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A phylogenetic analysis of woodpeckers and their allies using 12S, Cyt b, and COI nucleotide sequences (class Aves; order Piciformes)

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Abstract

Although the woodpeckers have long been recognized as a natural, monophyletic taxon, morphological analyses of their intra- and intergeneric relationships have produced conflicting results. To clarify this issue, and as part of a larger study of piciform relationships, nucleotide sequences for the 12S ribosomal RNA (12S; 1123 bp), cytochrome *b* (Cyt b; 1022 bp), and cytochrome oxidase c subunit 1 (COI; 1512 bp) mitochondrial genes were obtained from 34 piciform species that included 16 of the 23 currently recognized woodpecker genera (subfamily Picinae), three piculets (subfamily Picumninae), a wryneck (subfamily Jynginae), a honeyguide (family Indicatoridae), and three barbets (infraorder Ramphastides). Analyses were conducted on the individual and combined 12S, Cyt b, and COI sequences with maximum parsimony, neighbor-joining, maximum likelihood, and Bayesian algorithms. Based on the strong, congruent support among the different data partitions and models of sequence evolution, a highly resolved consensus of the relationships among woodpeckers and their allies could be formed. The monophyly of Indicatoridae + Picidae (infraorder Picides), Picidae, Picinae + Picumninae, and Picinae was strongly supported in all analyses. However, the tribes Colaptini, Picini, Campephilini, and Campetherini were shown to be paraphyletic as were the genera of Colaptes and Piculus. A revision of the tribal-level classification of woodpeckers is proposed and the importance of plumage convergence among woodpeckers is discussed.

Keywords: Woodpeckers; Picidae; Mitochondrial; Phylogeny; Evolution; Classification

1. Introduction

The woodpeckers have historically been recognized as a natural group on the basis of their unique suite of morphological specializations for excavating in trees; i.e., a protrusible barbed tongue with elongated hyoids, enlarged geniothyroid gland, stiffened retrices, zygodactyl feet, and thick cranium. The woodpeckers comprise approximately 218 species in 23 genera within the subfamily Picinae (sensu Short, 1982). They feed primarily on insects though in some genera fruits, seeds or sap can comprise a significant portion of the diet seasonally (e.g., *Picoides*) or year round (e.g., *Sphyrapicus* and *Melanerpes formicivorus*) (Winkler et al., 1995). Foraging modes vary from ground-foraging ant-feeding specialists (e.g., *Colaptes*, *Picus*), to arboreal, arthropod-gleaning generalists (e.g., *Meiglyptes*, *Hemicircus*), to specialized excavators for wood-boring beetles (e.g., *Campephilus*). Nest cavities are generally formed by excavating into trees although a few species form nest cavities in earthen embankments (e.g., *Geocolaptes olivaceous* and *Colaptes rupicola*). Woodpeckers are a cosmopolitan taxon with species on all continents except Australia and Antarctica. Their closest relatives, the piculets (subfamily Picumninae; three genera and 30 species) and wrynecks (subfamily Jynginae; one genus and two species), comprise the remainder of the family Picidae (Short, 1982). Both subfamilies feed primarily on insects by gleaning, probing, and light excavation. Like woodpeckers, piculets

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and wrynecks have enlarged mandibular glands (Goodge, 1972), elongated tongues and hyoids (Coues, 1903), and zygodactyl feet. Piculets have lightly barbed tongues and excavate their own nest cavities in trees but do not have the stiffened retrices of woodpeckers (Short, 1982). Wrynecks have neither stiffened retrices nor barbed tongues and, though cavity nesters, do not excavate their own nests. The monophyly of woodpeckers and the sister status of piculets and woodpeckers within a monophyletic family Picidae have never been seriously disputed. Notably however, relationships among the species and genera of woodpeckers have yet to be clearly established.

There are several possible explanations for their poorly resolved classification. Foremost is the rapid diversification of basal picine lineages from an ancestral species and the consequent difficulty of distinguishing synapomorphies that arose during these brief periods of common ancestry from subsequent parallel and convergent changes. Woodpeckers are also a morphologically uniform group with little information about the taxon's ancestral character states provided by their outgroups or scant fossil record. Thus speculative scenarios for transition series of ancestral to derived taxa have been difficult to test. Another difficulty is that both their external and internal morphological characters have been suspected as labile to convergence and of limited taxonomic utility (see Bock, 1963; Cody, 1969; Goodwin, 1968; Moynihan, 1968; Short, 1976). Thus each taxonomic revision has used a different suite of characters and formed novel groups of genera. However, since Hargitt's (1890) revision, two general classification schemes can be recognized. One scheme, based primarily on gross anatomy, groups genera by similar foraging modes while the other scheme, based mostly on plumage characters, groups the genera by similar appearances and geographic distributions. Thus, the taxonomic schemes of Hargitt (1890), Ridgway (1914), Burt (1930), Peters (1948), and Goodge (1972) all reached similar conclusions and relied on varying combinations of bill shape, toe length, and myology. The second taxonomic scheme, favored by Goodwin (1968) and Short (1982), joins taxa with similar plumage characteristics and geographic distributions into numerous lineages (tribes) that progress from primitive ground-foraging woodpeckers (i.e., *Melanerpes*, *Campethera*, and *Picus*) to increasingly specialized trunk-foraging taxa (i.e., *Picoides*, *Celeus*, *Campephilus*, *Reinwardtipicus*, and *Mulleripicus*).

Burt (1930) divided the woodpeckers of Middle and North America into two groups. One group, specialized for arboreal excavating, lacked the accessory semitendinous muscle and had a skull buttressed by infolded frontal bones. The other, less arboreal, group possessed the accessory semitendinous muscle but lacked infolded frontal bones. Peters (1948), in the first monographic revision of the woodpeckers since Hargitt (1890), divided them into two groups based on the relative lengths of the two outermost toes and the width of the nasal shelf. Goodwin (1968) noted that in Peters' scheme the predominantly ground-feeding woodpeckers were grouped together while he separated pairs of genera "whose geographic distribution, colour patterns, and general similarity suggest close relationships between them." Goodwin hypothesized that Peters' characters may be "rather labile adaptive characters" and developed a classification from plumage characters that were presumed to be more reliable. Goodge (1972) studied the myology of wrynecks, piculets, and woodpeckers from 24 genera and 47 species of the family Picidae and developed yet another set of taxonomic conclusions.

The most recent taxonomic revision of the woodpeckers was conducted by Short (1982) and was based primarily on similarities in plumage, behavior, and distribution. Short recognized 23 woodpecker genera grouped in the six tribes of Melanerpini, Campetherini, Colaptini, Campephilini, Picini, and Meiglyptini. Short's classification, which differs drastically from Peters (1948), neatly groups genera with similar plumages and geographic distributions into tribes. Thus, the Melanerpini are generally North and Middle American, the Campetherini are African, the Colaptini and Campephilini are South American, the Picini are Eurasian, and the Meiglyptini are Asian. In addition, genera with similar appearing species are grouped in tribes (e.g., *Dryocopus* and *Campephilus*, *Dinopium* and *Chrysocolaptes*, and *Veniliornis* and *Piculus*). Short believed that highly similar plumages are accurate indicators of common ancestry and very unlikely to have arisen by convergence.

In the past decade, phylogenetic studies with molecular character sets have conflicted with Short's taxonomic scheme (DeFilippis and Moore, 2000; Prychitko and Moore, 2000; Tennant, 1991; Weibel, 2001; Weibel and Moore, 2002a,b). In general, these studies found that: (i) *Colaptes* and *Piculus* are closely related and both genera are probably mutually paraphyletic, (ii) *Veniliornis* is a South American subclade of *Picoides*, and (iii) *Dryocopus* is closely related to a *Colaptes–Piculus* clade and is not closely related to *Campephilus*. In terms of the six tribes proposed by Short (1982) these results suggest that *Dryocopus* should be moved from Campephilini to Colaptini and that *Veniliornis* should be moved from Colaptini to Campetherini. These results also suggest that plumage convergence among woodpeckers has likely caused numerous errors in their generic-level classification. For example, *Veniliornis* species are generally much more similar in plumage to distantly related *Piculus* species than to their relatives in *Picoides* while *Dryocopus* species are more similar to *Campephilus* than to their relatives in *Colaptes* or *Piculus*. Unfortunately, these recent phylogenetic studies were limited to New World species representing only nine of the 23 woodpecker genera and used only a single New World piculet genus as an outgroup. The main purpose of our study is

therefore to expand the molecular data set in an attempt to resolve clearly the intergeneric relationships of woodpeckers. A secondary goal was to test the monophyly of the family Picidae and infraorder Picides.

