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The Impact of Parsimony Weighting Schemes on Inferred Relationships among Toucans and Neotropical Barbets (Aves: Piciformes)

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The development of new schemes for weighting DNA sequence data for phylogenetic analysis continues to outpace the development of consensus on the most appropriate weights. The present study is an exploration of the similarities and differences between results from 22 character weighting schemes when applied to a study of barbet and toucan (traditional avian families Capitonidae and Ramphastidae) phylogenetic relationships. The dataset comprises cytochrome *b* sequences for representatives of all toucan and Neotropical barbet genera, as well as for several genera of Paleotropical barbets. The 22 weighting schemes produced conflicting patterns of relationship among taxa, often with conflicting patterns each receiving strong bootstrap support. Use of multiple weighting schemes helped to identify the source within the dataset (codon position, transitions, transversions) of the various putative phylogenetic signals. Importantly, some phylogenetic hypotheses were consistently supported despite the wide range of weights employed. The use of phylogenetic frameworks to summarize the results of these multiple analyses proved very informative. Relationships among barbets and toucans inferred from these data support the paraphyly of the traditional Capitonidae. Additionally, these data support paraphyly of Neotropical barbets, but rather than indicating a relationship between *Semnornis* and toucans, as previously suggested by morphological data, most analyses indicate a basal position of *Semnornis* within the Neotropical radiation. The cytochrome *b* data also allow inference of relationships among toucans. Supported hypotheses include *Ramphastos* as the sister to all other toucans, a close relationship of *Baillonius* and *Pteroglossus* with these two genera as the sister group to an (*Andigena*, *Selenidera*) clade, and the latter four genera as a sister group to *Aulacorhynchus*. © 2000 Academic Press

INTRODUCTION

The appropriateness of various methods of phylogenetic analysis of molecular data has been widely discussed on both theoretical and empirical grounds. For instance, a number of studies have indicated that methods which take into account specific features of molecular evolution (e.g., variation in base composition and rate heterogeneity) may be more robust over a wider range of conditions (Huelsenbeck and Hillis, 1993; Kuhner and Felsenstein, 1994; Huelsenbeck, 1995; Schöniger and von Haeseler, 1995). In phylogenetic estimation using maximum-likelihood, as well as in many distance-based analyses, an explicit statement of a model of sequence evolution is fundamental. Within this statistical framework, specific tests can be performed to determine which of a finite set of models best fits the data at hand (Huelsenbeck and Rannala, 1997). This approach does require the assumption that the models examined are reasonable representations of the dynamics of sequence evolution. However, whatever mathematical representation of sequence evolution is settled on as appropriate, explicit criteria exist for choosing parameters and parameter values appropriate for particular analyses. Though parsimony analysis does not include an explicit evolutionary model, some information on the dynamics of sequence evolution can be incorporated by employing character and character-state transition weighting, potentially improving phylogenetic estimation. For instance, data from simulations (e.g., Bull *et al.*, 1993; Huelsenbeck and Hillis, 1993; Hillis *et al.*, 1994) and analysis of "known" phylogenies (e.g., Miyamoto *et al.*, 1994; Cunningham, 1997) suggest that weighting data during parsimony analysis increases phylogenetic accuracy. Weighting of data has been widely discussed in the literature, and many weighting schemes have been proposed (Farris, 1969; Neff, 1986; Wheeler, 1986, 1990; Williams and Fitch,

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1989; Albert and Mishler, 1992; Albert *et al.*, 1993; Knight and Mindell, 1993; Collins, Kraus, and Estabrook, 1994; Miyamoto *et al.*, 1994; Cunningham, 1997). Unfortunately, there has been little theoretical or empirical indication of which approaches to weighting might be most appropriate for analyzing data either generally or in any given situation. This ambiguity in defining optimal parsimony weighting schemes is in sharp contrast to choosing optimal models within the maximum-likelihood framework.

One approach to testing alternative weighting schemes is to assess their impact on congruence among phylogenetic estimates from multiple data sets which share the same historical signal (e.g., Miyamoto *et al.*, 1994; Hillis, 1995; Cunningham, 1997). However, this is impossible when only one data set is available for phylogenetic estimation. An alternative approach is to determine whether or not varying the weighting scheme used has a significant effect on phylogenetic inference (i.e., sensitivity analysis). It could be that with weakly structured or "noisy" data, each weighting scheme will yield a different hypothesis of relationship, but that none of these alternatives is strongly supported relative to the others. This would indicate a rather trivial influence of weighting on phylogenetic inference. On the other hand, if different weighting schemes yield different, strongly supported hypotheses of relationship, then the question of weighting becomes nontrivial, and indeed central, to the process of phylogeny reconstruction using parsimony. This type of assessment can be made by executing systematically a variety of analyses (to sample, probably in a biased fashion, the infinite universe of possible weighting schemes) and observing how those weighting schemes affect phylogenetic inference in terms of recovered topologies and estimated nodal support. This approach is not novel, having been applied, for instance, to the problem of Odontocete monophyly (Milinkovitch *et al.*, 1996; see also Friesen *et al.*, 1996). Here, we report the results of such an analysis of cytochrome *b* sequence data from toucans and barbets (Aves: Piciformes).

The barbets and toucans (suborder Ramphastoidea, *sensu* Peters, 1948) are a morphologically diverse group of approximately 119 species distributed throughout the tropics. The group contains such extremes of body size and ecology as the tiny (~10 g) terrestrial insectivorous tinkerbirds (genus *Pogoniulus*) to extremely large (~500 g) frugivorous toucans (genus *Ramphastos*). The group also exhibits significant variation in social behavior, ranging from territorial monogamy to cooperative breeding (e.g., *Stactolaema, Lybius,* and *Trachyphonus* [Grimes, 1976]; *Semnornis* [Restrepo and Mondragón, 1998]; and *Pteroglossus* [Skutch, 1958]). Knowledge of phylogenenetic relationships among these diverse genera will provide insight into the history of behavioral and morphological changes in the group. This suborder is also one of the few bird groups with a pantropical distribution (others include the Psittaciformes and Trogoniformes; Vuilleumier and Andors, 1993). Phylogenetic relationships within such clades and tests of congruence between them offer insights into the origins of continental avifaunas (Cracraft, 1973a,b). Relationships within these groups can also be informative at the continental scale in testing alternative explanations of diversification within tropical regions (e.g., Cracraft and Prum, 1988).

For these reasons, phylogenetic relationships among barbets and toucans have been the subject of a great deal of study and debate. At least two major questions have been asked with regard to barbet relationships, both involving the position of the toucans with respect to the barbets. First, monophyly of the barbets as traditionally defined (family Capitonidae, *sensu* Peters, 1948) has been called into question. Specifically, it was suggested on morphological grounds that the very distinctive toucans (family Ramphastidae, *sensu* Peters, 1948) are in fact a highly derived lineage of barbets, most closely related to the Neotropical barbets (*Capito, Eubucco,* and *Semnornis;* Burton, 1984; Prum, 1988), thus rendering the traditional Capitonidae paraphyletic. This hypothesis has also been corroborated by genetic analyses (Sibley and Ahlquist, 1990; Harshman, 1994; Lanyon and Hall, 1994). Second, based upon his analysis of the only data set with complete genus-level sampling within the Capitonidae to date, Prum (1988) suggested not only that barbets as a whole are paraphyletic but that Neotropical barbets are themselves paraphyletic, with *Semnornis* more closely related to the toucans than to other Neotropical barbets (Fig. 1).

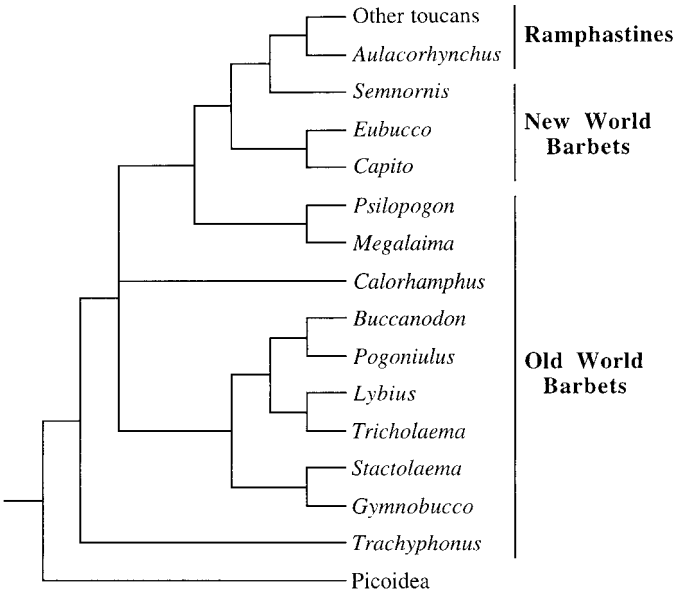

FIG. 1. Relationships among toucans and barbets as proposed by Prum (1988).

The monophyly of the toucans as a group has never been questioned, largely due to their shared distinctive bill morphology and behavior. Regarding relationships within the group, no single study has included data from every genus of the Ramphastidae, except the essentially nonphylogenetic survey of Haffer (1974). Among the phylogenetic hypotheses proposed to date (Haffer, 1974; Swierczewski and Raikow, 1981; Lanyon and Zink, 1987; Cracraft and Prum, 1988; Sibley and Ahlquist, 1990; Hackett and Lehn, 1997), the only intergeneric relationship which appears consistently without contradiction is the placement of *Pteroglossus* and *Baillonius* as sister taxa. One other hypothesis, consistent with three of the data sets and contested by only one (Swierczewski and Raikow, 1981), is the monophyly of the toucanets (this term will be taken to encompass the toucanets, araçaris, and mountain toucans; genera *Aulacorhynchus, Pteroglossus, Selenidera, Baillonius,* and *Andigena*) as the sister group to *Ramphastos.* In an attempt to resolve some of the ambiguities in both barbet and toucan intergeneric relationships, this study expands Lanyon and Hall's (1994) previous DNA sequence-based analysis to include cytochrome *b* sequences of all genera of toucans and Neotropical barbets, as well as adding several additional Paleotropical barbets.

METHODS

Taxon sampling. The sampling of Lanyon and Hall (1994) was expanded to include two members of each genus of toucans and Neotropical barbets (excepting *Aulacorhynchus,* for which only one species was sampled), as well as several additional genera of Paleotropical barbets (see Table 1). Based upon the results of morphological (Simpson and Cracraft, 1981; Swierczewski and Raikow, 1981; Prum, 1988) and molecular (Lanyon and Zink, 1987; Sibley and Ahlquist, 1990; Harshman, 1994) studies, sequences of the Picidae (Moore and DeFilippis, 1997) were included in analyses as an outgroup to the Ramphastidae (Table 1).

Amplification and sequencing of cytochrome b. Total genomic DNA was extracted from ~100 mg of each tissue sample using overnight proteinase K digestion in either (1) extraction buffer (100 mM tris, 10 mM Na_2–EDTA, 100 mM NaCl, 1% Na dodecyl sulfate, 10 mg/mL dithiothreitol [United States Biochemical], and 0.5 mg/mL proteinase K [Sigma Scientific]; pH 8.0) or (2) buffer supplied in an extraction kit (PureGene, Gentra Systems). The former samples were extracted twice with phenol/chloroform (25 phenol:24 chloroform:1 isoamyl alcohol) and once with chloroform and then concentrated using centrifugal dialysis in Centricon 100's (Amicon). The latter samples were treated according to manufacturer's instructions and resuspended after isopropanol precipitation in 50 µL ddH_2O. Subsequent to extraction, all samples were quantified by UV spectroscopy and diluted to 25 ng/µL final concentration. PCR amplification of cytochrome *b* was accomplished by a primary amplification of a majority of the gene using either of two primer pairs (B1a/B4 or B1a/B8) and subsequent reamplification of the entire initially amplified region and several segments (B1a/B2a, B3/B6, B5/B4, and L15507/B8; see Table 2) from a gel-purified sample (5 µL of initial PCR run on a 1% low-melting-point agarose [FMC] gel, excised, and melted in 200 µL ddH_2O) of this initial amplification. Primary amplifications were performed in 25-µL total reaction volumes (25–50 ng template DNA, 1× amplification buffer [1.5 mM final $MgCl_2$, Boehringer Mannheim], 0.5 U *Taq* polymerase [Boehringer Mannheim], 0.2 µM each primer, 80 µM each dNTP), with thermal cycling (MJ Research) parameters for initial amplifications as follows: an initial 3 min at 94°C, followed by 35 cycles of 15 s at 94°C, 30 s at 51°C, and 1 min at 72°C, followed by a final 3-min extension at 72°C. Reamplifications were performed in 50-µL volumes (using 2 µL of melted gel plug as template), with conditions as for primary amplifications, except for an increase of 2 degrees in annealing temperature and a reduction of extension time to 30 s. Reamplification products were purified by centrifugal dialysis using Microcon 100's (Amicon), twice adding 500 µL ddH_2O to the 50-µL reaction volume and spinning at 500*g* for 15 min. The resulting purified PCR product was quantified and diluted to 25 ng/µL final concentration. Purified PCR products were cycle-sequenced using 50–100 ng template, amplification or internal primers, and a PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Perkin–Elmer) according to the manufacturer's directions, except using 10-µL reaction volumes. Sequencing reactions were either phenol/chloroform extracted and precipitated according to manufacturer's specifications or purified using CentriSep columns (Princeton Separations, Princeton, NJ). Reactions were then electrophoresed on an ABI 377 automated sequencer (Applied Biosciences, Perkin–Elmer). Obtained sequences were 925 or 1074 bases in length, corresponding to positions 14991–15915 (or in several cases 16064) of the *Gallus* mitochondrial genome (Desjardins and Morais, 1990). All sequences obtained here (as well as those originally reported by Lanyon and Hall, 1994) have been submitted to GenBank (Accession Nos. AF123510–AF123532). Contig alignments were constructed using Sequencher (Genecodes, Ann Arbor, MI), and multiple alignments were made by eye using SeqPup (D. Gilbert, Indiana University, IN).

