A MITOCHONDRIAL DNA ANALYSIS OF THE TESTUDINE APOBARAMIN

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Abstract

Baraminology is a biosystematic discipline for reclassifying organisms within the young-earth creation model. The method is presently dependent on 15 theoretically-defined membership criteria that are designed to reveal patterns of phylogenetic discontinuity in nature. This survey examines the utility of the molecular criterion for resolving phyletic divisions. As a case study, the non-trionychoidea cryptodires (turtles with hard shells and retractable necks) have been analyzed with a panel of nine mitochondrial genes. Sequence comparisons with non-testudines supported a previous hypothesis that the turtles were apobaraminic or phylogenetically distinct from other vertebrates. Analyses within the testudines suggested the non-trionychoidea cryptodires were composed of at least two monobaramins including the Cheloniidae family and Gopherus *genus.*

Introduction

The theory of special creation suggests the biota is composed of phylogenetically distinct units. Marsh (1941) proposed the word baramin as a technical term for these groups. He hypothesized the baramin could be identified as a reproductively isolated and morphologically distinct entity. The observable processes of organic evolution were interpreted as a mechanism for propagating diversity within the baramin (Marsh, 1976). Jones (1972) and Siegler (1974; 1978) concluded such biological distinctions generally occur at the family level of taxonomic classification. A framework for systematically characterizing baraminic boundaries was provided by the introduction of discontinuity systematics (Remine, 1990; 1993). The method was offered as an alternative to traditional systematic approaches, which are inherently incapable of recognizing phyletic divisions. Bartz (1991) encouraged the adoption of discontinuity systematics, and Wise (1990) incorporated its terms and methods into the young-earth creation model.

A battery of membership criteria have been offered to elucidate the baramin (Wise, 1992a). The molecular criterion is of special interest because it allows the investigator to quantify phylogenetic gaps at a fundamental level. For years creationists have suggested such analyses could provide powerful evidence for their position. Marsh (1971) stated:

> One basic kind is unlike all other basic kinds because of its peculiar internal chemistry, the DNA of its genes. If different kinds are present we know these different chemistries are present also and effectively isolate one kind from another by bridgeless chemical abysses.

Denton (1985) demonstrated that the nested pattern of protein sequence diversity among major taxonomic assemblages was more consistent with a typological rather than macroevolutionary origin. Wise (1992b) suggested molecular similarities among adult organisms was evidence of a common Creator, much like a characteristic style is evidence of a certain artist. The current survey was designed to explore the utility of the molecular criterion for identifying phyletic divisions. In order to test previous systematic hypotheses the study has been placed within the context of turtle baraminology.

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The history of turtle systematics has been marked by a long search for natural groups (Gaffney, 1984). Since the baramin is hypothesized to be a real biological entity it is possible that turtle phylogeny can be more accurately understood in terms of discontinuity. The current viewpoint of Frair (1991) is that all turtles form a single phylogenetically related baramin. A hypothesis by Wise (1992a) is that turtles are composed of two unrelated baramins including the pleurodires (side-necked), the cryptodires (hidden-necked). An earlier suggestion of Frair (1984) and the recent conclusion of Wise (1992a) suggests turtles form four baramins including the pleurodires (side-necked), the trionychoids (soft-shells), the chelonioids (marine), the remaining cryptodires (an assortment of terrestrial and aquatic species).

Materials and Methods

Successive Approximation

Baraminology research involves a unique nomenclature that can be used to communicate the phylogenetic status of an organism or group of organisms. Since the goal of baraminic research is to recover the identity of phyletically distinct baramins it is necessary to prune an evolutionary tree. The tree can be approached from two directions (Figure 1). The root of a tree involves a unit called the apobaramin, which is defined as a group of organisms that may contain unrelated subgroups. Subtractive criteria such as stratomorphologic gaps are used to split these groups into smaller apobaramins. The tips of a tree involve the monobaramin, which is defined as a group of organisms related by common descent. Additive criteria such as the ability of organisms to hybridize are used to lump these phylogenetically related groups into larger monobaramins. The taxonomic distance between the apobaramin and monobaramin will decrease as the tree is pruned with more efficient criteria. The process of successive approximation theoretically will lead to an overlap in the apobaramin and monobaramin. The taxon constructed by this juncture is called the holobaramin, and is de-

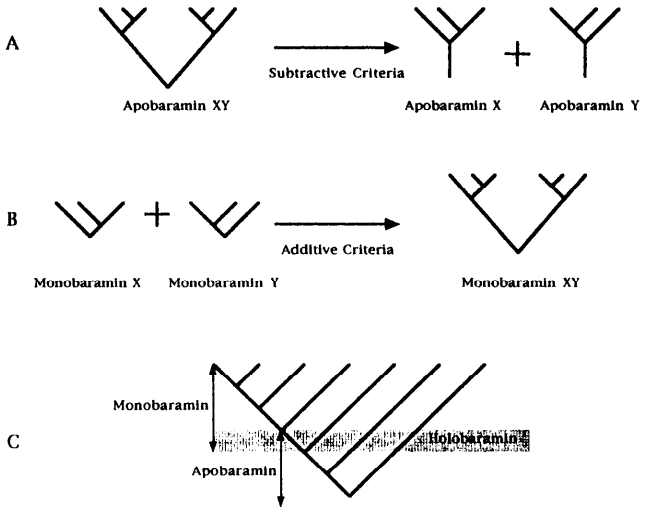

Figure 1. An illustrated description of successive approximation using baraminology nomenclature. The apobaramin is defined as a group of organisms containin phylogenetically unrelated subgrougs. Subtractive criteria are used to split an apobaramin into smaller apobaramins (A). The monobaramin is defined as a group of organisms related by common descent. Additive criteria are used to lump monobaramins into larger monobaramins (B). The taxon constructed by the juncture or overlap of an apobaramin and monobaramin is called the holobaramin, which is defined as a complete set of organisms related by common descent (C).

fined as a complete set of organisms related by common descent. It is the primary goal of baraminology to identify the holobaramin, which is an estimate of a created biological unit. One advantage of identifying holobaramins by successive approximation is that the technique allows for incomplete knowledge regarding the organism's biology. Researchers are not obligated to hypothesize beyond the resolving power of their criteria.

Character Selection

The interest in understanding biological phenomena from a molecular perspective has led to exponential growth in the number of reported DNA and protein sequences. The availability of this information through the internet provides baraminologists with a rich source of comparative data. It is suggested that DNA sequences have a greater baraminic utility than protein sequences. Due to the degeneracy of the genetic code a pair of identical protein sequences can have a divergent underlying DNA sequence (Figure 2). This property makes DNA a preferable molecule for biosystematic studies, which are designed to measure differences among organisms.

In the 1950s mitochondrial DNA (mtDNA) was discovered. Avise et al. (1987) noted that mtDNA was an ideal molecule for biosystematic analyses. It generally contains no pseudogenes, introns, or highly repetitive sequences, which tend to complicate biosystematic studies. For most organisms mtDNA was found to be maternally inherited, and did not undergo recombination or other genetic rearrangements. Due to its mode of inheritance mtDNA would be especially sensitive to population bottlenecks. Wilson et al. (1985) stated:

Sequence 1:	Thr	Arg	Leu	Gly
	ACC	CGA	UUA	GGG
Sequence 2:	Thr	Arg	Leu	Gly
	ACG	AGG	CUC	GGA

Figure 2. DNA sequences are more powerful for resolving subtle differences between molecules. In this example the protein sequences are identical, but the underlying DNA is 50% divergent.

> Because a single breeding pair of diploid animals contains four nuclear genomes and one transmissible mtDNA, a population that goes through an extreme bottleneck could lose all of its mtDNA variability and retain a significant fraction of its nuclear variability.

