The Auk 111(2):389–397, 1994

REEXAMINATION OF BARBET MONOPHYLY USING MITOCHONDRIAL-DNA SEQUENCE DATA

SCOTT M. LANYON AND JOHN G. HALL

Center for Evolutionary and Environmental Biology, Field Museum of Natural History, Roosevelt Road at Lake Shore Drive, Chicago, Illinois 60605, USA

ABSTRACT.—An 888-base-pair segment of the mitochondrial cytochrome-*b* gene was sequenced for New World barbets, Old World barbets, toucans, and several outgroup taxa. Toucans were consistently identified as the sister taxon of the New World barbets by a variety of analyses. These data are fundamentally different from earlier morphological analyses and the DNA-DNA hybridization study of these same taxa. Our results provide an independent confirmation of relationships proposed in both the morphological and molecular studies—that New World barbets and toucans are sister taxa with respect to Old World barbets. *Received 19 June 1992, accepted 25 November 1992.*

TRADITIONAL MORPHOLOGICAL analyses have disagreed on the phylogenetic relationships among the barbets (Capitonidae), a pantropical assemblage of frugivorous birds, traditionally placed in the Piciformes with woodpeckers, puffbirds, jacamars, and toucans (for references see Sibley and Ahlquist 1990). However, Burton (1984:433) concluded, based on comparative anatomy of the feeding apparatus in the Coraciiformes and Piciformes, that "it seems reasonable simply to regard toucans as a specialized group of barbets which have arisen and radiated in South America." Prum (1988) reassessed Burton's data and those of other workers (e.g. Swierczewski and Raikow 1981) and likewise concluded that New World barbets are more closely related to toucans than either taxon is to Old World barbets.

Sibley and Ahlquist's DNA-DNA hybridization data (1986) also confirmed Burton's findings. Having assumed a uniform rate of sequence divergence, Sibley and Ahlquist estimated that New World barbets and toucans shared a common ancestry independent of Old World barbets for about 20 to 30 million years. They placed the New World barbets within the toucan family Ramphastidae and divided the Old World barbets into two distinct families, the Megalaimidae and Lybiidae (Sibley and Ahlquist 1986, 1990).

The DNA-DNA hybridization data more directly reflect the magnitudes of genetic divergence of these taxa than do the morphological data. Consequently, the congruence of results of DNA-DNA hybridization and morphological studies was thought to be strong confirmatory evidence for barbet polyphyly. However, several systematists have recently questioned the usefulness of DNA-DNA hybridization data in phylogeny reconstruction (e.g. Sarich et al. 1989). These criticisms (e.g. Lanyon 1992) have centered on the quality of the data and their acquisition, as well as on the analytical limitations of distance data, for which only phenetic approaches are possible (Eldredge and Cracraft 1980). These uncertainties surrounding the DNA-DNA hybridization technique in general and Sibley and Ahlquist's (1990) data in particular, indicate that another kind of data set would be valuable in resolving the issue of barbet polyphyly.

The purpose of our study was to reexamine the issue of barbet polyphyly with a different kind of molecular data—mitochondrial DNA (mtDNA) sequences. While the DNA-DNA hybridization data are distance measurements involving the nuclear genome, the molecular data of our study are DNA sequences, which are character-based data that can be analyzed cladistically.

METHODS

Study organisms.—Two genera (or two congeneric species in the case of the New World Capitonidae) represented each group: New World barbets (Capitonidae, *Capito niger* and *C. dayi*); Old World barbets (Capitonidae, *Pogoniulus bilineatus* and *Lybius bidentatus*); and toucans (Ramphastidae, *Aulacorhynchus derbianus* and *Ramphastos tucanus*). A woodpecker (*Sphyrapicus varius*), a passerine (*Molothrus ater*), and the published DNA sequence of the chicken (*Gallus gallus*) mitochondrial genome (Desjardins and Morais 1990) were used for outgroup comparison.

DNA techniques.—A 50- to 100-mg tissue sample from

TABLE 1. Sequences and locations of amplification and sequencing primers used.

Primer description[a]	Identification tag	Sequence
L14841	B1	5'-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3'
H15149	B2	5'-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3'
L15042	B3	5'-ATCTGCATCTACCTACACATCGG-3'
H15767	B4	5'-GATGAATGGGTGTTCTACTGGTTG-3'
L15243	B5	5'-ACCCTAGTAGAATGAGCCTGAGG-3'

[a] Letters refer to light (L) or heavy (H) strands; numbers correspond to location of the 3' end of primer in human mtDNA sequence (Anderson et al. 1981).

each taxon was selected from the Field Museum frozen tissue collection, minced with a sterile blade and digested overnight at 37°C in extraction buffer (100 mM tris, pH 8.0, 10 mM Na_2EDTA, 100 mM NaCl, 1% sodium dodecyl sulfate, 10 mg/ml dithiothreitol [United States Biochemical], and 0.5 mg/ml proteinase K [Boehringer-Mannheim]). Whole-genomic DNA was extracted twice with equilibrated phenol, once with phenol/chloroform (1:1), and twice with chloroform (Maniatis et al. 1982) before being precipitated with ethanol.

