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Mitochondrial DNA phylogeny of the woodpecker genus *Veniliornis* (Picidae, Picinae) and related genera implies convergent evolution of plumage patterns

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The woodpecker genus *Veniliornis* comprises 12 species, all restricted to the New World tropics. The seemingly distantly related genus *Picoides* is broadly distributed in Eurasia and North America with two putative species, *P. lignarius* and *P. mixtus*, occurring in South America. The two genera are clearly distinct with respect to general plumage colouration and patterning as well as habitat utilization and thus traditionally have been placed in different tribes. Phylogenetic analyses of mtDNA sequences from the *COI* and cyt b genes indicated that both genera are reciprocally paraphyletic. The two South American species of *Picoides* belong to a clade comprising most species of *Veniliornis*, but *V. fumigatus* of Central and north-western South America belongs to a clade comprising species of *Picoides*. The mtDNA tree also indicated that *Veniliornis* is not closely related to the genus *Piculus,* as is implicit in current classifications. Misclassifications involving *Veniliornis* at both the generic and tribal levels appear to result from convergent evolution of plumage traits in specific forest types. We infer that the common ancestor of *Veniliornis* entered South America approximately at the time the Isthmus of Panama was formed, and diversification within South America was rapid.

ADDITIONAL KEYWORDS: biogeography – classification – molecular clock – New World – paraphyly – *Picoides* – *Piculus* – tropic.

INTRODUCTION

Species of the woodpecker genus *Veniliornis* are restricted to the New World tropics; ten of 12 species recognized by Short (1982) are found entirely in South America, and two species have distributions that extend into the southern-most region of Central America (Short, 1982; Winkler, Christie & Nurney, 1995; Winkler & Christie, 2002). The seemingly distantly related genus *Picoides* (as defined by Short, 1982) is the largest of all woodpecker genera; nine of its 33 species are distributed in North and Central America, two species in South America and the remaining 24 species in the Old World. Species assigned to each genus differ categorically with regard to ecology and overall appearance resulting from plumage colouration and pattern. *Veniliornis* species typically have more or less solidly coloured backs ranging from olivaceous green, tinted with golden and reddish hues in some species, to solid red in other species, and ventral aspects that are heavily barred with transverse patterns of green and off-white. With few exceptions, species of *Veniliornis* are found in tropical habitats characterized by dense vegetation (Short, 1982; Winkler *et al*., 1995). Consistent with their characterization as the pied woodpeckers, species of *Picoides*, in contrast, generally have black and white plumage marked with heavy barring dorsally and/or ventrally, and most species are partitioned ecologically among various woodland or savannah-like habitats.

The systematic relationships of these woodpecker genera are uncertain. Although not the earliest work, the classification developed by Short (1982) in his monumental monograph, 'Woodpeckers of the World', is perhaps the best starting point for discussing the

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history and logic of woodpecker classification germane to *Veniliornis* and *Picoides*. The true woodpeckers comprise the subfamily Picinae within the family Picidae (Order Piciformes). Short (1982) divided the Picinae into six tribes and assigned *Picoides* to the tribe Campetherini and *Veniliornis* to the tribe Colaptini. Short's Campetherini also includes the African genera *Campethera*, *Geocolaptes* and *Dendropicos*. In addition to *Veniliornis*, the Colaptini includes the genera *Piculus*, *Colaptes* (flickers) and *Celeus*; *Piculus* and *Colaptes* are restricted to the New World as are all species of *Celeus* except *C. brachyurus*, which occurs in southern Asia. Winkler & Christie (2002) speculated that *C. brachyurus* is actually a highly convergent offshoot of the Old World genus *Picus*, which makes more sense from a biogeographical perspective. While acknowledging some similarities between *Veniliornis* and *Picoides*, Short thought these superficial and suggested that they were primitive characters retained from an early ancestor common to the Campetherini and Colaptini and that *Veniliornis* was actually related to the colaptine genus *Piculus*. However, our recent DNA sequence-based studies of *Picoides* resulted in the surprising and strongly supported inference that the two species of *Veniliornis* included in the study as outgroup species formed a clade within the ingroup that was sister to a derived South American clade comprising *P. lignarius* and *P. mixtus* (Weibel & Moore, 2002a, 2002b). This result implied that Short's genus *Picoides* is paraphyletic and that at least some species of *Veniliornis* are misclassified at the tribal level. It is also possible, indeed likely, that *Veniliornis* is paraphyletic, but because we included only two species of *Veniliornis* in our earlier studies, we were unable to test this.

Few studies have focused on the systematics of these taxa, and within those studies that have been done there is little evidence and an absence of modern phylogenetic analysis that would have any bearing on the affinities of *Veniliornis*, with either *Picoides* or *Piculus*. Not surprisingly, varying classifications have been proposed, adopted and modified in works concerned with woodpecker systematics. Peters (1948) noted that the woodpeckers had not been monographed since Hargit's (1890) work. Taking guidance from Burt (1930), Peters (1948) divided the woodpeckers into two groups based on skull osteology and several other characters that appeared to be adaptations to arboreal vs. more terrestrial foraging. Among the species he put in his arboreal group were all the species that Short (1982) later lumped into the genus *Picoides* and all species of *Veniliornis*. In a study based on myology, Goodge (1972) noted that *Veniliornis* had no distinctive features and suggested that it might be a relatively recent offshoot of North American *Dendrocopos*, which would be consistent with Burt's (1930) inference and our earlier result. (*Dendrocopos* was subsumed by *Picoides* in Short's classification.) Goodwin (1968) suggested another possibility: an affinity between *Veniliornis* and the African genera *Dendropicos* and *Campethera.* Goodwin's suggestion was based on similarity, but he thought the similarity was more likely as a result of convergence than of common ancestry. Sibley & Monroe (1990) adopted Short's classification with the relevant exception that, following the suggestion of Ouellet (1977), they resurrected the genus *Dendrocopos* for the Eurasian species subsumed by Short's *Picoides*, leaving the North American species in the genus *Picoides*. It is unlikely that this is correct, however, because DNA sequence data strongly support the inference that the Eurasian lesser spotted woodpecker *Picoides minor* is the basal lineage in the clade of North American 'small' Picoides (Weibel & Moore, 2002a, b). Although noting this problem and a number of other shortcomings, Winkler *et al*. (1995) and Winkler & Christie (2002) adopted Sibley & Monroe's (1990) (and hence Short's) basic classification, but emphasized that a major revision was needed.

In establishing principles for his classification of woodpeckers, Short (1982) gave preference to plumage, and ecological and behavioural characters for inferring relationships, and this obviously underlies his classification. In overall appearance as determined by plumage, there are indeed striking similarities between species of *Veniliornis* and species of *Piculus*. Short interpreted these similarities as reflections of common ancestry. However, there has long been a suspicion that aspects of plumage and behaviour may be convergent in woodpecker species in certain ecological settings (Goodwin, 1968; Cody, 1969; DeFilippis & Moore, 2000; Weibel & Moore, 2002b; see Omland & Lanyon, 2000; Johnson & Lanyon, 2000; Dumbacher & Fleischer, 2001; Moyle, 2004; for examples from other avian groups). Perhaps the most interesting implication of the confused systematics of woodpeckers is that natural selection operating on the genetic variation available to woodpecker species has evolved similar but analogous phenotypes sufficiently often to have considerably confused their classification.

