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A molecular phylogenetic survey of caprimulgiform nightbirds illustrates the utility of non-coding sequences

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ABSTRACT

The order Caprimulgiformes comprises five bird families adapted to nocturnal activity. The order has been regarded as monophyletic, but recent evidence suggests that swifts and hummingbirds (Apodiformes) belong within it. To explore the group's phylogeny, we obtained more than 2000 bp of DNA sequence from the cytochrome *b* and c-*myc* genes for 35 taxa, representing all major lineages and outgroups. Non-coding sequences of the c-*myc* gene were unsaturated, readily alignable and contained numerous informative insertions and deletions (indels), signalling broad utility for higher level phylogenetics. A 12 bp insertion in c-*myc* links Apodiformes with owlet-nightjars, confirming paraphyly of the traditional Caprimulgiformes. However, even this rare genomic change is homoplasious when all birds are considered. Monophyly of each of the five traditional families was strongly confirmed, but relationships among families were poorly resolved. The tree structure argues against family status for *Eurostopodus* and *Batrachostomus*, which should be retained in Caprimulgidae and Podargidae, respectively. The genus *Caprimulgus* and both subfamilies of Caprimulgidae appear to be polyphyletic. The phylogeny elucidates the evolution of adaptive traits such as nocturnality and hypothermia, but whether nocturnality evolved once or multiple times is an open question.

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1. Introduction

The impact of molecular data on systematics has been dramatic. Insights from molecular phylogenies have revolutionized our thinking about evolutionary relationships, especially in poorly known groups. At the same time, it has become clear that short DNA sequences from a single gene will not be sufficient to obtain stable phylogenies for many groups. Single-gene trees may differ from each other and from the species tree under a variety of circumstances (Maddison, 1997; Kubatko and Degnan, 2007), and the resolving power of a particular gene will depend on how well its rate of evolution is matched to the structure of the tree in question (Braun and Kimball, 2002). Obtaining reliable, well-resolved phylogenies for many groups will require data from multiple genes evolving at a variety of rates (Rokas and Carroll, 2005). To this end, we here present mitochondrial and nuclear DNA sequence data for a broad sampling of a ubiquitous but little known order of birds.

The traditional avian order Caprimulgiformes comprises about 120 species intricately adapted to nocturnal, mostly insectivorous lifestyles. It includes some familiar birds, such as the nightjars (*Caprimulgus*) and nighthawks (*Chordeiles*), as well as many exotic and bizarre forms, such as the oilbird (*Steatornis*), a frugivorous, echo-locating troglodyte of South American jungles. Their nocturnal nature, cryptic plumage, secretive behavior, and generally tropical distributions make most caprimulgiforms difficult to study in life. They are consequently among the most poorly known groups of birds. Information concerning their taxonomy, distribution and life history has been spotty and scattered, but several recent treatises summarize available information (Cleere, 1998, 1999; Cohn-Haft, 1999; Holyoak, 1999a,b; Thomas, 1999; Holyoak, 2001).

The generally primitive state of caprimulgiform systematics is illustrated by the continuing discovery of new genera (Cleere et al., 2007) and new species (Louette, 1990; Lencioni-Neto, 1994; Safford et al., 1995; Sangster and Rozendaal, 2004). However, continued research on vocalizations and morphology further improves our understanding of nightbird species and species groups (Davis, 1962, 1978; Schulenberg et al., 1984; Fry, 1988; Cohn-Haft, 1993; Robbins et al., 1994; Robbins and Parker, 1997; Pratt, 2000; Cleere, 2002) and a number of pertinent molecular and morphological phylogenetic studies have now been published (Sibley and Ahlquist,

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1990; Mariaux and Braun, 1996; Brumfield et al., 1997; Johansson et al., 2001; Mayr, 2002; Mayr et al., 2003; Dumbacher et al., 2003; Barrowclough et al., 2006; Ericson et al., 2006; Larsen et al., 2007; Livezey and Zusi, 2007; Hackett et al., 2008).

Seminal works on caprimulgiform systematics were written by Sclater (1866a,b), Beddard (1886), Wetmore (1918), Peters (1940), and Tripepi et al. (2006). In addition, a detailed historical review was given by Sibley and Ahlquist (1990) and updated by later authors. Key features and questions are summarized here. The order includes five distinctive lineages, usually accorded family rank. These are the nightjars and nighthawks (Caprimulgidae; 89–90 species), frogmouths (Podargidae; 13 species), owlet-nightjars (Aegothelidae; 9 species), potoos (Nyctibiidae; 7 species), and oilbird (Steatornithidae; 1 species). Although the monophyly of each of these lineages has never been seriously questioned, Sibley and Ahlquist (1990) suggested erecting two new families based on deep divergences in their DNA hybridization data: Eurostopodidae for the nightjar genus *Eurostopodus*, and Batrachostomidae for the frogmouth genus *Batrachostomus*. This finding is consistent with a fossil record extending back to Eocene times for several groups (Mourer-Chauviré, 1982, 1987; Olson, 1987; Peters, 1991), which is as early as any modern bird families appear (James, 2005). Other molecular data are also consistent with these deep splits (Mariaux and Braun, 1996; Brumfield et al., 1997; Barrowclough et al., 2006; Cleere et al., 2007; Larsen et al., 2007).

Morphological diversity (Hoff, 1966) and genetic divergences (Sibley and Ahlquist, 1990; Mariaux and Braun, 1996) among the five lineages are even greater than those within them. In fact, accumulated divergence is so great that several analyses of molecular and morphological data failed to recover a close relationship among the five lineages (Johansson et al., 2001; Mayr, 2002; Chubb, 2004; Cracraft et al., 2004; Fain and Houde, 2004; Barrowclough et al., 2006; Ericson et al., 2006; Livezey and Zusi, 2007), leading to suspicions that the order might be polyphyletic. These suspicions were exacerbated by strong indications of a sister group relationship of owlet-nightjars with swifts and hummingbirds (Mayr, 2002; Mayr et al., 2003; Cracraft et al., 2004; Barrowclough et al., 2006; Ericson et al., 2006). However, studies sampling multiple nuclear genes and a larger number of taxa have now found strong support for monophyly of an expanded Caprimulgiformes with Apodiformes nested within it (Ericson et al., 2006; Hackett et al., 2008). A higher-order morphological phylogeny also recovered a clade consisting of these groups, but with Apodiformes sister to the traditional Caprimulgiformes (Livezey and Zusi, 2007). However, two of the five synapomorphies proposed by Livezey and Zusi for the traditional clade Caprimulgiformes were criticized by Mayr (2008; see also Discussion).

Generic and subfamily demarcations are in need of revision. The large genus *Caprimulgus* (>50 species), for example, appears to be a catch-all of distantly related, morphologically conservative lineages, but no satisfactory arrangement has yet been proposed (Sibley and Ahlquist, 1990; Cleere, 1998, 1999; Holyoak, 2001; Barrowclough et al., 2006; Larsen et al., 2007). Conversely, morphologically divergent lineages are represented by five small caprimulgid genera (*Macrodipteryx*, *Macropsalis*, *Uropsalis*, *Hydropsalis*, *Eleothreptus*) based on sexually selected characters that may or may not warrant generic distinction. Still other genera (e.g., *Nyctibius*, *Batrachostomus*) contain species that are older than many avian families, if even an approximate molecular clock can be applied (Mariaux and Braun, 1996; Cleere et al., 2007). Finally, although the standard division of Caprimulgidae into nightjar (Caprimulginae) and nighthawk (Chordeilinae) subfamilies has a morphological basis in the structure of the palate (desmognathous in Chordeilinae vs. schizognathous in Caprimulginae; Cleere, 1999) and the presence or absence of rictal bristles (Oberholser, 1914; Holyoak, 2001), the character states have not been polarized so it is not clear whether they support monophyly of either group. Both DNA hybridization and DNA sequence data strongly suggest that *Eurostopodus* species are sister to a clade including all other caprimulgids examined, and that relationships within the remaining caprimulgids are inconsistent with the standard caprimulgine/chordeiline split (Sibley and Ahlquist, 1990; Mariaux and Braun, 1996; Barrowclough et al., 2006; Larsen et al., 2007).

A well-resolved and well-supported phylogenetic tree for Caprimulgiformes would provide an organizing framework with which to understand their biology and evolutionary history. Toward that end, we here extend the cytochrome *b* sequence data set of Mariaux and Braun (1996) to include new ingroup and outgroup taxa, and add a second gene sequence from the nuclear locus c-*myc*. The c-*myc* gene is more slowly evolving than cytochrome *b*, providing better resolution deep in the tree (Harshman et al., 2003). Because it includes non-coding sequence elements that accommodate length variation more readily than protein-coding sequences, c-*myc* also provides a relatively large number of phylogenetically informative insertion/deletion (indel) characters. The expanded data set provides clear evidence that the traditional order Caprimulgiformes is paraphyletic, and allows us to address several of the systematic issues mentioned above.

