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Complex biogeographic scenarios revealed in the diversification of the largest woodpecker radiation in the New World

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ABSTRACT

Phylogenetic relationships and patterns of evolution within *Melanerpes*, one of the most diverse groups of New World woodpeckers (22–23 lineages), have been complicated due to complex plumages and morphological adaptations. In an attempt to resolve these issues, we obtained sequence data from four nuclear introns and two mitochondrial protein-coding genes for 22 of the 24 currently recognized species in the genus. We performed phylogenetic analyses involving Maximum Likelihood and Bayesian Inference, species-tree divergence dating, and biogeographic reconstructions. Tree topologies from the concatenated and species-tree analyses of the mtDNA and nDNA showed broadly similar patterns, with three relatively well-supported groups apparent: (a) the *Sphyrapicus* clade (four species); (b) the typical *Melanerpes* clade, which includes temperate and subtropical dry forest black-backed species; and (c) the mostly barred-backed species, here referred to as the "*Centurus*" clade. The phylogenetic position of *Melanerpes superciliaris* regarding the rest of *Melanerpes* is ambiguous as it is recovered as sister to the rest of *Melanerpes* or as sister to a group including *Sphyrapicus* + *Melanerpes*. Our species tree estimations recovered the same well-delimited highly-supported clades. Geographic range evolution (estimated in BioGeoBEARS) was best explained by a DIVALIKE + j model, which includes vicariance, founder effect speciation, and anagenetic dispersal (range expansion) as important processes involved in the diversification of the largest radiation of woodpeckers in the New World.

1. Introduction

The Great American Biotic Interchange (GABI) is a series of major biogeographic events with profound effects on New World's diversity (Simpson, 1980). Taxa dispersed between North and South America producing multiple range expansion, speciation, and extinction events during the closure of the Panamanian land bridge (Stehli and Webb, 1985). In recent years, time-calibrated phylogenies have aided to recognize the bridge closure as the fundamental GABI driver for many groups with different fossilization and dispersal capabilities (Smith and Klicka, 2010), and the documentation of cases of pre-closure dispersal events have indicated that GABI was a long and complex process dating back to the Oligocene-Miocene transition (Bacon et al., 2015).

Birds have shed light into the importance of the final GABI bridge closure for forest species, as well as on causes for temporal and ecological disagreements (Weir et al., 2009; Smith and Klicka, 2010). One less well-known aspect is the connection between North America, South America and the Caribbean, and whether islands played a significant role during GABI. For several passerine lineages, Hispaniola has been suggested as an ancestral area (Spellman et al., 2008; Lovette et al., 2010) with posterior events of colonization to both North and South America. For others, Caribbean endemics may represent relicts of ancient lineages that likely went extinct on the mainland (Benz et al., 2006; Barker et al., 2012, 2015). Thus, the Caribbean is a composite New World region with species from multiple origins with a complex biogeographic history including vicariance, land bridges, and overwater dispersal (Bond, 1948, 1963; Vázquez-Miranda et al., 2007).

Woodpeckers (Aves: Piciformes: Picidae) are a group of birds with a high species diversity, including 250 from 30 genera with

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a significant representation in the New World (e.g., Short, 1982; del Hoyo and Collar, 2014; Gill and Donsker, 2017). Most morphological and molecular analyses have demonstrated that Picidae is monophyletic (Webb and Moore, 2005; Benz et al., 2006; Fuchs et al., 2007; Dufort, 2016), with an evolutionary origin in the tropical regions of Eurasia in the mid-Tertiary, 45 million years ago (Ma), while the main diversification of modern lineages began about 13.4 ± 1.7 Ma (Benz et al., 2006; Fuchs et al., 2007; Dufort, 2016). In other studies, Neotropics and Afrotropics have been estimated as ancestral areas of distribution for woodpeckers and other Piciformes at approximately 50 Ma (Claramunt and Cracraft, 2015), however, particular biogeographic processes, such as dispersal to North America and the Caribbean are still largely unexplored.

Moreover, the recent increase in molecular systematic studies of woodpeckers (e.g., Benz et al., 2006; Fuchs et al., 2007, 2013; Dufort, 2016) have allowed a higher resolution in different taxonomic levels within the family, and have commonly shown that previously proposed phylogenetic relationships were incorrect (e.g., Benz et al., 2015). In spite of this, a major bias has been that few molecular studies in Picidae have included extensive species-level taxonomic sampling (but see Dufort, 2016); or in the case of morphological studies, some characters, such as coloration, have proved to be adaptations or convergences due to similar habitats or either as a result of social mimicry, leading to inconsistencies in the previous proposed phylogenies (e.g., Winkler et al., 1995; Winkler and Christie, 2002; Weibel and Moore, 2005; Fuchs et al., 2007; García-Trejo et al., 2009; Benz and Robbins, 2011). Thus, many subgroups within Picidae are not monophyletic (Dufort, 2016).

1.1. Study group

Species in the genus *Melanerpes*, one of the most diverse groups of woodpeckers, include approximately 22–24 lineages widely distributed in the New World, ranging from southeastern Canada to northern Argentina and the Caribbean islands. They inhabit deserts, tropical and temperate forests, and are ubiquitous in urban settings (Fig. S1.1 in Appendix 1; Gill and Donsker, 2017). However, complex plumages and morphological adaptations have made the assessment of phylogenetic relationships difficult. In addition, they are regarded as generalists because of a broad food flexibility, a condition that has been considered ancestral (Winkler and Christie, 2002). These hypotheses are supported by both the study of genes and osteology (Goodge, 1972; Webb and Moore, 2005), where *Melanerpes* and its putative sister group *Sphyrapicus* appeared as basal within Picinae (Short, 1982; Webb and Moore, 2005).

The genus *Melanerpes*, as well as the closely related genera *Sphyrapicus* and *Xiphidiopicus* (Overton and Rhoads, 2004), are all included in the tribe Melanerpini (*sensu* Winkler et al., 2014), which is restricted to the New World (Fig. S1.2). Geographic distribution of most species in this tribe is centered in the northern part of the American continent: all four species of *Sphyrapicus* (breeding distribution only) are distributed in temperate North America. The greatest diversity of *Melanerpes* (up to eight species) occurs in Mesoamerica, and *Xiphidiopicus* is restricted to Cuba and nearby islands (Winkler and Christie, 2002; del Hoyo and Collar, 2014). These distributional patterns, together with the distribution of fossils of both *Melanerpes* and *Sphyrapicus*, as well as other related fossil forms (e.g., Cracraft and Morony, 1969), suggest that Melanerpini (*sensu* Winkler et al., 2014) is of North American origin: fossils from the Middle Pleistocene have been found in North America, whereas fossils from the Late Pleistocene have been located in Brazil (Vuilleumier, 1984, 1985).

The biogeography of the insular taxa of *Melanerpes* (including *Centurus*) seems to be problematic. One idea is that Caribbean *Melanerpes* are expected to have a North American origin; however, this is ambiguous, given that some species are morphologically closer to the *Centurus* taxa in Mesoamerica (Bond, 1963, 1966), thus suggesting at least two dispersal events. Bond (1979) later suggested that the five species of Melanerpini in the Caribbean islands derived from at least five independent colonization events. Others have suggested instead that certain groups of birds (woodpeckers included) in the Caribbean may belong to monophyletic groups (see Ricklefs and Bermingham, 2001), implying a single colonization event and further differentiation, probably due to insular allopatric conditions in the region.

In an attempt to resolve the issues regarding the evolutionary relationships and historical biogeography of species currently assigned to the genus *Melanerpes*, we obtained sequence data from four nuclear introns and two mitochondrial protein-coding genes for 95% of currently recognized species in the genus (Supporting Information, Appendix 1). Specifically, and based on the phylogenetic framework, we tested the following biogeographic hypotheses: (1) a North American origin for Melanerpini (*sensu* Winkler et al., 2014), (2) a North American origin for the broad *Melanerpes* clade, (3) *Melanerpes* taxa in the Caribbean islands are derived from independent colonization events, and (4) diversification in *Melanerpes*, the largest radiation of woodpeckers in the New World, is related to GABI.

We performed phylogenetic analyses involving concatenation, species trees, divergence dating, and biogeographic reconstruction. Based on these analyses we provide scenarios for diversification and biogeography of the taxon that resulted in the current diversity, and briefly address the taxonomic implications.

2. Materials and methods

2.1. Taxon sampling

Tissue samples loans from the majority of the currently assigned species to *Melanerpes* were obtained from eight different scientific collections (see Appendix 1 and Acknowledgements). In all, we obtained 88 samples, 82 of which corresponded to 22 of the 24 recognized *Melanerpes* species; whenever possible, our sampling included more than two individuals per species. This intraspecific sampling scheme allowed us to assess species limits in some taxa with ample geographic variation (e.g., *Melanerpes aurifrons*). We only lack tissue samples from *M. pulcher* – a Colombian endemic that has been either considered a subspecies of *M. chrysauchen* (e.g. Short, 1982; Winkler et al., 1995) or a full species (del Hoyo and Collar, 2014) –, and from *M. flavifrons*, from the Atlantic Forest, for which we used published sequences. We also included four samples from the closely related genus *Sphyrapicus* (e.g. DeFilippis and Moore, 2000; Webb and Moore, 2005; Dufort, 2016). We did not include the Cuban endemic *X. percussus*, which in a previous molecular study, was found to be the sister taxon to *Melanerpes* (Overton and Rhoads, 2004). Although some mitochondrial CYTB sequences are available in GenBank, there are some concerns on their validity (Dufort, 2016).

For rooting purposes, we selected another member of the Melanerpini: *Dryobates [Picoides] scalaris*; as well as *Dryocopus lineatus*, a non-melanerpine taxon as outgroups, both members of taxonomic groups closely related to *Melanerpes* (Benz et al., 2006; Overton and Rhoads, 2004; Fuchs et al., 2013; Dufort, 2016).