The young-earth creation model assumes at least one extreme population bottleneck during the Flood. This catastrophic event would have reduced most terrestrial vertebrates to two or seven representatives per holobaramin. Thus, for holobaramins with strict maternal inheritance of mtDNA one might expect more variability to exist across the member's nuclear genomes rather than their corresponding mitochondrial genomes. It is possible that such a pattern could be blurred due to the higher mutation rate of mtDNA. However, turtles are noted for having extremely slow mtDNA mutation rates (Bowen, Nelson, and Avise, 1993; Avise, Bowen, Lamb, Meylan, and Bermingham, 1992).

Sequence Acquisition

Mitochondrial DNA sequences were extracted from the GenBank and European Molecular Biology Laboratories (EMBL) databases via the internet and from the literature. All available testudine sequences were reviewed, and those representing the most taxa (Table I) and containing the most characters were used for analysis. Two protein coding genes (PC: cytochrome *b,* NADH dehydrogenase subunit II) and seven nonprotein coding genes (NPC: 12S-rRNA, tRNAs for tryptophan, alanine, asparagine, cysteine, tyrosine, proline) were selected. The gene map (Figure 3) depicts the relative positions of sampled molecules within the human mitochondrial chromosome (Anderson et al., 1981).

Systematists frequently employ outgroup taxa to polarize their taxonomic characters against a presumptive ancestral state. Since baraminologists have not widely identified monobaramins it would be premature to focus on ancestral versus descendant characters. Outgroup taxa in this survey are defined as organisms that are taxonomically distinct from the group of interest. For example, to explore turtle/non-turtle relationships, sequences from five of the major vertebrate classes were accrued including: common carp *(Cyprinus carpio),* chicken *(Gallus gallus),* human *(Homo sapiens),* African clawed frog *(Xenopus laevis),* (GenBank/EMBL accession numbers: X02890, X52392, J01415, X61010). A variety of reptilian outgroups were chosen because no single reptile was represented by the selected molecules. These included: American alligator *(Alligator mississippiensis),* whiptail lizard

Table I. List of turtles included in this study.

Taxon	Number of Sequences	References
Bataguridae		
*Cuora amboinensis**	1	c
*Malayemys subtrijuga**	1	c
*Rhinoclemmys pulcherrima**	1	c
Cheloniidae		
*Caretta caretta**	7	a, f
Chelonia agassizi	1	a
*Chelonia mydas**	5	a, e
*Eretmochelys imbricata**	1	a
*Lepidochelys kempi**	1	a
Lepidochelys oliviacea	2	a
*Natator depressus**	1	a
Chelydridae		
*Chelydra serpentina**	1	a
Dermochelydae		
*Dermochelys coriacea**	1	a
Emydidae		
Clemmys marmorata	1	e
*Graptemys barbouri**	2	c, d
Graptemys caglei	1	d
Graptemys flavimaculata	1	d
Graptemys gibbonsi	1	d
Graptemys nigrinoda	1	d
Graptemys oculifera	1	d
Graptemys o. ouachitensis	1	d
Graptemys o. pseudogeographica	1	d
Graptemys pulchra	1	d
*Malaclemys terrapin**	4	c, d, f
Trachemys scripta	1	b
Testudinidae		
*Geochelone denticulata**	1	c
Geochelone gigantea	1	c
*Gopherus agassizii**	3	c
Gopherus berlandieri	1	c
Gopherus flavomarginatus	1	c
Gopherus polyphemus	2	c
*Manouria emys**	1	c
*Testudo horsfieldi**	1	c

[a]Bowen et al., 1993; [b]Hedges, 1994; [c]Lamb and Lydeard, 1994; [d]Lamb et al., 1994; [e]Kumazawa and Nishida, 1995; [f]Seutin et al., 1995.
*Taxon selected for dendrogram construction.

(Cnemidophorus uniparens), western landed gecko *(Coleonyx variegatus),* Hydrophiid sea snake *(Emydocephalus ijimae),* tuatara *(Sphenodon punctatus),* (GenBank/EMBL accession numbers: D31621, S71826; References: Kumazawa and Nishia, 1995; Seutin, Lang, Mindell, and Morais, 1994). Chimpanzee *(Pan troglodytes)* sequences were selected for comparisons with human mtDNA (GenBank/EMBL accession number: D38113).

Sequence Alignment

In order to quantify differences among molecules their sequences must be properly aligned. This task was accomplished with the computer program CLUSTAL W (Thompson, Higgins, and Gibson, 1994). Many of the problems encountered with sequence alignmen have been reviewed by DeSalle, Wray, and Absher

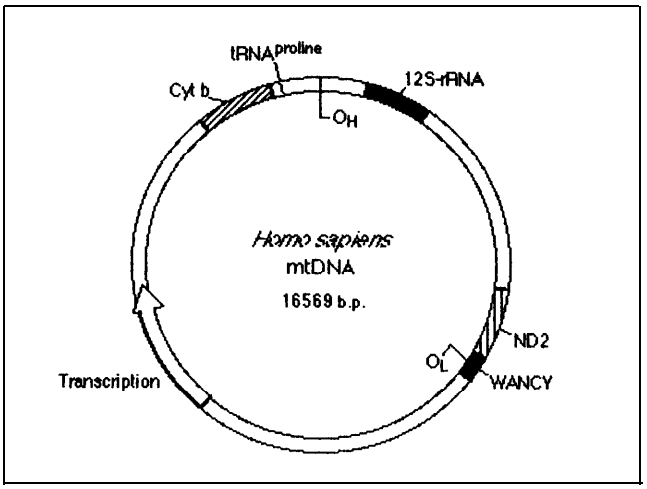

Figure 3. Gene map of human mitochondrial DNA showing relative positions of sampled genes.

(1994) and Wheeler (1994). Sequence alignment involves the identification of homologous nucleotide positions within homologous molecules, and is used as a basis for phylogenetic comparison. Some have suggested that sequence alignment cannot be treated separately from the construction of phyletic trees (Mindell, 1991; Feng and Doolittle, 1987; Hogeweg and Hesper, 1984). Since creationists do not assume all organisms or homologous molecules are related this approach has not been considered.

Sequence Analysis

When comparisons of nucleotide sequences are made, various differences can exist including substitutions and insertion/deletions or indels (Figure 4). Substitutions can be further divided into transition and transversion differences. A transition exists when a pyrimidine (T or C) is substituted by another pyrimidine; or a purine (A or G) is substituted by another purine. A transversion exists when a pyrimidine (T or C) is substituted by a purine (A or G) and vice versa. An indel exists when a nucleotide is inserted or deleted, which results in a longer or shorter sequence respectively. The relationship between transition differences and taxonomic distance is especially interesting. Substitutions between similar organisms are mostly transitions, and as taxonomic distance increases the proportion of transition differences decrease (Holmquist, 1983). Many

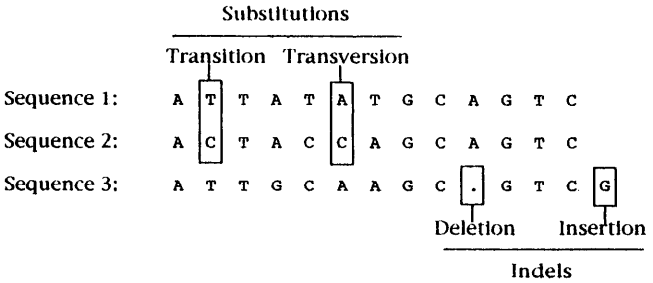

Figure 4. Diagram of three aligned DNA sequences illustrating nucleotide substitutions and indels.

explain this phenomena as homoplasy, and suggest that the genomes of divergent organisms are saturated with back mutations (Kraus and Miyamoto, 1991). Homoplasies are defined as similarities between organisms that are not a result of common descent. Wise provided a hypothesis for interpreting homoplasy within a baraminic context (1990, p. 351):

> When a large number of characters is examined cladistically in a polybaraminic group of organisms, a young-earth creationist would expect that homoplasies would be unavoidable and frequent between organisms of different holobaramins. Conversely, for the organisms in the group which are part of the same holobaramin, it should be possible to construct a cladogram with few or no homoplasies.

