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Phylogenetic Relationships among the Major Lineages of the Birds-of-Paradise (Paradisaeidae) Using Mitochondrial DNA Gene Sequences

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Complete mitochondrial cytochrome *b* gene sequences were determined from 12 species of the Australo-Papuan birds-of-paradise (Paradisaeidae) representing 9 genera. Phylogenetic analysis of these and 5 previously published sequences reveals a radiation of the main paradisaeinine lineages that took place over a relatively short evolutionary time scale. The core paradisaeinines are resolved as the monophyletic sister-group to the crow-like manucodines. The genus *Parotia* is basal to other paradisaeinines and is not closely related to the morphologically similar genera *Ptiloris* and *Lophorina*. Three major clades within the paradisaeinine ingroup include: (1) *Cicinnurus* and *Diphyllodes*, (2) *Ptiloris* and *Lophorina*, and (3) the genus *Paradisaea*. The monotypic genus *Seleucidis* is apparently closely related to clades (1) and (2). Cytochrome *b* sequences did not provide evidence for the monophyly of the sicklebill genera *Epimachus* and *Drepanornis*. The paradisaeid tree is characterized by short internodal distances. Thus, some clades cannot be strongly resolved by cytochrome *b* sequences alone.

INTRODUCTION

Species of the endemic Australo-Papuan birds-of-paradise (Paradisaeidae) exhibit perhaps the most extravagant radiation of plumage morphology, sexual dimorphism, ecological diversity, and complex courtship behavioral repertoires within passerine songbirds (Gilliard, 1969; Cooper and Forshaw, 1977; Diamond, 1986; Coates, 1990). Traditionally, about 20 genera have been recognized within the family, along with 40–44 species based on a polytypic biological species concept (Mayr, 1941, 1962; Gilliard, 1969; Beehler and Finch, 1985), or at least 90 diagnosably distinct species based on a phylogenetic species concept (Cracraft, 1992).

Early morphological studies of the evolutionary relationships of the birds-of-paradise postulated their close relationships to the bowerbirds, Ptilonorhynchidae (Bock, 1963). Initial molecular evidence, based on the analyses of DNA–DNA hybridization distance data, removed the bowerbirds to a more basal position within the corvine songbirds and instead supported the hypothesis that the crows, jays, and their allies (Corvidae) are the closest relatives of the paradisaeids (Sibley and Ahlquist, 1990). This arrangement has now been corroborated by phylogenetic analysis of mitochondrial DNA sequences (Helm-Bychowski and Cracraft, 1993). The latter study also provided support for monophyly of the polygynous, extravagantly plumaged so-called "core" birds-of-paradise (the paradisaeinines), and established the monogamous, unornamented "crow-like" manucodine paradisaeids as their sister-group when morphological characters are combined with the molecular data (Helm-Bychowski and Cracraft, 1993; see also Sibley and Ahlquist, 1985; Christidis and Schodde, 1992).

Intergeneric relationships of the paradisaeids are still a matter of controversy, and although a variety of opinions have been expressed based on broad morphological comparisons (Stonor, 1938; Mayr, 1945; Gilliard, 1969; Diamond, 1972; see Fig. 1), as well as molecular distance measurements among a handful of species (Schodde, 1976; Sibley and Ahlquist, 1985, 1990; Christidis and Schodde, 1992; see Fig. 1), relatively little consensus has been reached. Understanding these relationships is a matter of some importance in that they will provide a framework for understanding the tempo and mode of diversification within the family, as well as provide a basis upon which to assess the magnitude and direction of morphological, behavioral, and ecological changes (Harvey and Pagel, 1991; Brooks and McLennan, 1991).

With an improved understanding of the higher-level relationships of the paradisaeids and their allies, as well as increasing support for a sister-group relationship between the manucodines and the core birds-of-paradise, the stage is set for a more refined investiga-

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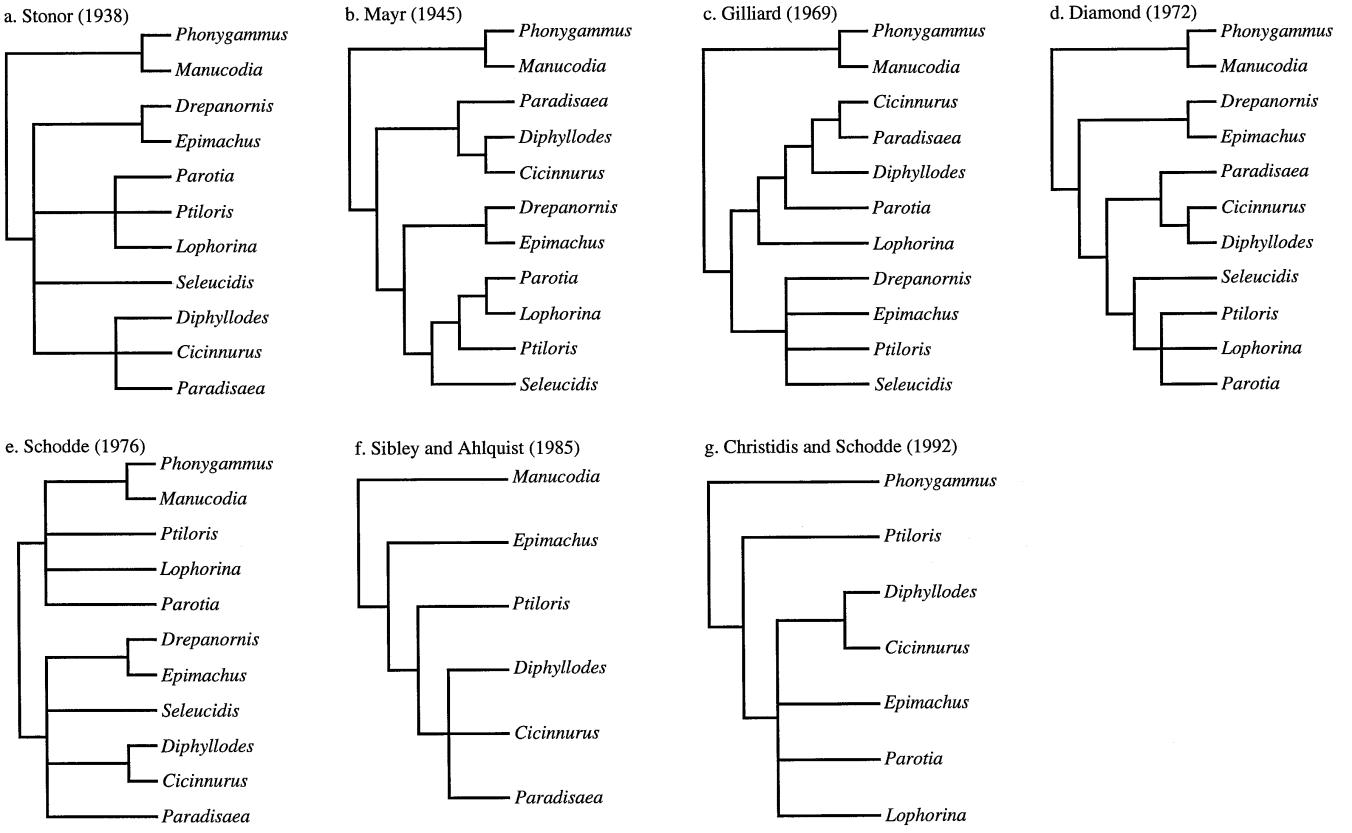

FIG. 1. Previous hypotheses of the evolutionary relationships among paradisaeid genera.

tion of the relationships within the paradisaeinines themselves. This is the first in a series of studies examining the phylogenetic relationships, patterns of diversification, and historical biogeography of the paradisaeids using molecular and morphological data sets. In this paper, we present new molecular evidence derived from complete mitochondrial cytochrome *b* gene sequences of nine genera of paradisaeids. Attention is directed to several important questions. First, do the genera classically understood to be "core" birds-of-paradise, i.e., the paradisaeinines, form a monophyletic assemblage? Second, does the molecular evidence offer support for the monophyly of several paradisaeinine clades proposed by previous investigators using traditional morphological characters? For example, are the sicklebills (*Drepanornis* and *Epimachus*) a natural group, and are *Cicinnurus* and *Diphyllodes* related as some workers have suggested? Third, what are the phylogenetic relationships among these clades and the genera *Lophorina, Seleucidis,* the riflebird complex (*Ptiloris*), *Parotia,* and the speciose genus *Paradisaea*? Finally, do the molecular data provide any insight into the relative ages of divergence and rates of evolution of the different lineages within the Australo-Papuan subregion, a radiation that appears to have begun perhaps 20–30 million years ago (Helm-Bychowski and Cracraft, 1993)?

