Molecular phylogeny and biogeographic history of the piculets (Piciformes: Picumninae)

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The subfamily Picumninae (piculets) includes 3 genera and 30 species of tiny and short-tailed woodpeckers with a pantropical distribution. Within the Picumninae, two cases of intercontinentally disrupted distributions at the genus level occur. The first one concerns the genus *Sasia* (one species in Africa and two in southeast Asia) while the second concerns *Picumnus* (one species in southeast Asia and 25 in South America). These disrupted distributions, as well as several morphological differences, have lead some authors to place the African representative of *Sasia* and the southeast Asian representative of *Picumnus* in their own monotypic genera (*Verreauxia* and *Vivia*, respectively). To address the taxonomic status and biogeographic history of the piculets, we sequenced 2676 bp of DNA from one mitochondrial (ND2) and two nuclear markers (myoglobin intron 2 and β -fibrinogen intron 7). Monophyly of Picumninae could not be recovered with confidence, while monophyly of *Sasia* and *Picumnus* were always strongly supported. Molecular dating analyses revealed that the splits both between the African and Indo-Malayan *Sasia* and between the New World and Old World *Picumnus* occurred at ca 7.9 Myr BP. This time corresponds to the beginning of the formation of the northern Hemisphere ice sheets and the accompanying expansion of grasslands throughout the world. The spread of open areas in the northern parts of Eurasia and America prevented gene flow between tropical forest birds, such as the piculets, in Africa, southeast Asia and South America, respectively.

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The avian subfamily Picumninae (Picidae) includes 30 species of small (9–11 cm) and short-tailed woodpeckers that are distributed in the tropics (Dickinson 2003). These birds climb tree trunks and mainly feed on ants and other small insects, or on large larvae. Current classification recognizes three genera in Picumninae, *Picumnus*, *Sasia* and *Nesocittes* (Dickinson 2003). Twenty-five out of the 26 recognized species in the genus *Picumnus* occur in South America while the remaining species, the speckled piculet *P. innominatus*, is endemic to southeast Asia. The South American species of

Picumnus form an assemblage of closely related species and superspecies that is supposed to have diversified recently, as inferred from their potential of interbreeding (Winkler and Christie 2002). The Asian species *innominatus* shares a synapomorphic tail pattern as well as a derived origin of the *musculus serratus anterior* (first thoracic rib only versus first thoracic rib and last cervical in other woodpeckers) with the New World members of *Picumnus* (Goodge 1972, Short 1982, Winkler and Christie 2002). The geographic distribution of *Picumnus* is unusual among birds and may only be paralleled in the

genera *Celeus* (Picidae) and *Spizaetus* (Accipitridae). Based mainly on the wide gap in geographic distribution the speckled piculet has sometimes been placed in a separate genus, *Vivia* Hodgson, 1837 (Wolters 1975–1982).

The Old World genus *Sasia* constitutes another example of an intercontinental distribution in Picumninae. Two of the three species of *Sasia* occur in tropical Asia (*abnormis* and *ochracea*), while the third is found in African rain forests (*africana*). The members of *Sasia* share several distinctive morphological features (e.g. bare area around the eye, round cross-section of the upper mandible) and the monophyly of the genus has never been questioned (Short 1982, Winkler and Christie 2002). Nevertheless, some peculiar morphological variation also occurs: *Sasia abnormis* and *S. ochracea* have ten tail feathers and lack the first toe, while *S. africana* has eight tail feathers and a first toe (albeit greatly reduced; Short 1988). Historically the African representative has been placed in a separate genus, *Verreauxia* Hartlaub, 1856 (Peters 1948).

The third genus of piculets is *Nesocittes*, which includes one species living on Hispaniola in the Antilles. This species is larger than the other piculets but shares with them the soft plumage, short bill and short tail. Other anatomical data indicate that *Nesocittes* may be closer to the Picinae than to other Picumninae (Goodge 1972).

The piculets constitute a group of birds with a highly interesting pattern of geographic distribution. A phylogenetic analysis of their relationships is expected to shed light on the evolution and biogeographic history of tropical forest birds in South America, Africa and southeast Asia. No such study has yet been published although Webb and Moore (2005) included three species of piculets (*Sasia abnormis*, *Picumnus aurifrons* and *P. cirratus*) in their molecular analysis. The results indicated that *S. abnormis* and the two *Picumnus* (*P. aurifrons* and *P. cirratus*) had been separated for a long period of time, but little biogeographic information can be extracted from that because of the sparse taxon sampling.

Herein, we study the relationships among piculets by using 2,676 bp obtained from two nuclear markers (myoglobin intron 2 and β -fibrinogen intron 7) and a mitochondrial protein-coding gene (ND2).

Material and methods

Taxonomic sampling

We sampled representatives of each biogeographic unit (Africa, Asia, South America). Unfortunately, we could not obtain any sample of the Antillean piculet *Nesocittes micromegas*. Several individuals were sequenced for the Old World taxa in order to investigate intra-

specific variability in the two genetic markers. We also included in our study several representatives of the Picinae in order to test the monophyly of the Picumninae and obtain independent data on inter-generic distances. We sampled Picinae genera from the three main clades of woodpeckers highlighted by Webb and Moore (2005). Trees were rooted with a representative of the honeyguides (*Indicator minor*, Indicatoridae), a family suggested to be the sister-group of the Picidae (Simpson and Cracraft 1981, Swierczewski and Raikow 1981, Sibley and Ahlquist 1990, Johansson and Ericson 2003, Webb and Moore 2005). Identifications and geographic origins of the samples and GenBank accession numbers are given in Table 1.

Laboratory procedure

We extracted DNA from frozen or alcohol preserved tissues (blood, liver, muscle) using a CTAB-based protocol (Winnepeninckx et al. 1993) with an overnight Proteinase K (0.1 mg.ml⁻¹) digestion. Myoglobin intron 2 was amplified with primers Myo2 or Myo2 Pi-F (5' CCT GTC AAA TAT CTG GAG GTA TG 3', this study), and Myo3F (Heslewood et al. 1998, Slade et al. 1993). The whole ND2 gene was amplified with primers L5219-Met and H6313-Trp for most of the samples (Sorenson et al. 1999). Additional primers, (L5758-ND2, H6681-CO1, Sorenson et al. 1999) were used for some samples that were difficult to amplify or sequence with L5219-Met and H6313-Trp. β -fibrinogen intron 7 was amplified with primers FIB-B17U and FIB-B17L (Prychitko and Moore 1997). The thermocycling conditions followed standard procedures for these genes (Prychitko and Moore 1997, Fuchs et al. 2004). Three microliters of the amplification products were electrophoresed on 1.5% agarose gel and visualized under UV light with ethidium bromide to check for the correct fragment size and to control for the specificity of the amplifications. PCR products were purified directly using the QiaQuick PCR Purification Kit (Qiagen, Holden, Germany) and cycle-sequenced using the CEQ Dye Terminator Cycle Sequencing kit (Beckman Coulter, Inc, Fullerton, CA, USA) or the 'BigDye[®] Terminator Cycle Sequencing Kit' (Applied Biosystems) in both forward and reverse directions with the same primers used for PCR amplifications, and finally ran on an automated CEQ2000 DNA Analysis System sequencer (Beckman Coulter, Inc, Fullerton, CA, USA) or an ABI PRISM[®] 3100 automated sequencer (Applied Biosystems). We obtained sequences from both strands of DNA for all taxa.

No length variations between alleles were detected for myoglobin intron 2 and β -fibrinogen intron 7. The occurrence of single nucleotide polymorphism (SNP) in the myoglobin intron 2 and β -fibrinogen intron 7

Table 1. List of taxa studied (following Dickinson 2003), tissue or voucher number and GenBank accession numbers. Abbreviations: AMNH, American Museum of Natural History, New York; LSUMZ, Museum of Natural Science, Louisiana State University; MNHN, Muséum National d'Histoire Naturelle, Paris; NRM, Swedish Museum of Natural History, Stockholm; ZMCU, Zoological Museum University of Copenhagen. References: 1 and 2; Prychitko and Moore (2000, 2003), and 3; Weibel and Moore (2002).

Species	Family	Sample no.	Origin	Myoglobin	Fibrinogen	ND2
Ingroup						
<i>Campephilus haematogaster</i>	Picidae	ZMUC 114730	Ecuador	DQ188143	AF240016 ¹	DQ188169
<i>Campepthera caroli</i>	Picidae	MNHN 03-04	Cameroon	DQ188157	DQ188131	DQ188183
<i>Colaptes auratus</i>	Picidae	AMNH PAC820	USA	DQ188152	AY082398 ²	DQ188178
<i>Dendrocopos leucotos</i>	Picidae	NRM 996095	Sweden	DQ188142	DQ188116	DQ188168
<i>Dendrocopos major</i>	Picidae	MNHN C29	France	DQ188153	DQ188127	DQ188179
<i>Dendrocopos minor</i>	Picidae	NRM 986593	Sweden	DQ188154	AF394321 ³	DQ188180
<i>Dryocopus martius</i>	Picidae	MNHN C30	France	DQ188140	DQ188114	DQ188166
<i>Jynx torquilla</i>	Picidae	MNHN 15-03	China	DQ188146	DQ188120	DQ188172
<i>Picoides mixtus</i>	Picidae	NRM 976765	Paraguay	DQ188151	AF394323 ³	DQ188177
<i>Picoides tridactylus</i>	Picidae	ZMUC 115007	Poland	DQ188138	AF394332 ³	DQ188164
<i>Picumnus aurifrons</i>	Picidae				AY082399 ²	
<i>Picumnus cirratus</i>	Picidae	NRM 996693	Paraguay	AY816219	DQ188124	AY816227
<i>Picumnus innominatus chinensis</i>	Picidae	MNHN 18-46	China	DQ188147	DQ188121	DQ188173
<i>Picumnus innominatus malayorum</i>	Picidae	MNHN 4-2H	Thailand	DQ188145	DQ188119	DQ188171
<i>Picumnus innominatus malayorum</i>	Picidae	MNHN 31-92	Lao RDP	DQ188144	DQ188118	DQ188170
<i>Picumnus rufiventris</i>	Picidae	ZMUC 114884	Ecuador	DQ188141	DQ188115	DQ188167
<i>Picumnus temminckii</i>	Picidae	ZMUC 115783	Paraguay	DQ188139	DQ188113	DQ188165
<i>Picus canus</i>	Picidae	MNHN 05-09	Lao RDP	DQ188156	DQ188130	DQ188182
<i>Picus viridis</i>	Picidae	MNHN C38	France	DQ188155	DQ188129	DQ188181
<i>Sasia abnormis</i>	Picidae	LSUMZ B-36428	Malaysia	DQ188137	DQ188111	DQ188163
<i>Sasia africana</i>	Picidae	MNHN 02-36	Cameroon	DQ188148	DQ188122	DQ188174
<i>Sasia africana</i>	Picidae	MNHN 03-05	Cameroon	DQ188149	DQ188123	DQ188175
<i>Sasia ochracea</i>	Picidae	MNHN 4-2F	Thailand	DQ188134	DQ188108	DQ188160
<i>Sasia ochracea</i>	Picidae	MNHN 4-2G	Thailand	DQ188133	DQ188107	DQ188159
<i>Sasia ochracea</i>	Picidae	NRM 947313	Vietnam	DQ188136	DQ188110	DQ188162
<i>Sasia ochracea</i>	Picidae	MNHN 05-43	Lao RDP	DQ188135	DQ188109	DQ188161
Outgroup						
<i>Indicator minor</i>	Indicatoridae	ZMUC 115456	Tanzania	DQ188132	DQ188106	DQ188158

sequences was suggested by the presence of double peaks. Those double peaks were coded using the appropriate IUPAC code. Absence of insertions, deletions and stop-codons in the reading frame of the protein-coding ND2 gene suggests that we did not amplify nuclear pseudogenes (Sorenson and Quinn 1998).

Multiple alignments of intron sequences were accomplished by hand modification after an initial alignment by Sequencher 4.1 (Gene Codes Corporation, Ann Arbor, MI, USA). Alignment of indels was straightforward for the two nuclear introns and indicated the presence of several insertion-deletions events (8 and 32 for myoglobin intron 2 and β -fibrinogen intron 7, respectively) among which 1 and 21 (myoglobin intron 2 and β -fibrinogen intron 7, respectively) were potentially informative. One region in the β -fibrinogen intron 7 sequences (nucleotides 461–473 in our alignment) was excluded for the phylogenetic analyses because it could not be aligned unambiguously. The alignments of the nuclear introns have been deposited in the EMBL database (accession numbers ALIGN_000924 and ALIGN_000925 for myoglobin intron 2 and β -fibrinogen intron 7, respectively). Gaps were treated as missing data in the analyses.

Phylogenetic analyses

Phylogenetic trees were estimated using parsimony (P), maximum likelihood (ML) and Bayesian inferences (BI) as implemented in PAUP* 4.0b10 (Swofford 2001) and MrBayes v. 3.0 (Huelsenbeck and Ronquist 2001). Likelihood models and parameters were estimated with Modeltest v. 3.6 (Posada and Crandall 1998), and MrModeltest (Nylander 2004) using the AIC criterion. P and ML analyses were performed using the heuristic tree bisection and reconnection branch-swapping (TBR) with 100 and 10 random addition replicates, respectively. Nodal support for P and ML was calculated using the non-parametric bootstrap (Felsenstein 1985; 1,000 and 100 replicates, respectively). ML bootstrap replicates were performed using PHYML (Guindon and Gascuel 2003). The three different gene regions we sequenced differ considerably in their properties and substitution dynamics (see results), we therefore did not perform combined ML and Bayesian analyses that assume a single model of evolution for the whole dataset. Accordingly, Bayesian analyses for the combined data set were performed freeing different parameters (base frequencies, rate matrix, shape parameter, proportion of invariable sites) to vary between the three partitions (ND2, myoglobin intron 2, β -fibrinogen intron 7). Four

Metropolis-coupled MCMC chains (three heated and cold) were run for 2×10^6 generations with trees sampled every 100 generations. The first 200,000 generations (2,001 trees) were discarded ('burn-in' period) and the posterior probabilities were estimated for the remaining saved generations. Three independent Bayesian runs initiated from random starting trees were performed for each data set and the log-likelihood values and posterior probabilities were checked to ascertain that the chains had reached stationarity.

Estimation of divergence times

Only a few fossils have been assigned to the woodpeckers so far. The earliest fossil record from the Pici (barbets and toucans, honeyguides, piculets and woodpeckers), a fragmentary distal end of a tarsometatarsus, is dated to be from the lower Oligocene of Belgium (Mayr and Smith 2001). Mayr (2001) also described a tarsometatarsus from the late Oligocene of Germany that closely resembles in size and proportions that of a cardinal woodpecker *Dendropicos fuscescens*. However, in both cases, a reliable assignment to any of the Pici family is not possible (Mayr 2001, Mayr and Smith 2001). A fossil feather preserved in amber constitutes thus the earliest fossil clearly assigned to the woodpecker family Picidae (Laybourne et al. 1994). This feather was identified to be closely related to the Antillean piculet *Nesocittes micro-megas* and estimated to be at least from the early Miocene (23.8 Myrs BP) and maximally from the upper Eocene (54.8 Myrs BP; Laybourne et al. 1994). The fact that we lack tissue of the Antillean Piculet prevented us to use this calibration point, even for specifying a minimum age for the Picinae-Picumninae split (see Results and Discussion). Instead, we used a calibration point based on biogeographic evidences. The calibration point used was the split between *Sasia ochracea* and *S. abnormis*. The distributional ranges of *S. ochracea* and *S. abnormis* are delimited by the Isthmus of Kra. Two important Isthmus of Kra Seaways probably occurred in the early Middle Miocene (24-13 Myrs BP), and in the early Pliocene (5.5-4.5 Myr BP; Woodruff 2003). These seaways would have separated the Sundaic and Indochinese biotas by a 30–100 kms wide gap of sea. Piculets are morphologically characterized by their very small size and round wings, which clearly not favour such oversea dispersal. Furthermore, these birds seems to be very philopatric as shown by a capture-recapture data, two females of *S. abnormis* being retrapped only 800 m from where they were originally mist-netted several years earlier (Winkler and Christie 2002). Therefore, the Isthmus of Kra Seaways probably have promoted the speciation between these two piculets and we here tentatively dated the *S. ochracea*-*S. abnor-*

mis split to have occurred 5 Myrs BP (as the mean between 4.5 and 5.5).

The applicability of the global molecular clock for all individual data sets was tested using the Likelihood Ratio Test ($-2 \ln A = 2(\ln \lambda_1 - \ln \lambda_2)$, where λ_1 is the likelihood of the restricted model) (Huelsenbeck and Rannala, 1997), which follows a Chi-squared distribution with $n-2$ degrees of freedom where n is the number of taxa. Divergence times were estimated using PAML 3.14 β (Yang 2003). A molecular clock was followed (see below) and we assigned a GTR+G model to each partition (ND2, myoglobin intron 2 and β -fibrinogen intron 7) for the dating analysis. The Bayesian tree obtained from the combined analyses of the three genes was used for the molecular clock analysis.

Results

The concatenation of the three gene regions resulted in a final alignment of 2676 bp. From the myoglobin intron 2, we obtained between 646 bp (*Dendrocopos minor*) and 682 bp (*Picoides tridactylus*), resulting in an alignment of 686 bp among which 76 were parsimony informative. Both Modeltest (Posada and Crandall 1998) and MrModeltest (Nylander 2004) selected the K80+G model as the best-fit model for the myoglobin data set. The ML parameters estimated by Modeltest were: base frequencies ($A=C=G=T=0.25$), ts/tv ratio = 2.5088, $\alpha = 0.6309$. Parsimony analyses yielded 16 equally parsimonious trees of 197 steps (consistency index = 0.85, retention index = 0.89). The strict consensus of the 16 equally parsimonious trees was identical to the Bayesian tree (mean score of the three runs: $-\ln = 2197.23$, SD = 0.73; Fig. 1). ML analyses yielded one tree ($-\ln = 2176.5033$) for which the topology only differ at two nodes from the Parsimony and Bayesian trees. These nodes were polytomized in the MP and Bayesian trees and resolved in the ML tree (none of them received significant support). The likelihood ratio test did not reject the hypothesis of a molecular clock for the myoglobin sequences (molecular clock: $-\ln = 2182.11$, $-2 \ln A = 11.21$, $df = 24$, $P = 0.99$).

From the β -fibrinogen intron 7, we obtained between 753 bp (*Picus canus*) and 888 bp (*Indicator minor*), resulting in an alignment of 968 bp. After exclusion of the zone where nucleotide alignment was ambiguous, the β -fibrinogen intron 7 data set was 954 bp long (261 characters were variable among which 138 were informative). Modeltest and MrModeltest selected the TVM and GTR model as the best-fit models for the β -fibrinogen data set, respectively. ML parameters for the TVM model estimated by Modeltest were: base frequencies (0.3024 0.1748 0.1794), rate matrix (1.1312 3.5509 0.8731 2.1501 3.5509). Parsimony analyses yielded 2 equally parsimonious trees of 310 steps

Fig. 1. Bayesian tree ($-\ln = 2197.23$ $SD = 0.73$) obtained from the nuclear myoglobin intron 2 gene under a K80+G model for nucleotide substitutions. Bootstrap proportions and posterior probabilities are indicated as P/ML/BI. Asterisks represent bootstrap values/posterior probabilities higher than 90% and 0.95, respectively. Dashes indicate that either an alternative node was found or that the node was supported by a bootstrap value/posterior probabilities lower than 50% and 0.6, respectively.

(consistency index = 0.92, retention index = 0.95). The strict consensus of the 2 equally parsimonious trees was identical to the ML and Bayesian trees (Fig. 2; ML tree: $-\ln = 3170.00$, Bayesian mean score of the three runs: $-\ln = 3194.93$, $SD = 0.24$). The likelihood ratio test did not reject the hypothesis of a molecular clock for the β -fibrinogen sequences (molecular clock: $-\ln = 3180.19$, $-2 \ln A = 20.38$, $df = 25$, $P = 0.73$).

The final alignment for the ND2 gene was 1,036 bp long, corresponding to the positions 5246 to 6281 of the chicken mitochondrial sequence (GenBank accession number X52392, Desjardins and Morais 1990). The number of parsimony informative sites was 445. Both Modeltest and MrModeltest selected the GTR+G+I model for the whole ND2 gene. The GTR+G+I model was also selected by MrModeltest for all three codon

positions. Modeltest parameters estimates for the ML analysis were as follows: base frequencies (0.3425 0.4317 0.0518), rate matrix (0.1101 7.2948 0.3164 0.0186 2.4163), α (0.9420), and proportion of invariable sites (0.4003). Parsimony analysis yielded one most parsimonious tree of 1660 steps (consistency index = 0.48, retention index = 0.62) that only differs at one node (the relative positions of *Picumnus* and *Sasia* with respect to Picinae) from the ML tree ($-\ln = 8299.20$). In neither case the node in question received bootstrap support. Both the partitioned by codon position (mean score of the three runs: $-\ln = 7860.21$, $SD = 0.26$) and non-partitioned (mean score of the three runs: $-\ln = 8321.41$, $SD = 0.16$) Bayesian analyses recovered similar trees (Fig. 3), the few discrepancies being nodes that were poorly supported, i.e., posterior probabilities

Fig. 2. Bayesian tree ($-\ln = 3194.93$, $SD = 0.24$) obtained from the nuclear β -fibrinogen intron 7 under a GTR model for nucleotide substitutions. Bootstrap proportions and posterior probabilities are indicated as P/ML/BI. Asterisks represent bootstrap values/posterior probabilities higher than 90% and 0.95, respectively. Dashes indicate that either an alternative node was found or that the node was supported by a bootstrap value/posterior probabilities lower than 50% and 0.6, respectively.

below 0.6 in one analysis, but collapsed in the other. The likelihood ratio test did not reject the hypothesis of a molecular clock for the ND2 sequences (molecular clock: $-\ln = 8314.90$, $-2 \ln A = 31.40$, $df = 24$, $P = 0.13$).

The combined analyses were performed without *Picumnus aurifrons* as we only had a fibrinogen sequence for this taxon. Parsimony (2,169 steps, 1 most parsimonious tree, consistency index = 0.57, retention index = 0.70), and Bayesian analyses (partitioned by gene: mean of the three runs: $-\ln = 13705.32$ $sd = 0.60$; partitioned by gene and codon position for ND2: mean of the three runs: $-\ln = 13244.31$ $sd = 0.82$) of the combined dataset yielded trees that are nearly identical to the individual gene trees (discrepancies

concern only nodes that were poorly supported in all analyses, such as the relative positions of *Sasia* and *Picumnus* with respect to the Picinae; Fig. 4).

Three main clades were recovered, corresponding to the genus *Sasia*, genus *Picumnus* and subfamily Picinae, respectively. These clades were all supported by bootstrap values of 100% and posterior probabilities of 1.0 (1–4). The mapping of the indels onto the trees reveals that monophyly of *Picumnus*, *Sasia* and Picinae were also supported by 2, 3 and 3 synapomorphic indels, respectively. *Sasia africana* was the sister-group of the two southeast Asian species (*S. abnormis* and *S. ochracea*) while the Southeast Asian *Picumnus innominatus* was the sister-group of the clade composed by the South American *Picumnus*. Both the African

Fig. 3. Bayesian tree ($-\ln = 8321.41$ $SD = 0.16$) obtained from the mitochondrial ND2 gene under a GTR + G + I model for nucleotide substitutions (non-partitioned by codon position). Bootstrap proportion and posterior probabilities are indicated as P/ML/BI non-partitioned/BI partitioned. Asterisks represent bootstrap values/posterior probabilities higher than 90% and 0.95, respectively. Dashes indicate that either an alternative node was found or that the node was supported by a bootstrap value/posterior probabilities lower than 50% and 0.6, respectively.

Sasia and the southeast Asian *Picumnus* are genetically divergent from their closest relatives (see Figs. 1–4).

The molecular dating analyses suggest that the African *Sasia* and the Old World *Picumnus* diverged from their closest relatives (Asian *Sasia* and New World *Picumnus*, respectively) at ca 7.9 MYA ($SD = 0.7$ and $SD = 0.8$, respectively).

Discussion

Picumninae

Monophyly of Picumninae has never been questioned because of the close morphological resemblance be-

tween these birds (Peters 1948, Wolters 1975–82, Winkler and Christie 2002, Dickinson 2003). None of our analyses strongly recovered the Picumninae as monophyletic. Instead, we found three well differentiated lineages within the Picidae: *Picumnus*, *Sasia* and the Picinae. In theory, this result could be due to that our taxonomic sampling did not allow us to test the monophyly of the presumed *Sasia*-*Picumnus* clade. This is very unlikely because we sampled several Picinae genera as well as a member of the subfamily Jynginae, the sister-group of the Picumninae-Picinae clade (Swierczewski and Raikow 1981, Pritchko and Moore 2003). An alternative hypothesis is that *Sasia* and *Picumnus* do not form a monophyletic assemblage. Non-monophyly of the *Sasia*-*Picumnus* clade seems

Fig. 4. Bayesian tree ($-\ln = 13705.32$ $SD = 0.60$) obtained from the combined data set with the best fit model assigned to each partition (ND2, myoglobin intron 2, β -fibrinogen intron 7). Bootstrap proportion and posterior probabilities are indicated as P/BI partitioned by gene/BI partitioned by codon position. Asterisks represent bootstrap values/posterior probabilities higher than 90% and 0.95, respectively. Dashes indicate that either an alternative node was found or that the node was supported by a bootstrap value/posterior probabilities lower than 50% and 0.6, respectively.

0.1 substitution / site

dubious, however, given that this clade is supported by several morphological synapomorphies (e.g. the loss of the coracoid head of the subcoracoideus and the presence of a fleshy insertion of the *musculus rhomboideus profundus* on the ilium; Goodge 1972). It should be noted that *Nesocittes* do not possess these two derived characters (Goodge 1972) and its relationships with respect to other piculets need to be further explored. Monophyly of the *Sasia-Picumnus* clade was also recovered by Webb and Moore (2005) with nearly significant posterior probabilities (0.94). Finally, a third hypothesis would be that Picumninae is in fact monophyletic, and that the *Picumnus-Sasia* split occurred more or less contemporaneously to the split between Picinae and Picumninae. This may be the most plausible hypothesis because our estimation of the divergence time for the *Picumnus-Sasia-Picinae* trichotomy is 15.5 Myrs BP ($SD = 1.3$), an age contemporary

with the Miocene climate optimum (Zachos et al. 2001). The hotter and more humid climate during this period probably favoured the formation of more complex ecological niches leading to more diverse foraging strategies among woodpeckers. Such a scenario is supported by the study of Styring and Hussin (2004) who showed that tropical rain forests in Malaysia offer an increasing variation in micro-habitats and ecological niches available to woodpeckers when compared to more temperate regions. The increased diversification in foraging strategies may in turn have led to a larger morphological and ethological variation between the three main lineages of woodpeckers leading to the *Picumnus*, *Sasia* and *Picinae* clades of today. Possibly, the systematic relationships between these lineages could be resolved by adding more sequence data and more taxa, especially the Antillean piculet (*Nesocittes micromegas*).

Picumnus

Monophyly of this genus has never been questioned but its pantropical distribution is unusual among birds and this has been used as an argument to recognize the genus *Vivia* for the Asian species. The merging of *Vivia* with *Picumnus* is supported by the general colouration pattern share, the laterally converging white bars on the outer tails feather and the white stripe on the central feather. Two other, often cited cases of pantropical distributions at the genus level in birds, *Celeus* (Picidae) and *Spizaetus* (Accipitridae; Dickinson 2003), may not be correct as both *Celeus* and *Spizaetus* are polyphyletic (Bunce et al. 2005, Lerner and Mindell 2005, Fuchs et al. unpub. data). The situation in *Picumnus* may thus be unique among birds. Our data strongly supported the monophyly of the genus *Picumnus* but the genetic distances between the Asian species and the four South American species are larger than those observed between traditionally recognized Picinae genera (see below). Given these data, retaining *P. innominatus* in the genus *Vivia* Hodgson, 1837 would appear, at a first instance, rather logical, but we do not advocate such a change as no morphological synapomorphy clearly define the remaining *Picumnus*. Our taxonomic sampling of South American *Picumnus* is also limited (four species representing three different superspecies). However, we can rule out the the Asian species is embedded within the New World radiation because the diversification of *Picumnus* in South American seems to be a rather recent event, as inferred from the several species complex (superspecies) currently recognized (e.g. Winkler and Christie 2002). Our estimation of the date of the first split within the two main South American lineages (3.0 Myr BP, $sd = 0.04$) suggests that the radiation of *Picumnus* in South America is attributable to the Plio-Pleistocene climatic oscillations and formations of river barriers throughout the Amazonian basin. As a consequence, we are confident that the Asian *Picumnus* represents a relict species that is the sister-group of the New World *Picumnus* radiation.

Sasia

The genus *Sasia* includes three species of tiny woodpeckers with disrupted distribution as one species (*S. africana*) is endemic to tropical Africa while the remaining two species (*S. abnormis* and *S. ochracea*) inhabits the Indo-Malayan Region. Due to some peculiar morphological differences such as number of toes and tail feathers (Short 1988), the African species was sometimes retained in its own genus, *Verreauxia* Hartlaub, 1856 (Peters 1948). All our analyses strongly supported the monophyly of the genus *Sasia* although the split between the African and the two Asian representatives is ancient (estimated herein to 7.9 Myrs BP). In fact, this predates the splits between many traditionally recognized genera in Picinae, e.g., the split

between *Dryocopus* and *Colaptes* which is here estimated to have occurred 5.2 Myr BP, $SD = 0.5$. Based both on morphological and genetic distinctness we resurrect the genus *Verreauxia* Hartlaub, 1856, for the African Piculet. The genus *Verreauxia* is defined by its lower number of rectrices (eight in *Verreauxia* versus ten in all other woodpeckers).

Biogeography

The identical estimates of the dating of the divergence between the African and Asian *Sasia* and the split between the Old World and New World *Picumnus* (7.9 Myr BP) suggests that the same external factor promoted the differentiation of these groups. The time estimates correspond to the start of the period of formation of the Northern Hemisphere ice sheet. The drier conditions may in turn have promoted the expansion of grasslands and the diversification of the C4 grasses (Zachos et al. 2001). The expanding grasslands resulted in large, non-forested areas in Eurasia and North America that prevented gene flow between tropical forest birds as the piculets in Southeast Asia and Africa on one hand, and Southeast Asia and South America on the other.

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