Phylogenetic analyses. All analyses were conducted using PAUP* (test version 4.0d65). Prior to phylogenetic analysis, sequences were evaluated for several properties known to affect such analyses. First, since heterogeneity in base composition is known to affect phylogenetic inference (e.g., Lockhart *et al.,* 1994; Yang and Roberts, 1995; Galtier and Gouy, 1998), variation

TABLE 1

Taxa Represented in This Study, Including Sources of Sequences and Tissues [Higher-Level Taxonomy Follows Prum (1988), and Taxon Sequences Follow Peters (1948) and Haffer (1974)]

Taxon	Source[a]	Citation
Family Ramphastidae		
Subfamily Trachyphoninae		
Trachyphonus darnaudii	KUMNH 86336 (OX-316)	This study
Subfamily Lybiinae		
Lybius bidentatus	Unvouchered zoo specimen (FM 4019)	Lanyon and Hall (1994)
Subfamily Pogoniulini		
Pogoniulus bilineatus	FM 346231 (1923)	*Ibid.*
Subfamily Megalaiminae		
Psilopogon pyrolophus	AMNH 22990 (PRS-676)	This study
Megalaima mystacophanos	KUMNH 88068 (OX-317)	*Ibid.*
Subfamily Capitoninae		
Capito dayi	SML86-169[b] (FM 7965)	Lanyon and Hall (1994)
	SML86-069[b] (FM 172)	This study
C. niger	FM 321056 (4021/6244/6674)	Lanyon and Hall (1994)
Eubucco richardsoni	LSUMNS 116711 (B5496)	This study
E. bourcierii tucinkae	APC-3100[c] (LSUMNS B10782)	*Ibid.*
Subfamily Semnorninae		
Semnornis frantzii	LSUMNS 138685 (B16019)	*Ibid.*
S. ramphastinus	ANSP 178078 (B7771)	*Ibid.*
Subfamily Ramphastinae		
Aulacorhynchus derbianus	FM 339643 (41/44/46)	Lanyon and Hall (1994)
Pteroglossus inscriptus	SML86-168[b] (FM 53)	This study
P. castanotis	ATP86-111[b] (FM 66)	*Ibid.*
Selenidera gouldii	ATP86-151[b] (FM 23)	*Ibid.*
S. spectabilis	LSUMNS 108250 (B2122)	*Ibid.*
Baillonius bailloni	LSUMNS 149882 (B20868)	*Ibid.*
Andigena hypoglauca	LSUMNS 105836 (B1856)	*Ibid.*
A. laminirostris	JCM86-08[d] (LSUMNS B7791)	*Ibid.*
Ramphastos vitellinus culminatus	SML86-196[b] (FM 54)	*Ibid.*
R. tucanus cuvieri	SML86-129[b] (FM 22/47/48)	Lanyon and Hall (1994)
Family Picidae		
Subfamily Picumninae		
Picumnus aurifrons	GB U83289	Moore and DeFilippis (1997)
Subfamily Picinae		
Colaptes auratus	GB U83282	*Ibid.*
C. rupicola	GB U83301	*Ibid.*
Piculus rubiginosus	GB U83292	*Ibid.*
Dryocopus pileatus	GB U83287	*Ibid.*
Sphyrapicus varius	FM 350792 (6243)	Lanyon and Hall (1994)
	GB U83295	Moore and DeFilippis (1997)
Veniliornis callonotus	GB U83297	*Ibid.*
V. nigriceps	GB U83299	*Ibid.*
Picoides villosus	GB U83293	*Ibid.*
Campephilus haematogaster	GB U83284	*Ibid.*

[a] Voucher number (tissue numbers in parentheses, if available; museum noted only if differing from voucher) or GenBank (GB) Accession. Museum abbreviations as follows: KUMNH, University of Kansas Museum of Natural History, Lawrence, KS; FM, The Field Museum, Chicago, IL; AMNH, American Museum of Natural History, New York, NY; LSUMNS, Louisiana State University Museum of Natural Science, Baton Rouge, LA; ANSP, Academy of Natural Sciences, Philadelphia, PA.

[b] Collector's number; voucher in the collection of the Museu Paraense Emílio Goeldi, Brazil.

[c] Collector's number; voucher in the collection of the Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos, Peru.

[d] Collector's number; voucher in the collection of the Museo Ecuatoriano de Ciencias Naturales, Ecuador.

among sequences in base pair composition was examined by codon position. Second, divergences among sequences were examined graphically, by codon position and category of substitution (transition or transversion). As noted by a number of authors (see especially Griffiths, 1997), the patterns of covariation among different components of interspecific sequence divergences (e.g., coding positions, transitions versus transversions) can be used to estimate the likelihood that superimposed changes have occurred in a given data set, at least when there is significant rate heterogeneity among the individual components examined. In analy-

TABLE 2

Primers Used in Amplification and Sequencing, Numbered According to Location in the *Gallus* Mitochondrial Genome (Desjardins and Morais, 1991)

Primer	Sequence (5′–3′)	Location and reference[a]
B1a	CCATCCAACATCTCAGCATGATGAAA	L14990 (Helm-Bychowski and Cracraft, 1993)
B3	ATCTGCATCTACCTACACATCGG	L15191 (Lanyon and Hall, 1994)
B5	ACCCTAGTAGAATGAGCCTGAGG	L15392 (Lanyon and Hall, 1994)
L15506	CTCACCTTCCTACACGAAACAGG	L15506 (Helm-Bychowski and Cracraft, 1993)
B2a	CCCCTCAGAATGATATTTGTCCTCA	H15298 (Helm-Bychowski and Cracraft, 1993)
B6	GCGTAGGCGAATAGGAAGTATCA	H15709
B4	ATGAAGGGATGTTCTACTGGTTG	H15916 (Edwards *et al.*, 1991)
B8	GGAGTCTTCAGTCTCTGGTTTACAAGAC	H16065 (Helm-Bychowski and Cracraft, 1993)

[a] L and H indicate identity with light and heavy strands, respectively.

ses of mitochondrial DNA, since transition substitutions appear to occur at much higher rates than transversion substitutions (Brown *et al.*, 1982; Aquadro *et al.*, 1984), transitions are usually plotted against transversions (or some scaled overall distance measure) to detect any plateau in transition differences at the highest levels of divergence. The existence of such a plateau is a fair indication that multiple transition substitutions have occurred at a given site. Since the occurrence of superimposed substitutions deteriorates the historical signal in a data set by increasing levels of homoplasy, *a priori* or *a posteriori* weighting schemes might be justified if this pattern is detected.

We performed a variety of parsimony analyses under various weighting schemes to assess the impact of weighting on phylogenetic inference for these data. Unfortunately, no criteria exist for choosing a set of near-optimal weighting schemes for a given data set; so, some arbitrary set must be chosen. We have elected to perform a series of weighting schemes in which we sequentially downweighted third positions as a whole on the one hand and third position transitions only on the other (including weights of 1, corresponding to equally weighted parsimony; other weights used were 0.50, 0.33, 0.25, 0.20, 0.17, 0.10, and 0.00). In addition to these analyses, we have performed analyses (1) weighting coding positions proportional to the inverse of the percentage of variable sites at those positions, (2) using the conservative weighting scheme proposed by Irwin *et al.* (1991) for cytochrome *b* sequences (this method ignores third position transition differences and codes the first position of leucine codons as pyrimidine [Y]), and (3) using Rodrigo's (1992) modification of Wheeler's (1990) combinatorial weights approach. Additionally, nucleic acid sequences were translated into protein sequences using the *Gallus* mitochondrial genetic code (Desjardins and Morais, 1990; implemented in MacClade v3.04, Maddison and Maddison, 1993) and analyzed under a parsimony criterion with equal weights. We note that the list of analyses that we performed is hardly exhaustive. For instance, we avoided use of a variety of six-category stepmatrix weighting schemes (Cunningham, 1997), as well as Williams and Fitch's (1989) dynamically weighted parsimony method, because these methods as described depend on parsimony reconstructions of the frequency of character state transitions. With skewed character state composition, as is typical for mitochondrial DNA (e.g., Kocher *et al.*, 1989), such reconstructions are known to be strongly biased (Collins *et al.*, 1994; Eyre-Walker, 1998) and would generate incorrect character-state transition weightings. During parsimony analyses, all searches were heuristic, using the tree-bisection-and-reconnection algorithm, with at least 10 (typically 50) random additions of taxa. Support for inferred relationships was evaluated using the bootstrap (Felsenstein, 1985) and for the most-parsimonious tree found with equal weights, the decay index (Bremer, 1994; generated using the converse constraint option of PAUP* for each node found in the most-parsimonious tree, employing heuristic searches with 10 random addition sequence replicates). Since distant outgroups are known to be a potential problem in phylogenetic inference (Smith, 1994), all phylogenetic analyses were executed both with Picidae as an outgroup to the Ramphastidae and without an outgroup.

Analyses under the maximum-likelihood criterion were preceded by an extensive evaluation of alternative models of sequence evolution using a fixed tree generated by the neighbor-joining method (Saitou and Nei, 1987) using Kimura two-parameter distances (Kimura, 1980). However, we note that the initial topology chosen did not appear to have a significant effect on the parameter values estimated (results not presented). Models evaluated included the F81 (Felsenstein, 1981), HKY85 (Hasegawa *et al.*, 1985), and general time-reversible (Yang, 1994), allowing for invariant sites, Γ-distributed rate heterogeneity, or a combination of both for each model (Gu *et al.*, 1995). Additionally, each model was evaluated both with and without the assumption of a molecular clock to determine the significance of rate heterogeneity among lineages (Felsenstein, 1981). Subsequent to model evaluation and selection, the maximum-likelihood tree was determined using a heuristic search with 10 random addition-sequence replicates, fixing the model parameters inferred for

the starting tree. Robustness of nodal support was evaluated using the bootstrap, with model parameters fixed across replicates, and a reduction to two random addition-sequence replicates per bootstrap replicate. As with parsimony analyses, maximum-likelihood analyses were performed both with and without the picid outgroup.

Subsequent to phylogenetic analysis, trees obtained under various optimization criteria were compared to previous estimates of relationship: for parsimony analyses, the Templeton (1983) and Compare-2 (Faith, 1991) tests were employed. Under the maximum-likelihood criterion, the test of Kishino and Hasegawa (1989) was used. The alternative topologies tested were the best trees under a given criterion, employing the following constraints: (1) barbet (nonramphastine) monophyly, (2) Neotropical barbet monophyly, (3) Paleotropical barbet monophyly, (4) Prum's (1988) estimate of relationships (Fig. 1), (5) *Trachyphonus* constrained as basal within Ramphastidae, and (6) *Semnornis* constrained as sister group to the ramphastines. The last two constraints are subsets of Prum's (1988) hypothesis of relationships and were included to evaluate their relative contribution to any incongruence between his estimate and those inferred from the current data set. All tests performed were one-tailed, because comparisons were between optimal trees under a given criterion and alternative (and therefore less optimal) trees under the various constraints (see above). For the Kishino–Hasegawa test, this correction guarantees that any nonsignificant result would remain nonsignificant under more appropriate topology comparison tests currently in development (N. Goldman, pers. comm.): significant results reported here might prove nonsignificant upon implementation of these tests.

RESULTS

A priori *assessment of data characteristics.* Though a complete 925-bp fragment was obtained from all samples (except for the sequence of *Baillonius,* which extends only 660 bp from primer B1a) in the current study, analysis of sequences obtained was limited to the original 888-bp fragment (corresponding to positions 15016–15903 of the *Gallus* mitochondrial genome) analyzed by Lanyon and Hall (1994) to avoid potential problems associated with missing data. Within the region examined, 45.1% of sites were variable, with 80.3% of the variable sites being parsimony informative. By codon position, 30.8% of first positions (66.3% of variable sites parsimony informative), 11.4% of second positions (57.1% of variable sites parsimony informative), and 92.9% of third positions (87.8% of variable sites parsimony informative) were variable. Overall, the vast majority of potential phylogenetic information (68.8% of all variable sites and 75.2% of all parsimony-informative sites) was at codon third positions. Levels of divergence between the taxa sampled varied from 4.6% between the two *Andigena* species to >21% between the *Capito* species and the picid outgroups (Table 3). An examination of transition divergence as a function of overall divergence among the barbets and toucans (Fig. 2) indicated that third positions experienced numerous superimposed transition substitutions, whereas third position transversions increased linearly with overall distance. First position transition substitutions gave some indication of saturation, since several ingroup comparisons were larger than ingroup/outgroup comparisons. Evidence of superimposed substitutions for other types of changes was somewhat difficult to interpret, given their relative rarity. Transition substitutions as a whole, and especially at third positions, showed evidence of saturation, which indicates that application of positional or substitutional weighting schemes might be appropriate.

