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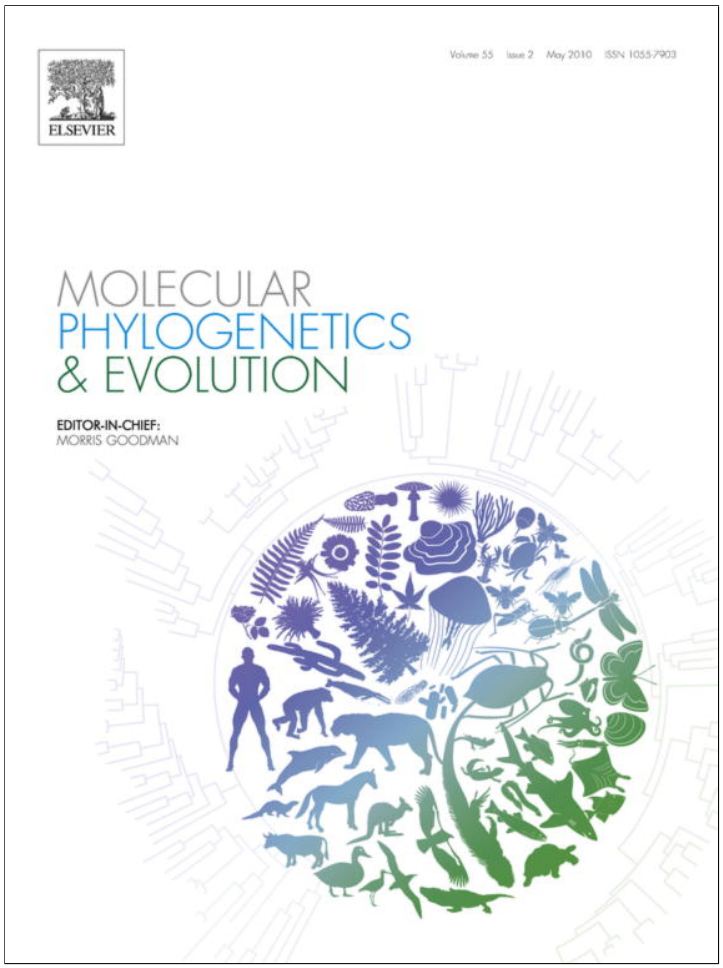

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A multi-gene estimate of phylogeny in the nightjars and nighthawks (Caprimulgidae)

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ABSTRACT

Caprimulgidae is a cosmopolitan family of nocturnal and crepuscular insectivorous birds comprising the nightjars, nighthawks, and relatives. Sexual selection and convergence or parallelism in plumage and behavior have made it difficult to discern evolutionary relationships in this group. In order to provide a framework for comparative studies of this family, a molecular phylogeny was reconstructed using mitochondrial cytochrome *b*, and nuclear c-*myc* and growth hormone DNA sequences. Likelihood, parsimony and Bayesian analyses agree in placing *Eurostopodus* species and *Caprimulgus enarratus*, a Malagasy endemic, as the earliest branches of the tree. The remaining taxa are divided among four well-supported clades, three in the New World and one in the Old World. Insertion/deletion events, common in non-coding sequences, provide additional support in resolving the phylogeny. Neither of the traditional subfamilies, Caprimulginae (nightjars) and Chordeilinae (nighthawks), is monophyletic, suggesting that the morphological specializations characterizing "nighthawks" evolved multiple times and the "nightjar" body plan is an old and conservative one. The large genus *Caprimulgus* is polyphyletic with respect to many other genera in the family, which are often defined by derived plumage traits that likely reflect sexual selection or ecological specialization. A taxonomic revision of the family is proposed based on the combined tree, including naming a new genus for *C. enarratus*.

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1. Introduction

Convergent evolution occurs when unrelated organisms develop similarities due to similar environmental or other evolutionary pressures. Such similarities may be mistaken for homologies, obscuring phylogeny. Molecular phylogenetic methods provide increasing evidence of convergent evolution in morphological adaptations. The cosmopolitan Caprimulgidae, a family of nocturnal or crepuscular birds (Cleere 1999), provides an excellent example. Due to their cryptic coloration, conserved appearance and poorly known behavior, traditional phylogenetic studies based on morphology and behavior have yet to resolve many systematic issues within this family (Cleere, 1998, 1999; Holyoak, 2001). This is not unexpected in a group such as Caprimulgidae, since morphological and behavioral characters may be subject to uniform selection for crypticity in many taxa, creating the potential for convergent or parallel evolution that can be misleading. Nevertheless, molecular studies (Sibley and Ahlquist, 1990; Mariaux and Braun, 1996; Barrowclough et al., 2006; Larsen et al., 2007; Braun and Huddleston, 2009) have begun to unravel the phylogeny of this family and shed light on these phenomena.

Caprimulgidae, with approximately 90 species, is the largest of five nocturnally adapted families in the traditional order Caprimulgiformes (although the traditional order is paraphyletic; see Braun and Huddleston, 2009 for a recent discussion). Caprimulgidae has traditionally been divided into two subfamilies: Caprimulginae (nightjars) and Chordeilinae (nighthawks) (Oberholser, 1914; Peters, 1940; Cleere, 1998, 1999; Holyoak, 2001). The cosmopolitan Caprimulginae are generally characterized by a schizognathous palate, long rictal bristles around the gape, and relatively short, rounded wings. They are crepuscular or nocturnal, and often forage by sallying after flying insects from the ground or exposed perches. In contrast, the Chordeilinae are restricted to the New World, have a desmognathous palate (but see Bühler, 1970), generally lack rictal bristles, have relatively longer, narrower wings, and are diurnal or crepuscular aerial hawkers (Oberholser, 1914; Cleere, 1998, 1999; Holyoak, 2001).

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Sixteen genera are currently recognized (Dickinson 2003), although as many as 51 genera have been named in the past (Peters, 1940; Cleere, 1998, 1999). Four genera are traditionally placed within Chordeilinae (*Chordeiles*, *Lurocalis*, *Nyctiprogne*, and *Podager*), whereas the other 12 genera (*Eurostopodus*, *Veles*, *Nyctidromus*, *Phalaenoptilus*, *Siphonorhis*, *Nyctiphrynus*, *Caprimulgus*, *Macrodipteryx*, *Hydropsalis*, *Uropsalis*, *Macropsalis*, and *Eleothreptus*) are usually placed within Caprimulginae. The composition of the two subfamilies, however, remains debatable, and molecular data suggest that one or both may not be monophyletic (Sibley and Ahlquist, 1990; Mariaux and Braun, 1996; Barrowclough et al., 2006; Braun and Huddleston, 2009). In the most recent monograph of Caprimulgiformes, Holyoak (2001) placed *Podager* in Caprimulginae because of the presence of rictal bristles, which are lacking in other chordeilines. *Eurostopodus* and *Veles* (=*Caprimulgus binotatus*) were moved to Chordeilinae because they lack rictal bristles. The presence or absence of rictal bristles requires further study because they are much reduced or vestigial in some taxa (KLH, pers. obs.). *Eurostopodus* shares other morphological affinities with Chordeilinae such as a square tail and narrow, pointed wings (Schodde and Mason, 1980; Holyoak, 2001).

Based on plumage, *Eurostopodus* has even been subsumed within *Caprimulgus* (e.g., Schodde and Mason, 1980), but molecular evidence has consistently shown *Eurostopodus* to be genetically divergent from all other caprimulgids (Sibley and Ahlquist, 1990; Mariaux and Braun, 1996; Barrowclough et al., 2006; Larsen et al., 2007; Braun and Huddleston, 2009). Sibley and Ahlquist (1990) suggested that *Eurostopodus* should be given family status, and Dickinson (2003) recognized the subfamily Eurostopodinae. With increased taxon sampling, Braun and Huddleston (2009) discovered a deep genetic divergence within *Eurostopodus*, and the possibility that the genus is not monophyletic. In fact, two of the seven species, *E. macrotis* and *E. temminckii*, have "ear tufts" and distinctive vocalizations, and were previously recognized in a separate genus, *Lyncornis*, the "eared nightjars" (Cleere, 1998). The deep divergence within *Eurostopodus* further complicates subfamilial division.

Several genera (e.g., *Eleothreptus*, *Hydropsalis*, *Macropsalis*, *Macrodipteryx*, *Uropsalis*) have only one or two species and are recognized on the basis of elaborate plumage characters such as elongated wing or tail feathers. These characters may be sexually selected (Cleere, 1998; Holyoak, 2001) and may be recently derived autapomorphies. Many other species, lacking distinguishing plumage features, are placed in the genus *Caprimulgus*, making it one of the largest of all avian genera, with 55–57 species (Cleere, 1998; Holyoak, 2001; Dickinson, 2003). The large size of *Caprimulgus*, combined with the notion that some other caprimulgid genera may be defined by autapomorphies, suggests that *Caprimulgus* may be a grab-bag of species with pleisiomorphic plumage characters. It has long been recognized that the genus may be polyphyletic (e.g., Cleere, 1999), and molecular studies (Sibley and Ahlquist, 1990; Barrowclough et al., 2006; Larsen et al., 2007; Braun and Huddleston, 2009) strongly indicate that this is true. To date, limited taxon sampling has precluded a comprehensive generic revision.

