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Evolutionary history of woodpeckers and allies (Aves: Picidae): Placing key taxa on the phylogenetic tree

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Abstract

We analyzed 2995 base pairs of nucleotide sequence data (nuclear β-fibrinogen intron 7 and mitochondrial cytochrome *b* and ND2 genes), using parsimony and model-based approaches to infer phylogenetic relationships of the woodpeckers and allies, yielding novel hypotheses for several critical gaps in the knowledge of picid phylogeny. We tested the monophyly of sub-families within the Picidae, and sampled from widely distributed and diverse genera (*Celeus*, *Colaptes*, *Dryocopus*, *Melanerpes*, *Picoides*, *Picumnus*, *Sasia*, *Piculus*, and *Picus*). Relationships of three poorly known Southeast Asian genera (*Dinopium*, *Reinwardtipicus*, and *Blythipicus*) were also examined, revealing unexpected sister relationships. All phylogenetic approaches recovered largely congruent topologies, supporting a monophyletic Picinae and paraphyletic Picumninae, with the monotypic piculet, *Nesoctites micromegas*, as sister to the Picinae. We report paraphyly for *Celeus* and *Piculus*, whereas the broadly distributed genera *Picumnus* and *Dryocopus* were supported as monophyletic. Our phylogenetic results indicate a complex geographic history for the Picidae, with multiple disjunct sister lineages distributed between the New World and Asia. The relationships and geographic distribution of basal picid lineages indicates an Old World origin of the Picidae; however, the geographic origin of the Picinae remains equivocal, as the sister relationship between the Caribbean *N. micromegas* and the true woodpeckers presents the possibility of a New World origin for the Picinae.

Keywords: Woodpecker phylogeny; Bayesian analysis; Biogeography; Picidae

1. Introduction

The woodpeckers (Picidae: Picinae) represent approximately 183 species in 24 genera (Winkler and Christie, 2002), and are a well-defined clade diagnosable by numerous morphological synapomorphies, including protrusible, barbed tongue with elongated hyoid apparati; enlarged geniothyroid gland; thickened cranium; failure of the nasal gland to enter the orbit; and stiffened rectrices (Burt, 1930; Goodge, 1972; Webb and Moore, 2005). Historical taxonomic treatments, based largely on phenotypic and behavioral characters, have considered the piculets (Picidae: Picumninae; 31 species in 3 genera) (Winkler and Christie, 2002) as the sister clade to the Picinae, while the wrynecks (Picidae: Jynginae; 2 species in *Jynx*) have been placed basal to the Picinae + Picumninae (Goodge, 1972). This sub-familial arrangement has seen recent confirmation from molecular phylogenetic analyses (Webb and Moore, 2005).

The Picidae has a cosmopolitan distribution with centers of species richness in the Neotropics and Southeast Asia, however, the clade does not cross Wallace's Line, and is absent throughout the Australo-Papuan region. Ecologically specialized foraging strategies and the unique capacity to extract insects from woody substrates has enabled the woodpeckers and allies to inhabit a diversity of habitats and may also facilitate their capacity to maintain high levels of sympatry through resource partitioning.

The morphological and behavioral adaptations required for these specialized modes of foraging are diverse, as illustrated by body mass, which ranges from 8 g piculets (*Sasia*)

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to >500 g woodpeckers (*Mullerpicus*, *Campephilus*). Despite this high level of specialization, several genera (*Celeus*, *Colaptes*, *Dendrocopos*, *Dryocopus*, *Melanerpes*, *Picoides*, *Picumnus*, *Sasia*, and *Picus*) are broadly distributed, encompassing dissimilar habitats on multiple continents. This impressive morphological and geographic diversity (Short, 1982) makes the clade ideal for biogeographic investigation and analyses of morphological evolution and innovation.

Imperative to such studies is a robust phylogenetic framework by which to guide inferences regarding geography and morphology (Lanyon, 1993). While previous studies based on external morphology and to a more limited degree internal anatomy (Burt, 1930; Goodge, 1972; Goodwin, 1968; Short, 1982) proposed several disparate hypotheses of inter-generic picid relationships, recent molecular studies (DeFilippis and Moore, 2000; Prychitko and Moore, 2000; Tennant, 1991; Webb and Moore, 2005; Weibel and Moore, 2002; Weibel and Moore, 2002) have begun to clarify the situation, detecting instances of convergent evolution and unexpected sister-taxon relationships. Nonetheless, the taxonomic breadth of these molecular studies has been constrained by lack of modern specimen material, leaving critical gaps in the knowledge of picid phylogeny.

In this contribution, we add several important taxa to the picture of woodpecker phylogeny, and examine the origins of the Picinae through more complete sampling of the Picumninae. Based on tissue samples from specimens acquired on recent collecting expeditions, we examine the relationships of the enigmatic and monotypic 'piculet' *Nesoctites micromegas*, and test for monophyly in *Picumnus* and *Celeus* through inclusion of *P. innominatus* and *C. brachyurus*, each the only non-Neotropical member of its genus. Additional key genera examined in this study include *Dinopium*, *Reinwardtipicus*, and *Blythipicus*, all poorly-known Southeast Asian taxa. Our results also serve as an independent test of previously proposed phylogenetic relationships, through use of genetic markers distinct from those used in previous studies (Webb and Moore, 2005). The result is a more complete phylogenetic framework for the Picidae, revealing novel, well-supported phylogenetic hypotheses, integral to interpreting the biogeographic history, and evolution of morphological novelties associated with the diverse behaviors of woodpeckers.

2. Methods

2.1. Taxonomic sampling

We sampled 46 picid species, representing 24 of 28 currently recognized genera (Table 1). Multiple species were selected within key genera (*Colaptes*, *Celeus*, *Dendrocopos*, *Piculus*, *Picus*, *Picumnus*, and *Dryocopus*) to test their monophyly and examine patterns of intercontinental genetic divergence. All three picumnine genera were sampled densely, including complete representation of *Nesoctites* and *Sasia*, and 18 of the 27 species of *Picumnus* (detailed phylogenetic results and taxonomic treatment of *Picumnus* will be presented elsewhere). At least two samples per taxon were sequenced for all picumnine taxa presented in this study, to confirm that sequences accurately represent the taxon of interest. We sampled outgroup taxa from two closely related piciform families (Ramphastidae: *Pteroglossus bailloni* and *Meglaima franklinii*; Indicatoridae: *Indicator exilis*), thought to constitute the closest relatives of the Picidae (Moyle, 2004; Sibley and Ahlquist, 1990; Webb and Moore, 2005).

2.2. Sequencing

Genomic DNA was extracted from frozen or ethanol-preserved tissue samples using proteinase K digestion under manufacturer's protocols (DNeasy tissue kit, Qiagen). A total of 2995 base pairs, spanning the mitochondrial cytochrome *b* (cyt *b*; 1065 bp) and NADH dehydrogenase subunit-2 (ND2; 1041 bp) genes, as well as the complete nuclear intron 7 of the β-fibrinogen (βFibI7; 728–889 bp) gene (see Table 2 for primer sequences), were amplified via PCR in 25 μl reactions using Amersham PureTaq RTG PCR beads (Amersham Biosciences). Thermocycle conditions for cyt *b* and ND2 included an initial 3 min at 94 °C, followed by 35 cycles of 20 s at 94 °C, 15 s at 53 °C, and 60 s at 72 °C, followed by a 7 min final extension at 72 °C. The βFibI7 thermocycle conditions differed from the above protocol only in having a lower annealing temperature of 50 °C.