Base pair composition varied significantly among coding positions of the gene, as well as among taxa. As in previous analyses of cytochrome *b* in birds (Kocher *et al.,* 1989; Edwards *et al.,* 1991; Kornegay *et al.,* 1993; Hackett, 1996; Nunn and Cracraft, 1996), composition bias was lowest in first positions of codons, highest in third positions, and intermediate in second positions (Table 4). As is typical for vertebrate mitochondrial DNA (Kocher *et al.,* 1989), composition of codon second positions was biased against adenine and thymine residues, whereas composition at third positions was strongly biased against guanine residues (3.4%) and somewhat less so against thymine residues (13.7%). Codon first positions exhibited essentially even base composition. Among ramphastid taxa, base composition at first and second codon positions was not significantly variable; however, third position base composition varied widely (Table 4). The observed variation was primarily among barbet genera, since comparisons among the Ramphastinae did not indicate significant heterogeneity. The most divergent sequences were those from the two species of *Capito,* both of which had frequencies of thymine residues distinctly higher than those of the other taxa examined (21.9–26.5% versus 13.7% for the Piciformes as a whole). Examination of a UPGMA analysis of Euclidean distances between taxa based upon third position base frequencies revealed two major clusters, one containing the two *Capito* species and the other containing the remaining taxa (analysis not presented). Clustering within the non-*Capito* taxa had no apparent correlation with phylogeny, as members of genera and subfamilies did not form coherent groups. Therefore, other than the distinctive *Capito* base composition, no other consistent taxonomic pattern was apparent.

Phylogenetic analyses under the parsimony criterion. Results of parsimony analyses are summarized in Figs. 3 and 4. All analyses conducted without the inclusion of an outgroup resulted in ingroup topologies identical to

TABLE 3

Cytochrome *b* Divergences among Toucans and Barbets

		[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]
Trachyphonus darnaudii	[1]	—	0.160	0.160	0.188	0.174	0.184	0.194	0.165	0.168	0.147	0.153	0.155	0.161	0.150	0.161	0.166	0.164	0.161	0.163	0.168	0.181
Lybius bidentatus	[2]	0.761	—	0.141	0.169	0.150	0.203	0.199	0.166	0.172	0.165	0.170	0.183	0.171	0.167	0.166	0.167	0.171	0.174	0.167	0.172	0.171
Pogoniulus bilineatus	[3]	1.165	1.177	—	0.175	0.155	0.200	0.195	0.174	0.173	0.169	0.180	0.171	0.173	0.172	0.163	0.158	0.156	0.161	0.165	0.179	0.179
Psilopogon pyrolophus	[4]	1.384	1.004	1.101	—	0.131	0.201	0.196	0.178	0.184	0.178	0.190	0.195	0.178	0.172	0.180	0.182	0.189	0.189	0.177	0.195	0.203
Megalaima mystacophanos	[5]	1.176	0.943	1.032	3.481	—	0.184	0.180	0.170	0.170	0.154	0.172	0.179	0.187	0.187	0.173	0.179	0.180	0.179	0.171	0.174	0.177
Capito dayi	[6]	1.224	1.198	1.341	1.553	1.376	—	0.128	0.177	0.164	0.165	0.175	0.175	0.176	0.164	0.179	0.174	0.181	0.170	0.170	0.158	0.210
C. niger	[7]	1.245	1.100	1.260	1.378	1.324	13.272	—	0.173	0.160	0.165	0.165	0.179	0.165	0.153	0.159	0.164	0.166	0.157	0.166	0.154	0.215
Eubucco richardsoni	[8]	0.889	0.813	1.008	1.357	1.343	2.598	2.358	—	0.087	0.148	0.149	0.155	0.163	0.156	0.156	0.152	0.158	0.158	0.151	0.153	0.200
E. bourcierii	[9]	0.962	0.871	0.921	1.360	1.275	2.491	2.236	4.710	—	0.147	0.146	0.157	0.151	0.157	0.158	0.164	0.160	0.158	0.153	0.151	0.198
Semnornis frantzii	[10]	1.030	0.820	1.033	1.292	1.185	2.078	1.768	1.362	1.345	—	0.089	0.134	0.152	0.143	0.148	0.134	0.142	0.146	0.154	0.145	0.179
S. ramphastinus	[11]	0.957	0.782	1.222	1.408	1.269	2.330	1.875	1.336	1.212	5.302	—	0.148	0.152	0.143	0.152	0.148	0.151	0.155	0.163	0.150	0.186
Aulacorhynchus derbianus	[12]	1.010	0.979	1.070	1.258	1.130	1.934	1.880	1.526	1.456	1.542	1.699	—	0.135	0.132	0.123	0.126	0.106	0.104	0.151	0.129	0.186
Pteroglossus inscriptus	[13]	1.000	1.012	1.258	1.308	1.323	2.215	2.177	1.680	1.484	1.857	1.913	3.450	—	0.076	0.122	0.120	0.121	0.118	0.145	0.134	0.193
P. castanotis	[14]	1.106	0.831	1.254	1.239	1.338	2.351	2.097	1.618	1.544	1.933	1.997	3.066	6.791	—	0.114	0.113	0.109	0.113	0.127	0.128	0.195
Selenidera gouldii	[15]	1.158	0.815	1.083	1.128	1.025	2.213	2.082	1.398	1.337	1.976	1.740	3.408	2.729	2.884	—	0.091	0.096	0.097	0.138	0.133	0.196
S. spectabilis	[16]	1.096	0.770	1.041	1.335	1.039	2.052	1.801	1.414	1.435	1.583	1.666	3.001	2.797	2.584	3.660	—	0.090	0.084	0.147	0.137	0.191
Andigena hypoglauca	[17]	1.069	0.839	1.069	1.188	1.102	2.312	2.200	1.518	1.451	1.729	1.723	3.094	2.968	3.041	8.757	4.925	—	0.045	0.136	0.134	0.191
A. laminirostris	[18]	1.041	0.856	1.115	1.188	1.090	2.114	1.984	1.517	1.433	1.700	1.695	2.848	2.633	2.851	7.077	4.499	20.017	—	0.139	0.133	0.187
Ramphastos vitellinus	[19]	1.159	0.788	1.109	1.179	1.131	2.620	2.373	1.600	1.670	1.799	1.847	2.149	2.243	2.346	2.452	2.472	2.485	2.367	—	0.092	0.196
R. tucanus	[20]	1.157	0.778	1.230	1.331	1.062	2.401	2.293	1.665	1.623	1.617	1.646	1.831	1.884	2.243	2.363	2.202	2.482	2.360	10.134	—	0.188
Sphyrapicus varius	[21]	1.131	1.075	1.166	1.456	1.106	1.102	1.043	1.033	1.057	1.026	1.048	1.035	1.177	1.243	1.032	1.084	1.033	0.988	1.261	1.044	—

Note. Overall divergence (*p*) is shown above the diagonal, and transition:transversion ratio is shown below.

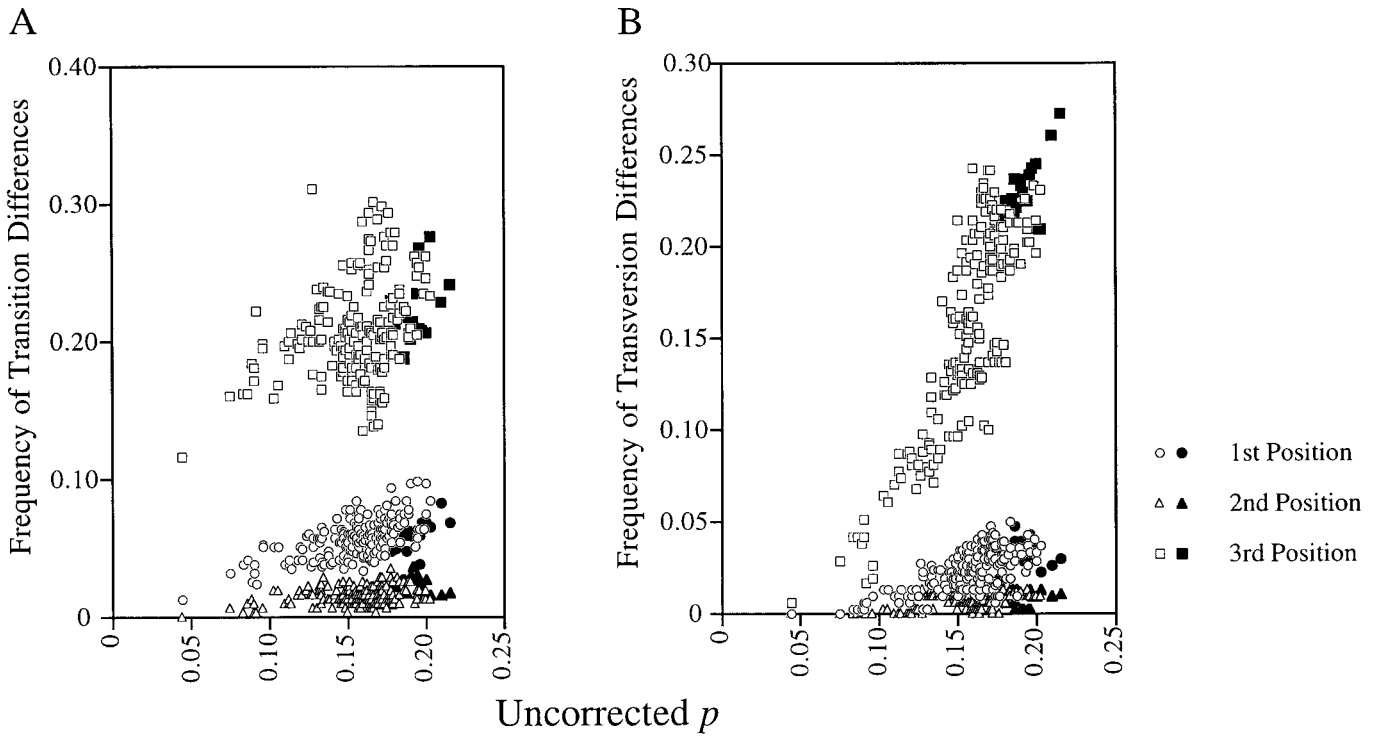

FIG. 2. Covariation of the frequency of transition (A) and transversion (B) differences with overall pairwise divergence. Comparisons among ingroup taxa are plotted with hollow symbols and those between ingroup taxa and a representative outgroup taxon (*Sphyrapicus*) as solid symbols.

TABLE 4

Base Composition by Coding Position, Composition Bias,[a] and Tests of Taxonomic Heterogeneity in Base Composition for Cytochrome *b* of Piciformes (χ^2 Not Presented for First or Second Position Comparisons, No Value Approached Significance)

	First Position				Second Position				Third Position			
Taxon	A	C	G	T	A	C	G	T	A	C	G	T
Trachyphonus darnaudii	0.2403	0.2955	0.2305	0.2338	0.1851	0.2727	0.1461	0.3961	0.3010	0.5696	0.0259	0.1036
Lybius bidentatus	0.2492	0.2963	0.2155	0.2391	0.1960	0.2838	0.1284	0.3919	0.3725	0.4941	0.0313	0.1022
Pogoniulus bilineatus	0.2382	0.2921	0.2214	0.2483	0.1961	0.2769	0.1288	0.3982	0.3539	0.4823	0.0059	0.1579
Psilopogon pyrolophus	0.2468	0.2662	0.2208	0.2662	0.1916	0.2662	0.1461	0.3961	0.3592	0.4240	0.0453	0.1715
Megalaima mystacophanos	0.2500	0.2955	0.2143	0.2403	0.1916	0.2727	0.1494	0.3864	0.3592	0.4693	0.0162	0.1553
Capito dayi	0.2362	0.2654	0.2265	0.2719	0.1867	0.2776	0.1445	0.3912	*0.2743*	*0.4426*	*0.0639*	*0.2193*
C. niger	0.2382	0.2821	0.2314	0.2483	0.1934	0.2846	0.1292	0.3927	*0.3024*	*0.4071*	*0.0253*	*0.2652*
Eubucco richardsoni	0.2435	0.2760	0.2175	0.2630	0.1851	0.2727	0.1429	0.3994	0.3430	0.5016	0.0129	0.1424
E. bourcierii	0.2386	0.2873	0.2159	0.2581	0.1851	0.2760	0.1429	0.3961	0.3333	0.5000	0.0129	0.1537
Semnornis frantzii	0.2403	0.2922	0.2175	0.2500	0.1916	0.2662	0.1461	0.3961	0.2945	0.5340	0.0162	0.1553
S. ramphastinus	0.2411	0.2930	0.2183	0.2476	0.1948	0.2630	0.1429	0.3994	0.2945	0.5663	0.0291	0.1100
All Barbets (11 spp.)	0.2420	0.2856	0.2209	0.2516	0.1906	0.2738	0.1408	0.3949	0.3260	0.4904	0.0260	0.1577
									$\chi^2_{(\text{Barbets})} = 107.19, P < 0.01$			
Ramphastinae (9 spp.)	0.2371	0.2866	0.2323	0.2441	0.1889	0.2798	0.1398	0.3915	0.2997	0.5499	0.0303	0.1201
									$\chi^2_{(\text{Ramphastinae})} = 15.27, P = 0.91$			
Ramphastidae (20 spp.)	0.2398	0.2860	0.2260	0.2482	0.1898	0.2765	0.1404	0.3934	0.3142	0.5171	0.0279	0.1408
									$\chi^2_{(\text{Ramphastidae})} = 154.58, P < 0.01$			
Picidae (10 spp.)	0.2325	0.2897	0.2396	0.2381	0.1887	0.2607	0.1473	0.4033	0.2997	0.5264	0.0449	0.1290
									$\chi^2_{(\text{Picidae})} = 41.13, P = 0.09$			
Piciformes (30 spp.)	0.2372	0.2873	0.2309	0.2446	0.1894	0.2709	0.1428	0.3969	0.3090	0.5204	0.0340	0.1366
	$\chi^2_{(\text{Piciformes})} = 13.66, P = 1.00$				$\chi^2_{(\text{Piciformes})} = 8.85, P = 1.00$				$\chi^2_{(\text{Piciformes})} = 219.16, P < 0.01$			
	$b = 0.0497$				$b = 0.2237$				$b = 0.4392$			

