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A Phylogenetic Study of the Malagasy Couas with Insights into Cuckoo Relationships

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The avian family Cuculidae (cuckoos) is a diverse group of birds that vary considerably in behaviors of interest to behavioral ecologists, e.g., obligate brood parasitism and cooperative breeding. The taxonomy of this group has historically been relatively stable but has not been extensively evaluated using molecular methods. The goal of this study was to evaluate phylogenetic relationships within the ecologically diverse genus *Coua* and the placement of *Coua* among major cuckoo lineages. We sequenced 429 bp of cytochrome *b* (cyt *b*) and 522 bp of ND2, both mitochondrial genes, for 26 species of cuckoos spanning 13 genera. We also included the enigmatic hoatzin (*Opisthocomus hoazin*) and used two *Tauraco* species as outgroups. ND2 exhibited higher rates of DNA sequence and amino acid substitution than cyt *b;* however, this did not greatly affect the overall levels of phylogenetic resolution and support provided by these two genes. Combined analyses produced two alternative phylogenies, depending on weighting scheme, both of which were fully resolved and were characterized by high bootstrap support. These phylogenies recovered monophyly for all of the traditional cuckoo subfamilies and indicated, with strong support, that the hoatzin is outside of Cuculidae. Within *Coua,* an arboreal and a terrestrial clade were identified. In contrast, habitat choice of *Coua* species did not greatly reflect the phylogeny.

Key Words: **molecular phylogeny; cytochrome *b;* ND2; Cuculidae; hoatzin.**

INTRODUCTION

The cuckoos (Aves, Cuculiformes, Cuculidae) are a diverse group of birds showing a wide range of morphologies and behaviors. Many species in this group are obligate brood parasites, and this behavior is thought to have evolved twice in cuckoos (Payne, 1997). In addition, several species breed cooperatively, which is relatively rare in birds. Hence, an understanding of the phylogeny of cuckoos will aid in understanding the evolution of the remarkable behavioral and ecological diversity in this group.

Cuckoo species occur on all continents, and several classification schemes have been proposed to divide this family into subgroups. Howard and Moore (1994) divide the cuckoos (Family: Cuculidae) into six subfamilies and place the hoatzin (*Opisthocomus hoazin*) in a monotypic family (Opisthocomidae). The classification of Sibley and Ahlquist (1990) is somewhat similar except that five of the six subfamilies of Howard and Moore (1994) are elevated to family status. In addition, Sibley and Ahlquist (1990) recognize *Opisthocomus* as a family within the Order Cuculiformes. Payne (1997) recognized six subfamilies: Cuculinae, Phaenicophaeinae, Centropodinae, Coccyzinae, Crotophaginae, and Neomorphinae. These subfamilies are slightly different from those of Howard and Moore (1994), and we use the classification scheme of Payne (1997) for convenience throughout this paper because it in general summarizes current taxonomic understanding of cuckoo relationships.

The systematic relationships among cuckoo lineages (subfamilies) is uncertain. We wanted to address several questions in particular: (1) is there evidence for monophyly of cuckoo subfamilies; (2) what are the relationships among the subfamilies; (3) does the enigmatic hoatzin fall within the cuckoos, as suggested by some studies (Sibley and Ahlquist, 1990; but see Avise *et al.,* 1994); (4) is the genus *Coua,* a group that shows considerable ecological variation (Urano *et al.,* 1994), monophyletic; and (5) what are the relationships within the genus *Coua?*

In this study, we wanted to determine the utility of mitochondrial protein-coding gene sequences in reconstructing a phylogeny for cuckoo lineages. Some authors (Meyer, 1994; Russo *et al.,* 1996) suggest that the high level of protein functional constraints on cytochrome *b* (cyt *b*) limits its utility in phylogeny reconstruction compared to that of some other mitochondrial

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protein-coding genes. We compare cyt *b* with NADH dehydrogenase subunit 2 (ND2) in terms of phylogenetic utility, rates of substitution, and magnitude of multiple substitution.

METHODS

DNA Sequencing

We extracted total genomic DNA from the tissue samples (no blood was used) listed in Table 1 using a Qiaquick Tissue Kit (Qiagen) with manufacturer's protocols. Samples of additional genera of cuckoos were largely unavailable. We performed PCRs on these extracts with the primers L14841 (Kocher *et al.,* 1989)–H4a (Harshman, 1996) for cyt *b* and L5758–H6313 (Johnson and Sorenson, 1998) for ND2. We conducted PCR amplifications in a Perkin–Elmer DNA Thermal Cycler 480 with the reactions conditions of one cycle of 3 min at 93°C, 1 min at 50°C, and 2 min at 72°C, followed by 35 cycles of 1 min at 93°C, 1 min at 52°C, and 1 min 20 s at 72°C. A 10-min extension at 72°C and a hold at 4°C followed these cycles. Reactions consisted of 1 to 5 µl DNA template, 3 µl of 10 µM solution of each primer, 3.9 ml of 25 mM $MgCl_2$, 2.5 µl of 20× reaction buffer (Epicentre Technologies), 1 µl dNTPs, 0.5 µl Thermo flavus polymerase (Epicentre), and 35 µl distilled water. After amplification, we purified PCR products using a Qiagen PCR Purification kit. We sequenced these purified products using an ABI Prism Dye Terminator Reaction Kit FS and manufacturer's protocols using the primers L14841–H15299 (Kocher *et al.,* 1989) for cyt *b* and L5758–H6313 for ND2. We purified sequenced products using Centrisep columns and manufacturer's protocols. Reactions were electrophoresed on an acrylamide gel using an ABI 377 Automated Sequencing machine. We resolved resulting chromatograms and aligned sequences between species using Sequencher 3.1 (GeneCodes). This produced a