All resulting double-stranded PCR products were purified with AMPure (Agencourt) magnetic bead purification, and visualized on a 1% agarose gel stained with ethidium bromide. Purified PCR products were cycle-sequenced with ABI Prism BigDye v3.1 terminator chemistry using the same primers as above. Two additional cycle sequencing primers were designed for cyt *b* (Table 2) to ensure that the 5′ and 3′ ends of the fragment were captured with accurate base calls. An internal heavy-strand primer was designed for ND2 (Table 2) to overcome the shorter read length experienced with the H6313 primer. Cycle sequencing products were purified using CleanSEQ (Agencourt) magnetic bead purification, and analyzed on an ABI Prism 3100 automated DNA sequencer (Applied Biosystems).

2.3. Phylogenetic analysis

Chromatograms of complimentary strands were reconciled in Sequencher 4.1 (Gencodes) and all sequence alignments were performed in Clustal X using default settings for gap opening and extension costs. Gaps resulting from indels in βFibI7 were scrutinized and corrected by eye.

We performed an incongruence length difference test (Farris et al., 1994) implemented in PAUP 4.0b10 (Swofford, 2000) by running 100 replicates of the partition homogeneity test, to test for potential incongruence in phylogenetic signal between genes, as these genes are known to exhibit distinct patterns and rates of nucleotide substitution (Prychitko and Moore, 2000; Sheldon et al., 2005). Pairwise distances were plotted for each gene to access possible

Table 1
Summary of taxa included in this study

Species	Country of origin	Source	Voucher #	Cyt *b* GenBank	ND2 GenBank	βFibI7 GenBank
Ingroup						
Jynx torquilla[a,b]	Myanmar	USNM	B05655	AY940803	DQ479151	AY082400
Sasia africana	Equatorial Guinea	KUNHM	8530	DQ479249	DQ479159	DQ479232
Sasia abnormis	Malaysia	LSUMNS	B36380	DQ479250	DQ479158	DQ479235
Sasia ochracea	Myanmar	USNM	B06057	DQ479252	DQ479160	DQ479233
Sasia ochracea	China	KUNHM	9952	DQ479251	DQ479161	DQ479234
Picumnus aurifrons	Brazil	USNM	B06874	DQ479244	DQ479152	DQ479236
Picumnus cirratus	Paraguay	KUNHM	2878	DQ479243	DQ479153	DQ479237
Picumnus exilis	Brazil	FMNH	399173	DQ479245	DQ479154	DQ479238
Picumnus spilogaster	Guyana	KUNHM	5851	DQ479248	DQ479157	DQ479239
Picumnus nebulosus	Uruguay	YPM	100972	DQ479247	DQ479156	DQ479240
Picumnus innominatus	China	KUNHM	6669	DQ479246	DQ479155	DQ479241
Nesoctites micromegas	Dominican Republic	KUNHM	8143	DQ479253	DQ479162	DQ479231
Nesoctites micromegas	Dominican Republic	KUNHM	8015	DQ479254	DQ479163	DQ479230
Melanerpes striatus[a]	Dominican Republic	KUNHM	8014	AF441652	DQ479165	DQ479216
Melanerpes aurifrons	Mexico	KUNHM	585	DQ479255	DQ479164	DQ479214
Sphyrapicus nuchalis	United States	KUNHM	2335	DQ479256	DQ479166	DQ479225
Campethera nivosa	Equatorial Guinea	KUNHM	8570	DQ479257	DQ479167	DQ479203
Campeth era cailliautii[a]	Equatorial Guinea	KUNHM	8634	AY940794	DQ479168	DQ479199
Dendrocopus hyperythrus	China	KUNHM	9939	DQ479258	DQ479169	DQ479207
Dendrocopus major	Italy	KUNHM	4496	DQ479259	DQ479170	DQ479210
Picoides pubescens	United States	KUNHM	7425	DQ479260	DQ479171	DQ479220
Dendropicos goertae	Ghana	LSUMNS	B39322	DQ479261	DQ479172	DQ479206
Celeus loricatus	Ecuador	LSUMNS	B11832	DQ479262	DQ479173	DQ479229
Celeus flavescens	Paraguay	KUNHM	304	DQ479263	DQ479174	DQ479228
Celeus brachyurus	Myanmar	USNM	B05706	DQ479264	DQ479175	DQ479198
Colaptes auratus[a,b]	United States	KUNHM	2534	AF441649	DQ479176	AY082398
Colaptes melanochloros	Paraguay	KUNHM	3418	DQ479265	DQ479177	DQ479202
Colaptes punctigula	Peru	KUNHM	963	DQ479266	DQ479178	DQ479204
Veniliornis fumigatus[a]	Mexico	KUNHM	1938	AY927217	DQ479179	DQ479226
Veniliornis kirkii[a]	Guyana	KUNHM	4042	AY927218	DQ479180	DQ479227
Piculus chrysochloros	Paraguay	KUNHM	2966	DQ479267	DQ479181	DQ479218
Piculus rubiginosus	Guyana	KUNHM	3926	DQ479268	DQ479182	DQ479221
Geocolaptes olivaceous[a]	South Africa	UWBM	71156	AY940801	DQ479148	DQ479212
Campephilus rubricollis	Guyana	KUNHM	1372	NS	DQ479184	DQ479205
Campephilus guatemalensis	Mexico	KUNHM	2017	DQ479269	DQ479183	DQ479200
Dryocopus lineatus[b]	Peru	KUNHM	799	DQ479270	DQ479186	AF240012
Dryocopus pileatus[a]	United States	KUNHM	6629	AY942885	DQ479187	DQ479211
Dryocopus martius	Austria	KUNHM	4539	NS	DQ479185	DQ479208
Picus canus	Russia	UWBM	74935	AY940808	DQ479145	DQ479222
Picus viridis	Russia	UWBM	61413	AY701056	DQ479146	DQ479223
Picus mentalis	Malaysia	LSUMNS	B36478	AY279265	DQ479188	DQ479219
Blythipicus pyrrhotis	China	KUNHM	B6759	DQ479271	DQ479189	DQ479196
Blythipicus rubiginosus	Malaysia	LSUMNS	B36283	NS	DQ479190	DQ479197
Reinwardtipicus validus	Malaysia	LSUMNS	B38653	DQ479272	DQ479191	DQ479224
Chrysocolaptes lucidus[a]	Philippines	USNM	B3704	AY940797	DQ479192	DQ479201
Dinopium shorii	Myanmar	USNM	B3291	DQ479273	DQ479193	DQ479209
Meiglyptes tristis	Malaysia	LSUMNS	B36356	DQ479274	DQ479194	DQ479217
Mulleripicus funebris[a]	Philippines	USNM	B3804	AY940805	DQ479195	DQ479215
Outgroup						
Indicator exilis	Equatorial Guinea	KUNHM	8503	DQ479242	DQ479150	DQ479213
Pteroglossus bailloni[a,b]	Paraguay	KUNHM	227	AY279305	DQ479149	AY279262
Megalaima franklinii[a,b]	China	KUNHM	10413	AY279269	DQ479147	AY279225

