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Biogeographic Origins of Goannas (Varanidae): A Molecular Perspective

Susan Fuller,*,† Peter Baverstock,* and Dennis King‡

*School of Applied Science, Southern Cross University, P.O. Box 157, Lismore, 2480, New South Wales, Australia; †Laboratoire de Biologie des Invertebres, INRA, 123 Boulevard Francis Meilland, B.P. 2078, 06606, Antibes Cedex, France; and ‡Western Australian Museum, Francis Street, Perth, 6000, Western Australia, Australia

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This project aims to clarify the phylogenetic relationships among the extant species of *Varanus* in order to elucidate the origins of Varanidae, using DNA sequences. Results obtained for a minimum of 662 nucleotides of 12S rRNA sequence data from each of 21 extant species of *Varanus* indicate that the Australian varanids form a single monophyletic clade and also suggest that within the Australian varanids, members of the subgenus *Odatria* (pygmy monitors) may form a clade separate from those in the subgenus *Varanus* (large monitors). The Asian species appear to be sister taxa to the Australian species, while the two African species investigated were most divergent, suggesting that the Varanidae are not Gondwanic in origin. Hypothesis testing analyses were performed and involved constraining the 12S sequence data according to previously described topologies and testing the difference using parametric and nonparametric statistics. The phylogeny generated using 12S sequence data was statistically different from previously described morphological trees, while there was some support for topologies based on chomosomal and immunological datasets. Overall, our results suggest that the Australian species may be derived from an Asian source and are, therefore, in agreement with the hypothesis based on the fossil record suggesting that Varanidae may be Asian in origin. © 1998 Academic Press

INTRODUCTION

The herpetofauna of Australia can be divided into two major elements; the old Gondwana faunal derivatives and the more recent invaders from the north (Hecht, 1975). Prior to the 1970s, when continental drift theories were not widely accepted, Australia's entire biota was assumed to have been derived by multiple invasions from Asia. For the most part, these assumptions were based on mainly noncladistic morphological classifications, the fossil record, and the present geographic distribution of the extant species. Correct biogeographical conclusions are more likely to result from well-tested cladistic relationships. This has been demonstrated by Baverstock and Donnellan (1990), who showed that the Australian lizard family Agamidae actually has a Gondwanic rather than an Asian origin.

The family Varanidae also provides an interesting biogeographic model. The 44 recognized extant species are distributed throughout the former Eurasia (the Middle East, southern USSR, and Asia) and parts of the former Gondwana continent (i.e., Africa and Australia). For a number of years, it has generally been accepted that varanids arose in Laurasia approximately 65 MYA (Hoffstetter, 1968) and subsequently radiated into Africa (Branch, 1982) and Australasia (Cogger and Heatwole, 1981) during the late Tertiary period. This conclusion was based primarily on two factors, namely, that the earliest known varanoid fossils came from North America, Europe, and Mongolia and that the majority of the living subgenera of the family Varanidae are found in Eurasia (Hecht, 1975). A Gondwanic origin was not considered likely because the present varanid distribution does not encompass South America. On the assumption that the earliest varanid fossils in Australia have been found and those that have been classified were correctly dated as coming from Miocene deposits (15 MYBP, Hecht, 1975), then the presence of varanids in South America is unlikely as it had already split (approximately 45–50 MYBP) from the Gondwanan landmass.

The first attempts to classify varanids into higher taxonomic groups were made by Mertens (1942a,b,c, 1958, 1963). Using osteological and external morphological characters, Mertens described 10 subgenera within the family. However, more recently several of Mertens' conclusions have been questioned. This is not surprising given the high degree of morphological conservativeness exhibited within the family (Pianka, 1995). Bohme (1988) proposed a phylogeny for 26 species of varanids, based on hemipeneal morphology (Fig. 1A), that showed two major radiations (Afro-Asian and Indo-Australian). Subsequently, Becker *et al.* (1989) proposed a phylogeny based on lung morphology (Fig. 1B) which was

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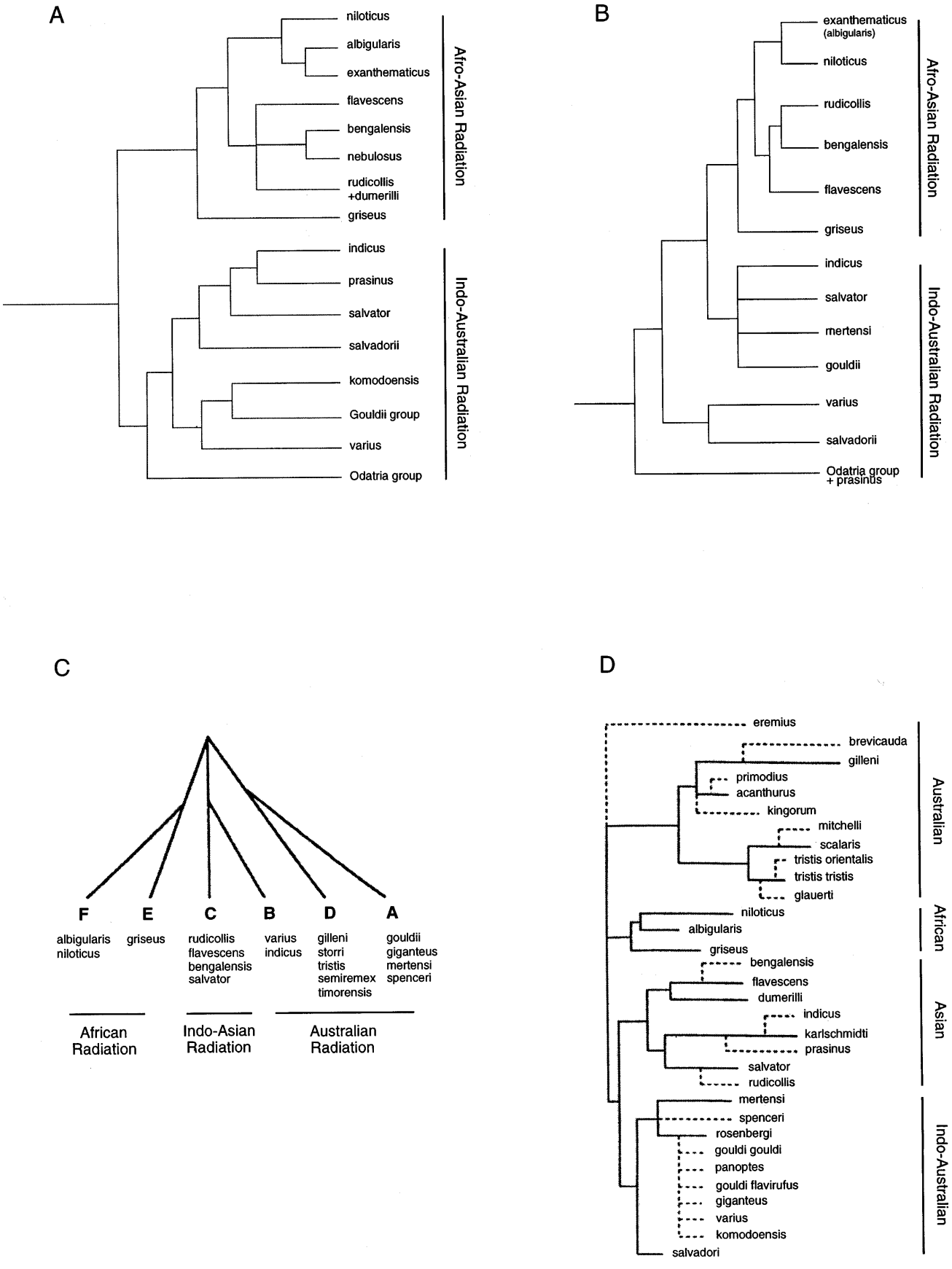

FIG. 1. Proposed phylogeny of varanids based on hemipeneal morphology (A) after Bohme (1988), lung morphology (B) after Becker *et al.* (1989), chromosomal morphology (C) after King and King (1975), and one-way (solid line) and reciprocal (dashed line) MC'F tests (D) after Baverstock *et al.* (1993).

largely (although not entirely) in agreement with that proposed by Bohme (1988).

The first phylogenies based on genetic data were obtained when King and King (1975) performed a karyological study on 16 of the 32 varanid species then described. Later, King (1990) extended this study to include 27 varanid species. These studies identified three distinct evolutionary lineages in *Varanus* and postulated that there were two independent invasions to Australia from an Asian source and the differentiation of a third radiation within Australia (Fig. 1C). Based on the absence of fossils from Africa before the Miocene and Pliocene (Clos, 1995; Hoffstetter, 1968), they also suggested that African taxa were the result of a recent Middle Eastern/Asian invasion. An electrophoretic analysis was undertaken by Holmes *et al.* (1975), but an insufficient number of markers were used to accurately resolve cladistic relationships.

