Phylogenetic Relationships of the Enigmatic Hoatzin (*Opisthocomus hoazin*) Resolved Using Mitochondrial and Nuclear Gene Sequences

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The hoatzin (*Opisthocomus hoazin*) is a bizarre, long-tailed, crested bird that inhabits the riparian lowlands of South America. Among its peculiar attributes are (1) microbial foregut fermentation to convert plant cellulose in consumed foliage into simple sugars, (2) a highly modified skeleton to accommodate its large crop, and (3) in the young of this species, wing claws at the wrist joint which are used to climb among the branches of the nest tree. Consequently, the taxonomic position of this unusual bird has perplexed systematists since its description over 200 years ago. Traditionally classified among the fowl-like birds (Galliformes), recent studies have favored its placement with the cuckoos (Cuculiformes: Cuculidae). To help resolve this systematic uncertainty, we sequenced six mitochondrial genes (cytochrome oxidase I, II, and III, ATPase 8, ATPase 6, and cytochrome *b*) and one nuclear gene (c-mos), totaling 5,487 base pairs. With this large data set and an appropriate range of outgroup taxa, we demonstrate that the hoatzin should not be classified among the cuckoos or Galliformes. Instead, our analyses indicate that the hoatzin is most closely related to the turacos (Musophagiformes: Musophagidae), a small family of arboreal, frugivorous birds inhabiting sub-Saharan Africa. This phylogenetic relationship is also supported by osteological, behavioral, and fossil evidence.

Introduction

DNA sequences provide systematists with a wealth of new characters to help resolve phylogenetic relationships that have proven intractable with other data sets. However, the task of constructing accurate phylogenies from sequence data is complicated by phenomena such as saturation and multiple hits, functional constraints on molecules, biased base composition among taxa, rate variation among sites, and the problems of inferring species trees from gene trees. Phylogenetic studies using gene sequences should utilize a large amount of sequence data (Cao et al. 1994; Hillis, Huelsenbeck, and Cunningham 1994; Cummings, Otto, and Wakeley 1995; Harlid, Janke, and Arnason 1997), relatively dense taxon sampling including relevant outgroups (Graybeal 1998), and appropriate models of sequence evolution (Huelsenbeck and Crandall 1997). When any of these critical elements are lacking, phylogenetic relationships of the ingroup taxa may be estimated inaccurately.

A classic case of conflicting hypotheses of phylogenetic relationships in avian systematics is provided by the hoatzin (*Opisthocomus hoazin*), which is unquestionably among the most bizarre and enigmatic of bird species in appearance, life history, and morphological specializations. This ungainly, long-tailed bird with a bright blue face and ragged crest occupies the tropical riparian habitats of South America (Sibley and Monroe 1990). Hoatzins breed communally, in a manner similar to that of the cooperatively breeding cuckoos (Crotophaginae; *Crotophaga* spp. and *Guira guira*). Nests are built over permanent watercourses, or along streams in flood. The nestling hoatzin may be best known for the presence of well-developed reptilian-like wing claws that it uses to climb among the branches of the nesting tree. When in danger, the young hoatzin drops into the water, then uses its fore- and hindlimb claws to clamber back into the vegetation once the threat has passed (Strahl 1987). Wing claws are usually lost as the bird matures but may be retained by some adults (Olson 1992).

The hoatzin feeds on young leaves, shoots, and twigs of trees and shrubs, but unlike other birds, it uses microbial foregut fermentation to convert plant cellulose to simple sugars (Domínguez-Bello et al. 1994). Its sternum is highly modified to accommodate a large crop and has a markedly flattened posterior margin which the hoatzin rests on a branch during digestion. Kornegay, Schilling, and Wilson (1994) and Kornegay (1996) discuss the biochemical properties, molecular genetics, and evolution of multiple genes encoding for digestive and calcium-binding lysozymes in the hoatzin.