Tissue sources: KUNHM, University of Kansas Natural History Museum and Biodiversity Research Center; LSUMNS, Louisiana State University Museum of Natural Science; USNM; United States National Museum of Natural History; UWBM, University of Washington Burke Museum.
NS = No sequence obtained.
[a] Cyt *b* sequence from Genbank.
[b] βFib7 sequence from Genbank.

saturation, while rate heterogeneity across lineages was tested using a likelihood ratio test (LRT), to examine the difference in likelihood scores for a ML topology with and without a molecular clock enforced. Twice the difference in log likelihood value was compared to a χ^2 distribution with $n-2$ degrees of freedom, where n = number of taxa.

Table 2
Summary of primers used in this study

Gene	Primer	Sequence
Cytochrome *b*	L14841[a]	5′-GCTTCCATCCAACATCTCAGCATGATG-3′
	H16065[b]	5′-GGAGTCTTCAGTCTCTGGTTTACAAGAC-3′
	Cyt*b*_WpIN_L[c]	5′-CTTCACCTTCCTCCACGAATCAGGCTC-3′
	Cyt*b*_WpIN_H[c]	5′-CCTGATTCGTGGAGGAAGGTGAAGTGGATT-3′
ND2	L5216[d]	5′-GGCCCATACCCCGRAAATG-3′
	H6313[d]	5′-CTCTTATTTAAGGCTTTGAAGGC-3′
	ND2INH-WP[c]	5′-GCTAGGGAGAGGAGTGTGAGTAT-3′
B-fibrinogen intron 7	FIB-BI7L[e]	5′-TCCCCAGTAGTATCTGCCATTAGGGTT-3′
	FIB-BI7U[e]	5′-GGAGAAAACAGGACAATGACAATTCAC-3′

[a] Kocher et al. (1989).
[b] Helm-Bychowski and Cracraft (1993).
[c] Designed by B.W. Benz for this study.
[d] Johnson and Sorenson (1998).
[e] Prychitko and Moore (1997).

Bayesian analyses were performed using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003), with a flat Dirichlet distribution for estimation of nucleotide substitution and base frequencies, and a default flat prior distribution for all other parameter estimation. The concatenated data set was partitioned by gene and codon position to optimize substitution model specificity, and parameters of data partitions were permitted to vary independently by unlinking partitions. All analyses were run for 2.0×10^6 generations with a random starting tree, and four Markov chains under default heating values, sampling every 100 generations, resulting in a total of 2.0×10^4 samples. Stationarity of the MCMC analyses was determined by plotting −lnL against generation time, and the "burn-in" trees sampled prior to stationarity were discarded. Multiple analyses were run for each partitioning strategy to avoid reaching misleading stationarity on local optima.

We conducted maximum parsimony (MP) and maximum likelihood (ML) analyses in PAUP 4.0b10 (Swofford, 2000) to further explore the data set and examine the robustness of the Bayesian results. Heuristic searches were used in all analyses, with 1000 random addition replicates and tree-bisection-reconnection (TBR) branch-swapping. Random addition replicates were reduced to 100 for ML analyses, and the number of rearrangements per replicate were restricted to 5000. In MP analyses, gaps resulting from indels in βFibI7 sequences were treated as single evolutionary events and coded as a fifth character state. Multiple MP analyses were run to explore the effect of excluding third position transition substitutions for cyt *b*, as this gene showed significant saturation at third position transitions. We used Modeltest 3.7 (Posada and Crandall, 1998) to determine the best-fit model of evolution and parameter estimates for the ML analysis via a hierarchical likelihood ratio test (hLRT) and Akaike Information Criterion (AIC). The General Time Reversible model of nucleotide substitution with a gamma distribution and invariable sites (GTR + I + G) was identified as the best fit model by the hLRT, while AIC selected the TVM + I + G model. Both models were implemented in separate ML analyses, however, we present the GTR + I + G ML results, as the two models returned nearly identical results.

Node support was determined via non-parametric bootstrap resampling, using 1000 random addition replicates and TBR branch-swapping for MP; bootstrap replicates were reduced to 100 for ML analyses. Support for alternative topologies within the Picumninae was tested using Shimodaira–Hasegawa (SH) tests to examine differences in −lnL values for *Sasia* + *Picumnus* vs. *Sasia* and *Picumnus* as independent lineages (Shimodaira and Hasegawa, 1999). A SH test was also performed to examine the effect of constraining *Nesoctites* within the Picumninae, as opposed to letting it form an independent lineage sister to the Picinae.

3. Results

3.1. Sequence attributes

The concatenated sequence alignment resulted in a data matrix of 3077 nucleotide characters for the 46 ingroup and 3 outgroup taxa (Table 3). In all, 1490 sites were variable, of which 1112 (74.6%) were phylogenetically informative. Aligned ND2 and cyt *b* sequences appeared to be of mitochondrial origin, rather than nuclear copies, as base composition across taxa was homogeneous, codon positions exhibited expected substitution rates, and overlapping fragments did not conflict. Nucleotide frequencies for cyt *b* and ND2 were cytosine-rich, but consistent with frequencies previously published for these genes in birds, as were relative percentages of informative sites per codon position (Sheldon et al., 2005; Webb and Moore, 2005). Base composition of βFibI7 was highly A–T rich, exhibiting approximately a 2:1 bias over G–C; again, this pattern corresponds closely to previously reported nucleotide frequencies for this gene in picids, and is expected given the non-random direction of mutation characteristic of non-coding gene regions (Li and Graur, 1991; Prychitko and Moore, 2000). The aligned βFibI7 sequences contained 22 autapomorphic indels, the largest of which include a 140 bp deletion in

Table 3
Attributes of sequence variation in three genes across the Picidae

Gene	Total sites	Variable sites	Informative sites	Variable sites by codon (Informative)			Nucleotide frequencies			
				1st %	2nd %	3rd %	%A	%C	%G	%T
Cytochrome *b*	1065	508 (47.7%)	427 (40.1%)	32.1 (22.8)	13.2 (5.6)	97.7 (91.8)	25.2	36.1	12.9	25.8
ND2	1041	618 (59.4%)	517 (49.7%)	50.7 (36.6)	28.8 (18.1)	98.6 (94.2)	29.7	38.2	8.9	23.3
β Fibrinogen 7	816[a] (935[b])	364 (38.8%)	168 (17.9%)				31.0	17.3	18.0	33.7

[a] Average length.
[b] Aligned length including indels.

Reinwardtipicus validus, and a 93 bp deletion in *Sasia africana*. Although βFibI7 sequences also contained a number of smaller indels, they were typically non-overlapping, allowing for unambiguous sequence alignment when adjusted by eye. With the exception of a 35 bp deletion shared between *Geocolaptes* and *Picus canus* + *P. viridis*, and a 6 bp deletion shared between the Malarpicini and *Melanerpes aurifrons*, the 24 synapomorhic indels were congruent with our phylogenetic results.