TABLE 1

List of Cuckoo Subfamilies, Genera, and *Coua* Species, Including Samples Used in This Study

Subfamily	Genera	Samples
Cuculinae	*Clamator, Pachycoccyx, Cuculus, Cercococcyx, Cacomantis, Chrysococcyx, Rhamphomantis, Surniculus, Caliechthrus, Microdynamis, Eudynamys, Scythrops*	*Cacomantis flabelliformis* (C-C627) *Cacomantis merulinus* FMNH 6635 *Cacomantis variolosus* FMNH 6655 *Cercococcyx montanus* FMNH 3584 *Cuculus fugax* FMNH 6669 *Cuculus saturatus* FMNH 6671 *Cuculus vagans* FMNH 6025 *Chrysococcyx klaas* FMNH 2051 *Chrysococcyx lucidus* C-C299 *Eudynamys scolopacea* C-MV1698 *Scythrops novaehollandiae* C-MV1627
Phaenicophaeinae	*Ceuthmochares, Phaenicophaeus, Carpococcyx,* *Coua* *gigas* *coquereli* *serriana* *reynaudii* *cursor* *ruficeps* *cristata* *verreauxi* *caerulea*	*Coua caerulea* FMNH 564 *Coua cristata* FMNH 528 *Coua cursor* FMNH 531 *Coua serriana* FMNH 703 *Coua reynaudii* FMNH 6172 *Coua ruficeps* FMNH 708
Centropodinae	*Centropus*	*Centropus phasianinus* C-JCW 034 *Centropus viridis* FMNH 527
Coccyzinae	*Coccyzus, Saurothera, Hyetornis, Piaya*	*Coccyzus americanus* FMNH 3360 *Coccyzus melacoryphus* FMNH 4742 *Piaya cayana* FMNH 4221 *Piaya minuta* FMNH 4218
Crotophaginae	*Crotophaga, Guira*	*Crotophaga major* FMNH 4797 *Guira guira* FMNH 3383
Neomorphinae	*Tapera, Dromococcyx, Morococcyx, Geococcyx, Neomorphus*	*Geococcyx californicus* BMNH X7470
Outgroup		*Opisthocomus hoazin* A *Tauraco corythaix* FMNH 8113 *Tauraco johnstoni* FMNH 2922

Note. Sources of tissue collections: FMNH, Field Museum of Natural History; BMNH, J. F. Bell Museum of Natural History; C, Les Christidis (Museum of Victoria, Australia); A, John Avise (University of Georgia, Athens).

data set consisting of 429 bp of cyt *b* and 522 bp of ND2 (GenBank accession nos. AF204974–AF205031).

Analysis

To compare rates and types of substitutions between cyt *b* and ND2, we computed overall percentage sequence divergence (pairwise percentage of mismatched sites) and transition and transversion differences for each codon position in pairwise comparisons using PAUP* (Swofford, 1998). We plotted the percentage sequence divergence for ND2 against that for cyt *b*. Furthermore, the percentage transition and transversion substitutions at each position in pairwise comparisons were plotted against total percentage sequence divergence to evaluate the potential for multiple substitutions in different codon positions in each gene. We computed the number of variable and informative sites for each gene and compared these between genes using a *z* approximation statistic (Milton and Arnold, 1990). We translated gene sequences and compared the proportion of variable amino acid sites as well as the mean amino acid substitution rates for both genes.

For all phylogenetic analyses we used two turacos (Order: Musiphagiformes) as a composite outgroup, since this order is traditionally considered the sister taxon to the Cuculiformes (Payne, 1997). To determine if cyt *b* and ND2 could be considered samples of the same underlying phylogeny (Bull *et al.,* 1993), we conducted a partition homogeneity test (Farris *et al.,* 1994, 1995; Swofford, 1998) using PAUP* (Swofford, 1998). Since this test indicated no significant incongruence between the two genes, we performed a limited number of the following analyses with the two genes separated and performed all of the analyses with the combined data set. To reconstruct the phylogeny we conducted 10 random addition replicate heuristic parsimony searches using PAUP* (Swofford, 1998). We used several different transversion weighting schemes to determine the sensitivity of the phylogeny to weighting, which included weighting transversions over transitions from 1:1 to 10:1. To determine the level of support for various nodes in the phylogenies, we conducted bootstrap analyses (Felsenstein, 1985) using 100 full heuristic bootstrap replicates.

