

The Great American Interchange in birds: a phylogenetic perspective with the genus *Trogon*

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Abstract

The 'Great American Interchange' (GAI) is recognized as having had a dramatic effect on biodiversity throughout the Neotropics. However, investigation of patterns in Neotropical avian biodiversity has generally been focused on South American taxa in the Amazon Basin, leaving the contribution of Central American taxa under-studied. More rigorous studies of lineages distributed across the entire Neotropics are needed to uncover phylogeographical patterns throughout the area, offering insights into mechanisms that contribute to overall Neotropical biodiversity. Here we use mitochondrial DNA sequence data and intensive geographical sampling from the widespread Neotropical avian genus *Trogon* to investigate the role of the GAI in shaping its phylogeographical history. Our results show that genetic diversity in *Trogon* exceeds the perceived biodiversity, and that the GAI resulted in lineage diversification within the genus. Despite greater diversity in South America, a Central American centre of origin with multiple and independent dispersals into South America is indicated. These dispersals were followed by the evolution of divergent lineages associated with the Andes Mountains and other South American geographical features. According to our phylogenetic reconstructions, several species, which were originally defined by morphological characters, are nonmonophyletic. In sum, our results elucidate the evolutionary history of *Trogon*, reveal patterns obscured by extant biodiversity, and serve as a biogeographical model to consider in future studies.

Keywords: biogeography, character mapping, Great American Interchange, Isthmus of Panama, Neotropics, phylogeography, *Trogon*

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Introduction

The completion of the Isthmus of Panama allowed for the exchange of previously isolated North and South American biotas. This 'Great American Interchange' (GAI) is recognized as having had a dramatic effect on biodiversity in both continents (Wallace 1876; Simpson 1940; Stehli & Webb 1985; Vrba 1992). Before the GAI, the biota of North America had general Holarctic affinities due to historic connections with Europe and Asia, while the biota of South America formed a distinct assemblage due to its 'splendid isolation' since the break up of Gondwanaland (Simpson 1980; Stehli & Webb 1985; Marshall 1988; Ricklefs 2002;

Morley 2003). This isolation was disrupted by the completion of the Isthmus of Panama, a protracted geological event that culminated in a complete land bridge between North and South America approximately 3 million years ago (Ma) (Coates & Obando 1996). The impact of the GAI on mammalian biodiversity throughout North and South America is well documented in the fossil record (Simpson 1980; Stehli & Webb 1985; Marshall 1988). The mammalian exchange was asymmetrical, with a greater number of genera dispersing from North America to South America. This asymmetry is predicted from MacArthur–Wilson equilibrium theory (MacArthur & Wilson 1967), because North America had a larger reservoir of genera at the time of the interchange (Marshall *et al.* 1982). Immigration of North American genera into South America was followed by rapid radiation, which created levels of diversity beyond predictions of simple equilibrium models (Marshall *et al.*

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1982). Hypothesized proximate factors for the diversification in South America include a combination of demographic, climatic, competitive, and ecological variables (Stehli & Webb 1985; Vrba 1992).

The impacts of the GAI on other vertebrate groups, including birds, are less understood due to a comparatively poor fossil record. Most hypotheses regarding the evolution of avian biodiversity in the Neotropics have focused on South America (Haffer 1997), and in general do not assess the impact of the GAI. These hypotheses evoke the effects of palaeogeography, river barriers, climate change, and ecotones to explain South American avian biodiversity, particularly among areas of endemism in the Amazon Basin. Cracraft & Prum (1988) were the first to investigate relationships among these areas within a phylogenetic framework through a cladistic analysis of morphological characters in three genera. More recent studies used DNA sequence data to elucidate phylogeographical patterns in the Amazon (Marks *et al.* 2002; Aleixo 2004; Cheviron *et al.* 2005; Eberhard & Bermingham 2005; Ribas *et al.* 2005; Aleixo 2006). These studies provided important insights regarding the evolutionary history of birds in South America. However, the role of the GAI in large-scale Neotropical avian biodiversity patterns remains unclear due to limited sampling in Central America. Additional studies with comprehensive geographical and taxonomic sampling of lineages with representation in both Central and South America are needed to assess the impact of the GAI on historical diversification in birds. Well-supported phylogenies for such groups can be used to infer the direction and timing of movement across the Isthmus of Panama, and the location of geographical structuring beyond the Amazon Basin.

The avian genus *Trogon* (Family Trogonidae) is a good model group for the study of the GAI and biogeographical patterns across the entire Neotropics. It contains 17 species (Sibley & Monroe 1990) that are collectively distributed from the southwestern USA to northern Argentina, with many species occurring on both continents. All species of *Trogon* are nonmigratory, and are regarded as relatively weak fliers that move only short distances (Collar 2001). Therefore, biogeographical patterns are not likely to be obscured by long-distance dispersal. Past phylogenetic research on the genus has been unable to explore intercontinental evolutionary patterns due to either incomplete sampling or a focus on the pantropical distribution of the entire family (Espinosa de los Monteros 1998; Johansson & Ericson 2005; Moyle 2005). Also, all three previous studies used one exemplar per species. This sampling scheme makes the assessment of intraspecific diversity and species monophyly impossible, and could produce a misleading phylogeny of the group (Omland *et al.* 1999). In this study all *Trogon* species were sampled and multiple representatives from disparate geographical

regions were included wherever possible. This thorough sampling should produce a more comprehensive phylogeny, better suited to evaluate the role of the GAI and diversification patterns across both Central and South America.

The specific goals of this study were: (i) to produce a well-supported molecular phylogeny of *Trogon* with broad geographical sampling of each species, (ii) to use the phylogeny to detect intercontinental biogeographical patterns and reconstruct the ancestral areas of lineages, and (iii) to evaluate the impact of the GAI on the evolutionary history of the genus.

Materials and methods

Sampling

We analysed 160 *Trogon* samples representing all 17 species to produce a phylogeny of the group (Appendix). Multiple samples from each species ($n = 2-19$) were analysed with a focus on maximizing geographical coverage, allowing the evaluation of intraspecific genetic diversity for most species. Representatives ($n = 29$) of all other genera in the family (*Apaloderma*, *Euptilotis*, *Harpactes*, *Pharomachrus*, and *Priotelus*) were used as outgroups. Samples were acquired through scientific collecting, loans from ornithology holdings at other research institutions, or sequences available in GenBank (Appendix).

Laboratory protocols

Total genomic DNA was extracted from all samples using a DNeasy Tissue Kit (QIAGEN) following the manufacturer's protocol. The mitochondrial DNA (mtDNA) NADH dehydrogenase 2 (ND2) gene was amplified via polymerase chain reaction (PCR) using the flanking primers L5215 (Hackett 1996) and HTrpC (Smithsonian Tropical Research Institute), and in some cases also with the internal primers L5758 and H5766 (Johnson & Sorenson 1998). Amplifications were done in 12.5 μ L PCRs under conditions described in a previous study (Klicka *et al.* 2005). PCR products were purified using the enzyme ExoSAP-IT (USB Corp.) following the manufacturer's protocol. We prepared 20 μ L sequencing reactions using a Big Dye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) with 0.5 μ L of Big Dye and 20-40 ng of purified PCR product. Sequencing reactions were purified using the CleanSEQ (Agencourt Bioscience) magnetic bead clean up, and analysed with an ABI 3100-*Avant* automated sequencer (Applied Biosystems). We used the program SEQUENCHER (Gene Codes) to unambiguously align complementary strands, check for gaps in the sequences, and translate the nucleotide sequences to check for the absence of stop codons in the gene sequences.

