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Tapping the woodpecker tree for evolutionary insight

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ABSTRACT

Molecular phylogenetic studies of woodpeckers (Picidae) have generally focused on relationships within specific clades or have sampled sparsely across the family. We compared DNA sequences of six loci from 203 of the 217 recognized species of woodpeckers to construct a comprehensive tree of intrafamilial relationships. We recovered many known, but also numerous unknown, relationships among clades and species. We found, for example, that the three picine tribes are related as follows (Picini, (Campephilini, Melanerpini)) and that the genus *Dinopium* is paraphyletic. We used the tree to analyze rates of diversification and biogeographic patterns within the family. Diversification rate increased on two occasions during woodpecker history. We also tested diversification rates between temperate and tropical species but found no significant difference. Biogeographic analysis supported an Old World origin of the family and identified at least six independent cases of New World-Old World sister relationships. In light of the tree, we discuss how convergence, mimicry, and potential cases of hybridization have complicated woodpecker taxonomy.

1. Introduction

The woodpeckers (Picidae) constitute a well-defined family whose members mostly peck on wood to extract insects and their larvae. Woodpeckers occupy a variety of habitats, but are highly specialized ecologically and behaviorally. Currently, 33 genera and 217 species are recognized (Dickinson and Remsen, 2013), and they occur in every major biogeographic region except Australasia, Madagascar and Antarctica. Because the family exhibits remarkable instances of convergence in plumage and behavior, and also intriguing biogeographic patterns, the group offers rich opportunities for research into associated evolutionary and ecological issues (Benz et al., 2015; Lammertink et al., 2016; Prum, 2014; Prum and Samuelson, 2012; Styring and Zakaria bin Hussin, 2004). However, a prerequisite for investigating the underpinnings of woodpecker ecology and evolution is a comprehensive, well-resolved estimate of phylogeny of the group (Sheldon and Whittingham, 1997). Although the phylogenetic position of the woodpecker family within birds as a whole–along with its closest relatives, the honeyguides (Indicatoridae) and barbets (Capitonidae, *sensu lato*)–is now well-established (Hackett et al., 2008; Jarvis et al., 2014; Prum et al., 2015), the relationships of many taxa within the family remain uncertain.

Numerous attempts have been made to reconstruct phylogenetic relationships within the Picidae (Benz et al., 2006; DeFilippis and Moore, 2000; Del-Rio et al., 2013; Dufort, 2015; Fuchs et al., 2013, 2008, 2007, 2006; Goodge, 1972; Prychitko and Moore, 1997, 2000; Short, 1982; Webb and Moore, 2005; Weibel and Moore, 2002; Winkler et al., 2014). However, most of these studies have focused on a single clade (e.g. Fuchs et al., 2017, 2008; Fuchs and Pons, 2015; Weibel and Moore, 2002) or sampled just a few taxa among major clades (Benz et al., 2006; Winkler et al., 2014). Such approaches lack the scope necessary to address evolutionary patterns across the whole family. The most comprehensive study to date is Dufort's (2015) super-matrix analysis of about 170 taxa based mainly on previously published DNA sequences. Unfortunately, large amounts of data from many species were missing in that study (68% of sequence data was missing in the total matrix among the species compared) and relationships within several clades remained unresolved. Regardless of such limitations, previous molecular studies of woodpecker phylogeny have improved our understanding substantially.

The woodpeckers are commonly divided into three subfamilies. Jynginae, the wrynecks, appears to be sister to all other woodpeckers (Benz et al., 2006; DeFilippis and Moore, 2000; Dufort, 2015; Webb and Moore, 2005; Winkler et al., 2014). They comprise just two species, *Jynx torquilla* and *J. ruficollis*, which possess numerous distinct morphological characters that set them apart from the rest of the family, including soft plumage, cryptic coloration, and an absence of characteristic rigid tail feathers (Goodge, 1972; Short, 1982; Winkler and

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Christie, 2002).

Picumninae, the piculets, comprises 29 species, divided into three genera, *Verreauxia*, *Sasia* and *Picumnus*. Piculets are morphologically distinct from the other woodpeckers, but share behavioral characteristics (like wood-tapping) with the rest of the family (Winkler and Christie, 2002). *Verreauxia* and *Sasia* differ from *Picumnus* in possessing bare skin around the eyes, reduction (*V. africana*) or absence (*S. abnormis* and *S. ochracea*) of the hallux, and absence of tail and crown stripes (Goodge, 1972; Short, 1982; Winkler and Christie, 2002). Interspecific relationships within *Sasia* and *Verreauxia* are well-resolved (Fuchs et al., 2006), but those within *Picumnus* remain obscure. Because *Picumnus* species are often rare and localized in distribution, several have not been included in molecular phylogenetic studies. Determining their relationships is further complicated by extensive hybridization among species (Dickinson and Remsen, 2013). In addition to its intrageneric uncertainties, *Picumnus'* relationship to the other two piculet genera has not been established. Some molecular studies place *Picumnus* as sister to *Sasia* and *Verreauxia* (Benz et al., 2006; Dufort, 2015; Webb and Moore, 2005), whereas others do not (Winkler et al., 2014), making the Picumninae paraphyletic. A fourth genus, the monotypic *Nesoctites,* used to be included within Picumninae, but *Nesoctites* is now generally believed to be the sister of Picinae and not a true piculet (Benz et al., 2006; Dufort, 2015; Fuchs et al., 2007).

Picinae, the typical woodpeckers, consists of 176 species in 29 genera, and their classification is also in flux. Using morphological similarities and geographic distributions, Short (1982) divided the subfamily into six tribes. His groupings disagreed with those of Goodge (1972), which was based on anatomical characters, in part because Goodge's (1972) arrangement required multiple and sometimes dramatic cases of convergent evolution in plumage and, thus, was not especially parsimonious. Moreover, neither of these early morphological assessments benefited from rigorous tree-building methodology. With the application of modern molecular methods, our knowledge of picine relationships has improved substantially, leading to the resolution of several of early disagreements and clarifying why it has been so difficult to discern woodpecker relationships from morphology alone (e.g., Benz et al., 2006; Dufort, 2015; Fuchs et al., 2013, 2008, 2007, 2006; Fuchs and Pons, 2015; Moore et al., 2011, 2006; Overton and Rhoads, 2006; Weibel and Moore, 2002). Currently, five tribes of Picinae are recognized: Nesoctitini, Hemicercini, Campephilini, Picini and Melanerpini (Dickinson and Remsen, 2013; Dufort, 2015). The commonly accepted arrangement has Nesoctitini (one species) diverging first from the rest of the picines, followed by Hemicercini (two species). However, relationships among and within the three remaining tribes, Campephilini, Picini, and Melanerpini, are not well-established.