Pairwise distances among ingroup and outgroup taxa varied over an order of magnitude. Uncorrected cyt *b* ingroup divergences ranged from 2.7% (*Picumnus spilogaster* to *P. cirratus*) to 21.3% (*Picumnus innominatus* to *Melanerpes striatus*), with an average sequence divergence of 14.4%. Uncorrected ND2 divergences among ingroup taxa ranged from 2.3% (*Picumnus spilogaster* to *P. cirratus*) to 22.5% (*Jynx torquilla* to *Colaptes punctigula*), while divergence across taxa averaged 17%. Uncorrected βFibI7 divergence ranged from 0.1% (*Sasia abnormis* to *S. ochracea*) to 10.1% (*Picumnus innominatus* to *Nesoctites micromegas*), averaging 6% across taxa. Although ND2 demonstrates overall higher rates of evolution, cyt *b* exhibited the greatest saturation, particularly in third position transitions (not shown), as has been observed and documented previously (Webb and Moore, 2005). Excluding third-position cyt *b* transitions had a negligible effect on node support, so the final MP analysis was run on the complete data set.

3.2. Phylogentic analysis

A partition homogeneity test was unable to reject congruence ($P = 0.55$) in phylogenetic signal among cyt *b*, ND2, and βFibI7, thus all analyses were performed on the combined 3-gene data set. We detected rate heterogeneity across lineages, as indicated by highly significant results ($P = 0.001$) of a Likelihood Ratio Test, invalidating assumptions of a molecular clock or clocklike evolution. The three phylogenetic methods used to explore the concatenated data set all converged on similar phylogenetic results, differing only at a few weakly supported nodes; hence for simplicity, we present the Bayesian and ML results.

A seven-partition Bayesian analysis resulted in a phylogenetic hypothesis of the Picidae that is supported with posterior probabilities of $\geqslant 95\%$ for all but 8 nodes (Fig. 1). In all analyses, monophyly of the Picinae was recovered with strong support; however, the Picumninae was consistently found to be paraphyletic, with *Nesoctites micromegas* placed as sister to the Picinae. A SH test confirmed that a monophyletic Picumninae (*Sasia*, *Picumnus*, and *Nesoctites*) was a significantly ($P = 0.0033$) worse explanation of the data as opposed to *Nesoctites* constituting an independent lineage sister to the Picinae. The wryneck *Jynx torquilla* was placed as sister to the Picumninae + Picinae clade, while the honeyguide, *Indicator exilis*, was strongly supported as the closest relative of the Picidae.

The monophyly of *Picumnus* was strongly supported in all analyses, with the Asian *P. innominatus* placed as sister to the Neotropical *Picumnus* radiation. Strong support was not recovered for the short node uniting *Sasia* and *Picumnus* (93% posterior probability), and SH tests confirmed this weak support, as *Sasia* + *Picumnus* was not a significantly better solution to the data, as compared with an arrangement in which they constitute separate lineages paraphyletic to the remainder of the woodpeckers ($P = 0.64$).

Within the Picinae, three clades were consistently recovered, corresponding to the Dendropicini, Megapicini, and Malarpicini clades proposed by Webb and Moore (2005). The Dendropicini ((*Melanerpes*, *Sphyrapicus*)(*Dendrocopos* (*Picoides*, *Veniliornis*))) was recovered with 100% posterior probability, although monophyly of *Dendropicos* was not supported, as it formed a polytomy within the (*Dendrocopos* (*Picoides*, *Veniliornis*)) clade. Sister to Dendropicini is the Megapicini (*Blythipicus*(*Campephilus* (*Chrysocolaptes, Reinwardtipicus*))) and the Malarpicini. The latter includes a diverse assemblage (*Dryocopus*, *Mullerpicus*, *Piculus*, *Colaptes*, *Celeus*, *Picus*, *Meiglyptes*, *Dinopium*, *Campethera*, and *Geocolaptes*) in several sub-clades. The relationship of the Megapicini to the Malarpicini was not strongly supported (73%), nor was the node linking *Campephilus* with *Chrysocolaptes* + *Reinwardtipicus* (63%).

We recovered strong support for an African *Campethera* + *Geocolaptes* clade as well as an Asian (*Dinopium* (*Meiglyptes*, *Celeus brachyurus*)) clade, but the relationship of this Southeast Asian lineage within the Malarpicini was unclear. A monophyletic *Picus* was recovered with strong support in all analyses. The remaining groups include a strongly supported *Mulleripicus* + *Dryocopus* as sister to (*Celeus*(*Colaptes*, *Piculus*)). We confirmed paraphyly for *Piculus* as noted by Webb and Moore (2005) and polyphyly for *Celeus*, with significant posterior probability.

While the results of the ML analysis were largely congruent with respect to topology and posterior probabilities recovered in the Bayesian analysis, three notable differences were recovered for nodes that were supported by significant

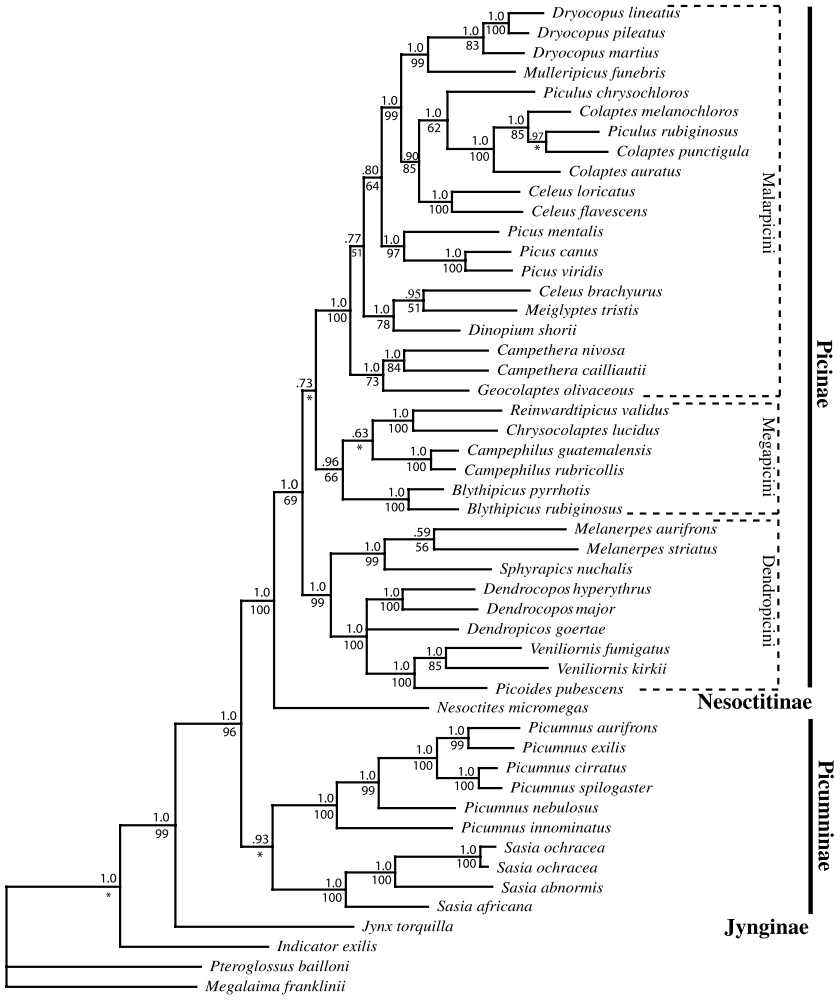

Fig. 1. Phylogenetic results depicting the evolutionary history of the woodpeckers and allies, as inferred from a seven-partition Bayesian analysis using the GTR + I + G model. Numbers above each node represent posterior probabilities, while Maximum Likelihood bootstrap values are indicated below each node, with * indicating less than 50% bootstrap support.