[a] $b = 2/3 \cdot \Sigma_i |c_i - 0.25|$; c_i = frequency of the ith base.

those found with the outgroup, indicating that the outgroup had little effect on the analysis other than providing a root. Results of analyses excluding outgroups are therefore not presented here. Analysis of the relationships of *Baillonius* was limited to *a posteriori* estimates because of the limited sequence obtained from this sample (see Discussion). Analysis of the remaining sequences using equally weighted parsimony yielded a single most-parsimonious ingroup tree (Fig. 4A; L = 1919, CI = 0.34 excluding uninformative characters [all CI's subsequently reported exclude uninformative characters]). Though many hypothesized relationships remained constant across analyses, some inferences of relationship among barbets and toucans varied significantly with weighting scheme employed. The features that remained fairly constant across analyses (refer to Fig. 3, nodes A–N) included generic monophyly (excepting *Selenidera*); sister group relationships between the generic pairs (*Capito, Eubucco*), (*Megalaima, Psilopogon*), and (*Andigena, Selenidera*) (though in some cases *Selenidera* was paraphyletic, see below); monophyly of the toucanets; a clade containing (*Capito, Eubucco*) and the ramphastines; monophyly of the Neotropical clade (*Semnornis, Capito, Eubucco,* and ramphastines); a sister group relationship between *Trachyphonus* and the Neotropical clade; and monophyly of toucans and barbets (Ramphastidae). Beyond this set of fairly consistent results, two major sets of relationships were obtained, generally corresponding to the type of weighting employed. Downweighting of third position changes, regardless of the type of change, consistently resulted in the following groups (Fig. 4B): a clade containing *Lybius, Pogoniulus, Megalaima,* and *Psilopogon,* with a sister group relationship between *Lybius* and the (*Megalaima, Psilopogon*) clade; monophyly of Prum's (1988) Ramphastinae; a sister group relationship between *Pteroglossus* and the (*Andigena, Selenidera*) clade; and monophyly of *Selenidera.* In the case of downweighting third position transition changes only, retaining transversion information, the relationships consistently obtained included (Fig. 4D) monophyly of Prum's (1988) Lybiinae (*Lybius, Pogoniulus*), a sister group relationship between the (*Lybius, Pogoniulus*) clade and the (*Trachyphonus,* Neotropical barbet, toucan) clade, a sister group relationship between *Ramphastos* and the (*Capito, Eubucco*) clade (i.e., nonmonophyly of the toucans, subfamily Ramphastinae), a sister group relationship between *Aulacorhynchus* and the (*Andigena, Selenidera*) clade, and paraphyly of the genus *Selenidera* (with *S. spectabilis* clustering with *Andigena*). Generally speaking, the transversion only, Irwin *et al.* (1991), and combinatorial weights analyses

Node	1	2.1	2.2	2.3	2.4	2.5	2.6	2.7	3.1	3.2	3.3	3.4	3.5	3.6	3.7	4	5	6	7.1	7.2	8.1	8.2	MIN	MAX	Node Description
A	100	99	99	100	99	100	99	98	100	100	100	100	100	100	100	100	98	100	100	100	100	100	98	100	*Semnornis*
B	100	100	100	100	100	99	99	91	100	100	100	100	100	100	100	100	100	100	83	78	100	94	78	100	*Eubucco*
C	100	100	100	100	100	100	100	92	100	100	100	100	100	100	100	100	100	100	93	87	100	95	87	100	*Capito*
D	100	100	100	100	100	99	99	96	100	99	100	100	100	99	96	88	99	100	100	97	99	99	88	100	*Andigena*
E	100	100	100	100	100	100	99	90	100	100	100	100	100	100	100	96	100	100	100	100	100	100	90	100	*Pteroglossus*
F	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	*Ramphastos*
G	99	99	96	95	93	96	87	77	100	100	100	100	100	100	100	100	98	100	100	100	98	98	77	100	(*Psilopogon* , *Megalaima*)
H	91	93	92	87	85	79	80	65	96	94	98	95	97	96	93	95	73	97	82	91	92	85	65	98	(*Capito* , *Eubucco* , *Semnornis* , Ramphastinae)
I	64	90	91	93	92	90	84	72	76	73	68	81	72	80	70	47	76	66	63	70	75	72	47	93	(H, *Trachyphonus*)
J	95	100	100	100	100	100	98	93	97	96	99	99	98	98	99	95	98	95	97	98	97	97	93	100	Ramphastoidea (Peters 1948)
K	43	64	67	65	64	76	66	69	59	63	71	65	78	71	83	84	65	85	69	61	59	76	43	85	(*Capito* , *Eubucco*)
L	46	64	69	78	78	71	78	74	64	71	71	72	78	71	71	80	54	79	40	79	68	58	40	80	(*Aulacorhynchus* , *Pteroglossus* , *Selenidera* , *Andigena*)
M	92	82	70	60	55	40	41	13	94	97	98	96	97	96	95	96	80	97	88	91	88	86	13	98	(*Selenidera* , *Andigena*)
N	75	70	63	57	50	55	34	12	86	79	85	84	82	80	84	82	35	85	54	59	74	57	12	86	(*Capito* , *Eubucco* , Ramphastines)
O	28	47	56	61	61	75	59	53	33	27	37	40	30	41	38	17	52	13	21	36	14	17	13	75	(*Lybius* , *Pogoniulus* , *Psilopogon* , *Megalaima*)
P	28	52	59	65	67	58	71	70	41	36	41	42	36	38	37	11	80	26	40	44	34	37	11	80	(*Pteroglossus* , *Selenidera* , *Andigena*)
Q	27	66	73	76	77	86	79	76	34	23	33	31	36	30	26	17	88	24	24	31	20	21	17	88	(*Lybius* , *Psilopogon* , *Megalaima*)
R	48	66	65	62	62	58	62	51	60	40	54	46	36	42	41	0	36	24	55	39	54	52	0	66	*Selenidera*
S	28	52	61	62	62	64	57	46	44	34	40	32	45	45	39	18	26	30	65	59	38	65	18	65	Ramphastinae
T	44	38	33	27	22	25	17	8	43	52	50	48	57	50	50	73	14	64	36	45	51	43	8	73	(*Aulacorhynchus* , *Selenidera* , *Andigena*)
U	50	22	18	14	13	8	11	9	52	57	50	52	52	54	55	72	8	58	51	59	65	69	8	72	(*Lybius* , *Pogoniulus*)
V	36	16	8	6	5	0	0	0	30	37	28	29	27	27	25	24	6	37	28	26	27	24	0	37	(*Trachyphonus* , *Lybius* , *Pogoniulus* , H)
W	50	34	35	38	36	40	36	41	38	59	45	52	64	57	57	52	62	72	43	57	44	44	34	72	(*Selenidera spectabilis* , *Andigena*)
X	43	32	23	15	12	11	0	0	45	50	44	58	40	47	53	73	14	59	8	16	47	13	0	73	(*Capito* , *Eubucco* , *Ramphastos*)
Y	0	0	0	7	11	5	13	19	0	0	0	0	0	0	0	0	0	0	17	7	0	25	0	25	(*Semnornis* , *Ramphastinae*)
Z	0	0	0	6	10	6	13	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	(*Pteroglossus* , *Selenidera*)
AA	28	12	8	9	9	5	6	0	19	16	16	14	10	13	0	0	10	6	9	0	18	0	0	28	(*Capito* , Ramphastinae)
BB	21	31	27	24	22	15	19	15	24	24	24	27	26	26	26	19	19	32	25	17	28	22	15	32	(*Pogoniulus* , *Trachyphonus* , H)
CC	0	7	7	7	8	9	12	19	0	7	8	7	8	7	0	6	11	0	7	14	5	0	0	19	(*Capito* , *Eubucco* , *Semnornis*)
DD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	13	(*Capito* , *Eubucco* , Ramphastinae [-*Aulacorhynchus*])
# IG Trees	1	2	1	1	1	1	1	1	3	1	1	1	1	1	2	3	1	2	8	8	1	1			

FIG. 3. Results of parsimony analysis under various weighting schemes. Analyses are numbered across the top and correspond to the following: 1, equally weighted parsimony; 2, downweighting third positions (0.50, 0.33, 0.25, 0.20, 0.17, 0.10, 0.00); 3, downweighting third position transitions (0.50, 0.33, 0.25, 0.20, 0.17, 0.10, 0.00); 4, transversion parsimony; 5, weighting codon positions by inverse of percentage variable; 6, Irwin *et al.*'s (1990) conservative weights; 7, analysis without *Capito* third positions (1, equally weighted parsimony; 2, eliminating third position transitions); and 8, combinatorial weights analysis (1, with and 2, without *Capito* third positions). Nodes found in analyses are labeled A–DD along the left and a brief description of the node is given on the right). Shading of a cell containing a node letter (along the left) indicates that that node contradicts barbet monophyly. Node letters that are shaded and in boldface italic contradict Neotropical barbet monophyly. Reading down from the top to the bottom, within a given analysis column, the numbers in each row indicate the percentage of bootstrap pseudoreplicates in which the corresponding node was recovered. The MIN and MAX columns (on the right) indicate the minimum and maximum bootstrap percentage for a given node across all analyses. Shading of cells within a column indicates that the corresponding node was present in the set of shortest trees found in the corresponding analysis. The number of equally parsimonious topologies of ingroup (not picid) relationship found for a given analysis is given along the bottom.

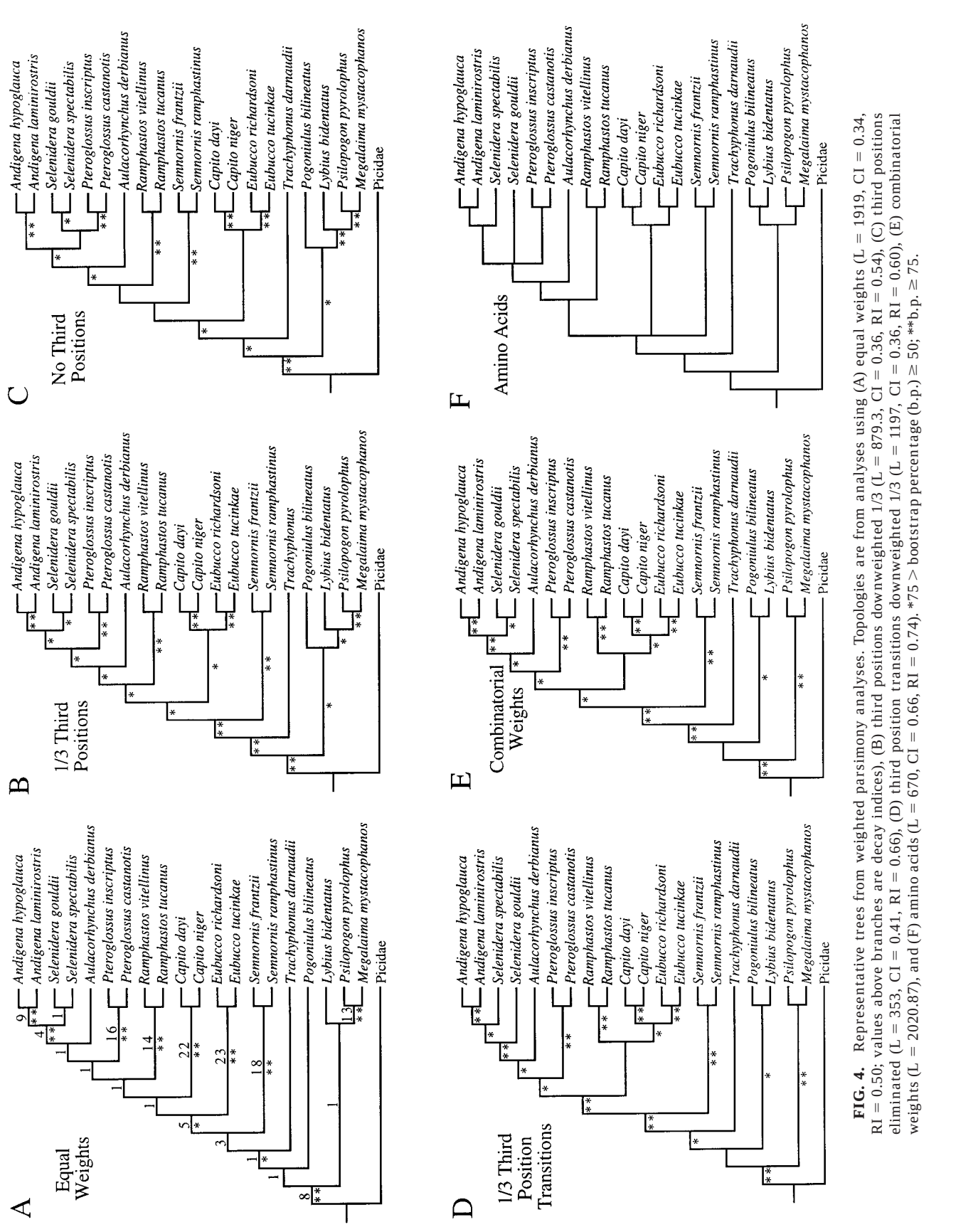

FIG. 4. Representative trees from weighted parsimony analyses. Topologies are from analyses using (A) equal weights (L = 1919, CI = 0.34, RI = 0.50; values above branches are decay indices), (B) third positions downweighted 1/3 (L = 879.3, CI = 0.36, RI = 0.54), (C) third positions eliminated (L = 353, CI = 0.41, RI = 0.66), (D) third position transitions downweighted 1/3 (L = 1197, CI = 0.36, RI = 0.60), (E) combinatorial weights (L = 2020.87), and (F) amino acids (L = 670, CI = 0.66, RI = 0.74). *75 > bootstrap percentage (b.p.) ≥ 50; **b.p. ≥ 75.

yielded results congruent with the third position transition downweighting analyses, indicating that the major effect of these schemes is the elimination of third position transitions. Weighting by the inverse of the proportion of variable sites gave results somewhat intermediate between positional and stepmatrix weighting schemes, though one relationship in this analysis was idiosyncratic (Fig. 3, node DD; *Aulacorhynchus* fell outside of a clade including the remainder of the Ramphastinae, *Capito,* and *Eubucco*). Another unusual pattern of relationships was found for the third position downweighting analyses using 0.1 and 0 weights (Figs. 3 and 4C). In these cases, a unique pattern of relationships among toucanets was observed, with *Selenidera* clustering with *Pteroglossus* rather than *Andigena* and *Aulacorhynchus* being sister to both. Additionally, these latter analyses supported a sister group relationship between *Semnornis* and the ramphastines. Finally, parsimony analysis of amino acid sequences using equal weights yielded 1200 equally parsimonious trees of length 254 (Fig. 4F; CI = 0.66 excluding uninformative characters, RI = 0.74). The 50% majority rule consensus of these trees is similar to results obtained from downweighting third positions, which is reasonable, given that most changes at first and second positions cause amino acid replacement, whereas third position changes cause relatively few.

