More Taxa, More Characters: The Hoatzin Problem Is Still Unresolved

Michael D. Sorenson,† Elen Oneal,*[1] Jaime García-Moreno,†[2] and David P. Mindell†‡*

*Department of Biology, Boston University; and †Museum of Zoology and ‡Department of Ecology and Evolutionary Biology, University of Michigan

The apparently rapid and ancient diversification of many avian orders complicates the resolution of their relationships using molecular data. Recent studies based on complete mitochondrial DNA (mtDNA) sequences or shorter lengths of nuclear sequence have helped corroborate the basic structure of the avian tree (e.g., a basal split between Paleognathae and Neognathae) but have made relatively little progress in resolving relationships among the many orders within Neoaves. We explored the potential of a moderately sized mtDNA data set (~5,000 bp for each of 41 taxa), supplemented with data from a nuclear intron (~700 bp per taxon), to resolve relationships among avian orders. Our sampling of taxa addresses two issues: (1) the sister relationship and monophyly, respectively, of Anseriformes and Galliformes and (2) relationships of the enigmatic hoatzin *Opisthocomus hoazin*. Our analyses support a basal split between Galloanserae and Neoaves within Neognathae and monophyly of both Galliformes and Anseriformes. Within Galliformes, megapodes and then cracids branch basally. Within Anseriformes, mitochondrial data support a screamer (Anhimidae) plus magpie goose (Anseranatidae) clade. This result, however, may be an artifact of divergent base composition in one of the two anatids we sampled. With deletion of the latter taxon, *Anseranas* is sister to anatids as in traditional arrangements and recent morphological studies. Although our data provide limited resolution of relationships within Neoaves, we find no support for a sister relationship between either cuckoos (Cuculiformes) or turacos (Musophagiformes) and hoatzin. Both mitochondrial and nuclear data are consistent with a relationship between hoatzin and doves (Columbiformes), although this result is weakly supported. We also show that mtDNA sequences reported in another recent study included pervasive errors that biased the analysis towards finding a sister relationship between hoatzin and turacos.

Introduction

Recent studies based on DNA sequence data contribute to an emerging consensus that the basal divergence among extant birds is between paleognaths (ratites and tinamous) and neognaths (all other extant birds) (Stapel et al. 1984; Groth and Barrowclough 1999; García-Moreno and Mindell 2000; van Tuinen, Sibley, and Hedges 2000; Braun and Kimball 2002; Edwards et al. 2002; Paton, Haddrath, and Baker 2002; García-Moreno, Sorenson, and Mindell 2003), as has become the conventional view based on morphology (Cracraft 1986, 2001; Cracraft and Clarke 2001; Livezey and Zusi 2001 [but see Woodbury 1998]). With this fundamental aspect of the avian tree now well resolved, molecular systematists are beginning to address the more difficult problem of sorting out the many neognath orders that apparently evolved in a relatively rapid radiation, whether before or after the K-T boundary (Feduccia 1995; Cooper and Penny 1997; Benton 1999; Waddell et al. 1999; Cracraft 2001; Dyke 2001; Paton, Haddrath, and Baker 2002). Recent efforts in this direction are the studies of Hughes and Baker (1999), Espinosa de los Monteros (2000), van Tuinen, Sibley, and Hedges (2000), van Tuinen et al. (2001), and Johansson et al. (2001). We focus here on two specific questions, providing a test case in which to explore the potential of a moderately sized mtDNA data set and somewhat larger sample of taxa to test hypotheses about avian ordinal relationships.

First, we address the sister relationship and monophyly, respectively, of Anseriformes and Galliformes. Various molecular and morphological analyses agree on the monophyly of Galloanserae (Cracraft 1981; Sibley and Ahlquist 1990; Caspers et al. 1997; Livezey 1997*a*; Mindell et al. 1997, 1999; Groth and Barrowclough 1999; García-Moreno and Mindell 2000; van Tuinen, Sibley, and Hedges 2000; Livezey and Zusi 2001; Braun and Kimball 2002; Paton, Haddrath, and Baker 2002), but other morphological studies (Ericson 1996, 1997) and a limited analysis of the nuclear c-*myc* gene (Ericson, Parsons, and Johansson 2001) failed to support this grouping. The basal position of Galloanserae within Neognathae also has been questioned on the basis of spinal cord morphology (Woodbury 1998). Olson and Feduccia (1980) argued that the fossil taxon *Presbyornis* provides evidence that waterfowl (Anseriformes) and flamingos (Phoenicopteriformes) were derived from ancestral shorebirds (Charadriiformes). This suggestion is inconsistent with DNA-DNA hybridization data (Sibley and Ahlquist 1990) and was explicitly tested and rejected in recent morphological analyses (Ericson 1997; Livezey 1997*a*), but DNA sequencing studies have not included all of the major lineages within Galloanserae or all of the other avian taxa (e.g., flamingos, charadriiforms) needed to fully test this hypothesis. Our interest in this question was stimulated also by preliminary analyses of 12S rRNA gene sequences in which screamers (Anhimidae) and magpie goose (Anseranatidae), which have long been recognized as anseriforms (Livezey 1997*a*, 1997*b*), grouped with cracids, either within the galliform clade or as a sister group to galliforms. Other recent DNA sequencing studies did not include both magpie goose and screamers (Sraml et al. 1996; Groth and Barrowclough 1999) or had too few data to resolve their relationships (Ericson, Parsons, and Johansson 2001).

[1] Present address: Department of Ecology and Evolutionary Biology, University of Michigan.

[2] Present address: Max Planck Research Centre for Ornithology, Vogelwarte Radolfzell, Radolfzell, Germany.

Key words: Anseriformes, avian systematics, Galliformes, Galloanserae, Neoaves, *Opisthocomus hoazin*.

E-mail: msoren@bu.edu.

Mol. Biol. Evol. 20(9):1484–1499. 2003
DOI: 10.1093/molbev/msg157
Molecular Biology and Evolution, Vol. 20, No. 9,

Table 1
List of Taxa Included in Analyses

Order	Family	Species
Struthioniformes	Struthionidae	*Struthio camelus*
	Rheidae	*Rhea americana*
Tinamiformes	Tinamidae	*Eudromia elegans*
Galliformes	Cracidae	*Crax rubra*
	Megapodiidae	*Alectura lathami*
	Megapodiidae	*Megapodius eremita*
	Phasianidae	*Gallus gallus*
	Numididae	*Acryllium vulturinum*
Anseriformes	Anhimidae	*Chauna torquata*
	Anseranatidae	*Anseranas semipalmata*
	Dendrocygnidae	*Dendrocygna arcuata*
	Anatidae	*Aythya americana*
Bucerotiformes	Bucerotidae	*Tockus erythrorhynchus*
Trogoniformes	Trogonidae	*Trogon curucui*
Coraciiformes	Coraciidae	*Coracias spatulata*
Coliiformes	Coliidae	*Colius striatus*
	Coliidae	*Urocolius macrourus*
Cuculiformes	Cuculidae	*Cuculus canorus*
	Centropodidae	*Centropus cupreicaudus*
	Coccyzidae	*Coccyzus erythropthalmus*
	Opisthocomidae	*Opisthocomus hoazin*
	Crotophagidae	*Crotophaga ani*
	Neomorphidae	*Neomorphus geoffroyi*
Psittaciformes	Psittacidae	*Neophema elegans*
	Psittacidae	*Nandayus nenday*
Musophagiformes	Musophagidae	*Musophaga violacea*
	Musophagidae	*Crinifer piscator*
Strigiformes	Strigidae	*Asio otus*
Columbiformes	Columbidae	*Columba leucocephala*
	Columbidae	*Treron sieboldii*
Ciconiiformes	Scolopacidae	*Scolopax minor*
	Burhinidae	*Burhinus senegalensis*
	Accipitridae	*Buteo jamaicensis*
	Falconidae	*Falco peregrinus*
	Phoenicopteridae	*Phoenicopterus ruber*
	Ciconiidae	*Mycteria americana*
	Ciconiidae	*Ciconia ciconia*
Passeriformes	Eurylaimidae	*Smithornis sharpei*
	Tyrannidae	*Sayornis phoebe*
	Corvidae	*Corvus frugilensis*
	Passeridae	*Vidua chalybeata*

NOTE.—Taxa are arranged in orders and families following Sibley and Ahlquist (1990). Details on genetic samples, voucher specimens, and GenBank accession numbers are available on the journal's Web site as online Supplementary Material.

Second, we address the relationships of the hoatzin (*Opisthocomus hoazin*). This unique South American bird has puzzled avian systematists since it was described over 200 years ago (see Sibley and Ahlquist [1990] for a historical review of its classification). Most often, the hoatzin has been placed with Galliformes (e.g., Peters 1934; Cracraft 1981), Cuculiformes (e.g., de Quieroz and Good 1988; Hedges et al. 1995; Hughes 1996) or Musophagiformes (e.g., Verheyen 1956; Hughes and Baker 1999; Hughes 2000). Sibley and Ahlquist (1973, 1990) concluded that the hoatzin is a derived cuckoo and nested it within Cuculiformes as the sister lineage of a clade comprising the New World ground cuckoos (Neomorphidae), guira cuckoo, and anis (Crotophagidae). A similar result was obtained by Hughes (1996) using behavioral and ecological characters, although a later analysis placed hoatzin with turacos (Hughes 2000).

Avise, Nelson, and Sibley (1994*a*), Hedges et al. (1995), and Mindell et al. (1997) analyzed DNA sequence data for the hoatzin but with limited sampling of taxa and/or characters and no clear resolution of its relationships. Johnson, Goodman, and Lanyon (2000) ruled out a placement of hoatzin within cuckoos but sampled too few outgroups to say more. In contrast, Hughes and Baker (1999) found strong support for a sister relationship between hoatzin and turacos (Musophagiformes) in a combined analysis of mitochondrial and nuclear DNA sequences and declared the hoatzin problem ''resolved.'' Hughes (2000) obtained the same result in an osteological analysis in which she identified 19 characters uniting hoatzin and turacos. With greater taxon sampling, our analysis provides a broader test of hoatzin relationships than in other recent studies. We also provide evidence that the sequences reported by Hughes and Baker (1999) contain numerous errors that biased their analysis towards finding a sister relationship between hoatzin and turacos (see *Results*).

Finally, we comment on some general issues in avian ordinal systematics and include tests of the monophyly of several avian orders, including Anseriformes, Galliformes, a broadly conceived Ciconiiformes (Sibley and Ahlquist 1990), and an inclusive Cuculiformes, comprising cuckoos, turacos, and hoatzin (de Quieroz and Good 1988; Hughes 2000).