2. Materials and methods

2.1. Tissue samples and data collection

Tissue samples from 34 nightbirds and putative relatives were obtained from our own fieldwork and from genetic resource collections of major museums (Table 1). The partial cytochrome *b* sequences of Mariaux and Braun (1996) were confirmed and extended to encompass the entire coding sequence. We used chicken (*Gallus gallus*) cytochrome *b* and c-*myc* sequences from GenBank (NC_001323 and J00889) as an unambiguous outgroup for phylogenetic analyses. Genomic DNA was extracted from tissues using methods described in Mariaux and Braun (1996) and Robbins et al. (2005). Cytochrome *b* and a portion of c-*myc* were amplified via PCR using the primers listed in Table 2 and methods similar to those described in Robbins et al. (2005) and Harshman et al. (2003), respectively. Some c-*myc* sequences required cloning, due to length heterozygosity. Sequences were generated on automatic sequencers using ABI Big Dye Ready-Reaction kits and manufacturer standard protocols, except the Big Dye was diluted 1:16. DNA sequences were deposited in GenBank under accession numbers shown in Table 1.

2.2. Sequence alignment and phylogenetic analyses

Cytochrome *b* sequences were aligned using ClustalW (Thompson et al., 1994). No internal gaps were needed. C-*myc* sequences were aligned using Clustal W as well, followed by manual adjustment by eye. A 39 bp segment near the 3′ end of the c-*myc* intron was excluded from further analyses, due to ambiguous alignment of a hypervariable poly-T element.

Because of the very different rates of evolution and mutational biases between nuclear and mitochondrial (mtDNA) genes, we explored the possibility of phylogenetic incongruence among loci before combining data (Bull et al., 1993; Swofford et al., 1996). We used the incongruence length difference test (Farris et al., 1995) as implemented in PAUP* (Swofford, 2002), where it is known as the partition homogeneity test.

Maximum parsimony (MP) tree searches were conducted for each gene separately and the two genes combined using PAUP* v. 4.0b10 (Swofford, 2002). We ran heuristic searches with equal weighting, 10 random sequence addition replicates, and tree

Table 1
Specimens examined. Taxonomy follows Dickinson (2003).

Common name	Scientific name	Voucher and/or tissue no.	Collector	GenBank Accession Nos.	
				Cytochrome *b*	c-*myc*
Mountain owlet-nightjar	*Aegotheles albertisi*	MVIC[a] E044	Schodde, R.	X95764	FJ588484
Barred owlet-nightjar	*Aegotheles bennettii*	MVIC[a] E636	Christidis, L.	X95774	FJ588482
Australian owlet-nightjar	*Aegotheles cristatus*	MVIC[a] W191	Baverstock, P.R.	X95775	FJ588483
Feline owlet-nightjar	*Euaegotheles insignis*	KUNHM[b] 95997	Mack, A.	FJ588456	EU738243
Philippine frogmouth	*Batrachostomus septimus*	CMNH[c] 36767 (B499)	Gonzales, D.	EF100673	EU738255
Marbled frogmouth	*Podargus ocellatus*	MVIC[a] C332	Christidis, L.	X95771	FJ588474
Papuan frogmouth	*Podargus papuensis*	MVIC[a] C876	Wombey, J.	X95772	FJ588475
Philippine nightjar	*Caprimulgus manillensis*	USNM[d] B06090	Ross, C.A.	FJ588443	FJ588463
Chuck-will's-widow	*Caprimulgus carolinensis*	USNM[d] 622565 (B16652)	Schmidt, B.K.	FJ588442	FJ588461
White-tailed nightjar	*Caprimulgus cayennensis*	USNM[d] 625369 (B11295)	Schmidt, B.K.	FJ588444	FJ588464
Blackish nightjar	*Caprimulgus nigrescens*	USNM[d] 586047 (B04478)	Robbins, M.B.	FJ588446	FJ588466
Great eared nightjar	*Eurostopodus macrotis*	USNM[d] 607328 (B03732)	Dickerman, R.W., and party	FJ588447	EU738292
White-throated nightjar	*Eurostopodus mystacalis*	MVIC[a] JWC129	Wombey, J.	X95779	FJ588468
Papuan nightjar	*Eurostopodus papuensis*	MVIC[a] E660	Schodde, R.	X95780	FJ588467
Common poorwill	*Phalaenoptilus nuttallii*	USNM[d] B00084	Braun, M.J.	X95770	FJ588462
Common nighthawk	*Chordeiles minor*	USNM[d] 586281 (B07837)	unknown	FJ588441	FJ588459
Sand-colored nighthawk	*Chordeiles rupestris*	ANSP[e] T2755	Sornoza, F.	X95778	FJ588460
Rufous-bellied nighthawk	*Lurocalis rufiventris*	ANSP[e] 4467	Davis, T.J.	FJ588445	FJ588465
Long-tailed potoo	*Nyctibius aethereus*	LSUMZ[f] B10877	Meyer, A.S.	X95781	FJ588473
Rufous potoo	*Nyctibius bracteatus*	LSUMZ[f] B4509	Cardiff, S.W.	X95765	EU738327
Great potoo	*Nyctibius grandis*	LSUMZ[f] B15415	Bates, J.M.	X95766	EU738328
Common potoo	*Nyctibius griseus*	USNM[d] 612299 (B00493)	1989 Bocas Expedition	X95767	FJ588472
Northern potoo	*Nyctibius jamaicensis*	KUNHM[b] B2116	netted	FJ588449	FJ588471
White-winged potoo	*Nyctibius leucopterus*	LSUMZ[f] B20315	Cohn-Haft, M.	X95768	FJ588469
Andean potoo	*Nyctibius maculosus*	LSUMZ[f] B271	Braun, M.J.	FJ588448	FJ588470
Oilbird	*Steatornis caripensis*	LSUMZ[f] B7474	Willard, D.E.	EF100675	FJ588476
Crimson topaz	*Topaza pella*	USNM[d] 625397 (B11959)	Schmidt, B.K.	FJ588450	FJ588477
Escudo hummingbird	*Amazilia tzacatl handleyi*	USNM[d] 607712 (B01021)	Parsons, T.J.	FJ588452	FJ588479
Bronzy hermit	*Glaucis aeneus*	USNM[d] B00319	1989 Bocas Expedition	FJ588451	FJ588478
Uniform swiftlet	*Aerodramus vanikorensis*	USNM[d] 608672 (B04039)	Angle, J.P. and Beehler, B.M.	FJ588453	EU738244
Chapman's swift	*Chaetura chapmani*	USNM[d] 609124 (B05266)	Braun, M.J.	FJ588454	FJ588480
Whiskered treeswift	*Hemiprocne comata*	USNM[d] 607338 (B03790)	Ross, C.A.	FJ588455	FJ588481
Barn owl	*Tyto alba*	USNM[d] 612697 (B06443)	James, R.C.	FJ588458	EU738388
Eastern screech owl	*Otus asio*	USNM[d] B27714	Braun, M.J.	FJ588457	FJ588485

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bisection-reconnection (TBR) branch swapping. Bootstrap analyses were performed with equal weighting, 1000 bootstrap pseudoreplicates, and 10 random sequence addition replicates for every pseudoreplicate. Weighted parsimony analyses (WMP) were performed in the same way with the following weights: c-*myc*—1st positions = 10.3, 2nd positions = 15.5, all other sites = 1; cytochrome *b*—1st positions = 3, 2nd positions = 10, and 3rd positions = 1.

For maximum likelihood (ML) analysis, models of sequence evolution for cytochrome *b*, c-*myc*, and a combination of both genes were evaluated using ModelTest 3.06 (Posada and Crandall, 1998). First, a neighbor-joining (NJ) tree was produced via PAUP* under the Jukes–Cantor model of evolution (Jukes and Cantor, 1969). Parameters were then calculated for fifty-six nested models of evolution on the NJ tree and the best model of sequence evolution was selected using the Akaike Information Criterion (AIC). This model and parameter values were fixed in a heuristic maximum likelihood search in PAUP* with 10 rounds of random sequence addition and TBR branch swapping. Model estimation was then repeated on the resulting tree. This process was repeated in a successive approximation approach (Sullivan et al., 1995; Swofford et al., 1996) until tree topology and model parameter values converged. The model and parameter values were then fixed for bootstrap analyses in PAUP*. In the cases of cytochrome *b* alone and c-*myc* alone, 100 pseudoreplicates with one random sequence addition at each pseudoreplicate were performed. For the combined analysis, we performed bootstrap analyses using 650 pseudoreplicates with one random addition at each pseudoreplicate.