2. Methods

2.1. Taxon sampling

To sample uniformly the diversity of woodpeckers, at least two genera were selected from each woodpecker tribe and, in most cases, two species were selected from each genus. The 34 species used in this study represent 16 of the 23 genera in the subfamily Picinae. More comprehensive sampling was precluded by the limited availability of specimens. In addition, species were chosen from a series of increasingly distant outgroups. Thus, members of the subfamilies Picumninae (*Sasia abnormis*, *Picumnus cirratus*, and *P. aurifrons*) and Jynginae (*Jynx torquilla*) were selected from within the family Picidae while members of Indicatoridae (*Indicator variegatus*) and Ramphastidae (*Capito niger*, *Pteroglossus flavirostris*, and *Megalaima haemacephala*) were selected from the order Piciformes. To insure that sequences were accurately attributed to the target species, DNA was isolated and sequenced from at least two individuals for each species. For each sequence, the tissue source, voucher number, and GenBank accession number are listed in Table 1.

2.2. Amplification and sequencing of 12S

Genomic DNA was extracted with Qiagen's DNeasy tissue kit following the manufacturer's protocol for animal tissue. Two microliters of DNA was used per 50 μl PCR (ca. 200 ng template DNA, 3 μl of 25 mM $MgCl_2$ [GibcoBRL], 5 μl of 8 mM dNTPs [Promega], 5 μl buffer [GibcoBRL 10× PCR buffer minus Mg], 5 μl of 5 pM light and heavy strand primers, 24.75 μl of ddH_2O, 0.25 μl of *Taq* DNA Polymerase [GibcoBRL 5 U/μl]). Double-stranded DNA amplification was achieved with primers L1263 (Quinn and Wilson, 1993) and H2546 (5′ CGA TCT GGT AGT ACG TTT TCC AT). The internal primers L1729 [=12Sa] (Kocher et al., 1989), L2021 (5′ CCC GCT AAA AAG ACA GGT CCA 3′), and H2152 [=12Sb] (Kocher et al., 1989) were occasionally used to amplify or sequence problematic samples. L and H refer to the light and heavy strands, and primer number refers to position of the 3′ base of the primer for chicken mtDNA (Desjardins and Morais, 1990). PCRs were run on a Perkin-Elmer 2400 thermocycler with an initial 2 min at 94 °C, followed by 30–35 cycles of 20 s at 94 °C, 20 s at 50 °C, 45 s at 70 °C, and a final 10-min extension at 70 °C. PCR products were cleaned with Wizard PCR Preps (Promega) or the QIAquick PCR purification kit (Qiagen). Sequencing was performed with the ABI Prism BigDye Terminator Cycle Sequencing Reaction Kit (Perkin-Elmer) using the PCR thermocycler profile. The manufacturer's sequencing protocol was followed except that 50–100 ng template DNA, 3.2 pmol primer, 1.5 μl of 5× BigDye buffer, and 1 μl of BigDye terminators were used in a 10 μl reaction volume. Electrophoresis and sequence readouts were on an ABI 100 model 377 automated sequencer (Applied Biosystems).

2.3. Amplification and sequencing of Cyt b and COI

The Cyt b and COI genes were amplified and sequenced following the protocols in DeFilippis and Moore (2000) and Moore and DeFilippis (1997).

2.4. Alignment and secondary structure

All sequences were manually aligned as light strand nucleotides with the software program ESEE (Cabot and Beckenbach, 1989; version 3.0s). The 12S sequences were initially aligned to form a piciform secondary structure model with reference to the secondary structure model of *Falco peregrinus* (Mindell et al., 1997). Gaps were inserted to: (i) maintain alignment of stem regions across taxa, (ii) maintain intramolecular base pairings predicted by the structural model, and (iii) maximize nucleotide identity across taxa in variable loop regions. To assess the veracity of this piciform secondary structure model the aligned sequences were subsequently compared to 130 avian 12S sequences obtained from GenBank, representing 61 families and 104 genera. Our piciform secondary structure alignment only required the insertion of gaps into all piciform sequences when aligned with the larger avian alignment (alignment available upon request). The 1005 sites in the piciform 12S alignment had an average of 35 gaps and 970 nucleotides (range = 956–984). The Cyt b and COI sequences had no indels, encoded open reading frames, and were readily aligned by reference to their start and stop codons.

2.5. Sequence editing

The 12S data set contained the complete gene sequence; the first nucleotide corresponds to position 1297 of the chicken mitochondrial genome (Desjardins and Morais, 1990). The Cyt b data set contained 1022 nucleotides, lacking 120 nucleotides from the 5′ end of the complete gene. The first nucleotide of this truncated sequence corresponds to position 15,013 of the chicken mitochondrial genome. For *Melanerpes erythrocephalus*, 112 nucleotides at the 3′ end of the gene were not sequenced because a gene rearrangement has eliminated the 3′ primer annealing site in tRNA-Pro. The COI data set contained the complete gene sequence of 1512 nucleotides, the first nucleotide corresponding to position 6645 of the chicken mitochondrial genome.

Table 1
List of species and tissue sources for 12S, Cyt b, and COI sequences used in this study

Species	Voucher number	Gene sequence	GenBank Nos.
Geocolaptes olivaceus	uwbm 53193	Cyt b, COI, 12S	AY940801, AY940780, AY940752
Colaptes atricollis	lsu 3581	Cyt b	AY940793
Colaptes atricollis	lsu 3582	COI, 12S	AY940773, AY940739
Colaptes auratus	wsu 86-10.1	Cyt b, COI	AY942881, AY942868
Colaptes auratus	wsu 81-7.1	12S	AY940740
Piculus rubiginosus	lsu 5162	Cyt b, COI, 12S	AY942889, AY942876, AY940767
Piculus rivolii	lsu 1274	Cyt b, COI, 12S	AY940812, AY940791, AY940766
Celeus elegans	lsu 4364	Cyt b, COI, 12S	AY940795, AY940774, AY940742
Celeus flavus	lsu 4385	Cyt b, COI, 12S	AY940796, AY940775, AY940743
Picus miniaceus	lsu 1195	Cyt b	AY940811
Picus miniaceus	lsu 1153	COI, 12S	AY940790, AY940765
Picus canus	uwbm 51783	Cyt b	AY940808
Picus canus	uwbm 51782	COI, 12S	AY940787, AY940762
Campethera cailliautii	ucop p691	Cyt b, COI, 12S	AY940794, AY944307, AY940741
Meiglyptes tukki	lsu 1162	Cyt b, COI	AY940807, AY940786
Meiglyptes tukki	lsu 1169	12S	AY940759
Mulleripicus funebris	ucop p703	Cyt b, COI, 12S	AY940805, AY940784, AY940757
Dryocopus pileatus	wsu 86w-3.4	Cyt b, COI	AY942885, AY942872
Dryocopus pileatus	wsu 86w-1.5	12S	AY940751
Dryocopus lineatus	wsu 95-1.3	Cyt b	AY940800
Dryocopus lineatus	lsu 6660	COI, 12S	AY940779, AY940750
Chrysocolaptes lucidus	ucop p969	Cyt b, 12S	AY940797, AY940745
Chrysocolaptes lucidus	usnm 607371	COI	AY940776
Campephilus haematogaster	lsu 2188	Cyt b, COI, 12S	AY942882, AY942869, AY940744
Campephilus melanoleucos	lsu 2802	Cyt b, COI, 12S	AY940798, AY940777, AY940746
Dendropicos griseocephalus	ucop p815	Cyt b, COI, 12S	AY942884, AY942871, AY940749
Dendropicos fuscescens	uwbm 471	Cyt b, COI, 12S	AY942883, AY942870, AY940748
Picoides albolarvatus	wsu 86w-14.5	Cyt b, COI, 12S	AY942887, AY942874, AY940760
Picoides villosus	wsu 86w-14.4	Cyt b	AY942890
Picoides villosus	wsu 86w-10.7	COI, 12S	AY942877, AY940768
Veniliornis nigriceps	lsu 8176	Cyt b, COI, 12S	AY942893, AY942880, AY940772
Veniliornis callonotus	lsu b5178	Cyt b, COI	AY942892, AY942879
Veniliornis callonotus	lsu b5175	12S	AY940771
Sphyrapicus varius	wsu 86w-14.8	Cyt b, COI	AY942891, AY942878
Sphyrapicus varius	wsu 85-2	12S	AY940770
Melanerpes carolinus	wsu 86w-1.4	Cyt b, COI, 12S	AY942886, AY942873, AY940755
Melanerpes erythrocephalus	wsu 86w-2.6	Cyt b, COI, 12S	AY940804, AY940783, AY940756
Picumnus aurifrons	lsu 18479	Cyt b	AY942888
Picumnus aurifrons	lsu 18254	COI, 12S	AY942875, AY940761
Picumnus cirratus	lsu 6701	Cyt b, COI, 12S	AY940809, AY940788, AY940763
Sasia abnormis	lsu 1151	Cyt b, COI, 12S	AY940813, AY940792, AY940769
Jynx torquilla	uwbm 49209	Cyt b, COI, 12S	AY940803, AY940782, AY940754
Indicator variegatus	fmnh 355280	Cyt b, 12S	AY940802, AY940753
Indicator variegatus	fmnh 356703	COI	AY940781
Megalaima haemacephala	lsu b20788	Cyt b, COI, 12S	AY940806, AY940785, AY940758
Capito niger	lsu b4430	Cyt b	AY940799
Capito niger	lsu b4805	COI, 12S	AY940778, AY940747
Pteroglossus flavirostris	lsu b3560	Cyt b, COI, 12S	AY940810, AY940789, AY940764

uwbm = University Washington Burke Museum; lsu = Louisiana State University Museum of Natural History; wsu = Wayne State University; ucop = University Copenhagen, Denmark; usnm = United States National Museum; fmnh = Field Museum of Natural History.