Another explanation for the high proportion of transitions among similar organisms rests on the molecular structure of purines and pyrimidines. Since transitions involve the substitution of structurally similar molecules, perhaps the bias towards similar organisms is indicative of a baramin-specific mutation. Members of a baramin are hypothesized to be morphologically and physiologically similar, though not identical (Marsh, 1976). Transitional mutations may reflect a process of limited variation at the molecular level.

Sequence differences are commonly corrected for the unequal occurrence of transitions and transversions (Kimura, 1980). Since these assumptions deserve more study from the creationist perspective this survey uses raw uncorrected sequence differences calculated as the number of differences divided by the total sequence length. The program CLUSTAL W was used to calculate total sequence differences, and the differences attributable to transitions and transversions. As a method of summarizing the diversity of sequence differences among taxonomic groups, a plot of taxonomic rank versus average percent sequence difference was constructed. The percent sequence difference for species was calculated as the average difference for a subspecies and geographical variants within a given species. Genus differences were calculated as the average difference of species within a given genus, and so on for higher taxonomic levels. Class differences were calculated as interorder comparisons of turtles with the reptilian outgroups. Subphylum differences represent turtle comparisons with the four remaining vertebrate outgroups. For taxonomic rank the classification of Gaffney (1988) was used.

Hierarchical patterns encoded in turtle mtDNA were illustrated with unrooted dendrograms for cytochrome *b* using distance and parsimony methods. A variety of clustering techniques exist for analyzing sequence data (Felsenstein, 1988), and a given topology (i.e. branching pattern) should be considered a hypothesis. Congruence among topologies using several techniques indicates the pattern is supported by the data. Statistical confidence can be assigned to a topology using the bootstrap resampling method (Felsenstein, 1985). The data set is randomly resampled and patterns that cannot be attributed to chance are recovered. Branches appearing at least 95% of the time are considered significant, but Hillis and Bull (1993) have noted that bootstrap proportions of 70% can be significant. The neighbor-joining algorithm (Saitou and Nei, 1987) was selected as the distance method because it is free from the assumptions of an evolutionary clock. CLUSTAL W was used to calculate neighbor-joining distances and evaluate the taxonomic patterns with 200 bootstrap iterations. Parsimony analysis was accomplished using the PAUP 3.1.1 computer package (Swofford, 1993). The aligned sequences were evaluated with a bootstrapped (200 iterations) 50% majority rule consensus tree using the heuristic search option, random addition of taxa, and TBR branch swapping. A pairwise matrix of homoplasies was calculated from the cladogram. The number of homoplasies divided by the total sequence length was used to explore the diversity of homoplasies among taxonomic levels. Since the computer time required to generate a dendrogram increases dramatically with the number of species involved, only one species from each genus that contained a cytochrome *b* sequence was selected for dendrogram construction (see Table I).

Scriptural Considerations

In baraminology the Scriptures can be used to generate biosystematic hypotheses. The Authorized Version mentions a tortoise *min* in Leviticus 19:29-30. Jones (1972) has argued that the Hebrew does not refer to the modern tortoise, but actually indicates a variety of lizard species. Regardless of the implied species, characteristics of the testudine baramin can be inferred from other Scripture. The ecological and morphological requirements that clean water swarmers contain fins and scales would categorize aquatic and marine turtles as unclean. The requirement that clean land swarmers have split hooves and display rumination would likewise categorize the terrestrial turtles as unclean. From Genesis 6 and 7 it is learned that unclean land vertebrates, such as the terrestrial turtle baramin, were preserved on the Ark in pairs. Many organisms including fish, invertebrates, and some amphibians were not required to be taken on the Ark (Jones, 1973; Woodmorappe, 1994). Marine turtles are designed to survive at sea, which indicates they would be excluded from the Ark. However, the geophysical upheavals associated with the Flood would have caused high mortality in free swimming marine turtle populations. It is doubtful that aquatic turtles could have survived an extended period outside the Ark. These species are much smaller than marine turtles and are weaker swimmers.

The diversity of turtle mtDNA from different habitats can be evaluated for patterns consistent with Biblical history. If modern turtles are monobaraminic and descended from a single pair preserved on the Ark, we might expect to observe an even distribution of mtDNA variability among the various aquatic, marine, and terrestrial species. In contrast, if modern

Table II. Summary of mitochondrial DNA sequence data used in this study.

Gene	Sequence Length[a]	Number of Variable Sites[b]	Number of Comparisons
Cytochrome *b*	213 b.p.	128 (0.60)	980
NADH dehydrogenase subunit II	164 b.p.	141 (0.86)	11
125-rRNA	64 b.p.	47 (0.73)	11
WANCY[c]	355 b.p.	204 (0.57)	26
$tRNA^{proline}$	52 b.p.	49 (0.94)	18

[a]b.p. = base pairs.
[b](proportion of variable sites).
[c]Gene cluster of five tRNAs with single-letter abbreviation of amino acid anticodon (W = Tryptophan, A = Alanine, N = Asparagine, C = Cysteine, Y = Tyrosine). Spacer sequences were not included in the analysis.

turtles are polybaraminic and descended from several pre-Flood monobaramins the mtDNA might reflect differing degrees of variability. Specifically, we would expect the marine turtles to display more mtDNA variability because the population bottleneck in these species could have been less extensive than in terrestrial species.

It has been asked whether thresholds in overall DNA similarity exist, which could be used to consistently identify baraminic boundaries (Remine, 1990). To explore this question the sequence differences among monobaraminic turtles have been compared with a biologically similar but baraminically distinct standard, human-chimpanzee sequences. Scriptures indicate these taxa are phylogenetically unrelated even though they share many similarities. Thus, if thresholds in overall DNA similarity exist we would expect sequence differences between humans and chimpanzees to be greater than or equal to the differences between monobaraminic turtles.

Results

Sequence Variation

The mtDNA sequences averaged a length of 169 bases with 113 variable positions (Table II). The WANCY gene cluster generated the longest sequence alignment, but was the least variable. Alternatively, $tRNA^{Proline}$ was the shortest sequence and the most variable. Cytochrome *b* was the most speciose gene available with 980 comparisons. It is preferable to analyze sequences at least several hundred bases in length (Graybeal, 1994) because longer sequences are analogous to larger sample sizes. The relatively short sequences used in this survey would indicate the data set was of minimal baraminic utility.

A plot of taxonomic rank versus average percent sequence difference summarizes the nucleotide comparisons (Figure 5). Four patterns are notable. First, NPC genes are more divergent at higher taxonomic levels (i.e. subtractive comparisons between turtles and outgroups), whereas PC genes are more divergent at lower taxonomic levels (i.e. additive comparisons within turtles). The large standard deviations associated with higher-level comparisons indicates an excess of

