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MOLECULAR PHYLOGENY OF TROGONIFORMES (AVES)

by

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A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

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Jesús Alejandro Espinosa de los Monteros Solís

Advisor: Dr. Joel L. Cracraft

The Trogoniformes is a well defined order of non-passerines. The higher-level phylogenetic relationships of the order are poorly understood. Many approaches have been used to address the relationships of trogoniforms, but the results do not show consistency. Additionally, the characters used to establish the divisions inside the Trogoniformes either overlap among taxa or are so ambiguous as to be impractical for forming monophyletic groups.

In this study, I investigated the phylogenetic relationships of the Trogoniformes based on cladistic analyses of DNA sequences of mitochondrial 12S ribosomal RNA (rRNA) and cytochrome-*b*.

Chapter 1 explores the higher-level relationships of Trogoniformes to several putative relatives. In this study 35 taxa were sequenced including trogons, owls, cuckoos, parrots, nightjars, hummingbirds, kingfishers, motmots, rollers, mousebirds, and woodpeckers. This analysis concluded that trogons are more closely related to mousebirds, parrots and cuckoos than to any other birds. Most analyses recovered the Coliiformes as the sister-taxon

to trogons, however, conflicting results did not allow a conclusive resolution of their higher-level affinities. The limited number of characters supporting internodal branches is best interpreted as a relatively rapid cladogenesis in their evolutionary history.

Chapter 2 examines the intergeneric relationships among trogons. The results show that the genera currently accepted are monophyletic. The New World genera were the sister-taxa to the Asian trogons, and the most basal clade was the African trogons. The phylogeny supports Africa as the ancestral area of trogons, and that the New World was invaded subsequently. Finally, patterns of plumage coloration are examined, giving special attention to the evolution of iridescent structures in feathers.

In Chapter 3 a model for the secondary structure of the 12S rRNA gene is presented. The model proposes the existence of 40 stems and 37 loops, arranged in the typical four helices suggested for the small subunit rRNA. The higher-order structures were used to build an alignment appropriate for phylogenetic analysis. The phylogeny recovered from this alignment was compared with trees inferred from alignments assembled with alignment software. The alignment based on the secondary structure is sensitive to positional covariation of stems. Nonetheless, the phylogeny recovered with this method was more congruent with relationships proposed with non-molecular data than were the phylogenies inferred from the use of alignment software packages.

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GENERAL INTRODUCTION

The Order Trogoniformes is a small uniform group of mainly tropical, forest-dwelling, frugivorous birds encompassing a total of 39 species (Table 1). These birds have been divided into six genera: *Apaloderma, Pharomachrus, Euptilotis, Priotelus, Trogon,* and *Harpactes* (Sibley and Monroe 1990).

The natural history of some species is well known (e.g. Skutch 1942, 1944, 1948). Trogons are brightly colored birds, often with metallic plumage, having short necks, broad stubby bills, long and truncate or square tipped tails, and in some species of quetzals (*Pharomachrus*), with long streaming tail coverts that far exceed the length of the tail. Most species show sexual dimorphism both in size and color pattern. These solitary forest birds are strictly arboreal. Trogons fly short distances following an undulated pattern. Trogons nest in natural cavities in decayed trees, termite or wasp nests, and frequently using old abandoned nests of parrots or large woodpeckers. The clutch size is from 2 to 4 eggs. The shell's coloration is often white, and in some species lightly blue, but never with dark spots or bands. Incubation and parental care are undertaken by both sexes. The chicks are nidicolous (i.e., they are born naked and with their eyes closed). The diet of trogons is composed of small fruits and insects, both of which they take while flying. The larger species can prey on snails and small vertebrates like lizards and frogs. Trogoniforms are distributed world-wide, but confined to tropical

areas. None of the species is migratory, but some of them undertake local movements.

The Trogoniformes is one of the orders of non-passerine birds in which there is relatively little variation in external morphology. Ridgway (1911, vol. V p. 731) gave the following diagnosis of the family Trogonidae:

> "Bill short (much shorter than head), broad basally (triangular in vertical profile), the culmen decurved and terminally more or less distinctly uncinate; maxillary tomium serrate subterminally (smooth in one American genus, *Pharomachrus*, and in most of the Old World forms); base of bill concealed by well-developed, curved, antrorse latero-frontal, pre-malar, and mental bristle-tipped feathers; feet weak, the tarsus much shorter than longest anterior toe, sometimes entirely feathered; anterior toes united for about the basal half; wing short, rounded, very concave beneath; primaries more or less falcate or subfalcate terminally, the tenth decidedly shortest; secondaries short; tail longer than wing, composed of twelve broad and nearly truncate rectrices; plumage dense and soft, easily detached; colors bright, brilliantly metallic in adult males, the under parts of body pure red, orange, or yellow; young usually spotted and without bright colors."

To complement Ridgway's description, a list of morphological characters used to characterize the order is given in Table 2. Perhaps the only autapomorphy that differentiates trogoniforms from other birds is their heterodactyl foot. In this condition, digits 1 and 2 are directed backward, whereas digits 3 and 4 are united for their basal half and directed forward. In other words, the hallux is the outer of the two hind toes, whereas in zygodactyl birds it is the inner one. This unusual arrangement of the toes is correlated with a distinctive arrangement of the deep plantar tendons. The flexor perforans, leading to the two front digits, is split into two parts. The

flexor hallucis is also divided, going respectively to the two posterior toes. Maurer (1977) called attention to some other important characters in the appendicular myology of trogoniforms. The M. extensor longus digiti majoris pars distalis, the acetabular and post-acetabular portions of M. iliotibialis lateralis, and M. flexor cruris lateralis pars accessorius are absent. The M. pubo-ischiofemoralis consists of one belly. In some sense, trogons are relatively primitive in terms of intrinsic muscles of the foot and possess all but M. adductor digiti II, M. adductor digiti IV, and M. lumbricalis. They also possess the ancestral condition of M. peroneus longus in which a tendon passes to M. flexor perforatus digiti III.

The Coraciiformes have been proposed by some authors as the most likely close relatives of trogons (e.g. Feduccia 1975; Maurer and Raikow 1981; Sibley and Ahlquist 1972, 1990). However, trogons share several myological features with other avian groups that are not present in coraciiform birds. Some of these differences with coraciiforms are: a type VII plantar tendon pattern, a tendinous M. pectoralis pars propatagialis longus, a scapular origin of M. deltoideus minor, a humeral anchor of M. triceps branchii, the insertion of M. flexor digitorum superficialis on the distal phalanx of digiti majora, a radial head to M. extensor digitorum communis, and the loss of the proximal tendon of M expansor secundariorum (Maurer 1977).

The monophyly of the order Trogoniformes is generally not questioned. However, the phylogenetic relationships within the order itself

are poorly understood, as are the relationships of trogons to other orders of birds. Therefore, I propose to use molecular data to explore three primary systematic questions:

A) What is the sister-group of the Trogoniformes?

B) What are the intergeneric relationships within trogons?

C) What are the relationships of the species within the order?

This dissertation will follow a paper format, with each chapter being written as an independent paper. Therefore, each chapter follows the guidelines of the journal to which the paper will be submitted. The references for each chapter are presented as a final section at the end of the dissertation.

Chapter 1 addresses the evolutionary relationships among the Trogoniformes and several other putative closely related groups of birds. The phylogenetic analysis is based on the complete cytochrome *b* gene and nearly complete 12S ribosomal RNA gene sequences for a total of 2102 base pairs. Maximum parsimony was used to reconstruct the phylogenetic hypotheses. In addition to the phylogenetic affinities of trogons, this chapter reviews the monophyly of several of the bird groups used.

Chapter 2 attempts to answer the second and third objectives of my dissertation. I used a total of 20 species of trogons for this analysis. Representatives of the Coliiformes, and Cuculiformes were used for character

polarization and rooting of the phylogeny. I chose these taxa based on the results obtained in chapter 1.

In the final chapter I present a model for the secondary structure of the 12S rRNA using the sequences obtained during the course of this study. The model was used to construct a multiple alignment and the phylogeny recovered was contrasted with phylogenetic hypotheses inferred from alignments built through alternative methods.

Table 1. Classification of Trogoniformes (Sibley and Monroe 1990).

Order	Trogoniformes	
Family	Trogonidae	
Subfamily	Apaloderminae	
		Apaloderma narina
		aequatoriale
		vittatum
Subfamily	Trogoninae	
Tribe	Trogonini	
		Pharomachrus mocino
		antisianus
		fulgidus
		auriceps
		pavoninus
		Euptilotis neoxenus
		Priotelus temnurus
		roseigaster
		Trogon massena
		melanurus
		clathratus
		comptus
		viridis
		bairdii
		citreolus
		melanocephalus
		mexicanus
		elegans
		collaris
		aurantiiventris
		personatus
		rufus
		surrucura
		curucui
		violaceus
Tribe	Harpactini	
		Harpactes reinwardtii
		fasciatus
		kasumba
		diardii
		ardens
		whiteheadi
		orrhophaeus
		duvaucelii
		oreskios
		erythrocephalus
		wardi

Table 2. Characters used to diagnose the order Trogoniformes (Sibley 1955).

Palate: schizognathous
Basipterigoid processes: present
Vomer: large
Sternal notches: two on each side
Aftershalft: large
Nostrils: holorhinal
Feet: heterodactyl
Gadow's deep plantar tendon arrangement type: VIII
Pelvic muscle formula: AX
Caeca: large
Carotids: left only
Syrinx: tracheo-bronchial
Primaries: 10
Secondaries: 11-12
Rectrices: 12
Oil gland: nude
Culmen: decurved and uncinate

CHAPTER 1

HIGHER-LEVEL PHYLOGENY OF TROGONIFORMES

Summary. The evolutionary relationships between the Trogoniformes and several other putative closely related groups of birds were studied using mitochondrial DNA sequences. The analysis was based on complete cytochrome-*b* and nearly complete 12S ribosomal RNA (rRNA) gene sequences for 35 taxa including trogons, owls, cuckoos, parrots, nightjars, hummingbirds, kingfishers, motmots, rollers, mousebirds, and woodpeckers. The Coraciiformes have been suggested as the nearest relatives to trogon, however, this hypothesis was not supported. The analyses concluded that trogons are more closely related to mousebirds, parrots and cuckoos than to coraciiforms. However, the conflicting results did not allow elucidation of higher-level phylogenetic affinities of trogons. A saturation analysis showed evident saturation in cytochrome-*b* third positions and 12S rRNA loops. After removing the saturated partitions the phylogeny supported the mousebirds (Coliidae) as the sister taxon to trogons. Phylogeny inconsistencies appeared to be attributable to an imbalance between the length of terminal and internodal branches. The limited number of characters supporting internodal branches seems to exemplify a relatively rapid cladogenesis at an early period in the evolutionary history of these birds.

Introduction

In 1880 Sclater wrote: "It will be generally allowed, I believe, by all ornithologists that the Systema Avium is not at present in a very satisfactory state." More than a hundred years later ornithologists are confronting the same predicament. Although, it has been estimated that we currently know more than 90% of the extant species of birds (e.g., Clements 1991), the phylogenetic relationships among them are poorly understood. The Order Trogoniformes is not an exception. From the earliest classification by Linnaeus until today, the Trogoniformes have been placed close to kingfishers, motmots, cuckoos, parrots, toucans, and rollers, among others.

A chronological outline of the history of the proposed affinities of the order Trogoniformes is presented in Table 1. An extensive review of the classification of these birds based on morphological studies was given by Sibley and Ahlquist (1990), therefore it will not be duplicated here. However, it is important to outline some of the factors that have made it difficult for systematists to understand the phylogenetic relationships between trogons and other groups of birds. Although numerous morphological characters have been used to explore the phylogenetic history of birds, most classifications have been based on the myological and skeletal structure of the hindlimb (Garrod 1874, Sclater 1880, Mayr and Amadon 1951). These characters have been used because of their structural diversity among non-

passerine birds. In the trogoniforms, a heterodactyl foot is the autapomorphy that clearly defines them as a monophyletic group. Trogons differ from other birds in that digits one and two are directed backward, whereas digits three and four are united for their basal half and directed forward. This configuration is different from the primitive zygodactyl condition in which the hallux (digit 1) is the outer of the two hind toes. The derived heterodactyl condition implies a distinctive organization of the deep plantar tendons. Thus, the flexor perforans, leading to the two front digits, is divided into two parts, and the flexor hallucis is divided to the two posterior toes (Maurer 1977). Another remarkable difference in the appendicular myology of trogons is the considerable reduction of several muscles of the leg. This apparent simplicity in the intrinsic muscles of the foot makes trogons appear relatively primitive. Yet another plesiomorphic condition present in trogons is the insertion of the M. peroneus longus onto the tendon of to M. flexor perforatus digiti III (Maurer and Raikow 1981). The configuration of the hindlimb muscles of trogons, which helps define the highly derived heterodactyl foot, is not shared with other birds and is not therefore informative with respect to relationships.

Besides foot structure, many different characters have been used to explore the phylogenetic relationships of trogons to other birds (Table 1). However, from Gadow's classification based on forty characters (Gadow 1892) to single character phylogenies (Gadow 1889, Verheyen 1960, Feduccia 1975),

morphological analyses have resulted in the conclusion that even though the characters used are all taxonomically useful to some degree, none of them is completely consistent and trustworthy. The apparent lack of congruence shown by morphology has led some systematists to apply a molecular approach to resolve the phylogenetic relationships of trogons.

In 1960, Sibley found that the electrophoretic pattern of the egg-white proteins of the Narina Trogon (*Apaloderma narina*) was relatively simple and similar to that of passerine birds. Subsequently, Sibley and Ahlquist (1972) compared the electrophoretic pattern of the egg-white proteins of *A. narina* with those of many other groups of birds, and suggested that it had similarities to coraciiforms in the mobility of the conalbumins and the dense Component 18. Based on the single ovalbumin region, however, the same analysis showed similarities to swifts, mousebirds, cuckoos, and passerine birds. A clear conclusion of egg-white protein electrophoresis was that the pattern of *Apaloderma* was quite different from those of Caprimulgiformes and Piciformes. In 1990, Sibley and Ahlquist approached the problem once again, this time using DNA-DNA hybridization data. In a first analysis they used *A. narina* as the tracer taxon, and found that *Trogon* and *Harpactes* were equidistant from *Apaloderma*, with the next closest taxon being two hornbills (Bucerotidae). Their results were not consistent, however, as a second analysis implied two owls and two nightjars were the closest taxa to trogons. When *Harpactes* was used as tracer taxon, an owl and a nightjar were again

the closest taxa. These apparently inconsistent results led them to the conclusion that (Sibley and Ahlquist 1990: 356): "the trogons have no close living relatives and, unless they are more closely related to owls, nightjars, or turacos, they are members of the cluster that includes the rollers, todies, hornbills, kingfishers, motmots and bee-eaters." Harshman (1994) reanalyzed the DNA-DNA hybridization data presented by Sibley and Ahlquist, but the tree he recovered for the assemblage of birds in which trogons were included was completely unresolved.

The main objective of the present study is to clarify the evolutionary history of the order Trogoniformes using a new molecular data set, namely mitochondrial DNA sequences. In addition, this study will also provide evidence bearing on a number of related taxonomic questions, particularly the monophyly of some of the major groups of non-passerine birds.

Materials and Methods

Data Collection. Variation in the rate of nucleotide substitution in DNA sequences occurs among sites (e.g., second vs. third position) and among the kinds of substitutions (e.g., transitions vs. transversions). In a general way, rapidly evolving changes like silent third positions or transition substitutions can help to resolve phylogenetic relationships among recently divergent taxa. On the other hand, more slowly evolving changes, like transversions or

amino-acid replacements, can be useful to infer phylogenies among older divergences. The cytochrome-*b* gene has proven to contain phylogenetic signal at several different levels. Using all phylogenetically informative characters contained in cytochrome-*b*, this gene has been used to address population and species problems (Smith and Patton 1991, Moritz et al. 1992). The cytochrome-*b* gene also has been used to study presumably older divergences by ignoring rapidly evolving changes (e.g., Edwards et al. 1991, Irving et al. 1991). At the same time, however, cytochrome-*b* has been shown to be uninformative for several higher-level phylogenetic problems (Graybeal 1993). The mitochondrial 12S rRNA gene, in contrast, shows a slower evolutionary rate than cytochrome-*b* and has been used to resolve extremely old divergences (Hay et al. 1995, Haise et al. 1995, Springer and Douzery 1996). Because they provide potentially complementary sets of character data to resolve relationships at different hierarchical levels, sequences from both cytochrome-b and 12S rRNA genes were used in this study.

Taxa Examined. Exploring the phylogenetic affinities of trogons required, in addition to an adequate sample of variation within trogons, an adequate sample of species from groups previously considered to be their close relatives. The complete sequence of cytochrome-*b* gene and nearly complete sequence of 12S rRNA gene, adding to a total of 2102 base pairs, were determined for 35 species of non-passerine birdsbelonging to 19 families

sensu Sibley and Ahlquist (1990) (Appendix I). The eleven major non-passerine groups included in this study were the Trogonidae (six species), Coraciiformes (seven species), Psittacidae (four species), Coliidae (three species), Piciformes (three species), Cuculiformes (three species), Strigidae (three species), Caprimulgidae (two species), Trochilidae (two species), Bucerotidae (one species), and Upupidae (one species) [From now on, I will use the expression "major groups" when referring to these eleven groups]. Tissue samples were obtained through grants from nine scientific collection (Appendix I). The cytochrome-*b* and 12S rRNA sequences for the chicken (*Gallus gallus*) were taken from Desjardins and Morais (1990). The 12S rRNA sequence for the Common Mallard (*Anas platyrhynchos*) was taken from Mindell et al. (1996). Finally, the cytochrome-*b* sequence for the Common Mallard was provided by Scott Stanley (unpubl. data). The chicken and mallard sequences were employed as outgroups to root the phylogeny.

DNA Extraction and Sequencing. Total genomic DNA was extracted from frozen tissue (muscle, liver and heart) using a Chelex 5% solution w/v following the protocol suggested by Singer-Sam et al. (1989). Target genes were amplified and isolated as single fragments using specifically designed PCR primers. This first amplification was conducted in Peltier-effect thermocyclers (MJ Research) following the parameters and conditions suggested by Nunn et al. (1996). PCR products were subjected to horizontal

electrophoresis in a 2% NuSieve low-melting point agarose gel (FMC Bioproducts). Gels were stained for 10 min. in a solution of 2 pg/ml of ethidium-bromide and visualized under UV light. The double-stranded DNA (dsDNA) products were cut directly from the gel and resuspended in 150 µl of ultra-pure water by heating to 73°C for 15 min. The genes were reamplified in subfragments of about 400 bp using internal oligonucleotide primers. Primers LPhe (L1243) 5'-CAAACAAAGCATGGCACTGAAG-3', and 12Sd (H1883) 5'-TTCGATTATAGAACAGGCTCCTC-3', were designed in our laboratory (numbered following the chicken mitochondrial genome [Desjardins and Morais 1990]). The remaining primers used are described elsewhere (Helm-Bychowski and Cracraft 1993, Knight and Mindell 1993). An air thermocycler (Idaho Technologies), was used to perform 40 µl amplifications of these subfragments in glass microcapillary tubes using standard buffers (Wittwer 1992). Subfragments were amplified using the following conditions: 2 sec at 94°C, 0 sec at 47°C, 15 sec at 71°C, for 35 cycles at slope 7. All PCR experiments were conducted along with positive and negative controls to test for contamination. Aliquots of 3 µl were visualized as described above. The remainder was purified to eliminate PCR primers, dNTPs, enzyme, and buffer components, using the GeneClean II kit (BIO 101 Inc.). Purified PCR products were subjected to cycle sequencing using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq DNA Polymerase FS, on the GeneAmp PCR System 9600 (Perkin Elmer).

Amplifications were performed in 6 µl reaction volumes containing 2.5 µl of the Prism kit reagent, 1 pmole of sequencing primer, 5 ng/µl dsDNA template, and 2 µl of ultra-pure water. Cycle sequencing was performed using the following conditions: 10 sec at 95°C, 5 sec at 50°C, 3 min at 60°C, for 32 cycles. The excess of Taq dideoxy terminators was removed with Centri-Sep spin columns (Princeton Separations) in a variable speed microcentrifuge at 2500 rpm for 2 min. Final purifications were dried in a vacuum centrifuge and resuspended in 2.5 µl of the loading buffer (6x deionized formamide, 1x 50 mM EDTA pH 8.0). Resuspended sequencing products were subjected to 4% polyacrylamide denaturing gel electrophoresis in the ABI Prism 377 DNA Sequencer (Perkin Elmer). Sequence files were analyzed with the aid of the program Sequencher v 3.0 (Gene Codes Corp., Ann Arbor, MI). A large degree of fragment overlap, as well as sequencing of both DNA strands ensured accurate data collection. Alignment of cytochrome-*b* sequences was by eye, but the 12S rRNA sequences were aligned using the program Malign (Wheeler and Gladstein 1992) and refined manually according to the proposed secondary structure for the 12S rRNA molecule (Springer and Douzery 1996, Houde et al. 1997, Mindell et al. 1997). A data set of alignment stable positions was produced by excluding ambiguous nucleotide sites (Gatesy et al. 1993).

Phylogenetic Analysis. Only alignment-stable regions within the data set

were used for phylogenetic analysis. Phylogenetic hypotheses were computed by maximum parsimony conducted with the program Phylogenetic Analysis Using Parsimony (PAUP 3.1.1; Swofford 1993). As the large number of taxa included in this study did not allow the use of exact searching algorithms, parsimony analyses were performed by heuristic searches. A hundred replicate searches with random addition of taxa were performed to eliminate input order bias. During all analyses, character polarization was established by outgroup comparison, and nucleotide transformations were considered unordered. Branch length was optimized by using delayed transformation (DELTRAN) which favors parallelisms over reversals. Branch swapping was made by the tree bisection reconnection algorithm (TBR). In those cases in which the solution included multiple equally parsimonious trees the signal was identified using strict consensus trees. Retention and consistency indexes were computed to evaluate the level of homoplasy in the most parsimonious tree. Robustness of the tree was examined by 300 bootstrap replications (Felsenstein 1985, Hillis and Bull 1993), as well as by branch support (Bremer 1988, 1994).

Saturation Analysis. A corrected distance matrix from the nucleotide sequences was computed to test for saturation effects (Arctander 1991, Maynard-Smith and Smith 1996). Correction was made with the program DNADIST from Phylip 3.5p package (Felsenstein 1993) using the Kimura two-

parameter model (Kimura 1980), assuming a 10:1 transition-transversion bias. This bias is considered a conservative estimate for birds (Kocher et al. 1990, Nunn and Cracraft 1996, Espinosa de los Monteros and Cracraft 1997).

Results

Variability in Cytochrome-b. The multiple alignment for the cytochrome-*b* sequences showed a continuous reading frame without indication of codon insertions or frameshifts. The reading frame for 34 taxa includes a total of 381 codons, and for three species of parrots (*Micropsitta finschi, Pionus senilis,* and *Psittrichas fulgidus*) the reading frame has 380 codons. In these three parrots, codon 380 has changed from tyrosine (TAC) to a stop codon (TAA). The termination codon for all the sequences was TAA as commonly found in the chicken mitochondrial protein-coding genes (Desjardins and Morais 1990). Recently, several studies have reported nuclear copies of mitochondrial genes especially cytochrome-*b* (Quinn 1992, Smith et al. 1992, Kornegay et al. 1993). These nuclear pseudogenes are characterized by frameshifts and nonsense codons, neither of which were apparent in the sequences obtained in the present study. Furthermore, given the fact that the complete cytochrome-*b* gene was isolated initially as a single fragment, including the tRNA-Thr and the last 90 bases for ND5 as flanking regions, I assume that the reported sequences are of mitochondrial origin only and do

not represent pseudogenous or translocated fragments that might be located elsewhere in the bird genome.

Across the 37 species included in this analysis the cytochrome-*b* contained 527 invariant positions (46.1%), 69 autapomorphic characters (6%), and 547 phylogenetically informative characters (47.9%). Variation was found mainly in third positions with 368 (59.8% of the polymorphic characters), followed by first positions with 167 (27.1%), and the least variable codon site was the second position with 81 (13.1%). Almost every third codon position was hit, most of this variation representing silent changes. Among the translated cytochrome-*b* protein sequences 144 (37.9%) of the 380 amino acid residues were variable. The highest incidence of hypervariable residues was located inside the fifth and eight transmembrane segments of the structural model for cytochrome-*b* (Howell 1989).

The distance differences shown in Table 2, constitute the mean value for all pairwise comparisons between species within two major groups (i.e., a mean value of 225 differences between Trogonidae and Coraciiformes is the arithmetic mean of the 42 pairwise comparisons).

Within trogons the total empirical number of substitutions was 183, and 61 for transversions (Table 2). Total divergence, transversions, and corrected distances identifies the Upupidae as the most distant taxon to trogons among the eleven groups studied (241, 119, and 29.8% respectively). Using total sequence divergence the closest taxon to trogons is the Psittacidae,

with 212 nucleotide substitutions. Examining only transversion differences the bucerotids are the closest to trogons, with 96 transversion substitutions.

Among major groups total uncorrected divergences range from 192 between Bucerotidae and Psittacidae to 243 between Upupidae and Strigidae. However, using only transversion substitutions the least variation was between Bucerotidae and Caprimulgidae (87 transversion differences). Based on pairwise corrected distances using Kimura's two parameter model (Table 3) the largest difference occurred between Upupidae and Strigidae (30.8%), whereas the smallest was between Bucerotidae and Caprimulgidae (21.8%).

The nucleotide compositional bias (C) in cytochrome-*b* with respect to codon position observed in this analysis (Table 4) is practically the same as that reported for other birds (Nunn and Cracraft 1996, Nunn et al. 1996) and for mammals (Irwin et al. 1991). The highest bias was observed in third positions (C=0.435), this codon position is rich in cytosine (45.6%), followed by adenine (37%), then thymine (13.4%), and very low in guanine (4%). A higher nucleotide compositional bias in third positions is an expected result, because the changes in nucleotides at this position tend to have a silent effect, therefore they must be the most variable. Second positions showed an intermediate bias (C=0.22) being rich in T (39.4%) and poor in G (12.9%), with intermediate percentages for C (27.1%) and A (20.6%). Finally, the smallest bias was observed in first positions (C=0.079). These sites are slightly rich in C (29.4%), poor in G (20.9%), and the proportions registered for A and T were

26.6% and 23.2%, respectively.

Variability in 12S rRNA. The fragment sequenced for the 12S rRNA contained several hypervariable regions that were characterized by multiple insertions and deletions, producing considerable variation in the length of the sequences. Although the multiple alignment for the 37 sequences had a length of 959 positions (excluding ambiguous alignments), the average number of nucleotides in the 12S rRNA was 905 bases (ranging from 897 in *Upupa epops* to 911 in *Geococcyx velox*). Using the proposed model for the secondary structure of the 12S rRNA molecule (Springer and Douzery 1996, Houde et al. 1997, Mindell et al. 1997), I was able to identify functional regions (i.e., stems and loops) in the sequences. Most of the stems and loops proposed in the core model for 49 species of mammals were easily identified in the nonpasserine 12S rRNA molecule. The major difference found was in the helix leading to stems 17 to 21. The number of stems and their lengths identified for birds in this region were too variable to be able to postulate homologies with the secondary structure model presented for vertebrates.

Of the 959 nucleotide positions aligned for the 12S rRNA matrix, 483 were invariant (50.4%), 64 were autapomorphic characters (6.7%), and 412 were phylogenetically informative characters (42.9%). Relatively higher variation was found in loops with 263 varying positions (55% of the polymorphic characters), followed by stems with 213 (45%). Ambiguous

alignments were produced by the occurrence of hypervariable fragments located mainly inside terminal loops.

Within trogons, the total nucleotide differences in the 12S rRNA sequences were 122, whereas only 36 transversion substitutions were found (Table 5). The closest taxon to trogons was Caprimulgidae with 157 nucleotide substitutions, while the two most distant taxa were Upupidae and Piciformes, each with 195 substitutions. Transversion differences ranged from 57 to the Cuculiformes to 76 to the Trochilidae. The difference between total and transversion distances may indicate a variable rate in nucleotide substitution. Finally, based on Kimura's corrected distances (Table 3), the closest taxon to trogons was Caprimulgidae (21.6%), and the most distant was Upupidae (29.1%).

Considering all eleven groups, Caprimulgidae and the outgroup (*Gallus-Anas*) are the most closely related taxa (141 nucleotide substitutions). Using total sequence divergence, Strigidae and Trochilidae were identified as the most divergent taxa, but the Strigidae and Upupidae exhibited the largest divergence based on transversion substitutions.

Table 6 reports nucleotide frequencies and compositional bias for stems and loops in 12S rRNA fragments. Of the two structural regions, loops presented the highest bias (C=0.265). Adenine, the most abundant nucleotide (41.4%), was followed by cytosine (28.7%), thymine (17.9%), and guanine (12%). An overabundance of adenine in loops has been explained by the

suggestion that the low polarity of adenine may favor hydrophobic interactions with proteins (Gutell et al. 1985). The nucleotide composition in stems was more uniform (C=0.075), being slightly richer in C (28.4%) and poorer in T (21.6%), with intermediate percentages for G (27.2%) and A (22.8%). Although there are some differences between the stems and loops in the 12S gene of non-passerine birds to those homologized for other organisms, the above results are consistent with previous reports (Springer et al. 1995, Springer and Douzery 1996, Houde et al. 1997, Mindell et al. 1997).

Phylogenetic Analysis. For maximum parsimony, the cytochrome-*b* and 12S rRNA sequences were subjected to independent as well as to combined analyses. In these analyses all phylogenetically informative positions were included and no deferential weight was assigned to any character. *Gallus* and *Anas* were specified as outgroups and roots of all tree searches. The results of these analyses are presented in Figure 1.

The analysis based on cytochrome-*b* alone resulted in seven equally parsimonious trees (4,080 steps long, 0.266 consistency index, and 0.317 retention index). The strict consensus tree was largely unresolved (Fig. 1a) with the coraciiform family Alcedinidae as the most basal taxon. Parrots (Psittacidae) were resolved as the sister-group of the trogons, and placed in a clade with three coraciiform families [((Momotidae, Todidae) Ceryliidae)]. A second resolved clade found in this tree was the hummingbirds (Trochilidae)

as the sister-taxon to the two piciform families (Ramphastidae and Picidae). Cytochrome-*b* sequences reveals that most of the major groups are monophyletic. The two exceptions were the coraciiforms, the families of which were scattered all over the tree, and the cuculiforms (Cuculidae, Centropidae, and Neomorphidae) which were paraphyletic, with the hoopoe (Upupidae) included within the cuculiform clade.

The phylogenetic analysis for the 12S rRNA produced three equally parsimonious trees of 2,672 steps in length, consistency index of 0.309, and retention index of 0.413. Although this gene produced fewer trees than the analysis of cytochrome-*b*, the strict consensus for these three trees was less resolved than that for cytochrome-*b*. Only two clades were identified in the consensus solution. In one, the hummingbirds were the sister-group to one of the cuculiform families (Centropidae), with their sister-taxon being the nightjars (Caprimulgidae). The other clade was formed by the hornbills (Bucerotidae) as the sister-group to the hoopoes, with the todies (Todidae) resolved as their closest relatives. Despite the lack of resolution in the consensus tree, most of the major groups were monophyletic (Fig. 1b). As in the cytochrome-*b* analysis, the two major groups that were not monophyletic in the 12S rRNA analysis were the coraciiforms and the cuckoos (Cuculiformes).

The global parsimony analysis, including all nucleotide positions for both genes, resulted in two equally parsimonious trees of 6,847 steps, 0.280

consistency index, and 0.347 retention index. The alternative phylogenetic hypotheses differ only in the relationships within trogons, therefore the strict consensus tree presented here is completely resolved (Fig. 1c). The most basal clade was formed by the hummingbirds and the nightjar as sister-taxa to each other. The rest of the cladogram was subdivided into two major subclades. The first was formed by the hoopoe plus the three cuculiform families, which in this cladogram are paraphyletic, as well as by their basal sister-group, the trogons. Within the second subclade three close associations between major groups can be distinguished: owls are most closely related to piciforms, hornbills are the sister-taxon to two coraciiform families (Coracidae, Meropidae), and mousebirds (Coliidae) are the closest taxon to parrots. As in the previous analyses the only major groups that were not recovered as monophyletic were the cuckoos and the coraciiforms. When 300 bootstrap replications were applied to this data set, only some of the major groups were supported by high bootstrap values, and none of the internodes had a bootstrap value greater than 50% (Fig. 1c).

Several studies have shown that by removing rapidly evolving changes, the phylogenetic signal can be improved for deep divergences (Edwards et al. 1991, Irving et al. 1991, Helm-Bychowski and Cracraft 1993). A transversion parsimony analysis including both genes was therefore conducted. The result produced seven equally parsimonious trees with length of 2,525 steps, 0.257 consistency index, and 0.448 retention index. The

strict consensus tree identified two coraciiform families (Meropidae, Coraciidae) as the most basal lineages of the tree (Fig. 1d). The remainder of the cladogram was poorly resolved. One major unresolved subclade included the hummingbirds, hoopoe, nightjars, and cuckoos. Two more subclades were delimited: in one, the mousebirds were the sister-group to the parrots, and in the other the hornbills were the sister-taxon to piciforms. Seemingly, removing all transitions from the analysis, instead of improving signal recovery, produced a loss in the structure at deeper nodes.

One of the attributes of DNA sequences is that at every nucleotide position there are only four possible character states, therefore multiple hits at a single site result in swapping nucleotides, eventually exhausting the chance of increasing variability. This saturation effect may confound phylogenetic analyses, because it increases the level of homoplasy. Empirical distances were plotted against Kimura distances to detect saturation in the different partitions of the sequenced genes.

Figure 2a shows the saturation pattern for transitions and transversions in cytochrome-*b*. It can be seen that transversions increase linearly while transitions accumulate faster and reach a plateau starting around 16%. A more precise saturation pattern is obtained from analyzing transition distances depending on codon position. The results show that the saturation rate was not the same for all sites (Fig. 2b). Second position transitions increased at a very slow rate and maintained a linear tendency.

First position transitions had a faster rate than second positions, but also increased more or less linearly. Finally, codon third positions diverged rapidly and plateaued at approximately 15%. The saturation zone was reached faster at third positions presumably because most of them represent synonymous sites.

A similar saturation analysis was conducted for the 12S rRNA sequences (Fig. 3). Plotting the empirical number of transitions and transversions in the complete gene against Kimura distance shows a linear path for both kind of changes. However a slight drop in the rate of transition accumulation is observed about 16% (Fig. 3a). When transitions were divided by structural regions for the 12S rRNA it was clear that transitions in stems maintain a linear path (Fig. 3b), but transitions in loops were saturated after 15% (Fig. 3c).

Based on this analyses I decided to eliminate the partitions that showed evident saturation (i.e., cytochrome-*b* third position transitions, and 12S rRNA transitions in loops, a total of 860 nucleotide positions). A global parsimony analysis of the modified data set produced one most parsimonious tree of 2,108 steps, consistency index 0.316, and retention index 0.415 (Fig. 4). In this phylogenetic hypothesis the mousebirds were the sister-taxon to the trogons, with their sister-group being the parrots, followed by the now-monophyletic cuckoos [((Cuculidae, Centropidae), Neomorphidae)], a clade containing three coraciiforms (hoopoes, hornbills and bee-eaters), then a clade

formed by the five remaining coraciiform families [((Momotidae, Todidae), ((Coraciidae, Alcedinidae), Cerylidae))]. The sister-group of all the above was a owl-piciform clade. Finally, at the base of the tree were the nightjars, followed by the most basal clade, the hummingbirds (Fig. 4).

The removal of the 860 nucleotide positions from the analysis produced a decrease in tree length of 4,739 steps relative to the initial combined analysis (Fig. 1C). This implies that the average character within the removed partitions had been hit 5.5 times, thus making them a major source of conflicting noise for phylogenetic analysis. However, even after removing these characters the bootstrap values for all the internal branches remained below 50% (Fig. 4). An alternative way of assessing phylogenetic signal is through a successive outgroup removal [phylogenetic signal here is defined as the tendency for monophyletic groups that are resolved by an analysis to continue to be resolved when either the data or the analytical method is altered]. This was done simply by removing from the analysis the taxa included in the outgroup, then recomputing the phylogenetic hypothesis, and rooting the cladogram at the most basal taxon as suggested by the previous tree. The successive outgroup removal analysis shows that with the exception of the positions of the bee-eater (Meropidae), the interrelationships among the major groups remained constant (Fig. 5).

Discussion

The phylogenetic signal in cytochrome-*b* and 12S rRNA genes showed a high sensitivity to data manipulation. Slight changes in the searching parameters resulted in alternative hypotheses of relationship. A possible explanation for these unstable results is that the heuristic searching algorithm used in the parsimony analysis might have reached a local optimum instead of finding the most parsimonious solution. In fact, when less than one hundred heuristic search replications were performed, the global parsimony analysis consistently recovered a tree that was nine steps longer than the most parsimonious tree presented here. An increment in the number of replications resulted in a broader number of possible topologies being explored. Without the use of exhaustive algorithms, however, one cannot be certain of finding the shortest tree for the data set. Furthermore, all the phylogenetic trees recovered during this study have relatively small internodal distances compared to the length of terminal branches (Fig. 4). For instance, the longest internodal branch in the most parsimonious tree recovered with the combined sequences is equal to four-fifths of the smallest terminal branch. In parsimony analysis, branch length is proportional to the amount of character support, therefore small internodal distances are the result of a limited number of synapomorphies for that particular node. A naive solution for this problem is to collect more data with the expectation

that these will increase the number of characters along short branches. Cummings et al. (1995) showed that individual genes, in general, have poor power for recovering entire genome trees. Their analyses led them to conclude that at least 8,000 contiguous nucleotide sites are required to reach a 95% chance of obtaining the same phylogeny as would be obtained with the entire mitochondrial genome.

An imbalance between internodal distances and terminal branches can increase the probability of rooting the network in different alternative positions (Wheeler 1990, Smith 1994). The outcome of this rooting sensitivity is the recovery of multiple equally parsimonious trees, and when their signal is condensed by strict consensus trees the result is sometimes highly unresolved. Nevertheless, short internodal branches may actually reflect a rapid pattern of avian diversification (a pattern sometimes called a "star phylogeny"). This rapid cladogenesis makes it difficult to recover the historical pattern within a lineage. Several apparent star phylogenies have been identified at different phylogenetic levels within birds (Kornegay et al. 1993, Helm-Bychowski and Cracraft 1993, Nunn and Cracraft 1996).

It must by stressed again that the goal of the present study has been to explore the higher-level phylogenetic affinities of trogons, and not to make an extensive review of the higher-level systematics of all birds. Moreover, this is not feasible using only two genes and a few representative species for the major groups of birds. An more complete understanding of the

phylogenetic history of birds will require detailed analyses of each group, and the integration of all available data (i.e., molecular, morphological, behavioral, etc.).

The phylogenetic relationships among the taxa that have been traditionally placed in the order Coraciiformes have been controversial. For instance, the monophyly of the order as well as of the families that compose it have been questioned. Also, the relationships among these families have not been established. Following Wetmore (1960) the order includes the families Alcedinidae, Todidae, Momotidae, Meropidae, Coraciidae, Brachypteraciidae, Leptosomidae, Upupidae, Phoeniculidae, and Bucerotidae. Recently, Sibley and Monroe (1990) proposed (1) to place Upupidae and Phoeniculidae into the order Upupiformes and to isolate Bucerotidae into Bucerotiformes, but (2) to keep Upupiformes and Bucerotiformes as sister groups that are closely related to the Coraciiformes.

The present study was unable to recover the coraciiforms as a monophyletic group. Nevertheless, two patterns were consistently recovered (Figs. 4 and 5): (a) the motmots and the todies were found to be more closely related to each other than to any other coraciiform family, and (b) the rollers (Coraciidae) and the kingfishers (Alcedinidae) were recovered as sister-groups. A stable result in both analyses was that Upupidae and Bucerotidae were closely related and usually placed as each other's sister-taxon, but never in the same clade as the other coraciiforms.

The order Cuculiformes encompasses nearly 150 species with highly heterogeneous morphology. As for many other groups of birds, the monophyly of cuckoos has been critically questioned (see Sibley and Ahlquist 1990 for an extensive review on the history of the various systematic arrangements of these birds). In the present study I obtained conflicting results regarding the phylogenetic relationships within the cuculiforms. Slight variations in the searching conditions used during the maximum parsimony analyses drastically changed the relationships among the three cuculiform families. Initial parsimony analyses recovered the cuculiforms as a paraphyletic group (Fig. 1), and only once the saturated partitions were removed did the cuckoos form a monophyletic clade (Figs. 4 and 5). These three cuculiform families represent relatively divergent groups within the order, and if cuckoos are indeed monophyletic, the fact that they were not identified as monophyletic in the parsimony analyses using all data (Fig. 1) is likely explained as an example of long-branch attractions. Hendy and Penny (1989) have demonstrated that the addition of taxa belonging to the problematic group can break up these long branches, and thus minimize the inconsistency problem. On the other hand, for a large data set the addition of new taxa can increase such problem (Kim 1996). Therefore, to obtain a more accurate understanding of the phylogenetic relationships of "cuckoos" would require one to narrow down the analysis to only a few representatives from taxa that are closely related to the cuckoos, and a more extensive sampling of

species presently included in cuculiform families. However, it is clear that such analysis goes beyond the objectives of the present study.

Finally, the results obtained in the present study clearly corroborate the monophyly of Trogoniformes. This hypothesis is significantly supported by 100% bootstrap and branch support of at least 19 in the global parsimony analysis (Fig. 5). Conflicting evidence does not allow definitive identification of their sister-taxon. Nevertheless, trogons seem to be more closely related to mousebirds, parrots, and possibly to cuckoos than to any other major group of birds considered in this analysis. A phylogenetically stable relationship the trogons to the mousebirds was recovered by the parsimony analysis once the saturated partitions in the data set were removed (Figs. 4 and 5). A close relationship between the trogons and the mousebirds is not a new idea. This suggestion has been made on the basis of morphological characters such as foot structure, intestinal convolutions, myology, and osteology (Sclater 1880, Fürbringer 1888, Gadow 1889, Beddard 1898). Most recently the electrophoretic patterns of the ovalbumin in the egg-white proteins of trogons showed consistent characteristics with those of the mousebirds (Sibley and Ahlquist 1972). One of the most commonly suggested groups as the sister-taxon to trogons has been the coraciiforms. In fact, some ornithologists have even classified trogons as a family within that order (Feduccia 1975, Maurer and Raikow 1981), but during this study no relationship with any coraciiform family was found.

Appendix I. List of specimens for the 35 species sequenced. Taxonomic scheme follows the classification proposed by Sibley and Monroe (1990). The data included are: collecting locality, specimen's reference number, and GenBank accession numbers for cytochrome-*b* and 12S rRNA genes respectively.

PICIDAE

Melanerpes carolinus (Red-bellied Woodpecker). USA; Illinois, Chicago. [1] JC-88-5-9-2. U89192, U89209.

Sphyrapicus varius (Yellow-bellied Sapsucker). Mexico; Queretaro, Jalpan. [2] BEHB-125. U89193, U89208.

RAMPHASTIDAE

Aulacorhynchus prasinus (Emerald Toucanet). Mexico; Oaxaca, Putla. [2] OMVP-705. U89191, U89207.

BUCEROTIDAE

Anthracoceros albirostris (Oriental Pied-hornbill). Singapore; Jurong Bird Park. [3] PRS-698. U89190, U89226.

UPUPIDAE

Upupa epops (Eurasian Hoopoe). USA; New York, Bronx Zoo. [1] PRS-282. U89189, U89213.

TROGONIDAE

Apaloderma vittatum (Bar-tailed Trogon). Tanzania; Iringa, Nyumbanitu. [4] P849. U89200, U89234.

Pharomachrus antisianus (Crested Quetzal). Ecuador; Napo. [5] ANS-P4835. U89204, U89235.

Euptilotis neoxenus (Eared Trogon). USA; Arizona, Cochise Co. [6] UA-16794. U89203, U89236.

Priotelus temnurus (Cuban Trogon). Cuba. [5] ANSP-5564. U89202, U89237.

Trogon personatus (Masked Trogon). Venezuela; Bolivar. [1] PRS-795. U89201, U89238.

Harpactes oreskios (Orange-breasted Trogon). East Malaysia; Sabah. [5]ANSP-1316. U89199, U89239.

CORACIIDAE

Coracias caudata (Lilac-breasted Roller). USA; New York, Bronx Zoo. [1]PRS-765a. U89184, U89225.

MOMOTIDAE

Momotus mexicanus (Russet-crowned Motmot). Mexico; Oaxaca, Cuicatlan. [2] OMVP-900. U89187, U89222.

Momotus momota (Blue-crowned Motmot). Mexico; Oaxaca, Tuxtepec. [2] MT-342. U89188, U89221.

TODIDAE

Todus todus (Jamaican Tody). Jamaica. [7] FM1354. U89186, U89223

ALCEDINIDAE

Ceyx erithacus (Black-backed Kingfisher). Singapore; Kent Ridge. [8]PRS-735. U89182, U89219

CERYLIDAE

Chloroceryle americana (Green Kingfisher). Mexico; Hidalgo, Tlanchinol. [2] BMM-680. U89183, U89220

MEROPIDAE

Merops nubicus (Northern Carmine Bee-eater). USA; New York, Bronx Zoo. [1] PRS-182. U89185, U89224

COLIIDAE

Colius striatus (Speckled Mousebird). USA; California, private aviary. [9] B20787. U89175, U89218

Colius leucocephalus (White-headed Mousebird). USA; Texas, Houston Zoo. [9] B20756. U89173, U89217

Colius colius (White-backed Mousebird). South Africa; Orange Free State. [1] GAV-385. U89174, U89216

CUCULIDAE

Cuculus fugax (Hodgson's Hawk-cuckoo). Singapore; Kent Ridge. [8]PRS-627. U89197, U89210

CENTROPIDAE

Centropus sinensis (Greater Coucal). Singapore; Thompsond Rd. [8]PRS-747. U89196, U89211

NEOMORPHIDAE

Geococcyx velox (Lesser Roadrunner). Mexico; Chiapas, Tacana. [2]BMM-885. U89198, U89212

PSITTACIDAE

Micropsitta finschii (Finsch's Pygmy-parrot). Solomon Is.; Rennell Ild. [1] MKL-87. U89176, U89232

Psittrichas fulgidus (Pesquet's Parrot). Captive bird. [1] PRS-669. U89177, U89231

Pionus senilis (White-crowned Parrot). Mexico; Hidalgo, Tlanchinol. [2]BMM-687. U89179, U89233

Amazona ventralis (Hispaniolan Parrot). Captive bird. [1] PRS-950. U89178, U89230

TROCHILIDAE

Chlorostilbon aureoventris (Glittering-bellied Emerald). Argentina; Buenos Aires. [1] PRS-1092. U89181, U89205

Amazilia tzacatl (Rufous-tailed Hummingbird). Mexico; Oaxaca, Tuxtepec. [2] MT-350. U89180, U89206

STRIGIDAE

Micrathene whitneyi (Elf Owl). Mexico; Queretaro, Peñamiller. [2]BEHB-202. U89170, U89229

Aegolius acadicus (Saw-whet Owl). USA; New York, Rockville. [1] PRS-

1194. U89172, U89227

Asio flammeus (Short-eared Owl). USA; New York, E Northport.

