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Mitochondrial DNA Evidence and Evolution in Varanoidea (Squamata)

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Varanoidea is a monophyletic group of anguimorph lizards, comprising the New World helodermatids, the Bornean earless monitor *Lanthanotus borneensis*, and the Old World monitors (*Varanus*). I use mitochondrial DNA sequences and extensive taxonomic sampling to test alternative hypotheses of varanoid relationships. The most parsimonious hypothesis confirms the monophyly of Varanoidea (*Heloderma, Lanthanotus*, and *Varanus*) and *Varanus*, as well as the sister-taxon relationship of *Varanus* and *Lanthanotus*. The relationships among *Varanus* species differ in several respects from previous hypotheses. Three major lineages are recognized within *Varanus*: an African clade basal to the rest of the group, an Indo-Asian clade, and an Indo-Australian clade. Within the last lineage, the endemic Australian dwarf monitors (*Odatria*) form a clade sister to the large Australian monitors (the *gouldii* group). Tests of the effects of rate heterogeneity and homoplasy demonstrate that putative process partitions of data are largely congruent with one another and contribute positive support to the overall hypothesis.

INTRODUCTION

Varanoidea is an ancient group of anguimorph lizards, comprising the two extant New World helodermatids (*Heloderma horridum* and *Heloderma suspectum*), the Bornean earless monitor (*Lanthanotus borneensis*), and the Old World monitors (*Varanus*). The earliest fossils that can be clearly assigned to this group include the Middle Cretaceous aigialosaurs (Carroll and deBraga, 1992); later varanoid fossils include the helodermatid-like monstersaurians *Gobiderma* and *Estesia* (Norell and Gao, 1997), the *Lanthanotus*-like *Cherminotus* (Gao and Norell, 2000), and the *Varanus*-like *Saniwides* and *Telmasaurus* (Pregill *et al.*, 1986). Currently, approximately 50 extant species of *Varanus* are recognized (Table 1). These species are found in Africa; central and southern mainland Asia and Malaysian and Indonesian islands (these may be roughly described as the Indo-Asian species); and Papua New Guinea and Australia (where more than half the species are found, henceforth referred to as the Indo-Australian species). All monitors share a basic body plan and several morphological synapomorphies, yet the impressive diversity achieved by *Varanus* within its rather conservative overall morphology makes it an ideal radiation for investigating and comparing the evolution of features such as gross morphology (e.g., Pianka, 1995), habitat and ecology (e.g., Losos and Greene, 1988), and physiology (e.g., Bartholomew and Tucker, 1964). The recent molecular studies of anguimorph lizards by Macey *et al.* (1997a, 1997b, 1999) suggest that these lizards may possess some features of interest

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TABLE 1
List of Extant Species of *Varanus*, Taken in Part from Kluge (m.s.) and Bennett (1995)

Species of *Varanus*	Subgenus	Voucher No.	Geography
acanthurus Boulenger, 1885	*Odatria*	AM R143881	N & Central Australia
albigularis (Daudin, 1802)	*Empagusia*	—	S Africa
auffenbergi[1] Sprackland, 1999	*Odatria*	—	
baritji King and Horner, 1987	*Odatria*	UMMZ 222676	N Australia
beccari[2a] (Doria, 1802)	*Odatria*	UMFS 10371	Aru Islands
bengalensis[3]	*Indovaranus*		SE Asia, Indonesian islands
bengalensis (Daudin, 1802)		PZ 300941	
nebulosis (Gray, 1831)		ROM 35017	
bogerti[2a] Mertens, 1950	*Euprepiosaurus*	—	Louisiade Archipelago
brevicauda Boulenger, 1898	*Odatria*	—	Australia
caerulvirens[2b] Ziegler, Böhme, and Phillipp, 1999	*Euprepiosaurus*	—	Indonesian islands
caudolineatus Boulenger, 1885	*Odatria*	—	Australia
cerambonensis[2b] Philipp, Böhme, and Ziegler, 1999	*Euprepiosaurus*	—	Indonesian islands
doreanus[2b] (Meyer, 1874)	*Euprepiosaurus*	UMFS 10296	Papua New Guinea
dumerilii (Schlegel, 1839)	*Tectovaranus*	UMFS 10375	SE Asia, Sumatra, Borneo
eremius Lucas and Frost, 1895	*Odatria*	AM R147247	Australia
exanthematicus (Bosc, 1792)	*Empagusia*	UMFS 10959	Central Africa
finschi[2b] Böhme and Ziegler, 1994	*Euprepiosaurus*	—	N Australia and S Papua New Guinea
flavescens (Hardwicke and Gray, 1827)	*Empagusia*	UF 67500	SE Asia
giganteus (Gray, 1845)	*Varanus*	UMFS 10960	Central Australia
gilleni Lucas and Frost, 1895	*Odatria*	AM R147264	Central Australia
glauerti Mertens, 1957	*Odatria*	UMFS 10370	NW Australia
glebopalma Mitchell, 1955	*Odatria*	UMMZ 218497	NW Australia
gouldii[4] (Gray, 1838)	*Varanus*	AM R123634	Australia
griseus	*Psammosaurus*		N Africa, Central Asia
caspius (Eichwald, 1831)		—	
griseus (Daudin, 1803)		UMMZ 221342	
koniecznyi Mertens, 1954		—	
indicus[2b] (Daudin, 1802)	*Euprepiosaurus*	AM 36431	N Australia, Papua
		AM 51525	New Guinea,
		AM R137997	Indonesian islands
jobiensis[2b] Ahl, 1932	*Varanus*	UMMZ 211713	Papua New Guinea
keithhornei (Wells and Wellington, 1985)	*Euprepiosaurus*	QM 70792	NE Australia
kingorum Storr, 1980	*Odatria*	UMMZ 219012	NW Australia
komodoensis Ouwens, 1912	*Varanus*	NZP*	Indonesian islands
melinus[2b] Böhme and Ziegler, 1997	*Euprepiosaurus*	UMFS 10164	Indonesian islands
mertensi Glauert, 1951	*Varanus*	AM R123877	N Australia
mitchelli Mertens, 1958	*Odatria*	UMMZ 210576	N Australia
niloticus (Linnaeus, 1766)	*Polydaedalus*	UMMZ 221377	S & Central Africa
olivaceus Hallowell, 1857	*Phillipisaurus*	UMMZ 210202	Luzon Island
panoptes[3,4]	*Varanus*		Australia, Papua New Guinea
horni Böhme, 1988		UMFS 10157	
panoptes (Gray, 1838)		UMMZ 210491	
rubidus Storr, 1980		—	
pilbarensis Storr 1980	*Odatria*	WAM R132659	W Australia
prasinus (Schlegel, 1839)	*Euprepiosaurus*	UMFS 10684	Papua New Guinea
primordius Mertens, 1942	*Odatria*	UMMZ 218495	N Central Australia
rosenbergi Mertens, 1957	*Varanus*	—	S Australia
rudicollis (Gray, 1845)	*Dendrovaranus*	UMMZ 210506	SE Asia, Borneo, Indonesian islands
salvadorii (Peters and Doria, 1878)	*Papusaurus*	UMFS 10294	Papua New Guinea

TABLE 1—Continued

Species of *Varanus*	Subgenus	Voucher No.	Geography
salvator[3]	*Varanus*		SE Asia, Borneo
andamanensis Deraniyagala, 1944		—	Philippines,
bivittatus (Kuhl, 1820)		UMFS 10670	Indonesian islands
cumingi Martin, 1838		UMFS 10369	
marmoratus (Wiegmann, 1834)		—	
nuchalis (Günther, 1872)		—	
salvator (Laurenti, 1768)		UMFS 10374	
togianus[5] (Peters, 1872)		UMFS 10298	
scalaris[6] Mertens, 1941	*Odatria*	UMMZ 218493	N Australia, S
		AM R138712	Papua New Guinea
semiremex Peters, 1869	*Odatria*	AZ-1	NE Australia
spenceri Lucas and Frost, 1903	*Varanus*	UMMZ 218500	Central Australia
spinulosis[2b] Mertens, 1941	*Euprepiosaurus*	—	Solomon Islands
storri Mertens, 1966	*Odatria*	AM R143912	N and Central Australia
telenesetes[2a] Sprackland, 1991	*Euprepiosaurus*	—	Louisade Archipelago
timorensis (Gray, 1831)	*Odatria*	WAM R107008	Timor, Semau, Savu
tristis (Schlegel, 1839)	*Odatria*	AM R143919	Australia
varius (Shaw, 1790)	*Varanus*	AM R133492	SE Australia
yemenensis Böhme, Joger, and Schätti, 1989	*Empagusia*	—	Yemen, Saudi Arabia
yuwonoi[2b] Harvey and Barker, 1997	*Euprepiosaurus*	UMMZ 225545	Halmahera Island

Note. If a voucher number is listed, the species was sampled for this study; dashes indicate that the species was not sampled. Voucher number institution key: AM, Australian Museum of Natural History; AZ, Australia Zoo; CAS, California Academy of Sciences; NZP, National Zoological Park (**komodoensis* specimen now at the Ueno Zoo, Japan); PZ, Philadelphia Zoo; QM, Queensland Museum; ROM, Royal Ontario Museum; UF, University of Florida; UMFS / UMMZ, University of Michigan Museum of Zoology; WAM, Western Australian Museum. Numbers by species names refer to the following comments:

[1] *auffenbergi* may be a synonym for *timorensis.*

[2] These species in the *prasinus* (2a) and *indicus* (2b) groups have recently been referred to the new subgenus *Euprepiosaurus* (Böhme *et al.*, 1994; Sprackland, 1994).