posterior probabilities. The sister relationship of the Asian *Meiglyptes* + *Celeus brachyurus* was recovered with 51% bootstrap support, while the basal arrangement of *Dinopium* sister to *Meiglyptes* + *Celeus brachyurus* received moderate bootstrap support of 78% (Fig. 1). The (*Colaptes melanochloros* (*Piculus rubiginosus*, *Colaptes punctigula*)) clade was also strongly supported in the Bayesian analysis, but was recovered as an unresolved polytomy in the ML analysis. Lastly, the basal position of *Indicator exilis* was unresolved, and formed a polytomy with a *Pteroglossus bailloni* + *Megalaima franklinii* arrangement that received 100% posterior probability; however, two synapomorphic indels shared between *I. exilis* and the Picidae support the Bayesian topology.

4. Discussion

4.1. Congruence in gene signal

Despite the disparity in patterns and rates of nucleotide evolution across the three genes used in this study, results of our partitioned Bayesian analysis were consistent with Webb and Moore (2005) at all nodes for which they reported significant posterior probabilities, however, our results differed significantly regarding the relationships of *Picus*, *Campethera*, *Geocolaptes*, and *Meiglyptes*. The 12S, CO1, and cyt *b* combined analysis from Webb and Moore recovered a weakly supported ((*Picus miniaceus*, *Meiglyptes*)(*Picus canus*, *Campethera*)) clade as sister to *Geocolaptes*. Conversely, our analyses indicate a strongly supported African clade of *Geocolaptes* + *Campethera*, as well as a monophyletic *Picus* clade (*Picus mentalis* (*P. canus*, *P. viridis*)). The unexpected placement of *Meiglyptes* as sister to "*Celeus*" *brachyurus* also differs from the results of Webb and Moore; however, given the morphological similarity of *C. brachyurus* to *M. tukki*, these species likely form a natural group.

4.2. Phylogeny and taxonomic implications

With the addition of 5 genera (*Dinopium*, *Reinwardtipicus*, *Blythipicus*, *Dendrocopos*, and *Nesoctites*) and several key species (*Celeus brachyurus*, *Picumnus innominatus*,

Dryocopus martius, and *Piculus chrysochloros*), our analyses yield a more complete picture of generic-level relationships within the Picidae, raising several issues of taxonomic importance and further illustrating paraphyly in 5 of the 6 tribes proposed by Short (1982).

The phylogenetic position of the Hispaniolan endemic *Nesoctites micromegas*, has long been recognized as enigmatic, as it shares several primitive characters with the piculets, but also exhibits derived features common to the Picinae (Goodge, 1972). Winkler and Christie (2002) suggested that it be placed in its own tribe, Nesoctitini, within the Picumninae. Our results unequivocally indicate that *N. micromegas* has been misallocated at the sub-familial level, which is further supported by a significant SH test, rejecting inclusion of *Nesoctites* within the Picuminae. Although its exclusion from the Picumninae is requisite, it is unclear whether *Nesoctites* should be treated as the basal member of the Picinae, or as a monotypic sub-family.

Nesoctites shares with the Picinae the derived feature of the nasal gland failing to enter the orbit (Goodge, 1972); however, it lacks the stiffened rectrices common to all picids, exhibits atypical foraging behavior, and engages in antiphonal duetting (Short, 1974), which is unique in the Picidae. Considering these features, as well as the lack of highly developed specialization for insect excavation in hard substrates (as in the Picinae), we tentatively advocate erecting a monotypic sub-family Nesoctitinae for this taxon.

Previously treated in two separate tribes (Short, 1982), the Dendropicini is composed of approximately 90 species distributed between four primary lineages, the New World Melanerpines and *Picoides* + *Veniliornis* clade, and the Old World genera *Dendrocopos* (Winkler and Christie, 2002) and *Dendropicos*, with the latter restricted to Africa. Although we sampled phenotypically and behaviorally distinct congeners within *Melanerpes* and *Dendrocopos*, these speciose genera require additional sampling, as we did not recover significant support for the monophyly of *Melanerpes*, and relationships within *Dendrocopos* may be more complex than previously thought, which is evidenced by a recent phylogenetic analysis suggesting that the monotypic genus *Sapheopipo* may be embedded within *Dendrocopos* (Winkler et al., 2005).

The Megapicini includes a morphologically diverse assemblage of 18 species (likely including *Hemicircus*) that were placed in three separate tribes under Short's (1982) taxonomy. Although we did not recover significant posterior probability or bootstrap support for a sister relationship between *Campephilus* and *Reinwardtipicus* + *Chrysocolaptes* (as in Webb and Moore, 2005), significant support for inclusion of *Campephilus* within the Megapicini does confirm the polyphyly of Short's arrangement. Furthermore, this phylogenetic arrangement is corroborated by several morphological characters (Webb and Moore, 2005). The phylogenetic position of *Reinwardtipicus* as sister to *Chrysocolaptes* is not unexpected given their morphological similarity and close geographic proximity. Although the two species of *Blythipicus* are phenotypically and behaviorally distinct from the other megapicines, their placement within the Megapicini is supported by significant posterior probability, as well an osteological data set from Webb (2002) and myological analyses in Goodge (1972). The Southeast Asian *Hemicircus* has yet to be included in a molecular phylogenetic study, however, Goodge (1972) demonstrated anatomical support for its placement in the Megapicini.

The remaining woodpecker diversity includes approximately 77 species distributed among several sub-clades comprising the Malarpicini. Inclusion of several Malarpicini taxa not examined in previous analyses resulted in two novel hypotheses. The Old World "*Celeus*" *brachyurus* is not placed with its New World congeners, but rather as sister to the Southeast Asian *Meiglyptes*; surprisingly, these two are placed as sister to *Dinopium*. Prior to Short (1982), "*C. brachyurus*" was placed in the monotypic genus *Micropternus*. Based on our molecular data and partial anatomical dissections by Goodge (1972), who concluded that *Micropternus* was related to*Meiglyptes*, *C. brachyurus* should be returned to *Micropternus*.