Methods

Taxa and DNA Sequencing

Taxa included in our analyses are listed in table 1. We include three paleognaths and root our trees between paleognaths and neognaths because this sister relationship is corroborated by a variety of molecular and morphological evidence (Cracraft 1986; Groth and Barrowclough 1999; García-Moreno and Mindell 2000; van Tuinen, Sibley, and Hedges 2000; Cracraft and Clarke 2001; Livezey and Zusi 2001; Braun and Kimball 2002; Edwards et al. 2002; Paton, Haddrath, and Baker 2002). Alternative rootings of the avian tree obtained in recent mtDNA studies (Härlid, Janke, and Arnason 1998; Härlid and Arnason 1999; Mindell et al. 1999; Haring et al. 2001; Johnson 2001) deserve further attention (see Braun and Kimball 2002; García-Moreno, Sorenson, and Mindell 2003), but this issue is beyond the scope of the present analysis. We include representatives of most major lineages in Galloanserae to test the monophyly of Anseriformes and Galliformes, respectively. A flamingo (*Phoenicopterus ruber*) and two ''charadriiforms'' (*Scolopax minor* and *Burhinus senegalensis*), as well as other taxa included in Ciconiiformes by Sibley and Ahlquist (1990), were sampled to test hypotheses that associate these taxa with waterfowl (e.g., Olson and Feduccia 1980; Hagey et al. 1990; Ericson, Parsons, and Johansson 2001). To test possible placements of hoatzin, we sampled two musophagids, all five cuculiform families recognized by Sibley and Ahlquist (1990), and a number of other avian orders, including those close to Cuculiformes and Musophagiformes in Sibley and Ahlquist's classification

(e.g., Coliiformes, Psittaciformes, and Strigiformes). We include two or more representatives for most of the orders we sampled to reduce branch lengths leading to terminal taxa. Sequences for nine of our 41 taxa were published previously (see online Supplementary Material).

DNA was isolated from 25 mg of muscle tissue using a QIAmp Tissue Kit (Qiagen). DNA for the two musophagids was obtained from the calamus of a single primary feather. For these two samples, we added 30 ml of 100 mg/ml dithiothreitol to the tissue digestion buffer (Cooper 1994). We sequenced a little over 5,000 bases of mtDNA per taxon in three separate regions. The small subunit (12S) ribosomal RNA gene and portions of the flanking tRNAs were amplified and sequenced in two overlapping fragments with primers L1263, H1859, L1754, and H2294. A contiguous region including a small portion of the large subunit (16S) rRNA gene, complete sequences of NADH dehydrogenase subunits 1 and 2 (ND1 and ND2), the first 36 bases of cytochrome oxidase subunit I (COI), and nine tRNA genes was amplified and sequenced with primers L3827, L4500, L5143, L5216, L5758, L6335, H4644, H5191, H5766, H6313, and H6681. Finally, a small portion of ND5, all of cytochrome *b*, and a portion of tRNA-threonine were amplified and sequenced with primers L14770, L14996, L15413, H15295, H15646, and H16064. Primer names refer to the strand and position of the 3′ base in the mtDNA sequence of *Gallus gallus* (Desjardins and Morais 1990). Most primers are revised versions of those described by Sorenson et al. (1999). Primer sequences are available online (http://people.bu.edu/msoren/primers.html) or by request from the first author.

Amplification and sequencing protocols were as in previous studies (Sorenson et al. 1999). We sequenced both DNA strands and reconciled forward and reverse sequences in Sequence Navigator (Applied Biosystems). Requisite precautions were taken to avoid the amplification of nuclear pseudogenes of mitochondrial origin (see Arctander 1995; Sorenson and Fleischer 1996; Zhang and Hewitt 1996), including the use of (1) muscle tissue rather than blood and (2) PCR primers with degenerate sites to prevent the preferential amplification of low copy number nuclear sequences (Sorenson and Quinn 1998). An apparent nuclear copy of the second half of ND2 in *Centropus cupreicaudus* did not match overlapping sequences for this taxon and contained an unusual 3-bp deletion. We obtained the mtDNA sequence for this taxon by amplifying a 2.8-kb fragment with primers L3827 and H6681 and then using this product as the template for amplification and sequencing of ND2.

Discrepancies between our sequences and those reported by Hughes and Baker (1999 [hereafter HB1999]) led us to replicate our results for hoatzin using two additional samples. We sequenced three different hoatzin samples for the mtDNA regions included in our analysis plus the regions sequenced by HB1999. The three tissues we used included the two used by HB1999 (LSUMNS B-10753 and B-10754). Additional primers used for this work were L6615, L7036, L7525, L7987, L8386, L8929, L9700, L10236, H7122, H7548, H8121, H8628, H9235, H9726, H10343, and H10884.

We also sequenced intron 9 of the nuclear phosphoenolpyruvate carboxykinase (PEPCK) gene (Cook et al. 1986; S. E. Stanley, personal communication). We used a nested PCR approach in which initial amplification using primers GTP1601F (5′-ACGAGGCCTTTAACTGGCAGCA-3′) and GTP1793R (5′-CTTGGCTGTCTTTCCGGAACC-3′) (S.E. Stanley, personal communication) provided the template for a second amplification using primers PEPCK9F (5′-GGAGCAGCCATGAGATCTGAAGC-3′) and PEPCK 9R (5′-GTGCCATGCTAAGCCAGTGGG-3′). Products were sequenced with the latter two primers. We determined PEPCK9 sequences for all taxa except *Corvus*, *Ciconia*, and *Rhea*. PEPCK9 sequences for most taxa comprised 658 to 700 nucleotides (including small portions of the flanking exons), except for *Neomorphus*, which was 1,014 nucleotides, due to a 333-bp insertion.

Details on genetic samples, voucher specimens, and GenBank accession numbers are available on the journal's Web site as online Supplementary Material.

Phylogenetic Analyses

Phylogenetic analyses were conducted in PAUP* versions 4.0b4-b10 (Swofford 2002) and POY (Gladstein and Wheeler 1996). Most of our analyses are based on a data set of 4,789 mtDNA characters that includes 30 positions with gap characters in one or more taxa but excludes regions of ambiguous alignment. Gaps were treated as a fifth character state in parsimony analyses. The excluded ''gap regions'' comprised approximately 350 nucleotides and 150 gap characters per taxon. We determined bootstrap values (Felsenstein 1985) using full heuristic searches and 500 randomly resampled data sets and Bremer support indices (Bremer 1988) using the program TreeRot (Sorenson 1999).

The failure of our study to corroborate the results of HB1999 led us to evaluate whether certain classes of DNA sequence characters or different kinds of character change provided conflicting evidence with respect to a number of specific phylogenetic hypotheses. In particular, we were interested in whether a high degree of homoplasy or convergent evolution in one class of characters (e.g., third codon positions in protein-coding genes) might obscure the phylogenetic signal in a more conserved class of characters. For the full data set and for each of four character partitions, we completed a series of parsimony analyses with increasingly severe down-weighting of transitions. Three of the character partitions were first, second, and third codon positions, respectively, whereas the fourth partition combined well-aligned portions of rRNA and tRNA genes. Although the different protein-coding genes in our analysis have somewhat different rates and modes of molecular evolution, we combined codon positions across genes, reasoning that functional constraints on sequence evolution are more similar within codon positions across genes than across codon positions within genes, at least for a set of mitochondrial genes.

After finding the most parsimonious (MP) tree for each character partition and weighting scheme, we evaluated the strength of evidence for or against specific phylogenetic

hypotheses in the following manner. If the clade of interest was present in the MP tree, we determined its Bremer support index (i.e., the number of extra steps required in the shortest tree without that clade [Bremer 1988]). If the specified clade was not present in the MP tree, we used a constrained parsimony search to determine the number of extra steps in the shortest tree that included the clade. This latter value has been defined as the local incongruence length difference (LILD) in the slightly different context of evaluating conflict among data partitions (Thornton and DeSalle 2000 [see also figure 6 in Johnson and Sorenson 1998]). As applied here, different data partitions in the current analysis may agree with each other, but all conflict with a previous hypothesis. Nonetheless, it is straightforward to determine for each data partition the number of extra steps required to obtain the previous hypothesis and we refer to this number as the LILD. As noted by Thornton and DeSalle (2000), Bremer support and LILD are similar and complimentary measures. A hypothesized clade has a positive Bremer value (and an LILD of 0) if it is present in the MP tree(s), whereas it has a positive LILD (and a Bremer of 0) if it is not present in the MP tree(s). Thus Bremer support and LILD, respectively, measure the strength of evidence for or against a particular clade on the same relative scale. We determined both Bremer and LILD values in the context of separate analyses for each data partition, rather than in a combined or simultaneous analysis. The latter approach is used in calculating partitioned Bremer support (Baker and DeSalle 1997). For each combination of character partition, weighting scheme, and search constraint, we completed 200 replicate heuristic searches with random addition of taxa and tree-bisection-reconnection (TBR) branch-swapping.

We completed two additional analyses to evaluate the extent to which inferred relationships varied among analyses using different approaches to tree reconstruction. First, we used optimization alignment (Wheeler 1996), as implemented in the program POY (Gladstein and Wheeler 1996), to analyze the full mtDNA data set, including regions with gaps. Although generally excluded from phylogenetic analyses, sequence regions that vary in length among taxa may contain valuable phylogenetic information (Giribet and Wheeler 1999; Simmons, Ochoterena, and Carr 2001; Sorenson and Payne 2001). Optimization alignment combines tree search and sequence alignment into a single process to find the most parsimonious combination of alignment and tree topology. We completed 100 replicate heuristic searches with random addition of taxa for both an equal weights parsimony analysis (gap cost = 1) and an analysis with transitions down-weighted by 50% (gap cost = transversion cost = 2, transition cost = 1). Other parameter settings in POY included −noquick, −slop=5, −checkslop=10, and −maxtrees = 5. Tree scores output by POY may slightly overestimate the actual tree length (see Sorenson and Payne 2001), so we examined all search replicates yielding trees within 10 steps of the best score for each weighting scheme.

Second, we conducted maximum-likelihood (ML) analyses in PAUP* based on well-aligned portions of the data set only and excluding 30 additional positions with a gap character in one or more taxa. We used Modeltest (Posada and Crandall 1998) to select a model of sequence evolution. The general time reversible (GTR) model, with unequal nucleotide frequencies, a proportion of invariant sites (I), and Γ-distributed rate variation among sites (the most highly parameterized model available in PAUP*) provided the best fit to the data. Parameter estimates for this model were obtained for the MP tree with 50% down-weighting of transitions, and these values were then fixed for subsequent tree searches, thereby reducing computation time. ML parameter estimates were as follows. Base frequencies: A = 0.3803, C = 0.3858, G = 0.068, and T = 0.1659. Relative transformation rates: A-C = 0.2212, A-G = 5.7677, A-T = 0.4893, C-G = 0.2091, C-T = 5.0518, G-T = 1.0000. Proportion of invariant sites: I = 0.3575. Shape parameter for the Γ distribution: $\alpha = 0.3970$. Forty heuristic searches with random addition of taxa were completed for the unconstrained ML analysis and 30 replicates were completed for each of several analyses constrained to find specified clades. The approximately unbiased test of Shimodaira (2002) as implemented in CONSEL (Shimodaira and Hasegawa 2001) was used to test for significant differences in likelihood between constrained and unconstrained trees.