All mismatches between conspecific gene sequences were checked to eliminate contaminant sequences and paralogous sequences of mitochondrial genes transferred onto nuclear chromosomes (nuclear–mitochondrial-transposed sequences or "numts"; Sorenson and Quinn, 1998). Substitutions that changed sequence length, created nucleotide polymorphisms, or had high background on sequencing readouts were scrutinized for possible numt contamination. Substitutions that disrupted the secondary structure of the ribosomal coding gene or formed stop codons in the reading frame of the protein coding genes would also indicate numt contamination. No numts were found.

2.6. Nucleotide exclusion/inclusion

For 12S, 128 sites were excluded from the analysis because they contained too many indels or substitutions to be homologously aligned; leaving a total of 877

nucleotide positions. Thus, for all analyses other than MP, the final data set contained 3411 nucleotides of concatenated 12S, Cyt b, and COI sequence. In the MP analyses, gaps were counted as a fifth character state. Stem nucleotides, though potentially subject to non-independent compensatory changes, were not excluded or down-weighted in the analyses for several reasons: (i) they are not the primary source of phylogenetic information (see results), (ii) elimination or down-weighting of all potentially non-independent nucleotide changes (e.g., stem compensations, tertiary interactions, compensatory coaxial stack changes, and basetriples) could be applied to most nucleotides in not only ribosomal coding but also protein coding genes, (iii) simplistic weighting schemes calculated from averages over entire genes are as arbitrary as equal weighting, (iv) estimates of stem-pair independence are confounded by partial, non-canonical compensation and delayed compensation (Gatesy et al., 1994). In the MP analyses for Cyt b and COI all third position transition substitutions were excluded because evidence of sequence saturation was observed. The occurrence of substitution saturation at third codon positions in Cyt b and COI was evaluated by plotting the number of pairwise differences against Jukes–Cantor distances (Fig. 1).

2.7. Phylogenetic analysis

All phylogenetic analyses were conducted with the software program Paup* (version 4.0b8, Swofford, 1999) except the Bayesian analysis. To determine if data sets could be combined in a single analysis, 100 replicates of the partition homogeneity test (Farris et al., 1994) were conducted on the gene partitions. A variety of substitution models and optimality criteria were also applied to each gene partition to assess the effect of data and model choice on tree topology and nodal support. In all maximum parsimony (MP) analyses, 100 replicate heuristic searches were conducted on starting trees obtained by stepwise addition of randomly ordered taxa subjected to tree bisection and reconnection (TBR) branch swapping. All other search options were set to default values. MP bootstrap tests followed the same search parameters except that each of the 500 replicates started from a random tree. Relative rate tests found no significant differences in evolutionary rates among lineages (data not shown).

For neighbor-joining (NJ) tree reconstruction (Saitou and Nei, 1987), general time-reversible (GTR) (Lanave et al., 1984; Rodriguez et al., 1990) distances were calculated for each gene and the concatenated sequences. NJ bootstrap tests used 500 replicates of GTR distance estimates.

For maximum likelihood (ML) analyses, a starting tree for estimating model parameters and conducting likelihood-ratio-tests was obtained by creating a 50% majority rule consensus of the MP and NJ trees from the three genes. A majority rule tree was chosen instead of the more conservative strict consensus tree because in Cyt b *Geocolaptes olivaceus* was anomalously placed deep inside of a clade that was strongly supported in all other analyses (data not shown). Nearly all of the clades in this starting tree were supported in all gene partitions and with high bootstrap values.

A series of nested likelihood-ratio-tests (LRT) (Goldman, 1993) were conducted to find the best-fit model of nucleotide substitution. We executed both "top-down" and "bottom-up" hierarchical LRTs similar to those of Posada and Crandall (2001) and in both cases found that the best model was the GTR + G + I with six rate categories for the discrete approximation to the gamma distribution (Yang and Kumar, 1996). The parameters estimated with the assumed starting tree and GTR + G + I model were then used to search for the ML branch lengths and tree topology using the same criteria as the MP heuristic search. The same search criteria were used for the ML and MP bootstrap tests except that only 100 replicates were conducted and the branch-swapping subroutine was eliminated.

An advantage of Bayesian phylogenetic analysis is that it allows specification of distinct evolutionary models for unique partitions of the data (Ronquist and Huelsenbeck, 2003), and more complex models generally improve accuracy of phylogenetic inference (Bollback, 2002; Posada and Crandall, 2001). Previous analyses have shown that patterns of nucleotide substitutions differ between stem and loop regions of the 12S rRNA gene (Webb, 2002), between codon positions of the protein coding genes, and between the 12S rRNA, Cyt b, and COI genes as a whole (DeFilippis and Moore, 2000; Moore and DeFilippis, 1997). Thus, three Bayesian analyses were performed using the computer program MrBayes (ver. 3.0B4, Ronquist and Huelsenbeck, 2003; Windows Version, http://morphbank.ebc.uu.se/mrbayes/). For the first analysis, we partitioned the data into three partitions, one representing each of the genes (12S, Cyt b, and COI). For the second analysis we created five partitions by further partitioning the 12S rRNA gene into loop regions, and paired nucleotides and unpaired nucleotides of the stem regions. For the third analysis, we created nine partitions by further partitioning the two protein coding genes into first, second, and third codon positions. For all partitions in all analyses, four character states were defined (ACGT, nucmodel = 4 × 4) except for the paired-stem partition of the five- and nine-partition analyses where 16 character states corresponding to the possible nucleotide pairings (A–G, A–T, etc.) in stems were defined (nucmodel = doublet). This configuration allows consideration of possible non-independent substitution of complementary bases in stems. The gamma-corrected (four rate categories) general-time-reversible model was specified for all partitions in all