2.2. Laboratory procedures

We extracted genomic DNA from all species from frozen or alcohol preserved tissues (muscle, heart or liver) using the DNeasy Extraction Kit (Qiagen), following the manufacturer's protocol. We targeted two protein-coding mitochondrial genes: ND2 - NAD dehydrogenase subunit 2, and CYTB - Cytochrome *B*; two autosomal introns: TGFB2 - Transforming growth factor 2 beta

subunit, intron 5, FIB7 - Beta fibrinogen, intron 7; and two z-linked introns: MUSK – muscle skeletal receptor tyrosine kinase gene, intron 3, and ACO1 – Aconitase 1, intron 9; for a total of five independent loci. These molecular markers were amplified via PCR (Polymerase chain reaction), in 20–25.0 μL reactions using GoTaq Green Mastermix (Promega, Madison, WI, USA) and Taq DNA polymerase (Vivantis Technologies, Selangor, Malaysia), using primers described in Table S1.1 and standard thermocycler protocols. Amplicons were purified using Agencourt AMPure PCR purification beads (Beckman-Coulter, Fullerton, CA, USA) following manufacturer's instructions. Sequencing was carried out using the BigDye v3.1 termination protocol (Applied Biosystems, Foster City, CA, USA) in an ABI 3730xl automated Sanger sequencer at the Beckman-Coulter Genomics Center (Danvers, MA, USA); as well as at the High-Throughput Genomics Unit Service of the University of Washington. Assembly of complementary strands and edition of chromatograms was carried out in GENEIOUS 7.0.6 (Biomatters, http://www.geneious.com/) and SEQUENCHER 4.7 (Gene-codes Inc., Ann Arbor, MI, USA). Each edited sequence was verified to be of avian origin and the correct locus by comparing them to reference sequences in the NCBI GenBank database using BLAST searches. Protein-coding genes (ND2 and CYTB) were checked for stop codons in the EMBOSS-TRANSEQ site (http://www.ebi.ac.uk/Tools/st/emboss_transeq/). Due to smaller effective population size (Ohta, 1972), birds and other taxa in islands are known to have higher rates of molecular evolution and substitutions under selection compared to mainland counterparts (Johnson and Seger, 2001; Smith and Klicka, 2013) that could lead to biased phylogenetic inferences. Protein-coding sequences were thus tested for selection signatures using four tests: Codon-based Fisher exact test of positive selection (Zhang et al., 1997), Tajima's D neutrality test (Tajima, 1989), Single Likelihood Ancestor Counting method (SLAC, Pond and Frost, 2005), and Mixed Effects Model of Evolution (MEME, Murrell et al., 2012). The first two are implemented in MEGA5 (Tamura et al., 2011) and the last two in HyPhy (Pond and Muse, 2005) through its online portal (Delport et al., 2010; http://datamonkey.org). These methods rely on calculating ω, also known as the dN/dS ratio (number of non-synonymous substitutions/number of synonymous substitutions; Kimura, 1977). Significant selection signatures were determined by P-values < 0.05 after a false discovery rate correction (FDR) for multiple comparisons in R3.1. When a codon was found to be under selection, it was tested for functional protein changes in PROVEAN (Choi and Chan, 2015) using a Genbank NCBI 2012 database and a threshold cutoff of −2.5 to detect deleterious or neutral protein changes. All sequences were aligned using MAFFT (Katoh et al., 2005), as implemented in Geneious 7.0.6 (Biomatters, http://www.geneious.com/), and CLUSTALX2 (Larkin et al., 2007) using default settings. Alleles bearing heterozygote sites were separated using *in silico* phasing in PHASE 2.1 (Stephens et al., 2001) by interconverting the original aligned FASTA files in SEQPHASE (Flot, 2010), running 100,000 generations with a burn-in of 10,000 using a 0.7 posterior probability. If alleles were under that probability threshold, we took one phased allele at random from the posterior following Ruegg et al. (2010). We tested for recombination between loci employing the Phi method (Bruen et al., 2006) in the program SPLITSTREE 4.3 (Huson and Bryant, 2006).

2.3. Phylogenetic inference

As our study includes both nuclear (nDNA) and mitochondrial loci (mtDNA), the probable effect of conflicting phylogenetic signal among these markers (Maddison, 1997; Edwards et al., 2007; Degnan and Rosenberg, 2009; Heled and Drummond, 2010) was explored by conducting separated analyses. These analyses showed different levels of support for the terminal branches, but both recovered a *Melanerpes* + *Sphyrapicus* monophyletic group. We therefore also conducted a concatenated analyses, as it has been suggested that nDNA genes may resolve phylogenetic relationships at deeper levels (Lin and Danforth, 2004). Analyses were thus based on separated datasets: concatenated (mtDNA + nDNA), mtDNA, and nDNA, as well as on individual nuclear loci. On all of these datasets, models of substitution and the appropriated partition schemes were selected using PARTITIONFINDER (Lanfear et al., 2012). For selection of both substitution models and partitioning schemes, we followed a strategy in which all models and partitions are compared and selected using the Bayesian Information Criterion, which favors less-parameterized schemes (Minin et al., 2003; Abdo et al., 2005). Partition schemes and selected models (Table 1) were used for analyses on each dataset as described below, except when otherwise stated. Trees were rooted using outgroups previously mentioned (Benz et al., 2006; Overton and Rhoads, 2004; Fuchs et al., 2013; Dufort, 2016).

Each dataset was analyzed using both Maximum Likelihood analysis (ML) as implemented in RAxML 7.2.6 (Stamatakis, 2006; Stamatakis et al., 2008) and Bayesian Inference (BI), using MRBAYES 3.2.6 (Ronquist et al., 2012). For ML analyses, we used the same partitioning schemes used in Bayesian analyses; all partitions were assigned the GTR + G model (Stamatakis et al., 2008) and support for the obtained clades was assessed with 1000 bootstrap replicates. For Bayesian analyses, we used eight Markov chain Monte Carlo (MCMC), which were run for 10^7 generations and sampling parameters every 1000 generations. Convergence and stationarity of the MCMC runs were assessed in two different ways. First, we used the average standard deviation of split frequencies (ASDSF), with 0.01 as an acceptable value for congruence between independent runs, as implemented in MRBAYES 3.2.6 (Ronquist et al., 2012). For the second, we used TRACER 1.5 (Rambaut and Drummond, 2007), in which values greater than 200 in the effective sample sizes (ESS; in our analyses, most were in the thousands) are obtained after stationarity and congruence are reached.

2.4. Species trees

For species tree estimation, we used the multispecies coalescent model (Heled and Drummond, 2010) implemented in *BEAST 1.8.2 (Drummond et al., 2012), as well as the quartet species-tree method for SNPs (single-nucleotide polymorphisms) singular value decomposition (Chifman and Kubatko, 2014) in SVDQUARTETS as implemented in PAUP*4.0a146 (Swofford, 2002). As species-tree methods require terminal taxa to be assigned a priori (Liu et al., 2009), we imposed 27 taxa (Including species in *Sphyrapicus*) from Winkler et al. (1995) to each individual allele. In order to reduce the complexity in species tree estimation and increase parameter convergence probability, we sampled two individuals and their respective haplotypes and alleles, since calculations do not benefit from adding extra individuals over number of loci (Drummond and Bouckaert, 2015). *BEAST species trees were run for 300×10^6 generations with a 10% burn-in sampling every 300,000 generations, under a Birth-Death speciation tree prior, and uncorrelated lognormal relaxed clocks for each locus. Maximum clade credibility species trees were summarized in TREEANNOTATOR 1.8 (Drummond et al., 2012). All Bayesian analyses were checked for convergence and ESS in TRACER 1.5 (Rambaut and Drummond, 2007; http://tree.bio.ed.ac.uk/software/tracer/).

We time-calibrated the species tree with the following avian substitution rates: ND2 (0.013 substitutions/lineage/million years; Arbogast et al., 2006); CYTB (0.01 substitutions/lineage/million years; Lovette, 2004); sex-linked loci (0.00195 substitutions/lineage/million years; Axelsson et al., 2004), and autosomal loci (0.00184 substitutions/lineage/million years; Axelsson et al., 2004), using a standard deviation of 0.45 and 0.1 for mtDNA and

Table 1
Partition schemes and models of substitution estimated for the phylogenetic analyses.

Gene	Length (bp)	Sequences sampled	Parsimony uninformative	Parsimony informative	Partition	Selected model
ND2	1041	88	55	486	1st Codon	HKY + I + G
					2nd Codon	HKY + I + G
					3rd Codon	GTR + G
Cytb	1051	88	36	399	1st Codon	GTR + G
					2nd Codon	K80 + I + G
					3rd Codon	HKY + I + G
MUSK	496	62	50	42	Musk	HKY + I + G
TGFB2	536	80	38	48	TGFB	HKY + I + G
ACO1	1092	27	95	52	Aco	HKY + I + G
FIB7	857	30	62	43	Fib7	HKY + I + G

nuclear loci, respectively (Smith and Klicka, 2010) for relaxed clock calibrations. Following Axelsson et al. (2004) and other avian general rates would suggest that Piciformes have similar evolutionary patterns to Galliformes, Palaeognathae, and other early lineage divergences in avian evolution, an assumption that would not correspond to higher substitution rates seen at the genomic level across Australaves and Coraciimorphae (Hackett et al., 2008; Jarvis et al., 2014; Prum et al., 2015). Woodpeckers however, have demographic and molecular patterns similar to Passeriformes that have shaped their evolutionary rates (Moore and DeFilippis, 1997). Thus, we also calibrated trees using locus-specific substitution rates (substitutions/lineage/million years) estimated from Hawaiian honeycreepers (Lerner et al., 2011): ND2 – 0.0145, CYTB – 0.007, TGFB – 0.00085, and FIB7 – 0.00095. There were no Z-linked loci surveyed for honeycreepers, however most rates were 50% of those from general rates (Lerner et al., 2011); therefore we halved the general avian Z-linked rate for ACOI and MUSK (0.000975).