To compare our results with phylogenies from other data sets, we first evaluated the likelihood of alternative topologies based on DNA–DNA hybridization (Sibley and Ahlquist, 1990) and behavioral, ecological, and morphological characters (Hughes, 1996). We used Kishino–Hasegawa tests (Kishino and Hasegawa, 1989) comparing our unweighted and transversion weighted trees and the alternative topologies. We estimated the best-fit-likelihood model for these tests by evaluating the likelihood of the unweighted parsimony tree under increasingly complex models of substitution. We used likelihood ratio tests to choose the simplest model that could not be rejected in favor of a more complex one. In this case, a general time-reversible model (six substitution types), using empirically estimated base frequencies, and a gamma distribution for rate heterogeneity (eight rate categories, shape parameter = 0.33) was the best-fit model. In each case we used an alternative topology that contained relationships proposed in that alternative but left relationships between taxa not included in the alternative topology as is in the transversion-weighted mtDNA sequence tree. A significant result indicates that an alternative topology provides a significantly worse fit to the data. To determine if mtDNA sequence data (this study) and behavioral/ecological data (Hughes, 1996) are in significant conflict with regard to the phylogeny of cuckoos, we conducted a partition homogeneity test (Farris *et al.,* 1994, 1995; Swofford, 1998) using only taxa in common to both studies and only informative characters. We performed a bootstrap analysis (Felsenstein, 1985) of Hughes (1996) behavioral/ecological data set using only the taxa in the partition homogeneity test comparison.

RESULTS

Sequence Variation and Evolution

For cyt *b,* 186 of 429 bases were variable and 152 of these were phylogenetically informative. ND2 had a larger proportion of variable (324 of 522) and phylogenetically informative (275) sites (both $P < 0.0001$). Percentage sequence divergence in pairwise comparisons ranged from 0.0 to 20.1% for cyt *b* and 0.6 to 29.5% for ND2. At divergences exceeding 8% for cyt *b,* ND2

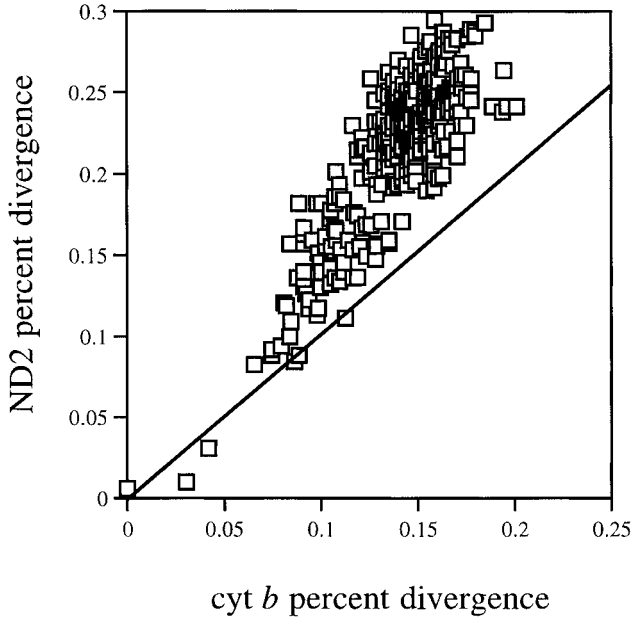

FIG. 1. Plot of percentage sequence divergence of pairwise comparisons for ND2 against cyt *b*. The line indicates the expected rates of divergence under equal substitution rates for both genes.

has a higher rate of detected substitution (Fig. 1), with ND2 pairwise divergences in general larger than pairwise cyt *b* divergences. While transition and transversion substitutions accumulate at similar rates at third positions for both genes (Fig. 2), an elevated rate of transition and transversion substitutions at first and second positions in ND2 over cyt *b* (not shown) can largely account for the overall increased substitution rate for ND2. An evaluation of the ratio of third position transition differences to transversion differences