Finally, we completed a separate parsimony analysis of the PEPCK9 sequences and a combined analysis of the PEPCK9 and mtDNA data. The PEPCK9 alignment was generally unambiguous but included numerous insertions and deletions, many of which involved multiple bases. Because contiguous gap characters may not represent independent evolutionary events (e.g., Simmons and Ochoterena 2000), we treated gap characters in the PEPCK alignment as missing data and added to the data matrix a new character for each unique indel, regardless of length. In cases where one indel was a subset of a larger indel in another taxon, we coded them as alternative character states in a single multistate character. To simplify this process, we excluded sequences of *Struthio* and *Eudromia*, which were quite divergent from other birds and more difficult to align. The alignment of two small regions (comprising from 7 to 10 and 6 to 11 nucleotides per taxon, respectively) was ambiguous, so we recoded each of these regions as a single multistate character to which we applied a step matrix specifying the number of steps required to change from one character state (i.e., unique sequence) to another (see Lutzoni et al. 2000). The PEPCK data set included 743 aligned nucleotide positions (excluding a 333-bp insertion in *Neomorphus*) plus 49 additional gap characters, 17 of which were parsimony informative. Double-peaks resulting from allelic variation were coded with standard IUPAC codes and treated as polymorphisms in the parsimony analysis.

Results

Our analyses consistently found strong support for several clades but variable relationships among orders within Neoaves. As an example, figure 1 shows the MP tree for well-aligned portions of the mtDNA data set in an analysis with transitions down-weighted by 50%.

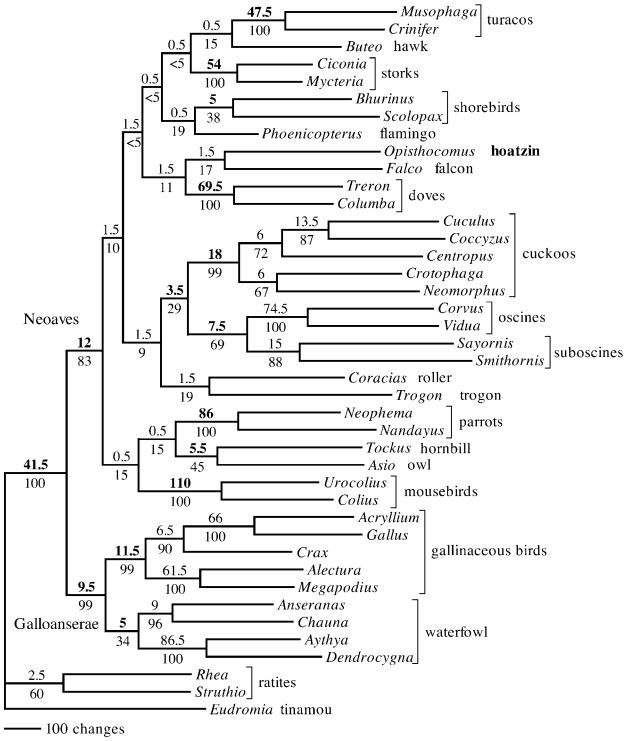

FIG. 1.—Most parsimonious tree for well-aligned regions of the mtDNA data set with transitions down-weighted 50% (tree length = 13,079.5; CI = 0.23). Bremer support indices and bootstrap proportions, respectively, are shown above and below each internal branch. Fractional Bremer values occur because transitions were given a weight of 0.5 in this analysis. Note the very low levels of support for many basal nodes within Neoaves. Branch lengths are proportional to the number of parsimony reconstructed changes under equal weights and ACCTRAN optimization.

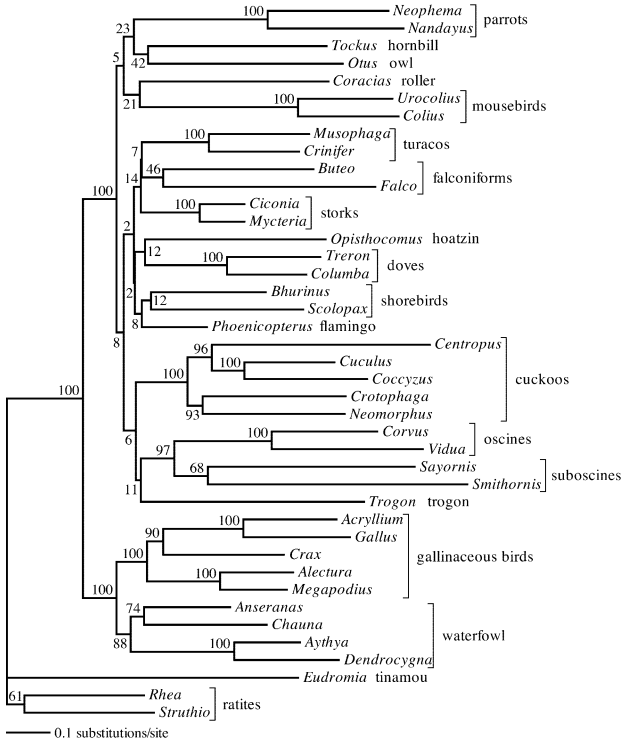

FIG. 2.—Best maximum-likelihood tree (−ln L = 76,748.64) found in 40 replicate heuristic searches with random addition of taxa. Bootstrap values, based on 100 replicate data sets and a single heuristic search per replicate, are shown above each node. See text for details on model parameters.

Our analyses are consistent with a basic division of Aves into three groups: Paleognathae, Galloanserae, and Neoaves (Cracraft and Clarke 2001). Assuming paleognaths as the outgroup, Galloanserae and Neoaves are sister taxa and constitute Neognathae. Monophyly of several orders is also well supported, including Galliformes, Coliiformes, Psittaciformes, Passeriformes, Cuculiformes, Columbiformes, and Musophagiformes. Anseriformes is also monophyletic in this analysis but with lower support values. The broadly conceived Ciconiiformes of Sibley and Ahquist (1990) is not supported, nor is monophyly of a falconiform clade (represented here by *Buteo* and *Falco*).

In contrast to relatively strong support for Galloanserae and Neoaves, almost all of the interordinal relationships within Neoaves are weakly supported and variable among analyses. In the analysis with 50% down-weighting of transitions, for example, most interordinal nodes have Bremer support values of only 0.5 to 1.5 and bootstrap values from less than 5% to 19% (fig. 1). Three nodes with somewhat higher Bremer support values correspond to sister relationships between a hornbill *Tockus* and an owl *Asio*, between Passeriformes and Cuculiformes, and between a thick-knee *Burhinus* and a woodcock *Scolopax*, the latter clade corresponding to the infraorder Charadriides in Sibley and Ahlquist (1990) or Charadriiformes in other avian classifications (e.g., Wetmore 1960). Only the first of these clades was found consistently in different analyses of the mtDNA data set.

The maximum-likelihood tree included all of the clades mentioned above, except for the sister relationship between Passerifomes and Cuculiformes (fig. 2). Relationships among taxa within Galliformes, Anseriformes, Passeriforms, and Cuculiformes, respectively, also were identical in parsimony and likelihood trees. Within Passeriformes, both oscines and subocines are monophyletic based on the taxa considered here. Within Cuculiformes, a New World ani *Crotophaga* and ground cuckoo *Neomorphus* are sister to a clade comprising a coucal *Centropus*, a New World nesting cuckoo *Coccyzus*, and an Old World brood parasite *Cuculus*. This topology is consistent with a comprehensive analysis of cuckoo relationships based on mtDNA 12S and ND2 sequences (Sorenson and Payne 2003) but differs from recent morphological studies (Hughes 1996, 2000). Other similarities between parsimony and likelihood analyses were a sister relationship between flamingo *Phoenicopterus* and two charadriiforms *Burhinus* and *Scolopax* and the failure to support Ciconiiformes as defined by Sibley and Ahlquist (1990). Also in contrast to parsimony analyses, the maximum-likelihood tree included a sister relationship between *Buteo* and *Falco*, corresponding to the infraorder Falconides in Sibley and Ahlquist (1990) or the order Falconiformes in most other classifications (e.g., Amadon and Bull 1988; Griffiths 1994). Other interordinal relationships differed among analyses.

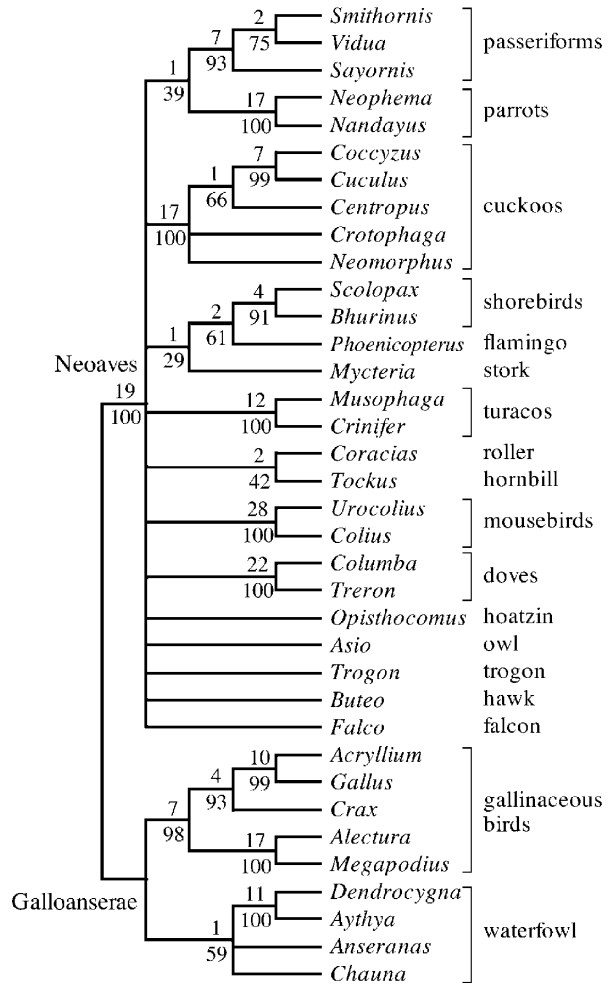

FIG. 3.—Strict consensus of 1,530 equally parsimonious trees of length 1,388 (CI = 0.62) for 38 taxa based on sequences of intron 9 of the PEPCK gene. Bremer support indices and bootstrap values are shown above and below nodes, respectively.

Optimization alignment tree searches incorporating gap regions yielded results similar to the parsimony analyses described above. The best equal weights trees include a sister relationship between hoatzin and *Trogon* or hoatzin and *Falco*. With transitions down-weighted 50%, the best trees included a sister relationship between hoatzin and *Trogon* or hoatzin and doves.

A separate analysis of PEPCK9 sequences yields a large number of equally parsimonious trees, the strict consensus of which includes most of the well-supported nodes in the mtDNA trees (fig. 3). Also consistent with some of the mtDNA analyses, the PEPCK9 tree includes a sister relationship between flamingo and two charadriiforms (*Burhinus* and *Scolopax*). The only results that differ from the mtDNA analyses are (1) a sister relationship between a roller *Coracias* and a hornbill *Tockus* and (2) lack of suboscine monophyly (i.e., *Sayornis* plus *Smithornis*), neither of which is strongly supported. Combined analyses of PEPCK and the much larger mtDNA data set yield results very similar to the mtDNA only analyses but with rearrangement of some basal nodes within Neoaves.