[1]PRS-1196. U89171, U89228

CAPRIMULGIDAE

Nyctiphrynus mcleodii (Eared Poorwill). Mexico; Oaxaca, Putla.

[2]MVP-7130. U89195, U89215

Caprimulgus vociferus (Whip-poor-will). USA; New Jersey, Essex Co.

[1] PRS-231. U89194, U89214

[1] Department of Ornithology, American Museum of Natural History, N. Y.

[2] Museo de Zoología, Universidad Nacional Autonoma de México.

[3] American Museum of Natural History. Donated by the Jurong Bird Park, Singapore.

[4] Institute of Zoology, Department of Population Biology, University of Copenhagen, Denmark.

[5] Genetic Resources Collection, Academy of Natural Sciences Philadelphia.

[6] Department of Ecology and Evolutionary Biology, University of Arizona.

[7] Bird Division, Field Museum of Natural History, Chicago.

[8] American Museum of Natural History. Donated by the Zoological Reference Collection, National University of Singapore.

[9] Collection of Frozen Tissues, Louisiana State University Museum of Natural Science.

Table 1. Phylogenetic affinities of the Trogoniformes as suggested by morphological characters.

Authority	Character used	Suggested affinities
Huxley (1867)	Palate structure	Cuckoos and coraciiforms
Garrod (1874)	Myology of hind limb	Puffbirds and bee-eaters
Sclater (1880)	Foot structure	Mousebirds, coraciiforms, nightjars, and piciforms
Stejneger (1885)	Foot structure	Piciforms, swifts and hummingbirds
Fürbringer (1888)	Morphology [a]	Coraciiforms and mousebirds
Gadow (1889)	Intestine convolutions	Swifts, hummingbirds and Mousebirds
Seebohm (1890)	Palate structure	Hoopoes and pigeons
Sharpe (1891)	Morphology [a]	Coraciiformes and cuckoos
Goodchild (1891)	Pterylosis	Swifts and hummingbirds
Gadow (1892)	Morphology [a]	Owls, swifts, hummingbirds and coraciiforms
Beddard (1898)	General myology	Mousebirds and coraciiforms
Chandler (1916)	Pterylosis	Hummingbirds
Mayr and Amadon (1951)	Morphology [a]	Nightjars and coraciiforms
Verheyen (1956)	Morphology [a]	Nightjars and owls
Verheyen (1960)	Vertebrae structure	Nightjars
Feduccia (1975)	Stapes structure	Kingfishers, Todidae, bee-eaters and Momotidae
Maurer and Raikow (1981)	Appendicular myology	Todies, motmots, bee-eaters and kingfishers

[a] Studies based on multiple morphological sources (e. g., osteology, coloration pattern, myology, etc).

Table 2. Pairwise average differences in cytochrome-*b* gene for the major taxa of non-passerine birds. Total uncorrected differences (tot) below the diagonal, transversion (tv) differences above the diagonal, and diagonal presents the average differences among the species represented for that taxon (tot-tv).

Taxa	1	2	3	4	5	6	7	8	9	10	11	12
1 Gallus-Anas	192-96	115	114	115	111	112	126	108	118	109	115	111
2 Strigidae	218	197-90	111	121	115	117	129	103	116	111	120	107
3 Coliidae	220	225	124-33	102	103	110	106	91	114	103	104	103
4 Psittacidae	213	221	203	149-56	117	110	124	100	122	101	108	103
5 Trochilidae	207	229	217	215	148-32	113	115	111	114	101	111	107
6 Coraciiformes [a]	211	225	214	205	220	192-97	123	100	111	106	109	108
7 Upupidae	241	243	228	223	229	233	—	111	117	115	107	119
8 Bucerotidae	214	211	200	192	219	196	217	—	103	87	104	96
9 Piciformes [b]	217	228	224	224	222	224	238	210	202-86	112	116	113
10 Caprimulgidae	204	215	214	205	209	211	225	194	218	142-35	103	99
11 Cuculiformes [c]	229	237	219	219	229	222	225	216	240	221	215-94	107
12 Trogonidae	229	230	221	212	227	225	241	213	233	220	227	183-61

[a] encompassing the families: Coracidae, Momotidae, Todidae, Alcedinidae, Cerylidae, and Meropidae.
[b] encompassing the families: Picidae, and Ramphastidae.
[c] encompasisng the families: Cuculidae, Neomorphidae, and Centropidae.

Table 3. Pairwise corrected distance using Kimuras two-parameter model for the major taxa of non-passerine birds. Corrected distance for cytochrome-*b* (cytb) below the diagonal, corrected distance for 12S rRNA (12S) above the diagonal, and diagonal presents the corrected distance among the species represented for that taxon (cytb-12S).

Taxa	1	2	3	4	5	6	7	8	9	10	11	12
1 Gallus-Anas	21.8-17.4	25.7	23.0	21.7	24.7	22.5	25.5	23.9	26.6	18.7	20.9	22.2
2 Strigidae	26.1	22.3-20.0	26.3	26.1	29.5	26.3	29.3	24.6	28.9	25.2	26.7	27.6
3 Coliidae	26.4	27.2	12.4-9.1	23.5	26.7	24.6	27.5	25.2	27.3	22.1	23.9	23.6
4 Psittacidae	26.2	27.8	24.3	16.3-11.2	25.8	23.5	26.5	22.2	26.8	19.9	23.9	24.4
5 Trochilidae	24.4	28.0	25.7	26.6	15.0-10.1	27.6	29.3	25.7	28.1	22.3	25.5	28.4
6 Coraciiformes	25.0	27.4	25.4	24.8	26.5	22.2-21.1	26.3	23.0	27.9	22.2	23.5	23.8
7 Upupidae	30.3	30.8	27.4	27.8	27.9	28.8	—	22.9	29.4	27.2	27.5	29.1
8 Bucerotidae	25.3	24.7	22.8	22.6	26.2	22.4	25.8	—	26.9	23.4	23.8	25.3
9 Piciformes	26.1	27.7	27.1	28.1	26.8	26.9	29.3	24.6	23.3-21.6	26.2	27.6	28.8
10 Caprimulgidae	23.9	25.6	25.1	24.6	24.3	24.9	27.2	21.8	26.0	14.3-7.3	19.1	21.6
11 Cuculiformes	27.9	29.4	26.0	26.9	27.8	26.6	26.9	25.5	29.8	26.2	25.0-18.9	22.2
12 Trogonidae	27.7	27.7	26.2	25.7	27.2	27.1	29.8	24.8	28.4	25.9	27.3	19.8-15.1

Table 4. Nucleotide composition, and base compositional bias in cytochrome *b*.

Taxon [a]	First position				Second position				Third position			
	C	T	A	G	C	T	A	G	C	T	A	G
M. carolinus	29.7	22.6	26.2	21.5	27.3	39.9	19.9	12.9	48.8	16.8	30.4	3.9
S. varius	29.7	23.1	25.2	22.0	27.0	40.2	19.9	12.9	49.1	15.7	32.5	2.6
A. prasinus	30.2	22.8	24.7	22.3	26.5	40.2	20.5	12.9	50.4	13.6	33.1	2.9
A. albirostris	31.0	21.8	26.5	20.7	27.0	39.4	20.5	13.1	52.5	6.8	34.6	6.0
U. epops	28.3	22.8	26.5	22.3	27.0	39.6	20.5	12.9	44.9	12.9	39.6	2.6
A. vittatum	28.6	23.4	25.7	22.3	26.8	39.9	20.7	12.6	42.8	17.1	37.8	2.4
P. antisianus	28.6	23.1	27.3	21.0	26.8	39.9	20.7	12.6	42.5	16.3	38.8	2.4
E. neoxenus	28.6	23.1	27.0	21.3	26.5	39.4	20.7	13.4	36.7	21.0	39.4	2.9
P. temnurus	28.6	23.6	26.0	21.8	27.3	39.6	20.5	12.6	40.7	16.0	37.5	5.8
T. personatus	27.6	24.7	27.0	20.7	26.0	40.4	21.0	12.6	43.0	15.2	37.3	4.5
H. oreskios	28.3	23.1	27.6	21.0	27.0	39.4	20.7	12.9	41.5	14.2	41.7	2.6
C. caudata	28.9	23.9	26.0	21.3	26.8	39.9	20.5	12.9	48.8	11.3	36.7	3.1
M. mexicanus	30.2	22.8	26.0	21.0	27.8	38.6	20.7	12.9	47.0	12.9	37.5	2.6
M. momota	30.2	23.1	25.7	21.0	27.6	38.6	21.0	12.9	48.6	11.3	36.0	4.2
T. todus	30.4	23.4	24.9	21.3	27.0	39.6	20.5	12.9	49.3	11.8	36.5	2.4
C. erithacus	29.9	23.1	26.0	21.0	27.6	39.4	20.7	12.3	36.3	24.1	27.6	12.0
C. americana	29.4	22.8	27.8	19.9	27.3	39.4	20.5	12.9	36.7	23.6	27.8	11.8
M. nubicus	31.0	22.0	27.0	19.9	27.6	39.9	19.4	13.1	53.5	14.7	27.6	4.2
C. striatus	29.1	24.1	26.5	20.2	27.6	38.1	20.7	13.6	44.4	11.8	37.8	6.0
C. leucocephalus	29.1	23.9	26.5	20.5	26.2	39.6	20.7	13.4	43.6	10.2	42.0	4.2
C. colius	28.6	24.1	26.8	20.5	27.8	38.3	21.0	12.9	45.4	9.7	40.4	4.5
C. fugax	27.3	24.1	27.8	20.7	27.0	39.4	20.7	12.9	46.7	9.4	40.2	3.7
C. sinensis	24.9	27.8	28.3	18.9	26.0	40.2	21.3	12.6	39.4	15.0	42.3	3.4
G. velox	28.6	23.1	30.2	18.1	27.6	39.1	20.7	12.6	46.2	12.9	39.6	1.3
M. finschii	31.1	22.6	25.5	20.8	28.2	38.4	20.8	12.6	50.0	10.0	36.6	3.4
P. fulgidus	30.5	23.4	25.8	20.3	27.6	38.7	20.8	12.9	49.2	8.7	40.0	2.1
P. senilis	30.0	23.2	26.8	20.0	27.6	38.9	20.8	12.6	51.1	7.4	37.9	3.7
A. ventralis	29.7	23.1	26.5	20.7	26.8	39.6	20.7	12.9	49.1	9.4	38.1	3.4
C. aureoventris	30.2	22.3	26.0	21.5	27.0	39.6	20.7	12.6	48.3	10.2	36.0	5.5
A. tzacatl	29.9	22.8	26.2	21.0	27.0	39.4	20.7	12.9	43.6	12.3	39.6	4.5
G. minutissimum	29.7	22.6	27.8	19.9	27.0	38.8	20.7	13.4	51.4	9.2	37.0	2.4
A. acadicus	29.9	21.3	28.1	20.7	27.3	39.1	20.7	12.9	42.8	10.5	41.7	5.0
A. flammeus	30.7	21.8	26.8	20.7	26.8	39.6	20.7	12.9	42.3	18.4	36.2	3.1
N. mcleodii	29.1	23.9	25.7	21.3	27.0	39.4	20.7	12.9	43.6	15.2	38.3	2.9
C. vociferus	29.7	21.8	26.8	21.8	26.8	39.6	20.7	12.9	44.6	13.4	40.2	1.8
Mean	29.4	23.2	26.6	20.9	27.1	39.4	20.6	12.9	45.6	13.4	37.0	4.0
Standard Deviation	1.2	1.1	1.1	0.2	0.5	0.6	0.3	0.3	4.4	4.1	3.9	2.3
Nucleotide Bias		0.079				0.22				0.435		

[a] Order and nomenclature as in Appendix I.

Table 5. Pairwise average differences in 12S rRNA gene for the major taxa of non-passerine birds. Total uncorrected differences (tot) below the diagonal, transversion (tv) differences above the diagonal, and diagonal presents the average differences among the species represented for that taxon (tot-tv).

Taxa	1	2	3	4	5	6	7	8	9	10	11	12
1 Gallus-Anas	134-55	70	66	62	69	61	72	63	71	52	60	61
2 Strigidae	180	152-53	78	76	76	72	84	64	81	67	68	71
3 Coliidae	165	181	78-22	66	79	64	70	70	77	60	67	64
4 Psittacidae	158	179	168	92-30	75	64	77	61	71	57	65	65
5 Trochilidae	174	198	183	178	86-16	72	70	60	78	68	67	76
6 Coraciiformes	163	182	175	168	190	156-52	66	56	73	57	60	58
7 Upupidae	177	193	188	180	197	182	—	63	76	75	72	74
8 Bucerotidae	171	174	178	162	182	168	164	—	62	59	53	61
9 Piciformes	184	192	187	184	190	191	196	187	158-46	70	72	74
10 Caprimulgidae	141	177	161	148	160	162	186	170	182	64-18	53	61
11 Cuculiformes	154	186	171	165	179	169	187	172	188	143	145-47	57
12 Trogonidae	161	190	170	173	193	172	195	180	195	157	161	122-36

Table 6. Nucleotide composition and base compositional bias in 12S rRNA.

Taxon [a]	Stems				Loops			
	C	T	A	G	C	T	A	G
M. carolinus	28.2	21.8	23.5	26.5	28.6	19.2	40.4	11.8
S. varius	27.5	22.6	23.4	26.5	32.2	16.8	38.9	12.1
A. prasinus	30.2	20.5	22.2	27.2	32.8	15.2	38.9	13.1
A. albirostris	29.4	20.6	22.1	27.9	30.6	18.4	37.0	14.0
U. epops	29.4	21.0	21.2	28.5	30.2	16.6	40.0	13.3
A. vittatum	28.9	21.2	21.9	28.0	24.3	21.5	41.8	12.4
P. antisianus	28.7	22.3	21.4	27.6	25.9	20.7	41.9	11.5
E. neoxenus	28.5	22.3	21.8	27.4	23.9	21.8	43.0	11.3
P. temnurus	29.6	20.9	21.3	28.1	23.7	22.3	42.1	11.9
T. personatus	27.4	23.0	21.7	27.9	25.1	20.9	41.8	12.2
H. oreskios	29.0	21.5	21.5	27.9	24.6	20.8	42.8	11.8
C. caudata	30.9	19.5	21.0	28.5	30.3	16.1	40.7	12.9
M. mexicanus	28.3	22.1	22.5	27.2	27.7	18.5	40.3	13.5
M. momota	28.2	21.9	22.4	27.5	28.3	17.4	40.3	14.0
T. todus	28.7	21.0	21.8	28.5	30.5	17.8	38.0	13.7
C. erithacus	28.6	21.8	22.4	27.1	28.5	18.3	41.2	12.1
C. americana	28.7	21.0	23.6	26.8	29.6	16.7	42.2	11.5
M. nubicus	27.9	21.9	21.9	28.3	29.7	15.8	38.6	16.0
C. striatus	25.2	23.7	25.9	25.2	27.0	18.8	44.3	09.9
C. leucocephalus	26.5	23.3	24.1	26.1	27.6	19.4	43.1	09.8
C. colius	26.7	23.1	24.6	25.6	26.7	19.2	43.4	10.7
C. fugax	26.0	23.8	24.5	25.7	25.6	20.0	42.0	12.5
C. sinensis	27.0	21.9	25.1	26.0	25.6	20.4	43.8	10.3
G. velox	26.4	23.2	23.5	26.9	29.3	20.0	39.8	10.9
M. finschii	28.9	21.0	23.6	26.6	32.2	13.8	44.4	09.5
P. fulgidus	29.7	20.7	24.4	25.2	34.2	12.9	42.4	10.4
P. senilis	29.1	21.1	23.2	26.6	32.5	14.9	41.7	10.9
A. ventralis	30.3	20.0	22.3	27.5	33.7	13.2	42.3	10.9
C. aureoventris	29.2	19.9	23.2	27.7	28.0	17.3	41.8	13.0
A. tzacatl	28.2	21.3	24.4	26.1	26.8	18.4	42.9	12.0
G. minutissimum	29.2	20.9	22.6	27.3	32.4	16.2	41.1	10.3
A. acadicus	29.1	20.0	20.9	30.0	29.6	15.7	39.9	14.7
A. flammeus	28.3	21.2	22.3	28.3	33.1	15.7	38.8	12.4
N. mcleodii	28.3	21.8	22.9	27.0	28.0	18.3	43.2	10.5
C. vociferus	28.5	21.2	23.3	27.0	26.4	18.9	43.2	11.4
Mean	28.4	21.6	22.8	27.2	28.7	17.9	41.4	12.0
Standard Deviation	1.2	1.1	1.2	1.0	2.9	2.4	1.8	1.5
Nucleotide Bias		0.075				0.268		

[a] Order and nomenclature as in Appendix I.

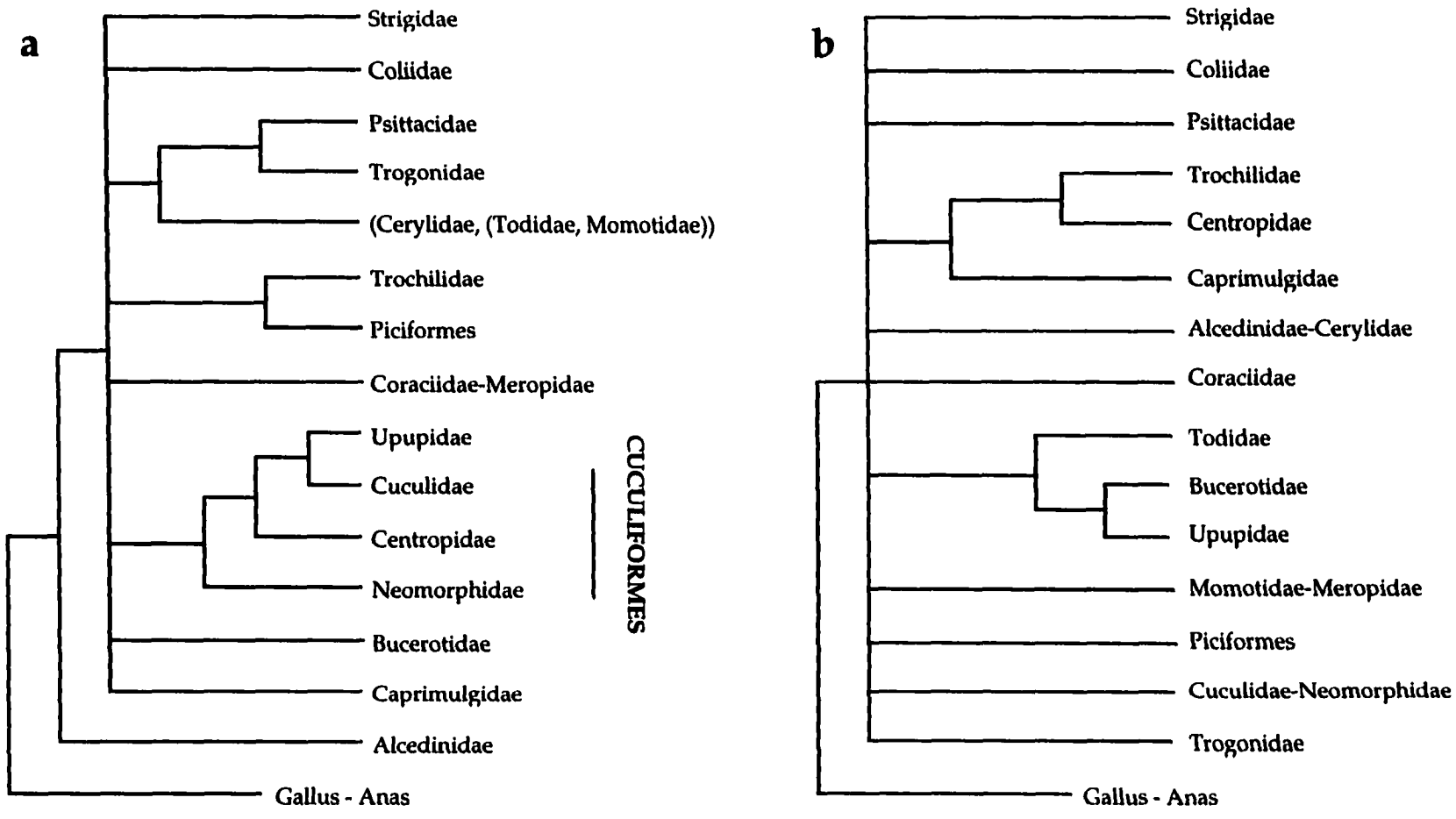

Fig. 1. Phylogenetic hypotheses obtained from maximum parsimony analyses. a) Strict consensus tree of seven equally parsimonious trees obtained from the global parsimony analysis for the cytochrome-b sequences (length: 4,080 steps, CI: 0.266, RI: 0.317). b) Strict consensus tree of three equally parsimonious trees obtained from the global parsimony analysis for the 12S rRNA sequences (length: 2,672 steps, CI: 0.309, RI: 0.413).

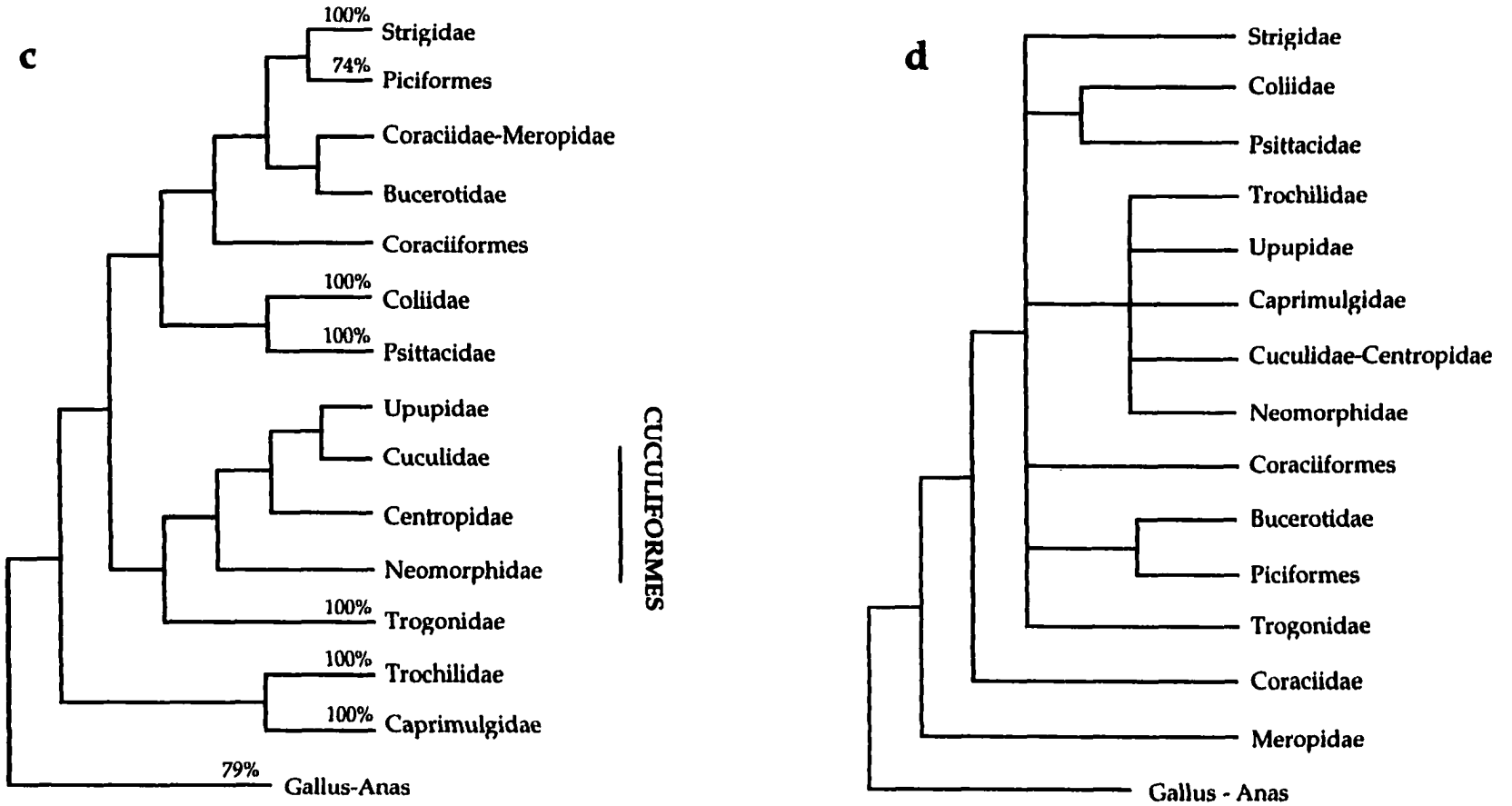

Fig. 1. (continued). c) Strict consensus tree of two equally parsimonious trees obtained from the global parsimony analysis for both genes (length: 6,847 steps, CI: 0.280, RI: 0.347), percentage are bootstrap values obtained for 300 replications. d) Strict consensus tree of seven equally parsimonious trees obtained from the transversion parsimony analysis for genes (length: 2,525 steps, CI: 0.257, RI: 0.448). All phylogenetic hypotheses were rooted to *Gallus gallus* and *Anas platyrhynchos* as the outgroup, and no differential weight was applied to informative characters.

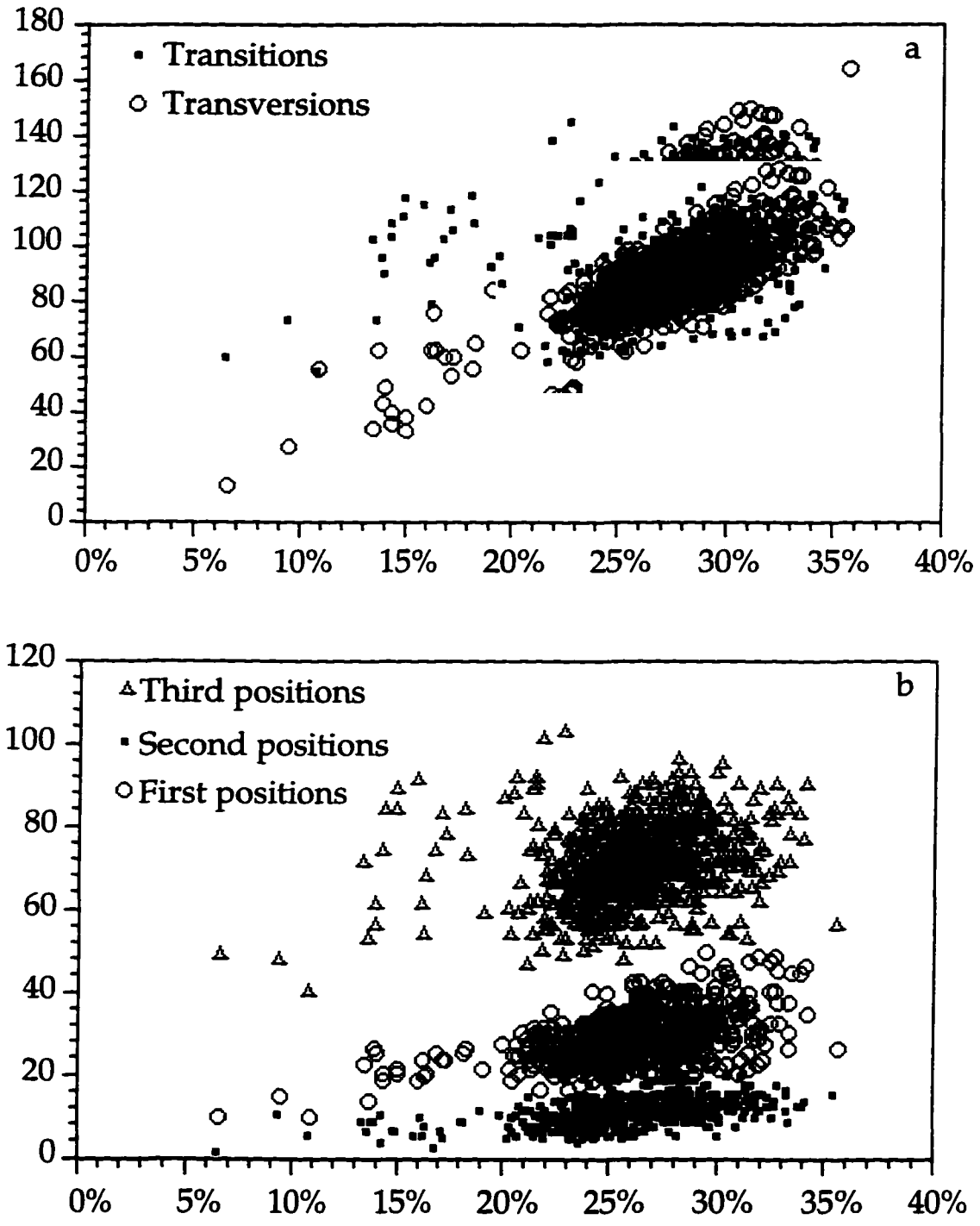

Fig. 2. Saturation curves for the cytochrome-*b* gene. Horizontal axes are corrected pairwise distances using Kimura's two parameter model, vertical axes are empirical numbers of transitions and transversion substitutions. a) Saturation curves for the entire gene, b) saturation curves for transitions only by codon position.

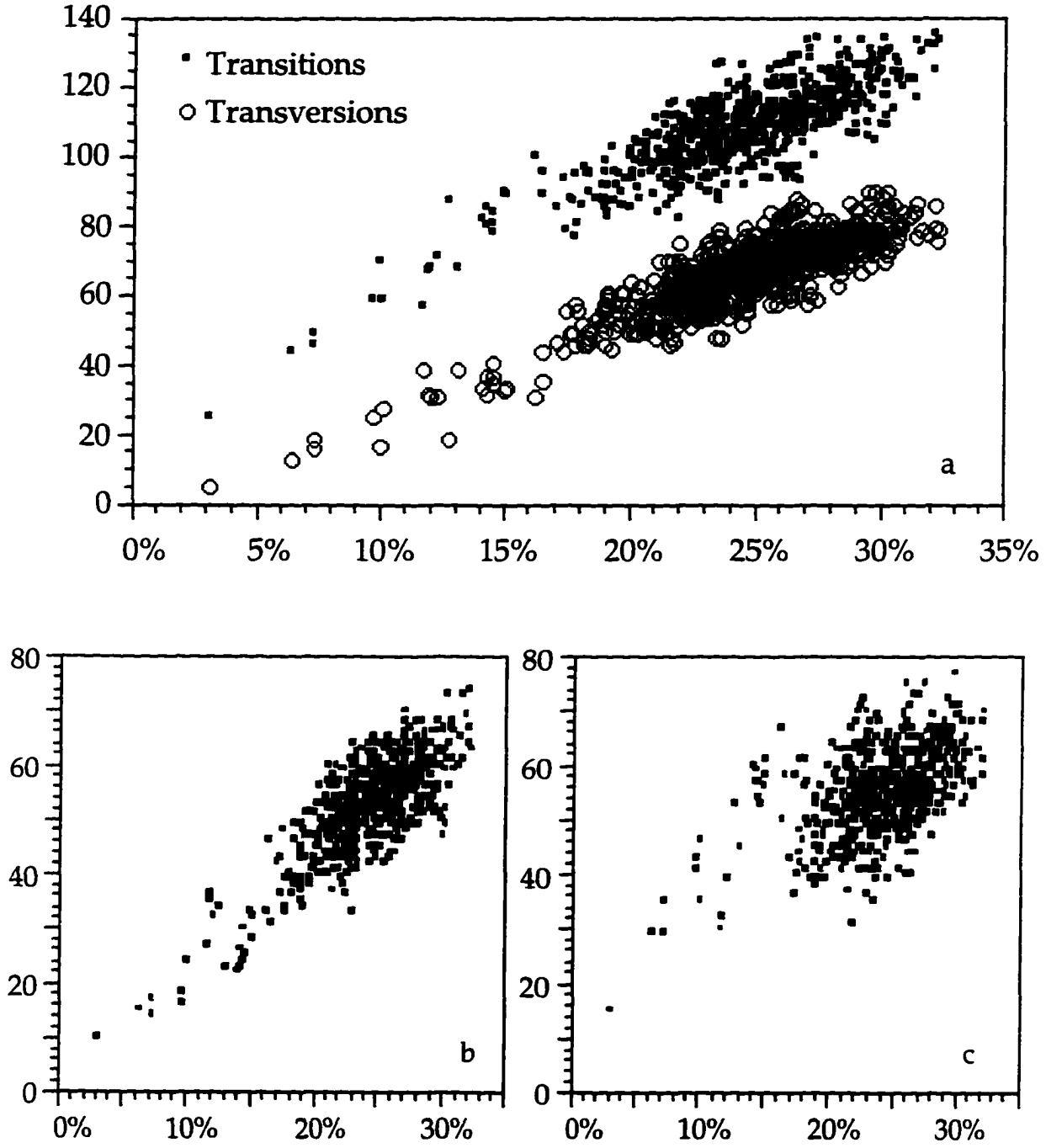

Fig. 3. Saturation curves for the 12S rRNA gene. Horizontal axes are corrected pairwise distances using Kimura's two parameter model, vertical axes are empirical numbers of transitions and transversion substitutions. a) Saturation curves for the entire gene, b) saturation curve for transitions only in stems, c) saturation curve for transitions only in loops.

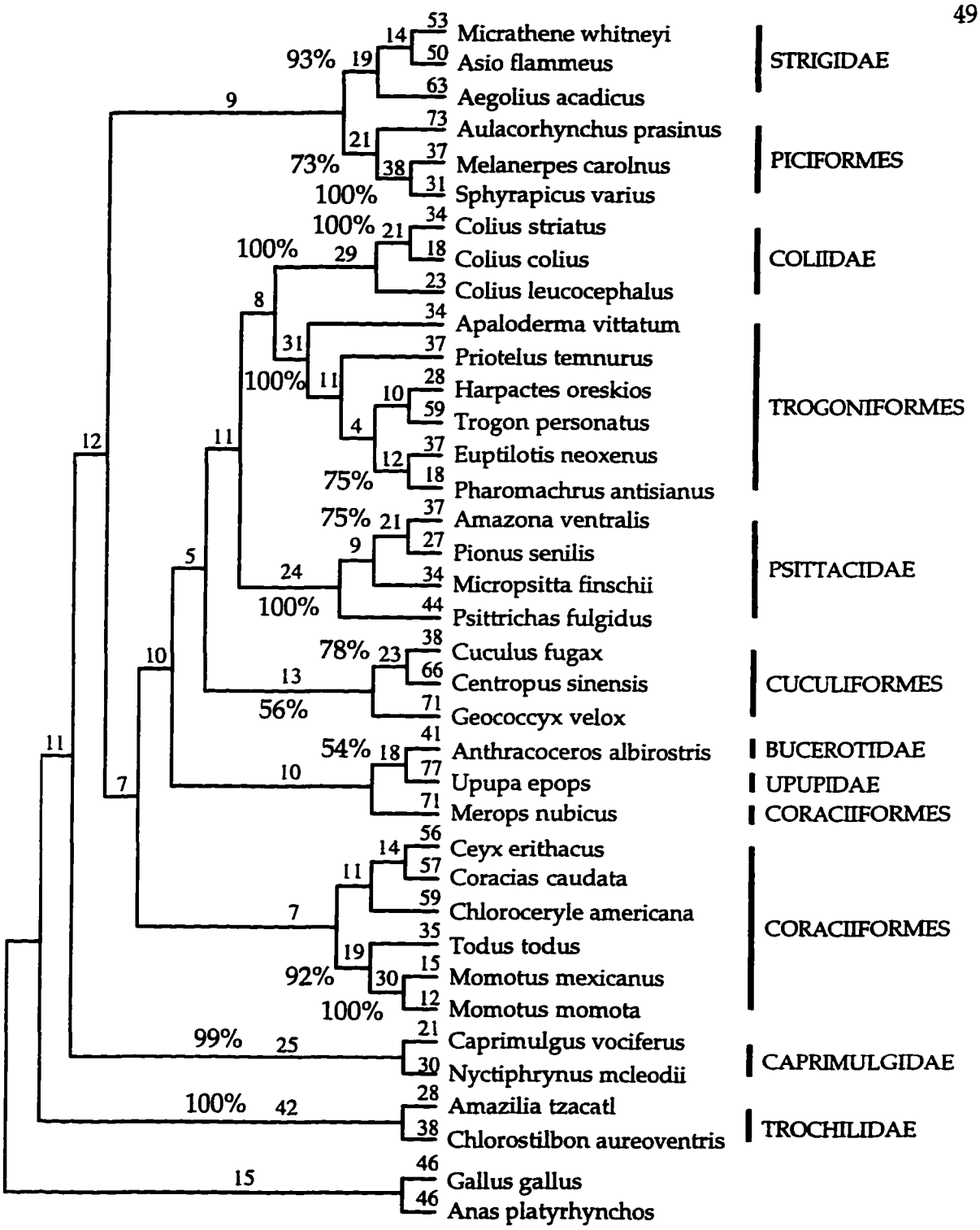

Fig. 4. Single most parsimonious tree for all the taxa included in this study, the tree was rooted to *Gallus gallus* and *Anas platyrhynchos* as the outgroup (length: 2,108 steps, CI: 0.316, RI: 0.415). This phylogenetic hypothesis was obtained after removing from the data set the saturated partitions as shown by the saturation analysis. Number above branches represent the branch length. Percentage are bootstrap values, only clades with bootstrap over 50% are indicated.

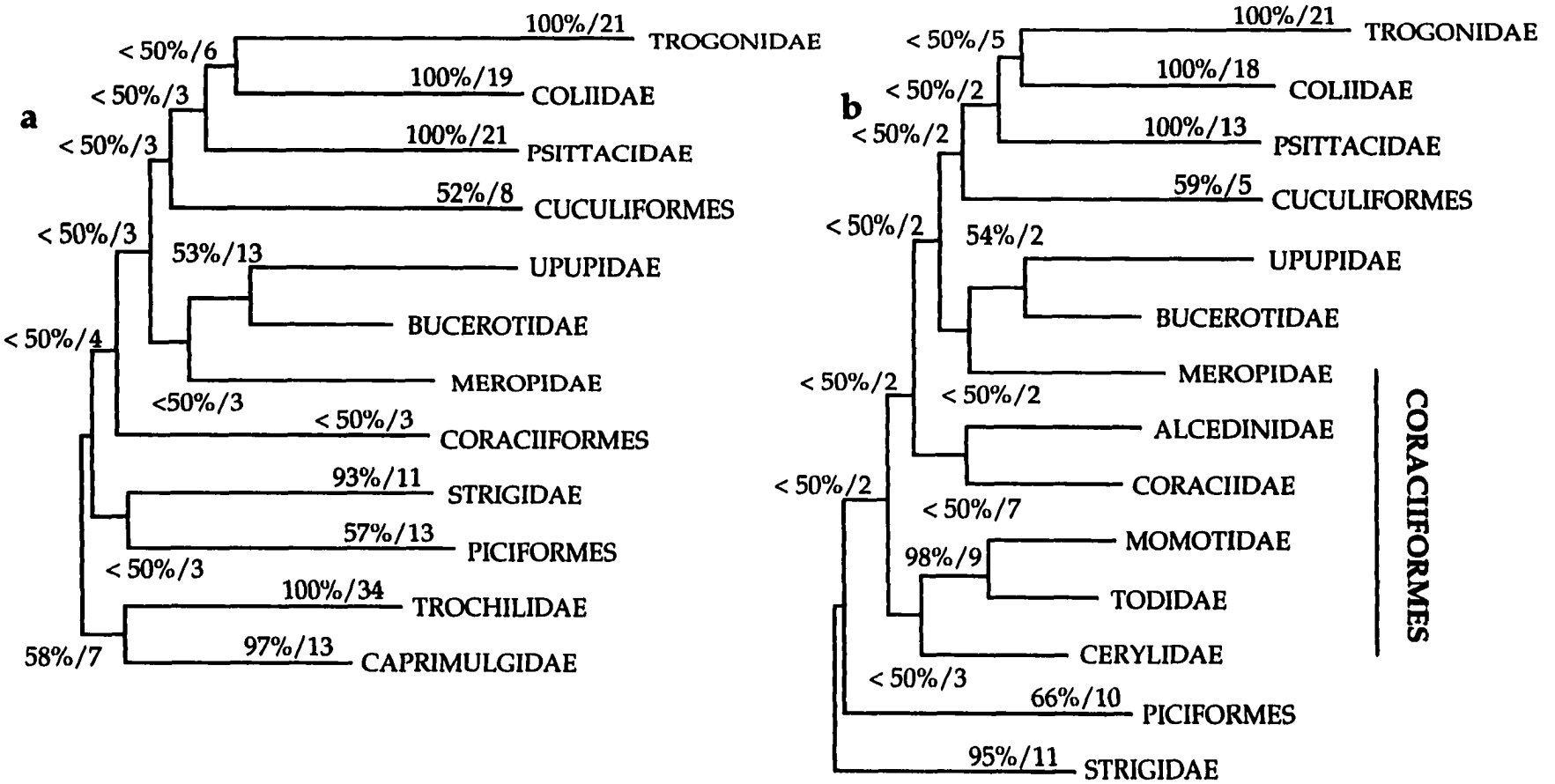

Fig. 5. Successive outgroup removal analyses. All trees were obtained from global parsimony analysis including both genes without saturated partition. Monophyletic branches were collapsed for detail on the species used refer to appendix I. Number above branches are bootstrap values and branch support respectively. a) Single most parsimonious tree (length: 1,989 steps, CI: 0.331, RI: 0.427) obtained after removing *Gallus gallus* and *Anas platyrhynchos*, the tree was rooted to the hummingbirds and the nightjars as suggested in figure 4. b) Single most parsimonious tree (length: 1,784 steps, CI: 0.352, RI: 0.423) obtained after removing the hummingbirds and the nightjars, the tree was rooted to the owls.

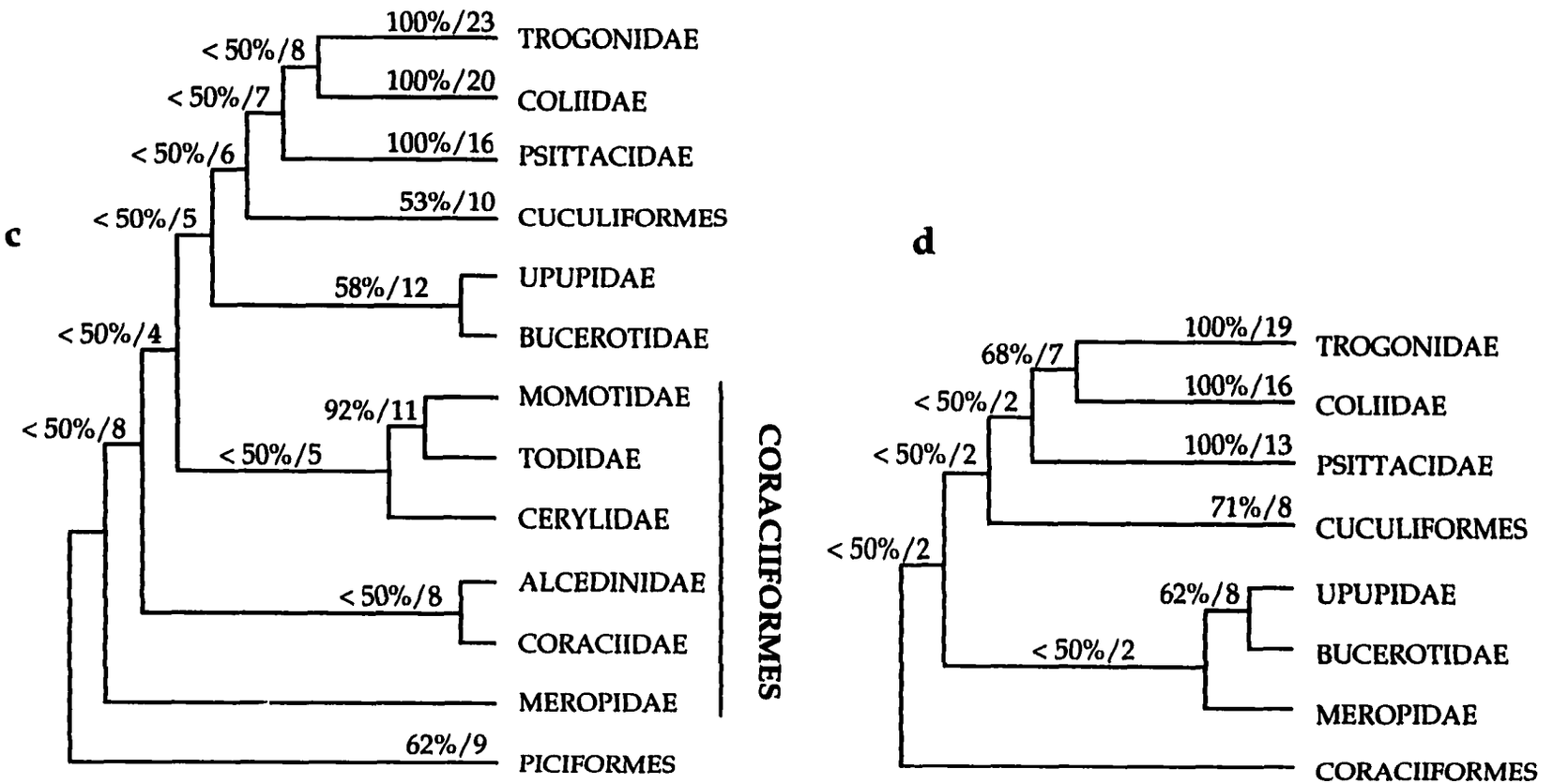

Fig. 5. (continued). c) Single most parsimonious tree (length: 1,584 steps, CI: 0.378, RI: 0.438) obtained after removing the owls, the tree was rooted to the piciforms. d) Single most parsimonious tree (length: 1,370 steps, CI: 0.409, RI: 0.450) obtained after removing the piciforms, the tree was rooted to the coraciiforms.