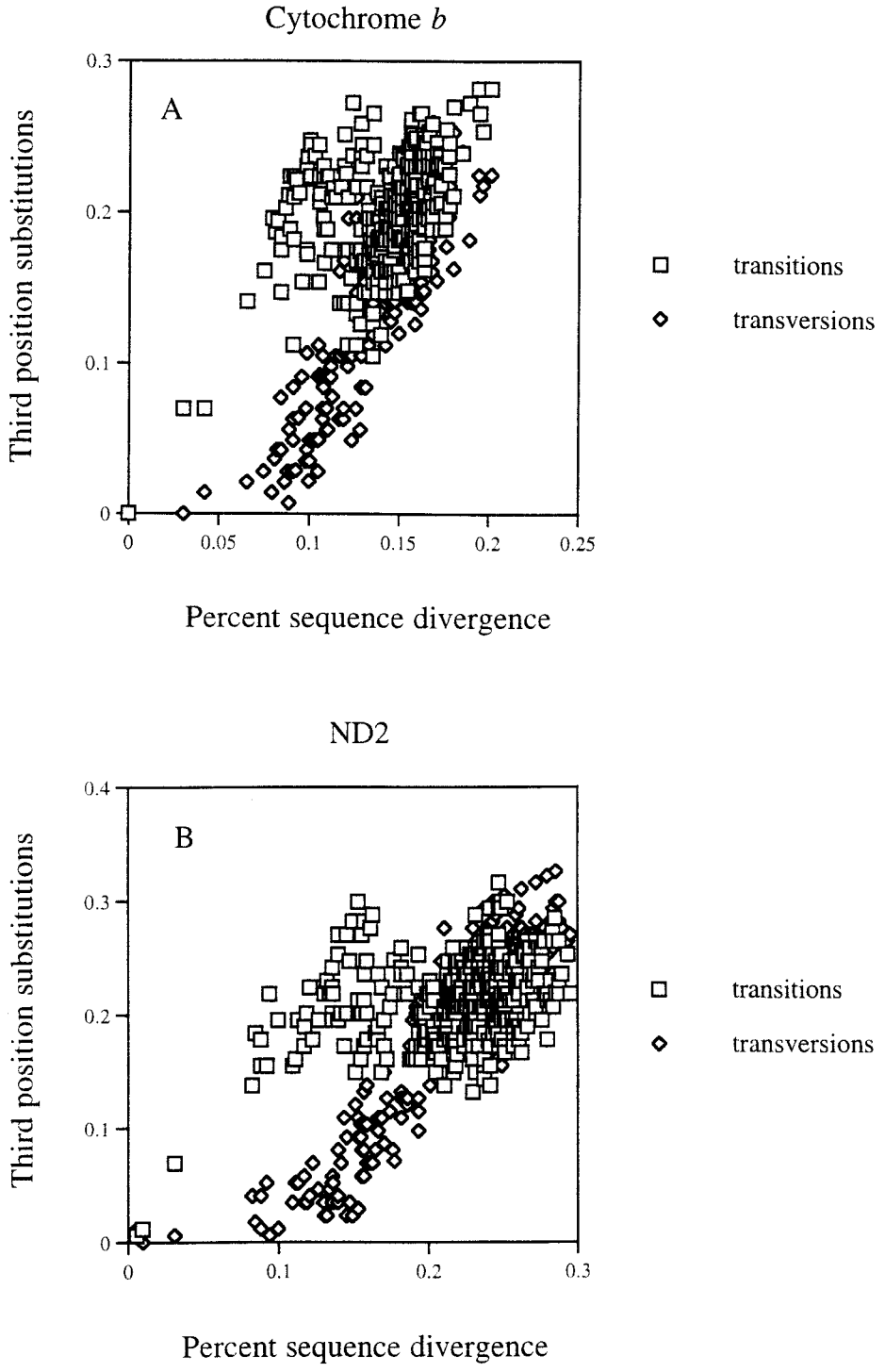

FIG. 2. Plot of third position transition and transversion substitutions against total percentage sequence divergence for each gene. (A) cyt *b;* (B) ND2.

(Sturmbauer and Meyer, 1992) revealed a transition/transversion ratio of approximately 5:1 for both genes. The ratio of the number of transitions reconstructed over the unweighted tree is 2.5 for cyt *b* and 2.2 for ND2. Presumably, these values are lower than the ratios estimated using Sturmbauer and Meyer's (1992) method because of unrecovered multiple substitutions for reconstructed changes (Johnson and Sorenson, 1998).

The differences in rates of substitution can largely be accounted for by differences in the rates of amino acid substitutions. Over the combined unweighted tree, cyt *b* shows a much lower average number of amino acid substitutions per amino acid residue (0.52) than does ND2 (2.36; Mann–Whitney U test, $P < 0.0001$). In addition, the proportions of variable amino acid sites were higher ($P < 0.0001$) for ND2 (59.2%) than they were for cyt *b* (24.5%).

Phylogeny

Unweighted parsimony analyses of the two genes independently resulted in three trees (length = 761, rescaled consistency index = 0.181) for cyt *b* and five trees (length = 1345, rescaled consistency index = 0.213) for ND2 (not illustrated herein). Of the nodes in the strict consensus tree for each gene, 14 were supported in over 50% of bootstrap replicates for cyt *b*, and 16 were supported in over 50% of bootstrap replicates for ND2. Nodes receiving over 60% bootstrap support were compatible between the cyt *b* and ND2 trees. A partition homogeneity test (Farris *et al.,* 1994, 1995; Swofford, 1998) indicated no evidence that the two data sets contained significant incongruence ($P = 0.90$); so, we combined the two data sets in the remaining analyses.