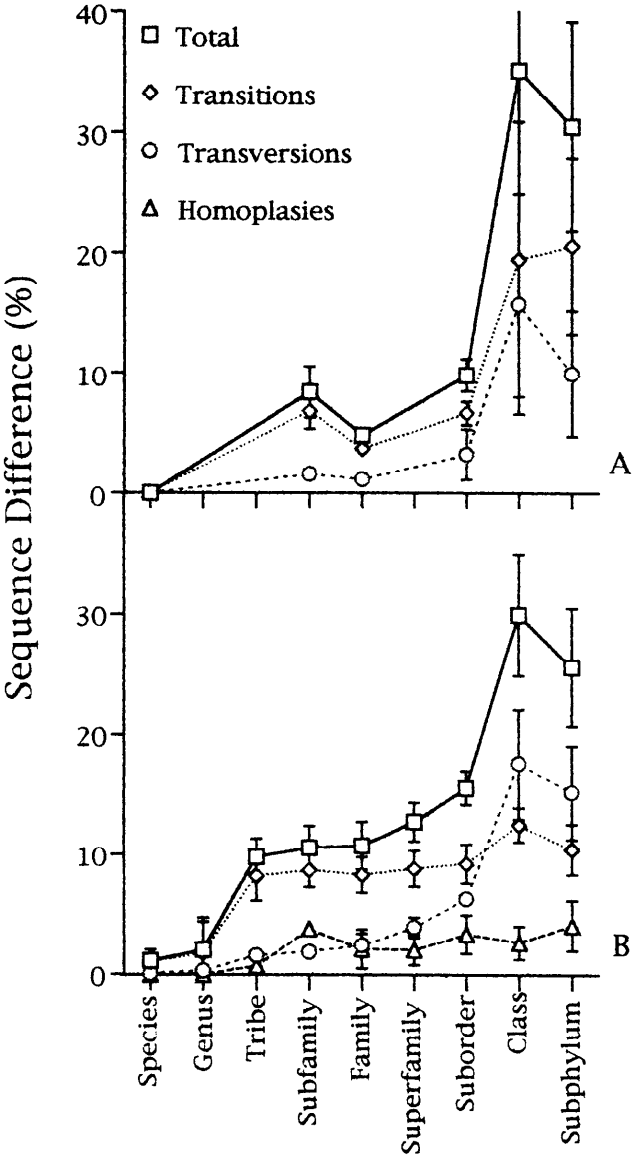

Figure 5. Sequence differences averaged (±SD) across taxonomic ranks for non-protein coding (A) and protein coding (B) mitochondrial genes.

variation at these levels. Secondly, for PC genes the transition and transversion lines intersect near the turtle outgroup boundary. Thirdly, for both PC and NPC genes there are two areas of large sequence divergence, which will be designated the tribe "hill" and class "mountain." Finally, the sequence differences attributable to homoplasies remain relatively constant beyond the level of subfamily.

Subtractive Comparisons

Holobaramin identification is accomplished by successive approximation of apobaramins and monobaramins (see Figure 1). The outgroups in this analysis were chosen to assess the relationship of turtles to non-turtles, which is a subtractive comparison for approximating apobaramins. The sequence differences between turtles and outgroups for cytochrome *b* and the

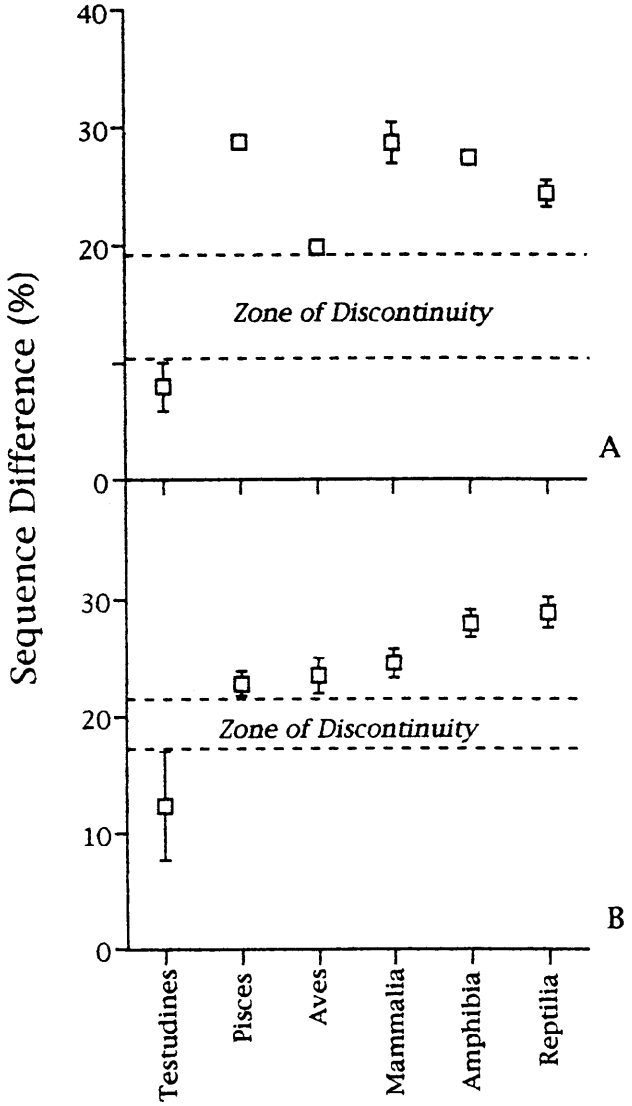

Figure 6. Sequence differences averaged (±SD) for turtle-turtle and turtle-outgroup comparisons using WANCY (A) and cytochrome *b* (B) genes.

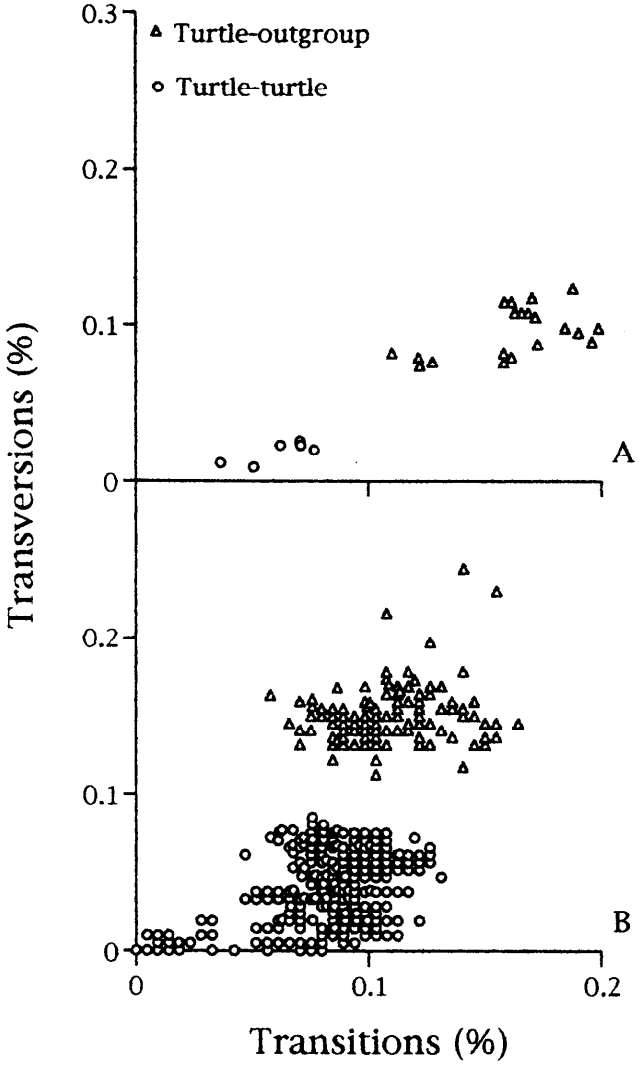

Figure 7. Transition versus transversion sequence differences for WANCY (A) and cytochrome *b* (B) genes.

WANCY gene cluster demonstrates a gap between these taxa (Figure 6). It is noted that for cytochrome *b* the turtle-reptile comparisons were more divergent than the other outgroup comparisons. Denton (1985, p. 293) noted such phylogenetic discrepancies commonly occur, "the molecules provide little support for this 'sequential' interpretation of the vertebrate classes." Both baraminologists and macroevolutionists have suggested that the differences between genes might not coincide with the differences between species, but given enough gene comparisons the historical patterns would become apparent (Patterson, 1987; Wise, 1992a). In this study nearly one-fourth (9/37) of the mitochondrial genes were sampled and macroevolutionary relationships were still not revealed.

As previously noted total sequence difference can be partitioned into transition and transversion differences. When these components are plotted against each other an intriguing pattern emerges (Figure 7). In addition to the two obvious clusters (one purely turtle, one purely outgroup) note the absence of transitory data points. There is a definite chemical abyss separating these two groups. In addition to the previously mentioned outgroups, 21 other vertebrates were compared to determine if the pattern was merely an artifact of small sample size. All comparisons confirmed the validity of the pattern (data not shown).

