Character Congruence and Phylogenetic Signal in Molecular and Morphological Data Sets: A Case Study in the Living Iguanas (Squamata, Iguanidae)

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The lizard family Iguanidae comprises eight living genera distributed throughout the New and Old World, and includes several island endemics. We reconstruct phylogenetic relationships among these genera using 90 previously published morphological characters, to which we add a molecular (mtDNA sequence) data set that includes 742 nucleotides of the ND4 gene and the complete sequences of the histidine, serine, and leucine tRNAs (217 nucleotides). Trees were initially constructed separately from these three data sets, and then tested for significant conflict in topologies that would suggest the influence of different evolutionary processes. The three data sets were then combined, and a single tree was obtained from the total evidence that permitted identification of potential sources of character incongruence. Several additional analyses of the combined data sets were repeated with sequential deletion of successive classes of homoplastic characters, and we show that the same single tree topology is recovered in most cases. However, part of the tree structure collapses when the matrix of combined characters is completely purged of all homoplastic characters. We argue that this extreme results in an unacceptable loss of phylogenetic information, and we present a single phylogenetic hypothesis for all living genera of iguanas. We show that this hypothesis is significantly more parsimonious than either of two previously published trees, and we discuss the evolution and biogeography of the Iguanidae based on the preferred hypothesis.

Introduction

There has been a profusion of studies designed to independently test existing phylogenetic hypotheses based on morphological or molecular data sets (see examples cited in Eemisse and Kluge 1993 and de Queiroz, Donoghue, and Kim 1995), and often these show that diverse data sets do not always yield the same estimates of phylogeny for the same organisms. Opinions differ on how to best extract phylogenetic signal in these cases. One approach involves generation of sequence-based phylogenetic hypotheses for comparisons to one or more preexisting trees, the construction of "fundamental cladograms" separately for each body of evidence, and the derivation from those of a consensus topology in cases of conflict (several consensus techniques are discussed in this context by Swofford 1991). The perceived strength of this approach is that consensus topologies, and the classifications derived from them, should be stable because they possess only concordant clades (Mickevich 1978). Many published studies have employed various measures of congruence between trees derived from morphological and molecular data (Brown et al. 1994; Titus and Larson 1995), or from different types of molecular data (Olmstead and Sweere 1994; other examples are given by de Queiroz, Donoghue, and Kim 1995).

An alternative method is to search for a single best-fitting hypothesis for all available evidence (reviewed in de Queiroz, Donoghue, and Kim 1995). This kind of analysis, undertaken for all characters combined (the "total evidence" approach of Kluge 1989), permits evaluation of alternative tree topologies on the basis of relative strength of evidence and consilience of characters (see also de Pinna 1991; Jones, Kluge, and Wolf 1993; Kluge and Wolf 1993; Chippendale and Wiens 1994). Two other arguments in favor of combined character analyses are that: (1) different data sets may provide phylogenetic signal at different but complimentary hierarchical levels, so that in combination they may substantially improve total resolution (Hillis 1987; Donoghue and Sanderson 1992) and (2) the signal within a single data set may be so weak that it is swamped by noise with individual analyses, but the total signal in the combined data set may be strong enough to "rise above" the noise and provide a robust solution (Barrett, Donoghue, and Sober 1991; but see Funk et al. 1995).

As a step toward resolution, Bull et al. (1993) advocated a "process partition" approach which involves a division of characters into two or more subsets that might have evolved under different constraints (see also de Queiroz 1993 and Miyamoto and Fitch 1995*a*). The rationale for this approach is that tree reconstruction methods may need to accomodate different evolutionary processes or constraints if partitioned data sets are shown to be strongly heterogenous. One test for heterogeneity is to obtain the optimal tree(s) for each data set, and then use an incongruence index to determine if variation in tree topologies is significantly greater than that expected from sampling processes within any single data set. If this cannot be demonstrated, then the data sets are assumed to show homogeneity, and can be combined for further phylogenetic analysis. One limitation of approaches using incongruence indices is that there is no objective cutoff point for making nonarbitrary decisions in borderline cases (Omland 1994).

In this study, we examine relationships among living genera of the Iguanidae by beginning with a process

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partition approach (Bull et al. 1993; Miyamoto and Fitch 1995*a*). We construct trees separately for three data sets (two molecular, one morphological), and then test these in all paired combinations for heterogeneity. Larson (1994) advocates the use of a conservative nonparametric test originally suggested by Templeton (1983) as a means for statistically evaluating whether the data significantly favor one topology over another. If trees are constructed from independent data sets and then tested by this method, a statistical statement can be made regarding whether incongruence is significant between the two, which partially overcomes the issue of a subjective cutoff point raised by Omland (1994). We then group all three data sets in a combined analysis and use the results of this reconstruction to evaluate levels of homoplasy in different data sets, and test these for significant differences in character conflict. The results from the taxonomic congruence and combined approaches are then tested for congruence to each other, and to previously published phylogenetic hypotheses. The evolution and biogeography of iguanas is then considered in the context of the best supported phylogenetic hypothesis derived from these approaches.

Review of Previous Phylogenetic Hypotheses for the Iguanidae

The lizard family Iguanidae, as defined by Frost and Etheridge (1989), includes all living genera of large herbivorous lizards collectively known as "iguanas." Eight living genera are currently recognized, seven of which are indigenous to the Americas, and all are characterized by relatively large body sizes and a number of adaptations to herbivory (Iverson 1980, 1982; Etheridge 1982; Troyer 1983; de Queiroz 1987*a*; Etheridge and de Queiroz 1988). Two genera, ***Amblyrhynchus*** (one species, the marine iguana) and ***Conolophus*** (two species, land iguanas), are endemic to the Galapagos Archipelago, while a third (*Cyclura,* the Caribbean rock and rhinoceros iguanas, containing about eight species) is endemic to a number of islands in the Caribbean. Mainland genera include the spiny-tailed iguanas (*Ctenosaura,* ca. 12 species; de Queiroz 19876, 1995) of Mexico and Central America; the green iguanas (Iguana, two species) of Mexico, Central and South America, and the Lesser Antilles; and the desert iguana (*Dipsosaurus,* one species) and chuckwallas (*Sauromalus,* with eight species; de Queiroz 1995) of the desert regions of northwestern Mexico and the southwestern United States (see maps in de Queiroz 1987a). The enigmatic genus ***Brachylophus*** contains two species endemic to the Fijian and Tongan Archipelagoes in the western Pacific (Gibbons 1981, 1985; Zug 1991). Although the monophyly of the Iguanidae is not questioned (Etheridge and de Queiroz 1988; Frost and Etheridge 1989), several alternative hypotheses of intergeneric relationships have been proposed (reviewed by de Queiroz 1987*a*), and are summarized in figure 1.

Avery and Tanner (197 1) described features of the head-neck skeleton and musculature, tongue, hemipenes, and a number of osteological measurements, and presented the hypothesis of relationships shown in figure 1*A*. A later description of variation in the musculature of the shoulder, brachium, and thigh regions of all genera was provided by Oldham and Smith (1983), but because no outgroup taxa were included in that study, the phylogenetic significance of the variation could not be assessed.

A more recent study by de Queiroz (1987*a*) was based on a detailed description of 95 morphological and osteological characters for representatives of all eight basal taxa (the living genera), and a cladistic maximum-parsimony analysis of generic relationships was proposed (fig. 1*B*). Note that under this hypothesis (1) it is equally plausible that either ***Bruchylophus*** or ***Dipsosaurus*** be considered the sister group of the remaining iguanids; (2) a large monophyletic "Iguanini" is recognized, but with unresolved relationships for ***Ctenosaura*** and ***Sauromalus*** (although these can be resolved on weak evidence to be the sister groups of the Iguanina and Amblyrhynchina, respectively); and (3) two monophyletic groups are nested within Iguanini on the basis of 4 (Iguanina) and 13 (Amblyrhynchina) synapomorphies, respectively. De Queiroz (1987*a*) also showed that, with the possible exception of ***Cyclura,*** which may be paraphyletic with respect to ***Iguana, the*** monophyly of all basal units (genera) was reasonably well supported.

Norell and de Queiroz (1991) extended this morphological analysis by adding the fossil taxa ***Armandisaurus explorator*** and ***Pumiliu novaceki* (**** indicates no evidence for monophyly), which resulted in a fully resolved tree (fig. 1*C*). ***Armandisaurus*** and ***Pumilia* are*** placed as sister taxa to ***Dipsosaurus*** and ***Iguana,*** respectively. As shown by Gauthier, Kluge, and Rowe (1988), the inclusion of fossils in analyses can modify phylogenetic conclusions based exclusively on extant taxa if the fossils have particular character states representing transformation stages no longer retained in extant species (see also Donoghue et al. 1989). In iguanas, the inclusion of ***Armandisaurus,*** either alone or with ***Pumilia****, fully resolves relationships; inclusion of ***Pumilia**** alone does not alter the topology presented in fig. 1*B*.

Methods and Materials

Taxa Sampled for this Study

Table 1 lists the species for which partial or complete data sets (mtDNA protein and tRNA sequences and the morphological characters summarized by Norell and de Queiroz 1991) were collected. Museum acronyms identify the following collections in which voucher specimens and/or tissue samples are maintained: BYU-M. L. Bean Life Science Museum, Brigham Young University; CAS—California Academy of Sciences; KU-Museum of Natural History, University of Kansas; MPM-Milwaukee Public Museum; and UNM-Museum of Southwestern Biology, University of New Mexico.