MATERIALS AND METHODS

Samples, DNA Extraction, and Additional Sequences

We sequenced the complete mitochondrial cytochrome *b* gene from 12 paradisaeid species representing 9 genera, 7 of which are not represented in previous work. In the case of *Lophorina superba, Paradisaea* (*raggiana*) *augustovictoriae,* and *Parotia lawesii* multiple individuals were sequenced [GenBank accession numbers; source of tissue (abbreviations: AM, Australian Museum; AMNH, Department of Ornithology frozen tissue collection, American Museum of Natural History; SP-J, field numbers of Stephen G. Pruett-Jones; NYZP, New York Zoological Park; ZSSD, Zoological Society San Diego)]: Superb Bird of Paradise, *L. superba* (U25732, SP-J 004; U25733, SP-J 005 and SP-J 060); Lawes' Parotia, *Paro. lawesii* (U25734, SP-J 106; U25735, SP-J 102 and SP-J 103); Wilson's Bird of Paradise, *Diphyllodes respublica* (U15200; NYZP/AMNH no number); King Bird of Paradise, *Cicinnurus regius* (U15201; ZSSD ISIS no. 489243); Twelve-wired Bird of Paradise, *Seleucidis melanoleuca* (U15202; NYZP/AMNH no number); Blue Bird of Paradise, *Paradisaea rudolphi* (U15203; SP-J 073); Red Bird of Paradise, *Paradisaea rubra* (U25736; NYZP/AMNH no number); Lesser Bird of Paradise, *Paradisaea minor*

(U25737; ZSSD ISIS No. 489237); Raggiana Bird of Paradise, *Paradisaea* (*raggiana*) *augustaevictoriae* (U25738, ZSSD ISIS No. 489241; U15204, ZSSD ISIS No. 281830); Buff-tailed Sicklebill, *Drepanornis albertisi* (U15205; SP-J 097); Brown Sicklebill, *Epimachus meyeri* (U15206; SP-J 090); and Curl-crested Manucode, *Manucodia comrii* (U15207; AM no number, from S. V. Edwards).

DNAs used for enzymatic amplification were extracted from liver, pectoral muscle, or whole blood. A minute piece of tissue was boiled for 15 min in 500 μl of a 5% w/v Chelex-bead suspension. Beads were pulse-centrifuged for a few seconds and 300 μl of the supernatant was removed as a source of template DNA for PCR.

In addition to the above taxa, sequences for the following were taken from a previous study (Helm-Bychowski and Cracraft, 1993): Trumpet Manucode, *Phonygammus keraudrenii* (GenBank Accession No. X74252), Black Sicklebill, *Epimachus fastuosus* (X74253), Paradise Riflebird, *Ptiloris paradiseus* (X74254), Magnificent Bird of Paradise, *Diphyllodes magnificus* (X74255), and a corvid, the Blue Jay, *Cyanocitta cristata* (X74258).

Mitochondrial Cytochrome b Gene Isolation, Amplification, and Sequencing

The complete gene and flanking regions were amplified and isolated as a single fragment using oligonucleotides L14857 5′-GGGTCTTTCGCCCTATCAAT-3′, chicken number (Desjardins and Morais, 1990), situated at the end of ND5 (designed for this study from a consensus of unpublished passerine ND5 sequences) and H15915 (Edwards and Wilson, 1990) in the tRNA-threonine gene following cytochrome *b* (the equivalent chicken position for this oligonucleotide is H16065). A four-step thermocycle was used to amplify this 1.2-kb fragment: 1 min at 94°C, 1 min at 40°C, 1 min at 60°C, 3 min at 72°C, for 35 cycles. Following electrophoresis in a 2% NuSieve low-melting point agarose gel containing 4 pg · ml^{-1} EtBr (Maniatis *et al.*, 1982), the mitochondrial fragment was plugged from the gel and resuspended in 300 μl water.

The following oligonucleotide primer pairs were used to amplify subfragments of the isolated gene: L14857–H15104, L14857–H15298, L14990–H15298, L15236–H15505, L15311–H15710, L15656–H16065 (see Helm-Bychowski and Cracraft (1993) and references therein for original oligonucleotide descriptions). An Idaho Technologies air-thermocycler (ATC) machine was used to perform 10-μl amplifications of double-stranded DNA (Wittwer *et al.*, 1989) using glass microcapillaries and standard buffers described elsewhere (Wittwer, 1992). Amplification conditions were 1 s at 94°C, 0 s at 48°C, 10 s at 72°C, and 35 cycles at slope 9 (fastest temperature ramping rate available for this machine) for all subfragments. DNA products were visualized on agarose gels and plugged as described above. In all experiments, concurrent negative and positive controls were made.

Single-stranded DNA was generated for sequencing using 1:100 dilutions of the sequencing primer in 50-μl amplification reactions together with 1 μl of the resuspended double-stranded DNA (Gyllensten and Erlich, 1988). These were performed in a Peltier-effect thermocycler (MJ Research) for each fragment with conditions 1 min at 94°C, 1 min at 52°C, and 2 min at 72°C for 35 cycles. Single-stranded DNA was concentrated by spin-dialysis (Millipore 30,000 NMWL) to 10 μl before sequencing. DNA sequencing was by the Sanger termination-dideoxy method (Sanger *et al.*, 1977) using Sequenase 2.0 (US Biochemical). Sequencing products were subjected to denaturing gel electrophoresis and autoradiography.

Phylogenetic Analysis

Cladistic analyses were performed using PAUP 3.1.1 (Swofford, 1993). Unordered character state changes were distributed using the DELTRAN (delayed transformation) optimization, which favors contemporaneous state changes (i.e., parallelisms over reversals). When exhaustive searches could not be made, 10 replicate heuristic searches were performed for each analysis and taxa were added in random order to minimize input order bias. Cladistic signal was assessed by the synapomorphic content of each branch as well as by bootstrap support determined by 200 replications (Felsenstein, 1985). Choice of initial outgroup followed the establishment of the corvids as sister-group to the paradisaeids (Helm-Bychowski and Cracraft, 1993). Analyses in which multiple equally most parsimonious trees were discovered were subjected to successive approximations character weighting based on the mean character consistency indices across trees (Farris, 1969; Carpenter, 1988).

Genetic Distances

To illustrate aspects of the accumulation of transition and transversion differences, corrected pairwise genetic distances were computed using the PHYLIP 3.4 program DNADIST (Felsenstein, 1991) set to the six-parameter maximum-likelihood model of DNA substitution and utilizing empirical nucleotide frequencies. In addition, for this computation relative substitution rates at different codon positions were set at 5:1:20 (positions 1:2:3) with a transition to transversion bias of 10:1.

RESULTS

Mitochondrial Cytochrome b Sequences

Mitochondrial cytochrome *b* sequences (1143 bp) of the 12 new paradisaeids (including two different haplotypes for 3 taxa) and 5 taxa from the literature (Helm-Bychowski and Cracraft, 1993) are aligned in Fig. 2

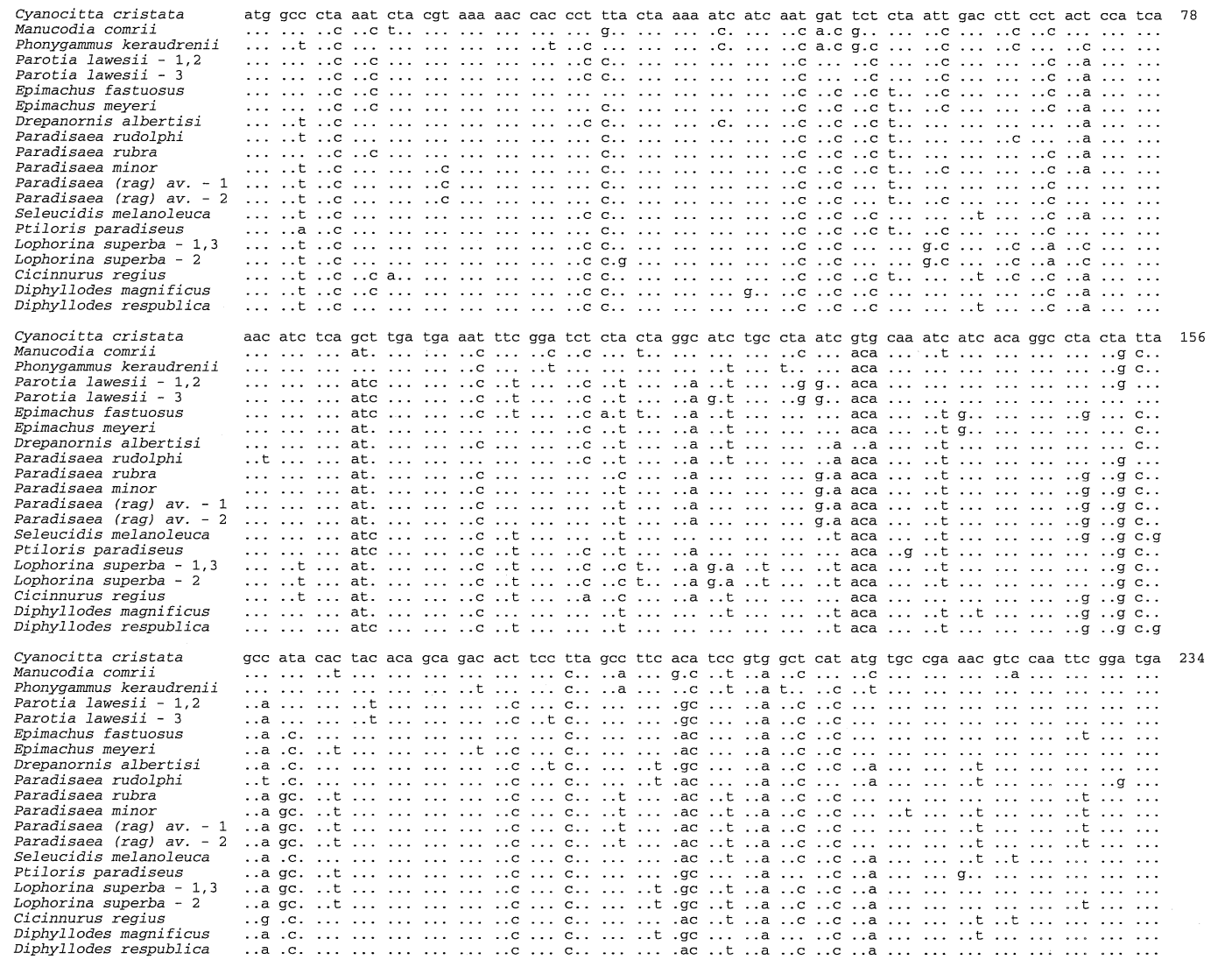

FIG. 2. Nucleotide sequences of the mitochondrial cytochrome *b* gene of paradisaeids aligned with that of *Cyanocitta cristata*. Where numbers follow taxon names these refer to different individuals sequenced (following order of appearance under Materials and Methods).