Bayesian analyses were performed using MrBayes 3.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). A partition was set for each gene with separate 6-parameter models. Four Markov chains were run with heating and swapping for 20 million generations each. Topology and model parameters were sampled every 100 generations and used to determine the posterior probabilities of clades and estimates of model parameters. The first 500 samples were discarded to allow for burn-in to the target distributions; default settings were used for all other options.

Hypotheses of monophyly derived from traditional classifications were examined by comparing globally optimal trees to the best trees that could be found compatible with each hypothesis. In each analysis, a traditional group was constrained to be monophyletic in PAUP*, and a new model of sequence evolution estimated by the method above. Then, a heuristic ML search with 10 random sequence addition replicates was conducted to find the optimal tree under that constraint. The difference in log likelihood of the constrained tree vs. the unconstrained tree was taken as a measure of support for a particular hypothesis of relationship or its alternative.

3. Results

3.1. Sequence analysis

The aligned data set comprises 2274 nucleotide sites from 35 taxa, about equally divided between cytochrome *b* and c-*myc*

Table 2
Main oligonucleotide primers used for PCR and sequencing.[a]

Primer	Sequence (5′–3′)	Reference
c-myc		
MYC-FOR-01[b]	TAATTAAGGGCAGCTTGAGTC	Harshman et al. (2003)
MYC-FOR-02[b]	TGAGTCTGGGAGCTTTATTG	Harshman et al. (2003)
MYC-FOR-03	AGAAGAAGAACAAGAGGAAG	Harshman et al. (2003)
MYC-FOR-04	AAAAGGCTAAAGTTGGAC	Harshman et al. (2003)
MYC-FOR-05	CACAAACTYGAGCAGCTAAG	Harshman et al. (2003)
MYC-REV-01[b]	CCAAAGTATCAATTATGAGGCA	Harshman et al. (2003)
MYC-REV-02[b]	TGAGGCAGTTTTGAGGTTCT	Harshman et al. (2003)
MYC-REV-03	CATTTTCGGTTGTTGCTG	This study
MYC-REV-04	GGCTTACTGTGCTCTTCT	Harshman et al. (2003)
MYC-REV-06	TTAGCTGCTCAAGTTTGTG	Harshman et al. (2003)
Cytochrome b[c]		
L14703[b]	GGMCAAAAMATTGCMTCYCAC	This study
L14764[b]	TGRTACAAAAAAATAGGMCCMGAAGG	Sorenson et al. (1999)
L14990	CCATCCAACATCTCAGCATGATGAAA	Kocher et al. (1989)
L15323	CCATGAGGACAAATATCATTCTGAGGTGC	Mariaux and Braun (1996)
H15298	CCCCTCAGAATGATATTTGTCCTCA	This study
H15730	GGGATTGAGCGTAGGATGGC	This study
H16060[b]	TTTGGYTTACAAGACCAATG	Robbins et al. (2005)

[a] Other ancillary primers were used to complete sequences of some taxa; their sequences are available from the authors.
[b] Amplification primers.
[c] Numbering of cytochrome *b* primers based on the *Gallus* mtDNA sequence (Desjardins and Morais, 1990).

(Table 3). All sites in cytochrome *b* code for protein, while only about half of c-*myc* sites do. The rest of c-*myc* is split between a 5′ intron and the 3′ untranslated region (UTR) of the gene. More than half of cytochrome *b* sites (51.3%) are variable, compared to one-third of c-*myc* sites (33.4%). Most variable cytochrome *b* sites are also phylogenetically informative (506 of 582 or 87%), while only 58% (221 of 378) of variable c-*myc* sites are.

Pairwise uncorrected sequence divergence is quite high in cytochrome *b*, ranging from 3.0 to 22.7% among ingroups (i.e., traditional caprimulgiforms) and from 13.6 to 25.1% in ingroup to outgroup comparisons. The c-*myc* gene is more slowly evolving, with ingroup distances ranging from 0.0 to 8.7% and ingroup–outgroup distances ranging from 4.9 to 13.8% (distances greater than 10.1% all involve *Gallus*). The relatively high ratios for both genes of informative to variable sites and ingroup to outgroup distances suggest that there are deep divergences within the ingroup, and that some ingroup lineages may rival outgroups in age. Normalized plots of pairwise distances suggest that cytochrome *b* is heavily saturated for substitutions among the deeper lineages (Fig. 1a). The numbers of transitions and transversions plateau quickly and eventually converge at larger distances. The intron and UTR elements of c-*myc* are evolving more rapidly than the exon, but none appear to be saturated within the ingroup (Fig. 1b and c; distances below 0.15). For the UTR, a cloud of points at higher distances gives a semblance of plateauing, but this may be unduly influenced by a single taxon, because all these points involve the outgroup *Gallus*.

Base composition varied between the two genes and among functional partitions within each gene (Table 4). Overall, base frequencies of c-*myc* were relatively uniform. The exon sequence was relatively low in T, and the 3′ UTR was low in G. However, no partition of the c-*myc* data differed significantly from homogeneity of base frequencies across taxa (Table 4). On the other hand, the cytochrome *b* sequences were high in C and low in G, and third codon positions were especially low in G and T. Both variable sites and third positions, which comprise the majority of variable sites for cytochrome *b*, were significantly heterogeneous in base composition across taxa. Most but not all of this heterogeneity appeared to be due to the inclusion of swifts and tree swifts, which had a very low proportion of T (mean = 0.072) at third positions. When those three taxa were deleted, third position heterogeneity was only marginally significant after multiple test correction (Table 4).

3.2. Length variation

Indels were absent from our sequences of cytochrome *b*, a mtDNA gene whose length is usually highly conserved. The aligned c-*myc* data, on the other hand, includes at least 54 inferred indels, ranging in length from 1 to 16 bp (Table 3). The high degree of sequence conservation in c-*myc* makes the alignment unambiguous for most indels. In the few cases for which there is more than one equally parsimonious placement, the alternative alignment generally would have little or no effect on phylogenetic inference. There is, however, one poly-T element near the intron–exon junction that is highly variable in length. This region (39 sites) was excluded from the dataset for phylogenetic analysis.

Fifty-one of the 53 alignable indels occur in non-coding regions and 28 of 53 are phylogenetically informative (Table 3). Of the two

Table 3
Summary of sequence data and tree statistics.

	Cytochrome *b*	c-*myc*				Total
		Intron b	Exon 3	3′ UTR	Indels	
Aligned length	1143	365	579	187	–	2274
Variable sites	586	192	123	66	–	967
Informative sites						
Coding (1/2/3)[a]	121/36/354	–	6/4/62	–	–	127/40/416
Non-Coding	–	113	–	39	–	152
Total	511	113	72	39	–	735
Indels						
Informative	0	18	2	9	–	28
Total	0	40	2	12	–	54
MP tree length	3304	390	271	93	29	–
Consistency index (CI)[b]	0.31	0.64	0.50	0.77	0.97	–
Retention index[b]	0.37	0.74	0.66	0.84	0.99	–
Rescaled CI[b]	0.11	0.47	0.33	0.65	0.96	–

[a] Coding (1/2/3) refers to first, second, and third codon positions.
[b] Consistency and retention indices for the various data partitions were calculated on the ML tree for the combined data.