Molecular studies have demonstrated the existence of extensive plumage convergence or parallelism, as well as potential mimicry within the Picinae (Benz et al., 2015, 2006; Lammertink et al., 2016; Prum, 2014; Prum and Samuelson, 2012). Morphological convergence is apparent between the Rufous Woodpecker (*Micropternus brachyurus*) of Asia and *Celeus* woodpeckers of South America, greater (*Chrysocolaptes*) and lesser (*Dinopium*) flamebacks of Asia, and the Helmeted Woodpecker (*Celeus galeatus*) and members of *Dryocopus*, making it difficult to determine phylogenetic relationships within the family by morphological comparisons alone (Benz et al., 2015, 2006; Fuchs et al., 2007; Lammertink et al., 2016; Prum, 2014). Compounding this problem, recent phylogenetic studies have also found that most tribes in the Picidae include Old and New World sister taxa (Benz et al., 2006; Dufort, 2015; Fuchs et al., 2013, 2007). Explaining such non-parsimonious distributions has proved difficult. Intercontinental dispersal (Benz et al., 2006; Fuchs et al., 2007) and ancient hybridization (Fuchs et al., 2013) have been suggested, but no well-supported rationale for these biogeographic pattern exists.

To address taxonomic uncertainties in the Picidae, we have reconstructed the phylogeny of the family by comparing DNA sequences in a nearly-complete matrix of six loci from 203 species. Using this tree, we address several evolutionary and ecological issues. These include: (1) rates and patterns of diversification in various clades, and how these may explain unusually great species richness in some geographic regions, such as Brazil (51 species) and Myanmar (40 species); (2) how hybridization might obscure relationships among some taxa; and (3) how convergence and potential mimicry may have played an important role in the evolution of woodpeckers. In future studies, the phylogeny can be used in quantitative examinations of woodpecker community assembly in locations where large numbers of species live in sympatry (Webb et al., 2002). A particularly promising location for such a study is Southeast Asia, where up to 15 species of woodpeckers can co-occur and an unusually rich stock of foraging data are available (Lammertink, 2004; Styring and Ickes, 2001; Styring and Zakaria bin Hussin, 2004). The phylogeny will also allow the quantitative analysis of morphological convergence in different regions where woodpeckers inhabit similar niches.

2. Materials and methods

We compared DNA sequences of 203 woodpecker species representing 93.5% of species recognized in Dickinson and Remsen (2013) (Table S1). We also sampled individuals from morphologically distinct populations in some polytypic species to test for monophyly. As outgroups, we included three species of *Indicator,* the woodpeckers' sister group (Hackett et al., 2008; Jarvis et al., 2014; Prum et al., 2015). The loci we compared were: mitochondrial protein-coding genes NADH dehydrogenase 2 (ND2), NADH dehydrogenase 3 (ND3), and ATP synthase 6 (ATP6); and nuclear autosomal myoglobin intron 2 (MB), autosomal transcription growth factor β 2 intron 5 (TGFβ2) and Z-linked muscle skeletal receptor tyrosine kinase intron 4 (MUSK). Sequences of these loci were obtained from three alternative sources: GenBank, preserved tissues, and toe-pads of museum specimens (Table S1).

We extracted total genomic DNA from frozen or alcohol preserved tissues or blood using DNEasy® Blood and Tissue Kit (Qiagen) following the manufacturers' protocol. DNA from toe-pads was extracted in a room dedicated to ancient DNA to avoid contamination of the samples with fresh DNA. We used the same extraction protocol for toe-pads as for the preserved samples but added 40 µl of dithiothreitol (DTT, 0.1 M) to facilitate tissue digestion. PCR amplifications were performed in 25 µl reactions using Taq DNA Polymerase (New England BioLabs Inc) and appropriate primers. Amplification consisted of 34 cycles at a denaturing temperature of 95 °C, an annealing temperature based on the primer pair used, and an extension temperature of 72 °C. We visualized the PCR products in 1.5% agarose gel stained with SYBR® Safe DNA Gel Stain (Invitrogen). Samples were sequenced at Beckman Coulter Genomics (Danvers, MA).

Sequences were assembled in Geneious 8.0.5 (Biomatters), manually checked for errors to identify ambiguous sites, and aligned using MUSCLE (Edgar, 2004) implemented in Geneious. Gene trees from each locus were generated using maximum likelihood (ML) in RAxML 8 (Stamatakis, 2014). Gene trees were used to check for congruence among sequences and to locate unusual signals in individual loci.

We used PartitionFinder 1.1.1 (Lanfear et al., 2012) with a BIC criterion and a greedy algorithm to find the best partitioning scheme for the data. Accordingly, mitochondrial loci were partitioned by codon position and nuclear loci by gene. We then used ML and Bayesian methods to build trees from the concatenated sequences. ML tree searches were conducted using RAxML 8 (Stamatakis, 2014) implemented through the CIPRES Science Gateway (Miller et al., 2010). Statistical support for the best tree topology was assessed using 1000 non-parametric bootstrap replicates in RAxML. Bayesian tree searches were conducted using MrBayes 3.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) through the CIPRES Science Gateway (Miller et al., 2010). Two parallel MCMC runs were implemented each with four chains of 10,000,000 generations sampled every 1000