Additive Comparisons

The hybridization criterion has been emphasized for grouping phylogenetically related taxa (Scherer, 1994). For n species in a given taxon there are $(n^2 - n)/2$ possible hybrids. Approximately 236 extant turtle species have been identified (Pritchard, 1979a), which amounts to 27,730 possible hybrids for the testudine order. A list of 84 turtle crosses has been compiled by Frair, but only 42 of these experiments involve interspecific comparisons or higher (Frair, personal communication). Among the turtles included in this study

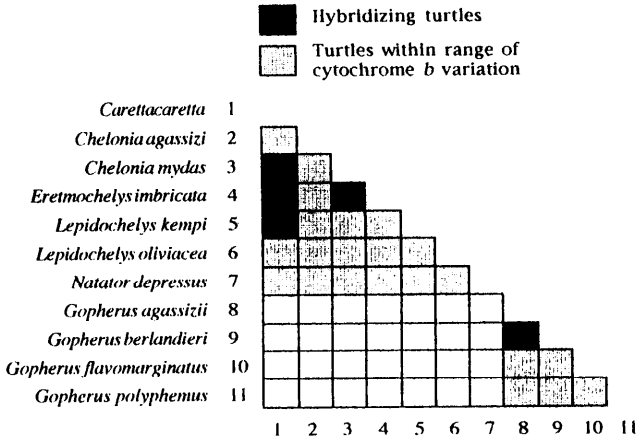

Figure 8. Hybridogram characterizing the Cheloniidae and *Gopherus* monobaramins.

six species are accurately known to hybridize: *Caretta caretta* x *Chelonia mydas, Eretmochelys imbricata, Lepidochelys kempi; Chelonia mydas* x *Eretmochelys imbricata; Gopherus berlandieri* x *Gopherus agassizii* (Conceicao, Levy, Marins, and Marcovaldi, 1990; Karl, Bowen, and Avise, 1995; Shaw, 1946; Wood, Wood, and Critchley, 1983; Woodbury, 1952). Turtles that are not known to hybridize, but fall within the range of cytochrome *b* sequence variation of hybridizing turtles can be lumped to form larger monobaramins. This approach was originally proposed by Remine (1990) and expounded by Wise (1970, 1992a) as the experimental or artificial variation criterion. Using this technique, two monobaramins were constructed including the complete Cheloniidae family, and the *Gopherus* genus (Figure 2). Among these turtles the distribution of cytochrome *b* sequence variation is peculiar (Figure 9). The bimodal appearance suggests two distinct assemblages of turtles can hybridize.

Clustering

The neighbor-joining dendrogram for cytochrome *b* DNA (Figure 10a) depicts branches that are proportional to total sequence differences. Turtles and outgroup taxa cluster as separate units with long branches leading to the later. The cladogram (Figure 10B) was notably congruent with the neighbor-joining dendrogram. The Testudinoidea cluster (i.e. Bataguridae + Testudinidae + Emydidae) was more highly resolved in the cladistic analysis, but received little bootstrap support. The Dermochelydae + Chelydridae cluster is probably artificial since the group received negligible bootstrap support for both dendrograms. A homoplasy index can be calculated for parsimony-based dendrograms as 1 - R/L, where R is the minimum number of synapomorphies and L is the length of the dendrogram (Kluge and Farris, 1969). The value has a range of 0 to 1 in order of increasing homoplasy, and is a goodness-of-fit measure indicating convergence in the data set.

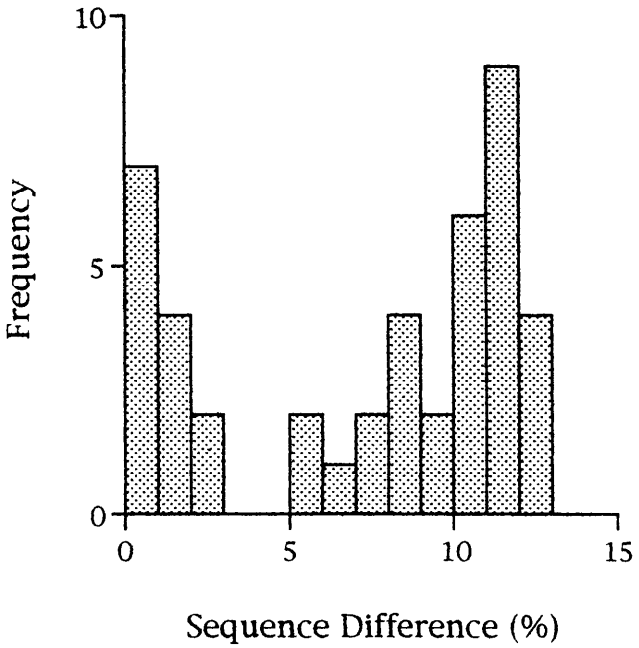

Figure 9. Frequency distribution of cytocbrome *b* sequence differences for monobaraminic turtles.

Table III. Comparisons of sequence differences among turtles, hybridizing turtles, and human-chipanzees.

	Average Sequence Difference (%)[a]				
Comparison	Cytb	ND2	12S	WANCY	$tRNA^{pro}$
All turtles	12.34 (4.67)	16.77 (0)	12.07 (0)	7.97 (2.08)	6.41 (5.55)
Hybridizing terrestrial turtles	3.99 (2.03)	—	—	—	—
Hybridizing marine turtles	7.38 (4.30)	—	—	5.92 (0)	6.41 (5.55)
Human-chimpanzee	9.39 (0)	8.54 (0)	4.69 (0)	2.93 (0)	3.92 (0)

[a](±SD).

The cladogram in Figure 10B required 467 steps and generated a homoplasy index of 0.597 as calculated by PAUP Excluding the outgroup taxa from the data set reduced the cladogram to 261 steps, but still maintained a large homoplasy index of 0.586.

Similarity Thresholds

Sequence comparisons between humans and chimpanzees are summarized in Table III. Only cytochrome *b* differences between monobaraminic turtles were less than human-chimpanzee differences. The variation among marine turtle WANCY and $tRNA^{Proline}$ sequences was approximately twice the value of human-chimpanzees. This data indicated that NPC genes may be unsuitable for evaluating threshold similarities, but a larger sample is necessary to make generalizable conclusions.