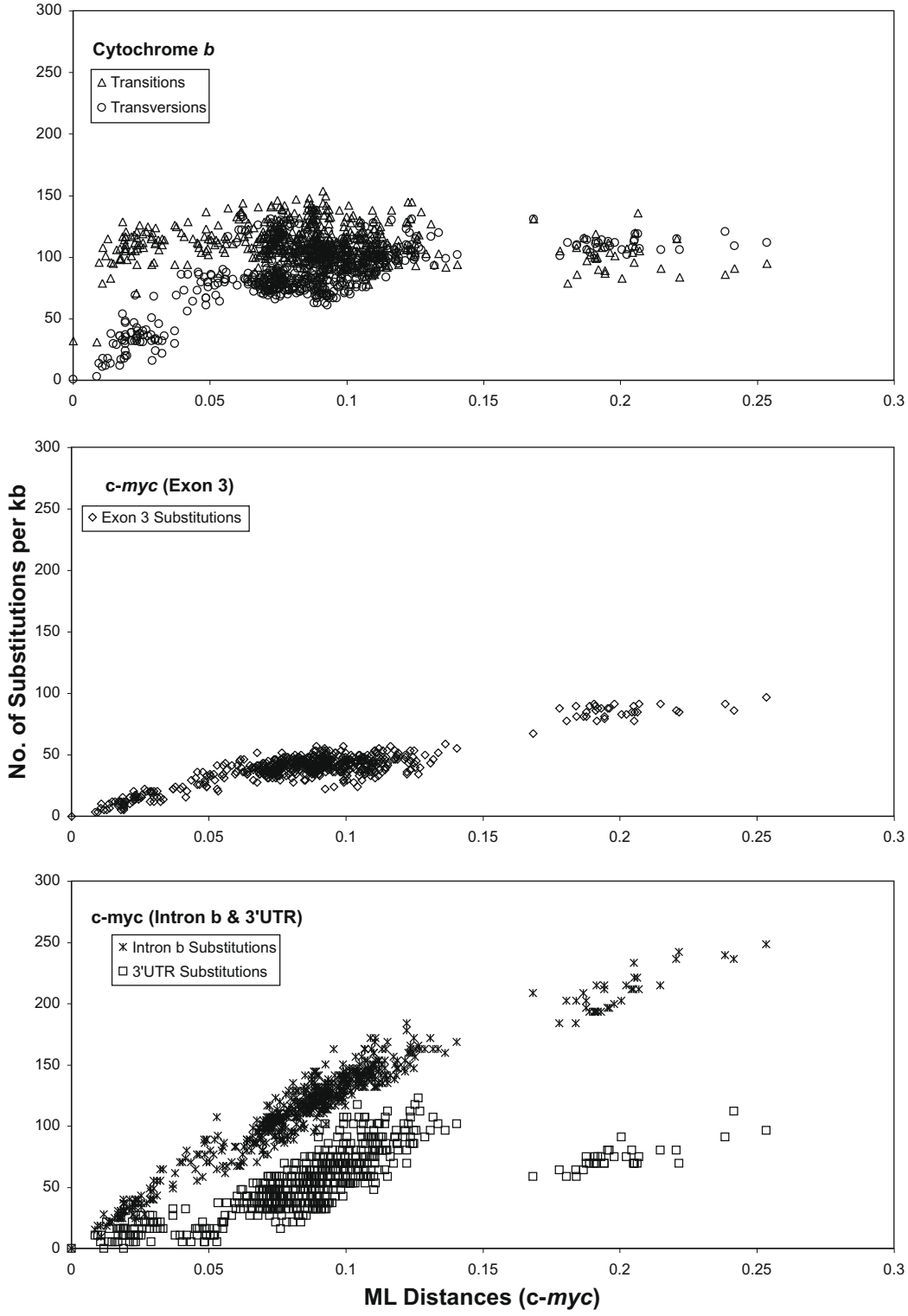

Fig. 1. Saturation plots of observed substitutions per kb in various data partitions vs. divergence of c-*myc* sequences estimated from the ML model for all pairwise comparisons among taxa.

non-coding regions, more indels occur per unit length in the intron than the 3′ UTR (11 vs. 6.4 per 100 bp), but the UTR has a much higher proportion of informative indels, so the number of informative indels per unit length is similar (4.7 per 100 bp of intron, 4.8 per 100 bp of UTR).

The direction of mutation can be inferred for 47 indels from their distribution among taxa on the optimal trees derived from substitutional variation. Twenty are insertions and 27 are deletions. Short indels of 1–3 bp are more common than long ones, and this is especially true for insertions (Fig. 2). Only one insertion is longer than 4 bp, while there are eight such deletions. The greater number and length of deletions results in a marked directionality in total length change: across the data set, a total of 99 bp have been deleted and only 44 bp have been inserted.

Table 4
Base composition of sequence data.

Data partition	Mean %A	Mean %C	Mean %G	Mean %T	Chi-square[a]	*P*-value[b]
c-myc						
All sites	29.9	22.9	23.9	23.3	18.6812	1.0000
Variable sites	27.5	23.5	25.7	23.3	45.5399	1.0000
Intron b	22.8	20.6	24.8	31.9	27.0078	1.0000
Exon 3	33.6	24.2	25.1	17.1	9.7654	1.0000
3′ UTR	31.0	22.6	18.5	27.9	8.5670	1.0000
Exon 3rd positions	25.0	28.7	28.8	17.5	24.6905	1.0000
Cytochrome b						
All sites	28.4	33.9	12.5	25.2	62.7774	0.9992
Variable sites	32.1	42.7	6.8	18.4	145.4238	0.0031
1st positions	26.8	29.5	20.9	22.7	19.2206	1.0000
2nd positions	20.7	26.9	13.0	39.4	4.8254	1.0000
3rd positions	37.7	45.2	3.6	13.5	207.7503	0.0000
3rd positions (minus swifts)	37.9	44.6	3.5	14.1	134.0671	0.0034

[a] The homogeneity of base frequencies across taxa was tested by chi-square.
[b] All *P*-values are shown prior to multiple test correction. Using the sequential Bonferroni procedure (Hochberg, 1988), the adjusted critical value over all tests was $\alpha < 0.005$.

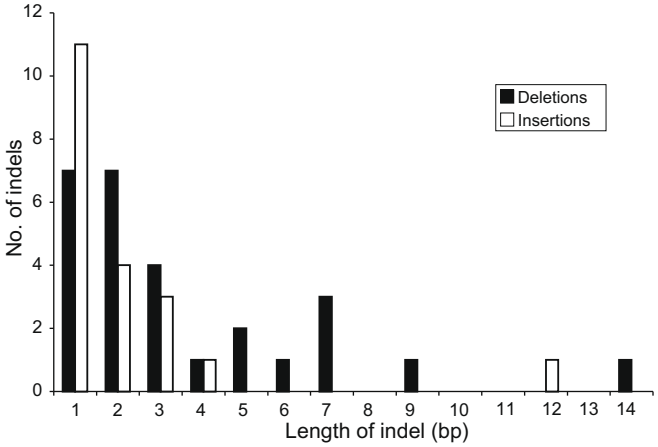

Fig. 2. Number of insertions and deletions of various lengths inferred in c-*myc* sequences. All indels that could be polarized are included (47 of 54).

3.3. Phylogenetic analyses—deeper nodes

When c-*myc* and cytochrome *b* datasets are analysed separately, both genes provide some phylogenetic resolution at various depths in the tree down to the family level (Fig. 3). In general, cytochrome *b* provided better resolution at the tips of the tree, while c-*myc* provided better resolution of deeper nodes, as might be expected from their relative evolutionary rates (Fig. 1). The phylogenetic signals from the two genes appear to be in good agreement. The topologies of the optimal trees from separate analyses of c-*myc* and cytochrome *b* are largely congruent, and there is low support for those nodes that are in conflict. A partition homogeneity test found no significant incongruence between the two genes ($P = 0.189$). We therefore combined the data for further analysis. Combining data from the two loci dictates a hybrid model of sequence evolution for ML analyses in PAUP* (Table 5), but separate models were applied to the two gene partitions in Bayesian analyses.

The resulting phylogeny has strong support for many traditional and some novel groupings (Fig. 4). Among the deeper nodes, all the traditional families of caprimulgiforms and outgroups were recovered with ML bootstrap support greater than 95% and Bayesian posterior probabilities (PP) of 1.0 (Fig. 4). All traditional families were further supported by one to four indel characters in c-*myc*. The traditional orders Apodiformes and Strigiformes received ML bootstrap support of greater than 90% and Bayesian PP of 1.0 (Fig. 4). A 1 bp indel in c-*myc* also supported apodiform monophyly. Most of these traditional groupings were also found in single gene analyses, albeit generally with lower support values (Fig. 3). The oilbird, *Steatornis*, grouped consistently with potoos (*Nyctibius*) in analyses of c-*myc* (Fig. 3) or combined analyses (Fig. 4), but with low support.

Conspicuously absent was support for the traditional order Caprimulgiformes. This group was not monophyletic in any analysis of single genes or combined data. "Outgroups" (swifts, hummingbirds, owls) were interspersed with "ingroups" in many analyses, but with inconsistent and weak associations (Figs. 3 and 4). Many basal branches were very short, indicating that there is little signal supporting them (e.g., Fig. S1). The one grouping that appears consistently is a clade linking Apodiformes (swifts and hummingbirds) with Aegothelidae (owlet-nightjars). This clade was found in all analyses of c-*myc*, and received relatively strong support in WMP, ML and Bayesian analyses (Fig. 3). It was also found in MP, WMP and Bayesian analyses of cytochrome *b* (not shown), but with weaker support. This nontraditional clade appears again in all analyses of the combined data, with 79% ML bootstrap support and Bayesian PP of 1.0 (Fig. 4). All apodiforms and aegothelids also shared a 12 bp insertion in the coding sequence of c-*myc* not found elsewhere in the data set, providing an apparent synapomorphy for the clade (see *Phylogenetic Utility of Introns*, *UTRs* and *Indels* below).

3.4. Phylogenetic analyses—shallow nodes

Within families, a number of interesting results emerge (Fig. 4). In Caprimulgidae, the large genus *Caprimulgus* is non-monophyletic. *Lurocalis* and *Phalaenoptilus* are found in well-supported clades with one or more *Caprimulgus* species. *Lurocalis* and *Chordeiles* are found in separate clades, with strong support values for one and a 3 bp insertion in the c-*myc* intron supporting the other. This topology renders both subfamilies, Caprimulginae and Chordeilinae, non-monophyletic. Support for each of these results comes from both cytochrome *b* and c-*myc* (Fig 3).

