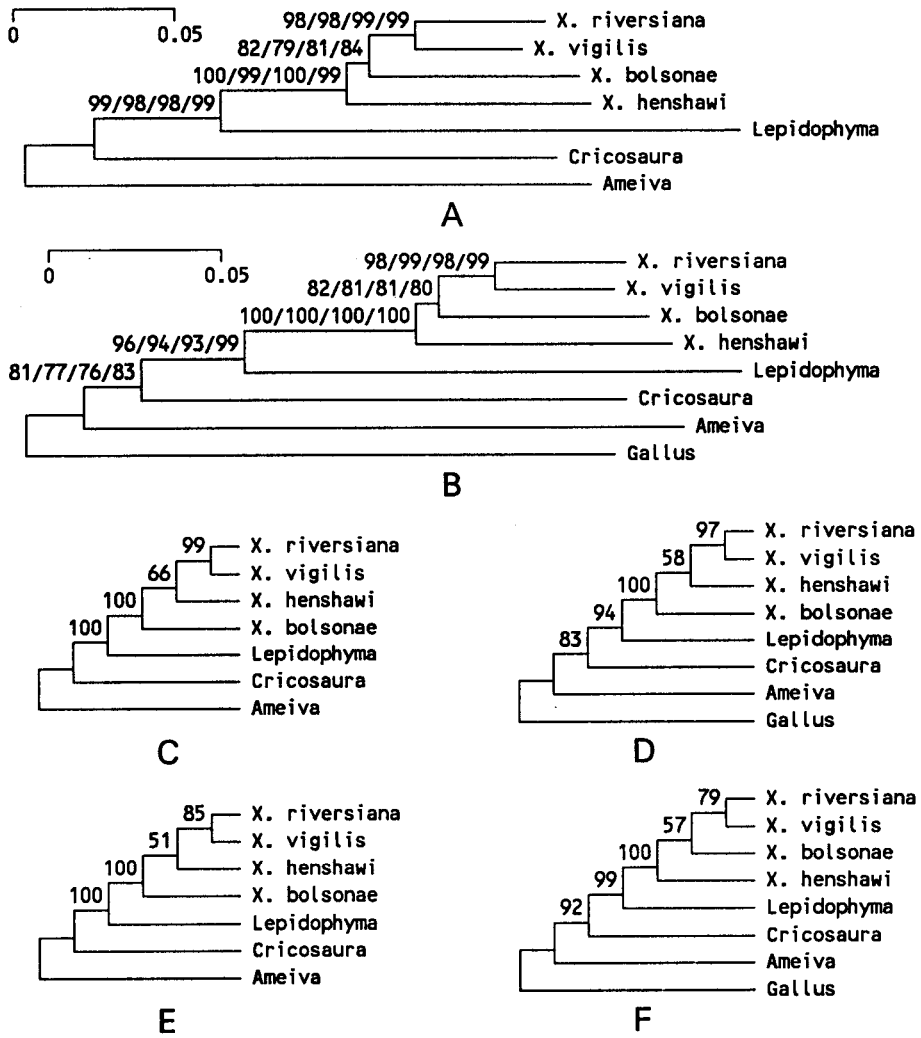


**FIG. 4.** Phylogenetic trees of xantusiid relationships obtained by analysis of all available sequence data from three genes (12S rRNA, 16S rRNA, and cyt b; 1028 sites total). All bootstrap values shown were obtained with 2000 replications. (A, B) Minimum evolution trees (Kimura 2-parameter distance) obtained by rooting with *Ameiva* and *Gallus*, respectively. For each node, there are three bootstrap *P*-values representing three separate analyses (JC, K, and T distances) followed by the branch-length significance value. (C–F) Maximum parsimony trees obtained by rooting with *Ameiva* (C, E) and *Gallus* (D, F), and by using either no weighting scheme (C, D), or by weighting transversions five times transitions (E, F).

and 4F), the monophyly of the family Xantusiidae was supported (76–92%). Tree lengths, consistency indexes, and consistency indexes excluding "uninformative" sites for parsimony (respectively) for the two unweighted maximum parsimony analyses are: 759, 0.80, 0.67 (Fig 4C) and 956, 0.75, 0.65 (Fig. 4D).

## DISCUSSION

Our examination of the two morphological characters supporting the alternative phylogeny of xantusiid genera found by Crother *et al.* (1986) has revealed the existence of serious problems with the morphological data set. We believe that there are additional problems with the use of that morphological data set for phylogenetic purposes, particularly in the determination of the outgroup character state(s), and conclude that it does not provide an acceptable basis for inferring xantusiid phylogeny.