Since analysis of sequence composition indicated significant heterogeneity among taxa at codon third positions, confined primarily to the two species of *Capito,* additional analyses of the data were performed. The compositional heterogeneity at third positions suggests that they should be eliminated from phylogenetic analysis using parsimony (since parsimony cannot incorporate corrections to take this factor into account). However, to eliminate or downweight third positions is to exclude a majority of the data (since the largest fraction of informative sites is in third positions) and, more importantly, to exclude a majority of the most potentially *informative* data (since third position transversions show little evidence of saturational effects). An alternative analysis which might avoid this difficulty, while still eliminating the majority of compositional heterogeneity, would be to eliminate all third positions of the *Capito* sequences alone. The results of such an analysis, using (1) equally weighted parsimony, (2) elimination of third position transitions, and (3) combinatorial weights, are presented in Fig. 5. The results of the three analyses are perfectly congruent with each other, though the two stepmatrix analyses offer more resolution, and the combinatorial weights analysis yields a completely resolved topology. The pattern of relationships inferred in this latter analysis has features in common with results of several of the previous parsimony analyses. The positioning of the (*Lybius, Pogoniulus*) and (*Megalaima, Psilopogon*) clades as successive sister taxa to the clade including *Trachyphonus,* the Neotropical barbets, and toucans is the same as that in most stepmatrix weighting schemes. The most notable difference between this analysis and the stepmatrix weighting analyses which include *Capito* third positions is the position of *Ramphastos,* which clustered with the (*Capito, Eubucco*) clade before those data were eliminated. That position would require paraphyly of the morphologically distinctive toucans and either multiple origins of the toucan morphotype or a reversal back to the barbet morphotype in the (*Capito, Eubucco*) clade. In the analysis without *Capito* third positions, *Ramphastos* clusters with the toucanets, forming a monophyletic Ramphastinae in agreement with third position downweighting analyses and requiring a single origin of the toucan morphotype.

Phylogenetic analyses under the maximum-likelihood criterion. Examination of likelihood scores for the neighbor-joining tree of Kimura two-parameter distances under various models of sequence evolution indicated that the most parameter-rich model currently available (general time-reversible with invariant sites and rates at variable sites following a Γ frequency distribution [abbreviated GTR + I + Γ]) offered a significant increase in likelihood over less complex models (versus Γ-distributed rates alone, $-2 \ln \Lambda = 343.4$, $df = 1$, $P \ll 0.01$; versus invariant sites alone, $-2 \ln \Lambda = 56.8$, $df = 1$, $P \ll 0.01$; versus HKY85 with invariant sites and Γ-distributed rates, $-2 \ln \Lambda = 58.2$, $df = 4$, $P \ll 0.01$). Additionally, likelihood ratio tests of the molecular clock indicated significant rate heterogeneity ($-2 \ln \Lambda = 43.3$, $df = 29$, $P < 0.05$). A heuristic search assuming the GTR + I + Γ model without a molecular clock and employing the parameter values found for the neighbor-joining tree yielded a single most likely tree (Fig. 6). Iteration of this process using the likelihood tree as the basis of parameter estimation did not alter the topology found. This tree is congruent with the strict consensus of the trees found under the parsimony criterion, excluding *Capito* third positions and eliminating third position transition substitutions (Fig. 5B; most notably, supporting toucan monophyly). However, the maximum-likelihood tree offers resolution of the polytomies found in the parsimony analysis, favoring a paraphyletic *Selenidera,* a relationship between *Pteroglossus* and the (*Andigena, Selenidera*) clade, and a placement of the southeast Asian barbet clade (*Megalaima* and *Psilopogon*) as sister taxon to all other barbets and toucans. Bootstrap analyses of these data under the maximum-likelihood criterion indicated strong support for nearly all nodes in the tree, with the exception of the (*Selenidera spectabilis, Andigena*) clade, the (*Pteroglossus, Selenidera, Andigena*) clade, and the sister group relationship between the (*Lybius, Pogoniulus*) and the (Neotropical barbet, toucan, *Trachyphonus*) clades.

Application of maximum-likelihood models that make the assumption of stationary base composition (as is

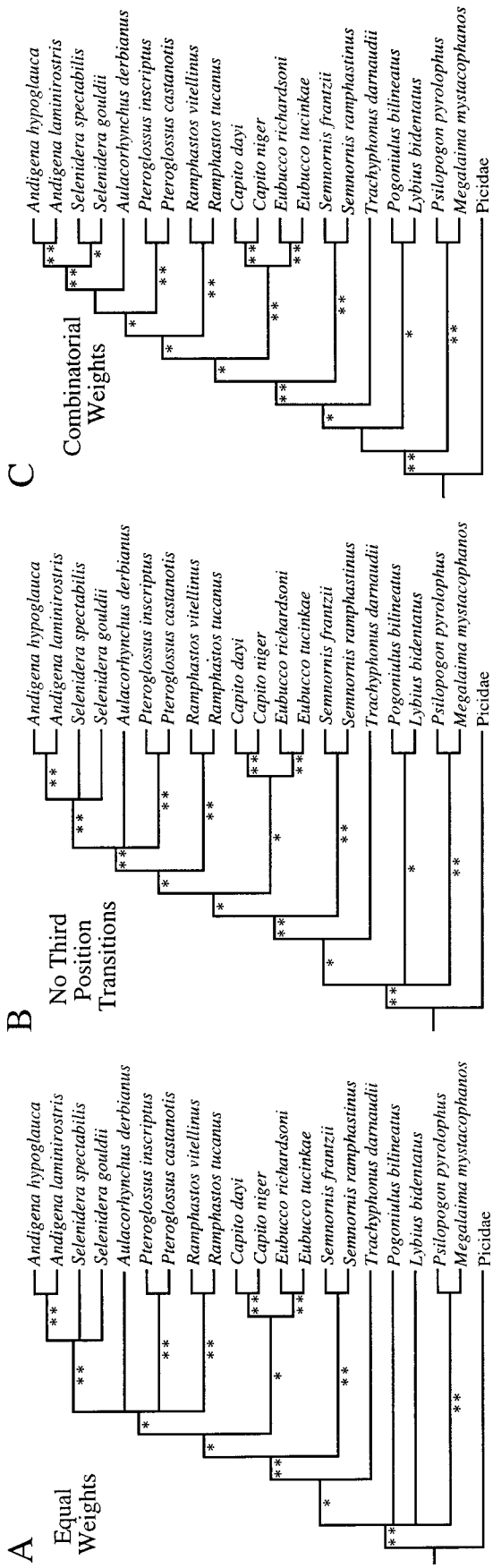

FIG. 5. Results of parsimony analyses with *Capito* third positions coded as missing. Topologies are from analyses with (A) equally weighted parsimony (8 ingroup trees, L = 1776, CI = 0.36, RI = 0.51), (B) elimination of third position transitions (8 ingroup trees, L = 809, CI = 0.37, RI = 0.68), and (C) combinatorial weights (1 ingroup tree, L = 1880.16). *** are as in Fig. 4.

standard for most implementations of likelihood search algorithms) to data that exhibit compositional heterogeneity could be problematic. In the current case, it is clear that sequences from the genus *Capito* are distinctive in their high proportion of thymine residues at third positions. One way of dealing with this difficulty is to elaborate the likelihood model used in order to take such heterogeneity into account (Yang and Roberts, 1995; Galtier and Gouy, 1998). However, this approach is costly in terms of computational load, and it is not currently feasible to perform tree searches, let alone parameter optimizations, with large numbers of taxa. As noted in the section on parsimony, another method of dealing with this problem is to drop the heterogeneous portion of the data from the analysis (e.g., Frati *et al.*, 1997). This approach was applied to the current data by dropping heterogeneous third positions from the analysis, reestimating the appropriate substitution model, and performing heuristic searches under that model. In this case, the GTR + I + Γ model did not offer a significant improvement over the HKY85 + I + Γ model ($-2 \ln \Lambda = 7.0$, $df = 4$, $P > 0.10$), though inclusion of both rate heterogeneity parameters continued to offer a significant improvement ($P \ll 0.01$). Apparently, the observed rate heterogeneity among taxa could not be attributed to changes in base composition, since a likelihood ratio test again rejected the molecular clock ($-2 \ln \Lambda = 65.0$, $df = 29$, $P \ll 0.01$). A heuristic search with the HKY85 + I + Γ model and parameters estimated using a neighbor-joining tree yielded three equally likely trees ($-\ln [\ell] = 2649.4$). The strict consensus of these three trees can be generated by collapsing the thick branch in Fig. 6 leading to the (*Capito, Eubucco,* ramphastine) clade. The three equally likely trees are the three possible resolutions of that trichotomy. As for parsimony analyses, a third approach was attempted, specifically, elimination of third position data from only those taxa which differ significantly from the others in base composition. The maximum-likelihood analysis was repeated using all positions but treating *Capito* third positions as missing data. This analysis resulted in a topology identical to that estimated without excluding *Capito* third positions, indicating a relative robustness of the method to the observed heterogeneity in base composition.

Tests of topological distinctness. The results of the Templeton, Compare-2, and Kishino–Hasegawa tests for comparisons between the relationships inferred from cytochrome *b* and the alternative hypotheses of relationship are summarized in Table 5. The only tree comparison found to be significant across all optimization criteria and test statistics was between the shortest (or most likely) trees and the tree inferred with the constraint of barbet monophyly. For the three weighted parsimony analyses, the Templeton test also distinguished between the shortest trees and the trees gener-

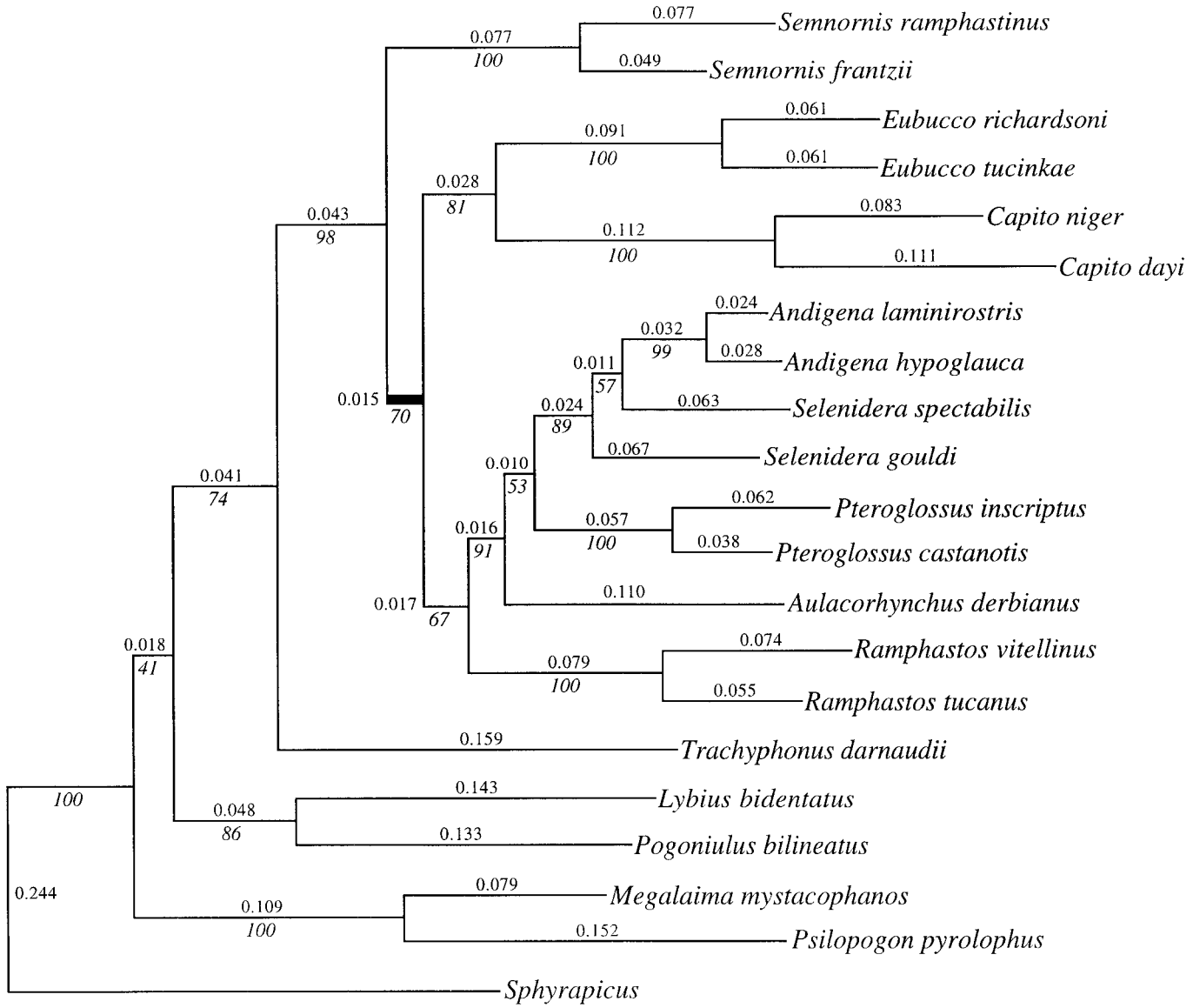

FIG. 6. Results of maximum-likelihood analysis of cytochrome *b* sequences ($-\ln(\ell) = 9110.0$; associated numbers are branch lengths). Topology is from a heuristic search under the general time-reversible model with invariant sites and Γ-distributed rates at variable sites (proportion of invariant sites $p_{iv} = 0.46$; $\alpha = 1.18$; R-matrix components $r_{ac} = 1.78$, $r_{ag} = 7.80$, $r_{at} = 1.11$, $r_{cg} = 0.38$, $r_{ct} = 9.55$, $r_{gt} = 1$; empirical base frequencies assumed stationary; molecular clock not enforced). The fractional values along branches indicate branch lengths in expected number of changes per character, and the italicized values below branches indicate the percentage of bootstrap pseudoreplicates in which the corresponding nodes were found. The thick branch collapses to a trichotomy when analyses are conducted excluding data from third positions.