To account for different topologies between concatenated methods and species trees during chronogram estimation, we constrained the ML/BI topology in BEAST using the same number of generations stated previously, with a starting ultrametric tree calibrated with time constrains from Dufort (2016); the product of eight fossil and geologic age constraints) using penalized likelihood (Sanderson, 2002) as implemented in the R package APE (Paradis et al., 2004) with eight different lambda values, a tolerance level of 1×10^{-8} and 500 iterations for cross validation.

SVDQUARTETS trees were obtained by SNPs from our nuclear loci evaluating all possible quartets with quartet Fiduccia-Mattheysses (QFM) amalgamation tree inference, multispecies coalescent tree model, and 1000 bootstrap replicates to assess significance. Bayesian phylogenies are usually depicted as a majority-rule consensus or maximum-clade credibility trees calculated from the posterior credibility tree distribution after burn-in. As those single-tree summaries often do not portray the complex variation of the posterior tree distribution due to gene-tree discordance, we supplemented our Bayesian phylogenies with posterior density 'cloudograms'. Cloudograms reveal dominant topologies and delimiting of clades regardless of allele sorting at different stages in the speciation process (Bouckaert, 2010). We constructed species-trees cloudograms with the posterior clade distribution with 'phangorn' (Schliep, 2011) in R 3.1 (R Core Team, 2014).

Branch support was estimated using a highly supported node (HSN) index (Vázquez-Miranda, 2014), with a custom function in R 3.1 (R Core Team, 2014). Briefly, this index calculates the number of nodes on a given tree with high statistical support over the total numbers of nodes. The HSN index goes from 0 to 1; 0 indicates that none of the nodes on a tree have significant support, and conversely a value of 1 indicates all nodes on a tree have significant support. We used 70% likelihood bootstrap replicates (Hillis and Bull, 1993) and 0.95 of posterior probability (Huelsenbeck et al., 2001) as thresholds.

2.5. Biogeographic history

Geographic range evolution in *Melanerpes* was estimated from our *BEAST concatenated tree using 'BioGeoBEARS' (BioGeography with Bayesian (and likelihood) Evolutionary Analysis in R Scripts; Matzke, 2013; http://cran.rproject.org/web/packages/BioGeoBEARS/index.html); which implements three different biogeographic models in a likelihood framework. Models implemented include DEC (Dispersal-extinction-cladogenesis; Ree and Smith, 2008), DIVALIKE (a likelihood version of DIVA; Ronquist, 1997), and BAYAREALIKE (a likelihood version of BAYAREA (Landis et al., 2013), all of which estimate rates of dispersal, extinction, cladogenesis, and vicariance. Additionally, a free parameter for the estimation of founder event speciation (+ *j*) can be added to any of the previous models included, creating DEC + *j*, DIVALIKE + *j*, and BAYAREALIKE + *j* models. All six models are fitted to the data, and the selection of the best model is accomplished through comparisons of their likelihood values and also using the Akaike Information Criterion (AIC) (Table 2). Results from BiogeoBEARS are visualized in charts showing either the single most-probable ancestral range at each node or as pie charts showing probabilities for all estimated ranges representing the average of all possible biogeographic histories under the selected model. Ambiguous probabilities in the nodes may be approached through Bayesian stochastic mapping (Nielsen, 2002; Ree, 2005; Revell, 2011), which samples the most probable biogeographic history along the different branches from a series of simulations depending on the dated tree, the selected model, the parameter estimates, and the observed geographic range (Landis et al., 2013). Simulations were run for calculation of 50 biogeographic stochastic maps under the model selected: DIVALIKE + *j*.

Table 2
Summary statistics for model selection in BioGeoBEARS. Abbreviations: log-likelihood (LnL), dispersal parameter (*d*), extinction parameter (*e*), founder effect parameter (*j*), and Akaike Information Criterion (AIC).

Model	LnL	# Parameters	*d*	*e*	*j*	AIC	AIC_wt
DEC	−93.91	2	0.012	0.012	0	191.8	1.10E−06
DEC + J	−81.28	3	0.0061	1.00E−12	0.067	168.6	0.12
DIVALIKE	−90.52	2	0.013	1.00E−12	0	185	3.30E−05
DIVALIKE + J	−79.4	3	0.0066	1.00E−12	0.053	164.8	0.82
BAYAREALIKE	−110.8	2	0.021	0.16	0	225.6	5.20E−14
BAYAREALIKE + J	−82.04	3	0.0052	1.00E−07	0.075	170.1	0.058

Based in the biogeographic divisions of Stotz et al. (1996), we defined nine geographic regions for our biogeographic analysis: (A) Boreal forests (mostly temperate forests on North America, north of Mexico), (B) Tropical Highland temperate forests (mountains from Mexico to the Andes of Colombia), (C) Tropical Lowland humid forests (eastern México to Venezuela and northwestern Ecuador), (D) Tropical Lowland Dry Forests (northwestern Mexico to northwestern Costa Rica; this area includes the Balsas Depression in Mexico), (E) Amazonia, (F) the Atlantic Forest, (G) the Central South America dry forests, (H) the Great Plains and southwestern North America deserts, and (I) the Caribbean islands.

3. Results

3.1. Sequence characteristics

Our concatenated alignment included 5073 base pairs (bp) from 2 mitochondrial and 4 nuclear genes. Of the total base pairs, 326 bp were parsimony-uninformative and 1070 bp were variable. Sequence characteristics as well as the number of sequenced samples per gene for the study group are shown in Table 1. All sequences are deposited in GenBank under accession numbers (KY284095-KY284153, KY288519-KY288613, KY302734-KY302800, KY313419-KY313431, and KY320184-KY320197).

We found no evidence of recombination in the six genes we sequenced (Phi test, *P*-values = 0.2–0.9 range). All of our tests of selection on protein-coding sequences did not find evidence for positive or diversifying selection. For CYTB (mean dN/dS ratio = 0.04, codon model fit LogL = −7091.65) paired *P*-values for the codon-based Fisher Exact Test were non-significant ($P = 1.0$), and Tajima's D was not significantly negative (1.30). MEME found two codons with signatures of diversifying selection but after a FDR correction these were not significant ($P > 0.72$). SLAC found no codons under positive selection. For ND2 (mean dN/dS ratio = 0.09, codon model fit LogL = −8468.08) paired *P*-values for the codon-based Fisher Exact Test were non-significant ($P = 1.0$), and Tajima's D was not significantly negative (1.09). MEME found eight codons with signatures of diversifying selection but after a FDR correction these were not significant ($P > 0.81$). SLAC found one codon (160) with non-synonymous changes (Threonine [T] to either Alanine [A] or Methionine [M]) suggesting a signature of positive selection ($P = 0.02$) but was not significant after a FDR correction ($P = 1.0$). PROVEAN detected no deleterious changes in either case (T > A = 0.59; T > M = 0.80). Several insertions and deletions (indels) were noted in our nDNA sequences. For FIB7, a 1-bp deletion was shared between our outgroup species, the *Sphyrapicus* species, *M. striatus*, all of the typical *Melanerpes* clade (see below), and *M. hypopolius*; four other deletions (4-bp non-consecutive) were shared between species in the ingroup regarding the outgroup (Fig. S1.3). In MUSK (Fig. S1.3), a 13-bp deletion was exclusive of *Sphyrapicus*; a 21-bp deletion united all *Melanerpes* species; a 10-bp deletion was shared between the ingroup species and *Dryobates scalaris*; and a 4-bp insertion was exclusive of *M. flavifrons*. In TGFB2, a deletion of 4-bp was shared between *M. carolinus* and *M. aurifrons*, a similar 4-bp deletion is an autapomorphy for the *Sphyrapicus* clade, and a 1-bp insertion is exclusive of *M. chrysogenys* (Fig. S1.5). Finally, in ACO1, a 6 bp insertion was detected in our sample of *M. carolinus*, relative to both the rest of ingroup and also the outgroup species (Fig. S1.6).

3.2. Phylogeny

Tree topologies from the phylogenetic analyses of the individual mtDNA (Fig. S1.7) and the combined nDNA (all 4 genes, Fig. S1.8), as well as the concatenated (mtDNA + nDNA) showed broad similarities, except for the position of *M. striatus* and species in the genus *Sphyrapicus* (Fig. 1). The phylogenetic position of *M. striatus* was ambiguous in these analyses: in the mDNA topology, samples of this taxon appear as a well-supported monophyletic group (Fig. S1.7), but either as a poorly-supported paraphyletic group in the ML nDNA topology (Fig. S1.8), or as monophyletic but poorly-supported in the BI (pp = 0.89); this discrepancy may be due to incomplete lineage sorting in nuclear markers (Price, 2008; Zink and Barrowclough, 2008). On the other hand, *M. striatus* was recovered as a well-supported monophyletic group in the concatenated analyses (mtDNA + nDNA), thus reinforcing that *Melanerpes* is a non-monophyletic genus, agreeing with previous results (see Dufort, 2016).

Despite these alternative locations of both *M. striatus* and the *Sphyrapicus* clade, three relatively well-supported groups were apparent in all of our phylogenetic analyses. These groups included: (a) the *Sphyrapicus* clade (four species); (b) the typical *Melanerpes* clade, which includes temperate and subtropical dry forest black-backed species (*M. candidus*, *M. lewis*, *M. herminieri*, *M. portoricensis*, *M. erythrocephalus*, *M. formicivorus*, and *M. cactorum*; and (c) a monophyletic group here referred to as the "*Centurus*" clade, which includes mostly barred-backed species (*M. hypopolius*, *M. radiolatus*, *M. chrysogenys*, *M. pygmaeus*, *M. rubricapillus*, *M. uropygialis*, *M. carolinus*, *M. superciliaris*, *M. santacruzi*, *M. aurifrons*, and *M. hoffmannii*), and black-backed species from humid tropical lowland forest (*M. pucherani*, *M. cruentatus*, *M. flavifrons*, and putatively *M. chrysauchen*). Both the *Melanerpes* and "*Centurus*" clades roughly correspond to formerly recognized genera (Peters, 1948; Selander and Giller, 1963). Further subdivisions were recovered within the "*Centurus*" clade, in which three additional monophyletic groups were apparent: (1) a clade including all samples of *M. hypopolius*; (2) a clade with all the barred-backed species from highly seasonal habitats (hereafter the *Centurus* clade); and (3) another clade with all mostly black-backed species from humid tropical lowland forests (hereafter the *Tripsurus* clade). *Centurus* and *Tripsurus* were recovered as sister, with *M. hypopolius* samples sister to both.