Lizards were either purchased from dealers (*Ctenosaura palearis, Ctenosaura similis,* and *Oplurus cuvi-*

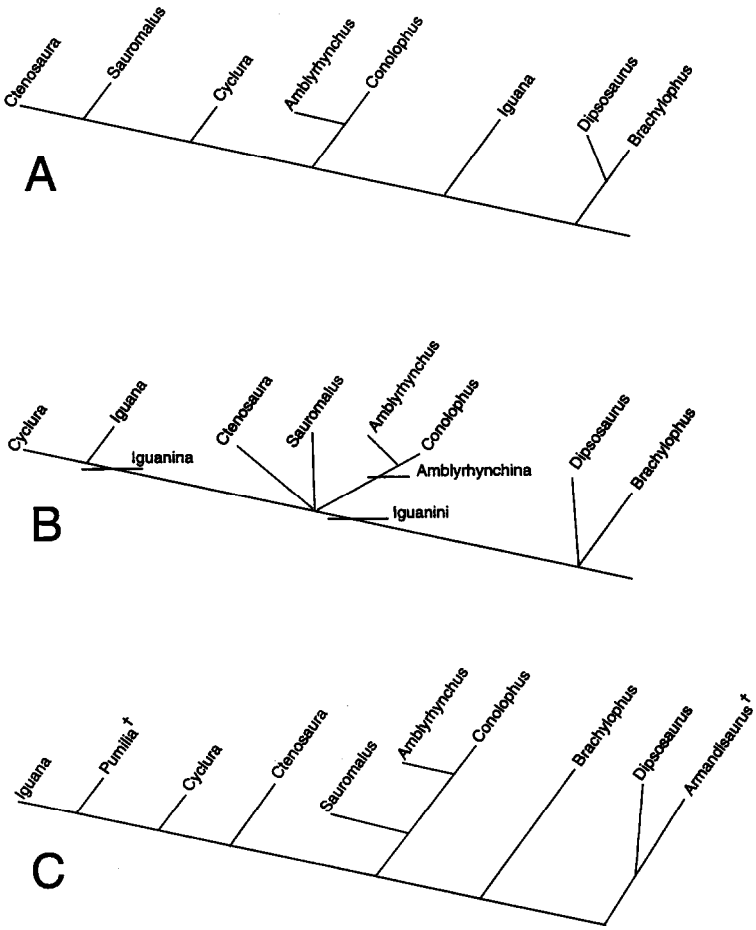

FIG. 1.—**Previously** proposed hypotheses of relationships among genera of the lizard family Iguanidae. A, Phylogeny proposed by Avery and Tanner (1971) on the basis of vaguely defined general similarities. ***B***, Phylogeny derived from the character-based cladistic analysis of de Queiroz (1987*a*).*C*, Phylogeny based on a reanalysis of the de Queiroz data set but with the inclusion of the fossil genera ***Armandisaurus*** and ***Pumilia*** (Norell and de Queiroz 1991).

eri) or collected from natural populations, and in most cases they were killed and prepared as standard museum voucher specimens. Selected tissues, including heart, liver, kidney, skeletal muscle, stomach-duodenum, and whole blood, were removed and frozen in the field in liquid nitrogen. In a few instances animals were not sacrificed, because they either were part of captive breeding colonies (both species of ***Cyclura***) or represented highly endangered natural populations (***Sauromalus varius***) from which individuals could not be permanently removed. In these cases, a small sample of blood was drawn from the caudal vein, and this served as a source of DNA. Taxonomic sampling within genera was usually opportunistic-species used were those legally available to us, although we sampled three of the four recognized species groups within ***Ctenosaura*** (L. Buckley, personal communication).

Laboratory Procedures

DNA was extracted by powdering approximately 100 μg of tissue in liquid nitrogen using a prechilled mortar and pestle. The powder was suspended in 500 μl of STE (0.1 M NaCl, 0.05 M Tris, 0.001 M EDTA; pH 7.5) and lysed by incubation in 20% SDS and 250 μg Proteinase K for 2 h at 55°C. Samples were then phenol-extracted and ethanol-precipitated (Sambrook, Fritsch, and Maniatis 1989), resuspended in 500 μl of water, and run on agarose gels to determine DNA concentrations.

Approximately 0.01 μg of DNA was used to amplify a 959-bp region of the mtDNA genome (beginning at position 11165 of the aligned bovine sequence of Anderson et al. 1982) with the ND4 and LEU primers originally described by Arévalo, Davis, and Sites (1994). Polymerase chain reaction (PCR) was performed using the following parameters: 93°C denaturation (30 s), 50°C annealing (30 s), and then ramping for 4 min into a 72°C extension (1 min) for 35 cycles. Double-stranded PCR products were ligated into the lambda Zap11 vector (Stratagene Cloning Systems), and all target DNAs were subcloned into pBluescript and sequenced by the method of Kraft et al. (1988) with various combinations of primers (table 2). For each clone, both strands of the target fragment were sequenced in almost all cases, and where this was not accomplished (***Oplurus***), the same strands were sequenced at least twice using primers with extensive overlap. Because ***Tuq*** polymerase results in observable mutation errors (Saiki et al. 1988; Tindall and Kunkel 1988; Keohavong and Thilly 1989), two

Table 1
Species, Museum Voucher Numbers (Where Relevant), and Geographic Sources of the Species Used in this Study

Species	Museum Accession Number	Approximate Locality
Phrynosomatidae		
Phrynosoma texanum[a]	—	Texas, U.S.A.
Sceloporus grammicus	BYU-38487	D.F., Mexico
Hoplocercidae		
Enyalioides laticeps	KU-212627	Amazonian Ecuador
Opluridae		
Oplurus cuvieri	"JWS-2905"	Madagascar
Iguanidae		
Amblyrhynchus cristatus	UNM-52884	Galapagos Islands, Ecuador
Brachylophus fasciatus	CAS-172524	Fiji
Conolophus subcristatus	UNM-52885	Galapagos Islands, Ecuador
Ctenosaura hemilopha	BYU-39709	Sonora, Mexico
Ctenosaura palearis	BYU-34667	Honduras
Ctenosaura similis	BYU-39457	Honduras
Cyclura n. nubila[b]	"890315"	Guantanamo Bay, Cuba
Cyclura ricordi[c]	"A2"	Hispaniola
Dipsosaurus dorsalis	BYU-39438	California, U.S.A.
Iguana delicatissima	BYU-43023	Dominica, Lesser Antilles
Iguana iguana	MPM-26176	St. Vincent, Lesser Antilles
Sauromalus obesus	BYU-39436	Arizona, U.S.A.
Sauromalus varius	"E603"	Isla San Esteban, Mexico

[a] Blood sample taken from animal caught and released in a natural population.
[b] Blood sample loaned from captive animal at San Diego Zoo.
[c] Blood sample loaned from captive animal at Indianapolis Zoo.

clones were sequenced per species, and three in one case in which there was a difference between clones. In this case (Iguana iguana), two clones differed at only a single base pair, and the third clone permitted a consensus sequence to be used. For ***Enyalioides laticeps,*** the amplified product was cut from a low-melting-point agarose gel (NuSieve 3:1; FMC Bioproducts, Rockland, Maine), and purified with GeneClean following the protocol descibed in the GeneClean manual (Bio 101, La Jolla, Calif.). Purified DNA was then ethanol precipitated and used directly for sequencing with a Sequenase version 2.0 kit (USB, Cleveland, Ohio).

Data Analysis

DNA sequences were input into the MacVector program (IBI-Kodak, version 3.5, 1991) and aligned against the bovine mtDNA sequence (Anderson et al. 1982), and also with sequences from lizards of the genus ***Sceloporus*** (Arévalo, Davis, and Sites 1994), using the CLUSTAL-V program (Higgins and Sharp 1989). The tRNA alignments were confirmed by alignment with conserved motifs in a large database compiled by M. Lynch (University of Oregon). Basic statistical summaries (base composition, numbers of transitions, transversions, etc.) of the sequences were computed with

Table 2
List of PCR and Sequencing Primers Used in this Study

Primer Label	Reference Positions	Sequence	Target Species
ND4	11165-11196	CACCTATGACTACCAAAAGCTCATGTAGAAGC	All taxa
MOR50	11235-11261	ATAATTCCATAGCCCCCTAG	*Enyalioides*
ND4Rev2	11384-11363	TTAATGATTTTAGATCTGTTTG	All taxa
IG-ND4#2	11329-11348	GAGGCATCGTAATAACCAGC	Most taxa
ND4#2	11358-l 1379	TACGACAAACAGACCTAAAATC	Most taxa
MOR225	11394-11413	TCCTCTTCAAGTCATATGGG	*Enyalioides*
MOR475dn	11649-l 1668	CATCAATCTTCTAGGAGAGC	*Enyalioides*
MOR475up	11668-l 1649	GCTCTCCTAGAAGATTGATG	*Enyalioides*
ND4Rev	11845-11828	TATTAGGAGATGTTCTCG	Almost all taxa
IG-His	11933-11913	TAATGTTTTTGTTAAACTAT	Most taxa
MOR850	11992-12013	GAACACCAAGAACTGCTAAT	*Enyalioides*
His	12002-l 1984	CACTGCCTAATGTTTTTGT	Almost all taxa
MOR800	12015-l 1999	GAGAATTAGCAGTTCTTGG	*Enyalioides*
LEU	12111-12086	CATTACTTTTTACTTGGATTTGCACCA	All taxa

Nom.-Reference positions are to the bovine sequence (Anderson et al. 1982), and primer sequences are given left-to-right from the 5' to 3' ends.

MEGA (version 1 .01; Kumar, Tamura, and Nei 1993). For phylogenetic analyses, each nucleotide was treated as an unordered character with four alternative states, while morphological characters were unordered and most were binary (12 were multistate). In the original studies (see descriptions in de Queiroz 1987*a* and modifications in Norell and de Queiroz 1991) a few characters were ordered, but our preliminary analyses showed no differences in tree topologies when these characters were treated first as ordered, and then as unordered. We therefore use the data matrix exactly as presented in the appendix of Norell and de Queiroz (1991), and treat all 90 characters as unordered.

Characters were polarized by using representatives of the families Hoplocercidae (*Enyalioides biceps),* Phrynosomatidae *(Phrynosoma texanum* and *Sceloporus grammicus;* sequences only), and Opluridae (*Oplurus cuvieri;* table 1) as alternative single outgroups (Donoghue and Cantino 1984). The deep cladistic structure of Iguanian lizards is poorly resolved (Frost and Etheridge 1989), and it is not possible at present to identify the sister group of the Iguanidae with any confidence. For this reason, the first round of analysis included all three alternative outgroups, and Phrynosomatidae was used to root trees both to evaluate the relationships of Hoplocercidae and Opluridae to the Iguanidae (all of these are complete for morphological and molecular characters) and to permit the basal taxa within Iguanidae to "float" as a test for monophyly of the ingroup (Nixon and Carpenter 1993).