CHAPTER 2

PHYLOGENETIC RELATIONSHIPS AMONG THE TROGONS (TROGONIFORMES)

ABSTRACT.— The order Trogoniformes is divided currently into six genera: *Apaloderma, Pharomachrus, Euptilotis, Priotelus, Trogon,* and *Harpactes.* For this study, the question of intergeneric relationships was addressed based on mitochondrial cytochrome-*b* and 12S ribosomal RNA genes. The results confirmed that the currently acepted genera represent monophyletic groups. A monophyletic clade encompassing the New World genera was the sister-group to Asian genus *Harpactes.* The sister-group of these clades, in turn, was the African genus *Apaloderma.* Within the New World clade, the sister-taxon of the genus *Trogon* was a clade formed by the Eared Trogon (*Euptilotis neoxenus*) and the Quetzals (*Pharomachrus*). The most basal genus within the New World clade was *Priotelus.* These results suggest an Old World (Africa-Europe) origin for trogons, with the New World representing a secondary area of diversification. Using the relationships inferred from the molecular data the evolutionary tendencies in the coloration pattern in the plumage of trogons were analyzed. Finally, an hypothesis for the evolution of iridescent structures in trogon feathers suggests an increase in the complexity of these structures towards the youngest lineages.

QUETZALS AND TROGONS (Order Trogoniformes) are one of the most colorful groups of birds. They have dense, lax plumage, and a well developed aftershaft. The color pattern of males is brown or metallic green with a blue-green gloss on the dorsal region; the head can be metallic green, black, gray, blue, violet, chestnut, pink, or red. In many species the upper part of the chest is separated from the lower part and the belly by a conspicuous white band. The lower chest, belly, and lower tail coverts can be red, yellow, or orange; the wings are completely black and the tail is black with very distinctive patterns of white. Females and juveniles are duller than adult males. Some species have colored bare periocular skin with tonalities ranging from light yellow to deep purple. The quetzals (*Pharomachrus*) are characterized by having a crested head, elongated upperwing coverts, and uppertail coverts exceeding the length of the tail, which in the Resplendent Quetzal (*P. mocino*) can reach up to 70 cm. The bill is short but broad basally, usually brightly colored, with a strongly arched culmen, and, in several species, having a serrated maxillary tomium. The wings are rounded and relatively small. The long tail is graduated, broad at the base, and truncated.

Morphological features said to be characteristic of the trogoniforms (Ogilvie-Grant 1892, Ridgway 1911, Sibley 1955, Sibley and Ahlquist 1990) include a schizognathous palate, basipterigoid processes that are always present, a large vomer, 15 cervical vertebra, two pairs of deep sternal notches, four to five pairs of ribs, a metasternum with four notches, separated

coracoids, a tracheo-bronchial syrinx, holorhinal nostrils having an ossified nasal septum, Gadow's type VIII deep plantar tendon arrangement, intestinal convolutions of type VI (Gadow 1889), pelvic muscle formula AX (Garrod 1873), a well developed caecum, left carotid artery only, a well defined spinal pterila extending from the nape to the oil gland, ten primaries, eleven to twelve secondaries, twelve rectrices, and a nude oil gland. All of these features, however, are found in varying combinations in other groups of birds also (Sibley and Ahlquist 1990). Perhaps the only autapomorphy that differentiates trogoniforms from other birds is their heterodactyl foot in which digits 1 and 2 are directed backward, whereas digits 3 and 4 are united for their basal half and directed forward. Thus, the hallux (digit 1) is the outer of the two hind toes, whereas in zygodactyl birds, in which digits 1 and 4 are reversed.

The trogoniforms are widely distributed in the tropics of both the Old and New Worlds. In the New World, they reach temperte latitudes in the Southwestern United States. Sibley and Monroe (1990) recognized a total of 39 species divided into six genera. The African trogons in the genus *Apaloderma* (three species) are endemic to mountain forests in Liberia, Nigeria, Cameroon, Angola, Zaire, Uganda, Kenya, Tanzania, Mozambique and Malawi. The second largest genus is *Harpactes* with eleven species that are found in SE Asia, from India and Sri Lanka to SE China, and south along the Malay Peninsula through Sumatra, Borneo, Java, and the Philippines.

The remaining genera are found in the Neotropical region. The monotypic genus *Euptilotis* is endemic to mountain forests of western Mexico and southern Arizona. The five species of the genus *Pharomachrus* are distributed from southern Mexicothrough Central America to South America in Peru, Bolivia, Colombia, Venezuela, Ecuador, and Amazonian Brazil. *Priotelus* includes two species endemic to the Caribbean islands of Cuba and Hispaniola. Finally, the largest genus in the order, *Trogon* (17 species), is distributed from southwestern New Mexico and SE Arizona southward through Mexico, Central and South America to northern Argentina.

Although the natural history of some trogons is well known (e.g., Skutch 1942, 1944, 1948), their phylogenetic relationships remain poorly understood. Systematic studies have been restricted mainly to descriptions of subspecies (e.g., Clark 1918, Zimmer 1948, Clancey 1959, Parkes 1970). Technical diagnoses of the different genera, moreover, do not clearly define the boundaries of these taxa; instead, genera and subgenera are primarily diagnosed using poorly defined morphological characters that frequently unite conflicting groups or are so ambiguous as to be useless for inferring monophyly. Characters such as the amount of feathers in the tarsi, serration of the tomium, presence of patches of bare skin, color and color patterns, and some skeletal features, have been used to infer relationships among trogons (Ogilvie-Grant 1892, Ridgway 1911, Clark 1918, Parkes 1970). The problem is that many of these characters show a greater range of variation within taxa

than among them. Trogoniforms are so uniform morphologically that some early classifications lumped most of the species in the genus *Trogon* and placed the few remaining species in *Pharomachrus* (Gould 1875). On the other hand, the use of the characters listed above has led other ornithologists to propose multiple subdivisions within trogoniforms. Swainson (1837), for example, recognized *Trogon, Harpactes, Apaloderma, Temnurus,* and *Calurus* (Resplendent Quetzal only); Ogilvie-Grant (1892) recognized the six currently accepted genera, but split the Asian trogons in two genera (*Harpactes* and *Hapalarpactes*); Sharpe (1900) divided the African trogons into *Hapaloderma* and *Heterotrogon;* Ridgway (1911) divided the genus *Trogon* into four different genera (*Trogon, Curucujus, Trogonurus,* and *Chrysotrogon*); and Peters (1945) split *Priotelus* into two genera (*Priotelus* and *Temnotrogon*) and the African trogons into two genera (*Apaloderma* and *Heterotrogon*).

The apparent lack of morphological characters to infer a reliable phylogeny for many groups of birds and other organisms has made systematists search for alternative sources of character-state data. The publication of the complete mitochondrial genome for the Domestic Fowl (*Gallus gallus*) by Desjardins and Morais (1990) facilitated the design of oligonucleotide primers to amplify any region in the avian mitochondrial genome. In addition, rapid developments in PCR and in DNA sequencing technologies have made the collection of DNA sequences a relatively easy

task, thus providing an important source of data for systematic studies for groups such as trogons that appear to lack morphological characters having the kind of variation needed for phylogenetic analysis.

The decision to use the mitochondrial cytochrome-*b* and 12S rRNA gene sequences in this study was based on the following considerations. Variation in nucleotide substitution rates in DNA sequences has been correlated with codon position, gene region, and substitution type. Thus, phylogenetic relationships among recently divergent taxa can be studied using rapidly evolving sites like silent, third codon positions, as well as transition substitutions in general. For txa that have diverged long ago, more slowly evolving changes like transversions or non-silent substitutions can be used. The cytochrome-*b* gene has been shown to contain phylogenetic signal at several different phylogenetic levels. Population and species problems have been addressed using all phylogenetically informative characters (Smith and Patton 1991, Moritz et al. 1992). Older divergences have been studied by ignoring the rapidly evolving changes (Edwards et al. 1991, Irving et al. 1991).

In contradiction to those studies, however, others have concluded that the information contained in cytochrome-*b* is inadequate to resolve some phylogenetic problems (Graybeal 1993, Avise et al. 1994). Other investigators, moreover, have suggested that analyses based on individual genes have a low probability of recovering entire genome trees. Thus, Cummings et al. (1995) proposed that at least 8,000 contiguous nucleotide sites would be required, in

order to reach a 95% chance of obtaining the entire genome phylogeny. As a consequence of this consideration the mitochondrial 12S rRNA gene was included in this study to complement the cytochrome-*b* data. The 12S rRNA gene has a slower evolutionary rate than cytochrome-*b*, making it suitable for providing characters to resolve deep divergences (Hay et al. 1995, Haise et al. 1995).

The main objective in the present paper is to address several basic questions concerning the systematics of trogons. First, does a molecular phylogeny support the current classification of Trogoniformes? And second, if so, what are the phylogenetic interrelationships among the genera? Once a corroborated phylogeny can be inferred, other questions about the biology and evolutionary history of trogons will be addressed.

METHODS

Taxa examined.– The complete sequence of the cytochrome-*b* gene and the nearly complete sequence of the 12S rRNA gene (over 2100 nucleotides in total) were determined for 20 species of trogons, including Narina Trogon (*Apaloderma narina*), Bar-tailed Trogon (*A. vittatum*), Crested Quetzal (*Pharomachrus antisianus*), Golden-headed Quetzal (*P. auriceps*), Pavonine Quetzal (*P. pavoninus*), Eared Trogon (*Euptilotis neoxenus*), Cuban Trogon (*Priotelus temnurus*), Black-tailed Trogon (*Trogon melanurus*), White-eyed

Trogon (*T. comptus*), White-tailed Trogon (*T. viridis*), Mountain Trogon (*T. mexicanus*), Elegant Trogon (*T. elegans*), Collared Trogon (*T. collaris*), Masked Trogon (*T. personatus*), Black-throated Trogon (*T. rufus*), Blue-crowned Trogon (*T. curucui*), Violaceous Trogon (*T. violaceus*), Diard's Trogon (*Harpactes diardii*), Philippine Trogon (*H. ardens*), and Orange-breasted Trogon (*H. oreskios*). In addition to trogons, the Lesser Roadrunner (*Geococcyx velox*), Hodgson's Hawk-cuckoo (*Cuculus fugax*), Greater Coucal (*Centropus sinensis*), Speckled Mousebird (*Colius striatus*), White-headed Mousebird (*C. leucocephalus*), and White-backed Mousebird (*C. colius*) were sequenced. These six species were employed as outgroups for character polarization and for rooting the phylogeny. Tissue samples were obtained from the Genetic Resources Collection of the Academy of Natural Sciences of Philadelphia, Institute of Zoology of the University of Copenhagen in Denmark, Department of Ornithology of the American Museum of Natural History, Museum of Natural Science of the Louisiana State University, Bird Collection of the University of Arizona, Museo de Zoología Facultad de Ciencias Universidad Nacional Autónoma de México, and Zoological Reference Collection National of the University of Singapore.

DNA extraction and sequencing.– Total genomic DNA was extracted from frozen tissue (muscle, liver and heart) using a Chelex 5% solution following the protocol suggested by Singer-Sam et al. (1989). Target genes were amplified and isolated as single fragments using specifically designed

PCR primers. This first amplification was conducted in Peltier-effect thermocyclers (MJ Research) according to the parameters and conditions suggested by Nunn et al. (1996). PCR products were subjected to horizontal electrophoresis in a 2% NuSieve low-melting point agarose gel (FMC Bioproducts). Gels were stained for 10 min. in a solution of 2 pg/ml of ethidium-bromide and visualized under UV light. The double-stranded DNA (dsDNA) products were cut directly from the gel and resuspended in 150 µl of ultra-pure water by heating to 73°C for 15 min. The genes were reamplified as subfragments of about 400 bp using internal oligonucleotide primers. Primers LPhe (L1243) 5'-CAAACAAAGCATGGCACTGAAG-3', and 12Sd (H1883) 5'-TTCGATTATAGAACAGGCTCCTC-3', were designed in our lab (numbered following the chicken mitochondrial genome [Desjardins and Morais 1990]). The remaining primers used are described elsewhere (Helm-Bychowski and Cracraft 1993, Knight and Mindell 1993). An air thermocycler (Idaho Technologies), was used to perform 40 µl amplifications of these subfragments in glass microcapillary tubes using standard buffers (Wittwer 1992). Subfragments were amplified using the following conditions: 2 s at 94°C, 0 s at 47°C, 15 s at 71°C, for 35 cycles at slope 7. All PCR experiments were conducted along with positive and negative controls to test for contamination. Aliquots of 3 µl were visualized as described above. The remainder was purified to eliminate PCR primers, dNTPs, enzyme, and buffer components, using the GeneClean II kit (BIO 101 Inc.). Purified PCR

products were subjected to cycle sequencing using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq DNA Polymerase FS, on the GeneAmp PCR System 9600 (Perkin Elmer). Amplifications were performed in 6 µl reaction volumes containing 2.5 µl of the Prism kit reagent, 1 pmole of sequencing primer, 5 ng/µl dsDNA template, and 2 µl of ultra-pure water. Cycle sequencing was performed using the following conditions: 10 s at 95°C, 5 s at 50°C, 3 min at 60°C, for 32 cycles. The excess of Taq dideoxy terminators was removed with Centri-Sep spin columns (Princeton Separations) in a variable speed microcentrifuge at 2500 rpm for 2 min. Final purifications were dried in a vacuum centrifuge and resuspended in 2.5 µl of the loading buffer (6x deionized formamide, 1x 50 mM EDTA pH 8.0). Resuspended sequencing products were subjected to 4% polyacrylamide denaturing gel electrophoresis in the ABI Prism 377 DNA Sequencer (Perkin Elmer). Sequence files were analyzed with the aid of the program Sequencher v 3.0 (Gene Codes Corp., Ann Arbor, MI). A large degree of fragment overlap, as well as sequencing both DNA strands ensured accurate data collection. Final alignment of cytochrome-*b* sequences was done by eye. Initial alignment of the 12S rRNA sequences was performed using the program Malign (Wheeler and Gladstein 1992). Structural regions in the 12S rRNA sequences were delimited according to the proposed secondary structure for the 12S rRNA molecule (Springer and Douzery 1996, Houde et al. 1997, Mindell et al. 1997).

Phylogenetic analysis.– All cytochrome-*b* nucleotide positions and only alignment-stable nucleotides of the 12S rRNA were used in maximum parsimony analyses conducted with the computer program Phylogenetic Analysis Using Parsimony (PAUP 3.1.1; Swofford 1993). Because of the relatively large number of taxa used in the analyses, it was necessary to use an heuristic algorithm for searching the tree space. Input order bias was minimized by performing one hundred replicate heuristic searches with random addition of taxa. During all analyses nucleotide transformations were considered unordered. Character states were optimized using delayed transformation (DELTRAN) which favors contemporaneous changes (i.e., parallelisms over reversals). Branch swapping was made by the tree bisection-reconnection algorithm (TBR). Retention and consistency indices were computed to evaluate the level of homoplasy in the most parsimonious tree. Finally, tree robustness was examined using 500 bootstrap replications (Felsenstein 1985, Hillis and Bull 1993), and branch support (Bremer 1988, 1994).

Genetic corrected distance.– Corrected pairwise distances for the nucleotide sequences were computed to test for saturation effects (Arctander 1991, Maynard-Smith and Smith 1996). Distances were computed with the program DNADIST from Phylip 3.5p package (Felsenstein 1993) using the Kimura's two-parameter model (Kimura 1980), assuming a 10:1 transition-transversion bias. This bias is considered a conservative estimate for birds

(Kocher et al. 1990, Nunn and Cracraft 1996, Espinosa de los Monteros and Cracraft 1997).

RESULTS

Sequences variability.– The combined sequences of the cytochrome-*b* and 12S rRNA genes resulted in an alignment of 2159 nucleotides. The cytochrome-*b* sequences contained 590 invariant positions (52%), 85 autapomorphic characters (7%), and 468 phylogenetically informative characters (41%). The variability within the three codon positions showed a pattern similar to that reported for other cytochrome-*b* genes (Edwards et al. 1991, Irwin et al. 1991, Graybeal 1993). Second positions were the least variable codon sites with 57 nucleotide substitutions (10% of the polymorphic characters), followed by first positions with 137 (25%). Third positions were the most variable with 362 (65%) nucleotide substitutions. Although the highest variation is present in third codon positions, only a few of these changes involve amino acid replacements. Among the translated sequences, a total of 118 (31%) of the 380 amino acids residues were variable. Based on the structural model for cytochrome-*b* proposed by Howell (1989), the highest incidence of hypervariable amino acid residues was located inside the transmembrane regions of the molecule, especially in the fourth, fifth and eight segments. This replacement pattern is consistent with that reported for

cytochrome-*b* in other organisms (Degli-Espoti et al. 1993).

Multiple insertions and deletions characterized the hypervariable regions within the 12S rRNA gene. This variability produced considerable differences in the length of the sequences obtained for the present study. Although the multiple alignment for the 26 taxa sequenced had a length of 1016 positions, the average number of nucleotides in the 12S rRNA ranged from 949 in *Priotelus temnurus* to 975 in *Apaloderma narina*. Functional regions (i.e., stems and loops) in the sequences were easily identified following the model proposed for the secondary structure of the 12S rRNA molecule (Springer and Douzery 1996, Houde et al. 1997, Mindell et al. 1997). The multiple alignment for the 12S rRNA sequences contained 590 invariant positions (58%), 72 autapomorphies (7%), and 354 phylogenetically informative characters (35%). The stem regions of the molecule were more conservative than the loops. Stems encompassed 315 of the invariant characters (53%), 20 of the autapomorphies (28%), and 134 of the phylogenetically informative characters (30%). Hypervariable fragments producing ambiguous alignments were located mainly inside the loop fragments.

Total empirical pairwise differences (in other words, combining both genes) between species of trogons ranged from 337 (16%) between *Trogon rufus* and *Apaloderma narina* and between *T. collaris* and *Pharomachrus antisianus*, to 87 (4%) between *P. antisianus* and *P. auriceps* (Table 1). The

smallest distance, between members of different genera, was 225 nucleotide substitutions (11%), between *Euptilotis neoxenus* and *P. pavoninus*. Excluding the distances between *Euptilotis neoxenus* and the three species of *Pharomachrus*, the difference between species belonging to different genera was over 275 nucleotide substitutions (13%). Similar patterns were observed when only transversion substitutions were analyzed. Transversion distances ranged from seven (0.3%) between *T. curucui* and *T. violaceus* to 117 (5%) between *A. vittatum* and *T. collaris*. Intergeneric transversion differences were extremely variable. For instance, within the genus *Trogon* transversion distances differed by an order of magnitude [78 (3%) between *T. viridis* vs. *T. collaris*, to seven (0.3%) between *T. violaceus* vs. *T. curucui*].

By analyzing the two genes individually, it is possible to examine the assumption of a differential rate of evolution between cytochrome-*b* and 12S rRNA. On the basis of the corrected distances using the Kimura two-parameter model (Table 2), it is clear that the cytochrome-*b* gene has a faster nucleotide replacement rate than the 12S rRNA gene. The biggest intergeneric difference for cytochrome-*b* was 23.3% (*T. mexicanus* vs. *H. oreskios*), slightly higher than the 19.8% found for 12S rRNA (*T. collaris* vs. *A. vittatum*). Similarly, the smallest intergeneric distance for cytochrome-*b* was 14.7% (*Euptilotis neoxenus* vs. *P. pavoninus*), nearly twice the 8.6% value found for the 12S rRNA (*E. neoxenus* vs. *P. antisianus*).

Variation in the transition-transversion ratio due to differences in

selection pressure within mitochondrial DNA produces a characteristic nucleotide bias especially at silent positions (Brown 1985, Sueoka 1988). The nucleotide composition and bias (*C*) in cytochrome-*b* reported for other birds (Nunn and Cracraft 1996, Nunn et al. 1996) and for mammals (Irwin et al. 1991) are almost identical to those observed in this study (Table 3). Because codon third position substitutions tend to be silent, and therefore the most variable, a higher nucleotide compositional bias was found (*C*=0.414). Third positions are rich in cytosine (42.4%), followed by adenine (38.6%), much lower in thymine (15.4%), and lowest in guanine (3.5%). Second positions have an intermediate bias (*C*=0.22), being rich in thymine (39.7%) and poor in guanine (12.8%), with intermediate percentages for cytosine (26.7%) and adenine (20.8%). First positions are the least biased (*C*=0.073), being relatively rich in cytosine (28.5%) and poor in guanine (20.8%), but intermediate in adenine and in thymine (27% and 23.7% respectively).

Of the two structural regions of the 12S rRNA, loops possessed the highest bias (*C*=0.191). The bias is mainly produced by an overabundance of adenine (39.2%), which is explained by the hypothesis that the low polarity of adenine may favor hydrophobic interactions with proteins (Gutell et al. 1985). Following adenine are cytosine (25.1%), then thymine (20.1%), and finally guanine (15.6%). A more uniform nucleotide bias is observed in stems (*C*=0.044), being slightly rich in cytosine (27%) and poor in adenine (23%), with intermediate percentages for guanine (26.7%) and thymine (23.7%). The

nucleotide composition and bias observed in the 12S rRNA sequences were consistent with those previously reported for mammals (Springer et al. 1995, Springer and Douzery 1996).

Several molecular studies have shown the existence of nuclear pseudogenes formed by translocated fragments of the mitochondrial genome, especially from cytochrome-*b* (Quinn 1992, Smith et al. 1992, Kornegay et al. 1993). Five observations indicate that the sequences used during this study were of mitochondrial origin only. (1) Nonsense codons for protein coding genes and frameshifts which are typical indicators of nuclear copies, were not apparent in the sequences obtained in the present study. (2) The genes were isolated initially as a single fragment including flanking regions of the tRNA-Thr and the last 90 bases of ND5 for cytochrome-*b*, and the 3' end of the tRNA-Phe for 12S rRNA. The isolation of entire genes minimizes the potential risk of amplifying smaller fragments that are more likely to be translocated into the nuclear genome. (3) The 381 amino acid codons forming the cytochrome-*b* were translated using the vertebrate mitochondrial code without ambiguities or intermediate stop codons. (4) The secondary structure deduced from the 12S rRNA sequences was entirely consistent with that proposed for other vertebrates (Springer and Douzery 1996, Houde et al. 1997, Mindell et al. 1997). (5) Finally, the nucleotide bias observed in the different gene partitions is nearly identical to those reported for other birds (Kornegay et al. 1993, Nunn and Cracraft 1996, Nunn et al. 1996).

Phylogenetic analysis.– A global parsimony analysis of the two combined sequences, including all substitutional variation regardless of position and type, resulted in three equally parsimonious trees of 3760 steps in length, consistency index of 0.407, and retention index of 0.5. The strict consensus tree (Fig. 1a) confirms that each genus within the Trogoniformes is a monophyletic group, but suggests that the relationships among genera are only partially resolved. The ten species of the genus *Trogon* are equally divided into two subclades that form one of the best supported lineages within the phylogeny. In one of the subclades (the "Violaceous subclade"), *T. curucui* is the sister-taxon to *T. violaceus*, their closest relative is *T. viridis*, and at the base of this subclade are *T. melanurus* and its sister-taxon *T. comptus*. The other subclade (the "Elegant subclade") is composed of *T. mexicanus*, *T. elegans*, *T. rufus*, *T. collaris*, and *T. personatus*. Excluding the last two species which are each other's closest relatives, the phylogenetic relationships in this subclade are unresolved. The sister-group of *Trogon* is *Priotelus* (the Cuban Trogon). The next node of the consensus tree is a polytomy formed by *Trogon-Priotelus*, *Pharomachrus-Euptilotis*, and by *Harpactes*. Within quetzals *Pharomachrus antisianus* and *P. pavoninus* are sister-species, their sister-group is *P. auriceps*, and the sister-taxon to the quetzals is the monotipic *Euptilotis neoxenus*. In the clade formed by the Asian trogons, *Harpactes diardii* is the sister-taxon to *H. ardens* and their sister-group is *H. oreskios*. The two African trogons (*Apaloderma*) the most

basal species in the cladogram are sister-taxa to the rest of the trogoniforms. Although the consensus tree shows an apparent lack of resolution, the nodes are supported with high bootstrap and branch support values at the genus level and above. Relatively low bootstrap and branch supports were registered for the clade comprising the Asian trogons (79% and 11, respectively) compared with values for the other genera.

The African trogons, quetzals, and New World Trogons were each identified in 100% of the bootstrap replications and each had a branch support of at least 27. In contrast to interspecific patterns, intergeneric relationships were poorly supported (bootstrap < 50%, and branch support < 3). The only exception was the lineage relating *E. neoxenus* to the quetzals which had a bootstrap value of 100% and a branch support of 23. Finally, the monophyly of the trogoniforms was highly supported, scoring bootstrap values of 100% even when the outgroup was not constrained to be monophyletic.

Using simulation analysis, Bull et al. (1993) showed that less accurate phylogenies are recovered when combining DNA partitions having different evolutionary rates, as compared to analysis of data sets consisting only of slowly evolving partitions of the genome. Following this suggestion, phylogenetic analysis was undertaken on the individual genes. A parsimony analysis of the alignment-stable regions within the 12S rRNA data set resulted in a single most parsimonious tree with a length of 1444 steps, consistency index of 0.468, and retention index of 0.601 (Fig. 1b). With respect

to intergeneric relationships the 12S rRNA tree was consistent with the consensus tree obtained in the combined analysis (Fig. 1a). In addition, many of the interspecific relationships were the same as for the combined analysis. The only exception was among quetzals, in which *P. pavoninus* was basal within the lineage and the sister-taxon to *P. auriceps*, and *P. antisianus*. Using the 12S rRNA data the Violaceous and the Elegant subclades were recovered fully resolved. The Violaceous subclade had the same relationships as in the combined analysis. The Elegant subclade, on the other hand, was resolved: *T. elegans* is the sister-taxon to *T. rufus*, their sister-group is a clade formed by *T. collaris* and *T. personatus*, and the sister-taxon to these two clades is *T. mexicanus*. Although the bootstrap value for the Asian trogons lineage (*Harpactes*) increased from 79% to 92%, none of the other nodes linking different genera received significative support based on the bootstrap or branch support values (excepting again, the *Euptilotis-Pharomachrus* lineage).

Using all phylogenetically informative characters of the cytochrome-*b* data set yielded two equally parsimonious trees of 2296 steps in length, consistency index of 0.372, and retention index of 0.43. The strict consensus tree of the two equally parsimonious solutions (Fig. 1c) was less resolved than the consensus tree resulting from combining the two genes. In addition, the values for the bootstrap and branch support were also lower.

The phylogenetic signal contained in the cytochrome-*b* data set allowed

me to resolve the species interrelationships within the genus *Trogon*. Relationships within the Violaceous subclade were congruent with the branching pattern of the two previous analyses, but the subclade was supported by a relatively low bootstrap value (77%). Species within the Elegant subclade, on the other hand, displayed an alternative set of relationships as compared to those resolved in the 12S rRNA analysis. For the cytochrome-*b* data set, the sister-taxon to *T. elegans* was *T. mexicanus*, and *T. rufus* was placed as the most basal taxon within the clade (i.e., the only difference is the topological shift between *T. mexicanus*, and *T. rufus*). A more problematic result was that the genus *Harpactes* was not recovered as a monophyletic lineage. *Harpactes diardii* and *H. ardens* were resolved as the sister-group to the *Euptilotis-Pharomachrus* clade, but the two most parsimonious trees had *H. oreskios* either as the sister-taxon to the rest of the trogons or as the sister-group of the two African trogons (*Apaloderma*). Most of the lack of resolution in the consensus tree is due to a conflictive rooting of the two networks by the outgroup. In one of the solutions the tree was rooted at *H. oreskios*, whereas in the other the outgroup rooted the tree at the internal branch between the genus *Trogon* and the rest of the trogoniforms (trees not shown).

One of the characteristics of genes with fast nucleotide substitution rates is that multiple hits result in increased levels of homoplasy, and substitution patterns eventually reach a saturation point where it is

impossible to generate further variability. As a consequence, homoplasy interferes with the true phylogenetic signal due to this saturation effect. Differences between patterns of variation result from differences in substitution processes. In protein coding genes like cytochrome-*b*, the possibility of nucleotide substitution is constrained by codon position or functional region and saturation seems to be a particular problem (Arctander 1991, Moritz et al. 1992, Nunn and Cracraft 1996). Empirical pairwise distances were plotted against Kimura distances to detect saturation in the different partitions of the genes used in this study. This analysis shows that the nucleotide substitution rate follows a linear relation in the 12S rRNA gene. Only in the cytochrome-*b* gene there is evidence of saturation. Figure 2 presents comparative curves for the accumulation of total substitutions depending on codon position in the cytochrome-*b*. Second positions show a clear linear pattern and had the slowest rate of nucleotide substitution (best fitted line slope= 0.77). The nucleotide replacement rate in first positions (slope= 1.81) was more than twice that for second positions, but the accumulation maintained essentially a linear pattern. The nucleotide substitution rate at third positions increases rapidly in relation to corrected distances, but after 13% of difference it begins to level-off into a zone of saturation.

On the basis of the foregoing analysis, I excluded the saturated partition of cytochrome-*b* (e.g., third position) from the data matrix and performed a

new parsimony analysis for the combined sequences of the two genes. The result from this analysis new was a single most parsimonious tree of 1971 steps in length, consistency index of 0.466, and retention index of 0.574 (Fig. 3). In this new phylogenetic hypothesis, all the genera were resolved as monophyletic and supported by high bootstrap values (>80%). The New World genera were retrieved as a monophyletic lineage, and their sister-taxon was *Harpactes* (Asian trogons). The most basal group was the African genus *Apaloderma*. Within the New World lineage, the two subclades of *Trogon* were recovered and the interspecies relationships were the same as those obtained for the 12S rRNA gene alone (Fig 1b). As before, the sister-group to *Trogon* was the lineage formed by *Euptilotis-Pharomachrus*. Finally, *Priotelus temnurus* (the Cuban Trogon) was the sister-taxon to the rest of the New World genera. This phylogenetic hypothesis remained constant even when the taxa included in the outgroup were changed. By removing the saturated partition within cytochrome-*b* a considerable amount of noise was eliminated, reducing the length of the tree by 1789 steps (i.e., from 3760 steps including all nucleotides, to 1971 steps without the 381 codon third positions). In spite of the noise elimination, however, the support values for the nodes linking different genera did not improve significantly. The nodes linking the New World genera to *Priotelus* and then to *Harpactes* are lost in trees one step longer than the most parsimonious solution.

Discussion

On the origin and distribution of Trogoniformes.– From the standpoint of biogeography, the trogoniforms have traditionally been an enigma because of their pantropical distribution (e.g., Darlington 1957). The current distribution of trogons coincides with the distribution of several other avian families (e.g., Anhingidae, Capitonidae, Heliornithidae, Jacanidae, Psittacidae), and other vertebrates (e.g., crocodilians, caecilians). Congruent biogeographic patterns between or among taxa can be explained by two processes: speciation of widespread taxa after a vicariant event, or multiple dispersal events. Vicariance is a more parsimonious explanation than dispersal because it is the simplest hypothesis with the fewest ad hoc assumptions to justify the distribution patterns. On the other hand, vicariance only can be supported by phylogenetic congruence, thus, without corroborated phylogenies for other taxa an explanation for the process responsible for the distribution pattern of trogons can not be offered at this point.

A problem in historical biogeography that can be addressed with the present data concerns the reconstruction of ancestral distribution areas. Early hypotheses about the evolution of trogons, pointed to the New World as the center of origin of these birds (Swainson 1837, Ridgway 1911). This idea was based on the high diversity of trogons in the Neotropics (64% of the species).

Cain (1971), however, argued that the area in which most species of a particular group are distributed may be a secondary center of diversification. Therefore, relative species richness may be false evidence for inferring the ancestral area of a taxon. Traditional vicariance biogeography assumes that the ancestral area must be considered as the sum of all the areas in which the group is found. Thus the observed distribution is explained by invoking vicariant events followed by speciation of the subpopulations. However, this implies that the distribution of the ancestor was more widespread than that of any of its descendants (Bremer 1992). The progression rule in phylogenetic biogeography proposes that the apomorphic species are more peripherally distributed than their plesiomorphic sister-species (Brundin 1981, 1988). Following this assumption, it may be possible to suggest an ancestral area and a series of dispersals to explain the current distribution of a group. Recently, Bremer (1992, 1995) has proposed a method for estimating the relative probability that different areas were part of the ancestral area of the group. This relative probability is computed based on a gain-loss ratio of individual areas after being coded as binary characters on a cladogram assuming irreversibility.

Applying Bremer's method to the cladogram proposed here (Fig. 3), Africa has a probability of 1.0, as it has been gained once on the *Apaloderma* lineage and then lost once on the clade encompassing the remaining genera. Asia and the New World each receive a probability of 0.5. Asia is gained on

the lineage leading to *Harpactes*, then is lost once in *Apaloderma*, and once in the New World clade. In the same manner, the New World is gained once in the clade form by the Neotropical genera, and then is lost once in *Apaloderma* and once in *Harpactes*. The idea of a Neotropical origin is, therefore, not supported. Instead, an African origin for trogons seems a reasonable conclusion, with the New World representing a secondary center of radiation. It is important to point out that these results do not suggest any particular dispersal or vicariant scenario. They only suggest an area that was most likely part of the ancestral area for trogons. It should be noted, however, that Bremer's method has been questioned by Ronquist (1994, 1995), who proposed a method based on standard character optimization using parsimony, and established the value at the root of the cladogram by Fitch optimization. Nonetheless, Ronquist's method as well as Brundin's progression rule, identify Africa as being the ancestral area of the Trogoniformes.

A further line of evidence supporting an origin in the Old World tropics comes from paleontology. The oldest known fossils within the family Trogonidae have been found in Tertiary deposits of Europe (Olson 1985). The earliest fossil comes from the middle Oligocene of Switzerland and has been referred to *Protornis glarniensis*. This species clearly shows the heterodactyl condition typical of the Trogonidae (Olson 1976). Another fossil species, *Paratrogon gallicus*, was recovered from lower Miocene deposits at Langy,

France (Olson 1976). In contrast, the oldest fossils discovered in the New World belong to two extant species (*Priotelus roseigaster* and *Trogon surrucura*), known from Pleistocene deposits from the Dominican Republic and Brazil respectively (Brodkorb 1971).

Considering that Africa is one of the largest continents, how can one explain that only 7% of the species of trogons are currently distributed there? In general, the overall biological diversity of Africa is relatively low compared to other tropical areas (Keast 1973). Identifying all the possible elements that determine the diversity patterns though time and space is extremely difficult. Many different physical, historical, and biological factors have been correlated with almost every pattern of variation in species diversity (Huston 1994). The principle of competitive exclusion (Hardin 1960) might be one of the factors that could be invoked as a causal reason for a lower number of species of trogons in Africa and Asia than in the Neotropics. Stated simply, competitive exclusion means that if ecologically similar species are competing for the same limiting resources they cannot coexist. The diversity of a single group is often low when competitors are present, and it has been interpreted that the less competitive forms are shifted away from the traits of the more aggressive competitor (Schluter 1988). In regard to feeding habits, all the Old World species of trogons are insectivorous, while the New World species base their diets on a mixture of fruits and animals. It seems that the fruit eating niches in the Old World are taken by avian families that are either poorly

represented or completely missing in the New World (e.g., Bucerotidae, Musophagidae, Pynonotidae, Irenidae). Consequently, a decrease in competition pressure may have resulted in radiation toward new niches in the New World.

Other factors that have been associated with species diversity are structural and climatic heterogeneity of the habitat (Huston 1994). At large scales heterogeneity in the environment allows the possibility of regional coexistence in spite of local extinction or displacement. In addition to this, patchiness of the habitat has a mayor effect on the number of functional types that occur within a local area (Schluter 1988). A great structural and climatic heterogeneity is generally correlated with more types of resources in an environment, which in turn might permit an increase of the number of species that can be present. The climate during the Quaternary were quite unstable, and this certainly affected the distribution of tropical biotas. The principal consequence from this climatic turnover during the late Cenozoic was a decrease of forest and a concomitant expansion of open woodlands, grasslands, and deserts (Potts and Behrensmeyer 1992). Although these were worldwide trends, the expansion of deserts and grasslands were especially important events in Africa (Bonnefille 1985). This possibly affected in a negative way the chances for radiation of forest birds such as trogons. Additionally, during phases of glacial aridity the rain forest in the Amazon and SE Asia shrank to isolated refugia (Roberts 1984), possibly constituting

favorable conditions for the speciation of the populations restricted in these areas. Finally, compared to SE Asia and the New World, the tectonic history of Africa was relatively stable during the last 40 million years without being affected by the rise of extensive mountain ranges. Thus, a high diversity in SE Asia and especially in the New World may be a direct consequence of the great orogenic activity during the late Cenozoic due to the collision of continental plates, and not the result of a long term history of the avifauna within such areas. Undoubtedly, to understand in detail all the possible interactions responsible for the differences in trogon diversity among Africa, Asia, and the New World will depend on more detailed studies.

Absolute dates of diversification for trogons are unknown due to the lack of an extensive fossil record. Nonetheless, the problem of dating can be approached through molecular studies. Several authors have suggested that approximate times can be estimated based on differences in nucleotide substitutions in mitochondrial DNA (Brown et al. 1982, DeSalle et al. 1987, Miyamoto and Boyle 1989, Irwin et al. 1991). This molecular clock hypothesis is based on the assumption that the average nucleotide substitution rate within a clade is constant throughout time. Earlier studies addressing the question of divergence time in mammals have estimated an average evolutionary rate of 10% in corrected cytochrome-*b* third positions per million years (Brown et al. 1982, Irwin et al. 1991). Other studies have concluded that this rate is substantially slower in birds (Helm-Bychowski

1984, Shields and Wilson 1987, Nunn et al. 1996).

The existence of a molecular clock has been seriously questioned because of evidence showing that different regions of the genome evolve at different rates in different lineages (Martin et al. 1992, Martin and Palumbi 1993). Most of these examples involve lineages whose species exhibit significant differences in body size, metabolic rate, or generation time. Trogons, by contrast, are rather uniform in body size and in generation time. The characteristics observed in the sequences of the cytochrome-*b* gene of trogons (Tables 1, 2, and 3) are similar to those observed in Procellariiformes, in which a rate of 1.58% and 2.86% for corrected third position per million years has been estimated (Nunn et al. 1996). Using these estimates, and assuming that the corrected third position divergence among the trogons is linear with respect to time, a rough date for the separation times between the genera of Trogoniformes can be suggested. The most recent divergence event occurred between the genus *Pharomachrus* and the genus *Euptilotis*, and can be estimated at 13.8 to 25 million years ago (Mya). If we compare these dates with others estimated for corvine birds (Helm-Bychowski and Cracraft 1993), we would have to conclude that trogoniformes represent a relatively old divergence within birds. A mean value of 51.5% for pairwise corrected third position divergence gives estimates of the date of origin for the Neotropical trogons between 18 and 32.6 Mya. The split of the African trogons with the rest of the genera (i.e., the first branching event within trogons) can be dated

between 19.7 to 35.6 Mya. Even with such broad estimates these data suggest that the radiation of the major clades of trogons, especially among Africa, Asia and the New World, may be characterized as a star phylogeny. If the major clades did diversify rapidly, that might be responsible for the problems in recovering a stable signal during cladistic analysis. Finally, trogons diverged from their sister-taxon (i.e., Coliiformes) between 25.5 to 46.1 Mya. This estimated date is consistent with the date suggested by the oldest fossils described for trogons (Olson 1975, 1985). A detail list of divergence times estimated for all the genera of Trogoniformes is presented in Table 4.

Coloration patterns.– Without question, one of the most attractive features of trogons is their plumage color. Indeed, the Resplendent Quetzal was considered a sacred bird in many pre-Columbian cultures of the New World because of its coloration pattern. Color patterns in birds are due both to individual feathers and to overlapping of groups of feathers that form the entire plumage. Color in feathers is caused by chemical pigments, structural features, or combinations of both. The brightest colors and more complex coloration patterns occur mainly on regions of the body that are involved during display (e.g., head, upper back, breast, tail, and external surface of the wings). Less exposed areas like the underparts of the body usually present dull colors and in most cases lack special patterns.

With the aid of a corroborated phylogeny it is possible to examine some of the evolutionary tendencies in the coloration pattern of trogons. Because

color and color patterns in trogoniforms have a strong sexual component, the following discussion is based mainly on male plumages.

For most of the species the ventral coloration pattern can be divided into two regions. The first is formed by the chin, throat and the upper portion of the breast which usually show the same color as the head or the back, while the second includes the lower portion of the breast and the belly which are typically red or yellow. In some species these two regions are clearly divided by a white band. This white band has evolved independently at least three times within the order, once in the Asian trogons (*H. diardii*) and twice in the genus *Trogon*. As suggested by the phylogenetic tree, this white band represents a synapomorphy for the Elegant subclade within the genus *Trogon* (Fig. 4a). As mentioned above, the belly of trogons can take two different colorations: red or yellow. The character state "red belly" can be interpreted as ancestral within the group. The derived condition "yellow belly" has appeared several times, once in the Asian trogons (*H. oreskios*) and twice independently in the genus *Trogon* (Fig. 4b).

A clear character on the tail feathers of trogons is the presence of white bands. Describing this character is complex because the pattern can change depending on sex and age. Nevertheless, the presence of this character can be interpreted to be the primitive condition for the group. Apparently, this character has evolved in two contrasting directions. It has transformed either from a single band located at the tip of the feather to multiple bands, or from

one band to no bands (Fig 4c).

Most of the species of trogons have multiple white spots on the secondary feathers, which are more evident in the males. The pattern created by these spots can go from a random distribution of tiny dots, to clearly defined white bands. The presence of any of these patterns is the plesiomorphic condition for trogons, whereas the apomorphic state is the loss of white pattern (i.e., secondaries plain black). This derived condition has appeared independently at least twice in the order, once in *T. viridis*, and once in the *Pharomachrus-Euptilotis* clade in which the loss of white pattern represents a synapomorphy (Fig. 4d).

The coloration pattern of the dorsal region can be divided into three general areas: the head, the back, and the rump. Brown back color in males is a synapomorphy for the Asian trogons. The presence of green back color is, then, clearly the plesiomorphic state for the order (Fig. 4e). The color of the other two dorsal regions (head and rump) are correlated with the color of the back, in fact the ancestral condition for both is that their color is the same as the color of the back. In other words, the plesiomorphic state for back color is brown in the Asian trogons, whereas it is green in the African and New World trogons. Head color presents at least two derived states: grey and violet. Violet heads have appeared twice in the New World genera, once in *Priotelus temnurus* and once in three species that are part of the Violaceous subclade within the genus *Trogon*, while gray heads have evolved at least

once within the Asian trogons (Fig. 4f).

Finally, the rump color for most of the species examined in this study was the same as the color of the back, the only exception was for three species. *Trogon melanurus*, *T. comptus*, and *T. viridis* have violet rumps that represent the only derived condition observed for this character (Fig. 4g).

Evolution of iridescent structures in trogon feathers.– Iridescence is the property of a structure to glitter with different colors depending on the angle from which that structure is observed (Fox and Vevers 1960). When a beam of light hits a membrane some of the light is reflected by its external surface, whereas some light penetrates the membrane until it is reflected by its lower surface. At the moment the light enters the membrane, the path of light is deflected and its wavelength is reduced depending on the density (refractive index) of the membrane. Finally, when the modified light rejoins the reflected light from the external surface of the membrane, the difference in wavelengths produces the reinforcement of a particular color. Usually, the reinforced wavelength has an metallic glow better known as iridescence color.

Iridescence is widespread in birds. Ducks, galliforms, doves, hummingbirds, birds-of-paradise, starlings and trogons are only a few of the groups in which iridescent patches can be seen. Iridescent colors are produced mainly in contour feathers, and more precisely in the barbules. In hummingbirds and nectariniids, the interference granules responsible for the production of iridescent colors are relatively uniform in design and

composition across the different species (Dorst 1951, Durrer and Villiger 1962). But for trogons, Durrer and Villiger (1966) described four different levels of structural complexity in these granules. Within trogons, the quetzals have the most complex kind of iridescence. Under electron microscopy, the barbules have from five to eight layers of elliptical platelets forming a continuous mosaic on the iridescent surface of the feather. These platelets are made of melanin filled with air capsules divided by extensions of the platelet wall. The layers of platelets are separated by layers of keratin with a thickness so uniform that its variation is less than 0.01μ. In the genus *Trogon*, the melanin granules are air-filled rods of nearly 1.25μ in length. These rods are packed so tightly that practically no keratin is left between them. Trogons are less brilliant than quetzals because the rod arrangement only allows the light to go though at a 30° angle, thereby producing a relatively weak iridescent effect (Durrer and Villiger 1966). The difference in color intensity between the species of trogons is due mainly to changes in the diameter of the rods. Rods with big diameters produce golden glows, while those with small diameters tend to produce blue tonalities. The other two structural patterns in the iridescent granules are variations from that described for the genus *Trogon*. In *Priotelus*, the total diameter of the rod is similar to that in *Trogon* (0.2μ), but the melanin wall is thicker in *Priotelus* than in *Trogon* (0.08μ, and 0.04μ respectively). Another difference between these two genera is that in *Priotelus* the melanin rods are clearly divided by keratin layers. Finally, in

Harpactes and *Apaloderma*, the diameter of the rods is larger than in *Priotelus* and the keratin layers dividing the rods are more than twice of those in *Priotelus* (Durrer and Villiger 1966).

If these four different configurations of iridescent granules found in the trogoniforms are mapped on the phylogeny obtained in the present study, one hypothesis for the evolution of these structures can be suggested. The least iridescent structures are found in the African and Asian trogons that are the most basal lineages (Fig. 6). These large rods divided by thick layers of keratin are relatively similar to the iridescent structures found in other birds (Dorst 1951, Durrer and Villiger 1962, 1966). In later lineages a reduction in the diameter of the rods and especially in the thickness of the melanin wall can be postulated. Concomitant with this, there is a reduction in the thickness of the keratin layer, an increment in the number of rods, and a displacement of the rods toward the external surface of the barbule. The more complex structures found in quetzals can be explained as a step further in the tendency towards increasing the number of rods on the surface of the barbule, finally leading to the fusion of multiple single air-filled chamber rods into elliptical platelets.

A phylogenetic classification of the genera of Trogoniformes.– Although all the phylogenetic hypotheses presented in the present study received relatively low bootstrap and branch support values, several of the relationships obtained were recovered consistently. Supported by high

bootstrap values (>90%), the different genera of trogons were always identified as monophyletic clades (except the Asian trogons when cytochrome-*b* was analyzed alone; Fig. 1b). The ten species of the genus *Trogon* were always divided in two subclades, the Violaceous subclade was consistently formed by the relationships: (((*T. curucui*, *T. violaceus*), *T. viridis*), (*T. melanurus*, *T. comptus*)). The remaining five taxa were included in the Elegant subclade. Although, the interspecific relationships in this subclade were not as stable as in the Violaceous subclade, *T. collaris* was always the sister-taxon to *T. personatus*. These two subclades were always supported by high bootstrap values (>75%). Another stable relationship consistently recovered was the lineage formed by the quetzals and their sister-taxon *E. neoxenus*. The union of these two lineages was supported in all the analyses by very high bootstrap values (>95%), and high branch support (>8). In addition to this, the distances between any pairwise comparison of *Euptilotis* with *Pharomachrus* were considerably smaller then the distance of any other pairwise comparison between two species of different genera (Table 1 and 2). The proposal here is that *Euptilotis neoxenus* should not be isolated in its own genus, but should be included within *Pharomachrus*. In three of the four phylogenies presented, the African trogons were the most basal taxon within trogoniforms, supporting a closer relationship of the Asian trogons to the New World Trogons.