Phylogenetic inference

Past research has indicated evidence of homoplasy in *Trogon* mtDNA sequence data as indicated by saturation plots and the large genetic distances among some taxa (Espinosa de los Monteros 1998; Moyle 2005). We used PAUP* 4.0b10 (Swofford 2002) to calculate uncorrected genetic distance matrices for all samples. Numerous paired *Trogon* samples had uncorrected genetic distances of over 20%, which is well above estimated saturation thresholds for ND2 (Hackett 1996; Johnson & Sorenson 1998). Phylogenetic reconstructions that do not correct for saturation can be prone to misleading results and problems with long-branch attraction (Bergsten 2005). Excessive homoplasy can be best accommodated by maximum likelihood (ML)-based analytical methods that incorporate the appropriate model of sequence evolution (Felsenstein 1978; Huelsenbeck & Hillis 1993; Hillis *et al.* 1994; Huelsenbeck 1995; Swofford *et al.* 2001), and for this reason we chose a priori to rely on ML as our primary means of obtaining phylogenetic hypotheses.

Maximum-likelihood reconstructions of phylogeny were completed according to the following procedures. We used MODELTEST 3.7 (Posada & Crandall 1998) to determine the model of molecular evolution for the complete gene sequence, and for each codon position. The Akaike information criterion option was chosen (Posada & Buckley 2004), and the GTR + I + Γ model was the best fit to the data in each case. We then used the program TREEFINDER (Jobb *et al.* 2004) to construct an ML phylogeny of the complete data set, and assess nodal support with 100 non-parametric bootstrap replicates. This program uses a fast sampling algorithm to estimate all parameters while exploring tree space, and its accuracy in phylogeny construction has been demonstrated to equal or exceed other commonly used programs (Jobb *et al.* 2004). We used this phylogeny to delimit *Trogon* lineages based on taxonomy, geography, or genetic distance (uncorrected pairwise distance > 0.7%). One sample from each such lineage was chosen to create a trimmed data set, which captures most of the genetic diversity within the group. MODELTEST 3.7 was used on the truncated data set (see above), and again the GTR + I + Γ model was the best fit to the data (complete gene and each codon position). For the trimmed data set, we used PAUP* to construct the best ML approximation of phylogeny using a successive approximations approach (Swofford *et al.* 1996; Sullivan *et al.* 2005). The initial search was completed with model parameters provided by MODELTEST, parameters were re-estimated using the resulting tree, and the process was repeated until the same tree was recovered in successive iterations. ML nodal support was assessed in TREEFINDER with 100 nonparametric bootstrap replicates, and nodes recovered in greater than 70% of the replicates were considered well supported.

Bayesian inference with the program MRBAYES 3.1.2 (Ronquist & Huelsenbeck 2003) was used to provide another measure of relationships and nodal support. Initial trial runs (ngen = 1 million, samplefreq = 100) with one cold and three heated chains were performed with and without partitions (by codon position). The first 2000 samples were discarded as 'burn-in', which produced a posterior distribution of 8000 topologies for each run. A 50% majority consensus was constructed from each distribution of topologies. Although the codon-partitioned analysis produced a posterior distribution with slightly higher likelihood scores, the consensus relationships among lineages were identical and the nodal support was very similar between analyses. Therefore, a longer analysis was completed without partitions to reduce the number of parameters and to maximize the amount of characters per partition. Two long runs (ngen = 10 million, samplefreq = 100) with one cold and three heated chains were completed, and the first 50 000 samples were discarded as burn-in to produce a conservative sample of the posterior distribution. A 50% majority consensus was constructed separately for each run, producing similar results. Therefore, we combined results from the two runs to produce a posterior distribution of 100 000 trees. A final 50% majority consensus was constructed, and nodes recovered in 95% or more of the topologies were considered well supported.

Ancestral area reconstruction

We used our resulting estimations of *Trogon* phylogeny to reconstruct the biogeography of ancestral nodes. Before the formation of the Isthmus of Panama, the last connection between Central and South America was a hypothesized proto-Antilles land bridge *c.* 80–60 Ma (Savage 1982; Iturralde-Vinent & MacPhee 1999). Molecular dating estimates the most recent common ancestor of *Trogon* to be approximately 20 million years old (Moyle 2005). Since the age of the genus is likely much younger than the last vicariant event between Central and South America (i.e. break up of proto-Antilles land bridge), vicariance is not a probable mechanism in explaining the current Central and South American distribution of this group. The sedentary nature of these species also de-emphasizes the role of long-distance dispersal between continents prior to the completion of the isthmus. These characteristics, along with our dense geographical and taxonomic sampling of the group, make ancestral area reconstruction an appropriate and useful tool for investigating the impact of the GAI on the biogeographical history of *Trogon*.

The biogeography of the GAI is more complex than a Central and South America dichotomy due to the geographical position of the Andes Mountains in northwestern South America. The Andes are a barrier to gene flow in a variety of Neotropical organisms (Brower 1994; Brumfield &

Capparella 1996; Zamudio & Greene 1997; Perdices *et al.* 2002; Cortes-Ortiz *et al.* 2003; Dick *et al.* 2003). The northern Andes rapidly uplifted between 5 Ma and 2 Ma (Gregory-Wodzicki 2000), making them both geographically and temporally proximate to the Isthmus of Panama. We therefore used four character states in scoring extant distributions of *Trogon* lineages in the ancestral area reconstruction analyses: Central America, Chocó-Darién, Andes Mountains, and *cis*-Andes. Central America was defined as west of the Panamanian state Darién. From the state of Darién to the western foothills of the Andes was considered the Chocó-Darién. The Andes Mountains character state was used for lineages confined to the Andes highlands, and everything east of the Andes were scored as *cis*-Andes. Using these character states allowed for a more comprehensive analysis of the role of the GAI in the historical biogeography of the group.

We then used simple parsimony (Fitch 1971) and stochastic mapping (Huelsenbeck *et al.* 2003) methodologies to hypothesize the biogeography of each node in the phylogeny estimated using the truncated data set. Simple parsimony (unordered, symmetric transformation matrix) reconstruction was done using MESQUITE 1.06 (Maddison & Maddison 2005) and the best ML topology. Stochastic mapping uses Bayesian statistics to simultaneously account for uncertainty in branch length and topology, and was done using SIMMAP 1.0 (Bollback 2006) with the last 5000 trees of the posterior distribution from each of the two long MRBAYES runs for a total of 10 000 trees. First, each node from the ML topology was characterized in the program using the constraints function. Then the two prior settings, bias and rate, were defined. The bias prior (π) is used in the estimation of the probability of changes between states. The α parameter of π is automatically set to 100 (equiprobable character states) by the program when there are more than two possible states. The rate prior (Γ) is user-defined, and is applied in estimating the rate of character changes. Trial runs explored a range of values for Γ parameters α and β , and had little effect on the posterior probabilities of ancestral reconstructions. Separate final runs were completed with two different parameter settings for Γ : $\alpha = 3$, $\beta = 2$ and $\alpha = 5$, $\beta = 5$. Each run consisted of 10 draws simulated from each tree to create a posterior probability of 100 000 samples. Finally, for every node within *Trogon*, the proportion of each character state in the posterior probability was calculated for both runs.

Timing of GAI dispersal events

We estimated the relative timing of the earliest dispersal events between Central and South America to evaluate their temporal relation to each other and the completion of the Isthmus of Panama. For each species, or species complex, we used the results of ancestral area reconstruction

analyses to identify the most basal dispersal events between Central and South America. These nodes correspond to estimates of the earliest intercontinental movements in *Trogon*, and represent the earliest participation of these species in the GAI. Subsequent dispersals and possible backcrossing through the isthmus were not considered in the dating analyses.

Timing historic events in the *Trogon* phylogeny is difficult due to an incomplete fossil record and a lack of knowledge regarding higher-level relationships. The fossil record of Trogoniformes is sparse, and is mainly comprised of records from Europe during the Oligocene and Eocene (Bordkorb 1971; Mayr 1999, 2005; Kristoffersen 2002). The relationship of these fossils to extant Trogoniformes is unclear, which prohibits their use as calibration points within the order. They can be used to place a minimum bound on the node between Trogoniformes and outgroup taxa (Moyle 2005); however, the depth of this node is uncertain since the closest relatives of Trogoniformes remains a contentious topic (Collar 2001).

