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Department of Vertebrate Zoology, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden

A re-evaluation of basal phylogenetic relationships within trogons (Aves: Trogonidae) based on nuclear DNA sequences

U. S. Johansson and P. G. P. Ericson

Abstract

The avian clade Trogonidae (trogons) consists of approximately 40 species distributed pantropically in the Neotropical, Afrotropical and Indomalayan zoogeographical regions.In this study, we evaluate the basal phylogenetic relationships within the trogons based on DNA sequences from three nuclear introns [myoglobin intron 2, β-fibrinogen intron 7 and glyceraldehydes-3-phosphodehydrogenase (G3PDH) intron 11]. In addition, previously published cytochrome *b* and 12S sequences were re-analysed and combined with the nuclear data set. The analysis of the three nuclear genes combined suggests a sister group relationship between the Afrotropical (*Apaloderma*) and Indomalayan (*Harpactes*) clades, whereas the Neotropical taxa (*Trogon*, *Pharomachrus*, and *Priotelus*) form an unresolved polytomy basal to these two groups. In addition, two of the three individual gene trees also support a sister group relationship between the Afrotropical and Indomalayan trogons. This is at odds with previously published studies based on mitochondrial sequence data and DNA–DNA hybridization. The third nuclear intron (G3PDH), however, suggests that the Afrotropical trogons are basal relative the other trogons. This was also suggested by the mitochondrial data set, as well as the analysis of the combined nuclear and mitochondrial data. Both of these conflicting hypotheses are supported by high posterior probabilities. An insertion in β-fibrinogen further supports a basal position of the Afrotropical clade. Analyses of the myoglobin intron with additional outgroups place the root differently and strongly support monophyly of each of the zoogeographical regions (including the Neotropics), and these three clades form a basal trichotomy. This suggests that that rooting is a serious problem in resolving basal phylogenetic relationships among the trogons.

Key words: Aves – Trogonidae – phylogeny – intron – myoglobin – β-fibrinogen – G3PDH – biogeography – Afrotropical – Neotropical – Indomalayan

Introduction

Trogons (Aves: Trogonidae) are a morphological homogeneous clade and consist of approximately 40 species distributed pantropically in the Neotropical, Afrotropical and Indomalayan zoogeographical regions. The majority of the species in this clade feed primarily on insects but some of the larger species (e.g. *Pharomachrus* – Quetzals) are to a large extent frugivorus. The clade is most diverse in the Neotropics, with approximately 25 species ranging from Arizona to northern Argentina. In contrast, only three species occur in the Afrotropical region south of Sahara and 11 species inhabit the Indomalayan region, ranging from southern India and the subtropical regions of the Himalayas southeastward to the Philippines and along the Malay Peninsula to Sumatra, Borneo, and Java.

Monophyly of Trogonidae is well supported by morphological data. All members of this clade have a heterodactyl foot in which digits I and II are directed backwards, while digits III and IV are directed forwards. This arrangement of the toes is unique to the trogons and not found in any other group of recent birds. The present distribution of trogons is restricted to the tropical and subtropical regions of South and Middle America, Africa, and Asia, but the fossil record indicates that they have had a wider distribution in the past. A heterodactyl tarsometatarsus has been found in the early Eocene (Ypresian) London Clay deposits in Walton-on-the-Naze in Essex, UK. This tarsometatarsus is also in its general appearance similar to recent trogons, indicating that members of this clade were present already 53 million years ago (Mayr 1999). In addition, a cranium from the latest Paleocene – earliest Eocene Fur Formation in Denmark also shows close similarity to recent trogons (Kristoffersen 2002) and thus further supports the presence of trogons in the Paleogene of Europe. A poorly preserved, incomplete skeleton with a heterodactyl foot is also known from the early Oligocene 'Glarner Fischerschiefer' of Matt, Switzerland (Olson 1976). Slightly younger is the Middle Oligocene (*c.* 33 million years ago) *Primotrogon wintersteini*, known from two specimens from Céreste in France (Mayr 1999, 2001). Both specimens have a heterodactyl foot and an overall morphology closely resembling recent trogons. However, *Primotrogon* lacks two skeletal synapomorphies in the coracoid shared by all extant trogons, and has been proposed to be the sister taxon to these (Mayr 1999). The youngest of the known European fossil trogons, *Paratrogon gallicus*, is known from two humeri from the Early Miocene (Aquitanian, *c.* 22 million years ago) of Saint-Gérand-le-Puy, France (Milne-Edwards 1867–1871, Mayr 1999).