Basal to all other caprimulgids, we find three exemplars of the genus *Eurostopodus* on two successive branches in both single gene and combined analyses (Figs. 3 and 4). However, the branching order of the two groups is reversed in single gene analyses. The cytochrome *b* divergence of *Eurostopodus macrotis* from the other *Eurostopodus* averages 17.8%, much higher than that found between most avian congeners. The three *Eurostopodus* are clearly associated with other caprimulgids by strong support values and

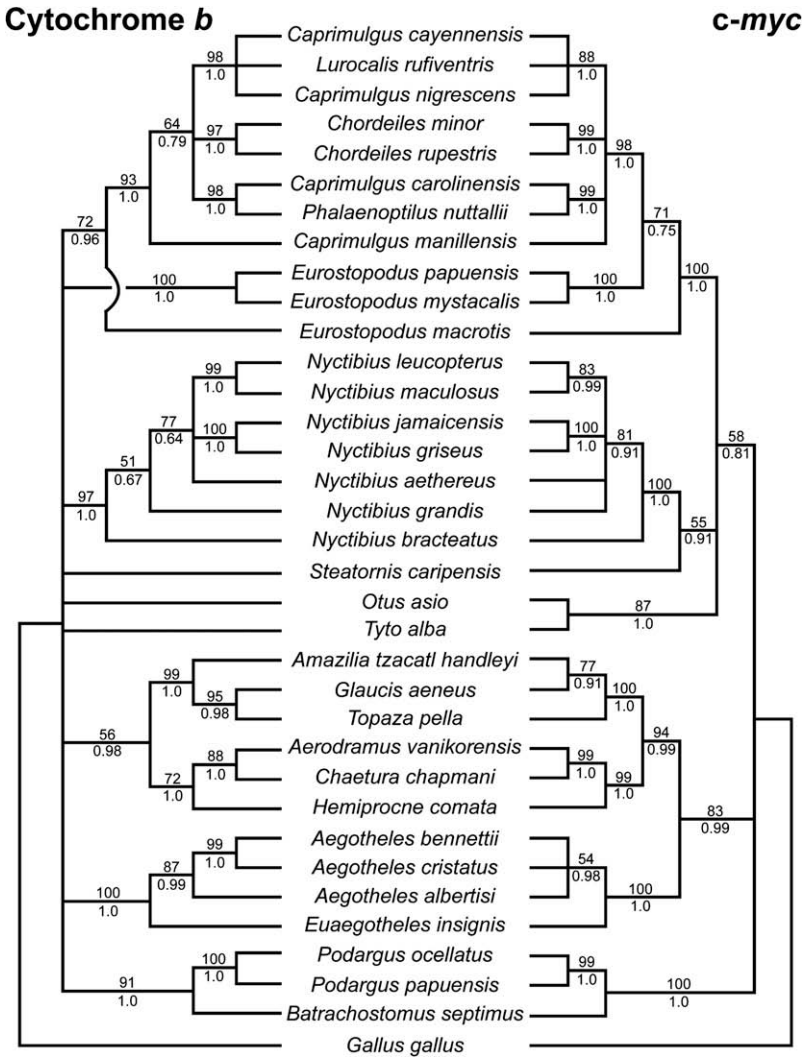

Fig. 3. Phylogenetic trees based on separate analyses of cytochrome *b* and c-*myc* sequences. ML bootstrap values are shown above the line and Bayesian posterior probabilities are shown below. Topologies are majority rule consensus trees from the ML bootstrap analyses (i.e., nodes with support below 50% are collapsed).

Table 5
Models of sequence evolution for various data partitions in ML analyses.

	Cytochrome *b* (GTR+I+Γ)[a]	c-*myc* (HKY+I+Γ)[b]	Combined (GTR+I+Γ)
Base frequencies			
A	0.3381	0.2839	0.3035
C	0.4493	0.2374	0.3587
G	0.0546	0.2412	0.1667
T	0.1580	0.2375	0.1711
Substitution rates			
A ↔ C	0.2092	1.0000	1.1592
A ↔ G	7.6057	3.4269	4.1531
A ↔ T	0.6518	1.0000	1.331
C ↔ G	0.3125	1.0000	0.291
C ↔ T	8.0419	3.4269	13.0189
G ↔ T	1.0000	1.0000	1.0000
Γ shape parameter (α)	0.4761	0.7106	0.4466
Proportion of invariant sites (*I*)	0.4118	0.3236	0.3785

[a] GTR—general time reversible (substitution rates symmetrical within types, free to vary between types).
[b] HKY—(Hasegawa et al., 1985).

three c-*myc* indels on the node that defines the family, but they are also clearly separated from other caprimulgids by strong support and a 7 bp deletion on the node that groups those taxa together. Most of the support linking *Eurostopodus* with other caprimulgids comes from c-*myc*; single gene analyses of cytochrome *b* showed weak signal for this clade (Fig. 3).

In the Nyctibiidae, we find two strongly supported pairs of small gray potoos, *griseus* + *jamaicensis* and *leucopterus* + *maculosus* (Fig. 4). These two pairs form a clade for which nodal support is less strong, but signal for that clade includes a 1 bp indel in c-*myc*. Basal to the small gray potoos, we find the two large potoos, *aethereus* and *grandis*, and the small rufous *bracteatus* on three successive branches with moderate support. Within the remaining caprimulgiform families, Podargidae and Aegothelidae, relationships of the sampled taxa are well-resolved, with some support coming from each gene.

In the Apodiformes, the tree swift, *Hemiprocne*, is sister to two apodid swifts, and three hummingbirds are sister to the swift clade (Fig. 4). Each of these nodes conforms to traditional relationships and receives support from both cytochrome *b* and c-*myc* (Fig. 3). Within hummingbirds, we find the strongest case of apparent conflict between genes in the current dataset. Cytochrome *b* supports a *Topaza* + *Glaucis* clade with 95% ML bootstrap, while c-*myc* supports a *Glaucis* + *Amazilia* clade with 77% ML bootstrap. Interestingly, the c-*myc* topology appears in combined analyses (Fig. 4), overriding the apparently strong signal from cytochrome *b*.