Unweighted parsimony analysis of the combined gene regions produced one fully resolved tree (Fig. 3). Of 27 nodes in this tree, 23 were supported in over 50% of bootstrap replicates and, in general, bootstrap values increased over analyses of either gene independently. In this tree, all of the subfamilies of Payne (1997) appeared as monophyletic and bootstrap support for the monophyly of each of these families exceeded 85% in each case. Support for the monophyly of cuckoos

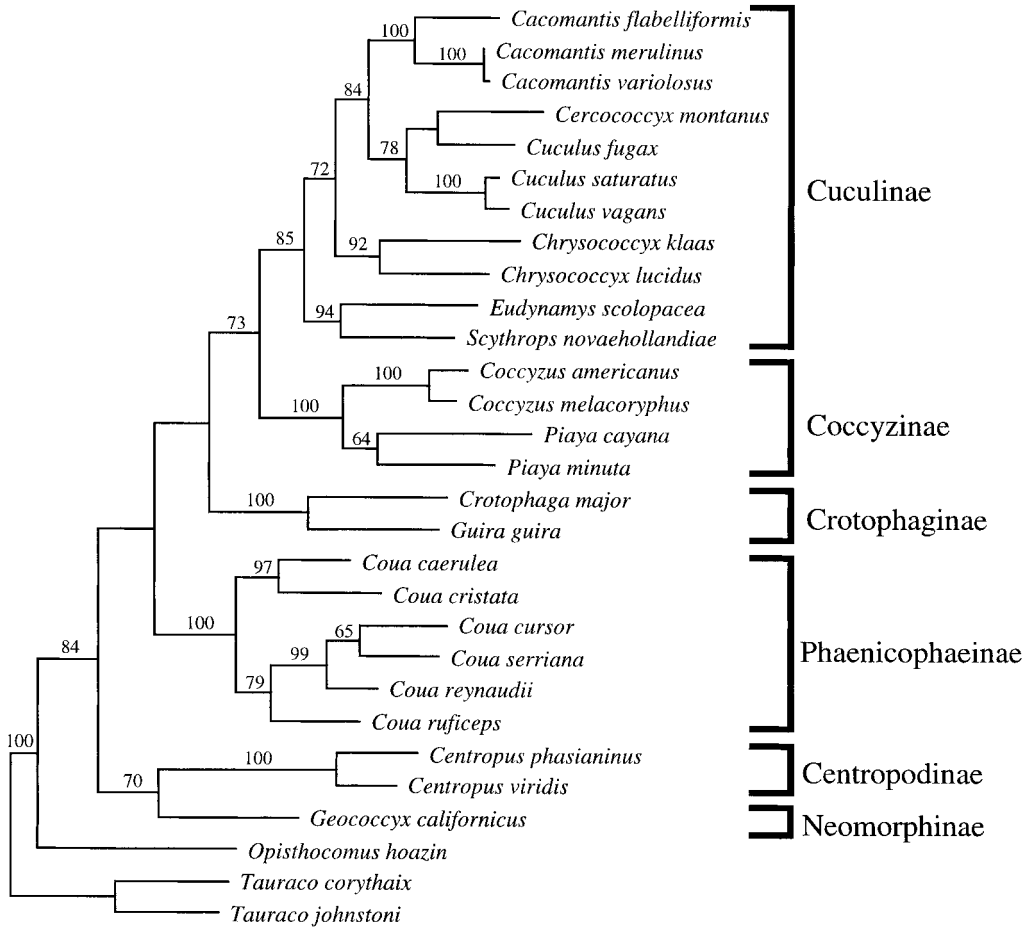

FIG. 3. Single most-parsimonious tree derived from combining gene regions and 1:1 or 2:1 weighting of transversions over transitions. For unweighted changes, length = 2112 and rescaled consistency index = 0.20. Bootstrap values from 100 full heuristic replicates are indicated above each branch. Subfamily designations are indicated along right margin.