First described 200 years ago as *Phasianus hoazin* by Müller (1776), the hoatzin has been placed most frequently among the fowl-like (Galliformes) birds (fig. 1*A*) in the monotypic Opisthocomidae (Fürbringer 1888; Peters 1934), likely due to its somewhat pheasant-like appearance. Despite much evidence to the contrary, this position has persisted in some more recent taxonomic classifications (Cracraft 1981; del Hoyo, Elliott, and Sargatal 1992). In contrast, other studies have supported the classification of the hoatzin with cuckoos (Cuculiformes; fig. 1*B*) based on similarities in osteology (De Queiroz and Good 1988), and mitochondrial (Avise, Nelson, and Sibley 1994) and nuclear gene sequences (Hedges et al. 1995). Additionally, the protein electrophoresis and DNA-DNA hybridization evidence of Sibley and Ahlquist (1973, 1990) indicated that the hoatzin was a Neotropical cuckoo most closely allied with the

Abbreviations: ANSP, Academy of Natural Sciences, Philadelphia; lnL, log-likelihood; ΔlnL, log-likelihood ratio; LSU, Louisiana State University; ML, maximum likelihood; NJ, neighbor-joining; QP, quartet puzzling; ROM, Royal Ontario Museum.

Key words: *Opisthocomus hoazin*, hoatzin, cuckoos, Cuculidae, turacos, Musophagidae.

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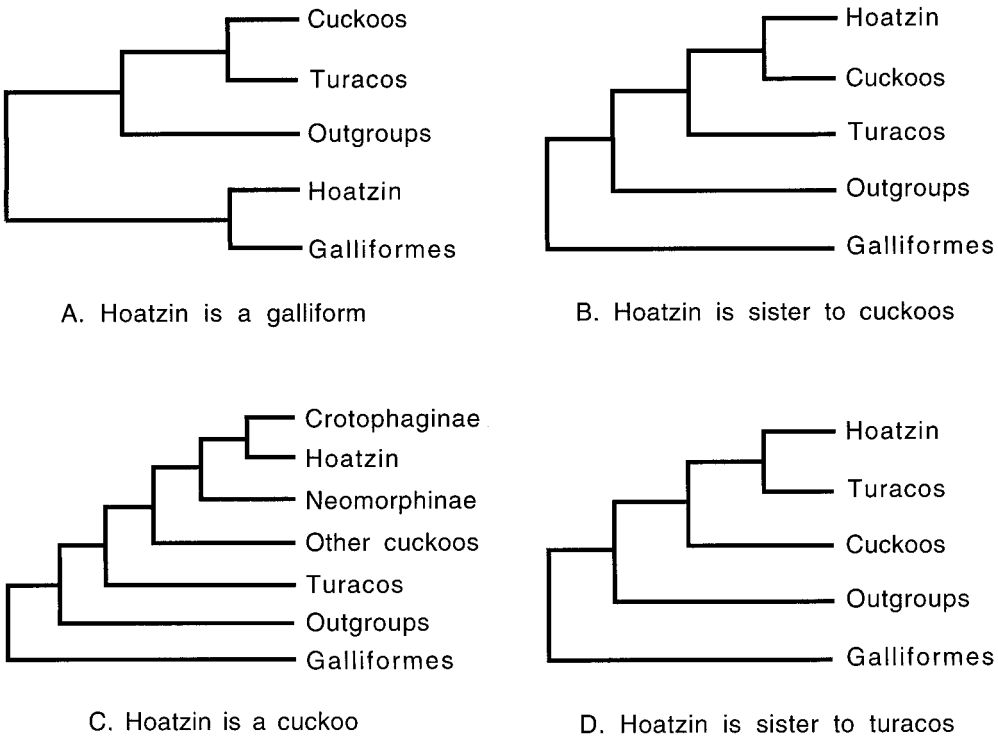

FIG. 1.—Alternative phylogenetic hypotheses of the relationships of the hoatzin. See table 2 for log-likelihood ratio tests of these trees versus the optimal ML tree for all positions in the DNA sequences.

roadrunners (e.g., Neomorphinae: *Geococcyx* spp.) and anis (Crotophaginae; fig. 1*C*). However, this association was questioned by Bock (1992), who suggested that the anisodactyl foot structure (three toes forward, one toe back) of the hoatzin was sufficient to exclude it from zygodactylous (two toes forward, two toes back) cuckoos.

A third but less popular hypothesis allied the hoatzin with turacos (Musophagidae; fig. 1*D*) on the basis of morphology (Pycraft 1895; Verheyen 1956) and behavior (Stegmann 1978). The turacos are a frugivorous family of 20 species that are restricted to the forests of sub-Saharan Africa (Sibley and Monroe 1990). The turacos have been placed among the Cuculiformes in most traditional (Linnaeus 1758; Fürbringer 1888; Peters 1940) and some contemporary (Cracraft 1981; Howard and Moore 1991) classifications. However, Sibley and Ahlquist (1990) suggested that turacos and cuckoos are not closely related. Consequently, some studies designed to establish the phylogenetic position of the hoatzin (e.g., Avise, Nelson, and Sibley 1994; Hedges et al. 1995) did not include turacos among taxa under consideration.