Using microcomplement fixation (MC'F), King *et al.* (1991) and Baverstock *et al.* (1993) examined 32 of the 44 species of *Varanus* and generated a phylogeny (Fig. 1D) composed of three major lineages (the African species, the subgenus *Odatria*, and the subgenus *Varanus*/Asian species). However, in spite of an unresolved polychotomy, their data provided strong evidence that the Australian varanids were at least diphyletic. With this scenario it would therefore be more parsimonious to postulate an Australian origin for Varanidae (with one invasion to Asia), rather than an Asian origin (with two invasions into Australia), therefore suggesting that the Varanidae may have arisen in Australia (i.e., have a Gondwanic origin). Furthermore, these proposed multiple invasions were dated using an albumin molecular clock at 25 MYA, at a time when Australasia was closer to Antarctica than to Asia (Flannery, 1989). A Gondwana origin is also more consistent with the high species diversity within Australia, where 27 of the 44 species are found.

Given the apparent contradictions concerning the origins and evolution of *Varanus*, it was the objective of this study to clarify the cladistic relationships within Varanidae and to elucidate the origins of Varanidae using DNA sequences. The molecular phylogeny was compared to phylogenies that have been previously proposed based on morphological, chromosomal, and immunological characters. The mitochondrial 12S rRNA gene was chosen for study because it appears to provide informative phylogenetic data (i.e., to contain sufficient character changes without being obscured by multiple substitutions at a single site (Hillis and Dixon, 1991; Graybeal, 1994)) for the resolution of species-level relationships.

MATERIALS AND METHODS

DNA Extraction and Sequencing

DNA was extracted from small amounts of liver, blood, or sloughed skin tissue from *Lanthanotus borneensis*, *Heloderma suspectum*, and the following species of *Varanus; V. niloticus, V. albigularis, V. bengalensis, V. olivaceus, V. salvator, V. dumerilli, V. prasinus, V. indicus, V. komodoensis, V. salvadorii, V. giganteus, V. mertensi, V. gouldii, V. varius, V. eremius, V. timorensis, V. pilbarensis, V. mitchelli, V. acanthurus, V. brevicauda,* and *V. tristis* (museum specimen identification details are given in the Appendix).

Extant platynotan squamates, including varanoid lizards (genera *Heloderma, Lanthanotus, Varanus*) and snakes, are considered monophyletic (Lee, 1997). On the basis of skull and postcranial skeleton morphology, *Lanthanotus* has been found to be intermediate in structure between *Heloderma* and *Varanus* and cladistically the sister group of *Varanus* among extant varanoids (Rieppel, 1980; Lee, 1997). Studies of hemipeneal morphology revealed that *Lanthanotus* and *Varanus* share a unique synapomorphy not found in *Heloderma*, therefore grouping these genera together (Branch, 1982). Extant members of the Varanidae have been placed in a single genus, which has generally been considered monophyletic (Pregill *et al.*, 1986). In the present study, therefore, *L. borneensis* and *H. suspectum* were chosen as outgroup taxa.

Each tissue was minced and digested in 500 µl of extraction buffer (100 mM NaCl, 50 mM Tris (pH 8.0), 10 mM EDTA (pH 8.0), 0.5% SDS, 0.2 mg Proteinase k) for 2.5 h at 55°C, with slight agitation. Purification was performed using two phenol:chloroform (1:1) extractions, a single chloroform extraction (Sambrook *et al.*, 1989), and one ethanol precipitation in 0.1 vol of 3 M sodium acetate and 2 vol of absolute ethanol. DNA was recovered after storing at −70°C for 15 min, centrifugation at 10500 rpm (Beckman J21M/E) for 5 min, and a final ethanol wash (70%). The DNA pellet was resuspended in 500 µl TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and stored at 4°C until used.

A segment of 12S rRNA approximately 380 bp in length (called 12S a/b) was amplified using polymerase chain reaction (PCR, Saiki *et al.*, 1988) with the primers developed by Kocher *et al.* (1989) and modified by M. S. Elphinstone. These primers were MT1091L (5′CAAACTGGGATTAGATACCCCACTAT3′) and MT1478H (5′-TGACTGCAGAGGGTGACGGGCGGTGTGT3′), where the numbers refer to the relative position in the human sequence (Kocher *et al.*, 1989). Subsequently, to increase phylogenetic resolution a second region of the 12S rRNA gene of approximately 400 bp (called 12S c/d) was amplified using primers defined by the authors, from the conserved region of the 12S rRNA gene across human, chicken, and frog. These primers were MT0698L (5′ATGCAAGCATCCGCACTCCCGTGA3′) and MT1076H (5′TTTAGGGCTAGGCATAGTGGGGTATCT3′). Individual PCR reactions were performed in a 25-µl final volume containing concentrations of deoxynucleoside triphosphate (dNTPs) of 100 µM, 100 nM each primer, Boehringer-Mannheim 1× *Taq* enzyme buffer (containing a final concentration of 1.5 mM $MgCl_2$), 0.5 units of *Taq* polymer-

ase (Boehringer-Mannheim), and 100 ng of template DNA in water. Temperature cycling (denaturation 93°C, annealing 52°C, and extension 75°C) was performed in a Minicycler thermal controller (MJ Research Inc.).

The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN GmbH), following the manufacturer's directions. The purified PCR products were eluted into 30 µl of sterile water and prepared for sequencing using the PRISM Ready Reaction Dye-Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems), following the manufacturer's directions. The extension products were sequenced using the Applied Biosystems Model 373A DNA Sequencing System. Each DNA fragment was sequenced from both the 3′ and 5′ ends, sometimes more than once in an effort to resolve any ambiguous positions. The sequences have been deposited in GenBank (Accession numbers AF004473 to AF004518).

Sequence Analyses

The sequences were aligned by eye using the computer program SeqEd 675 (v1.00A, Applied Biosystems). With the inclusion of the outgroup taxa, computer-assisted alignment was necessary using the program Clustal-W (Thompson *et al.*, 1994) using the default settings. The final output alignment for the 23 taxa was adjusted by eye.

Phylogenetic analysis involved both character-based methods (maximum-parsimony method (heuristic search), Swofford (1993), and maximum-likelihood method, Felsenstein, 1993) and distance-based methods (neighbor-joining method (Saitou and Nei, 1987) using Kimura's (1980) two-parameter distance estimate, Kumar *et al.*, 1993). The maximum-likelihood analyses were performed using the following parameters: empirical base frequencies and a default transition:transversion ratio (2:1) were used, the input order of sequences was not randomized, and global rearrangements were not employed. All phylogenetic analyses were based on equally weighted characters, with insertion/deletion events considered missing data. The robustness of the tree generated from maximum-parsimony analysis was evaluated using 500 bootstrap iterations (Felsenstein, 1985). The Bremer support index, which is the minimum number of extra steps required to break up a clade found on the most parsimonious tree, was also estimated to give an indication of the support for each node. The $g1$ statistic was calculated to determine whether the phylogenetic signal of the data was significantly greater than expected for random sequence (Hillis and Huelsenbeck, 1992).

Statistical Analyses

The maximum-likelihood method (Felsenstein, 1981), which finds the hypothesis that maximizes the probability of observing the data obtained, was primarily used for statistically testing alternative topologies against the one with the highest likelihood. This test, formulated by Kishino and Hasegawa (1989), uses the mean and variance of log-likelihood differences between trees, taken across sites. If the mean is more than 1.96 standard deviations different, then the trees are declared significantly different (Felsenstein, 1993). The 12S sequence data were constrained according to the alternative topologies proposed by previous studies based on lung (Becker *et al.*, 1989), hemipeneal (Bohme, 1988), chromosomal (King and King, 1975), and immunological (Baverstock *et al.*, 1993) data (Figs. 1A to 1D, respectively). The data were also analyzed after imposing the constraint that members of the subgenera *Odatria* and *Varanus* formed two independent monophyletic clades.

The strength of the best supported hypothesis generated under maximum parsimony was also evaluated by comparing its tree length to the lengths of alternative trees constrained to match the topologies shown in Figs. 1A to 1D. Statistical significance was assessed using the nonparametric Wilcoxon matched-pairs signed-ranks test suggested by Templeton (1983), following the method used by Sites *et al.* (1996) for sequence data. This procedure involved comparing the number of characters that undergo a different number of changes in the unconstrained versus alternative topologies, ranking these differences and assigning a sign to the rank (positive or negative depending on whether the difference favored the unconstrained or the alternative topology). If the sum of the positive ranks was very different from the sum of the negative, then the two topologies were considered significantly different. A normal approximation of the Wilcoxon test was used for large samples ($n > 25$), as outlined by Siegel (1956).