Phylogenetic analyses of mitochondrial cytochrome *b* and 12S DNA sequence data also support monophyly of the trogons (Espinosa de los Monteros 2000). In addition, monophyly of each of the clades *Pharomachrus*/*Euptilotis* (Neotropical), *Trogon* (Neotropical), *Apaloderma* (Afrotropical), and *Harpactes* (Indomalayan) has been supported by studies of these two genes (Espinosa de los Monteros 1998). However, relationships between these clades (and *Priotelus temnurus* – Neotropical) were poorly resolved in that study, with no bootstrap support exceeding 50%, and the basal topology differed depending on the various weighting schemes applied to the data set. Nevertheless, Espinosa de los Monteros (1998) favoured the topology that resulted from an analysis where the two genes were combined, but with the third codon positions in the cytochrome *b* gene excluded. In this tree each of the three zoogeographical regions were monophyletic with the Neotropical and the Indomalayan groups placed as sister groups, and the Afrotropical group placed basal to these. The same relationships between these regional groups have

previously been suggested by DNA–DNA hybridization data (Sibley and Ahlquist 1990), although, e.g. the Neotropical clades *Pharomachrus* and *Priotelus* were not included in that study.

An alternative arrangement, with the Neotropical trogons basal to the others was indicated by a preliminary analysis of sequence data obtained from the nuclear c-*myc* gene (Johansson 1998), but the bootstrap support for this arrangement did not exceed 50%.

Incongruence between different gene trees is commonly observed in phylogenetic studies (Doyle 1992; Page and Charleston 1997; Rokas et al. 2003). This incongruence can reflect different histories of the individual genes (e.g. through lineage sorting or hybridization and introgression). However, also taxon sampling, insufficient data (e.g. because of a combination of short internodes and slowly evolving genes), or excessive homoplasy can lead to a topology that differs from the species tree. The observation that a single gene tree may not accurately reflect the species tree stresses the importance of inclusion of more than one gene in phylogenetic studies to get a better estimate of the species tree.

In this study, we investigate the basal phylogenetic relationships within the trogons based on nucleotide sequences from three nuclear introns [myoglobin intron 2, β-fibrinogen intron 7 and glyceraldehyde-3-phosphodehydrogenase (G3PDH) intron 11]. In addition, we re-analyse the mitochondrial cytochrome *b* and 12S sequences deposited in GenBank (Espinosa de los Monteros 1998) and combine them with the nuclear gene sequences.

Materials and Methods

Taxon sampling and outgroup

The trogon clade is herein represented by 13 species; three from the Indomalayan region, two from the Afrotropical region, and seven from the Neotropical region (Table 1). The higher-level phylogenetic relationships within Neoaves (*sensu* Sibley et al. 1988) are poorly understood (e.g. Johansson et al. 2001; Mayr et al. 2003), and the sister clade of the trogons has not yet been convincingly identified. Several clades have been suggested, mostly different coraciiform taxa (e.g. Feduccia 1975; Maurer and Raikow 1981; Sibley and Ahlquist 1990), but other sister taxa have also been proposed, for example Coliidae – mousebirds (Espinosa de los Monteros 2000), Bucerotidae – hornbills (Johansson and Ericson 2003), and Steatornithidae – oilbird (Mayr 2003). Unfortunately, because of sequencing problems we were not able to obtain sequences for all of these taxa for all genes. Complete sequences for all genes were only obtained from two coraciiform taxa, a roller (*Coracias caudata* – Coraciidae) and a bee-eater (*Merops viridis* – Meropidae). Thus, in a first set of analyses these two taxa were used as outgroups (Table 1). However, to evaluate if the tree topology is sensitive to the choice of outgroup, we performed additional analyses on an expanded myoglobin data set that included representatives of additional groups, variously suggested closely related to trogons (*Alcedo atthis* – Alcedinidae, *Tockus erythrorhynchus* – Bucerotidae, *Colius striatus* – Coliidae, and *Steatornis caripensis* – Steatornithidae). This tree was rooted with *Alectura lathami* – Megapodiidae

DNA extraction, amplification, and sequencing

All amplifications were carried out with Ready-To-Go™ PCR Beads (Amersham Biosciences, Freiburg, Germany) as 25 μl reactions following the manufacturer's recommendations with a final concentration of each primer of 0.4 μM. Before sequencing, the PCR products were cleaned with QIAquick™ PCR Purification Kit (Qiagen®, Qiagen GmbH, Hilden, Germany). Sequencing reactions were done with Perkin Elmer Applied BioSystems PRISM terminator cycle sequencing kits with AmpliTaq FS polymerase with BigDye terminators, following the manufacturer's protocol. Both strands were sequenced for each gene and the multiple sequence fragments obtained by sequencing with different primers were assembled to complete sequences with SeqMan II™ (Dnastar Inc., Madison, WI, USA).

The specific primers for PCR and sequencing of each of the gene fragments are listed in Table 2. Myoglobin intron 2 was amplified either in a single amplification with Myo2 and Myo3F or as a nested PCR with Myo2 and Myo3 in the first amplification and Myo2 and Myo3F in the second amplification. The thermocycling conditions started with an initial 5 min denaturation at 94°C, followed by 40 cycles of 94°C for 40 s, 59°C for 40 s, and 72°C for 1 min, and completed with a final extension at 72°C for 5 min. The re-amplification was performed with identical times and temperatures, but run only for 22 cycles. Both strands of the gene were sequenced with Myo2 and Myo 3F as external primers and Myoint.c, Myoint.nc and Myoint.h2 as internal primers (Myoint.nc and Myoint.h2 on the complementary strand) (see also Norman et al. 1998; Irestedt et al. 2002; Johansson and Ericson 2003). The analysed region of this gene is approximately 700 bp covering the complete intron 2, including 13 and 10 bp of the flanking exons 2 and 3, respectively (Heslewood et al. 1998).