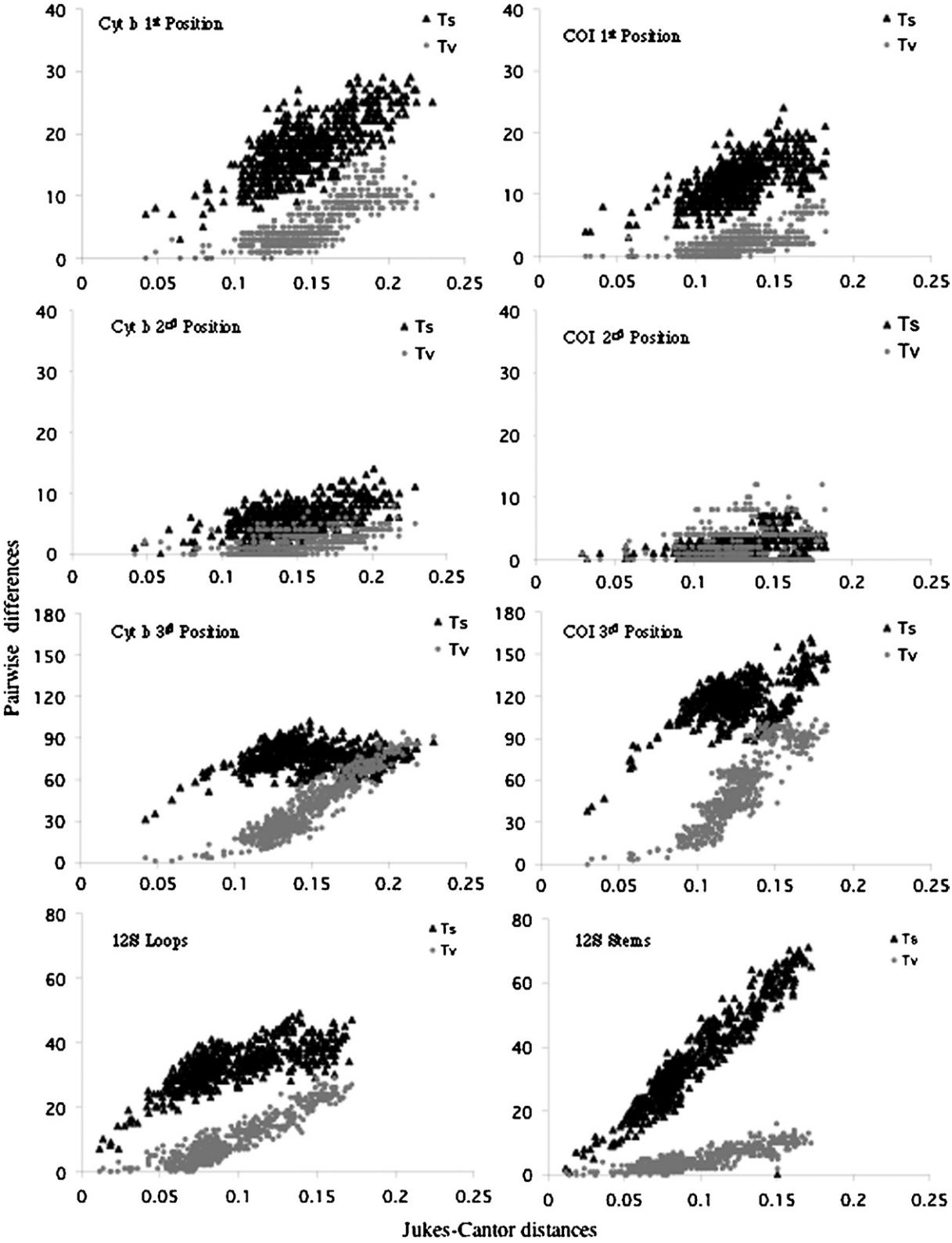

Fig. 1. Saturation plots for Cyt b, COI, and 12S transition and transversion substitutions. First, second, and third codon positions are plotted separately for Cyt b and COI. Loop and stem regions are plotted separately for 12S.

analyses. For the three-partition and five-partition analyses, the Monte Carlo Markov Chains (MCMC) were simulated for 500,000 generations and sampled every 100 generations; four chains were run and 250 initial trees were discarded (burn in). For the nine-partition analysis, the MCMC was simulated for 768,000

generations and sampled every 100 generations; four chains were run and 200 initial trees were discarded.

3. Results

3.1. Variability in 12S

Among the 877 nucleotide sites utilized for phylogenetic analysis, 38.3% were variable and 30.1% were potentially parsimony-informative. Double-stranded "stem" regions were more conserved than single-stranded "loop" regions; the percentage of invariant stem and loop sites being 67.5 and 50.5%, respectively. While loop regions accounted for only 34.3% of the gene they contained 45.2% of the total variable sites. Within these regions there was considerable variability in among-site-nucleotide diversity (Webb, 2002) and a ML estimate of among-site rate variability was greater for stems ($\alpha = 0.36$) than loops ($\alpha = 1.08$).

Saturation plots show that in 12S stem regions, number of pairwise differences for both transitions and transversions increases linearly with Jukes–Cantor (JC) distance (Jukes and Cantor, 1969; Fig. 1). In loop regions, transversions increase linearly over the entire range of distances while transitions plateau at approximately 10% sequence divergence. The average 12S JC distance was 9.9% among picine species (Table 2), 11.1% between picid subfamilies (data not shown), and 14.5% between piciform families (data not shown). Espinosa de los Monteros (2000) plotted transitional changes in loop regions versus Kimura distances for a group of more distantly related "higher non-passerine" orders and estimated that loop transitions begin to saturate at about 15% sequence divergence. However, our saturation plot of 164 avian 12S sequences (data not shown) shows no "plateau" in the number of transitions or transversions with increasing distances. This discrepancy is likely because in our analysis we excluded the most variable regions of the alignment; indicating that the exclusion of the most variable loop regions may remove much of the sequence saturation. For piciforms, transition substitution saturation should have little effect on distance estimates for sequences from different genera within a subfamily or possibly even different subfamilies within a family.

With increasing levels of taxonomic divergence and sequence saturation the observed ratio of transitions to transversions (Ts/Tv) is expected to deviate increasingly from the instantaneous ratio (Brown et al., 1982). Ts/Tv, calculated from observed differences in pairwise comparisons of complete 12S sequence alignments for eight avian orders with the software program Mega (Kumar et al., 1994), was higher in Piciformes ($=3.21$) than any other order investigated (range $= 2.93$–1.58). While such averages vary as a function of taxon sampling, the comparatively high average Ts/Tv ratio for piciforms suggests a relatively low level of sequence saturation. The instantaneous ratio was 7.6 for Piciformes (data not shown) versus 7.3 for gruiforms (Houde et al., 1997).

Among the variable, paired, stem nucleotides potentially half are non-independent changes that occurred to maintain base complementarity within a stem pair. Without a known phylogeny and history of nucleotide substitution, the exact number of compensatory changes cannot be known. However, a rough estimate of the frequency of compensatory changes among stem nucleotides was made as follows: (i) all parsimony-informative stem sites were identified, (ii) for a given informative site, the most common 5′ nucleotide state was assumed to be primitive and the less common state derived, (iii) 3′ pair members that formed canonical complements to the derived 5′ pair members were identified, (iv) if more than half of these 3′ nucleotides complemented the derived 5′ sites then the site was counted as a compensatory stem-pair change. This estimate was made on an alignment of 141 avian 12S sequences with 220 potentially informative characters; 101 of which were paired stem nucleotides. Of the 51 potentially informative 5′ stem-pair changes, eight did not have a compensatory change in the 3′ stem-pair member (likewise, of the 50 3′ stem-pair changes, seven were not compensated by 5′ changes). Thus, 84.3% of the potentially informative 5′ stem sites had a compensatory change in the 3′ stem-pair member and approximately 19.5% $[(101-15)\div 220\div 2]$ of the 220 potentially informative sites in our data set were non-independent, compensatory changes.

Among the 877 sites there was a slight excess of adenine and cytosine and a paucity of guanine and tyrosine nucleotides (31% A, 29% C, 21% G, and 19% T) (Fig. 2). Among the 128 discarded nucleotide sites, 11% were guanine, 28% adenine, and 50% cytosine. The nucleotide bias was less pronounced in stem regions (26% A, 28% C, 26% G, and 20% T) than in loop regions (41% A, 31% C, 11% G, and 17% T) though within stem regions the unpaired, bulged nucleotides showed the greatest bias of any subset (53% A, 26% C, 8% G, and 13% T). The increased abundance of adenines among unpaired nucleotides has been hypothesized to promote hydrophobic interactions with proteins (Gutell et al., 1985). An MP reconstruction of character state changes showed stems and loops to have similar substitution patterns except that transitions from A to G are noticeably less common in loops than in stems. The nucleotide proportions of

Table 2
Average Jukes–Cantor distances (±SD) among pairwise comparisons of 12S, Cyt b, and COI sequences with hypervariable regions excluded

	Among Picinae ($n = 325$)	Outgroups vs. Picinae ($n = 208$)	Among outgroups ($n = 28$)
12S	0.099 ± 0.019	0.160 ± 0.023	0.163 ± 0.029
Cyt b	0.128 ± 0.018	0.173 ± 0.019	0.178 ± 0.024
COI	0.113 ± 0.017	0.144 ± 0.018	0.147 ± 0.025

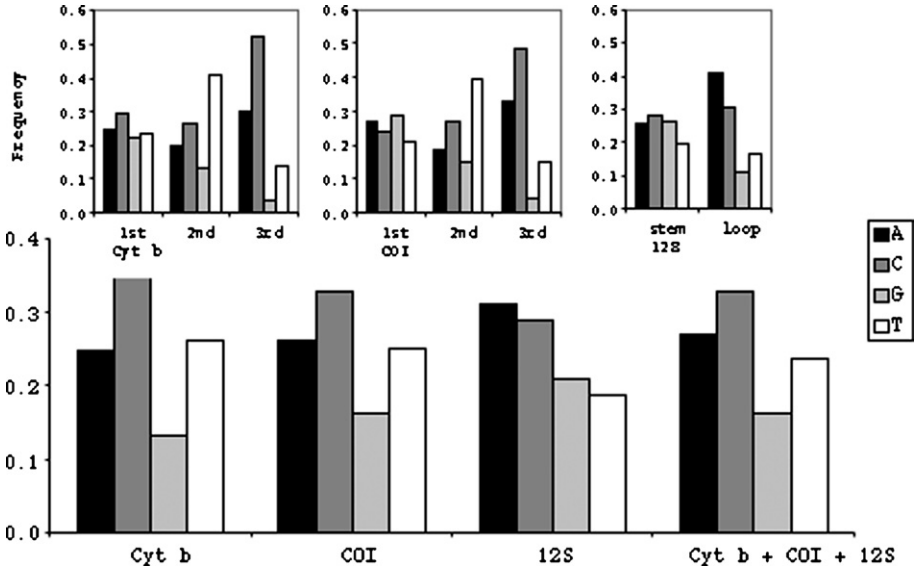

Fig. 2. Piciform nucleotide frequencies for Cyt b, COI, 12S, and the combined Cyt b, COI, and 12S sequences. Insets show nucleotide frequencies by codon positions or by stem and loop regions for each individual gene.

piciforms differed only slightly from those of a diverse array of 130 additional avian species and showed little variation among piciform subgroups (Webb, 2002).