RESULTS

MtDNA Sequence Variation in Varanus

The aligned 341-base sequence for the 12Sa/b fragment and the 360-base sequence for the 12Sc/d fragment, corresponding to positions L1140 and L750 in the human sequence, respectively, are given in Fig. 2. Some sections of DNA were excluded (indicated in Fig. 2 by the boldface segment (**C**) in each sequence); they could not be unambiguously aligned because insertions or

FIG. 2. DNA sequence data for the two segments of 12S rRNA for the 23 taxa. Note: Bold print indicates regions that were deleted because of ambiguity, a dot denotes identity with the first sequence, a dash denotes a gap, and a question mark denotes missing data. Abbreviations: Helode, *Heloderma suspectrum;* Lantha, *Lanthanotus borneensis;* V. nilo, *Varanus niloticus;* V. albi, *V. albigularis;* V. beng, *V. bengalensis;* V. dume, *V. dumerilli;* V. oliv, *V. olivaceus;* V. salt, *V. salvator;* V. pras, *V. prasinus;* V. indi, *V. indicus;* V. komo, *V. komodoensis;* V. giga, *V. giganteus;* V. goul, *V. gouldii,* V. mert, *V. mertensi;* V. sald, *V. salvadorii;* V. vari, *V. varius;* V. mitc, *V. mitchelli;* V. acan, *V. acanthurus;* V. brev, *V. brevicauda;* V. erem, *V. eremius;* V. pilb, *V. pilbarensis;* V. timo, *V. timorensis;* and V. tris, *V. tristis.*

12S a/b

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Helode AAAACAAAAT TATCCGCCAG AGAACTACGA GTGAAAAACT TAAAACTCAA AGGACTTGGC
Lantha .......CCC -T........ ..G.....T. .CA.C..G.. .......... ..........
V.nilo ........CC -..T....C. ....T...C. .C-.C.GG.. A......... .A......A.
V.albi ........CC -..T....C. ........C. .C-.CC.G.. A......... .A......A.
V.beng CC.....CCC -..T..G.C. ..G.G...C. .G-.C..G.. AG........ .A......A.
V.dume .T....?CCC -..T....C. ........C. .C-....G.. A......... .A......A.
V.oliv .C.....CCC -..T....C. ........C. .C-....G.. A......... .A......A.
V.salt .......CCC -..T....T. ........C. .C-.CG.G.. A......... .A......A.
V.pras TC.....CCC -..T....C. ....T...C. .C-.T..G.. A......... .A......A.
V.indi T.....GCCC -..T....C. ....T...C. .C-....G.. A......... .A......A.
V.komo .......CCC -..T....C. ........C. .C-.C..G.. A......T.. .A......A.
V.giga CT....GCCC -..T....C. ........C. .C-.C..G.. A......... .A......A.
V.goul CC.....CCA -.CT....C. ........C. .C-.C..G.. A......... .A......A.
V.mert TT.....CCC -..T....C. ........C. .C-.C..G.. A......... .A......A.
V.sald CG.G..GCCC -..T....C. ........C. .C-.C..G.. A......... .A......A.
V.vari T......CCC -..T....C. ........C. .C-.C..G.. A......... .A......A.
V.mitc T.G....CCC -.CT...... ....T...C. .C-.T..G.. A......... .A......A.
V.acan T......CCC -.CT....C. ..T.T...C. .C-.C..G.. A......... .A......A.
V.brev .......TCC -.......C. ........C. .C-.C..G.. AG........ .A......A.
V.erem .G.....CCC -.......T. ........T. .C-....G.. A......... .A......A.
V.pilb TC......CC -.CT....C. ........C. .C-.T..G.. A......... .A......A.
V.timo ...G...CCC -..T....C. ..G.....C. .C-.C..G.. A......... .A......A.
V.tris ......GCCC -..G....C. ........C. .C-.C..G.. A......... .A......A.

Helode GGTGCCCCAT ACTCAGCCTA GAGGAGCCTG TCCTATAATC GATAATCCAC GATAAACCTA
Lantha ....--.TC. ..C.CA-... .......... ........C. ....C...C. ...CC...C.
V.nilo ....--.TC. ..C.TA.... .......... .......... ....C..... ..CCT....G
V.albi ....--.TCC ..C.TA.... .......... .......... ....CC.... ...CC...CG
V.beng ....--.TC. ..C.TA.... .......... .......... ....CC.... ...CT...C.
V.dume ....--.TC. ..C.TA.... .......... .......... ....CC.... ...TC...C.
V.oliv ....--.TC. .TC.T..... .......... .......... ....C..... ...CC...CG
V.salt ....--.TC. ..C.TA.... .......... .......... ....CC.... ...CC...CG
V.pras ....--.TC. ..C.T..... .......... .......... ....T..... ....C...C.
V.indi ....--.TC. ..C.TA.... .......... .......... ....C..... ...CT...CG
V.komo ....--.TC. ..C.T..... .......... .......... ....C..... ...CC...CG
V.giga ....--.TC. ..C.T..... .......... .......... ....C..... ...CC...CG
V.goul ....--.TC. ..C.TA.... .......... .......... ....CC.... ...CC...CG
V.mert ....--.TC. ..C.T..... .......... .......... ....C..... ...CC...CG
V.sald ....--.TC. ..C.T..... .......... .......... ....CC.... ...CT...C.
V.vari ....--.TC. ..C.T..... .......... .......... ....CC.... ...CC...CG
V.mitc ....--.TC. ..C.TA.... .......... .T..T..... ....C..... ...CC...CG
V.acan ....--.TC. G.C..A.... .......... ....T..... ....C..... ...CT...C.
V.brev ....--.TT. ..C.TA.... .......... ....T..... ....C..... ...CC...CG
V.erem ....--.TT. ..C.T..... .......... ....T..... ....C..... ...TC...CG
V.pilb ....--.TC. ..C.TA.... .......... .T..T..... ....C..... ...TC...CG
V.timo ....--.TC. ..C.TA.... .......... .T..C..... ....C..... ...CT...CG
V.tris ....--.TC. ..C.TA.... .......... .T..T..... ....CC.... ...CC...CG

Helode ACCGCTTTTA GC-AAAATCA GCCTATATAC CGCCATCGCC AGTTTATCTT CTAAAAGATT
Lantha ...T..GC.. ..C..G.A.. .......... ....G...A. .......... AC......CC
V.nilo ...TT.AC.. ..--CC.... .......... ....G...AA ...A...TC. GC...GA..C
V.albi ...CT.AC.. ..--CC.C.. .......... ....G...AA ..CA....C. GC...G..C.
V.beng ...C..AC.. ..--.C.... .......... ....G...AA ...A....C. GC...G...C
V.dume ...A..AC.. ..--.CCA.. .......... ....G...AA ...A....C. GC...G...C
V.oliv ...A.CAC.. ..--.CCC.. .......... ....G...AA ...A....CC GC...G...C
V.salt ...A..AC.. ..--.CCC.. .......... ....G...AA ..AA....C. GC...G.GCC
V.pras ...A..AC.. ..--.TC... .T........ ....G...AA ...A....CC TC...G.CCC
V.indi ...ATCAC.. ..--.CCC.. .......... ....G...AA ...A....CC GC...G...C
V.komo ....TCAC.. .T-..CT... .......... ....G...AA ...G....CC TC...G..CC
V.giga .....CAC.. ..-..CT... .......... ....G...AA ...A....CC .C..GG...C
V.goul .....CAC.. ..-..CT... .......... ....G...AA ...A....CC .C..GG..C.
V.mert .....CAC.. ..-..TT... .......... ....G...AA ...A....CC .C..GG..CC
V.sald ...ATCAC.. ..-..CT... .......... ....G...AA ...A....CC TC...G..CC
V.vari ....TCAC.G ..-..CTC.. .......... ....G...AA ...A....CC TC...G..CC
V.mitc ...A.CAC.G ..-..CT... .......... ....G...AA ...A.....C .C..G...CC
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V.acan ...A..AC.. ..-..CTC.. ..... ....G...AA ...A....CC .C..GG..CA
V.brev .....CAC.. ..-..CT... ..... ....G...AA ...A....CC .C..G....G
V.erem ...A.CGC.. ..-..CT... ..... ....G...AA ...A....CC .C..G...CA
V.pilb ...A.CAC.. ..-..CT... ..... ....G...AA ...A.....C .C..G....G
V.timo ...ATCAC.G ..-..CTC.. ..... ....G...AA ...A....CC TC..G...CC
V.tris ...A.CAC.. ..-..CT... ..... ....G...AA ...G...T.C .C..G.A.C.