The complete intron 7 of the β-fibrinogen gene (approximately 900 bp) was amplified with FibB17U and FibB17L (Prychitko and Moore 1997) and the thermocycling conditions started with an initial 5 min denaturation at 94°C, followed by 35 cycles of 94°C for 45 s, 50°C for 1 min, and 72°C for 2 min, and completed with a final extension at 72°C for 5 min. For sequencing, primers B17U and B17L were used as external primers and in FibF10, FibR11 and B21U as internal primers (Table 2). In *Apaloderma vittatum* a poly-T stretch near the end of the sequence made the sequencing enzyme to stutter. This resulted in an unreadable sequence from that point for both the forward and reverse primers. As a result, the part just before and after this region is based on a single strand reading and the exact number of thymine residues is uncertain in this taxon.

The complete intron 11 of the glyceraldehyde-3-phosphodehydrogenase gene was amplified with G3PDH13b and G3PDH14b (Table 2), and the analysed part includes the complete intron (approximately 260–290 bp) and 36 and 18 bp of flanking exons 11 and 12, respectively (Fjeldså et al. 2003). The thermocycling conditions for the PCR started with an initial 5 min denaturation at 94°C, followed by 35 cycles of 94°C for 40 s, 57°C for 1 min, and 72°C for 1 min, and completed with a final extension at 72°C for 5 min. G3PDH14b were also used as sequencing primer together with G3PDHintL1 (Table 2, see also van Tuinen et al. 2001; Fjeldså et al. 2003).

The myoglobin, β-fibrinogen, and G3PDH sequences has been deposited in GenBank (GenBank accession numbers are given in Table 1).

Alignment

The sequences were aligned by eye in MegAlign™ (Dnastar Inc.). The alignment of the nuclear introns required insertion of several gaps in the sequences, but were relatively straightforward for the myoglobin and β-fibrinogen introns. The G3PDH sequences were more variable in length within the ingroup, but apparently homologous regions were often easily identified and the homology is uncertain only for a few nucleotide positions. The alignment of the three gene segments combined includes 2090 nucleotide positions (gaps included). The alignment of the 12S gene was based on the alignment in Espinosa de los Monteros (2003) with hypervariable regions of the loops excluded. All other gaps, in both the nuclear and mitochondrial data sets, were treated as missing data in the phylogenetic analyses.

Phylogenetic analysis

Phylogenetic relationships were estimated with Bayesian inference and Markov chain Monte Carlo (MCMC) in MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001). The gene partitions (myoglobin, β-fibrinogen, G3PDH, and the combined mitochondrial cytochrome *b* and 12S genes) were analysed both separately and combined, using the general-time reversal (GTR) model with an estimate of invariable sites

Table 1. Samples used in the study

Species	Zoogeographical region	Sample number	Owner	Myoglobin	Reference	β-Fibrinogen	Reference	G3P	Reference	cytochrome *b*	Reference	12S	Reference
Harpactes oreskios	Oriental	1316	ANSP	AY165827	2	AY600469	1	AY600484	1	U89199	4	U89239	4
Harpactes ardens	Oriental	P492	ZMCU	AY600499	1	AY600470	1	AY600485	1	U94796	4	U94810	4
Harpactes diardii	Oriental	1126	ANSP	AY600500	1	AY600471	1	AY600486	1	U94797	4	U94811	4
Apaloderma narina	Ethiopian	P1148	ZMCU	AY600502	1	AY600473	1	AY600488	1	U94798	4	U94812	4
Apaloderma vittatum	Ethiopian	P1340	ZMCU	AY600503	1	AY600474	1	AY600489	1	U89200	4	U89234	4
Pharomachrus auriceps	Neotropical	4825	ANSP	AY600504	1	AY600475	1	AY600490	1	U94799	4	U94813	4
Pharomachrus pavoninus	Neotropical	2689	ANSP	AY600505	1	AY600476	1	AY600491	1	U94800	4	U94814	4
Priotelus temnurus	Neotropical	5564	ANSP	AY600501	1	AY600472	1	AY600487	1	U89202	4	U89237	4
Trogon curucui	Neotropical	937172	NRM	AY600506	1	AY600477	1	AY600492	1	U94801	4	U94815	4
Trogon violaceus	Neotropical	P501	ZMCU	AY600507	1	AY600478	1	AY600493	1	U94802	4	U94816	4
Trogon melanurus	Neotropical	P494	ZMCU	AY165828	2	AY600479	1	AY600494	1	U94805	4	U94819	4
Trogon collaris	Neotropical	5531	FMNH	AY600508	1	AY600480	1	AY600495	1	U94808	4	U94822	4
Trogon mexicanus	Neotropical	16	FMNH	AY600509	1	AY600481	1	AY600496	1	U94809	4	U94823	4
Coracias caudata		750	NMWM	AY165807	2	AY600482	1	AY600497	1	U89184	5	U89225	5
Merops viridis		P935	ZMCU	AY165815	2	AY600483	1	AY600498	1				
Merops nubicus										U89185	5	U89224	5
Alcedo atthis		968171	NRM	AY165800	2								
Tockus erythrorhynchus		P487	ZMCU	AY165823	2								
Colius striatus		P398	ZMCU	AY233369	3								
Steatornis caripensis		B7474	LSUMZ	AY233371	3								
Alectura lathami		B20851	LSUMZ	AY165801	2								