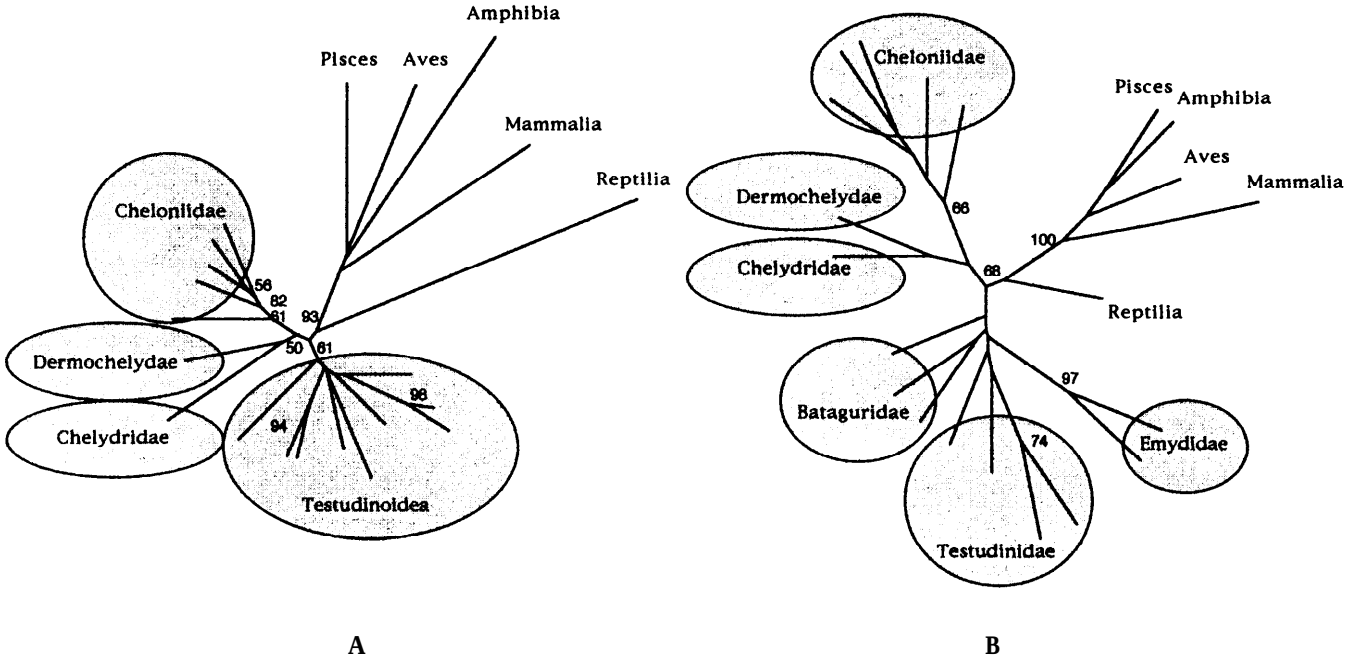

Figure 10. Unrooted dendrograms of cytochrome *b* constructed by distance (a) and parsimony (b) methods. The branch lengths are proportional to sequence differences in the neighbor-joining dendrogram. Numbers at nodes refer to bootstrap proportions. Only nodes recovered at least 50% of the time are reported. The ellipses denote natural groups within the testudine apobaramin.

Discussion

Systematic Hypotheses

Subtractive comparisons provided four lines of evidence supporting the hypothesis that turtles are apobaraminic:

(1) The class "mountain" of Figure 5 is indicative of a large gap between the PC and NPC mtDNA genes of turtles and other vertebrates.

(2) The transition and transversion intersection for PC genes between turtles and reptiles (Figure 5). Nucleotide substitutions between similar organisms are largely transitions, but transversions are predominant when turtles and outgroups are compared.

(3) Clearly definable gaps between turtles and outgroups as revealed by total sequence differences (Figure 6) and transition/transversion sequence differences (Figure 7). The data in Figure 6 also implies discordance between the mtDNA sequences of turtles and the supposed phylogenetic lineage of the vertebrates. If turtles had evolved from reptilian ancestry the reptilian sequences should have been more similar to the turtles than the other vertebrates.

(4) Topological congruence and strong bootstrap support for the separation of turtles and outgroups (Figure 10). The large homoplasy index calculated for the cladogram in Figure 10b was also supportive of phylogenetic discontinuity.

The exclusion of outgroup taxa from the parsimony analysis only reduced the homoplasy index by 0.011. The remaining high degree of convergence indicated that additional apobaraminic boundaries existed within the testudine apobaramin, and further challenged Frair's hypothesis (Frair, 1991) that turtles are holobaraminic. The gaps identified by the molecular data are corroborated by morphological and paleontological criteria. Pritchard stated:

> The first unquestionable turtles are of Triassic age, but despite the numerous primitive features of these early representatives, they shed little light on the evolution of the Order Testudines from its presumed cotylosaurian ancestors (1979a, p. 5).
> One can only speculate how the Proganocheiyidae evolved from the primitive reptile stem (1979b, p. 73)

In a cladistic survey of turtle morphology Gaffney and Meylan (1988, p. 160) noted, "Although there have been efforts to discover the 'ancestor' or closest relative of turtles, no consensus has been reached."

Hybridization is considered one of the most reliable additive membership criteria (Wise, 1992a). Since only 0.15% (42/27,730) of the possible turtle crosses have been reported it is likely that current turtle monobaramin estimates are very conservative. The Cheloniidae and *Gopherus* monobaramins were constructed based on reasonably strong data:

(1) Hybridization and cytochrome *b* sequence variation (Figure 8).

(2) Bootstrap supported clusters based on phenetic (Figure 10A) and cladistic (Figure 10B) dendrograms.

Monobaramin Characteristics

The increase in cytochrome *b* homoplasies at the level of subfamily suggested an appreciable amount of homoplasy can exist within monobaramins, which questions the hypothesis of Wise (1990). Separate analyses of marine turtle and *Gopherus* mtDNA yielded homoplasy indices of 0.230 and 0.159 respectively (Dutton, Davis, Guerra, and Owens, 1996; Lamb and Lydeard, 1994). The latter index is probably inflated because the study included the genus *Manouria* as an outgroup, which in this survey was 8.72% (± 0.97%) divergent from *Gopherus.* These data clearly indicate that convergent evolution can occur within the monobaramin.

Wise (1992a) postulated that members of a holobaramin might occupy similar habitats and trophic levels. Species of the Cheloniid monobaramin share a marine environment by partitioning its resources through different migratory patterns and food preferences (Hendrickson, 1980). Diverse trophic categories such as herbivory, omnivory, and carnivory are represented by the marine turtles. Conversely, the gopher tortoises occupy a more diverse habitat from the humid southeastern United States to the arid highlands of central Mexico. All taxa comprising the *Gopherus* monobaramin are herbivorous (Bury, 1982). These conflicting data indicate the ecologic and trophic membership criteria may be of little significance for defining baramins. However, these criteria can be used to provide a better understanding of monobaramin biology. Tyler (1996, p. 1) recently raised important questions regarding the range, of trophic variation within baramins:

> we are only beginning to address the question of how much variation can occur within a created Kind and, in particular, whether the genotype carries the information necessary for a herbivorous ancestor to develop a carnivorous descendant.

All members of vertebrate holobaramins were originally created as herbivores (see Genesis 1:30). The expression of carnivorous behavior appeared later, either after the Fall (Stambaugh, 1991) or after the Flood (Lambert, 1983). The observation that the Cheloniid monobaramin exhibits a variety of trophic categories is supportive of a large amount of created diversity within the holobaraminic gene pool.

The hypothesized turtle monobaramins are composed of endangered and threatened species (Bury, 1982; Bowen, Nelson, and Avise, 1993). Human activities are responsible for a majority of the excess mortality, but natural factors are also involved. This observation is significant in light of the fact that modern conservation measures discourage the production of interspecific hybrids (O'Brien and Mayr, 1991). Hybridization tends to obscure the boundaries of biological species, and modern conservation biology aims to protect species. The creationist position suggests that holobaramins have historically been the units of natural conservation, since the *min* not the species was preserved during the Flood (see Genesis 6:20, 7:14). Biologists have traditionally viewed hybrids in a negative context as unfit mutants, but recent data challenges this approach. Hybridization can result in rapid microevolution (Arnold, 1992) and increased fitness (Arnold and Hodges, 1995). If hybridization is a natural mechanism for generating diversity within the holobaramin, then the preservation of monobaramins could prove more efficient and successful than current procedures

Cytochrome *b* sequence variation was consistent with a polybaraminic origin for post-Flood turtles (Table III). The variation within monobaraminic marine turtles was nearly twice that for terrestrial species, which could indicate the population bottleneck caused by the Flood was more severe in terrestrial turtles. Alternatively, it could be argued that the observed sequence diversity is an artifact caused by taxonomic structure. Since comparisons within the Cheloniidae monobaramin (i.e. family) involve greater taxonomic distances than comparisons within the *Gopherus* monobaramin (i.e. genus) one would naturally expect a larger degree of sequence variation among the marine turtles.