Helode AAAAATAAAC ACAAAAGTTT T--------C ACTAGTACGT TAGGTCAAGG TGTAGCACAT
Lantha ..... .G..T..CCC C-------C. G...AA...A C..... ......TT..
V.nilo ..T...TG.. C...C...C. .A------C. ....AA.... C...C..... CA..A.TA..
V.albi GGT...TG.. T...C...C. C--------. ....AA.... C...C..... CA....T...
V.beng C.C...T.T. T...T..CCC A-------C. ....AA.... C...?..... GA...GTAT.
V.dume C.T...T.T. T...C..CCC GAA-----C. ....AA.... C..... CA....TA..
V.oliv T.T...T.T. T...C...C. C-----CGC. ....AA.... C...C..... CA....TA..
V.salt C.T...T.T. C...C..CCA CAT------. ....AA.... C...C..... CA....TA..
V.pras C.T...T.T. T...T..CCC C-------C. G..G.A.... C...C..... CA....TA.C
V.indi C.T...T.T. T...C....C ACA-----C. ....AA.... C...C..... CA....TT..
V.komo T.T...TTT. T...T...C. C-------C. G...AA.... C...C..... CA....TA..
V.giga T.T...TCT. TT..T...CC A-----CCC. C..GAA.... C...C..... CA....TA..
V.goul T.T...TTT. C......CCC A------CC. C...AA.... C...C..... CA....TT..
V.mert T.T...TTT. CT..T..G.C A------CC. C...AA.T.. C...C..... CA....TA..
V.sald T.T...TTT. T...C...CA C------CC. ....AA.... C...C..... CA....TA..
V.vari T.T...TTT. T...C...CC C---CCCCC. ....AA.... C...C..... CA....TA..
V.mitc C.T...TTT. T...C..CC. C-----CCC. G...AA.... C...C..... CA....TA..
V.acan C.T...TTT. CA..T...CC C-------C. ....AA.... C...C..... CA....TA..
V.brev C.T...TTT. C...T..CCC CG---CCCC. G...AA.... C...C..... CA....TA..
V.erem C.T...TTT. C...T....C .------CC. ....AA.... C...C..... CA....TA..
V.pilb C.T...TTT. C...T...C. ATCTCT-CC. ....AA.... C...C..... CA....TA..
V.timo C.T...TTT. T...C...C. CA-----CC. ....AA.... C...C..... CA....TA..
V.tris T.T...TTT. C...T...C. .-----CCC. .....A.... C...C..... CA....CA..

Helode AAAACGGTAA GAGATGGGCT ACATTTTCTA -TTC-AAGAA AATACGGAAA ACACAATGAA
Lantha G..GA..-.. ..... ......C... CCAACC..G. C.G...A... .T.TC.C...
V.nilo GT..A..-.. ..... ..... -C..AC.... C.C...A... .T.AC.....
V.albi GT.....-C. ..... .......T.. -..TAT.A.. ..C...A... .T.AT.....
V.beng GTTGA..-G. ..... .........? -?ATGC.... C.A...A... .T?AC.....
V.dume GT.GA..-C. ..... .........G -AG.AC.... C.A...A... GTGAC.....
V.oliv GT.GT..-C. ..... .........G -AAACC.... C.C...A... .T.GC.....
V.salt GT.GA..-C. ..... .........G -GCTAC.... C.A...A... .T.GC.....
V.pras GT.GT..-.. ..... .........G -AAACC.... C.....A... .TGGC.....
V.indi .TG.T..-C. ..... .........G -A..AC.... C.C...A... .T.GC.....
V.komo GTG....-C. ..... .........G -AC.AC.... C.C...A... .TGGT.....
V.giga GTGT...-C. ..... ..... -ACTAC.... C.....A..G .TGGCC....
V.goul GTG....-C. ..... ..... --C.CG.... C.....A..G .TGGCC....
V.mert GTG....-C. ..... ..... -A.TAC.... C.C...A..G .TGGCC....
V.sald GTG....-C. ..... ..... ACCGCC.... C.C...A... .T.GC.....
V.vari GTG....-T. ..... .....C...G -..TCC.A.G T.C...A... .TGGC.....
V.mitc GTGGT..-C. ..A..... ......C..T AAC.A...G. ......A..G .TGGT.....
V.acan GT..T..-C. ..... ..... -A.TG..... C.C...A..G .TGAT.....
V.brev GTGG...-C. ..... .........G -CA.TC.... C.C...A..G ...AT.....
V.erem GC.GT..-C. ..... .........G -A..AC.... C.....A..G .T.GC.....
V.pilb GTG.T..-T. ..A..... ......C... -ACTG...G. ......A..G GTGGC.....
V.timo GTG.T..-C. ..A..... ..... -A.AGC.... ..C...A..G .TGAC.....
V.tris GTG.T..-C. ..A..... .........G -A.TAC.... ......A..G GTGGT.....

Helode A--CTAGTG- TTTAAAGGCG GATTTAGTAG TAAAATTTAT A
Lantha --CACGA.A- ..A.....T. .......C.. ......AAGG .
V.nilo .-C..GT.AA ........A. .......C.. ...GGCGGG. .
V.albi .-CT..T.A- ..... .......C.. ...G.CGAGC .
V.beng T-?T.G?CA- ..??..... .?....???? ?????????? ?
V.dume .-C..GC.A- .......AT. .......C.. ...G..GA.A .
V.oliv .-C.A.C.A- ..C..... .......C.. ...G..GA.A .
V.salt .-C..GC.A- C.C.....T. .......C.. ...G..GA.A .
V.pras .-AT.GC.A- ........T. .......C.. ...G..GA.G .
V.indi .-C.CGC.A- ..C.....T. ..... ...G..GA.C .
```

FIG. 2—*Continued*

```
V.komo .-C...CCA- ..C.....A. .......C.. ...GGCGA.A .
V.giga .-C.C.CCA- .CCC....T. .......C.. ....G.GA.G .
V.goul .-CT.GCCA- .CCC....T. .......... ...G..GA.A .
V.mert .-T.CGCCAA .CCC....T. .......C.. ...GG.GG.A .
V.sald .-A..GCCA- ..CC....A. .......C.. ...G.CGA.G .
V.vari .-T..GTCA- ..CT....T. .......C.. ...G..GA.A .
V.mitc .CCT..CCA- .CC.....T. .......C.. ...G..GAGA .
V.acan .-C..GTCA- .CC.....T. .......C.. ...G..GA.A .
V.brev .-C...T.A- .CCT....T. .......C.. ...G..GA.A .
V.erem .-C.CGC.A- .CC..... ..... .....C.. ...GG.GA.A .
V.pilb .CC..GCCA- CCC.....T. .......C.. ...G..GA.A .
V.timo .CC..GTCA- CCCC..... ..... .....C.. ..GG.CGA.A .
V.tris .CCT..CCA- CCCC..... ..... .....C.. ...G..GA.A .
```

12S c/d

```
Helode GAACTCCGGA GCAAGTATCA GGCTC-AACA A-ATTGGCCC ATAACACTTT GCATCGCCAC
Lantha AGGGG-G... ...G..... .....GG.A. .CTCCA.... .CT....C.. ..CA.....
V.nilo TGGGA-.A.. ...G..... .....--... .TGC.A.... .......C.C ..CAA.....
V.albi T.C.A-.A.. ...G..... .....-.... .CG..A.... ..C....C.. ..CAA.....
V.beng TGCAA-GA.. ...G..... .....-.... .CG..A.... G.C....C.. ..CCA.....
V.dume TGCAA-TA.. ...GA..... .....-.... .CG..A.... .CT...TC.. ..C.A.....
V.oliv TGCAG-.A.. ...G..... .....-.... .CG..A.... .C.....C.. ..CAA.....
V.salt TGCAA-.A.. ...G..... .....-.... .CG..A.... .CC....C.. ..CCA.....
V.pras TGGAG-.A.. ..GG..... ...G.-.... .C...A.... .C.....C.. ..CAA.....
V.indi TGTAA-.A.. ...G..... .....-.... GCG..A.... C.C....C.. ..CAA.....
V.komo TGCAA-TA.. ...G..... ...C.-.... CTG..A.... .CC....C.C ..CAA.....
V.giga TGCAA-TA.. ...G..... .....-.... .CG..A.... .CT....C.. ..CAA.....
V.goul TGCAA-.A.. ...G..... .....-.... .CG..A.... ..T....C.. ..CAA.....
V.mert TGCAA-.A.. ...G..... ...C.-.... .TG..A.... ..T....C.. ..CAA.....
V.sald TGTAAT.A.. ...G..... ...C.-.... .CG..A.... ..C....C.. ..CAA.....
V.vari TGCAA-.A.. ...G..... ...C.-.... .CG..A.... .CC....C.. ..CAA.....
V.mitc T.TAG-.A.. ...G..... .....-.... .CG..A.... ..C....C.. ..CAA.....
V.acan TGTA.-TA.. ...G..... .....-.... .CG..A.... ..T....C.. ..CAA.....
V.brev TGCAA-.A.. ...G..... .....-.... .CG..A...A .CC....C.. ..TAA.....
V.erem TGTAA-.A.. ...G.C.... ...C.-.... .CG..A.... .CT..G.C.. ..CCAA....
V.pilb T.CAA-.A.. ...G..... .....-..T. .C...A.... ..T....C.. ..CAA.....
V.timo TGCAA-.A.. ...G..... .....-.... .CG..A.... .CC....C.. ..TAA.....
V.tris TGCAA-.A.. ...G..... .....-..A. .C...A.... .CC....C.. ..CAA.....