ANSP, Academy of Natural Sciences of Philadelphia; Louisiana State University, Museum of Natural Science; NMWM, National Museum of Namibia; NRM, Swedish Museum of Natural History, Department of Vertebrate Zoology; ZMCU, University of Copenhagen, Zoological Museum. References: 1, This study; 2, Johansson and Ericson (2003); 3, Mayr et al. (2003); 4, Espinosa de los Monteros (1998); 5, Espinosa de los Monteros (2000).

Table 2. Primers used for PCR and sequencing

Primer	Sequence (5′ to 3′)	Usage	Reference
Myoglobin			
Myo2	GCC ACC AAG CAC AAG ATC CC	PCR and external sequencing primer	Slade et al. (1993)
Myo3	CGG AAG AGC TCC AGG GCC TT	PCR and external sequencing primer	Slade et al. (1993)
Myo3F	TTC AGC AAG GAC CTT GAT AAT GAC TT	PCR and external sequencing primer	Heslewood et al. (1998)
Myoint.c	AGC CCT GGA GGA TCC ATT GG	Internal sequencing primer	Heslewood et al. (1998)
Myoint.nc	CCA ATG GAT CCT CCA GGG CT	Internal sequencing primer	Heslewood et al. (1998)
Myoint.h2	TCT AAA CTT GGA TAT TCA CAT	Internal sequencing primer	Irestedt et al. (2002)
β-Fibrinogen			
B17U	GGA GAA AAC AGG ACA ATG ACA ATT CAC	PCR and external sequencing primer	Prychitko and Moore (1997)
B17L	TCC CCA GTA GTA TCT GCC ATT AGG GTT	PCR and external sequencing primer	Prychitko and Moore (1997)
FibF10	GTT TAG ATT GGG GAA GAC ATA C	Internal sequencing primer (Trogonidae)	This study
FibR11	GAA ATG TAT GTC TTC CCC AAT C	Internal sequencing primer (Trogonidae)	This study
B21U	TGA TTG ATA ACT ACA ATT ACT TG	Internal sequencing primer (outgroup)	
G3PDH			
G3P13B	TCC ACC TTT GAT GCG GGT GCT GGC AT	PCR	Fjeldså et al. (2003), modified from van Tuinen et al. (2001)
G3P14B	AAG TCC ACA ACA CGG TTG CTG TA	PCR and external sequencing primer	Fjeldså et al. (2003), modified from van Tuinen et al. (2001)
G3PintL1	GAA CGA CCA TTT TGT CAA GCT GGT T	Internal sequencing primer	Fjeldså et al. (2003)

and a discrete Γ-distribution model of among site rate heterogeneity (invgamma). For each of the data partitions, five analyses were run (two with random starting trees, one with the Afrotropical clade being basal, one with the Neotropical clade basal, and one with the Indomalayan clade basal). In each analysis four Markov chains (three heated and one cold, temperature = 0.2) were run for 1 000 000 generations with trees sampled every 100th generation. The log likelihood values stabilized after approximately 5000 generations, and the posterior probabilities were calculated from the remaining 9950 trees.

Nodal supports were also evaluated under the parsimony criterion with a bootstrap analysis in PAUP* 4.0b10 (Swofford 2002). The support values were estimated with 1000 bootstrap replicates, each with 10 random additions of taxa. All analyses including the cytochrome *b* gene were performed both with transitions in the third codon position of this gene excluded (-3Ti) and with all characters equally weighted. Furthermore, as an alternative measure of nodal support, Bremer support values (Bremer 1988, 1994) were calculated in PAUP*.

Results

For the myoglobin intron 2 the uncorrected (p) sequence distances within Trogonidae range from 0.2% between *Pharomachrus auriceps* and *Pharomachrus pavoninus*, to 4.9% between *A. vittatum* and *Trogon collaris*. Between trogons and the outgroup the sequence distance in this intron ranges from 9.3 to 11.3%. Also for the β-fibrinogen intron the lowest pairwise divergence (0.4%) is found between *P. auriceps* and *P. pavoninus*. The highest sequence divergence between two trogons is found between *Harpactes diardii* and *P. temnurus* (9.5%), and between trogons and the outgroup sequence distances range from 15.0 to 20.1%. For the G3PDH intron the sequence distances range from 2.3% between *Trogon curucui* and *Trogon violaceus* to 9.1% between *Apaloderma narina* and *P. temnurus*. Between the trogons and the outgroup these figures range between 16.8 and 22%.