Our approach to dating dispersal events was to first use likelihood methods to estimate their temporal distribution. Relative timing estimates were calculated using the trimmed data set and the program r8s 1.71 (Sanderson 2003). In order to calculate confidence intervals, we generated 100 bootstrap replicate topologies in TREEFINDER with the sequence data and the successive approximations tree as a fixed topology. r8s was then run using these topologies with the PL and truncated Newton options. The node corresponding to the most recent common ancestor of all *Trogon* species was fixed to an arbitrary value of 1.0 on all replicates. The program calculated the average and 95% confidence interval for the age of each defined node of interest.

We then used a molecular clock to estimate the actual timing of events in relation to the completion of the Isthmus of Panama. Using molecular clocks to date events has many possible sources of error, including rate variation among lineages, clock calibration, saturation, and genetic polymorphism in ancestral populations (Edwards & Beerli 2000; Arbogast *et al.* 2002). These sources of error could be particularly troublesome with a single-locus study. Given these caveats, the time estimates of dispersal events should be considered coarse approximations. We used a rate calibration from cytochrome *b* (*cyt b*) data of Hawaiian honeycreepers (Fleischer *et al.* 1998) to estimate divergence dates. The rate was originally calibrated at 1.6% per million years with the Kimura 2-parameter model of molecular evolution, and was later calculated at 2.2% per million years for the more complex GTR + Γ model (Weir & Schluter 2004). Since this rate is based on *cyt b* evolution, its use on presumably faster-evolving genes such as ND2 may be inappropriate. To estimate the relative rates of *cyt b* and ND2, we compared GTR + Γ corrected pairwise distances for both of these genes for a subset of *Trogon* samples ($n = 14$),

and the rate of evolution for ND2 was 1.55 times the rate of *cyt b*. Similar relative rates of evolution for ND2 and *cyt b* have been documented in a variety of birds (Klicka *et al.* 2000; Voelker & Spellman 2003; Ribas *et al.* 2005; Sheldon *et al.* 2005; Benz *et al.* 2006). We therefore adjusted the molecular clock to 3.41% per million years, and applied this rate to pairwise GTR + Γ genetic distances of the trimmed data set to estimate the age of each defined node of interest.

Morphological character mapping

The taxonomy of *Trogon* has historically been based on morphological characters (Gould 1875; Ridgway 1911; Zimmer 1948; Collar 2001), and our analyses indicated multiple cases of nonmonophyly in *Trogon* species (see Results). We therefore mapped traditionally important morphological characters onto the molecular phylogeny to assess their phylogenetic utility. We used the program MACCLADE 4.03 (Maddison & Maddison 2000) to map eight prominent external morphological characters: female back colour (grey or brown), male head colour (green, blue, or black), bill colour (blue, yellow, or black), tail pattern (all black, black and white bars, white tips, or all white), iris colour (dark, white, yellow, or red), belly colour (red/orange or yellow), pectoral line (present or absent), and orbital ring (blue, yellow, red, or black). Each character was mapped onto the ML topology using simple, unordered parsimony. The number of step changes, retention index (RI), and consistency index (CI) were calculated for each character.

Results

Sequence characteristics and phylogenetic inference

The aligned sequences of the ND2 gene yielded 1041 base pairs, of which 599 were variable and 557 were phylogenetically informative. Nucleotide composition bias was evident in the matrix, with an excess of adenine and cytosine, and a deficiency of guanine ($A = 0.32$, $C = 0.31$, $G = 0.10$, $T = 0.27$), similar to results of a previous *Trogon* study using the same gene (Moyle 2005). Translation into amino acids failed to produce any stop codons, and no gaps were found in the sequences.

The phylogeny of the complete data set (Fig. S1, Supplementary material) splits *Trogon* into two major clades, and most nodes are well supported. Relationships among species generally correspond to those of a previous study that sampled 14 of the 17 *Trogon* species and used mitochondrial and nuclear sequence data (Moyle 2005). The 160 *Trogon* samples fall within 41 distinct mitochondrial lineages that characterize the diversity within *Trogon* (Fig. 1). Intraspecific diversity was high in many species, with multiple independent lineages inhabiting distinct geographical areas.

The observed intraspecific diversity among lineages revealed four cases of nonmonophyly in the current taxonomy (*collaris*, *melanurus*, *viridis*, and *violaceus*). Samples of *collaris* from Central America were more closely related to *aurantiiventris* than samples of *collaris* from South America. These species are very similar morphologically, with the main difference being that *collaris* and *aurantiiventris* have red and orange bellies, respectively. Two samples, one *collaris* and one *aurantiiventris*, in western Panama had incongruent mtDNA haplotypes and morphology (i.e. unexpected haplotypes given belly colour of specimens), suggesting hybridization in this area of sympatry. The species *bairdii* was embedded within *viridis*, rendering the latter paraphyletic. The species *melanurus* and *violaceus* were both polyphyletic. A clade of *melanurus* samples also contained the species *comptus* and *massena*, and the *violaceus* clade also included samples of *curucui* and *surrucura*. Each case of nonmonophyly was associated with species distributed in both Central and South America.

Ancestral areas and GAI dispersals

Both simple parsimony and stochastic mapping converged on the same result in the reconstruction of ancestral areas in *Trogon* (Fig. 2). The two stochastic mapping runs with different rate priors produced very similar posterior probabilities at each node; therefore, only the results from Γ parameter settings of $\alpha = 5$, $\beta = 5$ are reported in Fig. 2. Scoring the geographical position of the 41 mtDNA lineages revealed a bias toward South America (27 vs. 14 in Central America). Despite this bias, both parsimony and stochastic mapping (100% posterior probability) analyses reconstructed Central America as the ancestral area for the most recent common ancestor of *Trogon*. Shifts from Central to South America map onto the phylogeny in six separate clades (Fig. 2, nodes A–F), supporting multiple independent dispersal events between landmasses. The temporal order of these dispersal events is generally consistent between relative PL and molecular clock age estimates (Table 1). Age estimates for nodes A, B, E, and F tended to cluster together, covering an approximate range of dates from 1.3 to 4.0 Ma. Nodes C and D are comparatively older, with age estimates spanning dates from 4.2 to 9.7 Ma.

Character mapping

There was a considerable amount of variation in how the morphological characters traced the molecular phylogeny (Table 2, Fig. 3). Results from the three measures of phylogenetic signal (number of step changes, RI, and CI) were variable among and within characters. Female back colour was the only character that mapped perfectly onto the two major clades of *Trogon*. Other characters that appear