Helode CTCCCCACGG AT---ATCAG CAGTAATTAA TATTAGGCAA TTAGTG---- -AAAACTTGA
Lantha A..... .T--AAA... .....GC... C.......C. .AG.C.-AT- -C..G.C...
V.nilo A..... .T-AATA... ....G.C... C..... .A..CACCAA CT..G.....
V.albi A..... .T-ATTA... ....G..... C..... .A..CACCTA -C..G.....
V.beng A..... .T--ACA... ....G..... C..... .A...ACCT- -T.....
V.dume A..... .T--ACA... ....G.CC.. C.......C. .A..CACAT- -...G.....
V.oliv A..... .T-AACA... ....G.C... C..... .A..CATAT- -C..G.....
V.salt A..... .TGC-CA... ....G.C... C..... .G..CACATA -...G.....
V.pras A..... .--CAAG... ....G.CC.. C..... .A..CACAT- -C..G.....
V.indi A..... .T-AAAA... ....G.C... .T..... .A..CACAT- -C.....
V.komo A...T..... .T-CATA... ....G.CC.. C..... .A..CACAT- -C..G.....
V.giga A..... .TTT-CA... ....G.CC.. C..... .G..CACTC- -C..G..C..
V.goul A..... .TT-ACA... ....G.C... C..... .G..CAACC- -C.....C..
V.mert A..... .TT--AT... ....G.C... ..... .G...ACTC- -C.....C..
V.sald A..... .TT-ACA... ....G.CC.. C..... .A..CACAC- -...G.....
V.vari A..... .-CCATA... ....G.CC.. C..... .A..CATTC- -C..G.....
V.mitc A..... .T--AAT... ....G.C... C..... .A..C.CAC- -C..G.....
V.acan A..... .TAAACA... ....G.CC.. C..... .A...ACAC- -C.....
V.brev A..... .-AAACG... ....G.CC.. C..... .A..CACAC- -C..G.....
V.erem A..... .-AAACG... ....G.C.G. C.......T. .A..CACAC- -C..G.....
V.pilb A..... .TTAACA... ....G.CC.. C..... .A..CACAG- -C..G.....
V.timo A..... .T-AACA... ....G.C... C..... .A..CACAC- -C..G.....
V.tris A..... .T-AACA... ....G.C... C..... .A..CACAC- -C..G.....

