

# Phylogenetic relationships and rates of allozyme evolution among the lineages of sceloporine sand lizards

KEVIN DE QUEIROZ\*

Department of Zoology and Museum of Vertebrate Zoology, University of California, Berkeley, CA 94720, U.S.A.

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Parsimony analysis of characters derived from an electrophoretic survey of allozyme variation in the sceloporine sand lizards indicates that *Uma* is outside of a clade formed by the rest of the sand lizards and that *Cophosaurus* and *Holbrookia* share a more recent common ancestor with one another than either does with *Callisaurus*. Previous electrophoretic studies used phenetic clustering based on genetic distance data to assess relationships among these taxa. The resulting dendrograms were used to argue that *Holbrookia* is the sister group of all other sand lizards and that *Callisaurus* and *Cophosaurus* are sister taxa. When reanalysed using parsimony methods, the data from these previous studies are found to support the conclusions of the present study, namely, that *Uma* rather than *Holbrookia* is the sister group of all other sand lizards and that *Cophosaurus* is the sister taxon of *Holbrookia* rather than of *Callisaurus*. Relative rate tests indicate that the incongruencies between branching diagrams derived from phenetic clustering of genetic distances versus those derived from parsimony analysis of electrophoretic characters are attributable to increased rates of protein evolution in the *Holbrookia* lineage.

**KEY WORDS:**—Allozymes – *Callisaurus draconoides* – *Cophosaurus texanus* – electrophoresis – evolutionary rates – *Holbrookia lacerata* – *Holbrookia maculata* – *Holbrookia propinqua* – Wagner parsimony – phenetic clustering – phylogeny – relative rate test – *Uma exsul* – *Uma notata* – *Uma parapygas* – *Uma scoparia*.

## CONTENTS

Introduction . . . . .	334
Materials and methods. . . . .	334
Sampling . . . . .	334
Specimen preparation and electrophoresis . . . . .	334
Phylogenetic analysis . . . . .	335
Results . . . . .	336
Discussion . . . . .	342
Phylogenetic relationships . . . . .	342
Comparisons with previous studies . . . . .	348
Rates of protein evolution . . . . .	353
Acknowledgements . . . . .	355
References . . . . .	356
Appendices . . . . .	359

\*Present address: Division of Amphibians and Reptiles, U.S. National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, U.S.A.

The sceloporine sand lizards (Iguania) form a clade of *c.* ten extant species inhabiting arid and semi-arid habitats in western North America (Smith, 1946; Etheridge & de Queiroz, 1988; de Queiroz, 1989). Although monophyly of this informal taxon is well supported (Etheridge, 1964; Presch, 1969; Etheridge & de Queiroz, 1988; de Queiroz, 1989), and although it has been studied extensively in terms of both systematics and numerous other aspects of biology, the relationships among the various species and supraspecific taxa of sand lizards are disputed. In particular, disagreements exist concerning the primary dichotomy among the extant taxa and whether the greater earless lizard, *Cophosaurus texanus*, is the sister group of the other earless lizards, *Holbrookia*, or of the zebra-tail lizard, *Callisaurus draconoides* (e.g. Mittleman, 1942; Smith, 1946; Axtell, 1958; Norris, 1958; Savage, 1958; Earle, 1961, 1962; Clarke, 1965; Cox & Tanner, 1977; Adest, 1978; Blackburn, 1978). As part of a synthetic systematic analysis involving both morphological and biochemical data (de Queiroz, 1989), I undertook an electrophoretic survey of sand lizard allozymes in order to investigate phylogenetic relationships and rates of biochemical evolution within the sand lizard clade.

#### MATERIALS AND METHODS

##### *Sampling*

In order to obtain an adequate representation of variation both within and among conspecific populations, I employed a geographic sampling strategy (Buth, 1984). Sample sizes were limited to a maximum of 20 organisms per species because of constraints imposed by time and expense. I then attempted to obtain ten organisms from a single population and up to ten other organisms from one to ten additional populations from widely separated localities. For those species within which subspecies are recognized, an attempt was made to include representatives of as many of these subspecies as possible. Failure to achieve samples conforming to the above description for some species resulted from practical limitations. For example, I was able to secure only a single specimen of *Holbrookia lacerata*, and *Uma inornata* was not sampled because of its endangered status. Species, sample sizes, localities and specimen numbers for the sand lizards used in this study are given in Appendix 1.

Representatives of three successive outgroups were included in the electrophoretic comparisons for the purpose of determining ancestral character states. The three successive outgroups, according to the relationships proposed by Etheridge & de Queiroz (1988), are *Phrynosoma*, the *Sceloporus* group (*Sceloporus*, *Urosaurus*, and *Uta*) and *Petrosaurus*. I sampled these outgroups in such a way as to maximize the number of species, and thus presumably also the number of electromorphs, while constraining myself to ten, ten and five specimens from the three successive outgroups. Outgroup taxa, sample sizes, localities and specimen numbers are listed in Appendix 1.

##### *Specimen preparation and electrophoresis*

Tissues were dissected from freshly killed specimens and maintained at  $-76^{\circ}\text{C}$ . Tissue extracts from combined liver and either skeletal or smooth

TABLE 1. Proteins, presumptive genetic loci, and electrophoretic conditions used to study protein variation in the sceloporine sand lizards. Abbreviations for electrophoretic conditions are as follows: 1=LiOH, pH 8.2 (gel), pH 8.1 (tray), 3 h at 300 V; 2=PGI phosphate, pH 6.7, 4 h at 130 V; 3=phosphate-citrate, pH 7.0, 4 h at 100 V; 4=Poulik with EDTA, pH 8.7 (gel), borate, pH 8.2 (tray), 3 h at 250 V; 5=tris-citrate II with NADP, pH 8.0, 4 h at 130 V; 6=tris-citrate III, pH 7.0, 3 h at 180 V

Protein	Enzyme commission number	Locus	Electrophoretic conditions
Aconitate hydratase	4.2.1.3	Acon	1
Albumin	—	Alb	1, 5
Alcohol dehydrogenase	1.1.1.1	Adh-1	1
	1.1.1.1	Adh-2	1
Aspartate aminotransferase	2.6.1.1	Aat	4
Creatine kinase	2.7.3.2	Ck	5
Esterase	3.1.1.1	Est	1
Esterase D	3.1.1.1	Est-D	1
General protein	—	GP-1	1, 5
Glucose phosphate isomerase	5.3.1.9	Gpi	3
Glycerol-3-phosphate dehydrogenase	1.1.1.8	$\alpha$ -Gpd	1, 5
Isocitrate dehydrogenase	1.1.1.42	Icdh-1	5
	1.1.1.42	Icdh-2	5
L-Iditol dehydrogenase	1.1.1.14	Iddh	5
Lactate dehydrogenase	1.1.1.27	Ldh-1	4
	1.1.1.27	Ldh-2	4
Malate dehydrogenase	1.1.1.37	Mdh-1	6
	1.1.1.37	Mdh-2	6
Mannose-6-phosphate isomerase	5.3.1.8	Mpi	3
Tripeptide aminopeptidase	3.4.13	Pep-B	1
Peptidase C	3.4.13	Pep-C	1
Proline dipeptidase	3.4.13.9	Pep-D	1
Peptidase E	3.4.13	Pep-E	1
Peptidase S	3.4.13	Pep-S	1
Phosphoglucomutase	2.7.5.1	Pgm	6
6-phosphogluconate dehydrogenase	1.1.1.44	6-Pgd	5
Purine nucleoside phosphorylase	2.4.2.1	Np	4
Superoxide dismutase	1.15.1.1	Sod	1, 2

muscle (stomach and intestine), or both, were subjected to horizontal starch gel electrophoresis using standard techniques (Selander *et al.*, 1971; Harris & Hopkinson, 1976; Richardson, Baverstock & Adams, 1986). Twenty-eight loci were scored using the electrophoretic conditions listed in Table 1.

#### *Phylogenetic analysis*

Presumptive loci were treated as characters and different combinations or suites of alleles (allozymes) as character states (Buth, 1984; Mickevich & Mitter, 1981, 1982). I used only a single population as the OTU (terminal taxon) representing each species, specifically the population with a large sample (nine or ten specimens). Consequently, the combinations of alleles treated as character states represent true polymorphisms, rather than variation among populations. Information from other populations was used to determine whether it was appropriate to generalize these character states to the species as a whole. All multistate characters were treated as unordered.

Phylogenetic relationships were analysed according to the principle that shared, derived characters but not shared, ancestral ones are evidence of exclusive

common ancestry, that is, of monophyly (e.g. Hennig, 1966). Primitive and derived character states were determined using the method of outgroup comparison (Watrous & Wheeler, 1981; Farris, 1982; Maddison, Donoghue & Maddison, 1984). The ancestral state was determined by reconstructing the condition of the most recent common ancestor of the nearest outgroup and the ingroup according to the relationships proposed by Etheridge & de Queiroz (1988) using the method of Maddison *et al.* (1984).

The best estimate of phylogenetic relationships among the various sand lizards was considered to be the arrangement that maximized Wagner parsimony, that is, the arrangement that minimized the number of instances in which homoplasy had to be postulated in order to account for shared character states. I used Swofford's Phylogenetic Analysis Using Parsimony (PAUP, version 3.0) and Maddison and Maddison's MacClade (version 2.97.50) programs to find the tree(s) that satisfied this criterion. Exhaustive searches and searches using the branch and bound algorithm were conducted using PAUP, insuring that the shortest tree or trees were found.

## RESULTS

The various presumptive alleles and their distributions and frequencies among taxa are summarized in Table 2; data for the large populations only are presented in Table 3. These data formed the basis of the following characters and (unordered) character states. (For cases in which the ancestral condition could be determined, that condition is assigned state 0.)