with differences shown against the sequence from the outgroup *Cy. cristata*. No insertions or deletions in the 380-amino-acid coding sequence are present among these birds. In addition, differences from the avian gene order were not detected in the flanking ND5 and tRNA-threonine genes (Desjardin and Morais, 1990), although size and sequence variations were discovered in the intergenic spacers between ND5, cytochrome *b*, and tRNA-threonine (unpublished data). Termination codon usage was TAA as found most frequently in the chicken mitochondrial protein-coding genes (Desjardins and Morais, 1990) and phasianid cytochrome *b* genes (Kornegay *et al.*, 1993) except for *E. meyeri*, which has a TAG termination codon. Thus, the frequency of TAA and TAG termination codons in paradisaeids is similar to that found in phasianids. Based on the fact that the complete cytochrome *b* gene was isolated initially as a single fragment, that no frameshifts were observed, and that no nonsense codons were apparent, we assume that these sequences are of mitochondrial origin only and do not represent pseudogenous or translocated fragments that might be located elsewhere in these birds' genomes. Indeed, the isolation of a large initial fragment for further amplification experiments lessens the likelihood of amplifying and sequencing fragments of cytochrome *b* which may have other origins (e.g., see Kornegay *et al.*, 1993; Quinn, 1992; Smith *et al.*, 1992). We view this approach as superior to the isolation of an array of subfragments from a genomic extract, which may potentially have diverse noncontiguous origins, or be of a species-composite nature due to cross-contamination of amplification ex-

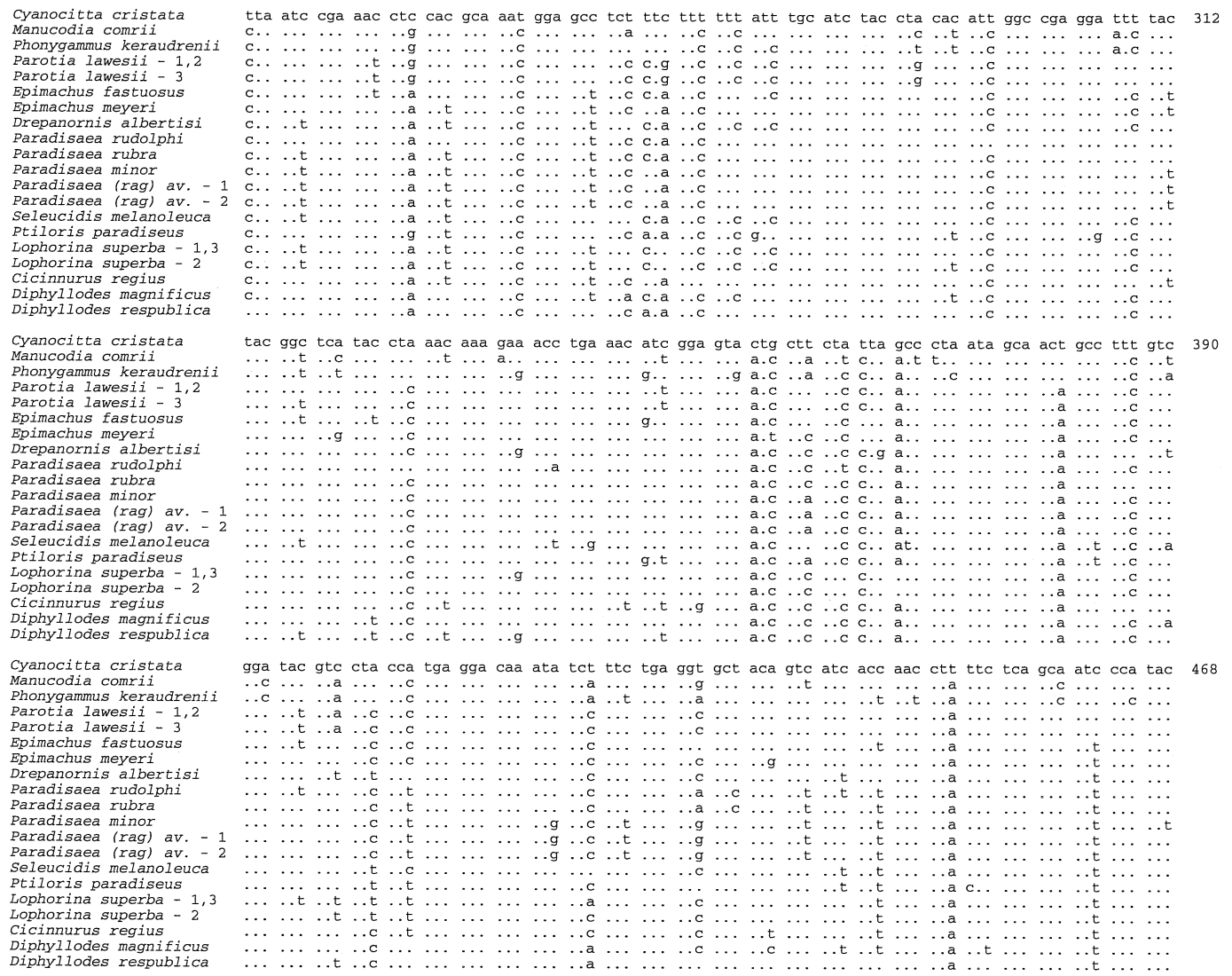

FIG. 2—*Continued*

periments (a fact which is not always noticeable if the overlapping regions happen to be conservative in sequence).

Base Compositional Bias and Saturation

All the sequences presented here exhibit similar compositional biases at different codon positions and across the entire gene (Table 1). The same pattern of codon position-dependent compositional bias in the cytochrome *b* gene appears to be present in all birds so far examined (e.g., almost identical biases are found within a nonpasserine group, the phasianids; Kornegay *et al.*, 1993). There is no major bias at first positions and the only notable bias at second positions is a consistent T over G, which is undoubtedly due to the leucine/isoleucine richness of cytochrome *b* and the invariant T nucleotides at second positions of the eight mitochondrial codons for these amino acids (Desjardins and Morais, 1990). As noted in other passerine birds (Edwards *et al.*, 1991; Helm-Bychowski and Cracraft, 1993), as well as in mammals (Irwin, 1991), the third positions of codons exhibit the most extreme compositional bias. This bias is consistent among these two vertebrate groups in being A and C rich and depauperate of G nucleotides (in this study only approximately 3% of third positions overall have G nucleotides).

Saturation, i.e., the occurrence of multiple substitutions at the same nucleotide sites, can be inferred by the fall in the typical transition bias. The transition substitution bias in mitochondrial DNA was established from observations of variation in vertebrates (e.g., in primates; Brown *et al.*, 1982), as well as invertebrates (e.g., *Drosophila;* de Salle *et al.*, 1987). The presence of this bias in avian mitochondrial DNA is