ated under the constraint of Paleotropical barbet monophyly. Also, the statistic was significant for the comparison to Prum's hypothesis under the 1/3 third position downweighting criterion and nearly so for the other weighting schemes. The behavior of the Compare-2 test was more idiosyncratic, though in general it found more significant differences between alternative topologies than the other two tests (57% of comparisons significant at the $\alpha = 0.05$ level, compared to 35% for the other two tests combined). The lack of significant conflict between the topologies inferred from sequence data and some previous hypotheses of relationship is somewhat surprising, given the high bootstrap percentages obtained for groupings which contradict those hypotheses (e.g., the relationship of [*Capito*, *Eubucco*] and the ramphastines to the exclusion of *Semnornis*). Other conflicts may simply be due to errors in rooting, with alternative placements of the root being nearly equivalent in tree length and likelihood (e.g., rooting between *Trachyphonus* and other barbets and toucans versus rooting at [*Megalaima*, *Psilopogon*]).

TABLE 5

Comparison of Optimal Topologies Estimated from Cytochrome *b* Sequences with Previous Estimates of Relationship within the Ramphastidae (*sensu* Prum, 1988)

Criterion	Weighting scheme	Best tree	Alternative topology[a] 1	2	3	4	5	6
Parsimony	Equal weights	1919 (1)	1938 (1) $P_T = 0.04$ $P_{C2} = 0.02$	1924 (2) $P_T = 0.18$ $P_{C2} = 0.08$	1927 (2) $P_T = 0.20$ $P_{C2} = 0.21$	1932 (2) $P_T = 0.11$ $P_{C2} = 0.01$	1923 (1) $P_T = 0.33$ $P_{C2} = 0.09$	1926 (2) $P_T = 0.13$ $P_{C2} = 0.07$
	1/3 Third positions	879.3 (2)	889.3 (3) $P_T < 0.01$ $P_{C2} = 0.01$	880.0 (1) $P_T = 0.32$ $P_{C2} = 0.43$	883.3 (1) $P_T = 0.02$ $P_{C2} = 0.01$	887.7 (1) $P_T = 0.03$ $P_{C2} = 0.01$	881.7 (1) $P_T = 0.18$ $P_{C2} = 0.19$	880.7 (1) $P_T = 0.16$ $P_{C2} = 0.32$
	1/10 Third positions	512.2 (1)	518.8 (2) $P_T = 0.01$ $P_{C2} = 0.01$	512.5 (1) $P_T = 0.12$ $P_{C2} = 0.37$	515.6 (3) $P_T = 0.01$ $P_{C2} = 0.05$	519.9 (1) $P_T = 0.07$ $P_{C2} = 0.01$	515.1 (1) $P_T = 0.36$ $P_{C2} = 0.13$	
	1/3 Third position transitions	1197.0	1216.3 (1) $P_T = 0.02$ $P_{C2} = 0.01$	1202.0 (1) $P_T = 0.39$ $P_{C2} = 0.01$	1204.7 (1) $P_T = 0.04$ $P_{C2} = 0.01$	1208.7 (2) $P_T = 0.06$ $P_{C2} = 0.02$	1201.7 (1) $P_T = 0.30$ $P_{C2} = 0.12$	1204.0 (2) $P_T = 0.19$ $P_{C2} = 0.01$
Maximum likelihood		9100.0	46.4 (14.0) $P < 0.01$	2.3 (3.3) $P = 0.25$	8.3 (6.3) $P = 0.10$	8.5 (8.5) $P = 0.16$	6.2 (8.1) $P = 0.23$	2.6 (3.1) $P = 0.20$

Note. Values given for parsimony are tree lengths (no. of trees in parentheses), *P* value of Templeton (1983) test (P_T), and *P* value of the Compare-2 test (P_{C2}, Faith, 1991; for comparisons of multiple equally parsimonious trees with these tests, the largest *P* value obtained is given). Values given for maximum-likelihood are $\Delta\ell$ between constrained and unconstrained topologies (standard deviation of difference s[$\Delta\ell$] in parenthesis), and *P* value for $\Delta\ell$/s($\Delta\ell$) calculated from the Student *t* distribution (Kishino and Hasegawa, 1989). *P* values for all tests are one-tailed (see Methods). The single blank cell indicates that the constraint tree was identical to the most-parsimonious tree for the 1/10th third position downweighting.

[a] Shortest trees found for the cytochrome *b* data, imposing the following constraints: 1, all barbets constrained as monophyletic; 2, Neotropical barbets constrained as monophyletic; 3, Paleotropical barbets constrained as monophyletic; 4, Prum's (1988) estimate of relationships constrained; 5, *Trachyphonus* constrained as basal within Ramphastidae; 6, *Semnornis* constrained as sister group to the Ramphastines.

DISCUSSION

Effects of weighting on phylogenetic inference. As noted under Introduction, approaches to character and character-state transition weighting have been widely discussed. The question of which weighting scheme is most appropriate for analysis of a particular data set is an issue, however, only if alternative weighting schemes significantly affect the results obtained. In the current case, the various approaches to data weighting clearly affected phylogenetic inference in terms of both topology and measures of nodal support (Figs. 3–5). The pairwise differences between individual topologies are small: in general, only a few nodes are in conflict. On the other hand, bootstrap percentages for individual nodes vary widely among analyses. As a measure of this variation, we examined the percentage of individual nodes that had bootstrap percentages in various analyses varying across arbitrary thresholds at 50 and 75. For instance, in Fig. 3 node O (defining monophyly of the Paleotropical taxa) had a bootstrap percentage as low as 13 in one analysis (Irwin *et al.*'s conservative weighting scheme) and one as high as 75 in another analysis (downweighting of third positions by 0.17). Overall, 48% of nodes crossed the 50% bootstrap support threshold (that is, had bootstrap percentages both greater and less than 50% in the set of analyses conducted), and 31% crossed the 75% support threshold. Nearly a third (27%) of all nodes inferred had bootstrap percentages lower than 50 in at least one analysis and greater than 75 in another analysis. If bootstrap percentages are taken as indications of support for a given node (e.g., Sanderson, 1989; Hillis and Bull, 1993), then it is clear that the choice of weighting scheme has an extreme effect on which nodes will be accepted as robust, regardless of the cutoff level chosen (50 or 75%).

The results of analyses performed in this study suggest that while the overall weighting scheme may have a significant impact on phylogenetic inference, the precise values used in weighting may be of less importance. Many studies have suggested downweighting transition substitutions in phylogenetic analyses of mitochondrial data sets, especially in cases of deep divergences in which multiple superimposed substitutions are likely (Irwin *et al.*, 1991; Edwards *et al.*, 1991; Knight and Mindell, 1993; Reeder, 1995; Griffiths, 1997), but few have indicated how strongly to downweight them. Three alternative suggestions have been to (1) eliminate them entirely (e.g., Irwin *et al.*, 1991; Miyamoto *et al.*, 1994; Griffiths, 1997), (2) downweight them by the estimated ratio of transversion and transition rates (e.g., Friesen *et al.*, 1996), or (3) weight all

substitutions relative to their inferred frequencies (e.g., Williams and Fitch, 1989; Wheeler, 1990; Knight and Mindell, 1993; Cunningham, 1997). This study suggests that, at least for the approximate levels of divergence and transition/transversion ratios observed here, the exact number used for downweighting transitions versus transversions does not matter a great deal. The average transition/transversion ratio for these data, as estimated by uncorrected sequence divergences for intrageneric comparisons, is 9.1 ($\pm$2.2 SE, $n = 7$). Analyses downweighting third position transitions from 1/3 through 1/10, bracketing the 1/9 value suggested by intrageneric comparisons, all yielded identical results (Fig. 3, analyses 3.2–3.6). Further, the analyses downweighting third position transitions by 1/2 and eliminating third position transitions entirely both yielded the same topology found in the other transition downweighting analyses as one of several equally parsimonious trees (Fig. 3, analyses 3.1 and 3.7). However, more complex character-state transition weighting schemes (in this study, combinatorial weights; Wheeler, 1990) did yield results slightly different from simple transition/transversion weighting, though the general pattern of relationships obtained was similar (Figs. 3 and 4). Overall, these results indicate that the precise ratio of weights used for transition versus transversion substitutions has little significant impact on the results of phylogenetic inference: weights between 0.5 and 0.0 yielded essentially identical results. The generality of this result across varying levels of sequence divergence and substitution characteristics (e.g., levels of compositional bias) should be assessed and documented (e.g., this study; Friesen *et al.*, 1996).

Phylogenetic relationships among toucans and barbets and biogeographic affinities of the Neotropical avifauna. Given that the choice of weighting scheme appears to have a significant impact on the relationships inferred and especially on the degree of confidence given to subsets of those relationships, selecting the most appropriate scheme becomes critical. What criterion should be used to determine the most appropriate weighting scheme? This question needs to be addressed from both theoretical and empirical perspectives. Unlike analysis of models within the maximum-likelihood framework, no single, objective criterion exists to guide the choice of a weighting scheme under the parsimony criterion. Unfortunately, little progress has been made to date in understanding the functional properties and relative merits of parsimony weighting schemes, despite the ubiquity of such schemes (but see, e.g., Cunningham, 1997). Since there are no *a priori* criteria for choosing an optimal parsimony weighting scheme for any single data set, we have used an *a posteriori* approach, the phylogenetic framework, to summarize the results of the phylogenetic analyses conducted here. As originally formulated, Lanyon's (1993) phylogenetic framework approach was a method for combining phylogenetic information from disparate data sources that were potentially heterogeneous in analytical method applied (e.g., character versus distance) and in taxonomic sampling into a summary of robust hypotheses of relationship. This method has two components: (1) the estimation of a measure of nodal "robustness" (e.g., jackknife or bootstrap support) for multiple data sets and (2) the use of combinable-component (semistrict) consensus (Bremer, 1990) to summarize robust estimates of phylogeny. The same procedure can be applied to multiple analyses of a single data set on the premise that each alternative analysis (or group of similar analyses) may allow the detection of phylogenetic signal in the data that might otherwise be obscured. If the various sets of relationships inferred are complementary and congruent, then all will be incorporated into the consensus, whereas detection of alternative conflicting sets of relationships yields ambiguity. This modification of phylogenetic framework technique has been termed Robust Taxonomic Congruence (Harshman, 1996). Application of this approach to the analyses summarized in Fig. 3, using a bootstrap percentage >75 as a criterion for recognizing robustness (exceeding the 70% level suggested by Hillis and Bull, 1993), yields the consensus topology in Fig. 7. This phylogenetic framework contains only three trichotomies. The first of these reflects ambiguity regarding monophyly of the genus *Selenidera*, the second regarding the monophyly of the ramphastines, and the third regarding the placement of *Pogoniulus*. All of these polytomies reflect conflict between the two major classes of results obtained using character and character state weighting. Interestingly, the first two of these conflicts disappear when *Capito* third positions are eliminated from analysis (Fig. 5). To reflect this, we have indicated the preferred resolution of these two polytomies in Fig. 7. The fact that we are presenting this metahypothesis as our summary of relationships among barbets and toucans as inferred under the parsimony criterion does not reflect our general preference for this approach in phylogenetic inference. Rather, it represents our response to the tension between believing that weighting can improve phylogenetic inference using parsimony and not having an objective criterion for choosing among weighting schemes. This compromise should be seen as a call for further study of the effects of weighting schemes in parsimony analysis and techniques for choosing among them.