Some of the individual nDNA topologies also supported some of the groups described above. In all of the four nuclear genes in our dataset (ACO1, TGFB2, FIB7, and MUSK), the *Sphyrapicus* clade was recovered consistently, indicating a deep genetic differentiation regarding the broad *Melanerpes* clade. Samples of *M. striatus* also appeared as monophyletic, suggesting deep genetic differentiation from both *Sphyrapicus* and the broad *Melanerpes* clade. Other groups received mixed support: the *Centurus* clade was recovered by FIB7, MUSK, and TGFB2 (Figs. S1.3–S1.6 in Appendix 1); the typical *Melanerpes* clade was recovered with high support only in the FIB7 tree (Fig. S1.3 in Appendix 1); *Tripsurus* was recovered (with low-support) only by MUSK (Fig. S1.4 in Appendix 1); and, the broad *Melanerpes* clade was recovered with high support only in ACO1 (Fig. S1.6 in Appendix 1).

Our concatenated (mtDNA + nDNA) phylogenetic analyses showed some differences in the evolutionary relationships of some species regarding the currently accepted morphological arrangement. Some taxa previously suggested to be members of superspecies or even conspecific were located in independent branches in the phylogeny. Most of these changes were located within the *Centurus* clade, with *M. pygmaeus*-*M. rubricapillus*, *M. uropygialis*-*M. hypopolius*, and *M. santacruzi*-*M. aurifrons*. Other changes involve the inclusion of *M. cactorum* within the typical *Melanerpes* clade, and the inclusion of *M. pucherani*, previously not considered part of the black-backed *Melanerpes*, in the *Tripsurus* clade (Short, 1985) within our "*Centurus*" clade. Finally, our results suggested some signs of incomplete lineage sorting in *M. hoffmannii*-*M. rubricapillus*. In the mtDNA and nDNA analysis, these species appeared as sister taxa, however, in the concatenated analyses, *M. rubricapillus*

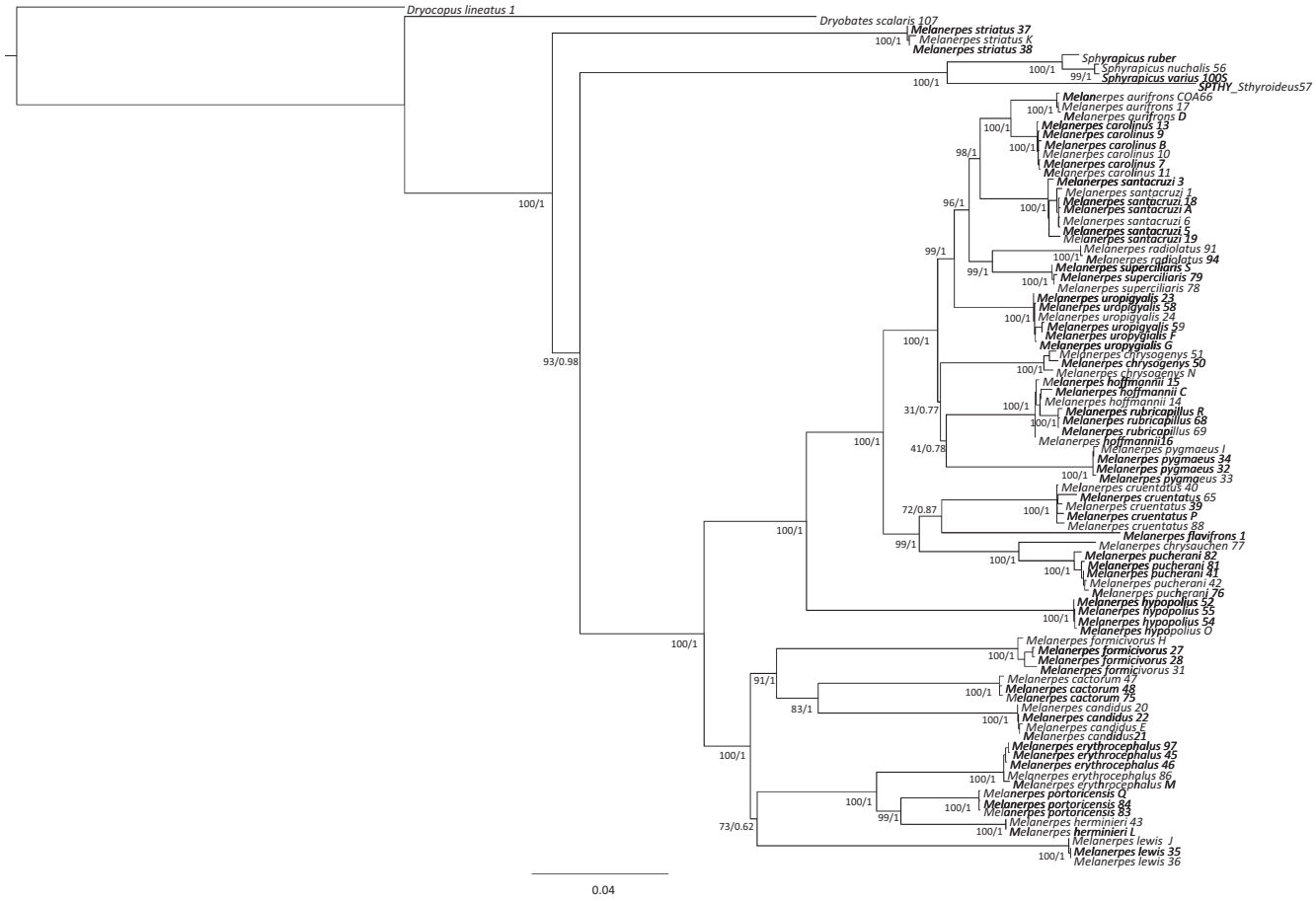

Fig. 1. Concatenated (mtDNA + nDNA) ML estimate of the phylogeny for *Melanerpes* woodpeckers. Numbers along branches refer to ML bootstrap support (before slash) and to BI posterior probabilities (after slash).

appeared as a monophyletic group, but embedded within *M. hoffmannii*, suggesting a probable recent speciation event.

3.3. Species tree

Our species tree estimations recovered the same five well-delimited highly-supported clades in our concatenated BI and ML estimations (Fig. 2): a *Sphyrapicus* clade, the *M. striatus* clade, the typical *Melanerpes* clade, a clade including all species formerly classified in *Tripsurus*, and a clade including all species formerly in the genus *Centurus*.

Species tree nodal support (Fig. 2,) was lower than on concatenated trees (Fig. 1). The HSN index on the full coalescent species trees from *BEAST (Fig. 2, HSN = 0.46) indicate that less than 50% of nodes received high statistic-support on species trees, compared to 77% of nodes from concatenation. The HSN index for the summary species tree from SVDQUARTETS was 59%. Lower HSN values between species trees and concatenated analyses suggest support values are inflated in 17–31% of nodes from concatenation. Using passerine rates from honeycreepers both concatenated and species trees had HSN values of 0.61, indicating that higher rates of molecular evolution also influence nodal support. However, most poorly supported nodes were concentrated towards the tips of the phylogeny on concatenated analyses whereas deeper nodes were unsupported in species trees. There were few topological differences between concatenation and species trees, although BI support values tended to be higher in the concatenated tree. The most important differences were on the placement of *M. striatus* from Hispaniola and *M. hypopolius* from the Balsas basin in southern Mexico. In full coalescent species trees (Figs. 2 and 3A) and SNP-based species tree (Fig. 3B), *M. striatus* was sister to a broad *Melanerpes* clade, whereas in the concatenated tree, this taxon was recovered as sister to the *Sphyrapicus* clade (Fig. 1); again, the ambiguous position of *M. striatus* suggests that *Melanerpes* is not monophyletic. Similarly, the position of *M. hypopolius* under full coalescence was as sister to a clade containing *Centurus* species, whereas in the concatenated and mtDNA trees this taxon appeared as sister to the *Centurus* + *Tripsurus* clade, rendering barred-backed species as paraphyletic if *Tripsurus* is recognized. These differences, along with most within-clade relationships were poorly supported by full coalescent and summary species-tree methods, thus not contradicting our results from concatenation.

3.4. Biogeography

Range evolution in *Melanerpes* was best explained by a DIVALIKE + *j* model, which has a chance of 82% of being the most probable among other models considered (Table 2). Although biogeographic interpretations should be taken with care, as some of our areas are large and include different islands (e.g., the Antilles, where both Puerto Rico and Guadeloupe are included) or a mixed biotic composition (e.g., North America), the biogeographic model selected includes vicariance, founder effect speciation, and anagenetic dispersal (range expansion) as important processes involved in the diversification of this large radiation of woodpeckers in the New World.