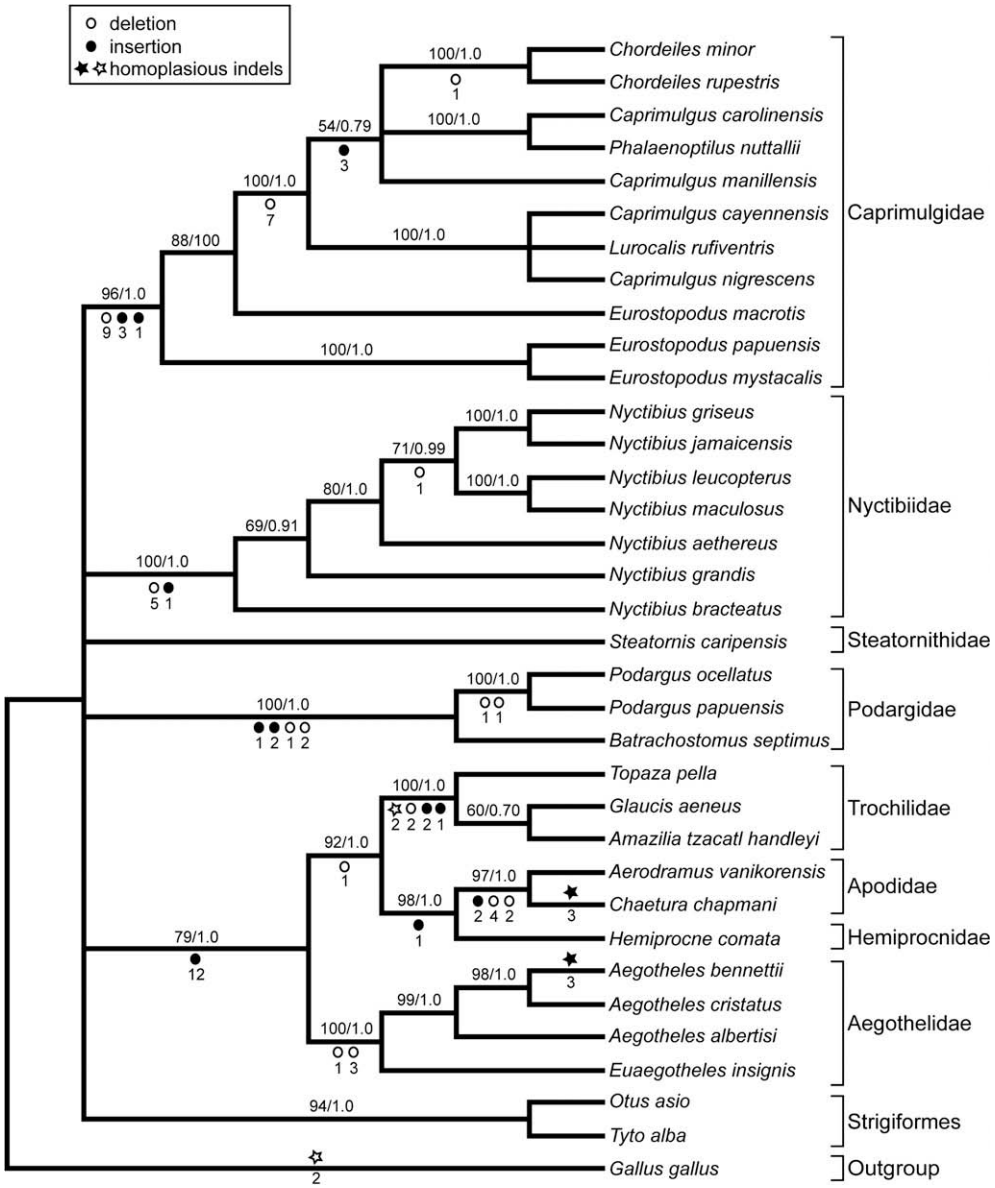

Fig. 4. Best estimate phylogeny based on combined analysis of cytochrome *b* and c-*myc*. Topology is the majority rule consensus tree from the combined ML bootstrap analyses (i.e., nodes with support below 50% are collapsed). Numbers above branches are ML bootstrap values (left) and Bayesian posterior probabilities (right). All phylogenetically informative indels were mapped onto the tree by parsimony. Open circles indicate deletions and filled circles indicate insertions, with indel length in bp given below. Open and filled stars denote two homoplasious indels discussed in the text.

3.5. Comparisons of alternative hypotheses

The traditional order Caprimulgiformes was non-monophyletic in all our optimal trees, as were the subfamilies Chordeilinae and Trochilinae, and the genera *Caprimulgus and Eurostopodus*. To assess whether the sequence data strongly contradicted monophyly of these traditional taxa, we compared the likelihoods of the datasets on the optimal tree to their likelihoods on the best alternative topologies found when these traditional groups were constrained to be monophyletic (Table 6). Monophyly of Caprimulgiformes requires a decrease of 28 log likelihood units for the combined data. Similarly, monophyly of either Chordeilinae or *Caprimulgus* would imply substantially reduced likelihood for all data partitions. The decrease in likelihood required to make either *Eurostopodus* or Trochilinae monophyletic is less dramatic, due in part to conflict between the two genes, but both genes support non-monophyly of these taxa.

Table 6
Comparison of alternative tree topologies.[a]

Constrained group[b]	Δ ln L[c]		
	Cytochrome *b*	c-*myc*	Combined
Caprimulgiformes monophyletic	13.04	17.69	28.40
Chordeilinae monophyletic	19.38	18.49	34.84
Trochilinae monophyletic	4.03	2.33	1.93
Caprimulgus monophyletic	44.78	36.98	78.41
Eurostopodus monophyletic	7.70	1.24	6.67

[a] Constrained and unconstrained ML heuristic tree searches were conducted in PAUP* (Swofford, 2002) as described in Section 2.

[b] Each constrained tree was compared to the respective optimal unconstrained ML tree topology for cytochrome *b* (ln $L = -14144.65509$), c-*myc* (ln $L = -5540.89994$) and combined datasets (ln $L = -20342.74842$).

[c] Numerical values of Δ ln L are the decreases in likelihood of the constrained versus the unconstrained trees.

3.6. Phylogenetic utility of introns, UTRs, and indels

The nuclear partition of our dataset (c-*myc*) has less homoplasy than the mitochondrial partition (cytochrome *b*), as indicated by consistency indices (Table 3). This is true for both the coding and non-coding elements of c-*myc*, with the non-coding elements showing the highest consistency indices.

The indel characters in the data set show very little homoplasy (Table 3). Of the 28 phylogenetically informative indels, 26 map to a single node on the optimal trees (Fig. 4), resulting in a high rescaled consistency index (RC = 0.96). The few indels that are homoplasious are either associated with repetitive elements, show sequence variation indicating independent origins, or both. Examples of homoplasious repetitive elements include the highly variable, 39 bp poly-T region that was excluded from phylogenetic analysis, and a GAA insertion near the beginning of the c-*myc* exon, which becomes the fourth in a string of directly repeated glutamic acid codons. This GAA insertion is inferred to have occurred twice on the optimal trees, once in a swift, *Chaetura chapmani*, and once in an owlet-nightjar, *Aegotheles bennetti* (Fig. 4). The only other indel that is homoplasious on the tree is a 2 bp insertion at intron position 181–182 that occurs in hummingbirds and in chicken. However, the inserted sequence is different (GT in hummingbirds, AT in chicken), suggesting an independent origin.

The 12 bp c-*myc* insertion that supports the clade of swifts, hummingbirds and owlet-nightjars is not homoplasious within the current dataset. However, when we examine all available c-*myc* sequences, it is found again in some barbets (Fig. 5). This insertion is associated with a mildly repetitive element; it forms a third copy of a tandem duplication found in all groups of birds and crocodilians. The evidence suggests that the barbet third copy arose independently of the swift/hummingbird/owlet-nightjar insertion. The barbet insertion is not found in other piciform birds (Fig. 5). Thus, it is phylogenetically nested in a way that suggests independent origin.

4. Discussion

4.1. Phylogenetic utility of introns, UTRs, and indels

The potential advantages of non-coding nuclear sequences for deeper level phylogenetics have been noted previously (e.g., Prychitko and Moore, 2003; Harshman et al., 2003, 2008; Mathee et al., 2007; Chojnowski et al., 2008; Hackett et al., 2008), and they certainly applied with the current dataset. The lower homoplasy found in c-*myc* vs. cytochrome *b* is due in part to the higher rate of evolution of mitochondrial genes (e.g., Table 5) and the relatively great time depth of the tree. In addition, cytochrome *b* sequence evolution is limited by strict constraints of protein function, such that relatively few sites are free to vary (Table 5). The combined result of these factors is that the variable sites in cytochrome *b* accumulate homoplasious substitutions on the deeper branches and are saturated with change (Fig. 1a). The c-*myc* exon shares with cytochrome *b* the functional constraints of all protein-coding sequences, but its slower evolutionary rate results in less saturation (Fig. 1) and less homoplasy (Table 3) in our dataset. The non-coding elements of c-*myc* (intron and 3′ UTR) have less functional constraint and intermediate evolutionary rates (Fig. 1). Consequently, they have less homoplasy than either of the coding elements (Table 3), and, in that sense, produce "better" phylogenetic characters.

The other great advantage of non-coding nuclear sequences is that they are much more likely to retain indel mutations. Indels arise through molecular processes distinct from substitutions. They are therefore expected to provide a largely independent source of phylogenetic information, and be relatively free of many of the caveats that apply to substitutions (Rokas and Holland, 2000). The indels in the present c-*myc* data fulfilled these expectations. They provided confirmation for many nodes in the tree, including some with marginal support from substitutions. They had very low homoplasy overall, and the ones that were

```
Aegothelidae
Aegotheles bennettii        ...TCT AGC ACA GAG TCC AGC ACA GAG TCC AGC ACA GAG ACA TCA GAA...
Aegotheles albertisi        ...TCT AGC ACA GAG TCC AGC ACA GAG TCC AGC ACA GAG ACA TCA GAA...
Aegotheles cristatus        ...TCT AGC ACA GAG TCC AGC ACA GAG TCC AGC ACA GAG ACA TCA GAA...
Euaegotheles insignis       ...TCT AGC ACA GAG TCC AGC ACA GAG TCC AGC ACA GAG ACA TCA GAA...
Apodiformes
Hemiprocne comata           ...TCC AGC ACA GAG TCC AGC ACA GAG TCC AGT ACA GAC ACA TCA GAA...
Aerodramus vanikorensis     ...TCC AGC ACA GAG TCC AGC ACA GAG TCC AGT ACA GAC ACA TCA GAA...
Chaetura chapmani           ...TCC AGC ACA GAG TCC AGC ACA GAG TCC AGT ACA GAC ACA TCA GAA...
Glaucis aeneus              ...TCC AGC ACA GAG TCT AGC ACA GAG TCC AGC ACA GAC ACA TCA GAA...
Topaza pella                ...TCC AGC ACA GAG TCT AGC ACA GAG TCC AGC ACA GAC ACA TCA GAA...
Amazilia tzacatl handleyi   ...TCC AGC ACA GAG TCT AGC ACA GAG TCC AGC ACA GAC ACA TCA GAA...
Other Nightbirds
Nyctibius griseus           ...TCC AGC ACA GAG TCC AGC ACA GAC --- --- --- --- ACA TCA GAA...
Podargus ocellatus          ...TCC AGC ACA GAG TCC AGC ACA GAG --- --- --- --- ACA TCA GAA...
Caprimulgus manillensis     ...TCC AGC ACA GAG TCC AGC ACA GAC --- --- --- --- ACA TCA GAA...
Steatornis caripensis       ...TCC AGC ACA GAG TCC AGC ACA GAC --- --- --- --- ACA TCA GAA...
Outgroups
Otus asio                   ...TCC AGC ACA GAG TCC AGC ACC GAC --- --- --- --- ACA TCA GAG...
Gallus gallus               ...TCC AGC ACA GAG TCC AGC ACA GAA --- --- --- --- GCA TCA GAG...
Piciformes
Megalaima virens            ...TCC AGC ACA GAG TCC AGC ACA GAG TCC AGC ACA GAC ACA TCA GAA...
Indicator maculatus         ...TCC AGC ACA GAG TCC AGC ACA GAC --- --- --- --- ACA TCA GAA...
Galbula albirostris         ...TCC AGC ACA GAG TCC AGC ACA GAC --- --- --- --- ACT TCA GAA...
Capito niger                ...TCC AGC ACA GAG TAC AGC ACA GAC --- --- --- --- ACA TCA GAA...
Dryocopus pileatus          ...TCG AGC ACA GAG TAC AGC ACA GAC --- --- --- --- ACA TCA GAA...
                               repeat 1        repeat 2        repeat 3
```