Monophyly of Anseriformes and Galliformes

All analyses based on the full mtDNA data set supported monophyly of Galliformes and a sister relationship between magpie goose *Anseranas* and a screamer *Chauna*. Anseriformes, however, was paraphyletic in some parsimony analyses of the mtDNA data set. Depending on the weighting scheme, trees in which Anhimides, comprising screamer and magpie goose (Sibley and Ahlquist 1990), or Anatidae, represented here by *Aythya* and *Dendrocygna*, was sister to Galliformes were equally parsimonious with trees in which Anseriformes was monophyletic. The ML tree (fig. 2), however, includes monophyly of anseriforms and galliforms, respectively, and like the parsimony analyses, a sister relationship between magpie goose and a screamer. The PEPCK analysis (fig. 3) also supported anseriforms and galliforms as monophyletic sister taxa but with three equally parsimonious arrangements of screamer, magpie goose, and anatids within Anseriformes. Both the mtDNA and PEPCK analyses provide strong support for relationships within Galliformes. Megapodiidae is basal, whereas Cracidae is sister to Phasianidae plus Numididae.

Relationships of the Hoatzin

The position of hoatzin was variable among analyses and the alternative placements found were weakly supported (e.g., fig. 1). In parsimony analyses, hoatzin was sister either to *Falco* or *Trogon*, depending on weighting scheme. In the ML tree, hoatzin is sister to the two columbiforms we sampled (fig. 2). A sister relationship with doves also was found in 74% of the MP trees in the PEPCK analysis. In other PEPCK trees, hoatzin was sister to the owl *Asio* or to a clade comprising parrots and passeriforms. In no case did we find a sister relationship between hoatzin and turacos. Our results contrast with those of HB1999 in which a comparable data set indicated very strong support for a sister relationship between hoatzin and turacos (93% support value based on ML-based quartet puzzling).

We analyzed different data partitions under a range of weighting schemes to explore whether evidence of a relationship between turacos and hoatzin might be present in particular subsets of the mtDNA data or in certain kinds of character change (fig. 4). None of these partitioned analyses supported a sister relationship between hoatzin and turacos, and the number of additional steps required to obtain this result (the LILD) was relatively large in all cases, particularly for second codon positions (fig. 4*a*). Down-weighting transitions had little effect on the relative magnitude of the LILD, suggesting that focusing on relatively conserved transversions also provided no evidence of a relationship between hoatzin and turacos. Similarly, none of the partitioned analyses suggested a relationship between hoatzin and cuckoos (fig. 4*b*).

In parsimony analyses of the mtDNA data, hoatzin was sister to *Trogon* except for analyses with intermediate down-weighting of transitions, in which it grouped with *Falco*. Partitioned analyses suggest that a relationship with *Trogon* was due primarily to second and third codon

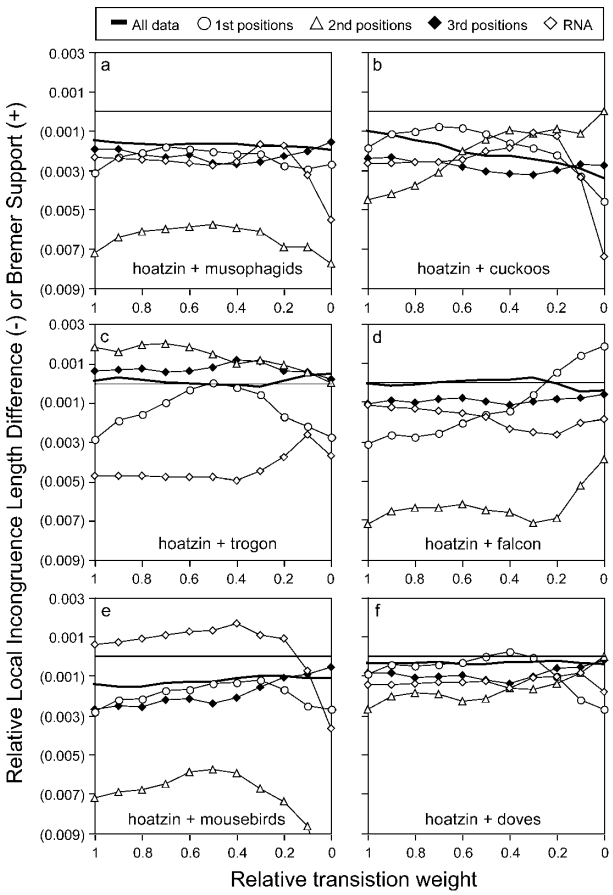

FIG. 4.—Bremer support and local incongruence length difference (LILD) indices for six alternative placements of the hoatzin (*a–f*). Values are plotted for each of 11 weighting schemes ranging from equal weights to transversion parsimony (relative transition weight = 0) and for each of five data partitions, including the full mtDNA data set (well-aligned portions only). The Bremer support index is plotted if the clade in question was present in the most parsimonious tree for a given data partition/weighting scheme, whereas the LILD is plotted if the clade in question was not present. To facilitate comparison, both Bremer support and LILD values are presented as a proportion of the unconstrained tree length for the given data partition/weighting scheme. See text for further explanation and discussion.

positions, whereas RNA characters conflicted with this result (fig. 4*c*). Similarly, whereas first codon position transversions suggest a relationship with *Falco*, this result seems to be contradicted by second codon positions (fig. 4*d*). A sister relationship between hoatzin and mousebirds (Colliiformes) was found in most analyses of RNA characters, but other data partitions conflicted with this result (fig. 4*e*). Finally, a sister relationship between hoatzin and doves (Columbiformes), the result obtained in our ML analysis, had relatively small LILD values (or in one case, positive Bremer support) for all data partitions and weighting schemes (fig. 4*f*). If *Trogon*, *Falco*, and *Buteo* (the other falconiform in our data set) are excluded, parsimony analyses of the full mtDNA data set yield a hoatzin plus doves clade regardless of weighting scheme.

Our failure to corroborate the results of HB1999 led us to compare cytochrome *b* sequences for taxa common to both data sets (cytochrome *b* was the only gene region sequenced in both studies). Sequences reported by HB1999 differ by at least 2.4% and by as much as 8.4% from our sequences for the same species (table 2). Differences in at least the *Opisthocomus* and *Neomorphus* sequences must reflect some form of error rather than intraspecific variation, because tissue samples from the same individual birds were used in both studies. Other sequences reported by HB1999 differ substantially from sequences reported in a variety of previous studies and show a ratio of transversion differences that is much higher than expected for intraspecific comparisons (table 2). In contrast, most of our sequences differ by less than 1% from previous sequences for the same taxa (table 2). Exceptions include *Opisthocomus* and *Coccyzus* sequences from Avise, Nelson, and Sibley (1994*a*), a study that was criticized for sequencing errors (Hackett et al. 1995), and *Colius* and *Trogon* sequences reported by Espinosa de los Monteros (1998, 2000). Nonetheless, our *Opisthocomus* and *Colius* sequences are more similar to sequences from these previous studies than are the sequences of HB1999. The *Trogon* sequences differ primarily by transitions, as would be expected for a comparison of somewhat divergent haplotypes within a species.

We obtained nearly complete sequence for both DNA strands by sequencing relatively small overlapping fragments and are confident in the accuracy of our sequences—an example of our results can be viewed online (http://people.bu.edu/msoren/research.html). We also confirmed the accuracy of our data by sequencing three different hoatzin samples, among which there were only 52 variable positions in 9,364 bp and only two differences involving transversions. The two samples from Peru (LSUMNS B-10753 and B-10754) differed by only eight transitions (0.09%). In contrast, our sequences for the Peru samples differ from those reported by HB1999 at 74 or 75 positions over 4,796 bases (1.5% to 1.6%), including 42 transversion differences (table 3).

Particularly problematic is that these apparent errors in the HB1999 data are not random with respect to the inference of phylogenetic relationships. In 38 of the 42 positions that differ by a transversion mismatch, the HB1999 hoatzin sequence has the same character state (purine versus pyrimidine) as their turaco sequences. In the other four positions, both purines and pyrimidines are found among the HB1999 turaco sequences. In contrast, the HB1999 hoatzin sequence has the same character state as cuckoos in only 23 of these 42 positions. In four positions, it differs from cuckoos, and in 15 positions, both purines and pyrimidines are found among the HB1999 cuckoo sequences. Discrepancies in the *Musophaga* cytochome *b* sequences show a similar pattern, with the HB1999 *Musophaga* and hoatzin sequences being more similar to each other than are our sequences for the same taxa (fig. 5). Considering three turacos (*Musophaga*, *Corythaixoides*, and *Corythaeola*), average uncorrected transversion distance between hoatzin and turacos for cyctochrome *b* is 8.2% using sequences from our study and Veron and Winney (2000) but only 6.0% using sequences from HB1999.

We reanalyzed the HB1999 data set using simple parsimony with equal weights for all characters and changes. Bootstrap support for the clade comprising

Table 2
Comparison of Cytochrome *b* Sequences from Different Studies

Species	Source of Other Sequence	Reference[a]	HB1999 Sequences Compared with Others			Our Sequences Compared with Others		
			Ts	Tv	bp[b]	Ts	Tv	bp[b]
Opisthocomus hoazin	This study		10	15	1045			
	U09257-9	1	36–52	20	961	41–53	8	961
Musophaga violacea[c]	This study		17	23	1041			
	AF102083	2	14	22	1017	0	0	1020
Corythaixoides concolor[c]	AH010178	2	23	10	819			
Corythaeola cristata[c]	AH010179	2	31	21	921			
Crinifer piscator	AF102101	2				3	4	925
Turaco persa[d]	AF102086	2	95	76				982
Neomorphus geoffroyi	This study		57	13	980			
Crotophaga ani	This study		19	20	1045			
Colius striatus	This study		68	20	1043			
	U89175	3	67	26	1043	41	10	1143
Struthio camelus	AF338715	4				0	0	1068
	Y12025	5				0	0	1068
Rhea americana	Y16884	6				6	0	1290
Eudromia elegans	AF338710	4				2	0	1276
	AY016016	7				3	2	1272
Trogon curucui	U94801	8				29	1	1143
Coccyzus erythropthalmus	U09266	1				38	29	961
Columba leucocephala	AF182689	9				0	0	1045
Falco peregrinus	U83307	10				6	2	1143
	X86746	11				4	1	1025
Pheonicopterus ruber	U08940	12				2	2	1009
Mycteria americana	U72779	13				0	2	1070
	U08949	12				1	2	1009
Sayornis phoebe	AF447613–4	14				3–4	0	1143

NOTE.—The number of transition (Ts) and transversion (Tv) differences between each pair of sequences is given. We used tissues from the same individual birds for *Opisthocomus hoazin* and *Neomorphus geoffroyi* as in Hughes and Baker (1999 [= HB1999]). For other taxa, different tissues were used. Comparisons limited to GenBank sequences greater than 900 bp in length and associated with a published study.