It is widely acknowledged than a useful classification should be based

on a corroborated phylogeny, and therefore the classification must reflect the historical relationships of the taxa encompassed within that group. Accordingly, I recommend the following phylogenetic classification for the genera of Trogoniformes:

ORDER Trogoniformes

FAMILY Trogonidae

Subfamily Apaloderminae

GENUS *Apaloderma*

Subfamily Trogoninae

Tribe Harpactini

GENUS *Harpactes*

Tribe Trogonini

GENUS *Priotelus*

GENUS *Trogon*

GENUS *Pharomachrus*

Sequences availability.– All the sequences used in this study have been deposited in GenBank and are available through the following accession numbers (cytochrome-b and 12S rRNA, respectively): *Apaloderma narina* (U94798, U94812), *A. vittatum* (U89200, U89234), *Pharomachrus antisianus* (U89204, U89235), *P. auriceps* (U94799, U94813), *P. pavoninus* (U94800, U94814), *Euptilotis neoxenus* (U89203, U89236), *Priotelus temnurus* (U89202, U89237), *Trogon melanurus* (U94805, U94819), *T. comptus* (U94804, U94818),

T. viridis (U94803, U94817), *T. mexicanus* (U94809, U94823), *T. elegans* (U94806, U94820), *T. collaris* (U94808, U94822), *T. personatus* (U89201, U89238), *T. rufus* (U94807, U94821), *T. curucui* (U94801, U94815), *T. violaceus* (U94802, U94816), *Harpactes diardii* (U94797, U94811), *H. ardens* (U94796, U94810), *H. oreskios* (U89199, U89239), *Geococcyx velox* (U89198, U89212), *Cuculus fugax* (U89197, U89210), *Centropus sinensis* (U89196, U89211), *Colius striatus* (U89175, U89218), *C. leucocephalus* (U89173, U89217), *C. colius* (U89174, U89216).

Table 1. Pairwise empirical differences for the combined sequences of cytochrome-*b* and 12S rRNA. Total differences below the diagonal, transversion differences above the diagonal.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1) H. oreskios	—	79	83	102	96	94	79	82	82	94	106	109	107	95	95	110	101	116	111	104
2) H. ardens	263	—	36	104	100	94	82	81	81	89	107	102	110	92	90	104	98	111	110	101
3) H. diardii	275	171	—	108	110	104	89	88	86	96	108	105	109	92	92	104	98	112	110	101
4) P. temnurus	316	311	304	—	105	107	89	78	90	96	109	106	108	92	89	107	100	108	103	100
5) A. narina	301	317	332	327	—	34	97	96	98	106	103	104	105	87	91	107	102	114	106	105
6) A. vittatum	285	306	329	310	181	—	99	102	98	110	104	103	108	92	94	112	108	117	112	106
7) P. auriceps	277	275	278	286	292	288	—	19	15	57	92	89	94	83	79	98	89	102	99	92
8) P. antisianus	289	287	300	268	303	293	102	—	24	62	97	92	99	84	88	103	92	109	98	95
9) P. pavoninus	292	284	298	292	312	294	87	130	—	56	91	88	93	84	84	98	91	108	99	96
10) E. neoxenus	296	297	308	322	342	329	231	233	225	—	106	105	108	97	93	104	101	107	107	99
11) T. curucui	317	310	321	333	335	340	312	326	314	331	—	7	33	41	43	67	61	76	72	64
12) T. violaceus	307	311	324	323	330	332	306	330	309	327	90	—	34	40	40	68	64	77	73	63
13) T. viridis	299	299	317	322	315	320	299	311	314	325	205	200	—	46	46	73	71	78	76	69
14) T. comptus	300	292	307	326	314	307	294	301	301	313	234	233	225	—	16	61	58	70	62	56
15) T. melanurus	306	295	313	308	321	300	279	299	294	301	226	210	210	123	—	58	55	65	63	53
16) T. elegans	314	326	327	326	344	324	316	319	317	318	282	280	255	258	256	—	42	33	32	26
17) T. rufus	302	313	328	321	337	326	300	305	299	309	271	263	253	254	241	188	—	45	46	38
18) T. collaris	307	325	326	322	346	352	323	337	334	329	276	267	238	261	251	188	199	—	39	20
19) T. mexicanus	333	314	330	326	333	345	322	321	326	325	265	272	264	266	256	197	192	195	—	30
20) T. personatus	321	326	341	310	330	340	313	324	318	319	279	270	244	271	248	195	186	155	190	—

Table 2. Kimura two parameter corrected distances, cytochrome-*b* below the diagonal, 12S rRNA above the diagonal.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1) H. oreskios	—	11.0	12.3	14.9	15.2	14.8	13.7	13.2	12.9	13.0	17.1	16.7	15.1	15.3	16.3	15.8	15.7	16.3	15.8	15.9
2) H. ardens	17.9	—	6.9	15.2	15.6	15.7	14.8	14.5	14.2	15.5	17.7	17.6	16.4	17.4	17.6	16.9	18.1	18.1	16.3	16.8
3) H. diardii	18.3	10.7	—	15.4	16.9	17.6	15.7	16.3	15.7	15.8	18.7	18.7	18.1	17.1	18.4	17.7	18.4	19.0	17.2	18.1
4) P. temnurus	21.6	20.7	19.7	—	15.8	15.1	14.1	14.6	13.7	14.3	17.1	16.9	16.2	16.6	15.9	16.2	16.6	17.0	16.5	14.8
5) A. narina	19.1	20.8	21.9	22.2	—	6.3	15.4	15.7	15.3	17.4	17.9	17.6	17.6	17.2	18.1	17.2	18.1	18.6	17.8	17.2
6) A. vittatum	17.4	19.2	20.7	20.7	12.2	—	15.9	15.5	15.3	16.8	18.5	18.3	18.4	17.2	18.1	18.0	19.5	19.8	18.9	18.0
7) P. auriceps	17.3	16.1	15.8	18.3	17.9	16.9	—	2.0	2.5	9.8	17.1	16.7	16.4	16.8	16.0	15.8	16.1	17.5	16.6	16.0
8) P. antisianus	19.2	17.8	17.9	15.5	19.0	17.9	7.8	—	2.5	8.6	17.1	17.5	16.7	16.7	16.1	15.5	15.6	18.0	17.0	15.9
9) P. pavoninus	19.9	17.7	18.2	19.4	20.5	18.1	5.9	10.4	—	9.4	16.9	17.0	15.9	16.9	16.0	15.2	15.1	17.4	17.3	15.4
10) E. neoxenus	20.6	18.2	19.5	22.9	22.7	21.6	15.0	16.4	14.7	—	16.5	16.8	16.1	17.0	15.9	14.8	15.6	16.1	15.6	15.5
11) T. curucui	19.8	18.4	19.0	22.1	21.2	21.4	19.0	20.9	19.4	22.3	—	3.0	7.7	10.3	9.6	12.5	12.6	12.4	10.5	11.1
12) T. violaceus	19.0	18.5	19.3	20.8	20.8	20.4	18.4	20.9	18.6	21.5	5.7	—	6.8	10.4	9.0	12.6	12.3	12.4	10.3	10.7
13) T. viridis	19.3	18.2	19.0	21.4	18.9	19.0	18.0	19.3	20.3	21.9	13.7	13.9	—	10.3	9.3	11.3	12.0	11.6	11.2	11.0
14) T. comptus	19.0	16.2	18.3	21.2	18.8	18.1	16.8	17.8	17.6	19.3	14.6	14.4	13.6	—	4.6	12.4	12.7	12.6	12.5	12.3
15) T. melanurus	18.8	16.3	17.8	19.4	19.0	16.5	15.5	18.1	17.4	18.6	14.4	13.1	12.8	7.6	—	12.5	12.7	12.4	11.7	12.5
16) T. elegans	20.6	21.0	20.5	21.9	23.1	19.9	20.7	21.5	21.4	22.1	18.7	18.3	16.6	15.8	15.4	—	6.0	6.6	8.0	6.8
17) T. rufus	19.0	18.2	19.9	20.7	21.2	18.7	18.2	19.4	19.0	20.1	17.2	16.5	15.7	15.0	13.4	13.4	—	6.5	6.8	6.2
18) T. collaris	19.4	20.1	19.4	20.6	22.3	22.1	20.2	21.7	21.9	22.4	18.2	17.1	14.4	16.1	15.0	12.7	14.3	—	7.4	5.1
19) T. mexicanus	23.3	20.2	21.5	21.5	21.1	21.9	20.8	20.3	20.7	22.3	18.6	19.7	17.9	16.7	16.2	12.4	13.1	12.8	—	6.9
20) T. personatus	21.4	21.1	22.0	21.0	21.3	21.9	20.1	21.7	21.4	21.5	19.7	18.9	15.6	17.4	14.5	13.3	13.0	10.5	12.7	—

Table 3. Nucleotide composition, and base compositional bias in cytochrome *b* and 12S rRNA genes.

	First position				Second position				Third position				Stems				Loops			
Taxon	C	T	A	G	C	T	A	G	C	T	A	G	C	T	A	G	C	T	A	G
C. striatus	29.1	24.1	26.5	20.2	27.6	38.1	20.7	13.6	44.4	11.8	37.8	6.0	24.0	25.3	26.4	24.2	26.3	18.8	41.5	13.5
C. leucocephalus	29.1	23.9	26.5	20.5	26.2	39.6	20.7	13.4	43.6	10.2	42.0	4.2	25.4	24.6	24.9	25.1	26.8	18.9	40.3	13.9
C. colius	28.6	24.1	26.8	20.5	27.8	38.3	21.0	12.9	45.4	9.7	40.4	4.5	24.9	25.1	25.1	24.9	26.8	18.9	40.1	14.2
C. fugax	27.3	24.1	27.8	20.7	27.0	39.4	20.7	12.9	46.7	9.4	40.2	3.7	25.3	24.7	25.3	24.7	24.6	20.3	38.8	16.2
C. sinensis	24.9	27.8	28.3	18.9	26.0	40.2	21.3	12.6	39.4	15.0	42.3	3.4	26.5	22.1	25.7	25.7	24.5	20.6	40.4	14.4
G. velox	28.6	23.1	30.2	18.1	27.6	39.1	20.7	12.6	46.2	12.9	39.6	1.3	26.2	23.4	24.2	26.2	26.5	20.0	38.6	14.8
H. oreskios	28.3	23.1	27.6	21.0	27.0	39.4	20.7	12.9	41.5	14.2	41.7	2.6	28.8	22.3	21.4	27.5	24.2	19.7	40.7	15.4
H. ardens	28.1	23.6	28.3	19.9	27.0	39.4	21.0	12.6	38.3	20.5	39.9	1.3	28.5	22.5	21.9	27.1	24.6	19.9	39.8	15.8
H. diardii	28.1	24.1	28.1	19.7	27.0	39.4	21.0	12.6	39.6	17.3	40.4	2.6	27.1	23.3	23.0	26.6	22.7	21.1	41.0	15.2
P. temnurus	28.6	23.6	26.0	21.8	27.3	39.6	20.5	12.6	40.7	16.0	37.5	5.8	29.6	21.6	21.9	26.8	24.2	21.3	38.4	16.1
A. narina	25.7	26.5	26.2	21.5	26.8	39.9	20.7	12.6	41.5	17.8	37.8	2.9	28.0	22.5	22.3	27.2	25.0	19.4	39.0	16.6
A. vittatum	28.6	23.4	25.7	22.3	26.8	39.9	20.7	12.6	42.8	17.1	37.8	2.4	28.0	22.0	22.8	27.2	25.2	19.0	39.2	16.6
P. auriceps	28.3	23.1	26.8	21.8	27.0	39.9	20.5	12.6	42.8	15.7	37.8	3.7	27.7	24.2	22.3	25.8	24.8	20.1	39.2	15.9
P. antisianus	28.6	23.1	27.3	21.0	26.8	39.9	20.7	12.6	42.5	16.3	38.8	2.4	28.2	23.6	22.2	26.0	25.1	19.9	39.0	16.1
P. pavoninus	28.6	22.8	27.6	21.0	27.0	39.6	20.7	12.6	45.4	13.9	37.3	3.4	28.0	23.4	22.5	26.1	25.7	19.1	39.2	15.9
E. neoxenus	28.6	23.1	27.0	21.3	26.5	39.4	20.7	13.4	36.7	21.0	39.4	2.9	27.9	23.6	22.2	26.3	24.4	20.6	39.6	15.4
T. curucui	28.9	24.1	26.8	20.2	26.2	40.2	20.7	12.9	40.9	18.1	36.7	4.2	25.3	25.3	22.8	26.6	25.2	20.5	38.2	16.0
T. violaceus	29.4	23.9	25.5	21.3	26.5	39.9	20.7	12.9	39.9	19.9	37.0	3.1	25.5	25.0	22.8	26.6	24.6	21.3	38.8	15.3
T. viridis	31.0	21.0	26.5	21.5	26.5	39.9	21.0	12.6	40.9	17.8	38.1	3.1	26.8	24.4	21.9	26.8	25.5	19.7	39.9	14.9
T. comptus	28.6	23.9	26.5	21.0	27.0	39.9	20.5	12.6	41.5	18.1	37.3	3.1	28.3	22.8	22.3	26.6	23.7	21.3	38.7	16.3
T. melanurus	30.4	22.3	24.7	22.6	26.8	39.9	20.7	12.6	42.0	18.1	37.8	2.1	26.6	24.5	22.5	26.4	24.4	20.9	38.8	15.9
T. elegans	28.9	23.4	26.5	21.3	26.0	40.4	21.0	12.6	43.3	15.0	36.0	5.8	26.8	24.3	22.7	26.2	25.5	20.1	37.9	16.5
T. rufus	29.4	22.8	27.0	20.7	26.2	40.2	21.0	12.6	47.5	11.8	36.0	4.7	26.6	24.2	22.8	26.4	26.0	19.3	38.3	16.4
T. collaris	28.6	23.6	27.0	20.7	26.2	40.2	20.7	12.9	43.0	14.2	40.4	2.4	27.1	23.8	22.7	26.3	26.0	20.4	37.7	15.8
T. mexicanus	28.6	23.4	27.6	20.5	26.2	40.2	21.0	12.6	44.1	14.2	37.5	4.2	26.3	24.7	22.5	26.6	24.7	21.4	38.3	15.6
T. personatus	27.6	24.7	27.0	20.7	26.0	40.4	21.0	12.6	43.0	15.2	37.3	4.5	27.4	23.6	21.9	27.1	24.9	21.0	38.3	15.8
Mean	28.5	23.7	27.0	20.8	26.7	39.7	20.8	12.8	42.4	15.4	38.6	3.5	27.0	23.7	23.0	26.3	25.1	20.1	39.2	15.6
Nucleotide Bias	0.073				0.22				0.414				0.044				0.191			

Table 4. Divergence time between the genera of Trogoniformes. Below diagonal are the maximum divergence times calibrated using a third positions nucleotide substitution rate of 1.58% per million years (pmy), and above diagonal are the minimum divergence times calibrated using a third positions nucleotide substitution rate of 2.86% pmy (Nunn et al. 1996).

Taxa	1	2	3	4	5	6	7	8
1) Harpactes	—	19.49	19.20	17.17	17.52	17.85	25.34	23.94
2) Priotelus	35.27	—	20.99	16.68	20.41	19.31	24.78	25.77
3) Apaloderma	34.75	38.00	—	18.15	21.05	18.91	26.30	23.49
4) Pharomachrus	31.08	30.19	32.85	—	13.83	18.56	24.81	25.21
5) Euptilotis	31.72	36.95	38.10	25.03	—	19.00	27.32	27.25
6) Trogon	32.30	34.95	34.22	33.60	34.40	—	24.27	25.25
7) Coliidae	45.86	44.86	47.61	44.91	49.46	43.94	—	22.07
8) Cuculiformes	43.33	46.65	42.52	45.64	49.33	45.70	34.94	—

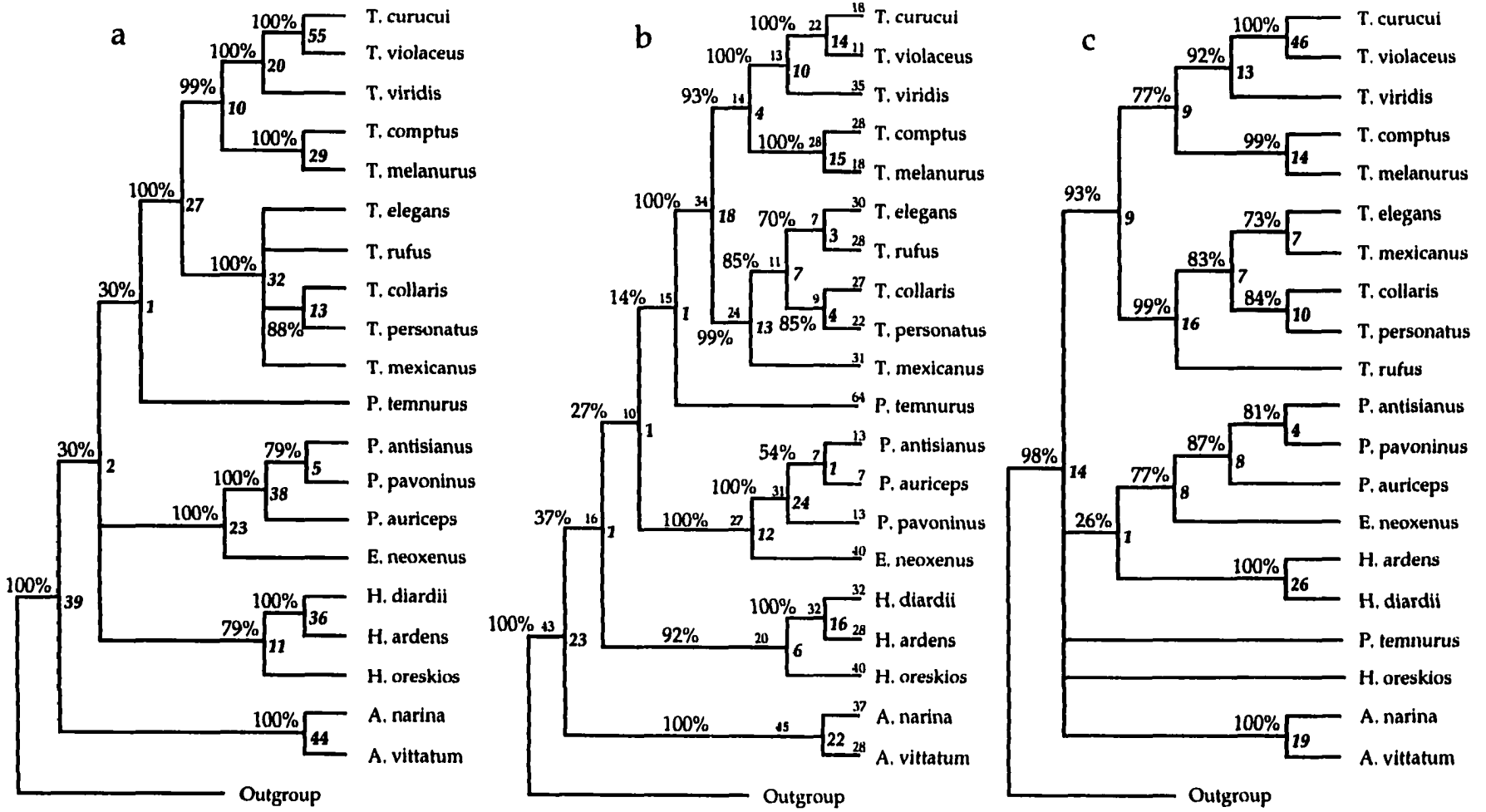

Fig. 1. Phylogenetic hypotheses recovered from parsimony analyses. a) Strict consensus tree from three trees obtained from the combined analysis of all nucleotide position of both genes (length: 3760; CI: 0.407; RI: 0.5). b) Single most parsimonious tree resulting from the sequences of the 12S rRNA (length: 1444; CI: 0.468; RI: 0.601). c) Strict consensus tree from two trees obtained from cytochrome-*b* only (length: 2296; CI: 0.372; RI: 0.43). Percentage is bootstrap values for 500 replications, number in front of nodes is branch support index, and number above branches are the branch length (tree b only).

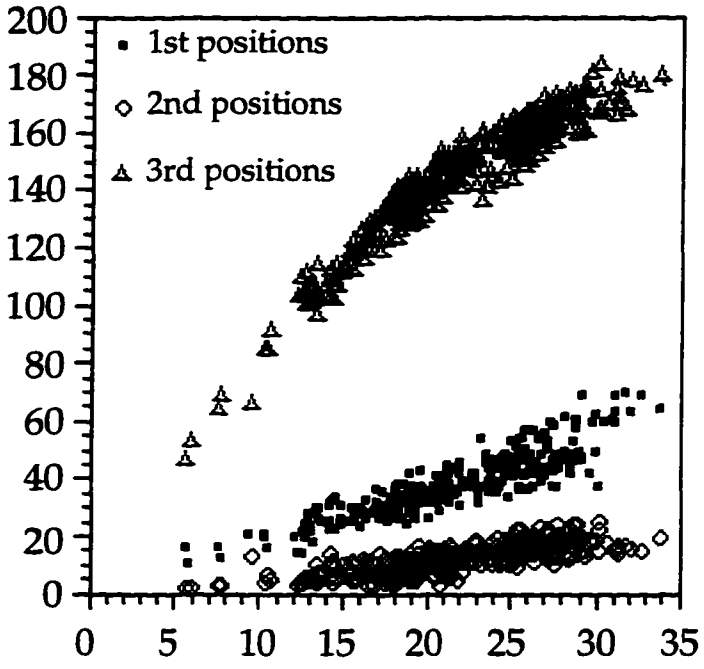

Fig. 2. Saturation plots for the cytochrome-b gene sequences constructed using empirical differences for each codon position as a function of corrected sequence divergence (%) and using the Kimura two parameter model with a 10:1 transitions transversion ratio.

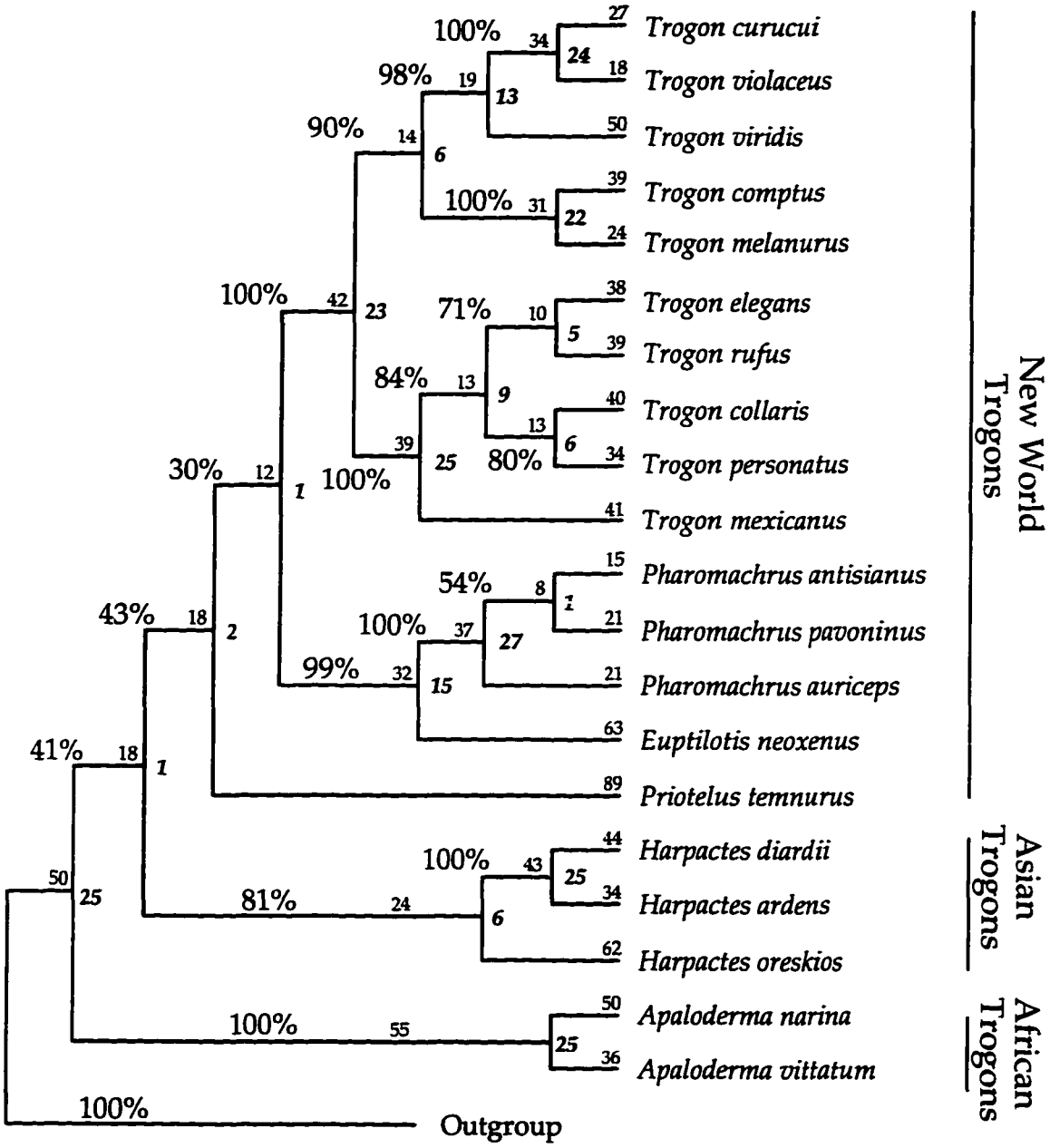

Fig. 3. Single most parsimonious tree obtained for a combined analysis of both genes after removing third positions from the cytochrome-*b* sequences (length: 1971; CI: 0.466; RI: 0.574). Percentages are the values of 500 bootstrap replications, numbers above branches are branch lengths, and numbers in front of the nodes are branch support values.

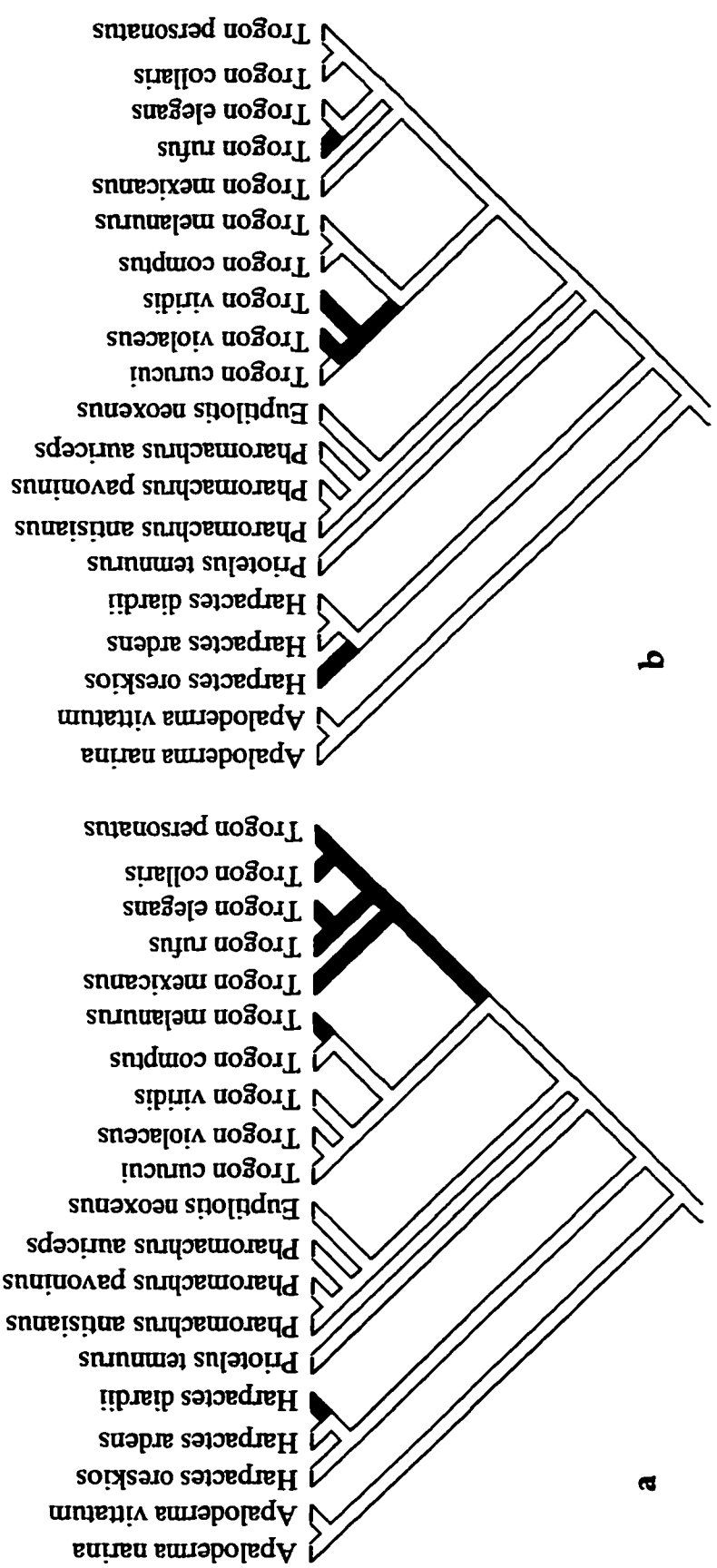

Fig. 4. Evolutionary tendencies in the coloration pattern for the feathers of trogons. a) White pectoral line dividing the chest and belly color: absent [white]; present [black]. b) Color of the belly: red [white]; yellow [black].

Fig. 4. (continued). c) Coloration pattern of tail feathers: one white spot at the tip of the feather [white]; multiple white bands [hatched]; no white pattern [black]. d) White spots on the secondary feathers: present [white]; absent [black].

Fig. 4. (continued). e) Color of the back: green [white]; brown [black]. f) Color of the head: same color as the back [white]; gray [hatched]; violet [black].

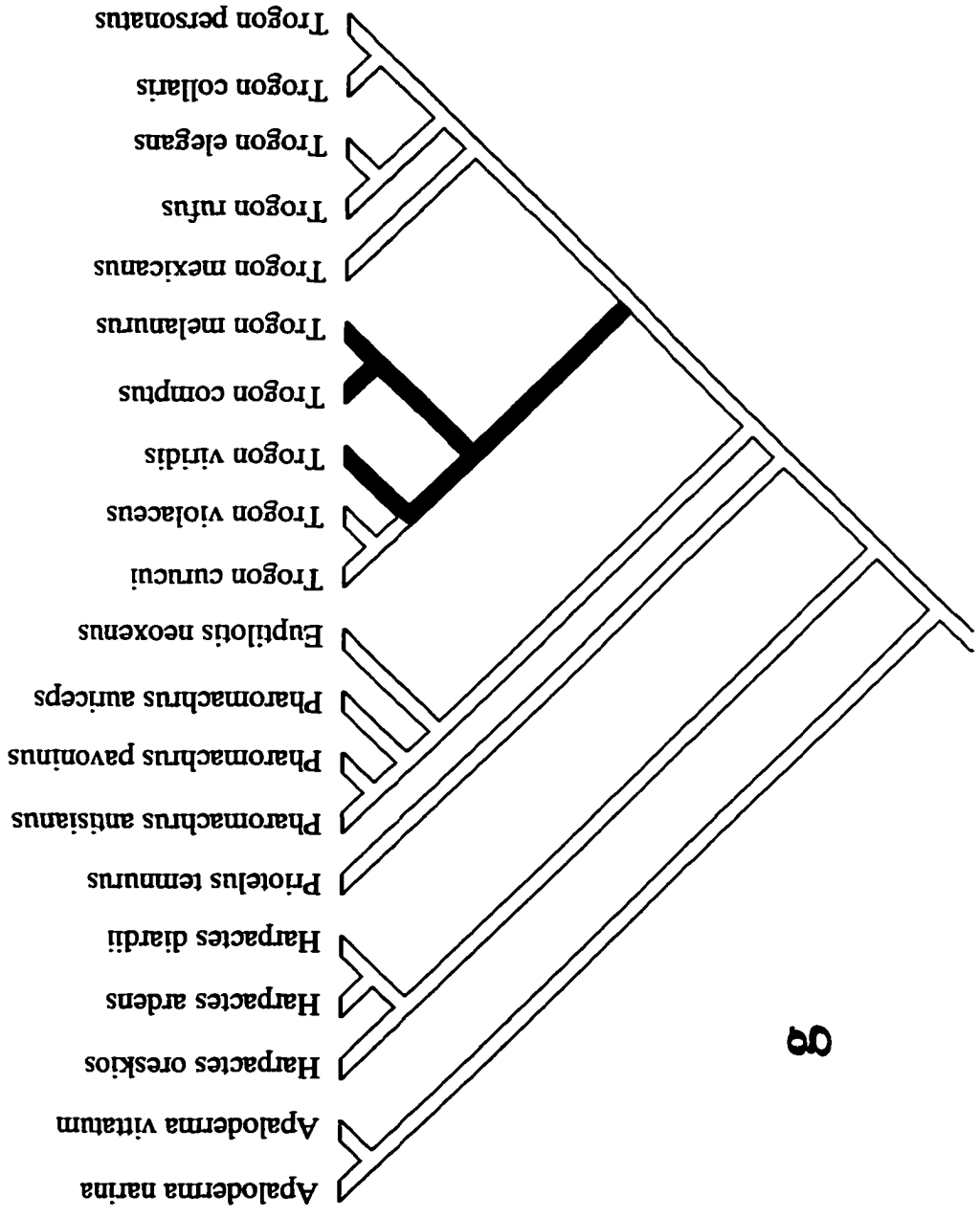

Fig. 4. (continued). g) Color of the rump: same color as the back [white]; violet [black].

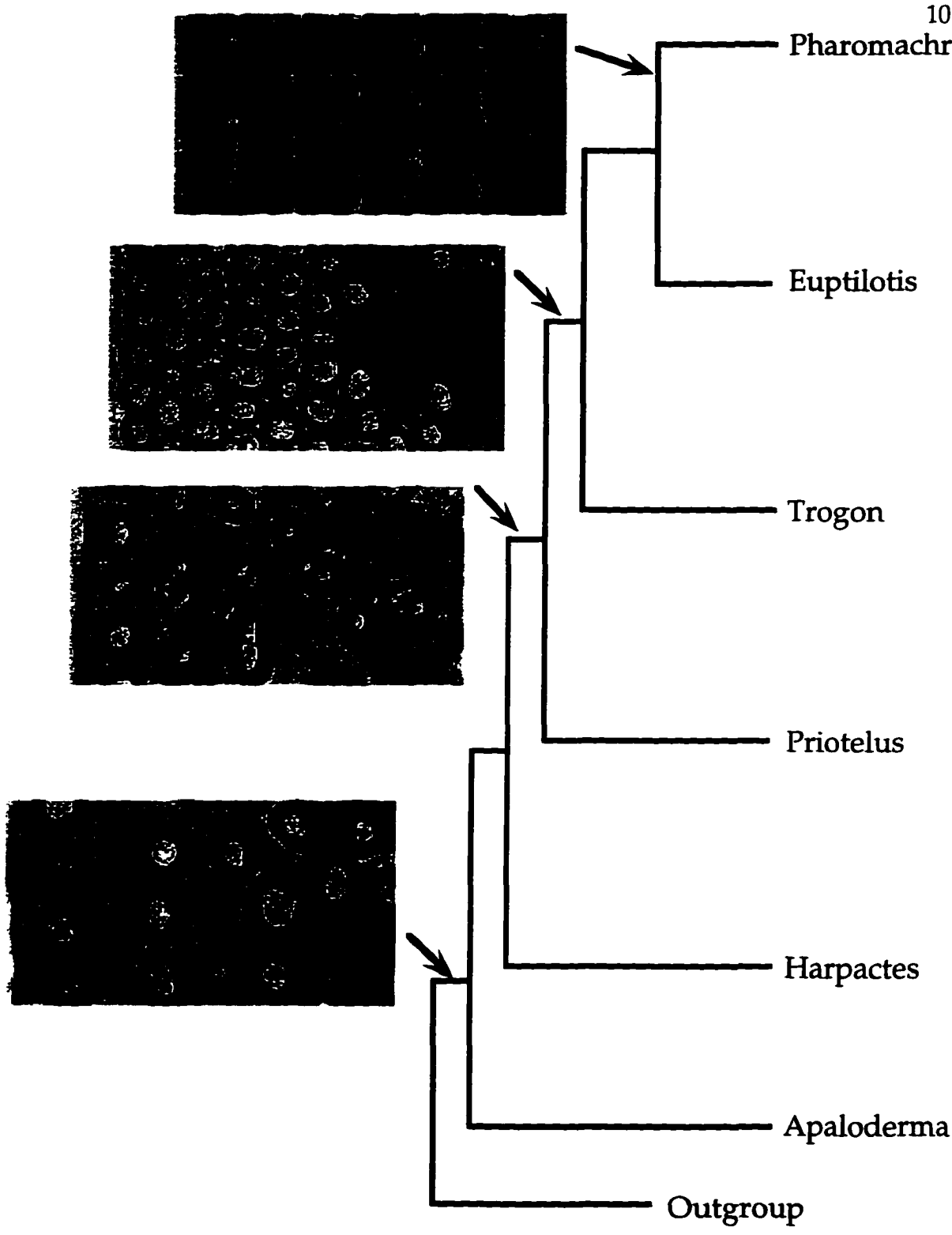

Fig. 5. Most parsimonious scenario for the evolution of iridescent structures in the feathers of trogons. Electron microscope photographs were scanned from Durrer and Villiger (1966). Photographs were taken using a 30,000:1 magnification scale.

CHAPTER 3

A MODEL FOR THE SECONDARY STRUCTURE OF THE 12S rRNA OF NON-PASSERINE BIRDS AND ITS POTENTIAL FOR ALIGNING SEQUENCES

Abstract.— A model for the secondary structure of the 12S ribosomal RNA (rRNA) gene of non-passerine birds is presented. Preliminary higher-order structures were delimited following the previously proposed model for vertebrates. Paired regions were refined according to a complementary base pairing criterion and compensatory mutations were considered as a further confirmation of the existence of these structures. The results suggest the presence of 40 stems, 20 internal loops, and 17 external loops, arranged in the typical four domains suggested for the small subunit rRNA. The higher-order structures recovered in the model were used to build a multiple sequence alignment appropriate for phylogenetic analysis. The phylogenetic tree recovered from this alignment was compared with cladograms inferred from alignments assembled using different alignment programs. Although, the alignment based on the secondary structure is sensitive to positional covariation of stems, the phylogeny recovered with this method resulted in relationships that are more congruent with nonmolecular data than the phylogenies inferred from alternative alignments.

The ultimate goal of phylogenetic inference is the determination of natural groups (i.e., monophyletic taxa). Such lineages can be recognized because their genetically inherited characters have led to the presence of shared derived features. Can be argued, therefore, that genomic information is the ultimate source of data for phylogenetic inferences. A reasoning like this has supported the development of biochemical techniques for obtaining data for systematic analyses (Barrowclough 1992).

The rapid development of PCR gene amplification and direct DNA sequencing technologies has allowed molecular systematists to accumulate a large amount of data in a relatively short period of time. However, the use of DNA sequences in phylogenetic reconstruction presents a series of problems. Not all genes are equally useful to address systematic questions at different levels in the taxonomic hierarchy. We must ponder whether the DNA sequences contain variation appropriate for the question posed (e.g., Smith and Patton 1991, Moritz et al. 1992, Edwards et al. 1991, Irving et al. 1991, Graybeal 1993, Avise et al. 1994), whether the compared sequences have a clear origin and do not represent pseudogenes or translocated fragments (e.g., Quinn 1992, Smith et al. 1992, Kornegay et al. 1993), and whether different genome partitions should be analyzed in combination or independently (e.g., Bull et al. 1993).

One of the most significant and neglected parts of any phylogenetic analysis is the use of a reasonable criterion for inferring primary homology

(*sensu* de Pinna 1991) especially in non-protein coding DNA sequences. One characteristic of DNA is that every nucleotide position there are four possible character states, which increases the difficulty in inferring homology among sequences. Fitch (1970) has defined two processes that can yield "homologous" sequences. If the putative homologous sequences can be traced back to a speciation event, the process is called orthology (i.e., real homology). In contrast, if they are traced back to a gene duplication event, the process is called paralogy. In addition to this, insertion and deletion events may confuse the positional homology of the individual nucleotide sites or amino acid positions. One way to overcome this last problem is the use of multiple sequence alignment software (e.g., Wheeler and Gladstein 1992, Thompson et al. 1994). These programs are based on algorithms that attempt to maximize the number of nucleotide matches between sequences using *a priori* gap and nucleotide transformation costs. The sequences are aligned by adding gaps, and the final alignment is the one that identifies the minimum possible cost.

Although, such procedures are intuitively appealing, they present several disadvantages. Most of the algorithms used are sensitive to the input order of the taxa, which can influence the nature of the homology hypotheses (Lake 1991). For some data sets the alignment programs can generate more than one alignment with the same lowest cost. In addition, even slight variations in the alignment parameters can yield alternative alignments.

Possibly the most disturbing problem of all, and the most difficult to resolve satisfactorily, is that the alignment parameters are determined by subjective criteria without regard to any biological or functional characteristic of the molecule. Furthermore, in some cases the final parameters are selected because the resultant multiple alignment recovers a phylogenetic hypothesis that agrees with preconceived ideas about the relationships of the taxa involved in the analysis.

Gatesy et al. (1993) have proposed a method for handling multiple minimum cost sequence alignments in which a consensus final alignment is obtained by discarding the alignment-ambiguous nucleotide sites. However, this method also does not take into account biological and functional features of the genes. Hickson et al. (1996) reported that results were significantly improved when higher-order structural information for the third domain of animal 12S ribosomal RNA (rRNA) was incorporated into phylogenetic analysis.

In the present study, I will present for the first time a model for the secondary structure of the complete mithochondrial 12S rRNA for non-passerine birds. This model will be used to infer the primary homology of the nucleotide positions and to assemble a multiple alignment for the sequences of non-passerine birds. This alignment will be used to recover the phylogenetic relationships among the genera of Trogoniformes, and the results will be compared with phylogenies inferred from alignments found

using multiple alignment programs.

Materials and Methods

Taxon sampling. A total of 56 sequences from non-passerine species were used to infer the secondary structure of the 12S rRNA molecule,. The taxa included the following 14 orders of non-passerine birds, Anseriformes (two species), Apodiformes (two species), Caprimulgiformes (three species), Coliiformes (three species), Coraciiformes (nine species), Cuculiformes (three species), Galliformes (one species), Gruiformes (one species), Piciformes (three species), Psittaciformes (four species), Rheiformes (one species), Strigiformes (three species), Tinamiformes (one species), and Trogoniformes (twenty species). Tissue samples were obtained from the following institutions: Genetic Resources Collection, Academy of Natural Sciences of Philadelphia; Institute of Zoology, University of Copenhagen in Denmark; Bird Division, Field Museum of Natural History; Department of Ornithology, American Museum of Natural History; Museum of Natural Science, Louisiana State University; Bird Collection, Department of Ecology and Evolutionary Biology, University of Arizona; Museo de Zoología, Facultad de Ciencias Universidad Nacional Autónoma de México; Zoological Reference Collection, National University of Singapore; and the Jurong Bird Park, Singapore.

DNA sequence collection. The chicken sequence (*Gallus gallus*) was

taken from Desjardins and Morais (1990), while sequences for six other species, the Common Mallard (*Anas platyrhynchos*), the Trumpeter Swan (*Cygnus buccinator*), the Greater Rhea (*Rhea americana*), the Undulated Tinamou (*Crypturellus undulatus*), the Common Nighthawk (*Chordeiles minor*) and the Eurasian Coot (*Fulica atra*) were taken from Mindell et al. (1996). The remaining 49 taxa used for this study (Table 1) were sequenced in our laboratory. Genomic DNA was extracted from frozen tissue (muscle, liver and heart) using a Chelex 5% solution w/v following the method suggested by Singer-Sam et al. (1989). To minimize the possible risk of amplifying translocated nuclear copies, the 12S rRNA gene was isolated and amplified as a single fragment using specifically designed PCR primers. The LPhe (L1243) 5'-CAAACAAAGCATGGCACTGAAG-3', and 12Sd (H1883) 5'-TTCGATTATAGAACAGGCTCCTC-3' oligonucletides, were designed in our lab (numbers follow the chicken mitochondrial genome [Desjardins and Morais 1990]). Other primers used have been described elsewhere (Knight and Mindell 1993). PCR amplification was conducted in Peltier-effect thermocyclers (MJ Research) following the parameters and conditions suggested by Nunn et al. (1996). All PCR experiments were conducted with positive and negative controls to test for contamination. Successful reactions were purified to eliminate PCR primers, dNTPs, enzyme, and buffer components, using the GeneClean II kit (BIO 101 Inc.). Purified PCR products were subjected to cycle sequencing using the ABI Prism Dye Terminator Cycle

Sequencing Ready Reaction kit with AmpliTaq DNA Polymerase FS, on the GeneAmp PCR System 9600 (Perkin Elmer). Sequencing products were subjected to 4% polyacrylamide denaturing gel electrophoresis in the ABI Prism 377 DNA Sequencer (Perkin Elmer). For all the taxa, both strands were sequenced to guarantee accuracy. All sequences have been deposited in GenBank under the following inclusive accession numbers: U89205-U89239 and U94810-U94823.

Inferring the 12S rRNA model. Comparative sequence analysis is probably the most common method used to infer higher-order structural models (e.g., Fox and Woese 1975, Glotz et al. 1981, Gorski et al. 1987, Gutell and Woese 1990, Hickson et al. 1996, Springer and Douzery 1996, Houde et al. 1997, Mindell et al. 1997). Five structural features in the rRNA molecule were recognized: a) stems, which are regions of complementary base pairing; b) bulges, which are unpaired bases within a stem; c) internal loops, which are unpaired regions of nucleotides between stems; d) terminal loops, which are unpaired regions of nucleotides delimited by the proximal and distal complementary strands of only one stem; and e) helices, which are a group of stems, internal and terminal loops, delimited by unpaired regions. To identify these structural features a preliminary multiple alignment of the 12S rRNA sequences was performed using the program MALIGN (Wheeler and Gladstein 1992). Putative helices, stems, and loops were delimited using as a template the proposed secondary structure for the mammalian 12S rRNA

molecule (Springer and Douzery 1996). Final structures were delimited manually according to the following criteria. Stems were formed by conventional Watson-Crick base pairs, and the only non-canonical pairing accepted was between G-U when they were located in the interior of the stem. Stems were delimited by bilateral unpaired regions of more than two base pairs. Compensatory base changes were considered as further evidence for accepting putative stems. Following Zuker (1989), terminal loops were formed by at least three nucleotides to maintain structural consistency in the contiguous stem. Finally, only those structures present in at least 75% of the taxa were considered to be part of the general model for the secondary structure of non-passerine 12S rRNA.