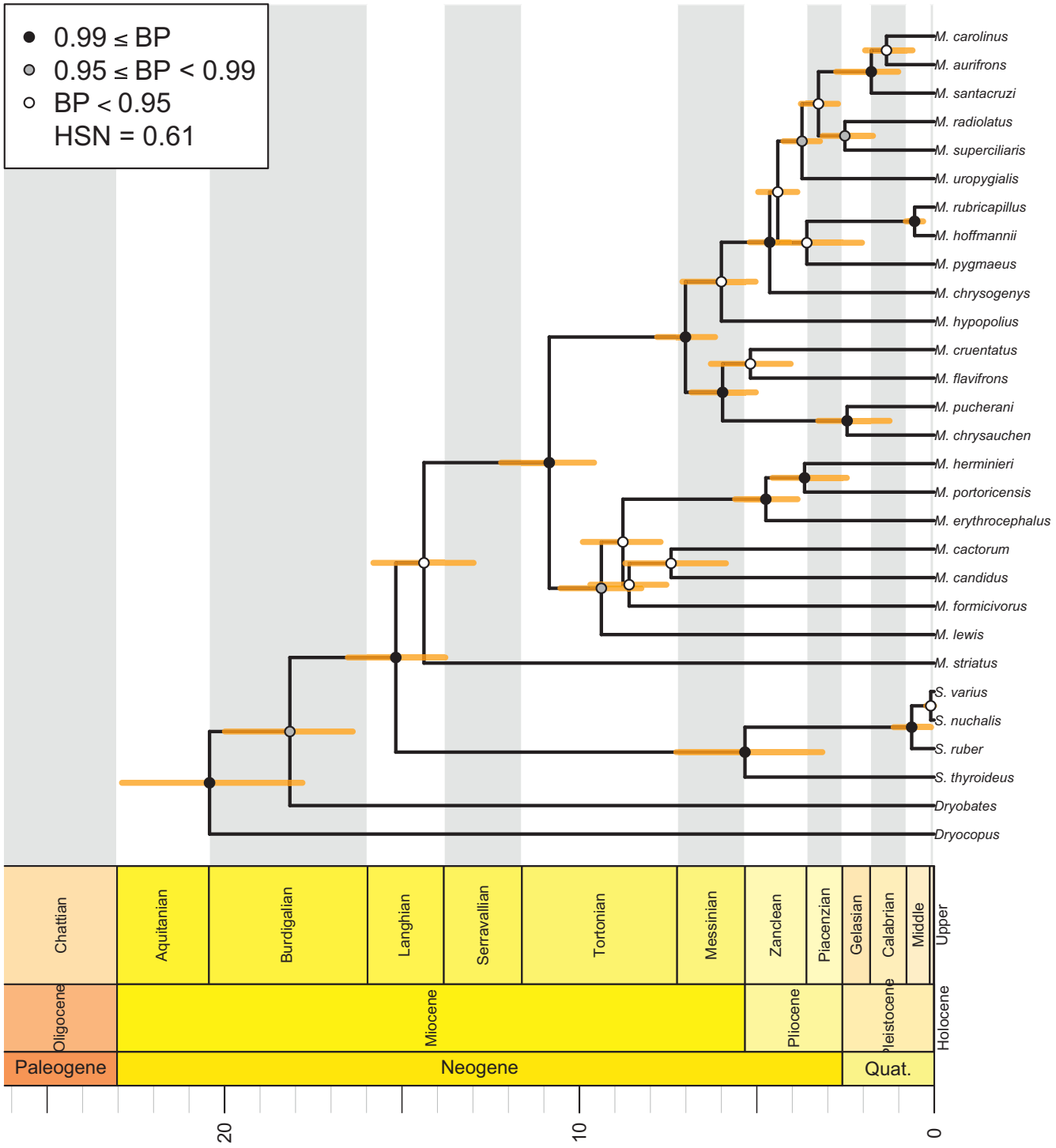

Fig. 2. Species tree chronogram from *BEAST calibrated with rates from Lerner et al. (2011). Orange node bars correspond to 95% highest posterior density (HPD). Node symbols correspond to posterior probabilities (see figure legend). Scale box is in millions years, and depicts geologic periods, epochs, and eras according to the ICS International Chronostratigraphic Chart (Cohen et al., 2013).

According to the selected biogeographic model, founder effect, anagenetic dispersal (range expansion), and vicariance have been drivers for the speciation and biogeography in our study group. Lineage diversification started in the Early to Middle Miocene (Fig. 4), with the split between *M. striatus* and a clade including *Sphyrapicus* + broad *Melanerpes* occurred about 15 Ma (range 17.2–13.1 Ma) in the temperate forests of North America and the Caribbean, for which the DIVALIKE + *j* model suggested a vicariant event. Other vicariant events occurred between the typical *Melanerpes* clade and the *"Centurus"* clade about 10.4 (range 12.1–8.9 Ma), within the typical *Melanerpes* clade about 7.7 Ma (range 9–6.5 Ma) during the Late Miocene, and also within the *"Centurus"* clade 6.4 Ma (range 7.6–5.3 Ma) Late Miocene Early Pliocene (Fig. 4). Following these results, the early vicariant split for *M. striatus* of Hispaniola decrease the potential number of colonization events to other Caribbean islands to at least two but

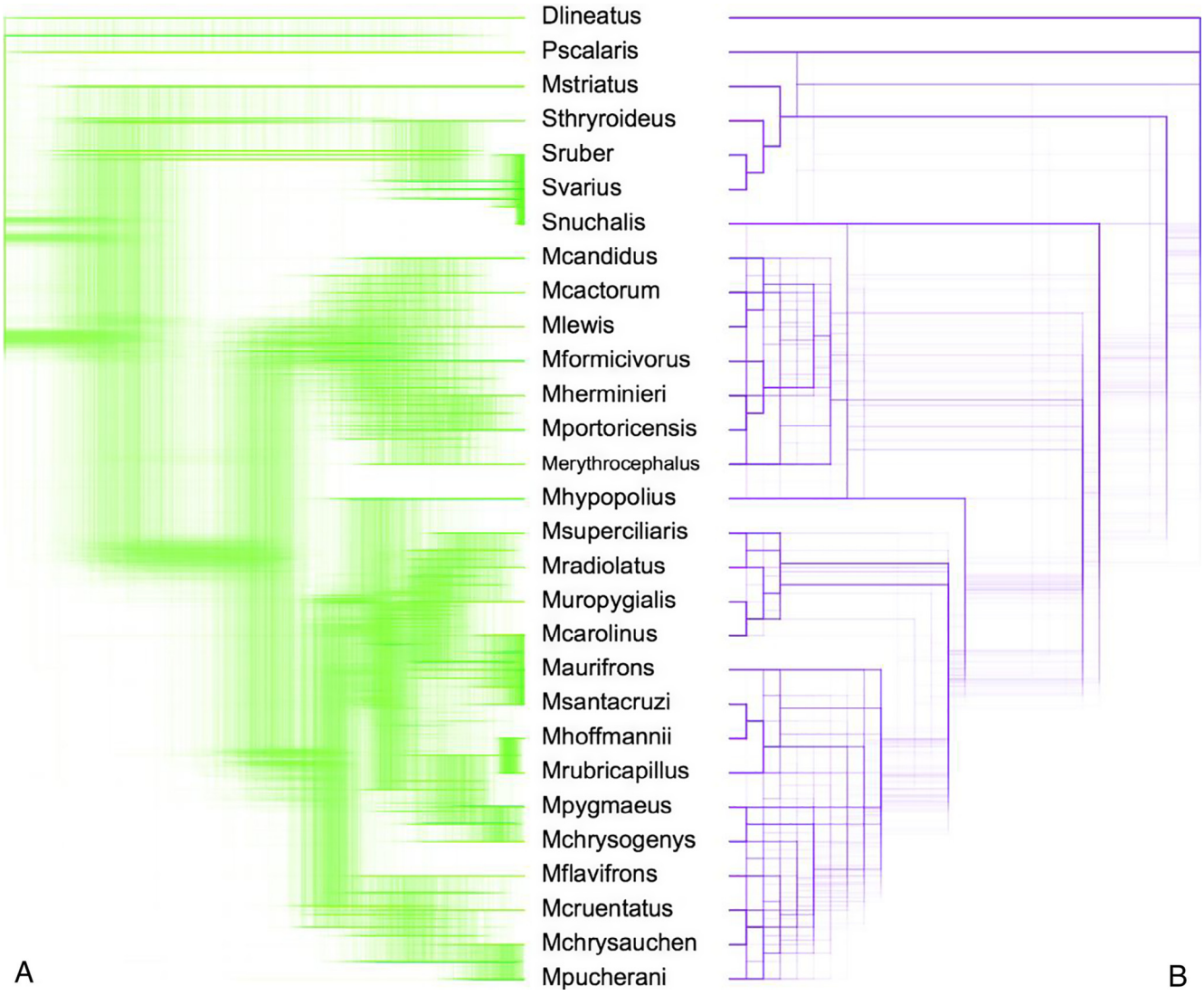

Fig. 3. Cloudograms from species trees. (A) Full coalescent posterior clade density of 1000 draws estimated in *BEAST (Fig. 1). (B) Bootstrap density of 1000 replicates estimated in SVDQUARTETS. Tip labels starting with an S correspond to the genus *Sphyrapicus*, those with an M to the genus *Melanerpes*. *Dryocopus lineatus* (Dlineatus) and *Dryobates scalaris* (Dscalaris) are outgroups.

more or less synchronic independent waves, as they occurred in two different clades: the typical *Melanerpes* clade about 4.5 Ma (range 5.8–3.3 Ma), and the *Centurus* clade about 2.9 Ma (range 3.7–2.2 Ma) in the Late Miocene-Early Pleistocene. Other founder effect speciation events suggested include the divergence in the Pliocene of *M. flavifrons* in the Atlantic Forest; of *M. pygmaeus* in the Yucatán Peninsula and nearby islands; of *M. rubricapillus* from dry forests of western Mesoamerica in the Middle to Late Pleistocene; and that of *M. santacruzi* from the southwestern deserts of North America in the Late Pliocene- Early Pleistocene. Finally, although most species are endemic to a single region in our biogeographic analysis, some anagenetic dispersal (range expansion) events are conspicuous. Those include the expansion of *M. formicivorus* to the highlands of Mesoamerica and the Colombian Andes during the Late Pliocene- Early Pleistocene, that of *M. santacruzi* also to the highlands of Mesoamerica and the Gulf of Mexico slope, and of *M. carolinus* to temperate forests in North America, both of these since at least the Middle to Early Pleistocene, respectively.