PAUP software (version 3.1; Swofford 1993) was used for the phylogenetic analyses, with minimum-length trees derived by branch-and-bound searches performed for all combinations of analyses for the entire data set (sequences plus morphological data). This search strategy was then repeated separately for the protein sequences (742 bp of ND4, including the 26-nt primer sequences identical for all taxa), the three tRNA genes combined (histidine, serine, and leucine; 217 bp total, including 26 identical primer sequences), and the 90 morphological characters. Initial searches were based on equal weighting of all characters, and the strength of phylogenetic signal was evaluated by the gl test for skewness (Hillis and Huelsenbeck 1992). Support for internal nodes was assessed by use of both a "support index" (SI; Bremer 1988) and, for selected analyses, a branch-and-bound bootstrap (Felsenstein 1985) with 1,000 replicates. The parsimony algorithm is preferred in this study because it provides the strongest criterion by which we can evaluate the influence of different subsets of characters on the final estimate of phylogeny, and it generally performs as well as alternatives (i.e., maximum likelihood, neighbor-joining, etc.) in recovering phylogenetic history under realistic conditions (Hillis, Huelsenbeck, and Cunningham 1994). The strength of the best supported hypothesis is evaluated by comparing its length to the lengths of alternative trees constrained to match the topologies shown in figure 1*A* and *C,* and testing for statistical significance using the nonparametric ranked-sign test suggested by Templeton (1983).

Results

MtDNA Sequence Variation in Iguanas

The entire sequence of the light strand of the mtDNA ND4-Leucine fragment and the morphological character matrix of Norell and de Queiroz (1991), aligned across all ingroup and outgroup taxa examined in this study, are available from the first author upon request. Sequences are available in GenBank under accession numbers U66224–U66239. A total of 742 new ND4 bases (247 codons, including the ND4 primer) are resolved, and table 3 summarizes base composition and transition/transversion (TI/TV) ratios at different levels of divergence for the ND4 coding region. Base composition was AC-biased across all positions (mean across all taxa = 32.1% for A and 33.0% for C), and also across all first (means = 37.9% and 28.7% for A and C, respectively) and all third base positions (means = 42.9% and 40.3% for A and C, respectively). The second position was characterized by a TC bias (41.3% and 30.0%, respectively), which is a common pattern in animal mitochondrial DNA (Naylor, Collins, and Brown 1995).

The transition/transversion (TI/TV) ratios are 0.97 and 1.36 at the first and second positions when averaged across all taxa, and shift toward a higher proportion of transitions when counted for ingroup taxa only (1.34 and 1.87, respectively), or within genera only (2.71 and 4.75, respectively). The first position may be saturated for transitions across all taxa, and the second position may approach saturation. This appears not to be the case among ingroup taxa, although the third position here saturates with transitions (TI/TV = 1.01). When considering only intrageneric variation in ND4 sequences, the majority of phylogenetically informative characters are third base transitions (TI/TV = 5.36, table 3).

Table 3 also shows base composition for different regions of the combined tRNA sequences, averaged across all taxa. The tRNAs are AT-biased when base composition is averaged across all positions, or summed over complementary strands of the accepting stems only, all other stems, or all loops. The loops are more AT-rich (39.7% and 28.4%, respectively) than the accepting stems (34.2% and 27.7%, respectively), which are slightly more AT-rich than the other stems (26.5% and 27.5%, respectively). When the tRNA genes are considered separately, the total number of changes is highest in histidine and lowest in leucine. In all three sequences, transversions are uncommon to absent in either the accepting stems or the other stems, but they are equal to (in serine) or exceed (histidine and leucine) the number of transitions in the loops.

Outgroup Structure and Monophyly of the Iguanidae

Figure 2 depicts trees obtained from both maximum-parsimony and neighbor-joining searches carried out for the DNA sequences only, across all ingroup and outgroup taxa, with the Phrynosomatidae being used to polarize all characters. The maximum-parsimony search recovers a monophyletic Iguanidae, albeit with only modest bootstrap (based on 2,000 replications) and Bre-

Table 3
Summary of Base Composition (Mean and Range) and Transition/Transversion Ratios (TI/TV) Averaged Across All Taxa

	ALL POSITIONS				FIRST				SECOND				THIRD			
ND4:	A	T	C	G	A	T	C	G	A	T	C	G	A	T	C	G
Mean.. .	32.1	23.8	33.0	11.1	31.9	16.8	28.7	16.5	15.5	41.3	30.0	13.3	42.9	13.3	40.3	3.5
Low....	29.2	21.2	27.7	10.0	35.0	14.3	25.7	15.2	14.8	39.7	24.7	12.7	35.6	7.2	24.9	0.0
High ..	34.5	29.0	36.3	12.4	39.7	21.1	31.2	19.0	16.5	44.3	31.2	14.0	51.5	25.8	49.8	7.2

	ACROSS ALL TAXA			INGROUP TAXA ONLY			WITHIN GENERA[a]		
	First	Second	Third	First	Second	Third	First	Second	Third
TI	60	30	186	59	28	167	38	19	177
TV. . . .	62	22	260	44	15	165	14	4	3
TI/TV	0.97	1.36	0.72	1.34	1.87	1.01	2.71	4.75	5.36

	ALL POSITIONS				ACCEPTING STEMS				ALL OTHER STEMS				ALL LOOPS			
tRNAs[b]:	A	T	C	G	A	T	C	G	A	T	C	G	A	T	C	G
Mean. ..	32.3	28.6	20.0	18.9	34.2	27.7	19.7	18.4	26.5	27.5	21.0	21.9	39.7	28.4	16.5	14.9
Low....	27.9	27.9	16.4	16.8	27.5	25.0	13.2	13.2	25.3	28.4	20.4	19.5	30.3	24.1	10.2	11.5
High ...	35.5	30.1	24.2	21.1	39.5	34.2	27.5	21.0	29.5	31.0	24.1	23.0	44.1	35.7	24.1	20.0

	HISTIDINE			SERINE			LEUCINE		
	Acc. Stem	Other Stems	Loops	Acc. Stem	Other Stems	Loops	Acc. Stem	Other Stems	Loops
TI	8	9	7	6	13	4	0	2	5
TV.	1	0	10	0	3	4	0	0	8
TI/TV	8.00	—	0.70	—	4.33	1.00	—	—	0.63

[a] Combined for averages of *Ctenosaura, Cyclura, Iguana,* and *Sauromalus.*
[b] Combined for all three genes.

mer support (71% and 3, respectively), and, with very weak support, groups the families Hoplocercidae and Opluridae as sister taxa (bootstrap proportion of <50%, Bremer index = 1; fig. 2A). This arrangement contradicts hypotheses derived from morphological data (Frost and Etheridge 1989), and because some distance-based/neighbor-joining algorithms correct for multiple hits (Saitou and Imanishi 1989; Rzhetsky and Nei 1992), we performed a neighbor-joining search (Saitou and Nei 1987) over pairwise distances estimated from Kimura two-parameter and Tamura-Nei models (Kimura 1980; Tamura and Nei 1993; data not presented). The single

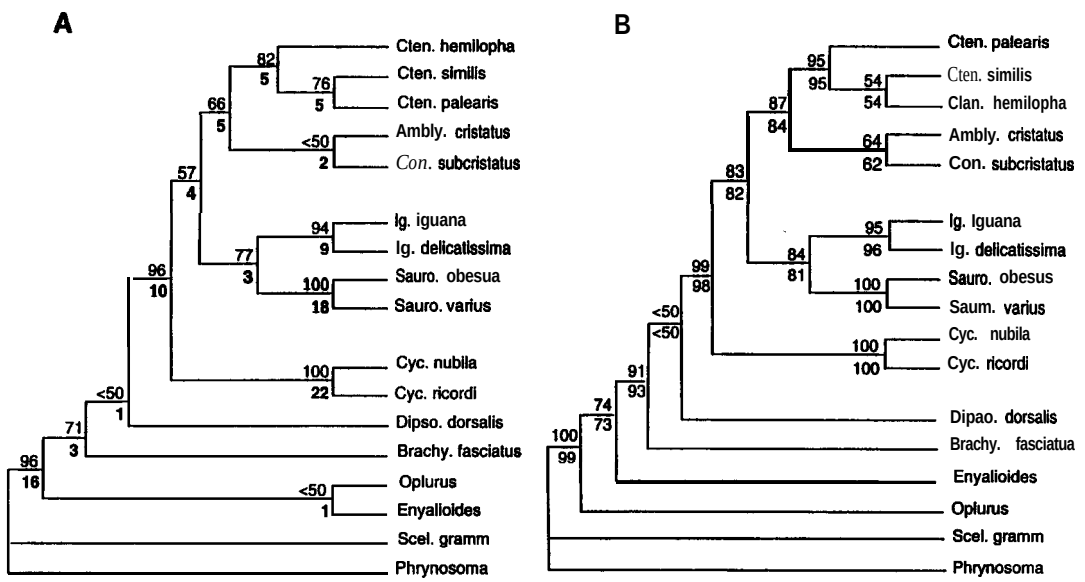

FIG. 2.—Maximum-parsimony tree *(A)* and neighbor-joining tree (B) obtained from MEGA searches of sequence dam sets for all ingroup and outgroup taxa combined. *A* represents the single shortest tree obtained for all taxa when rooted to the Phrynosomatidae, with tree length (TL), consistency index (CI, exluding uninformative characters), and retention index (RI) values of 1,364, 0.462, and 0.403, respectively. Support for the internal nodes is given by the branch-and-bound bootstrap proportion (above branch) and the Bremer support index (below). *B* represents the neighbor-joining tree obtained from the same data set, but the support indexes in this case are bootstrap proportions of Kimura two-parameter and Tamura-Nei distances, above and below each branch, respectively (see text).

tree obtained from these searches (fig. 2*B*) again recovers the Iguanidae as monophyletic over both Kimura distances, with bootstrap proportions of 91% and 93%, respectively. The outgroup Opluridae is placed basal to all other taxa (bootstrap proportions are >99% for both distances), and the Hoplocercidae is recovered as the first outgroup to the Iguanidae with bootstrap proportions of 74% and 73%, respectively. In subsequent analyses, we use Hoplocercidae and Opluridae alternatively as first and second outgroups, but we emphasize that because we have not sampled other taxa of possible relevance to the issue of which family is the sister taxon of the Iguanidae, our "first" and "second" outgroups are used in a relative context. Our results are consistent with the morphological data supporting a monophyletic Iguanidae (Frost and Etheridge 1989).