The set of relationships represented in Fig. 7 is strikingly similar to the results obtained using maximum-likelihood: the two topologies are in conflict only in two places. First, analyses under the maximum-likelihood criterion prefer a reconstruction with paraphyletic *Selenidera*, with *S. spectabilis* more closely related to *Andigena* than to *S. gouldi*. Second, maximum-likelihood analyses conflict with the parsimony

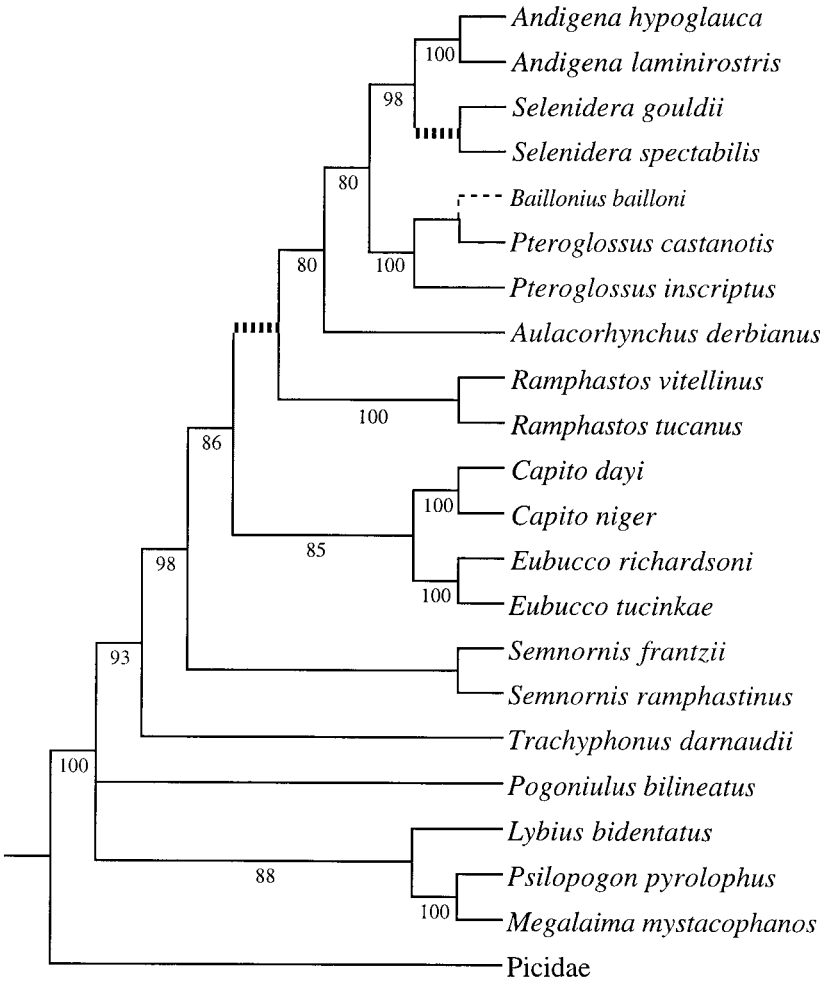

FIG. 7. Phylogenetic framework constructed from analyses summarized in Fig. 3, using a bootstrap percentage >75 as a criterion for robustness. Numbers below branches indicate the highest bootstrap percentage estimated in any analysis (see Fig. 3). The thick, barred branches highlight nodes that are not resolved in the 75% framework but which have a preferred resolution based upon analyses with *Capito* third position data eliminated from analysis. The position of *Baillonius* under equally weighted parsimony with the framework topology constrained is indicated by a dashed line.

framework in the arrangement of the Paleotropical taxa, with the former preferring a (*Lybius, Pogoniulus*) clade sister to the (Neotropical barbet, toucan, *Trachyphonus*) clade and the latter preferring a (*Lybius*, Asian barbet) clade (with the relationships of *Pogoniulus* unresolved). In both of these cases of conflict, the results of maximum-likelihood analyses are most similar to the results from parsimony analyses using stepmatrix weighting. As noted under Results, the maximum-likelihood topology is most similar to that found under parsimony with combinatorial weights, excluding data from *Capito* third positions, disagreeing only on the relative placement of *Pteroglossus* and *Aulacorhynchus* (though all results from equally weighted parsimony analyses without *Capito* third position data are congruent with the maximum-likelihood result). With these data, it appears that stepmatrix weighting methods appear to yield results most congruent with maximum-likelihood (especially when the effects of compositional heterogeneity can be reduced or eliminated). This is not to suggest that the convergence of results from these two methods necessarily indicates convergence on the true relationships of the taxa involved: both methods could be identically tracking the same misleading signal. However, at the least, this convergence suggests the elimination of method-specific effects on phylogenetic reconstruction as a possible explanation for incongruence with other data sources (e.g., morphology).

The relationships represented in Figs. 6 and 7 are similar to those proposed in several previous analyses of relationship within the Ramphastidae (Prum, 1988; Sibley and Ahlquist, 1990) in suggesting paraphyly of the traditional Capitonidae, as well as in other aspects (e.g., a close relationship between *Capito* and *Eubucco* and between *Megalaima* and *Psilopogon*). On the other

hand, the results presented here differ from previous estimates in a number of ways. In agreement with Prum (1988), our data indicate that not only are Neotropical barbets more closely related to toucans than to Paleotropical barbets but some Neotropical barbets are more closely related to toucans than to other Neotropical barbets. However, Prum's analysis suggested that *Semnornis* was more closely related to the toucans than to *Capito* and *Eubucco*, whereas we have found the reverse of this arrangement. Regarding the placement of *Trachyphonus*, our best estimates of phylogeny (and all individual analyses) support placement of the genus as the sister group to the Neotropical radiation, whereas Prum's (1988) analysis suggested a basal position within the family. Our parsimony framework suggests a close relationship of *Lybius* and (*Megalaima*, *Psilopogon*) to the exclusion of *Pogoniulus*, whereas our maximum-likelihood analyses and previous studies suggest a sister group relationship between *Lybius* and *Pogoniulus*. Much of the conflict between these analyses may be attributable to poor taxon sampling in the molecular analyses. Sibley and Ahlquist's (1990) analysis did not include two of the genera whose placement is most in contention (*Semnornis* and *Trachyphonus*), and the sequence data set suffers from poor sampling of Paleotropical taxa. It is possible that more thorough taxonomic sampling could help resolve some of these conflicts. Potentially, inclusion of morphological data in global analyses could yield more robust estimates of relationship (e.g., Helm-Bychowski and Cracraft, 1993). However, inclusion of Prum's morphological data in a total evidence analysis with the molecular data reported here yielded topologies identical to those obtained with the molecular data alone (result not presented), indicating that more extensive morphological character sampling might be necessary. The significance of apparent conflicts over basal barbet relationships is perhaps mitigated by the observation that statistical topology comparisons failed to detect a significant difference between the cytochrome *b* topologies and those inferred under various constraints consistent with Prum's (1988) proposed relationships (see Results).

Inferred relationships among genera of toucans and toucanets are somewhat more robust than those at higher levels. In all analyses that recovered ramphastine monophyly (the most likely hypotheses on gross morphological grounds), *Ramphastos* was reconstructed as sister to the remaining genera of the subfamily. Within the subfamily, *Andigena* and *Selenidera* are almost always reconstructed as sister taxa (with the lone exception being the analysis eliminating all third position data), generally with high bootstrap support (Fig. 3). The main point of contention among the various analyses is the relative placement of *Aulacorhynchus* and *Pteroglossus* with respect to the (*Andigena*, *Selenidera*) clade. Analyses that downweight all third position changes tend to support *Aulacorhynchus* as more basal, whereas all stepmatrix analyses support the reverse arrangement (Figs. 3 and 4). As might be expected, parsimony analysis of amino acid sequences produces a tree congruent with those from third position downweighting, with two unambiguously optimized amino acid substitutions supporting a (*Pteroglossus*, *Andigena*, *Selenidera*) clade (a Met–Thr substitution at amino acid position 227 of the gene and a Leu–Phe substitution at position 324). In contrast, there are no unambiguously optimized substitutions supporting the alternative arrangement. Finally, we examined the placement of *Baillonius bailloni* by constraining the topology in Fig. 7 (excluding *Baillonius*) and performing a parsimony analysis using equal weights including only data corresponding to the incomplete *Baillonius* sequence. The preferred placement of this sequence as sister to *Pteroglossus castanotis* is indicated in Fig. 7. Of 100 bootstrap replicates, 75 supported this placement. The relationships among ramphastine genera proposed here are completely congruent with the suggestions of Prum and Cracraft (1988) and Sibley and Ahlquist (1990), though each of these analyses proposed only a single node relevant to the current analysis. Our results also agree with Haffer (1974) and Lanyon and Zink (1987) on the basal position of *Ramphastos* within the family (*contra* Swierczewski and Raikow's [1981] suggestion of a [*Pteroglossus*, *Ramphastos*] relationship) but disagree in some aspects of the arrangement of toucanet genera. We find support for Haffer's suggestion of a relationship between *Pteroglossus* and *Baillonius* but none for his association of those genera with *Selenidera*. Our results also contradict Lanyon and Zink's (1987) preferred arrangement of *Andigena* as sister to *Aulacorhynchus* and *Selenidera* with *Pteroglossus*. Finally, Hackett and Lehn (1997) found *Baillonius* as the sister to *Pteroglossus* and not nested within it. Our arrangement of toucan genera is the first published hypothesis of relationships for the group based on an analysis of all genera. A thorough phylogenetic analysis of morphological character variation in the family would provide an interesting test of this result.

The pattern of relationships suggested by our analyses of these data has at least one notable biogeographic implication. Prum's (1988) phylogenetic hypothesis for genera of barbets indicated a sister group relationship between the Neotropical radiation and the barbets currently distributed in southeast Asia. Unfortunately, comparable hypotheses of phylogenetic relationships within other bird groups with pantropical distributions are few. One such, Espinosa de los Monteros' (1998) analysis of trogon relationships based on variation in cytochrome *b*, also suggested a relationship between a monophyletic Neotropical radiation and species currently distributed in southeast Asia. In contrast, all analyses reported here suggest a relationship of Neo-

tropical toucans and barbets with the genus *Trachyphonus*, which is currently limited in distribution to Africa. This contrasting pattern of relationships was also found in a study of parrot relationships (Miyaki *et al.*, 1998), though both density of taxon sampling and sequence lengths examined were insufficient for a robust test of alternative hypotheses. In all of these cases, support for a relationship of the Neotropical radiations with either Asia or Africa was relatively poor. Clearly, more extensive sampling of taxa and sequences will be necessary for establishing the directionality of this relationship in individual cases and for the Neotropical avifauna as a whole.

Effects of base composition heterogeneity on phylogenetic inference. One of the most interesting patterns found in this study is one of the consistent differences between analyses that downweighted all third position changes and those that downweighted only third position transitions: specifically, the placement of the *Ramphastos* sequences relative to the toucanets and (*Capito*, *Eubucco*). Consistently, stepmatrix weighting schemes failed to support monophyly of the toucans (subfamily Ramphastinae), a group distinguished by its derived, specialized bill morphology. On the grounds of parsimony alone, this seems unlikely, as it requires either two separate origins of the toucan bill morphology or its loss in a toucan-billed common ancestor of *Capito* and *Eubucco*. Interestingly, this conflict is resolved when the base composition heterogeneity at third positions in this data set is recognized. When third position data from the *Capito* sequences (which have an unusually high frequency of thymine residues relative to others in the data set) are eliminated, the monophyly of the ramphastines (and therefore a single origin of the toucan bill morphology) is supported. This result is also congruent with maximum-likelihood analyses of the data, though likelihood supports ramphastine monophyly both with and without *Capito* third position data included. This suggests that likelihood may be less sensitive than parsimony to violations of the assumption (explicit or otherwise) of stationary base composition. In general, the result obtained here suggests that identification of sequences with unusual base composition—a potential indicator of nonstationarity—could reveal systematic biases in phylogenetic inference which need to be addressed in analytical treatments of the data.

Utility of cytochrome b *in phylogenetic inference within the Ramphastidae.* Recently, the utility of cytochrome *b* for inferring relationships among divergent taxa has been questioned (Meyer, 1994; Moore and DeFillipis, 1997). It has been argued that the combination of the inherently high rate of mutation of mitochondrial DNA (Brown *et al.*, 1979), the strong degree of constraint which results in relatively low levels of observed amino acid divergence (Desjardins and Morais, 1990), and the strongly skewed base composition characteristic of the mitochondrial genome rapidly causes degradation of hierarchical signal in cytochrome *b*. Several authors have suggested guidelines for determining whether or not cytochrome *b* is likely to be useful in a given situation. Meyer (1994) suggested a window starting around 15–20% overall divergence in which reconstructions using cytochrome *b* are unlikely to be informative. The vast majority of comparisons in our analysis of ramphastid relationships fall within this range (Fig. 2). Moore and DeFilippis (1997) suggested that cytochrome *b* should be useful for taxa that diverge at a DNA–DNA hybridization dissociation metric $\Delta T_{50}H$ less than ~12.5. Examination of Sibley and Ahlquist's (1990) results indicates that basal ramphastid divergences are at $\Delta T_{50}H = 12.8$, again right at the limit of the gene's proposed area of maximal usefulness. As a more general rule applicable to all sequence data, Albert *et al.* (1993) suggested measuring the value of λ (the number of changes per character per branch on a phylogenetic tree), with values >0.1 indicating potentially serious degradation of phylogenetic signal. Calculation of λ for the cytochrome *b* gene on the tree derived from parsimony analysis using equal weights yields a value of 0.036. However, if one excludes first and second positions, this number rises to 0.087, very near Albert *et al.*'s (1993) proposed limit of potential usefulness. By all these measures, cytochrome *b* appears to be at the limit of its utility for inferring basal relationships among the Ramphastidae. Comparisons among genera of Ramphastinae are much less divergent, and the gene should, in theory, be providing reliable signal in this region of the topology. Even at higher levels, however, comparison of cytochrome *b* sequence data with the only other molecular data with comparable taxon sampling (Sibley and Ahlquist, 1990) suggests that phylogenetic signal within the gene is not entirely degraded. Specifically, transversion distances from sequence comparisons are strongly correlated with $\Delta T_{50}H$ (regression line for proportion of transversion differences as a function of $\Delta T_{50}H$: $p_{TV} = 0.005 \cdot \Delta T_{50}H + 0.023$; $r^2 = 0.95$, $n = 7$). It may be that choice of appropriate analytical techniques could expand the range of sequence divergence over which phylogeny can be inferred using cytochrome *b*, but some explicit, well-justified criteria need to be generated for choosing among techniques (e.g., among weighting schemes within parsimony). Congruence among data sets is one possible avenue for testing the utility of analytical techniques (Miyamoto *et al.*, 1994; Hillis, 1995; Cunningham, 1997). To address these questions, future studies of relationships among barbet genera should examine the variation in other genetic loci, as well as expanding sampling of cytochrome *b* from other paleotropical species and genera.