[3] These subspecies may represent distinct species.

[4] The nomenclatural changes to *flavirufus, gouldii,* and *panoptes* proposed by Böhme (1991) are not reflected because of counterarguments by H. Cogger and G. Shea (pers. comm.).

[5] *salvator togianus,* initially described as *togianus* (Peters, 1872), is probably a separate species (A. Kluge, pers. comm).

[6] AM R138712 was initially identified as *timorensis.*

in the study of specific mechanisms of molecular evolution, such as parallel evolution of duplication and deletion events.

However, the study of *Varanus* evolution has been hampered by a (largely) non-monophyletic subgeneric taxonomy and the lack of a well-sampled, well-corroborated phylogenetic hypothesis. Although reconstruction of monitor lizard history has been attempted from several sources of evidence, including karyotypes (King and King, 1975), electrophoretic phenotypes (Holmes *et al.*, 1975), male intromittant organ morphology (Branch, 1982; Böhme, 1988; Card and Kluge, 1995), skeletal elements (Estes *et al.*, 1988), ecological and physiological characteristics (Losos and Greene, 1988), lung morphology (Becker, 1991), DNA sequence data (Baverstock *et al.*, 1993; Fuller *et al.*, 1998), and various combinations of characteristics (King, 1990; King *et al.*, 1991; Sprackland, 1991), these studies differ considerably in their conclusions and no well-corroborated consensus phylogeny has emerged. Specific areas that need testing include the relationships among the African, Indo-Asian, and Indo-Australian species, whether the subgenera *Varanus* and *Odatria* are monophyletic, the relationships within Australian endemics (the large-bodied *gouldii* group and the small-bodied *Odatria*), and whether the subgeneric taxonomy reflects phylogeny.

The earliest studies of *Varanus* employing biochemical data include that of Holmes *et al.* (1975), who combined the chromosome work of King and King (1975)

with isozyme electrophoretic data to broadly test phylogeny, biogeography, and taxonomy. Using a phenetic analysis of a few characters, these investigators suggested that the African species and Indo-Australian species each arose from the Indo-Asian monitors of the *salvator* group (including *bengalensis*, *flavescens*, and *rudicollis*). In the African radiation, *griseus* (found in central Asia and Asia Minor as well as northern Africa) was considered to have given rise to *niloticus* and *exanthematicus*, which then radiated southward into Africa. In the Indo-Australian radiation, *Odatria* was thought to have initially colonized Australia and subsequently given rise to the *gouldii* group, with a possible second radiation into Australia by an *indicus–varius* group separately descended from the ancestors of *Odatria*. Because *prasinus* and its relatives were not included, Holmes *et al.* and King and King could not determine the karyomorph or isozyme affinities of this group, which also has an Indo-Australian distribution. Holmes *et al.* and King and King noted that the traditional taxonomy probably did not reflect natural groups within *Varanus*.

Sprackland (1991) focused primarily on the origin and monophyly of *Odatria* and whether species of the *prasinus* group were part of this assemblage, using the data of Holmes *et al.* (1975) combined with additional morphological and ecological characters. Based on his preferred cladogram, Sprackland suggested that the African species were basal to the rest of *Varanus* and that the Indo-Asian taxa formed a cline between the African species and the monophyletic Indo-Australian clade. *Odatria* was monophyletic and had possibly evolved from the *gouldii* group through a *salvator*-like ancestor (contra King and King, 1975). Sprackland's hypothesis also demonstrated that *prasinus* and its relatives were more closely related to *indicus* than *Odatria*. Sprackland's data were later reanalyzed by Card and Kluge (1995), who determined that his conclusions regarding *prasinus* and *Odatria* monophyly were corroborated, but that no resolution of the relationship between the *gouldii* group and *Odatria* could be obtained using this data set.

Baverstock *et al.*'s (1993) analysis also broadly examined relationships within *Varanus*. Based on reciprocal microcomplement fixation testing of a limited number of taxa, they identified three major clades of *Varanus*: an African clade, a clade of the large-bodied Indo-Asian and Indo-Australian taxa, and *Odatria*. Because outgroup material either was unavailable or failed to fix in cross-reactions with *Varanus*, the authors could not root their tree and the relationships among these lineages went undetermined. In the Indo-Asian and -Australian clade, the Indo-Australian species *mertensi*, *rosenbergi*, and *salvadorii* were sister to a clade of Indo-Asian species. Baverstock *et al.* hypothesized two separate invasions of Australia, as the Australian endemics (*Odatria* and the *gouldii* group) were not sister taxa. A phylogenetic hypothesis based on both reciprocal and one-way comparisons, with a more inclusive set of species, additionally suggested that Australia had been invaded at least three times and corroborated Sprackland's (1991) proposition that *prasinus* is closely related to the *indicus* complex rather than to *Odatria*. Baverstock *et al.* followed King and King (1975) in noting the shortcomings of the traditional subgeneric classification of *Varanus*.

More recently, DNA sequence data were used by Fuller *et al.* (1998) to attempt to resolve the biogeographic origins of *Varanus*. Their study was based on two nonoverlapping segments of the mitochondrial 12S ribosomal RNA gene (about 700 bp total) and included *Heloderma* and *Lanthanotus* as outgroup taxa. Parsimony analysis resulted in a single most parsimonious tree suggesting that the African species are sister to the rest of *Varanus*, the Indo-Asian species form a paraphyletic assemblage, and the Indo-Australian species are a clade. Subgenus *Varanus* (represented by the *gouldii* group and *indicus*, *salvator*, *komodoensis*, and *varius*) was polyphyletic, and *Odatria* was paraphyletic with the *gouldii* group nested within it, although log-likelihood ratio and nonparametric parsimony (Wilcoxon matched-pairs signed-ranks) tests suggested that the topology of the best overall hypothesis was not significantly worse than one in which each of the subgenera *Varanus* and *Odatria* were constrained to monophyly. Additionally, *prasinus* was shown to be related to *olivaceus* rather than to *indicus* or any odatrian species.

A consensus phylogeny based on these studies is largely unresolved. To date, there is no single well-supported hypothesis of relationships for Varanoidea and *Varanus*, and the current subgeneric taxonomy of *Varanus* (Table 1) apparently does not reflect phylogeny. Incomplete taxonomic sampling within studies

and differential sampling among studies have certainly contributed to these shortcomings, as many of the previously mentioned works sampled incompletely within *Varanus* or did not sample its nearest relatives. Thorough taxonomic sampling is necessary to ensure that the data are maximally informative, to reduce the potential for long-branch artifacts (Hillis, 1998; Graybeal, 1998), and to encompass a complete picture of history. The present study extensively samples the diversity of Varanoidea and results in a well-corroborated phylogenetic hypothesis that will further the investigation of the evolution and biogeography of these remarkable lizards.

MATERIALS AND METHODS

Taxonomic sampling. Forty-eight individuals of *Varanus* were sequenced, representing each of the currently recognized subgenera (see Table 1 for a list of species sampled, subgeneric classification, voucher numbers, and general geographic localities). Some species were sampled from different localities (*indicus*, *panoptes*, and the subspecies of *salvator*). A specimen initially misidentified as *timorensis* was reidentified as *scalaris*. Other varanoids sampled include the monotypic lanthanotid *L. borneensis* (no accession number), the taxon most closely related to *Varanus* (corroborated by both morphological and molecular data; e.g., Rieppel, 1980, and Fuller *et al.*, 1998), and the two extant helodermatids *H. horridum* (UMFS 10134) and *H. suspectum* (no accession number), considered to be sister to *Lanthanotus* + *Varanus* (Pregill *et al.*, 1986). Outgroup taxa *Anguis fragilis* (CAS 190559), *Anniella pulchra* (CAS B15p556), and *Elgaria kingi* (UMFS 10107) were selected to represent Anguioidea, the putative sister taxon to Varanoidea (Camp, 1923; reviewed by Rieppel, 1988).