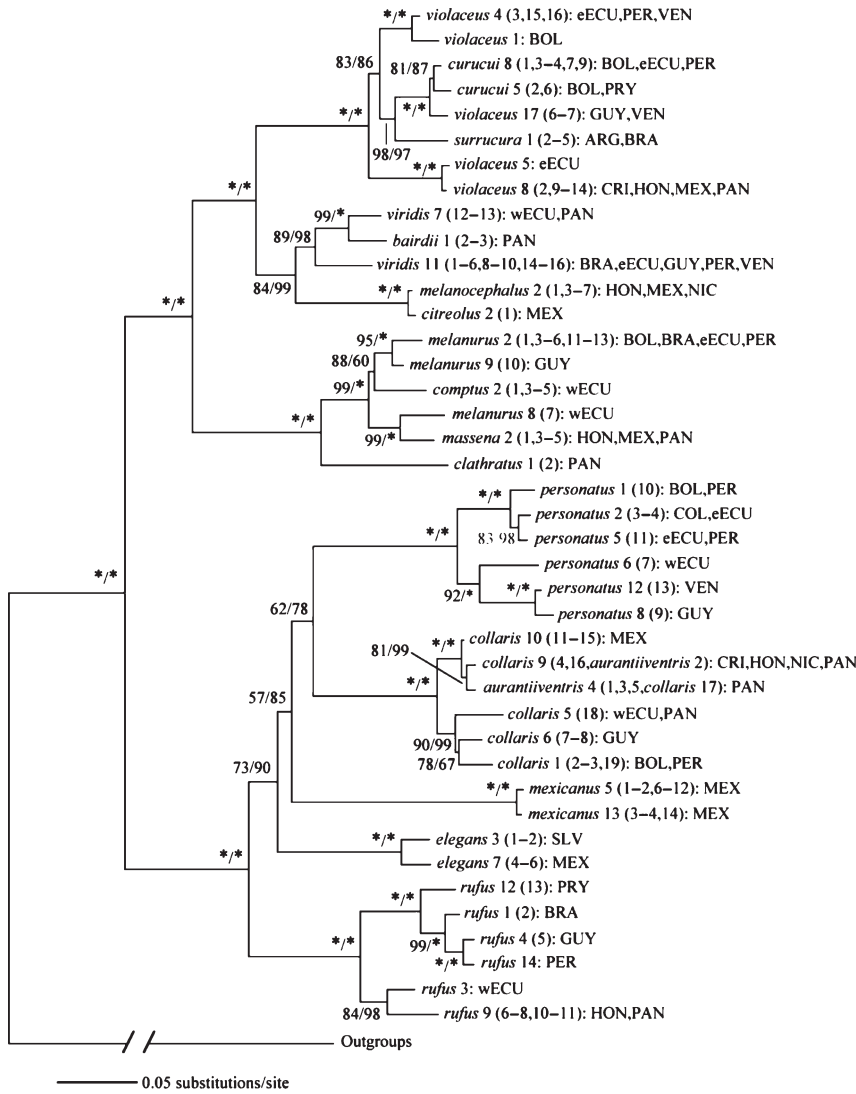


Fig. 1 Maximum likelihood phylogeny of the trimmed *Trogon* data set. The phylogeny is rooted with representatives of *Pharomachus*, *Euptilotis*, *Priotelus*, *Apaloderma*, and *Harpactes* (not shown). Numbers following species names refer to sample numbers (Appendix). The first number is the sample used to construct the phylogeny, and the numbers in parentheses indicate other samples that fall within that lineage. Countries where samples were collected follow sample numbers. Numbers on nodes correspond to maximum likelihood bootstrap (left) and Bayesian posterior probability (right) percentages. Asterisks represent values of 100%.

Table 1 Age estimates of dispersal events in the *Trogon* phylogeny

Node	Penalized likelihood*		Molecular clock†	
	Average	95% CI	Ave	95% CI
A	0.16	0.12–0.20	3.1	2.3–3.9
B	0.17	0.12–0.22	2.4	2.2–2.6
C	0.27	0.21–0.33	4.8	4.2–5.4
D	0.42	0.34–0.49	8.9	8.0–9.7
E	0.11	0.08–0.14	1.8	1.3–2.2
F	0.21	0.14–0.27	3.4	2.9–4.0

*Relative estimate based on setting of 1.0 for common ancestor of all *Trogon* species.

†Estimated ages in million years based on 3.41% per million year molecular clock.

Table 2 Summary statistics from mapping of *Trogon* morphological characters

Character	No. of step changes	RI	CI
Female back colour	1	1.00	1.00
Male head colour	3	0.91	0.67
Bill colour	3	0.90	0.67
Tail pattern	5	0.80	0.60
Iris colour	4	0.50	0.75
Belly colour	5	0.71	0.20
Pectoral line	4	0.40	0.25
Orbital ring colour	8	0.67	0.38

to provide phylogenetic signal include male head colour and bill colour. In contrast, belly colour, pectoral line, and orbital ring colour did a relatively poor job in tracking the phylogenetic relationships as determined by molecular data.

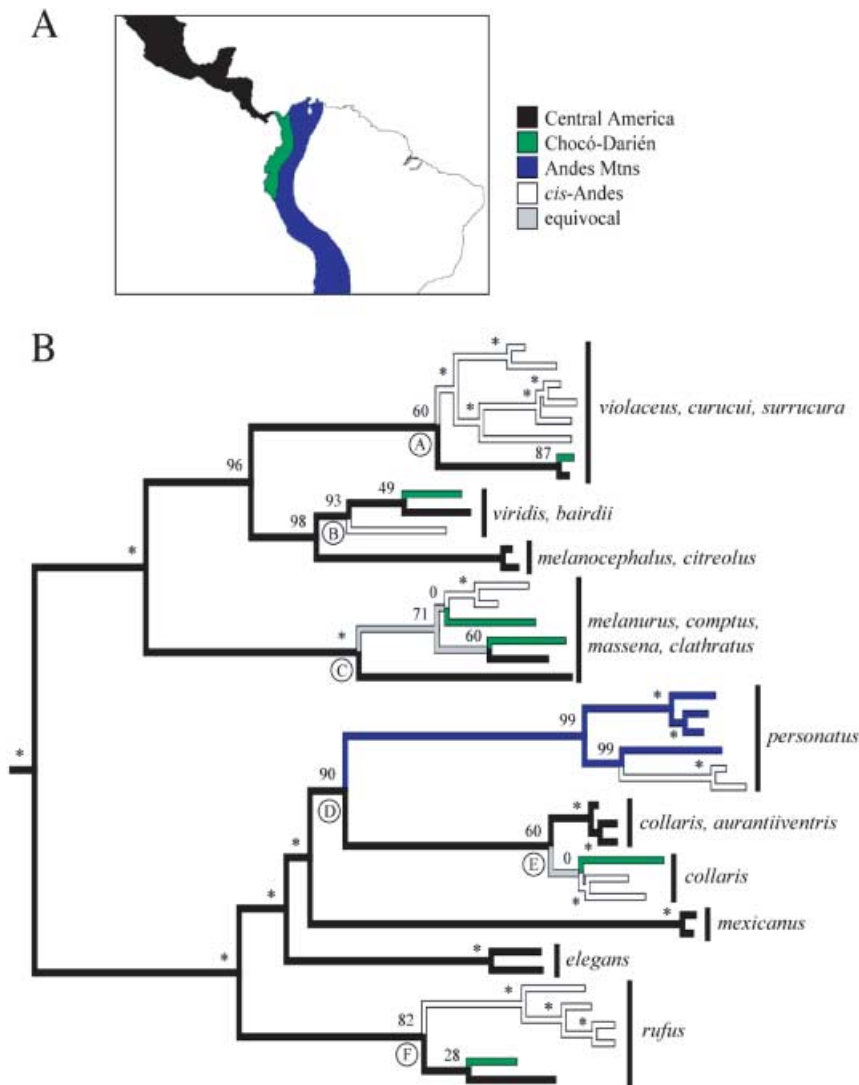


Fig. 2 Reconstruction of ancestral areas using the phylogeny of the trimmed *Trogon* data set. (A) Map of the distribution of the four character states used in the analyses. (B) Parsimony reconstruction of ancestral areas. Numbers on nodes correspond to the stochastic mapping posterior probability of the character state displayed. Asterisks represent values of 100%. Nodes labelled with letters are referred to in the text, and represent basal dispersal events from Central to South America.

Discussion

Thorough taxonomic and geographical sampling within *Trogon* allowed investigation into numerous aspects of the evolutionary history of the group. The sequence data produced a phylogeny that confidently describes the relationships among most species (Fig. 1). The inclusion of multiple individuals per species allowed the first assessment of intraspecific genetic diversity in *Trogon*, which revealed mtDNA nonmonophyly in multiple species. Comprehensive sampling of the entire genus throughout Central and South America permitted a reliable reconstruction of ancestral areas, which was used to infer the role of the GAI in the phylogeographical history of the group.