Alignments. Six sets of multiple alignments were compared to evaluate the potential usefulness of higher-order structures in rRNA as an objective criterion for establishing temporary homology before phylogenetic analysis. The first three multiple alignments were assembled with the parsimony-based alignment program MALIGN (Wheeler and Gladstein 1992). The only difference among the alignments was the assigned gap penalty value (i.e., 1, 2, and 4). The nucleotide substitution cost was maintained constant at one, without assigning differential weights to transition or transversion changes. Insertions and deletions were treated as evolutionarily independent events. The remaining alignment parameters were: score 4, build, alignswap, treeswap, keepaligns 100, keeptrees 100, and iter. The fourth

multiple alignment was constructed by removing alignment-ambiguous positions. These nucleotides were identified by contrasting the three previous minimum cost alignments and by following the suggested procedure of Gatesy et al. (1993). The fifth multiple alignment was built with the multiple sequence alignment program CLUSTAL W (Thompson et al. 1994). Pairwise alignment parameters used were: gap penalty 2, K-tuple 1, number of top diagonals 10, and window size 10. For multiple alignments the parameters used were fixed gap penalty 10, floating gap penalty 10, and toggle transitions (DNA) unweighted. The sixth and final multiple alignment was created using the secondary structure model to establish primary homology regions among the sequences. The alignment-ambiguous regions as indicated in the model (Fig. 1) were removed during phylogenetic analysis.

Phylogenetic analysis. Phylogenetic hypotheses were recovered by maximum parsimony conducted with the program Phylogenetic Analysis Using Parsimony (PAUP 3.1.1; Swofford 1993). The large number of taxa included in this study did not allow the use of exact searching algorithms, therefore, parsimony analyses were performed using heuristic searches. One hundred replicate searches with random addition of taxa were performed to eliminate input order bias. During all analyses, character polarization was established by outgroup comparison, and nucleotide transformations were considered unordered. Branch length was optimized using delayed

transformation (DELTRAN) which favors parallelisms over reversals. Branch swapping was done using the tree bisection reconnection algorithm (TBR). In those cases in which the solution included multiple equally parsimonious trees, the signal was identified using strict consensus trees. Retention and consistency indexes were computed to evaluate the level of homoplasy in the most parsimonious tree. Five hundred bootstrap replications (Felsenstein 1985), were performed to evaluate relative strength for the nodes in each phylogenetic hypothesis.

RESULTS

The 12S rRNA gene. The 3' terminal primer used was the 12Sb primer of Knight and Mindell (1993), which anneals inside the gene itself, thus all the sequences obtained lack the terminal of 22 nucleotides. Judging from previously published sequences this region is highly conservative and the absence of this small fragment should not have a significant effect on the results. The most variable regions in this gene are characterized by multiple insertions and deletions, producing a difference of 54 nucleotides between the shortest and the longest sequence. On average, the sequenced fragment has 938 nucleotides, ranging from 915 in *Aulacorhynchus prasinus* (Emerald Toucanet), to 969 in *Apaloderma narina* (Narina Trogon).

The nucleotide and base compositional bias (Prager and Wilson 1988) for the 49 taxasequenced in our laboratory is presented in Table 1. Compared

to other organisms, especially invertebrates (e.g., Vawter and Brown 1993), the nucleotide composition in the 12S gene is relatively uniform across non-passerine birds. A decreased level of variation in nucleotide composition has also been reported for mammals (Springer and Douzery 1996). Martin (1995) concluded that one of the factors that most influences base composition is changes in metabolic rate. Therefore some bias is highly expected among homoiotherms. The average base compositional bias (C) among the non-passerine 12S sequences was 0.137. The lowest nucleotide bias was registered for *Trogon violaceus* (C=0.098), whereas two species of parrots (*Psittrichas fulgidus* and *Micropsitta finschii*) showed the highest bias (C=0.204). Adenine was more abundant than any of the other nucleotides with an average of 300 (32%), followed by cytosine with 265 (28.3%), thymine with 191 (20.4%), and finally guanine with 181 (19.3%). Gutell et al. (1985) have suggested that an over abundance of adenine promotes hydrophobic interactions with proteins because adenine is the least polar of the four nucleotides.

The relative GC proportion ranged from 43.6% in the Speckled Mousebird (*Colius striatus*) to 51.3% in the Carmine Bee-eater (*Merops nubicus*). The values registered for the overall GC content of each sequence (Table 1) falls within the range of other known vertebrate 12S rRNA genes (e.g., Vawter and Brown 1993, Gutell 1994, Springer and Douzery 1996). Comparing structural regions, stems show a higher GC proportion (53.3%)

than loops (40.6%). Turner et al. (1988) have computed that the G-C bonds have a lower free energy value than do A-U or G-U pairs, therefore the high GC% in paired regions helps to maintain the structural cohesion of stems.

A model for the secondary structure of the 12S rRNA molecule. The model inferred from the 12S sequences of non-passerine birds displays the four domains (helices) typical of previously proposed models of small sub-unit (16S-like) molecules (Neef et al. 1990). Among the small structural elements, a total of 40 stems, 20 internal loops, and 17 terminal loops were recognized (Fig. 2). The first helix is formed by the 5' terminal sequence and the first 14 stems, the second helix encompasses stems 15 to 23, stems 24 to 36 constitute the third helix, and finally helix four includes stems 37 to 40 plus the 3' terminal sequence. A central core unpaired region unites the three first helices, while the fourth helix is linked by four unpaired nucleotides to the distal strand of stem 24 in helix three.

As with other slowly evolving genes, the 12S rRNA is characterized by highly conserved regions. These invariable fragments are not restricted to individual stems or loops, but rather usually include several of these structural elements (Fig. 1). Among the largest conserved regions observed throughout the non-passerine sequences are 24 contiguous nucleotides formed by the last eight bases of the distal strand of stem 24, the unpaired region between stem 24 and 37, the proximal strand of stem 37, the proximal strand of the internal loop between stem 37 and 38, and the first four bases of

the proximal strand of stem 38. A second conserved regions (23 bases long) encompasses the entire stem 21, the terminal loop at stem 21, the distal strand of the internal loop between stems 21 and 20, and the first three bases of the distal strand of stem 20. A third invariant region of over twenty nucleotides is formed by the last two bases of the distal strand of the internal loop between stems 38 and 37, the distal strand of stem 37, the unpaired region between stems 37 and 40, and at least the first eight bases of the proximal strand of stem 40. Many other conserved motifs have been recorded in a large range of taxa including both vertebrates and invertebrates. Dahlberg (1989) identified 23 bases that are involved in the formation of the decoding site (i.e., the entry site for the tRNA and the peptidyl site). All these positions are easily recognized in the non-passerine sequences (Fig 1). Although these bases are scattered throughout the primary structure of the 12S rRNA, once the gene is folded into a three dimensional structure the nucleotides come together (Springer and Douzery 1996).

In contrast to conserved motifs, the 12S rRNA gene of non-passerines has at least eight hyper-variable regions (Fig. 1). These regions are typified by multiple nucleotide insertions that make it impossible to identify common paired regions throughout the taxa. Five of the hyper-variable regions are located at terminal loops (at stems 8, 10, 22, 33, and 39), while the remaining three are situated within internal loops (the unpaired regions between stems 4 and 13, the first half of the distal strand between stems 17 and 16, and the

first half of the unpaired region between stems 34 and 35).

Other models that have been proposed for the 12S rRNA of other organisms present slight differences in the number of stems and length of the common stems. Some of these differences, especially compared to the model proposed for mammals (Springer and Douzery 1996), are summarized below.

The first stem has a length of five nucleotides, with the third base pair forming a non-canonical A-C bond (Fig 3a). Apparently A-C bonds are relatively frequent among avian species (Hickson et al. 1996). The proximal strand has a bulge after the non canonical bond. For most taxa the bulge is formed by one cytosine, and only in the ratites (*Rhea americana* and *Crypturellus undulatus*) and in the Trumpeter Swan (Mindell et al. 1996) the bulge is constituted by adenine.

In the general model proposed for mammalian 12S (Springer and Douzery 1996) the third stem is nine bases long. In contrast, the model for non-passerine birds suggests a length of ten nucleotides for stem 3 (Fig. 3a). For all of the bird species, the extra base-pair is a conventional Watson-Crick uracil and adenine. Although there is some variability in this stem, most of the changes in the proximal strand are compensated by changes in the complementary nucleotides in the distal strand.

Stem 5 is eight bases in length, two bases longer than in mammals. The addition of these extra bases is supported by compensatory changes. The only exception observed so far is in the three species of Piciformes

(*Aulacorhynchus prasinus*, *Sphyrapicus varius* and *Melanerpes carolinus*) in which this stem is seven bases long. The proximal strand of stem 5 shows two bulges formed by adenine in all the bird species (Fig 3a).

Among invertebrates, and especially in bacteria, there is an extra stem between the proximal strands of stems 5 and 6 (van de Peer et al. 1994). In all the non-passerine sequences it is possible to postulate a one-base pair bond between guanine and cytosine (Fig. 3a). A one-base pair bond is relatively simple to find within any unpaired region, therefore I have omitted this stem from the model. It should be mention that a non-canonical bond (U-G) can be postulated in birds which would support the existence of a small stem at this position.

For almost all the bird sequences, stem 8 is three base-pairs long. The exceptions are four species of trogons (*Pharomachrus antisianus*, *P. auriceps*, *P. pavoninus*, and *Euptilotis neoxenus*). In these birds the middle base in the distal strand is lost, leaving a stem of two base-pairs with a cytosine bulge on the proximal strand.

Van de Peer et al. (1994) also acknowledged the existence of a large stem adjacent to the distal strand of stem 6. In birds a two base-pair stem can be postulated in this area (Fig. 3a). The small number of nucleotides in this unpaired region, however, is less than the minimal number needed to form a stable terminal loop (Zuker 1989). Therefore, I have also omitted this stem in the general model for non-passerine birds.

Stem 14 for non-passerine birds has a length of seven base pairs (Fig. 3a). In other models, especially those proposed for mammals, this stem is shorter, ranging from 5 to 3 base-pairs (Springer and Douzery 1996).

Helix II has some of the most variable regions of the molecule (Fig. 3b). Apparently most of the positions between stems 16 to 19 have experienced multiple substitutions, making it difficult to hypothesize homologous paired regions among the different taxa. In the internal loop between stems 16 and 17 it is possible to construct at least one stem from two to five bases, but, instead of observing compensatory changes among the species the position of the stem is shifted. For this reason the general model shows a big internal loop in this region. A similar problem exists in the internal loop between stems 17 and 18. In this structure a stem of at least three base pairs can be built for nearly 50% of the taxa, wheras in the remaining birds the position of the stem is shifted. A stem in this area has been omitted because the common structure that has been identified in some of the species fails to meet the criteria established in this study.

Other minor differences observed in helix II are: a) stem 18 has four standard Watson-Crick bonds plus an unpaired middle region that can vary from one to two nucleotides; b) stem 19 varies from two to five base pairs.

Several models have identified a stem in the unpaired region between the proximal strands of stems 28 and 29 (Neefs et al. 1990, Hickson et al. 1996). In 73% of the species in this study it is possible to assemble a stem of at least

two bases (Fig 4a). The remaining taxa do not show compensatory changes or positional covariance, consequently I have not included this helix in the core model.

Stems 29 and 30 proposed for the non-passerine birds (Fig. 4a) are considered as one continuous structure in the model for mammals with multiple bulges in both the proximal and distal strands (Springer and Douzery 1996). Hickson et al. (1996) proposed at least eight alternative potential pairings for the nucleotides in this region. The arrangement chosen for the non-passerine birds corresponds to the structure that requires the minimal free energy of those presented by Hickson et al (1996).

Stem 31 in Springer and Douzery (1996) is formed by six nucleotide pairs but the two central bases do not form bonds with their complementary nucleotides. On the other hand, in non-passerines the six nucleotides form standard Watson-Crick unions (Fig. 4a).

Stem 36 in other models has a length of eight nucleotide pairs. In birds a deletion has occurred in the proximal strand, leaving only seven base pairs in this stem with a bulge on the distal strand (Fig 4a).

The terminal loop at stem 39 is highly variable especially in the regions adjacent to the stem, therefore the alignment of this part is extremely difficult. Across the sequences it is possible to find paired regions (Fig 4b), however the hyper-variable region inside the loop creates difficulties for finding a common structure in non-passerines.

Recovered phylogenies. The reconstructed alignments (i.e., using alignment software and the secondary structure model) were analyzed using exactly the same searching parameters on each of the independent parsimony analyses. As expected the alternative alignment methods produced slight differences in the final length of the multiple alignment. The alignment lengths ranged from 1031 to 803 positions after the alignment-ambiguous positions were excluded (Gatesy et al. 1993). All the inferred topologies yield the mousebirds (Coliiformes) to be the sister-group of trogons (Trogoniformes), and the cuckoos (Cuculiformes) to be the most basal clade. Although all the alignments recovered these three orders of non-passerine birds as monophyletic groups, alternative topologies showed differences in the generic interrelationships among the taxa, especially within the trogons.

The multiple alignment assembled with the program MALIGN, assuming an internal gap value of one, recovered four equally parsimonious trees of 1423 steps, with a consistency index (CI) 0.427, and a retention index (RI) 0.576. The strict consensus tree (Fig. 5a) yields the New World trogons as a monophyletic group, but relationships among the genera are not resolved. The sister-taxon of the New World trogons was the Asian trogons, and the African clade was at the base of the Trogoniformes.

When the alignment constructed with an internal gap penalty of two was used for phylogenetic analysis the result was a single most parsimonious tree (Fig. 5b). The tree had a length of 1473 steps, CI of 0.439, and RI of 0.586.

In contrast to the previous analysis, the New World trogons in this tree were paraphyletic. The sister-group to the genus *Trogon* was three species of African trogons (*Harpactes*), the sister-taxon to this clade was the Cuban Trogon (*Priotelus temnurus*), sister to them were the two African trogons (*Apaloderma*), and at the base of the trogons clade were the Quetzals (genera *Pharomachrus* and *Euptilotis*).

The last multiple alignment built with MALIGN (assuming an internal gap penalty of four) produced four equally parsimonius trees of length 1640, CI of 0.441, and RI of 0.586. The differences among these four trees involve only the relationships among the five species in the genus *Trogon* (Fig. 5c). The generic interrelationships recovered are exactly the same as those obtained in the previous analysis .

The next phylogenetic analysis was performed using the multiple alignment constructed with the program Clustal W. Figure 5d represents the strict consensus tree of two equally parsimonious trees of length 1529 steps, CI of 0.452, and RI of 0.563. As in the two previous analyses, the New World trogons were recovered as a paraphyletic group. The trogoniforms were divided into two large clades. One was formed exclusively by the species of the genus *Trogon*. The second group included Quetzals, Asian trogons, and a third subclade that encompassed the African trogons and the Cuban Trogon, but relationships among them were unresolved. Other alignment parameters were used to construct multiple alignments with the program

Clustal W. The parsimony analyses performed with these alignments recovered alternative conflicting trees (phylogenies not shown).

Alignment-ambiguous positions were determined by comparing the three minimal cost alignments assembled with MALIGN. Using this procedure a total of 226 characters were excluded leaving only the sites that were alignment invariant. A parsimony analysis based on the resultant matrix recovered four equally parsimonious trees (808 steps, CI 0.46, RI 0.622). In the strict consensus tree the relationships among the major groups were unresolved. Although the genera were recovered as monophyletic, the only resolved nodes at this level were the African trogons as the sister-group of the Cuban Trogon, and the Eared Trogon (*Euptilotis*) as the sister-taxon of *Pharomachrus* (Fig 6a).

Finally, after the multiple alignment based on the secondary structure model was subjected to phylogenetic analysis and it resulted in a single most parsimonious tree of 1444 steps in length, CI 0.468, and RI 0.601 (Fig. 6b). In this tree, African, Asian, and New World trogons were recovered as monophyletic groups. The sister-taxon to the genus *Trogon* was the Cuban Trogon, while their sister-group was the Neotropical Quetzals. The Asian trogons were the sister-clade to the New World trogons, and at the base of all was the African clade (*Apaloderma*).

Discussion

Assumptions of homology are critical to cladistic inference, thus when undertaking an analysis of molecular sequences lacking a reading frame, as much attention should be given to the construction of the multiple alignment as is given to the phylogenetic analysis itself. In this study, I applied some of the most commonly available methods for building multiple alignments and used these alignments to recover cladistic hypotheses. Finding unbiased criteria to evaluate the results is not a simple task. No matter how different any group of nucleotide sequences may be, it is always possible to align them. During the alignment process gaps are inserted in the sequences in an attempt to optimize some *a priori* criterion (e.g., maximize base matches, minimize cost, etc). In the end, the alternative alignments differ primarily in the number of phylogenetically informative sites. Tree descriptive measurements such as length, consistency index, or retention index are dependent on the number of characters, therefore a simple comparison of such values does not help assess the accuracy of the alignment method.

Perhaps the best way to evaluate the performance of the alternative alignments is to compare the relationships recovered with those alignments. All the different methods in this study produced alignments that recovered the different orders of birds as monophyletic groups. Furthermore, all the analyses were consistent in recovering the different genera of trogons as

natural groups. The major discrepancy among the trees was that in some of them the New World genera of trogons (i.e., *Trogon, Priotelus, Euptilotis,* and *Pharomachrus*) formed a monophyletic clade, while in others this assemblage was recovered as being paraphyletic. At this time, no rigorous cladistic analysis has been published about the phylogenetic relationships of trogons, consequently there is no solid evidence to support a monophyletic origin for the New World trogons. On the other hand, Clark (1918) found a series of morphological characters that apparently support a close relationship among the New World genera, and Evans (1899) reported many similarities in feather structures among these genera. Assuming that the New World trogons are monophyletic, the only alignments that were able to identify the "correct" relationships were those constructed with the secondary structure model (Fig. 6b) and with the alignment built using MALIGN assuming an internal gap penalty of one (Fig. 5a).

Apparently, all the different alignment methods that were compared in this study have shown some inconsistency in inferring primary homology in the sequences. All alignment software packages are extremely sensitive to closely positioned repetitive sequences (i.e., tandems), especially when one of them is more variable that the other. Alignment algorithms, working on pairwise comparisons, are also affected by order of taxon input. Removing alignment-ambiguous positions has two major problems: (a) applying all the combinations of alignment parameters to detect alignment-ambiguous

positions is not possible, and (b) the more alignments that are compared the more positions are removed, and in extreme cases not enough phylogenetically informative characters remain to address the phylogenetic questions. Also, as noted by Gatesy et al. (1993), excluding alignment-ambiguous sites does not necessarily guarantee the complete elimination of phylogenetic noise in the data.

Positional covariation and compensatory mutations are the two processes that maintain paired regions in the rRNA molecule. If the alignment is based on the secondary structure the positional homology of nucleotides may be easily misidentified when positional covariation occurs. Sometimes, when the sequences belong to closely related taxa, the alignment of hyper-variable regions can be achieved with some confidence using the secondary structure model. On the other hand, if the sequences are too divergent the alignment of these regions may be highly subjective.

The use of features derived from the secondary structure model for the 12S rRNA definitely facilitates the process of aligning multiple sequences. Alignments assembled throughout this procedure have objectivity in that primary inference of nucleotide homology is based on structural characteristics of the molecule, instead of on some ambiguous assumption for assigning penalty values. Although higher-order structural models are unavailable for some groups of organisms there are standard methodologies to infer them. Comparative sequence analysis is perhaps the most accessible

way to deduce a core model for the secondary structure of rRNAs. The first step is to align and compare a few closely related sequences to identify complementary frames that can imply the existence of paired regions. The presence of compensatory changes will provide further evidence to corroborate the validity of putative stems. Once this is done, more divergent sequences can be added to refine the different structural features of the molecule.

Improving the alignment of sequences becomes a relatively easy operation once a structural model is available. After comparing multiple sequences it becomes clear that the positional homology of some stems is not corroborated (Fig. 3 and 4). Another important consideration is the alignment-ambiguous nucleotide sites, nevertheless these problems are easier to examine and described with reference to structural models. The use of highly conservative regions for identifying the boundaries of variable frames is the first step for constructing an alignment based on structural information. After this is done, the small variable frames can be easily aligned using any computer program. In this study only the longest conservative motives for non-passerine birds have been presented. A more complete list of invariable structures for the 12S rRNA of vertebrates can be found in Kjer (1995), along with a detailed set of instructions for applying such structural information to raw data for recovering multiple alignments.

A detailed analysis of the model for the secondary structure of the 12S

rRNA in non-passerine birds has shown that nucleotide variation is widespread within the molecule, and it is not restricted to specific structures or regions. Consequently, applying a weighting scheme as broad as loop vs. stems may be inadequate for phylogenetic analysis. One of the assumptions of most phylogenetic reconstruction procedures is that characters are independent. Clearly, however, paired regions in the 12S molecule violate this assumption. Based on this fact it is important to derive some kind of weighting scheme that can accomodate this problem.

Nonetheless, inference of higher-order structural models for rRNA sequences can help understand the functional role of ribosomes during protein synthesis (Noller 1991). Also, secondary structure models allow us to estimate the rate and patterns of nucleotide substitution among the functional regions of the rRNA molecule (Vawter and Brown 1993). Finally, this study supports the conclusion that alignments based on structural features of the 12S rRNA gene can recover a phylogenetic hypothesis more congruent with morphological evidence than can alignments constructed in the absence of such models.

Table 1. Nucleotide composition, overall GC content, and bias (C) for the 49 non-passerine 12S rRNA genes sequenced.

Taxa	A	C	G	T	GC%	C	Taxa	A	C	G	T	GC%	C
Centropus sinensis	325	251	166	198	44.4	0.150	Colius leucocephalus	315	255	163	199	44.8	0.149
Cuculus fugax	308	243	175	203	45.0	0.124	Colius striatus	328	246	159	195	43.6	0.158
Geococcyx velox	298	267	174	203	46.8	0.133	Colius colius	318	252	166	195	44.9	0.150
Apaloderma narina	307	259	195	208	46.8	0.112	Ceyx erithacus	294	269	179	185	48.3	0.143
Apaloderma vittatum	304	256	188	203	46.7	0.118	Chloroceryle americana	301	275	174	174	48.6	0.165
Harpactes oreskios	303	255	183	198	46.6	0.126	Momotus momota	290	263	186	186	48.5	0.130
Harpactes ardens	306	259	190	202	46.9	0.121	Momotus mexicanus	290	265	190	180	49.2	0.133
Harpactes diardii	317	245	182	212	44.7	0.117	Todus todus	278	283	193	179	51.0	0.135
Priotelus temnurus	293	251	183	200	46.8	0.116	Merops nubicus	279	273	202	172	51.3	0.128
Euptilotis neoxenus	302	247	177	206	45.5	0.119	Coracias caudata	284	285	190	163	48.6	0.156
Pharomachrus antisianus	295	258	179	200	46.9	0.124	Anthracoceros albirostris	273	281	192	182	51.0	0.129
Pharomachrus auriceps	307	258	182	206	46.2	0.124	Upupa epops	282	280	190	174	50.8	0.143
Pharomachrus pavoninus	305	258	186	205	46.5	0.120	Amazona ventralis	297	300	174	151	51.4	0.197
Trogon personatus	295	248	184	204	46.4	0.111	Psittrichas fulgidus	316	303	170	159	49.9	0.204
Trogon curucui	304	245	188	218	45.3	0.100	Micropsitta finschii	317	288	163	159	48.6	0.204
Trogon violaceus	307	241	184	223	44.5	0.098	Pionus senilis	299	290	171	165	49.8	0.182
Trogon viridis	308	255	185	208	46.0	0.119	Amazilia tzacatl	312	259	174	182	46.7	0.155
Trogon comptus	306	251	188	209	46.0	0.112	Chlorostilbon aureoventris	301	269	186	170	49.1	0.154
Trogon melanurus	306	249	186	215	45.5	0.107	Aulacorhynchus prasinus	278	293	182	162	51.9	0.165
Trogon elegans	305	251	191	211	46.1	0.107	Sphyrapicus varius	287	281	175	181	49.3	0.153
Trogon rufus	310	256	190	204	46.5	0.119	Melanerpes carolinus	292	265	173	187	47.8	0.143
Trogon collaris	305	259	186	208	46.5	0.118	Aegolius acadicus	286	280	208	169	51.7	0.134
Trogon mexicanus	309	249	184	216	45.2	0.110	Asio flammeus	291	295	188	170	51.2	0.161
Nyctiphrynus mcleodii	308	266	171	186	47.0	0.155	Micrathene whitneyi	298	295	172	192	48.8	0.160
Caprimulgus vociferus	308	258	175	185	46.8	0.148							
MEAN	300	265	181	191	47.5	0.137							

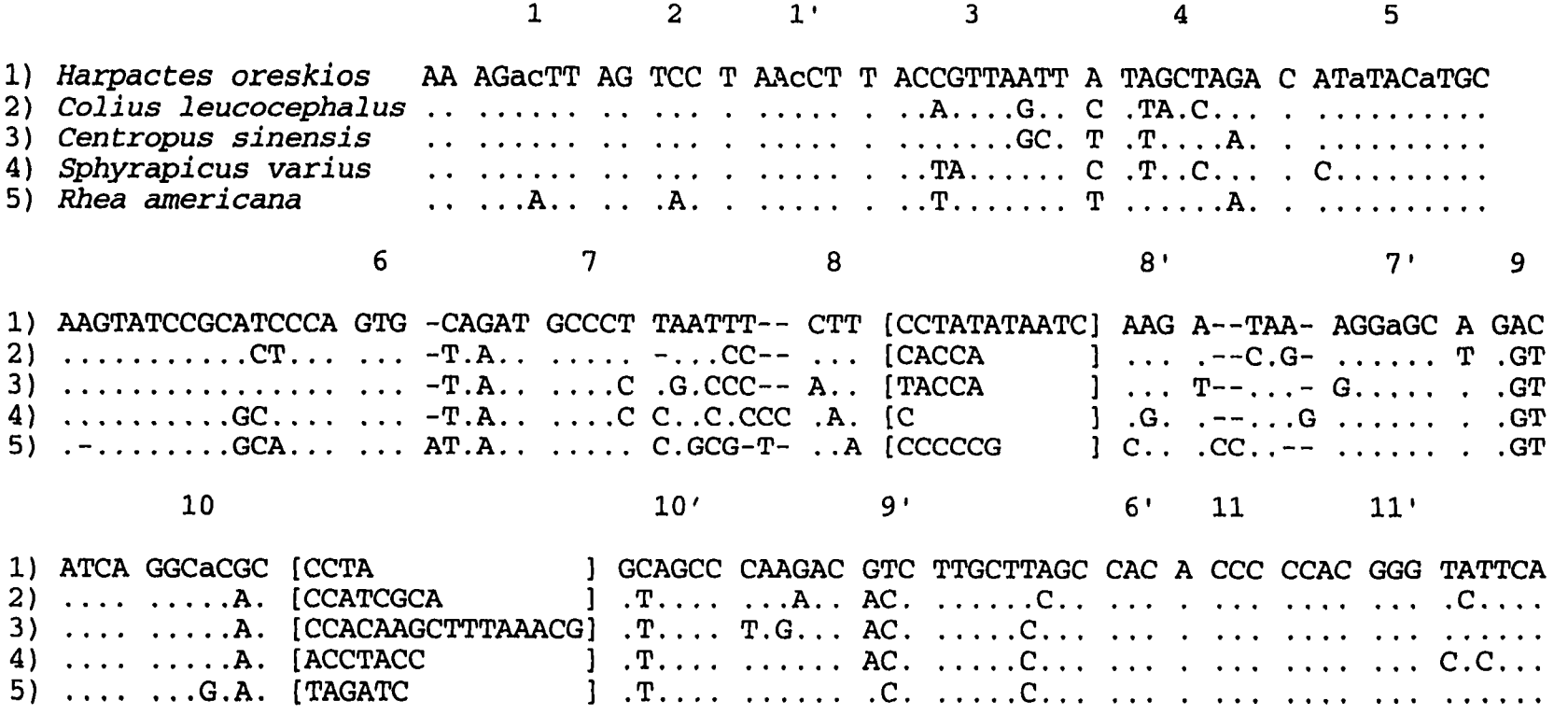

Fig. 1. Multiple alignment for the nucleotide sequences of five non-passerine 12S rRNA genes. Paired regions are indicated by numbers above the sequences, complementary distal sequences are indicated with a prime symbol. Bulges within stems are depicted by lower-cases. Hyper-variable regions are contained in brackets. Bases proposed that form the decoding site (Dahlberg 1989) are indicated by an asterisk. The alignment for all 56 sequences is available from the author.

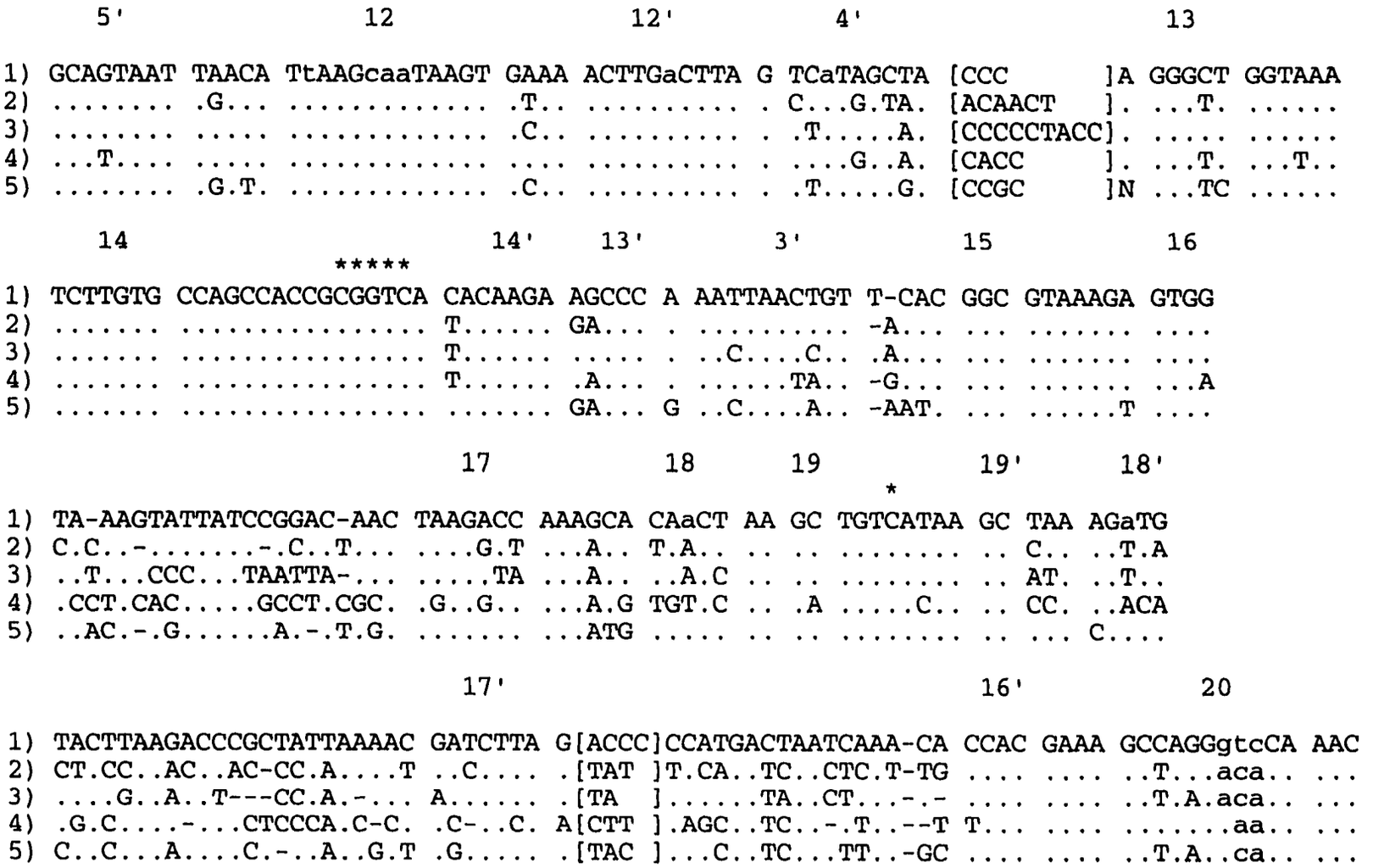

Fig. 1. (continued).

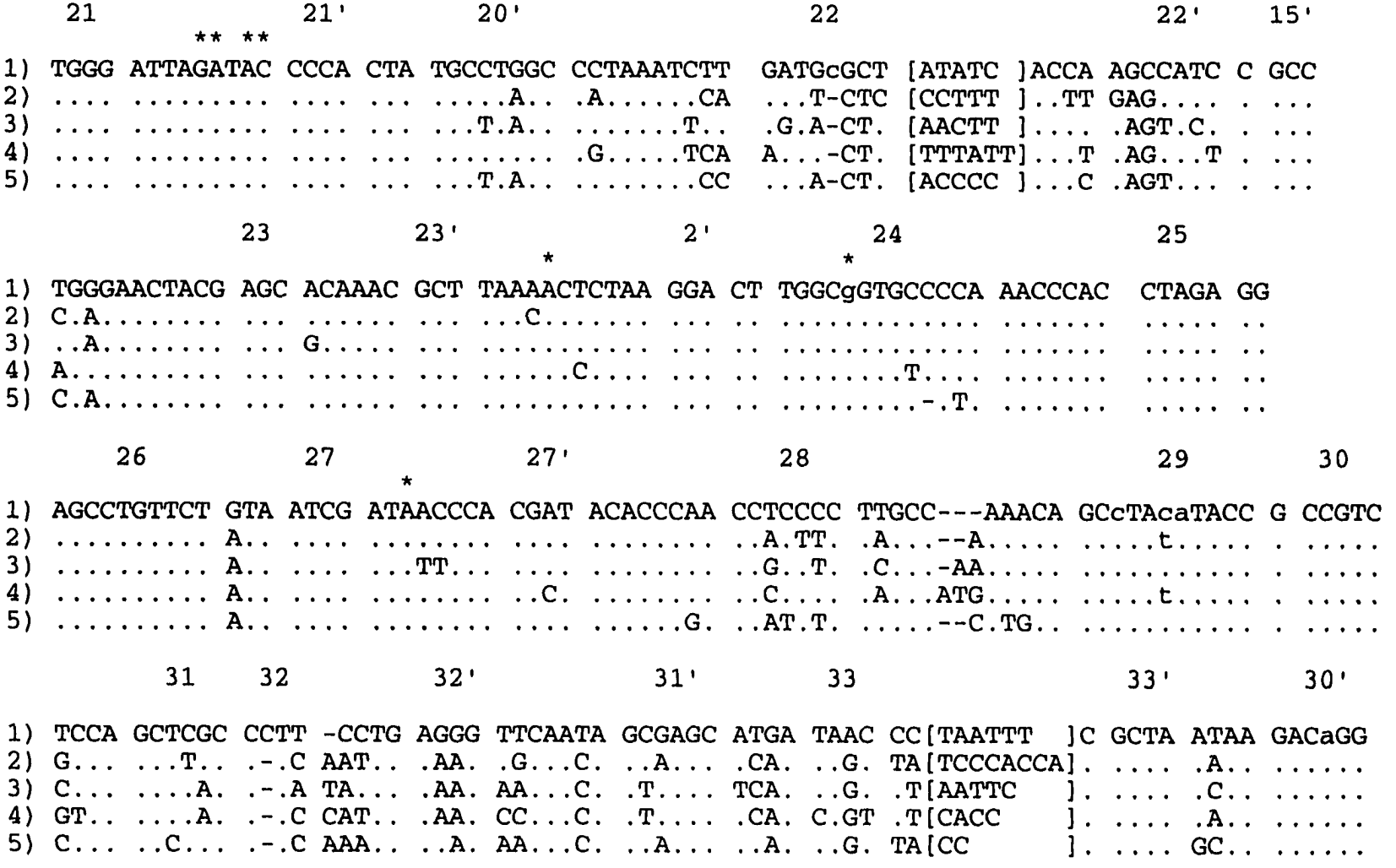

Fig. 1. (continued).

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          29'          28'          26'          34           34'

1) TCAA GGTATAGC TAAT GGGGTGG -A AGaAATGGGCT ACA TTTTCT AA-AGT AGAACA [CTTATTTTAA]AACGAAA
2) .... ........ CC.. .AA.... -. ........... ... ..C... .C-.A. ...GT. [CT        ].......
3) .... ........ .C.. .A..C.. -. ........... ... C.C... ..CCC. ....A. [C         ]-......
4) .... ........ C... ....G.. A. ........... ... ...... G.-CAC ...CA. [C         ].G.....
5) .... ........ AT.. .A.A... -. ........... ... ...... .G-TC. ...... [GC        ]C......

     35             35'                25'          36                    36'
                                      **                                          *
1) AGGGGCG TGAAAT- CGCCCCT AGAAGGCGGA TTTAG CAGTA AAAAGGG A-CAA-TAAAG CTCTcTTT AAGC-CGGCCC
2) .T....A ......- TT..... .......... ..... ..... ....AA. .-...-..G.. ..T.t... ...A-T.....
3) ..A.A.A .....A- A.T.T.. .......... ..... A.... ..G.... .TT..C..... .C...... ..AAC....T.
4) GA....C .....CC T....TC .....A.... ....T ..... ...CAT. .-.T.-.C.T. .CTGtC.. ....-......
5) GA....A .....-- .A...TC C......... ..... ..... ..GT... .-TC.-G.... .C.A.... ....-......

        24'              37         38           39
              *      * *       *
1) TGAGGCACGTaCA TACC GCCCGTC AC CCTCCTC G--CA GGCC [GACACTATAAAAC]ATAGC-TAATACA
2) ..GA......... .... ....... .. ....... A--T. T..T [TCCAATCAATA  ]...AT-...A...
3) ..G.A........ .... ....... .. ....T.. AAAA. A..T [TTACACCCACT  ]...AT-....TT.
4) ..G.A........ .... ....... .. ....... .--T. A..G [AACATTCCCT   ]T.CT.A...A...
5) ..AG......... .... ....... .. ....... A--.. A..T [ACCCTACTAA   ]...AA-......-

               39'          38'          37'           40                40'
                            *     **
1) [CTCACA  ] GGC-C AAA GATGAGG TAA GTCGtAAC AAGG TAAGTGTA?? ???? ?????????? ??????
2) [AATATTCC] A..TA T.. ..A.... C.. ........ .... .........?? ???? ?????????? ??????
3) [TACACC  ] A..-G .G. ....... ... ........ .... .........?? ???? ?????????? ??????
4) [CCAACC  ] A.T-. T.. ....... ... ........ .... .........?? ???? ?????????? ??????
5) [TACTATT ] A..-T ... ....... C.. ........ .... .........CC GGAA GGTGCACTTA GCACAC
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Fig. 1. (continued).

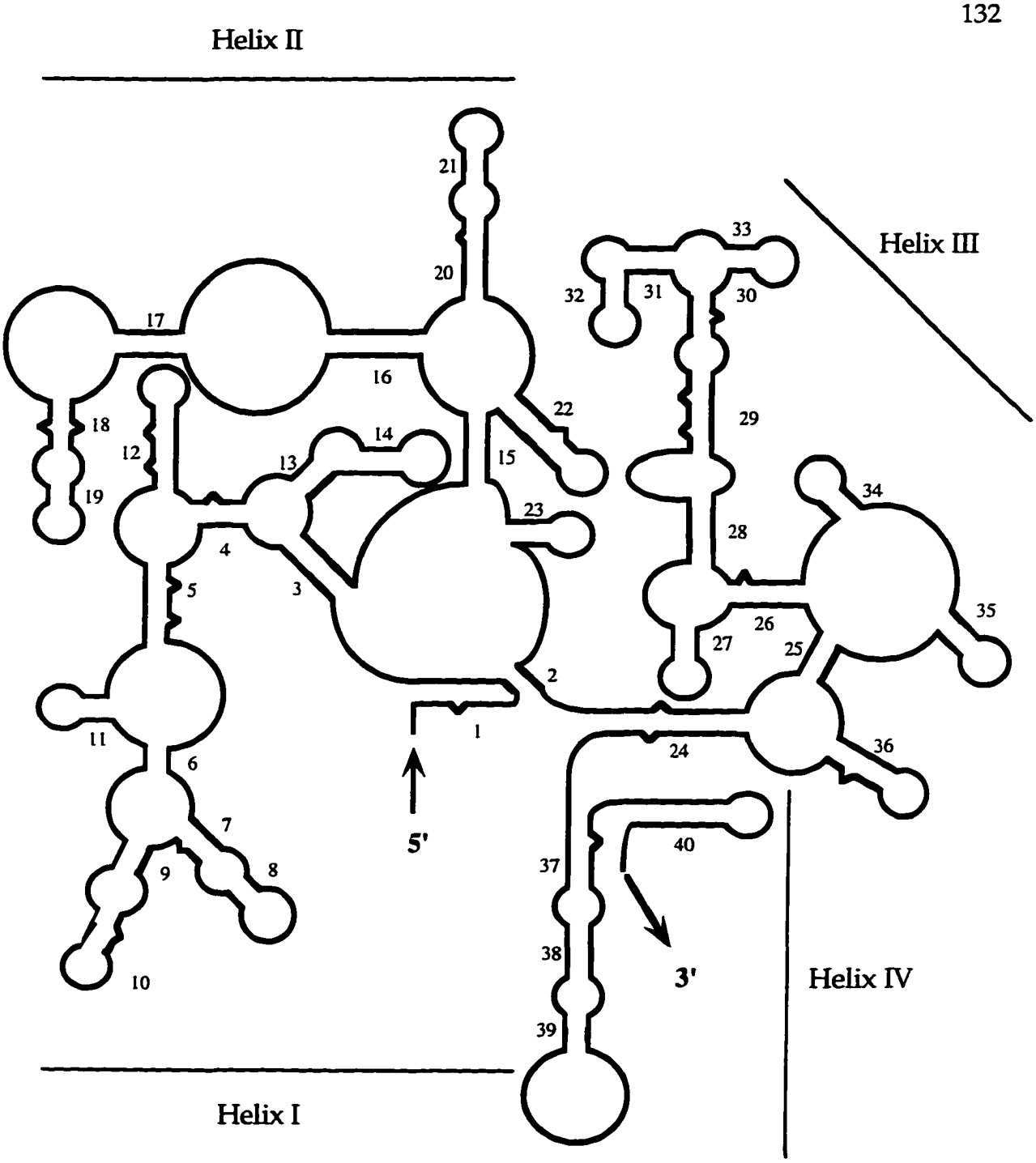


Fig. 2. Model for the secondary structure of 12S rRNA of non-passerine birds. Parallel lines represent stems, circles illustrate loops, and angular shapes in stems are bulges. Stems are numbered in sequential order from the 5′ to 3′ terminal ends. Detailed base pairing is shown in figures 3 and 4.

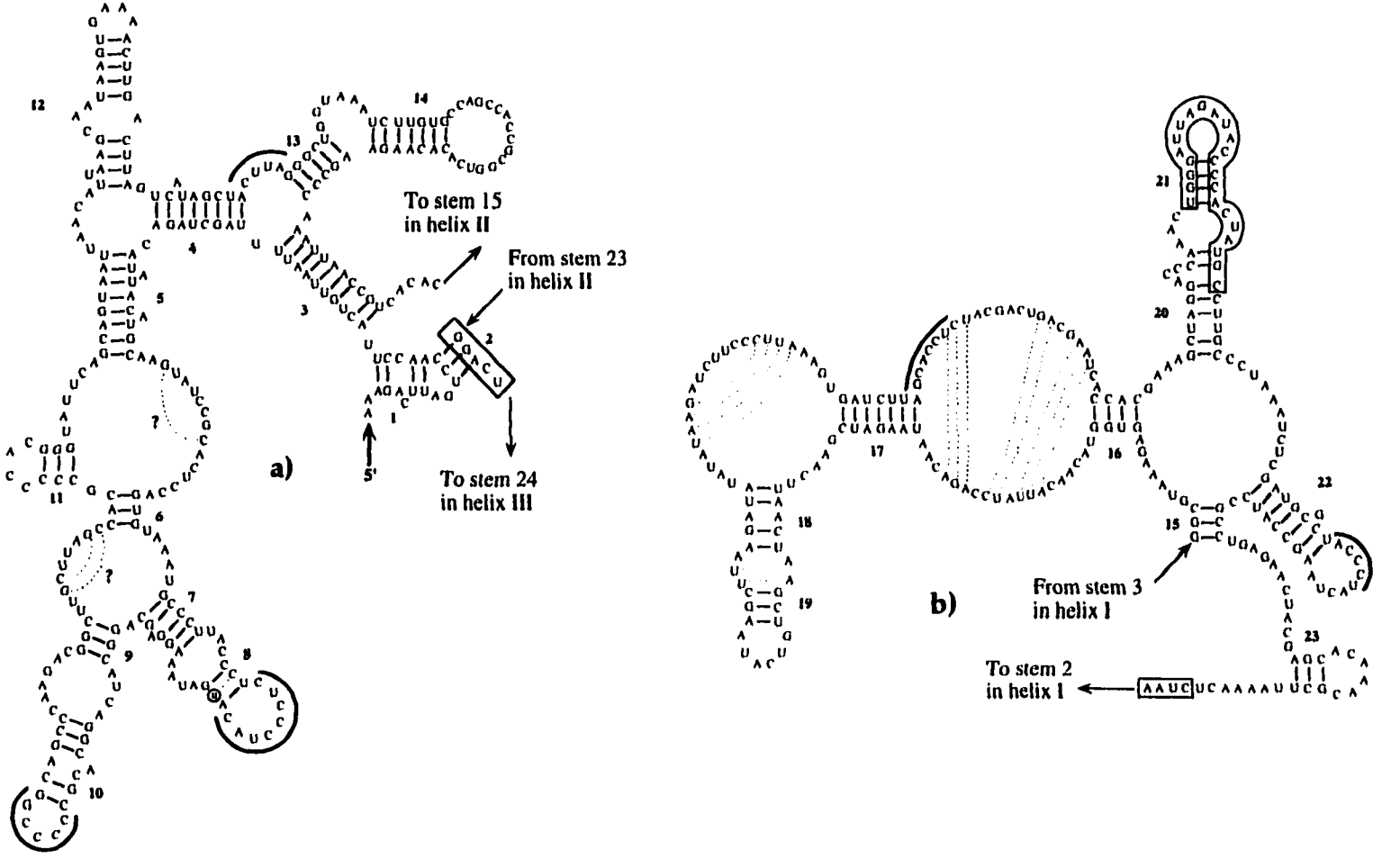


Fig. 3. Secondary structure models for a) Helix I, and b) Helix II of the 12S rRNA of non-passerine birds. The base sequence belongs to the Pavonine Quetzal (*Pharomachrus pavoninus*). Major conserved regions in all the taxa are enclosed in a box. Hyper-variable regions are marked by thick lines along the sequence. Dotted lines show potential paired regions that are not included in the model (see text). Uracil in stem eight was circled to indicate that this particular position is missing in quetzals but present in the remaining taxa.