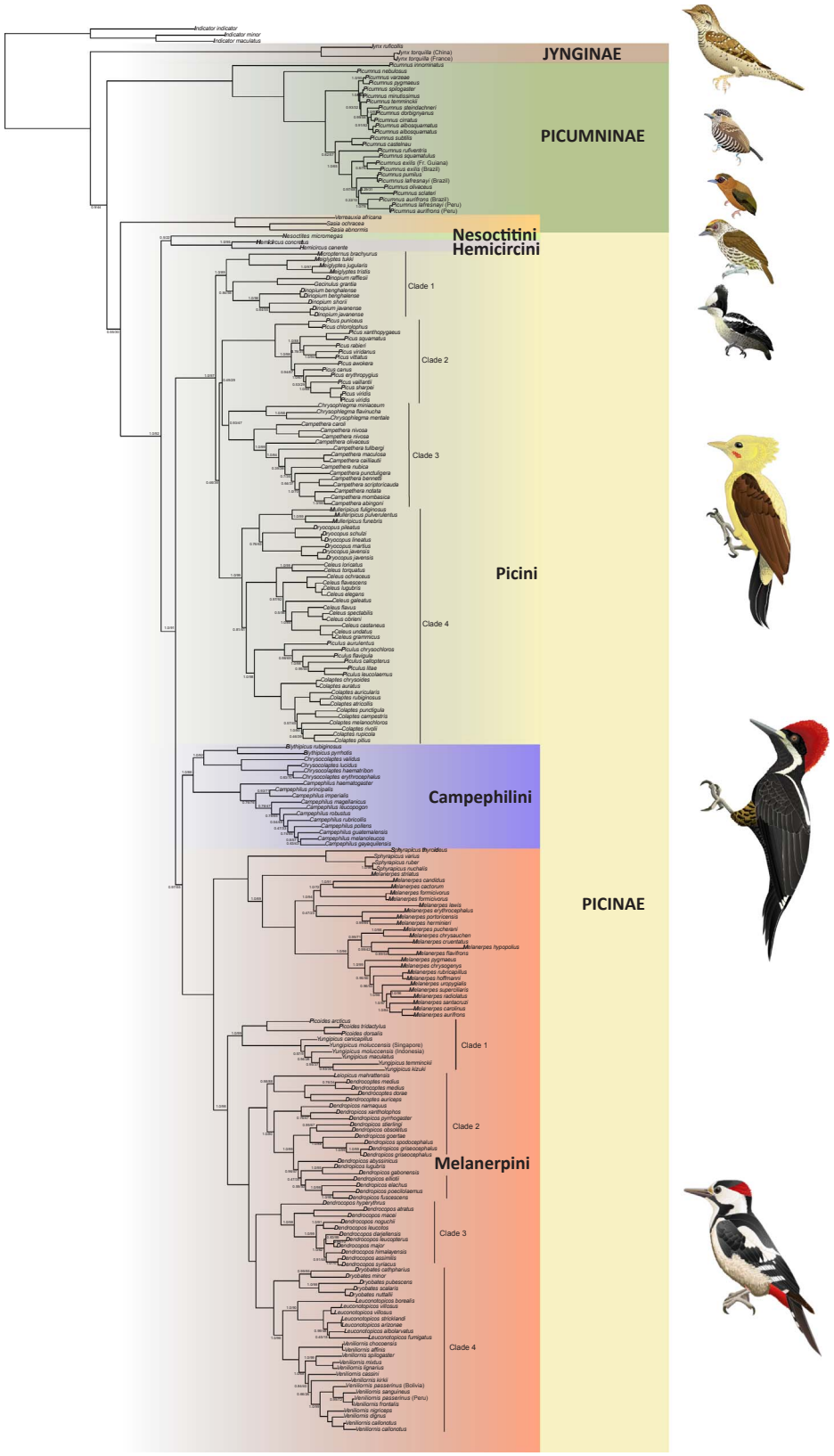


Fig. 1. Bayesian tree showing relationships among woodpeckers based on mtDNA and nuclear sequences. Posterior probabilities less than 1.0 and bootstrap values less than 100% are shown above or below branches, respectively. Subfamily and tribes are indicated by colored shading. Exemplar taxa of each subfamily and tribe are illustrated on the right. (Illustrations by Subir B. Shakya).

generations. We used Tracer 1.6 (Rambaut et al., 2014) to check for convergence among runs. A burnin of 25% was discarded from MrBayes runs and the remaining trees and associated data were summarized into a consensus tree.

We used BEAST 2.3 (Drummond et al., 2006, 2002) to generate a time-calibrated woodpecker tree. Divergence times were estimated using a relaxed clock and log normal distribution under a birth-death speciation model. Because fossils woodpeckers are scarce, we used established nucleotide substitution rates as calibration priors (Lerner et al., 2011). These priors were derived from Hawaiian honeycreepers

