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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