Though gaps were included as a fifth character state, their inclusion had little effect on overall sequence variability. Of the 877 sites, gaps contributed to only 10 variable sites and six parsimony-informative sites. Three of these parsimony-informative sites were due solely to the recognition of the gap character state. Eight of the 10 variable sites with gaps appear as mononucleotide indels and the rest as dinucleotide indels

3.2. Variability in Cyt b and COI

Of the 1022 Cyt b nucleotides, 47.3% were variable and 42.0% were parsimony informative. Among the first, second, and third codon positions the percentage of variable sites were, respectively, 30.8, 12.9, and 98.2%. The percentage of informative sites among the three positions were, respectively, 24.3, 7.6, and 94.1%. Of the 483 variable sites, 51.1% had synonymous substitutions. For the 1512 COI nucleotides, 40.5% were variable and 33.8% were parsimony informative. Among the first, second, and third codon positions the percentage of variable sites was, respectively, 17.4, 6.2, and 97.8%. The percentage of informative sites among the three positions were, respectively, 10.1, 2.4, and 88.9%. Of the 612 variable sites, 71.2% had synonymous substitutions.

Observed pairwise differences for each codon position of Cyt b and COI were plotted against JC distances to detect potential saturation among substitution types. For first and second codon positions, both transitions and transversions increase linearly with distance (Fig. 1). In the third codon position of each gene, transversions increase linearly over all distances while transitions plateau at approximately 12% sequence divergence. The average JC distance between species of the subfamily Picinae was 12.8% for Cyt b and 11.3% for COI (Table 2) while average JC distance between an outgroup species and the ingroup was 17.3% for Cyt b and 14.4% for COI. Transition substitutions at third codon positions are therefore excluded from all MP analyses. When third position transitions are included in the analysis, a tree with 19 of 31 nodes having bootstrap values >50% is produced whereas when they are excluded 26 of 31 nodes have bootstrap values >50% and the topology becomes nearly identical to that of the ML bootstrap tree.

Cyt b and COI had very similar nucleotide compositions and differed considerably from the composition of 12S (Fig. 2) though the three genes, when combined, had a more uniform nucleotide composition than any individual gene. Base frequencies for each codon position of these genes were similar to those found in previous studies (e.g., Espinosa de los Monteros, 2000). Thus, third codon positions showed an excess of C (ca. 50%) and paucity of G (ca. 4%) while first codon positions were least skewed. (See DeFilippis (1995) and Weibel (2001) for further analysis of Cyt b and COI substitutions patterns in woodpeckers.)

3.3. Gene congruence

Though the single genes varied somewhat in their MP and NJ trees, a partition homogeneity test indicated no significant difference among the genes ($p = 0.72$ for 12S vs. Cyt b vs. COI). Furthermore, although stochastic sampling error lowers the probability that single gene trees will recover the species phylogeny, in most trees the same clades were recovered and with high bootstrap support (data not shown). In all trees, the subfamily Picinae is

monophyletic and all congeneric species except *Colaptes*, *Piculus*, and *Picus* are sister taxa. For most trees, there is a monophyletic Picinae–Picumninae clade, Picidae clade, and Picides clade. Smaller clades that were found in all analyses included: *Piculus–Colaptes*, *Mulleripicus–Dryocopus*, *Picoides–Veniliornis*, and *Melanerpes–Sphyrapicus*. Most of the conflicts among the gene partitions were produced by a few taxa. Thus, removal of *Picus miniaceus*, *P. canus*, *Meiglyptes tukki*, *Campethera cailliautii*, and *Geocolaptes olivaceus* led to broad congruence in the tree topologies for each gene (data not shown).

Despite having the greatest number of parsimony-informative sites, the COI data produced the shortest MP tree (PI sites = 441, length = 906, CI = 0.421, RI = 0.545, uninformative characters removed in all comparisons). Cyt b had the second most informative sites and second shortest tree (PI sites = 365, length = 934, CI = 0.404, RI = 0.514) while 12S had the longest tree and lowest index values (PI sites = 264, length = 1170, CI = 0.391, RI = 0.493). The same trend in each gene's relative performance was shown by the number of most and nearly most parsimonious trees found after an heuristic search with the 100 random sequence additions. Thus, two maximum parsimony (MP) trees were found for COI. For Cyt b, there were three MP trees and one tree one step longer. For 12S, two MP trees were produced along with 13 trees one step longer and 15 trees two steps longer.

3.4. Combined gene analyses

A series of nested likelihood-ratio-tests found the best-fit model of nucleotide substitution to be the GTR + G + I with six rate categories for the discrete approximation to the gamma distribution. The ML estimate for the proportion of invariable sites was 0.525 and 0.792 for the gamma shape parameter. The ML estimated base frequencies were 0.332 A, 0.382 C, 0.110 G, and 0.176 T. The ML estimated rate matrix was AC = 0.593, AG = 11.527, AT = 1.672, CG = 0.420, and CT = 17.688.

With these parameter values, a single tree topology was found in all 10 random sequence addition replicates for ML heuristic searches. One hundred bootstrap

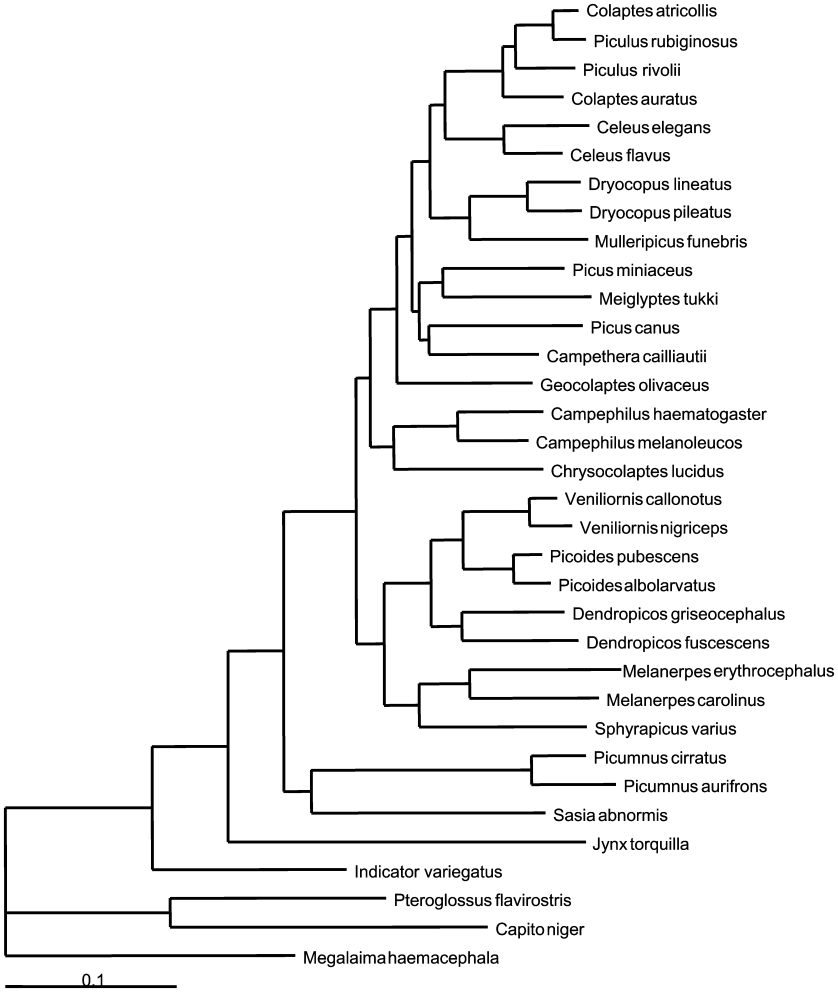

Fig. 3. Phylogenetic tree for nine-partition Bayesian analysis. Scale bar indicates substitutions/site.

replicates of the ML tree topology generated greater than 89% bootstrap support for the following taxa: infraorder Picides, family Picidae, subfamilies Picumninae + Picinae, subfamily Picinae, Short's tribe Melanerpini, and all genera except *Colaptes*, *Piculus*, and *Picus*. Notably, monophyly of Short's tribes Colaptini, Campetherini, Campephilini, Picini, and Meiglyptini was not supported by the ML tree.