Helode CCTAGCT-AT G----GTTAA CTGGGCCGGC AAATTTCGTG CCAGCAGCCG CGGTTATACG
Lantha .....T.-.. .A---CAAAC TAG...T... ..... ..... ......C...
```

FIG. 2—*Continued*

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V.nilo .......-.A AA-TTAAAA- --T..... ....C..... .......... ......C...
V.albi ....A..-.A AA-TTTTA-- TTT..... ....C..... .......... ......C...
V.beng .......-.A AA-TTCCCA- TTT..... ....C..... .......... ......C...
V.dume .......-.A ATACTCCT-T CTT..... ....C..... .......... ......C...
V.oliv ......C-.A AA-TTTCTC- --C..T.... .......... .......... ......C...
V.salt .......-.A TA-TTTT--C CTT..... ....C..... .......... ......C...
V.pras .......-.G AA-TTCC-CC CTC..T.... ....C..... .......... ......C...
V.indi .......-.A AA-TTCTTC- --T..... ....C..... .......... ......C...
V.komo ......C-.A AC----CACC CCA..... ....C..... .......... ......C...
V.giga .......-.A AA--CCCA-- TTT..... ....C..... .......... ......C...
V.goul .......-.A AACTTC---- TTT..... ....C..... .......... ......C...
V.mert .......-.A AACCTCC--- -TT..... ....C..... .......... ......C...
V.sald .......-.A AA-TTTT-CC C-C..... ....C..... .......... ......C...
V.vari .......-.A AA--CCC--T TTT..... ....C..... .......... ......C...
V.mitc .......-.A TACTTTTTCC CAT..... ....C..... .......... ......C...
V.acan ......C-.A AACTCC--CC C-T..... ....C..... .......... ......C...
V.brev ......C-.A AACTTCCAC- --T..T.... ....C..... .......... ......C...
V.erem .......-.A .ACTCCC--- --C..... ....C..... .......... ......C...
V.pilb .......-.A CACCCCTA-- --T..... ....C..... .......... ......C...
V.timo .......TCA CA-TT---CC C-T..... ....C..... .......... ......C...
V.tris ......C-.A CACTTT-ACC C-T..... ....C..... .......... ......C...

Helode AAAGGCCCAA AACAAGAAGC ATACGGCGTA AAGCGTGACT AGAATT---- -TTATCTTTG
Lantha ...A...... .....TGCT. C--..... ...T.....C .....A---- CCAG...A.T
V.nilo GG.....A.. ..T.T..TA- CA..... ...A....A. .TG.A.---- --...ACC.C
V.albi GG.....A.. ..T....TA- TA..... ...A.....A CA.GAG---- CCCGA.CC.T
V.beng GG.....AC. G.T..TCTA. TA..... ...A.....C .CGGAC---- --.T..CC..
V.dume GG.....A.. G.T.GC.CA- .A..... ...A.....A .T.GA.CTTC -TCCAACC.A
V.oliv G...A..A.. G.T.GC.GA. CA..... ...A....TC .A.GA.TTTC CCCCCACC.A
V.salt GG.....A.. G.T.GC.TA. TA..... ...A.....C .C.GA.TTT- --.CCACC.A
V.pras GG..A..G.. G.T.GC.CA. TA..... ...A.....C .C.GA.TT-- --..CACC.C
V.indi GG..A..A.. G.T.GC.CAT TA..... ...A.....C .C.GA.A--C CCCCAACC.C
V.komo GG.....A.. ..T..TTCA. CA..... ...A.....C .C.GA.T--- --CC.ACC.C
V.giga GG.....AG. G.T..CTT-. CG..... ...A.....C CC.GA.T--- --ACCACC.A
V.goul GG..A..A.. G.T..CCC-. CA..... ...A.....C .C.GA.TTT- --C.CACC.A
V.mert GG..A..A.. G.T..CTTC. TA..... ...A.....C .C.GA.T--- CCCCCACC..
V.sald GG.....A.. G.T..C.CA. C.T..... ...A..... .CG.A.T--- --.TC.CA.C
V.vari GGG....A.. ..T..T.TA. TA..... ...A.....C .C.GA.TT-- --CCCACC.T
V.mitc GG.....A.. G.T..T.C-. TA..... ...A..... TC.GA.---- --CTC.CCCA
V.acan GG.....AC. G.T..TTTAT .A..... ...A....TC CC.GA.TTTA TT.TCACC..
V.brev GG..A..A.. G.T..T.CA. CA..... ...A.....C .C.GA.T--- --.C.ACC..
V.erem GG.....A.. G.T..TTTA. CA..... ...A..... TT.GA.---- --CTCACC.A
V.pilb GG.....A.. G.T..C.CCT TA..... ...A..... CC.GA.---- --.TC.CC.A
V.timo GG.....A.. G.T..T.TC. CA..... ...A.....C CCCTAA---- -CCCCACC..
V.tris GG.....A.. G.T..T..T. CA..... ...A..... TC.GA.---- -CCCCACC..

Helode C---AGCTAT AAGGAGAAAT TAAAGTTAAG TAGTAAAATA CACTAACTAA AAGAATC-TT
Lantha .C-----.CT. .........C ....AC.... CT..... .CAAG..ATT .G...C.C-.
V.nilo AT-ACTAGGA CCT--A.... .GTGCC.C.. AGA.G....C .TAAGGTACC .G....TTC.
V.albi .--AACAGGA CCCC.A.... CTT.TC.C.. AGA.G...CC .TAAG.TATC .....C.C-.
V.beng .---CC.GGA TTTT-A...C .CT.TC.C.. AGA.G...CC .CAAG.TACC .....C.CCC
V.dume .CCAATTGGA TTTT-A...C ..T.TC.T.. AGA.G....C ..AAG.TATC .....C.CCC
V.oliv AT-GCC.GGA TTAC-A.... .TTGTC.C.. AGA.G....C .CAAG..ACC ......TC..
V.salt .C---A.GGA TTC-.A.... .CT.CC.C.. AGA......C .TAAGGTACC .....CTCC.
V.pras .--ATC.GGA TTC-.A.... .TC.CC.T.. AGA.G....C ..AAGGTACC .GA....C..
V.indi T--ACCTGGA CCCC-....C .TT.TC.C.. AGA.G....C .TAAG.TATT G....C.CCC
V.komo .---AT.GGA TTCT-A.... .TT.CC.T.. AGA.G....C .CAAG..ACC ....TCTCCA
V.giga .C--AC..GA TTCC-AG..C CCT.TC.C.. AGA.G....C .CAAG.TACC TG...CTCCC
V.goul .C-ACCAAGA TCCC-A.... .CTGTC.T.. AGA.G....C .CAAG..ACC CG...C.CCC
V.mert TC--AC.GGA CCCC-A.... CCT.TC.T.. AGA.G....C .CAAG.TATT C....C.CC.
V.sald .-----C.GGA TCTT-A.... .TT.CC.C.. AGA.G....C .TAAGGTATT C...GC.TCA
V.vari .---AC.GGA TTCC-A.... .TT.CC.T.. AGA.G....C .CAAG..ATC TG..CCTCCA
V.mitc .T--ACAGGA CCC-.A.... .CT.CC.C.. AGA.GG...C .CAAGGTA.C C....CTTCC
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FIG. 2—*Continued*

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V.acan .C--AT.GGA TTCC-AG... .TC.TC.T.. AGA.G...CC .CAAG.TATT .....C.CC.
V.brev .C--AC.AGA TCCC-..... CCTCCC.C.. AGA.G....C .CAAGC.ATC T....CTCC.
V.erem .C--AC.GGA TCCCC-.... .CT.CC.C.. AGA.G....C .CAAGTTATT .....C.CCC
V.pilb .T--ACAAGA CCCC-.G... .CTGCC.C.. AGA.G....C .CAAGG.AGC C.A..CTCC.
V.timo TT--CAAAGA TCCCC-...C CCTGTC.... AGA.G....C .CAAG..ACC TG...C.CCC
V.tris .T--CAAAGA CTC.-A.... .CT.CC.T.. AGA.G....C .CAAGGTACC T......C..

Helode AACC--CC-T TATGCA-AAT AATATTTCA- CTCACGAAAA ?TAAGAAAC? AACTAGG?TT
Lantha .-.AAT..-. ..AATTAAAA G.C....G.- .........G C........A .......A..
V.nilo C.A.AC..-. ACAC..---G GCC....A.- A........G TC.GA.T..A .......A..
V.albi ....AT..-C ..AATT---G GCC..C.A.A T........G C.GG..T..A .......A..
V.beng T.AAAC..-C A.AA..---G GCC..CC..- .......... TC.G..T..T .......A..
V.dume ..ATAT..-A C.AA..---G GT...CCT.- .......... TCTG..C..A .......A..
V.oliv C.A.AT..-A C.AA..---G GCC..CCA.- .........G C.CG..T..A ..?....A..
V.salt ..-.AT..-A C.AA..---G GCC..CCT.- .......... CC.G..C..A .......A..
V.pras C.G.AT..-C A.AAT.---G GCC..C.A.- T........G CC.G..C..A .......A.A
V.indi C.G.AT..-A C.AA..---G GCC..C.A.- .......... TC.G.TT..A .......A..
V.komo ..G.AT..-A C.AA..---G GCC..CCA.- .......... TCGG.GC..A ???????.??
V.giga T.A.AT..-G C.AAT.---G GCC..C.A.- .......... CC.G.TT..A .......A..
V.goul ..G.AT..-A C.AATT--AG GCC..C.G.- .......... CC.G.CCC.A ....???.??
V.mert ..A.AC..-A ..AT..---G GC...C.A.- .......... CC.G.TC..A .......A..
V.sald ..ATAT..TA AGAA..---G GCC..CCA.- .......... TCGG.GC... ?......A..
V.vari ..ATAT..CA CGAA..---G GCC..CCG.- .......... TCGG.GC..A .......A..
V.mitc .GT.AT..-. .CA...---G GCC..CCA.- .......... CCGG.GC..A .......A..
V.acan ..GAAT.TCG .CAA..---G .CC..C.T.- .......... CC.G..C..A .......A..
V.brev .GG.AT..CA CCAT..---G GCC..CCA.- .......... CC.G..C..A .......A..
V.erem .GGGAT.TCA CCCA..TCAG GCC..C.A?- ...??....? .????????. ???????.??
V.pilb .GT.AT..-C .CA.T.---G GCC..CCA.- .......... CC.G..C..A .......A..
V.timo C...CAATCC CCAA..---G GCC.CC.A.- .......... CCGG..C..A .......A..
V.tris .GA.AT..-C ACA...---A GCC..CCA.- .......... CCGG..T..A ..........
```

FIG. 2—*Continued*

deletions resulted in regions of variable length. For the total 12S rRNA segment (excluding ambiguous regions), there are 662 total aligned sites, 338 of which are variable and 229 of which are informative under parsimony conditions. The nucleotide composition of the total sequenced region was A = 34%, T = 18.9%, C = 28%, and G = 19.1%. For both segments of DNA, the percentage of transitions was plotted against the percentage of transversions (Fig. 3). Transitions outnumber transversions, except for a small number of comparisons involving the outgroup (i.e., at high levels of sequence divergence) and therefore this agrees with previous studies on animal mtDNA that report an initial high (>50%) transition bias which gradually decreases over time (Brown *et al.*, 1982; Hedges *et al.*, 1991). It is interesting to note the two distinct clusters of points in Fig. 3 which represent the ingroup and outgroup comparisons. The data in Fig. 3 do not exhibit a transition plateau (usually corresponding to 40–50% transitions), which is the point where multiple substitutions are occurring at the same site (Brown *et al.*, 1982), and therefore these data are useful for phylogenetic inference. Moreover, the 12S rRNA data were characterized by significant phylogenetic signal, as indicated by the $g1$ test for skewness ($g1 = -0.76$, $P < 0.01$).

To give an indication of the taxonomic levels where phylogenetic signal occurs, maximum-parsimony analyses were performed before and after removal of the outgroups. The deletion of both outgroups resulted in a single tree (length = 922 steps, retention index = 0.385) which was identical in structure to that obtained with the full dataset including the outgroups (single tree

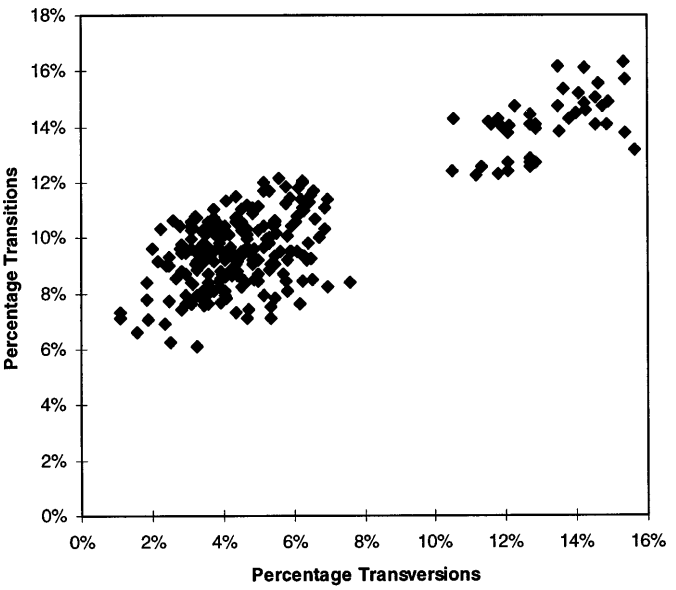

FIG. 3. Percentage of transitions versus percentage of transversions for the two sections of 12S rRNA (12S a/b, c/d) in the 23 taxa examined.

with length = 1156 steps, retention index = 0.395). If only one outgroup was used (e.g., *Heloderma*), a single tree was obtained that had the same structure but was longer (length = 1070 steps, retention index = 0.377). The topology generated from the maximum-parsimony analysis of the full 12S rRNA data set after 500 bootstraps is given in Fig. 4. Only bootstrap values of >50% are indicated on the tree.