We constructed a robust phylogeny of Caprimulgidae using molecular sequence data from more than 60% of caprimulgid species and 14 of 16 currently recognized genera. DNA sequences were collected from the entire mitochondrial DNA (mtDNA) cytochrome *b* gene (*MT-CYB*) and parts of two nuclear genes (c-*myc* or myelocytomatosis viral oncogene homolog [*MYC*] and growth hormone [*GH*]). *MT-CYB* is a rapidly evolving gene that is best for resolving diversification at the species level to subfamily or family level in birds (Moore and DeFilippis, 1997). *GH* included part of exons 2 and 3 and all of intron 2. *GH* has been developed as a probe of higher-level relationships among birds (Yuri et al., 2008), and its evolutionary rate is intermediate between *MT-CYB* and *MYC* in Caprimulgidae and relatives. *MYC* included part of intron b, all of exon 3, and part of the 3′ UTR. *MYC* is a well-studied, slowly evolving proto-oncogene that is useful for studying deep divergences in vertebrates (Braun et al., 1985; Graybeal, 1994; Ericson et al., 2000; Miyamoto et al., 2000; Harshman et al., 2003).

We asked two major questions regarding caprimulgid evolution: (1) Are the large genera *Caprimulgus* (55–57 species) and *Eurostopodus* (seven species) monophyletic? Non-monophyly could suggest that these birds have maintained a successful lifestyle and body plan while morphologically divergent forms arose from within the group through adaptation or sexual selection. (2) Are the two subfamilies, Chordeilinae and Caprimulginae, monophyletic? If both subfamilies are monophyletic, then aerial hawking and sallying, and the morphological adaptations associated with each foraging mode, may have each evolved only once in the family. If neither subfamily is monophyletic, then one or both of these suites of behavioral and morphological adaptations must have arisen multiple times through convergent or parallel evolution.

2. Materials and methods

2.1. Taxon sampling

We follow the taxonomy of Dickinson (2003) as the most recent comprehensive treatment of the family. Samples from 66 caprimulgid specimens were included in the study (Appendix A) representing 55 of 89 caprimulgid species and 14 of the 16 genera. Six additional specimens from four other caprimulgiform families were included as outgroups.

2.2. Laboratory methods

Genomic DNA was extracted from frozen tissues using a standard procedure of proteinase K digestion, phenol/chloroform extraction and ethanol precipitation (Sambrook et al., 1989; Mariaux and Braun, 1996). Phase-lock gel (Eppendorf, Westbury, NY) was used to aid phase separation during extraction. DNA was amplified via polymerase chain reaction (PCR) and sequenced for three gene regions: *MT-CYB*, *MYC*, and *GH*. Primers for PCR amplification and sequencing are listed in Table 1.

Amplification of *MT-CYB* was performed as follows: initial denaturation at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 10 min. Final concentrations were: 1x *Taq* DNA polymerase buffer (Promega), 2.5 mM $MgCl_2$ (Promega), 0.2 mM each dNTP, 0.2 μM each of primers L14764 and H16060, 0.05 U/μL *Taq* DNA polymerase (Promega) and 0.40 ng/μL template DNA.

Initial amplification of *MYC* used an initial denaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 30 s, 53 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 10 min. Final reaction concentrations were: 1x PCR buffer (supplied with TaKaRa HS), 1.75 mM $MgCl_2$, 0.2 mM each dNTP, 0.25 μg/μL bovine serum albumin (BSA), 0.2 μM each of primers MYC-F-01 and MYC-R-47, 0.05 U/μL *Taq* DNA polymerase (TaKaRa HS), and 0.5 ng/μL template DNA. A second amplification, using a 5′ nested primer, MYC-F-02, and 1 μL of 1:10 dilution of the initial PCR product, was performed at an annealing temperature of 55 °C. Final concentrations were 1x PCR buffer (GeneChoice), 1.7 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 μM each primers MYC-F-02 and MYC-R-47, and 0.05 U/μL *Taq* DNA polymerase (GeneChoice).

Touchdown PCR was performed for the initial amplification of *GH*. Final concentrations were 1x PCR buffer (GeneChoice), 2.0 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 μg/μL BSA, 0.1 mM tetramethylammonium chloride (TMAC), 0.2 μM each primers GH-

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Table 1
Primers used for amplification and sequencing.

Gene	Primer	Sequence 5′ to 3′	Reference
MT-CYB	L14764	TGTTACAAAAAAATAGGMCCMGAAGG	Sorenson et al. (1999)
MT-CYB	L15323	CCATGAGGACAAATATCATTCTGAGGTGC	Mariaux and Braun (1996)
MT-CYB	L15749	GCCATCCTACGCTCAATCCC	Braun and Huddleston (2009)
MT-CYB	H15295	TGATATTTGTCCTCATGG	Braun and Huddleston (2009)
MT-CYB	H15730	GGGATTGAGCGTAGGATGGC	Braun and Huddleston (2009)
MT-CYB	H16060	TTTGGYTTACAAGACCAATG	Braun and Huddleston (2009)
MYC	MYC-F-01	TAATTAAGGGCAGCTTGAGTC	Harshman et al. (2003)
MYC	MYC-F-02	TGAGTCTGGGAGCTTTATTG	Harshman et al. (2003)
MYC	MYC-F-03	AGAAGAAGAACAAGAGGAAG	Harshman et al. (2003)
MYC	MYC-F-05	CACAAACTYGAGCAGCTAAG	Harshman et al. (2003)
MYC	MYC-R-04	GGCTTACTGTGCTCTTCT	Harshman et al. (2003)
MYC	MYC-R-06	TTAGCTGCTCAAGTTTGTG	Harshman et al. (2003)
MYC	MYC-R-47	CTATAAAGACTTTATTAAAGGTATTTACAT	Kimball et al. (2009)
GH	GH-F874	CCTTCCCWGCCATGCCCCTTTCCAACC	Yuri et al. (2008)
GH	GH-R1925	TCCCTTCTTCCAGGTCCTTTART	Yuri et al. (2008
GH	GH-F897	TGTTTGCCAACGCTGTGCTGAGG	Yuri et al. (2008)
GH	GH-R1477	TACCGATTTCTGCTGGGCATCATCCTTC	Yuri et al. (2008)
GH	GH-INT2-F-04	CTCTRARARCAGTGGGAGATGGC	Yuri et al. (2008)
GH	GH-INT2-R-04	GCCATCTCCCACTGYTYTYAGAG	Yuri et al. (2008)
GH	GH-CAP-F-01	GTGAGAGGAAGACTTTTAGG	This study
GH	GH-CAP-R-01	CCTAAAAGTCTTCCTCTCAC	This study
GH	GH-CAP-F-02	GATGAGGAAAGGCTGAGGG	This study
GH	GH-CAP-R-02	CCCTCAGCCTTTCCTCATC	This study

F874 and GH-R1925, 0.04 U/μL *Taq* DNA polymerase (GeneChoice), and 0.40 ng/μL template DNA. Reaction conditions were: initial denaturation at 94 °C for 3 min followed by 40 cycles of 94 °C for 30 s, 70–61 °C for 30 s (decreasing annealing temperature by 1 °C per cycle in the first 10 cycles), and 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR products were diluted 1:10 and 1 μL of the dilution was used in a second nested amplification. Final concentrations were: 1x PCR buffer (GeneChoice), 1.5 mM $MgCl_2$, 1.7 mM each dNTP, 0.1 mM TMAC, 0.25 μM each primers GH-F897 and GH-R1477, and 0.025 U/μL *Taq* DNA polymerase (GeneChoice). Re-amplifications were performed as follows: 94 °C for 3 min followed by 30 cycles of 94 °C for 30 s, 66–62 °C for 30 s (reducing annealing temperature by 1 °C per cycle for the first five cycles), and 72 °C for 45 s, with a final extension at 72 °C for 10 min.

All PCR products were cleaned using PEG (polyethylene glycol) precipitation. Both strands of the purified PCR products were cycle sequenced with the amplification and internal primers listed in Table 1 and ABI BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Excess dye terminators were removed using Sephadex G-50 (Sigma, St. Louis, MO) filtration and a Millipore MultiScreen-HV plate (Millipore, Billerica, MA). Sequences were run on an ABI 3100 or 3130*xl* capillary DNA sequencer.

Due to length heterozygosity, some nuclear PCR products were cloned using the TOPO TA for Sequencing Cloning Kit (Invitrogen, Carlsbad, CA) to obtain a clean sequence. Clones from each PCR product were grown overnight in LB liquid culture and purified using an Eppendorf FastPlasmid Mini kit. Two clones of each PCR product were sequenced using primers listed in Table 1.

2.3. Data analysis

Sequences were edited and assembled into contiguous fragments using Sequencher 4.7 (GeneCodes). Alignments were initially performed using Clustal X v.1.83 (Thompson et al., 1997) with default parameters for gap opening and extension costs, and further improved manually using Se-Al v.2.0a11 (Rambaut, 1996). Base frequencies for each gene and gene partition were examined using the χ^2 test in PAUP*4.0b10 (Swofford, 2003) to determine if there was significant base compositional heterogeneity among taxa. A partition homogeneity or incongruence length difference test (ILD) (Farris et al., 1994) was also run in PAUP* to test for phylogenetic incongruence among the genes.