- (1) Acon: State 0 = allele a, 1 = b, 2 = c, 3 = bc, 4 = cd.
- (2) Alb: 0 = a, 1 = b, 2 = d, 3 = e, 4 = f.
- (3) Adh-1: 0 = a, 1 = ac.
- (4) Adh-2: 0 = d, 1 = bd.
- (5) Aat: 0 = c, 1 = e.
- (6) Ck: 0 = a.
- (7) Est: 0 = c, 1 = e, 2 = ce.
- (8) Est-D: 0 = e, 1 = f.
- (9) Gp-1: 0 = a, 1 = b, 2 = ac.
- (10) Gpi: 0 = c, 1 = b, 2 = d, 3 = f, 4 = cf.
- (11)  $\alpha$ -Gpd: 0 = a, 1 = b, 2 = d.
- (12) Icdh-1: 0 = c, 1 = ac, 2 = bc.
- (13) Icdh-2: 0 = a, 1 = c, 2 = bc.
- (14) Iddh: 0 = e, 1 = c, 2 = de.
- (15) Ldh-1: 0 = c.
- (16) Ldh-2: 0 = b, 1 = ab.
- (17) Mdh-1: 0 = d, 1 = b, 2 = c, 3 = e.
- (18) Mdh-2: 0 = b, 1 = a.
- (19) Mpi: 0 = b, 1 = a.
- (20) Pep-B: 0 = a, 1 = c, 2 = d, 3 = ce, 4 = ceh, 5 = bdei, 6 = gilm, 7 = gklm.
- (21) Pep-C: 0 = c.
- (22) Pep-D: 0 = f, 1 = ab, 2 = bc, 3 = cd, 4 = cf, 5 = fg, 6 = cd fgh.
- (23) Pep-E: 0 = a.
- (24) Pep-S: 0 = a, 1 = c, 2 = d, 3 = bcd.
- (25) Pgm: 0 = b, 1 = c.

TABLE 2. Allele frequencies of 28 presumptive loci among nine species of sand lizards and three outgroups. Scores for the outgroup columns indicate only those alleles present in the outgroups that are shared with one or more sand lizard taxa. Abbreviations: I, first outgroup; II, second outgroup; III, third outgroup; Call, *Callisaurus draconoides*; Coph, *Cophosaurus texanus*; Hola, *Holbrookia lacerata*; Homa, *Holbrookia maculata*; Hopr, *Holbrookia propinqua*; Umex, *Uma exsul*; Umno, *Uma notata*; Umpa, *Uma parapygus*; Umse, *Uma scoparia*

Locus	III	II	I	Call	Coph	Hola	Homa	Hopr	Umex	Umno	Umpa	Umse
Acon	a	a, b, c	b, c	c	c (0.75) d (0.25)	c (0.50) e (0.50)	c (0.61) b (0.39)	b	b	b (0.95) d (0.05)	b	a
Alb	?	?	?	b	e	c	d (0.97) g (0.03)	a	d	e	b	f
Adh-1	a	a, d?	a, c, e	a	a (0.98) b (0.02)	e	a (0.71) e (0.21) d (0.04) f (0.04)	a	a	a (0.95) c (0.05)	a	a
Adh-2	d	b, d	d	d (0.60) b (0.34) a (0.05)	d (0.95) c (0.05)	d	d (0.86) b (0.14)	d	d	d (0.98) b (0.02)	d	d
Aat	—	b, c	c, d	c (0.98) a (0.02)	e	d	e	e	c (0.96) b (0.04)	c	c	c
Clk	a	a	a	a	a	a	a	a	a	a	a	a
Est	—	a, c	f	c (0.84) e (0.05) f (0.05) h (0.05)	c (0.95) a (0.03) b (0.03)	a	c (0.68) a (0.18) d (0.05) b (0.03) g (0.03) h (0.03)	e	c	c	e	c
Est-D	e	e	e	f (0.98) h (0.02)	f (0.92) c (0.05) a (0.02)	f	f (0.92) b (0.03) d (0.03) g (0.03)	f	e	e	e	e
Gp-1	?	—	—	a	a (0.65) b (0.35)	b	b	b	a	a (0.84) d (0.16)	a	c (0.55) a (0.45)
Gpi	c	c, e	b, c	c	c (0.98) a (0.02)	f	f (0.55) e (0.45)	f	d	b	d	b

TABLE 2—continued

Locus	III	II	I	Call	Coph	Hola	Homa	Hopr	Umex	Umno	Umipa	Umsec
$\alpha$ -Gpd	a	a, b	a	b	b	b	b	b	d	a (0.97) c (0.03)	d	a
Icdh-1	—	c <sup>2</sup>	c	c (0.98) a (0.02)	c (0.95) a (0.05)	a (0.50) c (0.50)	c (0.82) a (0.10) b (0.08)	b (0.90) c (0.10)	c	c (0.98) a (0.02)	c	c
Icdh-2	—	c <sup>2</sup> , d	a	c	c	c	c (0.72) b (0.22) d (0.06)	c	a	a	a	a
Iddh	?	—	e	c	e	e	e (0.78) b (0.17) a (0.06)	e	e	e	e (0.90) d (0.10)	e
Ldh-1	b	a, b	c	c (0.98) b (0.02)	c	c	c (0.84) a (0.16)	c	c	c	c	c
Ldh-2	—	—	b	b	b	b	b	b	b	b	b (0.90) a (0.10)	b
Mdh-1	—	f	d	b (0.85) a (0.15)	b	c	c (0.79) f (0.10) g (0.10)	c	e	d	e	d
Mdh-2	b	b	b	a	b	b	b	b	b	b	b	b
Mpi	—	—	a, b	a	b (0.90) c (0.10)	b	b	b	b	b	b	b
Pep-B	?	?	?	d (0.42) e (0.28) i (0.18) b (0.10) j (0.02)	l (0.42) i (0.20) g (0.18) e (0.10) m (0.08) d (0.02)	i	k (0.26) i (0.24) m (0.16) e (0.13) g (0.13) l (0.08)	d	a	e (0.68) c (0.28) b (0.02) f (0.02)	c (0.70) e (0.25) h (0.05)	c (0.88) e (0.12)

Pep-C	—	c	—	c (0.90) a (0.10)	c	c (0.68) b (0.32)	c	c	c	c	c
Pep-D	—	—	—	f (0.45) g (0.42) h (0.08) e (0.05)	f	f (0.53) g (0.24) c (0.13) d (0.05) h (0.05)	f (0.90) c (0.10)	d (0.82) c (0.18)	c (0.88) b (0.12)	c (0.85) d (0.15)	a (0.78) b (0.22)
Pep-E	a	a	a	a	a	a	a	a	a	a	a
Pep-S	—	a, c d, e	c	c (0.95) d (0.02) e (0.02)	c	c (0.92) b (0.03) d (0.03) e (0.03)	c (0.75) b (0.15) d (0.10)	a	c	c	d
Pgm	—	b	—	b (0.98) a (0.02)	b	b	b	c	c	c	c
6-Pgd	—	d	d, e, h	d (0.65) a (0.35)	h	h (0.47) f (0.29) d (0.13) b (0.05) i (0.05)	f	j	j (0.70) g (0.20) k (0.10)	j	j
Np	d	b, d	b, d	b (0.88) a (0.05) c (0.05) d (0.02)	d	d	d	d	d	d	d
Sod	—	—	b	b (0.98) a (0.02)	d	d	b (0.95) c (0.05)	b	b	b	b

TABLE 3. Allele frequencies of 28 presumptive loci among eight populations representing eight species of sand lizards and three outgroups. Scores for the outgroup columns indicate only those alleles present in the outgroups that are shared with one or more sand lizard taxa. Abbreviations: I, first outgroup; II, second outgroup; III, third outgroup; Call, *Callisaurus draconoides*; Coph, *Cophosaurus texanus*; Homa, *Holbrookia maculata*; Hopr, *Holbrookia propinqua*; Umex, *Uma exsul*; Umno, *Uma notata*; Umpa, *Uma paraphysa*; Umnc, *Uma scoparia*. Alleles designated by boldface type were not found in other populations of the species and were not included in the allelic arrays recognized as character states

Locus	III	II	I	Call	Coph	Homa	Hopr	Umex	Umno	Umpa	Umnc
Acon	a	a, b, c	b, c	c	c (0.80) d (0.20)	c (0.83) b (0.17)	b	b	b	b	a
Alb	?	?	?	b	e	d (0.94) <b>g</b> (0.06)	a	d	e	b	f
Adh-1	a	a	a, e	a	a	a (0.50) e (0.50)	a	a	a	a	a
Adh-2	d	b, d	d	d (0.55) b (0.45)	d	d (0.75) b (0.25)	d	d	d	d	d
Aat	—	c	c	c	e	e	e	c	c	c	c
Clk	a	a	a	a	a	a	a	a	a	a	a
Est	—	c	—	c (0.90) e (0.10)	c	c (0.89) <b>d</b> (0.11)	e	c	c	c	c
Est-D	e	e	e	f	f (0.90) <b>c</b> (0.10)	f	f	e	e	e	e
GP-1	?	—	—	a	a	b	b	a	a	a	a (0.90) c (0.10)
Gpi	c	c, e	b, c	c	c	e (0.72) f (0.28)	f	d	b	d	b
$\alpha$ -Gpd	a	a, b	a	b	b	b	b	d	a	d	a
Icdh-1	—	c <sup>2</sup>	c	c	c (0.90) a (0.10)	c (0.89) a (0.11)	b (0.90) c (0.10)	c	c	c	c
Icdh-2	—	c <sup>2</sup> , d	a	c	c	c (0.78) b (0.11) <b>d</b> (0.11)	c	a	a	a	a
Iddh	?	—	e	c	e	e	e	e	e	e (0.90) d (0.10)	e





(26) 6-Pgd: 0 = f, 1 = j, 2 = ad, 3 = ce, 4 = gj, 5 = bdfh.

(27) Np: 0 = d, 1 = b.

(28) Sod: 0 = b, 1 = d, 2 = bc.

These 28 characters imply a minimum of 59 phylogenetic transformations. The distributions of these characters and character states among taxa are given in Table 4.

Wagner parsimony analysis of the data in Table 4 resulted in the identification of five trees of equal and minimum length [length = 63, consistency index (Kluge & Farris, 1969) = 0.937, 0.875 excluding uninformative characters] (Fig. 1). These trees differ in whether *Uma* is monophyletic (Fig. 1B, D, E) or paraphyletic (Fig. 1A, C). The trees in which *Uma* is monophyletic differ in the relationships of *Uma scoparia* and *Uma notata* to one another and to the presumptive *Uma paraphygas*-*Uma exsul* clade. Those in which *Uma* is paraphyletic differ in whether a clade composed of *Uma notata* and *Uma scoparia* or one composed of *Uma exsul* and *Uma paraphygas* is the sister group of the remaining sand lizards. The strict consensus tree of these five trees (Fig. 2) has a basal polytomy among the *Uma paraphygas*-*Uma exsul* clade, *Uma scoparia*, *Uma notata*, and a clade composed of the remaining sand lizards. The characters supporting the various presumptive and possible clades are listed in Appendix 2. That no unambiguous synapomorphies were identified for the sand lizard clade as a whole reflects the level of analysis (i.e. that the goal of this study was to identify clades *within* the group). It should not be interpreted as indicating that sand lizard monophyly is in doubt.