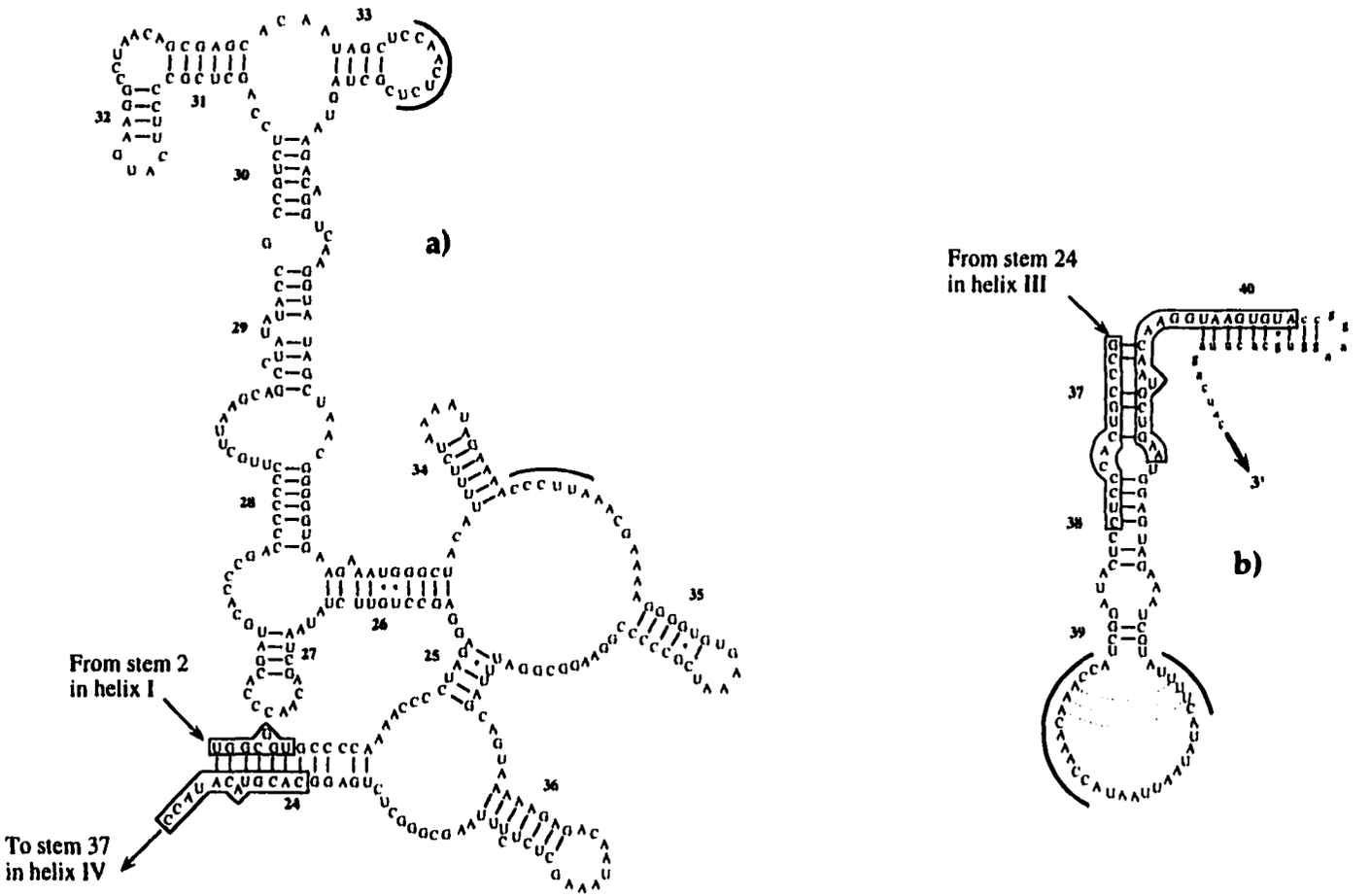


Fig. 4. Secondary structure models for a) Helix III, and b) Helix IV of the 12S rRNA of non-passerine birds. The base sequence belongs to the Pavonine Quetzal (*Pharomachrus pavoninus*). Major conserved regions in all the taxa are enclosed in a box. Hyper-variable regions are marked by thick lines along the sequence. Dotted lines show potential paired regions that are not included in the model (see text). Non-canonical Watson-Crick bonds common to all sequences are marked by an asterisk. To complement the missing fragment in the sequenced taxa (see text) the last 22 nucleotides of the Chicken (Desjardins and Morais 1990) were used in this figure (lower-cases in the 3′ terminal region).

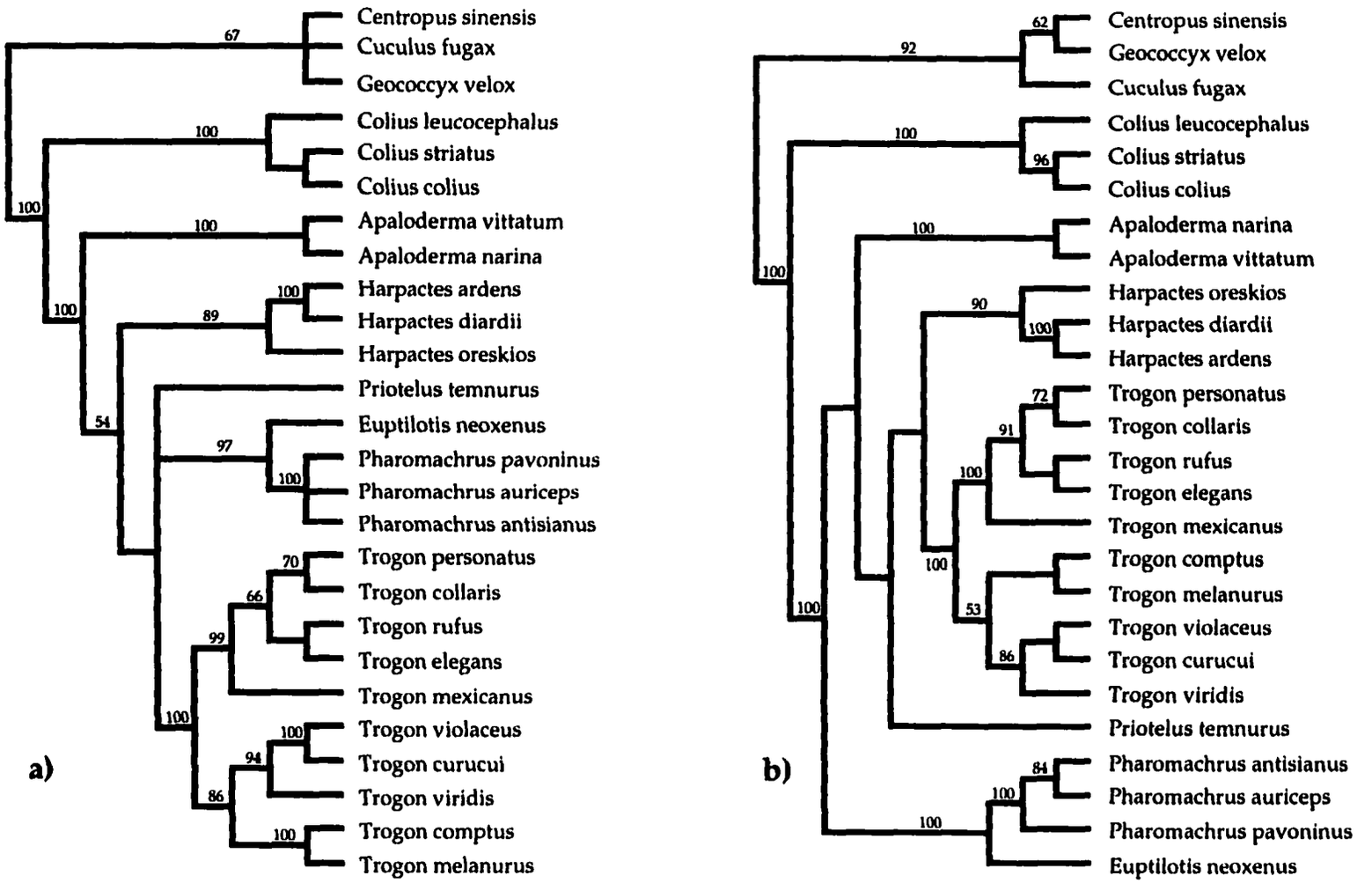
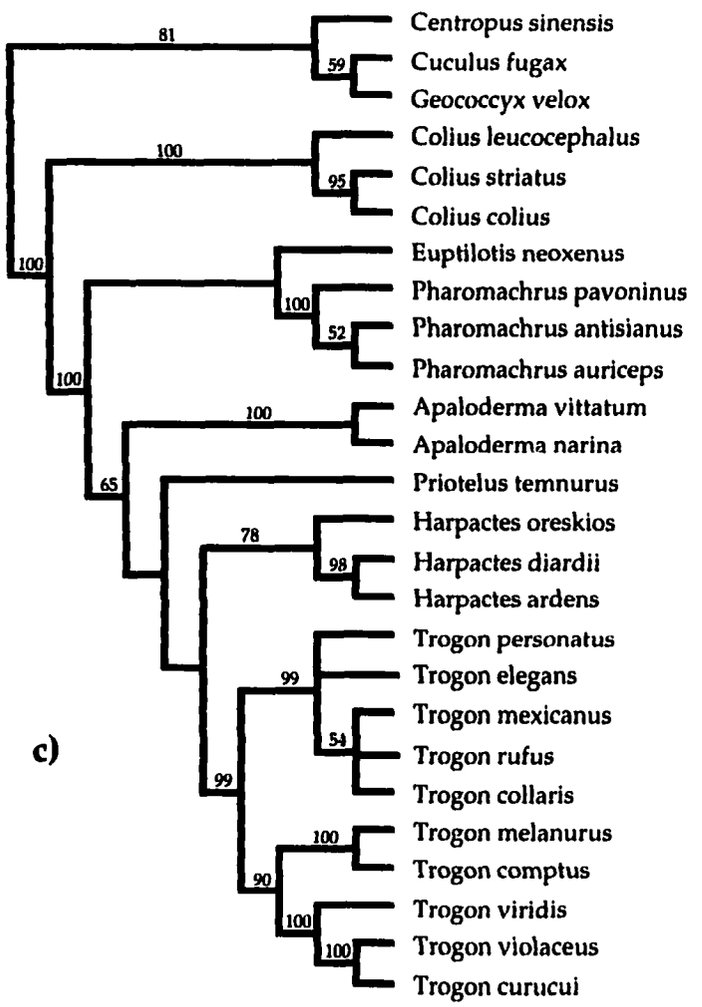


Fig. 5. Phylogenetic trees recovered from multiple alignments built using computer software. Numbers above branches are bootstrap percentages from 500 replications (only values over 50% are shown). Analyses were based on global parsimony, all trees were rooted to cuckoos, and no differential weight was applied to informative characters. a) MALIGN (gap penalty = 1): strict consensus tree of four trees, 1423 steps, CI: 0.427, RI: 0.576. b) MALIGN (gap penalty = 2): single most parsimonious tree 1473 steps, CI 0.439, RI 0.586.



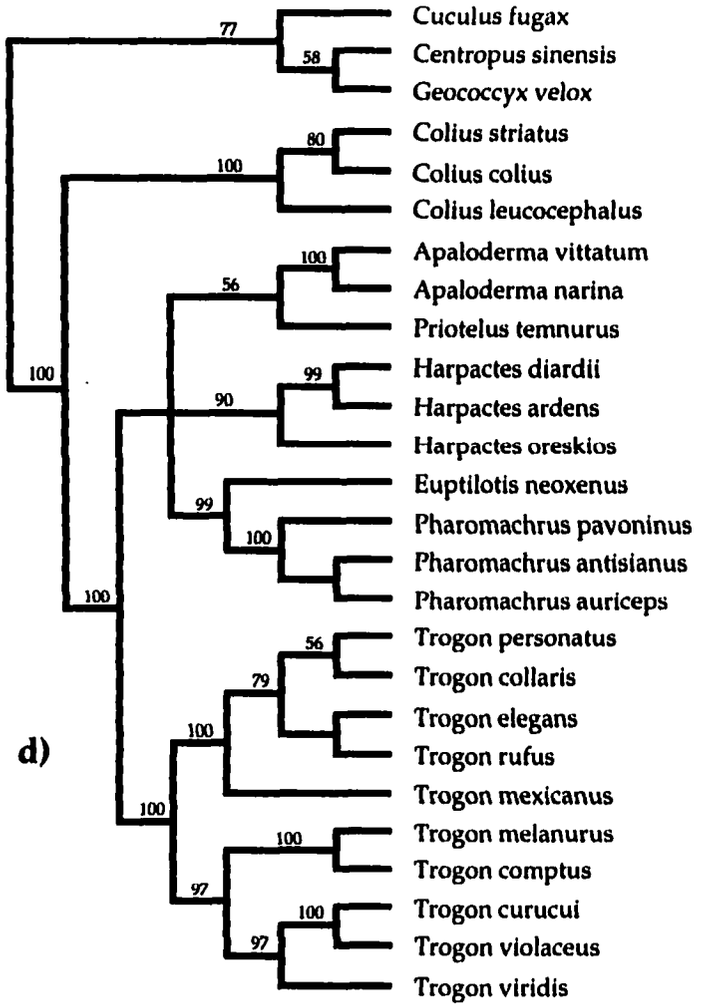


Fig. 5. (continued). Numbers above branches are bootstrap percentages from 500 replications (only values over 50% are shown). Analyses were based on global parsimony, all trees were rooted to cuckoos, and no differential weight was applied to informative characters. c) MALIGN (internal gap penalty = 4): strict consensus tree of four trees, 1640 steps, CI 0.441, RI 0.586. d) Clustal W: strict consensus tree of two trees, 1529 steps, CI 0.452, RI 0.563.

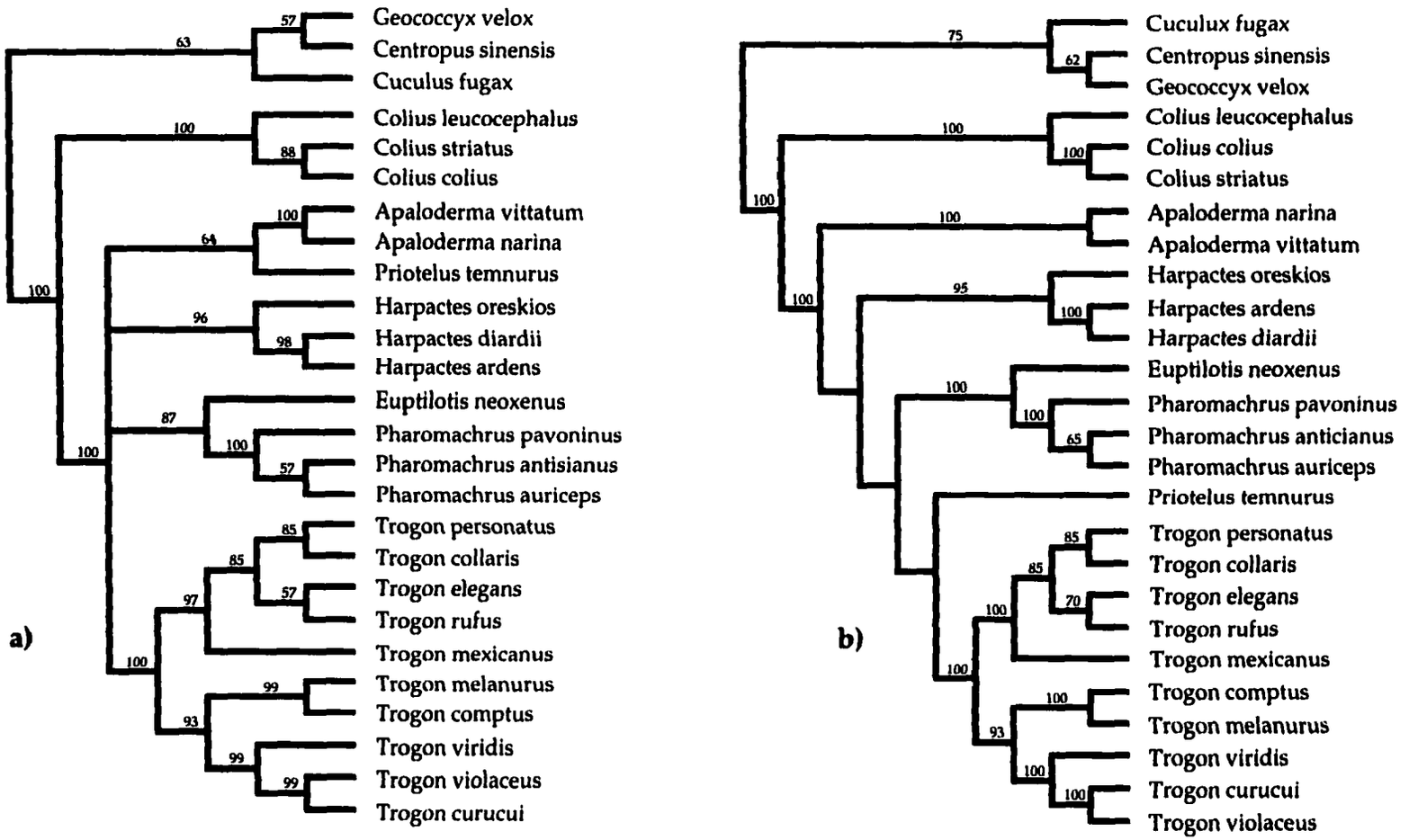


Fig. 6. Phylogenetic trees recovered from multiple alignments built by: a) removing alignment-ambiguous positions, and b) from the secondary structure of the 12S rRNA gene. Numbers above branches are bootstrap percentages from 500 replications (only values over 50% are shown). Analyses were based on global parsimony, both trees were rooted to cuckoos (root was not constrained as monophyletic), and no differential weight was applied to informative characters. a) Strict consensus tree of four trees, 808 steps, CI 0.460, RI 0.622. b) Single most parsimonious tree, 1444 steps, CI 0.468, RI 0.601.

CONCLUDING REMARKS

One of the most basic assumptions made in cladistic analysis is that only homologous characters are meaningful markers of phylogenetic relationships. The accuracy of a phylogenetic hypothesis recovered from molecular data is highly dependent on the accuracy of the sequence alignment. Different alignments can recover conflicting conclusions especially when the phylogenetic signal is weak. The approach of using secondary structure features apparently has improved the process for the alignment of non-protein coding sequences (12S rRNA). On the other hand, since both nucleotide substitutions and length variations accumulate as divergence time increases, the alignment based on the secondary structure is probably less important for closely related taxa that diverged recently, than for groups that diverged long ago. Although structural features can be used to improve the alignment of multiple sequences, the inference of primary homology for some nucleotides remains arbitrary (e.g., hyper-variable regions). A possible solution for this problem is perhaps the combined used of both a secondary structural model and of alignment programs. At the very least, once the alignment is built with alignment algorithms, higher-order features should be consulted to improve this alignment.

The present study is the first attempt to understand the phylogenetic relationships of the Trogoniformes using cladistic methods. Throughout this

study the results clearly corroborate the monophyly of the order Trogoniformes. In all the analyses this clade was significantly supported by bootstrap and branch support. In contrast, conflicting results among analyses do not allow definitive identification of the sister-taxon of trogons. The phylogenetic relationships recovered change substantially depending on the class of substitutions examined and on the taxa included in the analysis. Nevertheless, trogons seem to be more closely related to mousebirds, parrots, and possibly to cuckoos than to any other major group of birds considered in this study. A phylogenetically stable relationship of the trogons to the mousebirds was recovered once the saturated partitions in the data set were removed. A close relationship between the trogons and the mousebirds has been proposed previously based on morphological characters such as foot structure, intestinal convolutions, myology, and osteology (Sclater 1880, Fürbringer 1888, Gadow 1889, Beddard 1898). In addition, electrophoretic patterns of the ovalbumin components of the egg-white proteins of trogons showed characteristics consistent with those of the mousebirds (Sibley and Ahlquist 1972).

Many aspects of the relationships of trogons remain poorly understood. No comprehensive study of their anatomy has been undertaken, and analysis of morphological characters proposed by previous workers showed inconclusive results. The molecular results presented here also fail to identify close relatives of trogons in a consistent manner. Perhaps increasing

the number of morphological or molecular characters, or combining different data sets (e.g., molecules and morphology) will permit the recovery of a well supported hypothesis of trogon relationships.

The phylogenetic analysis of the intergeneric relationships among trogons resulted in a relatively stable topology. All the currently recognized genera were recovered as monophyletic, and in all cases they were supported by high bootstrap values (>90%). Within the genus *Trogon* two subclades were always recovered, one of these subclades was consistently formed by the relationships: (((*T. curucui*, *T. violaceus*), *T. viridis*), (*T. melanurus*, *T. comptus*)). The interspecific relationships in the second subclade were not as stable as in the first one; nonetheless, the remaining five species were consistently recovered as forming a monophyletic group. Within this second subclade the Collared Trogon (*T. collaris*) was always the sister-taxon to the Masked Trogon (*T. personatus*). These two subclades were always supported by high bootstrap values (>75%). Another relationship that was consistently recovered was the lineage formed by the quetzals (*Pharomachrus*) and their sister-taxon the Eared Trogon (*Euptilotis neoxenus*). The union of these two lineages was supported in all the analyses by very high bootstrap values (>95%), and high branch support (>8). In addition to this, the distances between any pairwise comparison of *Euptilotis* with *Pharomachrus* were considerably smaller then the distance of any other pairwise comparison between two species of different genera. These results strongly suggest that

Euptilotis neoxenus should not be isolated in its own genus, but should be included in *Pharomachrus*. Most analyses suggested that the African trogons (*Apaloderma*) were the most basal taxon within the order Trogoniformes, thus supporting a closer relationship of the Asian trogons (*Harpactes*) to the New World trogons. Several questions remain unanswered. Detailed studies at the species level are needed, especially for the Elegant Trogon (*T. elegans*) in which the populations distributed in Mexico and Central America have been considered separate species. Another species that certainly deserves attention is the Hispaniolan Trogon (*Priotelus roseigaster*) that externally resembles species of the genus *Trogon* more closely than it does the Cuban Trogon (*P. temnurus*). Until these and other similar problems are settled, the relationships presented here should be considered tentative.

APPENDIX I

Multiple alignment for the nucleotide sequences of non-passerine 12S rRNA genes. Paired regions are indicated by numbers above the sequences, and the complementary distal sequence is indicated with a prime symbol. Bulges within stems are depicted by lower-cases. Hyper-variable regions are contained in brackets. Bases proposed that form the decoding site (Dahlberg 1989) are indicated by an asterisk.

	1	2	1'
Harpactes oreskios	AA	AGacTT AG	TCC T AAcCT T
Harpactes ardens	..	....	... .
Harpactes diardii	..	....	... .
Priotelus temnurus	..	....	... .
Apaloderma narina	..	....	... .
Apaloderma vittatum	..	....	... .
Pharomachrus antisianus	..	....	... .
Pharomachrus auriceps	..	....	... .
Pharomachrus pavoninus	..	....	... .
Euptilotis neoxenus	..	....	... .
Trogon curucui	.G	....	... .
Trogon violaceus	.G	....	... .
Trogon viridis	..	....	... .
Trogon comptus	..	....	... .
Trogon melanurus	..	....	... .
Trogon elegans	..	....	... .
Trogon rufus	..	....	... .
Trogon collaris	..	....	... .
Trogon mexicanus	..	....	... .
Trogon personatus	..	....	... .
Colius colius	..	....	... .
Colius striatus	..	....	... .
Colius leucocephalus	..	....	... .
Centropus sinensis	..	....	... .
Cuculus fugax	..	....	... .
Geococcyx velox	..	....	... .
Micrathene whitneyi	.C	....	... C ..... .
Asio flammeus	..	....	... C ..... .
Aegolius acadicus	..	....	... C ..... .
Micropsitta finschii	..	....	... C ..... .
Psittrichas fulgidus	..	....	... .
Amazona ventralis	..	....	... .
Pionus senilis	..	....	... .
Amazilia tzacatl	..	..... .T	... .
Chlorostilbon aureoventris	..	....	... .
Ceyx erithacus	..	....	... .
Chloroceryle americana	..	....	... .
Coracias caudata	..	....	... .
Merops nubicus	.C	....	... .
Todus todus	..	....	... .
Momotus mexicanus	.G	....	... .
Momotus momota	.G	....	... .
Upupa epops	..	....	... .
Anthracoceros albirostris	..	....	... .
Aulacorhynchus prasinus	..	....	... .
Melanerpes carolinus	..	....	... .
Sphyrapicus varius	..	....	... .
Caprimulgus vociferus	..	....	... .
Nyctiphrynus mcleodii	..	....	... .
Chordeiles minor	..	....	... .
Gallus gallus	..	....	... .
Anas platyrhynchos	..	....	... .
Cygnus buccinator	..	...A.. ..	.A. . ...
Rhea americana	..	...A.. ..	.A. . ...
Crypturellus undulatus	..	...A.. ..	.A. . ...
Fulica atra	.C	....	... .

	3	4	5
H. oreskios	ACCGTTAATT	A TAGCTAGA	C ATaTACaTGC
H. ardens	....	C	. ...
H. diardii	....	C	. ...
P. temnurus	.......G..	T C.......	. ...
A. narina	.......G..	. ....C...	T .C........
A. vittatum	.......G..	. ....C...	T .C........
P. antisianus	..T.......	T	. ...
P. auriceps	..T.......	T	. ...
P. pavoninus	..T.......	T	. ...
E. neoxenus	....	T	. ...
T. curucui	..T....G..	C A...C...	. ...C......
T. violaceus	..T....G..	C A.....A.	. ...C......
T. viridis	....	T A.....A.	. ...C......
T. comptus	......G...	T A.....A.	. ...C......
T. melanurus	......G...	T A.....A.	. ...C......
T. elegans	.TA.......	T C.......	. ...C......
T. rufus	.TA.......	C	. ...C......
T. collaris	.TG.......	T C.......	T ...C......
T. mexicanus	.TA.......	C	T ...C......
T. personatus	.TG.......	T	T ...C......
C. colius	..A.......	C .TA.C.A.	. ...
C. striatus	..A....G..	T .TA.C.AG	T ....
C. leucocephalus	..A....G..	C .TA.C...	. ...
C. sinensis	.......GC.	T .T....A.	. ...
C. fugax	.......G..	T .T......	T ....
G. velox	.T.....G..	T .T....A.	. ...
M. whitneyi	....	C .T.....G	. .C........
A. flammeus	.......G..	C .TA.C...	. .C........
A. acadicus	....	. .T..C...	T .C........
M. finschii	....	C .T..CCA.	T ....
P. fulgidus	......T...	C .C..CCA.	T ....
A. ventralis	....	C .C...CA.	. ...
P. senilis	....	C .C...CA.	. ...
A. tzacatl	......G...	. .T..C.A.	. ...
C. aureoventris	......G...	. .T..CG..	. ...
C. erithacus	..A....G..	C .T..C...	T ....
C. americana	..T....G..	C .TA.C...	T ....
C. caudata	..T....GC.	T CT..C..G	. .C........
M. nubicus	..T....GC.	C .C....A.	T .C........
T. todus	.......GCC	C CT......	. ...
M. mexicanus	.......G..	T .T....A.	T .C........
M. momota	.......G..	G .T....A.	T .C........
U. epops	..A...GGCC	C CTAT....	. ...
A. albirostris	...A...G..	C .T.....G	T ....
A. prasinus	......GG..	C CTA...A.	. C..C......
M. carolinus	GTTA......	T .T..CGA.	T C.........
S. varius	..TA......	C .T..C...	. C.........
C. vociferus	....	T CT....A.	. ...
N. mcleodii	....	C CC....A.	. ...
C. minor	....	C .T......	. ...
G. gallus	T.TA..GG..	T .T......	. ...
A. platyrhynchos	..A...GG..	T .T......	. ...
C. buccinator	..G...GG..	T .T....A.	T TC........
R. americana	..T.......	T ......A.	. ...
C. undulatus	...A...GC.	. .CAT....	T ....
F. atra	..A.......	C .T..C.A.	. ...

```
                                                  6          7
H. oreskios          AAGTATCCGCATCCCA GTG -CAGAT GCCCT TAATTT--
H. ardens            ..........CT... ... -T.... ..... ....C.--
H. diardii           ..............  ... -T.A.. ..... ...CC.--
P. temnurus          ..........CT... ... -T.A.. ..... ......--
A. narina            ..........C.... ... -T.A.. ..... ..GGC.--
A. vittatum          ..........CT... ... -T.A.. ..... ..GAC.--
P. antisianus        ..........CT... ... -T.A.. ..... ..C-----
P. auriceps          ..........CT... ... -T.A.. ..... ..C-----
P. pavoninus         ..........CT... ... -T.A.. ..... ..C-----
E. neoxenus          ..........CG... ... -T.A.. ..... ...-----
T. curucui           .........GCT... ... -TGA.. ....C -..CC.--
T. violaceus         .........GC.... ... -TGA.. ....C -..CC.--
T. viridis           .........GC.... ... -..A.. ....C -..C..--
T. comptus           ....T.....CT... ... -TGA.. ....C -.GCC.--
T. melanurus         ..........CT... ... -TGA.. ....C -.GC..--
T. elegans           .........G..... ... -T.A.. ..... -...C.--
T. rufus             ..........C.... ... -TGA.. ....C -..CC.--
T. collaris          ..........C.... ... -TGA.. ....C -C..C.--
T. mexicanus         ..........C.... ... -TGA.. ....C -...C.--
T. personatus        ..........C.... ... -TGA.. ....C -......--
C. colius            .......T...CT... ... -T.A.. ....C -C.CAA--
C. striatus          ..........CT... ... -T.A.. ..... --..AA--
C. leucocephalus     ..........CT... ... -T.A.. ..... -...CC--
C. sinensis          .............. ... -T.A.. ....C .G.CCC--
C. fugax             .........GCT... ... -T.A.. ..... ...C-CT-
G. velox             .........GC.... ... -T.A.. ..... ...CACT-
M. whitneyi          ..........C.... ... -AGA.. ....- .CCGC---
A. flammeus          ..........C.... ... -T.A.. ....A .CCGC---
A. acadicus          ..........CT... ... -T.A.. ....C .--GC---
M. finschii          ..........C.... ... -..A.C ....C ...CCC--
P. fulgidus          ..........C.... ... -..A.C ..... C..CCC--
A. ventralis         ..........C.... ... -..A.C ..... -..AGCC-
P. senilis           ..........C.... ... -..A.C ..... C..ACCCC
A. tzacatl           ..........C.... A.. -T.A.. ....A ..GACAC-
C. aureoventris      .........GC.... ... -T.A.. ..... ...ACAC-
C. erithacus         .............. ... -T.A.. ....C -.GGC.T-
C. americana         ..........C.... ... -T.A.. ..... ..GCC.--
C. caudata           .........GC.... ... -T.A.. ....C C..CC.--
M. nubicus           ..........C.... ... -A.A.. ....C -.GGCAGC
T. todus             .........GC.... ... -T.A.. ....C C..CCC--
M. mexicanus         ..........CG... ... -T.A.. ..... .GG.CCC-
M. momota            ..........CG... ... -T.A.. ..... .GGCCCC-
U. epops             .......T..GC.... ... -T.A.. ..... C..C----
A. albirostris       ...C......GC.... ... -T.A.. ....A CGGC.C--
A. prasinus          .......T..GA...G ... -TGA.. ..... ..GAC.--
M. carolinus         .............. ... -T.A.. ....C ..GCCC--
S. varius            .........GC.... ... -T.A.. ....C C..C.CCC
C. vociferus         .......T..GAT... ... -T.A.. ..... -..ACCT-
N. mcleodii          .........GA.... ... -T.A.. ..... -..ACCT-
C. minor             .-........CT... ... AT.A.. ..... AGTAC-T-
G. gallus            .............. ... A-.A.. ....C C..AC---
A. platyrhynchos     .-........GC.... ... AT.A.. ..... C...-AG-
C. buccinator        .-........GC.... ... AT.A.C ..-.. CG.CCAC-
R. americana         .-........GCA... ... AT.A.. ..... C.GCG-T-
C. undulatus         .-........GCT... ... AT.A.. ..... CGCCCAT-
F. atra              .-........CT... ... AT.A.. ..... ..CCCC--
```

	8		8'	7'	9
H. oreskios	CTT	[CCTATATAATC]	AAG	A--TAA- AGGaGC	A GAC
H. ardens	...	[CCCTTCTAAT]	...	.--...-	G ...
H. diardii	...	[CCCTTATAAT]	...	.--C..-	G ...
P. temnurus	...	[TCTAATCT]	...	.--.GG-	. .G.
A. narina	...	[ATACTCATTT]	...	.--...-	G ...
A. vittatum	...	[ATATTTACTT]	...	.--..G-	. ...
P. antisianus	.C.	[CTCCCTAC]	.-.	.--...-	. .G.
P. auriceps	.C.	[CTCCCTAC]	.-.	.--...-	. .G.
P. pavoninus	.C.	[CTCTTTAC]	.-.	.--...-	. .G.
E. neoxenus	.C.	[CCCTAAAT]	.-.	.--...-	. .GT
T. curucui	...	[TCCAATCT]	...	.--...-	G ...
T. violaceus	...	[TCTAATCT]	...	.--...-	G ...
T. viridis	...	[CCTGACCC]	C..	.--...-	. ..T
T. comptus	...	[TCCAGCTT]	...	.--...-	. ...
T. melanurus	...	[TCCAGCTT]	...	.--...-	. ...
T. elegans	...	[CCTAACCA]	...	.--...- ...T..	G ...
T. rufus	...	[CCTGACCC]	...	.--CG.-	. ...
T. collaris	...	[CCTAACTC]	...	.--...- ...T..	. ...
T. mexicanus	...	[CTCAACCC]	...	.--C..- ...C..	G ...
T. personatus	...	[CCTTAATCC]	...	.--C..- ...T..	. ...
C. colius	...	[CGCCG]	...	.--C..-	T .GT
C. striatus	...	[CACCA]	...	.--C..-	T .GT
C. leucocephalus	...	[CACCA]	...	.--C.G-	T .GT
C. sinensis	A..	[TACCA]	...	T--...- G.....	. .GT
C. fugax	...	[ACT]	...	.--C..A	. .G.
G. velox	...	[ACT]	...	.--..GA	. .G.
M. whitneyi	.C.	[ATCAATTATC]	..T	.-GCTGG	. .GT
A. flammeus	.CC	[ACCCGCTTTA]	..T	G-GC.GA G.....	G .G.
A. acadicus	...	[GCAAATC]	...	---.CG- G.....	G .G.
M. finschii	...	[ACC]	...	.--C..A	. .G.
P. fulgidus	...	[ACT]	...	.--C..A	. .G.
A. ventralis	.CC	[ACC]	...	G--C.CT	. .G.
P. senilis	...	[GCC]	...	G--C.CT	. .G.
A. tzacatl	...	[ACC]	...	G--C..A	. .GT
C. aureoventris	...	[ACC]	...	.--C..A	. .GT
C. erithacus	...	[ACC]	...	.--...A	T .G.
C. americana	...	[ATA]	...	.--CG.A	C .G.
C. caudata	...	[ACC]	...	.--C..A C.....	T .GT
M. nubicus	...	[ACC]	...	G--C.CA G.....	. .G.
T. todus	...	[ATA]	...	G--A.GA	. .GT
M. mexicanus	...	[AAT]	...	.--...A	. .GT
M. momota	...	[AAC]	...	.--...A	. .GT
U. epops	..C	[CCCAAT]	G..	.--C..G	G .G.
A. albirostris	...	[ACC]	...	G--C.CA ...G..	. .G.
A. prasinus	...	[ACC]	...	.--C..G	C .G.
M. carolinus	.C.	[ACA]	.G.	.--C..G	. .G.
S. varius	.A.	[C]	.G.	.--...G	. .GT
C. vociferus	...	[ACC]	...	.--C..A	G .G.
N. mcleodii	...	[ACC]	...	.-AC..A	G .GT
C. minor	...	[AAT]	...	.GGC..A	. .G.
G. gallus	...	[TCTTCCC]	...	C--A..-	. .GT
A. platyrhynchos	.C.	[TCACCCC]	.G.	C--CTTA	G .GT
C. buccinator	..A	[CTCACCACC]	T..	GCC.TG-	G .GT
R. americana	..A	[CCCCCG]	C..	.CC..--	. .GT
C. undulatus	G.A	[CCTCACTT]	...	.----..G	G .GT
F. atra	..G	[CTCACTGAGG]	C..	GG--..G	. .G.

	10			10'
H. oreskios	ATCA	GGCaCGC	[CCTA	] GCA----GCC
H. ardens	T...	.....	[CCTA	] ...----...
H. diardii	....	.....	[CCTA	] ...----...
P. temnurus	....	.....	[CACA	] ...----...
A. narina	....	.....	[CCC	] ...----...
A. vittatum	....	.....	[CCCC	] ...----...
P. antisianus	....	.....	[CCCCG	] ...----...
P. auriceps	....	.....	[CCCG	] ...----...
P. pavoninus	....	.....	[CCCG	] ...----...
E. neoxenus	....	.....	[TCCG	] ...----...
T. curucui	....	.....	[TCCA	] ...----...
T. violaceus	....	.....	[TCCA	] ...----...
T. viridis	....	...g...	[TCCA	] ...----...
T. comptus	....	.....	[TTTA	] ...----...
T. melanurus	....	.....	[TTACA	] ...----...
T. elegans	....	.....	[TCCA	] ...----...
T. rufus	....	.....	[ACTA	] ...----...
T. collaris	....	.....	[TCCA	] ...----...
T. mexicanus	....	.....	[TTTA	] ...----...
T. personatus	....	.....	[TCCA	] ...----...
C. colius	....	.....A.	[CCACCGTA	] .T.----...
C. striatus	....	.....A.	[CCACCATA	] .T.----...
C. leucocephalus	....	.....A.	[CCATCGCA	] .T.----...
C. sinensis	....	.....A.	[CCACAAGCTTTAAACG	] .T.----...
C. fugax	....	.....	[TCCAGACACTT	] ...----...
G. velox	....	...g.A.	[CCCCCCCCCC	] .T.----...
M. whitneyi	....	.....A.	[CCTTCCCCCC	] .T.----...
A. flammeus	....	...g.A.	[GCACCTCCCCGCC	] .T.----...
A. acadicus	....	.....A.	[CTAGCCATCGCGG	] .T.----...
M. finschii	....	.....A.	[ATCAAC	] .T.----...
P. fulgidus	....	.....A.	[CCATC	] .T.----...
A. ventralis	....	...g.A.	[CTCT	] .T.----...
P. senilis	....	.....A.	[TTCT	] .T.----...
A. tzacatl	....	.....A.	[TAAGGCC	] .T.----...
C. aureoventris	....	.....A.	[CAACT	] .T.----...
C. erithacus	....	...g.A.	[TACAAA	] .T.ACC-...
C. americana	....	...t.A.	[CAACTA	] CT.TC--...
C. caudata	....	...t.A.	[CTAT	] .TG----...
M. nubicus	....	.....A.	[CAGCTT	] .T.----...
T. todus	....	.....A.	[TTCGTTCTTTTCCC	] .T.----...
M. mexicanus	....	.....	[CACAACT	] .T.----...
M. momota	....	...g.A.	[CACAGCC	] .T.----...
U. epops	....	...t...	[CGACCTATAACC	] .T.GGCT...
A. albirostris	....	.....	[CACCAT	] .TC-GCA...
A. prasinus	....	...g...	[CCC	] ...----...
M. carolinus	....	.....A.	[CCTCT	] .T.----...
S. varius	....	.....A.	[ACCTACC	] .T.----...
C. vociferus	....	.....A.	[CCAAAGAT	] .T.----...
N. mcleodii	....	.....A.	[CCAACACCAC	] .T.----...
C. minor	....	...G.A.	[TCAAAACT	] .T.----...
G. gallus	....	.....A.	[TCAGCA	] .T.----...
A. platyrhynchos	....	.....A.	[CCAAGCA	] .T.----...
C. buccinator	....	.....A.	[CCAAGTA	] .T.----...
R. americana	....	...G.A.	[TAGATC	] .T.----...
C. undulatus	....	...T.A.	[TCTT	] .T.----...
F. atra	....	.....A.	[CAAC	] .T.----...

	9'			6'	11		11'
H. oreskios	CAAGAC	GTC	TT***GC***TT-A***GC***	CAC	A	CCC	CCAC GGG
H. ardens	.....	T..	.....C-...	...	.	...	T... ...
H. diardii	.....	...	.....C-...	...	.	...	T... ...
P. temnurus	T.....	...	.....-...	...	.	...	....
A. narina	.....	...	.....-...	...	.	...	....
A. vittatum	.....	...	.....-...	...	.	...	....
P. antisianus	.....	.C.	.....-...	...	G	...	....
P. auriceps	.....	.C.	.....-...	...	.	...	....
P. pavoninus	.....	.C.	.....-...	...	.	...	....
E. neoxenus	.....	.C.	.....-...	...	.	...	....
T. curucui	.....	...	....C.-...	...	.	...	....
T. violaceus	.....	...	....C.-...	...	.	...	....
T. viridis	.....	...	....CA-...	...	.	...	....
T. comptus	.....	...	.....-...	...	.	...	....
T. melanurus	.....	...	.....-...	...	.	...	....
T. elegans	.....	...	.....-...	...	.	...	....
T. rufus	.....	...	.....-...	...	.	...	....
T. collaris	...A..	...	.....-...	...	.	...	....
T. mexicanus	.....	...	.....-...	...	.	...	....
T. personatus	.....	...	.....-...	...	.	...	....
C. colius	...A..	AC.	.....-C..	...	.	...	....
C. striatus	...A..	AC.	.....-T..	...	.	...	....
C. leucocephalus	...A..	AC.	.....-C..	...	.	...	....
C. sinensis	T.G...	AC.	.....C-...	...	.	...	....
C. fugax	.....	.C.	.....-...	...	.	...	....
G. velox	.....	.C.	....CC-...	...	.	...	....
M. whitneyi	.....	AC.	....ACT...	...	.	...	....
A. flammeus	.....	.C.	....ACT...	...	.	...	....
A. acadicus	.....	.C.	....ACC...	...	.	...	A... ...
M. finschii	.....	.C.	AC..-CC...	...	.	...	....
P. fulgidus	.....	AC.	AC..-CCC..	...	.	...	....
A. ventralis	.....	.C.	A...--AC..	...	.	...	....
P. senilis	.....	.C.	AC..-CAC..	...	T	...	....
A. tzacatl	...A..	AC.	.....C-...	...	.	...	....
C. aureoventris	.....	AC.	.....C-G..	...	.	...	....
C. erithacus	.....	.C.	.....-...	...	.	...	....
C. americana	.....	.C.	.....-...	...	.	...	....
C. caudata	.....	.C.	.....C-...	...	.	...	....
M. nubicus	...A..	.C.	.....-T..	...	.	...	....
T. todus	.....	AC.	....GC-...	...	.	...	....
M. mexicanus	T.....	AC.	.....-...	...	.	...	....
M. momota	T.....	AC.	.....-...	...	.	...	....
U. epops	A.....	.C.	.....C-...	...	C	...	....
A. albirostris	T.....	.C.	.....C-...	...	.	...	....
A. prasinus	..C...	.C.	.....C-...	...	.	...	....
M. carolinus	...A..	.C.	.....C-...	...	.	...	....
S. varius	.....	AC.	.....C-...	...	.	...	....
C. vociferus	.....	.C.	.....-...	...	.	...	....
N. mcleodii	.....	AC.	.....-...	...	.	...	....
C. minor	.....	.C.	.....-...	...	.	...	....
G. gallus	.....	.C.	.....A...	...	.	...	....
A. platyrhynchos	.....	.C.	....-.A...	...	G	...	....
C. buccinator	.....	.C.	.C...A-...	...	G	...	....
R. americana	.....	.C.	.....C-...	...	.	...	....
C. undulatus	...A..	AC.	....CC-...	...	.	...	....
F. atra	...A..	.C.	.....C-...	...	.	...	....

```
                                  5'                  12
H. oreskios          TATTCA GCAGTAAT TAACA TtAAGcaaTAAGT GAAA
H. ardens            ...... ........ ..... ............. ....
H. diardii           ...... ........ ...T. ............. ....
P. temnurus          ...... ........ ..... ............. ....
A. narina            ..C... ......G. ..... .........G... ....
A. vittatum          ...... ......G. ..... ............. ....
P. antisianus        ...... ........ ..... ............. ....
P. auriceps          ...... ........ ..... ............. ....
P. pavoninus         ...... ........ ..... ............. ....
E. neoxenus          ...... ........ ..... ............. .T..
T. curucui           ...... .....G.. ..... ............. ....
T. violaceus         ...... .....G.. ..... ............. ....
T. viridis           ...... .....G.. ..... ............. ....
T. comptus           ...... .....G.. ..... ............. ....
T. melanurus         ...... .....G.. ..... ............. .G..
T. elegans           ..C... .....G.. .G... ............. .T..
T. rufus             ..C... .....G.. .G... ............. ....
T. collaris          ..C... .....G.. .G... ............. ....
T. mexicanus         ..C... .....G.. ..... ............. ....
T. personatus        ..C... .....G.. .G... ............. ....
C. colius            ...... ........ ..... ............. .T..
C. striatus          ...... ........ ..... ............. .T..
C. leucocephalus     .C.... ........ .G... ............. .T..
C. sinensis          ...... ........ ..... ............. .C..
C. fugax             ...... ........ ..... ............. ....
G. velox             ...... ........ ..... ............. ....
M. whitneyi          ..CC.. ......G. ....C .........G... .C..
A. flammeus          ...... ......G. ..... ............C .C..
A. acadicus          ..C... ......G. .G... ..........G.C .C..
M. finschii          ..C... ........ ..... ..........G.C .T..
P. fulgidus          ..C... ........ ..... ..........G.C .C..
A. ventralis         ..C... ........ ..... .........GG.C .T..
P. senilis           ..C... ........ ..... ..........G.C ....
A. tzacatl           ..C... ........ ..... ............. .T..
C. aureoventris      ..C... ........ ..... ............. .C..
C. erithacus         ...... ........ ..... ......t..G... .C..
C. americana         C..... ........ ..... .........G... .T..
C. caudata           ..C... ......G. .G... .........G... .T..
M. nubicus           ...... ......G. AG... .........G... ....
T. todus             ..C... ........ ..... .........G... .T..
M. mexicanus         ...... ......G. .G..C .........G... ....
M. momota            ...... ......G. .G... .........G... ....
U. epops             C.A... ........ ..... .........G..C ....
A. albirostris       ...... ........ .G... ..G...t.....C ....
A. prasinus          CC.... .....G.C ..... ......c...... .C..
M. carolinus         ...... ........ ..... ............. ....
S. varius            C.C... ...T.... ..... ............. ....
C. vociferus         C.C... ........ C.... ............. .T..
N. mcleodii          ..C... ........ C.... ............. .C..
C. minor             ...... ........ ..... ............. .C..
G. gallus            ..C... ........ ....C ............. .T..
A. platyrhynchos     ...... ......G. ..... .........G... .C..
C. buccinator        ...... ......G. ..... .........G... .C..
R. americana         ...... ........ .G.T. ............. .C..
C. undulatus         .C.... ........ ....T ......t...... ....
F. atra              ...... ........ ..... .........G... ....
```

	12'		4'		
H. oreskios	ACTTGaCTTA	G	TCaTAGCTA	[CCC	]A
H. ardens	....	.	....	[TCA	].
H. diardii	....	.	.T.......	[TCA	].
P. temnurus	....	.	C........	[ACT	].
A. narina	....	.	....G.T..	[TCCCT	].
A. vittatum	....	.	....G....	[CCCTC	].
P. antisianus	....	.	....	[CTT	].
P. auriceps	....	.	....	[CTT	].
P. pavoninus	....	.	....	[CTTT	].
E. neoxenus	....	.	....	[CTT	].
T. curucui	....	.	.T..G...T	[ATAT	].
T. violaceus	....	.	.T..G...T	[ATAT	].
T. viridis	....	.	.T......T	[TGT	].
T. comptus	....	.	.T......T	[CCT	].
T. melanurus	....	.	.T......T	[TCT	].
T. elegans	G.........	.	........T	[TAT	].
T. rufus	G.........	.	........T	[TATT	].
T. collaris	G.........	.	.T......C	[CAC	].
T. mexicanus	G.........	.	.T......T	[TAT	].
T. personatus	G.........	.	.T......T	[AAT	].
C. colius	....	.	.T..G.TA.	[ATAATC	].
C. striatus	....	.	CT..G.TA.	[ACAACC	].
C. leucocephalus	....	.	C...G.TA.	[ACAACT	].
C. sinensis	....	.	.T.....A.	[CCCCCTACC	].
C. fugax	....	.	.......A.	[TCCC	].
G. velox	....	.	.T.....A.	[CCCTGCCCTC	].
M. whitneyi	...C......	.	C......A.	[CCCCTA	].
A. flammeus	G.........	.	.T..G.TA.	[CACCTCCCCCT	].
A. acadicus	G.........	.	....G..A.	[CATCCAGCCA	].
M. finschii	G.C.......	.	.T.G...A.	[CC	].
P. fulgidus	G.C.......	.	...A...G.	[TCC	].
A. ventralis	G.CC......	.	CT.G...G.	[CC	].
P. senilis	G.C.......	.	.T.G...A.	[TT	].
A. tzacatl	....	.	.T..G.TA.	[ACCCA	].
C. aureoventris	....	.	.T.CG..A.	[ACTTCT	].
C. erithacus	...C......	.	....G..A.	[CACTT	].
C. americana	...C......	.	....G.TA.	[CATTA	].
C. caudata	...C......	.	C..CG..A.	[CCACA	].
M. nubicus	....	.	.T.G...G.	[ATCC	].
T. todus	...C......	.	.......AG	[TAACC	].
M. mexicanus	...C......	.	.T.....A.	[CCTA	].
M. momota	...C......	.	.T.....A.	[CCTA	].
U. epops	G.........	.	C.....TAG	[AGATTA	].
A. albirostris	G.......C.	.	C......A.	[TGCT	].
A. prasinus	....	.	.T....TA.	[ACC	].
M. carolinus	....	.	...CG..A.	[CAT	].
S. varius	....	.	....G..A.	[CACC	].
C. vociferus	....	.	.T.....A.	[CCACCC	].
N. mcleodii	....	.	.T.....GG	[CCCCCC	].
C. minor	....	.	.T.....A.	[CACAT	].
G. gallus	....	.	C......A.	[CCC	].
A. platyrhynchos	...C......	.	........A.	[GCCTCCACCCA	].
C. buccinator	....	.	.T.....A.	[CAACTCATTTCCTTA	].
R. americana	....	.	.T.....G.	[CCGC	]N
C. undulatus	G.........	.	...C.ATG.	[TCC	].
F. atra	...CN.....	.	.T..G..A.	[CCCTAC	].