Taxonomic Congruence Among Disparate Data Sets

Figure 3 presents minimum-length trees produced by separate parsimony analyses for ND4 sequences (*A* and B), tRNA sequences (C and D), and the morphological data matrix (E and *F*). For reasons given above, all analyses are replicated using ***Enyalioides*** and then ***Oplurus*** as alternative outgroups. Trees obtained from ND4 sequences are characterized by significant phylogenetic signal ($g1 = -0.801$ [***Enyalioides***] and -0.832 [***Oplurus***], $P \ll 0.01$ in both), and show exactly the same topologies but with slightly different lengths (tree length [TL] = 731 [***Enyulioides***] and 735 [***Oplurus***]). All genera represented by two or more species (***Ctenosaura, Cyclura, Iguana,*** and ***Sauromalus) are*** recovered as monophyletic groups, and the two Galapagos genera (***Amblyrhynchus*** and ***Conolophus) are*** recovered as sister taxa (the Amblyrhynchina of de Queiroz 1987*a*). Both trees show strong support (Bremer index = 10+) for the Iguanini clade (fig. 1*B*) but with the Caribbean genus *Cyclura* as the sister taxon of the clade ((***Sauromalus*** + ***Iguana)*** + ***((Amblyrhynchus*** + ***Conolophus)*** + ***Ctenosaw-u)). The*** Fijian genus ***Bruchylophus*** is the sister group of this clade, and the desert iguana Dipsosaurus ***dorsalis*** is the basal member of the family. With the exception of these basal taxa (which are reversed), this topology is identical to those obtained in the more global searches described above (fig. 2A and B).

When the Phrynosomatidae is used as the outgroup (monophyletic relative to the ingroup; see Frost and Etheridge 1989), a single tree is recovered with a topology identical to those depicted in figure 3A and *B.* Phylogenetic signal is also strong in this data set ($g1 = -0.945$, $P \ll 0.01$), but the tree is much longer (TL = 841, consistency index [CI] = 0.508, and rescaled consistency index [RC] = 0.222; tree not shown).

Considerably less structure was evident in the tRNA sequences (fig. 3C and *D*), even though significant signal is also present in this subset of characters. Rooting to ***Enyalioides*** produces a single fully resolved tree of 73 steps ($g1 = -0.599$, $P < 0.01$), but this topology collapses with a single additional step (72 equally parsimonious trees are recovered at 74 steps), and the only structure retained includes (1) the monophyly of ***Sauromalus, (2)*** a ***(Bruchylophus*** + ***Dipsosaurus)*** clade; and (3) a ***(Conolophus (Ctenosaura similis (Ctenosaura hemilopha*** + ***Ctenosaura palueris)))*** clade. All three of these internal nodes collapse at 75 steps (1,043 trees). Substitution of ***Oplurus*** as the outgroup reveals approximately equal signal ($g1 = -0.669$, $P < 0.01$), but almost no resolution at the shortest tree length. Figure 3*D* shows a strict concensus of 118 equally parsimonious trees at 69 steps, and the two clades recovered [monophyletic ***Sauromalus*** and ***(Brachylophus*** + ***Dipsosaurus)]*** both collapse with two additional steps. Similar results were obtained when the Phrynosomatidae was used as the outgroup; despite strong signal ($g1 = -1.203$, $P \ll 0.01$), there was little resolution at the shortest tree length (119 trees at 87 steps, CI = 0.598, RC = 0.326; consensus tree not shown). These results all include the four informative gaps in the tRNA sequences, but analyses without these characters gave identical tree topologies. For this reason, all subsequent analyses include the four tRNA gap characters.

Morphological characters provided greater resolution than tRNA sequences, but less resolution than the ND4 sequences (fig. 3E and *F*). Rooting to ***Enyulioides*** recovered five trees with lengths of 104 steps, and statistically significant phylogenetic signal ($g1 = -1.913$, $P \ll 0.01$), and clearly separates the same basal taxa identified by the ND4 sequences (***Bruchylophus*** and ***Dipsosaurus***) from all others (Bremer index = 3, fig. 3*E*). ***Dipsosaurus*** is grouped with the fossil form ***Armandisaurus,*** in agreement with Norell and de Queiroz (1991), and this clade is basal to the ***(Bruchylophus*** + all other iguanas) clade. The unresolved polytomy nested within the basal groups again recovers all genera as monophyletic, and, in contrast to the tree derived from ND4 sequences, resolves the Galapagos genera as the sister clade of ***Sauromalus,*** albeit with weak support (Bremer index = 1). Results were similar when ***Oplurus*** was used as the outgroup; phylogenetic signal was strong ($g1 = -1.850$, $P \ll 0.01$), but 15 trees were recovered with a length of 104 steps. The topology of the consensus tree is identical to that produced from rooting to ***Enyalioides,*** except that the basal taxa ***Armandisaurus, Brachylophus,*** and ***Dipsosaurus are*** not resolved (fig. 3*F*). The absence of complete resolution in our analyses of morphological data relative to the single tree obtained by Norell and de Queiroz (1991) likely results from our outgroup substitution approach to character polarization, which did not allow for ambiguity. Norell and de Queiroz (1991) used as an outgroup a hypothetical ancestor constructed from the polarity decisions used by de Queiroz (1987*a*), which were based on the use of basiliscines, crotaphytines, morunasaurs, and oplurines as simultaneous outgroups. They found that 20 characters varied either within or among these to such an extent that polarity was considered indeterminate (see de Queiroz 1987*a* for details).

In summary, all data sets contain significant (tRNA sequences) to highly significant (ND4 sequences and the morphological characters) phylogenetic signal, and the ND4 sequences produce identical completely resolved trees with all three alternative outgroups. The tRNA genes have only a few informative characters (table 3),

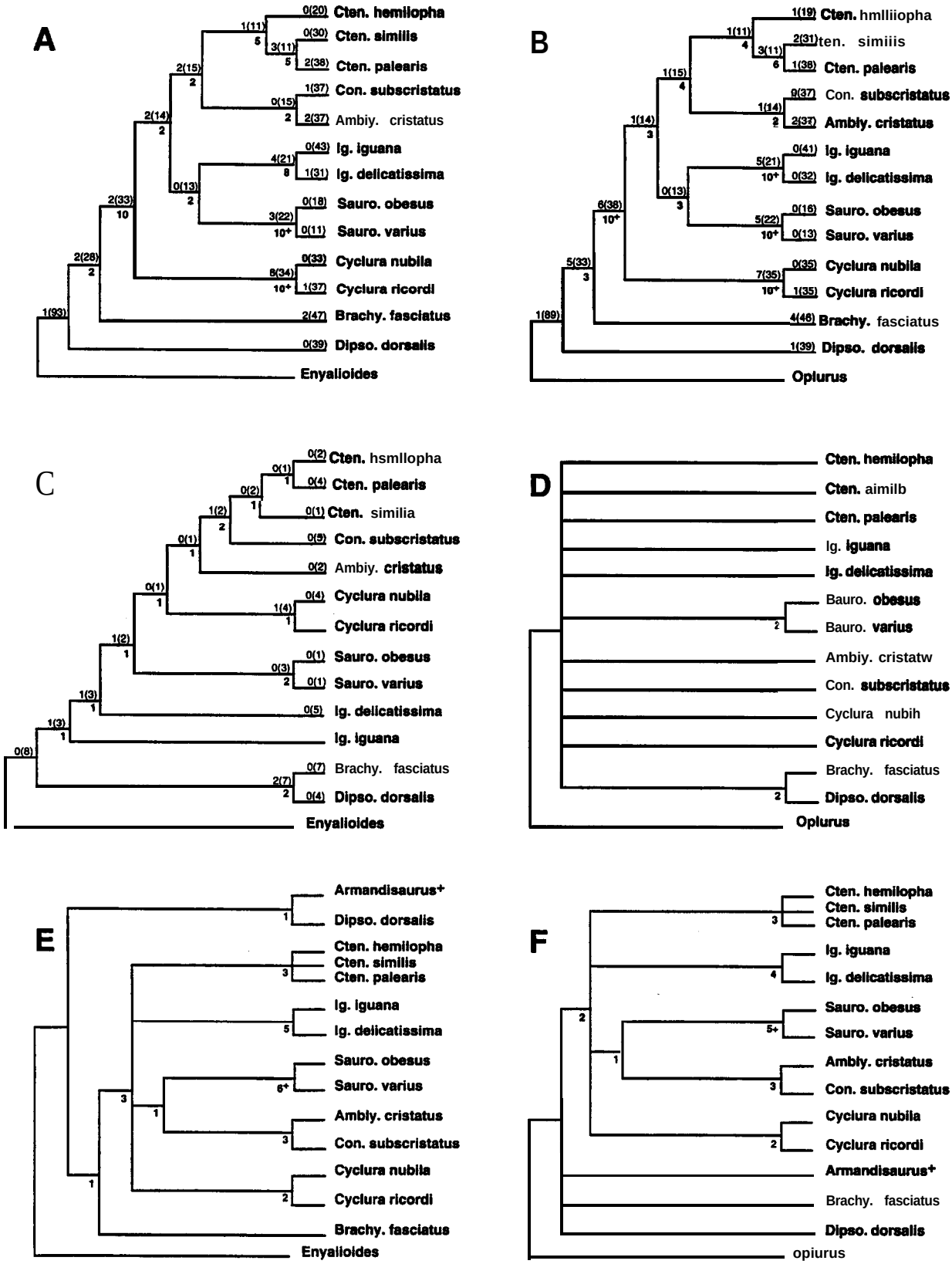

FIG. 3.-Maximum-parsimony trees obtained from PAUP branch-and-bound searches (with ACCTRAN character state optimizations) for each of the three data sets used in this study, and rooted to the alternative outgroups. *A* and *B* represent the single shortest trees for mtDNA ND4 sequences, with TL, CI, and RI of 731, 0.509, and 0.402 (*Enyalioides*), and 735, 0.524, and 0.419 (*Oplurus*), respectively. Equivalent values for the remaining trees are: C and D-results for tRNA sequences (three genes combined, including gap characters); TL = 73, CI = 0.548, and RI = 0.431 (*Enyalioides*); and TL = 69, CI = 0.565, and RI = 0.483 (*Oplurus*—strict consensus of 118 trees); *E* and F-results for morphological characters; TL = 104, CI = 0.731, and RI = 0.748 (*Enyalioides*—strict consensus of 5 trees); and TL = 104, CI = 0.721,

Table 4
Summary of Wilcoxon Ranked Sign Tests for All Paired Combinations **of Tree Topologies Derived for Each of the Three Data Sets**

Outgroup	Tree 1		Tree 2	n[a]	Ts[b]	P[c]
Enyalioides	*ND4*	*vs.*	morphology	27	81	<0.01
	morphology	vs.	ND4	6	6	NS
	ND4	vs.	tRNAs	49	589	NS
	tRNAs	vs.	ND4	10	10	NS
	tRNAs	vs.	morphology	4	8	NS
	morphology	vs.	tRNAs	21	8.5	<0.01
Oplurus	ND4	vs.	morphology	18	36	<0.05
	morphology	vs.	ND4	6	6	NS

[a] Number of characters that undergo different numbers of changes on the two trees being compared.