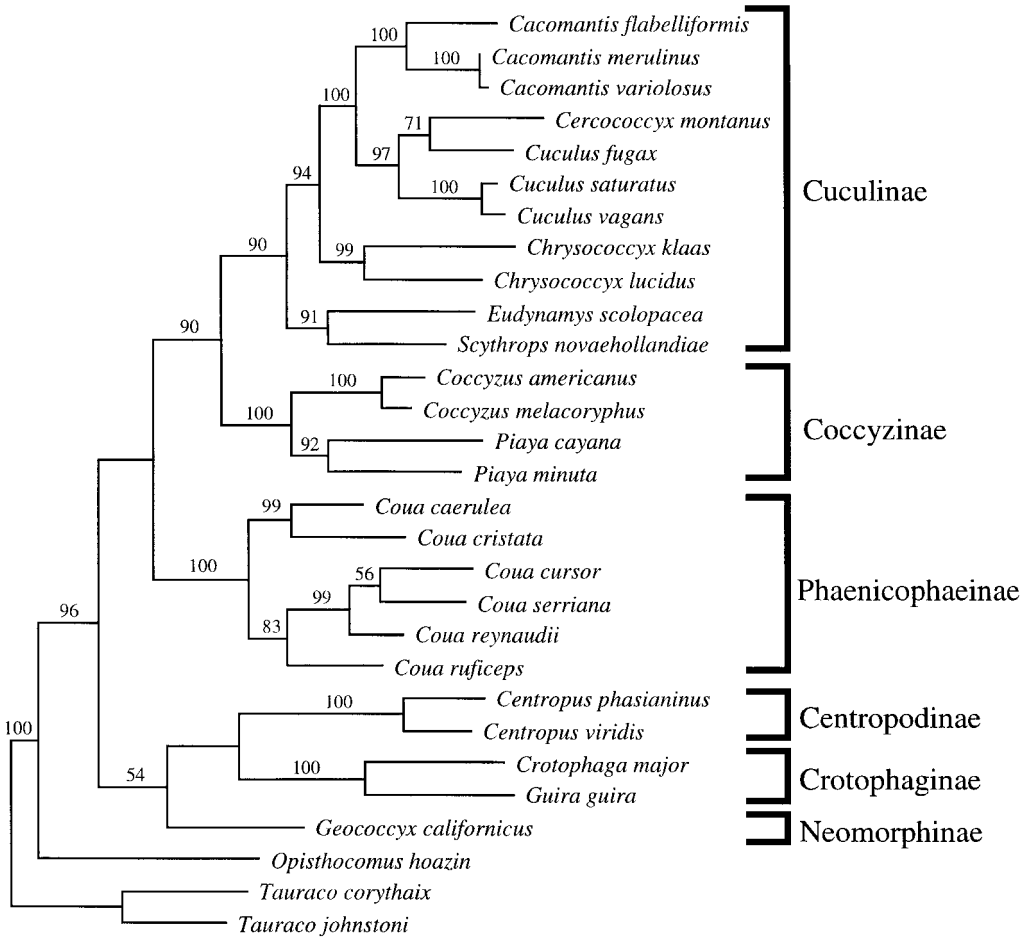

FIG. 4. Single most-parsimonious tree derived from combining gene regions and 3:1 to 10:1 weighting of transversions over transitions. For 3:1 weighted changes, length = 3335 and rescaled consistency index = 0.25. Bootstrap values from 100 full heuristic replicates are indicated above each branch. Subfamily designations are indicated along right margin.

(exclusive of *O. hoazin,* which appears as sister to the cuckoos) was 84%.

Because of the high potential for multiple transition substitution at third positions (Fig. 2), we evaluated the sensitivity of tree topology to alternate weighting schemes. Transversion weighting of 2:1 produced a single tree identical to the unweighted tree (Fig. 3). However, weighting transversions over transitions by 3:1 or higher produced an alternate topology (a single fully resolved tree, Fig. 4). This tree was also highly supported in bootstrap replicates and differed from the unweighted tree only by moving the Crotophaginae (*Crotophaga* and *Guira*) to be sister to the Centropodinae (*Centropus*), instead of sister to the Coccyzinae plus Cuculinae. Both of these alternative placements of Crotophaginae are not well supported in bootstrap replicates under either weighting scheme. In other respects, the tree generated by high transversion weighting is identical to the unweighted tree.

When we evaluated the likelihood of alternative topologies (DNA–DNA hybridization tree: Sibley and Ahlquist [1990]; behavioral/ecological/morphological trees: Hughes [1996]) using the mtDNA sequence data, we found that both the DNA–DNA hybridization tree and the behavioral/ecological/morphological trees were significantly worse (Kishino and Hasegawa, 1989) than the unweighted tree (all $P < 0.005$, Table 2). In contrast, the transversion-weighted topology was not sig-

TABLE 2

Tree Comparisons Using Likelihood Ratio Tests of the mtDNA Data Set

Tree	ln likelihood	SD (diff.)	*t* statistic	*P* value
Unweighted mtDNA[a]	−9849.7			
Weighted mtDNA	−9853.9	4.2	7.5	0.57
DNA hybridization	−9892.7	43.0	14.3	0.0027
Behavior/ecology	−9983.2	133.5	21.3	<0.0001
Behav./ecol./morph.	−9945.4	95.7	17.6	<0.0001

[a] Indicates tree with highest likelihood in comparisons.

nificantly worse ($P = 0.57$) than the unweighted topology under the maximum-likelihood model. Although the topology of the behavioral/ecological data resulted in a significantly worse likelihood for the mtDNA data, a partition homogeneity test revealed no significant conflict ($P > 0.10$) between mtDNA sequence and behavioral/ecological data sets. This is likely due to the fact that only six nodes for among-genus relationships (using the taxa in common to both data sets) showed bootstrap support over 50% in the behavioral/ecological data, and four of these nodes appeared in the mtDNA tree.

DISCUSSION

Given the high levels of homoplasy and potential for multiple substitutions in both the cyt *b* and ND2 genes, it is remarkable that both genes and the combined analysis produce such highly resolved and well supported trees for relationships among Cuculiformes. In the weighted topology, only 5 of 25 nodes are supported by less than 80% in bootstrap replicates. Other studies using twice the amount of sequence of cyt *b* and ND2 for similar numbers of taxa have recovered a lower proportion of nodes with high bootstrap support than that in the cuckoo phylogeny (Fig. 4) (e.g., Johnson and Sorenson, 1998; Johnson and Lanyon, 1999). This comparison suggests that the ability of mitochondrial protein-coding genes to recover a strongly supported phylogenetic history depends on the timing of speciation events in that phylogeny and on the patterns of DNA substitution.