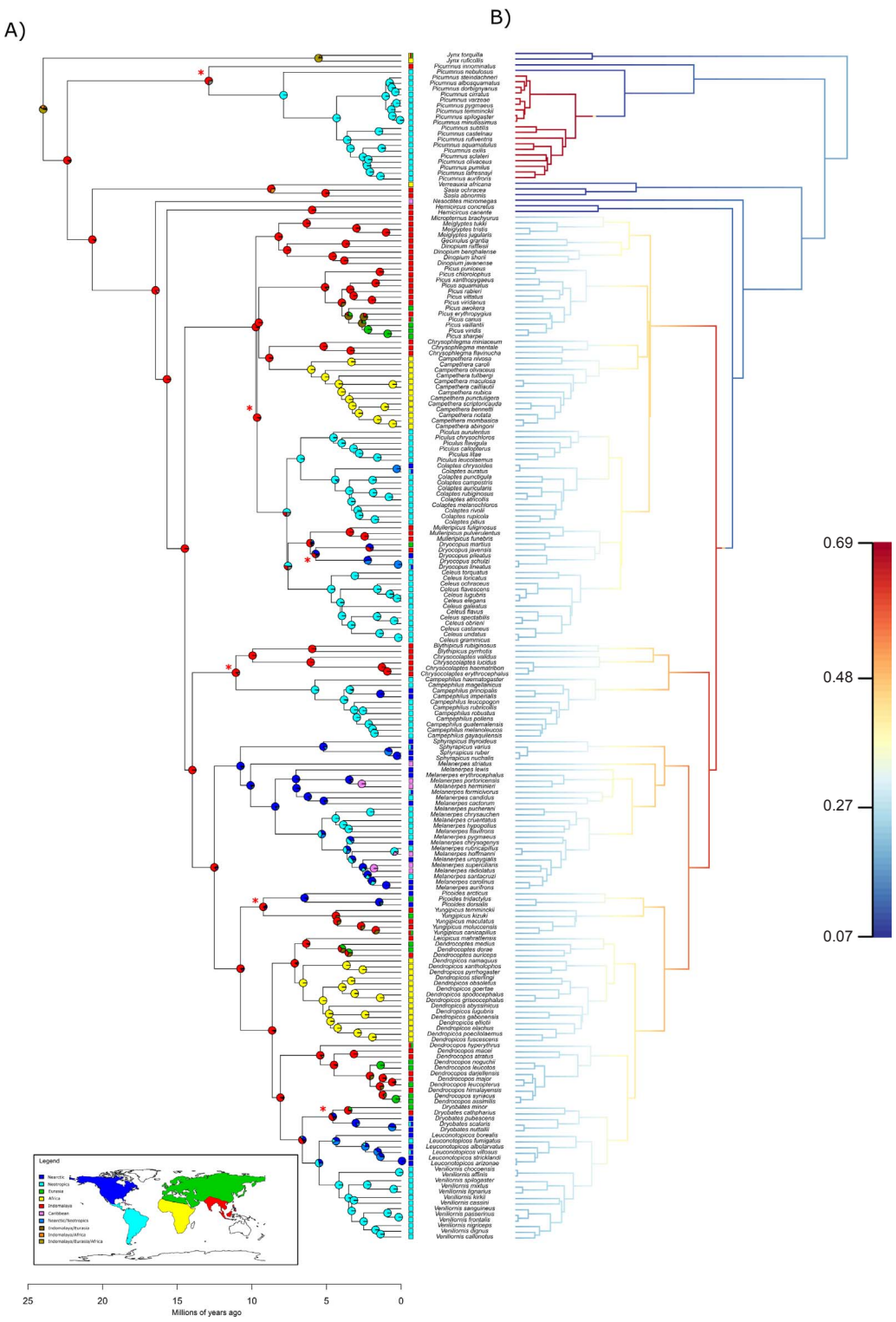


Fig. 2. A) Time-calibrated tree with woodpecker ancestral areas reconstructed in BioGeoBears generated from the DEC + J model. Color-coding is based on regions depicted in the map. * represents New World-Old Word transitions. B) Time-calibrated woodpecker tree with rates of diversification indicated by branch colors, determined using BAMM. (Time-tree calibration based on substitution rates of Lerner et al. (2011)).

and do not necessarily correspond to the rates of woodpeckers. Indeed, analysis by Weir and Schluter (2008) suggests Piciformes (n = 2) may have a slightly higher rate of molecular evolution than other avian lineages. Without fossils and other evidence, we have decided that using an existing calibration is better than using none at all, as long as the potential shortcomings are clear. For mitochondrial loci, we used a fixed substitution rate of 0.0063, 0.0016, and 0.058 for the 1st, 2nd, and 3rd codon positions respectively. For nuclear loci we used a fixed substitution rate of 0.0012 (averaged rate for nuclear loci used in Lerner et al., 2011). We also applied two other calibration points: 22.5 Ma from the fossil *Piculoides saulcetensis* representing the split between *Jynx* from the rest of the Picidae (De Pietri et al., 2011); and 5.0 Ma for the *Sasia abnormis/S. ochracea* split based on biogeographic evidence from the Isthmus of Kra (Fuchs et al., 2006). We used default priors for all other parameters and ran two independent MCMC chains for 50 million generations. Tracer 1.6 (Rambaut et al., 2014) was used to check for convergence among the runs, and TreeAnnotator from the BEAST package was used to generate maximum clade credibility trees after discarding 25% of trees as burn-in.

To estimate rates of diversification within woodpeckers, we used

BAMM 2.5.0 (Rabosky, 2014), performing multiple BAMM runs on the phylogeny for 30 million generations, sampling every 3000 generations. We used setBAMMpriors function in BAMMtools R package to generate prior values for the analysis. We used the BiSSE function as implemented in Diversitree (FitzJohn, 2012; FitzJohn et al., 2009; Maddison et al., 2007), while accounting for missing taxa (see below), to test the hypothesis that temperate species have higher speciation rates than tropical species due to the effect of climatic cycles during the Plio-Pleistocene. Species were coded as binary with no multistate coding allowed (Table S2). Some species (e.g. *Dendropicos fuscescens*) that are distributed in both tropical and temperate areas were coded as distributed in the region representing the largest portion of their distribution. We deemed this approach reasonable because in most cases these species were only marginally present in one or the other of the areas. To account for missing taxa we specified factors of 0.92 and 1, using the *sampling f* option in the makebisse function, representing the proportion of sampled species from tropical and temperate areas in our dataset, respectively. For diversification rate analyses we used the MCC tree from the time-calibrated analysis.

We used BioGeoBears to simulate ancestral area reconstructions for the family. We divided the world into six zoogeographic regions (Fig. 2) using approximate designations in Holt et al. (2013). All woodpecker species were assigned to one or more of the six zoogeographic regions (Table S3). We simulated ancestral area reconstructions on the MCC tree from time-calibrated analyses for all six models implemented in BioGeoBears: DEC, DEC + J, DIVA-like, DIVA-like + J, BayArea, and BayArea + J. Log-likelihoods and AICc scores were compared to determine the best-supported model.

3. Results

3.1. Data and phylogeny

We generated 787 new sequences, which are deposited in GenBank (Accession Numbers: MF766475-MF767261). Some of these are partial ATP6 sequences obtained from toe-pads, e.g., for *Hemicircus concretus* and *Meiglyptes jugularis*. Combined with 211 sequences downloaded from GenBank, the dataset consisted of 998 sequences representing six loci (4403 base pairs) from 203 species of woodpeckers (236 individuals, including multiple individuals of some species; Table S1). We also included sequences from three species of *Indicator* as outgroups.

PartitionFinder identified GTR + G + I as the preferred model for the 1st and 3rd codon positions of the mitochondrial loci (ND2, ND3 and ATP6). HKY + G + I was the preferred model for the 2nd codon position of the mitochondrial loci. For MUSK, HKY + G was the preferred model, and K80 + G was the preferred model for TGF and MB.

Both ML and Bayesian trees indicate that woodpeckers are monophyletic (Figs. S1 and 1). The ML and Bayesian trees are not entirely congruent, but incongruent groups have low bootstrap support (< 70%) and low posterior probabilities (< 0.90). Trees generated from mitochondrial and nuclear loci (Fig. S2) also show similar incongruences. For simplicity, we refer to the Bayesian tree below, unless otherwise indicated (Fig. 1).

All trees place wrynecks (Jynginae) as sister to all other woodpeckers. However, the Bayesian and ML trees conflict regarding the relationships among the piculets (Picumninae) and between the piculets and Picinae. In the Bayesian and ML trees, *Picumnus* and the clade comprising *Sasia* and *Verreauxia* are basal to the Picinae. However, it is unclear whether *Picumnus* and *Sasia/Verreauxia* form a monophyletic group or one or the other is sister to the Picinae. Among the members of *Picumnus,* the single Asian representative, Speckled Piculet (*P. innominatus*), is sister to all Neotropical piculets, and within the latter assemblage the Mottled Piculet (*P. nebulosus*) is sister to the rest. Among several closely related Neotropical *Picumnus* species, however, phylogeographic relationships are unclear and need further study. Some species in which we sampled multiple individuals (e.g. *P. aurifrons* and *P. lafresnayi*) are clearly not monophyletic.