The new sequence data from the 16S rRNA gene support the same generic relationships for xantusiid lizards as we obtained with the 12S rRNA and cyt b sequences (Hedges *et al.*, 1991). When combined, sequences from all three genes continue to support a robust phylogeny for xantusiid lizards. Virtually all nodes are statistically highly significant (98–100% confidence values) in all of the combined analyses with the teiid lizard *Ameiva* as outgroup, including neighbor-joining (different distance corrections) and maximum parsimony (unweighted, and transversions

weighted five times transitions). Bootstrap *P*-values remain quite strong (83–99%) when the more distant outgroup (*Gallus*) is included, although some values fall below the 95% level. The only exception involves the previously noted (Hedges *et al.*, 1991) poor resolution of branching order of *Xantusia bolsonae* and *X. henshawi*.

*Methods of Analysis*

Crother and Presch (1993) have criticized the methods of analysis used in our earlier study. Although we used distance analyses and maximum parsimony analyses in both of our studies, Crother and Presch (1993) have used only maximum parsimony in their reanalysis. Crother and Presch did not use the neighbor-joining method because it "yields only a single tree, an exact result only when the data are a perfect fit" (Jin and Nei, 1990).

The study cited by Crother and Presch, Jin and Nei (1990), actually showed that maximum parsimony is generally inferior to neighbor-joining in estimating the true topology in computer simulations involving different combinations of variables. Therefore, we fail to see why it was cited as a criticism of neighbor-joining. That the neighbor-joining method yields a single tree could be viewed as an advantage, rather than disadvantage, of the method. The true phylogeny being estimated is in fact a single tree, and if the single tree produced by neighbor-joining is the best estimate of that true topology, then it is a method superior to others. It has been demonstrated that in most cases, and especially those involving such small numbers of taxa, the neighbor-joining tree is also the minimum evolution tree (Nei, 1990), as was the case in this study.

Again referring to neighbor-joining, Crother and Presch state "because the method yields only a single tree, it does not allow for the examination of other less parsimonious, yet competing, hypotheses." Their use of the term "parsimonious" with the neighbor-joining method is unfortunate because it confuses two different methodologies. Maximum parsimony is not the criterion used to derive a neighbor-joining tree. Also, they fail to note that the comparison of alternative topologies is built into the algorithm for computing the neighbor-joining tree (Saitou and Nei, 1987; Nei, 1990). Furthermore, the minimum evolution method is available for those who wish to examine different, competing topologies (Nei, 1990; Rzhetsky and Nei, 1992). However, comparison of alternative topologies may not be very useful unless one can determine whether or not the alternatives are significantly different. Felsenstein (1988) reviews the several methods available to test statistical significance of different topologies. Additional methods have been published utilizing minimum evolution (Rzhetsky and Nei, 1992). Perhaps the simplest method to use is the bootstrap (Felsenstein, 1985), which can be applied to distance and parsimony analyses. If a parsimony analysis yields a highly significant grouping with the bootstrap method, one can expect that alternative groupings will not be found among the most-parsimonious trees, or even the near most-parsimonious trees.

*Tree Length*

Crother and Presch utilized tree length information to obtain the same result we obtained using the bootstrap. They found that the 12S rRNA data set and the combined (12S rRNA + cyt b) data set yielded statistically significant support for *Lepidophyma* + *Xantusia*, but that the cyt b data set (analyzed separately) did not. This is exactly what we reported, using the bootstrap method (Hedges *et al.*, 1991). We should also point out that the use of only 100 bootstrap replications by Crother and Presch is inappropriate for the purpose of determining statistical significance at the 95% level. The Hedges *et al.* (1991) study used 1000 replications, and since that time it has been determined that approximately 2000 replications are necessary for this purpose (Hedges, 1992), the number that we have used in this study.

*Insertions and Deletions*

Crother and Presch found that the inclusion or exclusion of insertions/deletions and ambiguities did not have an influence on the trees derived from the 12S rRNA or combined data sets, but did have an influence on the results of the cyt b analysis. This agrees with our finding (Hedges *et al.*, 1991) that the trees derived from the 12S rRNA and combined data sets had strong bootstrap support but that the cyt b trees did not. Therefore we do not see why this represents a criticism of our study.

Crother and Presch treated insertions and deletions as ambiguities in the parsimony analysis, and we have done this here. In reality, insertions and deletions are not "ambiguities" but potentially can contribute useful phylogenetic information. Although we did not use those data in our present analyses, it is interesting to note how many insertions/deletions unambiguously support the alternative hypotheses of generic relationships. There are five such cases, all located in the 12S rRNA data (Fig. 2). Three events (two, three, and four-base deletions) support *Lepidophyma* × *Xantusia* (sites 244–246; 359–362; 385–386), one one-base deletion supports *Cricosaura* + *Xantusia* (site 155), and one two-base deletion supports *Cricosaura* + *Lepidophyma* (sites 338–339). Thus, the insertion/deletion data appear to support the same phylogenetic relationships obtained with the nucleotide sites.