In this paper, we attempt to resolve the phylogenetic position of the hoatzin by employing a large data set comprising sequences from six mitochondrial genes and one nuclear gene, totaling over 5.4 kb of aligned sequence, to test the above hypotheses. We also use explicit models of DNA substitution which best fit the sequences obtained and include a range of sister group taxa (cuckoos, galliforms, turacos, and representatives from several nonpasserine orders) that have been suggested by previous studies.

Materials and Methods

Taxa

Representative taxa included the hoatzin (*Opisthocomus hoazin*; LSU B10753, B10754) and six cuckoo (Cuculidae) species from four of six cuculid subfamilies: fan-tailed cuckoo (*Cacomantis pyrrhophanus*: ROM AJB5638), golden-bronze cuckoo (*Chalcites lucidus*: ROM AJB5551, AJB5552), black-bellied cuckoo (*Piaya melanogaster*: ANSP 8348, 8603), smooth-billed ani (*Crotophaga ani*: ANSP 1468, LSU B11449), guira cuckoo (*Guira guira*: LSU B6625, ANSP LJ), and rufous-vented ground-cuckoo (*Neomorphus geoffroyi*: LSU B2319). Four turaco (Musophagidae) species were also used: green turaco (*Tauraco persa corythiax*: ROM IB63; *Tauraco persa schalowi*: ROM IB473), violet turaco (*Musophaga violacea*: ROM IB1638), go-away bird (*Corythaixoides concolor*: ROM MKP1413, MKP1427), and great blue turaco (*Corythaeola cristata*: LSU B19003).

The selection of other outgroup taxa was problematic. The DNA-DNA hybridization analyses of Sibley and Ahlquist (1990) brought into question the traditional grouping of cuckoos and turacos in the Cuculiformes. In addition, their Parvclass Passerae, which included cuckoos and other diverse taxa of unknown affinities, was unresolved at the ordinal level. If the hoatzin is sister to cuckoos, or is itself a cuckoo, then one of these enigmatic orders could be sister to the hoatzin-cuckoo

Table 1
Oligonucleotide Primers Used for Amplification, Listed from the 5′ End to the 3′ End

Primer	Position	Sequence
COIA[a]	6675–6695	AACYAACCACAAAGACATYGG
H8205[a]	8184–8205	GGTTCGATTCCTTCCTTTCTTG
L8205[a]	8184–8205	CAAGAAAGGAAGGAATCGAACC
LYSH[a]	9041–9058	TCTCTAGCTTAAAAGGCT
LYSL[a]	9034–9053	CAGCACTAGCCTTTTAAGCT
COIIIRH[a]	10148–10173	ATTATTCCGTATCGNAGNCCYTTTTG
A5REV[a]	9921–9943	TAATGGCACACCAAGCACACTCC
GLYH[a]	10729–10755	CCAGATTYTRAGATTGGAAGTCAATTG
b_1[a]	14965–14990	CCATCCAACATCTCAGCATGATGAAA
b_6[b]	16065–16089	GTCTTCAGTTTTTGGTTTACAAGAC
CMOS-L940[c]	924–944	GCCTGGTGCTCCATCGACTGG
CMOS-H1550[c]	1550–1570	GCAAATGAGTAGATGTCTGCT

NOTE.—The position of each primer is given relative to the published mtDNA and c-mos gene sequences of the domestic fowl (*Gallus gallus*; GenBank accession numbers X52392 and M19412).

[a] Source: O. Haddrath (personal communication).

[b] Source: T. Birt (in litt.).

[c] Source: Cooper and Penny (1997).

clade. Therefore, we also included in our analyses species from six other nonpasserine orders: domestic fowl (Galliformes, *Gallus gallus*: GenBank accession numbers X52392 and M19412); painted buttonquail (Gruiformes, *Turnix varia*: ROM AJB5640, IB43), great horned owl (Strigiformes, *Bubo virginianus*: ROM IB1780, IB2119), common nighthawk (Caprimulgiformes, *Chordeiles minor*: ROM IB1243, IB1685), chimney swift (Apodiformes, *Chaetura pelagica*: ROM IB1751, IB2165), and speckled mousebird (Coliiformes, *Colius striatus*: ROM MKP1484). *Turnix varia* was included because it was suggested to be paraphyletic with cuckoos and the hoatzin by Mindell et al. (1997).