Average sequence divergence among the varanid species studied here was high (0.138 ± 0.021), compared with that reported for the 12S rRNA gene between lizard species in the genus *Gallotia* (0.079 ± 0.018, Gonzales *et al.*, 1996). Sequence divergence within the family Varanidae ranged from 0.082 (for a comparison between two Australian species of the same subgenus) to 0.183 (for a comparison between an African and an Australian species). Average sequence divergence among the three varanoid families was 0.292 ± 0.011 for pairwise comparisons between Varanidae and Helodermatidae and 0.254 ± 0.011 for pairwise comparisons between Varanidae and Lanthanotidae. These values are quite low given that Hedges *et al.* (1991) found a 12S rRNA divergence estimate of 0.365 for comparisons between different genera within the lizard family Xantusiidae.

Phylogenetic Relationships

All Australian taxa represented, including both the subgenera *Odatria* and *Varanus,* are monophyletic. Within this group, members of the subgenus *Varanus* form two clades; the Asian–Australian taxa (*V. salvadorii, V. komodoensis,* and *V. varius*) and the solely Australian taxa (*V. gouldii, V. giganteus,* and *V. mertensi*). The Asian species investigated in this study were found to be the sister taxa to the Australian species, while the two African species were most divergent from the Australian taxa. In particular, those species that were in closer geographic proximity to Australia were more closely related to the Australian species. That is, *V. prasinus* (primarily from Papua New Guinea, but there have been isolated incidents of them occurring in the very north of Australia), *V. olivaceus* (Philippines), *V. salvator* (Indo-Malaysia, but with a single reported occurrence in northern Australia), and *V. dumerilli* (Borneo) are less divergent from the Australian species than *V. albigularis* and *V. niloticus* (Africa).

Hypothesis Testing

The 12S sequence data were constrained according to the relationships postulated in previous studies to enable the determination of whether the sequence data

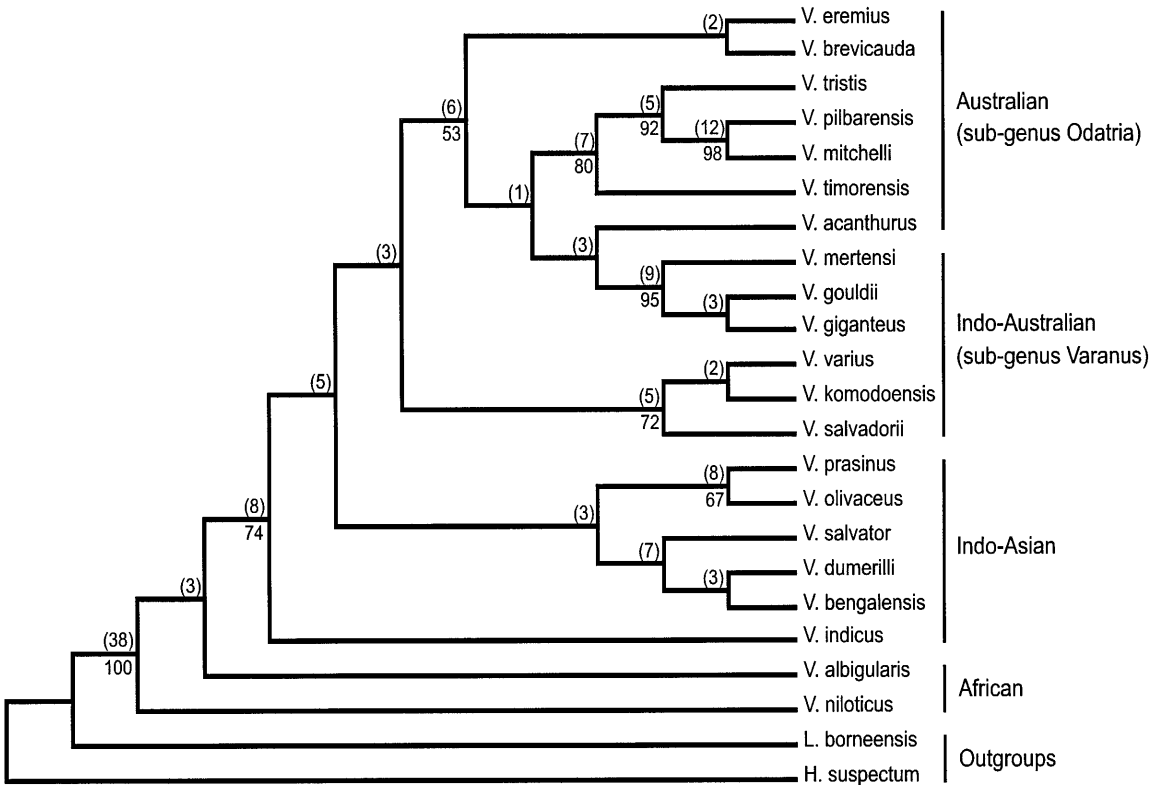

FIG. 4. Relationships among the 23 varanoid taxa examined, obtained by maximum-parsimony method. *H. suspectum* and *L. borneensis* were designated outgroups to root the tree. Numbers on the tree indicate the percentage (>50) of bootstrapped trees supporting each node (maximum parsimony, below branch) and the Bremer support index (in parentheses).

TABLE 1

Summary of Log-Likelihood Ratio Tests for the Comparison of Unconstrained and Constrained Maximum-Likelihood Topologies Generated from 12S Sequence Data

Comparison	No. of taxa	Log likelihood (LnL)	Differernce LnL	Standard deviation	Significantly worse ($P < 0.05$)
Best (ML)[a] tree vs monophyly *Odatria* and *Varanus*[b]	23	−6227.05			
		−6235.15	−8.10	9.93	No
Best tree vs hemipeneal (Fig. 1A)	19	−3928.04			
		−4019.42	−91.38	25.98	Yes
Best tree vs lung (Fig. 1B)	13	−2934.65			
		−2974.49	−39.83	13.76	Yes
Best tree vs chromosome (Fig. 1C)	13	−3133.99			
		−3156.79	−22.79	9.15	Yes
Best tree vs one-way MC'F (Fig. 1D)	17	−4193.23			
		−4306.82	−113.59	27.00	Yes
Best tree vs two-way MC'F (Fig. 1D)	9	−2891.64			
		−2920.14	−28.50	13.11	Yes

[a] ML, maximum likelihood. Negative log-likelihood values simply indicate that the corresponding probability is less than 1 (Felsenstein, 1993).
[b] Constraint of the subgenera *Odatria* and *Varanus* into two monophyletic clades.

supported these hypotheses. Using maximum-likelihood methods, all the previous phylogenies were statistically different ($P < 0.05$) from that produced from 12S data (Table 1). The investigation of character change differences in constrained versus unconstrained maximum-parsimony topologies (to statistically evaluate whether the data significantly favor one maximum-parsimony topology over another) revealed that the chromosome and MC'F phylogenies were statistically similar ($P > 0.05$) to the phylogeny generated from sequence data (Table 2). Once again, the topologies based on morphological characters were significantly different ($P < 0.05$) from the maximum-parsimony topology from sequence data (Table 2).

Finally, although there was not strong bootstrap support for the separation of the *Odatria* and *Varanus* species into two independent clades, log-likelihood ratio (Table 1) and nonparametric parsimony (Table 2) tests did indicate that the topology was not significantly worse ($P > 0.05$) if this constraint was imposed.

TABLE 2

Summary of Wilcoxon Matched-Pairs Signed-Ranks Tests for the Comparison of Unconstrained and Constrained Maximum-Parsimony Topologies Generated from 12S Sequence Data

Comparison	No. of taxa	Tree length[a]	n[b]	Test statistic	P (two-tailed probability)[c]
MP[d] topology vs monophyly *Odatria* and *Varanus*[e]	23	1156	50	553	>0.05
		1163			
MP topology vs hemipeneal (Fig. 1A)	19	860	59	577	<0.05
		885			
MP topology vs lung (Fig. 1B)	13	633	38	210	<0.05
		651			
MP topology vs chromosome (Fig. 1C)	13	594	50	552	>0.05
		601			
MP topology vs one-way MC'F (Fig. 1D)	17	770	34	210	>0.05
		780			
MP topology vs two-way MC'F (Fig. 1D)	9	437	29	145	>0.05
		447			

[a] The tree length, i.e., number of steps.
[b] The number of characters that undergo different number of changes in the two trees being compared.
[c] The two-tailed probability associated with the normal distribution for large sample approximation, i.e., $n > 25$ (Siegel, 1956).
[d] The most parsimonious tree.
[e] Constraint of the subgenera *Odatria* and *Varanus* into two monophyletic clades.