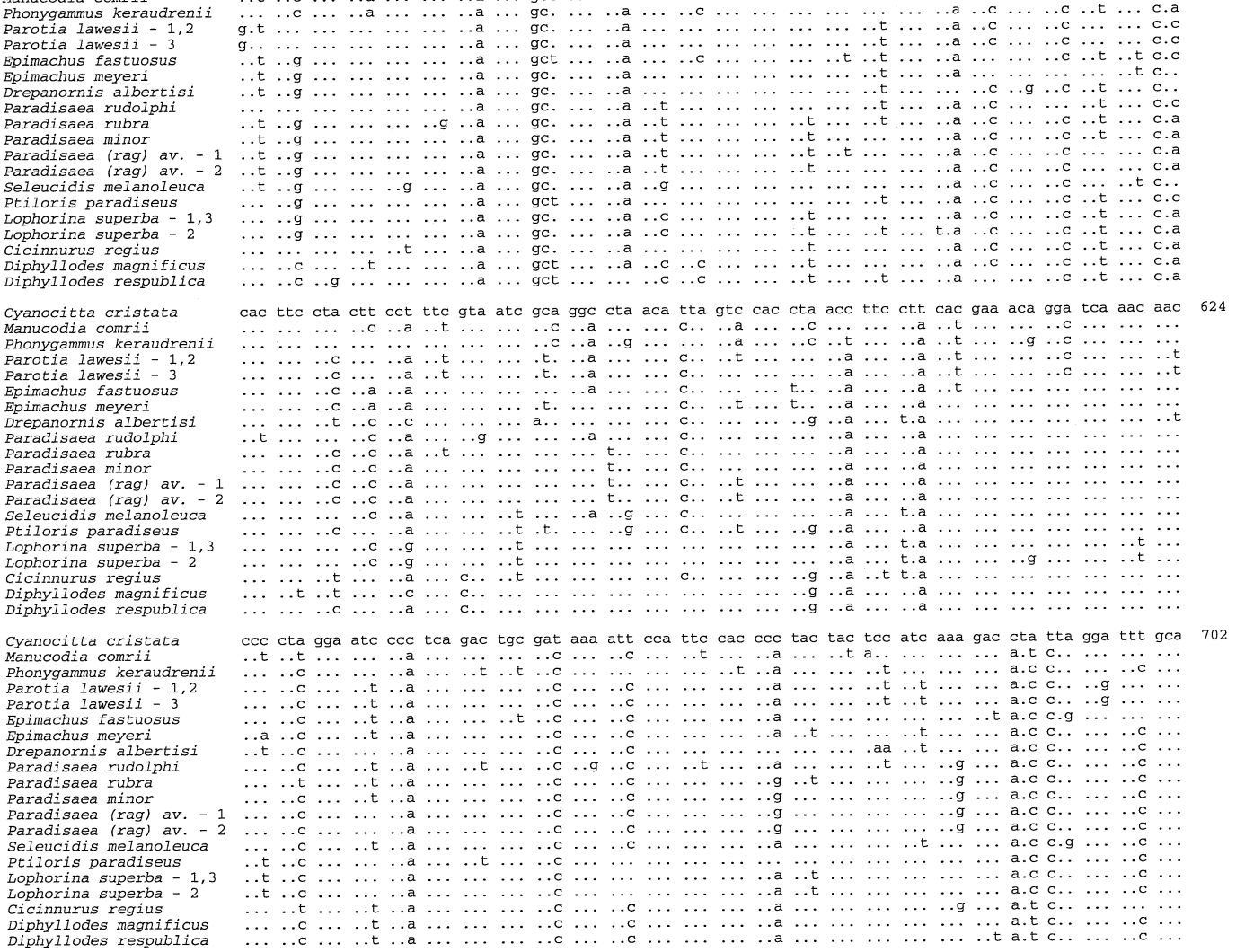

FIG. 2—*Continued*

now also well established (Kocher *et al.*, 1989; Edwards and Wilson, 1990) and is particularly noticeable at lower systematic levels in birds (Smith *et al.*, 1991). Among the bird-of-paradise species examined, the pairwise transition bias ranged from approximately 1.4 between distant relatives such as an outgroup versus a paradisaeinine (e.g., *M. comrii* vs *Paro. lawesii*) to 15.5 between recently diverged paradisaeinine congenerics (e.g., *E. fastuosus* vs *E. meyeri*). Intraspecifically there are few transversion differences, for example none occur between the *Para.* (*raggiana*) *augustovictoriae* haplotypes. These observations are consistent with the hypothesis that transversions accumulate gradually and effectively mask the transition differences that occurred previously. The growing transversion differences lead to a plateauing of observed transition differences as the limited number of sites that are able to vary eventually swap nucleotide classes (i.e., purine to pyrimidine or vice versa), exhausting those sites that might previously have incurred transition changes. This effect can be seen in Fig. 3, which shows the relationship of pairwise corrected genetic distance (which takes into account the occurrence of multiple hits) to the pairwise empirical numbers of transitions and transversions. Transitions grow quickly in relation to corrected distance while transversions increase more linearly, a situation which has been found in other birds (Arctander, 1991) as well as in vertebrate groups such as salamanders (Moritz *et al.*, 1992). The clear separation of the paradisaeinine comparisons (to the lower left in the graph) from the outgroup manucodine/*Cy. cristata* comparisons (to the upper right) verifies

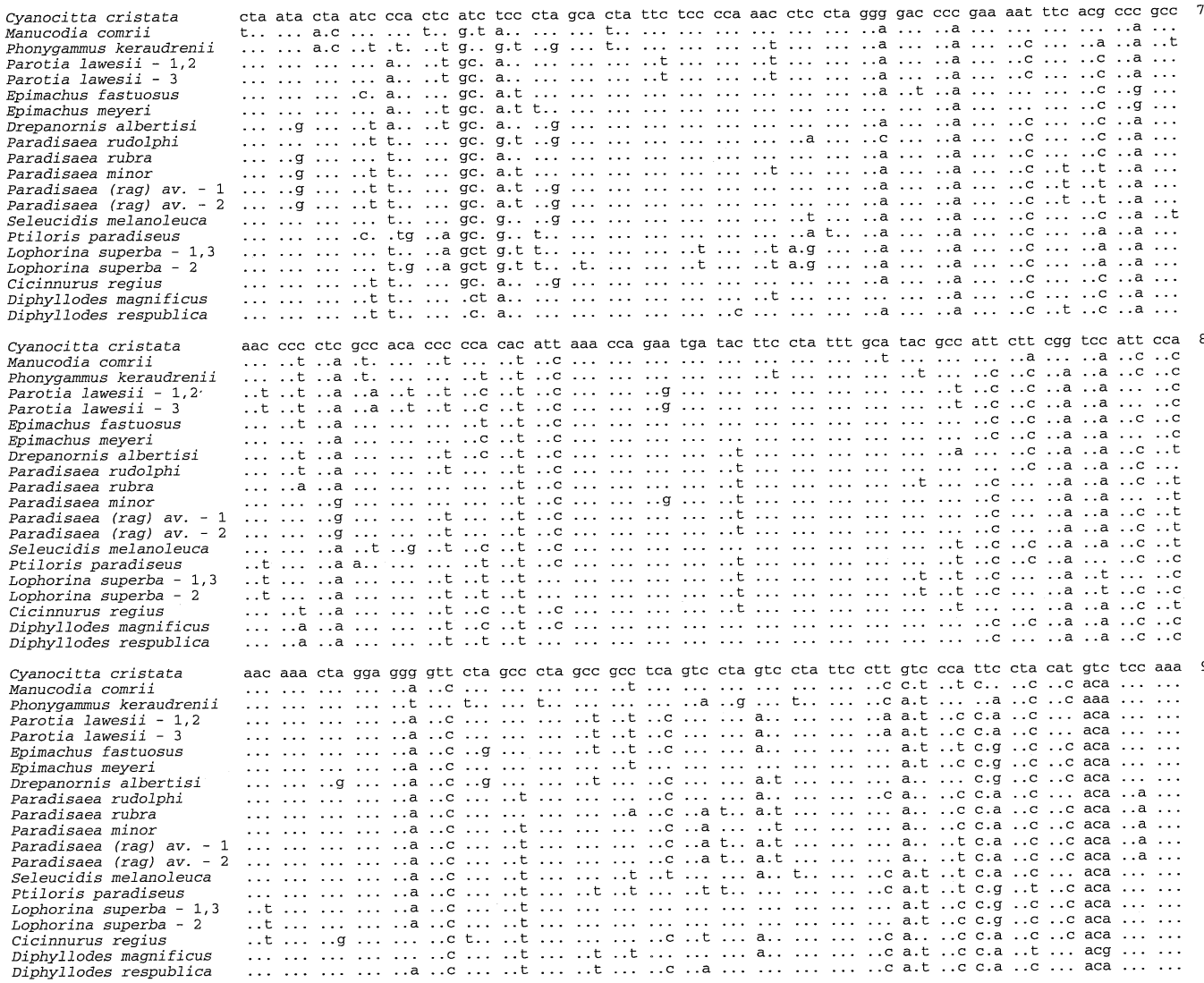

FIG. 2—*Continued*

the existence of site saturation when comparisons to these relatively distant outgroups are made.

Sequence Comparisons among Species

Complete pairwise percentage differences (uncorrected) and the numbers of transition and transversion substitutions among the birds-of-paradise and the corvid outgroup are shown in Table 2. Among these birds the pairwise percentage difference ranges from 0.26% (intraspecific) to 16.80% (outgroup vs paradisaeinine), i.e., from 3 to 192 pairwise differences in the complete gene sequences. It is noteworthy that the percentage difference (11.02%) between the two morphologically similar manucodine species is only slightly less than the largest pairwise comparison involving paradisaeinine taxa (a maximum of 11.55% between *Lophorina superba* and *E. fastuosus*) and exceeds the majority of inter-paradisaeinine comparisons.