	13	14		14'
			*****	
H. oreskios	GGGCT	GGTAAA	TCTTGTG CCAGCCACCGCGGTCA	CACAAGA
H. ardens	C	.....	....A....	.....
H. diardii	.....	.....	....T.	.....
P. temnurus	.....	.....	....	.....
A. narina	C	.....	....	G.....
A. vittatum	C	.....	....	G.....
P. antisianus	.....	.....	....	.....
P. auriceps	.....	.....	....	.....
P. pavoninus	.....	.....	....	.....
E. neoxenus	.....	.....	....T.	.....
T. curucui	C	.....	....	T.....
T. violaceus	...TC	.....	....	T.....
T. viridis	C	.....	....	T.....
T. comptus	C	.....	....	T.....
T. melanurus	C	.....	....	T.....
T. elegans	.A..C	.....	....	T.....
T. rufus	.A..C	.....	....	T.....
T. collaris	.A..C	.....	....	T.....
T. mexicanus	.A..C	.....	....	T.....
T. personatus	.A..C	.....	....	T.....
C. colius	...T.	.....	....	T.....
C. striatus	...T.	.....	....	T.....
C. leucocephalus	...T.	.....	....	T.....
C. sinensis	.....	.....	....	T.....
C. fugax	...T.	.....	....	T.....
G. velox	...TC	.....	....T.....	.....
M. whitneyi	C	.....	....	T.....G
A. flammeus	...TC	.....	....	.....
A. acadicus	...T.	.....	....	T.....
M. finschii	...TC	.....	....	T.....
P. fulgidus	C	.....	....	.....
A. ventralis	...T.	.....	....	T.....
P. senilis	...T.	.....	....	T.....
A. tzacatl	.....	...T..	....G.....C..	T.....
C. aureoventris	.....	...T..	....G.....	T.....
C. erithacus	...TC	.....	....	T.....
C. americana	...T.	.....	....	T.....
C. caudata	...TC	.....	....	T.....
M. nubicus	.....	.....	....G.....T.	T.....
T. todus	...T.	.....	....	.....
M. mexicanus	C	.....	....	.....
M. momota	C	.....	....	.....
U. epops	...T.	.....	....	.....G
A. albirostris	...T.	.....	....	.....
A. prasinus	...T.	.....	....	.....
M. carolinus	...T.	.....	....	T.....
S. varius	...T.	...T..	....	T.....
C. vociferus	C	.....	....	T.....
N. mcleodii	C	.....	....	T.....
C. minor	...TC	.....	.T.....	T.....
G. gallus	...T.	.....	....	T.....
A. platyrhynchos	...TC	.....	....	T.....
C. buccinator	...T.	.....	....	.....
R. americana	...TC	.....	....	.....
C. undulatus	C	.....	....	T.....
F. atra	.....	.....	....	T.....

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                          13'          3'                    15
H. oreskios               AGCCC A AATTAACTGT ---T-CAC-- GGC GTAAAGA
H. ardens                 G.... . .......... ---C-...-- ... .......
H. diardii                G.... . .......A.. ---C-...-- ... .......
P. temnurus               G.... . .......... ---.A...-- ... .......
A. narina                 G.... . .......... ---CAT..-- ... .......
A. vittatum               G..T. . .......... ---CAT..-- ... .......
P. antisianus             ..... . .......C.. ---CA...-- ... .......
P. auriceps               ..... . .......C.. ---CAT..-- ... .......
P. pavoninus              ..... . .......C.. ---CA...-- ... .......
E. neoxenus               ..... . .......... ---.A...-- ... .......
T. curucui                G.... . ......T... ---CAT..-- ... .......
T. violaceus              GA... . ......T... ---.AT..-- ... .......
T. viridis                G.... . .......C.. ---CA...-- ... .......
T. comptus                G.... . ..CC...... ---CATC.-- ... .......
T. melanurus              GA... . ...C...... ---.A...-- ... .......
T. elegans                G..T. . .......CA. ---AA...-- ... .......
T. rufus                  G..T. . .......CA. ---.A...-- ... .......
T. collaris               G..T. . ..C....CA. ---.A...-- ... .......
T. mexicanus              G..T. . ........A. ---.A...-- ... .......
T. personatus             G..T. . .......CA. ---.ATG.-- ... .......
C. colius                 .A... . .......... ----ATG.-- ... .......
C. striatus               .A... . .......... ----AT..-- ... .......
C. leucocephalus          GA... . .......... ----A...-- ... .......
C. sinensis               ..... . ..C....C.. ---.A...-- ... .......
C. fugax                  ..... . .......... ---AA...-- ... .......
G. velox                  GA... . .......... ---AA...-- ... .......
M. whitneyi               ..... . .......C.. ----AT..-- ... .......
A. flammeus               GA... . .......... ---CAT..-- ... .......
A. acadicus               GA... . .G.....A.. ----AG..-- ... ......G
M. finschii               GA... . .......C.. ----AA..-- ... .......
P. fulgidus               GA... . .......CAC ----A...-- ... .......
A. ventralis              .A... . .......C.. ----A...-- ... .......
P. senilis                .A... . .......... ----A...-- ... .......
A. tzacatl                GA... . ..CC...G.C ---AA...-- ... .......
C. aureoventris           .A... . ..CC...G.C ---AG...-- ... .......
C. erithacus              GA... . .......C.. ---CA...-- ... .......
C. americana              .A... . .......C.. ---CA...-- ... ......C
C. caudata                GA... . .G.....C.. ----A.G.-- ... .......
M. nubicus                ..... . .G........ ---CAGC.-- ... .......
T. todus                  .A... . .G........ ----ATG.-- ... .......
M. mexicanus              G.... . .......... ----A...-- ... .......
M. momota                 G.... . .......... ----A...-- ... .......
U. epops                  .A... . .G.C...... ---.A.C.-- ... .......
A. albirostris            .A... . ..C...TC.. ----ATG.-- ... .......
A. prasinus               .A... . ...C...... ----A...-- ... .......
M. carolinus              .A... . ......TAAC ----G...-- ... .......
S. varius                 .A... . ......TA.. ----G...-- ... .......
C. vociferus              GA... . .......... ---AAT..-- ... .......
N. mcleodii               G.... . .......... ---AAT..-- ... .......
C. minor                  GA... . .......C.. ---AA...-- ... .......
G. gallus                 .A... . ...C..TA.C ---.A.C.-- ... .......
A. platyrhynchos          GA... . ...C...... CC-.A...GA ... .......
C. buccinator             .A... . ...C...C.C CCA.AA..CC ... .......
R. americana              GA... G ..C....A.. ----AAT.-- ... ......T
C. undulatus              G.... . .G....TG.. ----ATT.-- ... ......C
F. atra                   ....T . .......C.. -------...CC ... .......
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	16	17
H. oreskios	GTGG TA-AAGTA-TTATCCGGAC-AAC	TAAGACC
H. ardens	.... ..G..-C.-.....A.GA-G..	..G...T
H. diardii	.... ..G..-C.-.....AAGA-G..	.....T
P. temnurus	.... ..C..-CC-C.G..TTTTT-...	.....T.
A. narina	.... ..TG.-..-.....GAAGT-...	.....T.
A. vittatum	.... C.TG.-A.-C....AAAG.-...	.....
P. antisianus	.... ..C..-C.-.....A...-...	.....T.
P. auriceps	.... ..C..-C.-.....A...-...	.....TT
P. pavoninus	.... ..C..-C.-.....A...-...	.....TT
E. neoxenus	.... ..T..-C.-.....A...-...	.....TT
T. curucui	.... .TT.G-C.-.....TAAGT-...	..G..TT
T. violaceus	.... .TT..-C.-.....TAA.T-...	.....T.
T. viridis	.... ..C..-..-..G..TAA.T-...	..G..T.
T. comptus	.... ..C..-..-.....TAAGT-...	....G..
T. melanurus	.... ..C..-.T-C.....AAGT-G..	....GT.
T. elegans	.... ..T..-C.-.....T.AGG-...	.....T.
T. rufus	.... ..T..-C.-.....T.A.T-...	..G..T.
T. collaris	.... ..T..-..-.....T.A.T-...	..G..T.
T. mexicanus	.... ..T..-C.-.....TAA.T-...	..G..T.
T. personatus	.... ..T..-..-.....TAA.T-...	..G..T.
C. colius	.... ..C..-CG-C....-AC..T...	....G.T
C. striatus	.... C.C..-C.-A....-AA..T...	..G.G.T
C. leucocephalus	.... C.C..-..-.....-.C..T...	....G.T
C. sinensis	.... ..T...CC-C...TAATTA-...	.....TA
C. fugax	.... ..TGC.C.-.....ACAGT-.C.	...A.T.
G. velox	.... ..TTT...-...C.TCT..-..T	...A.T.
M. whitneyi	.... G.CC.TA.T.....TTT--...	..GAG.T
A. flammeus	.... AGTC.-C.-C.....TC.A-G..	..GAGTT
A. acadicus	.... C.CTT.C.-G.....CCCA-...	..G.GTG
M. finschii	.... ACCC.AA.AA....TAA..C..T	.....
P. fulgidus	.... -CCC..G.-C....ACA..C...	.....TT
A. ventralis	.... CCCC..-.-C...TAAAGTAG.T	.....T.
P. senilis	.... .CCC..-.-C...TA.AGTAG.T	.....TT
A. tzacatl	...A C.CT.-C.-A.....ACCA-.GT	..G..TT
C. aureoventris	...A C.CT.-C.-A.....AC.G-GG.	GGG.G..
C. erithacus	.... CCC-CA.C-C...T-AT..C...	..G...T
C. americana	.... CCC.CACTCA....ACA.T-...	..G.GTA
C. caudata	.... ..CTGT.C-C....ACA..-...	..GA.T.
M. nubicus	.... .C-C....-..G..A.CC.-...	..G.GTT
T. todus	.... CCTCC..T-A....ACC..-...	..G...T
M. mexicanus	.... .CT.CACCCC....AT.C.-G..	.....
M. momota	.... CCT.CACCCC....AT.CT-G..	.....
U. epops	.... --GT.C..CC....-AC.A-G..	..TAG..
A. albirostris	.... ..CC.-CG-A.....T.-.-...	..G.G..
A. prasinus	.... .C-C.AACAC.....TC.TT...	..G...T
M. carolinus	...A .TA..CAT-C....GTTC.TGT.	.G..G..
S. varius	...A .CCT.CAC-.....GCCT.CGC.	.G..G..
C. vociferus	.... C.TG.-.G-.....ACA..-...	.....T.
N. mcleodii	.... ..C..-.G-.....AC.TT-...	.....T.
C. minor	.... ..TC.-.G-A....ACA..-...	.....T.
G. gallus	.... CCAC.-.G-.....T.C..C.G.	.....TT
A. platyrhynchos	.... ..AG.-.GCC.....TAC.T...	.....T.
C. buccinator	.... ..A..-.GCC.....TAG.T...	.....T.
R. americana	.... ..AC.-.G-.....A.-.T.G.	.....
C. undulatus	.... A.AC.-.G-C....TAA..-...	..G....
F. atra	.... ..ACT-..-C...-TA-T.T...	..G..T.