In comparing substitution patterns and rates of cyt *b* with ND2 in cuckoos, we found that substitutions accumulate at similar rates at low pairwise cyt *b* divergences (<8%); however, at higher divergences a larger proportion of substitutions are apparent in ND2. In dabbling ducks (Anatidae), Johnson and Sorenson (1998) found similar rates of DNA and amino acid substitution in cyt *b* and ND2 up to at least 13% for both genes. In contrast, in blackbirds (Icteridae) ND2 pairwise divergences were higher than those of cyt *b* above 5% cyt *b* sequence divergence (Johnson and Lanyon, 1999). Reasons for differences in relative substitution rates in cyt *b* and ND2 across avian groups are unknown but merit further investigation.

While third positions accumulate changes at similar rates in both genes, amino acid substitutions occur approximately four times faster in ND2 than in cyt *b,* resulting in a higher rate of transition and transversion substitution at first and second positions. Although increased constraints on cyt *b* amino acid residues have been noted and suggested to influence the ability of cyt *b* sequences to recover phylogenetic relationships (Meyer, 1994; Russo *et al.,* 1996), these constraints do not appear to greatly influence the overall levels of resolution and support for trees derived from cyt *b* versus ND2 in this study. The number of nodes which showed bootstrap support over 50% in unweighted cyt *b* analyses was only two less than that for ND2, which might be expected, given that the ND2 sequence was approximately 100 bp longer than the cyt *b* sequence. Combining the two gene regions greatly increased overall resolution and support for phylogenetic trees.

The overall levels of sequence divergence between cuckoo lineages is remarkable with respect to intraordinal variation in birds. Within cuckoos (Cuculidae), the maximum sequence divergence for ND2 is 29.5% and for cyt *b* it is 17.7%. The maximum ND2 divergence is larger than that between chicken (Galliformes) and waterfowl (Anseriformes) (24.8% [Johnson, unpublished]), considered by many authors (e.g., Sibley and Ahlquist, 1990) to be among some of the oldest bird lineages. The high sequence divergences between all the cuckoo subfamilies suggests that this avian group is extremely old, assuming that molecular substitution is relatively constant across avian groups. The ancient origins of cuckoos has also been suggested by Sibley and Ahlquist (1990), who found DNA–DNA hybridization distances to be relatively large (mean delta $T_{50}H$ for cuckoo basal split is 17.6; that for Galliformes vs Anseriformes is 22.9).

The phylogeny derived from the combined data supports the monophyly of the six subfamilies recognized by Payne (1997), as represented in this study. We included multiple representatives of eight genera in this study and seven of these were monophyletic. The paraphyly of *Cuculus* (with respect to *Cercococcyx*) is not supported in over 50% of bootstrap replicates in the unweighted tree and is relatively weakly supported (71%) in the combined weighted tree. In any case, *Cercococcyx* is usually considered to be a close relative of *Cuculus* (Payne, 1997). Although our study contained slightly less than half of recognized cuckoo genera, it is noteworthy that we generally recovered traditional classification schemes for this group. While unsampled genera may prove to cause currently recognized subfamilies to be paraphyletic, our molecular study increases our confidence that previous classifications will for the most part be upheld.

In general, relationships among cuckoo subfamilies are poorly supported, with one exception: Coccyzinae as the sister to Cuculinae. The sister relationship between this New and Old World group, respectively, is strongly supported (90% bootstrap in weighted tree, Fig. 4). Using cyt *b* sequences from a limited number of species of Cuculiformes and Galliformes, Avise *et al.* (1994) were unable to resolve whether *Opisthocomus* is more closely related to Cuculiformes or Galliformes. Sibley and Ahlquist (1990) placed *Opisthocomus* within cuckoos as sister to Crotophaginae and Neomorphinae based on DNA–DNA hybridization. In contrast, cyt *b* and ND2 sequences support a position for hoatzin (*O. hoazin*) outside of cuckoos (Cuculidae). This relation-

ship is strongly supported in all weighting schemes (bootstrap values >80%). While in this analysis with *Tauraco* as an outgroup, *Opisthocomus* appears as sister to the cuckoos, we interpret this result only to mean that *Opisthocomus* is not a member of Cuculidae. Identification of the correct placement for this enigmatic genus will require sampling of many other avian orders that are potential candidates for a closer relationship with *Opisthocomus.*

Comparing our phylogeny to the DNA–DNA hybridization phylogeny of Sibley and Ahlquist (1990), we find support for several of their nodes. For example, the sister relationship between *Crotophaga* and *Guira* is supported by our study. In addition, monophyly of a group containing *Cuculus, Cacomantis, Chrysococcyx, Eudynamys,* and *Scythrops* is common between our study and Sibley and Ahlquist's tree. In addition, Sibley and Ahlquist place *Piaya* and *Coccyzus* as close relatives and with a group containing the subfamily Cuculinae, which is supported by mtDNA. However, there are topological differences between the tree of Sibley and Ahlquist and our tree relating mainly to the placement of *Centropus.* We have no way of evaluating the strength of support for Sibley and Ahlquist's conclusions in the DNA–DNA hybridization data, as they provide no measure of nodal support for their conclusions.