Fig. 5. Twelve bp insertion in c-*myc* coding sequences of apodiform, aegothelid, and some piciform birds. Piciform sequences are from Hackett et al. (2008).

homoplasious were all associated with repetitive elements, a clear signal that those regions could be expected to be hypermutable. Patterns of indel evolution, with short indels more common than long ones and deletions more common than insertions, were similar to those found in other recent studies of birds and mammals (Fain and Houde, 2004; Johnson, 2004; Mathee et al., 2007), although the ratio of deletions to insertions found here (1.35/1) was lower than the same ratio for beta-fibrinogen intron 7 in doves (6/1; Johnson, 2004) or three introns in mammals (3/1; Mathee et al., 2007). This ratio may vary from gene to gene, as crocodilian c-*myc* sequences also had a lower ratio (1/1; Harshman et al., 2003).

Phylogeneticists have often avoided non-coding sequences for fear of uncertainty or subjectivity in alignment. It is clear from our dataset and others (Prychitko and Moore, 2003; Harshman et al., 2003; Fain and Houde, 2004; Ericson et al., 2006; Mathee et al., 2007; Hackett et al., 2008) that such sequences can be useful over larger phylogenetic distances than previously supposed. Algorithmic advances promise to make alignment of non-coding sequences more straightforward and objective (e.g., Katoh et al., 2005; Loytynoja and Goldman, 2008; Liu et al., 2009). Needed now are methods to model the evolution of indels and allow their frequency in relation to substitutional changes to be estimated. It seems clear from our data (Fig. 2) and other studies (Johnson, 2004; Mathee et al., 2007) that useful models with relatively few parameters can be devised to estimate indel evolution on fixed alignments of moderately sized datasets. This would allow indels to be incorporated directly in maximum likelihood or Bayesian tree searches, fully utilizing the information content of non-coding sequences.

4.2. Phylogeny and systematics—deep nodes

Among the deeper nodes in the phylogeny, all traditional families and orders, with the exception of Caprimulgiformes, were recovered with strong support in the combined analyses. Most were also supported by one or more indel synapomorphies. Thus, all of these taxa are likely to represent monophyletic clades, reflecting traditional notions of avian relationships.

The novel association of Aegothelidae with Apodiformes reported here has been found by the majority of recent relevant studies. With regard to morphological evidence, Mayr (2002) and Mayr et al. (2003) found this clade based on morphological data sets of up to 89 characters and 29 taxa, and highlighted the cruciform origin of the splenius capitis muscle as an "unambiguous synapomorphy" for the group (Mayr et al., 2003). Burton (1971) first reported the shared cruciform origin of the splenius capitis muscle, but did not consider it evidence of relationship, doubtlessly because that would have seemed a radical proposal at the time. The extinct Aegialornithidae may well represent a link between Apodiformes and Caprimulgiformes (Collins, 1976), particularly Aegothelidae (Mayr, 2002). Indeed, Collins' proposition that the Aegialornithidae are "representatives of a caprimulgiform lineage that later gave rise to the swifts" now seems prescient.

On the other hand, Livezey and Zusi (2007), analysing a morphological dataset of 2954 characters scored for 150 bird lineages in a parsimony framework, found the Apodiformes to be sister to the traditional order Caprimulgiformes, including Aegothelidae. The discrepancy may be a due to a rooting issue; because Aegothelidae is basal in Livezey and Zusi's Caprimulgiformes, shifting the root by one node will produce an aegothelid–apodiform clade. However, Mayr (2008) also criticized two of Livezey and Zusi's five "diagnostic apomorphies" for Caprimulgiformes, namely beak morphology and presence of the tapetum lucidum. Mayr (2008:66) found it "incomprehensible" that Livezey and Zusi assigned the same character state for beak morphology to Podargidae, Steatornithidae, Caprimulgidae and Aegothelidae, but a different one to Apodidae and Hemiprocnidae, whose beaks are "extremely similar" to those of Aegothelidae. Mayr also found Livezey and Zusi's coding of the tapetum lucidum as present for all members of Caprimulgiformes an "unacceptable generalization", citing the lack of firm evidence. In fact, after detailed study, Martin et al. (2004) found "no evidence of a tapetum in either the retina or choroid" of oilbird eyes. The tapetum is a reflective structure responsible for brilliant eyeshine and increased sensitivity to light in caprimulgids and many other nocturnal vertebrates (Nicol and Arnott, 1974; Ollivier et al., 2004). We have not found a clear statement in the literature on whether podargids and aegothelids have eyeshine or a tapetum of any kind, but potoos must certainly have a reflective structure responsible for their brilliant orange eyeshine, which can be seen at great distances (van Rossem, 1927; Braun, pers. obs.).

The molecular evidence for an aegothelid–apodiform clade has been consistent, and can now be considered nearly unassailable, having been found in numerous nuclear genes, a mitochondrial gene and indels. While the relationship was not detected in DNA hybridization studies (Sibley and Ahlquist, 1990), it is clear in retrospect that it was beyond the resolving power of that technique. Support for this clade has been reported in substitutional variation of at least 16 nuclear genes (Mayr et al., 2003; Cracraft et al., 2004; Ericson et al., 2006; Barrowclough et al., 2006; Hackett et al., 2008) and one mitochondrial gene (cytochrome *b*; this study). In general, these studies have found weak to moderate support in analyses of individual genes. Support values become truly convincing when multiple genes are analysed in an ML framework (Hackett et al., 2008). The possibility that base compositional artifacts could produce this grouping was specifically examined and rejected by Barrowclough et al. (2006) and in the present study.

Finally, indel support for an aegothelid–apodiform clade is found in the 4 codon c-*myc* insertion discussed here and previously (Braun and Huddleston, 2001; Mayr et al., 2003; Cracraft et al., 2004), and a 5 codon deletion in RAG-1 (Barrowclough et al., 2006). The concordance of signal for this clade from nuclear sequence data, mitochondrial sequence data and indels is reassuring, as these are essentially independent lines of evidence.

An aegothelid–apodiform clade renders the traditional order Caprimulgiformes non-monophyletic. But would a broader grouping, including all the traditional caprimulgiform and apodiform families, be monophyletic? Livezey and Zusi (2007) found morphological evidence for such a clade, although Mayr et al. (2003) did not. Most molecular studies to date have not produced convincing support for or against this grouping (Sibley and Ahlquist, 1990; Mayr et al., 2003; Cracraft et al., 2004; Fain and Houde, 2004; Barrowclough et al., 2006; this study). Ericson et al. (2006) did recover this clade, but did not address the possibility of inflation of its posterior probability, a common problem in Bayesian analyses (Alfaro and Holder, 2006). Hackett et al. (2008) found strong support for a caprimulgiform + apodiform clade in combined ML analyses of 19 genes, but that clade was present in only 3 of 19 single gene analyses. Thus, while these results are promising, a rigorous examination of this node to exclude potential artifacts, such as long branch attraction, base compositional bias, and gene tree problems is in order (e.g., Harshman et al., 2008).