*Transitions and Transversions*

In our earlier study, we examined the transition bias in our data set and concluded that transitions had not yet reached saturation. We performed the distance

analyses in that study with a standard multiple-hit correction (Jukes–Cantor), and in this study with two additional types of corrections (a Kimura correction for transition/transversion bias, and a Tamura correction for transition/transversion bias and base composition bias), all designed to compensate for the phenomenon of multiple-hits. All three corrections yielded trees with identical topologies and nearly identical bootstrap *P*-values (Figs. 3 and 4).

There is no satisfactory multiple-hit correction for discrete-parsimony analysis. Crother and Presch have used a common method whereby transversions are given a greater weight than transitions, inversely proportional to their frequency of occurrence. For comparative purposes, we have also done those analyses (Figs. 3 and 4). However, criticism of this weighting approach has been made elsewhere (Hedges and Maxson, 1992). The criticism is summarized below.

A transition will convey the same phylogenetic information as a transversion among sequences that have not diverged substantially. In greatly diverged sequences, multiple hits will obscure that phylogenetic information. Therefore, the problem is one of scaling: the distance corrections are scaled, the parsimony weighting method is not. The latter method lowers the information content of the data set by down-weighting many transitions that are potentially informative for phylogeny, especially among closely related taxa. The net result in a phylogenetic study is to reduce the total number of sites and thus to reduce phylogenetic resolution. If transitions are deleted, or given a small weight (1:10), a data set can be reduced by 50% or more in terms of the effective number of contributing sites, and the bootstrap *P*-values also will be reduced. Furthermore, the focus on transition bias ignores the fact that transversions also undergo multiple hits. To our knowledge, no one, including Crother and Presch, has determined how to correct for multiple hits in transversions in a parsimony analysis.

In this study, weighting of transversion/transitions had no effect on the intergeneric relationships, and both weighted trees for the complete data set (Figs. 4E and 4F) support a *Lepidophyma–Xantusia* sister group relationship at high bootstrap *P*-values (99–100%).

*Consistency Index*

We reported CI values for each of the parsimony analyses in our earlier study, as is typically done in nearly all studies employing parsimony, and Crother and Presch correctly note the inconsistency regarding CIs including/excluding uninformative sites. However, we used the bootstrap method, not the CI, for comparison of trees and we did not "discuss" the CI values. We agree with Crother and Presch that use of the CI for comparison of trees is problematical, and therefore we fail to see why those authors have chosen to use the CI to make comparisons.

*Morphological and Molecular Synthesis*

We believe that the details presented in this paper clearly establish that we do not treat morphological data or phylogenies with "disdain," but that we have well-founded reasons for concluding that there are serious problems with the existing morphological data for xantusiids. In their effort to combine the morphological and molecular data in a single analysis, Crother and Presch feared that the small number of morphological characters would be "swamped" by the much larger number of molecular characters (sites). To prevent this from happening, they assigned weights to the morphological characters in inverse proportion to their contribution to the combined data set.

Among the flaws of this arbitrary approach is that the fewer the number of characters in a data set, the greater is the weight assigned to each of them. This means that a morphological data set of, for example, only four characters would be weighted equal to DNA sequence data for the entire genome. We believe that this is an unacceptable method. Crother and Presch noted this problem with the approach but chose to use it nonetheless.

In order to derive a weighting scheme for the two data sets, Crother and Presch used the total number of nucleotide sites in our earlier study (709), but counted only the morphological characters "informative" for parsimony. Although they count 17 such characters for their data set (Crother *et al.*, 1986: their Table 1), we count only 9 (13 if the characters are treated as ordered). In addition, the parsimony analysis utilizes only those "informative" characters and therefore it is unclear why all nucleotide sites were counted. Of the 709 sites, there are only 168 parsimony informative sites. Their weighting ratio (morphological characters: molecular characters) was 40:1, based on the incorrect counts of 709:17. The correct ratio is 168:13, or approximately 13:1. Although we point out the errors in their calculations, we do not advocate use of this approach.

We concur with Swofford's (1991) arguments that more is to be gained by comparing phylogenies derived from molecules with those based on morphology, rather than by combining the data sets into a single analysis. Where significantly different topologies are found, it is especially important to gather new data, and to reexamine the characters that appear to be responsible for the incongruencies, rather than to analyze combined data in the hope of gaining support for a particular phylogenetic hypothesis.

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