Initially, a 307-base-pair (bp) segment of the mitochondrial cytochrome gene was amplified with the "universal" oligonucleotide primers (B1 and B2, Kocher et al. 1989; Table 1). Double-stranded amplifications (dsPCR) in 25 μl volumes were performed in 0.5 ml microcentrifuge tubes in 10 mM tris, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 150 μM each deoxynucleotide (dNTP), 0.5 μM each amplification primer, and 0.6 units *Taq* polymerase (Perkin Elmer). Genomic DNA (10–1,000 ng) was added to the reaction mixture after it had been overlayed with mineral oil. Reaction mixtures were subjected to 30–35 cycles of denaturation at 93°C (1 min), annealing at 52°C (1 min), and extension at 72°C (2 min), in a variable-temperature block (M J Research). The first cycle was preceded by a 3-min denaturation step, and the last cycle was followed by a 3-min extension time.

A portion (5 μl) of the dsPCR product was run on a 3% NuSieve agarose gel (FMC) in 40 mM tris-acetate buffer, pH 8.0, at 60 V. After staining the gel with ethidium bromide, the amplified PCR product was excised from the gel and melted in 300 μl water. This solution was diluted 1:20 and 2.4 μl was used as template in a 100-μl asymmetric PCR for the purpose of generating single-stranded DNA template for direct sequencing (Gyllensten and Erlich 1988). Our amplification conditions were similar to those for dsPCR except that dNTP concentrations were reduced to 50 μM and the concentration of one of the primers was reduced to 25 nM. Following 45 cycles of amplification, excess primers, salts, and free nucleotides were removed by three cycles of centrifugal dialysis (Centricon-30, Amicon). An aliquot of the washed and concentrated PCR product was then sequenced by the dideoxy method (Sanger et al. 1977) with a commercially available kit (Sequenase, United States Biochemical) with the oligonucleotide primer that had been limiting in the second stage PCR. The sequencing reactions were loaded on a 6% 60 × 20 cm, field-gradient polyacrylamide gel, and run for about 3 h at 55 W. Gels were fixed in a 5% methanol/5% acetic acid solution, dried, and exposed to *x*-ray film (Kodak X-OMART AR), which was then developed. The DNA sequence obtained from sequencing one strand was confirmed by sequencing the complementary strand.

An additional segment of the cytochrome-*b* gene was amplified with primers, B3, which is based on a consensus of several bird sequences and B4, which targets a 726-bp fragment overlapping that of the fragment produced with B1 and B2 (Table 1). Then, single-stranded amplification products were produced and sequenced with primers B3, B4, and B5. These primers' sequences were designed from known sequences of several bird cytochrome-*b* genes. Alternatively, the double-stranded amplification product was freed of remaining nucleotides and primers with a glass-powder suspension (GeneClean, Bio101, Inc.) and sequenced directly using the protocol of Thein (1989), modified by the addition of 10% dimethyl sulfoxide to annealing, labeling, and termination reactions. The sequence for an 888-bp region of the cytochrome-*b* gene was obtained for all seven taxa using B1 through B5 as sequencing primers. The cytochrome-segment data were readily aligned by visual inspection; no insertions or deletions were observed.

Data analysis.—A variety of options exists for analyzing sequence data. These approaches vary in their underlying assumptions about how DNA sequences evolve (e.g. rate constancy, substitution and codon bias among taxa, etc.). We elected to analyze the data set in several ways. Using different tree-building algorithms permits identification of phylogenetic hypotheses that are consistently supported by a data set despite the different assumptions inherent in each algorithm.

First, data were analyzed using PAUP (version 3.0L; Swofford 1990). All uninformative nucleotide sites (i.e. invariant sites or sites with only plesiomorphic or autapomorphic character states) were removed from the data set prior to analysis. This manipulation does not alter the resulting topology but does ensure a

meaningful measure of homoplasy. When this analysis resulted in multiple equally parsimonious trees a strict consensus of the alternatives was produced. All characters were treated as unordered and unweighted.