In contrast to Webb and Moore's (2005) results, we recovered strong support for monophyly of the widely distributed and phenotypically diverse *Picus*. This result was further supported by two indels spanning14 and 94 base-pair deletions in the βFibI7 sequences. Nonetheless, additional taxon sampling across the morphological and geographic diversity of this genus will be required to confirm its monophyly.

Finally, as noted by Webb and Moore (2005), we confirmed paraphyly in *Colaptes* and *Piculus* through inclusion of additional taxa including the type species of *Piculus* (*P. chrysochloros*). Consequently, *Piculus* should be defined more narrowly to include only *P. chrysochloros*, *P. leucolaemus*, *P. flavigula*, and *P. aurulentus*; given their likely close relationships with taxa studied, *P. simplex*, *P. callopterus*, and *P. litae* would fall into this group. The remainder of *Piculus*, including *P. rubiginosus* and *P. rivolii*, and likely *P. auricularis* (given its close association with *P. rubiginosus*), should be reallocated to *Colaptes*.

4.3. Evolution of morphology and behavior

The diverse suite of evolutionary novelties enabling woodpeckers' unique foraging mode have undoubtedly played an integral role in their diversification and success as a near-global clade of birds. Although additional taxon sampling and associated detailed morphological analyses will be required to examine the evolutionary sequence and origin of these characters, several generalities are now apparent from our results. The phylogenetic position of *Nesoctites* suggests that the earliest woodpeckers were likely relatively small in size, lacked stiffened rectrices, and were strictly arboreal. If extant piculets are representative of the ancestral form, excavating holes and communication via drumming evolved basally to the Picinae + Picumninae, as these behaviors are not present in

Jynx (Winkler and Christie, 2002). Two independent evolutionary origins of large size are evidenced by the distant relationships of *Mulleripicus* + *Dryocopus* and *Campephilus*. Although several genera exhibit occasional terrestrial foraging habits (e.g., *Picus*, *Colaptes*, and *Campethera*), strictly terrestrial foraging and nesting habits has evolved only twice-in the monotypic African *Geocolpates* and in the South American *Colaptes rupicola* (Winkler and Christie, 2002). The closely related *C. campestris* and *C. pittius* also forage almost exclusively on the ground, but generally exhibit terrestrial nesting only when appropriate trees are not available. Examples of plumage convergence among the woodpeckers are numerous, the most impressive of which we confirmed with placement of *Dinopium shorii* as sister to *Meiglyptes* + "*Celeus*" *brachyurus*, whereas the nearly identically plumaged *Chrysocolaptes lucidus* is positioned distantly in the Megapicini.

Previous systematic treatments relied heavily upon plumage and behavioral characters, but generally avoided anatomical data sets, as it was assumed these characters were under strong selection and would likely be misleading (Short, 1982). Conversely, our phylogenetic hypothesis closely corroborates the anatomical based groupings proposed by Goodge (1972), and it is now evident that foraging behaviour is fairly labile across genera and in some cases, among closely related congenerics. While several hypotheses have been put forth regarding the potential forces driving plumage convergence and behavioral lability among picids, none have yet been tested (Styring and bin Hussin, 2004; Weibel and Moore, 2005).

4.4. Biogeography

The phylogenetic hypotheses developed herein provide a rich basis for examining the historical biogeography of a near-global clade of birds. Although the resulting picture is complex, encompassing several chronologically distinct events, the Laurasian geographic patterns shared among three separate clades suggest a common historical route of continental interchange. This section, then, presents a preliminary geographic exploration of picid phylogenetic history.

In his 1982 monograph, Short hypothesized a New World origin for the Picidae, based on the observation that the Neotropics are rich in woodpecker diversity, especially the basal picumnine piculets. In contrast, our data indicate an Old World origin for the Picidae; however, given the broad Afro-Asian distribution of the honeyguides (*Indicator*) and wrynecks (*Jynx*), it remains unclear whether the Picidae arose in Africa or Asia. What has become evident is the possibility of an Asian origin of *Picumnus*, and its subsequent dispersal to and radiation in the Neotropics (Fig. 2A). Marginal support (93% posterior probability) uniting *Sasia* and *Picumnus* does not, however, entirely eliminate the possibility of an alternative scenario, as the results of a SH test could not reject *Sasia* and *Picumnus* as independent lineages in favor of the topology presented in Fig. 1.

Although *P. innominatus* bears the characteristic black-white lateral tail pattern and several morphological characters shared by the Neotropical piculets, monophyly of *Picumnus* is unexpected, given that these small picids do not exhibit the capacity for strong flight required in long distance dispersal, and historical evidence of a temperate North American piculet has not been found. Even more intriguing is the basal position of *P. nebulosus* within the Neotropical *Picumnus*, as this species is restricted to a small area in Southeastern Brazil and Uruguay; what might be a more expected sister taxon, *P. olivaceous* of Central America (data not shown), is actually embedded within the *P. aurifrons* and *P. exilis* clade. Genetic divergence between *P. innominatus* and *P. nebulosus* is relatively shallow (12.9%; ND2 uncorrected *p*-distance) suggesting a recent split that would have required considerable dispersal, possibly via a Beringian land bridge event.

The historical connection between Asian and New World clades is further reflected by relationships within the Megapicini (Fig. 2B), and the *Dryocopus* + *Mulleripicus* clade (Fig. 2C), both of which exhibit relatively shallow genetic divergences between their respective Asia and New World component taxa. The Neotropical *D. lineatus* is separated from its temperate sister taxon, *D. pileatus*, by 6.6% (ND2 uncorrected *p*-distance) sequence divergence, indicating a more recent split in comparison to 10.0% sequence divergence between *D. pileatus* and *D. martius* (widely distributed across much of temperate Europe and Asia). The placement of *Blythipicus* basal to *Campephilus* + *Chrysocolaptes* also illustrates this pattern, with 6.4% sequence divergence separating the Neotropical *Campephilus guatemalensis* and *C. rubricollis*, whereas approximately 12% sequence divergence separates *Chrysocolaptes* from *Campephilus*, and 14.7% separating *Blythipicus* from *Chrysocolaptes*.

The unexpected, but well-supported, position of the Hispaniolan 'piculet' *Nesoctites* as sister to the Picinae has potentially critical implications for interpreting the biogeographic origins of the Picinae. A contour feather preserved in amber provides fossil evidence of the presence of this lineage on Hispaniola from at least 25 million years ago, with older estimates ranging to the Eocene at ~55 million years ago (Laybourne et al., 1994). Although no fossil evidence of a piculet has been reported from the New World, *Nesoctites* may represent a relictual lineage that was historically widespread, but has managed to persist only in the Caribbean. Regardless, a sister-group relationship of *Nesoctites* and the Picinae presents evidence for the possibility of a New World origin of the Picinae.