Maximum parsimony (MP) analyses were performed on individual genes, pairwise gene combinations, and all genes concatenated using PAUP*. A heuristic search of 1000 random taxon addition search replicates and TBR branch swapping was performed with MulTrees "on" and all characters equally weighted. Non-parametric bootstrap analyses were run to test the robustness of the trees. For *MT-CYB*, gene pairs and combined data, 1000 bootstrap pseudo-replicates were performed with 100 random sequence additions per pseudo-replicate. However, initial analyses of the nuclear genes and combined nuclear genes found large numbers of equally parsimonious trees and it was necessary to limit the number of trees saved in the bootstrap analyses. In those cases, 100 bootstrap pseudo-replicates were performed with 20 random additions per pseudo-replicate and a limit of 100 trees (NCHUCK = 100 CHUCKSCORE = 1) for swapping in each random addition pseudo-replicate.

Models for maximum-likelihood (ML) analyses were selected in a successive-approximations approach (Swofford et al., 1996; Sullivan et al., 2005). Starting trees were obtained using neighbor-joining with Jukes–Cantor distances in PAUP*. The best model estimated with Modeltest 3.7 (Posada and Crandall, 1998) using the Akaike information criterion (AIC) was then used in a heuristic tree search in PAUP* of 10 random addition replicates with TBR branch swapping. Model estimation was then repeated on the resulting tree. This process was repeated until tree topology and model parameter values converged. Final models and parameter values for each gene are shown in Table 2. Nodal support values were estimated with GARLI 1.0 (Zwickl, 2006) using the best-fit model with model parameters fixed, and 100 ML bootstrap pseudo-replicates with one stepwise addition per pseudo-replicate.

Bayesian analyses were performed using MrBayes v.3.1 (Huelsenbeck and Ronquist, 2001). Four MCMC chains were run simultaneously for 10,000,000 generations and sampled every 500 generations. Data collected from the first 1,000,000 generations (2000 trees) were discarded as the burn-in. A general-time-reversible model of sequence evolution with invariable sites and gamma

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Table 2
Parameters used in maximum-likelihood analyses.

Gene	Model[d]	Bases				α[a]	I[b]	Rates[c]				
		A	C	G	T			A↔C	A↔G	A↔T	C↔G	C↔T
MT-CYB	GTR + I + Γ	0.321	0.448	0.065	0.167	0.672	0.462	0.521	14.967	1.369	0.388	20.159
MYC	TVM + I + Γ	0.316	0.205	0.214	0.264	0.750	0.489	0.649	4.830	0.260	0.788	4.830
GH	TVMef + Γ	0.250	0.250	0.250	0.250	0.710	-	1.043	3.804	0.550	0.946	3.804
MYC + MT-CYB	GTR + I + Γ	0.301	0.359	0.165	0.176	0.597	0.531	0.874	5.213	1.210	0.238	18.749
Combined	GTR + I + Γ	0.274	0.296	0.206	0.224	0.458	0.377	1.024	4.022	0.762	0.421	9.625
Nuclear (MYC + GH)	TVM + I + Γ	0.273	0.232	0.240	0.255	0.721	0.304	0.938	4.258	0.440	0.950	4.258

[a] Γ shape parameter.
[b] Proportion of invariable sites.
[c] Rates are relative to G↔T.
[d] GTR = general-time-reversible model; TVM = transversional model; TVMef = transversional model with equal base frequencies.

distribution of rate heterogeneity (GTR + I + Γ) was used with model parameters estimated independently for each data partition. For *MT-CYB*, the analysis was performed with the data partitioned by first, second, and third base positions. For *MYC*, the analysis was performed with three data partitions: intron, exon, and 3′UTR. The dataset was partitioned by exon and intron sequences for the *GH* analysis. For combined analyses, the dataset was partitioned by gene.

To test prior hypotheses of relationships, constrained ML tree searches were run on each of the individual gene datasets as well as the combined and pairwise gene datasets. Trees were constrained for monophyly of each of the subfamilies under both the Holyoak (2001) classification and the traditional classification (Peters, 1940; Cleere, 1998, 1999). Additional tree searches were run in which *Caprimulgus* and *Eurostopodus* were each separately constrained to be monophyletic. An ML analysis was performed using PAUP* and the best-fit model previously described with model parameters optimized for the constrained tree using Modeltest. A heuristic search for the best constrained tree was performed with 10 random sequence additions.

3. Results

3.1. Sequence analyses

The concatenated dataset of all genes had an aligned length of 4226 base pairs (bp). A total of 47 bp were excluded from analysis as ambiguously aligned (see below). All analyses of the 72 ingroup plus outgroup taxa were conducted on the remaining 4179 characters.

MT-CYB was 1143 bp in length in all but two taxa, *Nyctidromus albicollis* and *Caprimulgus enarratus*, which both showed a single codon deletion near the 3′ end at positions 1135–1137. Further amplification and sequencing using different pairs of primers yielded similar results, suggesting the products were from authentic mtDNA rather than nuclear copies. Since the deletions maintain the reading frame, the sequences do not appear to be pseudogenes. Length variation at the 3′ end of *MT-CYB* has been found in other birds (Groth, 1998; Cicero and Johnson, 2001; Randi et al., 2001).

MYC had an aligned length of 1318 bp, with individual sequences ranging in length from 1230–1271 bp. Two poly-nucleotide tracts totaling 47 bp (29 in the intron and 18 in the 3′ UTR) were subsequently excluded from all analyses as too variable to be unambiguously aligned. This resulted in 1271 aligned bp for analysis (324 bp from intron b, 575 bp from exon 3, and 372 bp from the 3′ UTR).

GH had an aligned length of 1765 bp (32 bp from exon 2, 1659 bp from intron 2, and 74 bp from exon 3). Individual sequences ranged from 781–923 bp in length for all but 17 taxa, which had lengths of 1566–1594 bp due to the presence of a long insertion of ~769 bp in the intron which was identified as being a chicken repeat 1 (CR1) retrotransposon.

Base composition of each gene was homogeneous across all taxa when calculated based on parsimony informative characters using the χ^2 test in PAUP* (Swofford, 2003). Consistent with base compositions found in other vertebrates (Johns and Avise, 1998), *MT-CYB* was low in G's (0.05) and high in C's (0.46), particularly in third base positions (0.47). *MYC* was low in T's (0.18) in the exon and low in C's (0.15) in the 3′ UTR. These base compositions are also consistent with those found in other vertebrates (Miyamoto et al., 2000). *GH* exons were low in G's (0.03).

The three genes are evolving at different rates. As expected, *MT-CYB* (mean ML distance among ingroup taxa = 0.39) is evolving faster than either of the nuclear genes. The slowest gene was *MYC* (mean ML distance = 0.03) with *GH* (mean ML distance = 0.06) between the other two genes. *MT-CYB* third base positions (mean ML distance = 2.77) are evolving at a much faster rate than first and second base positions (mean ML distance = 0.04). The 3′ UTR (mean ML distance = 0.01) of *MYC* is evolving slightly slower than coding exon 3 (mean ML distance = 0.02), and both are evolving much slower than intron b (mean ML distance = 0.06). Exons (mean ML distance = 0.02) of *GH* are evolving at a slower rate than the intron (mean ML distance = 0.07).

In general, substitution types for all genes were biased toward transitions. Neither nuclear gene, *MYC* or *GH*, was saturated. Among *MT-CYB* sequences, however, there was evidence of saturation at all codon positions. As distances got larger, there were as many apparent transversions as transitions in third base positions. At these distances, multiple hits have occurred at many sites and substitution saturation for *MT-CYB* may be obscuring phylogenetic signal.

3.2. Incongruence length difference (ILD) test

An ILD test (Farris et al., 1995) of all data indicated significant conflict in phylogenetic signal among the genes ($p = 0.003$). Pairwise analyses indicated that *GH* was the source of the conflict ($p = 0.001$ for both), particularly due to the CR1 insertion. In fact, significance decreased ($p = 0.04$) when the insertion was excluded from the ILD test. As a result, separate phylogenetic analyses were conducted on each gene, and on a combined *MT-CYB* and *MYC* dataset. For comparison, all three datasets were also combined for analysis, as well as the nuclear genes, because some studies suggest the ILD test may be too conservative (Cunningham, 1997; Darlu and Lecointre, 2002; Hipp et al., 2004).