Picinae monophyly is well supported, as is the monophyly of four of the five picine tribes. Nesoctitini (*Nesoctites*) and Hemicercini (*Hemicircus*) are recovered as sister groups, albeit with low support, and they are consistent outgroups to the other tribes. Picini appears to be sister to the clade comprising Campephilini and Melanerpini (BS = 91%; PP = 1.0). However, reciprocal monophyly and a sister-group relationship between Campephilini and Melanerpini, although well supported in the Bayesian tree (PP = 0.98), are not well-supported in the ML tree (BS = 55%). The low support stems, in part, from disagreement between mitochondrial and nuclear ML trees: the mitochondrial tree indicates with low support (BS = 67%) that Campephilini and Melanerpini are each monophyletic and sister taxa, whereas the nuclear data do not support Campephilini monophyly.

For convenience of discussion, Picini may be divided into four main clades (marked in Fig. 1). Clade 1 comprises *Dinopium*, *Gecinulus*, *Meiglyptes* and *Micropternus*, all from South and Southeast Asia. *Micropternus* is sister to *Meiglyptes,* and together they are sister to the clade of *Dinopium* and *Gecinulus. Dinopium,* however, is not monophyletic; *D. rafflesii* is sister to *G. grantia.* Clade 2 consists of the genus *Picus,* a group of medium to large-sized species found in SE Asia and the Palearctic. Clade 3, which is not as strongly supported as the others, comprises two sister groups, the large African genus *Campethera* and the smaller Asian *Chrysophlegma.* Clade 4 consists mostly of New World genera (*Colaptes*, *Piculus*, *Celeus* and part of *Dryocopus*) and some Old World woodpeckers (*Mulleripicus* and the rest of *Dryocopus*). The large-bodied *Mulleripicus* woodpeckers of South and Southeast Asia are sister to *Dryocopus* of the Old and New worlds, and together they may be sister to the remaining three genera of New World Picini (branch support is low). The northern Philippine species *M. funebris* is more closely related to *M. pulverulentus* of the Southeast Asia than to the southern Philippine species *M. fuliginosus.* In the first ever molecular assessment of *D. schulzi*, we find it closely related to *D. lineatus.* Among the remaining genera, *Colaptes* is sister to *Piculus. Celeus* (including the former *Dryocopus galeatus*) is monophyletic, but its sister relationship is unclear.

Campephilini consists of three genera of large-bodied species with an Asian-New World distribution. *Campephilus* occurs in the New World and is sister to the group comprising *Chrysocolaptes* and *Blythipicus* of South and Southeast Asia. Within *Campephilus*, *C. haematogaster* is sister to all the other species.

The predominantly New World Melanerpini consists of a diverse array of species, some of which have consistently confounded taxonomists. Two major clades are evident. The first includes *Sphyrapicus* and *Melanerpes,* both of which are monophyletic. The smallest interspecific distance occurs between *S. nuchalis* and *S. ruber* (ND2 sequences: 0.96% divergence). Within *Melanerpes*, *M. striatus* of Hispaniola is sister to the rest of the genus. The remaining *Melanerpes* are divided into two clades corresponding to the presence or absence of underpart barring, each with seven or eight species.

The second clade within Melanerpini, often referred to as 'Dendropicini', consists of many species with uncertain relationships. Four principal clades are delimited to simplify discussion (marked in Fig. 1). Clade 1 comprises two genera, *Picoides* and *Yungipicus,* and is sister to the remaining three clades. Clade 2 includes the African *Dendropicos,* Asian *Leiopicus,* and Eurasian *Dendrocoptes.* Clade 3 comprises *Dendrocopos,* a well-characterized group from Eurasia. Clade 4 consists of *Dryobates*, *Leuconotopicus,* and *Veniliornis. Dryobates* is sister to *Leuconotopicus* and *Veniliornis*. *Leuconotopicus arizonae* hardly differs from *L. stricklandi* (ND2 sequence 99.90% identical). *Veniliornis* is monophyletic and consists of Central and South American woodpeckers. *Veniliornis affinis* is sister to *V. chocoensis,* and together they are sister to the rest of the genus. *Veniliornis passerinus* is paraphyletic.

The phylogeny in Fig. 2 provides an approximate time scale, but we emphasize that that calibration was based largely on a passerine rate (Lerner et al., 2011), and thus the accuracy of the dates may be unreliable.

Table 1
Support of woodpecker ancestral areas by various models in BioGeoBears.

Model	LNL	Num params	D	E	J	AICc	AICc_wt
DEC	−251.1	2	0.0073	1.0e−12	0	506.2	7.2e−10
DEC + J	−229	3	0.0029	1.0e−12	0.018	464.1	1.00
DIVALIKE	−275	2	0.011	1.8e−09	0	554.1	2.8e−20
DIVALIKE + J	−242.6	3	0.0039	1.0e−12	0.019	491.3	1.2e−06
BAYAREALIKE	−320.7	2	0.0037	0.095	0	645.4	4.4e−40
BAYAREALIKE + J	−250.5	3	0.0025	1.0e−07	0.023	507.2	4.4e−10

3.2. Ancestral area reconstruction and diversification rates

Ancestral areas were simulated using six models in BioGeoBears. DEC+J was supported as the best model (log-likelihood: −229, AICc: 464.1) for our data (Fig. 2a, Table 1). The Old World (Asia + Africa + Europe) was recovered as the family's area of origin. At least six transitions have occurred between the New and Old world.

BAMM analysis identified two shifts in the rate of Picidae diversification as the most-likely configuration (PP = 0.43), followed by (less likely) three rate shifts (PP = 0.20). For the model with two rate shifts, one occurred within the Neotropical piculet clade and the other at the base of the Picinae (Fig. 2b). The results suggest that the rate of diversification is still high within the piculets, but that the Picinae rate slowed after an initial burst. BiSSE analyses suggested that temperate species have slightly higher speciation rates than tropical species (Fig. S3). However, the posterior distributions of the two parameters overlap to such a degree that the rate difference is not significant. Our analyses also revealed that the transition rate from temperate to tropical areas is about twice as likely as the reverse, with very limited overlap in the posterior distribution.

4. Discussion

In terms of taxa and DNA sequences compared, this study represents the most comprehensive molecular effort to date to estimate woodpecker phylogeny, and it substantially improves our understanding of picid intrafamilial relationships. Using the phylogeny (Fig. 1) and time-calibrated trees (Fig. 2), we discuss the biogeographic ramifications of woodpecker diversification patterns, potential evolutionary significance of plumage convergence, and the likely effects of hybridization on woodpecker relationships.