		18		19	*	19'	
H. oreskios	AAAGCA---	CAa-CT	AA	GC	TGTCATAA	GC	TAA
H. ardens	......---	...-..	..	..	....	..	...
H. diardii	...A..---	...-..	..	..	....	..	...
P. temnurus	...CTG---	...-..	.G	..	....	..	...
A. narina	...ATT---	..g-..	..	..	C.......	..	...
A. vittatum	...A.T---	...-..	..	..	....	..	...
P. antisianus	G..CT.---	T..-..	..	..	....	..	.T.
P. auriceps	G..CT.---	T..-..	..	..	....	..	...
P. pavoninus	G..CT.---	T..-..	..	..	....	..	...
E. neoxenus	...CTG---	T..-..	..	..	....	..	...
T. curucui	...CTG---	T..-..	G.	..	....	..	.G.
T. violaceus	...CTG---	T..-..	G.	..	....	..	...
T. viridis	...CTG---	...-..	G.	..	....	..	...
T. comptus	...CTG---	...-..	G.	..	....	..	.G.
T. melanurus	...CTG---	T..-..	G.	..	....	..	.T.
T. elegans	...TTG---	T..-..	G.	..	....	..	...
T. rufus	...TTG---	T..-..	G.	..	....	..	...
T. collaris	...TTG---	...-..	G.	..	....	..	.T.
T. mexicanus	...TTG---	T..-..	G.	..	....	..	...
T. personatus	...TTG---	T..-..	G.	..	....	..	...
C. colius	G..A..---	T.A-.C	..	..	C.......	..	C..
C. striatus	...A..---	T.A-.C	..	..	C.......	..	C..
C. leucocephalus	...A..---	T.A-..	..	..	....	..	C..
C. sinensis	...A..---	..A-.C	..	..	....	..	AT.
C. fugax	G..ATG---	T..-..	G.	..	C.......	..	CT.
G. velox	...A.G---	T.g-TC	..	.T	....	..	.C.
M. whitneyi	..GAT.---	T..-.C	..	..	C.......	..	CC.
A. flammeus	...AT.---	...-.C	..	..	....	..	CC.
A. acadicus	...A..---	...-.C	..	..	C.......	..	.T.
M. finschii	...C..CCA	...-.A	C.	..	.....C..	..	.C.
P. fulgidus	...C..---	..gc.C	..	..	C.......	A.	CC.
A. ventralis	...C.T---	...c..	..	..	C....C..	..	CC.
P. senilis	...CTT---	...c.C	..	..	C....C..	..	CT.
A. tzacatl	...A..---	...-..	G.	..	C.......	..	AC.
C. aureoventris	G..A..---	...-..	G.	..	A.......	..	AT.
C. erithacus	...ATG---	T..-.C	G.	..	....	..	CT.
C. americana	...ATG---	...-..	..	..	....	..	C..
C. caudata	...AT.---	.G.-..	G.	..	C.......	..	CC.
M. nubicus	G..A.G---	TGc-..	G.	..	....	..	.T.
T. todus	G..A.G---	...-..	G.	..	....	..	...
M. mexicanus	...CTG---	.G.-.C	..	..	....	..	CC.
M. momota	...CTG---	.G.-.C	..	..	....	..	.C.
U. epops	...ATG---	T..-T.	..	..	....GC..	..	.T.
A. albirostris	...ATG---	TT.-..	G.	..	....	..	CC.
A. prasinus	...TTG---	...-TC	G.	..	....	..	CT.
M. carolinus	...A..---	TGc-..	G.	..	C....C..	..	CC.
S. varius	...A.G---	TGT-.C	..	.A	.....C..	..	CC.
C. vociferus	G..AT.---	...-T.	G.	..	....	..	.T.
N. mcleodii	G..AT.---	...-T.	G.	..	....	..	.T.
C. minor	...A..---	..G-T.	G.	..	....	..	.T.
G. gallus	...ATG---	...-.C	..	..	....	..	CT.
A. platyrhynchos	...ATG---	...-..	..	..	....GC..	..	AC.
C. buccinator	...ATG---	...-..	..	..	....	..	CC.
R. americana	...ATG---	...-..	..	..	....	..	...
C. undulatus	...ATG---	..T-.C	..	..	.......T	..	CT.
F. atra	....T.---	...-..	..	..	....	..	CC.

```
                          18'                                  17'
H. oreskios       AGaTG TACTTAAGACCCGCTATTAAAAC GATCTTA
H. ardens         ..... ...C...A...AA.......G.. .......
H. diardii        ..... ...C...AG..AA-......G.. A......
P. temnurus       ..... C.TC....G...T.CC.A..... .......
A. narina         T.... C..C......TAT..C.A..G.. .......
A. vittatum       ..... C.TC......TAC..C.A..G.. .......
P. antisianus     ....A ..TA.....T.TT.CC.....GT .......
P. auriceps       ....A ..TA.....T.TT.CC.....GT .......
P. pavoninus      ....A ..TA.....T.TT.CC.....GT .......
E. neoxenus       ..... CCTA...A...AC..CC.....T .......
T. curucui        ....A C......A...AC..C..... .......
T. violaceus      ..... C......A...AC..C..... .......
T. viridis        ..... C..C...A...AT..T..... .......
T. comptus        ..... C..........AC.C..... ..C....
T. melanurus      ..... C.........T.AC..... ..C....
T. elegans        ....A C..C.......AC.CC.C....T .......
T. rufus          ..... C..........AT.CC.C....T .......
T. collaris       ..... C..........GC.CC.C....T .......
T. mexicanus      ..... C..........AC..C.C..G.T .......
T. personatus     ..... C.T.......AC.CC.C....T .......
C. colius         ..T.A .T.C....CA.AC-CC.A..... ..C.C..
C. striatus       ..T.A CC.G...ACT.AC-CC.A..... ..C.C..
C. leucocephalus  ..T.A CT.CC..AC..AC-CC.A....T ..C....
C. sinensis       ..T.. ....G..A..T---CC.A.-... A......
C. fugax          ..c.A C......AG..AC-C.......T .......
G. velox          CAC.A CCTC...A.T.AC-CC.-.-C.. ...T...
M. whitneyi       T..GA ..A.A..-.A..C.C.....C.T ..CTC..
A. flammeus       ..... C...A..-.A..ATC..C...G. ..C.C..
A. acadicus       G.g.. ...C...-.G..A.C.C....GT ..C.C..
M. finschii       .AgCA C.TCA..-G...AACC.A..... .......
P. fulgidus       ..... CC.CG..-....AACC.A..... .......
A. ventralis      ..G.C CT.CA..-G...AACC.A..... .......
P. senilis        G.G.C CC.CG..-....AACC.A..... .......
A. tzacatl        ..... .G.AG....A.-C....A...G- A..A.CC
C. aureoventris   ..... .GTAG....A.TC.A.GA..-GT T.AGCCC
C. erithacus      ....A C..G...-..T.A.CC.C..C.T ....C..
C. americana      ....T C..A...-C...C..... A.C.C..
C. caudata        ..cC. C..A....-T..A.CC.A..... ....C..
M. nubicus        ..GCC CGAAC...-.T...CCCC...G. ..C.C..
T. todus          ..gC. C..AG..-.T....CC....C.T ....C..
M. mexicanus      GAgC. C.TA....-...CACC..... .......
M. momota         GAgC. C.TA....-...CACC....G.. ..A....
U. epops          GAc.A C..AC.G.--A.CACC--..--. T..AG.C
A. albirostris    T.tCA C..A.....T.TCTAGC.....T ..C.C..
A. prasinus       C.... C..CC..-....C.AT.A.C.TA ..-.C..
M. carolinus      ...CA ..........---.C.ATAA.T-T. AC-..C.
S. varius         ..ACA .G.C....-...CTCCCA.C-C. .C-..C.
C. vociferus      .A... ........G...C.CC....C.. .....C.
N. mcleodii       .A... ...........-A.CC.C..C.. .....C.
C. minor          .A... .GTC.......-A.CC.C..N.. ..C....
G. gallus         ....C C..C...-....AACCC-...T. C......
A. platyrhynchos  ..... C..C...-..A.A.C..C..G.T .......
C. buccinator     ..... C..C...-..A.A.C.....G.T .......
R. americana      C.... C..C...A....C.-..A..G.T .G.....
C. undulatus      ..... -..ACC.T.A..TAC..A..TTT .G..CC.
F. atra           T.... ...C...A...AC.-C.A..TG. ....C..
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16'

H. oreskios	G[ACCC	]CCATGACTAATCAAA-CA	CCAC	GAAA
H. ardens	.[TCTC	]AT.C..T....T...-..	....	....
H. diardii	A[CATC	]TT.C..TA.....-T.	....	....
P. temnurus	.[CCTA	]ATC.TGA.T.A.T..-T.	....	....
A. narina	.[TCCAT	]T..C...G..CT...-..	....	....
A. vittatum	.[CTCAACCTC	]A..C...GG.CT...-.G	....	....
P. antisianus	.[CACCT	].T.C....G.CG..T-..	....	....
P. auriceps	.[CACCC	].T.C....G.CG.GT-..	....	....
P. pavoninus	.[CACTT	].T.C....G.CG..T-..	....	....
E. neoxenus	.[CATCC	].TGC...CT....G.-..	....	....
T. curucui	.[CTC	]......TCT....C.-T.	....	....
T. violaceus	.[CCT	]......TCT.CT.C.-T.	....	....
T. viridis	.[CCC	].A....TCT.CT.C.-..	....	....
T. comptus	A[CCAC	]......TAT.CT.T.-T.	....	....
T. melanurus	.[CCCC	].T....TAT.CT.T.-T.	....	....
T. elegans	.[AGCA	]...C...CT.C..TC-T.	....	....
T. rufus	.[AACA	].......CC....TC-T.	....	....
T. collaris	.[AACA	]......TCT....TC-T.	....	....
T. mexicanus	.[GACA	]......TCT..T.TC-TG	....	....
T. personatus	.[AGCA	]......TCT....TT-T.	....	....
C. colius	.[TAC	]T..C..TAG.C.T.C-T.	....	....
C. striatus	.[TAT	]T..C..TA..C.C.T-TG	....	....
C. leucocephalus	.[TAT	]T.CA..TC..CTC.T-TG	....	....
C. sinensis	.[TA	]......TA..CT...-.-	....	....
C. fugax	.[CAAAT	]TAT...TA.....G.--.	....	C...
G. velox	A[GATTTTT	]T.T..GTA..C..G.C.T	....	....
M. whitneyi	.[AAAT	]..CC..TAC.A...CC.C	....	....
A. flammeus	.[TTTCAC	].ACG...A..A-..C-.C	....	....
A. acadicus	.[CAACGCA	]...C..TGG.-....-.T	....	....
M. finschii	A[CAC	].T.A...CTT..CC.-.C	....	....
P. fulgidus	.[CAC	]T..C...CC.C.CC.-.C	....	T...
A. ventralis	A[CAC	].A.C...CC...CC.-.C	....	....
P. senilis	A[CAC	]TA.C...CT.C.CT.-.T	....	....
A. tzacatl	T[AACTA	]A......CT.ATT.C-TG	T...	....
C. aureoventris	C[AGCCAA	]G..C...CT.ATT.C-TG	T...	....
C. erithacus	.[CAC	]TA..A.T.G.-.TGC-.G	....	....
C. americana	.[CACTA	]A..A....C.-.CCT-.G	....	....
C. caudata	.[TCCC	]...C..T...-.C..-GC	....	....
M. nubicus	.[CAAC	]TTCC......-.C..-.C	....	....
T. todus	.[TGCC	]TT....TCT.C.CCC-TC	....	....
M. mexicanus	.[CGC	]TT.C..T...-..T.-..	....	....
M. momota	.[CGC	]TT.C..T...-..T.-..	....	....
U. epops	.[	].A.C...C..CACTC-.C	....	....
A. albirostris	.[TGCC	]..GC...C..CT...-.T	....	....
A. prasinus	.[CAC	].A.G...CG.-.C..-.C	....	....
M. carolinus	A[CTT	].A.C..TC..-.CG.-AC	T...	....
S. varius	A[CTT	].AGC..TC..-.T..--T	T...	....
C. vociferus	.[CAC	].A....AC...TTC.CT.	....	....
N. mcleodii	.[CACT	]TA....TCT.-TTC.C..	....	....
C. minor	.[TAC	].A.........TTN.CAG	....	....
G. gallus	.[CCT	].A.C..T....TTT.-AC	....	....
A. platyrhynchos	.[AAAC	]TAGC..T....TTG.-AC	....	....
C. buccinator	.[GAAC	]TA.C....G..TT..-AC	....	....
R. americana	.[TAC	]...C..TC...TT..-GC	....	....
C. undulatus	.[TAC	]TA.A.......T.T.-.G	....	....
F. atra	.[CAC	]TA.C..TC..CT...-..	....	T...

	20	21		21'		
				** **		
H. oreskios	GCCAGGgtcCA	AAC	TGGG	ATTAGATAC	CCCA	CTA
H. ardens	.....c...	...	....	.....	....	...
H. diardii	.....ac...	...	....	.....	....	...
P. temnurus	..T....c...	...	....	.....	....	...
A. narina	..T...act..	...	....	.....	....	...
A. vittatum	..T...ac...	...	....	.....	....	...
P. antisianus	..T...ac...	...	....	.....	....	...
P. auriceps	..T...ac...	...	....	.....	....	...
P. pavoninus	..T...ac...	...	....	.....	....	...
E. neoxenus	..T...ac...	...	....	.....	....	...
T. curucui	..T....c...	...	....	.....	....	...
T. violaceus	..T....c...	...	....	.....	....	...
T. viridis	..T....c...	...	....	.....	....	...
T. comptus	..T...ac...	...	....	.....	....	...
T. melanurus	..T...ac...	...	....	.....	....	...
T. elegans	..T....c...	...	....	.....	....	...
T. rufus	..T...a....	...	....	.....	....	...
T. collaris	..T...ac...	...	....	.....	....	...
T. mexicanus	..T....c...	...	....	.....	....	...
T. personatus	..T....c...	...	....	.....	....	...
C. colius	..T.T.aca..	...	....	.....	....	...
C. striatus	..T.T.aca..	...	....	.....	....	...
C. leucocephalus	..T...aca..	...	....	.....	....	...
C. sinensis	..T.A.aca..	...	....	.....	....	...
C. fugax	..T...aca..	..T	....	.....	....	...
G. velox	..T.....a..	...	....	.....	....	...
M. whitneyi	.....c...	...	....	.....	....	...
A. flammeus	...T...ct..	...	....	.....	....	...
A. acadicus	.....c...	...	....	.....	....	...
M. finschii	.....ca..	...	....	.....	....	...
P. fulgidus	..T..Aa.a..	...	....	.....	....	...
A. ventralis	.....ca..	...	....	.....	....	...
P. senilis	.....aca..	...	....	.....	....	...
A. tzacatl	..T.A.aca..	...	....	.....	....	...
C. aureoventris	....A.aca..	...	....	.....	....	...
C. erithacus	..T....c...	...	....	.....	....	...
C. americana	.....a..	...	....	.....	....	...
C. caudata	..T....ca..	...	....	.....	....	...
M. nubicus	..TGA...t..	...	....	.....	....	...
T. todus	..T....ca..	...	....	.....	....	...
M. mexicanus	..T.....a..	...	....	.....	....	...
M. momota	..T.....a..	...	....	.....	....	...
U. epops	..T...aca..	...	....	.....	....	...
A. albirostris	.....aca..	...	....	.....	....	...
A. prasinus	...G..aaa..	...	....	.....	....	...
M. carolinus	.....ga..	...	....	.....	....	...
S. varius	.....aa..	...	....	.....	....	...
C. vociferus	..T....ca..	...	....	.....	....	...
N. mcleodii	..T...aca..	...	....	.....	....	...
C. minor	.....aNa..	N.N	....	.....	....	...
G. gallus	..T...ac...	...	....	.....	....	...
A. platyrhynchos	.....c...	...	....	.....	....	...
C. buccinator	.....c...	...	....	.....	....	...
R. americana	..T.A..ca..	...	....	.....	....	...
C. undulatus	....A..aa..	...	....	.....	....	...
F. atra	....A.aca..	...	....	.....	....	...

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                    20'                    22
H.oreskios          TGCCTGGC CCTAAATCTT GATGcGCT [ATATC      ]ACCA
H.ardens            ........ .......... ........ [GCCCC      ]....
H.diardii           ........ .......... ........ [ACTCT      ]....
P.temnurus          .....A.. .T.....TC. ........ [ACTCT      ]....
A.narina            .....A.. .......... ........ [ACTCCT     ]...T
A.vittatum          .....A.. .......... ....t... [ACTCCC     ]...T
P.antisianus        .....T.. .........C ........ [ACCCT      ]..T.
P.auriceps          .....T.. .........C ........ [ACCCT      ]..T.
P.pavoninus         .....A.. .........CC ........ [ACCCT      ]..TG
E.neoxenus          .....T.. .........C. ........ [ACCCT      ]..TT
T.curucui           .....A.. TA......C. A....... [ACATTC     ]....
T.violaceus         .....A.. TA......C. A....... [GCATTC     ]....
T.viridis           .....A.. TA......C. A....... [ATATTC     ]....
T.comptus           .....A.. .A......C. ....t... [ATATTC     ]..T.
T.melanurus         .....A.. .A......C. ....t... [ACATTC     ]..T.
T.elegans           .....A.. .........C ........ [ATACTC     ]....
T.rufus             .....A.. .G......CC ........ [ATACTT     ]....
T.collaris          .....A.. .........C. ........ [ACACTC     ]....
T.mexicanus         .....A.. .........C. A....... [ACACTC     ]....
T.personatus        .....A.. .T......C. ........ [ATACTC     ]....
C.colius            .....A.. .A......CA ...C-CTC [CCCTC      ]...T
C.striatus          .....A.. .A......CA ...C-CTC [CACCC      ]...T
C.leucocephalus     .....A.. .A......CA ...T-CTC [CCTTT      ]..TT
C.sinensis          ...T.A.. .......T.. .G.A-CT. [AACTT      ]....
C.fugax             .....A.. .......... ...A-CT. [AACCTT     ]....
G.velox             .....A.. .......... .G..-T.. [CACCCT     ]..T.
M.whitneyi          ........ .........C ....-CTC [TCCATA     ]..T.
A.flammeus          ....A... .........C. A...-CTC [CCACC      ]....
A.acadicus          .....A.. .A......CC ....-T.C [ACTATCACCC]....
M.finschii          .....A.. .......... ....-TT. [CCAAC      ]..A.
P.fulgidus          .....A.. .........C. ....-TT. [TCCAC      ]..AC
A.ventralis         ........ .......... ....-CT. [CCCAG      ]..AC
P.senilis           ........ .......... ....-T.. [CCCAA      ]..AC
A.tzacatl           .....A.. .T........ ..CA-CT. [CCTAG      ]....
C.aureoventris      ...T.... .T........ ..CA-CT. [CCCCA      ]..T.
C.erithacus         .....A.. .........A AG..-CT. [ATGCTAC    ]....
C.americana         ........ .........A ....-TTC [ACCACCC    ]....
C.caudata           .....A.. .........CA ....-T.C [ATTCC      ]..T.
M.nubicus           ...TCA.. .........AC AG..-TT. [TCCCT      ]..T.
T.todus             .....A.. .......... ...A-CT. [ACCCC      ]....
M.mexicanus         .....A.. .......T.A ..C.-CT. [ACCCT      ]..TG
M.momota            .....A.. .......T.A ....-CT. [ACCCT      ]..TG
U.epops             .....A.. .........A ....-CT. [CCATCC     ]....
A.albirostris       .....A.. .......... ....-TT. [TCCGC      ]..T.
A.prasinus          ....CT.. .........C. .G..-C.C [TTACT      ]....
M.carolinus         ........ .G.T...TCA A...-CT. [TCTT       ]...T
S.varius            ........ .G.....TCA A...-CT. [TTTATT     ]...T
C.vociferus         .....A.. .A.......C ..CA-CT. [GTTTA      ]....
N.mcleodii          .....A.. .........C. .....-CT. [ACTTA      ]....
C.minor             ........ .........C. ...A-CT. [ACTTA      ]....
G.gallus            .....A.. .........A ...A-C.. [CCCATC     ]..AC
A.platyrhynchos     ........ .......... ...A-CT. [ACCCT      ]...G
C.buccinator        ........ .......... ...A-CT. [ACTTT      ]...G
R.americana         ...T.A.. .........CC ...A-CT. [ACCCC      ]...C
C.undulatus         ...T.... .........A .....-CT. [AACCT      ]....
F.atra              ...T.... .........A ...A-CT. [CTTCCCC    ]..T.
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	22'		15'		23		23'
H. oreskios	AGCCATC	C	GCC	TGGGAACTACG	AGC	ACAAAC	GCT
H. ardens	.....	.	...	.....	...	.....	...
H. diardii	.....	.	...	.....	...	.....	...
P. temnurus	.....	.	...	.....	...	.T....	...
A. narina	.....	.	...	.....	...	.....	...
A. vittatum	.....	.	...	.....	...	.....	...
P. antisianus	.....	.	...	..A.....	...	.....	...
P. auriceps	.....	.	...	..A.....	...	.....	...
P. pavoninus	.....	.	...	..A.....	...	.....	...
E. neoxenus	..T....	.	...	..A.....	...	.....	...
T. curucui	.....T	.	...	....G.....	...	.....	...
T. violaceus	.....T	.	...	.....	...	.....	...
T. viridis	.....T	.	...	.....	...	.....	...
T. comptus	.....T	.	...	.....	...	.....	...
T. melanurus	.....T	.	...	.....	...	.....	...
T. elegans	.....T	.	...	.....	...	.....	...
T. rufus	.....T	.	...	.....	...	.....	...
T. collaris	.....T	.	...	....T.....	...	.....	...
T. mexicanus	.....T	.	...	.....	...	.....	...
T. personatus	.....T	.	...	.....	...	.....	...
C. colius	GAGT...	.	...	C.A...T....	...	.....	...
C. striatus	GAGT...	.	...	C.A.....	...	.....	...
C. leucocephalus	GAG....	.	...	C.A.....	...	.....	...
C. sinensis	.AGT.C.	.	...	..A.....	...	G.....	...
C. fugax	.AGT...	.	...	C.A.....	...	.....	...
G. velox	..A..C.	.	...	..A.....	...	.....	...
M. whitneyi	GAG....	.	...	A.AAT.....	...	.....	...
A. flammeus	GAG...T	.	...	A...C.....	...	.....	...
A. acadicus	G.A....	.	...	A.A.C.....	...	.....	...
M. finschii	.AA....	.	...	..A.....	...	.....	...
P. fulgidus	.AA....	.	...	C.A.....	...	.....	...
A. ventralis	.AA....	.	...	C.A.....	...	.....	...
P. senilis	..A....	.	...	C.A.....	...	.....	...
A. tzacatl	.AGTG..	.	...	..A.....	...	.....	...
C. aureoventris	.AGTG..	.	...	..A.....	...	.....	...
C. erithacus	.AG..C.	.	...	A.A.T.T....	...	.....	...
C. americana	.AA....	.	...	A.A...T....	...	.....	...
C. caudata	GAA....	.	...	..A.....	...	.....	...
M. nubicus	.AA..C.	.	...	C.A.....	...	.....	...
T. todus	.AGT...	.	...	..A...T....	...	.....	...
M. mexicanus	.AG.C..	.	...	..A.T.T....	...	.....	...
M. momota	.AG.T..	.	...	C.A.T.T....	...	.....	...
U. epops	.AG....	.	...	C.A.....	...	.T....	...
A. albirostris	.AA....	.	...	C.A.G.....	...	.....	...
A. prasinus	GAG..C.	.	...	C...G.....	...	.....	...
M. carolinus	.AG...T	.	...	A.....T....	...	.....	...
S. varius	.AG...T	.	...	A.....	...	.....	...
C. vociferus	.AGTG..	.	...	C.A.....	...	.....	...
N. mcleodii	.AGT...	.	...	..A.....	...	.....	...
C. minor	.AGT...	.	...	..A.....	...	.....	...
G. gallus	.TGT...	.	...	..A.....	...	.....	...
A. platyrhynchos	.AGT...	.	...	ATAT.....	...	.....	...
C. buccinator	.AGT...	.	...	A.A.....	...	.....	C..
R. americana	.AGT...	.	...	C.A.....	...	.....	...
C. undulatus	.AG...T	.	...	C.A.....	...	.T....	...
F. atra	.AGT...	.	...	..A.....	...	.....	...

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                                    2'          24
                                    *           *
H. oreskios          TAAAACTCTAA GGA CT TGGCgGTGCCCCA AACCCAC
H. ardens            ........... ... .. ............. .......
H. diardii           ........... ... .. ..........T. .......
P. temnurus          ......C.... ... .. .........T.. ..T....
A. narina            ......C.... ... .. .......A..T.. .......
A. vittatum          ......C.... ... .. .........T.. ..T....
P. antisianus        ........... ... .. ............. ..A....
P. auriceps          ........... ... .. ............. ..A....
P. pavoninus         ........... ... .. ............. ..A....
E. neoxenus          .G......... ... .. ............. ..A....
T. curucui           ......C.... ... .. ........TT... .....C.
T. violaceus         ......C.... ... .. ........TT... .....C.
T. viridis           ......C.... ... .. ........TT... .....C.
T. comptus           ......C.... ... .. ........T.... .......
T. melanurus         ......C.... ... .. ........TT... .......
T. elegans           ......C.... ... .. ........TT... ..T....
T. rufus             ......C.... ... .. ........TT... .......
T. collaris          ......C.... ... .. ........TT... .......
T. mexicanus         ......C.... ... .. ........TT... .......
T. personatus        ......C.... ... .. ............. .......
C. colius            ........... ... .. ............. .......
C. striatus          ........... ... .. .......A..... .......
C. leucocephalus     ...C....... ... .. ............. .......
C. sinensis          ........... ... .. ............. .......
C. fugax             ........... ... .. ..........T. .......
G. velox             ........... ... .. ........T.... .......
M. whitneyi          ........... ... .. ............. .......
A. flammeus          ......C.... ... .. .......A...T. .......
A. acadicus          .G......... ... .. ............. ..T....
M. finschii          ........... ... .. ...........T. .......
P. fulgidus          ........... ... .. ...........T. C......
A. ventralis         ........... ... .. ...........T. .......
P. senilis           ........... ... .. ...........T. .......
A. tzacatl           ........... ... .. ..........T.. .......
C. aureoventris      ........... ... .. ............. .......
C. erithacus         ........... ... .. ........T.... .......
C. americana         .G......... ... .. .......A..... .......
C. caudata           ........... ... .. ........T..T. C......
M. nubicus           .G......... ... .. ............. --.....
T. todus             ........... ... .. ............. .......
M. mexicanus         ........... ... .. ........T.T.. G......
M. momota            ........... ... .. ........T.T.. G......
U. epops             .G......... ... .. .........T.T. .......
A. albirostris       ........... ... .. ............. .......
A. prasinus          ......C.... ... .. ........T.... .....C.
M. carolinus         ......C.... ... .. ........T.... .......
S. varius            ......C.... ... .. ........T.... .......
C. vociferus         ........... ... .. ............. .......
N. mcleodii          ........... ... .. ............. .......
C. minor             ........... ... .. ........T.... .......
G. gallus            ........... ... .. ............. .......
A. platyrhynchos     ........... ... .. ........-..T. .......
C. buccinator        ........... ... .. .........-... .......
R. americana         ........... ... .. .........-.T. .......
C. undulatus         ........... ... .. .........-.T. .......
F. atra              ........... ... .. .........T... .......
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	25		26	27		27'
					*	
H. oreskios	CTAGA	GG	AGCCTGTTCT	GTA	ATCG	ATAACCCA	CGAT
H. ardens	.G...	..	....	...	....	....	....
H. diardii	.....	..	....	...	....	....	....
P. temnurus	.....	..	....	A..	....	....	....
A. narina	.....	..	....	A..	....	....	....
A. vittatum	...A.	..	....	...	....	....	....
P. antisianus	.....	..	....	A..	....	.C......	....
P. auriceps	.....	..	....	A..	....	.C......	....
P. pavoninus	.....	..	....	A..	....	.C......	....
E. neoxenus	.....	..	....	A..	....	.C......	....
T. curucui	.....	..	....	A..	....	....T...	....
T. violaceus	.....	..	....	A..	....	....T...	....
T. viridis	.....	..	....	A..	....	....T...	....
T. comptus	.....	..	....	...	....	....	....
T. melanurus	.....	..	....	A..	....	....	....
T. elegans	.....	..	....	A..	....	....	....
T. rufus	.....	..	....	A..	....	....	....
T. collaris	.....	..	....	A..	....	....	....
T. mexicanus	.....	..	....	A..	....	....	....
T. personatus	.....	..	....	A..	....	....T...	....
C. colius	.....	..	....	...	....	....	....
C. striatus	.....	..	....	...	....	....	....
C. leucocephalus	.....	..	....	A..	....	....	....
C. sinensis	.....	..	....	A..	....	...TT...	....
C. fugax	.....	..	....	...	....	....	..C.
G. velox	.....	..	....	...	....	....	....
M. whitneyi	.....	..	.......C..	A..	....	....	....
A. flammeus	.....	..	.......C..	A..	....	....	....
A. acadicus	.....	..	.......C..	...	....	.C......	....
M. finschii	.....	..	....	A..	....	....T...	....
P. fulgidus	.....	..	....	A..	....	....	....
A. ventralis	.....	..	....	A..	....	....	....
P. senilis	.....	..	....	A..	....	....	....
A. tzacatl	.....	..	....	A..	....	....T...	..C.
C. aureoventris	.....	..	....	A..	....	.......G	..C.
C. erithacus	.....	..	....	A..	....	....	....
C. americana	.....	..	.......C..	A..	....	....	....
C. caudata	.....	..	....	A..	....	....	....
M. nubicus	.....	..	....	A..	....	....	....
T. todus	.....	..	.......C..	A..	....	....	....
M. mexicanus	.....	..	.......C..	A..	....	....	....
M. momota	.....	..	.......C..	A..	....	....	....
U. epops	.....	..	....	A..	....	..G.....	..C.
A. albirostris	.....	..	....	...	....	....	....
A. prasinus	.....	..	....	...	....	....	..C.
M. carolinus	.....	..	....	A..	....	....	..C.
S. varius	.....	..	....	A..	....	....	..C.
C. vociferus	.....	..	....	A..	....	....T...	....
N. mcleodii	.....	..	....	A..	....	....	....
C. minor	.....	..	....	A..	....	....	....
G. gallus	.....	..	....	A..	....	....T...	....
A. platyrhynchos	.....	..	....	...	....	..G.T...	....
C. buccinator	.....	..	....	AC.	....	....T...	....
R. americana	.....	..	....	A..	....	....	....
C. undulatus	.....	..	....	A..	....	...C....	....
F. atra	.....	..	....	...	....	....	....

	28		29
H. oreskios	ACACCCAA	CCTCCCC	TTGCC---AAACA	GCcTAcaTACC
H. ardens	.T.....	.....	.....---.GG..	.....
H. diardii	.T.....	.....	.....---.G...	.....
P. temnurus	G.....	..C....	.....---.G...	.....
A. narina	G....TG.	..C.TT.	.....---.....	.....
A. vittatum	G....T..	..C.TT.	.....---.....	.....
P. antisianus	G.....G.	..C....	....T--T..G..	.....t.....
P. auriceps	G.....G.	..C....	.....---T..G..	.....t.....
P. pavoninus	G.....G.	.....	.....---T..G..	.....t.....
E. neoxenus	.....G.	..C....	.....---T.....	.....
T. curucui	.....G.	....T..	.....---AT.T..	.....
T. violaceus	.T....G.	....T..	.....---AT.T..	.....
T. viridis	.....G.	....T..	.....---AC.T..	.....
T. comptus	G.....G.	.....	.....---AC.-..	.....
T. melanurus	.....G.	.....	.....---AC.-..	.....
T. elegans	.....G.	.....	...T.--AC.T..	.....
T. rufus	.....G.	.....	.....--AC.C..	.....
T. collaris	.T....G.	.....	...T.--AC.C..	.....a.....
T. mexicanus	G.....	.....	...T.--AT.T..	.....
T. personatus	.T....G.	.....	.....--AC.C..	.....
C. colius	.....T..	..A.TT.	.A...-GC.....	.....t.....
C. striatus	.....T..	..A.TT.	.A...--A.....	.....t.....
C. leucocephalus	.....	..A.TT.	.A...--A.....	.....t.....
C. sinensis	.....	..G..T.	.C...-AA.....	.....
C. fugax	.....	..GT.T.	.G...T-G..G..	.....
G. velox	C.....	..GAT..	.A...TAA..T..	.....t.....
M. whitneyi	.....	..G..T.	.....--A..-..	..t.....
A. flammeus	.....G.	..A..T.	.....--A.G-..	.....
A. acadicus	.....G.	..A....	.A..AC-G..-..	.....t.....
M. finschii	.....G.	..A.TT.	.C...--A..G..	.....
P. fulgidus	.....G.	..A.TT.	.....--A.....	.....
A. ventralis	C.....G.	..A.TT.	.....--A.....	.....
P. senilis	C.....G.	..A.TT.	.....--A.....	.....
A. tzacatl	.....	..G.T.T	....T-TGCT-..	.....
C. aureoventris	.....G.	..G.T.T	.....-CACT-..	.....
C. erithacus	.....G.	..G....	.G...--A..G..	.....
C. americana	.....G.	..A....	.A...--CG.G..	.....
C. caudata	G.....	.....	.....--A..G..	.....
M. nubicus	.....	..GTT..	.....--A..G..	.....
T. todus	C.....	..G.TT.	.A...--AG.G..	.....
M. mexicanus	G.....G.	..A.T..	.A...--AG.G..	.....
M. momota	G.....G.	..ATT..	.A...--A..G..	.....
U. epops	.....	..C..T.	....---AG.G..	.....
A. albirostris	.....	..C.TT.	.....--A..G..	.....t.....
A. prasinus	.....C.	..C.T..	.....--C..G..	.....
M. carolinus	.....	.....	....AAT-.....	.....t.....
S. varius	.....	..C....	.A...ATG.....	.....t.....
C. vociferus	C.....	..G.TT.	.....--AG....	.....
N. mcleodii	T.....	..G.TT.	.....--A..G..	.....
C. minor	.T.....	..G....	.....--A..G..	.....
G. gallus	T.....	..A....	.....--AGC...	.....
A. platyrhynchos	CA.....	..G....	.....-GA.C...	.....
C. buccinator	TA.....	..A....	.....--A.C...	.....
R. americana	.....G.	..AT.T.	.....--C.TG..	.....
C. undulatus	T.....	..AT...	.C...-CC.....	.....
F. atra	.T.....	..C.T..	.....-CA..G..	.....

	30	31	32	32'
H. oreskios	G CCGTC	TCCA GCTCGC	CCTT	-CCTG A-GGG
H. ardens	. .....	.A..	....	-.T.. .-A..
H. diardii	. .....	.A..	....	-.T.. .-A..
P. temnurus	. .....	....	...C	-T... .-A..
A. narina	. .....	....	...C	-.... .-A..
A. vittatum	. .....	....	...C	-.... .-A..
P. antisianus	. .....	....	....	-.A.. .-A..
P. auriceps	. .....	....	....	-.A.. .-A..
P. pavoninus	. .....	....	...C	-.A.. .-A..
E. neoxenus	. .....	....	....	-TA.. .-A..
T. curucui	. .....	....	...-	-A... .-A..
T. violaceus	. .....	....	...-	-A... .-A..
T. viridis	. .....	....	....	TA... .-...
T. comptus	. .....	....	....	-A... .-A..
T. melanurus	. .....	.T..	....	AA... .-...
T. elegans	. .....	....	....	CA... .G...
T. rufus	. .....	....	.TC.	CA... .-.A.
T. collaris	. .....	....	....	CG... .-...
T. mexicanus	. .....	....	....	TA... .-...
T. personatus	. .....	....	....	CG... .-A..
C. colius	. .....	G...	.-..	AAT.. .-.A.
C. striatus	. .....	GT.. ...T..	.-..	AAT.. .-.A.
C. leucocephalus	. .....	G... ...T..	.-.C	AAT.. .-AA.
C. sinensis	. .....	C... ....A.	.-.A	TA... .-AA.
C. fugax	. .....	G... ..C.A.	.-.C	C.... .-AA.
G. velox	. .....	G... ..C.A.	.-.C	ATT.. .-AA.
M. whitneyi	. .....	GT.. .TC.A.	.-.C	--A.. .-.A.
A. flammeus	. .....	G... ..C.A.	.-..	--T.. .-AA.
A. acadicus	. .....	G... ....A.	.-.C	-.... .-.A.
M. finschii	. .....	A... ..C.A.	.-.C	C-T.. .-.A.
P. fulgidus	. .....	GT.. ..C.A.	.-.C	C-G.. .-.A.
A. ventralis	. .....	C... ..C.A.	.-.C	T-G.. .-.A.
P. senilis	. .....	C... ..C.A.	.-.C	T-A.. .-.A.
A. tzacatl	. .....	G... .TC.A.	.-.A	T.T.. .-AA.
C. aureoventris	. .....	G... .TC.A.	.-.A	T.... .-.A.
C. erithacus	. .....	A... .TC.A.	.-..	C.... .-AA.
C. americana	. .....	A... ..C.A.	.-.C	-AT.. .-AA.
C. caudata	. .....	G... ...TA.	.-CC	CAA.. .-A..
M. nubicus	. .....	GT.. ..C.A.	.-..	TA... .-AA.
T. todus	. .....	G... ..C.A.	.-.C	C.... .-.A.
M. mexicanus	. .....	G... ....A.	.-.C	CA... .-.A.
M. momota	. .....	G... ....A.	.-.C	CA... .-.A.
U. epops	. .....	G... ..C.A.	.-..	C.... .-AA.
A. albirostris	. .....	G... ..C.A.	.-.C	C.T.. .-.A.
A. prasinus	. .....	G... ..C.A.	.-.C	A.A.. .-TA.
M. carolinus	. .....	GT.. ..C.A.	.-.C	CA... .-.A.
S. varius	. .....	GT.. ....A.	.-.C	CAT.. .-AA.
C. vociferus	. .....	G... ..C.A.	.-.C	-AT.. .-.A.
N. mcleodii	. .....	GT.. ..C.A.	.-.C	-AT.. .-.A.
C. minor	. .....	G... ..C.A.	.-.C	-AT.. .-.A.
G. gallus	. .....	G... ..C.A.	.-.C	TAA.. .-AA.
A. platyrhynchos	. .....	G... ..C.A.	.-.C	GAA.. .-.A.
C. buccinator	. .....	G... ..C.A.	.-.C	GAA.. .-.A.
R. americana	. .....	C... ..C...	.-.C	AAA.. .-.A.
C. undulatus	. .....	G... ..C...	.TAC	-TGAA .-.A.
F. atra	. .....	C... ....A.	.-.G	C.... .-TA.

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                                  31'          33
H. oreskios          -TTCAATA GCGAGC ATGA TAAC CC[TAATTT  ]C
H. ardens            -.C..... ...G.. .... ..G. ..[CGATTCT ].
H. diardii           -.C..... ...... .... ..G. ..[CTATTAT ].
P. temnurus          -CCA.GC. ..A... G.A. ..G. TA[CT      ].
A. narina            -.G...C. ...... .CA. ..G. .T[CAAACTTT].
A. vittatum          -.CT..C. ...... .CA. ..G. .T[TGAATCAT].
P. antisianus        -CCT..C. ...... .CA. ..G. T.[CAACTCT ].
P. auriceps          -CCT.... ...... .CA. ..G. T.[CAACTCT ].
P. pavoninus         -CCT..C. ...... .CA. ..G. ..[TAACTCT ].
E. neoxenus          -CCT..C. ...... .CA. ..G. ..[AGCTCTA ].
T. curucui           -CCT.... ...... .CA. ..G. ..[AAGCCTT ].
T. violaceus         -.CT..C. ...... ..A. ..G. .T[AAGCCTT ].
T. viridis           -.CT..C. ...... ..A. ..G. ..[GAGCCTT ].
T. comptus           -.....C. ...G.. TCA. ..G. .A[AAATCTT ].
T. melanurus         -.CT..C. ...... TCA. ..G. .A[CAATCTT ].
T. elegans           -CC...C. ...... GCA. ..G. ..[CTATCTC ].
T. rufus             -CC...C. ...... GCA. ..G. .T[CAACCC  ].
T. collaris          -CC...C. ...... GCA. ..G. ..[CACTACTC].
T. mexicanus         -CCT..C. ...... ..A. ..G. ..[CACCCC  ].
T. personatus        -CC...C. ...... GCA. ..G. ..[CC      ].
C. colius            -.A..GC. ..A... .CA. ..G. .A[CTACACG ].
C. striatus          -.G...C. ..A... .CA. .... .A[CCACG   ].
C. leucocephalus     -.G...C. ..A... .CA. ..G. TA[TCCCACCA].
C. sinensis          -AA...C. .T.... TCA. ..G. .T[AATTC   ].
C. fugax             -AAT..C. .T.G.. .CA. ..G. ..[TC      ].
G. velox             -GA...C. .T.... .CA. C.G. ..[TATCC   ].
M. whitneyi          -CC...C. .T.GT. .CA. ..GT .A[CCAACC  ].
A. flammeus          -.GT..C. .T.GA. .CA. ..G. ..[CAATC   ]T
A. acadicus          -.....C. .T.... GCA. G.GT TG[AACC    ].
M. finschii          -.A...C. .T.... CCA. C.G. .A[CTATC   ].
P. fulgidus          -.A...C. .T.... CCA. C.G. ..[ATCAACC ].
A. ventralis         -CA...C. .T.... CCA. C.G. ..[CCACTC  ].
P. senilis           -CA..... .T.... TCA. ..G. ..[ACACCC  ].
A. tzacatl           -AAT..C. .T..A. .CA. C.G. .T[CAACCA  ].
C. aureoventris      -AA...C. .T.G.. ..A. C.G. ..[TAACCA  ].
C. erithacus         -.C.C.C. .T.GA. .CA. .... ..[AAACC   ].
C. americana         -AC.T.C. .T.... .CA. C.G. ..[CT      ].
C. caudata           -AG.C.C. .TA... GAA. ..G. .T[TCT     ].
M. nubicus           -AC..CA. .T.G.. .CA. ..GT TG[CGCCCTA ].
T. todus             -CG..GC. .T.... .CA. ..G. AT[CTAAC   ].
M. mexicanus         -CA..... .T.... .CA. C.GT .A[AAATC   ].
M. momota            -CAT..C. .T.... .CA. C.G. ..[AAAATC  ].
U. epops             -.A.C.C. ..CG.. .CA. C.GT ..[CC      ].
A. albirostris       -CA...C. .T.G.. CCA. ..GT ..[CCAACCCC].
A. prasinus          -.A...C. .T.GA. .CA. C..T TT[CCT     ].
M. carolinus         -.C...C. .T.... ..A. ..G. TT[CACC    ].
S. varius            -CC...C. .T.... .CA. C.GT .T[CACC    ].
C. vociferus         -CA...C. .T.G.. TAA. ..G. ..[TAC     ].
N. mcleodii          -CAT..C. .T.... TAA. ..G. ..[CTAAAC  ].
G. gallus            -AA...C. .T.... TCA. ..G. ..[CT      ].
A. platyrhynchos     -CG...C. .T.G.. GCA. C.G. A.[CC      ].
C. buccinator        -CA...C. .T.GA. .CA. ..G. AT[CC      ].
R. americana         -AA...C. ..A... ..A. ..G. TA[CC      ].
C. minor             -.AT..C. .T.... CAA. ..G. ..[CCCAC   ].
C. undulatus         ---T.... ...GT. ..A. ..GT ..[AT      ].
F. atra              TC...... .T.... .CA. ..G. A.[CT      ].
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	33'		30'		29'		28'
H. oreskios	GCTA	ATAA	GACaGG	TCAA	GGTATAGC	TAAT	GGGGTGG
H. ardens	....	....	T.....	....	....	....	....
H. diardii	....	....	......	....	....	....	....
P. temnurus	....	.C..	......	....	....	....	....
A. narina	....	G...	......	....	....	....	.AA.G..
A. vittatum	....	G...	......	....	....	....	.AA.G..
P. antisianus	....	G...	......	....	....	...C	....
P. auriceps	....	G...	......	....	....	...C	....
P. pavoninus	....	G...	......	....	....	...C	....
E. neoxenus	....	.C..	......	....	....	.G..	....
T. curucui	....	.C..	......	....	....	....	....A..
T. violaceus	....	.C..	......	....	....	....	....A..
T. viridis	....	GC..	......	....	....	....	....A..
T. comptus	....	.C..	......	....	....	....	....
T. melanurus	....	.C..	......	....	....	....	..A....
T. elegans	....	.A..	......	....	....	....	....
T. rufus	....	.A..	......	....	....	....	....
T. collaris	....	.A..	......	....	....	....	....
T. mexicanus	....	.A..	......	....	....	....	....
T. personatus	....	.A..	......	....	....	....	....C..
C. colius	....	.A..	......	....	....	CC..	.AA....
C. striatus	....	.A..	......	....	....	CC..	.AA....
C. leucocephalus	....	.A..	......	....	....	CC..	.AA....
C. sinensis	....	.C..	......	....	....	.C..	.A..C..
C. fugax	....	G...	......	....	....	.T..	...AC..
G. velox	....	GC..	......	....	....	.C..	..ATC..
M. whitneyi	A...	....	......	....	....	CC..	.A..C..
A. flammeus	....	.A..	......	....	....	CT..	.A.....
A. acadicus	A...	....	......	..G.	....	.C..	....C..
M. finschii	....	.C..	......	....	....	CT..	.AA....
P. fulgidus	A...	.C..	......	....	....	CT.C	.AA....
A. ventralis	....	....	......	....	....	CC.C	.AA....
P. senilis	....	....	......	....	....	CC.C	.AA....
A. tzacatl	....	.A..	......	....	....	CC..	..A.C..
C. aureoventris	....	.A..	......	....	....	CC..	A.A.C..
C. erithacus	....	.C..	......	....	....	.C..	.T..C..
C. americana	....	.C..	......	....	....	C..C	....
C. caudata	....	.C..	......	....	....	CC..	....C..
M. nubicus	A...	GC..	......	....	....	C...	..ACG..
T. todus	....	GC..	......	....	....	CC.C	..A.G..
M. mexicanus	....	....	......	....	....	C...	..AT...
M. momota	....	.C..	......	....	....	C...	..AT...
U. epops	A...	....	......	....	....	CC..	.A..G..
A. albirostris	A...	....	......	....	....	CC..	.AA.G..
A. prasinus	.T..	.C..	......	....	....	CT..	....G..
M. carolinus	....	.A..	......	....	....	CC..	....G..
S. varius	....	.A..	......	....	....	C...	....G..
C. vociferus	....	.C..	......	....	....	.C..	.AA.C..
N. mcleodii	....	.C..	......	....	....	CC..	.AA.C..
C. minor	....	.C..	......	....	....	CC..	....C..
G. gallus	....	....	......	....	....	CT..	....
A. platyrhynchos	....	....	......	....	....	CT..	...AC..
C. buccinator	....	....	......	....	....	CT..	..A....
R. americana	....	GC..	......	....	....	AT..	.A.A...
C. undulatus	A...	....	......	....	....	CC..	...A...
F. atra	....	G...	......	....	....	CC..	..A.G..

	26'	34	34'
H. oreskios	-A AGaAATGGGCT	ACA TTTTCT	AA-AGT-- AGA-ACA
H. ardens	-.	... .	..-.A.-- ...-.T.
H. diardii	-.	... .	.T-.A.-- ...-.T.
P. temnurus	-.	... .	.G-CA.-- ...-.T.
A. narina	-.	... ..C...	..-.A.AG ...-.AT
A. vittatum	-.	... ..C...	..-.A.AG ...-.AT
P. antisianus	-.	... .	..-.A.-- ...-.A.
P. auriceps	-.	... .	..-.A.-- ...-.T.
P. pavoninus	-.	... .	..-.A.-- ...-.T.
E. neoxenus	-.	... .	..-.A.-- ...-...
T. curucui	-.	... .	..-.A.-- ...-.T.
T. violaceus	-.	... .	..-.A.-- ...-.T.
T. viridis	-.	... .	..-.A.-- ...-.T.
T. comptus	-.	... .	..-GA.-- ...-...
T. melanurus	-.	... .	..-GA.-- ...-...
T. elegans	-.	... .	..-.A.-- ...-.T.
T. rufus	-.	... .	..-.A.-- ...-.T.
T. collaris	-. ..t.....	... .	..-.A.-- ...-.T.
T. mexicanus	-.	... .	..-.A.-- ...-.T.
T. personatus	-.	... .	..-.A.-- ...-.T.
C. colius	-.	... .	.T-.A.-- ...-...
C. striatus	-.	... .	.T-.A.-- ...-...
C. leucocephalus	-.	... ..C...	.C-.A.-- ...-GT.
C. sinensis	-.	... C.C...	..CCC.-- ...-.A.
C. fugax	-.	... .	..C.A.-- ...-.A.
G. velox	-. ..t.....	... ..C...	.CC.A.AG ...-...
M. whitneyi	-. ...G.....	... C.C...	..-.A.-- ...-GA.
A. flammeus	-. ...G.....	... ..C...	GG-CA.C- ...-G..
A. acadicus	-. ...G.....	... ..C...	..-.A.-- ...-GA.
M. finschii	-.	... .	..-.A.-- ...T.A.
P. fulgidus	-.	... .	..C...-- ...-.T.
A. ventralis	-.	... .	..-.A.-- ...-...
P. senilis	-T	... .	..-.A.-- ...-...
A. tzacatl	-.	... .	.T-.C.-- ...-.A.
C. aureoventris	-.	... .	..-CC.-- ...-.A.
C. erithacus	-.	... ..C...	..-.A.-- ...-G..
C. americana	-. ...G.....	... .	..-.A.-- ...-.A.
C. caudata	-.	... ..C...	..-CA.-- ...-GA.
M. nubicus	-.	... .	..-G..-- ...-...
T. todus	-. ...G.....	... .	..-CC.-- ...-.A.
M. mexicanus	-. ...G.....	... .	..-.A.-- ...-...
M. momota	-. ...G.....	... .	..-.A.-- ...-...
U. epops	-C	... ..AC..	..-CT.-- ..G-TT.
A. albirostris	-.	... ...C..	..-TA.-- ...-.A.
A. prasinus	-.	... .	..-CC.-- ...-C..
M. carolinus	A.	... .	G.-CAC-- ...-CA.
S. varius	A.	... .	G.-CAC-- ...-CA.
C. vociferus	-.	... .	..-CA.-- ...-.A.
N. mcleodii	-.	... .	.C-CA.-- ...-.A.
C. minor	-.	... .	..-CC.-- ...-.A.
G. gallus	-G	... .	..-CA.-- ...-...
A. platyrhynchos	-.	... ..CC..	.TGCA.-- ..G-G..
C. buccinator	-.	... ..CC..	.TACA.-- ..G-G..
R. americana	-.	... .	.G-TC.-- ...-...
C. undulatus	-.	... .	..-TA.-- ...-C..
F. atra	-.	... .	..-.A.-- ...-.A.

35

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H. oreskios          [CTTATTTTAA]AACGAAA AGGGGCG TGAAAT---
H. ardens            [CCCGACA   ].C..... .....T. .....C---
H. diardii           [CCCTTCA   ]....... G...AT. .....C---
P. temnurus          [ACCCA     ]G..... .....T. ......---
A. narina            [CTTCTTATTA].....  G..... ......---
A. vittatum          [TTTTTATTA ]G..... G..... ......---
P. antisianus        [CCCTTA    ]....... .....T. ......---
P. auriceps          [AACTCTA   ]....... ...A... ......---
P. pavoninus         [AACTCTA   ]....... ....... ......---
E. neoxenus          [AAATTTTA  ]....... ...A.T. ......---
T. curucui           [TCCTTTT   ]C..... ..A..TA ......--T
T. violaceus         [TCCTTTT   ]T..... ..A..TA ......--T
T. viridis           [TTATCTC   ]....... ..A..T. .....C--C
T. comptus           [TTCTTT    ].....G ..A...A ......--T
T. melanurus         [TCCTTT    ]G.....G ..A...A ......--T
T. elegans           [TTTCTT    ]....G.G .T...T. ......--T
T. rufus             [TCTCTT    ]....G.. ..A.... ......--T
T. collaris          [CTCCTT    ]....... ..A..T. ......--C
T. mexicanus         [CCCTTT    ]....G.. ..A.AT. ......--T
T. personatus        [TCCCTT    ]....G.. ..A..T. ......--T
C. colius            [CT        ]....... .A....A ......---
C. striatus          [TT        ]C..... ...A.TA ......--T
C. leucocephalus     [CT        ]....... .T....A ......---
C. sinensis          [C         ]-....... ..A.A.A .....A---
C. fugax             [TC        ]....... ..A.ATA .....A---
G. velox             [          ]--..... ....A.A .....C---
M. whitneyi          [CCC       ]T...G.C G..AC.A .....A---
A. flammeus          [CC        ]C..... GA.A..A .....CACC
A. acadicus          [TCT       ]....G.C G..... ......--C
M. finschii          [CTC       ]C..... ......C ......--C
P. fulgidus          [TCA       ]....... ......C .....C--C
A. ventralis         [CCC       ]....... ......C .....C--C
P. senilis           [CCC       ]....... ..A...C .....C--C
A. tzacatl           [TT        ]T..... G.....A ......---
C. aureoventris      [C         ]T..... G...A.A .....----
C. erithacus         [AC        ]C...G.. G....TA ......--C
C. americana         [AC        ]C..... G.A...A .....C---
C. caudata           [CCACC     ]....... G..... .....C---
M. nubicus           [ACC       ]....G.. ...A..A .....C---
T. todus             [CCCC      ]....G.T GA.A..A ......---
M. mexicanus         [A         ]C..AG.. ....A.C .....C---
M. momota            [A         ]C..AG.. ....A.C .....C---
U. epops             [          ]C..... G...C.A .....G--C
A. albirostris       [CCC       ]T..... ......A .....----
A. prasinus          [CA        ]C..... .A.A..C .....C---
M. carolinus         [T         ]....... GA..A.C .....T--C
S. varius            [C         ].G..... GA....C .....C--C
C. vociferus         [CC        ]C.....C ......A ......---
N. mcleodii          [          ]C...G.C G.....A ......--C
C. minor             [CC        ]N...G.. ....A.A .....C---
G. gallus            [          ]....... .A..AT. .....C--C
A. platyrhynchos     [G         ]C...G.. ..AA.TA .....C---
C. buccinator        [          ]C...G.. ..AA.TT .....T---
R. americana         [GC        ]C..... GA....A .....----
C. undulatus         [CACCC     ]T..T... C.-.A-A .....----
F. atra              [TCC       ]....... ......C .....----
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                    35'              25'        36
                          **
H. oreskios      CGCC-CCT AGAAGGCGGA TTTAG CAGTA AAAAG-GG
H. ardens        .A..-... G......... ..... ..... .....-A.
H. diardii       .AT.-.TC G......... ..... ..... .....-..
P. temnurus      .A..-... G......... ..... ..... ...G.-A.
A. narina        .T..-..C G......... ..... ..... ...G.-A.
A. vittatum      ....-... T......... ..... ..... ...G.-A.
P. antisianus    ....-..C G......... ..... ..... .....-A.
P. auriceps      ....-... G......... ..... ..... ....T-A.
P. pavoninus     ....-..C G......... ..... ..... .....-A.
E. neoxenus      .A.T-... G......... ..... T.... ..G..-A.
T. curucui       TA.T-T.. .......... ..... ..... .....-A.
T. violaceus     TA..-T.. .......... ..... ..... .....-A.
T. viridis       TA..-T.. G......... ..... ..... .....-A.
T. comptus       TA..-T.. G......... ..... ..... .....-A.
T. melanurus     TA..-T.. G......... ..... ..... .....-A.
T. elegans       .A..-... G......... ..... ..... .....-A.
T. rufus         .A..-T.. G......... ..... ..... .....-A.
T. collaris      .A..-... .......... ..... ..... .....-A.
T. mexicanus     .A..-T.. G......... ..... ..... .....-A.
T. personatus    .A..-... .......... ..... ..... ...G.-A.
C. colius        TA..-... .......... ..... ..... ..T.A-A.
C. striatus      TA..-... .......... ..... ..... ....A-A.
C. leucocephalus TT..-... .......... ..... ..... ....A-A.
C. sinensis      A.T.-T.. .......... ..... A.... ..G..-..
C. fugax         TAT.-... .......... ..... ..... ..G.A-A.
G. velox         T.G.-... .......... ..... A.... ..G.A-..
M. whitneyi      AC..-... .-........ ..... ..... ..G.-T..
A. flammeus      T...-.TC .......... ..... ..... ..G.-T..
A. acadicus      AC..-... .-........ ..... ..... ..G..TA.
M. finschii      T...-... .......... ..... ..... ..G..-A.
P. fulgidus      T...-..C .......... ..... ..... ..G..-A.
A. ventralis     T...-..A .......... ..... ..... ..GC.-..
P. senilis       T...-..A .......... ..... ..... ..GC.-..
A. tzacatl       TC..-..C .......... ..... ..... ..GT-T..
C. aureoventris  .T..-..C .A........ ..... ..... ..GT-T..
C. erithacus     T...-..C .A........ ..... ..... .GTC.-.A
C. americana     T.T.-A.C G......... ..... ..... ...T.-..
C. caudata       ....-..C .......... ..... ..... ..GC--A.
M. nubicus       T...-..C .......... ..... ..... ...C.-A.
T. todus         T..T-.TC G......... ..... ..... .GGC.-..
M. mexicanus     AAT.-... ......T... ..... ..... ..G..-..
M. momota        AAT.-... ......T... ..... ..... ..GG.-..
U. epops         T.T.-..C G......... ..... T.... ..GG.-..
A. albirostris   .T..-... G.....T... ..... ..... ..GT.-..
A. prasinus      G...-.T. ......A... ..... ..... ...CA-C.
M. carolinus     .AT.-.TC .......... ..... ..... .G.CA-TA
S. varius        T...-.TC .....A.... ....T ..... ...CA-T.
C. vociferus     T...-..C .......... ..... ..... ..G..-..
N. mcleodii      G...-..C .....T.... ..... ..... ..GT.-A.
C. minor         T...-... G....-.... ..... ..... ..GC.-..
G. gallus        ....-.T. ......A... ..... ..... ..GT.-A.
A. platyrhynchos T..T-T.. ......A... ..... ..... ..GC.-..
C. buccinator    T..T-T.. G.....A... ..... ..... ..GT.-..
R. americana     .A..-.TC C......... ..... ..... ..GT.-..
C. undulatus     TA..-.T. .......... ..... ..... ..GT.-.A
F. atra          TA..C... ......A... ..... T.... ..GG.-A.
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                                        36'
                                                                *
H. oreskios        A-CAA-TA------AAG CTCTcTTT AAGC-CGGCCC
H. ardens          .-...-..------... ...C.... ..A.-T.....
H. diardii         .-T..-..------T.. ....t... .....-T...T.
P. temnurus        .-T..-..------... ...CtC.. .....-T.....
A. narina          .-...-G.------... ...Ca... .....-T.....
A. vittatum        .-T..-G.------... ...Ca... .....-T.....
P. antisianus      .-...-..------... ........ .....-G...T.
P. auriceps        .-...-..------... ........ .....-T...T.
P. pavoninus       .-...-..------... ........ .....-....T.
E. neoxenus        .-T..-..------... ........ .....-T...T.
T. curucui         .-.T.-..------... ....a... ...A-......
T. violaceus       .-.T.-..------... ....a... ...A-......
T. viridis         .-.T.-..------... ........ ...A-......
T. comptus         .-.C.-..------G.. ........ ...A-....T.
T. melanurus       .-.C.-..------... ........ ...A-......
T. elegans         .-...-..------G.. ....a... ...A-T...T.
T. rufus           .-...-..------G.. ....a... .....-T...T.
T. collaris        .-...-..------G.. ....a... .....-T.....
T. mexicanus       .-.T.-..------... ....a... .....-T.....
T. personatus      .-...-..------G.. ....a... .....-......
C. colius          .-T..-..------.T. ..T.t... ...A-T......
C. striatus        .-...-..------..A ..T.t... ...A-......
C. leucocephalus   .-...-..------G.. ..T.t... ...A-T......
C. sinensis        .TT..C..------... .C...... ..AAC....T.
C. fugax           .-T..-.G------... ........ ..AT-T......
G. velox           .-...-..------... ..T....A ..AT-T......
M. whitneyi        .CTC.-..------..A .CAC.... ..AA-A......
A. flammeus        .G.C.-..------.CA TC...... ...A-T......
A. acadicus        CCTC.-..------..A ..AC.... ..CA-A......
M. finschii        .-...-.C------... .C...C.. .....-T..T..
P. fulgidus        .-...-.C------... ........ ..A.-...T..
A. ventralis       .-...-.C------... .C.C.... .....-...T..
P. senilis         .-...-.C------... .C.C.... .....-...T..
A. tzacatl         .-.C.-C.------.C. .CA.-C.. ..AGCT......
C. aureoventris    .-.C.-C.------.T. .CAC-C.. ..AACT......
C. erithacus       .-T..-G.------.T. TCTAaC.. ..A.-T......
C. americana       .-...-G.------.T. .C.C.... .....-....T.
C. caudata         .-...-A.------G.. .CTA.... .....-......
M. nubicus         .-TT.-G.------T.. ...G-... ..AGCT......
T. todus           .-T..-..------... .C.C.C.. ..A.-....T.
M. mexicanus       .-TT.-A.------G.. .C.C.... .....-T......
M. momota          .-TT.-A.------G.. .C.C.... .....-......
U. epops           .-...-..------.TA ...C.... ..A.-A......
A. albirostris     .-...-.C------.C. .C.C.... .....-......
A. prasinus        .-...-.C------... .CTGt... .....-......
M. carolinus       .-.C.-CC------.T. ..TG.C.. ..A.-T......
S. varius          .-.T.-.C------.T. .CTGtC.. .....-......
C. vociferus       .-T..-.T------... .C.C.... ...A-....A.
N. mcleodii        .-T..-.C------... .C.C.... ...A-....A.
C. minor           .-...-.C------... .C.C.... ...A-....A.
G. gallus          .-TC.-..CCCCCT... ...A.... ...A-....T.
A. platyrhynchos   .-...-..------... ...G.... .....-......
C. buccinator      .-...-..------G.. .CTA.... .....-......
R. americana       .-TC.-G.------... .C.A.... .....-......
C. undulatus       .-TC.-G.------.T. TC.A.... ..A.-T......
F. atra            .-T..-G.------.T. .C.C..-. ..A.T......
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	24'		37		38
	*	* *		*	
H. oreskios	TGAGGCACGTaCA	TACC	GCCCGTC	AC	CCTCCTC
H. ardens	.AG.....	....	.....	..	.....
H. diardii	.AG.....	....	.....	..	.....
P. temnurus	.....	....	.....	..	.....
A. narina	.....	....	.....	..	.....
A. vittatum	.....	....	.....	..	.....
P. antisianus	.....	....	.....	..	.....
P. auriceps	.....	....	.....	..	.....
P. pavoninus	.....	....	.....	..	.....
E. neoxenus	.....	....	.....	..	.....
T. curucui	..G.A.....	....	.....	..	.....
T. violaceus	..G.A.....	....	.....	..	.....
T. viridis	..G.A.....	....	.....	..	.....
T. comptus	..G.A.....	....	.....	..	.....
T. melanurus	..G.A.....	....	.....	..	.....
T. elegans	..G.A.....	....	.....	..	.....
T. rufus	..G.A.....	....	.....	..	.....
T. collaris	..GAA.....	....	.....	..	.....
T. mexicanus	..G.A.....	....	.....	..	.....
T. personatus	..G.....	....	.....	..	.....
C. colius	..G.....	....	.....	..	.....
C. striatus	..G..T.....	....	.....	..	....T..
C. leucocephalus	..GA.....	....	.....	..	.....
C. sinensis	..G.A.....	....	.....	..	....T..
C. fugax	..G.....	....	.....	..	.....
G. velox	..G.A.....	....	.....	..	.....
M. whitneyi	..G.A.....	....	.....	..	.....
A. flammeus	..G..T.....	....	.....	..	.....
A. acadicus	..G.....	....	.....	..	.....
M. finschii	.AG.....	C...	.....	..	....T..
P. fulgidus	.AG.A.....	....	.....	..	.....
A. ventralis	.AG.....	....	.....	..	.....
P. senilis	.AG.....	....	.....	..	.....
A. tzacatl	C.G.....	....	.....	..	.....
C. aureoventris	C.G.....	....	.....	..	.....
C. erithacus	..G.A.....	....	.....	..	.....
C. americana	..G..T.....	....	.....	..	.....
C. caudata	.AG.A.....	....	.....	..	.....
M. nubicus	..G.....	....	.....	..	.....
T. todus	..G.....	....	.....	..	.....
M. mexicanus	..G.....	....	.....	..	.....
M. momota	..G.....	....	.....	..	.....
U. epops	..GA.....	....	.....	..	.....
A. albirostris	..G.....	....	.....	..	.....
A. prasinus	..G.A.....	....	.....	..	.....
M. carolinus	.AG.A.....	....	.....	..	.....
S. varius	..G.A.....	....	.....	..	.....
C. vociferus	..G.....	....	.....	..	.....
N. mcleodii	..G.A.....	....	.....	..	....T..
C. minor	..G.A.....	....	.....	..	.....
G. gallus	.....	....	.....	..	....T..
A. platyrhynchos	.AG.....	....	.....	..	.....
C. buccinator	..G.....	....	.....	..	.....
R. americana	.AG.....	....	.....	..	.....
C. undulatus	.AG.....	....	.....	..	.....
F. atra	..GA.....	....	.....	..	....T..

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H. oreskios        G--CA GGCC [GACACTATAAAAC]ATAGC--TAAT-ACA
H. ardens          A--.. ...T [AGCAAATTAACC ]...A.--....-T..
H. diardii         A--.. A... [AACAAACTAATC ]...AT--....-T..
P. temnurus        A--A. .... [ATTAAACAAAAC ]...A.--....-...
A. narina          A--T. ...T [ATCAGATTAAACT]...A.--....-T..
A. vittatum        A--.. ...T [ATCAAATTAGACT]...A.--....-T..
P. antisianus      A--T. ...T [ACCAAACAAACC ]...AT--....-.T.
P. auriceps        A--.. ...T [ACCAAACAAACT ]...A.--....-.T.
P. pavoninus       A--.. ...T [ACCAGATAAACC ]...A.--....-GT.
E. neoxenus        A--.. .... [ATTAAATAAACT ]...T.--....-.TG
T. curucui         .--T. ...T [ACAAATAAACC  ].....--....-.TG
T. violaceus       .--T. ...T [ACAAATAACCC  ].....--....-.TG
T. viridis         A--.. ...T [ACAAATAAACC  ]...A.--....-.TC
T. comptus         A--.. .... [ACAAATAGATC  ]...AT--....-...
T. melanurus       A--.. ...T [ACAAATAAATC  ]...C.--....-.T.
T. elegans         A--.. ...T [ACAAATAAATT  ]...A.--....-GT.
T. rufus           .--.. ...T [ACAAATTAAATG ].....--....-..G
T. collaris        A--.. ...T [ACAAATAAATT  ].....--....-GTG
T. mexicanus       A--.. ...T [AACAAATAATT  ]....T--....-.T.
T. personatus      A--.. ...T [ACAAATAAATT  ]...A.--....-G-.
C. colius          A--T. T..T [ACCCAACAATG  ]...CT--....-...
C. striatus        .--T. TA.T [GCCCACCAATA  ]...CT--....-...
C. leucocephalus   A--T. T..T [TCCAATCAATA  ]...AT--...A-...
C. sinensis        AAAA. A..T [TTACACCCACT  ]...AT--....-TT.
C. fugax           A--.. A..T [CCAAGTTTAC   ]...A.--....-G.C
G. velox           A--.. A... [CAACTTCACA   ]...C.--....-..T
M. whitneyi        A--.. A..T [ACCCTTCCCA   ]...A.--....-..C
A. flammeus        .--.. A..T [ACTCCCCCC    ]...AA--....-..C
A. acadicus        .--.. A..T [ACCGTCACCCT  ]...A.--....-G.-
M. finschii        AT-A. .... [CCCAACACC    ]C..A.--....-C...
P. fulgidus        A--T. A..T [ACCCCACACA   ]C..AG--....-C..
A. ventralis       A--.. A... [CCTACAAACCCA ]C..A.--....-.A.
P. senilis         A--.. A... [CCCATAAACACA ]T..A.--....-...
A. tzacatl         .--T. A..T [ACCAACATAA   ]G..A.--....-C..-
C. aureoventris    A--.. A..T [ATTAACACAA   ]G..A.--....-C.T-
C. erithacus       A--.. A... [ACCAACCTCT   ]...AT--....-..-
C. americana       A--.. A..T [ACCCATGC     ]...A.--....-..-
C. caudata         .--.. A..T [ACACGATCCCAA ]G..A.--..----..G
M. nubicus         A--.. A..T [GCAGCCCATG   ]G..A.--....-C..G
T. todus           A--T. A..T [ACACCCAAAC   ]..GA.--.G..-..C
M. mexicanus       A--.. A... [CTAATACCCT   ]G..AACC.T..-...
M. momota          A--.. A... [CTAATACCCT   ]G..AACC....-...
U. epops           .--.. A... [CATTCCACAGC  ]...AT--....-..C
A. albirostris     A--.G A..T [ACTCCTCTCC   ]....A--....-...
A. prasinus        A--.. A... [ACAAGCGCCC   ]C..A.--....-CC..
M. carolinus       .--.. A..A [ACACCTCTCC   ]TC.C.A-..G-A..T
S. varius          .--T. A..G [AACATTCCCT   ]T.CT.A-...-A...
C. vociferus       AA-A. A..T [ATCAAACCCT   ]...A.T-....-...
N. mcleodii        AA-A. A..T [CCACCAAACCCT ]...A.T-....-...
C. minor           AACA. A..T [GCCTAAGCCCC  ]...AT-TC....-...
G. gallus          A--.. A... [ATCAACATCA   ]...AA--..TAT..T
A. platyrhynchos   A--T. A... [ACACCCCCAC   ]...A.--....-..-
C. buccinator      A--A. A..T [ATATCCCAC    ]...A.--....-..-
R. americana       A--.. A..T [ACCCTACTAA   ]...AA--....-..-
C. undulatus       A--T. A..A [ACTAACCCA    ]...CA--....-..C
F. atra            A--.- A..C [CCCAACACCT   ]G..A.--...C-..-
```

		39'		38'		37'
				*	**	
H. oreskios	[CTCACA]	GGC-C	AAA	GATGAGG	TAA	GTCGtAAC
H. ardens	[TCCACA]	A..-T	...	.....	...	.....
H. diardii	[CCGACA]	A..-T	...	.....	...	.....
P. temnurus	[CCTACA]	A..-.	...	.....	...	.....
A. narina	[CCACCCA]	A..-.	...	.....	...	.....
A. vittatum	[CCATTAA]	A..-.	...	.....	...	.....
P. antisianus	[CTTTTA]	T..-T	...	.....	...	.....
P. auriceps	[CTCTTA]	T..-T	...	.....	...	.....
P. pavoninus	[CTTTTA]	T..-T	...	.....	...	.....
E. neoxenus	[CTCATA]	A..-T	...	..C....	...	.....
T. curucui	[CCTATA]	A..-.	...	.....	...	.....
T. violaceus	[CCTATA]	...-.	...	.....	...	.....
T. viridis	[TCTATA]	A..-.	...	.....	...	.....
T. comptus	[CCTAAA]	A..-.	...	.....	...	.....
T. melanurus	[CCTAGA]	A..-.	...	.....	...	.....
T. elegans	[TCTAAA]	A..-.	G..	.....	...	.....
T. rufus	[TATATAAA]	A..-.	...	..C....	...	.....
T. collaris	[TCTAAA]	A..-.	...	.....	...	.....
T. mexicanus	[TCCAAATA]	A..-.	...	..C....	...	.....
T. personatus	[GTCCAAA]	A..-.	...	.....	...	.....
C. colius	[AATAACC]	A..TA	T..	..A....	C..	.....
C. striatus	[ACTAGCC]	AAAAA	T..	..A....	...	.....
C. leucocephalus	[AATATTCC]	A..TA	T..	..A....	C..	.....
C. sinensis	[TACACC]	A..-G	.G.	.....	...	.....
C. fugax	[CTTCAA]	A..-T	...	.....	...	.....
G. velox	[CTACCGTA]	...-T	...	..C....	...	.....
M. whitneyi	[ACCACC]	A..-T	...	.....	...	.....
A. flammeus	[CCCTCAA]	A..-T	...	.....	...	.....
A. acadicus	[GCTAACA]	A..-T	...	.....	...	.....
M. finschii	[ATCACCAC]	A..-T	...	..C....	...	.....
P. fulgidus	[ACAAAT]	A..-.	...	.....	C..	.....
A. ventralis	[ACTAAT]	A..-.	...	..A....	...	.....
P. senilis	[ACCAGC]	A..-.	...	..A....	...	.....
A. tzacatl	[CAGTTTCC]	A..-.	...	..-C...	...	.....
C. aureoventris	[CATCTTC]	A..-.	...	.....	...	.....
C. erithacus	[CCCAAT]	T..-T	G..	.....	...	.....
C. americana	[CCAACAT]	AA.-T	G..	..C....	...	.....
C. caudata	[CTCAACCT]	...-T	...	..C....	...	.....
M. nubicus	[CGCAACAT]	.A.-.	...	..A....	...	.....
T. todus	[CTCTAG]	A..-T	G..	.....	...	.....
M. mexicanus	[CTCCTTC]	.A.-.	...	.....	C..	.....
M. momota	[CCTCTCC]	...-T	...	.....	C..	.....
U. epops	[ACACACT]	C..-T	G..	.....	...	.....
A. albirostris	[CCCGTT]	A..-T	G..	..C....	...	.....
A. prasinus	[AAAATC]	A..-T	G..	..C....	C..	.....
M. carolinus	[GCAATT]	A..-T	...	.....	...	.....
S. varius	[CCAACC]	A.T-.	T..	.....	...	.....
C. vociferus	[TAATC]	A..-.	...	..C....	...	.....
N. mcleodii	[TTAATT]	A-.-.	...	..C....	...	.....
C. minor	[CCAACT]	A..-.	.G.	.....	...	.....
G. gallus	[TCCCCTCCC]	...-T	...	..C....	C..	.....
A. platyrhynchos	[CACGTAAA]	T..-.	...	.....	...	..-.....
C. buccinator	[CATTAACA]	C..-T	...	.....	...	.....
R. americana	[TACTATT]	A..-T	...	.....	C..	.....
C. undulatus	[CTCTCAA]	A..-T	...	.....	...	.....
F. atra	[GCCCACCG]	...-.	...	..C....	...	.....

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                                40                  40'
H. oreskios          AAGG TAAGTGTA?? ???? ?????????? ??????
H. ardens            .... ........?? ???? ?????????? ??????
H. diardii           .... ........?? ???? ?????????? ??????
P. temnurus          .... ........?? ???? ?????????? ??????
A. narina            .... ........?? ???? ?????????? ??????
A. vittatum          .... ........?? ???? ?????????? ??????
P. antisianus        .... ........?? ???? ?????????? ??????
P. auriceps          .... ........?? ???? ?????????? ??????
P. pavoninus         .... ........?? ???? ?????????? ??????
E. neoxenus          .... ........?? ???? ?????????? ??????
T. curucui           .... ........?? ???? ?????????? ??????
T. violaceus         .... ........?? ???? ?????????? ??????
T. viridis           .... ........?? ???? ?????????? ??????
T. comptus           .... ........?? ???? ?????????? ??????
T. melanurus         .... ........?? ???? ?????????? ??????
T. elegans           .... ........?? ???? ?????????? ??????
T. rufus             .... ........?? ???? ?????????? ??????
T. collaris          .... ........?? ???? ?????????? ??????
T. mexicanus         .... ........?? ???? ?????????? ??????
T. personatus        .... ........?? ???? ?????????? ??????
C. colius            .... ........?? ???? ?????????? ??????
C. striatus          .... ........?? ???? ?????????? ??????
C. leucocephalus     .... ........?? ???? ?????????? ??????
C. sinensis          .... ........?? ???? ?????????? ??????
C. fugax             .... ........?? ???? ?????????? ??????
G. velox             .... ........?? ???? ?????????? ??????
M. whitneyi          .... ........?? ???? ?????????? ??????
A. flammeus          .... ........?? ???? ?????????? ??????
A. acadicus          .... ........?? ???? ?????????? ??????
M. finschii          .... ........?? ???? ?????????? ??????
P. fulgidus          .... ........?? ???? ?????????? ??????
A. ventralis         .... ........?? ???? ?????????? ??????
P. senilis           .... ........?? ???? ?????????? ??????
A. tzacatl           .... ........?? ???? ?????????? ??????
C. aureoventris      .... ........?? ???? ?????????? ??????
C. erithacus         .... ........?? ???? ?????????? ??????
C. americana         .... ........?? ???? ?????????? ??????
C. caudata           .... ........?? ???? ?????????? ??????
M. nubicus           .... ........?? ???? ?????????? ??????
T. todus             .... ........?? ???? ?????????? ??????
M. mexicanus         .... ........?? ???? ?????????? ??????
M. momota            .... ........?? ???? ?????????? ??????
U. epops             .... ........?? ???? ?????????? ??????
A. albirostris       .... ........?? ???? ?????????? ??????
A. prasinus          .... ........?? ???? ?????????? ??????
M. carolinus         .... ........?? ???? ?????????? ??????
S. varius            .... ........?? ???? ?????????? ??????
C. vociferus         .... ........?? ???? ?????????? ??????
N. mcleodii          .... ........?? ???? ?????????? ??????
C. minor             .... ........CC GGAA GGTGCACTTA GCATAT
G. gallus            .... ........CC GGAA GGTGCACTTA GACTAC
A. platyrhynchos     .... ........CC GGAA GGTGTACTTA GAATAC
C. buccinator        .... ........CC GGAA GGTGTACTTA GAATAT
R. americana         .... ........CC GGAA GGTGCACTTA GCACAC
C. undulatus         .... ........CC GGAA GGTGTACTTA GCATAT
F. atra              .... ........CC GGAA -GTGCACTTA GCACAA
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