3.3. Caprimulgid monophyly and basal taxa

The traditional family Caprimulgidae (including *Eurostopodus*) was monophyletic with strong support in all analyses (Fig. 1) except

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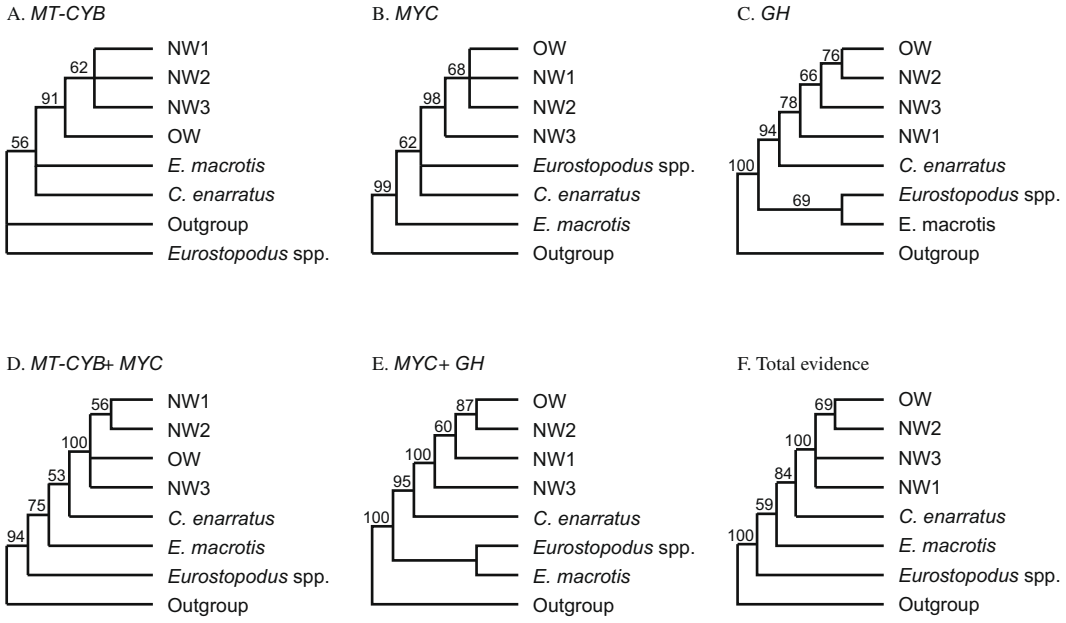

Fig. 1. Skeleton trees showing positions of the main clades in maximum-likelihood analyses of various data partitions (A) *MT-CYB*, (B) *MYC*, (C) *GH*, (D) *MT-CYB* + *MYC*, (E) *MYC* + *GH*, (F) Combined (*MT-CYB* + *MYC* + *GH*). Numbers on branches indicate ML bootstrap > 50% from GARLI analyses. NW=New World, OW=Old World.

MT-CYB, where three *Eurostopodus* spp. failed to group together with the other Caprimulgidae (Fig. 1A). However, these are the most divergent ingroup taxa for all genes, and homoplasy in *MT-CYB* may be obscuring phylogenetic signal at that depth of the tree.

The four *Eurostopodus* spp. and *Caprimulgus enarratus* were early-branching caprimulgids in all analyses. These five species are therefore referred to as "basal taxa". There were two distinct groups within the genus *Eurostopodus*: one consisting of *E. macrotis* and another containing *E. papuensis*, *argus*, and *mystacalis*. The average ML distance over all genes between the two groups is 0.12, whereas the average ML distance amongst *E. papuensis*, *argus*, and *mystacalis* is 0.07. The order of branching of the two groups varied among genes and in combined analyses, without strong support for any specific topology. The two groups clustered together in *GH* and nuclear (*MYC* + *GH*) analyses with low support (Fig. 1C and E), but not in *MT-CYB*, *MYC*, and combined analyses (Fig. 1A, B, D, F, and Fig. 2). Therefore, monophyly of this genus remains unresolved. *Caprimulgus enarratus* was sister to all remaining caprimulgids with strong support in GH and nuclear trees (Fig. 1C and E), but the optimal topology for *MT-CYB* differed (Fig. 1A). This difference is likely due to saturation of signal at this genetic distance in *MT-CYB*, and it results in lowered support for the clade in combined analyses.

3.4. Core caprimulgids

All other caprimulgids form a clade with strong support in all analyses. ML bootstrap support for this core caprimulgid clade ranged from 78% to 100% (Figs. 1 and 2). Within this clade, there are four main groups found consistently in all single gene analyses, but with varying degrees of support. These groups include one Old World (OW) and three New World (NW) clades. When the genes are combined, support for each of these main groups becomes quite strong (ML bootstrap 90–100%; Bayesian posterior probability 1.00). However, the relationships among these clades remain unresolved, as the exact topology varies among the genes and in combined analyses (Figs. 1 and 2).

3.5. Old World clade

This clade includes all African, Asian, and European taxa sampled, aside from the basal taxa. Support for this clade is very strong ranging from 97% to 100% ML bootstrap in single gene and combined analyses (Fig. 2). The two *Macrodipteryx* spp. are sister in all analyses and are embedded deeply within this clade, although *M. vexillarius* was at one time considered a separate genus, *Semeiophorus* (Cleere, 2003). *Caprimulgus fossii* and *C. climacurus* were at one time classified in a separate genus, *Scotornis* (Peters, 1940), and we find them sister in all analyses. These taxa are part of a larger clade that is present in all analyses and includes *C. europaeus*, *C. rufigena*, and an unidentified caprimulgid. *Caprimulgus europaeus* is sister to *C. rufigena* with strong support in all analyses. The sequences of the unidentified *Caprimulgus* sp. are not closely similar to other species in this study, nor to the *MT-CYB* sequences of *C. inornatus* or *C. fraenatus* published by Larsen et al. (2007). Based on collecting locality, the unidentified *Caprimulgus* sp. may be *C. tristigma*, *C. natalensis*, or *C. ruwenzorii*, which were not sampled in this study.

3.6. New World 1

The New World 1 clade (NW1) is composed of *Nyctiphrynus*, *Phalaenoptilus*, *Siphonorhis*, and a group of *Caprimulgus* spp. All of these *Caprimulgus*, with the exception of *C. rufus*, are found in North and Central America. *Siphonorhis*, a West Indian island endemic, is basal to all taxa within this group in all analyses except in *MT-CYB* and *MYC* parsimony analyses.

Caprimulgus vociferus vociferus, *C. v. arizonae*, and *C. saturatus* form a group in all analyses except those based on *MYC*, but their inter-relationships remain unresolved. This suggests that these three taxa should be given equivalent taxonomic status.

The group *C. salvini* plus *C. carolinensis* and *C. rufus* is found with varying degrees of support in all analyses except those based solely on *MYC* (not shown, but see Han, 2006). This group, along with *C. ridgwayi*, forms a strong clade of species with non-overlapping breeding distributions.

3.7. New World 2

New World 2 (NW2) is composed of two nighthawk genera, *Chordeiles* and *Podager*. This clade occurs with high support in all single gene and combined analyses. All analyses place *P. nacunda*

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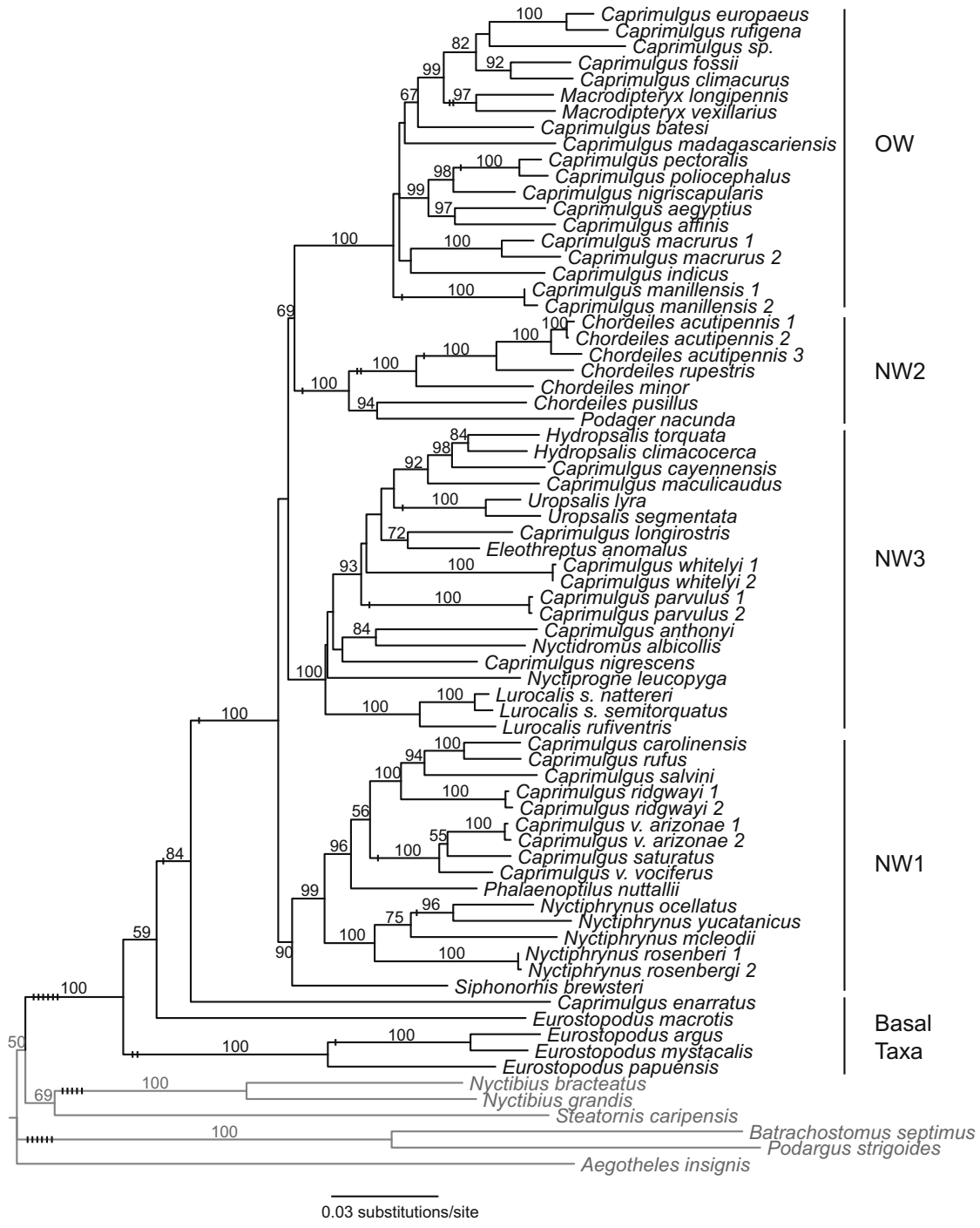

Fig. 2. ML phylogram from PAUP* of analysis of all data combined ($-\ln L = 35532.27$). Outgroups are indicated in gray. Numbers on branches indicate ML bootstrap > 50% from GARLI analysis. Bars indicate presence of indels with a CI = 1. Autapomorphic indels are not shown. NW = New World, OW = Old World, BT = basal taxa.

as sister to *C. pusillus* except for those based on *MT-CYB* where instead, *P. nacunda* branches basally to all *Chordeiles* species (not shown, but see Han, 2006). *Chordeiles acutipennis* is sister to *C. rupestris* in all analyses, with the exception of *MYC* where these two species form a polytomy with *C. minor*. The separation of NW2 from *Lurocalis* and *Nyctiprogne* makes Chordeilinae non-monophyletic.

3.8. New World 3

NW3 is primarily found in South America, and comprises caprimulgine genera *Hydropsalis*, *Uropsalis*, *Eleothreptus*, *Nyctidromus*, and *Caprimulgus* (in part), and the chordeiline genera *Nyctiprogne* and *Lurocalis*. *Hydropsalis* spp. are sister to *C. cayennensis* in all analyses except *GH*, where the relationships are unresolved. Note that *Caprimulgus* spp. are interspersed with other genera (Fig. 2).

3.9. Constrained search results

To test monophyly of current genera and subfamilies, we compared likelihoods of constrained tree topologies to the likelihoods of optimal unconstrained tree topologies. When the genus *Caprimulgus* was constrained to be monophyletic, and when both the traditional (Peters, 1940; Cleere, 1999) and Holyoak's (2001) sub-

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families were constrained to be monophyletic, the results indicate a large decrease in likelihood (Table 3), thus rejecting monophyly. There was, however, only a small decrease in likelihood when *Eurostopodus* was constrained to be monophyletic (Table 3), leaving monophyly of this group still in question.

3.10. Indel support

There were a total of 123 insertion/deletion (indel) characters found using simple indel coding (Simmons and Ochoterena, 2000) as implemented in SeqState (Müller, 2005). Indel character state changes on our combined ML tree (Fig. 2) were determined using DESCRIBETREES in PAUP* with ACCTRAN optimization (APOLIST = YES). In general, deletions were more common than insertions by more than two to one (Fig. 3). Short indels (1–3 bp) were more common than long ones, with larger indels (>12 bp) occurring only sporadically.

Nineteen indel characters were homoplasious, requiring two or more changes on the combined ML tree (consistency index [CI] <1). Of the remaining indel characters, 34 were perfectly consistent, requiring only a single change on the tree, while the other 70 were autapomorphic. The indels with consistency index = 1 that are not autapomorphic were mapped onto the combined ML tree (Fig. 2). All but two of these indels mapped to nodes that were strongly supported in analyses of substitutional variation (100% ML bootstrap). One of the two indels united *Nyctiphrynus ocellatus* and *N. yucatanicus* (1 bp; 96% ML bootstrap) while the other supported *Caprimulgus enarratus* + core caprimulgids (7 bp; 84% ML bootstrap).

A large insertion (769 bp), representing CR1 (chicken repeat 1), is present in *GH* intron 2 in most OW taxa with the exception of

Table 3
Differences in likelihoods (Δln *L*) between optimal and alternative tree topologies[a].

Hypothesis	MT-CYB	MYC	GH	Combined
Holyoak Chordeilinae monophyletic[b]	120.20	94.92	88.64	287.61
Chordeilinae monophyletic[c]	36.87	26.44	20.84	76.50
Holyoak Caprimulginae monophyletic[d]	182.04	142.27	190.44	490.83
Caprimulginae monophyletic[e]	191.91	131.66	165.02	470.67
Eurostopodus monophyletic[f]	8.14	1.26	–	2.90
Caprimulgus monophyletic[g]	356.18	223.05	257.48	840.34

[a] Constrained ML heuristic tree searches were conducted in PAUP* using models of evolution previously estimated for unconstrained tree searches (Table 2) with parameters optimized using Modeltest 3.7. Each constrained tree was compared to the respective optimal unconstrained ML tree for (a) *MT-CYB* (−ln *L* = 18051.08), (b) *MYC* (−ln *L* = 5901.17), (c) *GH* (−ln *L* = 9967.12), and (d) total combined datasets (−ln *L* 35532.27). Δln *L* values shown are the decreases in likelihood of the constrained versus the unconstrained trees.

[b] ((*Chordeiles* [6 OTUs], *Eurostopodus* [4 OTUs], *Lurocalis* [3 OTUs] *Nyctiprogne*)).

[c] ((*Chordeiles* [6 OTUs], *Lurocalis* [3 OTUs] *Nyctiprogne*, *Podager*)).

[d] ((*Caprimulgus* [36 OTUs], *Eleothreptus*, *Hydropsalis* [2 OTUs], *Macrodipteryx* [2 OTUs], *Uropsalis* [2 OTUs], *Nyctidromus*, *Nyctiphrynus* [5 OTUs] *Phalaenoptilus*, *Podager*, *Siphonorhis*)).

[e] ((*Caprimulgus* [36 OTUs], *Eleothreptus*, *Eurostopodus* [4 OTUs], *Hydropsalis* [2 OTUs], *Macrodipteryx* [2 OTUs], *Uropsalis* [2 OTUs], *Nyctidromus*, *Nyctiphrynus* [5 OTUs], *Phalaenoptilus*, *Siphonorhis*)).

[f] ((*E. argus*, *E. macrotis*, *E. mystacalis*, *E. papuensis*)).

[g] ((*Caprimulgus* [36 OTUs])).

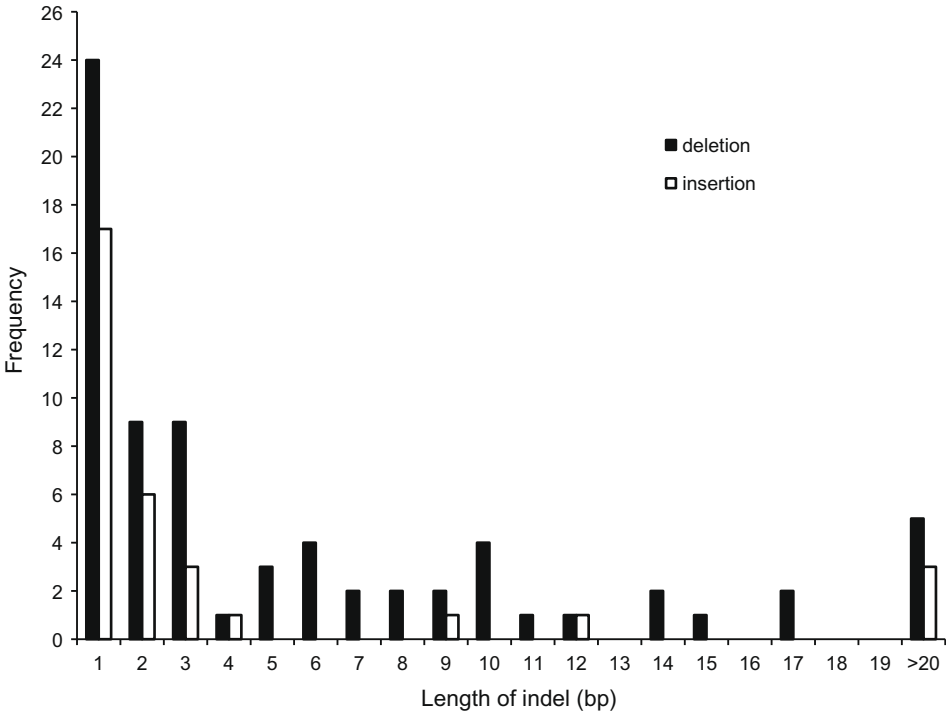

Fig. 3. Frequency distribution of indels by length. Only indels with a CI = 1 are included.