DNA extraction, amplification, and sequencing. Genomic DNA was extracted from fresh, frozen, or ethanol-preserved muscle or liver tissue or buffer-preserved blood samples with QIAamp tissue kits (Qiagen) as per the manufacturer's instructions. One extract that did not immediately amplify (*kingorum*) was cleaned of proteins and other PCR inhibitors by centrifugation through a Centricon-30 column (Amicon); after this treatment, the extract amplified normally.

The 2.8-kb region sequenced spans the 3′ end of the 16S rRNA gene to the 5′ region of CO-1 and includes the complete sequences of ND-1, ND-2, and nine tRNAs ($tRNA^{Leu}$, the "IQM" cluster of $tRNA^{Ile}$, $tRNA^{Gln}$, and $tRNA^{Met}$, and the "WANCY" cluster of $tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, and $tRNA^{Tyr}$). To further minimize the chances of amplifying nuclear copies, the entire 2.8-kb target region was amplified using long PCR protocols and primers L3827 and H6681 (Sorenson *et al.*, 1999). Smaller fragments were subsequently amplified using standard PCR protocols from the long piece of DNA using primer pairs L3827–H4644, L4500–H5191, L4951–H5760, and L5601–H6681 (see Sorenson *et al.*, 1999, for primer sequences). The *Varanus*-specific primers L4951 (5′-CCTCCTCTG AAAACAATTTCTCCC-3′), H5760 (5′-GATGAGGA GTGCTATTGGGGC-3′), and L5601 (5′-TGACTMCC AGAAGTHCTTCAAGG-3′) respectively replace the primers L5216, H5766, and L5758 of Sorenson *et al.* (1999), providing a better match to template DNA and a more substantial region of overlap between contiguous fragments. DNA from two species, *flavescens* and *primordius*, did not amplify across the entire region, and primers L3827 and H5766 (Sorenson *et al.*, 1999) were used for long-product amplification; the sequence for these taxa therefore terminates at about halfway through ND-2. The lack of amplification might be attributed to poor DNA quality for *flavescens*, in which the tissue sample was somewhat degraded, but is less explicable in *primordius*, as a sample of a different individual also did not amplify using any primer to the 5′ side of H5766.

PCR products were electrophoresed through agarose gels, visualized with ethidium bromide staining, removed from the gel, and purified using a QIAamp gel extraction kit (Qiagen). Light and heavy strands were both sequenced using dye terminators (Perkin–Elmer) and analyzed with an ABI 377 automated sequencer. Primer sequences were trimmed from chromatograms and the individual sequences were assembled by overlapping each sequenced PCR product into a single contiguous segment. Protein-coding genes were identified and aligned by inferred amino acid sequences, and tRNAs were identified and aligned by stem and loop sections of the secondary structure in each sequence (Lynch, 1996). All sequences can be found in GenBank (AF407488–AF407541).

Phylogenetic analysis. The 2.8-kb sequences were parsed into 13 gene sequences for analysis (partial 16S rRNA, ND-1, ND-2, nine tRNAs, and partial CO-1). The short region of 16S sequenced comprised stem regions constant across all taxa and highly variable loop regions and was therefore excluded from the analysis. Intergenic spacer regions were excluded from all analyses, as were uninformative characters and sites where alignment was nontrivial (primarily tRNA loops), leaving 1474 parsimony-informative sites. The few (32) gapped regions that remained were considered to be potentially informative (as suggested by Giribet and Wheeler, 1999), and gaps were treated as a fifth base.

For the primary analysis, all informative sites were weighted equally (for rationale see Results and Discussion). Because one region of a sequence pairs with another region on the same strand in stem and loop structures, as are found in tRNAs, it could be argued that a change in one base in one stem region must necessitate a compensatory change in the pairing site of the second region, and therefore to count each pair of a stem region would have artificially inflated the number of informative characters. However, mutations resulting in stem mispairings are not unusual in animal mitochondrial tRNAs (Lynch, 1996, pers. obs.), so sequences of both strands in a given stem were used. Data were analyzed simultaneously in PAUP* 4.0b4 (Swofford, 1999) with 1000 heuristic search replicates; initial trees were randomly generated and swapped using tree bisection–reconnection. Jackknife values were calculated with PAUP* emulating JAC resampling (Farris *et al.*, 1996) for 1000 replicates with 20 heuristic searches per replicate. Bremer support values (Bremer, 1994) were calculated using TreeRot (Sorenson, 1999) and PAUP* with 20 heuristic searches per node examined. Searches were also run in which third codon position transitions were weighted to zero and in which third positions were eliminated altogether. Heuristic and jackknife searches for these two analyses were computed as above with 100 replicates.

Two separate data partitions were analyzed using the incongruence length difference test (ILD, Mickevitch and Farris, 1981; Farris *et al.*, 1994, 1995) as implemented by XARN (Farris, 1996) and the partition homogeneity test in PAUP*. The first partition tested for incongruence among first, second, and third codon positions in the protein-coding genes, and the second tested for incongruence among genes (ND-1, ND-2, CO-1, and the combined tRNAs). The Mickevitch/Farris (M/F) incongruence statistic was calculated by determining the shortest length for each partition and the number of extra steps needed to find the best fitting cladogram in the combined analysis (as per Mickevitch and Farris, 1981; Kluge, 1989). For analyses of most character sets in each partitioned analysis, minimum length was calculated with 100 heuristic replicates with the same settings as used for the simultaneous analysis. One of the data classes, CO-1, had a small number of informative characters (16); minimum and reconstructed length for CO-1 was calculated using tree bisection–reconnection swapping on the most parsimonious tree obtained from the simultaneous analysis. The length obtained by this procedure is not guaranteed to be minimal, but an overestimate of the shortest length for that partition can only overestimate the M/F statistic, and the small number of steps that the CO-1 partition contributes means that a small difference in this number will not significantly affect the value of the statistic.

RESULTS AND DISCUSSION

Sequence Identity

Mitochondrial DNA sequences are known to have transferred to nuclear DNA in many taxa (Zhang and Hewitt, 1996; Sorenson and Fleischer, 1996), potentially confounding to phylogenetic analysis if nuclear pseudogenes are analyzed along with true mitochondrial genes. The methods employed and results obtained in this study indicate that the sequences are indeed mitochondrial in origin. The use of mitochondria-enriched tissue, mitochondrial-sequence-specific primers, and an initial amplification of a large segment of DNA helps ensure that these sequences are not nuclear (Sorenson and Quinn, 1998). The absence of premature stop codons in ND-1 and ND-2 suggests that these regions produce functional mRNA transcripts, and the stable secondary structures of the tRNAs also suggest that these tRNAs function in translating mRNA. In addition, no sequence differences are found between contiguous, overlapping PCR products for any species,

TABLE 2
Summary of Base Composition of 52 Taxa (Excluding *flavescens* and *primordius*, which Lack Sequence for the Second Half of ND-2 and WANCY tRNAs)

	A	T	C	G
Range	0.29–0.39	0.19–0.28	0.25–0.43	0.06–0.12
Mean	0.33	0.23	0.36	0.08

demonstrating that a single long product was initially amplified. The absence of unusually short branches on the most and near-most parsimonious hypotheses also implies that no nuclear sequences were accidentally recovered. The base composition of each taxon for the sequenced region is biased against guanine (Table 2), found to be typical of anguid mitochondrial protein-coding and tRNA genes, but not nuclear genes (e.g. Macey *et al.*, 1999).

tRNA^{Cys} *and the Origin of Light-Strand Replication*

In all *Varanus* sequenced, the last three bases of the origin of light-strand replication overlap with the first three bases of the aminoacyl (AA) stem in tRNA^{Cys}, as has been observed throughout squamates (Macey *et al.*, 1997b, 1999). The 3′-GCC-5′ region that initiates light-strand elongation in *Mus* (Brennicke and Clayton, 1981) is present; the 3′-GBCCB-5′ consensus sequence suggested by Macey *et al.* (1997b) to be related to the 3′-GGCCG-5′ necessary for genome replication in *Homo* (Hixson *et al.*, 1986) is different among most genera sequenced (Table 3). In all *Varanus* species, the D-arm of tRNA^{Cys} has been replaced by a nonpairing region of 3–7 bp. *E. kingi* has a single basepairing that may represent a truncated stem, also noted by Macey *et al.* (1999). *Lanthanotus* has a 3-bp stem, while *Ang. fragilis*, *Ann. pulchra*, and both species of *Heloderma* have a 4-bp stem (Macey *et al.* reported slightly different numbers of stem bases for *Anniella* and *H. suspectum*). The 4-base tandem repeat reported by Macey *et al.* (1997b, 1999) to occur in and around the D-arm for *griseus* does not appear to be necessarily conserved in *Varanus*; the specimen of *g. griseus* sequenced for this study lacks this specific repeat (Fig. 1), and other *Varanus* lack a >2-bp evenly spaced repeat entirely. The African and Asian species of *Varanus* also have the 6-bp stem and 2-bp loop of the T-arm in tRNA^{Cys} noted by Macey *et al.* (1999); however, the Australian species generally have shorter stems, varying from 3 to 5 bases, and larger loops, varying from 1 to 7 bases.