In nearly all single gene and combined ML analyses three clades were consistently formed. *Campephilus* and *Chrysocolaptes* were almost always joined as sister taxa though their position among other woodpeckers varied from the basal-most taxon to sister group of either of the two remaining subclades. The remaining woodpeckers typically formed the following clades: (*Picus*, *Campethera*, *Meiglyptes*, *Geocolaptes* ((*Mulleripicus*, *Dryocopus*)(*Celeus*, (*Colaptes*, *Piculus*)))) and ((*Melanerpes*, *Sphyrapicus*)(*Dendropicos* (*Picoides*, *Veniliornis*))). In all analyses except Cyt b, the nested hierarchy of ((*Melanerpes*, *Sphyrapicus*)(*Dendropicos* (*Picoides*, *Veniliornis*))) was well supported by bootstrap replicates. In Cyt b, *G. olivaceus* weakly grouped with (*Picoides*, *Veniliornis*) while (*Melanerpes*, *Sphyrapicus*) formed the basal-most clade of woodpeckers. The ML tree showed only three poorly resolved areas: (i) *Picumnus*' sister group relationship to either *Sasia* or Picinae, (ii) the position of (*Campephilus*, *Chrysocolaptes*) within Picinae, and (iii) the relationships among *Picus miniaceus*, *P. canus*, *Campethera*, *Meiglyptes*, and *Geocolaptes*.

The three-partition, five-partition, and nine-partition Bayesian trees were topologically identical to each other (Fig. 3) and to the ML tree except for *P. miniaceus* and *M. tukki* joining as sister taxa, which is a group supported in many of the single gene analyses and receiving a posterior probability of 68% in the nine-partition analysis. Posterior probabilities varied only slightly, on average among the three Bayesian trees (Fig. 4).

The two MP trees differed from the Bayesian ML tree only in having the relationship between *Sasia* and *Picumnus* unresolved (data not shown). The topologies and support values of the MP, ML, and Bayesian trees were very similar. The GTR-NJ tree differed from the other trees only in the relationships among *Picus*, *Meiglyptes*, *Campethera*, and *Geocolaptes* (data not shown). In terms of bootstrap support, the GTR-NJ tree differed only in weakly supporting a relationship between *Geocolaptes* and *Campethera* and leaving the relationship of the *Dryocopus–Mulleripicus* clade to the *Celeus–Colaptes–Piculus* clade unresolved.

Therefore, within the subfamily Picinae three woodpecker clades repeatedly appeared in our phylogenetic results. One clade contained all woodpeckers belonging to the genera of *Colaptes*, *Piculus*, *Celeus*, *Dryocopus*, *Mulleripicus*, *Meiglyptes*, *Picus*, *Campethera*, and

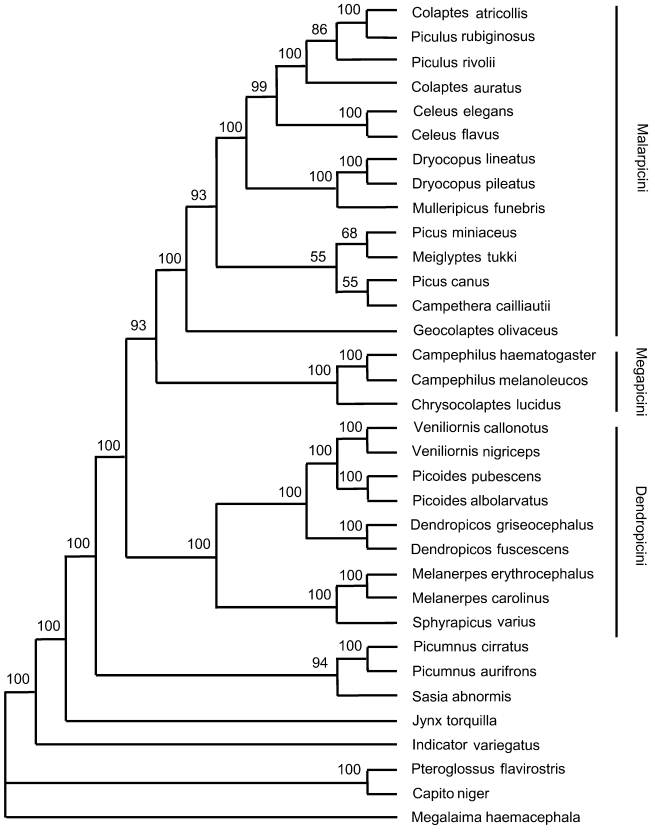

Fig. 4. Posterior probabilities for nine-partition Bayesian analysis.

Geocolaptes. The second clade contained the genera *Picoides*, *Veniliornis*, *Dendropicos*, *Melanerpes*, and *Sphyrapicus* while the third consistently supported clade contained *Campephilus* and *Chrysocolaptes*.

4. Discussion

4.1. Congruence among data partitions

As a single linkage group, the 12S, Cyt b, and COI mitochondrial gene sequences are expected to produce identical gene trees that trace a common genomic and taxonomic history. The different partitions may, however, lead to the reconstruction of different gene trees when stochastic factors and deterministic biases overwhelm the shared phylogenetic signal in the sequences. The partition homogeneity test showed that these data partitions did not support significantly different histories ($p = 0.72$ for 12S vs. Cyt b vs. COI). Since increasing sample size generally improves estimation procedures, the best estimate of the genome's phylogenetic history is expected to be produced from the largest set of nucleotides, which is the set of combined gene sequences (DeFilippis and Moore, 2000; Hillis and Wiens, 2000; Saitou and Nei, 1986).

We believe the best estimate of relationships among the 34 taxa is that of the Bayesian tree based on the concatenated gene sequences (Fig. 3). In the nine-partition Bayesian analysis, 27 of 31 nodes are supported by posterior probabilities greater than 90% (Fig. 4). The three most weakly supported nodes involve relationships within the single clade containing *Meiglyptes tukki*, *Campethera cailliautii*, *Picus miniaceus*, and *P. canus*. The fourth sub-90% node involves *Piculus rivolii* (86%). The ML, MP, and NJ analyses of the concatenated sequences produced tree topologies very similar to this Bayesian tree topology; differing only in the relationships among nodes with weak posterior probabilities. The individual gene trees were largely congruent with the results of the Bayesian analysis on the concatenated sequences although none of them duplicated that tree topology.

The Bayesian tree is congruent with other data sets as well. For example, very similar tree topologies are produced with data sets for the nuclear β-fibint7gene (Prychitko and Moore, 2000); allozymes and mtDNA restriction sites (Tennant, 1991); osteology (Webb, 2002); and myology (Goodge, 1972; but not Swierczewski, 1997).

4.2. Classification and monophyly

The classification for the order Piciformes proposed by Sibley and Ahlquist (1990) was supported by all subsets of this study. When the concatenated piciform sequences were rooted by a passeriform or galliform outgroup the honeyguide always joined with the Picidae clade (data not shown).

A monophyletic family Picidae was formed in all subsets of this study as *Jynx torquilla* was always sister taxon to the piculet–woodpecker clade. The unnamed clade containing all piculets and woodpeckers was also monophyletic in all subsets of this study. However, a monophyletic subfamily Picumninae received weak support in the ML, NJ, and MP bootstrap analyses. With nearly equal frequency, *Sasia abnormis* was either sister group to a *Picumnus*–Picinae clade or grouped with *Picumnus* to form a monophyletic Picumninae. The short internode shared by Asian *Sasia* and South American *Picumnus* and the long terminal branches apparent in the Bayesian tree (Fig. 3) indicate that these lineages diverged early following, at most, a short period of common ancestry. The monophyly of the piculets should be more completely investigated with additional sequences from *Nesoctites micromegas*, *Sasia ochrea*, *S. africana*, and *Picumnus innominatus*.

The subfamily Picinae was monophyletic in all analyses. However, in contrast to Short's identification of six woodpecker tribes, we found three distinct, strongly supported woodpecker clades. On the basis of the mitochondrial sequence analyses reported here and an unpublished osteological data set (Webb, 2002) we propose the recognition of the following three woodpecker tribes: Malarpicini, Dendropicini, and Megapicini. The tribe Malarpicini will contain all woodpeckers in *Colaptes*, *Piculus*, *Celeus*, *Dryocopus*, *Mulleripicus*, *Dinopium*, *Meiglyptes*, *Picus*, *Campethera*, and *Geocolaptes*. The tribe Dendropicini will contain *Picoides*, *Veniliornis*, *Dendropicos*, *Melanerpes*, *Sphyrapicus*, *Xiphidiopicus*, and *Sapheopipo*. The tribe Megapicini will contain *Campephilus*, *Chrysocolaptes*, *Reinwardtipicus*, and *Blythipicus*. The relationships of *Hemicircus* and *Gecinulus* to other woodpeckers remain enigmatic and they are placed *incertae sedis* within the subfamily Picinae.

While three well-supported tribes of woodpeckers can be defined, the relations *among* these new tribes are ambiguous. The Bayesian analyses show relatively strong support for a sister group relationship between the Megapicini and Malarpicini tribes (nine-partition and five-partition = 93%, three-partition = 80%), though other analyses weakly support either Megapicini or portions of Dendropicini as the sister group to Malarpicini. More rarely, as with β-fibint7 sequences, Dendropicini and Megapicini are united as sister groups with weak support (Prychitko and Moore, 2000). Equally ambiguous evidence for the taxonomic relationships among these tribes is found with allozymes, external morphology, and myology.