[a] References: 1, Avise, Nelson, and Sibley 1994*a*; 2, Veron and Winney 2000; 3, Espinosa de los Monteros 2000; 4, Haddrath and Baker 2001; 5, Härlid, Janke, and Arnason 1997; 6, Härlid, Janke, and Arnason 1998; 7, Cooper et al. 2001; 8, Espinosa de los Monteros 1998; 9, Johnson and Clayton 2000; 10, Griffiths 1997; 11, Seibold and Helbig 1995; 12, Avise, Nelson, and Sibley 1994*b*; 13, Slikas 1997; 14, Cicero and Johnson 2002.

[b] Number of base pairs compared.

[c] Our turaco sequences and those of Veron and Winney (2000) have one codon less than most other birds near the end of cytochrome *b*, whereas the sequences reported by HB1999 are identical to the other birds in their data set (in fact, the last eight bases of cytochrome *b* are identical for all taxa in the HB1999 data set).

[d] The *Tauraco persa* sequence reported by HB1999 (AF168115) appears to be a mislabeled cuckoo sequence based on phylogenetic analysis of cytochrome *b* sequences only. Interestingly, this sequence is more similar to hoatzin than are any of the other cuckoo sequences in HB1999.

hoatzin and musophagids was 68%. When we replaced the hoatzin sequences reported by HB1999 with our hoatzin sequences, bootstrap support for a hoatzin plus musophagid clade declined to 21%, a value that may still be inflated due to errors in the HB1999 turaco sequences.

Using our data set, we evaluated alternative phylogenetic hypotheses for the hoatzin using both maximum likelihood and parsimony. In general, there is a strong correlation between the Δ ln L for a given hypothesis in the mtDNA analysis and the number of extra steps (the LILD) in parsimony analyses of either mtDNA or PEPCK sequences (table 4). Hoatzin is clearly not a galliform or a derived New World cuckoo. Whereas the number of extra steps required to unite hoatzin with turacos or cuckoos is large, neither hypothesis is significantly rejected in a likelihood framework. This is nonetheless a very different result from the bootstrap analysis of HB1999, which suggested strong support for hoatzin plus turacos and significant rejection of a hoatzin plus cuckoo clade (see their table 2). Our bootstrap analyses also suggest little or no support for placing hoatzin with either turacos or cuckoos (table 4).

Base Composition

MtDNA base composition varies widely among the taxa in our study (fig. 6), and this variation may contribute to some of our results. For example, both *Tockus* and *Asio* have a relatively high proportion of C and low proportion of A nucleotides in their mtDNA sequences and are sister taxa in our mtDNA analyses (using both MP and ML) but not in our PEPCK analysis. In contrast, the two charadriiforms we sampled (*Scolopax* and *Burhinus*) are relatively divergent in base composition, and this group was only weakly supported in analyses of the mtDNA data set (again using both MP and ML), whereas PEPCK yielded relatively strong support for a charadriiform clade (fig. 3). The base composition of hoatzin is not particularly unusual, but is similar to that of *Trogon*, the taxon with which it grouped in most parsimony analyses of the mtDNA data set. Hoatzin is only slightly closer to doves in base composition than to turacos and, on average, is somewhat more divergent from cuckoos, which are divergent from other birds in having a higher proportion of A and lower proportion of C.

Table 3
Relative Divergence of Hoatzin Sequences from Turaco and Cuckoo Sequences Reported by HB1999

	Differences Between Hoatzin Sequences[a]		Average Number of Tv Differences Between Sequences					
			Alternative Hoatzin Sequences versus HB1999 Turaco Sequences			Alternative Hoatzin Sequences versus HB1999 Cuckoo Sequences		
Gene	Ts[b]	Tv[b]	This study	HB1999	Difference	This study	HB1999	Difference
COI	8	7	71.3	64.3	7	93.5	89.5	4
COII	3–4[c]	5	50	46.3	3.7	50.8	49.2	1.6
COIII	4–5[c]	8	43.7	36.3	7.4	55.5	49.5	6
Cyt *b*	10	15	75	62.3	12.7	88.3	83.7	4.6
atp 8[d]	4	4	16.7	12.7	4	28.3	25.7	2.6
atp 6	2–3[c]	3	52	49	3	73.5	72.5	1
All	32–33	42	308.7	271	37.7	390	370	20

NOTE.—We compare our hoatzin sequences with those reported by Hughes and Baker (1999 [= HB1999]) and compare hoatzin sequences from both studies with the turaco and cuckoo sequences reported by HB1999.

[a] This study versus HB1999.

[b] Ts: transition (C-T or A-G); Tv: transversion.

[c] We observed a single transition difference between B-10753 and B-10754 in each of three genes.

[d] The HB1999 *Opisthocomus* sequence lacks a 3-bp insertion in ATPase 8 that is present in all three of our hoatzin sequences.

Discussion

Our analyses are consistent with recent morphological analyses and a growing number of molecular studies in dividing modern birds into three main clades: Paleognathae, comprising ratites and tinamous; Galloanserae, comprising galliforms and anseriforms; and Neoaves (or Plethornithae [Groth and Barrowclough 1999]), comprising all other birds (see Cracraft and Clarke 2001 for a review). Although our analysis did not include nonavian outgroups, other recent studies suggest that the root of the avian tree lies between Paleognathae and all other birds (Neognathae) (Groth and Barrowclough 1999; García-Moreno and Mindell 2000; van Tuinen, Sibley, and Hedges 2000; Livezey and Zusi 2001; Braun and Kimball 2002; Edwards et al. 2002; Paton, Haddrath, and Baker 2002), a rooting that is compatible with our results. Monophyly of both Galloanserae and Neoaves rules out suggested relationships between hoatzin and galliforms or between waterfowl and shorebirds or flamingos.

Galloanserae

Within Galliformes, our analyses show strong support for a sister relationship between megapodes and all other galliforms. This differs from DNA-DNA hybridization results in which cracids and megapodes were sister taxa (Sibley and Ahlquist 1990) but is consistent with morphological studies (Livezey and Zusi 2001) and other recent DNA sequencing studies (Ericson, Parsons, and Johansson 2001; Dimcheff, Drovetski, and Mindell 2002). In contrast, monophyly of Anseriformes was weakly supported and relationships among a screamer, magpie goose, and anatids were variable among analyses. Separate analyses of RNA characters (rRNA plus tRNA) nested the screamer or screamer plus magpie goose within galliforms as the sister taxon of cracids, suggesting some conflict within the mtDNA data set. PEPCK sequences and the mtDNA ML analysis, however, both support monophyly of Anseriformes and Galliformes. Divergence of both screamers and magpie goose near the base of the anseriform radiation (fig. 2) likely contributes to the weak support for Anseriformes in our analyses.

Within Anseriformes, all analyses of the full mtDNA data set suggest a sister relationship between a screamer and magpie goose (see also Sibley and Ahlquist 1990; Mindell et al. 1997). This result conflicts with traditional classifications and recent morphological analyses in which screamers are sister to a clade comprising magpie goose and anatids (Livezey 1997*a*, 1997*b*). Nuclear gene sequences also support this traditional arrangement (Cra-

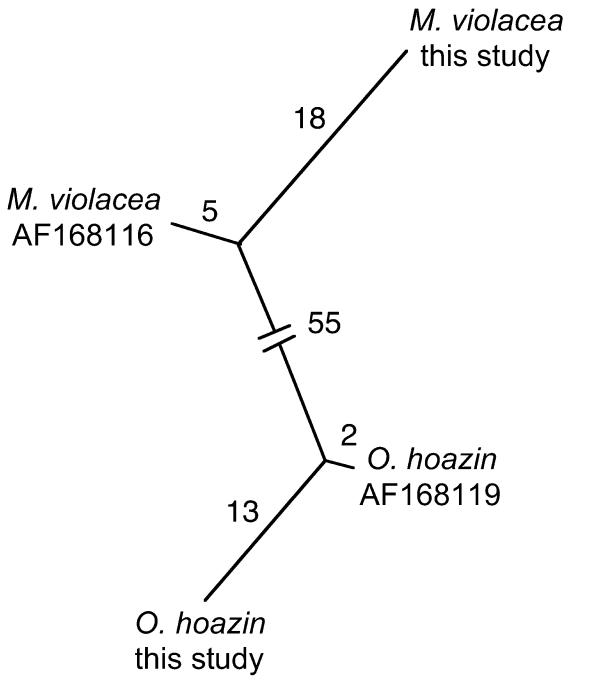

FIG. 5.—Graphic illustration of differences among cytochome *b* sequences for a turaco *Musophaga violacea* and hoatzin *Opisthocomus hoazin* as determined in this study and by Hughes and Baker (1999). Transversion differences only are shown for 1,045 positions of cytochrome *b*. Our sequences for these two taxa differ by 86 transversions, whereas the Hughes and Baker (1999) sequences differ by only 62 transversions.

Table 4
Comparison of Alternative Phylogenetic Hypotheses Under ML and MP Criteria

Hypothesis	−ln L	Δ ln L	*P*-value	mtDNA LILD	PEPCK LILD	Bootstrap Values		
						ML	mtDNA	PEPCK
Hoatzin with doves (ML tree)	76748.64	—	—	7	0[a]	12	5	34
Hoatzin with turacos	76755.30	6.66	0.42	29	1	0	0	<1
Hoatzin with cuckoos	76758.73	10.09	0.41	20	3	0	0	0
Hoatzin with (*Neomorphus*, *Crotophaga*)	76851.85	103.21	<0.001	53	21	0	0	0
Hoatzin with galliforms	76890.76	142.12	<0.001	82	29	0	0	0
''Cuculiformes'' incl. turacos, cuckoos, hoatzin	76774.76	26.12	0.14	45	4	0	0	0
''Ciconiiformes'' (Sibley and Ahlquist 1990)	76754.88	6.24	0.15	20	5	0	0	0
Screamers sister to other anseriforms	76751.16	2.52	0.43	13	0[b]	17	4	26

NOTE.—Trees constrained to include the specified group were compared with the ML tree using the approximately unbiased test of Shimodaira (2002). *P*-values for the last two hypotheses were determined in separate tests. The LILD (number of extra steps in the shortest tree consistent with each hypothesis) is in comparison with the equal weights parsimony trees for each data set. Bootstrap values are the proportion of replicates that included the listed clade.

[a] Topology found in 74% of 1,530 MP trees for PEPCK9.

[b] Topology found in 33% of 1,530 MP trees for PEPCK9.

craft et al. 2003). Divergent base composition in *Aythya* (fig. 6), one of the two anatids we sampled, may account for the inconsistent mtDNA results. When *Aythya* is excluded, magpie goose is sister to *Dendrocygna* rather than the screamer *Chauna* in both parsimony and likelihood analyses of the mtDNA data.