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Caprimulgus manillensis and *C. madagascariensis*. This insertion provides support for a particular resolution of the weakly supported nodes at the base of the OW clade. CR1 is a short interspersed repetitive DNA element that belongs to a family of non-long terminal repeat (non-LTR) retrotransposons (Stumph et al., 1981; Chen et al., 1991; Haas et al., 1997). Retroposed elements such as CR1 have been useful characters for phylogenetic analyses (Shedlock and Okada, 2000; Shedlock et al., 2004; Farwick et al., 2006; Kriegs et al., 2007). Therefore, the 769 bp insertion provides good evidence for uniting all taxa in which it is present, and placing *C. manillensis* and *C. madagascariensis* at the base of the clade. Our trees based on substitutional variation do not have this topology, but the two taxa are separated by short internodes with no support (e.g., Fig. 2).

4. Discussion

4.1. Phylogenetic insights and general conclusions

The genetic data presented here confirm some traditional groupings of caprimulgids but also provide many novel relationships. Caprimulgidae is monophyletic with respect to outgroup taxa although the two traditional subfamilies, Caprimulginae and Chordeilinae (Peters, 1940), are not monophyletic. The alternative subfamily circumscriptions suggested by Holyoak (2001) are also non-monophyletic. *Podager*, which Holyoak (2001) considered to be caprimulgine, groups together with *Chordeiles* in the NW2 clade, while *Eurostopodus*, which Holyoak considered a chordeiline, is an early radiation among all other Caprimulgidae. This suggests that rictal bristles, which Holyoak (2001) used to define subfamilies, are not informative diagnostic characters at that level. Several molecular studies have previously indicated that the subfamilies are not monophyletic (Sibley and Ahlquist, 1990; Mariaux and Braun, 1996; Barrowclough et al., 2006; Larsen et al., 2007; Braun and Huddleston, 2009) and Whitney et al. (2003) suggested *Nyctiprogne* could be more closely related to some caprimulgines than chordeilines.

This study supports the finding that *Eurostopodus* branches basally to all other Caprimulgidae (Barrowclough et al., 2006; Larsen et al., 2007). Although *C. enarratus* is also an early lineage, the nuclear gene data suggest that it is sister to the core caprimulgids. The addition of three *Eurostopodus* taxa in our study reveals that the genus may be paraphyletic. Large genetic distances exist between the *E. argus*, *E. papuensis*, *E. mystacalis* clade and *E. macrotis*, which differs from the other three species in having ear tufts. Based on the presence of these distinctive ear tufts and its southeastern Asia distribution, we presume *E. temminckii* is aligned with *E. macrotis*, as has traditionally been suggested (Peters, 1940; Cleere, 1998, 1999; Holyoak, 2001).

All basal taxa are found in Australasia and Madagascar, suggesting the Indian Ocean region as a possible origin of Caprimulgidae. Early Tertiary fossils of caprimulgids are very scarce and fragmentary (Mourer-Chauviré, 1988; Olson, 1999; Mayr, 2005), and therefore do not provide evidence to support or refute this idea. The enigmatic *Caprimulgus enarratus* is endemic to dense forests in northwestern and eastern Madagascar and is likely a relict lineage. Indeed, the more derived position of the other Malagasy endemic, *C. madagascariensis*, within the OW clade suggests that it stems from a second, more recent colonization.

The three New World and one Old World clades in the core caprimulgids were also found by Barrowclough et al. (2006) based on the *RAG*-1 gene and Larsen et al. (2007) using *MT-CYB*. The internal topology of each of those clades was largely congruent between studies as well, providing independent evidence that the phylogeny presented here will prove robust.

In all analyses, *Caprimulgus* is polyphyletic, with species found in each of the well-supported major clades. *Caprimulgus* has traditionally been a catchall for any caprimulgids lacking striking plumage characters, and revision is long overdue (Sibley and Ahlquist, 1990; Cleere, 1998, 1999; Holyoak, 2001; Barrowclough et al., 2006). The multi-gene phylogeny presented here provides clear evidence that can be used to begin this process.

There are strong biogeographical groupings within the core caprimulgids. While the inter-relationships among the OW and three NW clades remain unresolved, the optimal topology from the combined analysis suggests a radiation in the New World from which the Old World clade was derived. More data are needed to corroborate this pattern, but it is consistent with the findings of Barrowclough et al. (2006). Most caprimulgid species missing from our study are from Africa and Asia. Whether all of those taxa belong to the same major OW clade remains to be seen.

4.2. Evolution of distinctive morphological traits

Non-monophyly of the subfamilies indicates that their distinct aerial foraging modes and associated morphological adaptations arose through convergent evolution. Based on the results of this study, the characters used to define the traditional subfamilies are homoplasious. Many of these characters appear to be tracking the foraging niches of the taxa more closely than their phylogeny. For example, rictal bristles, which are present in most nightjars but absent in most nighthawks, must have been gained or lost multiple times. They are likely the result of trophic adaptations to aerial foraging by sallying from a perch. They may be pleisiomorphic for the family or have arisen in the common ancestor of core caprimulgids and *C. enarratus*. Rictal bristles are present in all other major caprimulgiform lineages (oilbird, owlet-nightjars, frogmouths, and Rufous Potoo [*Nyctibius bracteatus*]), but absent in *Podargus* frogmouths and most potoos (Cleere, 1998). It has been suggested that the pectinate middle claw of caprimulgids may function as a comb to straighten the rictal bristles after preening (Cleere 1998). However, the presence or absence of a pecten does not correlate perfectly with rictal bristles, since it is apparently absent in other families with rictal bristles (Cleere, 1998), and present in some caprimulgid genera without rictal bristles (e.g., *Eurostopodus*, *Chordeiles*, and *Nyctiprogne*; MBR, pers. obs.).

Most caprimulgid lineages that forage by hawking insects on the wing (i.e., the nighthawk niche) have either reduced or absent rictal bristles. These lineages include *Nyctiprogne* (NW3), *Lurocalis* (NW3), and *Chordeiles* (NW2). The internode that separates *Nyctiprogne* and *Lurocalis* at the base of the NW3 clade is very short and weakly supported in our combined tree. Using *RAG-1*, Barrowclough et al. (2006) found 98% ML bootstrap support for this node, but the logdet bootstrap was below 50%. The morphological similarities of these genera suggest that they may be sister taxa rather than paraphyletic, and it is not yet clear that available genetic data can eliminate that possibility. If they are sister, only one transition to a nighthawk niche would be required in NW3.

The Nacunda Nighthawk (*Podager*) is embedded within *Chordeiles* (NW2), and generally forages on the wing like other nighthawks, yet has "strong rictal bristles" (contra Cleere, 1998; Holyoak, 2001). If gain or loss of bristles is equally probable, then either *Podager* regained them after loss in the ancestor of NW2, or retained them as the primitive state while they were lost independently by *C. pusillus* and the ancestor of the other three *Chordeiles* species. The presence of reduced or vestigial bristles on most *Chordeiles* spp. would suggest the latter is more likely. The absence of bristles in *Eurostopodus*, however, may be primitive or derived, depending on the state in the common ancestor of Caprimulgidae. Either way, comparative data on diet and foraging mechanics can

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now be interpreted in a phylogenetic framework to help understand the function and evolution of rictal bristles.

The rampant polyphyly now apparent in *Caprimulgus* indicates that the "nightjar" body plan is an old and successful one, which has been maintained in many caprimulgid lineages. From it, divergent morphologies have apparently arisen through trophic adaptation (e.g., "nighthawks", discussed above) or sexual selection for highly divergent plumage traits. Indeed, many of the other currently recognized genera contain only one or two species and are based on striking plumage features such as modified wing or tail feathers. For example, the genus *Macrodipteryx* consists of two species of Afro-tropical nightjars, *M. longipennis* and *M. vexillarius*. Breeding males are characterized by elongated second primaries, which are used for sexual display-flights during the breeding season (Cleere, 1998; Holyoak, 2001). Males are thought to attract females by displaying at leks, suggesting they are polygynous (Cleere, 1998; Holyoak, 2001). In fact, the modified primaries of the two *Macrodipteryx* spp. are rather distinct from each other, and *M. vexillarius* has at times been placed in a separate genus *Semeiophorus* (see Cleere, 2003 for a review). Given the sister status of these taxa, and their nesting within the OW clade, it seems very likely that the elongation and modification of these feathers evolved relatively rapidly in response to sexual selection. The genera *Eleothreptus* and *Veles* are also characterized in part by modified wing feathers.

Similarly, in the genera *Hydropsalis*, *Uropsalis*, and *Macropsalis*, males are characterized by having elongated and modified tail feathers. It is likely these elongated tail feathers are sexually selected. Several species are known to display at communal leks or display the tail prominently during courtship (Cleere, 1998; Holyoak, 2001).

4.3. Taxonomic recommendations

A major taxonomic revision is now warranted. The phylogeny presented here is well resolved and in substantial agreement with all previous molecular work on the family (Sibley and Ahlquist, 1990; Mariaux and Braun, 1996; Barrowclough et al., 2006; Larsen et al., 2007; Braun and Huddleston, 2009). The taxon sampling is broad and includes all morphologically divergent lineages.

In proposing a new classification, we seek first to have all named taxa represent monophyletic groups. Second, we seek stability of the named taxa; whenever possible, we retain currently recognized taxa, and when more than one partitioning scheme for a clade is plausible, we opt for the one we believe is more likely to remain viable in the face of new data.