All Bayesian analyses for a certain gene partition, irrespective of starting tree, converged on an identical tree topology, with only minor differences in posterior probabilities. The analysis of the three nuclear introns combined resulted in a highly supported tree with only one node with posterior probabilities less than 1.00 and two with bootstrap support less than 100% (Fig. 1). These highly supported nodes include: (1) Monophyly of the Indomalayan *Harpactes*; (2) Monophyly of Neotropical *Pharomachrus*; (3) Monophyly of the Neotropical

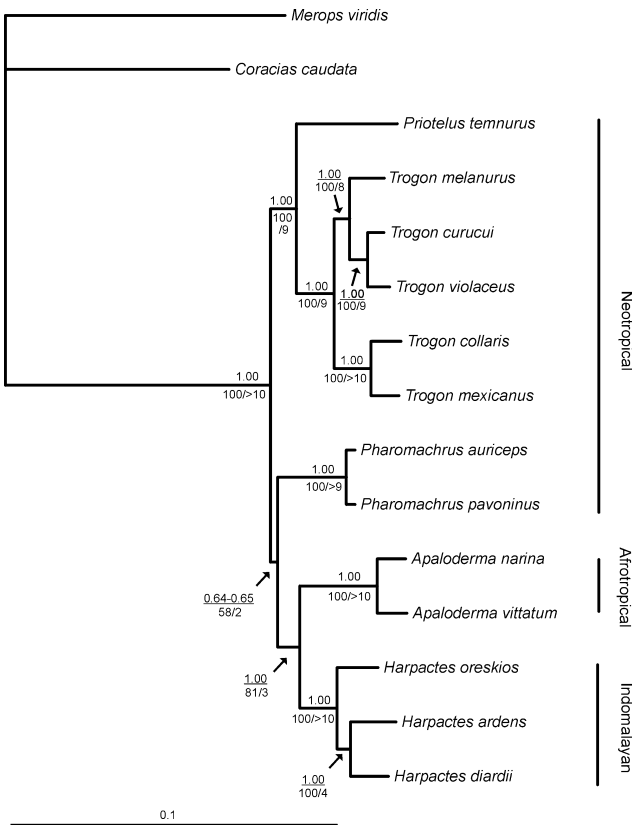

Fig. 1. The 50% majority-rule consensus tree obtained from the Bayesian inference analysis of the combined data set of the three nuclear introns (myoglobin intron 2, β-fibrinogen intron 7 and G3PDH). Nodal supports are indicated in front of the nodes (posterior probabilities from the Bayesian analysis are given above branches and bootstrap/Bremer support from the parsimony analysis below)