## DISCUSSION

### *Phylogenetic relationships*

Every grouping appearing on one or more of the minimum length trees is supported by at least one character. Nevertheless, the support for some of these groupings as clades is ambiguous because of equally parsimonious alternative interpretations of character transformation. This kind of ambiguity applies to all of those groupings seen on some but not all of the minimum length trees.

Although *Uma* is paraphyletic on some of the minimum length trees, monophyly of this taxon should not be considered in doubt. Monophyly of *Uma* is supported unambiguously by at least eight morphological characters (de Queiroz, 1989). Furthermore, the results of the present electrophoretic study do not call the monophyly of *Uma* into question because they are interpretable as an artifact of the ambiguous nature of unordered multistate characters.

All of the characters that are interpretable as potential synapomorphies of part of *Uma* (either *exsul* and *paraphygas* or *notata* and *scoparia*) and the clade composed of *Callisaurus*, *Cophosaurus* and *Holbrookia*, are ambiguous. Two of these characters (20 and 22) imply virtually nothing about relationships among the various sand lizards because they are unpolarized and exhibit different states in all or almost all of the terminal taxa. Any state of these characters that exists in any of the terminal taxa of a possible group on a tree is interpretable as originating in the stem lineage of that group.

A third character (2) has three states (1, 2 and 3) that each occur in a single terminal taxon within *Uma* and in a single terminal taxon within the clade

TABLE 4. Distribution of character states of 28 electrophoretic characters among eight species of sand lizards and their common ancestor. A question mark means that the state is unknown

Taxon	Character																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
<i>Callisaurus draconoides</i>	2	1	0	1	0	0	2	1	0	0	1	0	1	1	0	0	1	1	1	5	0	5	0	0	1	0	2	1	0
<i>Cophosaurus texanus</i>	4	3	0	0	1	0	0	1	0	0	1	1	1	0	0	0	1	0	0	6	0	0	0	1	0	3	0	0	
<i>Holbrookia maculata</i>	3	2	1	1	1	0	0	1	1	4	1	1	2	0	0	0	2	0	0	7	0	6	0	3	0	5	0	1	
<i>Holbrookia propinqua</i>	1	0	0	0	1	0	1	1	1	3	1	2	1	0	0	0	2	0	0	2	0	4	0	3	0	0	0	2	
<i>Uma exul</i>	1	2	0	0	0	0	0	0	0	2	2	0	0	0	0	0	3	0	0	0	0	3	0	0	1	1	0	0	
<i>Uma notata</i>	1	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3	0	2	0	1	1	4	0	0	
<i>Uma paraphygas</i>	1	1	0	0	0	0	1	0	0	2	2	0	0	2	0	1	3	0	0	4	0	3	0	1	1	1	0	0	
<i>Uma scoparia</i>	0	4	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	1	0	1	0	0	2	1	1	0	0	
Ancestor	?	?	?	0	0	0	?	0	?	0	0	0	0	0	0	0	0	0	?	?	?	?	0	?	?	?	?	0	

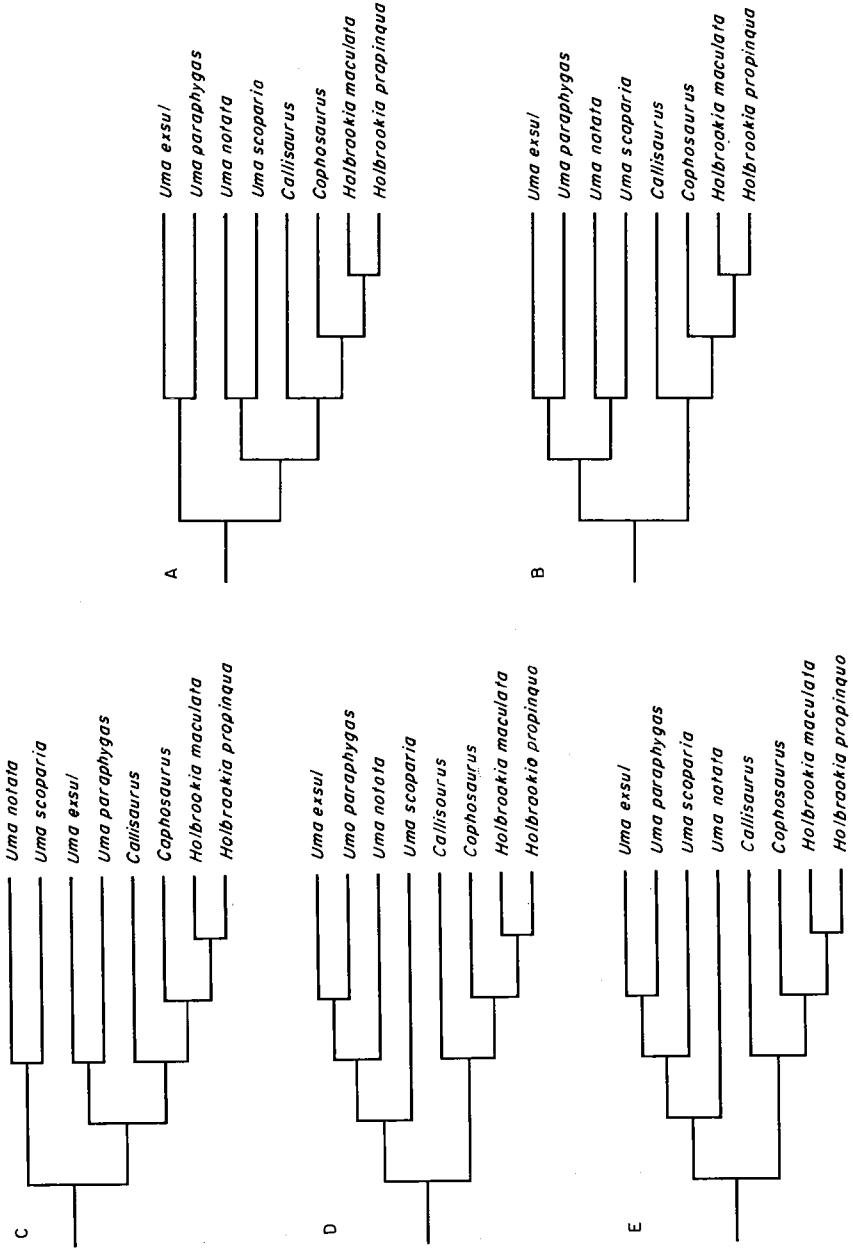


Figure 1. The five minimum length trees resulting from Wagner parsimony analysis of 28 electrophoretic loci coded as characters (total length = 63; consistency index = 0.937; 0.875 for informative characters only).

composed of *Callisaurus*, *Cophosaurus* and *Holbrookia*. The unpolarized and unordered condition of this character enables the length of the tree to be minimized by interpreting any state shared by two or more taxa on different sides of the primary dichotomy within a presumptive clade as the ancestral state for that clade. Because of the structure of the stable part of the tree based on other characters, and because none of the electrophoretic characters supports the monophyly of *Uma* unambiguously, placing a subclade of *Uma* in which one member possesses one of the shared states of this character as the sister group of the clade composed of *Callisaurus*, *Cophosaurus* and *Holbrookia* and having this shared state arise in the common ancestor of the clade thus formed is one possible arrangement that minimizes tree length. Support for such a grouping, however, is highly tenuous. First, only two out of six taxa in the supposed clade actually possess the character state. Second, although this kind of interpretation is possible for each of three different shared states, the different states imply two contradictory ways in which *Uma* might be paraphyletic (Fig. 1A, C). Third, this is only one of several possible optimizations that minimize the number of character state transformations, and alternative optimizations are compatible with monophyly of *Uma*. This character (Alb) apparently exhibits a high degree of homoplasy within the sand lizards (Appendix 2).

Two remaining characters (11 and 17) can be interpreted as supporting (ambiguously) paraphyly of *Uma*, but they can be interpreted parsimoniously as favouring only one of the two trees in which *Uma* is paraphyletic (Fig. 1C). In both of these characters, *Uma notata* and *Uma scoparia* retain the primitive state, and both *Uma exsul* and *Uma paraphygus* have a derived state that differs from the derived state (character 11) or states (17) found in the other sand lizards. Because the order among the derived states is unknown, it is possible that all of the derived states are part of a single transformation series, in which case *Uma exsul* and *Uma paraphygus* would be the sister group of the clade composed of *Callisaurus*, *Cophosaurus* and *Holbrookia*. It is also possible, however, that the derived states in *Uma exsul* and *Uma paraphygus* represent transformations from the plesiomorphic condition independent of those in *Callisaurus*, *Cophosaurus* and *Holbrookia*, in which case these characters do not support the conclusion that *Uma* is paraphyletic.

A similar situation exists in the case of trees indicating paraphyly of the presumptive clade known informally as the ocellated sand lizards (de Queiroz, 1989). Monophyly of this group, composed of the taxa *Uma notata*, *Uma scoparia* and *Uma inornata* (the last not sampled in this study), is supported unambiguously by ten morphological characters (de Queiroz, 1989). Although the electrophoretic characters by themselves allow the possibility that this taxon is paraphyletic, this conclusion is not actually supported by any of them.

Only two characters (20 and 22) have states that are potentially interpretable as evidence of a close relationship between *Uma exsul*, *Uma paraphygus* and *Uma notata*, and these same characters are two of the four with states that are potentially interpretable as evidence of a close relationship between *Uma exsul*, *Uma paraphygus* and *Uma scoparia*. Both of these characters, and one of the other two that is potentially interpretable as evidence of the latter grouping (character 2), are highly ambiguous in that *Uma notata* and *Uma scoparia* possess different states and these states also differ from the state (character 22) or states (characters 2 and 20) found in *Uma exsul* and *Uma paraphygus*. The absence of

unambiguous electrophoretic evidence supporting monophyly of the clade composed of *Uma notata* and *Uma scoparia*, and the unordered nature of these states, allows every state present in a presumptive clade composed of *Uma exsul*, *Uma parapygas* and either *Uma notata* or *Uma scoparia* to be interpreted as potentially originating in the stem lineage of that group. Other interpretations are possible, however, including support for the monophyly of the presumptive *Uma notata*-*Uma scoparia* clade. Indeed, examination of the allelic composition of these states reveals that in one of the characters (22) *Uma notata* and *Uma scoparia* share what appears to be a derived allele (b) not seen in *Uma exsul* and *Uma parapygas*.