Within the Malarpicini, a clade containing *Colaptes*, *Piculus*, *Mulleripicus*, *Dryocopus*, and *Celeus* is consistently reconstructed and can be designated as the subtribe Dryocolaptes. This clade was reconstructed in the

majority of the single gene and concatenated analyses, and it had a posterior probability of 100% in the three Bayesian trees. Short (1982), though placing these genera in different tribes, sequentially listed *Piculus*, *Colaptes*, *Celeus*, and *Dryocopus* in his classification to indicate their close relationship while Peters (1948) and Goodge (1972) loosely grouped these genera together with other members of the Malarpicini. Additional support for the monophyly of Dryocolaptes comes from the vocalizations, geographic distributions, and ant-specialized diets these taxa share.

Although the Malarpicini was well-supported, the relationships *among* the members of this tribe were often poorly resolved. In the concatenated sequence analyses, *G. olivaceus* was placed at the base of this clade in the ML and Bayesian trees while the ML and NJ bootstrap analyses showed its position within the Malarpicini as unresolved. In the individual gene analyses it tended to pair with *C. cailliautii*. Short (1982) considered *Geocolaptes* to be a derived campetherine woodpecker but Peters (1948) placed it as the first genus in a series that includes all of the Malarpicini. Osteologically, it groups within the *Colaptes–Piculus* subclade; presumably as a result of morphological convergence among ground-feeding woodpeckers (Webb, 2002). It most likely belongs either to a clade of Old World woodpeckers containing portions of *Picus* and *Campethera* or is basal to all other members of Malarpicini. It is unlikely to be closely related to *Colaptes* or a member of the predominantly New World subtribe Dryocolaptes.

The genus *Picus* contains distinct subclades of crested, yellow-naped species (ca. *Chrysophlegma*) and non-crested, red-crowned species (ca. *Gecinus*) and is likely a polyphyletic genus (Goodwin, 1968). In no analyses were *Picus* (*Chrysophlegma*) *miniaceus* and *P.* (*Gecinus*) *canus* joined as sister taxa. These taxa were variably allied with *Campethera*, *Meiglyptes*, *Geocolaptes*, or *Dryocopus* within Malarpicini. The polyphyly of this genus was also weakly supported in the osteological data set.

The genera of *Colaptes* and *Piculus* are very likely paraphyletic. The Bayesian analyses of the concatenated sequences showed *C. atricollis* and *P. rubiginosus* to be sister taxa, to the exclusion of *C. auratus* and *P. rivolii*, with posterior probabilities of 100%. Their paraphyly likewise received nearly 100% bootstrap support in all single gene analyses. Moore, using full-length Cyt b and COI sequences (unpubl. data), has found that the genus *Colaptes*, sensu lato (s.l.), should include the superspecies *Piculus rubiginosus* and *P. rivolii* and that a monophyletic *Piculus*, sensu stricto (s.s.), contains only *P. leucolaemus*, *flavigula*, *chrysochloros*, and *aurulentus*. Tennant (1991), Moore and DeFilippis (1997), and Prychitko and Moore (2000) found similar evidence for the paraphyly of these genera.

In contrast to Short's classification, *Mulleripicus* and *Dryocopus* appear to be sister taxa as they were joined in all single-gene analyses, had posterior probabilities of 100% in the Bayesian analyses of the concatenated sequences and high bootstrap support in the concatenated-sequence MP, NJ, and ML trees. Peters (1948) merged the New World *Ceophloeus* with the Old World *Dryocopus* and the monophyly of this expanded genus (*Dryocopus*, s.l.) has been confirmed (Swierczewski, 1997; Moore, unpubl.). The genus *Dinopium*, which shares a Southeast Asian distribution with *Dryocopus* and *Mulleripicus* and looks similar to many New World *Dryocopus* species, was found to be their sister group in Webb's (2002) osteological study.

The relationships of *Meiglyptes* and *Campethera* are enigmatic. In all analyses they grouped with Old World taxa within the Malarpicini and, like *Geocolaptes*, possibly form a clade of Old World woodpeckers containing portions of *Picus* and *Campethera*. They are unlikely to be members of the predominantly New World Dryocolaptes subtribe. The monophyly of the genus *Campethera* has not been tested in any recent studies and its status should remain tentative as it contains such distinct subclades.

The tribe Dendropicini contains all woodpeckers belonging to *Picoides*, *Veniliornis*, *Dendropicos*, *Xiphidiopicus*, *Sphyrapicus*, *Melanerpes*, and *Sapheopipo*. *Picoides*, *Veniliornis*, *Dendropicos*, *Sphyrapicus*, and *Melanerpes* formed a well-supported clade in all analyses except for the Cyt b data set where *Melanerpes* and *Sphyrapicus* were placed as basal members of the Picinae (*Xiphidiopicus* and *Sapheopipo* were not sequenced and their inclusion in this tribe is based on other sources of information). The genera of Dendropicini have historically been grouped together though in varying combinations by different authors. Peters (1948) sequentially listed the genera as *Melanerpes*, *Sphyrapicus*, *Veniliornis*, *Dendropicos* (s.s.), *Picoides*, *Sapheopipo*, *Xiphidiopicus*, and "*Dendropicos*" [= *Polipicus*, *Mesopicos*, *Thripias*] though *Melanerpes* was put in a separate subgroup because of its short hind toe and narrow nasal shelf. Goodge (1972) placed them into the three closely related groups of: (i) *Melanerpes* + *Sphyrapicus*, (ii) *Mesopicos* + *Dendrocopos* + *Picoides* + *Sapheopipo* + *Dendropicos* + *Veniliornis* + *Thripias*, and (iii) *Xiphidiopicus*. Swierczewski (1997) followed Peters' scheme by separating *Melanerpes* from the remaining clade members. Short (1982) produced the most distinct classification by placing the taxa in three different tribes: (i) *Melanerpes*, *Sphyrapicus*, and *Xiphidiopicus* comprised Melanerpini, (ii) *Dendropicos* (s.l.) and *Picoides* (s.l.) partially comprised Campetherini, and (iii) *Veniliornis* partially comprised Colaptini. Numerous molecular studies have contradicted Short's hypotheses and found a close relationship between these taxa (DeFilippis and Moore, 2000; Tennant, 1991; Weibel and Moore, 2002b).

Melanerpes (*Centurus*) *carolinus* and *M. erythrocephalus*, though formerly placed as separate genera, were

found to be sister taxa in all analyses. Tennant (1991) found monophyly among nine *Melanerpes* species to the exclusion of two *Sphyrapicus* and seven *Picoides* species. However, the monophyly of the genus *Melanerpes* should not be assumed until it is demonstrated that species of *Dendropicos* or *Xiphidiopicus* do not fall within this clade and more melanerpine species are included in an appropriate phylogenetic analysis. In all analyses, *Sphyrapicus* was the sister taxon of *Melanerpes* and this clade had a posterior probability of 100% in the Bayesian analysis of the concatenated sequences. Peters (1948) and Swierczewski (1997) separated *Melanerpes* and *Sphyrapicus* though most studies found them to be closely related taxa (DeFilippis and Moore, 2000; Goodge, 1972; Prychitko and Moore, 2000; Short, 1982; Tennant, 1991). In Webb's (2002) osteological study *Sphyrapicus*, *Melanerpes*, and *Xiphidiopicus* did not form a clade but were sequentially placed among the basalmost members of the Picinae. We suspect that sequence data will show *Xiphidiopicus* to form a clade with *Melanerpes* and *Sphyrapicus* as suggested by Short.

The remaining members of the Dendropicini, *Dendropicos*, *Picoides*, and *Veniliornis*, form a strongly supported clade in all analyses except Cyt b (where *Geocolaptes* joins this clade). Within Dendropicini the current generic-level taxonomy is largely incorrect. Tennant (1991) and Weibel and Moore (2002a,b) have previously indicated that the genus *Picoides* (s.l.) is paraphyletic; the South American species of *Picoides* (*mixtus* and *lignarius*) being the sister taxa of *Veniliornis*. The genus may further be paraphyletic with respect to the genus *Dendropicos* as a ML tree of COI, Cyt b, and β-fibint7 sequences from 17 species of *Picoides* placed *D. fuscescens* among a group of Old World *Picoides* species (Weibel and Moore, 2002b). The species of *Dendropicos fuscescens* and *D.* (*Mesopicos*) *griseocephalus*, despite formerly being placed in separate genera, were joined as sister taxa with high bootstrap support in all analyses. However, the monophyly of this genus should remain tentative until a more comprehensive analysis of the relations among Old World species of *Picoides* and *Dendropicos* is completed.