Hoatzin

In reviewing the history of classification of the hoatzin, Sibley and Ahlquist (1990) made clear the need for ''reason ... to supplant tradition'' (p. 378) in avian systematics. Unfortunately, progress in solving the hoatzin problem seems to have been hindered both by traditional points of view about its relationships and conclusions based on insufficient or erroneous data. Although Sibley and Ahlquist's (1972, 1973) results for egg-white proteins could not be replicated by Brush (1979), the earlier result apparently influenced their interpretation of DNA-DNA hybridization data for hoatzin. The latter were of poor quality because of technical problems with the hoatzin sample and provided essentially no useful information about hoatzin relationships (F. Sheldon, personal communication). Data quality was again an issue in the DNA sequencing studies of Avise, Nelson, and Sibley (1994*a* [see Hackett et al. 1995]) and Hughes and Baker (1999), as shown above.

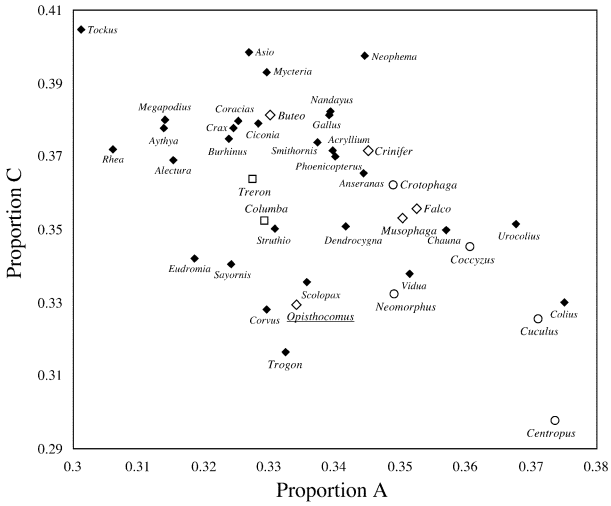

FIG. 6.—Base composition for variable positions (n = 2610) in the mtDNA data set. We plot proportions of A (adenine) and C (cytosine) because they show the greatest variation among taxa. See text for discussion.

Strong support for a sister relationship between hoatzin and turacos in HB1999 can be attributed primarily to sequencing errors and perhaps to a lesser extent the more limited sample of taxa in their study. Differences between the studies are not due to the inadvertent sequencing of nuclear copies or ''numts'' (see Quinn 1992; Arctander 1995; Zhang and Hewitt 1996; Sorenson and Quinn 1998). This explanation would require that the many different primer pairs used in each study consistently amplified mtDNA in one study and nuclear homologs in the other. In addition, large numbers of transversion differences (tables 2 and 3) are inconsistent with expectations for intraspecific comparisons of mtDNA and numts. Sequences transposed to the nucleus experience a reduced rate of evolution such that most differences between mtDNA and numt accumulate in the mtDNA copy (Zischler et al. 1995; Sorenson and Fleischer 1996; Bensasson et al. 2001). This means numts may be more similar to ancestral sequences, which is the case for sequences reported by HB1999 (see fig. 5), but comparison of mtDNA and numts from the same species should also yield a typically high transition-transversion ratio (e.g., Lopez et al. 1994).

Errors in the HB1999 data set generally increased the similarity of their sequences, but increased disproportionately the similarity of hoatzin and turaco sequences (table 3 and fig. 5). Given very low support values for interordinal relationships within Neoaves generally (fig. 1), alternative topologies may turn on a very small number of characters. An error rate of 1.5% (i.e., 74 characters in a 4,796 character data set) easily overwhelms this kind of analysis, particularly when the errors involve transversion differences that carry more weight in model-based phylogenetic analyses. The conflicting results of our study and that of HB1999 highlight the need for accurate sequence data and replication in molecular systematics—especially for monotypic taxa such as the hoatzin.

In our analyses, the position of hoatzin as well as other basal relationships within Neoaves were not well resolved. Although we were not able to reject all previous

hypotheses for the hoatzin in a maximum-likelihood framework, neither did we find any compelling evidence that hoatzin is closely related to either turacos or cuckoos. These clades required a large number of extra steps in parsimony analyses and were never or almost never recovered in bootstrap analyses using either MP or ML (table 4). Analysis of individual data partitions across a range of transition weights also suggests the lack of any phylogenetic signal for a relationship between hoatzin and either turacos or cuckoos (fig. 4). Similarly, a recent morphological analysis of avian lice found that *Osculotes*, a genus specific to hoatzin, was the sister taxon of a large clade of lice, none of which occur on either cuckoos or turacos (Smith 2001).

If anything, our data suggest a sister relationship between hoatzin and doves (Columbiformes). This was the result of our ML analysis and parsimony analyses in which a few long-branched taxa (*Trogon*, *Falco*, and *Buteo*) were excluded. With all taxa included, a hoatzin-plus-doves clade never required more than a small number of extra steps in comparison to the MP tree (fig. 4*f*). Finally, a hoatzin-plus-doves clade was present in 74% of the MP trees from our analysis of PEPCK intron 9. This potential congruence of mitochondrial and nuclear data is noteworthy and suggests that a relationship between hoatzin and doves deserves further testing by both molecular and morphological systematists. After galliforms, cuckoos, and turacos, doves are mentioned most frequently in Sibley and Ahlquist's (1990) review of hoatzin systematics, presumably reflecting at least some similarity perceived by early taxonomists. Of course, our result is not strongly supported and is conditional on the taxa included in our analysis—for example, we sampled none of the diverse set of taxa included in Gruiformes by Sibley and Ahlquist (1990).

Problems and Prospects in Avian Molecular Systematics

As in other recent molecular studies (Groth and Barrowclough 1999; van Tuinen, Sibley, and Hedges 2000; van Tuinen et al. 2001; Johansson et al. 2001), we find support for Neoaves but poor resolution of relationships within this clade. Strong support for the deeper split between Neoaves and Galloanserae makes clear that this difficulty is not simply a problem of reconstructing ancient divergences. Rather, the potential to resolve relationships with molecular data is a function of internode length relative to the time depth of the divergences involved. Many avian orders clearly diverged from each other in a *relatively* rapid radiation, raising the question of whether the base of Neoaves represents a genuine polytomy that simply cannot be resolved (see Stanley and Cracraft 2002; Poe and Chubb, in review).

Based on our analyses, we think molecular sequence data continue to have significant utility for testing hypotheses about interordinal relationships within Neoaves. Our approach of analyzing different data partitions under various weighting schemes (i.e., fig. 3), for example, provides a fairly convincing rejection of hypotheses relating hoatzin to either cuckoos or turacos, even though these hypotheses were not statistically rejected in a ML framework. The placement of hoatzin remains uncertain, however, and this kind of result may be a recurring problem for avian ordinal systematics—certain hypotheses will be relatively easily rejected, but their alternatives may remain poorly supported. One potential positive result at the interordinal level was the sister relationship between flamingo and two charadriiforms found in both our PEPCK and our mtDNA analyses. This result is not necessarily inconsistent with van Tuinen et al. (2001), in which flamingos and grebes were strongly supported as sister taxa. The addition of a grebe to our data set would be needed to further test this interesting hypothesis.

Our analysis of data subsets with different modes of evolution is potentially valuable as a heuristic approach to testing hypotheses and discovering conflicts or systematic biases within a larger data set. This approach, however, partitions characters into classes that are still demonstrably heterogenous (see below) and fails to capture the potentially complex interaction of characters in an analysis of the full data set. A sister relationship between hoatzin and *Falco*, for example, is supported by the full mtDNA data set when transitions are down-weighted 40% to 70%, even though none of the individual data partitions support this result (fig. 4*d*). Although far more demanding computationally, this approach also could be implemented in a ML framework, calculating Δ lnL for ML trees with and without a particular clade (see Lee and Hugall 2003). Ideally, such analyses would fit an appropriate model of sequence evolution to each data partition independently. Although consistent failure (or conversely strong support) of a particular hypothesis across data partitions is of significant interest, combined analyses of all available character data should be given priority in the development and testing of phylogenetic hypotheses (e.g., Baker and DeSalle 1997).

A continuing problem in higher-level avian systematics will be variation in base composition among lineages, a form of nonindependent character evolution that can bias phylogenetic analyses (e.g., Naylor and Brown 1998; Foster and Hickey 1999; Sorenson and Payne 2001). Convergent base composition in unrelated taxa may obscure historical relationships, whereas divergent base composition inherited from a common ancestor may inflate the apparent support for a natural group (e.g., Sefc, Payne, and Sorenson 2003). With respect to the competing hypotheses of turacos, cuckoos, or doves as the sister group of hoatzin, small differences in overall base composition (see fig. 6) seem unlikely to overwhelm all other information in the data set. Anseriforms and galliforms, for example, show a broad range of base composition, yet still form a well-supported clade, as do the four passeriforms we sampled. In contrast, the hornbill-plus-owl (*Tockus*-plus-*Asio*) clade in our mtDNA analyses and unexpected relationships within Anseriformes (see above) may be artifacts of variation in base composition among taxa.

Significant variation in base composition is evident in most data sets covering broad taxonomic samples, and failure to account for this variation is perhaps one of the most significant shortcomings of commonly used models of sequence evolution. ML methods that incorporate lineage-specific base composition have been developed (Galtier and Gouy 1995, 1998; Yang and Roberts 1995)

and applied in avian studies (Haddrath and Baker 2001; Paton, Haddrath, and Baker 2002). The current implementation of this method effectively allows only the evaluation of candidate trees and not searches of tree space and therefore was impractical given the number of taxa in our analysis. Other complications in real sequence data include variation in the substitution rate matrix among nucleotide positions and lineage-specific differences in the rate matrix even within individual positions (Lopez, Casane, and Philippe 2002), as may occur in a ''covarion'' process in which substitution patterns at a site depend on changes in neighboring sites (Fitch 1971; Galtier 2001). In our experience with large data sets, application of model selection criteria (e.g., as implemented in Modeltest [Posada and Crandall 1998]) almost always results in selection of the most complex model tested (e.g., GTR + I + Γ)—this begs the question of how much additional complexity might be justified statistically and to what extent more complex models will affect or improve phylogenetic inference (see Sanderson and Kim 2000; Buckley and Cunningham 2002).

Recent molecular analyses of avian orders, including ours, are still quite limited in terms of the number of taxa and characters considered. Increased taxon sampling may improve the resolution of relationships within Neoaves by allowing better reconstruction of ancestral character states and in effect reducing the relative depth of divergence for basal internodes. Recent studies strongly suggest benefits from greater taxon sampling (Pollock et al. 2002; Zwickl and Hillis 2002), perhaps in part because it reduces potential biases associated with some of the complexities of sequence evolution discussed above. Unfortunately, breaking long branches is not an option for monotypic taxa such as the hoatzin, except for the addition of relevant fossil material to morphological studies.