Biogeographic patterns for the species tree were generally similar to those obtained for the concatenated tree (Fig. S1.9). The only significant difference was the location of *M. striatus* regarding the phylogenetic location of both the broad *Melanerpes* clade and the *Sphyrapicus* clade. The selected biogeographic model for the species tree also corresponded to DIVALIKE + *j*, and in this case, at least three colonization events in the Caribbean spanning three different clades were indicated: The first one involved *M. striatus* to Hispaniola, whereas the other two corresponded to those already described for the typical *Melanerpes* and the *Centurus* clades in the concatenated tree (see above). Chronograms estimated with general avian rates had on average 40% shorter times compared to those from passerines rates (Fig. 5). Times estimated using the latter rates largely overlapped with divergences calculated using multiple fossils and biogeographic calibration points (Dufort, 2016), suggesting general rates might not be appropriate in groups with high rates of evolution such as Piciformes. In either case all our timeframes are more recent than major geologic events in the Caribbean. On the mainland younger time estimates from general rates would suggest that many splits between sister species happened after the final closure of the Isthmus of Panama, whereas more appropriate rates place them the dynamic and older connections between North and South America (Bacon et al., 2015).

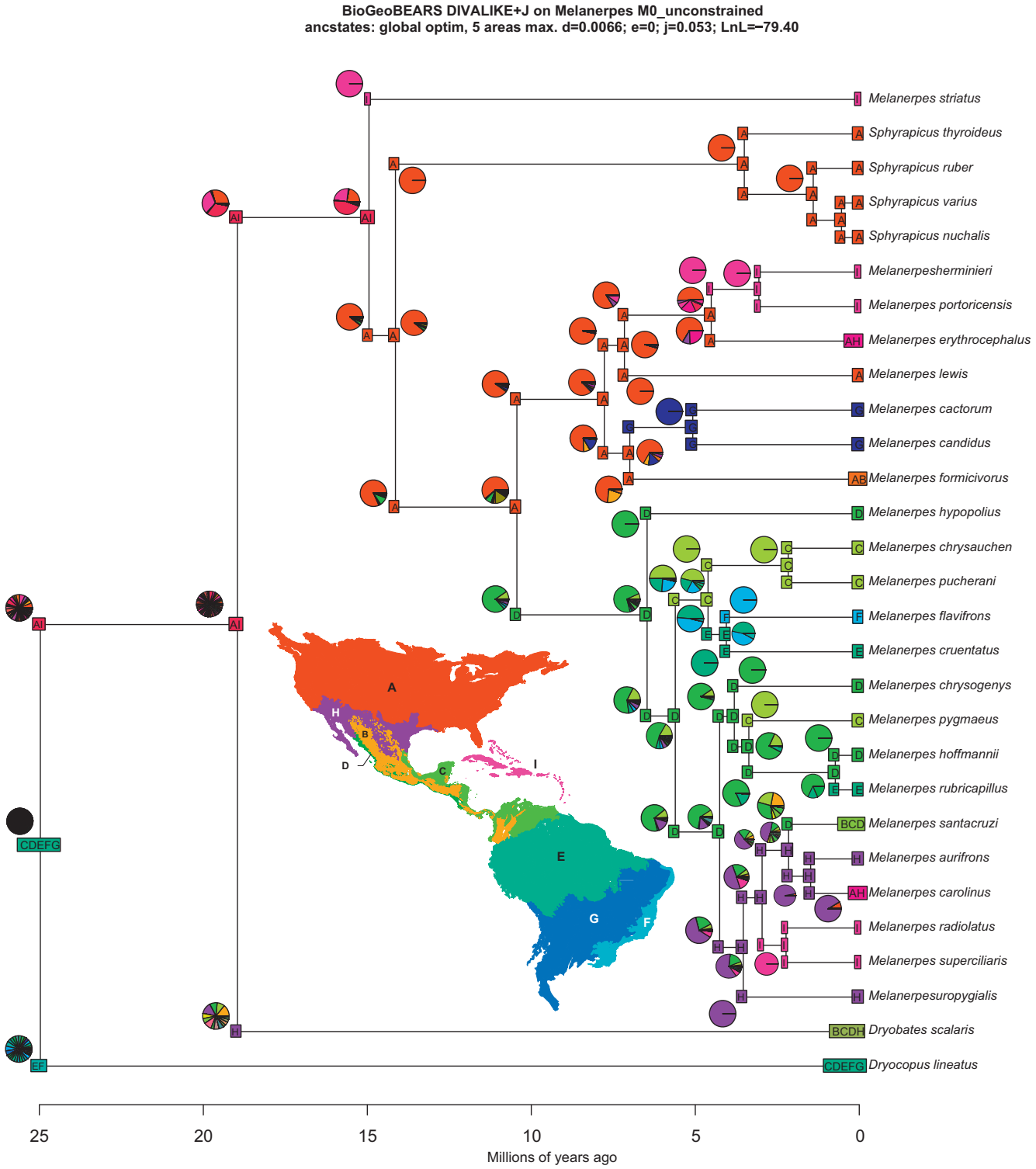

Fig. 4. Biogeographic estimation on the concatenated tree using model DIVALIKE + *j* (−LnL = −79.48), as implemented in BiogeoBEARS. Time-calibrated phylogeny estimated in *BEAST. Colors correspond to coded areas (see inset). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

4.1. Phylogeny

Due to a high differentiation in phenotypic and morphological characters, several species currently included in *Melanerpes* have been assigned to at least six other genera: *Melanerpes*, *Centurus*, *Tripsurus*, *Linneopicus*, *Trichopicus*, *Asyndesmus*, *Balanosphyra*, *Chryserpes*, and *Leuconerpes* (Short, 1982; Selander and Giller, 1963; Winkler and Christie, 2002; Leonard and Heath, 2010). Posterior studies added some behavioral traits (Skutch, 1943, 1948), suggesting that the inclusion of different character datasets may help in the resolution of conflicting phylogenetic relationships within this genus. However, despite extensive differentiation, all species