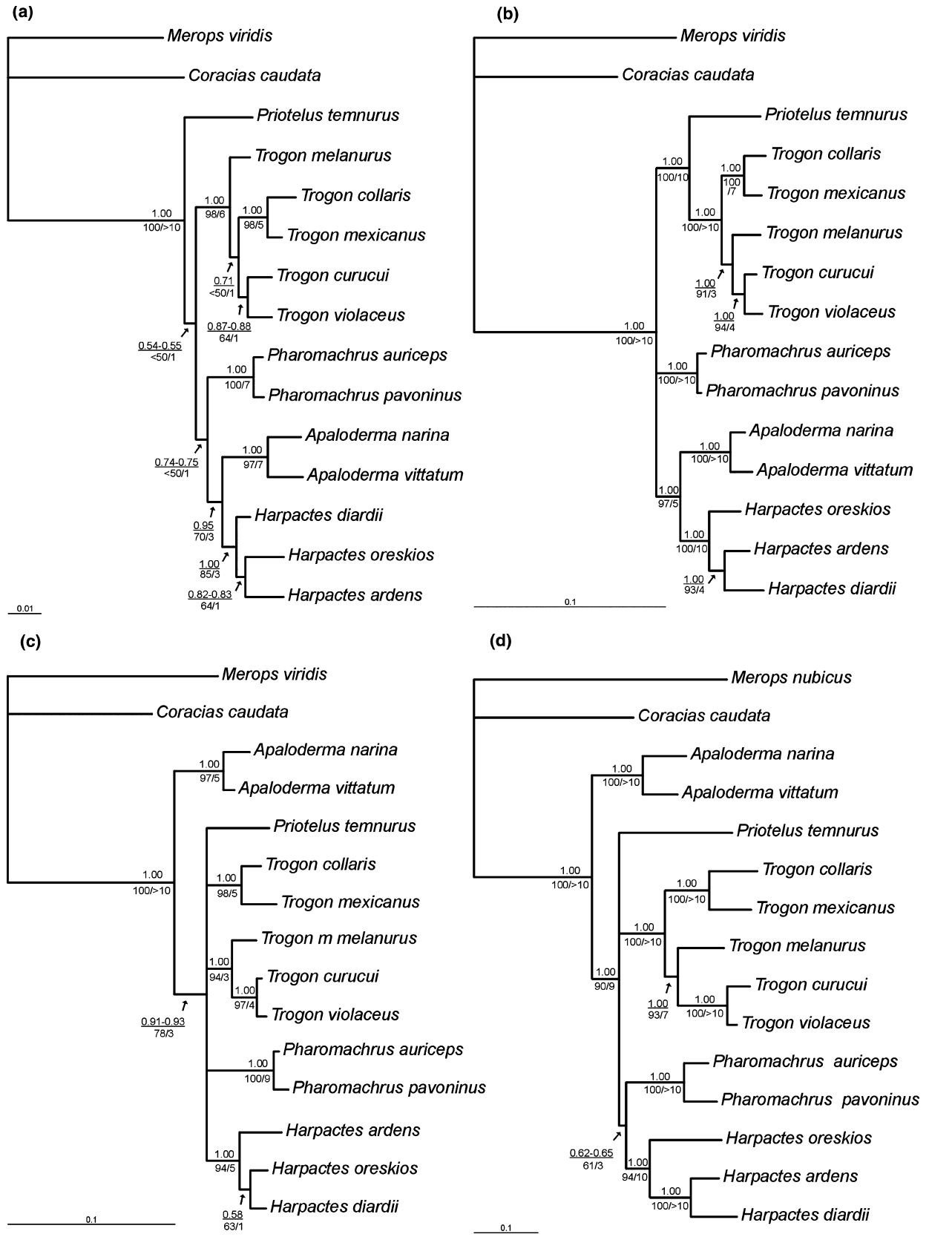

Fig. 2. The 50% majority-rule consensus tree obtained from the Bayesian inference analysis of the individual gene trees (a) myoglobin intron 2, (b) β-fibrinogen, (c) G3PDH, and (d) cytochrome *b* (transitions in third positions excluded) and 12S combined. Nodal supports are indicated in front of the nodes (posterior probabilities from the Bayesian analysis are given above branches and bootstrap/Bremer support from the parsimony analysis below)

Trogon; and (4) Monophyly of *Apaloderma* in the Afrotropical region. All these clades are also present in each of the individual gene trees (Fig. 2). Furthermore, a sister group relationship between *Priotelus* and *Trogon* is highly supported in the combined nuclear tree (Fig. 1), although this clade is not present in all gene trees (Fig. 2). Most notably, in this combined nuclear tree the trogons from the Afrotropical zoogeographical region (*Apaloderma*) are placed as sister group to the Indomalayan clade (*Harpactes*), similar to what is indicated by the nuclear c-*myc* gene (Johansson 1998; Contre Espinosa de los Monteros (1998) and Sibley & Ahlquist (1990)). This association has a posterior probability of 1.00 and bootstrap support of 86% in the parsimony analysis. However, this tree does not support monophyly of the Neotropical trogons. Instead, *Pharomachrus* is placed as the sister group of *Apaloderma* and *Harpactes*. This is, however, the weakest supported node in this tree, and the posterior probabilities varied in the five analyses between 0.64 and 0.65 and the bootstrap support for this association is 58%.

Of the individual gene trees, both myoglobin and β-fibrinogen (Fig. 2a, b) support a sister group relationship between the Afrotropical (*Apaloderma*) and Indomalayan (*Harpactes*) trogons with relatively high support (myoglobin: posterior probability 0.95, bootstrap 70%, β-fibrinogen: posterior probability 1.00, bootstrap 97%). However, the third nuclear intron, G3PDH, place the Afrotropical clade basal relative all other trogons (posterior probability 0.92, bootstrap 78%), but is otherwise quite unresolved (Fig. 2c).

The placement of *Priotelus* as the sister group of *Trogon*, as indicated by the combined analysis, is only supported by the β-fibrinogen gene tree (Fig. 2b). The position of *Priotelus* is essentially unresolved by the other two nuclear introns, although the Bayesian analysis of the myoglobin intron places *Priotelus* as sister group of all other trogons (posterior probability 0.55). Furthermore, in this tree the Neotropical *Pharomachrus* is placed not as sister to the other Neotropical trogons, but as sister to the Afrotropical/Indomalayan clade. However, also for this grouping the posterior probability is low (0.75) and in the parsimiony analysis this relationship and the basal position of *Priotelus* is unsupported (bootstrap support < 50%). In both the β-fibrinogen and G3PDH gene trees the position of *Pharomachrus* is unresolved (Fig. 2b, c).

The analysis of the mitochondrial data set (cytochrome *b* and 12S) (cf. Espinosa de los Monteros 1998) resulted in yet another topology (Fig. 2d). Similar to the G3PDH gene tree, *Apaloderma* is placed basal relative the other trogons (posterior probability 1.00, bootstrap 90%), but in the mitochondrial gene tree a few more nodes received support and each of the clades *Trogon*, *Pharomachrus*, *Harpactes* and *Apaloderma* are monophyletic. The basal position of *Apaloderma* was also found by Espinosa de los Monteros (1998), but receive even higher support in this study (bootstrap support 90% compared with 40% in Espinosa de los Monteros 1998). However, depending whether transitions of the third position of cytochrome *b* is excluded or not, the relationships between *Pharomachrus*, *Trogon*, *Priotelus*, and *Harpactes* are resolved differently. In the equally weighted analysis these four clades are placed in an unresolved polytomy, whereas *Pharomachrus* and *Harpactes* are placed as sisters when transitions in third positions are excluded (Fig. 2d), although this latter association has a very low support (posterior probability 0.62–0.65, bootstrap 61%).