Phylogeny of Varanus

A single most parsimonious cladogram results from simultaneous analysis of all 1474 informative characters (Fig. 2, CI = 0.246, RI = 0.487). This hypothesis is generally well supported. Bremer support and consistency and retention indices were determined for the separate genes and from different functional regions of the genes; these measures demonstrate that all site classes contribute support, and homoplasy in this data

TABLE 3
Aligned Consensus Structures of the Stem and Loop Region of the O_L

Taxon	5′ stem		3′ stem
Anguis and *Elgaria*	CTTCTCCCGTT	(7)	AACGGGAG**AAG**CCCCGGA
Anniella	CTTCTCCCGTT	(10)	AACGGGAG**AAG**CCCGGGA
Heloderma	CTTCTCCCGCC	(6)	AACGGGAG**AAG**TTCCGGG
Lanthanotus and *Varanus*	CTTCTCCCGT$_{2-7}$	(R$_{5-12}$)	A$_{2-7}$CGGGAG**AAG**YCCAGGG

Note. The numbers in parentheses indicate the number of bases in the loop. Subscript numbers show the variation in stem size in *Varanus* and *Lanthanotus*. The total length of the O_L varies from 28 (*Heloderma*) to 44 (*varius*). In *Lanthanotus* and some *Varanus*, G replaces the 5′-most A in the 3′-most stem. Underline indicates the 5′-CGG-3′ region demonstrated by Brennicke and Clayton (1981) to initiate light-strand replication in *Mus*; dotted underline indicates the 5-base region reported by Macey *et al.* (1997b) to complement the heavy-strand sequence 3′-GGCCG-5′ in tRNA^{Cys} necessary for light-strand replication (Hixson *et al.*, 1986). Boldface indicates the overlap between the 3′-most O_L stem and tRNA^{Cys}. Some variable sites in the stem are labeled with standard one-letter code: R = A, G; Y = C, T.

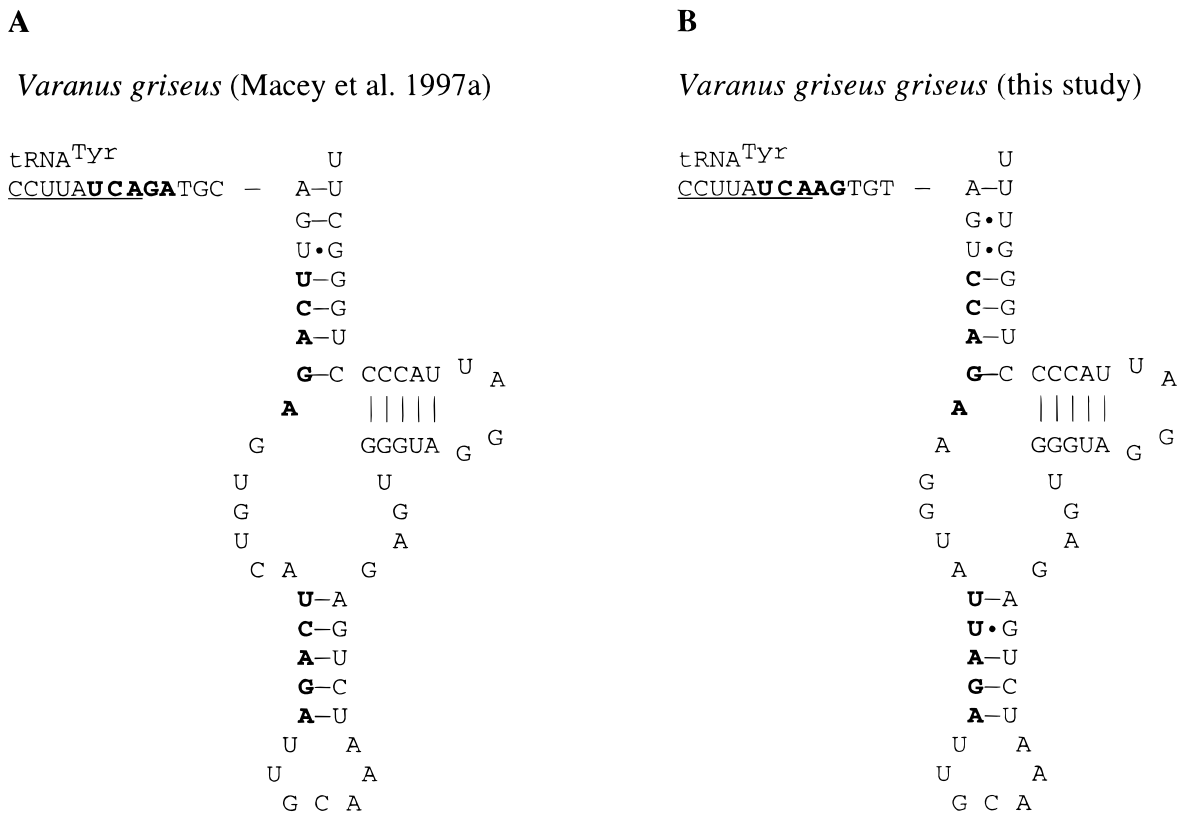


FIG. 1. Inferred secondary structure of tRNA^{Cys} for two individuals of *Varanus griseus* (A, Macey *et al.*, 1997a, Fig. 4; B, this study) represented as 5′ to 3′ RNA transcript and showing the D-arm replacement loop typical of squamates. Underlined region represents the adjacent aminoacyl stem of tRNA^{Tyr}. Boldface indicates tandem repeats found by Macey *et al.* (1997a) lacking in the individual sequenced for this study.

set is attributable mainly to differences within the recognized classes of data, not between them (Table 4). *Varanus* is strongly supported as monophyletic (Bremer support = 105, jackknife support = 1.00), as is the close relationship between *Lanthanotus* and *Varanus* (Bremer support = 51, jackknife support = 1.00) and the monophyly of Varanoidea (*Heloderma*, *Lanthanotus*, and *Varanus*; Bremer support = 42, jackknife support = 1.00).

Within *Varanus*, three major lineages (African, Indo-Asian, and Indo-Australian) are delimited. The African species form a group sister to the rest of *Varanus*, while the Indo-Asian clade is sister to the Indo-Australian clade. The Indo-Asian group, weakly supported as monophyletic, comprises two distinct clades, labeled A and B in Fig. 2. Group A includes terrestrial Asian forms, such as *bengalensis*, and the water monitors of the *salvator* complex. The subspecies of *salvator* form a

FIG. 2. Most parsimonious hypothesis based on simultaneous analysis of 1474 informative characters (length = 11,274, CI = 0.246, RI = 0.487), with species ranges noted (NT, Northern Territory (Australia); PNG, Papua New Guinea). Outgroup taxa not shown. Boldface line indicates the *Varanus* clade, in which only the species name is given. Numbers above branch are Bremer support; numbers below the branch are the percentage node recovery in a parsimony jackknife analysis (see Materials and Methods). Indo-Asian group A consists of mainland Asian and southeast island species, while those in Indo-Asian group B are found primarily in New Guinea (with the exception of *olivaceus*, which is found only in the Philippines).

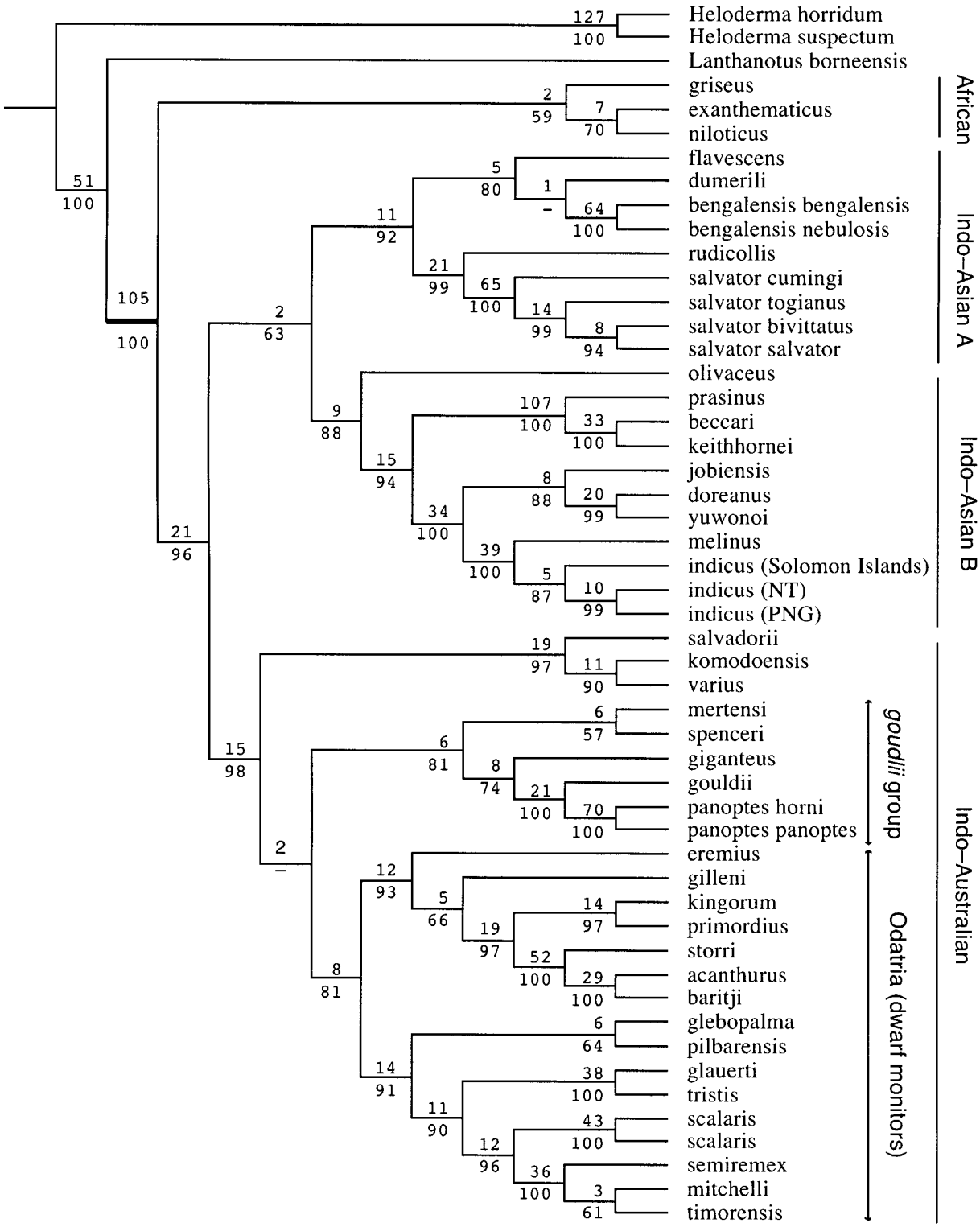


TABLE 4
Indices of Each of the Two Partition Sets

Site class	*n*	Steps	BI	CI	RI
First positions	356	2,067	213	0.322	0.552
Second positions	203	905	201	0.316	0.587
Third positions	651	6,991	635	0.206	0.430
tRNA stems	241	1,152	208	0.301	0.589
tRNA loops	24	159	31	0.258	0.524
ND-1 (total)	502	4,284	547	0.224	0.459
ND-2 (total)	691	5,507	456	0.253	0.484
CO-1 (total)	16	172	45	0.198	0.469
tRNAs (total)	265	1,311	240	0.296	0.582
Simultaneous	1474	11,274	1288	0.246	0.487

Note. In the first set, data are partitioned by the presumed function of sites within genes (five categories) and in the second data are partitioned among genes (four categories). Indices were calculated from the most parsimonious hypothesis using combined data. Rows show indices for each category in the partition: *n*, number of parsimony-informative sites; steps, length on the most parsimonious hypothesis; BI, Bremer support (fractional values have been rounded); CI and RI, ensemble consistency and retention indices, respectively.

distinct and strongly supported monophyletic clade (Bremer support = 65, jackknife = 1.00). In Group B, the herbivorous *olivaceus* is sister to a clade that includes the arboreal *prasinus* group and the mangrove monitors (*indicus* complex) (Bremer support = 15, jackknife support = 0.94). The mangrove monitors form a distinct lineage (Bremer support = 34, jackknife = 1.00) that includes the species *doreanus*, *jobiensis*, *melinus*, and *yuwonoi*, all of which were initially described as subspecies of *indicus*.

The Indo-Australian species are well supported as a single lineage (Bremer support = 15, jackknife = 1.00), within which three major clades may be discerned. The lace monitor *varius*, restricted to eastern Australia, is not closely related to the other large Australian monitors, but rather to the clade including the Komodo dragon *komodoensis* (found on Flores, Komodo, and nearby small islands) and the crocodile monitor *salvadorii* (endemic to New Guinea). This group is sister to the Australian endemics, which includes both large- (*giganteus* and the *gouldii* group) and small-bodied lizards (subgenus *Odatria*). The low support value for the node that unites the *gouldii* group and *Odatria* reflects a tree two steps longer in which the *gouldii* group forms a clade with the (also large-bodied) *komodoensis–salvadorii–varius* clade. In either case, *Odatria* appears to be the monophyletic (Bremer support = 8, jackknife = 0.81) sister taxon to a clade of much larger relatives. Within *Odatria*, there is strong support for monophyly of the spiny-tailed group (*acanthurus*, *baritji*, *kingorum*, *primordius*, and *storri*, BI = 19, jackknife = 0.97; this group is also noted by Baverstock *et al.* (1993)). The reidentification of the "*timorensis*" from Australia's Northern Territory is corroborated by this specimen's close relationship to *scalaris* rather than to a correctly identified *timorensis* from Timor.

Comparison to Previous Phylogenetic Hypotheses

The network of relationships among major groups suggested by King and King (1975) and Holmes *et al.* (1975) is similar to the network in my phylogeny (Fig. 2), although the rooting and therefore inferred direction of chromosome change are quite different. These authors recognize the *gouldii* group (their group A, the Australian members of subgenus *Varanus*, with some intragroup allozyme variation), a group including *indicus* and *varius* (B, not recovered in the present study), a *salvator* group (C, including *bengalensis*, *flavescens*, and *rudicollis*), *Odatria* (D), and separate *griseus* (E) and *niloticus–exanthematicus* (F) groups. King and King (1975, p. 104) infer the direction of evolution by estimating the "primordial form" to be the most common, simplest karyomorph, as represented by group C, with 7 large metacentric chromosome pairs, 1 subacrocentric large chromosome pair, and 12 acrocentric microchromosome pairs. According to King and King's network of relationships, 4 of the large chromosomes have accumulated 11 pericentric inversions and tend toward acrocentricity, while the microchromosomes have independently evolved metacentricity twice. The slightly different network proposed by Holmes *et al.* (1975) requires one less inversion in the large chromosome 5. Given their hypothesized ancestral condition in *Varanus*, mapping onto my cladogram requires 11 inversions in the large chromosomes and 3 in the microchromosomes; however, most lizard species have metacentric rather than acrocentric microchromosomes (King and King, 1975), and if the ancestral condition is metacentricity, then only 2 inversions are required.

The hypotheses resulting from the analyses of Baverstock *et al.* (1993), Card and Kluge (1995), and Fuller *et al.* (1998) are all similar in several ways, although the differences in taxonomic sampling render them not directly comparable. In general, they identify an African group, at least two separate Indo-Asian groups, the *gouldii* group, and *Odatria*. Baverstock *et al.*'s (1993) unrooted hypothesis has a basal polytomy of four groups: the African species, the Indo-Asian and large Indo-Australian species, *Odatria* (excluding *eremius*), and *eremius* alone; the relationships within these groups are generally similar to those found by my DNA analysis. Card and Kluge's (1995) consensus tree has too many polytomies to suggest relationships among the larger groups, although the close relationships they identify between *prasinus* and *indicus* and between *Odatria* and the large Indo-Australian species are corroborated by my hypothesis. The hypothesis of Fuller *et al.* (1998), also based on mitochondrial DNA sequences, is similar to mine in several respects, such as the placement of the African species sister to all other *Varanus* species, resolution of two Indo-Asian clades, and resolution within the Indo-Australian taxa (*salvadorii–komodoensis–varius* sister to the *gouldii* group + *Odatria*). Differences include their placement of *indicus* outside my Indo-Asian B group and *acanthurus* (an odatrian) sister to the *gouldii* group.