4.1. Phylogeny

Our phylogenetic estimate supports many of the relationships observed in previous studies (Benz et al., 2006; Dufort, 2015; Fuchs et al., 2013, 2008, 2007; Winkler et al., 2014), and it sheds light on relationships of previously unstudied and traditional taxonomically recalcitrant taxa. However, some issues remain unresolved. Here we highlight new discoveries and incongruities, and unresolved problems.

Benz et al. (2006), Dufort (2015) and Fuchs et al. (2006; comparing nuclear sequences) found Picumninae to be monophyletic, whereas Winkler et al. (2014) and Fuchs et al. (2006; comparing mtDNA sequences) found it to be polyphyletic. Our study does not resolve this issue. The Bayesian tree places *Picumnus* as sister to the clade containing *Verreauxia, Sasia* and Picinae (PP = 0.9); the ML tree places *Sasia* and *Verreauxia* as sister to a clade containing *Picumnus* and the Picinae (BS = 47%). Sequencing more *Picumnus* taxa and non-coding nuclear loci, and applying improved models, should help to resolve this issue, if it can be resolved (Poe and Chubb, 2004; Reddy et al., 2017).

Within Picinae, Nesoctitini and Hemicircini each appear to be monophyletic and, in turn, sister to the rest of the subfamily. The remaining Picinae are divided into three clades as follows: (Picini, (Campephilini, Melanerpini)). This arrangement differs from most previous studies. Webb and Moore (2005), Benz et al. (2006), and Winkler et al. (2014) found Melanerpini to be the sister of Picini and Campephilini, whereas Dufort (2015) found Campephilini as sister to Melanerpini and Picini. Our results agree with the tree obtained by Fuchs et al. (2013) when they removed Fibrinogen beta chain intron 7 (*FGB7*) and *MUSK* from their dataset. Fuchs et al. (2013) found a conflict among their gene trees, and suggested that introgression could have played a role in the incongruence. Although we did not compare *FGB7*, we did include *MUSK*. Even so, our results still support the topology Fuchs et al. (2013) produced without *MUSK* and *FGB7*.

Within Picini, *Dinopium* is paraphyletic because *D. rafflesii* is sister to *Gecinulus grantia* Morphologically, *D. rafflesii* resembles other *Dinopium* woodpeckers, except that it has plain brownish rather than black-and-white striped underparts, and its females do not have spotted crests as in *Dinopium*. In respect to these characters, and also wing coloring and red crests, *D. rafflesii* is most similar to *Gecinulus* species.

Our study strongly supports a monophyletic *Dryocopus,* with *Mulleripicus* as its sister group. This finding contrasts with those of Winkler et al. (2014) and a similar arrangement by Dufort (2015), who found that *Mulleripicus* is sister only to the Asian members of *Dryocopus* and not the New World species. Also, *M. funebris* and *M. fuliginosus* of the Philippines are not sister taxa (see Dufort, 2015), even though they were at one time considered conspecific. *M. funebris* is more closely related to *M. pulverulentus* of mainland Southeast Asia than to *M. fuliginosus* of the southern Philippines.

Melanerpine relationships in our study are similar to those obtained by earlier investigators (Benz et al., 2006; Dufort, 2015; Fuchs et al., 2013; Navarro-Sigüenza et al., 2017). The Melanerpini is divided into two clades, each with seven or eight species, corresponding to the presence or absence of underpart barring. This finding matches the result of Navarro-Sigüenza et al. (2017), except for differences in the location of some species between trees (e.g., *M. hypopolius* and *M. superciliaris*). *Leuconotopicus arizonae* is closely related to *L. stricklandi*, despite differences in morphology, behavior, and habitat (Davis, 1965; Ligon, 1968). *Veniliornis affinis* is sister to *V. chocoensis,* and together they are sister of the rest of *Veniliornis.* This arrangement contradicts that of Dufort (2015), who found *V. affinis* to be sister to *V. callonotus* and part of the *V. passerinus* complex.

4.2. Biogeography

Our results confirm one of the most curious features of woodpecker distribution, namely the sister relationship between several Old World and New World taxa (Fig. 2a). We find at least six Old World-New World pairs. These pairings fall into two categories. One group includes species whose members have disjunct distributions in two tropical regions. For example, *Picumnus innominatus* of Southeast Asia is sister to all New World piculets, and no piculets intervene between Manchuria (the northernmost limit of *P. innominatus*) and Central America. A similar pattern occurs in Campephilini; Asian taxa are restricted to the tropical forests of Southeast Asia, and the New World taxa are restricted to Central and South America. The second group of woodpeckers includes such genera as *Dryobates*, *Dryocopus*, *Picoides* (*sensu lato*) and *Leiopicus*, which occur contiguously across the North America and Eurasia.

These trans-continental distributions most likely result from a

combination of multiple dispersal and range contraction events. Vicariance by continental movement is generally too old to account for modern patterns, but Pleistocene glacial events could have split populations between North American and Asia. Most woodpecker groups, even tropical taxa, probably had wider distributions in the Miocene, when tropical climate extended well into the Northern Hemisphere (Morley, 2000). With subsequent cooling of the Earth in the Pliocene, and especially during the glacial cycles of the Pleistocene, many taxa would have been forced to lower latitudes in Asia, Africa, and Central and South America. The resulting tropical groups in the Old and New worlds, and Africa and Southeast Asia, would then have been cut off from one another. Following glacial retreat in the Pleistocene, some of the groups would have reoccupied higher latitudes of the Northern Hemisphere; others would have remained in the tropics. Such taxic movements in response to climate change on a global scale explain the multiple cross-continent relationships among woodpeckers, as well as many other taxa of similar age and distribution, including trogons, barbets, bee-eaters, rollers, mousebirds, todies and motmots (e.g., Feduccia, 1999; Ksepka and Clarke, 2009; Mayr, 2014; Sheldon et al., 2015). In the case of woodpeckers, however, one caveat is that several Northern Hemisphere taxa, unlike obligate tropical families, are cold tolerant, and these taxa probably moved relatively easily along northern corridors connecting the New and Old worlds. Such a scenario is also suggested by our best-supported BioGeoBears model, DEC + J, in which initial dispersal during times of continental interconnectivity is followed by extirpation after climatic cycles in the area around Beringia, leading to two subpopulations that evolved independently (Fig. 2a).

The wide distribution and potential for multiple dispersal events of woodpeckers might imply they are good colonizers. However, woodpeckers have never colonized (or persisted in) Madagascar, New Guinea, Australia, Oceania, and most major oceanic island groups (except the Caribbean, Ryukyu Islands and the Philippines). Only three species, *Mulleripicus fulvus*, *Dendrocopos moluccensis*, and *D. temminckii*, have crossed Wallace's Line, and no species have expanded any further east than Wallacean islands, even though about 29 species occur in the Greater Sundas, just west of the Line. On a smaller geographic scale, it is striking that the green woodpecker (*Picus viridis*) has not colonized Ireland or Corsica, which lie only a few tens of kilometers from source populations, and it does not cross the Strait of Gibraltar, which is only 14 kilometers wide (Pons et al., 2011). The limited propensity of woodpeckers to disperse successfully over water favors scenarios of taxon expansion, contraction, and vicariance in response to climate changes, rather than multiple intercontinental dispersal events to explain their current distribution. This view, however, must be weighed against the possibility that woodpeckers disperse readily, but are inevitably outcompeted by resident species (especially hole-nesters) or depredated in newly colonized areas.

The origin of the family Picidae remains a mystery. Short (1982) suggested that woodpeckers originated in the Neotropics because of the abundance of Neotropical piculets, a presumably ancient group. Early Miocene feathers preserved in amber from the Caribbean (Laybourne et al., 1994) suggest that woodpeckers, perhaps closely related to *Nesoctites,* were present in the New World before most modern woodpecker lineages diversified. However, some of the earliest diverging woodpecker lineages (*Jynx*, *Verreauxia* and *Sasia*) are exclusively Old World forms. Moreover, most piciform fossils are from the Old World, dating to the Oligocene (Cracraft and Morony, 1969; De Pietri et al., 2011). Our BioGeoBears analyses also favored an Old World origin (Fig. 2a), but it is difficult to determine the center of origin from the modern distribution of such a widespread family. That will depend on knowing the origin of higher nonpasserines as a whole (Claramunt and Cracraft, 2015), which in turn will depend on more fossil evidence and improved dating of paleontological events.

4.3. Diversification of woodpeckers

BAMM analysis identified two shifts in rates of woodpecker diversification, one within the Neotropical piculet clade and another early in Picinae evolution (Fig. 2b). The theoretical framework behind BAMM has recently been criticized (Moore et al., 2016; also see Rabosky et al., 2017). Taking this into consideration, we do not to put too much emphasis on the actual values generated by the analysis, but rapid diversification in these two parts of the woodpecker tree makes sense because of numerous branches and short internodes that occur there. These tree sections are also where molecular phylogenetic studies have had the greatest difficulty resolving topology, suggesting the species diverged from one another in a relatively short time.

Because of disparate levels of diversity between (fewer) temperate and (more) tropical woodpeckers, we also compared diversification rates between the two regions. The abundance of species in the tropics has often been attributed to higher speciation rates (Hillebrand, 2004; Ricklefs, 2006; Rolland et al., 2014). However, temperate regions have endured intense climatic cycles during the Plio-Pleistocene, potentially causing more isolation events and higher diversification rates than in the tropics (Botero et al., 2014). Although woodpecker diversity in the tropics is greater than in temperate regions, our data does suggest (albeit not significantly) that speciation rates in the temperate regions have been higher than those in the tropics (Fig. S3).

4.4. Hybridization and introgression within species complexes

Hybridization in woodpeckers is a well-known phenomenon (Howell, 1952; Short, 1965; Winkler and Christie, 2002) and hybrid zones have been studied in several groups: e.g., subspecies of Northern Flicker (*Colaptes auratus*) (Moore and Buchanan, 1985; Short, 1965), sapsuckers (*Sphyrapicus ruber*, *S. nuchalis* and *S. varius*) (Billerman et al., 2016; Howell, 1952; Johnson and Johnson, 1985; Scott et al., 1976), and *Melanerpes* (M. *carolinus/M. aurifrons*) (Smith, 1987). Hybridization has also been noted between piculet species (Dickinson and Remsen, 2013; McCarthy, 2006), and several cases are documented in *Dryobates* and *Leconotopicus* (McCarthy, 2006). Hybridization and differential introgression could have important consequences at the molecular level, especially in obscuring phylogenetic relationships (e.g. Benz and Robbins, 2011; Fuchs et al., 2013). For example, Benz and Robbins (2011) showed that introgression between *Celeus elegans* and *C. lugubris* likely led to incongruent mitochondrial and nuclear gene trees and, thus, problems in discerning relationships between these two taxa.

In our tree, difficulties in resolving relationships among *Picumnus* species are probably due to hybridization. We found (with restricted sampling) that Peruvian *P. lafresnayi* is sister to Peruvian *P. aurifrons*, and Brazilian *P. lafresnayi* is sister to Brazilian *P. pumilus*. Such a result would be expected if the taxa in question have hybridized, although other explanations are possible, such as misidentification caused by morphological convergence. Greater taxic sampling and amounts genetic data will be required work out relationships among several *Picumnus* species. We also encountered cases of likely hybridization in *Veniliornis.* Our mtDNA data indicate that *V. passerinus* of Bolivia is sister to *V. frontalis* (Fig. S2a), whereas *V. passerinus* of Peru is sister to the clade comprising *V. passerinus* (Bolivia), *V. frontalis* and *V. sanguineus*. Nuclear data, however, indicate that the two *V. passerinus* (Bolivia and Peru) are sister taxa (Fig. S2b). This nuclear-mtDNA disagreement suggests introgression between *V. passerinus* and either *V. frontalis* or *V. sanguineus*. As in *Picumnus*, better taxic and genetic sampling are required to investigate the population genetic dynamics of these taxa effectively.

4.5. Convergence, parallelism, or mimicry in woodpeckers

Superficial resemblance of syntopic woodpecker species belonging to different clades is a common occurrence. The most striking example

occurs between two genera, *Chrysocolaptes* (Campephilini) and *Dinopium* (Picini). The smaller *Dinopium* species resemble the larger *Chrysocolaptes* species, and this resemblance is extended even to sexual dimorphism and geographic (subspecific) variation. For example, in Sri Lanka, where the red-backed *C. lucidus stricklandi* replaces the yellow-backed *C. l. guttacristatus* from mainland India, the red-backed *D. benghalense psarodes* replaces the yellow-backed *D. b. benghalense* from the mainland (Freed et al., 2015). Other cases of convergence in plumage occur between *Celeus galeatus* (formerly *Dryocopus galeatus*) and *Dryocopus* species (Benz et al., 2015; Lammertink et al., 2016), *Meiglyptes* and *Hemicircus*, and *Colaptes* and *Piculus* (Benz et al., 2006; Fuchs et al., 2008; Prum, 2014; Short, 1982). In most of these cases, the convergent taxa are not each other's closest relatives, but their ranges coincide. Interestingly, such cases of convergence are not restricted to species of woodpeckers, but also between woodpeckers and other species in other families. For example, the buff-spotted woodpecker (*Campethera nivosa*) of West Africa appears to mimic the spotted honeyguide (*Indicator maculatus*) (O. Johnson and R. Terrill, pers. comm.).

Short (1982) suggested that similarities among woodpecker species evolved because of social dominance and close taxonomic relationships among the similar species. He argued that species look similar to one another to avoid interspecific competition during breeding and nesting seasons. Similarly, Prum (2014) suggested the woodpeckers' version of mimicry (i.e., non-Müllerian, non-Batesian) is caused by social dominance interactions, and he provided a model to explain the process based on the aptly named Hairy-Downy Game, referring to the similar looking hairy (*Leuconotopicus villosus*) and downy (*Dryobates pubescens*) woodpeckers. In this scheme, one species, usually the smaller species mimics the plumage of a second dominant species to avoid attack by the dominant species. He also argued that the smaller species has the advantage of being able to fool predators into believing it is the larger species, thus reducing predatory attacks. A final factor that could drive mimicry, or at least remarkable similarity, is parallel evolution. Closely related species of woodpeckers are expected to share genetic and developmental architecture, allowing the expression of similar plumages, perhaps as simply as turning on a genetic or developmental switch (Adams, 2010; Marroig and Cheverud, 2005; Schluter, 1996). However, for hairy and downy woodpeckers, Weibel and Moore (2005) rejected parallel evolution as an explanation of phenotypic similarity due to strong dissimilarities in juvenile plumage contrasting with strong similarities in adult plumage. The likelihood of parallel evolution harkens back to Short's recognition of mimicry occurring in "close taxonomic" relatives, but most woodpecker mimic-pairs are not especially closely related. Detailed genetic, ecological, and behavioral studies are needed to test these alternative hypotheses explicitly.

4.6. Missing taxa

Fourteen woodpecker species (and a few recently split taxa) were not compared in our study. These missing taxa are mainly members of genera that were otherwise well represented (except *Xiphidiopicus*), and their exclusion from the study should have a minor impact on the overall shape of the woodpecker tree. Nevertheless, their absence prevents the determination of some higher-level relationships.

Five species of *Picumnus* are missing from the study: *P. fuscus*, *P. limae*, *P. fulvescens*, *P. granadensis* and *P. cinnamomeus*. *Picumnus fuscus* is considered a close relative of *P. aurifrons* based on similarities in song (Parker and Rocha, 1991). *Picumnus limae* and *P. fulvescens* are potentially closest to *P. nebulosus,* and *P. granadensis* is probably nearest to *P. olivaceus* (Winkler and Christie, 2002). No closest relative of *P. cinnamomeus* has been suggested (Winkler and Christie, 2002). A sixth taxon, *P. nigropunctatus,* was described by Zimmer and Phelps (1950), who noted that it did not closely-resemble any other species, but was roughly similar to *P. exilis.* Currently it is considered synonymous with *P. e. salvini* (Dickinson and Remsen, 2013).

In the Picini, the missing taxa are: *Dinopium everetti*, *Gecinulus viridis*, *Mulleripicus fulvus*, *Colaptes fernandinae*, *Piculus simplex*, and *Dryocopus hodgei*. We did, however, compare close relatives of most of these species. *Dinopium everetti* was recently split from *D. javanense* (Collar, 2011; Winkler and Christie, 2002). *Gecinulus viridis* is most similar to *G. grantia*, with which it can hybridize (Round et al., 2012), and both are presumably close to *Dinopium rafflesii*. *Mulleripicus fulvus* is either closest to *M. fuliginosus* (Winkler and Christie, 2002) or *M. pulverulentus* (Dufort, 2015). Moore et al. (2011) found that *Colaptes fernandinae* is sister to the rest of *Colaptes*. *Piculus* simplex is thought to form a superspecies with *P. leucolaemus* and '*P.*' *auricularis* (Winkler and Christie, 2002); however, Moore et al. (2011) found '*P.*' *auricularis* to be a member of *Colaptes* not *Piculus*. Thus, the exact position of *P. simplex* remains uncertain. Finally, *Dryocopus hodgei* is closest to *D. javensis* (Dufort, 2015; Winkler and Christie, 2002).

In the Campephilini, *Chrysocolaptes festivus* is missing from our analysis. It is potentially most closely related to *C. lucidus,* along with several other recently split species: *C. xanthocephalus*, *C. strictus*, *C. guttacristatus,* and *C. stricklandi*. The relationships among these taxa have been reviewed recently (see Winkler and Christie, 2002; Collar, 2011; Winkler et al., 2014).

In the Melanerpini, the missing taxa are: *Melanerpes pulcher*, *Xiphidiopicus percussus*, *Veniliornis maculifrons*, *Dendrocopos analis* and *D. ramsayi*. *Melanerpes pulcher* of Columbia's Magdalena Valley was originally considered a subspecies of *M. chrysauchen* and is likely close to that species. *Xiphidiopicus percussus* of Cuba does not resemble any species on the island or adjacent mainland, and its phylogenetic position has been questioned many times. Olson (1972) suggested that it and another distinct woodpecker, *M. striatus*, are close relatives deserving their own genus, *Chryserpes*. Cytochrome-*b* sequencing comparisons also connected *Xiphidopicus* and *Melanerpes* (Overton and Rhoads, 2006). *Veniliornis maculifrons* is considered closely related to *V. passerinus* (Moore et al., 2006). The last two missing species, *D. analis* and *D. ramsayi*, were recently split and are likely close to *D. macei* and *D. maculatus*, respectively (Winkler and Christie, 2002).

4.7. Conclusions

Our estimate of woodpecker phylogeny supports many, if not most, relationships found in previous studies, but also provides new insights, such as the possible polyphyly of the Picumninae and the sister relationships of Picini and Melanerpini/Campephilini. It also indicates that the picinine genus, *Dinopium* is paraphyletic. Analyses of diversification patterns indicate two rate increases, one within the Neotropical piculet clade and another early in picine evolution. Also, temperate species may have a slightly higher rate of diversification than tropical species but this difference was not statistically significant. Analyses of biogeographic patterns indicate that at least six instances of New World/Old World sister relationships occur in the family, and the likely center of woodpecker origin is the Old World.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2017.09.005.

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