DNA Extraction, Amplification, and Sequencing

Genomic DNA was extracted from liver tissue in a solution of 0.1% SDS, 100 mM Tris-HCl (pH 8.0), 100 mM NaCl, 10 mM EDTA, and 10 mg/ml proteinase K. After 12 h at 55°C, the extract was purified using Tris-HCl-saturated buffered phenol and a chloroform/isoamyl solution.

Concatenated sequences 3,768 bases in length, comprising the mitochondrial genes cytochrome oxidase I, cytochrome oxidase II, ATPase 8, ATPase 6, and cytochrome oxidase III, were obtained by amplifying four overlapping fragments via the polymerase chain reaction using the following primer pairs: COIA, H8502; L8205, LYSH; LYSL, COIIIRH; and A5REV, GLYH. Separate amplifications yielded sequences of 1,071 bases of the mitochondrial cytochrome *b* gene (primers b_1 and b_6) and 648 bases of the nuclear gene c-mos (primers CMOS-L940 and CMOS-H1550; see table 1). Cycle sequencing was performed with the Thermosequenase DYEnamic direct cycle sequencing kit (Amersham) and fluorescently labeled universal M13-tailed primers (LI-COR). Sequences were run on a LICOR 4200 bidirectional automated sequencer using the manufacturer's recommended protocols. Sequences were assembled using the computer program ESEE 3 (Cabot and Beckenbach 1989), and their identities were checked with amino acid alignment to homologous genes in *G. gallus*.

Phylogenetic Analysis

We constructed trees from nucleotide sequences by the maximum-likelihood (ML) method using PAUP* 4.0b2a (Swofford 1998). The ML analysis was further extended by quartet puzzling (QP) using PUZZLE 4.0.2 (Strimmer and von Haeseler 1996) and QP options in PAUP. QP computes ML values for all possible quartets of taxa and combines the resulting topologies into an overall tree. It also efficiently calculates support values analogous to bootstrap values for internal branches in a tree based on 1,000 puzzling steps.

In preliminary QP analyses, chi-square tests comparing the nucleotide composition of each sequence to the frequency distribution assumed in the likelihood model indicated that four taxa failed to meet the model assumptions of compositional stationarity. Subsequent QP analyses examining first, second, and third codon positions separately revealed that this heterogeneity occurred only at the third codon position. Consequently, trees were also constructed using only first and second codon positions. By the same criteria, amino acid compositions of all taxa were in accordance with the likelihood model. Gamma distribution parameters (all positions: $\alpha = 0.2$; first and second positions: $\alpha = 0.1$) estimated from the data set using PUZZLE indicated rate heterogeneity in nucleotide substitution among sites, and thus we employed four gamma rate categories to correct for substitution rate bias in all ML and QP analyses. Transition/transversion ratios, nucleotide frequencies, and purine/pyrimidine transition rates were also determined empirically using PUZZLE.

We used the Tamura-Nei 93 (TN93; Tamura and Nei 1993) model of substitution for analyses using PAUP* and PUZZLE. This model allows for inequality of base frequencies, transition and transversion rates, and rates of substitution among sites, as exhibited by our data. TN93 also accounts for unequal purine/pyrimidine transition rates (2.29 for our data), making it the preferred model. The appropriateness of TN93 was further demonstrated using log-likelihoods (lnL) as out-

lined by Huelsenbeck and Crandall (1997). These authors demonstrate that the differences in lnL scores of topologies resulting from alternate models can be used to predict the most suitable model for a specific data set. TN93 consistently produced topologies with higher lnL values than other models of substitution for our data. Trees were also constructed with the neighbor-joining (NJ) method with gamma distribution using TN93 distances.