The purposes of this study were: (1) to clarify the evolutionary relationships among species classified as *Veniliornis* and, in so doing (2) to test further the hypothesis that this genus is reciprocally paraphyletic with the genus *Picoides* and, implicitly (3) to determine the appropriateness of assigning these genera to the tribes Colaptini and Campetherini, respectively. To achieve these goals, we estimated a phylogeny, based on the mitochondrial protein coding genes cytochrome oxidase I (*COI*) and cytochrome b (cyt b) that included ten of the 12 species of *Veniliornis* recognized by Short (1982), plus species of *Picoides*, *Piculus* and outgroup species sufficient to test these hypotheses.

We then used this phylogeny, in conjunction with information on the biogeography and ecology of member species, the geological history of Central and South America, and a molecular clock, to formulate a hypothesis on the origin and diversification of *Veniliornis*.

MATERIAL AND METHODS

Gene sequencing and sequence alignment

Total DNA was extracted from frozen muscle, liver, or kidney tissues with the Qiagen DNeasy Tissue Kit according to the manufacturer's specifications. The mitochondrial genes *COI* and cyt b were PCR amplified by the methods described in Kocher *et al*. (1989), Edwards, Arctander & Wilson (1991), Moore & DeFilippis (1997), and DeFilippis & Moore (2000), using the primers listed in Weibel & Moore (2002a). Amplified products were cleaned with the Promega Wizard Prep Kit. Double-stranded PCR products (*COI*: 1551 of 1551 bases and cyt b: 1029 of 1143 bases) were sequenced at the Wayne State University Molecular Core Facility using an Applied Biosystems ABI 100 model 377 automated sequencer with the Big Dye Terminator Reaction. Sequences were aligned by eye using the sequence-editing computer program ESEE (Cabot & Beckenbach, 1989). Because both cyt b and *COI* are protein coding genes with high conservation at the amino acid level, alignment of their DNA sequences across species is trivial; no insertions or deletions were observed.

Taxic sampling

Specimens used in this study are listed in Table 1. All species classified by Short (1982) as *Veniliornis* were included in the study except *V. sanguineus* and *V. maculifrons* because tissue specimens were not available. We also included a specimen of *V. chocoensis*, generally considered a distinct species (Peters, 1948; Sibley & Monroe, 1990; Winkler *et al*., 1995; Winkler & Christie, 2002), but considered as a subspecies of *V. affinis* by Short (1974, 1982). *P. lignarius* and *P. mixtus* were included because our previous work suggested that they are more closely related to species of *Veniliornis* than they are to other species of *Picoides* (Weibel & Moore, 2002a, b). Four species of *Piculus* were included to test the hypothesis that *Veniliornis* belongs in the tribe Colaptini through a recent common ancestor with this genus (Short, 1982). Additional species of *Picoides* were included because a clade within this assemblage, or the assemblage as a whole, is likely to be the sister group of *Veniliornis* (DeFilippis & Moore, 2000; Prychitko & Moore, 2000; Weibel & Moore, 2002a, b; Webb & Moore, 2005). Similarly, *Colaptes* (represented by *Colaptes auratus*) likely shared a recent common ancestor with at least one species of *Piculus*, *Pl. rubiginosus* (DeFilippis & Moore, 2000; Prychitko & Moore, 2000; Webb & Moore, 2005), but at this juncture relationships among species comprising the genus *Piculus* are unclear. *C. auratus* was included to facilitate rooting of the *Piculus* clade (or clades). *Dryocopus pileatus* appears to be basal to the *Colaptes–Piculus* clade, and the piculet *Picumnus aurifrons* represents the sister subfamily of the true woodpeckers (Moore & DeFilippis, 1997; Prychitko & Moore, 2000; Webb & Moore, 2005), and thus served to root the tree as a whole.

Where possible, we determined the DNA sequences for two specimens of each species and compared the sequences to ascertain that the sequences used in the phylogenetic analyses were not PCR contaminants. This was possible for all species except *V. affinis*, *V. cassini*, *V. chocoensis*, *V. kirkii* and *P. lignarius*. Twenty sequences representing 12 species were new; the remaining sequences have been determined in previous studies (see Table 1; Moore & DeFilippis, 1997; DeFilippis & Moore, 2000; Prychitko & Moore, 2000; Weibel & Moore, 2002a, b).

Phylogenetic analysis

Maximum parsimony (MP) and maximum likelihood (ML) phylogenetic analyses were performed using the computer program package PAUP* (beta version 4.0, Swofford, 1998) following the methods described by Weibel & Moore (2002a, b). Bayesian (BA) analyses were performed using the computer program package MrBayes 3.0 (Ronquist & Huelsenbeck, 2003). We used the computer program package Modeltest (Posada & Crandall, 1998, 2001) on the concatenated *COI* plus cyt b dataset to provide guidance in selecting appropriate nucleotide substitution models for analyses that required model specification (ML, BA). Modeltest selected the GTR + I + G model, consistent with our previous studies that indicate that in woodpeckers both *COI* and cyt b have unequal nucleotide frequencies, very heterogeneous substitution rate matrices, substantial rate variation among sites and a substantial frequency of highly conserved (invariant) sites; these are the criteria of the GTR + I + G model (Moore & DeFilippis, 1997; DeFilippis & Moore, 2000; Prychitko & Moore, 2000; Weibel & Moore, 2002a).

The MP analysis was performed using equally weighted characters and a heuristic search with TBR branch swapping and 30 random-addition replicate datasets. This served as the parsimony-based tree for comparison with the ML and BA trees and as the initial user tree in the ML tree search.

A search for the ML tree with fitted parameter estimates is computationally overwhelming for this many

Table 1. List of species (Order Piciformes, Family Picidae) used in this study

Species*	Common name (WP = woodpecker)	Locale	Museum†	Voucher number	Intraspecific divergence‡		GenBank Accession number§	
					COI	cyt b	COI	cyt b
Subfamily Picinae								
Tribe Colaptini								
Veniliornis affinis	Red-stained WP	Loreto, Peru	LSU	5045			AY927189	AY927209
V. callonotus	Scarlet-backed WP	Lambayeque, Peru	LSU	5178			AF394305	AF389336
V. cassini	Golden-collared WP	Amazonas, Peru	LSU	20214			AY927190	AY927210
V. chocoensis	Choco WP	Esmeraldas, Ecuador	UC	p1347			AY927191	AY927211
V. dignus	Yellow-vented WP	Carchi, Ecuador	UC	p713	0.39	0.79	AY927193	AY927213
V. dignus	Yellow-vented WP	Pasco, Peru	LSU	8043	–	–	AY927192	AY927212
V. frontalis	Dot-fronted WP	Provincia de Tucuman, Argentina	UWBM	1799	0.13	0	AY927194	AY927214
V. frontalis	Dot-fronted WP	Provincia de Tucuman, Argentina	UWBM	54403	–	–	AY927195	AY927215
V. fumigatus	Smoky-brown WP	Cajamarca, Peru	LSU	32360	0.07	0.40	AY927196	AY927216
V. fumigatus	Smoky-brown WP	Cajamarca, Peru	LSU	32970	–	–	AY927197	AY927217
V. kirkii	Red-rumped WP	Maniba/Guayas, Ecuador	UC	p718			AY927198	AY927218
V. nigriceps	Bar-bellied WP	Pasco, Peru¶	LSU	8176			AF394306	AF389337
V. passerinus	Little WP	Bolivia	WSU	96 W-4.2	0.06	0.10	AY927199	AY927219
V. passerinus	Little WP	Bolivia	WSU	96 W-4.3	–	–	AY927200	AY927220
V. spilogaster	White-spotted WP	Provincia de Misiones, Argentina	UWBM	1883	0	0.49	AY927202	AY927222
V. spilogaster	White-spotted WP	Caaguazu, Paraguay	LSU	25914	–	–	AY927201	AY927221
Piculus chrysochloros	Golden-green WP	Loreto Departmento, Peru	LSU	4296	3.75	4.42	AY927184	AY927204
Pl. chrysochloros	Golden-green WP	Chiquitos, Departmento Santa Cruz, Bolivia	FMNH	334419	–	–	AY927183	AY927203
Pl. flavigula	Yellow-throated WP	Loreto Departmento, Peru	LSU	4831	2.50	1.90	AY927186	AY927206
Pl. flavigula	Yellow-throated WP	Rondonia, Cochoeira Nazare, Brazil	FMNH	389784	–	–	AY927185	AY927205
Pl. leucolaemus	White-throated WP	Panama	LSU	2130	0.50	0.20	AY927187	AY927207
Pl. leucolaemus	White-throated WP	Panama	LSU	2134	–	–	AY927188	AY927208
Pl. rubiginosus	Golden-olive WP	Lambayeque, Peru	LSU	5222			AF272591	U83292
Colaptes auratus	Northern flicker	Kentucky, USA	WSU	86–1.8			AF272582	U83301
Tribe Campetherini								
Picoides albolarvatus	White-headed WP	California, USA	WSU	86 W-14.1	0	0.31	AF394273	AF389302
P. albolarvatus	White-headed WP	California, USA	WSU	86 W-14.5	–	–	AF394274	AF389303

P. borealis	Red-cockaded WP	Florida, USA	FSU	209–1	1.01	0.60	AF394277	AF389306
P. borealis	Red-cockaded WP	Florida, USA	FSU	314–3.1	–	–	NA	AF389307
P. borealis	Red-cockaded WP	Florida, USA	FSU	314–3.2	–	–	AF394278	AF389308
P. leucotos†	White-backed WP	Moscovskaya Oblast, Russia	UWBM	49580	0	0.20	AF394282	AF389312
P. leucotos†	White-backed WP	Moscovskaya Oblast, Russia	UWBM	49608	–	–	AF394283	AF389313
P. lignarius	Striped WP	Santa Cruz, Bolivia	LSU	6593			AF394284	AF389314
P. major†	Great spotted WP	Irkutskaya Oblast, Russia	UWBM	51700	0.14	0.59	AF394286	AF389316
P. major†	Great spotted WP	Krasnoyarskiy Kray, Russia	UWBM	51755	–	–	AF394287	AF389317
P. minor	Lesser spotted WP	Khabarovskiy Kray, Russia	UWBM	47225	0.33	0	AF394288	AF389318
P. minor	Lesser spotted WP	Khabarovskiy Kray, Russia	UWBM	47226	–	–	AF394289	AF389319
P. mixtus	Checkered WP	Provincia de Corrientes, Argentina	UWBM	810	0.21	0.10	AF394290	AF389320
P. mixtus	Checkered WP	Provincia de Corrientes, Argentina	UWBM	816	–	–	AF394291	AF389321
P. nuttallii	Nuttall's WP	California, USA	WSU	86 W-13.1	0.37	0	AF394292	AF389322
P. nuttallii	Nuttall's WP	California, USA	WSU	86 W-13.3	–	–	AF394293	AF389323
P. pubescens	Downy WP	Alabama, USA	WSU	86 W-2.3	0.13	0.50	AF394294	AF389324
P. pubescens	Downy WP	Texas, USA	WSU	86 W-5.5	–	–	AF394295	AF389325
P. scalaris	Ladder-backed WP	New Mexico, USA	WSU	86 W-8.2	0	0.20	AF394296	AF389326
P. scalaris	Ladder-backed WP	Arizona, USA	WSU	86 W-11.7	–	–	AF394297	AF389327
P. stricklandi	Strickland's WP	Arizona, USA	WSU	88 W-2.2		0.23	NA	AF389328
P. stricklandi	Strickland's WP	Arizona, USA	UA	16860		–	AF394298	AF389329
P. villosus	Hairy WP	Arizona, USA	WSU	86 W-10.7	0.32	2.39	AF394301	AF389332
P. villosus	Hairy WP	California, USA	WSU	86 W-14.4	–	–	AF394302	AF389333
Tribe Campephilini								
Dryocopus pileatus	Pileated WP	Texas, USA	WSU	86 W-3.4			AF272585	U83286
Subfamily Picumninae								
Tribe Picumnini								
Picumnus aurifrons	Bar-breasted Piculet	Santa Cruz, Bolivia	LSU	18254			AF272589	U83289

*All *Picoides* sequences and *Veniliornis callonotus* and *V. nigriceps* sequences for *COI* and cyt *b* were obtained from Weibel & Moore (2002a).

†FMNH, Field Museum of Natural History; FSU, Florida State University (F. James); LSU, Louisiana State University Museum of Natural Science; UA, University of Arizona; UC, University of Copenhagen Avian Blood Bank; UWBM, Burke Museum at University of Washington; WSU, Wayne State University (W.S. Moore).

‡Percent sequence divergence between at least two conspecific specimens (see also Weibel & Moore, 2002a).

§A single specimen for a species serves as the template sequence for combining conspecific specimens. The template sequence is selected based on completeness and fewest ambiguous sites, and missing data in the template sequence are filled using the homologous overlapping sequence from the second conspecific specimen. Sites with different nucleotides across conspecific sequences were considered ambiguous and scored as missing data in the synthetic sequence. NA, not available; the specimen could not be sequenced.

¶Note: The locale for *V. nigriceps* was mistakenly published as La Paz, Bolivia in Weibel & Moore (2002a, 2002b).

operational taxonomic units and nucleotide characters; thus, we used an approximate search strategy modified from Frati *et al*. (1997) and Weibel & Moore (2002a, b). An initial topology was generated by MP. With this topology fixed as a user tree, parameters for the GTR rate matrix, proportion of invariable sites (I), and Γ-distribution shape parameter (α) were all estimated under a ML criterion. To complete the ML tree search, these parameters were then fixed at the estimated values and a heuristic search was conducted for the ML topology (with TBR branch swapping, and ten random-addition replicate datasets).

Bootstrap analyses were performed on the MP tree with 1000 replicate datasets and on the ML tree with 100 replicate datasets with the model parameters (rate matrix, α and I) fixed at the values used in the ML topology search.

A feature of BA in MrBayes 3.0 is that the program allows more detailed specification of the substitution model (Ronquist & Huelsenbeck, 2003). It was established in previous studies and the Modeltest analysis that *COI* and cyt b evolve at different overall rates in woodpeckers and that there is substantial rate variation among codon sites (DeFilippis & Moore, 2000). Accordingly, in our most parameterized BA model, we specified six partitions: 1st, 2nd and 3rd codon positions for each of the two genes, with the GTR + I + G model specified for each partition. A Markov Chain Monte Carlo (MCMC) simulation was initiated with a random tree; four chains were run for 1 400 000 generations using empirical base frequencies; a tree was sampled every 1000 generations for a total of 1400 trees. Examination of the likelihood values over the course of the simulation indicated that the sampling process found a stable distribution considerably before 100 000 generations. Conservatively, we discarded the first 100 trees ('burn-in', Huelsenbeck & Ronquist, 2001), representing the initial 100 000 generations, from the sampling distribution.

RESULTS

All sequences were archived in GenBank (see Table 1). Extensive matching overlap in fragments and pairing of conspecific taxa in the preliminary phylogenetic analysis indicated that for all specimens these sequences were not contaminates. The total length of the concatenated (see below), aligned sequences was 2580 nucleotides. We were conservative in 'calling' nucleotides, scoring a given nucleotide as unknown if it was ambiguous either as a result of background noise or conflict between overlapping fragments. With the exception of *V. chocoensis*, no more than 147 of the maximum 2580 nucleotides were scored as ambiguous. Unfortunately, only a single specimen of *V. chocoensis* was available to us, and the DNA we extracted from this specimen was somewhat degraded. Consequently, the number of unambiguously called nucleotides for this sequence was 1897, 683 nucleotides less than was the total concatenated length. Wishing to be conservative in our analysis, we excluded this sequence from our main analysis, but did an additional set of analyses which were identical in all respects except that the *V. chocoensis* sequence was included.

Sequencing two specimens for each species allowed a limited comparison of intraspecific variation as well as authentication of the sequences. As with *Picoides* species (Weibel & Moore, 2002a), intraspecific sequence divergence was low among *Veniliornis* species for both genes (< 0.4% for *COI* and < 0.8% for cyt b, Table 1). Intraspecific sequence divergence was higher between the two specimens of *Pl. chrysochloros* (3.8% for *COI* and 4.4% for cyt b) and of *Pl. flavigula* (2.5% for *COI* and 1.9% for cyt b), probably as a result of the geographically disparate locales from where the specimens were collected. To reduce the number of 'uncalled' nucleotides for each species in the phylogenetic analyses, sequences for pairs of specimens were combined to form single 'synthetic' sequences to represent the species, following the protocol of Weibel & Moore (2002a). Phylogenetic analysis of the more complete synthetic sequences should improve estimates of statistical support for interspecific nodes without biasing inferred relationships because the divergence between specimen pairs was low. (Previously published single sequences from six species were used directly, i.e. without synthesis (Moore & DeFilippis, 1997; DeFilippis & Moore, 2000). These sequences were verified against sequences from a second specimen with the exception of *COI* from *V. callonotus* and *V. nigriceps*; the other four species were *Pl. rubiginosus*, *C. auratus*, *Dryocopus pileatus* and *Picumnus aurifrons*.)

Weibel & Moore (2002a) showed that *COI* and cyt b have evolved similarly among *Picoides* species with respect to nucleotide base composition and substitution rates at synonymous sites, which is where most substitutions occur. Moreover, phylogenetic analyses based on the individual genes produced similar trees with no statistically significant conflict. Thus, the two datasets (*COI* and cyt b) were combined to form an aggregate DNA sequence dataset of 2580 nucleotide sites for phylogenetic analysis (see Bull *et al*., 1993; Huelsenbeck, Bull & Cunningham, 1996).

The ML tree for the main analysis is presented in Figure 1, and includes the complete concatenated dataset except for the *V. chocoensis* sequence. The ML tree served as a reference for comparison with the MP and BA topologies, which differed in minor ways. Every node that occurred in the ML tree occurred in either the MP or the BA tree, and most occurred in

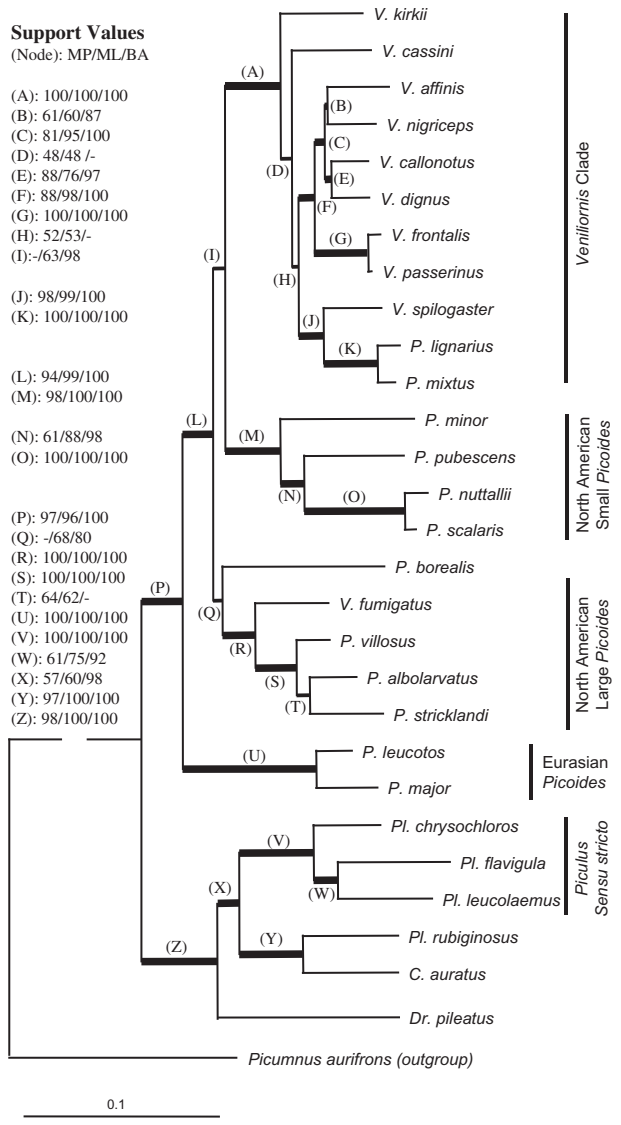

Figure 1. Maximum likelihood tree. Thick lines indicate internodes that also occurred in both the maximum parsimony (MP) and Bayesian (BA) tree; thin lines indicate internodes that occurred in only one additional tree, either the MP or the BA tree, in addition to the ML tree. Support values listed to the left are bootstrap proportions for MP (1000 replicates) and ML (100 replicates) and credibility values for BA (1300 trees sampled). Branch lengths are estimated proportions of nucleotides substituted based on the GTR + Γ +I model. The scale at the bottom indicates 10% divergence along a branch.

both. In Figure 1, nodes that occurred in all three trees are indicated by thick branches and those that occurred in only one tree in addition to the ML tree are indicated by thin branches. Bootstrap proportions and BA credibility values are tabulated to the left of the tree. Generally, all trees grouped species similarly, and most nodes were well supported in all trees. Statistical support is indicated by bootstrap values of at least 70%, a value roughly equated with a 95% probability that the node is real based on a four-taxon simulation study (Hillis & Bull, 1993). For the BA tree, the credibility values of nodes were higher. These values are the percentages of the 1300 trees sampled from the MCMC simulation that contained the specific clades and are estimates of the posterior probabilities of those clades in the actual tree (Huelsenbeck & Ronquist, 2001). We considered BA credibility values of 95% or greater to be statistically significant, but view this as a rough guide given recent evidence that BA posterior probabilities based on sampling from a MCMC appear to be biased on the high side (Yoshiyuki, Glazko & Nei, 2002; Simmons, Pickett & Miya, 2004). As an example for interpreting Figure 1, Node I, which represents the common ancestor of *Veniliornis* and the clade of North American 'small' *Picoides,* occurred in the ML tree with a bootstrap proportion of 63% and in the BA tree with a credibility value of 98%, but did not occur in the MP tree.

To avoid confusion resulting from disparities between the classification implicit in our phylogenies and previous classifications, we state two major results at this juncture and then refer to two redefined taxa throughout the remainder of our discussion. The first result is that the genera *Veniliornis* and *Picoides* were found to be reciprocally paraphyletic: the common ancestor of all species assigned to the genus *Veniliornis* (Node L in the ML tree, Fig. 1; note that *V. fumigatus* clustered with the clade of North American 'large' *Picoides*) also gave rise to several species now classified as *Picoides*. Node L occurred in all trees with support values of 94%, 99% and 100% for MP, ML and BA trees, respectively. As suggested in our previous studies (Weibel & Moore, 2002a, 2002b), which included only two species of *Veniliornis*, the two South American species of *Picoides*, *P. lignarius* and *P. mixtus*, are derived species within a clade otherwise comprising only species classified as *Veniliornis*. These two species shared an inferred common ancestor with *V. spilogaster* (Node J), with support values of 98%, 99% and 100%, respectively, and a more ancient common ancestor with all species of *Veniliornis* (Node A), except *V. fumigatus,* with support values of 100% for all three trees. The second result is that *V. fumigatus* was found to be an early divergent lineage of a clade informally referred to as the North American 'large' Picoides by Weibel & Moore (2002a, b). This inference was implicit in Nodes R and S, which had 100% support values in all three trees. *V. fumigatus* apparently evolved its *Veniliornis*-like plumage traits independently of those in the common ancestor of the major clade of *Veniliornis*. These results would make further discussion potentially con-

fusing because we must refer by name to various clades in the tree that do not conform to traditional nomenclature. Thus, throughout the remainder of this paper, unless stated otherwise, we use the term *Veniliornis* to refer to the clade that includes all species of *Veniliornis,* except *V. fumigatus*, plus *P. lignarius* and *P. mixtus*; conversely, we implicitly include *V. fumigatus* when we refer to the clade of North American 'large' *Picoides*.

The three topologies differed in relatively minor ways and the differences were not statistically significant. The MP tree (2938 evolutionary steps, –ln L = 16565.721) could be derived from the ML tree (2942 evolutionary steps, –ln L = 16561.446) by interchanging the clade of North American 'large' *Picoides* with the clade of North American 'small' *Picoides* and then further moving *P. borealis* to the base of the *Veniliornis* clade. Neither of these changes involved significantly supported nodes. The BA tree (2950 evolutionary steps, –ln L = 16565.707) could be derived from the ML tree by pairing *V. kirkii* and *V. cassini* as sister species and attaching this bi-membered clade as sister to the *spilogaster–lignarius–mixtus* clade. To test the null hypothesis that these three topologies are equally good explanations of the data, we performed the Shimodaira–Hasegawa log-likelihood ratio test (Shimodaira & Hasegawa, 1999) using the FULLOPT option (Swofford, 1998). This is a bootstrapping procedure in which the model parameters are optimized for each bootstrap replicate, and it is an appropriate test when the topologies to be compared are chosen a posteriori; here we chose to compare the ML, MP and BA trees after they had been found by the phylogenetic analyses. The null hypotheses were all accepted, further confirming that we cannot infer that one of these three topologies represents the more likely evolutionary history of these species compared with the other two (1000 replicates, MP vs. ML, $P = 0.42$; BA vs. ML, $P = 0.42$).

Relationships within *Veniliornis* were defined by ten internodes in the ML tree (Fig. 1), including the ancestral node for the clade, and eight of the ten occurred in both the MP and BA trees as well. The two exceptions were Nodes D and H, which were also weakly supported by bootstrap proportions. If these two nodes were collapsed, four basal lineages remained: (1) *V. kirkii*, (2) *V. cassini*, (3) the clade comprising six species bracketed by *V. affinis* and *V. passerinus*, and (4) the tri-membered clade comprising *V. spilogaster* and the two misclassified species of *Picoides*.

The surprising inference that *V. fumigatus* is a basal lineage in the clade of North American 'large' *Picoides* was strongly supported. The two specimens had nearly identical sequences for both genes, making it unlikely that we had a contaminant or chimeric sequence; moreover, the inferred common ancestor (Node R) with 'large' *Picoides* was supported by bootstrap proportions or credibility values of 100% for all three trees.

As in our previous studies, there remains the question of the relationship of the clades of North American 'small' and 'large' *Picoides* species to each other and to *Veniliornis*. In the ML and BA trees, the North American 'small' *Picoides* clade was sister to the *Veniliornis* clade and the North American 'large' *Picoides* clade was basal (Fig. 1), but in the MP tree the positions of the clades of the 'smalls' and 'larges' were reversed. Node I is essential in this inference; it was supported by a 98% credibility value in the BA analysis but only by a 63% bootstrap proportion in the ML analysis. Because of the insignificant bootstrap support for Node I in the ML tree, the tendency for BA credibility values to be inflated and the insignificant bootstrap support for the alternative relationship in the MP tree, we think this relationship should be considered unresolved. The four species of *Piculus* clearly belong to a clade exclusive of the *Picoides–Veniliornis* clade. The three species representing Short's *Piculus s.s.* (*Pl. chrysochloros*, *Pl. leucolaemus* and *Pl. flavigula*) formed a strongly supported clade (Node V) that was sister to a bi-membered clade comprising *C. auratus* and *Pl. rubiginosus* (Node Y).

We did not include the *V. chocoensis* sequence in the main analysis because we wanted to be conservative with the presentation of our analysis. Although we had only one sequence for this species and 683 of the maximum 2580 nucleotides were missing, comparison of this sequence with those of other species of *Veniliornis* and phylogenetic analyses that included *V. chocoensis* gave every indication that the sequence is authentic. When the sequence was aligned with those of other species, the mismatches appeared uniformly, randomly distributed along the length of the concatenated sequence, which would not be the case if it were a chimeric sequence. Inclusion of the sequence in the phylogenetic analysis had little impact on the topology or levels of support determined in the main analysis: *V. chocoensis* joined the *Veniliornis* clade with strong statistical support (MP bootstrap = 92%, ML bootstrap = 99%, BA credibility = 100%), and was the basal lineage but with marginal statistical support (MP bootstrap = 73%, ML bootstrap = 56%, BA credibility = 93%). Also, *V. cassini* and *V. kirkii* became sister species, and this bi-membered clade was sister to the *V. spilogaster–Picoides* clade, but statistical support was weak in both cases.

DISCUSSION

Of the genes that have been studied to date, mitochondrial encoded genes are arguably best suited for

resolving the phylogenetic history of avian groups less than approximately 5 Myr in age (Moore & DeFilippis, 1997; Moore, Smith & Prychitko, 1999), which is roughly the time frame over which *Veniliornis* and related species of *Picoides* have diversified (see below). Nuclear gene introns are perhaps the most obvious alternative sources of sequence for resolving relationships of this antiquity, but our comparison in *Picoides* of β-fibrinogen intron 7 (*β-fibint 7*) with mitochondrial *COI* and cyt b showed that the mitochondrial encoded genes provide a stronger phylogenetic signal at this level of evolutionary history (Weibel & Moore, 2002b). Thus, mitochondrial genes would seem to be the best choice of genetic marker for resolving relationships among genera and tribes of woodpeckers. Consistent with this are the high bootstrap proportions and estimated posterior probabilities for most nodes (Fig. 1) and the general congruence of topologies among trees generated by the different phylogenetic methods.

A potential shortcoming of mitochondrial genes is that they are inherited as a single linkage group and provide only one independent estimate of the species tree; therefore, it is possible that a specific gene tree does not reflect the species tree because of lineage sorting or hybridization. However, because of maternal inheritance and haploidy, the mitochondrial genome has a lower effective population size and a higher probability of tracking the species tree than does a nuclear gene with regard to lineage sorting (Moore, 1995).

Another potential problem that would lead to fallacious inferences is that of amplifying and sequencing a contaminant sequence (Hackett *et al*., 1995; Edwards & Arctander, 1996, 1997). Particular caution must be exercised with PCR methods, and one should be suspicious when the resultant phylogeny differs in salient details from conventional beliefs about the systematics of the group, as is the case here. The strategy we adopted of sequencing two specimens for each species, when possible, greatly reduced the chance of making this mistake. Three species in our study attached to the inferred tree in strikingly unconventional positions: *P. lignarius*, *P. mixtus* and *V. fumigatus*. We sequenced two specimens of *P. mixtus* and *V. fumigatus*; divergence between the duplicate-specimen sequences was low, as expected for conspecifics. Only a single *P. lignarius* sequence was available to us, but it attached to the tree in the most plausible way – as the sister species of *P. mixtus* but not very distant from it. In sum, we believe that the tree in Figure 1 accurately portrays the evolutionary history of the included species because it was based on genes appropriate for the time frame, statistical support for individual nodes was generally high, the mitochondrial-genome tree has a high probability of tracking the species tree, and we took precautions against inclusion of contaminant sequences in the analysis.

Before turning to relationships of direct concern in this study, the lingering uncertainty of the sister group of the South American radiation of *Veniliornis* must be discussed briefly. The ML and BA trees (Fig. 1) placed the clade of 'small' *Picoides* as sister to *Veniliornis*, whereas the clade of 'large' *Picoides* occupied this position in the MP tree (not shown). The ML and BA trees are consistent in this regard with the ML tree reported in our previous study, which included *β-FibInt 7* as well as the same two mitochondrial genes, but included fewer species of *Veniliornis* (Weibel & Moore, 2002b). Levels of statistical support leave this inferred relationship in limbo: the sister group relationship of the 'small' *Picoides* clade with *Veniliornis* was not significantly supported in the ML tree (Node I, 63%), but the estimated posterior probability for this node in the BA tree was 98%. We caution that studies have shown BA credibility values to be biased on the high side. Similarly, the bootstrap proportion for a *Veniliornis*–'small' *Picoides* node was only 58% in our previous study based on *β-FibInt 7* plus the two mitochondrial genes (1000 replicates of a neighbour-joining bootstrap, Weibel & Moore, 2002b). On the other hand, the 'large' *Picoides*–*Veniliornis* sister-group relationship was not significantly supported in the MP (58%) analyses. It is disappointing that the enlarged taxon sample did not help to resolve this issue, but with three analyses favouring one inference and two favouring another, none with strong statistical support, we must continue to consider the relationship between the 'small' *Picoides*, 'large' *Picoides*, and South American *Veniliornis* clades as an unresolved trichotomy. It is likely that additional sequence from mitochondrial genes would resolve this relationship.

Turning to the questions of misclassification at the levels of genera and tribes, it is evident that the genera *Picoides* and *Veniliornis* are reciprocally paraphyletic: *P. lignarius* and *P. mixtus* should be assigned to a taxon with all species of *Veniliornis* except *V. fumigatus,* which should be assigned to *Picoides*; statistical support was consistent and strong among all trees for these inferences. With regard to his classification of *Veniliornis* at the tribal level, Short (1982) noted similarities between species of *Veniliornis* and *Piculus* in plumage colouration, and although he did not split *Piculus* nominally, he noted a long recognized division within *Piculus* into '*Chloronerpes*', which has some affinity to the flickers (*Colaptes*) and a residual group he called *Piculus s.s.* Short thought *Veniliornis* had some affinity with the latter. The similarities are indeed striking and involve solid olivaceous-green colouration of the back and neck, tinged with varying red and yellow tones, and sexual dimorphism involving red crowns. Thus, he assigned *Veniliornis* to the

tribe Colaptini, along with the genera *Colaptes* and *Piculus*.

We did not include representatives of the *Piculus s.s.* group in our previous studies but included three species (*Pl. flavigula, Pl. leucolaemus and Pl. chrysochloros*) here to test the possibility that *Veniliornis* should be included in the Colaptini through a relationship with this group. It is clear from the tree (Fig. 1) that the affinity of *Veniliornis* is with *Picoides* and not *Piculus*; the relevant nodes were inferred by all analytical methods and the statistical support was strong in all cases.

In a recent phylogenetic study focused on higher level relationships among woodpeckers based on three mitochondrial genes, *12S-rRNA*, *COI* and cyt b, Webb & Moore (2005) proposed dividing the woodpeckers (Picinae) into three tribes, Malarpicini, Dendropicini and Megapicini, which represent the three major lineages that diverged early and abruptly from the primordial woodpecker. Our study is consistent with that proposed classification, and to the extent that our results are directly relevant, substantiates it: *Veniliornis* belongs in the Dendropicini, along with *Picoides*, and did not descend from the common ancestor of the Malarpicini. The Malarpicini derives it name from the fact that most member species have a sexually dimorphic malar stripe, which is apparently important in sex recognition. Species of *Veniliornis* do not have sexually dimorphic malar stripes, whereas species of *Piculus* do; this is further evidence that *Veniliornis* is not related to Short's colaptine woodpeckers.

Focusing now on relationships among species presently assigned to the genus *Veniliornis (*e.g. Short, 1982), in our analyses *V. fumigatus* was consistently inferred to be an early lineage in the clade of North American 'large' *Picoides* rather than a member of the *Veniliornis* clade. This is surprising for two reasons: first, it strongly resembles in plumage appearance species typical of *Veniliornis*, although it does lack ventral barring, which is characteristic of the genus; second, it is basal to a triad of North American species including *P. villosus*, *P. albolarvatus* and *P. stricklandi*. If it is true that *Picoides* originated in Eurasia and spread to North America and then South America, this would imply by parsimony that *V. fumigatus* originated as a lineage in North America, came to occupy a range in Central and South America and evolved a plumage appearance analogous to that of true species of *Veniliornis*. It is interesting that the range of *V. fumigatus* extends substantially farther north in Central America and Mexico, as far north as the Tropic of Cancer, compared with any other species assigned to *Veniliornis*, and that it is in limited sympatry, or nearly so, with both *P. villosus* and *P. stricklandi* (Winkler *et al*., 1995). Also, the somber, humid forest habitat of *V. fumigatus*, typically in the lowlands, is quite distinct from the more xeric habitats of either species of *Picoides*, except *P. villosus sanctorum*, the southernmost subspecies, which occurs in wet, epiphyte-laden forests of Costa Rica and Panama. Remarkably, this subspecies has lost much of the wing spotting prevalent in other subspecies of *P. villosus* and has evolved a fumigated (smoky-brown) colouration of its ventral plumage, seemingly parallel to that seen in *V. fumigatus*. (Winkler *et al*., 1995; Winkler & Christie, 2002 provide colour plates and range maps; Short, 1982 provides colour plates.)

The remaining species of *Veniliornis* plus the two species of *Picoides* noted above, *P. lignarius* and *P. mixtus*, formed a strongly supported clade with bootstrap proportions and credibility values of 100% (Node A in Fig. 1). Unfortunately, relationships among *Veniliornis* species were not fully resolved. The uncertainty stems from the variable positions of *V. kirkii* and *V. cassini* and manifested as low support values for Nodes D and H (Fig. 1) and as some incongruence of the MP and ML trees, which were identical, with the BA tree. In the BA tree, *V. kirkii* and *V. cassini* joined as sister species and this bi-membered clade was sister to the *V. spilogaster–P. lignarius–P. mixtus* clade, but the credibility values supporting these inferences, 71% and 86%, respectively, were not significant. Thus, there was no conflict between the BA tree and the other two trees involving statistically supported nodes. Short (1982) considered *V. kirkii*, and *V. cassini* members of an allospecies along with *V. maculifrons* (not included in our study) and *V. affinis*. Although not supported at a level of statistical significance, our analyses consistently placed *V. affinis* in a derived clade as the sister species of *V. nigriceps*, separated from either *V. cassini* or *V. kirkii* by two strongly supported nodes (F and C), whereas *V. cassini* and *V. kirkii* appeared more basal. When *V. chocoensis* was brought into the analyses, it too assumed a basal but uncertain position (not shown). Short (1974) considered *V. chocoensis* to be a subspecies of *V. affinis*, although historically it has been considered a relative of *V. cassini* (Todd, 1919; Peters, 1948) and was maintained as a distinct species by Winkler *et al*. (1995). We recommend caution in drawing conclusions about the relationships of these lineages. That *V. kirkii*, *V. cassini* and *V. chocoensis* are basal lineages of the genus is plausible because they are all lowland species that collectively occupy the north-western corner of South America. Thus, their biogeography is consistent with the hypothesis that the common ancestor of *Veniliornis* entered South America from the north across the Isthmus of Panama. *V. kirkii*, in particular, is the only species whose range extends onto the Isthmus of Panama. It is also doubtful that *V. affinis* forms a monophyletic group

with *V. kirkii* and *V. cassini* and thus doubtful that these species should be considered an allospecies. For the time being, however, these alternatives should be considered as no more than tentative hypotheses. We believe they could be tested by generating additional sequence data and by expanding the taxa sampled to include greater intraspecific variation. The relationships among the other species within *Veniliornis* were consistent among trees and strongly supported statistically. These are apparent in Figure 1 and will be discussed in the context of the evolutionary scenario below.

The relationships of the two species not included in our study, *V. sanguineus* and *V. maculifrons*, remain uncertain. Short (1982) thought *V. sanguineus* has no very close relatives, but based on its small size and other traits he suggested that it is related *to V. passerinus*. We think this is the most plausible hypothesis in the absence of DNA sequence data. The relationship of *V. maculifrons* is even less certain. Its range is restricted to a small coastal region of eastern South America just north of the Tropic of Capricorn. Short (1982) considered it a member of an allospecies with *V. kirkii*, *V. cassini* and *V. affinis*. Our results indicate that *V. affinis* is not closely related to either *V. kirkii* or *V. cassini*, and from a biogeographical perspective, *V. maculifrons* is more plausibly related to *V. affinis* than it is to the former two species. This is because its range appears to overlap that of *V. affinis*, or at least it is in close proximity, whereas the ranges of *V. kirkii* and *V. cassini* are remote from that of *V. maculifrons*. Short (1982) also noted similarities of *V. maculifrons* with *V. passerinus* and *V. spilogaster*, both of whose ranges overlap with, or are in close proximity to, that of *V. maculifrons*. DNA sequence data from *V. sanguineus* and *V. maculifrons* is likely to have the potential to resolve these uncertainties.

Reconstruction of the evolutionary history of the genus *Veniliornis* and of woodpeckers on a broader scale is the long-term goal of our research program and was the motivation for this study. However, doing this for *Veniliornis* is well beyond the scope of this paper because it would require detailed analyses of geographical ranges, anatomical, behavioural and ecological traits for each species, and a thorough molecular clock analysis; then, this must all be considered in the context of the geological history of South America, especially the emergence of the Isthmus of Panama and the uplift of the Andes. Nonetheless, we believe a 'coarse-focus' reconstruction can be proposed reasonably at this time, and that it would be useful in guiding further studies of the evolution of the numerous animal and plant groups that span the two continents.

We used a molecular clock calibration of 2.0% mtDNA sequence divergence between species per Myr (Klicka & Zink, 1997; Moore *et al*., 1999) to infer an approximate time for diversification of the *Picoides–Veniliornis* complex. Genetic distances were estimated with the Tamura–Nei formula and left Γ-uncorrected for rate variation among sites so that we could use an earlier calibration (Moore *et al*., 1999). Referring to Figure 1, we estimate that divergence of the ancestral *Veniliornis* lineage (Node A) from the common ancestor with either the 'small' or 'large' *Picoides* ancestral lineage occurred approximately 5.1 Mya, presumably in North or Central America because this date antecedes the emergence of the Isthmus of Panama and both potential sister groups are restricted to the northern continent. We further hypothesize that the common ancestor entered South America via the Isthmus of Panama, which emerged approximately 3.5 Mya (Coates & Obando, 1996), and began to diversify initially in lowland forests. This timing is correlated with the 'great American faunal interchange' (Marshall *et al*., 1979; Vuilleumier, 1984; Webb, 1985). The apparent basal lineages, *V. kirkii* and *V. cassini*, are lowland, humid forest species (as is *V. chocoensis*), but it is possible that the ancestor was adapted to more arid woodlands as there is some evidence that the land bridge supported woodlands that were more xeric than is now the case (Webb, 1985; Zamudio & Greene, 1997). It is of interest in this context that the lineages of both North American 'small' and 'large' *Picoides* that occur in Central America are adapted to relatively arid woodlands (*P. scalaris*, *P. stricklandi* and *P. villosus*).

The diversification of *Veniliornis* appears to have begun as the ancestral lineage(s) entered South America approximately 3.3 Mya; this estimate is based on the average molecular-clock time between the two basal lineages, *V. kirkii* and *V. cassini*, and the remaining clade (Node H). We hypothesize that the predominantly olivaceous, solid dorsal plumage prevalent in *Veniliornis* evolved in the common ancestor or independently in several of the early lineages as they adapted to humid, somber, tropical-forest habitats. Early diversification appears to have been rapid as evidenced by several short internodes (D, H, F, J, C, B and E). Rapid diversification was likely driven by one or both of two causes, the accelerated uplift of the Andes, especially the northern Andes, in the late Pliocene and early Pleistocene (see Haffer, 1974; Zamudio & Greene, 1997; Lamb, 2004 for reviews), and invasion by these lineages of a vast, heavily forested continent devoid of woodpeckers or other species competent to occupy scansorial, wood-excavating, insectivorous ecological niches. Diversification in *Veniliornis* was associated with marked ecological divergence. For example, the clade comprising *V. affinis*, *V. nigriceps*, *V. callonotus* and *V. dignus* contains an Amazonian lowland, tall rainforest spe-

cies (*V. affinis*), an Andean, high-elevation, humid forest species (*V. nigriceps*), an Andean mid-elevation species (*V. dignus*) and an arid lowland, tropical scrub species (*V. callonotus*). Although the ranges of these species need to be mapped in greater detail, sister species appear usually to have parapatric or near parapatric distributions separated along strong elevational and/or ecological gradients. Divergence between *V. passerinus* and *V. frontalis* was more recent, approximately 0.35 Mya. *V. Passerinus* is widely distributed among diverse habitats in the Amazon basin to 1200 m in elevation, whereas *V. frontalis* inhabits humid, transitional forests on the Andean slopes up to 2000 m in the border region of Bolivia and Argentina; they are in limited sympatry (Winkler *et al*., 1995).

Misclassified *P. lignarius* and *P. mixtus* are sister species and together are the sister group of *V. spilogaster*. These relationships were strongly supported statistically. This is a relatively derived trio of species with ranges geographically distant from where the ancestral lineage presumably entered South America. *P. lignarius* has a disjunct distribution with a population in west-central Bolivia and one in the southern Andes of Chile and Argentina. (Our specimen was from Bolivia.) *V. spilogaster*, like *P. lignarius*, occurs in a diversity of habitats over its range in south-eastern South America from southern Brazil to north-eastern Argentina and appears to be partially sympatric with *P. mixtus*. The latter species is more restricted to arid woodland habitats. Given that *lignarius* and *mixtus* were misclassified as *Picoides*, it is not surprising that their plumage patterns resemble those of many species of *Picoides* with dorsal patterns of transverse barring and checkering, as opposed to the solid dorsal colouration characteristic of *Veniliornis*, except the dark barring and spotting of *lignarius* and *mixtus* is more olivaceous than pure black as is common in *Picoides*. *V. spilogaster*, the sister species of the *lignarius–mixtus* clade, is actually very similar in overall plumage pattern to these two species, but appears darker because there is a proportional increase in the percentage of dark pigmentation. Assuming that the ML tree in Figure 1 is correct, the most parsimonious explanation for the evolution of pied vs. solid dorsal plumage patterns is that the pied pattern is primitive in the New World *Picoides–Veniliornis* complex, solid plumage evolved in the common ancestor of *Veniliornis* and the pied pattern re-evolved (i.e. is a reversal) in the common ancestor of the *spilogaster–lignarius–mixtus* clade. However, because two critical nodes (D and H) were not significantly supported, we cannot exclude the possibility that the pied pattern is a symplesiomorphy (i.e. a retained primitive character state).

Phylogenies for woodpeckers based on DNA sequences from mitochondrial and nuclear genes are highly congruent with each other (Prychitko & Moore, 1997, 2000; Weibel & Moore, 2002b; Webb & Moore, 2005) and with phylogenies based on allozymes (Tennant, 1991), but substantially incongruent with phylogenies implied by current classification, which is based primarily on plumage characteristics. Character incongruence of this magnitude (species assigned to the wrong genera and genera assigned to the wrong tribes) is suggestive of important underlying evolutionary phenomena, specifically, some form of selection leading to convergence of plumage phenotype. In some cases, the selection driving convergence may result from interspecific territoriality favouring a common plumage pattern (Cody, 1969). Although this is possibly a factor driving convergence among species of *Veniliornis* and *Piculus*, another, simpler hypothesis is plausible: specifically, the solid, dark olivaceous-green back plumage, lightly over-tinted with red and golden-yellow is cryptic in the generally dark, tropical forests of South and Central America where these species occur. As an example which is consistent with Short's (1982) observation, spotting the crimson-mantled woodpecker *Piculus rivolii* in the Yungas forests of the Andean slopes of Bolivia, where the canopy is draped with mosses and dotted with epiphytic plants, is remarkably difficult (W.S.M., pers. observ.). While molecular phylogenies implicate selection as a driving force in woodpecker plumage evolution, hypotheses about the nature of the selective forces remain to be tested. An equally intriguing and completely unanswered set of questions concerns the nature of genetic variation that underlies adaptive plumage patterns that seemingly 'blink' on and off over the evolutionary history of the radiation. Do genetic 'modules' that evolved in ancestral species lie dormant in the genomes of descendant species to be later restored by a few simple mutational differences in derived species, or do the developmental programs arise de novo in each species that expresses a seemingly common plumage phenotype?

Finally, a major long-term objective of our DNA sequence-based studies is to revise the classification of the true woodpeckers (subfamily Picinae) so that it portrays the evolutionary history of the group. There is considerable work to be done, and we prefer to postpone a complete revision until this work is complete. However, Webb & Moore (2005) suggested that the genera comprising the Picinae be grouped into three tribes rather than six as in Short's (1982) classification, with each of the three tribes corresponding to one of the three lineages that emerged early in woodpecker evolution. Consistent with that classification and the conclusions reached in this paper, the genera *Veniliornis* and *Picoides* will be assigned to the tribe

Dendropicini (along with *Dendropicos*, *Melanerpes*, *Sphyrapicus*, *Xiphidiopicus*, and *Sapheopipo*). It will be necessary to reclassify the assemblage of species comprising *Picoides*, but we postpone doing so because of the complexity of the assemblage and because many Eurasian species have not yet been sampled. In the case of *Veniliornis*, although *V. sanguineus* and *V. maculifrons* have not yet been included in DNA-based analyses and *V. chocoensis* needs to be sampled further, it is apparent that there is a strongly supported monophyletic group comprising 14 species that should be named *Veniliornis*. The genus comprises *V. chocoensis*, *V. kirkii*, *V. cassini*, *V. affinis*, *V. nigriceps, V. callonotus*, *V. dignus*, *V. frontalis*, *V. passerinus*, *V. spilogaster*, *V. lignarius*, *V. mixtus*, *V. sanguineus* and *V. maculifrons*; *V. fumigatus* should be reclassified as *P. fumigatus. V. lignarius* and *V. mixtus* are renamed from *Picoides* to *Veniliornis*. The type species, by subsequent designation, should be *Veniliornis sanguineus,* designated as *Picus sanguineus* by Gray, 1855 (American Ornithologists' Union, 1998). Although *V. sanguineus* was not included in our DNA-based analysis, plumage and morphological similarities strongly suggest that it is a member of the clade we have defined as *Veniliornis*.

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