Because synonymous transition substitutions occur much more frequently in mtDNA than synonymous transversions or amino-acid substitutions, the former are more likely to contain high degrees of homoplasy. Consequently, only transversion information was considered in an initial cladistic analysis (i.e. transversion parsimony). An additional parsimony analysis was performed using the original four-base data set.

To estimate the reliability of the topologies resulting from each of these three analyses, ingroup taxa and characters were manipulated using jackknifing (Lanyon 1985) and bootstrapping (Felsenstein 1985) procedures, respectively. These manipulations determine the degree to which the topology is dependent on the taxonomic and character composition of the data set.

Results

Figure 1 summarizes the cytochrome-DNA sequence data obtained by PCR and direct sequencing. For several reasons, these data probably represent mitochondrial genomic sequences and not mtDNA introduced into the nuclear genome, as has been reported for the human genome (e.g. Kamimura et al. 1989). First, only a PCR product of the predicted size is ever observed. Second, there is no evidence for two bases at some positions in the sequence, which would occur if a significant proportion of the amplified DNA were a nuclear pseudogene originally of mitochondrial genomic origin. Third, the occurrence of such a pseudogene in any of these taxa would be reflected in a relaxation of the constraints on the acceptance of substitutions in first and second positions of codons and in a lower substitution bias in favor of transitions at silent sites; no evidence for this phenomenon was found.

Patterns of DNA sequence divergence.—Of the 888 sites, 321 (36%) were variable for at least one of the seven taxa. Potentially about one-half of these variable sites (188) are phylogenetically informative. Most variable sites occur at the third positions of codons, where substitutions are mostly synonymous. Proportionately few potentially informative substitutions occur in first positions (18%), and only seven (4%) are found in the most conservative second-codon position. These observations are consistent with other studies of this gene in birds (Kocher et al. 1989, Edwards and Wilson 1990, Edwards et al. 1991). Among these potentially informative sites, nearly two-thirds of substitutions are transitions; this ratio rises to 75% for all sites exhibiting at least one substitution among these seven taxa.

This mitochondrial gene segment is highly biased in base composition (first position bias = 0.055; second position bias = 0.236; third position bias = 0.414), as has been noted for animal mitochondrial DNA (mtDNA) in general (Brown 1985). This is manifested as a guanine deficiency in the second- and especially third-codon positions. As has been noted for other avian taxa, a paucity of thymines in silent third-position codons also contributes to this bias. Table 2 also shows that third-position base composition may vary among toucans, Old World barbets, and New World barbets.

These taxa exhibit a strong bias for transition substitutions (Fig. 2), in agreement with comparisons of other vertebrate mtDNAs (e.g. Moritz et al. 1987). The frequency of transition substitutions decreases with the total number of substitutions among all pairwise comparisons of taxa, whereas the frequency of transversions increases. If the total number of substitutions is approximately proportional to time since divergence, these data indicate that transitions have effectively saturated variable sites in this segment of the cytochrome-*b* gene for these taxa. These data also suggest the likelihood of multiple transition substitutions at synonymous sites with transversions having accumulated at a slower rate (cf. Irwin et al. 1991).

Patterns of amino-acid replacement.—The majority of amino-acid substitutions among these taxa are of a conservative nature, consisting primarily of interchanges among similar hydrophobic residues (e.g. valine, isoleucine, leucine) in transmembrane segments of the cytochrome-*b* sequence (e.g. residues 43, 47, 118, 161; Howell 1989). The other substitutions are also exchanges between residues with similar physical properties (e.g. threonine and serine). These patterns and sites of amino-acid substitutions are consistent with those found in a recent study of the cytochrome-*b* gene in mammals (Irwin et al. 1991). One particular substitution in *Capito* species is notable, however, in that the tyrosine residue at position 104—conserved in all vertebrates examined, as well as in most other life forms (Howell 1989, Kocher