Amino acid sequences were analyzed using QP and NJ with gamma distribution. In the QP analysis, the data set was initially partitioned into mtDNA (amino acids 1–1613: $\alpha = 0.16$) and nucDNA (amino acids 1614–1829: $\alpha = 0.35$) sequences to apply appropriate substitution models, mtREV24 (Adachi and Hasegawa 1996*b*), and JTT (Jones, Taylor, and Thornton 1992) and Dayhoff (Dayhoff, Schwartz, and Orcutt 1978), respectively. However, Cao et al. (1994) demonstrated that the mode of evolution of some mitochondrial genes is better described by models usually applied to nuclear proteins, such as JTT. Therefore, we calculated lnL scores for the partitioned data set and each gene separately to determine which of the three models was the most appropriate.

To test alternative hypotheses of hoatzin relationships, we conducted lnL ratio (ΔlnL) tests (Kinshino and Hasegawa 1989) for optimal trees based on nucleotide sequences from our study and from competing trees using PUZZLE. Tree branches from optimal trees were subsequently rearranged using RETREE (Felsenstein 1993) to simulate other hypotheses of hoatzin relationships (i.e., sister to cuckoos or to Galliformes). Estimated bootstrap probabilities for optimal and alternative trees were derived using MOLPHY (Adachi and Hasegawa 1996*a*). We also used the likelihood mapping option in PUZZLE to perform a four-cluster analysis on nucleotide and amino acid sequences. This algorithm can identify the phylogenetic relationships among taxon clusters and provide quantitative support for both optimal and alternative groupings (Strimmer and von Haeseler 1996). The hoatzin was placed in one cluster. Three additional clusters were constructed to represent avian groups in which the hoatzin has been previously classified: (1) cuckoos, (2) turacos, and (3) galliforms and others, represented in this study by *G. gallus, T. varia, B. virginianus, C. minor, C. pelagica,* and *C. striatus.*

Results

Aligned sequences from the seven genes included in this study totaled 5,487 bp (mtDNA = 4,839 bp; nucDNA = 648 bp) and had a transition/transversion ratio of 2.35. The base composition of these concatenated sequences reflected primarily the known underrepresentation of G in vertebrate mtDNA (A = 28.4%, C = 31.3%, G = 15.7%, T = 24.6%). The numbers of variable sites (excluding uninformative sites) were 1,882 (33.8%) for all positions and 498 (14.0%) for only first and second codon positions. Amino acid sequences had 288 (15.7%) variable sites. Sequence divergence values among key groups of taxa ranged from 12.3% among turacos, to 16.3% among cuckoos, to 17.5% from the hoatzin to turacos, to 20.7% from the hoatzin to cuckoos, to 22.0% from the hoatzin to *G. gallus.* All sequences have been deposited in GenBank under accession numbers AF168009–AF168119.

Irrespective of the method of phylogeny reconstruction, all trees based on nucleotide sequences placed the hoatzin in the basal position of a well-supported clade with turacos and not among or basal to cuckoos (fig. 2). In the QP tree, only 46 (1.9%) of the 2,380 quartets analyzed were unresolved, indicating that the sequences contain a strong phylogenetic signal. The ML and QP trees produced using first and second positions in codons were identical to the all-positions tree shown in figure 2 except for the placement of outgroups. Removal of the third positions also lowered the support value for the hoatzin-turaco clade to 78% from 93% in the all-positions tree. Thus, significant phylogenetic signal is retained at third positions among these taxa, and the topology of the tree is not affected by the moderate compositional heterogeneity at these positions in outgroup taxa. When the phylogenetic analysis was confined to the hoatzin and its traditionally hypothesized sister groups (cuckoos, turacos, and galliforms), the support value for the hoatzin-turaco clade increased to 98% (fig. 3).

Trees constructed using amino acid sequences were less well resolved. The NJ tree supported the hoatzin-turaco-cuckoo clade but differed from figure 2 in the positions of other taxa. QP analyses of amino acids were not informative. Our assay of models for the mitochondrial proteins indicated that combined genes were best described using mtREV24. Individually, COII, COIII, and cytochrome *b* had significantly better lnL scores using mtREV24. There were no significant differences in lnL scores for the mtREV24 and JTT models of substitution for COI, ATPase 6 and 8, and c-mos. The Dayhoff model (Dayhoff, Schwartz, and Orcutt 1978) produced poorer lnL scores in all cases and was discarded from subsequent analyses. Thus, the mtREV24 model could be reasonably applied to our entire data set. Unfortunately, all attempts to optimize models used for protein analysis failed to produce a tree that was resolved above the family level and, therefore, could not provide any indication of hoatzin relationships.