DISCUSSION

Phylogenetic Relationships

All of the Australian species examined in the present study form a monophyletic clade. The subgenus *Varanus* is composed of two clades which are not directly related, one consisting of *V. salvadorii, V. komodoensis,* and *V. varius* and a second with *V. gouldii, V. giganteus,* and *V. mertensi.* A similar distinction was found in previous research based on chromosome morphology (the differentiation of lineages A and B in the phylogeny of King and King (1975), Fig. 1C) and lung morphology (Becker *et al.* (1989), Fig. 1B). Initially, King and King (1975) proposed that the *V. gouldii* group (A) was derived from the *Odatria* because they shared an unusual karyotype. However, with the addition of more taxa to the analysis, this conclusion was discounted (King, 1990). Hemipeneal studies (Branch, 1982; Bohme, 1988) also challenged the original suggestion of King and King (1975) that the *V. gouldii* group arose from the *Odatria. V. komodoensis,* although found only in Indonesia, has been considered a member of the Australian subgenus *Varanus* since Mertens (1958) originally described the subgeneric relationships of *Varanus.* It is possible that this Indonesian population represents a relict population from a past significantly larger distribution that encompassed Australia or it may simply be the result of a past invasion from Australia. Interestingly, *V. salvadorii,* which is distributed throughout Papua New Guinea, appears to be closely related to *V. komodoensis* and *V. varius* and may also be a relictual population that once linked *Varanus* populations in Indonesia to populations in Australia. The positioning of *V. salvadorii* with Asian species in a separate clade, on the basis of hemipeneal morphology, is at odds with both MC'F and 12S sequence data findings.

The Asian species investigated in this study were found to be paraphyletic with the Australian species, while the two African species were most divergent from the Australian taxa. In their MC'F study, Baverstock *et al.* (1993) found that the Asian taxa were most closely allied with the Australian subgenus *Varanus* group and similarly, the phylogeny based on chromosome morphology (King and King, 1975; King, 1990) revealed that the Asian species were most similar to the *V. komodoensis/V. varius* group. In the present study, the African taxa were found to be more closely related to the Asian species than to the Australian species and this is consistent with previous interpretations based on hemipeneal and lung morphology (Figs. 1A and 1B). It was not possible to establish the African species' relationships based on chromosome and MC'F data (Figs. 1C and 1D) because the trees were unrooted.

Hypothesis Testing

One of the objectives of this study was to assess how well the 12S sequence data supported the relationships found in previous studies. The hypothesis testing analysis performed using maximum-likelihood procedures suggested that all the previously proposed varanid phylogenies (i.e., Figs. 1A to 1D) were statistically different to the 12S sequence data phylogeny. This analysis also revealed that the *Odatria* and *Varanus* could be separated into two monophyletic clades without significantly altering the topology. Conversely, the nonparametric hypothesis testing analyses revealed that the phylogeny based on the 12S sequence data was more congruent with phylogenies generated using genetic characters (i.e., Figs. 1C and 1D) as opposed to morphological characters (i.e., Figs. 1A and 1B). Once again, the separation of the *Odatria* and *Varanus* into two clades was supported using the nonparametric statistic. The difference between the two sets of results can be attributed to the inherent differences associated with the two types of models (parametric versus nonparametric). Templeton (1983) suggested that parametric models, such as maximum likelihood, may not be robust to even small departures from the assumptions used to generate the parametric model and it may often be the case that these assumptions are not biologically appropriate. Equally, it should be acknowledged that Templeton's nonparametric model will not perform well when convergent events are common. Taking into consideration these provisos, the results from the hypothesis testing analysis should be considered indicative of possible similarities between different topologies, but not conclusive.

Biogeographic Considerations

If the family Varanidae arose in Asia, then we would expect that the Asian species would be paraphyletic and that the highest species diversity of the family would occur in the area of origin (i.e., Asia). According to MC'F data, the first prediction regarding paraphyly was not true (Baverstock *et al.,* 1993). Moreover, extant Varanidae reach their highest species diversity in Australia. The MC'F results also indicated that the Australian varanids were diphyletic. An Australian (Gondwanic) origin (as suggested by Hutchinson and Donnellan (1993)) may, therefore, offer a more parsimonious explanation than an Asian origin with two invasions into Australia.

Based on the 12S data, the Asian species are paraphyletic and therefore support the view that the ancestral group was probably Asian. There is strong fossil evidence to support the hypothesis that Africa was colonized by *Varanus* within the last 20 MYBP (Clos, 1995). The only other fossil remains from Africa that are definitely *Varanus* have been found in recent strata (McDowell and Bogert, 1954) and fossil and living varanids are absent from the island of Madagascar. These populations do not, therefore, appear to be Gondwanaland relicts and are more likely the result of an independent radiation from an Asian source. This

hypothesis is not contradicted by the results of the present study.

The results of the combined MC'F/chromosome analysis of King (1990) indicate that the major lineages within the extant Varanus diverged no more than 40 MYBP. Such a finding places the source of the Australian members of this taxa within the context of an Asian rather than Gondwanic origin. Furthermore, varanids are usually considered a late Tertiary invader of Australia because the earliest Australian fossils were recorded from the middle Miocene of southern Australia (Pianka, 1995) and are, therefore, not within a Gondwanic timescale. From the 12S data, we can quite confidently propose that the Australian subgenera *Odatria* and *Varanus* have arisen from an Asian source and are not Gondwanic in origin.

King and King (1975) found that there were three radiations in Australia: (1) an initial radiation by the subgenus *Odatria,* (2) the *V. gouldii* group arising from the *Odatria,* and (3) a recent invasion of the *V. indicus* morph from southeast Asia into northern Australia, resulting in the *V. varius* morph. With the addition of more taxa, King (1990) concluded that in fact the *V. gouldii* group did not originate from the *Odatria.* The 12S data have confirmed that the *V. varius* group forms a clade separate to the *V. gouldii* group and the *Odatria.*

Plate tectonic theory suggests that Australia and Papua New Guinea were connected during the Oligocene, then were totally separated by a broad seaway in the Miocene, and then recently connected in the Pleistocene (Flannery, 1989). The 12S data suggest that the Papuan species, *V. prasinus* and *V. indicus,* are distantly related to the Australian species and therefore the common ancestry may go back approximately 30 MY.

The phylogeny of the Varanidae generated using 12S sequence data was broadly similar to that proposed by Baverstock *et al.* (1993) based on MC'F data, with the major difference between the two phylogenies being that in this study the Australian species appear to form a single, monophyletic assemblage (although this conclusion requires further sequence data to confirm it). Neither phylogeny produced from morphological characters (i.e., Becker *et al.,* 1989; Bohme, 1988) was in close agreement with the sequence data phylogeny. In conclusion, the phylogeny proposed here supports an Asian origin for the Australian varanid subgenera. Although the overall origin for the genus still remains unclear, the 12S data do not in any way support a Gondwanic origin.

APPENDIX: MUSEUM SPECIMEN IDENTIFICATION DETAILS

Varanus niloticus (South Australian Museum (SAM), No. unknown; location, Africa), *V. albigularis* (SAM, No. unknown; Africa), *V. bengalensis* (Florida Museum of Natural History, No. UF30225; Thailand), *V. olivaceus* (Dallas Zoo, No. 825893; Philippines), *V. salvator* (SAM, No. unknown; Indo-Malaysia), *V. dumerilli* (Santiago Zoo, No. unknown; Borneo), *V. prasinus* (Australian Museum (AM), No. R124774; Papua New Guinea (PNG)), *V. indicus* (AM, No. R124576; PNG/North Australia), *V. komodoensis* (Santiago Zoo, No. unknown; Indonesia), *V. salvadorii* (SAM, No. unknown; PNG), *V. giganteus* (SAM, No. R20988; South Australia), *V. mertensi* (AM, No. R126199; Western Australia), *V. gouldii* (Southern Cross University, No. A08, New South Wales, Australia), *V. varius* (SAM, No. R23277, South Australia), *V. eremius* (Western Australian Museum (WAM), No. R102398; Western Australia), *V. timorensis* (WAM, No. R101564; Timor), *V. pilbarensis* (WAM, No. R125766; Western Australia), *V. mitchelli* (SAM, No. unknown; Northern Territory, Australia), *V. acanthurus* (SAM, No. R29309; Western Australia), *V. brevicauda* (SAM, No. R36239; Northern Territory, Australia), *V. tristis* (WAM, No. R77061; Western Australia), *Lanthanotus borneensis* (Cincinatti Zoo, No. unknown; Borneo), *Heloderma suspectum* (University of Texas Museum, No. unknown; North America).

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