Phylogenetic Analysis

Cladistic analysis of all substitutional variation, regardless of position and type, among the 19 haplotypes from 16 paradisaeid taxa and the single corvid outgroup taxa resulted in a single most parsimonious tree of 1036 steps (Fig. 4). Although there was marginally decreasing overall tree length, no change occurred in the branching pattern when either one or both individual haplotypes were included from the species represented by multiple individuals (*L. superba, Para.* (*raggiana*) *augustovictoriae,* and *Paro. lawesii;* analyses not shown). There were 399 variable sites and of these 295 were phylogenetically informative (73.9%) and 104

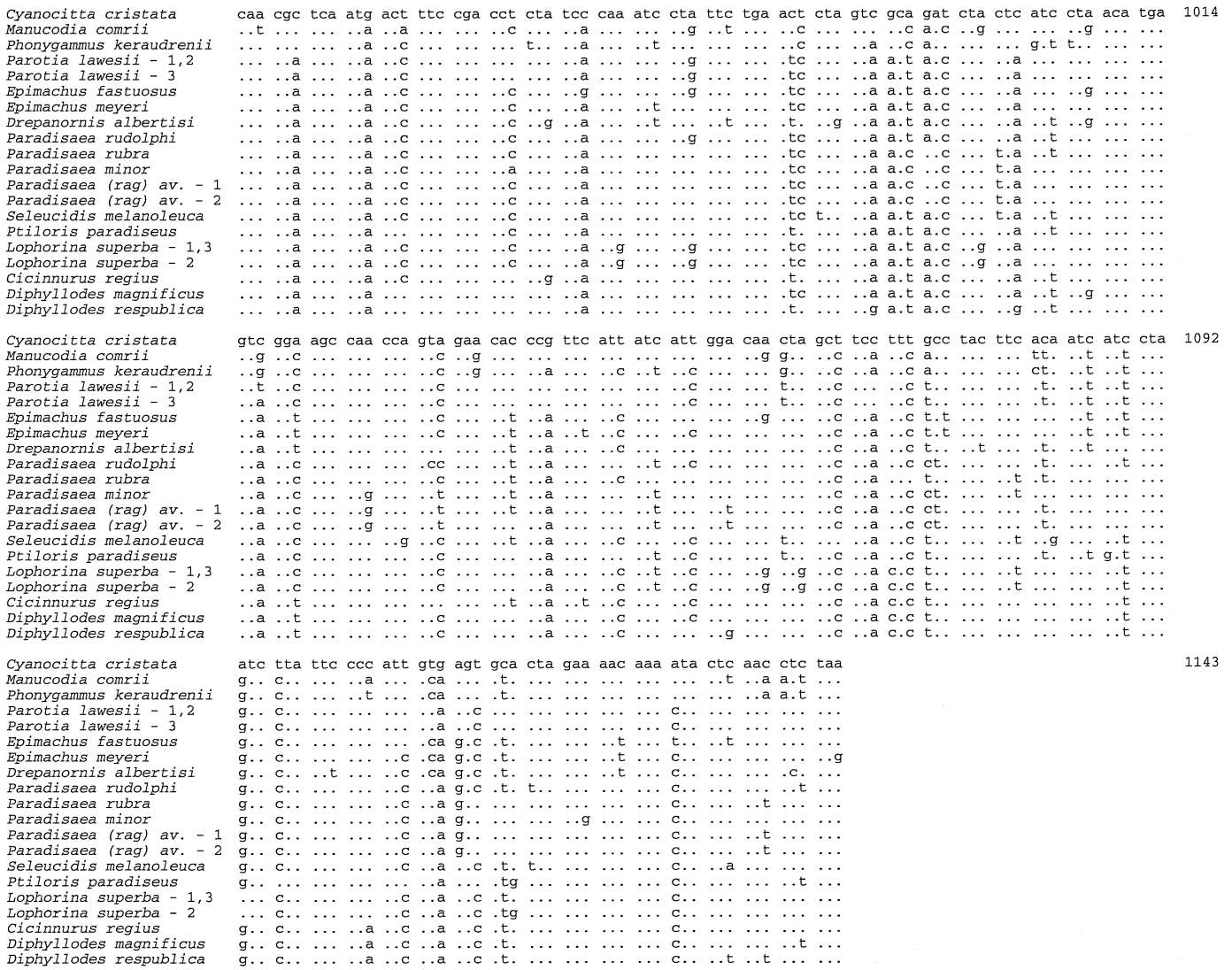

FIG. 2—*Continued*

autapomorphic (26.1%). The distribution of variable sites across codon position was, as expected, biased toward third positions: 74.2% at third (mostly synonymous), 20.6% at first (mainly leucine synonymous C/T first position changes), and only 5.3% occurring at second positions.

Paralleling the results of previous studies (e.g., Helm-Bychowski and Cracraft, 1993), our primary analysis with a corvid outgroup supports the hypothesis that the paradisaeids can be subdivided into two distinct lineages, the manucodines, now represented by both genera *Phonygammus* and *Manucodia,* versus the "core" birds-of-paradise, the paradisaeinines. In cladistic terms both lineages are strongly supported by relatively large, and nearly equal, numbers of derived character changes (48 and 50 synapomorphies, respectively). A bootstrap analysis also indicates a very high level of support for these two groups. Given these results, the paradisaeid division now seems robustly established.

Our data suggest that the first lineage which branched within the core paradisaeinines was the genus *Parotia,* which forms a sister-group to the remaining genera. The next branch contains the two large, dark-plumaged *Epimachus* sicklebills. Not surprisingly, these morphologically quite similar species form a sister-taxa relationship, but they are not resolved as the sister-taxon of the other genus of sicklebill, represented in our study by a single species, *Dr. albertisi,* even though morphological characters suggest a close relationship (Cracraft, unpublished data). *Dr. albertisi* occupies a position between the more basal *Epimachus* and all remaining core paradisaeinines.

It is worth noting, however, that an alternative tree supporting a monophyletic sicklebill group (i.e., both *Epimachus* and *Drepanornis*) can be formed at 1039

TABLE 1

Empirical Base Composition and Computed Base Compositional Bias (Prager and Wilson, 1988) of the Mitochondrial Cytochrome *b* Gene of Paradisaeids and the Corvid *Cyanocitta cristata*

Species	Codon Position															All				
	First					Second					Third									
	G	A	T	C	Bias[a]	G	A	T	C	Bias[a]	G	A	T	C	Bias[a]	G	A	T	C	Bias[a]
Diphyllodes respublica	81	103	80	117	0.009	49	79	156	97	0.056	11	163	53	154	0.156	141	345	289	368	0.032
Diphyllodes magnificus	81	102	79	119	0.010	50	78	156	97	0.056	8	167	51	155	0.168	139	347	286	371	0.033
Cicinnurus regius	81	103	82	115	0.008	49	79	156	97	0.056	11	163	60	147	0.143	141	345	298	359	0.031
Seleucidis melanoleuca	83	101	85	112	0.005	49	79	157	96	0.057	14	161	54	152	0.146	146	341	296	360	0.029
Ptiloris paradiseus	88	97	83	113	0.005	50	78	158	95	0.058	11	164	56	150	0.150	149	339	297	358	0.027
Lophorina superba - 1,3	87	98	82	114	0.006	50	78	156	97	0.056	12	166	56	147	0.148	149	342	294	358	0.028
Lophorina superba - 2	87	98	83	113	0.005	50	78	157	96	0.057	15	162	57	147	0.138	152	338	297	356	0.026
Paradisaea (ragg) av. - 1	86	98	83	114	0.006	49	79	157	96	0.057	13	163	57	148	0.143	148	340	297	358	0.028
Paradisaea (ragg) av. - 2	86	98	83	114	0.006	49	79	157	96	0.057	13	163	54	151	0.149	148	340	294	361	0.028
Paradisaea minor	87	97	82	115	0.006	49	79	156	97	0.056	14	165	55	147	0.145	150	341	293	359	0.028
Paradisaea rubra	86	98	83	114	0.006	49	79	156	97	0.056	9	170	47	155	0.174	144	347	286	366	0.031
Paradisaea rudolphi	84	100	81	116	0.007	49	79	157	96	0.057	8	171	55	147	0.162	141	350	293	359	0.031
Drepanornis albertisi	83	102	80	116	0.008	50	78	155	98	0.054	15	160	57	149	0.138	148	340	292	363	0.028
Epimachus fastuosus	85	101	84	111	0.005	49	79	154	99	0.054	12	162	55	152	0.149	146	342	293	362	0.029
Epimachus meyeri	85	100	83	113	0.005	49	79	156	97	0.056	9	166	47	159	0.173	143	345	286	369	0.032
Parotia lawesii - 1,2	84	101	80	116	0.008	50	78	158	95	0.058	11	161	46	163	0.169	145	340	284	374	0.031
Parotia lawesii - 3	85	100	80	116	0.008	50	78	158	95	0.058	10	163	47	161	0.170	145	341	285	372	0.031
Phonygammus keraudrenii	88	101	87	105	0.002	49	79	157	96	0.057	11	157	59	154	0.144	148	337	303	355	0.027
Manucodia comrii	86	105	82	108	0.005	49	78	157	97	0.058	13	157	55	156	0.146	148	340	294	361	0.028
Cyanocitta cristata	88	94	89	110	0.003	49	78	158	96	0.059	15	153	59	154	0.134	152	325	306	360	0.026
Mean	85.05	99.85	82.55	113.55	0.006	49.35	78.55	156.60	96.50	0.057	11.75	162.85	54.00	152.40	0.152	146.15	341.25	293.15	362.45	0.029
Standard deviation	2.31	2.60	2.44	3.22	0.002	0.49	0.51	1.05	1.00	0.001	2.24	4.25	4.27	4.86	0.013	3.73	5.09	5.91	5.59	0.002
%	22.32	26.21	21.67	29.80		12.95	20.62	41.10	25.33		3.08	42.74	14.17	40.00		12.79	29.86	25.65	31.71	

Note. Where numbers follow taxon names these refer to different individuals sequenced (following order of appearance under Materials and Methods).