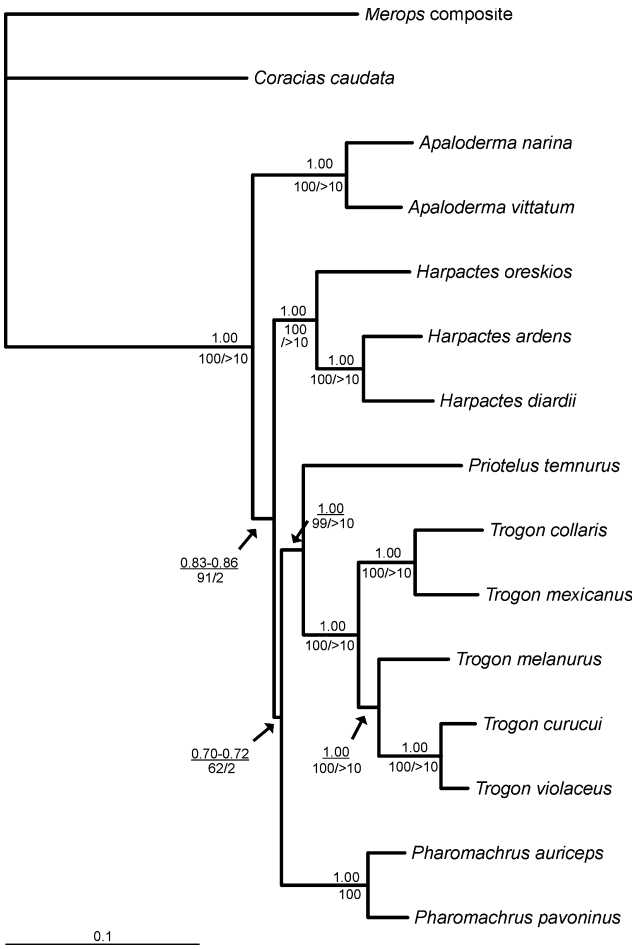

Fig. 3. The 50% majority-rule consensus tree obtained from the Bayesian inference analysis of the combined nuclear (myoglobin intron 2, β-fibrinogen intron 7 and G3PDH) and mitochondrial [cytochrome *b* (transitions in third positions excluded) and 12S] data set. Nodal supports are indicated in front of the nodes (posterior probabilities from the Bayesian analysis are given above branches and bootstrap/Bremer support from the parsimony analysis below)

When the nuclear and mitochondrial data are analysed together as a single data set, yet another topology emerges (Fig. 3). Neither the combined intron tree nor the mitochondrial gene tree in their self support the monophyly of the Neotropical trogons, but when these two partitions are analysed together the monophyly of this clade receive weak support (posterior probability 0.70–0.72, bootstrap 62%). Within the Neotropical clade, *Priotelus* is placed as sistergroup to *Trogon*. The Indomalayan clade is placed as the sister group to the Neotropical trogons, and the Afrotropical clade is, similar to the mitochondrial and the G3PDH gene trees, placed basal relative these two clades (posterior probability 0.83–0.86, bootstrap 91%).

Discussion

The sequence data in this study do not support an undisputable basal phylogeny of the trogons. In the combined nuclear and mitochondrial analysis the Neotropical trogons are monophyletic and placed as sister groups to the Indomalayan clade, and the Afrotropical trogons are placed basal relative to these (Fig. 3). However, basal relationships are poorly sup-

Table 3. Number of informative characters in the nuclear genes compared with the mitochondrial data set

	Myoglobin (intron 2)	β-Fibrinogen (intron 7)	G3PDH (intron 11)	Nuclear genes combined	Mitochondrial (cyt *b* and 12S)
Total number of nucleotides	730	953	407	2090	2055
All taxa					
Number of variable nucleotides	163 (22%)	350 (37%)	141 (35%)	654 (31%)	820 (40%)
Number of apomorphic nucleotides	92 (12%)	185 (19%)	75 (18%)	352 (17%)	235 (11%)
Number of 'parsimony informative' nucleotides	71 (10%)	165 (17%)	66 (16%)	302 (14%)	585 (28%)
With outgroups excluded					
Number of variable nucleotides	90 (12%)	201 (21%)	83 (20%)	374 (18%)	699 (34%)
Number of apomorphic nucleotides	53 (7%)	95 (10%)	40 (10%)	188 (9%)	193 (9%)
Number of 'parsimony informative' nucleotides	37 (5%)	106 (11%)	43 (10%)	186 (9%)	506 (25%)

ported and the individual gene trees support different basal relationships. Two of the gene trees (myoglobin and cytochrome *b*/12S) indicate, albeit with very low posterior probabilities, non-monophyly of the Neotropical trogons. Furthermore, myoglobin and β-fibrinogen indicates that the Afrotropical and Indomalayan clades are sisters and place the Neotropical trogons basal relative them. G3PDH and cytochrome *b*/12S, on the other hand, place the Afrotropical clade basal relative the other trogons.