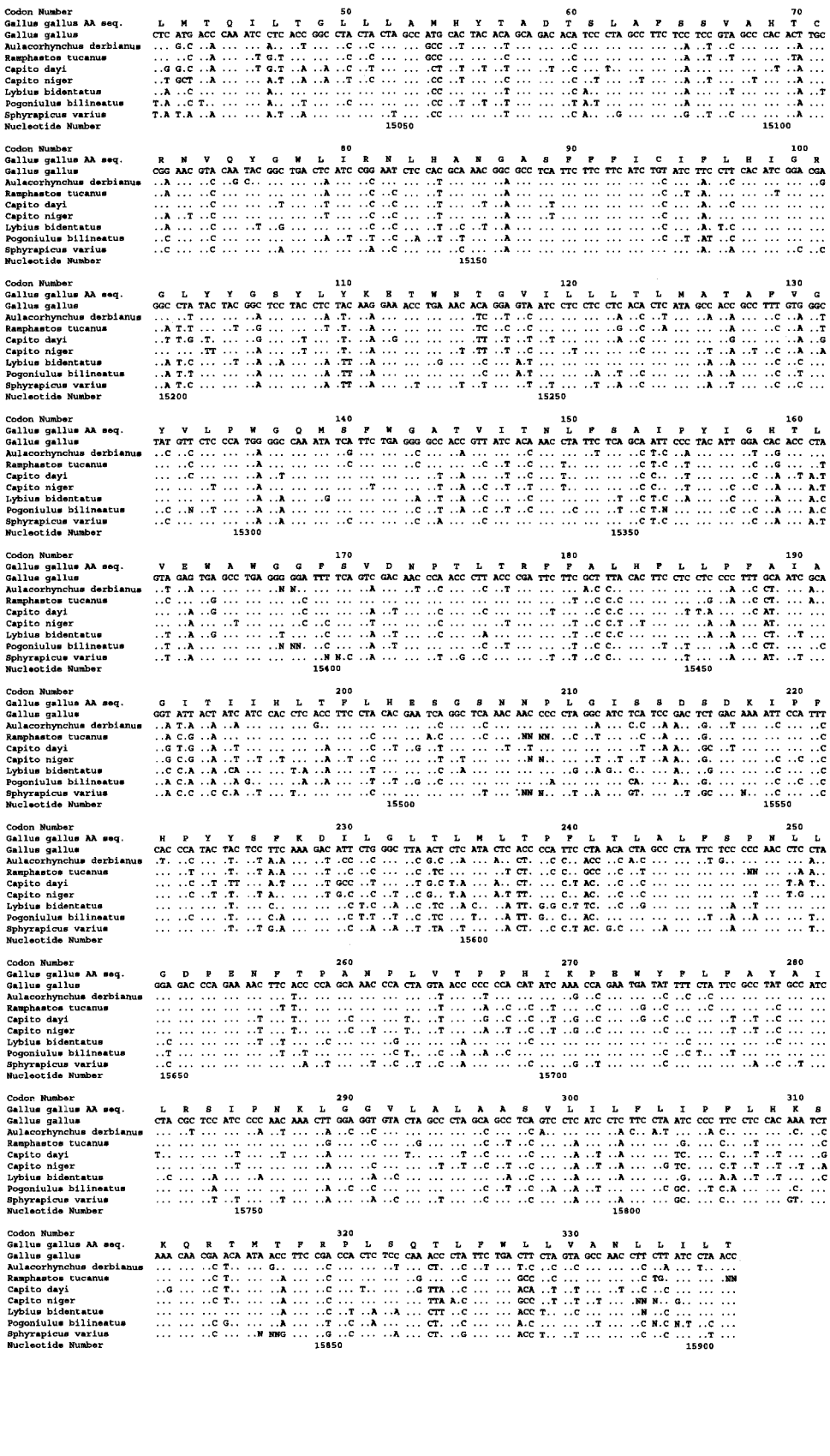
Codon Number
Gallus gallus AA seq.
Gallus gallus
Aulacorynchus derbianus
Ramphastos tucanus
Capito dayi
Capito niger
Lybius bidentatus
Pogoniulus bilineatus
Sphyrapicus varius
Nucleotide Number
15050
15100
15150
15200
15250
15300
15350
15400
15450
15500
15550
15600
15650
15700
15750
15800
15850
15900

TABLE 2. Base composition at first-, second-, and third-codon positions (light strand).

Taxon	Base[a]			
	G	A	T	C
First-codon position				
Aulacorhynchus derbianus	0.220	0.247	0.224	0.308
Ramphastos tucanus	0.227	0.241	0.234	0.298
Capito niger	0.231	0.238	0.248	0.282
C. dayi	0.226	0.236	0.273	0.266
Pogoniulus bilineatus	0.221	0.238	0.248	0.293
Lybius bidentatus	0.215	0.249	0.239	0.296
Sphyrapicus varius	0.235	0.232	0.235	0.297
$\bar{x}$	0.225	0.240	0.243	0.291
SD	0.0069	0.0060	0.0158	0.0136
Second-codon position				
Aulacorhynchus derbianus	0.128	0.189	0.402	0.280
Ramphastos tucanus	0.137	0.195	0.401	0.267
Capito niger	0.129	0.193	0.393	0.285
C. dayi	0.128	0.193	0.395	0.284
Pogoniulus bilineatus	0.128	0.196	0.399	0.277
Lybius bidentatus	0.128	0.196	0.392	0.284
Sphyrapicus varius	0.129	0.190	0.405	0.276
$\bar{x}$	0.130	0.193	0.398	0.279
SD	0.0033	0.0028	0.0049	0.0062
Third-codon position				
Aulacorhynchus derbianus	0.017	0.308	0.108	0.566
Ramphastos tucanus	0.041	0.266	0.119	0.573
Capito niger	0.024	0.303	0.265	0.408
C. dayi	0.064	0.277	0.220	0.439
Pogoniulus bilineatus	0.003	0.355	0.157	0.485
Lybius bidentatus	0.031	0.373	0.102	0.495
Sphyrapicus varius	0.027	0.331	0.150	0.491
$\bar{x}$	0.030	0.316	0.160	0.494
SD	0.0193	0.392	0.612	0.0605