[a] $B = (4/3) \sum_{i=1}^{4} (b_i - 0.25)^2$.

steps or 3 more than the most parsimonious tree (in this constrained topology, some rearrangement of the branching pattern does occur among the remaining apical paradisaeinine groups).

Within the remaining core paradisaeinines two major clades are resolved, although character support and bootstrap support for both of these are relatively weak.

The first clade is composed of the genera *Diphyllodes, Cicinnurus, Seleucidis, Ptiloris,* and *Lophorina* (shown topmost in Fig. 4). Among these five genera, *Diphyllodes* and *Cicinnurus* are sister-taxa, as are *Ptiloris* and *Lophorina.* The distinctive Twelve-wired Bird-of-Paradise, *S. melanoleuca,* is resolved as the sister-taxon to the *Ptiloris–Lophorina* clade. A less parsimonious tree of length 1037 (i.e., a single step longer than the most parsimonious tree) switches the origin of *Seleucidis* within this first clade from that shown in Fig. 4 to a neighboring position basal to the *Diphyllodes–Cicinnurus* lineage. In addition, shifting *Seleucidis* to a position basal to all four genera (*Diphyllodes, Cicinnurus, Ptiloris,* and *Lophorina*) requires a minimal tree of 1040 steps (4 more than the most parsimonious and with no rearrangements occurring outside of the clade).

The second major paradisaeinine clade is composed solely of members of the genus *Paradisaea,* represented in our study by four species, *Para. rudolphi, Para. rubra, Para. minor,* and *Para.* (*raggiana*) *augustovictoriae.* Branching at the base of these four taxa, *Para. rudolphi* has a considerably greater number of autapomorphic characters than the remaining species and is interpreted as having arisen very soon after the origin of the clade. Perhaps as expected from plumage comparisons, the highly ornately plumed *Para. minor* and *Para.* (*raggiana*) *augustovictoriae* are close relatives and occur apically within the *Paradisaea* clade, with the less extravagantly plumed *Para. rubra* occurring at an intermediate position between these two taxa and *Para. rudolphi.*

Various authors have noted the sensitivity of the resolution of ingroup relationships to the choice of outgroup taxa (e.g., Wheeler, 1990; Helm-Bychowski and Cracraft, 1993; Smith, 1994). This problem is com-

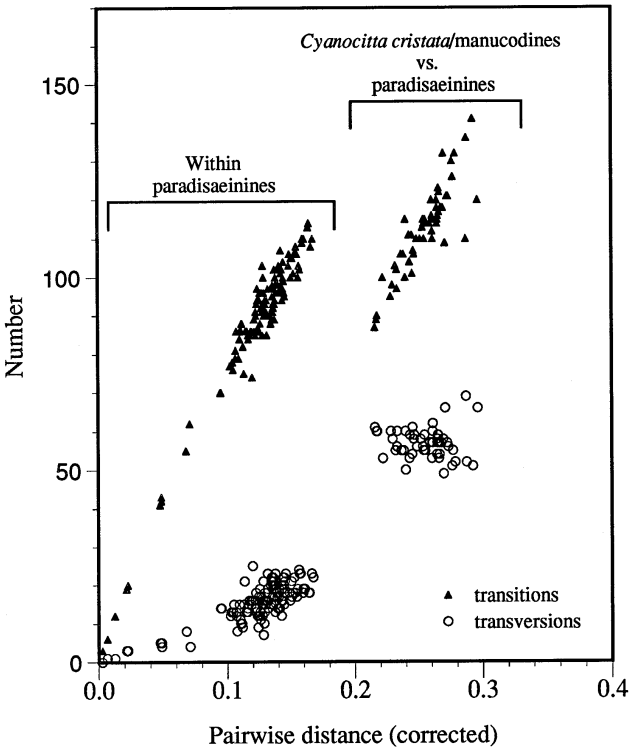

FIG. 3. Graph of corrected pairwise distance versus empirical numbers of transition and transversion substitutions between cytochrome *b* gene sequences in this study. Distances were computed using DNADIST and the maximum-likelihood model of nucleotide substitution (Felsenstein, 1991).

pounded when phylogenetic resolutions of the ingroup taxa are characterized by short internodal distances in comparison to the terminal branch lengths (Lanyon, 1988; Smith, 1994), a situation present in our paradisaeid tree (Fig. 4). The resolution of paradisaeid ingroup relationships may also be highly affected by the relatively long branch of the corvid outgroup (e.g., see Felsenstein, 1978; Smith *et al.*, 1992). In an effort to assess the potential effect that a relatively distant outgroup might be having on the relationships within the paradisaeinines, a second analysis was performed with *Cyanocitta* excluded and the manucodines instead utilized to root the tree. Figure 5a shows the strict consensus of two equally most parsimonious trees. Because the two trees are substantially different from one another in their resolution of relationships among the core paradisaeinine clades, the relationships appear unresolved in the consensus. This result is most likely caused by the low character support for the internal branches. However, a single round of successive approximations character-weighting, based on the mean character consistency indices across the two trees (Farris, 1969; Carpenter, 1988), results in a tree that is identical in branching pattern with the analysis shown in Fig. 4.

It has been argued that pairwise transversion differences exhibit an approximately linear relationship to age of divergence (Miyamoto and Boyle, 1989; Irwin *et al.*, 1991). Using values calculated from Table 2, the two manucodines and the corvid *Cyanocitta* were nearly equidistant from the paradisaeinines (mean

TABLE 2

Uncorrected Pairwise Percentage Difference (above Diagonal) and Numbers of Transition/Transversion Substitutions (below Diagonal) for the Complete (1143 bp) Cytochrome *b* Gene

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*Diphyllodes respublica*	-	5.51	7.35	8.49	9.80	9.71	10.32	9.45	9.36	9.36	8.66	9.80	10.15	8.92	9.54	9.80	9.89	14.09	13.56	14.44
2	*Diphyllodes magnificus*	55/8	-	7.35	8.22	9.62	9.27	9.71	10.24	10.15	9.89	9.19	8.66	9.01	9.54	8.92	9.45	9.71	13.39	13.65	15.05
3	*Cicinnurus regius*	70/14	70/14	-	8.66	10.76	9.80	10.24	8.75	8.84	8.75	7.96	8.40	8.75	10.41	9.19	10.06	10.32	15.22	15.05	15.57
4	*Seleucidis melanoleuca*	82/15	79/15	86/13	-	9.80	9.45	9.97	9.45	9.54	9.71	9.01	9.01	9.36	9.62	9.27	9.45	9.36	14.44	15.14	16.45
5	*Ptiloris paradiseus*	92/20	88/22	105/18	97/15	-	9.54	9.27	10.24	9.97	10.67	10.15	10.24	10.67	10.15	9.27	9.54	9.97	13.82	14.00	15.84
6	*Lophorina superba* - 1,3	90/21	85/21	91/21	90/18	96/13	-	1.14	10.32	10.06	9.80	4.71	10.15	10.32	11.29	9.80	10.85	10.94	14.44	14.79	16.10
7	*Lophorina superba* - 2	96/22	89/22	97/20	97/17	94/12	12/1	-	10.59	10.32	10.24	9.80	10.59	10.76	11.55	10.24	11.46	11.55	14.87	15.05	16.80
8	*Paradisaea (ragg) av.* - 1	92/16	97/20	84/16	93/15	101/16	99/19	103/18	-	0.26	2.01	4.02	7.87	9.54	10.15	9.10	11.20	11.46	15.40	14.70	15.31
9	*Paradisaea (ragg) av.* - 2	91/16	96/20	85/16	94/15	98/16	96/19	100/18	3/0	-	1.92	4.11	7.96	9.62	10.24	9.01	10.94	11.20	15.14	14.44	15.22
10	*Paradisaea minor*	92/15	94/19	85/15	97/14	105/17	92/20	98/19	20/3	19/3	-	4.11	8.14	10.06	10.06	8.66	10.85	10.94	15.40	15.31	15.40
11	*Paradisaea rubra*	86/13	88/17	76/15	89/14	97/19	91/20	93/19	41/5	42/5	43/4	-	7.79	8.84	9.10	8.05	9.97	10.06	15.49	14.17	15.05
12	*Paradisaea rudolphi*	89/23	74/25	75/21	89/14	96/21	94/22	100/21	77/13	78/13	81/12	77/12	-	9.19	9.89	9.10	9.62	9.89	14.52	14.26	15.22
13	*Drepanornis albertisi*	98/18	85/18	86/14	94/13	106/16	95/23	101/22	93/16	94/16	100/15	86/15	86/19	-	9.62	8.40	10.41	10.15	15.31	15.31	15.84
14	*Epimachus fastuosus*	86/16	91/18	107/12	103/7	102/14	110/19	114/18	102/14	103/14	102/13	91/13	98/15	100/10	-	5.77	8.49	8.22	14.87	13.12	15.84
15	*Epimachus meyeri*	93/16	86/16	93/12	97/9	94/12	95/17	101/16	90/14	89/14	86/13	79/13	87/17	86/10	62/4	-	8.31	8.40	14.79	13.74	14.35
16	*Parotia lawesii* - 1,2	91/21	85/23	98/17	96/12	90/19	100/24	108/23	109/19	106/19	106/18	96/18	90/20	104/15	88/9	84/11	-	0.61	13.82	12.95	14.79
17	*Parotia lawesii* - 3	93/20	89/22	102/16	96/11	96/18	102/23	110/22	113/18	110/18	108/17	98/17	94/19	102/14	86/8	86/10	6/1	-	14.09	13.04	14.96
18	*Phonygammus keraudrenii*	106/55	100/53	115/59	115/50	103/55	111/54	115/55	123/53	120/53	122/54	121/56	110/56	118/57	115/55	114/55	102/56	106/55	-	11.02	16.27
19	*Manucodia comrii*	95/60	98/58	110/62	116/57	100/60	110/59	112/60	110/58	107/58	116/59	101/61	104/59	109/66	90/60	97/60	87/61	89/60	103/23	-	15.66
20	*Cyanocitta cristata*	106/59	115/57	121/57	136/52	130/51	132/52	141/51	118/57	117/57	118/58	114/58	120/54	132/49	126/55	111/53	113/56	114/57	120/66	110/69	-