Studies of avian relationships are shifting from mitochondrial to nuclear sequence data (e.g., Groth and Barrowclough 1999; Johansson et al. 2001; Barker, Barrowclough, and Groth 2002), presumably on the logic that more conserved nuclear genes will provide better resolution of deep divergences. We think there is a need, however, for direct comparisons of the efficacy of comparable mtDNA and nuclear data sets for much larger samples of taxa before all effort is diverted from one genome to the other. The potential to use mitochondrial and nuclear data as independent sources of character information argues for the continued collection of both. Given the magnitude of the avian orders problem, comprehensive analyses of relationships within avian families and orders may be more productive for individual researchers in the short term. If these efforts are minimally coordinated, a database of comparable mitochondrial and nuclear sequence data applicable to avian interordinal relationships will be generated.

Acknowledgments

For providing tissues for genetic analysis, we thank Les Christidis (Museum Victoria and Australian National Wildlife Collection), Fred Sheldon and Donna Dittman (Louisiana State University Museum of Natural Science), Mike and Ali Lubbock (Sylvan Heights Waterfowl, North Carolina), Yoram Yom-Tov and Tamar Dyan (Tel Aviv University Research Zoo); Robert Payne and Janet Hinshaw (University of Michigan Museum of Zoology), and Jimmy Pichner (Minnesota Zoo). Scott Stanley shared his genomic sequence of the PEPCK gene in *Gallus gallus* and provided the outer primers for intron 9. Jackie Moustakas and Kristina Sefc assisted with data collection. Horst Wagner kindly assisted with the construction of a parallel computing cluster used in our phylogenetic analyses. Funding was provided by a National Science Foundation grant to D.P.M. and M.D.S.

References Cited

Amadon, D., and J. Bull. 1988. Hawks and owls of the world. Proc. West. Found. Vert. Zool. **3**:295–357.

Arctander, P. 1995. Comparison of a mitochondrial gene and a corresponding nuclear pseudogene. Proc. R. Soc. Lond. B Biol. Sci. **262**:13–19.

Avise, J. C., W. S. Nelson, and C. G. Sibley. 1994*a*. Why one-kilobase sequences from mitochondrial DNA fail to solve the hoatzin phylogenetic enigma. Mol. Phylogenet. Evol. **3**: 175–184.

———. 1994*b*. DNA sequence support for a close phylogenetic relationship between some storks and New World vultures. Proc. Natl. Acad. Sci. USA **91**:5173–5177.

Baker, R. H., and R. DeSalle. 1997. Multiple sources of character information and the phylogeny of Hawaiian Drosophilids. Syst. Biol. **46**:654–673.

Barker, F. K., G. F. Barrowclough, and J. G. Groth. 2002. A phylogenetic hypothesis for passerine birds: taxonomic and biogeographic implications of an analysis of nuclear DNA sequence data. Proc. Roy. Soc. Lond. B Biol. Sci. **269**:295–308.

Bensasson, D., D. X. Zhang, D. L. Hartl, and G. M. Hewitt. 2001. Mitochondrial pseudogenes: evolution's misplaced witnesses. Trends Ecol. Evol. **16**:314–321.

Benton, M. J. 1999. Early origins of modern birds and mammals: molecules vs. morphology. Bioessays **21**:1043–1051.

Braun, E. L., and R. T. Kimball. 2002. Examining basal avian divergences with mitochondrial sequences: model complexity, taxon sampling, and sequence length. Syst. Biol. **51**:614–624.

Bremer, K. 1988. The limits of amino-acid sequence data in angiosperm phylogenetic reconstruction. Evolution **42**:795–803.

Brush, A. H. 1979. Comparison of egg-white proteins: effect of electrophoretic conditions. Biochem. Syst. Evol. **7**:155–165.

Buckley, T. R., and C. W. Cunningham. 2002. The effects of nucleotide substitution model assumptions on estimates of nonparametric bootstrap support. Mol. Biol. Evol. **19**:394–405.

Caspers, G. J., D. U. de Weerd, J. Wattel, and W. W. de Jong. 1997. Alpha-crystallin sequences support a galliform/anseriform clade. Mol. Phylogenet. Evol. **7**:185–188.

Cicero, C., and N. K. Johnson. 2002. Phylogeny and character evolution in the *Empidonax* group of tyrant flycatchers (Aves: Tyrannidae): a test of W. E. Lanyon's hypothesis using mtDNA sequences. Mol. Phylogenet. Evol. **22**:289–302.

Cook, J. S., S. L. Weldon, J. P. Garcia-Ruiz, Y. Hod, and R. W. Hanson. 1986. Nucleotide sequence of the mRNA encoding the cytosolic form of phosphoenolpyruvate carboxykinase (GTP) from the chicken. Proc. Natl. Acad. Sci. USA **83**:7583–7587.

Cooper, A. 1994. DNA from museum specimens. Pp. 149–165 *in* B. Herrmann and S. Herrmann, eds. Ancient DNA: recovery and analysis of genetic material from paleontological,

archaeological, museum, medical, and forensic specimens. Springer, New York.
Cooper, A., C. Lalueza-Fox, S. Anderson, A. Rambaut, J. Austin, and R. Ward. 2001. Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. Nature **409**:704–707.
Cooper, A., and D. Penny. 1997. Mass survival of birds across the Cretaceous-Tertiary boundary: molecular evidence. Science **275**:1109–1113.
Cracraft, J. 1981. Toward a phylogenetic classification of the recent birds of the world (class Aves). Auk **98**:681–714.
———. 1986. The origin and early diversification of birds. Paleobiology **12**:383–399.
———. 2001. Avian evolution, Gondwana biogeography and the Cretaceous-Tertiary mass extinction event. Proc. R. Soc. Lond. B Biol. Sci. **268**:459–469.
Cracraft, J., F. K. Barker, M. J. Braun et al. (14 co-authors). 2003. Phylogenetic relationships among modern birds (Neornithes): toward an avian tree of life. *In* J. Cracraft and M. J. Donoghue, eds. Assembling the Tree of Life. Oxford University Press, New York (in press).
Cracraft, J., and J. Clarke. 2001. The basal clades of modern birds. Pp. 143–156 *in* J. Gauthier and L. F. Gall, eds. New perspectives on the origin and early evolution of birds. Proceedings of the International Symposium in Honor of John H. Ostrom. Peabody Museum of Natural History, Yale University, New Haven, Conn.
de Quieroz, K., and D. A. Good. 1988. The scleral ossicles of *Opisthocomus* and their phylogenetic significance. Auk **105**:29–35.
Desjardins, P., and R. Morais. 1990. Sequence and gene organization of the chicken mitochondrial genome—a novel gene order in higher vertebrates. J. Mol. Biol. **212**:599–634.
Dimcheff, D. E., S. V. Drovetski, and D. P. Mindell. 2002. Phylogeny of Tetraoninae and other galliform birds using mitochondrial 12S and ND2 genes. Mol. Phylogenet. Evol. **24**:203–215.
Dyke, G. J. 2001. The evolutionary radiation of modern birds: systematics and patterns of diversification. Geol. J. **36**:305–315.
Edwards, S. V., B. Fertil, A. Giron, and P. J. Deschavanne. 2002. A genomic schism in birds revealed by phylogenetic analysis of DNA strings. Syst. Biol. **51**:599–613.
Ericson, P. G. P. 1996. The skeletal evidence for a sister-group relationship of anseriform and galliform birds—a critical evaluation. J. Avian Biol. **27**:195–202.
———. 1997. Systematic relationships of the palaeogene family Presbyornithidae (Aves: Anseriformes). Zool. J. Linn. Soc. **121**:429–483.
Ericson, P. G. P., T. J. Parsons, and U. S. Johansson. 2001. Morphological and molecular support for nonmonophyly of the Galloanserae. Pp. 157–168 *in* J. Gauthier and L. F. Gall, eds. New perspectives on the origin and early evolution of birds. Proceedings of the International Symposium in honor of John H. Ostrom. Peabody Museum of Natural History, Yale University, New Haven, Conn.
Espinosa de los Monteros, A. 1998. Phylogenetic relationships among the trogons. Auk **115**:937–954.
———. 2000. Higher-level phylogeny of Trogoniformes. Mol. Phylogenet. Evol. **14**:20–34.
Feduccia, A. 1995. Explosive evolution in tertiary birds and mammals. Science **267**:637–638.
Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**:783–791.
Fitch, W. M. 1971. Rate of change of concomitantly variable codons. J. Mol. Evol. **1**:84–96.
Foster, P. G., and D. A. Hickey. 1999. Compositional bias may affect both DNA-based and protein-based phylogenetic reconstructions. J. Mol. Evol. **48**:284–290.
Galtier, N. 2001. Maximum-likelihood phylogenetic analysis under a covarion-like model. Mol. Biol. Evol. **18**:866–873.
Galtier, N., and M. Gouy. 1995. Inferring phylogenies from DNA sequences of unequal base compositions. Proc. Natl. Acad. Sci. USA **92**:11317–11321.
———. 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.
García-Moreno, J., and D. P. Mindell. 2000. Rooting a phylogeny with homologous genes on opposite sex chromosomes (gametologs): a case study using avian CHD. Mol. Biol. Evol. **17**:1826–1832.
García-Moreno, J., M. D. Sorenson, and D. P. Mindell. 2003. Congruent avian phylogenies inferred from mitochondrial and nuclear DNA sequences. J. Mol. Evol. (in press).
Giribet, G., and W. C. Wheeler. 1999. On gaps. Mol. Phylogenet. Evol. **13**:132–143.
Gladstein, D., and W. C. Wheeler. 1996. POY: phylogeny reconstruction via direct optimization. American Museum of Natural History, New York.
Griffiths, C. S. 1994. Monophyly of the Falconiformes based on syringeal morphology. Auk **111**:787–805.
———. 1997. Correlation of functional domains and rates of nucleotide substitution in cytochrome *b*. Mol. Phylogenet. Evol. **7**:352–365.
Groth, J. G., and G. F. Barrowclough. 1999. Basal divergences in birds and the phylogenetic utility of the nuclear RAG-1 gene. Mol. Phylogenet. Evol. **12**:115–123.
Hackett, S. J., C. S. Griffiths, J. M. Bates, and N. K. Klein. 1995. A commentary on the use of sequence data for phylogeny reconstruction. Mol. Phylogenet. Evol. **4**:350–353.
Haddrath, O., and A. J. Baker. 2001. Complete mitochondrial DNA genome sequences of extinct birds: ratite phylogenetics and the vicariance biogeography hypothesis. Proc. R. Soc. Lond. B Biol. Sci. **268**:939–945.
Hagey, L. R., C. D. Schteingart, H. T. Tonnu, S. S. Rossi, D. Odell, and A. F. Hofmann. 1990. β-phocacholic acid in bile; biochemical evidence that the flamingo is related to an ancient goose. Condor **92**:593–597.
Haring, E., L. Kruckenhauser, A. Gamauf, M. J. Riesing, and W. Pinsker. 2001. The complete sequence of the mitochondrial genome of *Buteo buteo* (Aves, Accipitridae) indicates an early split in the phylogeny of raptors. Mol. Biol. Evol. **18**:1892–1904.
Härlid, A., and U. Arnason. 1999. Analyses of mitochondrial DNA nest ratite birds within the Neognathae: supporting a neotenous origin of ratite morphological characters. Proc. R. Soc. Lond. B Biol. Sci. **266**:305–309.
Härlid, A., A. Janke, and U. Arnason. 1997. The mtDNA sequence of the ostrich and the divergence between paleognathous and neognathous birds. Mol. Biol. Evol. **14**:754–761.
———. 1998. The complete mitochondrial genome of *Rhea americana* and early avian divergences. J. Mol. Evol. **46**:669–679.
Hedges, S. B., M. D. Simmons, M. A. M. Vandijk, G. J. Caspers, W. W. Dejong, and C. G. Sibley. 1995. Phylogenetic relationships of the hoatzin, an enigmatic South American bird. Proc. Natl. Acad. Sci. USA **92**:11662–11665.
Hughes, J. M. 1996. Phylogenetic analysis of the cuculidae (Aves, Cuculiformes) using behavioral and ecological characters. Auk **113**:10–22.
———. 2000. Monophyly and phylogeny of cuckoos (Aves, Cuculidae) inferred from osteological characters. Zool. J. Linn. Soc. **130**:263–307.
Hughes, J. M., and A. J. Baker. 1999. Phylogenetic relationships of the enigmatic hoatzin (*Opisthocomus hoazin*) resolved using mitochondrial and nuclear gene sequences. Mol. Biol. Evol. **16**:1300–1307.