Support for the hoatzin-turaco clade (fig. 1*D*) was further demonstrated using ΔlnL tests (table 2). Relative to the optimal tree constructed using all sites in codons (fig. 2), ΔlnL values for topologies in which the hoatzin was sister to cuckoos (fig. 1*B*), was itself a cuckoo (fig. 1*C*), or was a galliform (fig. 1*A*) were at least two standard deviations worse. This was also the case for first and second positions. The estimated bootstrap probability for the optimal tree was 0.9860. Values for alternative topologies were 0.0010, 0.0005, and 0.0130 (table 2).

Likelihood mapping provided additional support for the hoatzin-turaco sister relationship. In quartet trees, support for this sister relationship was provided by 86% (all nucleotide positions), 61% (first and second positions), and 52.4% (aa; mtDNA only) of the quartets. In

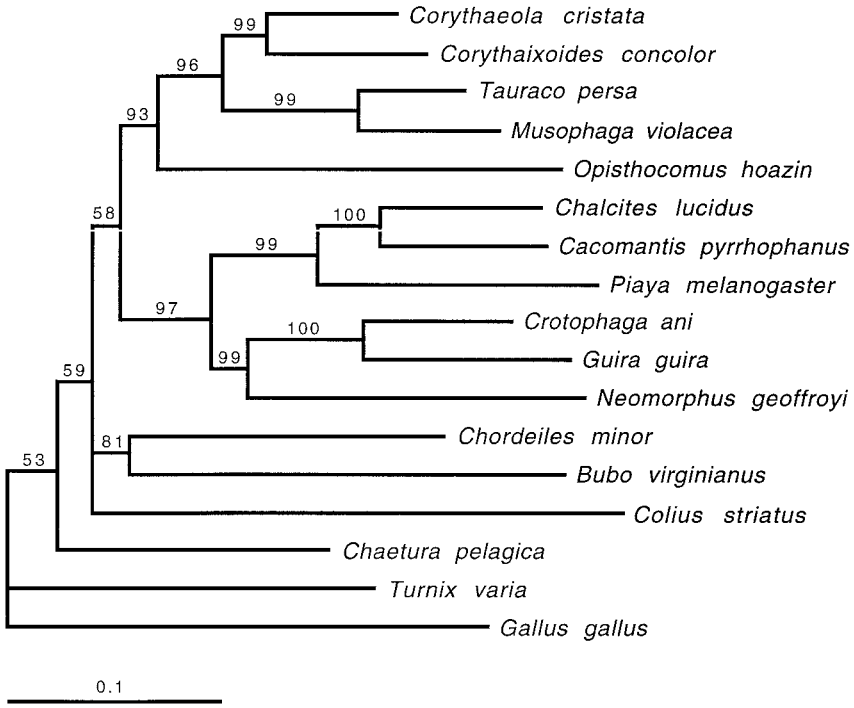

FIG. 2.—Phylogenetic relationships of the hoatzin, four turaco (Musophagidae) species, six cuckoo (Cuculidae) species, and other species representing six avian orders inferred from DNA sequences from portions of six mitochondrial genes (cytochrome oxidase I, II, III, ATPase 6 and 8, cytochrome *b*) and one nuclear gene (c-mos), totaling 5,487 bp. Tree was constructed using QP. QP support values are indicated on internodes. The optimal ML tree differed only in the positions of outgroup taxa.