The allure of dating historical speciation events from molecular phylogenies has resulted in the now-customary practice of implementing a 'molecular clock' to translate sequence divergences into specific dates for splitting events (Klicka and Zink, 1997; Weir and Schluter, 2004; Zink et al., 1995). However, given known variation in avian molecular evolutionary rates (Lovette, 2004), lack of precise calibration points for the piciforms, and rate heteroge-

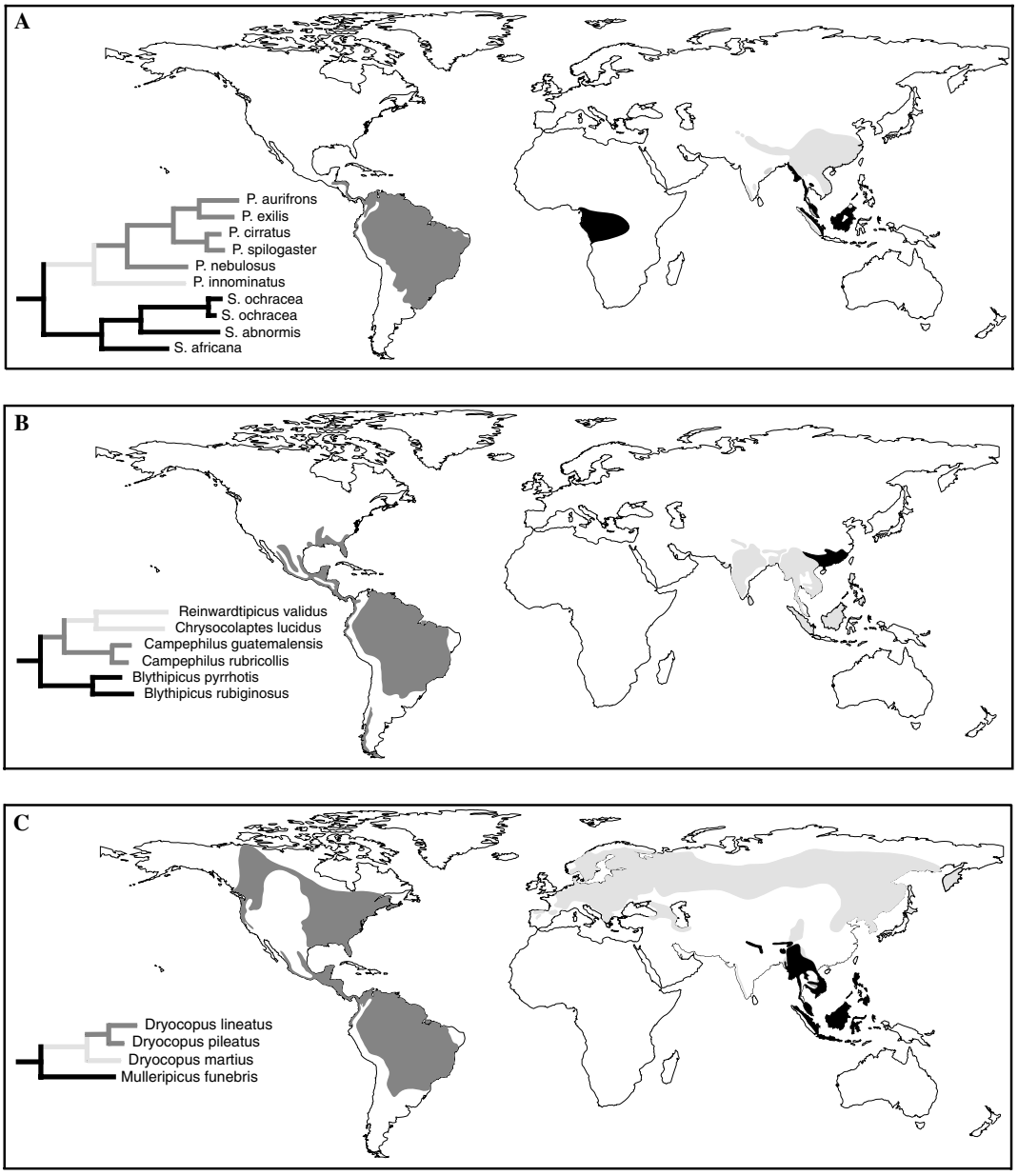

Fig. 2. Distributions of three picid clades that exhibit Southeast Asian—New World sister relationships. (A) *Picumnus* + *Sasia*; (B) *Reinwardtipicus*, *Chrysocolaptes*, *Campephilus*, and *Blythipicus*; (C) *Dryocopus* + *Mulleripicus*. Shaded regions depict generic level distributions, some of which overlap in Southeast Asia.

neity observed in our molecular data, we avoid the temptation of dating splitting events precisely based on molecular divergence calibrations. We do note, however, that many of the sequence divergences at the base of the Asia-America splits noted above are not particularly substantial, suggesting that these divergences are not extremely old (i.e., for Gondwanan splits to be a useful explanation).

Seeking a more recent explanation for an Asian-American connection, we note the relatively northerly extent of many Asian tropical/subtropical bird species' ranges, in many cases reaching as far north as Beijing. Considering the somewhat warmer climate that apparently occurred in the Miocene-Pliocene (Billups and Schrag, 2002), subtropical and temperate Asian species may have been able to disperse eastward into North America. Similar Laurasian patterns are evident in several plant groups (Donoghue et al., 2001) as well as a number of mammalian clades, most notably, the series of intercontinental migrations that apparently took place among the Felidae in the late Miocene (Johnson et al., 2006). If this scenario is reasonable, in some cases, we may see traces left behind, such as the broadly distributed *D. pileatus*; in others, most notably *Picumnus*, the hypothetical North American populations that would have had to exist are not witnessed by any evidence whatsoever.

Finally, within the woodpecker radiation, several subradiations are endemic to particular continents. For example, *Piculus* + *Colaptes* is restricted to the Americas,

"*Celeus*" *brachyurus* + *Dinopium* + *Meiglyptes* to Asia, and *Campethera* + *Geocolaptes* to Africa. These clades clearly indicate *in situ* diversification within continents.

5. Conclusions

This contribution adds several key taxa to the emerging picture of picid phylogenetic relationships. In particular, addition of the Asian "representatives" of *Celeus* and *Picumnus* shows the former to be a member of an independent clade, but the latter indeed to constitute the sister lineage to the New World *Picumnus*. Perhaps most importantly, we place the enigmatic "piculet" *Nesoctites* on the tree, as sister to the Picinae; this result both changes the interpretation of body size evolution in the group, and presents some serious challenges for interpreting the biogeographic history of the woodpeckers. In comparison with the closely related barbets that exhibit continental patterns of in situ diversification, the geographic history of the woodpeckers and allies is considerably more complex, and will require additional taxon sampling to elucidate fully these historical patterns.

Three woodpecker genera (*Xiphidiopicus*, *Hemicircus*, and *Gecinulus*) remain unrepresented in molecular studies. Their addition to the tree could change some of the interpretations presented herein, although likely not changing overall patterns. More detail is needed within *Dendrocopos*, *Picus*, *Piculus*, and *Melanerpes*, as each represents a diverse and possibly historically heterogeneous assemblage. Also needed in several cases are species-level phylogenetic studies and phylogeographic analyses to elucidate finer-scale patterns within the group.