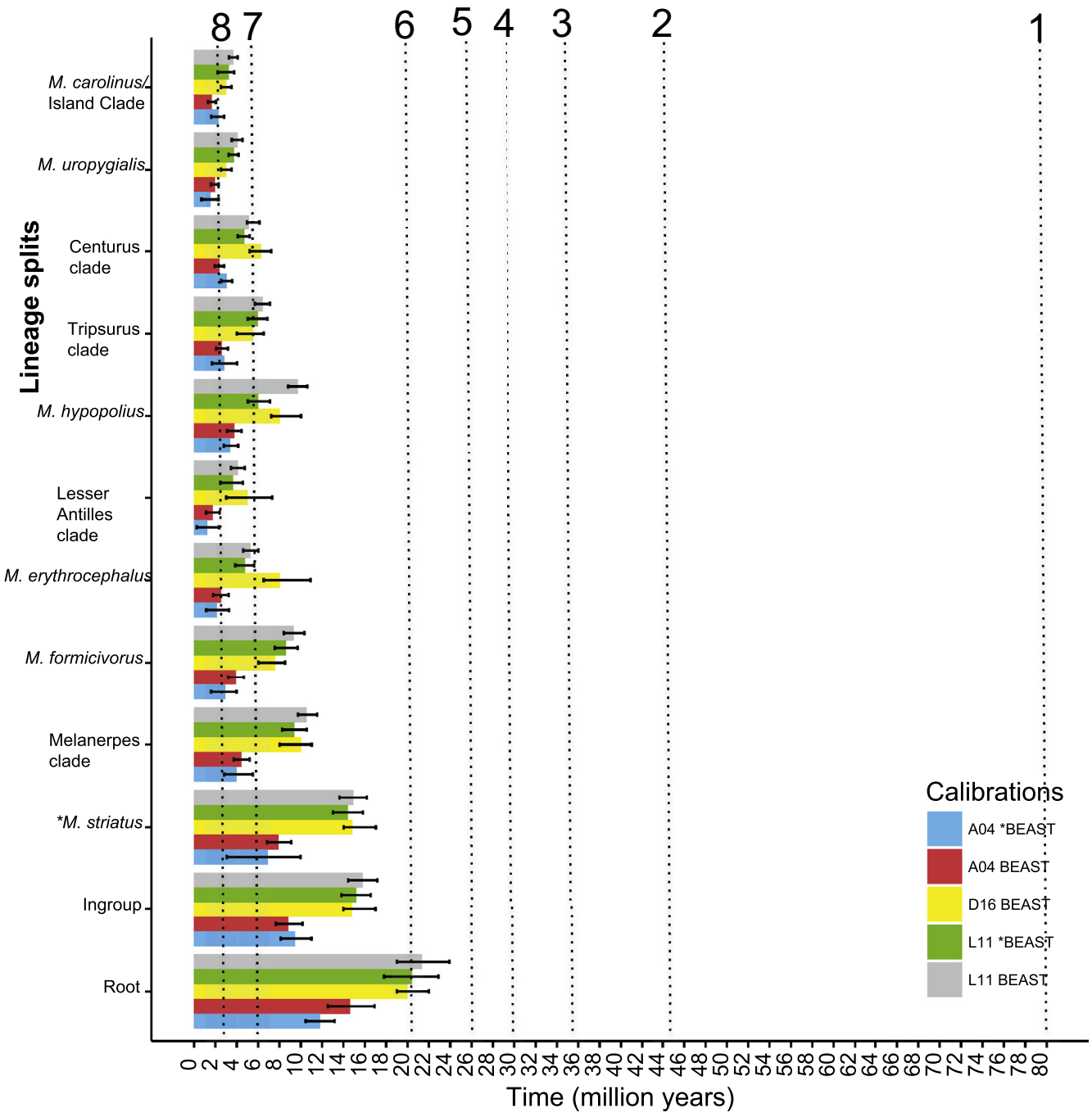

Fig. 5. Lineage split times for ten clades of interest with different calibration estimates. Horizontal axis indicates time of divergence in million years before present. The ambiguous position of *Melanerpes striatus* is marked with an asterisk (*). Blue bars correspond to *BEAST species-tree calibrations using general avian rates from Axelsson et al. (2004), red bars correspond to a BEAST concatenated calibrations using rates from Axelsson et al. (2004), yellow bars correspond to divergence times in Dufort (2016), green bars correspond to *BEAST species-tree calibrations from calibration using passerine avian rates from Lerner et al. (2011), and grey bars correspond to a BEAST concatenated calibrations using rates from Lerner et al. (2011). Dashed lines indicate the start of important geologic/biogeographic events: (1) Proto-Antillean formation[1], (2) Rise of the Lesser Antilles[1], (3) Uplift of GAARlandia (Greater Antilles-Aves Ridge[1]), (4) Drowning of GAARlandia[1], (5) Drift of Jamaica[1], (6) Significant symmetrical waves of dispersal during the Great Biotic American Interchange[2] (GABI), (7) Andean uplift[1] and Asymmetrical dispersal from South America during GABI[2], (8) Final closure of the Isthmus of Panama[2] (Iturralde-Vinent and MacPhee, 1999; Bacon et al., 2015). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were later lumped in *Melanerpes* (Short, 1982), on the basis that some species show intermediate morphological and phenotypic traits perceived as bridging the variation extremes between the two main groups (i.e., black-backed species and barred-backed species), as well as on observations of non-exclusive behavioral traits (see Selander and Giller, 1963). Recent molecular studies on woodpeckers have revealed that *Melanerpes* (in a broad sense, see above) is a monophyletic group, whose sister taxon is either the genus *Sphyrapicus* or the Cuban endemic *Xiphidiopicus percussus* (Overton and Rhoads, 2004; García-Trejo et al., 2009; Dufort, 2016).

Some of the formerly recognized genera presently included within the broad *Melanerpes* were apparent in our concatenated and species-tree analyses, which recovered four clades congruent

with phenotypic and morphologic studies mentioned above. The genetic structure in our analysis recovered a typical *Melanerpes* clade (black-backed species, but including *M. cactorum*, a barred-back species probably convergent with "*Centurus*"), and a "*Centurus*" clade (barred-backed and mostly black-backed species, Fig. 6); additionally, within the "*Centurus*" clade, we recovered a monophyletic group including all the lowland humid forest species previously included in *Tripsurus*. In contrast to previous studies, two other groups, the *Sphyrapicus* clade and *M. striatus* appeared as sister to a broad *Melanerpes*. For this latter, support values were low, suggesting other phylogenetic arrangements. However, these results add to the increasing evidence towards the generic distinctiveness and recognition of the genus *Chryserpes* (Miller, 1915; Selander and Giller, 1963; Olson, 1972; Dufort, 2016), as well as to the close relationship of *Sphyrapicus* to *Melanerpes*. The lack of evidence for selection skewing the phylogenetic position of island endemics suggests that, at least for the genes and taxa we surveyed, the possibility of higher evolution rates on islands (Johnson and Seger, 2001; Smith and Klicka, 2013) is not affecting Melanerpini woodpeckers.

At the species level, the allocation of some species seems to erode the support of clinal variations for the current taxonomic

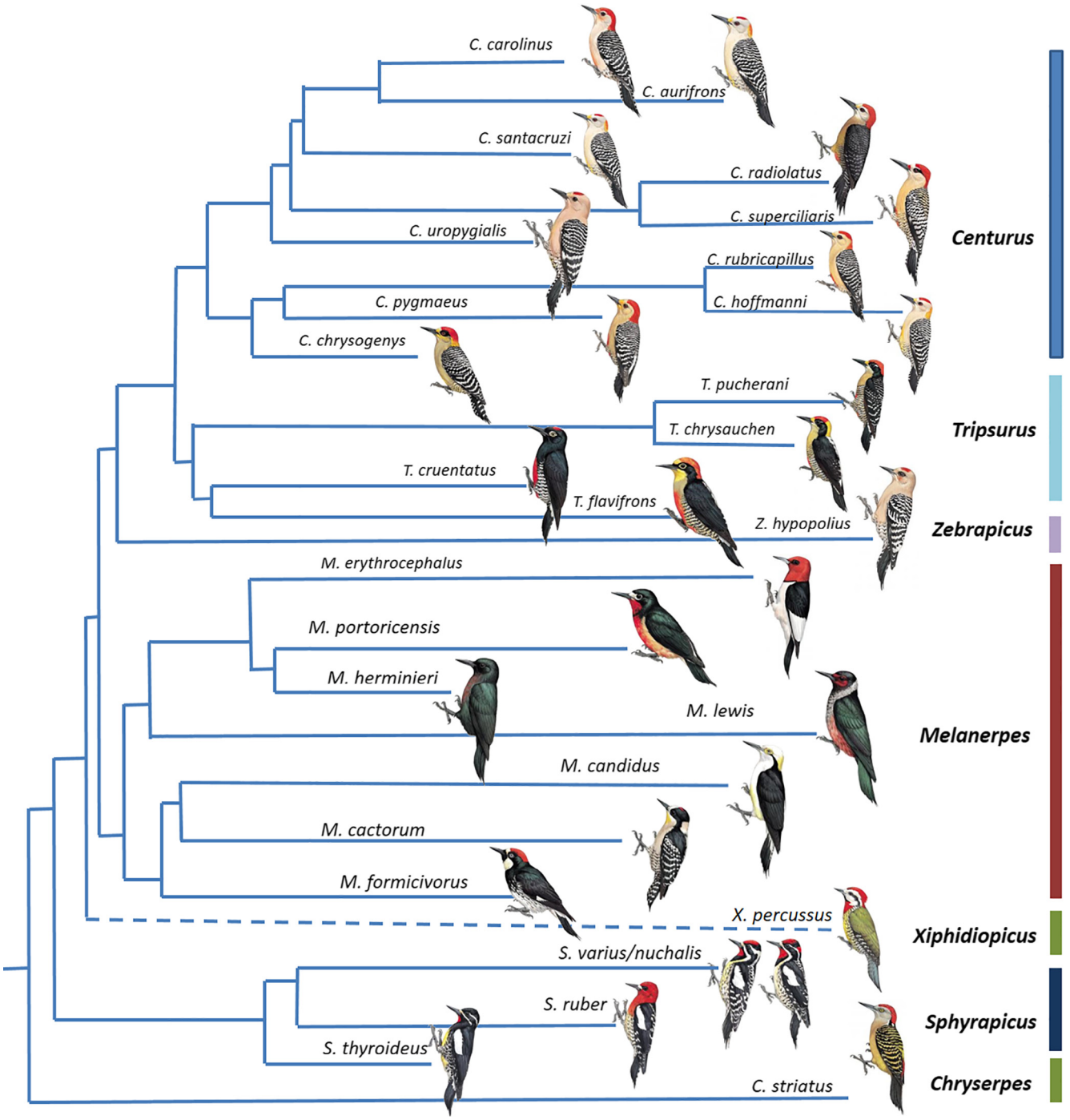

Fig. 6. Simplified phylogeny and taxonomy for *Melanerpes*, *Sphyrapicus* and allies based on the concatenated ML tree. Dashed line depicts the putative position of *Xiphidiopicus* sensu Overton and Rhoads (2004). Branch lengths modified for illustration purposes only. Illustrations reproduced with permission from the Handbook of the birds of the World Alive (http://www.hbw.com). Individual illustration credits are depicted in Supplementary file S3.

arrangement of *Melanerpes*, as some members with traits considered diagnostic for their inclusion in some of the morphological/phenotypic-based groups were included in a different clade in the concatenated analyses. This is the case of *M. cactorum*, which has been suggested to be either a probable member of *Centurus* (Short, 1985) or included with no obvious close relatives in the typical *Melanerpes* clade; we recovered it as sister to *M. candidus*, as previously suggested (García-Trejo et al., 2009). The other relevant example is provided by our "*Centurus*" clade in the concatenated species tree, in which the barred-backed *M. hypopolius*, appears as sister to both the mostly black-backed *Tripsurus* clade and the barred-backed *Centurus* clade.

4.2. Biogeography

Diversification in the broad *Melanerpes* clade occurred since the Late Miocene and continued throughout the Pleistocene, apparently driven by broad climatic changes in North America (Fig. 4), as well as by significant geological changes, namely the closure of the Panama Isthmus (Weir et al., 2009; Smith and Klicka, 2010; Smith et al., 2012; Bacon et al., 2015). In this period, climatic changes lead to an expansion of temperate savannas and grasslands in central North America and a continued trend to the reduction in the extent of the warm-temperate forests in eastern and western North America (Cerling et al., 1997; Graham, 1999; Pound et al., 2012). Towards the Pliocene, grasslands increased their extent as a consequence of a cooling trend coming from the Miocene (Cerling et al., 1997; Graham, 1999). These changes enhanced the differentiation within the broad *Melanerpes* clade, for which the biogeographic model indicated a founder event, likely represented by both the increase in the extent of the grasslands and the aridification of the Mexican Plateau (Jaeger et al., 2005) as the responsible for the cladogenesis between the typical *Melanerpes* clade and the "*Centurus*" clade, thus promoting the evolution of a temperate-forest lineage and a tropical-forests lineage. Within the tropical forests "*Centurus*" clade, a further cladogenetic event in the Late Miocene (about 6.4 Ma, range 7.6–3.4 Ma) led to the separation of the black-barred *Tripsurus* clade in lowland humid forest and the barred-backed *Centurus* clade inhabiting dry tropical forests. These time estimates are congruent with colonization events to South America as seen in mammal (Stadelmann et al., 2007; Patterson and Costa, 2012), avian (Weir et al., 2009; Smith and Klicka, 2010; Smith et al., 2012), and insect taxa (Zhang et al., 2017).