[b] The test statistic.

[c] The two-tailed probability levels obtained from table 30 in Rohlf and Sokal (1981).

and produce either very weakly resolved trees or multiple alternatives (fig. *3C* and *D,* respectively). These genes appear not be be saturated, however (table 3), and are perhaps not properly matched to the levels of divergence being addressed within the Iguanidae. The morphological characters provide more resolution than the tRNA genes, but less than the ND4 sequences, and show substantially different placement of selected genera. However, the incongruence among the three different tree topologies, and especially between those based on ND4 sequences and morphological characters, does not by itself mean that these data sets are in significant conflict. Visual inspection of the topologies alone does not permit a rigorous evaluation of the possibility that different data sets are actually estimating the same genealogy within the limits of sampling error. Here we use the nonparametric method proposed by Templeton (1983; Wilcoxon ranked sign test) to test whether the most parsimonious topology(ies) obtained from each data set constitute suboptimal topologies for the other data sets.

Table 4 summarizes results of all pairwise Wilcoxon tests carried out to evaluate the alternative topologies depicted in figure 3. In all comparisons, "Tree 1" is the most parsimonious topology for the data set in that column and "Tree 2" is the alternative (constrained) against which the most parsimonious tree is being compared. Note that, because of the poor resolution for the tRNA sequences when rooted to *Oplurus,* four combinations of tests were not carried out for this outgroup. Results show that, for both outgroups, the tree topologies generated from the ND4 sequences are significantly more parsimonious than those produced from the morphological data ($P < 0.01$ and $P < 0.05$ for *Enyalioides* and *Oplurus,* respectively). In contrast, the most parsimonious trees produced from the morphological data set are not significantly better than those generated from the ND4 sequences for either outgroup. In other words, the morphological tree topologies are suboptimal (i.e., significantly less parsimonious) for the ND4 sequences, but the reverse is not true. Nevertheless, these data sets are judged to contain significant conflicts with respect to at least part of the phylogenetic topologies being compared (they display taxonomic incongruence). Similarly, there is also significant conflict between the most parsimonious tree derived from morphological characters, when *Enyulioides* is used as the outgroup, relative to that derived from the tRNA sequences ($P < 0.01$). In contrast, the two molecular tree topologies (ND4 vs. tRNA sequences) are not in significant conflict with each other (table 4).

Character Congruence in the Combined Data Set

In this round of analyses, all three data sets were combined, each character was treated as an independent unit of phylogenetic information, and all characters were weighted equally. We did not use the Phrynosomatidae in this analysis because of the large number of missing characters (i.e., the morphological matrix, fig. 2). Figure 4 depicts minimum-length trees derived from parsimony analyses of the combined data sets and rooted to the two alternative outgroups for which complete molecular and morphological data sets were available. In both cases, strong phylogenetic signal was present in the total data set (g1 = − 1.168, $P \ll 0.01$ for *Enyalioides;* gl = − 1.176, $P \ll 0.01$ for *Oplurus),* and topologies were identical and of similar length (TL = 913 for *Enyalioides* and 915 for *Oplurus).* Two trees were generated from each analysis and in both cases the alternatives switched the basal positions between *Brachylophus* and *Dipsosuurus.* Internal to these basal taxa, both tree topologies matched those produced from analysis of the ND4 sequences alone (fig. 3A and B). This suggests that the inclusion of morphological and tRNA sequence data may have obscured signal present in the ND4 gene, which was sufficient to resolve the basal positions of *Brachylophus* and *Dipsosuurus.* However, given the likelihood of third-position saturation in the ND4 sequence (table 3), which would be most serious at these basal nodes, we treat this issue in greater detail in the *Discussion.*

We evaluated heterogeneity among data sets by first combining the ND4 and tRNA sequences (these were judged not to be in significant conflict with each other; see fig. 3 and table 4), and then testing the relative levels of homoplasy in the molecular vs. the morphological data sets (which do show conflict; fig. 3 and table 4). Table 5 summarizes a contingency χ^2 test for these two character classes, for each outgroup, respectively. Incongruence is evaluated for individual characters against

←

and RI = 0.741 (*Oplurus*—strict consensus of 15 trees). In these searches, all characters were equally weighted, and support for the internal nodes in each tree is summarized as follows: numbers above the branches represent the total number of synapomorphies (in parentheses) and the total number of unambiguous synapomorphies (changes appearing only once on the tree) for each branch; the bold number below each line is the Bremer index for each.

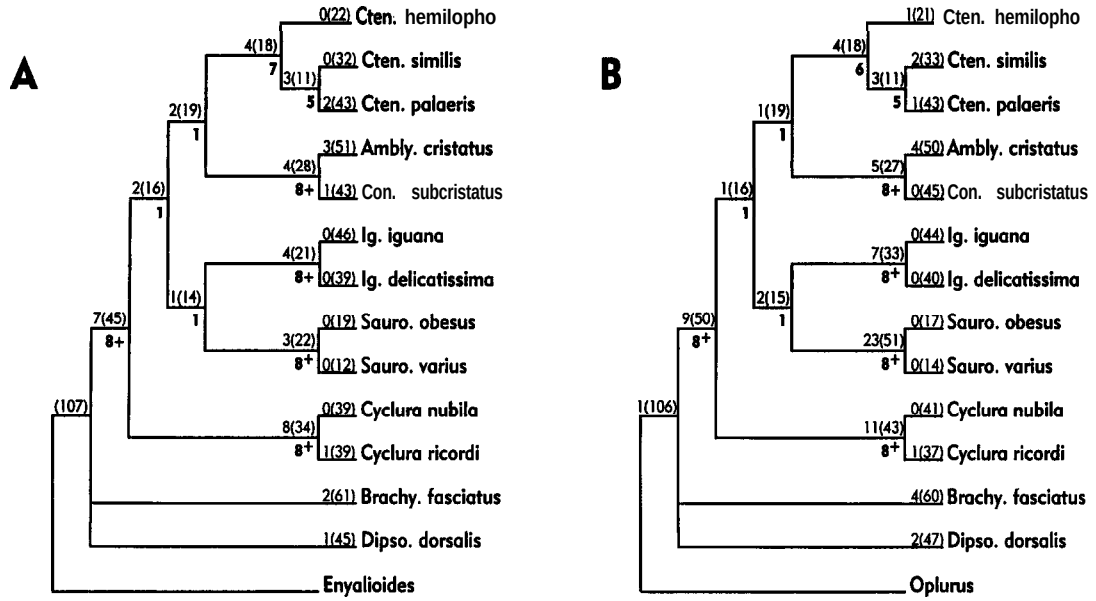

FIG. 4.-Maximum-parsimony trees obtained from PAUP branch-and-bound searches (under ACCTRAN character state optimization) for all three data sets combined. *A* and *B* represent the strict consensus of the two shortest trees recovered when *Enyalioides* or Oplurus were each used as outgroups, respectively. For each of these, the TL, CI, and RI are: 913, 0.529, and 0.441 *(Enyalioides);* and 915, 0.541, and 0.451 (*Oplurus*), respectively. Numbers on branches and at nodes are defined as in figure 3.

the topologies generated from the combined data sets (fig. 4), in the manner described by Larson (1994). All phylogenetically informative characters are included and categorized according to observed levels of homoplasy.

For both outgroups, there is significant heterogeneity between the molecular and morphological characters with respect to levels of homoplasy ($P \ll 0.01$ in both; table 5). In the case of *Enyalioides,* for example, the most common of the 258 phylogenetically informative molecular characters are those with one (n = 118; 45.7%), two (n = 63; 24.4%), or three-plus (n = 47; 18.2%) homoplasies, while consistent characters (i.e., those with a ci = 1.0) were the least common (n = 30; 11.6%). In contrast, the majority of the 58 informative morphological characters were either consistent (n = 34; 58.6%) or showed one homoplasy (n = 20; 34.5%), while characters with two (n = 3; 5.2%) or three-plus (n = 1; 1.7%) homoplasies were uncommon to rare (table 5). Patterns were similar for trees rooted to *Oplurus.*

We corrected for significant heterogeneity between data sets by sequentially deleting the groups of homoplastic characters. Figure 5 summarizes analyses in which successive groups of characters were deleted, beginning with the most homoplastic and sequentially deleting the less homoplastic character sets (table 5). Because all previous analyses have produced identical topologies when outgroups were varied, we confined this

Table 5
Contingency Table Evaluating Levels of Homoplasy in Morphological Versus Molecular Characters for the Topologies Favored by Parsimony Analyses of the Combined Data Sets (Fig. 4*A* and *B*)

	Consistent O[a] (E[b])	1 Homoplasy O (E)	2 Homoplasies O (E)	3+ Homoplasies O (E)	Total
A. Oplurus					
Sequences	35 (52.3)	117 (116.2)	62 (53.9)	43 (36.5)	259
Morphology	26 (10.7)	23 (23.8)	3 (11.0)	1 (7.5)	53
Total	63	140	65	44	312
χ^2 = 16.819, $P \ll 0.01$ 0.01[3]					
B. Enyalioides					
Sequences	30 (5 1.9)	118 (112.0)	63 (53.5)	47 (38.9)	258
Morphology	34 (11.7)	20 (25.2)	3 (12.0)	1 (8.8)	58
Total	64	138	66	48	318
χ^2 = 16.812, $P \ll 0.01$ 0.01[3]					

[a] O = observed
[b] E = expected.