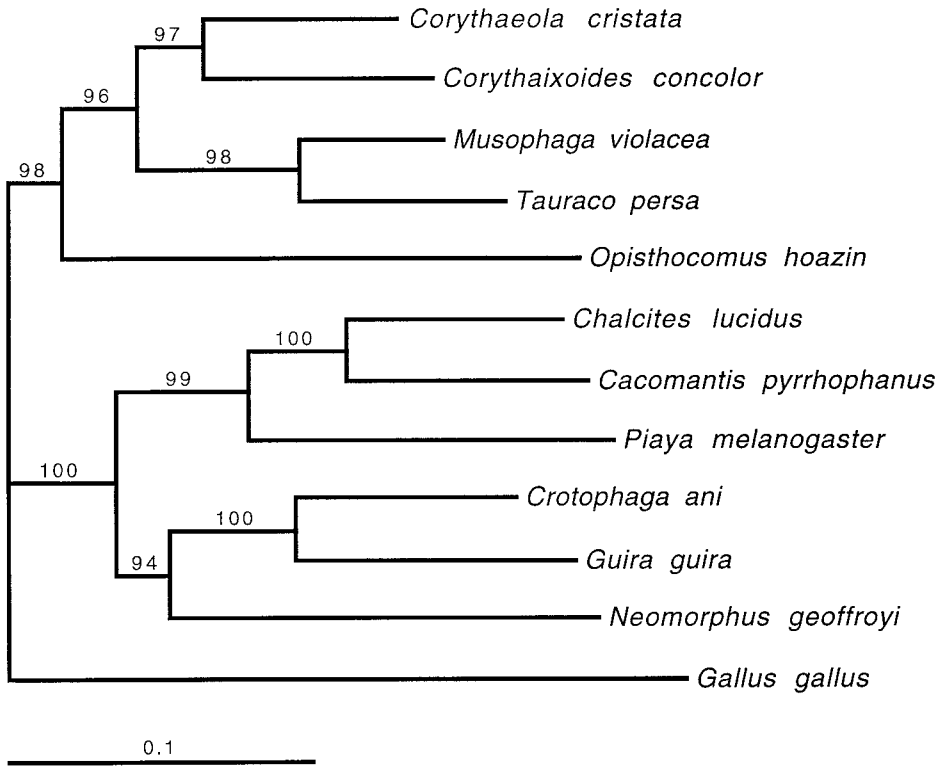

FIG. 3.—Phylogenetic relationships of the hoatzin, turacos, cuckoos, and *Gallus gallus.* The tree was constructed using ML, with QP support values indicated on the internodes.

Table 2
Log-Likelihoods for the Optimal Maximum-Likelihood Tree and the Differences in Log-Likelihoods (ΔlnL ± SE) of Alternative Trees Relative to that of the Optimal Tree

Tree	All Positions ΔlnL ± SE	First and Second Positions ΔlnL ± SE	pBoot[a]
Hoatzin is sister to turacos (Verheyen 1956; this study; fig. 1*D*)	(−39,417.23)	(−13,809.08)	0.9860
Hoatzin is sister to cuckoos (Hedges et al. 1995; Mindell et al. 1997; fig. 1*B*)	−25.86 ± 8.82	−14.29 ± 6.67	0.0010
Hoatzin is a cuckoo (Sibley and Ahlquist 1973, 1990; fig. 1*C*)	−135.47 ± 21.57	−56.29 ± 14.36	0.0005
Hoatzin is a galliform (Peters 1934; Cracraft 1981; fig. 1*A*)	−37.97 ± 12.89	−25.32 ± 12.73	0.0130

NOTE.—All alternative trees are significantly worse ($P < 0.5$) than optimal tree. The all-positions optimal tree obtained with quartet puzzling is illustrated in figure 2, and alternative trees are shown in figure 1.

[a] Estimated bootstrap probability for all-positions trees derived using MOLPHY (Adachi and Hasegawa 1996*a*).

contrast, the hoatzin-cuckoo sister relationship had 0% and 11% support for all positions and for first and second positions, respectively. Support for a sister relationship with other groups was also lower, at 13.9% of quartets for all positions and 27.8% for first and second positions. Likelihood mapping based on nucDNA amino acid sequences was not considered because 78.0% of the data were unable to provide support for any cluster arrangement.

Discussion

Bock (1992) listed the taxonomic affinities of the hoatzin among the most vexing problems in avian macrosystematics. It is so divergent morphologically, its sister relationships have remained unclear for more than 200 years. As a result, it has been allied with Galliformes in 17, turacos in 4, and cuckoos in 8 major classifications. In addition, the hoatzin has been placed in the monotypic order, Opisthocomiformes, 12 times (Sibley and Ahlquist 1973, 1990). Modern studies attempting to resolve this systematic uncertainty have met with varying success, partly because they have been unduly influenced in their choice of outgroups by DNA-DNA hybridization results (Sibley and Ahlquist 1990) or by limited taxon sampling.