Ancestral area reconstructions indicate a Central American origin for *Trogon* and multiple, independent dispersals into South America (Fig. 2). The GAI has created a scenario where the current area of highest diversity (South America)

is not the area of origin for the genus. This refutes early hypotheses that link centres of diversity with ancestral regions (Adams 1902; Matthew 1915), and supports the need for comprehensive phylogenies and more complex analyses to determine areas of origin (Posadas *et al.* 2006). The higher extant *Trogon* diversity in South America is the result of GAI dispersal into, and subsequent diversification within, South America. Past research demonstrates that this pattern was difficult to detect without a full phylogenetic analysis of the genus. Haffer (1967) hypothesized an Amazon origin for the *melanurus–comptus–clathratus–massena* complex with three 'consecutive invasions' west of the Andes. Our *Trogon* phylogeny refutes this hypothesis by placing the node separating *clathratus* from the other species as basal and strongly supporting a Central American origin for this complex (Figs 1 and 2). A molecular assessment of *rufus*, which did not include other *Trogon* samples, hypothesized an Amazonian origin for the species (Brumfield & Capparella

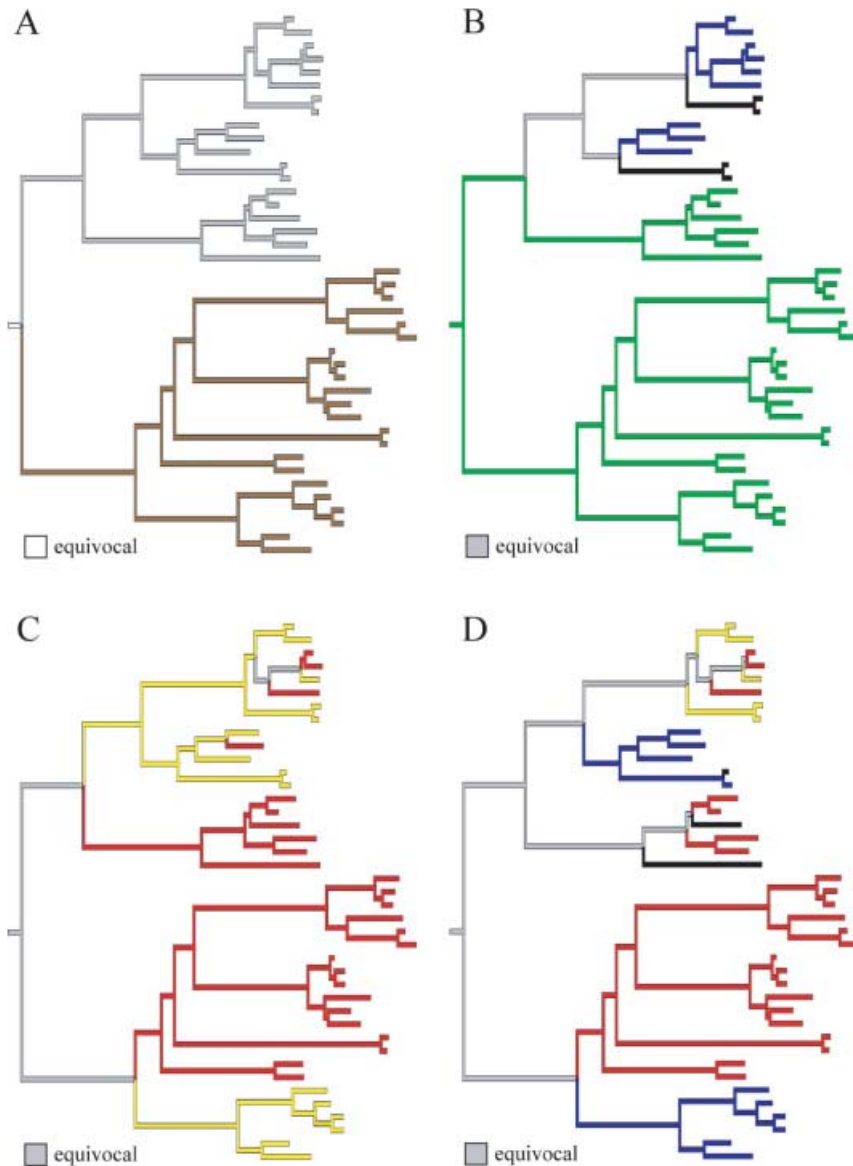


Fig. 3 Parsimony mapping of *Trogon* (A) female back, (B) male head, (C) belly, and (D) eye ring colours using the maximum-likelihood phylogeny of the trimmed *Trogon* data set. Branch colours represent the character coding used for each trait.

1996). The more comprehensive analyses presented here do not support this hypothesis. The pattern of dispersal followed by diversification is common in the mammalian GAI, where a number of North American immigrants into South America (e.g. cricetid rodents, canid carnivores, and horses) underwent a 'significant and apparently rapid diversification' (Marshall *et al.* 1982). Nonmutually exclusive theories proposed to explain this diversification pattern in mammals evoke competitive superiority (Stehli & Webb 1985) and the ability to exploit unoccupied niches and adaptive zones (Patterson & Pascual 1972).

Theories explaining the diversity of birds in South America, particularly in the Amazon Basin, often focus on the role of barriers as mechanisms of diversification (Haffer 1997). These barriers are hypothesized to have formed

through sea-level fluctuations (Emsley 1965), development of rivers (Capparella 1991), Pleistocene climatic changes (Haffer 1969), or suture zones across ecotones (Endler 1982). While all of these mechanisms might have played a role in *Trogon* diversification in South America, it is important to note that the GAI and dispersal from Central America was the first step of this diversification process. The importance of the GAI in shaping New World mammalian diversity is well documented due to the fossil record; however, the GAI is not often cited as a major influence in New World avian diversity patterns, which is likely due to the comparatively poor fossil record for birds. Results from this study suggest that the GAI may have played a larger role in Neotropical avian diversification than has been recognized, and enforce the need for systematic and biogeographical

studies to include Central American forms to gain a full understanding of historical diversification patterns in South American biota.

The ability of birds to fly might suggest that the GAI for birds may not hinge as strongly on the completion of the Isthmus of Panama as compared to less volant organisms. Some large terrestrial mammals were able to disperse between continents prior to the completion of the isthmus by island-hopping (Stehli & Webb 1985; Marshall 1988), so the notion that some birds did the same is not unreasonable. *Trogon* species are nonmigratory and not known as strong fliers (Collar 2001). They are absent from most islands that are close to the mainland (Collar 2001), occurring only on Trinidad, Isla Tres Marias, and a few islands off of the coast of Quintana Roo in Mexico. It is therefore reasonable to assume that *Trogon* ancestors were not active in long-distance dispersal prior to the completion of the Isthmus of Panama. The timing of dispersal events supports this notion (Table 1). The general consistency of age estimates in numerous, independent lineages suggests that several dispersal events were approximately coincident in time. Date estimates for nodes A, B, E, and F are in the vicinity of the proposed date of 3 Ma for the completion of the Isthmus of Panama, and a more sophisticated molecular calibration that better accounts for saturation and ancestral population size and polymorphism would likely make these time estimates more recent (Arbogast *et al.* 2002; Garcia-Moreno 2004). For these weakly dispersing birds, it is more parsimonious to conclude that the clustered date estimates are associated with the completion of the Isthmus of Panama rather than multiple and contemporaneous long-distance dispersals over water. The older age estimates for nodes C and D could be the product of imprecise dating estimations, long-distance dispersal, or the use of a proposed earlier land bridge connecting Central and South America (Bermingham & Martin 1998).