Taxonomy

I agree with Baverstock *et al.* (1993, p. 629), who note that the taxonomy of *Varanus*, as proposed by Mertens (1942), has little to commend it in light of recent evidence. Currently 10 subgenera are officially recognized, of which 2 (*Varanus* and *Odatria*) comprise many species, 1 (*Empagusia*) comprises a few species, and 7 (see below) are monotypic. In addition, I recognize Böhme *et al.*'s (1994) and Sprackland' s (1994) resurrection of subgenus *Euprepiosaurus* although the name has yet to regain its formal status.

Subgenus *Varanus* includes large Australian and some Indonesian taxa and is clearly polyphyletic. The Australian species of subgenus *Varanus* (excluding *varius*) are monophyletic (the *gouldii* group), but each of the other species in this subgenus (*komodoensis*, *varius*, and the *salvator* complex) is more closely related to other lineages than they are to one another. Monophyly of *Odatria* is strongly supported if Mertens' placements of *prasinus* in *Odatria* (1942) and *mitchelli* in subgenus *Varanus* (1958) are reassessed; the removal of *prasinus* and the inclusion of *mitchelli* are clearly warranted if taxonomy is to reflect well-corroborated relationships. *Glebopalma* is also an odatrian, despite osteological characters that suggest its affinity with subgenus *Varanus* rather than *Odatria* (A. Kluge, pers. comm.). As *flavescens* is related to other Asian species rather than *exanthematicus* (to which it bears a superficial resemblance), *Empagusia* is polyphyletic. All monotypic subgenera (*Dendrovaranus* for *rudicollis*, *Indovaranus* for *bengalensis*, *Papusaurus* for *salvadorii*, *Phillipinosaurus* for *olivaceus*, *Polydaedalus* for *niloticus*, *Psammosaurus* for *griseus*, and *Tectovaranus* for *dumerili*) are clearly associated with other species, and the use of these names at the subgeneric rank should be reconsidered. Many sources of evidence corroborate monophyly of *Euprepiosaurus*, which comprises the *prasinus* and *indicus* species groups (Sprackland, 1991, 1994; Böhme *et al.*, 1994).

Homoplasy and Data Congruence

The problem of homoplasy in sequence data is of increasing concern to systematists as the potential for within-data incongruence has become more apparent. Homoplasy as measured by incongruence can have several possible causes and is thought to inevitably result when evolutionary rates at different functional sites are heterogeneous. However, a comparison of the consistency and retention indices shows that no given partition set performs notably better or worse than any other. In neither partition analysis is incongruence more pronounced between given partitions sets than it is within a partition set, and the null hypothesis (that each of the partition sets is biased toward different tree topologies) is rejected by the ILD test (codon partition, M / F = 0.0197, real ILD = 1.0, $P < 0.001$; gene partition, M / F = 0.0165, real ILD = 1.0, $P < 0.001$). Partition homogeneity performs a similar test from the opposite point of view (the null hypothesis being that the data are homogeneous across partitions), and in this test the null hypothesis cannot be rejected for either partition ($P = 0.38$ and $P = 0.32$, respectively; see Allard *et al.* (1999) for a discussion of how ILD and partition homogeneity test results compare). A graph of the partitioned Bremer support value of each node for the most parsimonious hypothesis (Fig. 3) demonstrates

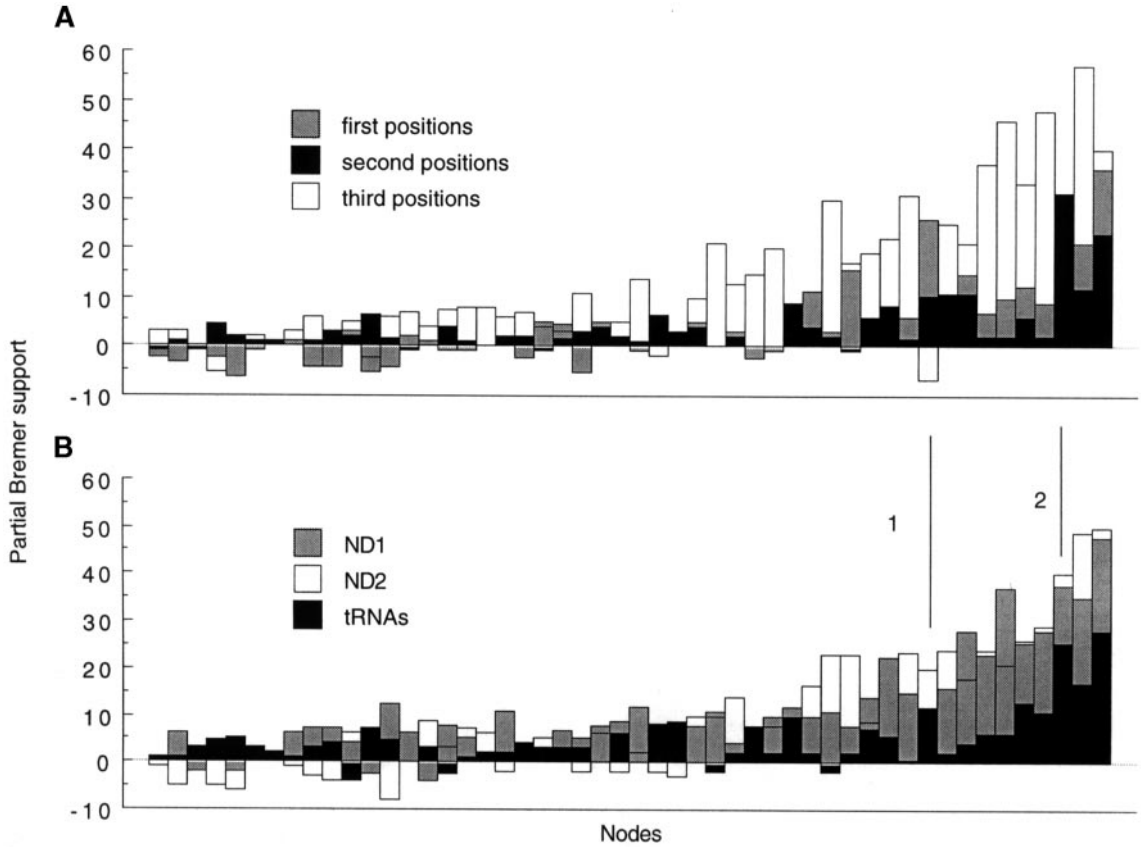
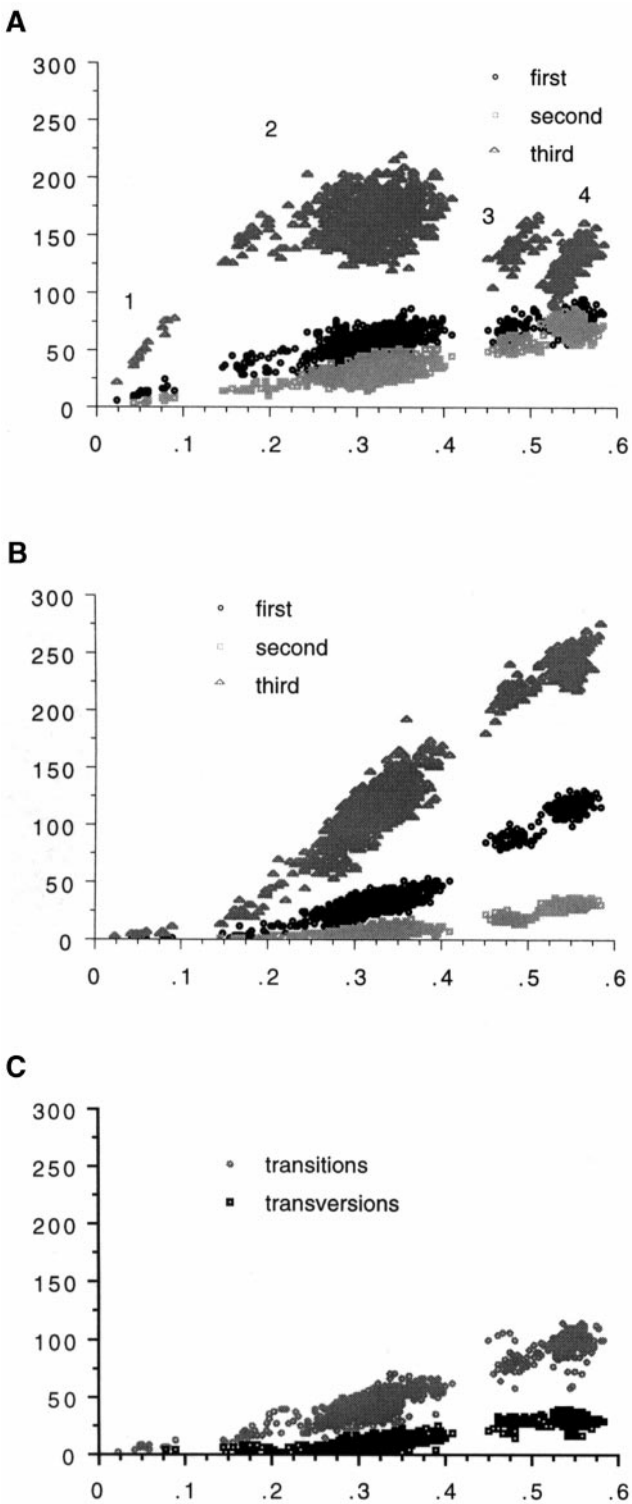