While large genetic distances separate *Eurostopodus* from all other caprimulgids, erecting the family Eurostopodidae (Sibley and Ahlquist, 1990) or dividing the family into two subfamilies, Eurostopodinae and Caprimulginae (Barrowclough et al., 2006), now seems inadvisable. The traditional family is clearly monophyletic (Barrowclough et al., 2006; Hackett et al., 2008), and subdividing it at this time would be problematic because the discovery of two divergent lineages in *Eurostopodus* makes monophyly of that group uncertain. To be certain of monophyly, at least four families or subfamilies would have to be erected.

Among the basal taxa, *Eurostopodus macrotis* is substantially differentiated from other sampled *Eurostopodus* species. We believe it merits being placed into a separate genus, for which the name *Lyncornis* Gould 1838 is available. Although *E. temminckii* was not sampled, it is likely sister to *E. macrotis* since the two species share vocal and morphological similarities such as the presence of "ear tufts" (Cleere, 1998). It too should be placed in the resurrected *Lyncornis*. *Caprimulgus enarratus* is more divergent from the core caprimulgids than any of the four major clades are from each other. Therefore, it should be assigned to a new genus, a name for which we propose below.

Within core caprimulgids, the four strongly supported major clades (NW1–3, OW) provide a natural partitioning scheme, but numerous taxa need to be reassigned to reflect the non-monophyly of the current genus *Caprimulgus*. Additionally, a number of small or monotypic genera should be subsumed. *Caprimulgus* Linnaeus 1758 should be restricted to the Old World clade, since the type specimen for the genus is *C. europaeus*. *Macrodipteryx* should be subsumed within *Caprimulgus*. While the primaries of breeding male *Macrodipteryx* are spectacularly modified, they may have evolved rapidly under intense sexual selection. To maintain *Macrodipteryx* as a separate genus would require erection of at least six other genera within the OW clade, with little guarantee of monophyly for most of them.

No tissue samples were available for *Veles*, an Afro-tropical nightjar, or 19 other Old World species currently treated in *Caprimulgus*. We provisionally assign all of these taxa to *Caprimulgus* as well. Two of them, *C. inornatus* and *C. fraenatus*, were sequenced by Larsen et al. (2007) for *MT-CYB*, and clearly would fall within the OW clade as defined here. Given the surprising insights that molecular data have yielded thus far, any of the unsampled taxa might turn out to be misplaced in *Caprimulgus* as here restricted, but nothing yet known about their morphology or biogeography suggests that they will. It is encouraging that our tree is consistent with most of the superspecies and species groups proposed by Fry (1988) and Cleere (1999).

All NW2 species should be assigned the genus *Chordeiles* Swainson 1831, which has priority over the monotypic *Podager* (Peters, 1940). *C. gundlachii*, unsampled in this study, surely belongs here. Whitney et al. (2003) made a compelling case that the recently described *C. vielliardi* should be reassigned to *Nyctiprogne*, and we assume that it will fall in NW3 once sequenced.

The NW3 clade, which currently is subdivided into seven genera, should be subsumed into *Hydropsalis* Wagler 1832, as it has priority over the other names (Peters, 1940). An unsampled eighth genus, *Macropsalis*, will presumably fall into this clade, and we assign it to *Hydropsalis* as well. While this course may seem drastic, the polytomy at the base of NW3 makes any other course less palatable due to the difficulty of defining monophyletic groups. If this polytomy can be resolved, the taxa currently in *Nyctiprogne* and *Lurocalis* may deserve generic status, either separately or together. *Caprimulgus candicans* has been shown to be sister to *Eleothreptus* based on morphology (Cleere, 2002) and genetics (Larsen et al., 2007), so it also belongs to *Hydropsalis*. We provisionally assign *Nyctiprogne* (*Chordeiles*) *vielliardi* to *Hydropsalis*, based on the conclusions of Whitney et al. (2003), and *C. maculosus* and *C. hirundinaceus* to this genus based on their South American distributions. Some may find the proposal to lump eight avian genera into one surprising, especially given that some of the small genera are morphologically distinctive, but we believe that careful consideration of the evidence will convert initial skeptics.

Within NW1, *Siphonorhis* is clearly basal to the rest of the clade and should continue to be recognized. Additionally, *Nyctiphrynus*, which is robustly placed sister to the rest of the taxa within this clade, can remain as currently constituted. All other taxa should be assigned to the genus *Antrostomus* Bonaparte 1838, which has priority among available names for this clade (Peters, 1940). It may be argued that the Common Poorwill, *Phalaenoptilus*, deserves generic status because of its remarkable physiology (it is the only bird known to hibernate; Cleere, 1998; Holyoak, 2001), and such treatment may be feasible in the future. At this time, following that course would require two more genera to insure monophyly on the combined tree. We provisionally assign *Caprimulgus badius*, *cubanensis*, and *noctitherus* to *Antrostomus* based on their Central American and West Indian distributions. *C. sericocaudatus* is found in

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South America, but we assign it to *Antrostomus* based on its placement in a species group with *salvini* and *badius* by Cleere (1999).

4.4. A new genus for Caprimulgus enarratus

The large genetic divergence of *C. enarratus* from all other caprimulgids was unanticipated, but is consistent with the long isolation of many Malagasy endemics. The species has always been placed in *Caprimulgus*, although Cleere (1999) commented that its systematic position deserved further study. Based on our results, it should be assigned to a new genus, for which we propose the name

Gactornis (Han, Robbins, and Braun) new genus.

Type species – Caprimulgus enarratus Gray 1871.

Diagnosis – A caprimulgid defined by its large genetic divergence in both nuclear and mtDNA from all other genera and species of caprimulgids studied thus far. No defining morphological characters for the genus are yet known. The single species, *enarratus*, appears to be unusually quiet for a caprimulgid; its song is as yet unknown (Cleere, 1999; Xeno-Canto Foundation, 2000–2010).

Etymology – *Gactornis* is formed from the four single letter abbreviations (G, A, C, T) for the nucleotides of DNA (guanine, adenine, cytosine, thymine) and the Greek word *ornis* (bird). It refers to the fact that the distinctiveness of the lineage only became apparent upon examination of the nucleotide sequence of its DNA. *Gactornis* is masculine, so the scientific name of the single species becomes *Gactornis enarratus*. Its English name should remain Collared Nightjar.

5. Conclusion

Since the first molecular data on Caprimulgidae appeared (Sibley and Ahlquist, 1990), it has been clear that a major revision of the family would be required. Our work builds on two decades of accumulated genetic data (Mariaux and Braun, 1996; Barrowclough et al., 2006; Larsen et al., 2007; Braun and Huddleston, 2009), which informed our sampling design. It has become clear that for caprimulgids, as well as many other bird families, achieving a well resolved and robust phylogeny requires sampling multiple genes and as many species as possible.

The proposed classification decreases the total number of genera from 16 to 9. Several taxa remain unsampled, especially from the Old World, and these might foster additional revisions as their genetic data become available. A number of polytomies in the tree remain, and will require more sequence data to resolve. Nevertheless, our results present a major advance in the understanding of the evolutionary relationships within the Caprimulgidae. Our tree provides the basis for a more natural and stable classification, and comparative studies on trait evolution.

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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.2010.01.023.

References

Barrowclough, G.F., Groth, J.G., Mertz, L.A., 2006. The RAG-1 exon in the avian order Caprimulgiformes: phylogeny, heterozygosity, and base composition. Mol. Phylogenet. Evol. 41, 238–248.

Braun, M.J., Deininger, P.L., Casey, J.W., 1985. Nucleotide sequence of a transduced *myc* gene from a defective feline leukemia provirus. J. Virol. 55, 177–183.

Braun, M.J., Huddleston, C.J., 2009. A molecular phylogenetic survey of caprimulgiform nightbirds illustrates the utility of non-coding sequences. Mol. Phylogenet. Evol. 53, 948–960.

Bühler, P., 1970. Schädelmorphologie und Kiefermechanik der Caprimulgidae (Aves). Z. Morph. Tiere. 66, 337–399.

Chen, Z.-Q., Ritzel, R.G., Lin, C.C., Hodgetts, R.B., 1991. Sequence conservation in avian CR1: an interspersed repetitive DNA family evolving under functional constraints. Proc. Natl. Acad. Sci. U.S.A. 88, 5814–5818.

Cicero, C., Johnson, N.K., 2001. Higher-level phylogeny of New World vireos (Aves: Vireonidae) based on sequences of multiple mitochondrial DNA genes. Mol. Phylogenet. Evol. 20, 27–40.

Cleere, N., 1998. Nightjars: A Guide to Nightjars, Nighthawks and Their Relatives. Yale University Press, New Haven.

Cleere, N., 1999. Family Caprimulgidae (Nightjars). In: del Hoyo, J., Elliott, A., Sargatal, J. (Eds.), Handbook of the Birds of the World, Barn Owls to Hummingbirds, vol. 5. Lynx Edicions, Barcelona, pp. 302–386.

Cleere, N., 2002. A review of the taxonomy and systematics of the sickle-winged and white-winged nightjars (Caprimulgidae). Bull. Br. Ornithol. Club 122, 168–179.

Cleere, N., 2003. The pennant-winged nightjar *Macrodipteryx vexillarius* (Caprimulgidae), its generic status, synonyms and types. Bull. Br. Ornithol. Club 123, 181–186.