[a] G = guanine, A = adenine, T = thymine, C = cytosine.

et al. 1989, Edwards and Wilson 1990, Edwards et al. 1991, Irwin et al. 1991)—is replaced by phenylalanine in these barbets. If this unique replacement substitution occurs in other barbet or toucan taxa, the character may be of value in sorting out the evolutionary histories of toucans, barbets, and related avian fauna.

Cladistic analysis of transversion substitutions.—Our analysis supports a monophyletic assemblage containing New World barbets and toucans. An exhaustive search for the most-parsimonious tree, with *Sphyrapicus varius* as the outgroup, identified a single shortest tree of length 139 (*CI* = 0.647 after removal of uninformative characters) for the 90 potentially informative transversions (Fig. 3A). The next-shortest tree is five steps longer. Use of either *Molothrus ater* or *Gallus gallus* as the outgroup results in the same topology. Seventeen unreversed transversions (*CI* = 1.0) support a clade containing toucans and New World barbets. An additional seven transversions characterized by consistency indices less than 1.0 also define this assemblage.

This branching sequence also identifies the two Old World barbets as sister taxa. A jackknife manipulation of ingroup taxa indicates that monophyly of the Old World barbets is weakly

←

Fig. 1. Sequences of the 888-bp segment of cytochrome-*b* gene used. Dots indicate sequence identity to published chicken sequence. Also indicated is corresponding chicken amino-acid sequence. Codon number and nucleotide number, indicated above and below the sequences, respectively, refer to chicken mtDNA (Desjardins and Morais 1990). N = not determined.

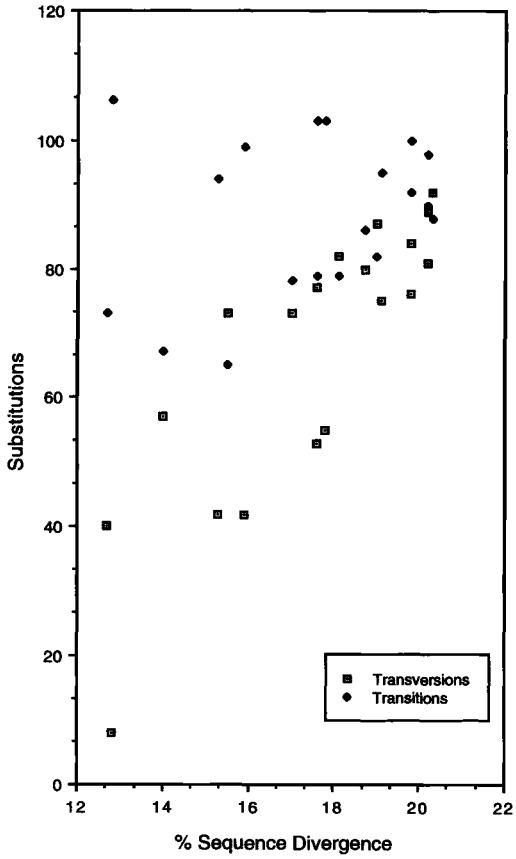

Fig. 2. Patterns of nucleotide substitutions in 888-bp cytochrome-*b* gene segment among capitonid taxa, ramphastid taxa, and a woodpecker. Points represent pairwise comparisons of taxa with respect to frequencies of transitions and transversions versus percent sequence divergence.