The only other character bearing on potential paraphyly of the *Uma notata*-*Uma scoparia* group (character 26) has one state in *Uma exsul*, *Uma parapygas* and *Uma scoparia* and another in *Uma notata*. The character is unpolarized and unordered, but if the state present in the first three taxa is derived relative to that present in the fourth, then the former would be potential evidence of a close relationship among *Uma exsul*, *Uma parapygas* and *Uma scoparia*. Nevertheless, because the states are unordered, either state could be derived relative to the other. If the state in *Uma notata* is derived from that seen in *Uma exsul*, *Uma parapygas* and *Uma scoparia*, then this character does not imply that the *Uma notata*-*Uma scoparia* group is paraphyletic. In fact, examination of the allelic composition of the states suggests that it is more reasonable to interpret the state seen in *Uma notata* as the derived state. This state consists of two alleles, g and j, the former of which is found in no other sand lizard or any of the outgroups examined and for that reason appears to be autapomorphic for *Uma notata*. In contrast, the state found in the other three taxa consists of a single allele, j, which is also found in *Uma notata*. If this allele is derived, then the simplest explanation for its distribution is that it arose in the common ancestor of all *Uma*.

Neither paraphyly of *Uma* nor paraphyly of the ocellated sand lizards occurs on all minimum length trees. Even considering individually the trees on which these arrangements occur, the potentially supporting characters have alternative minimum step interpretations. Only two of the groups not common to all five minimum length trees have unambiguous interpretations of character transformation in their stem lineages on any of the trees. A monophyletic *Uma* has an unambiguous transformation in its stem, state 1 of character 10 (Gpi, allele b), on the trees in which one or the other of *Uma notata* or *Uma scoparia* is the sister group of *Uma exsul* and *Uma parapygas* (Fig. 1D, E). This interpretation, however, requires that the state in *Uma exsul* and *Uma parapygas* is derived relative to that in *Uma notata* and *Uma scoparia*. Alternatively, the state in *Uma notata* and *Uma scoparia* may be derived relative to that in *Uma exsul* and *Uma parapygas*, or both states may be independent transformations from the primitive state. In the latter case, monophyly of *Uma* is not supported.

The possible *Uma notata*-*Uma scoparia* clade has an unambiguous transformation in its stem lineage involving the same character and state (Gpi, allele b) on both trees in which *Uma* is paraphyletic (Fig. 1A, C). Both the state in *Uma notata* and *Uma scoparia* and that in *Uma exsul* and *Uma parapygas* are derived relative to the ancestral sand lizard, but their order of derivation relative to one another is unknown. If the possible *Uma notata*-*Uma scoparia* clade is not the sister group of the presumptive *Uma exsul*-*Uma parapygas* clade, then independent derivation of the two states from the ancestral condition and hence a

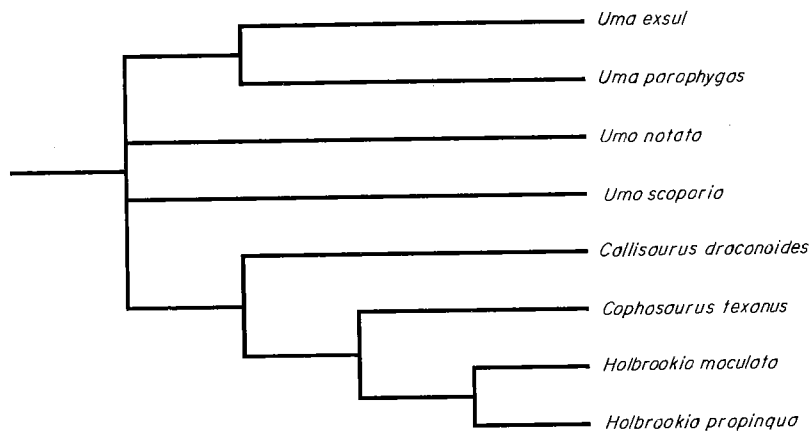


Figure 2. Strict consensus tree derived from the five minimum length trees in Fig. 1.

transformation in the stem lineage of the *Uma notata*–*Uma scoparia* clade minimizes the number of character state transformations. However, if the *Uma notata*–*Uma scoparia* clade is the sister group of the *Uma exsul*–*Uma paraphygas* clade (Fig. 1B), then the transformation to state 1 in the common ancestor of *Uma notata* and *Uma scoparia* is ambiguous because it may be the intermediate stage in a transformation series between the ancestral sand lizard condition and the state in *Uma exsul* and *Uma paraphygas*. If so, then an equally parsimonious interpretation is that it originated in the stem of the clade of all *Uma* rather than that of the possible *Uma notata*–*Uma scoparia* clade.

In summary, the alternative arrangements on the five minimum length trees result not from character incongruence but from ambiguities stemming from alternative interpretations of transformation among states of unordered characters. Although certain relationships are not ruled out, neither are they supported by the characters. Many of the electrophoretic characters suffer to some degree from this kind of ambiguity.

Even those clades common to all the minimum length trees are not necessarily supported unambiguously in terms of characters, as in the case of the presumptive clade of *Uma exsul* and *Uma paraphygas*. Although every one of the five minimum length trees has at least one character supporting this presumptive clade unambiguously, there is no single character that can be interpreted as an unambiguous synapomorphy on all of the minimum length trees.

Despite these difficulties, every dichotomous node common to all five trees (Fig. 2) has at least one character that unambiguously transforms in its stem on each of the minimum length trees. Furthermore, for each of those dichotomous nodes with no branches terminating in a species of *Uma*, every one of the two or more characters that unambiguously transforms in its stem lineage does so on each of the five trees. The unambiguously supported relationships are (1) that *Callisaurus*, *Cophosaurus* and *Holbrookia* share a common ancestor not shared with *Uma*, (2) that *Cophosaurus* and *Holbrookia* share a common ancestor not shared with *Callisaurus* or *Uma* and (3) that *Holbrookia maculata* and *H. propinqua* share a common ancestor not shared with other sand lizards, in other words, that *Holbrookia* is monophyletic.

Monophyly of various of the terminal taxa is also supported by unambiguous

characters; however, this evidence should be viewed with caution. Some species are represented by only a single population, the character states of which cannot necessarily be generalized to the entire species. It is significant, however, that widely separated populations of the polytypic species *Callisaurus draconoides* share derived characters supporting monophyly of this taxon, for example, Iddh (allele c), Mdh-2 (a), Mpi (a) and Np (b).

The results of the present study contradict a widely held view (e.g. Axtell, 1958; Norris, 1958; Earle, 1961, 1962; Clarke, 1965; Adest, 1978) that the greater earless lizard, *Cophosaurus texanus*, shares a more recent common ancestor with the zebratail lizard, *Callisaurus draconoides*, than with the other earless lizards, *Holbrookia*. Consequently, these results also contradict a related hypothesis in which the derived 'earless' condition in *Cophosaurus* and *Holbrookia* is viewed as the result of homoplasy in the form of evolutionary parallelism. Given that *Cophosaurus* and *Holbrookia* are sister taxa, there is no reason to postulate parallelism to account for the shared, derived earless condition. Instead, the simplest explanation is that the earless condition originated in the common ancestral lineage shared exclusively by these two taxa.

#### *Comparisons with previous studies*

Several electrophoretic studies of sceloporine sand lizards have been done previously (Guttman, 1970; Adest, 1977, 1978, 1987). In an early study, Guttman (1970) found that species representing all four genera of sand lizards possessed the same mobilities in three different fractions (bands) of haemoglobin. He contrasted this situation with results obtained for 40 other species of lizards, in which he found only two cases of electrophoretically identical haemoglobins involving "two closely related pairs of *Sceloporus* and two xantusiids" (Guttman, 1970: 572). Guttman (1970) offered five possible explanations for the high degree of similarity among sand lizard haemoglobins but considered the most likely to be that the sand lizards are more closely related than their taxonomy indicates. In that the haemoglobin electromorphs studied by Guttman were invariant among the sand lizards, his results neither support nor contradict those of the present study.

Adest (1977, 1978, 1987) performed extensive studies on electrophoretic variation in the sceloporine sand lizards, both within and among currently recognized genera and species. Of particular significance to the present study are his analyses of relationships among the species of *Uma* (Adest, 1977) and his analysis of intergeneric relationships (Adest, 1978).

In his study of relationships among the species of *Uma*, Adest (1977) proposed that *Uma exsul* is the sister group of all other species of *Uma*. Although this proposal is contradicted by the results of the present study, in which *Uma exsul* appears to be the sister group of *Uma paraphygas*, the data upon which Adest's conclusions were based are ambiguous. Adest's proposed phylogeny differs from the relationships among the species of *Uma* on all minimum length trees found in the present study only in the position of the root. Because no outgroup taxa were included in his study, the position of the root implied by his data is unknown. (Phenograms, such as that presented by Adest, are rooted only in the sense that the branch at the last clustering level specifies the 'bottom' of the dendrogram; they are not rooted on the basis of information about ancestral states.)



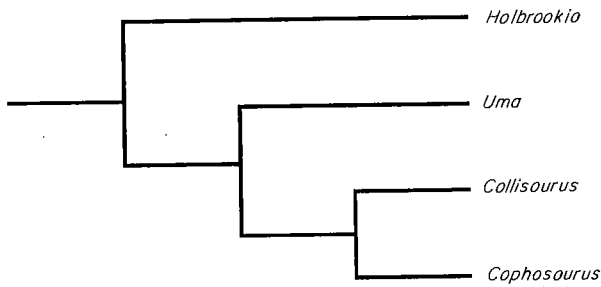


Figure 3. Topology of dendrograms constructed by Adest (1978) based on analyses of data from three electrophoretic studies using the UPGMA and the method of Fitch & Margoliash (1967). The diagram is a simplified composite of four dendrograms, some of which include multiple taxa of *Holbrookia* and some of which do not include *Uma*.

Consequently, it is also unknown whether his data imply relationships contradictory to those proposed in the present study. In any case, the evidence for a sister group relationship between *Uma exsul* and *Uma parapygas* in the present study is tenuous in that none of the characters supporting this relationship does so on all minimum length trees (see above).