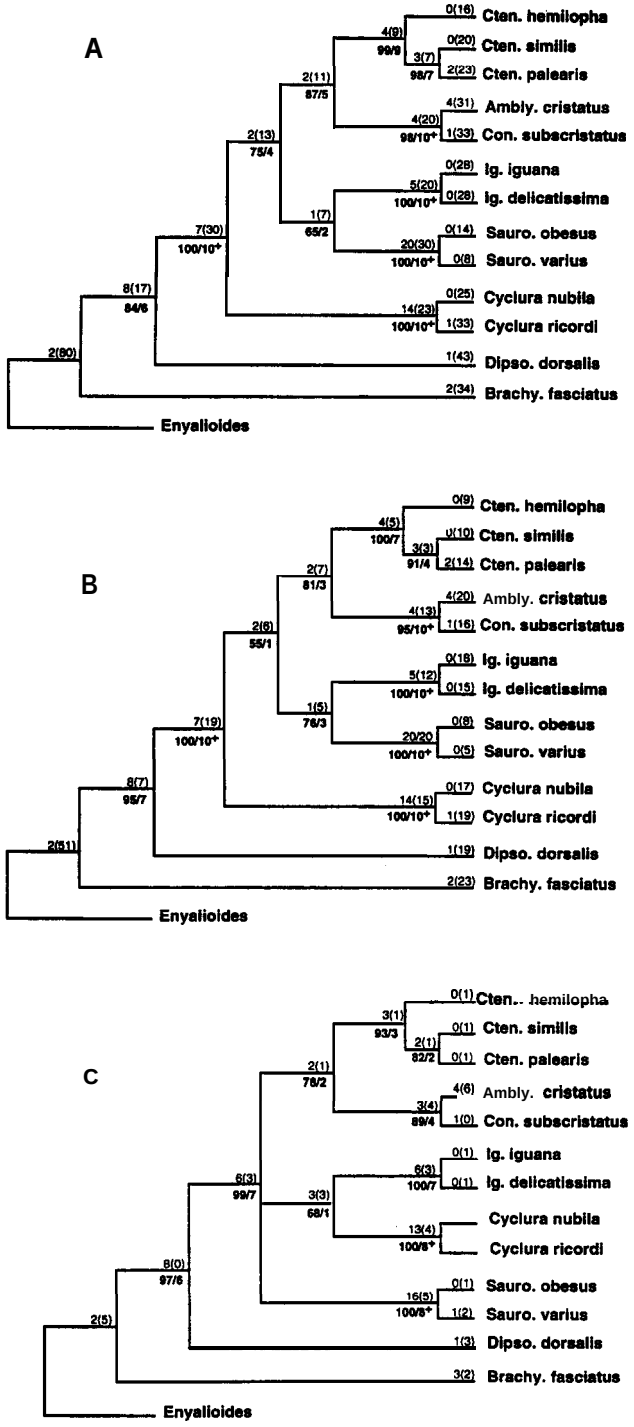

FIG. S.-Maximum-parsimony trees obtained from PAUP branch-and-bound searches (under ACCTRAN optimization) in subsets of the combined data set, all rooted to *Enyalioides (see* text). ***A,*** Single most parsimonious tree obtained from the matrix from which all characters with three or more homoplasies are deleted (TL = 685, CI = 0.591, RI = 0.506). ***B,*** Single most parsimonious tree obtained from matrix from which all characters with two and three-plus homoplasies are deleted (TL = 445, CI = 0.652, RI = 0.582). ***C,*** Consensus of two most parsimonious trees obtained from the matrix of consistent characters only (TL = 128, CI = 0.797, RI = 0.798). Numbers above branches are defined as in figure 3, while bold numbers below are the bootstrap proportions (1,000 branch-and-bound replicates) and support indexes, to the left and right of the slanted lines, respectively.

next round of analysis to the use of ***Enyalioides*** alone. This is justified because ***Enyalioides*** is the "first" outgroup to the Iguanidae (fig. 2); tree lengths are shorter when ***Enyalioides*** is the outgroup, relative to ***Oplurus*** and the Phrynosomatidae, and there is substantially better ingroup resolution with the tRNA sequences for ***Enyalioides*** (compare fig. 3C and ***D***).

When only the most homoplastic characters are deleted from the analysis (the "3+ homoplasies" column in table 5) a single tree is recovered (fig. 5*A*) with a topology similar to that recovered from either the ND4 sequences alone (fig. 3A) or the complete data set (fig. 4A). The exception is the placement of ***Brachylophus*** and ***Dipsosaurus.*** Phylogenetic signal is strong ($g1 = -1.234$, $P \ll 0.01$), and support for almost all interior nodes appears to be strong, whether estimated by bootstrap values, Bremer indexes, or overall patterns of character congruence. All genera are very strongly supported as monophyletic, as is the clade Amblyrhynchina (***Amblyrhynchus*** + ***Conolophus),*** the sister group relationship ***(Ctenosaura*** + Amblyrhynchina), the sister group position of ***Cyclura*** to ***((Zguana*** + ***Sauromalus)*** + ***(Ctenosaura*** + Amblyrhynchina)) in the Iguanini, and the basal placement of ***Dipsosaurus*** internal to ***Brachylophus,*** which reverses their position as inferred from the ND4 sequences alone (fig. 3A). The hypothesized ***(Iguana*** + ***Sauromalus)*** clade receives the least support, with a boostrap value of 65, a Bremer index of 2, and seven of eight synapomorphies showing some level of homoplasy.

When both intermediate and highly homoplastic characters (the "2" and "3+" columns in table 5) are deleted from the analysis, the same topology is recovered (fig. 5B) with strong phylogenetic signal ($g1 = -1.391$, $P \ll 0.01$). The strength of support increases for some nodes, assessed by either bootstrap proportions, Bremer indexes, or the ratio of consistent to homoplastic characters ***(Dipsosaurus*** + all other iguanids to the exclusion of ***Brachylophus),*** and decreases for others ***((Zguana*** + ***Sauromalus),*** (Amblyrhynchina + ***Ctenosaura),*** and especially the node joining these two clades [fig. 5*B*]).

The final iteration of this series of analyses included only the subset of 64 consistent characters (table 5), and significant phylogenetic signal was retained ($g1 = -1.475$, $P \ll 0.01$) in the two equally parsimonious trees recovered (the consensus tree is depicted in fig. 5*C*). These trees differ from the previous two in placement of the genera ***Cyclura, Iguana,*** and ***Sauromalus.*** Both trees consistently recover a ***(Cyclura*** + ***Iguana)*** clade (the Iguanina defined by de Queiroz, 1987a; fig. 1*B*), albeit with weak support by some measures (fig. 5*C*), and then alternately place ***Sauromalus*** either as the sister taxon to this clade ***[(Sauromalus (Cyclura*** + ***Iguana))],*** or as basal to all other iguanas internal to ***Dipsosaurus [(Sauromalus ((Cyclura*** + ***Iguana)*** (Amblyrhynchina + ***(Ctenosaura)))].*** However, when the "consistent characters only" matrix is forced onto the topology depicted in figure 5A and ***B, the tree*** is only a single step longer than the most parsimonious solution (TL = 129, CI = 0.791, and RI = 0.791), and the two tree

Table 6
Summary of Wilcoxon Ranked Sign Tests for Selected Paired Combinations of Tree Topologies Derived from Different Data Sets

Tree 1 (Length)		Tree 2 (Length)	*n*	Ts		*P*
ND-4 only (731)	vs.	Combined-A (734)	25	150		NS
Combined-A (685)[b]	vs.	ND4-only (692). .	28	254		NS
Combined-B (445)[c]	vs.	ND4-only (453). . .	14	2	=	0.05
Combined-A (685)	vs.	Av-Tann (714)[e] . . .	47	276	a	0.01
Combined-A (685)	vs.	Nor-deQ (708)[f] . . .	51	400	a	0.05
Combined-B (445)	vs.	Av-Tann (464). . .	31	96	a	0.01
Combined-B (445)	vs.	Nor-deQ (462)	33	132	a	0.01
Combined-C (1 28)[d]	vs.	Av-Tann (146). . . .	18	24	≅	0.01
Combined-C (128)	vs.	Nor-deQ (140). . . .	15	225	<	0.05

[a] Tree in figure 3A.
[b] Tree in figure 5A.
[c] Tree in figure 5B.
[d] Tree in figure 5C.
[e] Tree in figure 1A.
[f] Tree in figure 1C.

topologies are not in significant conflict as assessed by the Wilcoxon rank sum test (n = 5, test statistic (Ts) = 5, NS). Similarly, no significant differences are evident when the larger (deleting 3+ homoplasies only) or intermediate sized (deleting 2 and 3+ homoplasies) data sets are constrained to either topology derived from the "consistent characters only" data matrix (data not shown).

The Preferred Hypothesis

Here we ask whether or not the tree topologies derived from the three subsets of the combined data (fig. 5) are significantly more parsimonious than the fully resolved alternative hypotheses derived either from the ND4 sequences alone (fig. 3A and *B*) or from those published by Avery and Tanner (1971) (fig. 1*A*) and Norell and de Queiroz (1991) (fig. 1*C*). Table 6 summarizes the pairwise Wilcoxon tests for these comparisons, and reveals four important points. First, the ND4-based tree topology, which places *Dipsosaurus* basal to all other taxa, is not significantly more parsimonious than the alternative arrangement (*Brachylophus* basal to all) obtained from the combined data sets from which the "3+" and "2 and 3+" homoplasies are deleted (fig. 5A and B). Second, the reverse is true only for the combined data set that does not include the "3+" homoplastic characters; the "combined A" tree in table 6 is not significantly more parsimonious than the "ND4-only" alternative. However, when the "combined B" data set (2 and 3+ homoplasies deleted) is tested against the ND4 tree, the former is significantly more parsimonious (P = 0.05; table 6).

Third, the single most parsimonious trees derived from subsets of the total combined data set which exclude either the highly homoplastic (3+) or the intermediately and highly homoplastic (2 and 3+) characters, are significantly ($P < 0.05$) or highly significantly ($P \ll 0.01$) more parsimonious than either the Avery–Tanner or the Norell–de Queiroz hypotheses. Finally, the alternative topologies derived from the small subset of consistent characters (fig. 5*C*), which are not in significant conflict with those derived from the larger subsets of the combined character matrix (fig. 5*A* and *B*), are also significantly more parsimonious than either the Avery-Tanner or the Norell-de Queiroz topologies (table 6). We consider the best supported hypothesis of intergeneric relationships to be the topology depicted in figure 5*A* and *B (see also fig.* 2*B*), but we recognize that the weaker support for the alternative placement of *Cyclura* and *Sauromalus (fig.* 5*C*) cannot be unequivocably rejected with available evidence.