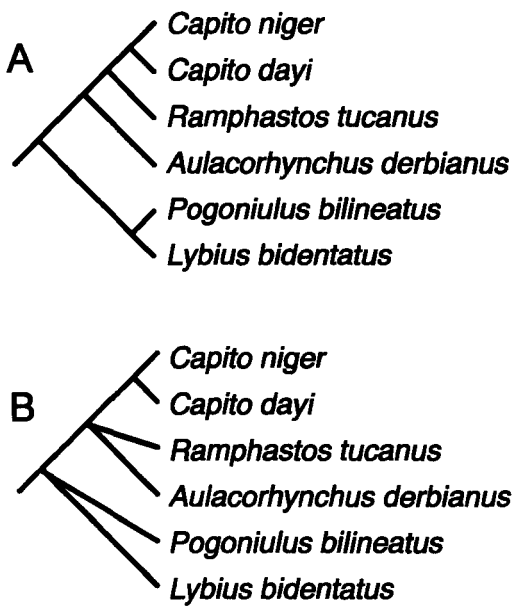

Fig. 3. Parsimony analysis of cytochrome-*b*-sequence data for barbets and toucans: (A) single most-parsimonious tree identified by PAUP exhaustive-search option; (B) strict-consensus tree derived from jackknifing taxa with subsequent analysis by PAUP exhaustive-search option. Polychotomies indicate portions of topology unstable to this manipulation.

supported by the data. This node is not retained in all pseudoreplicate trees (Fig. 3B).

Analysis of these data also suggest that toucans are polyphyletic. One genus appears to be the sister taxon of the New World barbets, with the other toucan genus as the sister taxon to the *Ramphastos–Capito* clade. However, this hypothesis is only marginally supported by the data. Monophyly of the two toucan taxa is supported when the two Old World barbets are used instead of *S. varius* as outgroup taxa—even when a jackknife manipulation is performed.

Cladistic analysis of complete data set.—Analysis of the complete data set, including transition data, produces a single most-parsimonious tree of 407 steps for the 188 potentially informative nucleotide sites. The resulting topology is identical to that derived from the analysis restricted to transversion data (Fig. 3A). The topology is unaffected by use of different outgroup taxa, the next most-parsimonious trees are a minimum of three steps longer, and jackknifing of ingroup taxa results in no change to the topology.

Discussion

The results of this investigation clearly corroborate the morphological and DNA-DNA hybridization findings of Prum (1988) and Sibley and Ahlquist (1990), respectively. Regardless of whether we examined transversions or all character-state changes, analyzed the data cladistically, used all ingroup taxa, or applied a jackknife manipulation of taxa to these analyses, toucans and New World barbets were consistently identified as being more closely related to one another than either were to Old World barbets. Indeed, support for this hypothesis departed significantly from chance expectations if the data set were actually uninformative about this evolutionary event or if the true phylogeny was actually a trichotomy rather than a dichot-

omy. Seventeen transversions ($CI = 1.0$) and 7 additional homoplasious transversion apomorphies support the hypothesis that New World barbets and toucans are sister taxa with respect to Old World barbets. Twenty-seven unreversed base substitutions (both transitions and transversions) support this hypothesis.

The product of an exhaustive PAUP analysis is the shortest unrooted network (or a set of equally shortest unrooted networks). This network must be rooted to be interpretable as a phylogeny. How dependent are the results of our study on the selection of the three outgroup taxa used in this study to root this network? Of the three possible rooted three-taxon statements for toucans, Old World barbets, and New World barbets, the unrooted networks derived from the DNA sequence data rule out one alternative. It is impossible to create a monophyletic assemblage containing all barbets from these networks. The conclusion that the Capitonidae is polyphyletic is independent of the choice of outgroups.

In contrast, identification of the sister taxon of the Ramphastidae requires rooting the network. As indicated above, the use of any of the three outgroup taxa places the root between Old World barbets and toucans, thereby identifying the latter as the sister taxon to the New World barbets.

The analysis of different types of data—mtDNA sequence, DNA-DNA hybridization, and morphological—confirms barbet polyphyly and the existence of a New World barbet and toucan clade. Testing phylogenetic hypotheses for congruence with "independent" data sets is a useful way of estimating the reliability of those hypotheses. In this instance, our data and the morphological analyses support the view that DNA-DNA hybridization technology has been useful in identifying evolutionary relationships. However, this conclusion does not imply an acceptance of the DNA-DNA hybridization results in their entirety. The phylogeny of Sibley and Ahlquist (1990) does not represent a single hypothesis, but rather a set of hypotheses. The merits of each hypothesis must be considered independently.