Sampling of *Trogon* species distributed in Central and South America revealed intercontinental phylogeographical patterns. In the lowland-inhabiting *rufus*, *viridis*-*bairdii* complex, and *violaceus*-*curucui*-*surrucura* complex, the most basal phylogeographical break is associated with the Andes (Figs 1 and 2). In each case, there is also a secondary, more recent dispersal event from Central America to the Chocó-Darién that does not cross the Andes. This recurring pattern suggests that the permeability of the Andes as a barrier has decreased over time. When *Trogon* ancestors for these groups first dispersed from Central to South America, the northern Andes were still rising (Gregory-Wodzicki 2000), and they were able to cross or circumvent the mountains. As the mountains rose, they become a more formidable barrier for these species, and subsequent dispersals into South America are restricted to the Chocó-Darién on the west side of the Andes. Reconstructing ancestral areas on the phylogeny did not detect any definitive dispersal

events from South to Central America (Fig. 2). It is possible that *Trogon* niche space in lower Central America is saturated (nine of the 17 extant species occur in Panama), subsequently making dispersal in this direction more difficult.

Patterns in external morphology and cryptic geographical genetic structure have misled traditional taxonomy of *Trogon*. Characters such as belly colour, tail patterns, presence of a pectoral line, and colours of soft parts (e.g. iris, orbital ring, bill) have figured prominently in the taxonomy of *Trogon* (Gould 1875; Ridgway 1911; Wetmore 1972). Plumage patterns on the tail and wing panel are hypothesized to play a role in species recognition (Collar 2001), but empirical evidence supporting these ideas is lacking. Three non-monophyletic species (*melanurus*, *viridis*, and *violaceus*) have conserved morphologies across distinct lineages separated by the Andes and the Isthmus of Panama. In each case, one or more of these lineages is most closely related to another species with diagnosable differences in morphological characters. This demonstrates that some morphological characters are labile with respect to phylogeny, which is a recurring theme in avian phylogenetic studies (Kennedy *et al.* 2000; Omland & Lanyon 2000; Burns *et al.* 2003; Pereira & Baker 2005; Weckstein 2005). However, there is also a possibility that the discordance between the mtDNA phylogeny and morphology could be an artefact of introgression (Ballard & Whitlock 2004), which cannot be tested with the available data. A more thorough review of character evolution in this genus is needed to fully evaluate the relationship between morphology and phylogeny.

Collectively, these results demonstrate that the GAI was an important mechanism in the historical diversification of *Trogon*, the details of which had been masked by incomplete molecular phylogenetic study and incorrect taxonomy. The GAI and dispersal into South America has often been cited as a mechanism driving Neotropical mammalian diversity, but is not often suggested as a prominent cause of patterns in Neotropical avian diversity. Well-supported phylogenies with complete sampling of widespread avian groups like the genus *Trogon* can provide data that are lacking in the fossil record, and be used to better assess the importance of the GAI in shaping Neotropical avian diversity.

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Supplemental material

The following supplementary material is available for this article:

Fig. S1 Maximum likelihood phylogeny of the complete *Trogon* data set.

This material is available as part of the online article from:

<http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-294X.2007.03647.x>

(This link will take you to the article abstract).

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Appendix

Data for specimens used in the study.

Genus	Species/sample no.	Museum*	Specimen	Locality
<i>Trogon</i>	<i>aurantiiventris</i> 1	STRI	PA-TAU145	Panama: Bocas del Toro
	<i>aurantiiventris</i> 2	STRI	PA-TAU194	Panama: Bocas del Toro
	<i>aurantiiventris</i> 3	MBM	GMS1012	Panama: Veraguas, 3 km WSW of Santa Fe
	<i>aurantiiventris</i> 4	MBM	GMS1034	Panama: Veraguas, Chilarges
	<i>aurantiiventris</i> 5†	LSUMNS	B26405	Panama: Chiriquí, Burica Peninsula
	<i>bairdii</i> 1	STRI	PA-TBA383	Panama: Chiriquí, Burica Peninsula
	<i>bairdii</i> 2	STRI	PA-TBA384	Panama: Chiriquí, Burica Peninsula
	<i>bairdii</i> 3	STRI	PA-TBA385	Panama: Chiriquí, Burica Peninsula
	<i>citreolus</i> 1	CNAV	PO02919	Mexico: Michoacan
	<i>citreolus</i> 2	CNAV	PE25988	Mexico: Oaxaca
	<i>clathratus</i> 1	USNM	B02029	Panama: Bocas del Toro, Los Planes
	<i>clathratus</i> 2	USNM	B02304	Panama: Bocas del Toro, Los Planes
	<i>collaris</i> 1	LSUMNS	B22827	Bolivia: La Paz, Cerro Asunta Pata
	<i>collaris</i> 2	LSUMNS	B12471	Bolivia: Santa Cruz, Arroyo del Encanto
	<i>collaris</i> 3†	LSUMNS	B9341	Bolivia: Pondo, Nicolas Suarez
	<i>collaris</i> 4	LSUMNS	B35769	Costa Rica: Cartago, 11 km SW of Pejibaye
	<i>collaris</i> 5	ANSP	2032	Ecuador: Manabi, Cerro San Sebastian
	<i>collaris</i> 6	USNM	B10636	Guyana: Acari Mountains
	<i>collaris</i> 7	USNM	B10733	Guyana: Acari Mountains
	<i>collaris</i> 8	USNM	B10782	Guyana: Acari Mountains
	<i>collaris</i> 9	MBM	GAV1965	Honduras: Atlantida, 19 km WSW of La Ceiba
	<i>collaris</i> 10	FMNH	394271	Mexico: Guerrero, Sierra de Atoyac
	<i>collaris</i> 11	MZFC	HGOSLP140	Mexico: Hidalgo, El Coyol
	<i>collaris</i> 12	FMNH	394272	Mexico: Oaxaca, San Gabriel Mixtepec
	<i>collaris</i> 13	FMNH	394273	Mexico: Oaxaca, San Gabriel Mixtepec
	<i>collaris</i> 14	MZFC	OVMP0703	Mexico: Oaxaca, Santa Ana del Progreso
	<i>collaris</i> 15	FMNH	393987	Mexico: Veracruz, Sierra de Santa Martha
	<i>collaris</i> 16	MBM	DAB1316	Nicaragua: Matagalpa, 10 km N of Matagalpa
	<i>collaris</i> 17	USNM	B01545	Panama: Chiriqui, Los Planes
	<i>collaris</i> 18	LSUMNS	B2141	Panama: Darien, 6 km NW of Cana
	<i>collaris</i> 19	FMNH	397885	Peru: Madre de Dios, Alto Madre
	<i>comptus</i> 1†	LSUMNS	B29953	Ecuador: Pichincha, Pedro Vicente Maldonado
	<i>comptus</i> 2	ANSP	2297	Ecuador: Esmeraldas, 20 km NNW of Alto Tambo
	<i>comptus</i> 3	ANSP	2349	Ecuador: Esmeraldas, 20 km NNW of Alto Tambo
	<i>comptus</i> 4	ZMUC	132166	Ecuador: Pichincha, Rio Pizare
	<i>comptus</i> 5	ZMUC	132167	Ecuador: Pichincha, Rio Pizare
	<i>curucui</i> 1	FMNH	394463	Bolivia
	<i>curucui</i> 2	FMNH	394464	Bolivia
	<i>curucui</i> 3†	AMNH	JJW266	Bolivia: Santa Cruz
	<i>curucui</i> 4	ANSP	2733	Ecuador: Sucumbios, Laguna de Layato Cocha
	<i>curucui</i> 5	LSUMNS	B25715	Paraguay: Alto Parana, Cerro Leon
	<i>curucui</i> 6‡	UMMZ	227501	Paraguay
	<i>curucui</i> 7	FMNH	320981	Peru: Madre de Dios, Hacienda Amazonia
	<i>curucui</i> 8	FMNH	433225	Peru: Madre de Dios, Moskitana
	<i>curucui</i> 9	LSUMNS	B10564	Peru: Ucayali, 65 km ENE of Pucallpa
	<i>elegans</i> 1	FMNH	434015	El Salvador: Ahuachapan, Parque Nacional El Imposible
	<i>elegans</i> 2†	FMNH	434013	El Salvador: Ahuachapan, Canton Concepcion
	<i>elegans</i> 3	FMNH	434014	El Salvador: Sonsonate, Canton Las Lajas
	<i>elegans</i> 4	MZFC	DUET042	Mexico: Michoacan, La Verdura
	<i>elegans</i> 5	MZFC	QRO189	Mexico: Queretaro, Laguna de la Cruz
<i>elegans</i> 6	MZFC	QRO486	Mexico: Queretaro, El Chuveje	
<i>elegans</i> 7	MBM	JK03280	Mexico: Jalisco, Sierra de Bolanos	
<i>massena</i> 1	MBM	JK01022	Honduras: Atlantida, 19 km WSW of La Ceiba	
<i>massena</i> 2	MZFC	B1935	Mexico: Campeche	
<i>massena</i> 3	MZFC	B2073	Mexico: Campeche	
<i>massena</i> 4	MZFC	CHIMA221	Mexico: Oaxaca, Chalchijapa	