FIG. 3. Graph showing partitioned branch support at nodes for the two partition sets: (A) first, second, and third codon positions, and (B) ND-1, ND-2, and the nine tRNAs combined (CO-1 not included). Both graphs are ordered by increasing total branch support on the total evidence tree. Line 1 indicates the node for Varanoidea; line 2 indicates the *Varanus* node. Positive numbers indicate that the given partition contributes to support at that node; negative numbers indicate that the given partition favors an alternative hypothesis.

that, at most nodes, support from each partition was positive; however, some conflict between partitions can be seen at nodes in which support is positive for one character set and negative for another.

As an extension of the rate heterogeneity argument, incongruence in a phylogenetic hypothesis is also thought to necessarily result from the more rapidly changing sites being saturated with change. That data are saturated is often used as a basis for downweighting, excluding, or transforming data before a phylogenetic analysis is performed, the rationale being that such data have suffered multiple substitutions at each site and the phylogenetic signal has been overwritten (e.g. Swofford *et al.*, 1996). A plot of the transitions and transversions for the different partitions reveals that most of the data do not have a profile typical of saturated data, with the exception of third position transitions (Fig. 4), in which the amount of change is no longer positively correlated with increasing genetic distance.

When transitions in the third codon positions are weighted to zero, the resulting two equally parsimonious hypotheses differ from that of the simultaneous analysis in placing the *gouldii* group as sister to the *salvadorii–komodoensis–varius* clade, resolving a slightly different relationship of *glebopalma* and *pilbarensis* in *Odatria* and producing uncertain resolution among *salvator bivittatus, s. salvator,* and *s. togianus*. When third codon positions are eliminated altogether, two different most parsimonious hypotheses result, the consensus of which results in additional resolution loss within *Odatria*. Jackknife support lessens or disappears for



several intermediate and recent nodes in both of these searches. These results are not especially surprising given that the third positions contribute nearly half of the informative characters and more than half of the total tree length (Table 3). Change is indisputably more frequent in third positions than in first or second codon positions or tRNAs.

However, relative frequency of change alone is not a reliable predictor of phylogenetic informativeness (Björklund, 1999; Källersjö *et al.*, 1999; Sennblad and Bremer, 2000), and percentage sequence divergence or saturation curves alone may not reflect whether phylogenetic structure remains present. When taxonomic sampling includes both recent and ancient nodes, as does the present analysis, rapidly changing characters (such as the third codon positions) will provide support to the relatively recent nodes, while the more slowly changing sites (such as the second codon positions) will support the deeper nodes. That the rapidly changing characters may be relatively incongruent at deeper nodes does not necessarily interfere with overall tree resolution, or overwhelm the analysis with incongruence, as long as other characters are present to resolve these older nodes (for review see Allard *et al.*, 1999). In the present analysis, the tRNA and first and second codon changes are providing the needed support at the more ancient nodes, such as those delimiting Varanoidea and *Varanus* (Fig. 3).

The sequence data used in this study demonstrate that heterogeneous evolutionary rates are not necessarily detrimental to phylogenetic analysis. The lower CI and RI values for third positions (Table 3) indicate that these data are more homoplastic than other classes of characters, and the saturation curve (Fig. 4) demonstrates that at least transitions in the third codon position occur frequently enough to have been replaced multiple instances in the time since the divergence

FIG. 4. Graphs of transitions (A) and transversions (B) at each codon position and transitions and transversions in the tRNAs (C). Note four distinct groups of points, which represent different groups of taxa: (1) closely related taxa, such as the *indicus* and *salvator* complexes, and the two individuals of *scalaris* and *panoptes*; (2) *Varanus* species compared to other *Varanus* species; (3) *Lanthanotus* compared to *Varanus*; (4) *Varanus* compared to *Heloderma* and the outgroup taxa.

of anguioids and varanoids. Nevertheless, when the rapidly evolving characters are eliminated from the analysis, several equally parsimonious hypotheses result and support at many nodes is drastically diminished. This reduction in both resolution and support indicates that rapidly evolving regions can contribute positively to a hypothesis, a fact not immediately evident if data are eliminated *a priori* and not first analyzed in a phylogenetic context.

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REFERENCES

Allard, M. W., Farris, J. S., and Carpenter, J. M. (1999). Congruence among mammalian mitochondrial genes. *Cladistics* **15,** 75–84.

Bartholomew, G. A., and Tucker, V. A. (1964). Size, body temperature, thermal conductance, oxygen consumption and heart rate in Australian varanid lizards. *Physiol. Zool.* **37,** 341–354.

Baverstock, P. R., King, D., King, M., Birrell, J., and Krieg, M. (1993). The evolution of species of the Varanidae: Microcomplement fixation analysis of serum albumins. *Aust. J. Zool.* **41,** 621–638.

Becker, H. O. (1991). The lung morphology of *Varanus yemenensis* (Böhme, Joger and Schatti) and its bearing on the systematics of the Afro-Asian monitor radiation. *Mertensiella* **2,** 29–37.

Björklund, M. (1999). Are third positions really that bad? A test using vertebrate cytochrome b. *Cladistics* **15,** 191–197.

Böhme, W. (1988). Zur Genitalmorphologie der Savria; Functionelle und stammesgeschichtliche Aspekte. *Bonn. Zool. Beitr.* **40,** 27–56.

Böhme, W. R., Horn, H.-G., and Ziegler, T. (1994). Zur Taxonomie der Pazifikwarane (*Varanus indicus*-Komplex): Revalidierung von *Varanus doreanus* (A. B. Meyer, 1874) mit Beschreibung einer neuer Unterart. *Salamandra* **30,** 119–142.

Branch, W. R. (1982). Hemipeneal morphology of platynotan lizards. *J. Herpetol.* **16,** 16–38.

Bremer, K. (1994). Branch support and tree stability. *Cladistics* **10,** 295–304.

Brennicke, A., and Clayton, D. A. (1981). Nucleotide assignments of alkali-sensitive sites in mouse mitochondrial DNA. *J. Biol. Chem.* **256,** 10613–10617.

Camp, C. L. (1923). Classification of the lizards. *Bull. Am. Mus. Nat. Hist.* **48,** 289–435.

Card, W., and Kluge, A. G. (1995). Hemipeneal skeleton and varanid systematics. *J. Herpetol.* **29,** 275–280.

Carroll, R. L., and deBraga, M. (1992). Aigialosaurs: Mid-Cretaceous varanoid lizards. *J. Vert. Paleo.* **12,** 66–86.

De Salle, R., and Brower, A. V. Z. (1997). Process partitions, congruence, and the independence of characters: Inferring relationships among closely related Hawaiian *Drosophila* from multiple gene regions. *Syst. Biol.* **46,** 751–764.

Estes, R. K. (1983). The fossil record and early distribution of lizards. *In* "Advances in Herpetology and Evolutionary Biology" (A. Rhodin and K. Miyata, Eds.), pp. 365–391. Museum of Comparative Zoology, Cambridge, MA.