How much sequence is sufficient?—We obtained DNA sequence data using the universal primers of Kocher et al. (1989) before acquiring the full data set of 888 base pairs. Given the time and expense of sequencing, we asked if this initial data set would have been sufficient to reconstruct the phylogeny for this group of taxa. To address this question we examined the distribution and number of potentially informative transversions in the ingroup for the initial 273-bp fragment, using Lake's evolutionary parsimony method (1987). This approach is specifically designed to increase the probability of identifying the correct phylogeny when evolutionary rates within the ingroup are extremely variable. However, evolutionary rates may be nearly equivalent given that the taxa analyzed in our study are relatively closely related. If they are, subtracting Lake's correction factors (components H, J, L, N, O, and S) from his three parsimony terms (X, Y, and Z) does not necessarily yield the best estimate of the number of transversions supporting each of the three alternative three-taxon statements. If evolutionary rates are equivalent, adding these, as well as Lake's components u, v, and w to the relevant parsimony term may give a better estimate:

$$X = E + u + H + J, \quad (1)$$

$$Y = F + v + L + N, \quad (2)$$

and

$$Z = G + w + O + S. \quad (3)$$

This manipulation is equivalent to recoding DNA sequence data as binary characters (purine vs. pyrimidine) as discussed above. Because all components are added, it is not appropriate to test X, Y, and Z for departure from zero as Lake (1987) suggested. Rather, the test must be for departure from an expected value. If the true phylogeny for three taxa is a trichotomy and evolutionary rates in the three lineages are roughly the same, the number of transversions supporting each of the three possible sister-taxon arrangements should be equivalent in number—that is, one-third of the potentially informative transversions. Conversely, each of the three alternative hypotheses should be contradicted by two-thirds of the potentially informative transversions. Therefore, an estimate of the strength of support for a dichotomous node can be obtained by determining whether the number of synapomorphies supporting that node significantly exceeds the number expected if the true phylogeny were a trichotomy. Note that the expected character-state distribution would be identical if instead of assuming a trichotomy we assume that the data set is actually uninformative, with all shared character states

TABLE 3. Comparison of potentially informative transversions to random expectations for 273-bp of cytochrome-*b* for three alternative three-taxon statements. See text for details.

New World barbet (NW)	Old World barbet (OW)	Number of transversion synapomorphies	
		((T, NW)OW)	((T, OW)NW) + ((NW, OW)T)
Toucan (T): *Aulacorhynchos derbianus*			
Capito dayi	*Pogoniulus bilineatus*	23 (11.7)[a]	12 (23.3)
C. dayi	*Lybius bidentatus*	27 (13.0)	12 (26.0)
C. niger	*P. bilineatus*	24 (12.7)	14 (25.3)
C. niger	*L. bidentatus*	28 (13.7)	13 (27.3)
Toucan (T): *Ramphastos tucanus*			
C. dayi	*P. bilineatus*	28 (13.3)	12 (26.7)
C. dayi	*L. bidentatus*	33 (15.0)	12 (30.0)
C. niger	*P. bilineatus*	30 (14.7)	14 (14.7)
C. niger	*L. bidentatus*	35 (16.0)	13 (32.0)

[a] Number of observed transversion synapomorphies supporting indicated three-taxon statement(s). Values in parentheses are expected number of transversion synapomorphies if true phylogeny for toucans, Old World barbets, and New World barbets is a trichotomy. $P < 0.05$ (*G*-test) in all eight cases.

the result of retention of the primitive character state found in the outgroup or independent acquisition of a base by two or more ingroup taxa.

For the six ingroup taxa, there are eight possible combinations of one toucan, one New World barbet, and one Old World barbet. Table 3 shows the distribution of potentially informative transversions (as identified by outgroup comparison with *S. varius*). For all eight comparisons, New World barbets and toucans share significantly more transversion synapomorphies within the initial 273-bp fragment than expected by chance ($P < 0.05$, *G*-test). Consequently, we conclude that the initial 273-bp obtained using the "universal primers" of Kocher et al. (1989) were sufficient to address the question of barbet polyphyly. It is encouraging that such a small fragment can provide a strong phylogenetic signal.

The finding that barbets are paraphyletic is of interest to students of evolutionary processes. To us the corroboration of paraphyly by this study raises intriguing questions regarding the dramatic differences in the rate of morphological change in related lineages. Why has the lineage leading to toucans experienced such rapid evolution in bill shape and size compared to barbets? On the other hand, the various barbet lineages are so similar that some workers (e.g. Burton 1984, Short and Horne 1985) prefer to retain them as a paraphyletic assemblage to designate their status as an evolutionary grade. Why have the barbets—evolving separately in South America and the Old World since the late Cretaceous—retained their highly similar morphologies, behaviors, habitat and food requirements?

ACKNOWLEDGMENTS

We acknowledge NSF grant BSR-8614240, the Eppley Fund, San Diego Zoo, David Willard, Julian Kerbis, Paulo Vanzolini, Laurent Ntahusa, Andre Niyokindi, Leif Davenport, Peter Trenchard, the Museu de Zoologia-Universidade de Sao Paulo, and the I.N.E.C.N./Peace Corps Biodiversity Project for assistance in the acquisition of tissue samples used in this study. For support of the laboratory work, we acknowledge the contributions of the Ellen Thorne Smith fund. Finally, for assistance in the laboratory, we acknowledge Pamela Austin.