Appendix Continued

Genus	Species/sample no.	Museum*	Specimen	Locality
<i>Trogon</i>	<i>massena</i> 5	STRI	PA-TMS315	Panama: Bocas del Toro
	<i>massena</i> 6	STRI	PA-TMS84	Panama: Chiriqui, Burica Peninsula
	<i>massena</i> 7	STRI	PA-TMS273	Panama: Colon, Achioté
	<i>massena</i> 8	MBM	JK04273	Panama: Colon, Achioté
	<i>massena</i> 9†	LSUMNS	B28530	Panama: Colon, Achioté
	<i>melanocephalus</i> 1	MBM	JK01020	Honduras: Atlantida, 15 km W of La Ceiba
	<i>melanocephalus</i> 2	MBM	JK01035	Honduras: Atlantida, 15 km W of La Ceiba
	<i>melanocephalus</i> 3	MBM	JK01041	Honduras: Atlantida, 15 km W of La Ceiba
	<i>melanocephalus</i> 4	MZFC	B2014	Mexico: Campeche
	<i>melanocephalus</i> 5	MZFC	CONA99009	Mexico: Campeche, Rancho Tachich
	<i>melanocephalus</i> 6†	KU	547	Mexico: Queretaro
	<i>melanocephalus</i> 7	MBM	DAB1854	Nicaragua: Granada, Laguna Blanca
	<i>melanurus</i> 1	LSUMNS	B14826	Bolivia: Santa Cruz, Velasco
	<i>melanurus</i> 2	FMNH	391999	Brazil: Mato Grosso, Alta Floresta
	<i>melanurus</i> 3	FMNH	392000	Brazil: Mato Grosso, Alta Floresta
	<i>melanurus</i> 4	FMNH	389729	Brazil: Rondonia, Cachoeira Nazare
	<i>melanurus</i> 5†	LSUMNS	B31379	Brazil: Rondonia, 90 km E of Vila Nova Mamore
	<i>melanurus</i> 6	ANSP	3263	Ecuador: Sucumbios, Imuya Cocha
	<i>melanurus</i> 7	ANSP	4634	Ecuador: Esmeraldas, Cabecera de Bilsa
	<i>melanurus</i> 8	ANSP	4683	Ecuador: Esmeraldas, Cabecera de Bilsa
	<i>melanurus</i> 9	ANSP	8244	Guyana: Iwokrama Reserve
	<i>melanurus</i> 10	ANSP	8642	Guyana: Iwokrama Reserve
	<i>melanurus</i> 11	LSUMNS	B2500	Peru: Loreto, Rio Napo
	<i>melanurus</i> 12	FMNH	397887	Peru: Madre de Dios, Alto Madre
	<i>melanurus</i> 13	FMNH	433226	Peru: Madre de Dios, 13 km NNW of Atalaya
	<i>mexicanus</i> 1	MZFC	BMM656	Mexico: Hidalgo, Tlanchinol
	<i>mexicanus</i> 2	MZFC	HGOSLP027	Mexico: Hidalgo, Cerro Jarros
	<i>mexicanus</i> 3	FMNH	343219	Mexico: Jalisco, Sierra de Manantlan
	<i>mexicanus</i> 4	FMNH	393988	Mexico: Jalisco, Sierra de Manantlan
	<i>mexicanus</i> 5	FMNH	394275	Mexico: Mexico, Ocuilón
	<i>mexicanus</i> 6	MZFC	OVMP0319	Mexico: Oaxaca, El Aguacate
	<i>mexicanus</i> 7	MZFC	OVMP0436	Mexico: Oaxaca, Cerro Piedra Larga
	<i>mexicanus</i> 8	MZFC	OVMP0463	Mexico: Oaxaca, Cerro Piedra Larga
	<i>mexicanus</i> 9	MZFC	QRO280	Mexico: Queretaro, El Pemoche
	<i>mexicanus</i> 10	MZFC	QRO467	Mexico: Queretaro, Bomintza
	<i>mexicanus</i> 11	MZFC	CONA450	Mexico: San Luis Potosi, San Nicholas de los Montes
	<i>mexicanus</i> 12	MZFC	HGOSLP424	Mexico: San Luis Potosi, San Nicholas de los Montes
	<i>mexicanus</i> 13	MBM	JK03279	Mexico: Jalisco, Sierra de Bolanos
	<i>mexicanus</i> 14†	FMNH	343220	Mexico: Jalisco
	<i>personatus</i> 1	ZMUC	115519	Bolivia: Cochabamba, Cocapata
	<i>personatus</i> 2	ZMUC	134954	Colombia, Antioquia
	<i>personatus</i> 3	ZMUC	134972	Colombia, Antioquia
	<i>personatus</i> 4	USNM	B03099	Ecuador: Sucumbios, Cocha Seca
	<i>personatus</i> 5	ANSP	3791	Ecuador: Zamora Chinchipe, Cajanuma
	<i>personatus</i> 6	ANSP	506	Ecuador: Esmeraldas, Maldonado
	<i>personatus</i> 7	ANSP	559	Ecuador: Esmeraldas, Maldonado
	<i>personatus</i> 8	USNM	B15882	Guyana: Mount Roraima
	<i>personatus</i> 9	LSUMNS	B48503	Guyana: Kopinang Mountain
	<i>personatus</i> 10	FMNH	397889	Peru: Madre de Dios, Alto Madre
	<i>personatus</i> 11	LSUMNS	B421	Peru: Piura, Cruz Blanca
<i>personatus</i> 12	LSUMNS	B7596	Venezuela: Amazonas, Cerro de la Neblina	
<i>personatus</i> 13†	AMNH	GBF2125	Venezuela: Amazonas	
<i>rufus</i> 1	FMNH	389730	Brazil: Rondonia, Cachoeira Nazare	
<i>rufus</i> 2	FMNH	389731	Brazil: Rondonia, Cachoeira Nazare	
<i>rufus</i> 3	ANSP	2216	Ecuador: Esmeraldas	
<i>rufus</i> 4	ANSP	8471	Guyana: Iwokrama Reserve	
<i>rufus</i> 5	ANSP	8477	Guyana: Iwokrama Reserve	