The tribe Megapicini contains all woodpeckers belonging to the genera of *Campephilus*, *Chrysocolaptes*, *Reinwardtipicus*, and *Blythipicus*. In the Bayesian tree of the concatenated sequences *Campephilus* and *Chrysocolaptes* were joined as sister taxa with a posterior probability of 100%. In Webb's (2002) osteological analysis these two genera were joined with *Reinwardtipicus* (*Chrysocolaptes*) *validus* with 90% bootstrap support and this clade was in turn sister to *Blythipicus rubiginosus*. Both Peters (1948) and Goodge (1972) allied these species, along with *Hemicircus*, as the "ivory-billed" woodpeckers. Swierczewski (1997) found *Chrysocolaptes* and *Campephilus* to be sister taxa but did not study *Reinwardtipicus*, *Blythipicus*, or *Hemicircus*. In contrast, Bock (1963), Goodwin (1968), and Short (1982) allied *Campephilus* and *Dryocopus* because of their similar plumages and geographic distributions. Short removed *Reinwardtipicus validus* from the genus *Chrysocolaptes* and allied it with *Blythipicus*; remotely joining all three genera in the tribe Picini along with *Picus*, *Dinopium*, *Gecinulus*, and *Sapheopipo*. Although the molecular evidence for the tribe Megapicini is limited, the concurrence among the myological and osteological data sets suggests that sequence data will place *Reinwardtipicus*, *Blythipicus* and, possibly, *Hemicircus* within this tribe.

The remaining genera of *Hemicircus*, *Gecinulus*, and *Sapheopipo* have not been included in any molecular analyses and their taxonomic relationships are problematic. On the basis of Webb's (2002) osteological studies, *Hemicircus* is the sister taxon to all other woodpeckers. Consistent with this position is their notably piculet-like tail with short, round-edges and slightly stiffened retrices, their piculet-like foraging behavior (Ali and Ripley, 1987; Short, 1973, 1982), and fruit eating habits (Winkler et al., 1995). However, Goodge (1972) found the myology of *Hemicircus* to be most similar to that of *Blythipicus* and they are in turn similar to other "ivory-billed" genera (*Campephilus*, *Chrysocolaptes*, and *Reinwardtipicus*). Peters (1948) also listed *Hemicircus* next to the "ivory-billed" woodpeckers and, more generally, among the woodpeckers with wide nasal shelves and elongated fourth toes, a group that corresponds to our Dendropicini and Megapicini (though excluding *Melanerpes*). Support for the schemes of Goodge and Peters comes from the absence of a malar stripe in all of Megapicini and Dendropicini, and the presence of crests in the Megapicini. In contrast, Short (1982) placed *Hemicircus* with *Meiglyptes* and *Mulleripicus* in the tribe Meiglyptini after noting their similar body forms, plumages, and behaviors. We advocate that *Hemicircus* be placed *incertae sedis* within the subfamily Picinae.

Goodge (1972) found the myology of *Sapheopipo* to be very similar to that of *Picoides* (s.l.) and noted two shared myological characters that may define subclades in the tribe Dendropicini (Webb, 2002). Short (1982) placed *Sapheopipo* within his tribe Picini and saw it as a derivative of a lineage leading to *Blythipicus* and *Reinwardtipicus*. Goodwin (1968) thought it was "an offshoot of *Dendrocopos* [=*Picoides*] stock" and especially of *D. leucotos*. However, the similar wing, crown, vent, and belly coloration cited as evidence by Goodwin could equally well associate *Sapheopipo* with *Melanerpes* or *Blythipicus*. Peters (1948) listed *Sapheopipo* after *Picoides* because of its wide nasal shelf and elongated fourth toe. Despite its general *Picus*-like appearance, several characters (i.e., nasal shelf, fourth toe, malar, and stripe) seem to exclude it from belonging within the Malarpicini. These characters, along with its rufous-tinged plumage and *Blythipicus*-like vocalizations, seem to ally it to the Megapicini. However, it lacks the specialized central

retrices of that tribe and has the red vent, red belly wash, sexually dimorphic crown-coloration, and myological novelties of some of the Dendropicini. We advocate that Peters' arrangement be followed and tentatively place *Sapheopipo* (*incertae sedis*) within Dendropicini.

Peters (1948) placed *Gecinulus* rather incongruously between the genera of *Dinopium* and *Meiglyptes*. All of these genera have narrow nasal shelves, short fourth toes, and, in *Gecinulus* and some *Dinopium* species, only three toes. Goodwin (1968) saw them as a possible link between the "green woodpeckers of the genus *Picus*" and the green-backed *Dinopium rafflesii* while Short (1982) associated *Gecinulus* with *Sapheopipo*, *Dinopium*, and *Picus* in the tribe Picini. We advocate that *Gecinulus* be placed *incertae sedis* within the Picinae although their lack of a malar stripe may indicate that they do not belong to Malarpicini.

4.3. Plumage evolution in woodpeckers

The utility of plumage characters for tribal-level diagnoses of woodpeckers has been an issue of frequent dispute (Bock, 1963; Cody, 1969; Goodge, 1972; Goodwin, 1968; Short, 1982). According to our phylogenetic results, many plumage characters in woodpeckers have undergone repeated instances of convergent gain or loss. For example, a nuchal crest has been independently gained or lost on several occasions as it is found within subgroups of Megapicini, Malarpicini, and variably within *Dryocopus*, *Picus*, *Campethera*, *Picoides*, and *Dendropicos*. Another character, dorsal patterning, varies from barred to solid in many genera and produces an inaccurate diagnosis for the paraphyletic genera of "*Colaptes*" (barred) and "*Piculus*" (solid).

While plumage characteristics can undoubtedly inform phylogenetic hypotheses, they appear to have little utility for determining phylogenetic relationships among woodpecker genera. In fact, few plumage characters uniquely delimit woodpecker clades. Rather, a small suite of plumage characters seems to have been repeatedly modified in each genus independently; a case similar to that seen in New World orioles of the genus *Icterus* (Omland and Lanyon, 2000). For example, while fine ventral barring can be used to diagnose the "*Veniliornis–Piculus* clade" (Short, 1982) the genera are not closely related and the character sporadically appears in many other genera.

The results of our molecular phylogenetic analyses and other studies (Webb, 2002; Weibel and Moore 2002a, 2002b) strongly suggest that several remarkable cases of plumage convergence have occurred. The most impressive examples involve the remarkable resemblance of *Dinopium javanense* to *Chrysocolaptes lucidus* and of *Dryocopus lineatus* to *Campephilus melanoleucos* and *C. guatemalensis*. None of these species-pairs are closely related. However, if *Chrysocolaptes* is considered to be an Asian *Campephilus* species and *Dinopium* an Asian *Dryocopus* species then the convergence between species of *Campephilus* (s.l.) and *Dryocopus* (s.l.) has occurred in both South America and Asia. Another instance of plumage convergence occurs with the similar appearing but distantly related species of *Picoides pubescens* and *P. villosus* (Weibel and Moore, 2002b). Yet other possible cases of plumage convergence are between *Hemicircus canente* and *Meiglyptes jugularis* and between *Celeus brachyurus* and *Blythipicus pyrrhotis*. The similarities of all these species-pairs are more likely due to convergence than the mere retention of ancestral plumage patterns because it is in sympatry that their plumages become most similar.

Cody (1969) proposed that in ecologically competitive species, plumage convergence could promote a spatial separation of their activities or, in the extreme case, promote a mutually beneficial interspecific territoriality. His model required that the species be territorial, or at least show intrasexual aggressive behavior, and predicted that the "similarities in appearance affect only those characteristics involved in visual or vocal aggressive displays… [and] these similarities are often less pronounced or even absent where the two species are allopatric. Sympatry is necessary for their maintenance." Citing several of the above-mentioned woodpecker species-pairs, he discussed how species with specialized-foraging modes and morphologies could compete for similar food resources and partition this limited niche via interspecific territoriality rather than habitat or character displacement. Notably, *Campephilus* species aggressively respond to recorded calls of *Dryocopus lineatus* in areas of sympatry (Moore pers. obs.; also see Kilman, 1972). In the case of woodpeckers, it is possible that they compete for suitable nest cavity substrate, which may be a limiting resource for all species.

Diamond (1982) developed a different model for plumage convergence in interspecifically aggressive ecological competitors. He noted that in sympatry the plumages of Old World orioles (genus *Oriolus*, family Oriolidae) repeatedly converge on those of friarbirds (genus *Philemon*, family Meliphagidae) and that the mimics tend to be spared from attack by the larger models. Notably, the very similar appearing species-pairs of *Dinopium–Chrysocolaptes*, *Dryocopus–Campephilus*, *Hemicircus–Meiglyptes*, and *Picoides pubescens–P. villosus* all have distinct size disparities.

The forces that have led to the evolution of the complex, variable plumages of woodpeckers are poorly understood and in need of more focused study. While their diverse plumages can be partly attributed to selection for cryptic-coloration and sexual recognition markings (Short, 1971), it may be that the variable plumages have more often been the result of selection for interspecific recognition markings (Cody, 1969; Diamond, 1982).

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