Note. Where numbers follow taxon names these refer to different individuals sequenced (following order of appearance under Materials and Methods).

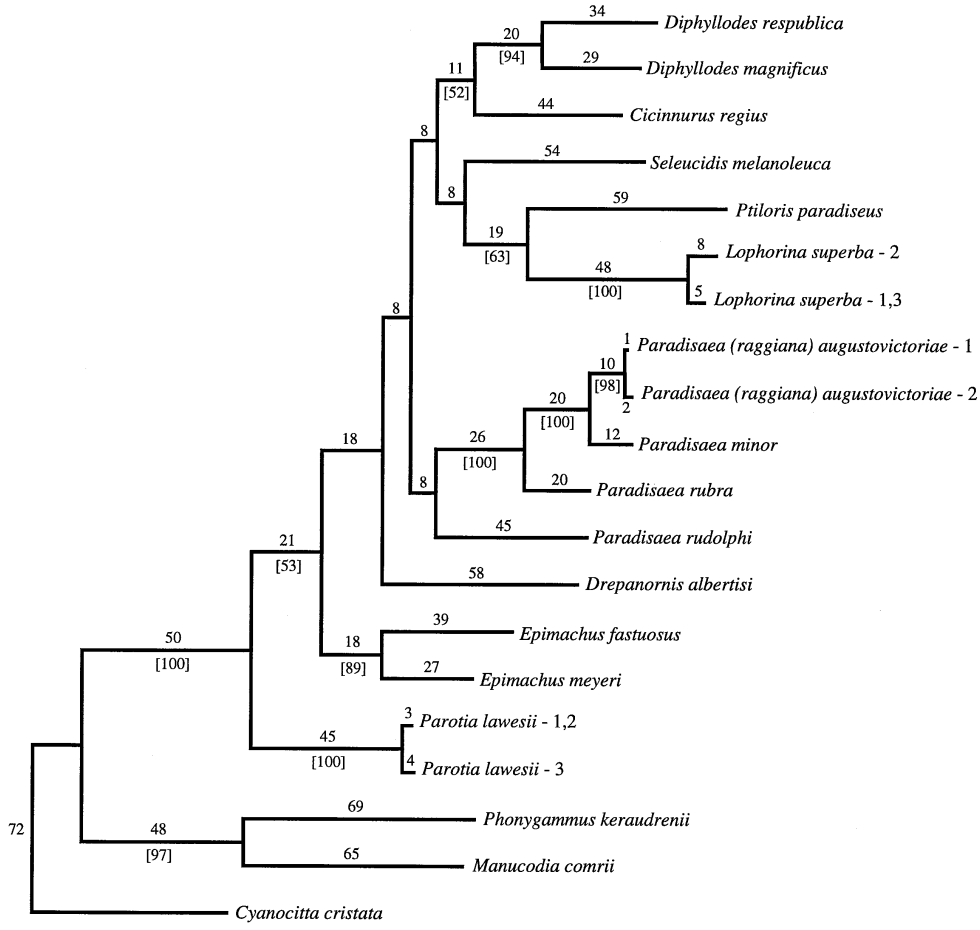

FIG. 4. Phylogram representing the single most parsimonious tree rooted to *Cyanocitta cristata* as an outgroup (L = 1036; C.I. = 0.419, excluding uninformative characters). Steps on tree shown above and bootstrap support (>50%) in brackets shown beneath the branches. Where numbers follow taxon names these refer to different individuals sequenced (following order of appearance under Materials and Methods).

transversion percentage difference: manucodines/paradisaeinines = 5.02%, n = 34; corvid/paradisaeinines = 4.80%, n = 17), suggesting that the three lineages diverged at nearly the same time (see also Helm-Bychowski and Cracraft, 1993). Given that manucodines and *Cyanocitta* were approximately equidistant from the paradisaeinines, both groups were excluded from the data set and a third analysis was undertaken with *Parotia* designated as the outgroup. Figure 5b shows the strict consensus of two equally most parsimonious trees. In addition to resolving some clades found in the manucodine-outgroup analysis (monophyly of *Diphyllodes–Cicinnurus* and *Paradisaea* spp.), the genera *Ptiloris* and *Lophorina* now form a clade, and the *Epimachus* sicklebills occupy a sister-group relationship relative to the remaining paradisaeinines. Both these results were found in the analysis using *Cyanocitta* as an outgroup (Fig. 4). Following a single round of iterative reweighting, a most parsimonious tree was found with an identical branching pattern to that shown in Fig. 4.

DISCUSSION

Cytochrome b Sequences and Paradisaeid Relationships

The phylogenetic hypothesis supported by cytochrome *b* sequence data (Fig. 4 and subsequent analyses) can be compared with previous conceptions of relationships for the taxa examined in this study (Fig. 1). The hypotheses of earlier workers were generally not supported by rigorous character analysis, and genera were united instead on the basis of overall similarities in plumage patterns or behavior. Stonor (1938) compared plumage characteristics, feather tract morphology (pterylosis), and skull shape of paradisaeids to produce the first general concept of generic interrelationships (Fig. 1a), though many of his characters occur outside the group and paradisaeid monophyly itself was not established. A more resolved phylogenetic hypothesis for paradisaeid genera was proposed by Mayr (1945), but again, supporting data for