Appendix *Continued*

Genus	Species/sample no.	Museum*	Specimen	Locality
<i>Trogon</i>	<i>rufus</i> 6	STRI	HA-TRR74	Honduras: Atlantida, La Ceiba
	<i>rufus</i> 7	STRI	PA-TRR252	Panama: Bocas del Toro
	<i>rufus</i> 8	LSUMNS	B2109	Panama: Darien, 6 km NW of Cana
	<i>rufus</i> 9	MBM	GMS975	Panama: Panama, Cerro Jefe
	<i>rufus</i> 10	MBM	JK04197	Panama: Veraguas, Restingue
	<i>rufus</i> 11†	LSUMNS	B26564	Panama: Colon, 17 km NW of Gamboa
	<i>rufus</i> 12	ZMUC	115780	Paraguay: Alto Parana, Estancia Itabo
	<i>rufus</i> 13	ZMUC	115779	Paraguay: Canindeyu, Estancia Jimenez
	<i>rufus</i> 14	LSUMNS	B27391	Peru: Loreto, 84 km WNW of Contamana
	<i>surrucura</i> 1	USNM	B05982	Argentina: Misiones, Posadas
	<i>surrucura</i> 2	USNM	B05987	Argentina: Misiones, Posadas
	<i>surrucura</i> 3	USP	X7	Brazil: Sao Paulo, State Park Bananal
	<i>surrucura</i> 4	USP	X9	Brazil: Sao Paulo, 30 km N of Ubatuba
	<i>surrucura</i> 5†	LSUMNS	B35558	Brazil: Sao Paulo, 30 km NW of Ubatuba
	<i>violaceus</i> 1	LSUMNS	B18257	Bolivia: Santa Cruz, Velasco
	<i>violaceus</i> 2	FMNH	393038	Costa Rica
	<i>violaceus</i> 3	ANSP	3289	Ecuador: Francisco de Orellana, Zancudo Cocha
	<i>violaceus</i> 4	ZMUC	113903	Ecuador: Sucumbios, Rio Lagarto Cocha
	<i>violaceus</i> 5	ANSP	5154	Ecuador: Loja, 10 km E of El Limo
	<i>violaceus</i> 6	USNM	B04344	Guyana: Berbice River
	<i>violaceus</i> 7	ANSP	8664	Guyana: Iwokrama Reserve
	<i>violaceus</i> 8	MBM	GAV1688	Honduras: Copan, 15 km ENE of Copan Ruinas
	<i>violaceus</i> 9	MBM	JK01036	Honduras: Atlantida, 19 km WSW of La Ceiba
	<i>violaceus</i> 10	MZFC	HGOSLP166	Mexico: Hidalgo, El Coyol
	<i>violaceus</i> 11	MZFC	QRO338	Mexico: Queretaro, Rio Tancuilin
	<i>violaceus</i> 12	MZFC	CONA297	Mexico: San Luis Potosi, San Nicolas de los Montes
	<i>violaceus</i> 13	LSUMNS	B26531	Panama: Colon, 17 km NW of Gamboa
	<i>violaceus</i> 14	STRI	PA-TYI654	Panama: Darien, Puerto Pina
	<i>violaceus</i> 15	LSUMNS	B27592	Peru: Loreto, 79 km WNW of Contamana
	<i>violaceus</i> 16	LSUMNS	B7561	Venezuela: Amazonas, Cerro de la Neblina
	<i>violaceus</i> 17†	AMNH	ROP258	Venezuela: Bolivar
	<i>viridis</i> 1	FMNH	391274	Brazil: Amapa, Fazenda Casimiro
	<i>viridis</i> 2	FMNH	391275	Brazil: Amapa, Fazenda Casimiro
	<i>viridis</i> 3	FMNH	392704	Brazil: Para, Monte Alegre
	<i>viridis</i> 4	FMNH	389732	Brazil: Rondonia, Cachoeira Nazare
	<i>viridis</i> 5	FMNH	389733	Brazil: Rondonia, Cachoeira Nazare
	<i>viridis</i> 6	ZMUC	114931	Ecuador: Sucumbios, Lunbaqui
	<i>viridis</i> 7	ANSP	4659	Ecuador: Esmeraldas, Cabecera de Bilsa
	<i>viridis</i> 8	ANSP	8463	Guyana: Iwokrama Reserve
	<i>viridis</i> 9	ANSP	8576	Guyana: Iwokrama Reserve
	<i>viridis</i> 10	USNM	B11315	Guyana: Linden Highway
	<i>viridis</i> 11	USNM	B11332	Guyana: Linden Highway
	<i>viridis</i> 12	LSUMNS	B28774	Panama: Colon, Achiote
	<i>viridis</i> 13	STRI	PA-TVI630	Panama: Darien, Puerto Pina
	<i>viridis</i> 14	LSUMNS	B4209	Peru: Loreto, 90 km N of Iquitos
	<i>viridis</i> 15	LSUMNS	B7385	Venezuela: Amazonas, Cerro de la Neblina
	<i>viridis</i> 16†	AMNH	SC931	Venezuela: Amazonas
<i>Apaloderma</i>	<i>aequatoriale</i> 1†	ANSP	11460	Equatorial Guinea
	<i>narina</i> 1	FMNH	434493	Democratic Republic of Congo
	<i>narina</i> 2†	AMNH	RWD21159	Liberia
	<i>narina</i> 3	MBM	JK01506	Malawi
	<i>narina</i> 4	FMNH	390084	South Africa
	<i>vittatum</i> 1	FMNH	439090	Malawi
	<i>vittatum</i> 2	FMNH	439094	Malawi
	<i>vittatum</i> 3	FMNH	438534	Mozambique
	<i>vittatum</i> 4†	FMNH	384820	Uganda
<i>Euptilotis</i>	<i>neoxenus</i> 1†	AMNH	PRS2606	USA

Appendix Continued

Genus	Species/sample no.	Museum*	Specimen	Locality
<i>Harpactes</i>	<i>ardens</i> 1†	FMNH	429208	Philippines
	<i>diardii</i> 1†	ANSP	1128	Borneo
	<i>duvaucelii</i> 1†	LSUMNS	B38592	Borneo
	<i>erythrocephalus</i> 1†	AMNH	PRS2170	Vietnam
	<i>oreskios</i> 1†	ANSP	1316	Borneo
	<i>orrhophaeus</i> 1†	LSUMNS	B38633	Borneo
<i>Pharomachrus</i>	<i>antisianus</i> 1†	LSUMNS	B22820	Bolivia
	<i>antisianus</i> 2	FMNH	397882	Peru
	<i>auriceps</i> 1	FMNH	97883	Peru
	<i>auriceps</i> 2†	LSUMNS	B3533	Peru
	<i>fulgidus</i> 1	ZMUC	115750	Colombia
	<i>moccino</i> 1	MBM	DAB1262	Nicaragua
	<i>pavoninus</i> 1	ANSP	2689	Ecuador
	<i>pavoninus</i> 2	FMNH	397884	Peru
	<i>pavoninus</i> 3†	LSUMNS	B5033	Peru
<i>Priotelus</i>	<i>roseigaster</i> 1†	KU	6363	Dominican Republic
	<i>roseigaster</i> 2	STRI	RD-TRO1	Dominican Republic
	<i>temnurus</i> 1†	ANSP	5564	Cuba

*AMNH, American Museum of Natural History; ANSP, Academy of Natural Sciences Philadelphia; CNAV, Colección Nacional de Aves, Universidad Nacional Autónoma de México; FMNH, Field Museum of Natural History; KU, University of Kansas Natural History Museum; LSUMNS, Louisiana State University Museum of Natural Sciences; MBM, Marjorie Barrick Museum of Natural History; MZFC, Museo de Zoología 'Alfonso L. Herrera'; STRI, Smithsonian Tropical Research Institute; UMMZ, Museum of Zoology, University of Michigan; USP, Universidade de São Paulo, Brazil; ZMUC, Zoological Museum University of Copenhagen.

†Moyle 2005.

‡Sorenson *et al.* 2003.