In the past decade, several studies have demonstrated that the hoatzin is not a gallinaceous bird. Sibley and Ahlquist (1990) used DNA-DNA hybridization to classify the hoatzin with the cuckoos (Cuculiformes, their study), adjacent to roadrunners (Neomorphinae) and anis (Crotophaginae). Furthermore, they concluded that turacos and cuckoos were not sister taxa and subsequently moved turacos (Musophagiformes) into a different superorder (Strigimorphae). Avise, Nelson, and Sibley (1994) sequenced 961 bp of the mitochondrial gene cytochrome *b* but could not resolve the phylogenetic position of the hoatzin using a variety of analyses. More recently, Hedges et al. (1995) used two mitochondrial genes (12S and 16S rRNA) and one nuclear gene (α-crystallin) to strongly support a sister relationship between the hoatzin and cuckoos. Unfortunately, turacos were not included among the taxa under investigation in either Avise, Nelson, and Sibley (1994) or Hedges et al. (1995).

De Queiroz and Good (1988) examined the pattern and number of dried scleral ossicles and also suggested that the hoatzin was more closely related to cuckoos than to Galliformes. However, their results were inconclusive in determining the hoatzin's sister taxon and could be equally persuasive in supporting a hoatzin-cuckoo or hoatzin-turaco relationship. A phylogenetic study of avian orders by Mindell et al. (1997), based on 859 bp of mitochondrial 12S rRNA, was also inconclusive in this regard. They found the hoatzin and Hartlaub's turaco (*Tauraco hartlaubi*) to be monophyletic using equal character weighting but paraphyletic under a 5:1 weighting of transitions to transversions. An additional analysis using transversion parsimony with a small subset of the original taxa indicated that the hoatzin and the black-billed cuckoo *(Coccyzus erythropthalmus*) were sister taxa. Mindell et al. (1997) concluded that the hoatzin was indeed sister to cuckoos because the latter analysis was more appropriate. Nevertheless, the different topologies generated from the data set illustrate the problems associated with the use of different taxon sampling and different weighting schemes. Regardless, it is likely that the amount of sequence data used by Mindell et al. (1997) was simply not large enough to adequately resolve the phylogenetic position of the hoatzin.

In sharp contrast, our phylogenetic analyses of the much longer DNA sequences from protein-coding genes in both organellar and nuclear genomes strongly support a sister relationship between the hoatzin and turacos, not cuckoos. The greater support for key nodes in trees afforded by longer sequences is in line with other molecular systematic studies (Cao et al. 1994; Hillis, Huelsenbeck, and Cunningham 1994; Cummings, Otto, and Wakeley 1995; Harlid, Janke, and Arnason 1997) and points to the power of these large data sets (and appropriate taxon sampling) in resolving problematical relationships. Earlier taxonomists relying predominantly on morphological characters first suggested that the hoatzin was allied with turacos. Pycraft (1895) noted similarities between the hoatzin and turacos in their pterylosis, or feather tract patterns. Additionally, Verheyen (1956) listed 50 osteological characters that united the hoatzin and turacos and consequently placed them in the same order (Musophagiformes) in his classification. Stegmann (1978) noted that both young turacos and young hoatzins use their wings and the claws of digits I and II for climbing among the branches of the nesting tree long

before their flight feathers have fully developed. Both taxa share a characteristic retardation of growth of the outer primaries that facilitates this form of locomotion. Stegmann (1978) added that although the wing and associated structures of the hoatzin more closely resemble those of cuckoos, if the taxonomic importance of these characters and the peculiarity of their ontogenetic development are considered, the hoatzin should be allied with the turacos.

The sister relationship between the hoatzin and the turacos presents some interesting biogeographical considerations given their respective endemicity in South America and Africa, but it has been well established that current distribution patterns of many bird species bear little resemblance to those of the past (Olson 1985). Furthermore, fossils attributable to the Opisthocomiformes and Musophagiformes have been found outside of the present ranges of the extant members—in eastern Columbia (Miller 1953) and southern Argentina (Cracraft 1971) and in France (Ballmann 1970), Germany (Ballmann 1972), and northern Africa (Olson 1985), respectively. Interestingly, elements of both taxa are combined in the Lower Eocene fossil *Foro panarium* of Green River, Wyoming. This species has a skull and mandible most like the hoatzin but shows some similarities to turacos in postcranial skeletal elements (Olson 1992). This is not to suggest that *F. panarium* represents the ancestor of the hoatzin-turaco radiation, because there is another contemporary fossil more closely associated with the modern hoatzin (Cracraft 1971). Rather, it indicates the existence of a lineage of birds that may have shared an ancestor with the proto-hoatzin-turaco, a species that has since been obscured by a distant point of divergence and subsequent adaptation to highly specialized lifestyles.

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