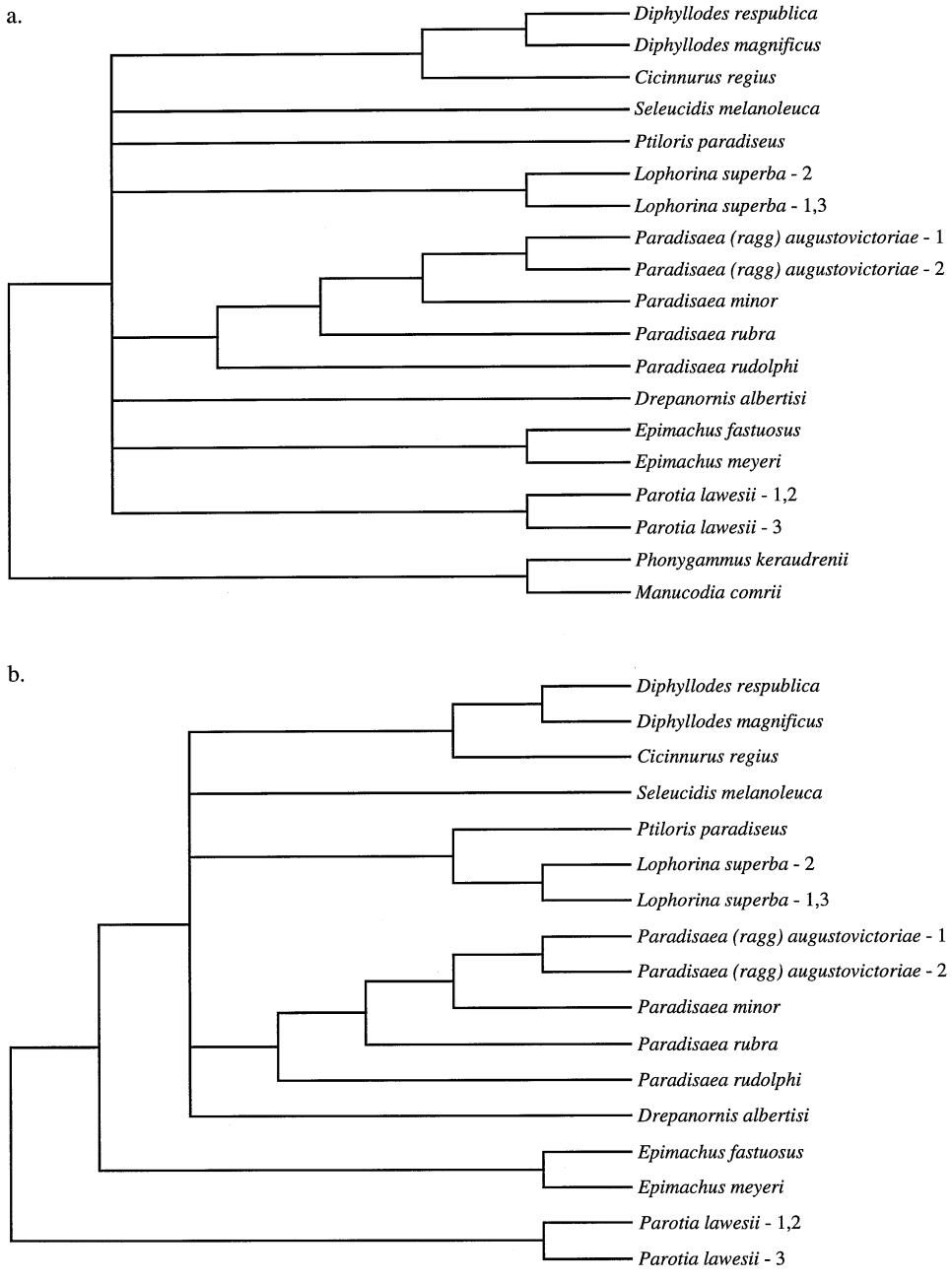

FIG. 5. (a) Strict consensus of two most parsimonious trees rooted to the manucodines as an outgroup (L = 916; C.I. = 0.439, excluding uninformative characters). (b) Strict consensus of two most parsimonious trees rooted to *Parotia* as an outgroup (L = 717; C.I. = 0.459, excluding uninformative characters). Where numbers follow taxon names these refer to different individuals sequenced (following order of appearance under Materials and Methods).

his generic groupings were not provided (Fig. 1b). Gilliard (1969), relying primarily on his knowledge of behavior, proposed a picture of intergeneric relationships that is largely unresolved (Fig. 1c). Shortly thereafter, Diamond (1972) provided a detailed phylogeny of paradisaeid genera using explicit characters to describe the groups, but many of the characters are demonstrably primitive and were sometimes used to support quite unrelated groups (Fig. 1d). Schodde (1976) proposed a phylogenetic framework for the paradisaeid genera which differed from previous workers in that it was expressed as a network, and like previous studies, little evidence was presented (Fig. 1e). Recently, two studies using molecular data have been published: the first based on one-way DNA hybridization distances (Sibley and Ahlquist, 1985; Fig. 1f) and another using a cladistic analysis of protein electrophoretic data (Christidis and Schodde, 1992; Fig. 1g), although both of these

studies included only a small number of paradisaeinine taxa.

Several general patterns of relationships emerge from previous studies, particularly the detailed treatment of the paradisaeids and proposed reclassification by Diamond (1972), which are consistent with the results from our mitochondrial cyt *b* phylogeny.

As demonstrated in a previous study (Helm-Bychowski and Cracraft, 1993), the polygynous, plumage-ornamented core paradisaeinines form a sister-group to the monogamous, unornamented, crow-like manucodines, represented by *Phonygammus* and *Manucodia*. The morphological distinctiveness of manucodines was noted by Bock (1963) and, so far, our molecular data from a wide selection of representative genera support this major division of the Paradisaeidae.

Within paradisaeinines the cyt *b* phylogeny indicates a close relationship between the genera *Diphyllodes* and *Cicinnurus*. In contrast to the remarkable plumage and behavioral differences between these genera, similarities have been identified in their skull and feather tract morphology (Stonor, 1938; Bock, 1963). For example, both genera possess elongated curved central rectrices, with barbs restricted to a single side of the shaft and which cross only at the base of the feathers, a condition found in no other paradisaeinines.

The cyt *b* phylogeny indicates a well-supported sister-group relationship between *Ptiloris* and *Lophorina*. This result is not unsurprising considering the close similarities in female plumage of both genera as well as common elements of plumage ornamentation in the males, for example, the presence of a ventral irridescent blue-green breast shield (Diamond, 1972). The evolutionary position of the monotypic genus *Seleucidis* has been more problematic, mostly due to the unique feather ornamentation of this species. However, skull morphology and feather tract morphology, as well as female plumage patterns, indicate an affinity with the core paradisaeinines (Stonor, 1938; Bock, 1963; Diamond, 1972) and this is verified by the cyt *b* phylogeny which places this taxon at, or near, the base of a clade comprising *Diphyllodes, Cicinnurus, Ptiloris,* and *Lophorina*.

The cyt *b* results provide good evidence for the monophyly of the speciose paradisaeinine genus *Paradisaea*. The lineage leading to perhaps the most divergent species in this genus, *Para. rudolphi*—which differs from other *Paradisaea* most notably in an overall blue plumage color as well as apparently being territorial and nonpolygynous (LeCroy, 1981)—branched off very early in the evolution of this group and was followed by lineages leading to progressively more highly ornamented species.

A close relationship has often been proposed between the genera *Parotia, Lophorina,* and *Ptiloris* because of their supposed plumage similarities (e.g., Diamond, 1972). The cyt *b* results in part support this hypothesis, with *Lophorina* and *Ptiloris* occurring as sister-groups. However, the data indicate that *Parotia* is not closely allied with either of these genera and instead occupies a basal position within the paradisaeinines, the first time such a relationship has been proposed. It is perhaps worth pointing out that *Parotia* does possess unique plumage ornamentation within the paradisaeinines, for example, wire-like plumes on the head and a feathered "skirt" surrounding the body. In addition, all members of the genus *Parotia* clear discrete display areas on the forest floor, a type of courtship behavior not performed by the arboreally displaying genera *Ptiloris* and *Lophorina*.

The sicklebill genera *Drepanornis* and *Epimachus* have long been considered close relatives based on striking morphological similarities of the bill, skull, and feather tracts (Stonor, 1938; Bock, 1963). So similar are the members of the two genera that Diamond (1972) ventured a comprehensive genus *Epimachus* to include all sicklebill species. However, in this analysis the cyt *b* data do not support the monophyly of these birds. This result could be influenced, in part, by the large number of autapomorphic characters on the branch to *Dr. albertisi*. Such characters might create random similarities to character states of other taxa and thus affect placement of *Drepanornis* on the tree (Felsenstein, 1978). The addition of the only missing sicklebill species from our study, *Drepanornis bruijnii*, might improve phylogenetic resolution by reducing the number of potentially homplastic characters along this branch.

The overall relationships among the major paradisaeinine lineages presented above should be considered tentative. Based on cyt *b* data the relationships among basal regions of the phylogeny are resolved relatively weakly, with small numbers of synapomorphies and consequently depressed bootstrap support for these branches. Ongoing studies of other paradisaeinine genera, the addition of other mitochondrial gene sequences, along with data incorporated from morphological investigations in progress will possibly alter some of the conclusions.

The Tempo of Paradisaeid Diversification

A number of authors have hypothesized a linear relationship between age of divergence and the accumulation of transversion differences in mitochondrial genes (Brown *et al.,* 1982; de Salle *et al.,* 1987; Miyamoto and Boyle, 1989; Irwin *et al.*, 1991). If this is approximately the case, the data of Table 2, along with the phylogenetic hypothesis of Fig. 4, can be used to draw inferences about the tempo of diversification within paradisaeids. It is clear from a comparison of Table 2 and Fig. 4, however, that if the relationships shown in Fig. 4 are indeed correct, there must have been significant differences in the rate of change in cytochrome *b* along the lineages of paradisaeids, and consequently it must also

be true that transversion distances are not reliable indicators of age of divergence. On the other hand, cytochrome *b* sequences by themselves may not be capable of resolving relationships across a particular clade. The relationships shown in Fig. 4 might therefore be incorrect to one degree or another, which would lead us to misinterpret the data on transversion differences and what they imply about the tempo of diversification. As a consequence of these caveats, the following conclusions should be considered tentative.

Given the assumption that the rate of cyt *b* transversion accumulation is relatively uniform within birds-of-paradise overall, the data suggest that the divergence between the two major lineages, the manucodines and the paradisaeinines, is old and occurred perhaps more than 20 million years ago (see Helm-Bychowski and Cracraft, 1993). Within the manucodines the divergence between *Manucodia* and *Phonygammus* was also relatively ancient, more so than current discussions of paradisaeid relationships seem to imply (e.g., Diamond, 1972, pp. 307–308). Indeed, the data included here suggest that this divergence is as old as the basal radiation of the core birds-of-paradise, the paradisaeinines.

With respect to the taxa included in this study, the contrast between long terminal branches and short internal branches leading to the main paradisaeinine lineages indicates a relatively rapid cladogenesis at an early point in the evolutionary history of these birds [similar results are apparent among the galliform birds (Kornegay *et al.*, 1993), as well as in the deeper branches of the corvine assemblage (Helm-Bychowski and Cracraft, 1993)]. Several divergences, for example, all seem to be fairly coincident in time: *Diphyllodes* versus *Cicinnurus, Ptiloris* versus *Lophorina,* and *Para. rudolphi* versus the other *Paradisaea.*

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