Estes, R., de Queiroz, K., and Gauthier, J. (1988). Phylogenetic relationships within Squamata. *In* "Phylogenetic Relationships of the Lizard Families" (R. Estes and G. Pregill, Eds.), pp. 119–281. Stanford Univ. Press, CA.

Farris, J. S. (1996). XARN. Naturhistoriska riksmuseet, Stockholm.

Farris, J. S., Albert, V. A., Källersjö, M., Lipscomb, D., and Kluge, A. G. (1996). Parsimony jackknifing outperforms neighbor-joining. *Cladistics* **12,** 99–124.

Farris, J. S., Källersjö, M., Kluge, A. G., and Bult, C. (1994). Testing significance of incongruence. *Cladistics* **10,** 315–319.

Farris, J. S., Källersjö, M., Kluge, A. G., and Bult, C. (1995). Constructing a significance test for incongruence. *Syst. Biol.* **44,** 570–572.

Fuller, S., Baverstock, P., and King D. (1998). Biogeographic origins of goannas (Varanidae): A molecular perspective. *Mol. Phylogenet. Evol.* **9,** 294–307.

Gao, K., and Norell, M. A. (2000). Taxonomic composition and systematics of Late Cretaceous lizard assemblages from Ukhaa Tolgod and

adjacent localities, Mongolian Gobi Desert. *Bull. Am. Mus. Nat. Hist.* **249,** 1–118.

Giribet, G., and Wheeler, W. (1999). On gaps. *Mol. Phylogenet. Evol.* **13,** 132–143.

Graybeal, A. (1998). Is it better to add taxa or characters to a difficult phylogenetic problem? *Syst. Biol.* **47,** 9–17.

Harvey, M. B., and Barker, D. G. (1998). A new species of blue-tailed monitor lizard (genus *Varanus*) from Halmahera Island, Indonesia. *Herpetology* **54,** 34–44.

Hillis, D. M. (1998). Taxonomic sampling, phylogenetic accuracy, and investigator bias. *Syst. Biol.* **47,** 3–8.

Hixson, J. E., Wong, T. W., and Clayton, D. A. (1986). Both the conserved and divergent 5′-flanking sequences are required for initiation at the human mitochondrial origin of light strand replication. *J. Biol. Chem.* **261,** 2384–2390.

Holmes, R. S., King, M., and King, D. (1975). Phenetic relationships among varanid lizards based upon comparative electrophoretic data and karyotypic analyses. *Biochem. Syst. Ecol.* **3,** 257–262.

Källersjö, M., Albert, V. A., and Farris, J. S. (1999). Homoplasy *increases* phylogenetic structure. *Cladistics* **15,** 91–93.

King, D., King, M., and Baverstock, P. (1991). A new phylogeny of the Varanidae. *Mertensiella* **2,** 211–219.

King, M. (1990). Chromosomal and immunogenetic data: A new perspective on the origins of Australia's reptiles. *In* "Cytogenetics of Amphibians and Reptiles" (E. Olmo, Ed.), pp. 153–180. Birkhäuser Verlag, Basel.

King, M., and King, D. (1975). Chromosomal evolution in the lizard genus *Varanus* (Reptilia). *Aust. J. Biol. Sci.* **28,** 89–108.

Kluge, A. G. (1989). A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). *Syst. Biol.* **38,** 7–25.

Kumazawa, Y., Ota, H., Nishida, M., and Ozawa, T. (1996). Gene rearrangements in snake mitochondrial genomes: Highly concerted evolution of control-region-like sequences duplicated and inserted into a tRNA cluster. *Mol. Biol. Evol.* **13,** 1242–1254.

Losos, J. B., and Greene, H. W. (1988). Ecological and evolutionary implications of diet in monitor lizards. *Biol. J. Linnean Soc.* **35,** 379–407.

Lynch, M. (1996). Mutation accumulation in transfer RNAs: Molecular evidence for Muller's ratchet in mitochondrial genomes. *Mol. Biol. Evol.* **13,** 209–220.

Macey, J. R., Larson, A., Anajeva, N. B., and Papenfuss, T. J. (1997a). Replication slippage may cause parallel evolution in the secondary structure of mitochondrial transfer RNAs. *Mol. Biol. Evol.* **14,** 30–39.

Macey, J. R., Larson, A., Anajeva, N. B., Fang, Z., and Papenfuss, T. J. (1997b). Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. *Mol. Biol. Evol.* **14,** 91–104.

Macey, J. R., Schulte, J. A., II, Larson, A., Tuniyev, B. S., Orlov, N., and Papenfuss, T. J. (1999). Molecular phylogenetics, tRNA evolution, and historical biogeography in anguid lizards and related taxonomic families. *Mol. Phylogenet. Evol.* **12,** 250–272.

Mertens, R. (1942). Die Familie der Warane (Varanidae). Dritte Teil: Taxonomie. *Abh. Senck. Naturf. Ges.* **466,** 235–391.

Mertens, R. (1958). Bemerkungen über die Warane Australiens. *Senck. Biol.* **39,** 229–264.

Mickevitch, M. F., and Farris, J. S. (1981). The implications of congruence in *Menidia. Syst. Zool.* **30,** 351–370.

Miyamoto, M. M., and Cracraft, J. (1991). Phylogenetic inference, DNA sequence analysis, and the future of molecular systematics. *In* "Phylogenetic Analysis of DNA Sequences" (M. M. Miyamoto and J. Cracraft, Eds.), pp. 3–17. Oxford Univ. Press, New York.

Norell, M. A., and Gao, K. (1997). Braincase and phylogenetic relationships of *Estesia mongoliensis* from the Late Cretaceous of the Gobi Desert and the recognition of a new clade of lizards. *Am. Mus. Novitates* **3211,** 1–25.

Pesole, G., Gissi, C., De Chirco, A., and Saccone, C. (1999). Nucleotide substitution rate of mammalian mitochondrial genomes. *J. Mol. Evol.* **48,** 427–434.

Pianka, E. (1995). Evolution of body size: Varanid lizards as a model system. *Am. Nat.* **146,** 398–414.

Pregill, G. K., Gauthier, J. A., and Greene, H. W. (1986). The evolution of helodermatid squamates, with a description of a new taxon and an overview of Varanoidea. *Trans. San Diego Soc. Nat. Hist.* **21,** 167–202.

Rieppel, O. (1980). The phylogeny of anguimorph lizards. *Denkschr. Schweiz. Naturforsch. Ges.* **94,** 94–86.

Rieppel, O. (1988). The classification of the Squamata. In "The Phylogeny and Classification of the Tetrapods, Volume 1: Amphibians, Reptiles, and Birds" (M. J. Benton, Ed.), pp. 261–293. Clarendon, Oxford.

Sennblad, B., and Bremer, B. (2000). Is there a justification for differential a priori weighting in coding sequences? A case study from *rbcL* and Apocyanaceae s. 1. *Syst. Biol.* **49,** 101–113.

Sorenson, M. D. (1999). TreeRot, version 2. Boston University, Boston, MA.

Sorenson, M. D., Ast, J. C., Dimcheff, D. E., Yuri, T., and Mindell, D. P. (1999). Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Mol. Phylogenet. Evol.* **12,** 105–114.

Sorenson, M. D., and Fleischer, R. C. (1996). Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. *Proc. Natl. Acad. Sci. USA* **93,** 15239–15243.

Sorenson, M. D., and Quinn, T. W. (1998). Numts: A challenge for avian systematics and population biology. *The Auk* **115,** 214–221.

Sprackland, R. (1991). The origin and zoogeography of monitor lizards of the subgenus *Odatria* Gray (Sauria: Varanidae): A re-evaluation. *Mertensiella* **2,** 240–252.

Sprackland, R. (1994). Rediscovery and taxonomic review of *Varanus indicus spinulosis*, Mertens, 1941. *Herpetofauna* **24,** 33–39.

Swofford, D. L. (1999). PAUP*: Phylogenetic Analysis Using Parsimony (beta version). Sinauer, Sunderland, MA.

Swofford, D. L., Olsen, G. J., Waddell, P. J., and Hillis, D. M. (1996). Phylogenetic inference. *In* "Molecular Systematics" (D. M. Hillis, C. Moritz, and B. K. Mable, Eds.), 2nd ed., pp. 407–514. Sinauer, Sunderland, MA.

Wheeler, W. (1996). Optimization alignment: The end of multiple sequence alignment in phylogenetics? *Cladistics* **12,** 1–9.

Zhang, D.-X., and Hewitt, G. M. (1996). Nuclear integrations: Challenges for mitochondrial DNA markers. *TREE* **11,** 247–251.