Given the previous well-supported picid phylogeny (Webb and Moore, 2005), the additions to knowledge that this study provides make a strong statement for the importance of continued general collecting of ornithological specimens on a global scale. New specimen material, rich in information both in the sense of actual data content (Peterson et al., 2005; Soberón, 1999) and in materials actually preserved, is critical in keeping the 'library of life' that are world systematics collections current, useful, and serving as a basis for future innovative studies (Winker, 1996). That these same specimen materials can be used in studies of plumage variation, morphology, parasite evolution, and molecular systematics, suggests that partial documentation (e.g., photos, blood, or feather samples) are simply insufficient as 'new' ornithological specimens.

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References

Billups, K., Schrag, D.P., 2002. Paleotemperatures and ice volume of the past 27 myr revisited with paired Mg/Ca and $^{18}O/^{16}O$ measurements on benthic foraminifera. Paleoceanography 17, 1–11.

Burt, W.H., 1930. Adaptive modifications in the woodpeckers. Univ. Calif. Publ. Zool. 32, 455–524.

DeFilippis, V.R., Moore, W.S., 2000. Resolution of phylogenetic relationships among recently evolved species as a function of amount of DNA sequence: an empirical study based on woodpeckers (Aves: Picidae). Mol. Phylogenet. Evol. 16, 143–160.

Donoghue, M.J., Bell, C.D., Li, J., 2001. Phylogenetic patterns in Northern Hemisphere plant geography. Int. J. Plant. Sci. 162, S41–S52.

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

Goodge, W.R., 1972. Anatomical evidence for phylogenetic relationships among woodpeckers. Auk 89, 65–85.

Goodwin, D., 1968. Notes on woodpeckers (Picidae). Bull. Br. Mus. Nat. Hist. (Zoology) 17, 1–44.

Helm-Bychowski, K., Cracraft, J., 1993. Recovering phylogenetic signal from DNA sequences: relationships within the corvine assemblage (class Aves) as inferred from complete sequences of the mitochondrial DNA cytochrome-*b* gene. Mol. Biol. Evol. 10, 1196–1214.

Johnson, W.E., Eizirik, E., Pecon-Slattery, J., Murphy, W.J., Antunes, A., Teeling, E., O'Brien, S.J., 2006. The late miocene radiation of modern Felidae: a genetic assessment. Science 311, 73–77.

Johnson, K.P., Sorenson, M.D., 1998. Comparing molecular evolution in two mitochondrial protein coding genes (cytochrome *b* and ND2) in the dabbling ducks (Tribe: Anatini). Mol. Phylogenet. Evol. 10, 82–94.

Klicka, J., Zink, R.M., 1997. The importance of recent ice ages in speciation: a failed paradigm. Science 277, 1666–1669.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablancha, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196–6200.

Lanyon, S.M., 1993. Phylogenetic frameworks-towards a firmer foundation for the comparative approach. Biol. J. Linn. Soc. 49, 45–61.

Laybourne, R.C., Deedrick, D.W., Hueber, F.M., 1994. Feather in amber is earliest New World fossil of Picidae. Wilson Bull. 106, 18–25.

Li, W.-H., Graur, D., 1991. Fundamentals of Molecular Evolution. Sinauer Associates, Sunderland, Mass.

Lovette, I.J., 2004. Mitochondrial dating and mixed support for the "2% rule" in birds. Auk 121, 1–6.

Moyle, R.G., 2004. Phylogenetics of barbets (Aves: Piciformes) based on nuclear and mitochondrial DNA sequence data. Mol. Phylogenet. Evol. 30, 187–200.

Peterson, A.T., Cicero, C., Wieczorek, J., 2005. Free and open access to distributed data from bird specimens: why? Auk 122, 987–990.

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

Prychitko, T.M., Moore, W.S., 2000. Comparative evolution of the mitochondrial *b* gene and nuclear β-fibrinogen intron 7 in woodpeckers. Mol. Biol. Evol. 17, 1101–1111.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Sheldon, F.H., Whittingham, L.A., Moyle, R.G., Slikas, B., Winkler, D.W., 2005. Phylogeny of swallows (Aves: Hirundinidae) estimated from nuclear and mitochondrial DNA sequences. Mol. Phylogenet. Evol. 35, 254–270.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.

Short, L.L., 1982. Woodpeckers of the World. Delaware Museum of Natural History, Greenville, Delaware.

Short, L.L., 1974. Habits of three endemic West Indian woodpeckers (Aves, Picidae). Am. Mus. Novit. 2549, 1–44.

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

Soberón, J., 1999. Linking biodiversity information sources. Trends Ecol. Evol. 14, 291.

Styring, A.R., bin Hussin, M.Z., 2004. Foraging ecology of woodpeckers in lowland Malaysian rain forests. J. Trop. Ecol. 20, 487–494.

Swofford, D.L., 2000. PAUP‘: Phylogenetic Analysis Using Parsimony (* and other methods). Ver 4.0b4a. Sinauer Associates, Sunderland, Massachusetts.

Tennant, M.R., 1991. Phylogenetic systematics of the Picinae. Ph.D. dissertation, Wayne State University, Detroit, Michigan.

Webb, D.M., 2002. Morphological and molecular evolution of the order Piciformes with emphasis on the woodpeckers of the world (subfamily Picinae). Ph.D. Dissertation, Wayne State University, Detroit, Mich.

Webb, D.M., Moore, W.S., 2005. A phylogenetic analysis of woodpeckers and their allies using 12S, Cyt *b*, and COI nucleotide sequences. Mol. Phylogenet. Evol. 36, 233–248.

Weibel, A.C., Moore, W.S., 2002. Molecular phylogeny of a cosmopolitan group of woodpeckers (genus *Picoides*) based on COI and cyt *b* mitochondrial gene sequences. Mol. Phylogenet. Evol. 22, 65–75.

Weibel, A.C., Moore, W.S., 2002. A test of a mitochondrial gene-based phylogeny of woodpeckers (genus *Picoides*) using an independent nuclear gene, β-fibrinogen intron 7. Mol. Phylogenet. Evol. 22, 247–257.

Weibel, A.C., Moore, W.S., 2005. Plumage convergence in *Picoides* woodpeckers based on a molecular phylogeny, with emphasis on convergence in Downy and Hairy woodpeckers. Condor 107, 797–809.

Weir, J.T., Schluter, D., 2004. Ice sheets promote speciation in boreal birds. Proc. R. Soc. B 271, 1881–1887.

Winker, K., 1996. The crumbling infrastructure of biodiversity: the avian example. Conserv. Biol. 10, 703–707.

Winkler, H., Christie, D.A., 2002. Family Picidae (woodpeckers). In: del Hoyo, J., Elliot, A., Sargatal, J. (Eds.), Handbook of the Birds of the World, Vol. 7, Jacamars to Woodpeckers. Lynx Editions, Barcelona.

Winkler, H., Kotaka, N., Gamauf, A., Nittinger, F., Haring, E., 2005. On the phylogenetic position of the Okinawa Woodpecker (*Sapheopipo noguchii*). J. für Ornithol. 146, 103–110.

Zink, R.M., Rowher, S., Andreev, A.V., Dittmann, D.L., 1995. Trans-Beringia comparisons of mitochondrial DNA differentiation in birds. Condor 97, 639–649.