Adest (1978) conducted three separate electrophoretic studies of sand lizard intergeneric relationships. In the first, he compared *Callisaurus*, *Cophosaurus* and three species of *Holbrookia* at 19 loci. In the second study, he compared *Callisaurus*, *Cophosaurus*, *Uma*, a sample of *Holbrookia maculata* from Arizona (representing the subspecies *H. m. thermophila*) and pooled samples of *H. maculata* from New Mexico, Oklahoma and Texas (representing the subspecies *H. m. flavilenta* and *H. m. maculata*) at 30 loci. In the third study, he compared representatives of all four sand lizard genera and the outgroup *Sceloporus poinsettii* at 16 loci.

Adest calculated pairwise Nei genetic distances (Nei, 1972) for all three data sets and constructed dendrograms from these distances using unweighted pair-group arithmetic average (UPGMA) clustering (e.g. Sneath & Sokal, 1973). In addition, he used the method of Fitch & Margoliash (1967) to construct a dendrogram from the genetic distances derived from the third data set. The dendrograms resulting from all of these analyses are identical in topology (Fig. 3). In all cases *Callisaurus* and *Cophosaurus* cluster together, the *Callisaurus-Cophosaurus* group clusters with *Uma* (when that taxon was included in the study) and the basal partition within the sand lizards is between the *Callisaurus-Cophosaurus-Uma* cluster and a cluster composed of various OTUs within *Holbrookia*.

Although Adest noted the poor fit (in terms of percent standard deviation) between the Fitch-Margoliash tree and the original distance matrix, he also noted the high concordance among the dendrograms (in terms of topology), and he interpreted these dendrograms as phylogenetic trees. One of Adest's major conclusions was that *Callisaurus* and *Cophosaurus* are sister taxa. This conclusion and his conclusion that *Callisaurus* and *Cophosaurus* are closer to *Uma* than to *Holbrookia* are fundamentally at odds with the conclusions of the present study. In the present study, all trees best fitting the data indicate that *Cophosaurus* is the sister group of *Holbrookia* rather than of *Callisaurus* and that the species of *Uma*

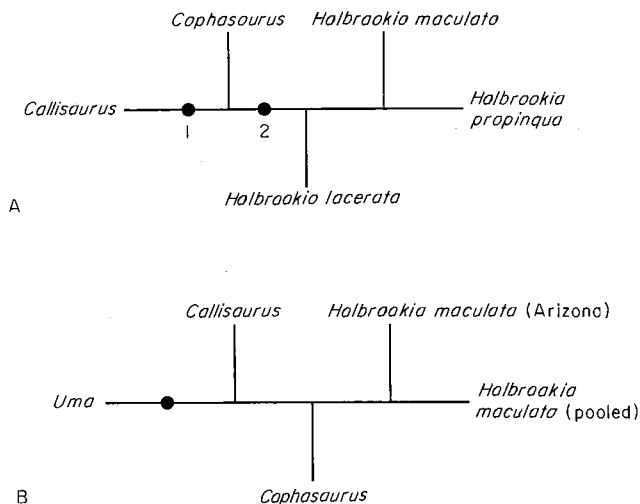


Figure 4. Minimum length unrooted trees resulting from Wagner parsimony analysis of: A, 19 electrophoretic loci from Adest's (1978) first study of sand lizard intergeneric relationships (total length = 24; consistency index = 1.000, 1.000 excluding uninformative characters); B, 30 electrophoretic loci from Adest's (1978) second study of sand lizard intergeneric relationships (total length = 46; consistency index = 0.978, 0.889 excluding uninformative characters). For A, dots indicate alternative placements of the root that will give rooted trees corresponding with the tree derived from Wagner parsimony analysis of my electrophoretic data (1) and Adest's phenogram (2). For B, the dot indicates the placement of the root that will give a rooted tree corresponding with the tree derived from Wagner parsimony analysis of my electrophoretic data. No root placement will give the same tree as Adest's dendrogram. Branch lengths in both diagrams bear no relationship to the number of changes occurring along them.

are outside of the clade stemming from the most recent common ancestor of *Callisaurus*, *Cophosaurus* and *Holbrookia* (Figs 1, 2).

Because of the discrepancies between Adest's conclusions and mine, his analysis deserves critical examination. I argue that these discrepancies result not from incongruencies between Adest's data and my own but from differences in their interpretation resulting from the use of different analytical methods. As it turns out, parsimony analysis of Adest's data gives results that contradict his conclusions and instead support the conclusions of the present study. Furthermore, this outcome is understandable as a consequence of peculiarities of the data that render the analytical methods used by Adest inappropriate as methods for reconstructing phylogenetic relationships.

Adest's first electrophoretic study of intergeneric relationships included only *Callisaurus*, *Cophosaurus* and three species of *Holbrookia*. I coded these data as characters treating the loci as characters and the allelic arrays as unordered character states. An exhaustive search using PAUP yielded a single tree of minimum length (Fig. 4A). This tree is unrooted because no outgroup was included in the study. The unrooted tree is equivalent both with Adest's phenogram (Fig. 3) and with the phylogenetic tree of the present study (Fig. 2). This equivalence exists because for this subset of sand lizards Adest's dendrogram and mine do not differ in terms of connections but only in the position of the root. Although rooting at the midpoint of the branches connecting the most divergent taxa is sometimes used as an alternative to

outgroup rooting, this method assumes homogeneity of evolutionary rates (Farris, 1972). This assumption is inappropriate in the present context, because unequal rates of evolution may be responsible for the differences between dendrograms resulting from phenetic clustering and those resulting from parsimony analysis (see below).

As with the previous analysis, I coded the data from Adest's second study as characters treating the loci as characters and the allelic arrays as unordered character states. Again, an exhaustive search using PAUP yielded a single minimum length tree (Fig. 4B). Adest's second study also did not include an outgroup, and therefore the tree is unrooted.

With the inclusion of *Uma*, the unrooted tree corresponding with the rooted tree based on parsimony analysis of my electrophoretic data differs from the unrooted tree corresponding with Adest's dendrograms. Consequently, the reanalysis of Adest's second data set bears on a choice between the alternative hypotheses. The unrooted tree resulting from parsimony analysis of Adest's second data set is incompatible with Adest's dendrograms. The only root placement that will result in a clade formed by *Callisaurus*, *Cophosaurus* and *Uma* will have *Callisaurus* the sister group of *Uma* rather than of *Cophosaurus*, and no root placement will result in *Callisaurus* and *Cophosaurus* being sister groups. In contrast, the unrooted tree resulting from Wagner parsimony analysis of Adest's second data set is topologically identical with the unrooted tree corresponding with the rooted tree based on parsimony analysis of my electrophoretic data. Rooting the unrooted tree resulting from reanalysis of the data from Adest's second study along the branch connecting *Uma* to the rest of the tree gives the topology of the tree resulting from parsimony analysis of my electrophoretic data.

Although based on the smallest samples of loci (16) and of organisms for each taxon (one or two), Adest's third study of intergeneric relationships of the sand lizards included outgroup taxa with which trees can be rooted. This is significant because, in contrast with unrooted trees, rooted trees are interpretable as phylogenies, and furthermore, being more restricted statements (a single unrooted tree corresponds with several different rooted trees), they allow greater discrimination between alternative phylogenetic hypotheses.

Using *Sceloporus poinsetti* and *Phrynosoma platyrhinos* as outgroups, I reconstructed the condition of the outgroup node (Maddison *et al.*, 1984) and used this hypothetical ancestor to root the tree(s) in my reanalysis of Adest's third data set. (G. Adest kindly supplied me with his data on *P. platyrhinos*, which were not included in his dissertation.) An exhaustive search of trees using PAUP revealed a single rooted tree of minimum length (Fig. 5; length = 19, CI = 0.895 for all characters, 0.778 for informative characters only). This tree differs from Adest's dendrograms based on the same electrophoretic data in two ways, first, in having *Uma* outside of a clade made up of the remaining sand lizards rather than being the sister group of a presumptive *Callisaurus-Cophosaurus* clade and second, in having *Cophosaurus* as the sister group of *Holbrookia* rather than of *Callisaurus*. In other words, when considering the taxa common to both studies, the tree resulting from parsimony analysis of characters based on the data from Adest's third study is identical to the tree resulting from a similar analysis of my electrophoretic data.

Adest also presented a dendrogram based on the Nei genetic distances derived

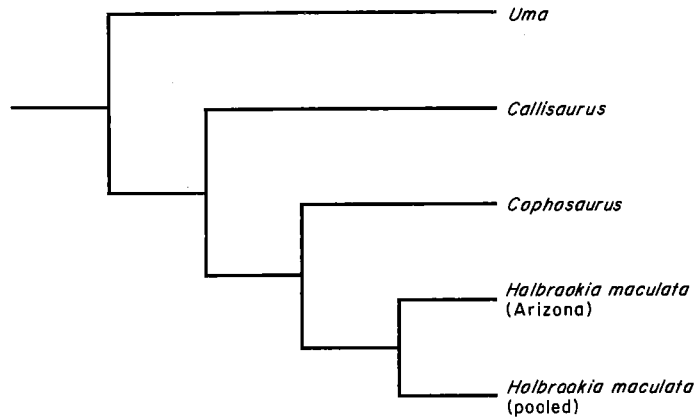


Figure 5. Minimum length rooted tree resulting from Wagner parsimony analysis of 16 electrophoretic loci from Adest's (1978) third study of sand lizard intergeneric relationships (total length = 19; consistency index = 0.895, 0.778 excluding uninformative characters).

from his third study using the method of Fitch & Margoliash (1967). This dendrogram has the same branching structure as his phenogram for the same data. Although the Fitch-Margoliash method does not assume constant evolutionary rates, the dendrogram presented by Adest is not the best estimate of sand lizard phylogeny judged by percent standard deviation, the criterion employed in that method. Reanalyses of the data upon which Adest's dendrogram is based (Adest, 1978: table 14) using the FITCH algorithm in Felsenstein's PHYLIP package (version 3.2) revealed that this dendrogram has an average percent standard deviation (APSD) of 12.37. I found two dendrograms with lower APSDs: one in which the relationships are those favoured by character analysis of my electrophoretic data (Fig. 2) and similar reanalysis of Adest's data (Fig. 5) and a second differing only in that the positions of *Callisaurus* and *Uma* are reversed. The APSDs for those dendrograms are 11.47 and 10.82, respectively.