Discussion

Our results show that significant phylogenetic signal is present in all three data sets, and that the tRNA and ND4 sequences are not in significant conflict with each other, but that these do conflict with the morphological data. Further, our preferred hypothesis (fig. 5*A* and *B*) is significantly more parsimonious than the topologies previously published by Avery and Tanner (1971) and Norell and de Queiroz (1991). The early study of Avery and Tanner (197 1) based a hypothesis of relationships on mean length-width ratios of bones, with an arbitrary cutoff of 40 or fewer points between means of the same bones indicating a close relationship. These kinds of ratios were used in an unspecified way to construct the phylogenetic tree presented in figure 1*A*. Aside from vague tree-construction methods, character selection in this study did not control for within-species variation or allometric growth (see critique by de Queiroz 1987*a*, pp. 8-9, 133–134), and in our opinion the topology presented by Avery and Tanner (1971) cannot be taken to have strong support.

The studies of de Queiroz (1987a) and Norell and de Queiroz (1991) both employed character-based parsimony reconstruction methods, and the demonstrated conflict between their trees and those derived from our molecular and combined analyses suggests distorted phylogenetic signal in the molecular and/or morphological data sets. This issue may be further compounded by the relatively weak support for some internal nodes in fig. 5*A* and *B*, which allows for alternative placement of *Cyclura* and *Sauromalus* when all homoplastic characters are removed (fig. 5*C*). All of these problems may arise from a number of causes which are not mutually exclusive, including (1) closely spaced speciation events followed by long isolation of daughter lineages, which will produce excessively long terminal branches relative to the internal branches and possible "long branch attraction" (Felsenstein 1978; Hendy and Penny 1989); (2) failure of a single locus phylogeny to track the species phylogeny (Neigel and Avise 1986; Pamilo and Nei 1988); (3) a mismatch of rates of character evolution relative to the divergence events being investigated, which may lead to substitutional saturation of some molecular characters (Larson 1994); (4) misdiagnosis of alternative character states, which may be especially likely when only "instantaneous" morphological characters are used (versus ontogenetic transformations; de Queiroz 1985), and/or when molecular characters with a limited number of alternative states are used (as is possibly

the case with second codon positions; Naylor, Collins, and Brown 1995); (5) strong bias in base composition among taxa, which may affect the performance of parsimony methods (Collins, Kraus, and Estabrook 1994); and/or (6) nonindependence of characters, which may be more of an issue for molecular characters than previously thought (Miyamoto and Fitch 1995*b*; Myers, Lundrigan, and Tucker 1995). We consider the first four of these possible sources of error, and acknowledge that we cannot fully address others in this study.

Short Internal Versus Long Terminal Branches

Whether assessed by bootstrap proportions, the Bremer index, or the number of apomorphic characters (both consistent and homoplastic), support for some internal branches in our preferred hypothesis is clearly weaker than for the others. For example, the (Iguana + *Sauromalus*) clade is supported by bootstrap proportions of 65% and 76% in the cladograms in figure 5*A* and *B*, respectively, and in these same trees the deeper branch uniting this clade to the (Amblyrhynchina + *Ctenosaura*) clade is supported by bootstrap proportions of 75% and 55%, respectively. All other internal branches are supported by bootstrap proportions of at least 84% and 81%, respectively, and the majority are above 90%. These patterns are generally similar for the other measures of branch support given in fig. 5. Closely spaced splitting appears to be at least part of the reason for relatively fewer derived character states supporting some internal branches in the trees in figure 5*A* and *B*, but we cannot rule out the possibility of inadequate sampling of characters appropriately matched to these events. The number and combination of morphological characters resolved by de Queiroz (1987u) and Norell and de Queiroz (1991), in combination with the tRNA and ND4 sequences collected in this study, appear to provide a sufficient number of conservative characters to stabilize most of the internal nodes in a consistently recovered topology. Deletion of the 3+ homoplastic characters does not alter tree topology (fig. 5*A* and B), and stabilizes the basal positions of *Bruchylophus* and *Dipsosaurus*, and the monophyly of all other iguanas relative to *Dipsosuurus (the* Iguanini in fig. 1*B*). Within the Iguanini, there are a large number of informative characters in the combined data set supporting unambiguous monophyly for the genera *Ctenosaura, Cycluru, Iguana,* and *Sauromalus*, monophyly of the Amblyrhynchina *(Amblyrhynchus + Conolophus),* and monophyly for the (Amblyrhynchina + *Ctenosaura*) clade (fig. 4A and *B).* A large number of these characters are highly homoplastic third-base transitions in the ND4 codons (tables 3 and 5), and their removal still leaves all of these groups intact, with increased values for the support indices on the internal nodes (include groups like the Amblyrhynchina, with very long terminal branches, compare indices in the tree topologies in figs. 4A and 5A). Successive deletion of more inclusive subsets of homoplastic characters dramatically reduces the number of steps on all terminal branches while only slightly altering the internal support indices. However, support for the Iguanini is reduced with deletion of the 2 and 3+ classes of homoplasies, and this node collapses if all homoplastic characters are deleted (compare tree topologies in fig. 5*A* and *B* with that in fig. 5*C*). While we cannot completely escape the possibility of short internal branches in the Iguanidae, we can show that the underlying phylogenetic signal is strong enough to minimize the probability of grouping of taxa on the basis of nucleotide identity by parallelism in long terminal branches. The combination of morphological and molecular data sets in this case appears to provide complimentary phylogenetic signals at different hierarchical levels (Hillis 1987; Donoghue and Sanderson 1992), and the hypervariable characters are unlikely to suggest the same incorrect topology (Farris, Kluge, and Eckardt 1970). The same topology is also recovered by neighbor-joining analyses of distance estimates over all sequence data based on algorithms that correct for multiple hits (Saitou and Imanishi 1989; Rzhetsky and Nei 1992) (fig. 2B).

Davis (1993) has shown that, when there is inconsistency among characters, the groups resolved are the products of complex interactions among them, and that character support for the monophyly of a group need not derive from unambiguous (consistent) synapomorphies only. More recently, Sullivan (1996) has shown that, despite significant incongruence, combining data sets can produce more robust phylogeny estimates when they differ in evolutionary rates. The general patterns of homoplasy evident in the combined data set presented here recover groups congruent with those identified by the consistent and low-homoplasy subsets of characters, so that strong structure emerges from their combined analysis despite the conflicts identified by the process partition approach. However, the tree based on combined data minus the 3+ classes of homoplastic characters is not significantly more parsimonious than the tree obtained from the ND4 sequences alone (table 6). The same tree derived from the combined data minus the 2 and 3+ homoplastic characters is significantly more parsimonious than the ND4-only tree (table 6), indicating that a combined approach makes the strongest case for an alternative tree when the most homoplastic characters are removed. This allows the differences identified by process partitions to be accomodated in the combined character analysis, in this case by deletion (see Chippindale and Wiens 1994; Wiens and Chippindale 1994; and Sullivan 1996 for further discussion). Acceptance of the alternative tree topology presented in fig. 5*C* requires deletion of all characters showing any homoplasy, which denies any value to support based on overall patterns of congruence recovered from the inclusion of these characters. We conclude that the "single" and "two homoplasy" classes of characters contain high signal-to-noise ratios, and their removal leads to unacceptable distortion of tree topologies (fig. 5*C*).

Gene Phylogeny Versus Species Phylogeny

Another possible source of character conflict between the morphological and sequence data sets used in this study is incongruence of the mtDNA phylogeny with the species phylogeny, which may occur if lineage

sorting of alternative haplotypes in polymorphic ancestral populations has not proceeded to the point of reciprocal monophyly in daughter lineages (Neigel and Avise 1986; Pamilo and Nei 1988).

Moore (1995) has recently shown that the probability of coalescence of a gene tree and a species tree increases with decreasing effective population size (N_e) for neutral markers, and because N_e for the mtDNA locus is one fourth the size of that for a single-copy nuclear locus when the sex ratio is 1: 1, these are precisely the conditions under which alternate mtDNA haplotypes should have a relatively high probability of quickly sorting to reciprocal monophyly in lineages diverging from a polymorphic ancestor. Estimates of N_e are not available for any species of iguanas, but many are characterized by territorial behavior and polygynous mating systems (which contribute to a high variance in male reproductive success) and/or genetically subdivided metapopulation structures (Christian and Tracy 1982; Werner 1982; Carothers 1984; Lamb, Jones, and Avise 1992). These features of iguana population biology should effectively reduce N_e further, and thus decrease coalescence times. We suggest that the probability of discordance between the mtDNA gene tree and the species tree is low for this group, and that sequence from conserved regions of the mtDNA genome would strengthen deep node support in our preferred topology.

Character Saturation

The possibility of character saturation and mismatch of evolutionary rates with the divergence times being investigated here is most likely for the ND4 sequence data set (tables 3 and 5). However, a number of unambiguous synapomorphies present in the ND4 sequence provide resolution deeper in the trees than the levels of conflict (fig. 3A and B), and the strength of support for these deep branches is approximately equal to that typical for more nested internal nodes in these same trees. If ND4 substitution saturation was the primary explanation for lack of congruence between molecular and morphological data sets in some parts of the trees, then deep level resolution would not be expected in the saturated data set.

Character Misdiagnosis and Limited Alternative States

Of the 95 morphological characters originally described by de Queiroz (1987*a*, pp. 100–105), 74 were skeletal and 2 1 were internal or external nonskeletal features. All but eight of the skeletal characters were binary (seven of the eight multistate characters had three states, and one had four). Two of the 21 nonskeletal characters had three states; all others were binary. Several of each of these classes of characters were qualitative and coded in such a manner that ambiguities might arise. For example, the alternative states for the labial process of the coronoid bone (de Queiroz 1987*a*, #36 on p. 102) are scored as (A) small, (B) medium, or (C) large. Further, many characters are polymorphic within the basal taxa (the eight genera recognized by Etheridge 1982), and thus could not be counted as unambiguous synapomorphies for these clades. Finally, the majority of characters were "instantaneous" in the sense that they contained no information on ontogenetic transformations (de Queiroz 1985), and most were scored from adults. These limitations may contribute to problems of homology assessment, character nonindependence, and subjectivity in scoring that frequently characterize morphological data sets (Swofford et al. 1996), and should serve to introduce caution into phylogenetic conclusions inferred from them. The modified version of this data set presented by Norell and de Queiroz (1991) was subject to the same limitations.