Hughes (1996) presented cladistic analyses based on behavioral, ecological, and morphological data. We find that the combined behavioral/ecological/morphological tree, as well as the behavioral/ecological tree (Fig. 5), of Hughes is significantly less likely than our unweighted topology using our mtDNA sequence data (Table 2). However, a partition homogeneity test between Hughes' (1996) behavioral/ecological data and our mtDNA data indicated no significant conflict. This is likely due to the low number of characters in the behavioral/ecological data set and the low levels of bootstrap support for the topology derived from that data set. Hughes (1996) did find support for a relationship between *Coccyzus* and Cuculinae, which was similar to that in our study. However, we found strong support for *Piaya* as the sister to *Coccyzus,* while Hughes (1996) placed *Piaya* as sister to all other cuckoos (Fig. 5). It is likely that the exclusion of *Piaya* from Coccyzinae in Hughes' (1996) study results from nonindependence between several characters relating to brood parasitism in the ecological/behavioral data set.

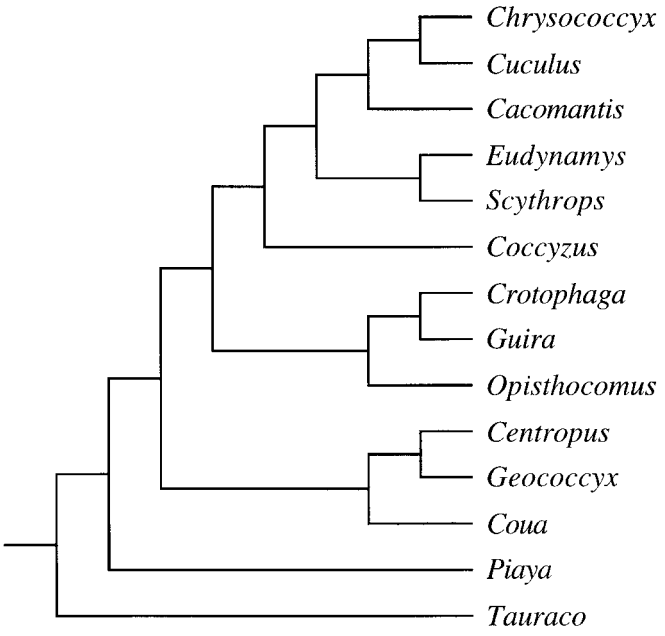

FIG. 5. Phylogeny of Hughes (1996) based on behavioral/ecological characters. Only genera that overlap with the genera in our study are included.

The traditional interpretation of the evolution of obligate brood parasitism within the cuckoos is that it evolved twice independently: once within all Cuculinae and once within some members of Neomorphinae (Payne, 1997). Because we included only one (non-brood-parasitic) member of Neomorphinae (*Geococcyx*) in our study, we are not able to evaluate this contention. We have included several members of the traditional Cuculinae and find strong support for the monophyly of this group, lending support to the conclusion of a single origin of brood parasitism in the ancestor of Cuculinae. Hughes (1996) claimed that *Coccyzus,* a facultative brood parasitic lineage, fell within a clade of obligate brood parasites (including Cuculinae and the parasitic members of Neomorphinae). Hughes concluded that obligate brood parasitism arose only once in cuckoos and facultative brood parasitism in *Coccyzus* was derived from obligate brood parasitism. However, we found strong support for a sister relationship between *Piaya* and *Coccyzus. Piaya* is not known to be a brood parasite of any kind, and this would suggest that either *Piaya* completely lost brood parasitism or that *Coccyzus* gained facultative brood parasitism independently of the evolution of brood parasitism in other cuckoos. Further evaluation of the phylogenetic relationships of *Tapera* and *Dromococcyx* (obligate brood parasitic members of Neomorphinae) is needed to potentially distinguish between these two hypotheses.

The genus *Coua* appears to have no close living relatives within the genera that we sampled. The genetic divergence between *Coua* and other cuckoos ranges from 20 to 28% for ND2, suggesting that this endemic Malagasy genus has been diverging from other cuckoos for a considerable time. The sister taxon to *Coua* is uncertain, but the unweighted tree places Cuculinae + Coccyzinae + Crotophaginae as its sister while the weighted tree places only Cuculinae + Coccyzinae as the sister to *Coua.*

The members of the genus *Coua* show a broad range of ecological adaptations. These include, for example, species that are principally arboreal and those that are

largely terrestrial (Urano *et al.,* 1994; Langrand, 1995). Furthermore, virtually all of the extant nine *Coua* species have distinct habitat distributions, occurring in either the dry forests in the west or the humid forests in the east. The only species that occurs in both habitats is *C. cristata.* We sequenced six of the nine species and the unweighted and weighted topologies show an identical topology for the species of *Coua. Coua caerulea* and *C. cristata* are the only arboreal forms that we sampled and these two species form a clade separate from the other species. The third arboreal species in this genus, *C. verreauxi,* is not represented in our current data set. All of the other four species for which we have sequences are terrestrial and form the sister clade to the arboreal species. Thus, on the basis of this study, it appears that there are two distinct lineages within the genus *Coua:* one arboreal and the other terrestrial. Species within each clade show a mixture of dry and humid forest habitat preferences, indicating little phylogenetic component to habitat preference.

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