Founder effect speciation was an important process in the diversification of our broad *Melanerpes*, as it seems to have occurred extensively in the study group. Our model suggests a biogeographic scenario in which members spanning two or three different clades within the broad *Melanerpes* (*M. striatus*, typical *Melanerpes*, and *Centurus*) underwent one or two independent colonization events to the Caribbean islands, sharply contrasting with previous ideas in which each one of the five insular species dispersed independently (Bond, 1979), or that all insular endemics were monophyletic (Overton and Rhoads, 2004). In this case, phylogenetic relationships and probable source areas for Caribbean species agree with the biogeographic proposal in birds Bond (1979) and in weevils (Zhang et al., 2017). On the other hand, the geographic distribution of these two clades in the Caribbean suggests that the presence of *M. striatus* in Hispaniola may have prevented the establishment of closely related woodpecker species in the island due to a priority effect (see MacArthur, 1972), or to extinction of representatives of the typical *Melanerpes* and *Centurus* clades.

South America was also colonized in the Late Miocene by members of the typical *Melanerpes* and the "*Centurus*" clade (including *Centurus* and *Tripsurus*) in at least three different times. Once again from North America, the typical *Melanerpes* clade reached central and eastern South America, where the differentiation of the two species (*M. candidus* and *M. cactorum*) seems to coincide with the final uplift of the Cerrado region (reviewed in da Silva, 1997) and the expansion of dry forests (Pennington et al., 2004). This is further supported by diversification events of plant lineages (Simon et al., 2009) and molecular studies in several mammal lineages characteristic of open habitats (reviewed in Carmignotto et al., 2012).

The expansion of dry forests and open habitats in South America, produced separation of the humid lowland forests, setting the stage for divergence of the *Tripsurus* clade (included in "*Centurus*"), for which our biogeographic model suggested a founder effect speciation event from Mesoamerican dry forests in the Late Miocene. This separation led to the evolution of taxa in the Atlantic forest and Amazonia, a pattern supported by a wide range of vertebrate lineages (e.g., Costa, 2003; Siedchlag et al., 2010; Thomé et al., 2010; Patel et al., 2011); and an apparent backcrossing event to Mesoamerica in the Pleistocene (range 2–4.2 Ma), seemingly coinciding with the uplift of the Panama Isthmus, a period in which lowland humid forests presented cycles of expansion and contraction, enhancing the speciation of several bird lineages (Smith et al., 2012). This diversification pattern is highly similar to that found for *Myotis* bats, for which an early colonization of South America 10–7 Ma was followed by a northward backcrossing to North America by the Late Miocene and Early Pleistocene (Stadelmann et al., 2007).

For the *Centurus* clade, differentiation occurred mostly in Mesoamerica, although their members have dispersed and differentiated in the Caribbean (see above) and in northern South America (*M. rubricapillus*), coinciding again with the Panamanian Isthmus closure and the GABI (range 4.3–6.6 Ma). In this clade, climatic changes of the Late Pliocene-Early Pleistocene apparently favored a third important process in the evolution of *Melanerpes*: anagenetic dispersal (i.e., range expansion; Ree, 2005; Ree and Smith, 2008; Matzke, 2012). Range expansion is expected to occur to contiguous areas or habitats, thus differs from founder effect speciation as this latter appears as an unexpected event. In this clade, most range expansion events occurred in a small set of species, which seems to have dispersed to contiguous and new environments, such as deserts (*M. uropygialis*, *M. aurifrons*), humid lowland forests (*M. santacruzi*, *M. pygmaeus*), and North American temperate forests (*M. carolinus*). These range expansion processes suggest that sympatry in some melanerpines, especially those in Mesoamerica, may be secondary in origin.

Different rates of evolution yielded contrasting estimates of diversification time in Melanerpini woodpeckers. Even though those time estimates rely on secondary calibrations due to the lack of ingroup fossil data, they largely match direct calibrations in Picidae (Dufort, 2016). Most major geologic events in the Caribbean are much older than the timeframe in our phylogenies regardless of which rates were used, suggesting the Proto-Antillean vicariance and Aves Ridge connection (Iturralde-Vinent and MacPhee, 1999; Vázquez-Miranda et al., 2007) likely did not play a major role for the diversification of this group. Mainland biogeographic scenarios on the other hand were more ambiguous because in many cases credibility intervals did not overlap. However, due to the dynamic connection between North and South America during GABI for the last 20 million years (Bacon et al., 2015) it is complicated to determine the effects of different mutation rates for many clades within the ingroup. Passerine rates yielding older splitting times suggest that many sister species diverged during the dynamic dispersal event influx during the Miocene-Pliocene, whereas younger times would indicate that Melanerpini woodpeckers were able to bridge the Isthmus of Panama only after it was permanently closed. Our findings emphasize the importance of using appropriate models of evolution to obtain a more accurate picture in historical biogeography.

4.3. Taxonomic implications

A common and extended phenomenon in woodpeckers is plumage convergence (Bock, 1963; Cody, 1969; Short, 1982; Winkler et al., 1995; Winkler and Christie, 2002; Weibel and Moore, 2005; Benz et al., 2015). Although the evolutionary basis for this extended plumage convergence is still not well understood (see Prum and Samuelson, 2012; Prum, 2014), a frequent result has been a high level of uncertainty in the phylogenetic relationships within the woodpeckers, from the species level (e.g. *Chrysocolaptes*; Collar, 2011) to the generic level (e.g. *Dryobates-Veniliornis*, Weibel and Moore, 2005; *Celeus-Dryocopus*, Benz et al., 2015). A more thorough and detailed analysis of color variation in the taxon (the broad *Melanerpes* used herein) will be published elsewhere (Benites et al., in prep.).

Previous taxonomic schemes in *Melanerpes* were mainly based on phenotypic characters. Initial classifications recognized several genera; however, most of these were dropped in posterior classifications (see Peters, 1948) for which the concept of clinal variation led to the recognition of only one highly variable genus (Short, 1982). This latter scheme has prevailed, despite some studies have claimed the clear differentiation of some of the generic taxa warranting recognition, as *Chryserpes* (see Olson, 1972), and *Centurus* (Selander and Giller, 1963). Our concatenated and species-tree analyses suggested a very different arrangement from those based on morphology. The molecular scheme clearly suggests that the clinal variation criterion of Short (1982) may not apply in *Melanerpes* and *Sphyrapicus*, as some clades either include members of the two main phenotypic groups (as in the typical *Melanerpes*), or are sister to a different phenotypic group (as *M. hypopolius* to the "*Centurus*" clade).

At the generic level, genetic-based analyses have shown that *M. striatus* from Hispaniola is either sister to a clade including *Melanerpes* and *Sphyrapicus* (see Dufort, 2016), or sister to *Melanerpes*, rendering *Melanerpes* as paraphyletic thus warranting the recognition of *Chryserpes* (Miller, 1915). The rest of the species were grouped in a broad *Melanerpes* clade, in which two groups were recovered: the typical *Melanerpes* clade and the "*Centurus*" clade. The typical *Melanerpes* clade includes taxa for which several genera were erected when described, acknowledging their phenotypic distinction. Included in here are the formerly recognized genera *Leuconerpes*, *Trichopicus*, *Asyndesmus*, *Linneopicus*, and *Balanosphyra*, all with solid black upperparts, except for *M. cactorum*, which characters led some to believe this taxon was allied with the barred-backed *Melanerpes* (Short, 1985). Based on our concatenated phylogeny, the typical *Melanerpes* are sister to a clade of mostly black-backed and barred-backed species here called "*Centurus*" due to the paraphyletic arrangement of the barred-backed taxa. "*Centurus*" includes three main subgroups that may be recognized. The first includes the barred-backed *M. hypopolius*, which is basal to two sister monophyletic groups: a group of mostly black-backed humid lowland-forest taxa, and a radiation of barred-backed taxa ranging from temperate forests in eastern North America to deserts and humid lowland forests in Mesoamerica. The arrangement of the "*Centurus*" clade prevents the application of the clinal variation, as *M. hypopolius* is sister to a clade that contains both the mostly black-backed species and barred-backed species. We here propose the recognition of *Tripsurus* for the mostly black-backed humid lowland-forest species, a monophyletic group that have radiated after entering a new adaptive zone (Mayr, 1974), and also the recognition of *Centurus* for the barred-backed species, a well differentiated group that have mostly radiated in dry lowland forests. The recognition of both *Tripsurus* and *Centurus* would require a generic name for *M. hypopolius*, a dry forest species endemic to central Mexico with diagnostic facial plumage differences (white forehead and grey eye rings), for which the name *Zebrapicus* (Malherbe, 1849) is available.

At the species level, most changes involve the sister relationships for some species, which are different from those proposed on morphological grounds. Our analyses revealed that some taxa merged into a superspecies, are actually not closely related. This is the case of *M. pygmaeus* and *M. rubricapillus* (Short, 1982), *M. hypopolius-M. uropygialis* (Peters, 1948), and some of taxa that have been included into the superspecies *carolinus*, such as *M. hoffmannii* and *M. santacruzi* (Short, 1982; Selander and Giller, 1963). Also within this "superspecies", taxa included show either all-red crowns or red-and-yellow crowns. This led to the inclusion of the Central American *M. hoffmannii*, which is highly similar to the North American *M. aurifrons*. Our study showed that *M. hoffmannii* is rather sister to *M. rubricapillus*. Regarding additional species limits, our results add to previous studies supporting the tropical *M. santacruzi* as a lineage that should be recognized as a full species, as it is sister to a clade containing both *M. carolinus* and *M. aurifrons* (García-Trejo et al., 2009; Dufort, 2016).

Our results suggest that a full rearrangement of the taxonomy based in our phylogenetic results may be performed. The proposal should try to maintain the most stability in generic names, but at the same time maximizing information on the evolutionary relationships of the taxa involved and the patterns of variation of additional characters (morphology, behavior, ecology). Clearly, the clinal variation as the main criterion for the taxonomic arrangement in *Melanerpes* has led to confusion in the proposed schemes in an admittedly difficult group in which plumage convergence has been extensive (Benites et al., in prep.). We believe that an integral scheme in which genetic divergence, morphology, and behavior should be used for the recognition of the generic limits, as in this case, the largest radiation of woodpeckers in the New 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.2017.04.013.

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