Similarly, the sequences presented here may also be subject to many of the same limitations. Homology assessment is reasonably secure for most base positions, because the ND4 region readily aligns with those of other vertebrates due to the conserved codon structure, and because the indels in the tRNA sequences are very small and widely scattered across all tRNA genes used. Nevertheless, mitochondrial tRNA sequences have seldom been the focus of phylogenetic studies (Simon et al. 1994), and our approach may not fully capitalize on the complexities and constraints imposed by secondary structure. More complex models for ribosomal gene evolution have been discussed for assessing phylogenetic information content (Kjer 1995; Sullivan, Holsinger, and Simon 1995; Hickson et al. 1996), and application of these should be extended to tRNA sequences.

In spite of these potential biases and those we have not addressed, there are substantial areas of agreement between our preferred hypothesis and that presented by Norell and de Queiroz (1991). We summarize these points, and then assess the strength of support for conflicting clades in light of limitations in character scoring or interpretation.

Agreement and Conflict Between Trees

All analyses support the deep basal positions for ***Brachylophus*** and ***Dipsosaurus,*** although de Queiroz (1987*a*) originally could not confidently decide which of these was basal (fig. 1*B*), and our favored hypothesis reverses the arrangement obtained by Norell and de Queiroz (1991) from the inclusion of the fossil ***Armandisaurus (fig.*** 1*C*). ***Armandisaurus*** is recovered as the sister taxon to ***Dipsosaurus,*** and on the basis of its retention of the presumed plesiomorphic condition for the interpterygoid vacuity, this clade stabilizes as the sister group of a ***(Brachylophus*** + Iguanini) clade (fig. 1*C*). Our combined data sets, minus the most homoplastic characters, appear to provide stronger support for a ***(Bruchylophus (Dipsosaurus*** + Iguanini)) arrangement, in which eight unambiguous synapomorphies defined the ***(Dipsosaurus*** + Iguanini) clade (all topologies in fig. 5). We did not include ***Armandisaurus*** in these analyses to shorten computer time, and because most of the characters are missing. Unless one can justify heavily weighting the alternate state of the interpterygoid vacuity represented in ***Armandisaurus, we*** think the strength of the evidence favors our hypothesis.

All analyses consistently recover the Iguanini as monophyletic, and the combined analyses show very

strong support for this group regardless of which index is considered or which combination of data sets is used (see all topologies in figs. 4 and 5). In addition, de Queiroz (1987*a*) demonstrated moderate to strong support for the monophyly of all genera of the Iguanidae, with the possible exception of *Cycluru.* The distribution of some morphological characters suggested that *Cycluru* might be paraphyletic with respect to the genus Iguana, which shares derived features of cephalic scutellation and widening of the parabasisphenoid with some but not all species of *Cyclura.* However, the overall distribution of derived characters in these and other genera contradict each other, and de Queiroz (1987a) only provisionally accepted the monophyly of *Cycluru.* Our analyses provide strong support for monophyly of both of these and all other genera from which multiple species were sampled (figs. 3*A*, 3*B*, 4, and 5), and further suggest that *Cycluru* and *Iguana* are not sister taxa (see all fully resolved topologies except that presented in fig. 5*C*).

In summary, the different studies agree on the basal positions of *Bruchylophus* and *Dipsosaurus* (albeit with different placement), the monophyly of the Iguanini, and the monophyly of *Cyclura* and Iguana, as well as all other genera. Important areas of conflict center on placement of the genera *Ctenosaura* and *Sauromalus,* as well as the monophyly of Iguanina (fig. 1*B*).

De Queiroz defined the clade "Iguanina" *(Cycluru + Iguana; fig.* 1*B*) on the basis of four synapomorphies, but two of these-the shape of the surangular bone and the crown patterns of the posterior marginal teeth-are possibly diagnostic of more inclusive groups containing *Ctenosuuru* and *Sauromalus,* respectively (de Queiroz 1987*a,* p. 168). The clade Iguanina is thus supported by only two unambiguous synapomorphies: (1) the articulation of the squamosal to the dorsal end of the tympanic crest of the quadrate, and (2) a narrowly constricted cristae ventrolaterales region of the parabasisphenoid behind the pterygoid process. In contrast, support for a clade Iguanini in which *Cycluru* is basal and *(Iguana + Suuromalus)* is internally nested (figs. 3*A*, 3*B*, 4, 5*A*, and 5B) is supported by a larger number of characters. In the topology presented in figure 5*A*, 13 synapomorphies unite all genera of Iguanini to the exclusion of *Cycluru.* Two of these are consistent-both third-base transversions-and 11 are homoplastic and include first- and third-base transitions, transversions, and sites in which both kinds of substitutions have occurred, one second-base transition, and one tRNA site in which both substitutions have occurred. Support for the (Iguana + *Suuromalus)* clade within this group is based on one consistent (structure of the pelvic girdle) and seven homoplastic characters (five third-base and one second-base sites for both transitions and transversions, and one transition in the histidine tRNA). The total numbers of synapomorphies decline when the two highest homoplasy classes of characters are deleted (fig. 5*B*), but this increases the bootstrap value/Bremer index for the *(Iguana + Cycluru)* clade from 65/2 to 76/3. The Iguanina clade is recovered by restricting analysis to consistent characters only, but bootstrap and Bremer support for this clade is relatively weak (68/1; fig. 5*C*). Before final acceptance of either hypothesis, however, the conflicting morphological and molecular characters highlighted here require reexamination, and additional markers appropriate to this level of divergence should be assayed.

One other strikingly different result is our consistent recovery of the genus *Ctenosaura* as the sister group of the Amblyrhynchina, rather than the (*Sauromalus* + Amblyrhynchina) arrangement presented by de Queiroz (1987*a*) and Norell and de Queiroz (1991) (fig. 1 *B* and *C*). This second arrangement is supported by two morphological characters: (1) reduced labial exposure of the angular bone and (2) short second ceratobranchials. Our combined data set that excludes the "3+ homoplasy" characters (fig. 5A) supports the (Amblyrhynchina + *Ctenosauru)* alternative on the basis of two consistent (third-base transitions) and 11 homoplastic characters (first-base transitions and transversions, a second-base transversion, all combinations of third-base substitutions, both classes of histidine tRNA substitutions, and a derived crown pattern on the tricuspid posterior marginal teeth). More importantly, this clade is consistently recovered in the ND4 sequences (fig. 3A and *B)* and in all four mixes of both the combined morphological-plus-molecular data sets (figs. 4 and 5), and its support is not confined to any particular class of characters. This relationship was proposed by Higgins (1978), but without strong documentation. We conclude that the available evidence strongly favors monophyly for the *(Ctenosauru* (*Amblyrhynchus* + *Conolophus*)) topology.

Biogeography

Several genera within the Iguanidae are endemic to island archipelagoes widely separated by open ocean. The genus *Bruchylophus,* endemic to the Fijian and Tongan Archipelagoes (Gibbons 1981, 1985; Pregill and Dye 1989; Zug 1991), is a striking example. Species of *Bruchylophus are* characterized by small clutch sizes (3-6 eggs) coupled with unusually long incubation periods (125–250+ days; Arnett 1979; Gibbons and Watkins 1982) and, like other iguanas, they are large, herbivorous, and possess functional nasal glands for excretion of excess salts (Cogger 1.974). These features may have preadapted the original ancestor of *Bruchylophus* to colonize oceanic islands in the western Pacific by a long-distance rafting event; the South Equatorial Current will carry items from the eastern (Galapagos) to the western Pacific (Fiji; see Gibbons 1985, p. 136). However, the relationship of *Brachylophus* with South American taxa is paralleled by similar patterns in other animal and plant groups (Croizat 1958), and this pattern may reflect former terrestrial landscapes that have moved across parts of the Pacific (Nur and Ben-Avraham 1982; Craw and Page 1988). If this scenario is geologically plausible, one need not postulate the trans-Pacific rafting event hypothesized by Cogger (1974) and Gibbons (1981).

Within the Iguanini, the earliest cladogenic event split the common ancestor of *Cycluru* from the ancestor of the remaining genera, and was associated with either a vicariant origin of *Cycluru* in the Caribbean or its sub-

sequent dispersal there. Hedges, Hass, and Maxson (1992) date the separation of the proto-Antilles at 70-80 MYA, and hypothesize that most elements of the extant endemic Antillean herpetofauna arose more recently by over-water colonization. However, the geological and biological history of the Greater Antilles is extremely complex (Rosen 1985; Woods 1989), and rigorous biogeographic study will be necessary to separate autochthonous from allochthonous elements of the biota.

The two genera endemic to the Galapagos Archipelago, ***Amblyrhynchus*** and ***Conolophus,*** may offer another example of more recent tram-Pacific colonization of oceanic islands, or a former geological connection between the Galapagos hotspot and terranes that now comprise parts of western Central and South America. The morphological studies support a sister group relationship for these taxa and confirm the earlier immunological study of Wyles and Sarich (1983). These investigators suggested a divergence time between these two genera of 15–20 MYA. If this date is accurate, then the divergence of these genera predates the ages of the oldest extant Galapagos islands (White, McBirney, and Duncan 1993). However, these genera may still have originated in the Galapagos if their original ancestor colonized one of the older, submerged seamounts east of the present islands (Carson 1992). Radiometric dating of these drowned islands places the oldest at about 10–11 Myr, relative to the age of about 3 Myr for the oldest above-surface island (Christie et al. 1992), but these authors suggest that islands have existed throughout the 80–90-Myr history of Galapagos hotspot activity. If so, an ancient divergence between land and marine iguanas can still be accommodated by postulating the original colonization event to an older, now submerged, island. Patterns of genetic divergence in other endemic Galapagos reptiles range from those showing extensive morphological radiation accompanied by little divergence (i.e., giant tortoises of the genus ***Geochelone;*** Marlow and Patton 198 1; Patton 1984) to those showing radiations characterized by moderate to pronounced genetic differentiation (i.e., lizards of the genera ***Phyllodactylus*** and ***Tropidurus;*** Wright 1983). Thus, speciation times among Galapagos endemics may be extended beyond the age of the extant islands (Christie et al. 1992).

The radiation of ***Sauromalus*** on islands in the Sea of Cortez is poorly understood, and the geological and biological histories of that region are also extremely complex (Grismer 1994). Further tests of the relationships within and among all genera of the Iguanidae should aid the identification of common features of iguana biology that permit either successful colonization of, or vicariant origins on, oceanic islands.

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