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REFERENCES

Albert, V. A., and Mishler, B. D. (1992). On the rationale and utility of weighting nucleotide sequence data. *Cladistics* **8:** 73–83.

Albert, V. A., Chase, M. W., and Mishler, B. D. (1993). Character-state weighting for cladistic analysis of protein-coding DNA sequences. *Ann. Missouri Bot. Gard.* **80:** 752–766.

Aquadro, C. F., Kaplan, N., and Risko, K. J. (1984). An analysis of the dynamics of mammalian mitochondrial DNA sequence evolution. *Mol. Biol. Evol.* **1:** 423–434.

Bremer, K. (1990). Combinable component consensus. *Cladistics* **6:** 369–372.

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

Brown, W. M., George, M., Jr., and Wilson, A. C. (1979). Rapid evolution of animal mitochondrial DNA. *Proc. Natl. Acad. Sci. USA* **76:** 1967–1971.

Brown, W. M., Prager, E. M., Wang, A., and Wilson, A. C. (1982). Mitochondrial DNA sequences of primates: Tempo and mode of evolution. *J. Mol. Evol.* **18:** 225–239.

Bull, J. J., Huelsenbeck, J. P., Cunningham, C. W., Swofford, D. L., and Waddell, P. J. (1993). Partitioning and combining data in phylogenetic analysis. *Syst. Biol.* **42:** 384–397.

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.

Collins, T. M., Kraus, F., and Estabrook, G. (1994). Compositional effects and weighting of nucleotide sequences for phylogenetic analysis. *Syst. Biol.* **43:** 449–459.

Collins, T. M., Wimberger, P. H., and Naylor, G. J. P. (1994). Compositional bias, character-state bias, and character-state reconstruction using parsimony. *Syst. Biol.* **43:** 482–496.

Cracraft, J. (1973a). Continental drift, paleoclimatology, and the evolution and biogeography of birds. *J. Zool.* **169:** 455–545.

Cracraft, J. (1973b). Vertebrate evolution and biogeography in the Old World Tropics: Implications of continental drift and palaeoclimatology. *In* "Implications of Continental Drift to the Earth Sciences" (D. H. Tarling and S. K. Runcorn, Eds.), Vol. 1, pp. 373–393. Academic Press, New York.

Cracraft, J., and Prum, R. O. (1988). Patterns and processes of diversification: Speciation and historical congruence in some neotropical birds. *Evolution* **42:** 603–620.

Cunningham, C. W. (1997). Is congruence between data partitions a reliable predictor of phylogenetic accuracy? Empirically testing an iterative procedure for choosing among phylogenetic methods. *Syst. Biol.* **46:** 464–478.

Desjardins, P., and Morais, R. (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., Arctander, P., and Wilson, A. C. (1991). Mitochondrial resolution of a deep branch in the genealogical tree for perching birds. *Proc. R. Soc. London B* **243:** 99–107.

Espinosa de los Monteros, A. (1998). Phylogenetic relationships among the trogons. *Auk* **115:** 937–954.

Eyre-Walker, A. (1998). Problems with parsimony in sequences of biased base composition. *J. Mol. Evol.* **47:** 686–690.

Faith, D. P. (1991). Cladistic permutation tests for monophyly and nonmonophyly. *Syst. Zool.* **40:** 366–375.

Farris, J. S. (1969). A successive approximations approach to character weighting. *Syst. Zool.* **18:** 374–385.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* **17:** 368–376.

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

Frati, F., Simon, C., Sullivan, J., and Swofford, D. L. (1997). Evolution of the mitochondrial cytochrome oxidase II gene in Collembola. *J. Mol. Evol.* **44:** 145–158.

Friesen, V. L., Baker, A. J., and Piatt, J. F. (1996). Phylogenetic relationships within the Alcidae (Charadriiformes: Aves) inferred from total molecular evidence. *Mol. Biol. Evol.* **13:** 359–367.

Galtier, N., and Gouy, M. (1998). Inferring pattern and process: Maximum-likelihood implementation of a nonhomogeneous model of DNA sequence evolution for phylogenetic analysis. *Mol. Biol. Evol.* **15:** 871–879.

Griffiths, C. S. (1997). Correlation of functional domains and rates of nucleotide substitution in cytochrome *b*. *Mol. Phylogenet. Evol.* **7:** 352–365.

Grimes, L. G. (1976). Co-operative breeding in African birds. *In* "Proceedings of the 16th International Ornithological Congress" (H. J. Frith and J. H. Calaby, Eds.), pp. 667–673. Australian Academy of Sciences, Canberra.

Gu, X., Fu, Y., and Li, W.-H. (1995). Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. *Mol. Biol. Evol.* **12:** 546–557.

Hackett, S. J. (1996). Molecular phylogenetics and biogeography of tanagers in the genus Ramphocelus (Aves). *Mol. Phylogenet. Evol.* **5:** 368–382.

Hackett, S. J., and Lehn, C. A. (1997). Lack of genetic divergence in a genus (*Pteroglossus*) of Neotropical birds: The connection between life-history characteristics and levels of genetic divergence. *Ornithol. Monogr.* **48:** 267–279.

Haffer, J. (1974). Avian speciation in South America. *Publ. Nuttall Ornithol. Club* **14.**

Harshman, J. (1994). Reweaving the tapestry: What can we learn from Sibley and Ahlquist (1990)? *Auk* **111:** 377–388.

Harshman, J. (1996). "Phylogeny, Evolutionary Rates, and Ducks," Unpublished Ph.D. dissertation, Univ. of Chicago.

Hasegawa, M., Kishino, M., and Yano, T. (1985). Dating the human–ape split by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **22:** 160–174.

Helm-Bychowski, K., and Cracraft, J. (1993). Recovering phylogenetic signal from DNA sequences: Relationships within the corvine assemblage (Class Aves) as inferred from complete sequences of the mitochondrial DNA cytochrome-*b* gene. *Mol. Biol. Evol.* **10:** 1196–1214.

Hillis, D. M. (1995). Approaches for assessing phylogenetic accuracy. *Syst. Biol.* **44:** 3–16.

Hillis, D. M., and Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* **42:** 182–192.

Hillis, D. M., Huelsenbeck, J. P., and Cunningham, C. W. (1994). Application and accuracy of molecular phylogenies. *Science* **264:** 671–677.

Huelsenbeck, J. P. (1995). The robustness of two phylogenetic meth-

ods: Four-taxon simulations reveal a slight superiority of maximum likelihood over neighbor joining. *Mol. Biol. Evol.* **12:** 843–849.

Huelsenbeck, J. P., and Hillis, D. M. (1993). Success of phylogenetic methods in the four-taxon case. *Syst. Biol.* **42:** 247–264.

Huelsenbeck, J. P., and Rannala, B. (1997). Phylogenetic methods come of age: Testing hypotheses in an evolutionary context. *Science* **276:** 227–232.

Irwin, D. M., Kocher, T. D., and Wilson, A. C. (1991). Evolution of the cytochrome *b* gene of mammals. *J. Mol. Evol.* **32:** 128–144.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16:** 111–120.

Kishino, H., and Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Mol. Evol.* **29:** 170–179.

Knight, A., and Mindell, D. P. (1993). Substitution bias, weighting of DNA sequence evolution, and the phylogenetic position of Fea's viper. *Syst. Biol.* **42:** 18–31.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Paabo, S., Villablanca, F. X., and Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* **86:** 6196–6200.

Kornegay, J. R., Kocher, T. D., Williams, L. A., and Wilson, A. C. (1993). Pathways of lysozyme evolution inferred from the sequences of cytochrome *b* in birds. *J. Mol. Evol.* **37:** 367–379.

Kuhner, M. K., and Felsenstein, J. (1994). A simulation comparison of phylogeny algorithms under equal and unequal evolutionary rates. *Mol. Biol. Evol.* **11:** 459–468.

Lanyon, S. M. (1993). Phylogenetic frameworks: Towards a firmer foundation for the comparative approach. *Biol. J. Linn. Soc.* **49:** 45–61.

Lanyon, S. M., and Hall, J. G. (1994). Reexamination of barbet monophyly using mitochondrial-DNA sequence data. *Auk* **111:** 389–397.

Lanyon, S. M., and Zink, R. M. (1987). Genetic variation in Piciform birds: Monophyly and generic and familial relationships. *Auk* **104:** 724–732.

Lockhart, P. J., Steel, M. A., Hendy, M. D., and Penny, D. (1994). Recovering evolutionary trees under a more realistic model of sequence evolution. *Mol. Biol. Evol.* **12:** 605–612.

Maddison, W. P., and Maddison, D. R. (1993). "MacClade: Analysis of phylogeny and character evolution, Version 3.05," Sinauer, Sunderland, MA.

Meyer, A. (1994). Shortcomings of the cytochrome *b* gene as a molecular marker. *Trends Ecol. Evol.* **9:** 278–280.

Milinkovitch, M. C., LeDuc, R. G., Adachi, J., Farnir, F., Georges, M., and Hasegawa, M. (1996). Effects of character weighting and species sampling on phylogeny reconstruction: A case study based on DNA sequence data in Cetaceans. *Genetics* **144:** 1817–1833.

Miyaki, C. Y., Matioli, S. R., Burke, T., and Wajntal, A. (1998). Parrot evolution and paleogeographical events: Mitochondrial DNA evidence. *Mol. Biol. Evol.* **15:** 544–551.

Miyamoto, M. M., Allard, M. W., Adkins, R. M., Janacek, L. L., and Honeycutt, R. L. (1994). A congruence test of reliability using linked mitochondrial DNA sequences. *Syst. Biol.* **43:** 236–249.

Moore, W. S., and DeFilippis, D. R. (1997). The window of taxonomic resolution for phylogenies based on mitochondrial cytochrome *b*. *In* "Avian Molecular Evolution and Systematics" (D. P. Mindell, Ed.), pp. 83–119. Academic Press, San Diego.

Neff, N. A. (1986). A rational basis for *a priori* character weighting. *Syst. Zool.* **35:** 110–123.

Nunn, G. B., and Cracraft, J. (1996). Phylogenetic relationships among the major lineages of the birds-of-paradise (Paradisaeidae) using mitochondrial DNA gene sequences. *Mol. Phylogenet. Evol.* **5:** 445–459.

Peters, J. L. (1948). "Check-List of Birds of the World," Vol. VI, Harvard Univ. Press, Cambridge, MA.

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.

Reeder, T. W. (1995). Phylogenetic relationships among Phrynosomatid lizards as inferred from mitochondrial ribosomal DNA sequences: Substitutional bias and information content of transitions relative to transversions. *Mol. Phylogenet. Evol.* **4:** 203–222.

Restrepo, C., and Mondragón, M. L. (1998). Cooperative breeding in the frugivorous toucan barbet (*Semnornis ramphastinus*). *Auk* **115:** 4–15.

Rodrigo, A. G. (1992). A modification to Wheeler's combinatorial weights calculations. *Cladistics* **8:** 165–170.

Saitou, N., and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4:** 406–425.

Sanderson, M. (1989). Confidence limits on phylogenies: The bootstrap revisited. *Cladistics* **5:** 113–130.

Schöniger, M., and von Haeseler, A. (1995). Performance of the maximum likelihood, neighbor joining, and maximum parsimony methods when sequence sites are not independent. *Syst. Biol.* **44:** 533–547.

Sibley, C. G., and Ahlquist, J. E. (1990). "Phylogeny and Classification of Birds," Yale Univ. Press, New Haven, CT.

Simpson, S. F., and Cracraft, J. (1981). The phylogenetic relationships of the Piciformes (Class Aves). *Auk* **98:** 481–494.

Skutch, A. F. (1958). Roosting and nesting of araçari toucans. *Condor* **60:** 201–219.

Smith, A. B. (1994). Rooting molecular trees: Problems and strategies. *Biol. J. Linn. Soc.* **51:** 279–292.

Swierczewski, E. V., and Raikow, R. J. (1981). Hind limb morphology, phylogeny, and classification of the Piciformes. *Auk* **98:** 466–480.

Templeton, A. R. (1983). Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and apes. *Evolution* **37:** 221–244.

Vuilleumier, F., and Andors, A. V. (1993). Avian biological relationships between Africa and South America. *In* "Biological Relationships between Africa and South America" (P. Goldblatt, Ed.), pp. 289–328. Yale Univ. Press, New Haven, CT.

Wheeler, Q. D. (1986). Character weighting and cladistic analysis. *Syst. Zool.* **35:** 102–109.

Wheeler, W. C. (1990). Combinatorial weights in phylogenetic analysis: A statistical parsimony procedure. *Cladistics* **6:** 269–275.

Williams, P. L., and Fitch, W. M. (1989). Finding the minimal change in a given tree. *In* "The Hierarchy of Life" (B. Fernholm *et al.*, Eds.), pp. 453–470. Elsevier Press, Amsterdam.

Yang, Z. (1994). Estimating the pattern of nucleotide substitution. *J. Mol. Evol.* **39:** 105–111.

Yang, Z., and Roberts, D. (1995). On the use of nucleic acid sequences to infer early branchings in the tree of life. *Mol. Biol. Evol.* **12:** 451–458.