LITERATURE CITED

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.

BROWN, W. M. 1985. The mitochondrial genome of animals. Pages 95–130 *in* Molecular evolutionary genetics (R. J. MacIntyre, Ed.). Plenum Press, New York.

BURTON, P. J. K. 1984. Anatomy and evolution of the feeding apparatus in the avian orders Coraciiformes and Piciformes. Bull. Br. Mus. (Nat. Hist.) Zool. 47:331–443.

DESJARDINS, P., AND R. MORAIS. 1990. Sequence and gene organization of the chicken mitochondrial

genome. A novel gene order in higher vertebrates. J. Mol. Biol. 212:599–634.

EDWARDS, S. V., P. ARCTANDER, AND A. C. WILSON. 1991. Mitochondrial resolution of a deep branch in the genealogical tree for perching birds. Proc. R. Soc. Lond. B Biol. Sci. 243:99–107.

EDWARDS, S. V., AND A. C. WILSON. 1990. Phylogenetically informative length polymorphism and sequence variability in mitochondrial DNA of Australian songbirds (*Pomatostomus*). Genetics 126: 695–711.

ELDREDGE, N., AND J. CRACRAFT. 1980. Phylogenetic patterns and the evolutionary process. Columbia Univ. Press, New York.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.

GYLLENSTEN, U. B., AND H. A. ERLICH. 1988. Generation of single-stranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLA-DQA locus. Proc. Natl. Acad. Sci. USA 85:7652–7656.

HOWELL, N. 1989. Evolutionary conservation of protein regions in the protonmotive cytochrome-*b*, and their possible roles in redox catalysis. J. Mol. Evol. 29:157–169.

IRWIN, D. M., T. D. KOCHER, AND A. C. WILSON. 1991. Evolution of the cytochrome-*b* gene of mammals. J. Mol. Evol. 32:128–144.

KAMIMURA, N., S. ISHII, M. LIANDONG, AND J. W. SHAY. 1989. Three separate mitochondrial DNA sequences are contiguous in human genomic DNA. J. Mol. Biol. 210:703–707.

KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PAABO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in mammals: Amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86:6196–6200.

LAKE, J. A. 1987. A rate-independent technique for analysis of nucleic acid sequences: Evolutionary parsimony. Mol. Biol. Evol. 4:167–191.

LANYON, S. M. 1985. Detecting internal inconsistencies in distance data. Syst. Zool. 34:397–403.

LANYON, S. M. 1992. [Review of] Phylogeny and classification of birds. A study in molecular evolution. Condor 94:304–307.

MANIATIS, T., E. F. FRITSCH, AND J. SAMBROOK. 1982. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

MORITZ, C., T. E. DOWLING, AND W. M. BROWN. 1987. Evolution of animal mitochondrial DNA: Relevance for population biology and systematics. Annu. Rev. Ecol. Syst. 18:269–292.

PRUM, R. O. 1988. Phylogenetic interrelationships of the barbets (Aves: Capitonidae) and toucans (Aves: Ramphastidae) based on morphology with comparisons to DNA-DNA hybridization. Zool. J. Linn. Soc. 92:313–343.

SANGER, F., S. NICKLEN, AND A. R. COULSON. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463–5467.

SARICH, V. M., C. W. SCHMID, AND J. MARKS. 1989. DNA hybridization as a guide to phylogeny: A critical analysis. Cladistics 5:3–32.

SHORT, L. L., AND J. F. M. HORNE. 1985. Social behavior and systematics of African barbets (Aves: Capitonidae). Pages 255–278 *in* Proceedings of the international symposium on African vertebrates (K.-L. Schuchmann, Ed.). Zoologisches Forschungsinstitut und Museum Koenig, Bonn.

SIBLEY, C. G., AND J. E. AHLQUIST. 1986. Reconstructing bird phylogenies by comparing DNA's. Sci. Am. 254(2):82–92.

SIBLEY, C. G., AND J. E. AHLQUIST. 1990. Phylogeny and classification of birds. A study in molecular evolution. Yale Univ. Press, New Haven, Connecticut.

SWIERCZEWSKI, E. V., AND R. J. RAIKOW. 1981. Hind limb morphology, phylogeny, and classification of the Piciformes. Auk 98:466–480.

SWOFFORD, D. 1990. PAUP. Phylogenetic analysis using parsimony, version 3.0. Illinois Natural History Survey, Champaign.

THEIN, S. L. 1989. A simplified method of direct sequencing of PCR amplified DNA with Sequenase T7 DNA polymerase. United States Biochemicals Comments 16:8–9.