There are several reasons for conflicting gene trees, some of which include different evolutionary histories of the genes. Although it is not possible to exclude the possibility that the genes included in this study have different histories, technical causes, e.g. insufficient data or choice of outgroups, may also affect the topology. Although the concatenated alignment of the nuclear genes included 2090 bp, only 654 bp were variable, whereof 352 (53%) were apomorphic (Table 3). In other words, only 14% of the characters are 'potentially parsimony informative', and if the outgroups are excluded, this figure decreases to 9%. Compared with the mitochondrial data set, which also is just over 2000 bp, the proportion of 'parsimony informative' characters is 28 and 25% with and without the outgroups, respectively (Table 3). However, the highly supported nodes indicate that this is not necessarily the problem.

It is also possible that misplacement of the root can cause the different hypotheses of basal relationships. After adding additional outgroups to the myoglobin data set, *Merops* and *Coracias*, the two taxa used as outgroups in the combined gene analysis, were placed as the sister clade to the trogons, together with *Alcedo* and *Tockus*. *Colius* and *Steatornis* are both placed basal relative to the trogon-coraciiform clade. This tree (not shown) differs in two important respects compared with that in which only *Merops* and *Coracias* are used as outgroups (cf. Fig. 2a). First, monophyly of the Neotropical trogons has a high posterior probability (0.96). Second, no support was found for a sistergroup relationship between the Afrotropical and Indomalayan clades, and the three zoogeographical regions are placed in a basal trichotomy. Thus, the tree seems to be sensitive to the choice of outgroup and it appears that the long lineage leading to the closest relative of the trogons, compared with relative short internodes connecting the three zoogeographical regions, is a serious problem in resolving the basal phylogeny.

Based on these data alone it is not possible to discriminate between the different hypotheses of basal phylogenetic relationships. However, one of the indels in the β-fibrinogen gene (a deletion of three basepairs) is shared between the Neotropical and Indomalayan clades, whereas no indel supports monophyly of an Afrotropical–Indomalayan clade, adding additional support for a basal position of the Afrotropical trogons suggested by the combined nuclear and mitochondrial data set, as well as the mitochondrial and G3PDH gene trees.

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Zusammenfassung

Eine Überprüfung der basalen phylogenetischen Verwandtschaftsbeziehungen innerhalb der Trogons (Aves, Trogonidae) mit Hilfe von nuklearen DNA-Sequenzen

Die Vogel-Ordnung der Trogonidae (Trogons) besthet aus ungefähr 40 Arten mit pantropischer Verbreitung in den neotropischen, afrotropischen und indomalaysischen zoogeographischen Regionen. Inder Untersuchung ermitteln wir die basalen phylogenetischen Verwandschaftsbeziehungen innerhalb der Trogons aus den DNA-Sequenzen dreier nuklearer Introns (Myoglobin Intron 2, β-Fibrinogen Intron 7 und Glyceraldehyd-3-Phosphodehydrogenase (G3PDH Intron 11). Auβerdem wiederholten wir die Analyse der Cytochrom-b- und der 12S-Sequenzen und kombinieren sie mit den Daten aus der Kern-DNA. Die gemeinsame Analyse der drei Kerngene weist auf eine Schwestergruppenbeziehung zwischen den afrotropischen (*Apaloderma*) und indomalaysischen (*Harpactes*) Claden hin, während die neotropischen Taxa (*Trogon*, *Pharomachrus* und *Priotelus*) eine night auflösbare Verzweigung basal zu diesen zwei Gruppen zeigen. Auβerdem unterstützen zwei der drei Bäume der einzelnen Kernsequenzen eine Geschwistergruppenbeziehung zwischen den afrotropischen und den indomalaysischen Trogons. Das weicht von früher publizierten Untersuchungen mit Hilfe von mitochondrialen Sequenzen und durch DNA–DNA-Hybridisierung deutlich ab. Das dritte nukleare Intron (G3PDH) stellt hingegen die afrotropischen Trogons relativ zu den anderen Trogons an die Basis. Das zeigte sich auch bei der Verwendung des mitochondrialen Datensatzes allein und bei der gemeinsamen Analyse der mitochondrialen und nuklearen Daten. Eine Insertion im β-Fibrinogen unterstützt ebenfalls die basale Position der afrotropischen Gruppe. Die Analyse des Myoglobin-Introns führt zu einer unterschiedlichen

Wurzelung bei der Verwendung unterschiedlicher Außengruppen und unterstütz deutlich eine Monophylie jeder der drei zoogeographischen Regionen (einschließlich der neotropischen Region) und zeigt eine basale Trichotomie der drei Gruppen. Dies läßt erkennen, daß die Wurzelung ein ernstes Problem in der Auflösung der basalen Phylogenie der Trogons darstellt.

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Author's address: U.S. Johansson (for correspondence), Department of Ecology and Evolution, University of Chicago, 1101 East 57th Street, Chicago, IL 60637, USA. E-mail: ulf@uchicago.edu. P.G.P. Ericson, Department of Vertebrate Zoology, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden.