A new classification of the Pied Woodpeckers assemblage (Dendropicini, Picidae) based on a comprehensive multi-locus phylogeny

Jérôme Fuchs *, Jean-Marc Pons

UMR 7205 Institut de Systématique, Evolution, Biodiversité, CNRS, MNHN, UPMC, EPHE, Sorbonne Universités, Département Systématique et Evolution, Muséum National d'Histoire Naturelle, CP 51, 57 rue Cuvier, F-75231 Paris Cedex 05, France
UMS MNHN/CNRS 2700, Outils et Méthodes de la Systématique Intégrative (OMSI), Muséum National d'Histoire Naturelle, 57 rue Cuvier, F-75231 Paris Cedex 05, France

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ABSTRACT

The pied woodpecker assemblage historically included the widespread genera *Picoides* and *Dendrocopos*. The assignment of species to either of these two genera has for long puzzled systematists due to their overall plumage similarity. Recent molecular studies not only suggested that both of these genera are not monophyletic, but also that four other genera, the African *Dendropicos* the South American *Veniliornis* and two Asian monospecific genera (*Hypopicus* and *Sapheopipo*) are nested within the *Dendrocopos–Picoides* clade. Yet, our current understanding of the phylogeny and taxonomy of this group is still very partial because several distinctive Old World species that have been assigned to different genera throughout their taxonomic history have not been sampled yet. Here, using DNA sequence data gathered from four loci, we reconstructed a species level phylogeny of the Indo-Malayan and Palearctic Pied Woodpeckers to understand the phylogenetic relationships and biogeographic history of the Eurasian species with respect to African and New World lineages. Our phylogenetic analyses revealed nine strongly supported clades within the Dendropicini. Noticeably, two species that had disputed affinities at the genus level clustered in clades with species from the same biogeographical region: the Brown-backed Woodpecker (*D. obsoletus*) is nested in *Dendropicos* and the Arabian Woodpecker (*D. dorae*) is related to two Eurasian species, the Brown-fronted (*D. auriceps*) and Middle-spotted woodpeckers (*D. medius*). The nine clades have a strong biogeographic component and very few dispersal event among bioregions occurred. For example, the African species formed a clade, suggesting that only one dispersal event is needed to explain the presence of Dendropicini in Africa. Based on our phylogenetic results, we propose a new classification of the Dendropicini that recognizes nine genera.

1. Introduction

The systematics of woodpeckers (Picidae) has been primarily based on plumage characters because of the lack of clear-cut differences in other phenotypic traits (Short, 1982). Yet, recent molecular studies highlighted striking cases of plumage convergence among unrelated woodpecker genera (Benz et al., 2006; Moore et al., 2006; Fuchs et al., 2007), suggesting that overall similarity in plumage pattern is particularly misleading for taxonomy in woodpeckers. The primary factors driving convergence are uncertain with hypotheses including adaptation to habitat (humid tropical forest, dryer temperate habitats, Moore et al., 2006) or

convergence occurring due to mimicry and/or inter-specific territoriality (Weibel and Moore, 2005). Hence the woodpeckers' taxonomy needs to be thoroughly revised in line with the evolutionary relationships that emerged from molecular phylogenetic studies.

The pied woodpecker assemblage historically included the widespread genera *Picoides* and *Dendrocopos*. The assignment of species to either of these two genera was problematic due to their overall plumage similarity (Table 1). Recent molecular work changed our understanding of the taxonomy and evolution of this group with the inclusion of genera (e.g. *Dendropicos*, *Sapheopipo*, *Veniliornis*) that were never thought to be related to *Dendrocopos*/*Picoides* (Weibel and Moore, 2002a, b; Webb and Moore, 2005; Winkler et al., 2005; Benz et al., 2006; Moore et al., 2006; Fuchs et al., 2007, 2013). These new relationships prompted the recognition of a new clade, the Dendropicini (sensu Webb and Moore, 2005 but not including *Sphyrapicus* and *Melanerpes*). The

* Corresponding author at: UMR7205 Institut de Systématique, Evolution, Biodiversité, CNRS, MNHN, UPMC, EPHE, Sorbonne Universités, Département Systématique et Evolution, Muséum National d'Histoire Naturelle, CP 51, 57 rue Cuvier, F-75231 Paris Cedex 05, France.
E-mail address: fuchs@mnhn.fr (J. Fuchs).

Table 1
Historical classifications among members of the pied woodpeckers assemblage.

	Peters (1948)	Short (1982)	Winkler and Christie (2002)	Dickinson (2003)
Veniliornis	*fumigatus, spilogaster, passerinus, frontalis, maculifrons, affinis, chocoensis, cassini, kirkii, callonotus, sanguineus, dignus, nigriceps*	*fumigatus, spilogaster, passerinus, frontalis, maculifrons, cassini, affinis* (including *chocoensis*) *kirkii, callonotus, sanguineus, dignus, nigriceps*	*fumigatus, spilogaster, passerinus, frontalis, maculifrons, affinis, chocoensis, cassini, kirkii, callonotus, sanguineus, dignus, nigriceps*	*fumigatus, spilogaster, passerinus, frontalis, maculifrons, cassini, affinis* (including *chocoensis*) *kirkii, callonotus, sanguineus, dignus, nigriceps*
Dendropicos	*fuscescens, stierlingi, elachus, abyssinicus, poecilolaemus, gabonensis, lugubris*	*fuscescens, stierlingi, elachus, abyssinicus, poecilolaemus, gabonensis, lugubris, elliotii* (including *johnstoni*), *goertae, spodocephalus, griseocephalus, namaquus, xantholophus, pyrrhogaster*	*fuscescens, stierlingi, elachus, abyssinicus, poecilolaemus, gabonensis, lugubris, elliotii* (including *johnstoni*), *goertae, spodocephalus, griseocephalus, namaquus, xantholophus, pyrrhogaster*	*fuscescens, stierlingi, elachus, abyssinicus, poecilolaemus, gabonensis, lugubris, elliotii* (including *johnstoni*), *goertae* (including *spodocephalus*), *griseocephalus, namaquus, xantholophus, pyrrhogaster,* **obsoletus**
Polipicus	*elliotii, johnstoni*	Lumped in *Dendropicos*	Lumped in *Dendropicos*	Lumped in *Dendropicos*
Mesopicos	*goertae* (including *spodocephalus*), *griseocephalus*	Lumped in *Dendropicos*	Lumped in *Dendropicos*	Lumped in *Dendropicos*
Thripias	*namaquus, xantholophus, pyrrhogaster*	Lumped in *Dendropicos*	Lumped in *Dendropicos*	Lumped in *Dendropicos*
Dendrocopos	*major, leucopterus, syriacus, assimilis, himalayensis, darjellensis, medius, leucotos, cathpharius, hyperythrus, auriceps, hyperythrus, macei, mahrattensis, minor, canicapillus, kizuki, moluccensis, maculatus, temminckii,* **obsoletus, dorae***, albolarvatus, villosus, pubescens, borealis, nuttallii, scalaris, arizonae, stricklandi, mixtus, lignarius*	Lumped in *Picoides*	*major, leucopterus, syriacus, assimilis, himalayensis, darjellensis, medius, leucotos, cathpharius, auriceps, atratus, macei, mahrattensis, minor, canicapillus, kizuki, moluccensis, maculatus, temminckii,* **dorae***, hyperythrus,* **obsoletus**	*major, leucopterus, syriacus, assimilis, himalayensis, darjellensis, medius, leucotos, cathpharius, auriceps, atratus, macei, mahrattensis, minor, canicapillus, kizuki, moluccensis, maculatus, temminckii,* **dorae**
Hypopicus	Lumped in *Dendrocopos*	Lumped in *Picoides*	Lumped in *Dendrocopos*	*hyperythrus*
Picoides	*tridactylus, arcticus*	*tridactylus, arcticus, albolarvatus, villosus, pubescens, borealis, nuttallii, scalaris* (including *arizonae*), *stricklandi, mixtus, lignarius, major, leucopterus, syriacus, assimilis, himalayensis, darjellensis, medius, leucotos, cathpharius, auriceps, atratus, macei, mahrattensis, minor, canicapillus, kizuki, moluccensis, maculatus, temminckii,* **dorae***, hyperythrus,* **obsoletus**	*tridactylus, arcticus, albolarvatus, villosus, pubescens, borealis, nuttallii, scalaris* (including *arizonae*), *stricklandi, mixtus, lignarius*	*tridactylus, arcticus, albolarvatus, villosus, pubescens, borealis, nuttallii, scalaris, arizonae, stricklandi, mixtus, lignarius*
Sapheopipo	*noguchii*	*noguchii*	*noguchii*	*noguchii*

phylogenetic relationships among the New World species have been thoroughly assessed over the last fifteen years (Weibel and Moore, 2002a,b, 2005, Moore et al., 2006). These authors highlighted three primary clades among New World species with most of the relationships among species and the underlying biogeographic implications being resolved. This involved, for example, the fact that South America was colonized twice by members of the pied woodpeckers clade, on a first occasion by *V. fumigatus* and on a second occasion by the *Veniliornis–P. mixtus/P. lignarius* clade (Moore et al., 2006).

This situation highly contrasts with our current knowledge concerning the relationships among the Palearctic and Indo-Malayan species and their relationships with members of the Dendropicini from Africa or the New World, as a thorough sampling of Palearctic and Indo-Malayan species was never achieved. The genus *Hypopicus* was included in Benz et al. (2006) and was found to be related to *Dendrocopos major* but, as the aim was to reconstruct a higher level phylogeny of woodpeckers, its exact relationships to other *Dendrocopos* species are still unclear. Two species, the Brown-backed Woodpecker *Dendropicos obsoletus*, endemic to the northern savannah of Africa, and the Arabian Woodpecker, *Dendrocopos dorae,* endemic to the Arabian Peninsula, have never been included in a molecular phylogeny. Both these species have occasionally been placed into other genera; *D. obsoletus* is sometimes considered to be related to *Dendrocopos* whereas *D. dorae* is sometimes considered to be nested within the African endemic genus *Dendropicos* (del Hoyo et al., 2014). Of the chosen taxonomic arrangement depends our understanding of the biogeographic history of the African Picinae (genera *Dendropicos* and *Campethera/Geocolaptes*) as a whole. As currently understood, the African Picinae are the result of two colonization events from Indo-Malaya (Fuchs et al., 2007). Yet, if *Dendropicos obsoletus* appears to be part of the Indo-Malayan or Palearctic *Dendrocopos* group and if *D. dorae* is related to *Dendropicos* and thus dispersed from Africa to Arabia, which is considered to be part of the Palearctic bioregion, the number of faunistic exchanges between Indo-Malaya-Palearctic and Africa would increase to four.

Limitations in our understanding of the phylogeny and taxonomy of the Old World pied woodpeckers also prevail at lower taxonomic levels, where the degree of differentiation of several taxa (e.g. *D. assimilis* or *D. leucopterus*) from their likely closest relatives (*D. syriacus* or *D. major*, respectively), and thus their biological status, is unclear.

Here, we reconstruct a species level phylogeny of the Indo-Malayan and Palearctic pied woodpeckers with the primary objective to understand in more detail the relationships and biogeography of Eurasian species with respect to African and New World lineages. Based on our results, which are highly congruent at the higher level with results from previous studies, we propose a new taxonomic arrangement for this clade of woodpeckers.

2. Materials and methods

2.1. Sampling

We sampled representatives from all currently recognized species within the Dendropicini (Winkler and Christie, 2002; Dickinson, 2003), with two exceptions. The first exception involves the genus *Veniliornis*, for which we sampled six out of the ten recognized species; the six sampled species (*kirkii, cassini, fumigatus, nigriceps, callonotus* and *passerinus*) represent the primary lineages within the genus (Moore et al., 2006). The second exception involves the African genus *Dendropicos*, for which only six out of the fourteen recognized species were included (Dickinson, 2003). The six sampled species represent all recognized subgenera and primary lineages based on another study with dense taxon sampling of African taxa at the subspecies level (Fuchs, Bowie, Carre and Pons, in preparation). Whenever possible we included multiple individuals per recognized species but, as our work mostly focused on Old World taxa, we did not necessarily include all divergent subspecific lineages from Nearctic taxa (e.g. *Picoides villosus* Klicka et al., 2011; Graham and Burg, 2012) in our analyses. Trees were rooted with the White-browed Piculet (*Sasia ochracea*) and members of the Malarpicini were included as proximate outgroups (Webb and Moore, 2005; Benz et al., 2006; Fuchs et al., 2006, 2007, 2008, 2013). The individuals included in the present study are listed in Supplementary Table 1.

2.2. Laboratory protocols

DNA was extracted from blood and tissue (muscle, liver) using the Quiagen extraction kit (Qiagen, Valencia, CA) following the manufacturer's protocol. We extracted the DNA from toe pad in a room dedicated to ancient DNA to avoid contamination of the museum samples by fresh DNA. We used the same extraction protocol as for the fresh samples and also added 40 µl of dithiothreitol (DTT, 0.1 M) to facilitate the digestion of these tissues.

We analyzed DNA sequence data from one mitochondrial protein coding gene (ATP6) and three autosomal introns (Myoglobin – MB – intron-2, Beta Fibrinogen – FGB – intron-5 and Transforming Growth Factor beta – TGFb2 – intron-5). All primers used to amplify and sequence the fresh samples are listed in Supplementary Table 2. To amplify the DNA from toe pad samples, we defined species-specific or species-group-specific primers for the mitochondrial locus. For the nuclear data, the primers were less lineage-specific due to the lower number of diagnostic sites. All primers used to amplify and sequence DNA from toe pad samples are detailed in Supplementary Table 3. The thermocycling conditions included a hotstart at 94 °C, an initial denaturation at 94 °C for 3 min, followed by 35–40 cycles at 94 °C for 40 s, 52–60 °C for 30 s, and 72 °C for 30–60 s, and was completed by a final extension at 72 °C for 5 min. Purified PCR products were cycle-sequenced using the Big Dye terminator chemistry (ABI, Applied Biosystems) in both directions with the same primers used for PCR amplification and run on an automated ABI 3100 DNA sequencer. Heterozygous sites in nuclear loci were coded using the appropriate IUPAC code. Sequences newly generated for this study have been deposited in Genbank (www.ncbi.nlm.nih.gov; Accession Numbers KR049266–KR049522). The alignments were straightforward, owing to the low number of insertion–deletions events and the conserved flanking sequences of the indels.

2.3. Data analyses

We used the program Phase v2.1.1 (Stephens et al., 2001; Stephens and Donnelly, 2003), as implemented in DNAsp 5.0 (Librado and Rozas, 2009) to infer the allelic phase for each nuclear locus. Several runs, using different seed values were performed. We used the recombination model and ran the iterations of the final run 10 times longer than the other runs. We considered the estimate from Phase v2.1.1 as the best estimate for phase probability for the downstream species tree analyses. For sequences that were heterozygous in length, we compared the ambiguous 5′-end with the unambiguous 3′-end of the forward and reverse sequences to resolve the placement and composition of gaps and the linkage of polymorphisms to those gaps (Peters et al., 2007). For the concatenated analyses, we used the unphased sequence data.

The best-fitting model of nucleotide substitution, using Bayesian Information Criterion (BIC) and the greedy algorithm, was determined using PartitionFinder (Lanfear et al., 2012). For the mitochondrial locus, the most supported number of partitions was determined from an initial set of three partitions (each codon position). As each of the nuclear DNA markers was non-coding, these alignments were not tested for partitioning.

Gene trees and concatenated trees were reconstructed using the Bayesian method implemented in MrBayes 3.2 (Ronquist et al., 2012). Bayesian analyses for two concatenated data sets (nuclear and mitochondria, and nuclear only) were performed allowing the different parameters (base frequencies, rate matrix, shape parameter, transition/transversion ratio, proportion of invariable sites) to vary between the different partitions (using the *prset ratepr = variable* and *unlink* commands, i.e. mixed-models analyses, Nylander et al., 2004). We used an exponential mean of 100 and 500 for the branch length priors for the ATP6 gene and the three nuclear loci, respectively; these prior settings improved the convergence of the analyses. The concatenated analyses were run using an exponential mean of 100 for the branch length prior. Default values were used for all other priors. The value of the heating parameter T was adjusted to obtain state swap frequencies between the cold and heated chains in the 20–70% range. Four Metropolis-coupled MCMC chains (one cold and three heated) were run for ten to twenty-five million iterations with trees sampled every thousand iterations. The number of iterations discarded before the posterior probabilities were estimated (i.e. the length of the 'burn-in' period) varied between analyses. Bayesian posterior probabilities were calculated from the remaining iterations. Using random starting trees, two independent runs were performed; we ensured that the potential scale reduction factor (PSRF) approached 1 for all parameters and that the average standard deviation of split frequencies was less than 0.01. We also used Tracer v1.6 (Rambaut and Drummond, 2007) to ensure that convergence was reached for the posterior distributions of the parameter estimates, ensuring that the effective sample size (ESS) of these estimates was >200.

Species tree were reconstructed using the coalescent based model implemented in *Beast (Heled and Drummond, 2009). Runs were 25×10^8 iterations. Given that we could not amplify nuclear data from all taxa and loci, especially for species for which the source of DNA was toe pads, and that *Beast requires at least one sequence per locus for each species, our sampling for the species tree analyses was reduced when compared to the concatenated analyses. Four species had to be excluded from the species tree analyses (*D. assimilis, D. cathpharius, D. namaquus, D. temminckii*). This strategy, however, would still allow a meaningful comparison among methods to assess the support for the relationships among the primary lineages. Species tree analyses were performed for the nuclear and nuclear/mitochondrial data sets. We used the same substitution model settings and partitioning scheme as in the MrBayes 3.2 analyses. We used a normal prior distribution for the ATP6 rate that corresponds to the rate obtained by Lerner et al. (2011). The analyses that used an empirically plausible rate

converged faster than assuming a fixed rate for the one of the loci but we insist on the fact that our aim was not to discuss the divergence times among Dendropicini.

3. Results

3.1. Mitochondrial topology

We obtained the complete ATP6 sequence for all species except *Dendropocos assimilis*, for which the central fragment is missing. No haplotypes were shared among species. Both the non-partitioned analyses and partitioned by codon position analyses converged on very similar topologies and clade posterior probabilities (Fig. 1); the only exception involved the relationship of the two three-toed species (*P. arcticus* and *P. tridactylus*) which was either sister to the clade formed by the small sized East Asian and Indo-Malayan species (non-partitioned) or sister to all other members of the Dendropicini and Melanerpini (partitioned). In both the non-partitioned and partitioned analyses, the Dendropicini were paraphyletic as the two Melanerpini we sampled were nested within it, although support values were low in both cases. Our analyses revealed nine well supported clades (Posterior Probability; PP: 0.97–1.0; Fig. 1) but the relationships among those clades did not receive strong support. None of the four polytypic genera were monophyletic.

The first group to emerge included small species (*D. kizuki*, *D. temminckii*, *D. moluccensis*, *D. maculatus*, *D. canicapillus* PP: 1.0/1.0) that are endemic to East Asia and Indo-Malaya.

The two disjunct subspecies of *D. moluccensis* were not directly related; instead they clustered with the species distributed closest to them: *D. m. nanus* (India) is the sister group of the mainland *D. canicapillus* whereas *D. m. grandis* (Indonesia) is the sister species of *D. maculatus* (Philippines). Within the latter species, substantial genetic differentiation was found between the two sister-sub-species (6.1%, uncorrected p-distances). The type specimen of *Dendrocopos nanus gigantiusculus* (UMMZ 147913) was related to *D. canicapillus* and not to *D. m. nanus*, in accordance with recent views (e.g. Dickinson, 2003). The second well supported clade included the two three-toed species (*P. arcticus* and *P. tridactylus*). The third primary clade (PP: 1.0/1.0) was itself subdivided into three primary lineages. The first lineage consisted of all South American species (*Veniliornis* and *P. mixtus*/*P. lignarius*, PP: 1.0/1.0) with the exception of *V. fumigatus*, which was nested in the second lineage with four North-American species (*P. borealis*, *P. albolarvatus*, *P. stricklandi*, *P. villosus*; PP: 1.0/1.0). Finally the third lineage included three closely related North-American species (*P. pubescens*, *P. nuttallii* and *P. scalaris*) as well as two Old World species (*D. minor* and *D. cathpharius*). The seventh primary lineage (PP: 0.97/0.97) included all African taxa from the genus *Dendropicos* as well as four Eurasian species among which *D. auriceps*, *D. dorae* and *D. medius* formed the eighth strongly supported lineage (PP: 1.0/1.0). The last Eurasian species, *D. mahrattensis* from this clade clustered with *D. namaquus* and *D. pyrrhogaster* but this relationship was not well supported. The Brown-backed Woodpecker (*D. obsoletus*) was nested in an entirely African sub-clade along with *D. griseocephalus*, *D. elliotii* and *D. fuscescens* (PP: 1.0/1.0). Finally the last strongly supported primary clade (PP: 1.0/1.0) consisted of eleven Palearctic and Indo-Malayan species of large size (Fig. 1). Two monophyletic genera were nested in this clade, *Hypopicus* and *Sapheopipo* with the latter taxon being the sister species of *D. leucotos* (uncorrected p-distances: 3.3%). The initial splits within the eleven species clade involved Indo-Malayan species (*D. atratus*/*D. macei*, *H. hyperythrus*, *D. darjellensis*), suggesting that the Palearctic species (*D. major*/*D syriacus* clade) are derived from the tropical Indo-Malayan species. The level of divergence among species within the *D. major*/*D syriacus* clade was small,

averaging 3% (minimum: *D. syriacus*/*D. assimilis*: 0.4%; maximum *D. leucopterus*/*S. noguchii*: 5.3%), suggesting a recent origin for all these species.

3.2. Nuclear loci

Our success with sequencing the nuclear loci was variable and in some cases we could only obtain partial nuclear sequences (*D. assimilis*/*D. leucopterus*) or even no nuclear sequences at all (e.g. *D. temminckii*). The final alignment of the nuclear loci was 1088 bp (MB), 565 bp (TGFb) and 606 bp (FGB). The rather unusual alignment length for MB was due to a synapomorphic insertion of 374 bp between *P. nuttalii*, *P. pubescens* and *P. scalaris*. The sharing of nuclear alleles among species was not uncommon for the three loci. For example, one myoglobin allele was shared among up to six different species (*P. villosus*, *P. lignarius*, *P. mixtus*, *V. callonotus*, *V. cassinii*, *V. kirkii*) that are not necessarily related in the ATP6 tree and one TGFb2 allele was shared between *D. canicapillus* and *D. kizuki*.

The individual nuclear locus trees showed very limited resolution among members of the Dendropicini (Fig. 2a–c), yet significant support was achieved for some species groups. Those include the clade formed by *P. arcticus*/*P. tridactylus* and the small Asian species (MB, PP: 0.97), and the clade formed by all Dendropicini except those species (TGFB2, PP: 1.0). At the higher level, MB provided very little support and resolution and most well supported deep nodes included three to five species. In contrast, TGFb2 had a lower number of alleles shared among species and a higher number of well supported deep nodes; these well supported nodes included a clade with all large Indo-Malayan *Dendrocopos* (*D. leucopterus*/*D. himalayensis* PP: 0.99), a clade with all South American taxa but *V. fumigatus* (PP: 1.0) which was in a larger group together with *P. borealis*, *P. villosus*, *V. fumigatus*, *P. strick-landii* and *P. albolarvatus* (PP: 0.98). Concerning the two species with disputed affinities, *D. obsoletus* was nested within a monophyletic *Dendropicos* (PP: 0.97), as sister to *D. griseocephalus* (PP: 0.95) in MB, a result that was also found in TGFb2 (PP: 0.97). The Arabian Woodpecker *D. dorae* was related to *D. auriceps* and *D. medius* in both loci (MB PP: 1.0; TGFb2 PP: 0.98).

Overall, the nuclear and mitochondrial trees were very congruent, and some nodes supported in the mitochondrial locus were found in the nuclear intron trees, even if not with strong support (e.g. monophyly of the small East Asian/Indo-Malayan *Dendrocopos* in TGFb2).

The trees resulting from the analyses of the concatenated unphased nuclear data sets and phased nuclear species tree analyses were very similar (Fig. 3A and B), with exceptions only involving nodes that received weak support in one of the analyses.

3.3. Concatenated and species tree analyses

The trees resulting from the concatenated and species tree analyses were well resolved, with most higher-level groups and the back bone topology (that is, genera or sub-genera) receiving posterior probabilities of 1.0 (Fig. 4A and B). Concatenating the mitochondrial and nuclear sequences usually resulted in an increase in support values and resolution. For example, significant support was recovered for the monophyly of the African taxa (including *D. obsoletus*; PP: 0.96) in the concatenated analyses whereas this monophyly, although often present in gene trees, was never significantly supported in individual loci.

4. Discussion

Our study provides a new robust phylogeny and for the first time assesses the relationship of some taxa with uncertain
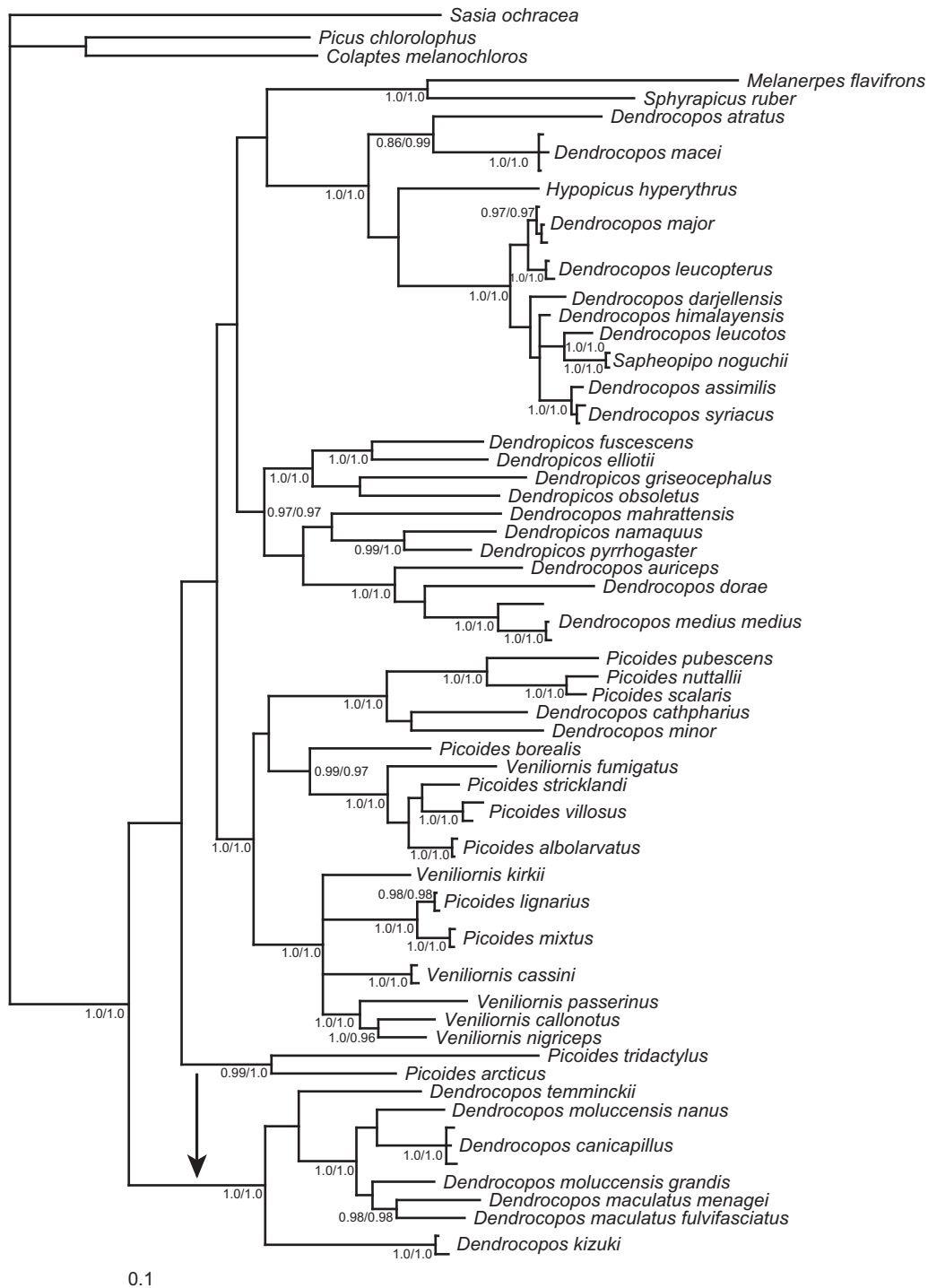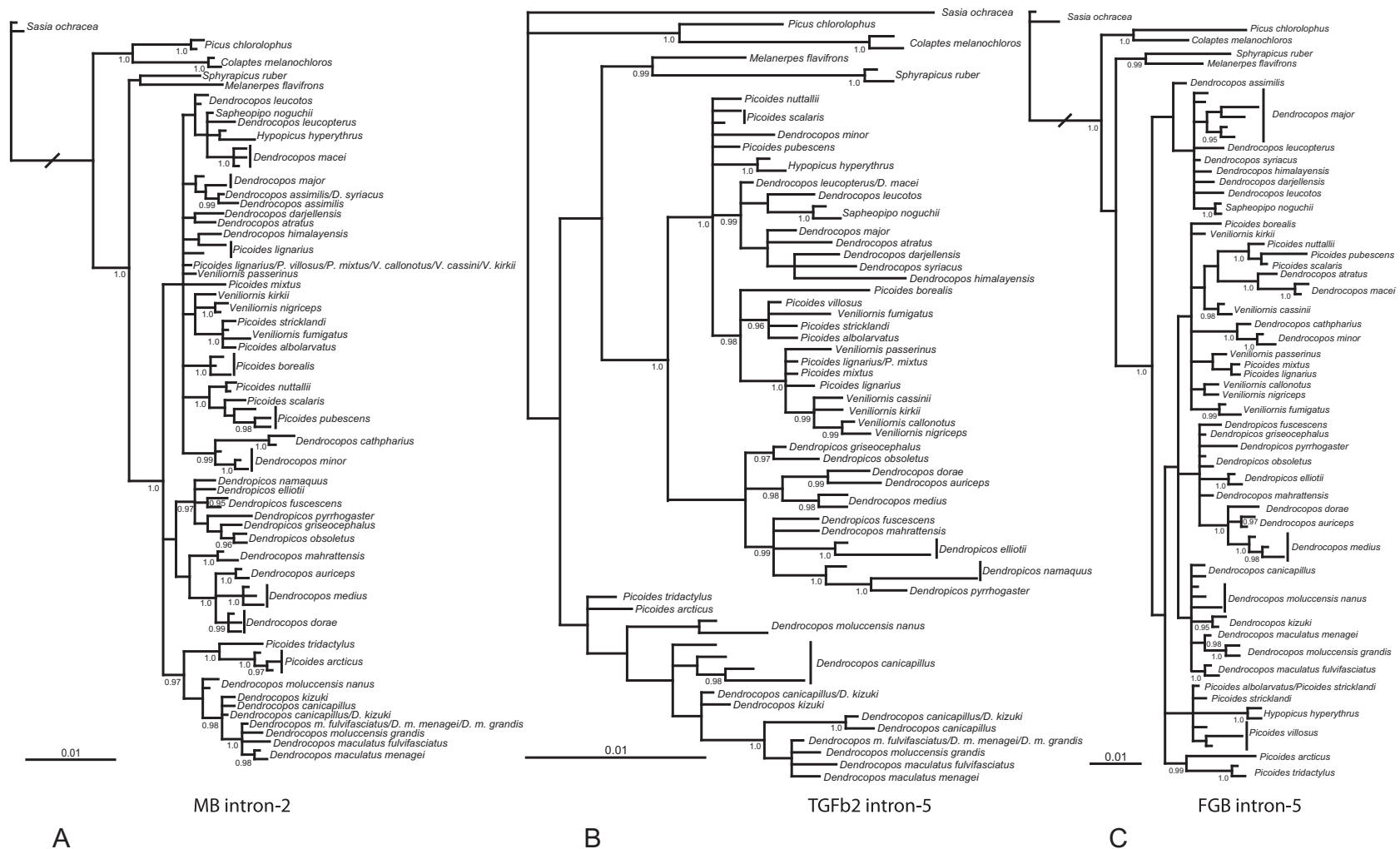
Fig. 1. 50% Majority rule consensus trees obtained from the partitioned analyses of the ATP6 locus. Posterior probabilities (non-partitioned/partitioned) are indicated when greater than 0.95. The trees resulting from the partitioned and non-partitioned analyses were very similar; the arrow indicates the position of the *P. arcticus/P. tridactylus* clade in the non-partitioned analyses.

affinities (e.g. *Dendropicos obsoletus, Dendrocopos dorae*). Here, we discuss the taxonomic and biogeographic implications of our results concerning dispersal across major biogeographical areas.

The most divergent, and strongly supported lineage found within the Dendropicini includes five nominal species (*D. kizuki, D. temminckii, D. moluccensis, D. maculatus, D. canicapillus*) of small to moderate size that are endemic to East Asia and Indo-Malaya. Our results suggest that the current species diversity is clearly underestimated in this group. First, one species, *Dendropicos*

moluccensis, was not recovered as monophyletic. Among birds, *D. moluccensis* has a distribution pattern that is at odds with distribution patterns recognized in other Indo-Malayan species. Indeed, one subspecies group (*D. m. nanus*) is restricted to India, Pakistan, Bangladesh and Sri Lanka whereas the other subspecies group is found in Southern Thailand, Borneo and the Lesser Sundas (*D. m. moluccensis* and *D. m. grandis*). The distribution of these two subspecies is interrupted by the distribution of *D. canicapillus* (India, Russia, China, Thailand, Laos, Vietnam).

Fig. 2. 50% Majority rule consensus trees obtained from the analyses of the three phased nuclear introns (MB intron-2 (A), TGFb2-intron-5 (B), FGB intron-5 (C)). Only unique alleles were retained for the analyses but alleles were sometimes shared among species. Posterior probabilities are indicated when greater than 0.95. Branch length leading to the outgroup for MB and FGB was reduced for graphical purposes.
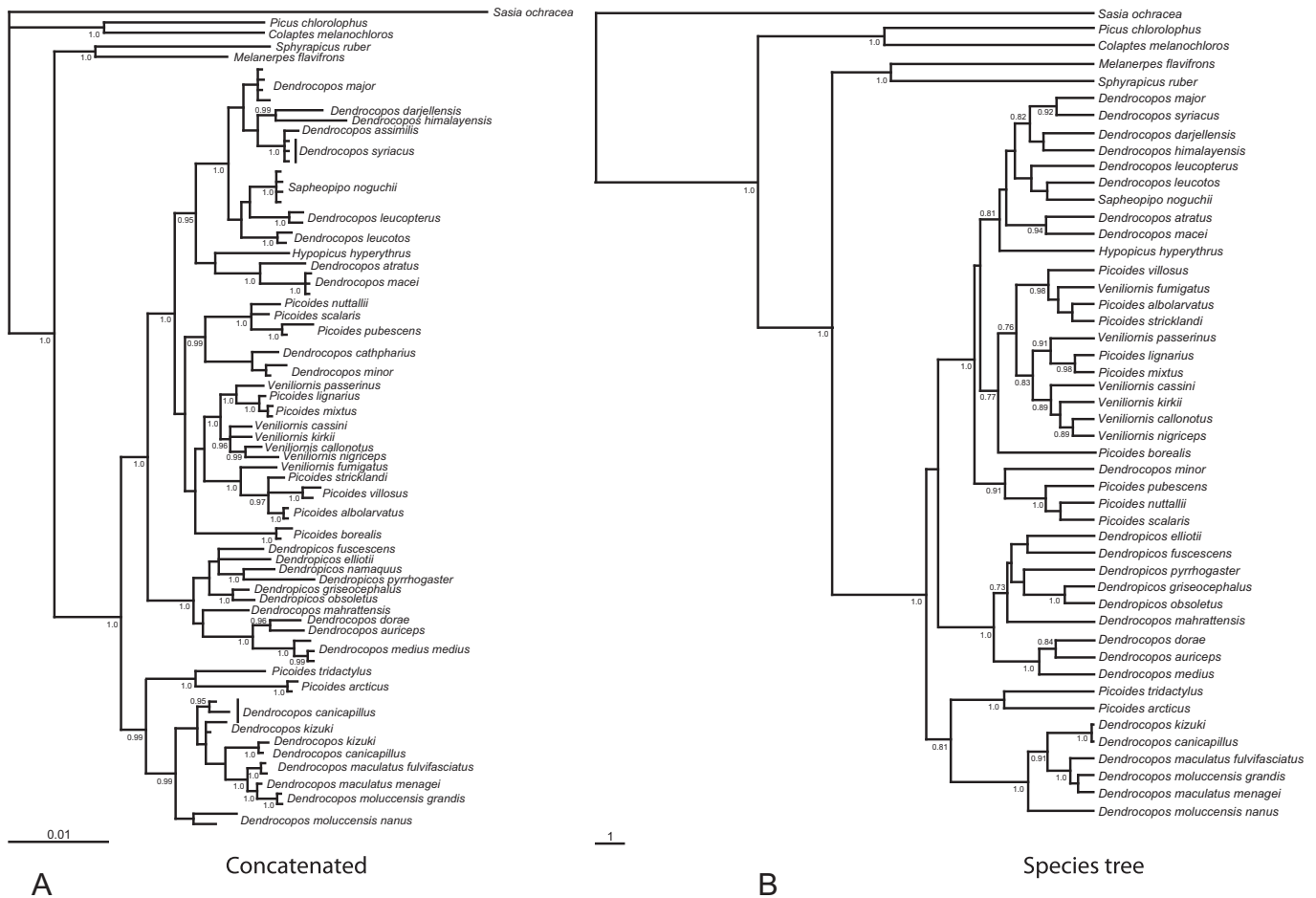
Fig. 3. 50% Majority rule consensus tree obtained from the analyses of the three concatenated unphased nuclear introns (partitioned by locus; A); and species tree analyses using the phased nuclear alleles (*Beast, B). Posterior probabilities are indicated when greater than 0.95 (concatenated) or 0.70 (species tree). The two topologies were very similar and differences only involved poorly supported nodes.

Ripley (1982) and Inskipp et al. (1996) treated *nanus* as a different species, a distinction that Greenway (1943) also considered plausible. Our analyses suggest an even more complex pattern as the populations from the Lesser Sundas are more closely related to *D. maculatus* (Philippines) whereas the relationships of the Indian group (*D. m. nanus*) are unresolved at the base of the clade that includes *D. canicapillus* and *D. maculatus*/*D. m. grandis*. Hence the relationships inferred from our study makes more sense from a biogeographic point of view than from what could be inferred from traditional taxonomy, as continental taxa are closely related whereas the Sundas populations are related to the species from the Philippines. From the phylogenetic pattern recovered (non monophyly) and the level of DNA sequence divergence, it appears reasonable to split *D. moluccensis* into two species, *D. nanus* and *D. moluccensis*. The DNA divergence between the two *D. maculatus* subspecies we sampled is very high (6.1%) and may suggest that this taxon is in fact composed of several biological species. Such a high level of genetic differences between avian populations from the Philippines has already been highlighted (Oliveros and Moyle, 2010; Hosner et al., 2014). Yet, a clear understanding of the number of biological species in *D. maculatus* would first necessitate a comprehensive sampling at the population/subspecies level before proposing any split. We also recovered very little genetic differentiation across the distribution of *D. canicapillus* despite having sampled a substantial spectrum of its range (Russia, China and India). We included the holotype of *Dendrocopos nanus gigantiusculus* (UMMZ 147913) that is now considered to be a junior

synonym of the subspecies *D. c. semicoronatus* (Dickinson, 2003). Our analyses confirmed this current taxonomic treatment as the specimen UMMZ 147913 was more closely related to *D. canicapillus* than to *D. m. nanus*.

Our analyses not only confirmed the relationships of the clade formed by the small Indo-Malayan species with the three toed woodpecker species (*P. arcticus* and *P. tridactylus*) but also its basal position within the Dendropicini. This large clade is then sister to the core Dendropicini which itself divided itself into three primary clades with a strong biogeographic component.

The first clade consists of all African taxa (genus *Dendropicos*) as well as four Palearctic and Indo-Malayan species (*D. auriceps*, *D. medius*, *D. dorae*, *D. mahrattensis*). The African taxa were monophyletic in both the concatenated and species tree analyses, although the support was, in some cases, only moderate. The Brown-backed Woodpecker *D. obsoletus*, one of the taxa that has been assigned to different genera, is clearly nested in *Dendropicos*; all analyses supported a closer relationship to the Olive Woodpecker (*Dendropicos griseocephalus*) than to any other African species. Hence our data unambiguously support the inclusion of *obsoletus* in *Dendropicos*. The other species with disputed affinities, the Arabian Woodpecker *Dendrocopos dorae*, clustered with the Middle-spotted (*D. medius*) and Brown-fronted (*D. auriceps*) Woodpeckers in a strongly supported clade in all analyses. The relationship among these three species is still unresolved. The relationship of the last species from this clade, the Yellow-crowned Woodpecker (*D. mahrattensis*), are uncertain as it varies
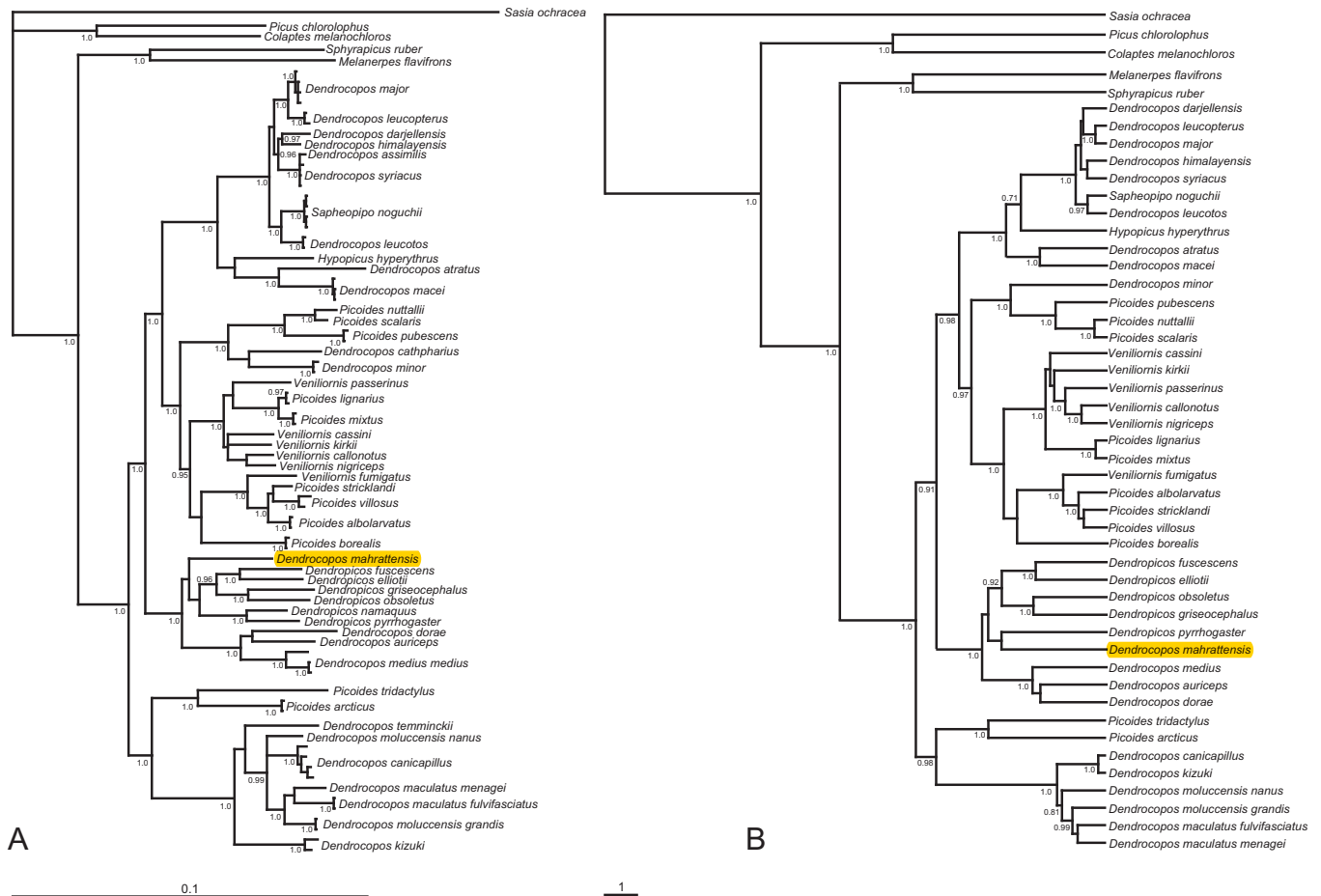
Fig. 4. 50% Majority rule consensus tree obtained from the analyses of the concatenated nuclear introns and mitochondrial locus (partitioned by locus and codon position – six partitions; A), and species tree analyses using the phased nuclear alleles and the mitochondrial locus (*Beast, B). Posterior probabilities are indicated when greater than 0.95 (concatenated) or 0.70 (species tree). The two topologies were very similar and differences only involved poorly supported nodes.

among loci or between analytical methods (concatenated or species tree). Indeed, *D. mahrattensis* appears sister to the African *Dendropicos* radiation, sister to the Palearctic/Indo-Malayan group, or even sister to the African species *D. pyrrhogaster* but always without strong support and being rather divergent. Most of the uncertainties concerning the monophyly of the African taxa involve *D. pyrrhogaster/D. namaquus* as the four other sampled species cluster together with strong support. In a previous study that focused on the higher-level relationships among the woodpecker tribes, we included three species from that clade: *mahrattensis*, *pyrrhogaster* and *fuscescens* (Fuchs et al., 2013). The two African species clustered together with strong support in the species tree analyses with *mahrattensis* being sister to these two species. Hence, the monophyly of the African taxa was supported in Fuchs et al. (2013). The fact that we could not recover the monophyly of the African taxa here could thus be explained by a soft polytomy as Fuchs et al. (2013) used 16 loci in their analysis. No species from the *D. auriceps–D. dorae–D. medius* clade was sampled by Fuchs et al. (2013) so no further conclusions concerning the relationships among *D. mahrattensis*, *Dendropicos* and the *D. auriceps-D. dorae-D. medius* clade could be made.

The second primary clade includes all *Picoides* and *Veniliornis* that are distributed in the New World as well as two Old World species, *Dendrocopos minor* and *Dendrocopos cathparius*. This mostly New World clade divides in three subclades for which the species composition and relationships are very similar (differences involved weakly supported nodes) to those extensively reported in

previous studies, including the fact that South America was colonized twice by members of the Dendropicini (Weibel and Moore, 2002a, b, Moore et al., 2006). Hence we will not discuss the phylogenetic relationships among the members of the New World clade. Recently, Winkler et al. (2014) suggested that *D. cathparius* is the sister-species of *D. minor*. Our results, derived from a different *D. cathparius* specimen, confirm this relationship that is also supported by a synapomorphic one base pair deletion in MB. The Scarlet-breasted Woodpecker, *D. cathparius*, was traditionally considered to be related to *D. darjellensis*, based on similar plumage patterns although the vocalizations differ (Winkler and Short, 1978). Our analyses revealed another case of striking plumage convergence in woodpeckers. Yet, we cannot ascertain if this convergence in plumage pattern of these partially sympatric species is due to the sharing of the same ecological constraints (tropical habitat, *Veniliornis*, Moore et al., 2006) or mimicry of the smaller species (*cathparius*) to match the pattern of the larger species (*darjellensis*), as possibly seen between *P. pubescens* and *P. villosus* (Weibel and Moore, 2005).

Finally, the last clade includes eleven species of large size that are found in Indo-Malaya and the Palearctic. The monophyly of this clade was supported in all analyses. The three species (*hyperythrus*, *atratus* and *macei*) that are distributed in the highland pine and oak forest of the Indo-Malayan bioregion are basal in this assemblage and well differentiated from each other (ATP6 uncorrected divergence: 10.6–13.2%). The remaining eight species, mostly found in the Palearctic bioregion, cluster together in a clade where the

maximum genetic divergence between a species pair is 5.3% (*D. leucopterus* and *S. noguchii*). Hence this pattern suggests that (1) tropical species are rather old and that few opportunities of speciation existed for them in Indo-Malaya and (2) the Palearctic clade had higher speciation rates. This pattern is in accord with recent studies that showed that speciation and extinction rates are higher at high latitudes than in the tropics (Weir and Schluter, 2007). In the Palearctic clade, our analyses revealed that the *D. major* superspecies *sensu* Winkler and Christie (2002; *D. major, D. leucopterus, D. assimilis, D. syriacus, D. himalayensis*) also includes *D. darjellensis*, a species traditionally thought to be related to *D. cathparius* (see above). We found very little divergence (0.4%) between the Sind (*D. assimilis*) and Syrian (*D. syriacus*) Woodpeckers, a level of divergence that is found at the intra-specific level in closely related species in our data set (e.g. *D. major* 0.3%). These two parapatrically distributed taxa are sometimes considered conspecific and are known to hybridize (Winkler and Christie, 2002). The Great-spotted and White-winged Woodpeckers were recovered as sister species in all our analyses. These two species are also known to hybridize, with one taxon (*tianshanicus*) even considered to represent offspring of mixed pairs (Winkler and Christie, 2002). The two species differ by 1.2% divergence in the ATP6 locus. The possibility of hybridization among the Palearctic species appears to be relatively common as hybrids identified using molecular data have also been noticed between *D. major* and *D. syriacus* (Michalczuk et al., 2014).

5. Classification

Proposing a new classification based on a phylogeny should be based on congruent results from several independent studies. The pied woodpeckers, especially the New World members, have received substantial attention over the last decade. The multiple phylogenies that have been proposed (Weibel and Moore, 2002a,b; Winkler et al., 2005; Moore et al., 2006, this study) are highly congruent regarding the topology and relationships of species. Hence, we think that our phylogeny, which includes all species that have never been sampled before, could serve as the support for a new taxonomy of the pied woodpeckers. Of course, several options do exist to keep genera monophyletic. The simplest solution would be to lump all species into one genus, *Picoides* Lacépède, 1799. However, this classification scheme would ignore the strong biogeographic structure of the clade or morphological differences across groups. We here propose to recognize nine genera for the pied woodpecker assemblage. We consider this scheme as the best option as it allows for taxonomic stability since two of the long recognized genera (*Dendropicos* and *Veniliornis*) are kept but also highlights the differences among groups (geography and size). A new taxonomic classification of the pied woodpeckers largely in accordance with our proposition has been recently proposed by Winkler et al. (2014). The main differences with our taxonomic proposition concerns the genus assignment of *dorae, auriceps, medius* and *mahrattensis*. We here propose to assign *medius, auriceps* and *dorae* to *Dendrocoptes* and not to the genus *Leiopicus*, as suggested by Winkler and Christie (2002) and Winkler et al. (2014), and *mahrattensis* to *Leiopicus*. In our classification, the genus *Leiopicus* is thus monospecific. The recognition of *Dendrocoptes* is justified by the uncertainties concerning the relationships of *mahrattensis*, the type species of *Leiopicus*, in our analyses. If further studies confirm the sister-group relationship between *mahrattensis* and the three *Dendrocoptes* species, we would recommend lumping *Dendrocoptes* into *Leiopicus*. Last, we choose to restrict the genus *Picoides* to the northern species *tridactylus* and *arcticus*. These species formed a distinct group which was sister to a group formed by the small East Asian and Indo-

Malayan species included in *Picoides* by Winkler et al. (2014) and assigned to the genus *Yungipicus* in the present study.

We below detail the species that we recommend to attribute to each of these genera using the species recognized by Dickinson (2003).

Picoides Lacépède, 1799. Species *arcticus, tridactylus*
Yungipicus Bonaparte, 1854. Species *canicapillus, kizuki, moluccensis* (including *nanus*), *maculatus, temminckii*. Based on the topology, we would recommend elevating *nanus* to species status.
Dryobates Boie, 1826. Species *cathpharius, minor, pubescens, scalaris, nuttallii*
Leuconotopicus Malherbe, 1845. Species *albolarvatus, borealis, fumigatus, stricklandi, villosus*
Veniliornis Bonaparte, 1825. Species *spilogaster, passerinus, frontalis, maculifrons, cassini, affinis* (including *chocoensis*), *kirkii, callonotus, sanguineus, dignus, nigriceps, lignus, mixtus*
Leiopicus Bonaparte, 1854. Species *mahrattensis*
Dendrocoptes Cabanis and Heine, 1863. Species *auriceps, medius, dorae*
Dendropicos Malherbe 1849. Species *fuscescens, stierlingi, elachus, abyssinicus, poecilolaemus, gabonensis, lugubris, elliotii, goertae* (including *spodocephalus*), *griseocephalus, namaquus, xantholophus, pyrrhogaster, obsoletus*
Dendrocopos Koch, 1816. Species *atratus, macei, hyperythrus, major, leucopterus, syriacus, assimilis, himalayensis, darjellensis, leucotos, noguchii*

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.2015.03.016.

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