The hypothesis that *Callisaurus* and *Cophosaurus* are closest relatives has been advocated by various authors (Axtell, 1958; Norris, 1958; Earle, 1961, 1962; Clarke, 1965; Adest, 1978). Not only is this hypothesis contradicted by the electrophoretic data as a whole, it is not supported by any individual electrophoretic characters. In my study, *Callisaurus* and *Cophosaurus* share a derived state for each of four characters (Est-D,  $\alpha$ -Gpd, Icdh-2, and Mdh-1). In three of these, the derived state shared by *Callisaurus* and *Cophosaurus* is also found in one (Icdh-2) or both (Est-D,  $\alpha$ -Gpd) species of *Holbrookia*. The fourth (Mdh-1, allele b) is unique to *Callisaurus* and *Cophosaurus*, but *Holbrookia* possess an alternative derived state. Because the relative order of these two states is unknown, it is possible that the allele shared by *Callisaurus* and *Cophosaurus* is ancestral relative to that seen in *Holbrookia*. Therefore, even if interpreted as evidence for a sister group relationship between *Callisaurus* and *Cophosaurus*, the evidence is ambiguous. In Adest's third study, the only one in which character polarities can be determined, *Callisaurus* and *Cophosaurus* share derived alleles in only two characters. In one of these ( $\alpha$ -Gpd) the derived allele is also shared with *Holbrookia*, and in the other (Gp-4), the derived allele is also shared with *Uma*.

There are no known electrophoretic characters unambiguously supporting a sister group relationships between *Callisaurus* and *Cophosaurus*.

#### *Rates of protein evolution*

The discrepancies between the results of my analyses of Adest's data and those of his own analyses are attributable to the use of analytical methods on his part that are inappropriate for reconstructing phylogenetic relationships under conditions that apparently exist within the sand lizard clade. In order for phenetic clustering methods, including the UPGMA employed by Adest (1978), to give accurate estimates of phylogenetic relationships, the data must have been generated by equal rates of evolutionary divergence among lineages (Colless, 1970; Farris, 1971; Felsenstein, 1988). Although such clocklike evolution is sometimes hypothesized for molecular characters, rate variations among lineages have been demonstrated, and such variations are acknowledged even by proponents of the so-called molecular clock hypothesis (e.g. Wilson, Carlson & White, 1977).

The existence of variation in evolutionary rates among the lineages within a study group can be tested prior to analysis of their phylogenetic inter-relationships by making comparisons with an outside reference taxon, a procedure known as the relative rate test (Sarich & Wilson, 1967a, b). Relative rate tests can be employed to assess the appropriateness of interpreting dendrograms produced by phenetic clustering as phylogenetic trees. Subjecting Adest's data to such tests reveals not only the existence of rate variations but also that the particular variations explain the discrepancies between phenograms and minimum length trees derived from the same data. The particular patterns of rate variation thus revealed are consistent with the phylogeny based on parsimony analysis of both my and Adest's electrophoretic data; they are incompatible with the interpretation of Adest's phenograms as phylogenetic trees.

Relative rate tests reveal that the Nei genetic distances used by Adest to construct phenograms of sand lizard relationships have been generated by unequal evolutionary rates. Only the last of Adest's three data sets includes taxa outside the sand lizard clade, *Sceloporus* and *Phrynosoma*, which are necessary for relative rate tests. If rates of evolution have been equal among the various lineages of sand lizards, then the genetic distances between either one of these outgroups and each of the sand lizard taxa should be roughly equal. This is not the case. Using *Sceloporus* as the outside reference taxon, the largest Nei genetic distance (to *Holbrookia maculata thermophila*) is 56% greater (1.91/1.22) than the smallest distance (to either *Callisaurus* or *Cophosaurus*; *Uma* and pooled populations of *Holbrookia maculata* exhibit intermediate distances to *Sceloporus*) (Adest, 1978: table 14). The Nei genetic distances between *Sceloporus* and all sand lizards are high (minimum 1.22), however, and the Nei genetic distance coefficient is less accurate at high distances (Nei, 1972).

*Phrynosoma* is more closely related to the sand lizards than is *Sceloporus* (Presch, 1969; Etheridge & de Queiroz, 1988); the former taxon also exhibits smaller genetic distances to the various sand lizards. Using *Phrynosoma* rather than *Sceloporus* as the outside reference taxon in a relative rate test, apparent rate variations are even greater. The distances between *Phrynosoma* and *Holbrookia*

(1.01 and 1.03 to *H. m. thermophila* and the pooled sample of *H. maculata*, respectively) are at least 80% greater than the distances between *Phrynosoma* and *Callisaurus*, *Cophosaurus* and *Uma* ( $d = 0.56$  for each comparison). *Holbrookia* appears to have experienced greater rates of protein evolution than the other lineages of sand lizards since their most recent common ancestor.

In fact, Adest (1978) postulated variation in rates of protein evolution in the sand lizards. His assessment, however, was based on interpreting his data in the context of his hypothesized phylogeny, which was based on phenetic clustering of genetic distance data. Acceptance of this hypothesized phylogeny led Adest to postulate relatively lesser rather than greater rates of evolution in the *Holbrookia* lineage. Adest's conclusions about both relationships and rates of evolution are undermined by his interpretation of a phenogram as a phylogenetic tree when the data do not satisfy the condition of constant evolutionary rates required for such an interpretation. Furthermore, his conclusion about rates of evolution is contradicted by the results of relative rate tests, which indicate that *Holbrookia* has undergone greater rather than lesser amounts of change compared with other lineages of sand lizards.

The particular rate variations that exist among sand lizard lineages explain the discrepancies between the results of Adest's phenetic analyses and my phylogenetic conclusions based on parsimony analyses of the same data. As demonstrated by relative rate tests, the lineage leading to *Holbrookia* apparently has undergone greater rates of protein evolution than those leading to the other extant sand lizards. If the discrepancy in evolutionary rates is sufficiently great, then *Holbrookia* should be more distant phenetically (i.e. in terms of genetic distance) from all other sand lizards than these other taxa are from one another, and this situation should hold even though some of these other taxa, in particular *Cophosaurus*, share a more recent common ancestor with *Holbrookia* than with one another. In other words, a sufficiently greater rate of protein evolution in *Holbrookia* would explain, for example, why *Cophosaurus* clusters phenetically with *Callisaurus* rather than *Holbrookia*, even though *Cophosaurus* shares a more recent common ancestor with *Holbrookia* than with *Callisaurus*.

Although not useful for relative rate tests because they do not include outgroups, Adest's first two studies, in addition to his third study, bear on the hypothesis that *Cophosaurus* and *Holbrookia* are sister taxa but are dissimilar because of increased rates of divergence in the latter taxon. This hypothesis implies that although phenetic distances between *Callisaurus* and *Cophosaurus* may be smaller than those between either taxon and *Holbrookia*, provided that rates of evolution in the *Callisaurus* and *Cophosaurus* lineages have been similar (which they have, see below), the genetic distances between *Cophosaurus* and *Holbrookia* should be smaller than those between *Callisaurus* and *Holbrookia*. This prediction is upheld. For all three of Adest's electrophoretic studies, the Nei genetic distances between *Cophosaurus* and various OTUs representing *Holbrookia* are consistently smaller than those between *Callisaurus* and these same OTUs representing *Holbrookia* (Table 5).

These patterns of electrophoretic similarity can be accounted for in the context of a phylogeny in which *Callisaurus* and *Cophosaurus* are sister taxa by postulating, as Adest did, greater rates of evolution in *Callisaurus* relative to *Cophosaurus*. This interpretation, however, is contradicted by relative rate tests. The Nei genetic distance for the comparison between *Callisaurus* and *Sceloporus* is

TABLE 5. Comparison of Nei genetic distances from various OTUs representing *Holbrookia* to *Cophosaurus* on the one hand and to *Callisaurus* on the other. The data are divided into three blocks corresponding with three separate studies conducted by Adest (1978) and presented in his tables 15, 16, and 17, respectively

OTU representing <i>Holbrookia</i>	Genetic distance to:	
	<i>Cophosaurus</i>	<i>Callisaurus</i>
<i>H. maculata</i>	0.43	0.85
<i>H. propinqua</i>	0.44	0.74
<i>H. lacerata</i>	0.84	1.10
<i>H. maculata</i> (Arizona)	0.44	0.69
<i>H. maculata</i> (pooled)	0.45	0.71
<i>H. maculata</i> (Arizona)	0.47	0.98
<i>H. maculata</i> (pooled)	0.40	0.73

identical to that between *Cophosaurus* and *Sceloporus* ( $d = 1.22$  for both comparisons), indicating similar rates of evolution in these two lineages for the loci sampled by Adest (1978: table 17). The same is true for the genetic distances between each of these two sand lizard taxa and *Phrynosoma*, which again have identical values ( $d = 0.56$ ). Two other things should be remembered. First, the existence of rate variations invalidate an assumption implicit in the interpretation of a phenogram as a phylogenetic tree, thus undermining the phylogenetic hypothesis upon which this Adest's interpretation was based. Second, the evidence of shared, derived characters contradicts this hypothesis of relationships. Rate variations among the lineages of sceloporine sand lizards apparently have been sufficiently great to cause phenetic clustering to yield an incorrect phylogenetic tree.

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#### Note added in proof

Two problems have come to my attention subsequent to the acceptance of this paper. The first concerns the greater genetic distance between *Callisaurus* and *Holbrookia* than between *Cophosaurus* and *Holbrookia* and its bearing on the alternative phylogenetic hypotheses. Although relative rate tests using *Sceloporus* and *Phrynosoma* as outgroups indicate similar rates of evolution in the *Callisaurus* and *Cophosaurus* lineages, accepting the phylogeny advocated in the present paper implies that it is also appropriate to use *Uma* as an outgroup to the clade stemming from the most recent common ancestor of *Callisaurus*, *Cophosaurus* and *Holbrookia*. Contrary to the previous results, a relative rate test using *Uma* as the outgroup indicated that the *Callisaurus* lineage has undergone greater divergence than the *Cophosaurus* lineage since their most recent common ancestor.

The discrepancy between the results obtained using different outgroups is attributable to two loci (6-Pgdh and Mpi). These loci exhibit a pattern of allelic distribution implying that the ancestral state for the sand lizard clade both differs from the state or states observed in *Phrynosoma* and *Sceloporus* and is retained in *Uma* and *Cophosaurus* but not in *Callisaurus*. The changes in the *Callisaurus* lineage are undetectable using *Phrynosoma* and *Sceloporus* as outgroups because relative to these outgroups they represent overlaid substitutions. When *Uma* is used as the outgroup, the changes in the *Callisaurus* lineage are detected because no intervening substitution occurs along the branches between *Uma* and the most recent common ancestor of the remaining sand lizards. Consequently, the *Callisaurus* lineage is seen to exhibit greater divergence than the *Cophosaurus* lineage since their most recent common ancestor. The existence of greater divergence in the *Callisaurus* lineage relative to the *Cophosaurus* lineage makes it impossible to distinguish between the alternative phylogenetic hypotheses on the basis of Adest's (1978) table of genetic distances. That is to say, both hypotheses now predict a greater distance between *Callisaurus* and *Holbrookia* than between *Cophosaurus* and *Holbrookia*.