Johansson, U. S., T. J. Parsons, M. Irestedt, and P. G. P. Ericson. 2001. Clades within the 'higher land birds', evaluated by nuclear DNA sequences. J. Zool. Syst. Evol. Res. **39**:37–51.

Johnson, K. P. 2001. Taxon sampling and the phylogenetic position of passeriformes: evidence from 916 avian cytochrome b sequences. Syst. Biol. **50**:128–136.

Johnson, K. P., and D. H. Clayton. 2000. Nuclear and mitochondrial genes contain similar phylogenetic signal for pigeons and doves (Aves: Columbiformes). Mol. Phylogenet. Evol. **14**:141–151.

Johnson, K. P., S. M. Goodman, and S. M. Lanyon. 2000. A phylogenetic study of the Malagasy couas with insights into cuckoo relationships. Mol. Phylogenet. Evol. **14**:436–444.

Johnson, K. P., and M. D. Sorenson. 1998. Comparing molecular evolution in two mitochondrial protein coding genes (cytochrome *b* and ND2) in the dabbling ducks (Tribe: Anatini). Mol. Phylogenet. Evol. **10**:82–94.

Lee, M. S. Y., and A. F. Hugall. 2003. Partitioned likelihood support and the evaluation of data set conflict. Syst. Biol. **52**:15–22.

Livezey, B. C. 1997*a*. A phylogenetic analysis of basal Anseriformes, the fossil *Presbyornis*, and the interordinal relationships of waterfowl. Zool. J. Linn. Soc. **121**:361–428.

———. 1997*b*. A phylogenetic classification of waterfowl (Aves: Anseriformes), including selected fossil species. Ann. Carnegie Mus. **66**:457–496.

Livezey, B. C., and R. L. Zusi. 2001. Higher-order phylogenetics of modern Aves based on comparative anatomy. Neth. J. Zool. **51**:179–205.

Lopez, J. V., N. Yuhki, R. Masuda, W. Modi, and S. J. O'Brien. 1994. Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. J. Mol. Evol. **39**:174–190.

Lopez, P., D. Casane, and H. Philippe. 2002. Heterotachy, an important process of protein evolution. Mol. Biol. Evol. **19**: 1–7.

Lutzoni, F., P. Wagner, V. Reeb, and S. Zoller. 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. Syst. Biol. **49**:628–651.

Mindell, D. P., M. D. Sorenson, D. E. Dimcheff, M. Hasegawa, J. C. Ast, and T. Yuri. 1999. Interordinal relationships of birds and other reptiles based on whole mitochondrial genomes. Syst. Biol. **48**:138–152.

Mindell, D. P., M. D. Sorenson, C. J. Huddleston, H. Miranda, A. Knight, S. J. Sawchuk, and T. Yuri. 1997. Phylogenetic relationships among and within select avian orders based on mitochondrial DNA. Pp. 213–247 *in* D. P. Mindell, ed. Avian molecular evolution and systematics. Academic Press, New York.

Naylor, G. J. P., and W. M. Brown. 1998. Amphioxus mitochondrial DNA, chordate phylogeny, and the limits of inference based on comparisons of sequences. Syst. Biol. **47**:61–76.

Olson, S. L., and A. Fedducia. 1980. *Presbyornis* and the origin of the Anseriformes (Aves: Charadriomorphae). Smith. Contr. Zool. **323**:1–24.

Paton, T., O. Haddrath, and A. J. Baker. 2002. Complete mitochondrial DNA genome sequences show that modern birds are not descended from transitional shorebirds. Proc. R. Soc. Lond. B Biol. Sci. **269**:839–846.

Peters, J. L. 1934. Checklist of birds of the world, Vol. 2. Museum of Comparative Zoology, Cambridge, Mass.

Pollock, D. D., D. J. Zwickl, J. A. McGuire, and D. M. Hillis. 2002. Increased taxon sampling is advantageous for phylogenetic inference. Syst. Biol. **51**:664–671.

Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics **14**:817–818.

Quinn, T. W. 1992. The genetic legacy of mother goose: phylogeographic patterns of lesser snow goose *Chen caerulescens* maternal lineages. Mol. Ecol. **1**:105–117.

Sanderson, M. J., and J. Kim. 2000. Parametric phylogenetics? Syst. Biol. **49**:817–829.

Sefc, K. M., R. B. Payne, and M. D. Sorenson. 2003. Phylogenetic relationships of African sunbird-like warblers: moho *Hypergerus atriceps*, green hylia *Hylia prasina* and tithylia *Pholidornis rushiae*. Ostrich **74**:8–17.

Seibold, I., and A. J. Helbig. 1995. Evolutionary history of New and Old World vultures inferred from nucleotide sequences of the mitochondrial cytochrome *b* gene. Philos. Trans. R. Soc. Lond. B Biol. Sci. **350**:163–178.

Shimodaira, H. 2002. An approximately unbiased test of phylogenetic tree selection. Syst. Biol. **51**:492–508.

Shimodaira, H., and Hasegawa, M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics **17**:1246–1247.

Sibley, C. G., and J. E. Ahlquist. 1972. A comparative study of the egg-white proteins of non-passerine birds. Bull. Peabody Mus. Nat. Hist. **32**:1–131.

———. 1973. The relationships of the hoatzin. Auk **90**:1–13.

———. 1990. Phylogeny and classification of birds. Yale University Press, New Haven, Conn.

Simmons, M. P., and H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. **49**:369–381.

Simmons, M. P., H. Ochoterena, and T. G. Carr. 2001. Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analyses. Syst. Biol. **50**:454–462.

Slikas, B. 1997. Phylogeny of the avian family Ciconiidae (storks) based on cytochrome *b* sequences and DNA-DNA hybridization distances. Mol. Phylogenet. Evol. **8**:275–300.

Smith, V. S. 2001. Avian louse phylogeny (Phthiraptera : Ischnocera): a cladistic study based on morphology. Zool. J. Linn. Soc. **132**:81–144.

Sorenson, M. D. 1999. TreeRot. Version 2. Boston University, Boston, Mass.

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

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

Sorenson, M. D., and R. B. Payne. 2001. A single, ancient origin of obligate brood parasitism in African finches: implications for host-parasite coevolution. Evolution **55**:2550–2567.

———. 2003. Molecular systematics: cuckoo phylogeny inferred from mitochondrial DNA sequences. *In* R. B. Payne. Bird families of the world: cuckoos. Oxford University Press, Oxford (in press).

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

Sraml, M., L. Christidis, S. Easteal, P. Horn, and C. Collet. 1996. Molecular relationships within Australian waterfowl (Anseriformes). Aust. J. Zool. **44**:47–58.

Stanley, S. E., and J. Cracraft. 2002. Higher-level systematic analysis of birds: current problems and possible solutions. Pp. 31–43 *in* R. DeSalle, G. Giribet, and W. Wheeler, eds. Molecular systematics and evolution: theory and practice. Birkhäuser Verlag, Basel, Switzerland.

Stapel, S. O., J. A. M. Leunissen, M. Versteeg, J. Wattel, and W. W. de Jong. 1984. Ratites as oldest offshoot of avian stem—evidence from μ-crystallin A sequences. Nature **311**:257–259.

Swofford, D. L. 2002. PAUP*: phylogenetic analysis using

parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Mass.
Thornton, J. W., and R. DeSalle. 2000. A new method to localize and test the significance of incongruence: detecting domain shuffling in the nuclear receptor superfamily. Syst. Biol. **49**:183–201.
van Tuinen, M., D. B. Butvill, J. A. W. Kirsch, and S. B. Hedges. 2001. Convergence and divergence in the evolution of aquatic birds. Proc. R. Soc. Lond. B Biol. Sci. **268**:1345–1350.
van Tuinen, M., C. G. Sibley, and S. B. Hedges. 2000. The early history of modern birds inferred from DNA sequences of nuclear and mitochondrial ribosomal genes. Mol. Biol. Evol. **17**:451–457.
Verheyen, R. 1956. Note systématique sur *Opisthocomus hoazin* (St. Müller). Bull. Inst. Roy. Sci. Nat. Belgique **32**:1–8.
Veron, G., and B. J. Winney. 2000. Phylogenetic relationships within the turacos (Aves, Musophagidae). Ibis **142**:446–456.
Waddell, P. J., Y. Cao, M. Hasegawa, and D. P. Mindell. 1999. Assessing the Cretaceous superordinal divergence times within birds and placental mammals by using whole mitochondrial protein sequences and an extended statistical framework. Syst. Biol. **48**:119–137.
Wetmore, A. 1960. A classification for the birds of the world. Smithson. Misc. Coll. **139**:1–37.
Wheeler, W. C. 1996. Optimization alignment: the end of multiple sequence alignment in phylogenetics? Cladistics **12**:1–9.
Woodbury, C. J. 1998. Two spinal cords in birds: novel insights into early avian evolution. Proc. R. Soc. Lond. B Biol. Sci. **265**:1721–1729.
Yang, Z. H., and D. Roberts. 1995. On the use of nucleic acid sequences to infer early branchings in the tree of life. Mol. Biol. Evol. **12**:451–458.
Zhang, D.-X., and G. M. Hewitt. 1996. Nuclear integrations: challenges for mitochondrial DNA markers. Trends Ecol. Evol. **11**:247–251.
Zischler, H., H. Geisert, A. vonHaeseler, and S. Pääbo. 1995. A nuclear fossil of the mirochondrial D-loop and the origin of modern humans. Nature **378**:489–492.
Zwickl, D. J., and D. M. Hillis. 2002. Increased taxon sampling greatly reduces phylogenetic error. Syst. Biol. **51**:588–598.

Scott Edwards, Associate Editor

Accepted April 24, 2003