The taxonomic implications of the aegothelid + apodiform clade were discussed by Sangster (2005) and Barrowclough et al. (2006). Two possibilities are to (1) transfer Aegothelidae to Apodiformes, or (2) recognize a supraordinal taxon that includes swifts, hummingbirds and owlet-nightjars. However, the probable monophyly of a grouping of all five traditional caprimulgiform families plus Apodiformes is pertinent to this discussion. If that larger clade is indeed monophyletic, expanding Caprimulgiformes to include

swifts and hummingbirds is a third possibility. More molecular data relevant to this question are likely to be forthcoming, and it seems prudent to delay taxonomic changes until sufficient data are available to make stable ones.

The association of *Steatornis* with Nyctibiidae seen here was also found by Hackett et al. (2008). Although the signal for it is not strong, this grouping deserves further scrutiny. It is interesting biogeographically, given that the current ranges of both taxa are restricted to the Neotropics. However, both taxa apparently occurred more widely in the past (Mourer-Chauviré, 1982, 1987; Olson, 1987).

Livezey and Zusi (2007) proposed the new suborder Hemiprocni on the basis of their morphological analysis suggesting that tree swifts are sister to a swift + hummingbird clade. Both our c-*myc* and cytochrome *b* sequence data reject that notion, and a 1 bp indel in c-*myc* also conflicts with it. In fact, all molecular evidence to date strongly supports the traditional idea that swifts are sister to treeswifts to the exclusion of hummingbirds (Sibley and Ahlquist, 1990; Chubb, 2004; Barrowclough et al., 2006; Ericson et al., 2006; Hackett et al., 2008).

4.3. Phylogeny and systematics—shallow nodes

At the family level, Sibley and Ahlquist (1990) recommended recognition of new families for *Eurostopodus* (=Eurostopodidae) and *Batrachostomus* (=Batrachostomidae) based on large DNA hybridization distances to other genera in Caprimulgidae and Podargidae, respectively. While that suggestion received support from mtDNA distance data (Mariaux and Braun, 1996), it is now complicated by the discovery of additional deep nodes in these families. The deep divergence within *Eurostopodus* reported here would require recognition of another new family if Sibley and Ahlquist's (1990) suggestion were followed. It would seem more reasonable to recognize the genus *Lyncornis* (to include at least the eared taxa *macrotis* and *temmincki*) as suggested by Cleere (1998, p.174), and retain both *Eurostopodus* and *Lyncornis* in Caprimulgidae. These taxa surely form a monophyletic group with other caprimulgids (Barrowclough et al., 2006; this study) despite the relatively deep molecular divergences among them. Similarly, the description of a new frogmouth genus, *Rigidapenna*, with large molecular divergences from both *Podargus* and *Batrachostomus* (Cleere et al., 2007), would require erection of another new family if Batrachostomidae were recognized. Because all these taxa are frogmouth-like in body form and habits, it seems more useful to retain them in Podargidae, which is surely monophyletic (Barrowclough et al., 2006; Cleere et al., 2007; this study).

Within Caprimulgidae, our data are consistent with previous evidence that several long-standing taxa are probably not monophyletic. These include the genera *Caprimulgus* and *Eurostopodus*, and the subfamilies Caprimulginae and Chordeilinae (Sibley and Ahlquist, 1990; Mariaux and Braun, 1996; Barrowclough et al., 2006; Larsen et al., 2007). However, it is not yet possible to propose a comprehensive revision of the family; this will require more extensive taxon sampling and probably more data, preferably from other genes (Barrowclough et al., 2006).

Within Nyctibiidae, our data confirm and extend the conclusions of Mariaux and Braun (1996) based on 656 bp of cytochrome *b* and Brumfield et al. (1997) based on isozymes. Two pairs of small gray potoos (*griseus* + *jamaicensis*, *leucopterus* + *maculosus*) belong to a well-supported clade. The larger *aethereus* and *grandis*, and the small rufous *bracteatus* are all more divergent, but their relationships are not well-resolved by the present data. The large genetic divergences among these taxa suggest that one or more new generic designations may be in order, but we prefer to wait until the phylogeny is well-resolved, so as to propose a classification that reflects their evolutionary history as accurately as possible.

The conflict between cytochrome *b* and c-*myc* on hummingbird relationships is intriguing. Each gene provides fairly strong support for one of two mutually exclusive groupings. However, neither of these groupings conforms to traditional notions of hummingbird relationships, as *Glaucis* is usually placed in a separate subfamily, Phaethorninae, with a few genera of hermits, while all other hummingbirds are placed in Trochilinae. Interestingly, the topology supported by c-*myc* is the one found in all combined analyses, even though the single gene support values were higher for the cytochrome *b* topology. We suspect that the cytochrome *b* support values may be inflated by the base compositional heterogeneity found in apodiform cytochrome *b*. The c-*myc* topology, with *Topaza* and relatives basal to all other hummingbirds, was also found in a recent analysis involving two mitochondrial genes, ND2 and ND4, and two nuclear genes, adenylate kinase 1 and beta-fibrinogen (McGuire et al., 2007).

4.4. Evolution of adaptive traits—nocturnality

Was the common ancestor of Caprimulgiformes + Apodiformes nocturnal? The exact topology of an expanded clade consisting of Caprimulgiformes + Apodiformes has important implications for the evolutionary history of nocturnal adaptation. While nocturnal activity is found in many groups of birds, only a few major lineages have adopted a largely nocturnal lifestyle—caprimulgiforms, owls and kiwis are the principal examples. The finding that Aegothelidae is the sister group of Apodiformes suggests that the diurnal swifts and hummingbirds, with their remarkable powers of flight, may be derived from a nocturnal ancestor. This suggestion is reinforced by the apparent monophyly of Caprimulgiformes + Apodiformes (Ericson et al., 2006; Hackett et al., 2008). This scenario implies that many adaptations to nocturnality were gained in their common ancestor and subsequently lost in Apodiformes. However, it is important to note that the basal branching structure of the Caprimulgiformes + Apodiformes is not yet well-resolved. If the root of this clade actually falls between Aegothelidae + Apodiformes and the rest of the caprimulgiform families, then it is equally parsimonious to suppose that there were two independent transitions to nocturnality rather than a loss of nocturnality by Apodiformes. More sequence data are needed to resolve the basal branching structure and clarify this issue.

Whether the transition to nocturnality occurred once or multiple times in an expanded Caprimulgiformes is particularly relevant in light of frequent observations of their morphological and genetic diversity, especially in traits that may be adaptive for nocturnal activity. For example, the tapetum lucidum, as mentioned above, is apparently absent in oilbirds (Martin et al., 2004). Instead, increased photosensitivity is achieved by a unique banked structure of the retina, in which rod photoreceptors are stacked three layers deep. Reflective structures (tapeta) in the eyes of nocturnal vertebrates are morphologically diverse and probably evolved independently in a number of lineages (Ollivier et al., 2004). Thus, whether the eyeshine of potoos is produced by a structure homologous to that of caprimulgids (Nicol and Arnott, 1974), and whether frogmouths and owlet-nightjars even have tapeta are open questions.

There is also considerable variation in the morphology of the cerebellum, telencephalon and Wulst, brain structures related to behavior and vision (Iwaniuk and Hurd, 2005; Iwaniuk et al., 2006; Iwaniuk and Wylie, 2006). The owlet-nightjar sequence for arylalkylamine *N*-acetyltransferase, a gene potentially associated with nocturnal activity, fails to form a clade with other caprimulgiform sequences in phylogenetic analyses (Fidler et al., 2004). Although found in no other bird, echolocation has arisen twice in this group, in oilbirds and *Aerodramus* swiftlets, as an adaptation to nesting in caves (Lee et al., 1996). Considerable variation in the structure of the main arteries near the heart has also been

noted (Glenny, 1953). Understanding of the evolutionary history of all these traits will be improved by a well-resolved phylogeny.

4.5. Evolution of adaptive traits—hypothermia

Hypothermic responses are well-known in both caprimulgiform and apodiform birds. The literature on facultative hypothermia in birds was reviewed by McKechnie and Lovegrove (2002). Data existed for 29 families in 11 orders at the time. While the capacity for shallow to moderate hypothermia is widespread, extreme hypothermia, during which body temperature may be reduced by 20 °C or more, was reported in only three families: Apodidae, Trochilidae, and Caprimulgidae. Current evidence indicates that these families all belong to a single clade, Caprimulgiformes + Apodiformes (Ericson et al., 2006; Hackett et al., 2008). Thus, extreme hypothermia, which is associated with daily torpor and/or hibernation, seems to be a derived trait of this group. Most families of this clade are known to have the capacity for at least moderate hypothermia (reduction in body temperature of >10 °C). Telemetric studies of free ranging birds indicate that facultative hypothermia is used routinely in Aegothelidae, Podargidae, and some Caprimulgidae (Brigham et al., 2006).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.08.025.

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