Method Reliability

Wise (1992a; 1992b) noted that not all molecules would be useful for identifying phylogenetic discontinuity. The effects of a common Creator, optimally efficient structural and functional motifs, common metabolic needs, etc. would tend to reduce a molecule's holobaramin specificity. The baraminic utility of a molecular data set is dependent on several factors including: the type of molecule selected, sequence length, and the proportion of variable sites within the molecule. When these parameters are maximized the efficiency of the molecular discontinuity criterion should increase.

It is crucial to develop a statistical framework for evaluating molecular data from a baraminic perspective. Wise (1992a) had suggested ANOVA and principal components analysis, but these techniques are not used in molecular studies. A related technique called ordination analysis (Higgins, 1992) might prove useful, and a least-squares method for evaluating the statistical significance of sequence differences has been published (Tyson and Fieldes, 1992; Tyson, 1992). While multicriterial approaches for resolving baraminic relationships should prove most efficient, molecular data sets will be of prime importance. Fitch and Atchley (1987) documented that among life history, molecular, and morphological characters the molecules recovered the correct phylogeny for a group of laboratory mice of known geneaology.

Summary

Successive approximation has been shown to be a conservative approach for identifying holobaramins that is limited by the baraminic utility of the data set. In this study holobaraminic relationships were unresolved because there was no juncture of the apobaramin with the monobaramin. Further research with more powerful data will be required to accurately approximate the holobaramin. An apobaraminic boundary was characterized, which separated the turtles from other vertebrates. Two biologically significant monobaramins have been identified including: the Cheloniidae or marine turtle monobaramin and the *Gopherus* or desert tortoise monobaramin. The boundaries elucidated by molecular data suggested the homoplasy criterion was sensitive to monobaraminic divisions, while the baraminic utility of the ecologic and trophic criteria was questioned. Several practical techniques have been outlined for analyzing sequence data, which are widely available through the internet. The application of these methods to a variety of organisms will advance our knowledge about a creation shaped in perfection, but now marred in decay.

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References

CRSQ-Creation Research Society Quarterly.

Anderson, S., A. T. Bankier, B. G. Barrell, M. H. L. de Bruijn, A. R. Coulson, J. Drouin, I. C. Eperon, D. P. Nierlich, B. A. Roe, F. Sanger, P. H. Schreier, A. J. H. Smith, R. Staden, and I. G. Young. 1981. Sequence and organization of the human mitochondrial genome. *Nature* 290:457-465.

Arnold, M. L. 1992. Natural hybridization as an evolutionary process. *Annual Review of Ecology and Systematics* 23:237-261.

_____ and S. A. Hodges. 1995. Are natural hybrids fit or unfit relative to their parents? *Trends in Ecology and Evolution* 10(2):67-71.

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* 18:489-522.

_____ B. W. Bowen, T. Lamb, A. B. Meylan, and E. Bermingham. 1992. Mitochondrial DNA evolution at a turtle's pace: Evidence for low genetic variability and reduced microevolutionary rate in the testudines. *Molecular Biology and Evolution* 9(3):457-473.

Bartz, P. A. 1991. A refinement of biosystematics which reflects baraminic variation. *CRSQ* 28:18-20.

Bowen, B. W., W. S. Nelson, and J. C. Avise. 1993. A molecular phylogeny for marine turtles: Trait mapping, rate assessment, and conservation relevance. *Proceedings* of *the National Academy of Science* USA 90:5574-5577.

Bury, R. B. (editor). 1982. North American tortoises: Conservation and ecology. U.S. Department of the Interior, Fish and Wildlife Service. Washington, D. C.

Conceicao, M. B., J. A. Levy, L. F. Marins and M. A. Marcovaldi. 1990. Electrophoretic characterization of a hybrid between *Eretmochelys imbricata* and *Caretta caretta* (Cheloniidae). *Comparative Biochemistry and Physiology* 97B(2):275-278.

Denton, M. 1985. Evolution: A theory in crisis. Burnett Books. Bethesda, MD.

DeSalle, R., C. Wray and R. Absher. 1994. Computational problems in molecular systematics. In: Scheirwater, B., B. Streit, G. P. Wagner, and R. DeSalle (editors), Molecular ecology and evolution: Approaches and application. Birkhauser. Basel, Switzerland. pp. 353-370.

Dutton, P. H., S. K. Davis, T. Guerra, and D. Owens. 1996. Molecular phylogeny for marine turtles based on sequences of the ND4-leucine tRNA and control regions of mitochondrial DNA. *Molecular Phylogenetics and Evolution* 5(3):511-521.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39(4):783-791.

_____ 1988. Phylogenies from molecular sequences: Inference and reliability. *Annual Review of Genetics* 22:521-565.

Feng, D. and R. F. Doolittle. 1987. Progressive sequence alignment as a prerequisite to correct phylogenetic trees. *Journal of Molecular Evolution* 25:351-360.

Fitch, W. M. and W. R. Atchley. 1987. Divergence in inbred strains of mice: A comparison of three different types of data. In: C. Patterson (editor), Molecules and morphology in evolution: Conflict or compromise? Cambridge University Press. Cambridge, England. pp. 203-216.

Frair, W. 1984. Turtles: Now and then. In: Proceedings of the North-coast Bible-Science Conference. Minneapolis, MN. pp. 33-38.

_____ 1991. Original kinds and turtle phylogeny. *CRSQ* 28:21-24.

Gaffney, E. S. 1984. Historical analysis of theories of Chelonian relationship. *Systematic Zoology* 33(3):283-301.

_____ and P. A. Meylan. 1988. A phylogeny of turtles. In: Benton, M. J. (editor), The phylogeny and classification of the tetrapods. Volume 1. Clarendon. Oxford, England. pp. 157-219.

Graybeal, A. 1994. Evaluating the phylogenetic utility of genes: A search for genes informative about deep divergences among vertebrates. *Systematic Biology* 43(2):174-193.

Hedges, S. B. 1994. Molecular evidence for the origin of birds. *Proceedings* of *the National Academy of Science* USA 91:2621-2624.

Hendrickson, J. R. 1980. The ecological strategies of sea turtles. *American Zoologist* 20:597-608.

Higgins, D. G. 1992. Sequence ordinations: A multivariate analysis approach to analysing large sequence data sets. *CABIOS* 8(1):15-22.

Hillis, D. M. and J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42:182-192.

Hogeweg, P. and B. Hesper. 1984. The alignment of sets of sequences and the construction of phyletic trees: An integrated method. *Journal of Molecular Evolution* 20:175-186.

Holmquist, R. 1983. Transitions and transversions in evolutionary descent: An approach to understanding. *Journal of Molecular Evolution* 19:134-144.

Jones, A. J. 1972. Boundaries of the *min:* An analysis of the Mosaic lists of clean and unclean animals. *CRSQ* 9:114-123.

_____. 1973. How many animals on the ark? *CRSQ* 10:102-108.

Karl, S. A., B. W. Bowen and J. C. Avise. 1995. Hybridization among the ancient mariners: Characterization of marine turtle hybrids with molecular genetic assays. *Journal of Heredity* 86(4):262-268.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111-120.

Kluge, A. G. and J. S. Farris. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* 18:1-32.

Kraus, F. and M. M. Miyamoto. 1991. Rapid cladogenesis among the pecoran ruminants: Evidence from mitochondrial DNA sequences. *Systematic Zoology* 40:117-130.

Kumazawa, Y., and M. Nishida. 1995. Variations in mitochondrial tRNA gene organization of reptiles as phylogenetic markers. *Molecular Biology and Evolution* 12(5):759-772.

Lamb, T., and C. Lydeard. 1994. A molecular phylogeny of the gopher tortoises, with comments on familial relationships within the testudnoidea. *Molecular Phylogenetics and Evolution* 3(4): 283-291.

_____ C. Lydeard, R. B. Walker, and J. W. Gibbons. 1994. Molecular systematics of map turtles *(Graptemys):* A comparison of mitochondrial restriction site versus sequence data. *Systematic Biology* 43:543-559.

Lambert, G. R. 1983. Was the pre-flood animal kingdom vegetarian? *CRSQ* 20:88.

Marsh, F. L. 1941. Fundamental biology. Self-published. Lincoln, NB.

_____. 1971. The Genesis kinds in the modern world. In: W. E. Lammerts (editor), Scientific studies in special creation. Baker Book House. Grand Rapids, MI. pp. 136-155.

_____. 1976. Variation and fixity in nature. Pacific. Omaha, NE.

Mindell, D. P. 1991. Aligning DNA sequences: Homology and phylogenetic weighting. In: Miyamoto, M. M. and J. Cracraft (editors), Phylogenetic analysis of DNA sequences. Oxford University Press. New York. pp. 73-89.

O'Brien, S. J. and E. Mayr. 1991. Bureaucratic mischief: Recognizing endangered species and subspecies. *Science* 251:1187-1188.

Patterson, C. 1987. Introduction. In: C. Patterson (editor), Molecules and morphology in evolution: Conflict or compromise? Cambridge University Press. Cambridge, England. pp. l-22.

Pritchard, P. C. H. 1979a. Encyclopedia of turtles. T. F, H. Publications. Neptune City, NY.

_____. 1979b. Taxonomy, evolution, and zoogeography. In: Harless, M. and H. Morlock (editors), Turtles: Perspectives and research. John Wiley. New York. pp. l-42.

Remine, W. J. 1990. Discontinuity systematics: A new methodology of biosystematics relevant to the creation model. In: Walsh, R. E. and C. Brooks (editors), Proceedings of the second international conference on creationism. Volume 2. Creation Science Fellowship. Pittsburgh, PA. pp. 207-213.

_____. 1993. The biotic message: Evolution versus message theory. St. Paul Science. St. Paul, MN. 538 pp.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.

Scherer, S. 1994. Basic types of life. In: Walsh, R. E. (editor), Proceedings of the third international conference on creationism. Volume 2. Creation Science Fellowship. Pittsburgh, PA. pp. 467-483.

Seutin, G., B. F. Lang, D. P. Mindell, and R. Morais. 1994. Evolution of the WANCY region in amniote mitochondrial DNA. *Molecular Biology and Evolution* 11(3):329-340.

Shaw, C. E. 1946. An anomalous pacific loggerhead turtle from the northwestern coast of Baja California. *Herpetologica* 3:123-124.

Siegler, H. 1974. The magnificance of kinds as demonstrated by canids. *CRSQ* 11:94-97.

_____. 1978. A creationist's taxonomy. *CRSQ* 15:36-38, 11.

Stambaugh, J. 1991. Creation's original diet and the changes at the fall *Creation Ex Nihilo Technical Journal* 5(2):130-138.

Swofford, D. L. 1993. Phylogenetic analysis using parsimony (PAUP Version 3.1.1). Illinois Natural History Survey. Champaign.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673.4680.

Tyler, D. J. 1996. Herbivores, carnivores and the created order. *Creation Matters* 1(3):1-2.

Tyson, H. 1992. Relationships between amino acid sequences determined through optimum alignments, clustering, and specific distance patterns: Application to a group of scorpion toxins. *Genome* 35:360-371.

_____ and M. A. Fieldes. 1992. A set of BASIC programs to evaluate relationships among protein sequences by optimum alignment and distance matrix analysis. *Computer Methods and Programs in Biomedicine* 18:61-72.

Wheeler, W. C. 1994. Sources of ambiguity in nucleic acid sequence alignment. In: Scheirwater, B., B. Streit, G. P. Wagner, and R. DeSalle (editors), Molecular ecology and evolution: Approaches and application. Birkhauser. Basel, Switzerland. pp. 323-352.

Wilson, A. C., R. L. Cann, S. M. Carr, M. George, U. B. Gyllensten, K. M. Helm-Bychowski, R. G. Higuchi, S. R. Palumbi, E. M. Prager, R. D. Sage, and M. Stoneking. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society* 26:375-400.

Wise, K. P. 1990. Baraminology: A young-earth creation biosystematic method. In: Walsh, R. E. and C. L. Brooks (editors), Proceedings of the second international conference on creationism. Volume 2. Creation Science Fellowship. Pittsburgh, PA. pp. 345-358.

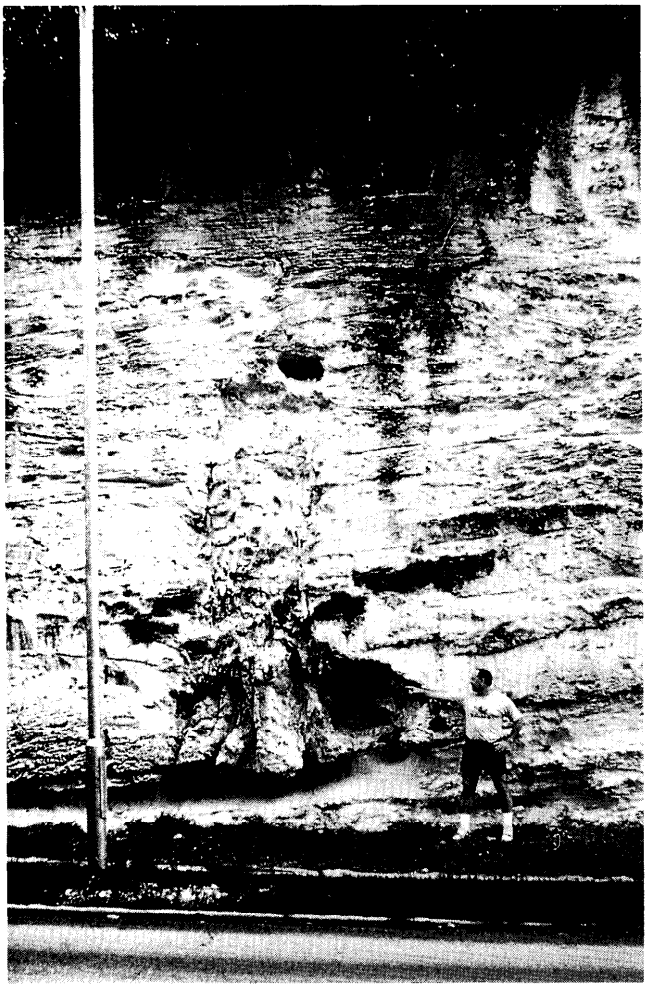
_____ 1992a. Practical baraminology. *Creation Ex Nihilo Technical Journal* 6(2):122-137.

_____. 1992b. Creation polycladism: A young-earth creation theory of biogenesis. In: Proceedings of the 1992 Twin-Cities creation science association, Genesis Institute, and Northwestern College. Minneapolis-St. Paul, MN. pp. 204-210.

Wood, J. R., F. E. Wood and K. Critchley. 1983. Hybridization of *Chelonia mydas* and *Eretmochelys imbricata. Copeia* 3:839-842.

Woodbury, A. M. 1952. Hybrids of *Gopherus berlandieri* and *G. agassizii. Herpetologica* 8:33-36.

Woodmorappe, J. 1994. The biota and logistics of Noah's ark. In: Walsh, R. E. (editor), Proceedings of the third international conference on creationism. Volume 2. Creation Science Fellowship. Pittsburgh, PA. pp. 623-631.



Polystrate tree cast in oolitic dune ridge, New Providence Island, Bahamas. This tree cast reveals the rapid build-up of carbonate sand from probably just one storm. Vast amounts of ooids would be available for dune development with a drop in sea-level during the Ice Age Timeframe. Current climatic models for the Pleistocene fail to provide the storm energy necessary to account for this rapid build-up of carbonate ooids and their subsequent lithification. Young-earth atmospheric studies support storm tracks and associated energies necessary to account for the rapid burial of this tree by migrating ooids. Photograph and caption by Carl R. Froede, Jr. See *CRSQ* 3:21-25 for an article on polystrate fossil trees.