The second problem concerns the method of coding and analysing allelic data. Treating the arrays of alleles observed in different taxa as unordered character states, which I did, potentially ignores some of the available phylogenetic information. Specifically, it ignores information about shared alleles—a problem that is particularly acute when allelic arrays differ only in the presence/absence of rare alleles. Swofford & Berlocher (1987, *Systematic Zoology*, 36: 293–325)

developed a parsimony method that avoids this problem by incorporating information on allele frequencies. I analysed the data in Table 3 for eight sand lizard species with this method using D. L. Swofford's **FREQPARS** program (version 1.0). Because the program's heuristic algorithm is not guaranteed to find the most parsimonious solution(s) even for small numbers of taxa, I simply compared the (unrooted) tree favoured in my previous analysis with the tree favoured by Adest (1978) and the tree produced by the heuristic algorithm. The tree favoured in this comparison was the same one favoured in the previous analysis.

## APPENDIX 1

Species, sample sizes, localities, and specimen numbers for specimens used in the electrophoretic study of allozyme variation. Abbreviations: KdQ, field series of the author; MVZ, Museum of Vertebrate Zoology, University of California at Berkeley; ROM, Royal Ontario Museum, Toronto; USC, University of Southern California Field Series.

## Sand lizards:

*Callisaurus draconoides rhodostictus* (10): U.S.A., California, Imperial Co., 0.5 mi N S-2 on Shell Canyon Rd., vic. Ocotillo (ROM 13776–13779, 13828–13831, 13833, 13841). *C. d. ventralis* (1): U.S.A., Arizona, Pima Co., Santa Catalina Mts., mouth Pima Canyon, vic. Tucson (MVZ 214732). *C. d. rhodostictus* (1): U.S.A., California, Inyo Co., Argus Mts., Darwin Wash (MVZ 214740). *C. d. myurus* (1): U.S.A., Nevada, Mineral Co., SW shore Walker Lake (KdQ 207). *C. d. rhodostictus* (1): México, Baja California, 6.8 mi N Cataviña on Hwy 1 (ROM 14066). *C. d. crinitis* (1): México, Baja California, dunes N Guerrero Negro (MVZ 199440). *C. d. carmenensis* (1): México, Baja California Sur, Santa Agueda (ROM 14452). *C. d. draconoides* (1): México, Baja California Sur, El Coro (MVZ 214731). *C. d. bogerti* (1): México, Sinaloa, Playas N Mazatlan (ROM 14992). *C. d. brevipes, bogerti*, or *inusitatus* (Specimens from this area have been referred to all three subspecies.) (1): México, Sonora, Bahía Kino, 1.8 mi on dirt rd. to Punta Chueca from Bahía Kino (ROM 15024). *C. d. brevipes* (1): México, Sonora, Alamos, Río Cuchujaqui (ROM 14929).

*Cophosaurus texanus scitulus* (10): México, Durango, Bolson de Mapimí, Laboratorio del Desierto, 40 km NE Ceballos, 26°41'N, 103°45'E (ROM 15103–15109, 15157–15159). *C. t. texanus* (2): México, Nuevo Leon, Cadereyta, 17.3 mi NE and 2.5 mi N Cadereyta rd. to Santa Isadora (ROM 15379–15380). *C. t. scitulus* (2): U.S.A., Arizona, Cochise Co., 1.9 mi W New Mexico border on I-10 (MVZ 214724–214725). *C. t. scitulus* (2): U.S.A., Arizona, Yavapai Co., 3 mi N Hillside (MVZ 200010–200011). *C. t. scitulus* (2): U.S.A., Texas, Hudspeth Co., scenic lookout N side I-10, 4 mi W Van Horn (MVZ 214709–214710). *C. t. texanus* (2): U.S.A., Texas, Travis Co., South San Gabriel River at Hwy 183 (MVZ 214727, 214729).

*Holbrookia lacerata lacerata* (1): U.S.A., Texas, no further data (MVZ 214876). *Holbrookia maculata approximans* (9): México, Durango, Bolson de Mapimí, Laboratorio del Desierto, 40 km NE Ceballos, 26°41'N, 103°45'E (ROM 15129–15133, 15189–15190, 15192–15193). *H. m. thermophila* (2): México, Sonora, Alamos, 5 mi E Navojoa (ROM 14934–14935). *H. m. approximans* (2): México, Zacatecas, 11 mi NE Concepcion Del Oro turnoff on Hwy 54 (USC 5611; ROM 15341). *H. m. flavilenta* or *thermophila* (The specimen has been skeletonized and can no longer be identified to subspecies.) (1): U.S.A., Arizona, Cochise Co., vicinity of Nicksville (MVZ 200016). *H. m. flavilenta* (1): U.S.A., Arizona, Cochise Co., 3.5 mi SE junction rd. to Ft. Bowie Natl. Hist. Site on Hwy 186 (MVZ 214872). *H. m. flavilenta* (2): U.S.A., Arizona, Cochise Co., junction I-10 and Hwy 666 N (MVZ 214808–214809). *H. m. maculata* (2): New Mexico, Chaves Co., 0.9 mi S Hwy 380, 37.0 mi E Rosewell (junction Hwy 285) MVZ 214803–214804. *Holbrookia propinqua propinqua* (10): U.S.A., Texas, Nueces Co., dunes N Access Rd. 3, Mustang Island (MVZ 214818–214823, 214857, 214860–214861, 214933).

*Uma exsul* (10): México, Durango, Hwy 47, rd. to Nazas and Rodeo (ROM 15304–15313). *U. exsul* (1): México, Coahuila, Bilbao dunes, vic. Viesca (ROM 15323). *Uma notata rufopunctata* (10): México, Sonora, 42 km SW Sonoita on Hwy 8 (to Puerto Peñasco), turn off to microondas San Pedro (ROM 13860–13863, 13865–13870). *U. n. rufopunctata* (7): México, Sonora, 17 km NE Puerto Peñasco on Hwy 8 (ROM 13916–13920, 13923–13924). *U. n. notata* (3): U.S.A., California, Imperial Co., E edge Algodones Dunes, 0.9 mi W junction I-10 and S-34 (MVZ 214798–214800). *Uma paraphygas* (10): México, Durango, Bolson de Mapimí, Laboratorio del Desierto, 40 km NE Ceballos, 26°41'N, 103°45'E (ROM 15080–15089). *Uma scoparia* (10): U.S.A., California, San Bernardino Co., vic. Pisgah Lava Flow, 10 mi W Newberry Springs on National Trails Hwy (ROM 14625, 14627–14634; MVZ 214787). *U. scoparia* (10): U.S.A., California, San Bernardino Co., SE edge Kelso Dunes (MVZ 214788–214797).

## Outgroups:

First outgroup (6 species out of 13). *Phrynosoma cornutum* (1): U.S.A., Texas, Pecos Co., 12.8 mi E jct Hwy 285 on I-10 (MVZ 214669). *Phrynosoma coronatum* (3): México, Baja California Sur, 37.6 mi N La Paz on Hwy 1 (KdQ 58); México, Baja California, 11.4 rd mi W Bahía de Los Angeles (KdQ 59); México, Baja California, 42.5 mi S El Rosario on Hwy 1 (KdQ 60). *Phrynosoma douglasi* (1): U.S.A., Arizona, Pima Co., Santa Catalina Mts., jct. Mt. Lemon Hwy and Oracle-Mt. Lemon rd. (KdQ 56). *Phrynosoma modestum* (1): U.S.A., Texas, Pecos Co., rest area N side I-10 at jct. Hwy 385 (MVZ 214668). *Phrynosoma platyrhinos* (3): U.S.A., Nevada, Washoe Co., 8.0 mi S jct. Hwy 446 (Nixon) on Hwy 447 (MVZ 214671); U.S.A., California, Kern Co., 12.1 mi NE California City on rd. to Red Mountain (MVZ 214828); U.S.A., California, Kern Co., 8.5 mi NE California City on rd. to Red Mountain (MVZ 214670). *Phrynosoma solare* (1): U.S.A., Arizona, Pima Co., Santa Catalina Mts., mouth Pima Canyon, vic. Tucson (MVZ 214667).

Second outgroup (10 species out of 81). *Sceloporus clarki* (1): U.S.A., Arizona, Pima Co., Santa Catalina Mts., mouth Pima Canyon (MVZ 214875). *Sceloporus graciosus* (1): U.S.A., New Mexico, Chaves Co., 0.9 mi S Hwy 380, 37.0 mi E Rosewell (junction Hwy 285) (MVZ 214698). *Sceloporus jarrovi* (1): U.S.A., Arizona, Cochise Co., Huachuca Mts., vic. Clark Spring (MVZ 214694). *Sceloporus olivaceus* (1): U.S.A., Texas, Travis Co., South San Gabriel R. at jct. Hwy 183 (MVZ 214677). *Sceloporus magister* (1): U.S.A., Nevada, Washoe Co.,

6.7 mi NW jct. Hwy 447 (Nixon) on Hwy 446 (MVZ 214826). *Sceloporus undulatus* (1): U.S.A., Arizona, Cochise Co., 3.1 mi SE jct. rd. to Ft. Bowie Natl. Hist. Site on Hwy 186 (MVZ 214874). *Sceloporus virgatus* (1): U.S.A., Arizona, Cochise Co., Chiricahua Mts., 5.8 mi E jct. Hwy 181 on Pinery Canyon Rd. (MVZ 214871). *Urosaurus graciosus* (1): U.S.A., California, San Bernardino Co., N edge Pisgah Lava Flow (KdQ 28). *Urosaurus ornatus* (1): U.S.A., Arizona, Cochise Co., Chiricahua Mts., vic. Cave Creek Canyon (KdQ 44). *Uta stansburiana* (1): U.S.A., Nevada, Washoe Co., 8.0 mi S jct. Hwy 446 (Nixon) on Hwy 447 (MVZ 214826).

Third outgroup (1 species out of 2). *Petrosaurus thalassinus* (5): México, Baja California Sur, El Coro (MVZ 190074–190078).