Cunningham, C., 1997. Can three incongruence tests predict when data should be combined? Mol. Biol. Evol. 14, 733–740.

Darlu, P., Lecointre, G., 2002. When does the incongruence length difference test fail? Mol. Biol. Evol. 19, 432–437.

Dickinson, E.C. (Ed.), 2003. The Howard and Moore Complete Checklist of the Birds of the World, third ed. Princeton University Press, Princeton, New Jersey.

Ericson, P.G.P., Johansson, U.S., Parsons, T.J., 2000. Major divisions in oscines revealed by insertions in the nuclear gene c-myc: a novel gene in avian phylogenetics. The Auk 117, 1069–1078.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–319.

Farris, J.S., Kallersjo, M., Kluge, A.G., Bult, C., 1995. Constructing a significance test for incongruence. Syst. Biol. 44, 570–572.

Farwick, A., Jordan, U., Fuellen, G., Huchon, D., Catzeflis, F., Brosius, J., Schmitz, J., 2006. Automated scanning for phylogenetically informative transposed elements in rodents. Syst. Biol. 55, 936–948.

Fry, C.H., 1988. Skulls, songs and systematics of African nightjars. Pan-African Ornithol. Congr. 6, 105–131.

Graybeal, A., 1994. Evaluating the phylogenetic utility of genes: a search for genes informative about deep divergences among vertebrates. Syst. Biol. 43, 174–193.

Groth, J.G., 1998. Molecular phylogenetics of finches and sparrows: consequences of character state removal in cytochrome *b* sequences. Mol. Phylogenet. Evol. 10, 377–390.

Haas, N.B., Grabowski, J.M., Sivitz, A.B., Burch, J.B.E., 1997. Chicken repeat 1 (CR1) elements, which define an ancient family of vertebrate non-LTR retrotransposons, contain two closely spaced open reading frames. Gene 197, 305–309.

Hackett, S.J., Kimball, R.T., Reddy, S., Bowie, R.C.K., Braun, E.L., Braun, M.J., Chojnowski, J.L., Cox, W.A., Han, K.-L., Harshman, J., Huddleston, C.J., Marks, B.D., Miglia, K.J., Moore, W.S., Sheldon, F.H., Steadman, D.W., Witt, C.C., Yuri, T., 2008. A phylogenomic study of birds reveals their evolutionary history. Science 320, 1763–1768.

Han, K.-L. 2006. Molecular systematics of nightjars and nighthawks (Caprimulgidae). M.S. Thesis, University of Maryland.

Harshman, J., Huddleston, C.J., Bollback, J.P., Parsons, T.J., Braun, M.J., 2003. True and false gharials: a nuclear gene phylogeny of Crocodylia. Syst. Biol. 52, 386–402.

Hipp, A.L., Hall, J.C., Sytsma, K.J., 2004. Congruence versus phylogenetic accuracy: revisiting the incongruence length difference test. Syst. Biol. 53, 81–89.

Holyoak, D.T., 2001. Nightjars and Their Allies: The Caprimulgiformes. Oxford University Press, New York.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Author's personal copy

Johns, G.C., Avise, J.C., 1998. A comparative summary of genetic distances in the vertebrates from mitochondrial cytochrome *b* gene. Mol. Biol. Evol. 15, 1481–1490.

Kimball, R.T., Braun, E.L., Barker, F.K., Bowie, R.C.K., Braun, M.J., Chojnowski, J.L., Hackett, S.J., Han, K.-L., Harshman, J., Heimer-Torres, V., Holznagel, W., Huddleston, C.J., Marks, B.D., Miglia, K.J., Moore, W.S., Reddy, S., Sheldon, F.H., Smith, J.V., Witt, C.C., Yuri, T., 2009. A well-tested set of primers to amplify regions spread across the avian genome. Mol. Phylogenet. Evol. 50, 654–660.

Kriegs, J.O., Matzke, A., Churakov, G., Kuritzin, A., Mayr, G., Brosius, J., Schmitz, J., 2007. Waves of genomic hitchhikers shed light on the evolution of gamebirds (Aves: Galliformes). BMC Evol. Biol. 7, 190–200.

Larsen, C., Speed, M., Harvey, N., Noyes, H.A., 2007. A molecular phylogeny of the nightjars (Aves: Caprimulgidae) suggests extensive conservation of primitive morphological traits across multiple lineages. Mol. Phylogenet. Evol. 42, 789–796.

Mariaux, J., Braun, M.J., 1996. A molecular phylogenetic survey of the nightjars and allies (Caprimulgiformes) with special emphasis on the potoos (Nyctibiidae). Mol. Phylogenet. Evol. 6, 228–244.

Mayr, G., 2005. The Paleogene fossil record of birds in Europe. Biol. Rev. 80, 1–28.

Miyamoto, M.M., Porter, C.A., Goodman, M., 2000. C-*myc* gene sequences and the phylogeny of bats and other eutherian mammals. Syst. Biol. 49, 501–514.

Moore, W.S., DeFilippis, V.R., 1997. The window of taxonomic resolution for phylogenies based on mitochondrial cytochrome *b*. In: Mindell, D.P. (Ed.), Avian Molecular Evolution and Systematics. Academic Press, San Diego, pp. 83–119.

Mourer-Chauviré, C., 1988. Le gisement du Bretou (Phosphorites du Quercy, Tarn-et-Garonne, France) et sa faune de vertébrés de l'Eocène supérieur. II Oiseaux. Paleontographica (A) 205, 29–50.

Müller, K., 2005. SeqState: primer design and sequence statistics for phylogenetic DNA data sets. Appl. Bioinform. 4, 65–69.

Oberholser, H.C., 1914. A monograph of the genus Chordeiles Swainson, type of a new family of goatsuckers. U.S. Natl. Mus. Bull. 86, 1–123.

Olson, S.L., 1999. Early Eocene birds from eastern North America: a faunule from the Nanjemoy Formation of Virginia. V. Div. Mineral Res. Pub. 152, 123–132.

Peters, J.L., 1940. Checklist of Birds of the World. Harvard University Press, Cambridge.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Rambaut, A., 1996. Se-Al: sequence alignment editor. Version 2.0a11. Program at: http://evolve.zoo.ox.ac.uk/.

Randi, E., Lucchini, V., Hennache, A., Kimball, R.T., Braun, E.L., Ligon, J.D., 2001. Evolution of the mitochondrial DNA control region and cytochrome *b* genes and inference of phylogenetic relationships in the avian genus Lophura (Galliformes). Mol. Phylogenet. Evol. 19, 187–201.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual, second ed. Cold Spring Harbor Laboratory Press.

Schodde, R., Mason, I.J., 1980. Nocturnal Birds of Australia. Lansdowne Editions, Melbourne.

Shedlock, A.M., Okada, N., 2000. SINE insertions: powerful tools for molecular systematics. BioEssays 22, 148–160.

Shedlock, A.M., Takahashi, K., Okada, N., 2004. SINEs of speciation: tracking lineages with retroposons. Trends Ecol. Evol. 19, 545–553.

Sibley, C.G., Ahlquist, J.E., 1990. Phylogeny and Classification of Birds: A Study in Molecular Evolution. Yale University Press, New Haven, London.

Simmons, M.P., Ochoterena, H., 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49, 369–381.

Sorenson, M.D., Ast, J.C., Dimcheff, D.E., Yuri, T., Mindell, D.P., 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Mol. Phylogenet. Evol. 12, 105–114.

Stumph, W.E., Kristo, P., Tsai, M.-J., O'Malley, B.W., 1981. A chicken middle-repetitive DNA sequence which shares homology with mammalian ubiquitous repeats. Nucl. Acids Res. 9, 5383–5397.

Sullivan, J., Abdo, Z., Joyce, P., Swofford, D.L., 2005. Evaluating the performance of a successive-approximations approach to parameter optimization in maximum-likelihood phylogeny estimation. Mol. Biol. Evol. 22, 1386–1392.

Swofford, D.L., 2003. PAUP* – Phylogenetic Analysis Using Parsimony (* and other methods), Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Swofford, D.L., Olsen, G.J., Waddell, P.J., Hillis, D.M., 1996. Phylogenetic inference. In: Hillis, D.M., Moritz, C., Mabel, B.K. (Eds.), Molecular Systematics. Sinauer Associates, Sunderland, Massachusetts, pp. 407–514.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res. 24, 4876–4882.

Whitney, W.M., Pacheco, J.F., da Fonseca, P.S.M., Webster, R.E., Kirwan, G.M., Barnett, J.M., 2003. Reassignment of *Chordeiles vielliardi* Lencioni-Neto, 1994, to *Nyctiprogne* Bonaparte, 1857, with comments on the latter genus and some presumably related chordeilines (Caprimulgidae). Bull. Br. Ornithol. Club 123, 103–112.

Xeno-Canto Foundation, 2000–2010. Xeno-Canto Africa. Accessed 12 January 2010. Available from: http://www.xeno-canto.org/africa/.

Yuri, T., Kimball, R.T., Braun, E.L., Braun, M.J., 2008. Duplication and accelerated evolution of growth hormone gene in passerine birds. Mol. Biol. Evol. 25, 352–361.

Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. Dissertation, The University of Texas at Austin.