## APPENDIX 2

Electrophoretic characters supporting various presumptive and possible clades of sand lizards. Unambiguous characters are those originating (under minimum step optimization) in the common ancestor of the taxa under which they are listed on all minimum length trees. Ambiguous characters are those interpreted as originating in the common ancestor of the taxa under which they are listed under some but not all minimum step optimizations or on some but not all minimum length trees. Homoplastic characters are marked with an 'H'.

Presumptive clades (common to all minimum length trees):

Sand lizards (entire):

Unambiguous (0):

None identified.

Ambiguous (10):

1 (1); Acon: b.

2 (1, 2 or 3); Alb: b, d or e.

7 (0); Est: c.

9 (0); Gp-1: a.

20 (0, 1, 2, 3, 4, 5, 6, or 7); Pep-B: a, c, d, ce, ceh, bdei, gilm, or gklm.

21 (0); Pep-C: c.

22 (0, 1, 2, 3, 4, 5, or 6); Pep-D: f, ab, cb, cd, cf, fg, or cdfigh.

24 (1); Pep-S: c.

25 (0 or 1); Pgm: b or c.

26 (0, 1, 2, 3, 4, or 5); 6-Pgd: f, j, ad, ce, gj, bdfh.

*Uma exsul* and *Uma paraphygas*:

Unambiguous (0):

None identified.

Ambiguous (6):

2 (1H or 2H); Alb: b or d.

10 (2); Gpi: d.

11 (2);  $\alpha$ -Gpd: d.

17 (3); Mdh-1: e.

20 (0 or 4); Pep-B: a or ceh.

22 (3); Pep-D: cd.

*Callisaurus*, *Cophosaurus*, and *Holbrookia*:

Unambiguous (2):

8 (1); Est-D: f.

13 (1); Icdh-2: c.

Ambiguous (6):

11 (1);  $\alpha$ -Gpd: b.

17 (1); Mdh-1: b.

20 (2, 5, 6, or 7); Pep-B: d, bdei, gilm, or gklm.

22 (0, 4, 5, or 6); Pep-D: f, cf, fg, or cdfigh.

25 (0); Pgm: b.

26 (0, 2, 3, or 5); 6-Pgd: f, ad, ce, bdfh.

*Cophosaurus* and *Holbrookia*:

Unambiguous (2):

5 (1); Aat: e.

12 (1); Icdh-1: ac.

Ambiguous (4):

2 (0, 2H, or 3H); Alb: a, d, or e.

20 (2, 6, or 7); Pep-B: d, gilm, or gklm.

22 (0, 4, or 6); Pep-D: f, cf, cdfigh.

26 (0, 3, or 5); 6-Pgd: f, ce, or bdfh.

*Holbrookia maculata* and *Holbrookia propinqua*:

Unambiguous (3):

9 (1); Gp-1: b.

17 (2); Mdh-1: c.

24 (3); Pep-S: bcd.

Ambiguous (6):

2 (0 or 2H); Alb: a or d.

10 (3 or 4); Gpi: f or ef.

20 (2 or 7); Pep-B: d or gklm.

22 (4 or 6); Pep-D: cf or cdfigh.

26 (0 or 5); 6-Pgd: f or bdfh.

28 (1 or 2); Sod: d or bc.

*Uma exsul*:

- Unambiguous (1):  
24 (0); Pep-S: a.  
Ambiguous (2):  
2 (2H); Alb: d.  
20 (0); Pep-B: a.

*Uma paraphygas*:

- Unambiguous (3):  
7 (1H); Est: e.  
14 (2); Iddh: de.  
16 (1); Ldh-2: ab.  
Ambiguous (2):  
2 (1H); Alb: b.  
20 (4); Pep-B: ceh.

*Uma notata*:

- Unambiguous (0):  
None identified.  
Ambiguous (4):  
2 (3H); Alb: e.  
20 (3); Pep-B: ce.  
22 (2); Pep-D: bc.  
26 (4); 6-Pgd: gj.

*Uma scoparia*:

- Unambiguous (3):  
1 (0); Acon: a.  
9 (2); Gp-1: ac.  
24 (2); Pep-S: d.  
Ambiguous (3):  
2 (4); Alb: f.  
20 (1); Pep-B: c.  
22 (1); Pep-D: ab.

*Callisaurus draconoides*:

- Unambiguous (7):  
1 (2); Acon: c.  
4 (1H); Adh-2: bd.  
7 (2); Est: ce.  
14 (1); Iddh: c.  
18 (1); Mdh-2: a.  
19 (1); Mpi: a.  
27 (1); Np: b.

- Ambiguous (4):  
2 (1H); Alb: b.  
20 (5); Pep-B: bdei.  
22 (5); Pep-D: fg.  
26 (2); 6-Pgd: ad.

*Cophosaurus texanus*:

- Unambiguous (1):  
1 (4); Acon: cd.  
Ambiguous (4):  
2 (3H); Alb: e.  
20 (6); Pep-B: gilm.  
22 (0); Pep-D: f.  
26 (3); 6-Pgd: ce.

*Holbrookia maculata*:

- Unambiguous (4):  
1 (3); Acon: bc.  
3 (1); Adh-1: ac.  
4 (1H); Adh-2: bd.  
13 (2); Icdh-2: bc.  
Ambiguous (6):  
2 (2H); Alb: d.  
10 (4); Gpi: cf.  
20 (7); Pep-B: gklm.  
22 (6); Pep-D: cdfigh.  
26 (5); 6-Pgd: bdfh.  
28 (1); Sod: d.

*Holbrookia propinqua*:

- Unambiguous (2):  
7 (1H); Est: e.  
12 (2); Icdh-1: bc.  
Ambiguous (6):  
2 (0); Alb: a.  
10 (3); Gpi: f.  
20 (2); Pep-B: d.  
22 (4); Pep-D: cf.  
26 (0); 6-Pgd: f.  
28 (2); Sod: bc.

## Possible clades (present on only some minimum length trees):

*Uma*

- Unambiguous (0):  
None identified.  
Ambiguous (5):  
10 (1 or 2); Gpi: b or d.  
20 (0, 1, 3, or 4); Pep-B: a, c, ce, or ceh.  
22 (1, 2, or 3); Pep-D: ab, bc, or cd.  
25 (1); Pgm: c.  
26 (1 or 4); 6-Pgd: j or gj.

*Uma exsul, Uma paraphygas, Callisaurus, Cophosaurus, and Holbrookia*

- Unambiguous (0):  
None identified.  
Ambiguous (5):  
2 (1 or 2); Alb: b or d.  
11 (1 or 2);  $\alpha$ -Gpd: b or d.  
17 (1 or 3); Mdh-1: b or c.  
20 (0, 2, 4, 5, 6, or 7); Pep-B: a, d, ceh, bdei, gilm, or gklm.  
22 (0, 3, 4, 5, or 6); Pep-D: f, cd, cf, fg, or cdfigh.

*Uma notata, Uma scoparia, Callisaurus, Cophosaurus, and Holbrookia*

- Unambiguous (0):  
None identified.  
Ambiguous (3):  
2 (3); Alb: e.  
20 (1, 2, 3, 5, 6, or 7); Pep-B: c, d, ce, bdei, gilm, or gklm.  
22 (0, 1, 2, 4, 5, or 6); Pep-D: f, ab, bc, cf, fg, or cdfigh.

*Uma exsul, Uma paraphygas, and Uma notata*

- Unambiguous (0):  
None identified.  
Ambiguous (2):  
20 (0, 3, or 4); Pep-B: a, ce, or ceh.  
22 (2 or 3); Pep-D: bc or cd.

*Uma exsul, Uma paraphygas, and Uma scoparia*

- Unambiguous (0):  
None identified.  
Ambiguous (4):  
2 (1H, 2H or 4); Alb: b, d, or f.  
20 (0, 1, or 4); Pep-B: a, c, or ceh.  
22 (1 or 3); Pep-D: ab or cd.  
26 (1); 6-Pgd: j.

*Uma notata and Uma scoparia*

- Unambiguous (0):  
None identified.  
Ambiguous (4):  
2 (3H or 4); Alb: e or f.  
10 (1); Gpi: b.  
20 (1 or 3); Pep-B: c or ce.  
22 (1 or 2); Pep-D: ab or bc.

*Note added after publication*

The problem I pointed out in the note added in proof concerning the amounts of divergence in the *Callisaurus* and *Cophosaurus* lineages was raised in the context of the data from the third of Adest's three studies. It is worthwhile to consider this problem further for two reasons. First, because the genetic distance values depend on the particular set of loci sampled, the problem may not apply to the other two studies. Second, it has occurred to me that there is a way to correct for the unequal amounts of divergence in the *Callisaurus* and *Cophosaurus* lineages so that the data from the third study can be used to test the alternative phylogenetic hypotheses.

Because Adest's first study did not include *Uma*, it is unknown whether the problem applies in this case. Consequently, the genetic distances obtained in this study cannot be used to test the alternative phylogenetic hypotheses. In the second study, the genetic distance between *Uma* and *Cophosaurus* is slightly larger than that between *Uma* and *Callisaurus*. In other words, the problem does not apply, and the original conclusion is unchanged. The smaller genetic distances between *Cophosaurus* and *Holbrookia* relative to those between *Callisaurus* and *Holbrookia* are consistent with the results of the parsimony analyses but contradict the results of the UPGMA analysis. The problem arises only in the third study. Because the *Callisaurus* lineage has experienced a greater amount of divergence than the *Cophosaurus* lineage in this set of loci, the fact that the genetic distances between *Cophosaurus* and *Holbrookia* are smaller than those between *Callisaurus* and *Holbrookia* cannot be taken at face value as evidence supporting the parsimony tree and contradicting the UPGMA tree. Nevertheless, the distances can be corrected so that the data can still be used to test the alternative hypotheses. The genetic distance between *Uma* and *Callisaurus* (0.58) is 0.21 greater than the distance between *Uma* and *Cophosaurus* (0.37). Therefore, one can correct for the unequal divergences in the *Callisaurus* and *Cophosaurus* lineages by subtracting 0.21 from the comparisons between *Callisaurus* and *Holbrookia*, and then comparing the corrected values with those for the comparisons between *Cophosaurus* and *Holbrookia*. When this is done, the distances between *Callisaurus* and *Holbrookia maculata* (Arizona) (0.77) and *Holbrookia maculata* (pooled) (0.52) are still greater than the distances for the comparisons involving *Cophosaurus* and the same two OTUs representing *Holbrookia* (0.47 and 0.40, respectively). This finding is consistent with the results of the parsimony analysis but contradicts those of the UPGMA analysis.