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Molecular phylogenetics and biogeography of Neotropical piping guans (Aves: Galliformes): *Pipile* Bonaparte, 1856 is synonym of *Aburria* Reichenbach, 1853

Erwin T. Grau[a], Sérgio Luiz Pereira[a,b,*], Luís Fábio Silveira[c,d], Elizabeth Höfling[c], Anita Wajntal[a]

[a] *Departamento de Biologia, Universidade de São Paulo, Caixa Postal 11461, CEP 05422-970, São Paulo, Brazil*
[b] *Department of Natural History, Royal Ontario Museum, 100 Queen's Park, Toronto, Canada M5S 2C6*
[c] *Departamento de Zoologia, Universidade de São Paulo, Caixa Postal 11461, CEP 05422-970, São Paulo, Brazil*
[d] *Museu de Zoologia da Universidade de São Paulo, Caixa Postal 42494, CEP 04218-970, São Paulo, Brazil*

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Abstract

The Cracidae are Neotropical galliform birds with 11 genera currently recognized. To investigate the questioned validity of *Pipile* Bonaparte, 1856 and the monotypic *Aburria* Reichenbach, 1853 as separate genera, we gathered data from 2727 bp of mitochondrial DNA (cytochrome *b*, ND2 and control region) and 151 osteological characters. Our phylogenetic analyses of DNA sequences indicated that *Aburria aburri* is embedded within *Pipile*. Also, genetic distances between *Aburria* and any *Pipile* species are equivalent to the distances estimated for other congeneric cracid species, which genus status is not doubtful. Although the osteological characters do not have phylogenetic signal to solve the phylogenetic relationships at species level, five synapomorphies were found for *Aburria* and *Pipile*. Therefore, we suggest that *Pipile* should be merged with *Aburria*, which is the oldest described genus. We estimated that speciation in this group occurred in the Plio-Pleistocene, concordant with other birds, primates and rodents that have similar geographic distribution, and proposed a diversification hypothesis based on the occurrence of sea transgressions and the formation of the Amazon Lagoon. Therefore, we conclude that these palaeogeographic events may have contributed to Neotropical taxa diversification to a greater extent than previously suspected.

Keywords: *Aburria*; *Pipile*; Phylogenetic analysis; mtDNA; Osteological characters; Biogeography

1. Introduction

The family Cracidae (Aves, Galliformes) includes curassows, guans, and chachalacas, found mainly in the Neotropical region. Most species are restricted to forested areas, and some can be used as bioindicators of habitat quality (Brooks and Strahl, 2000). Due to loss of habitat and extensive hunting, many species and subspecies are threatened, and one is considered extinct in the wild (BirdLife International, 2004; Silveira et al., 2003). Currently, 11 genera, 50 species, and over 60 subspecies are recognized in this family that still has taxonomic problems, especially at and below species level (Brooks and Strahl, 2000; del Hoyo et al., 1994). Efforts to understand their evolutionary history are underway. For example, the sister relationship of Cracidae to Phasianidae (*latu sensu*) is now well corroborated by molecular and morphological data (Dimcheff et al., 2002; Dyke et al., 2003; Silveira, 2003) and phylogenetic relationships at the genus level have been proposed recently using nuclear

* Corresponding author. Fax: +1 416 586 5553.
E-mail address: sergio.pereira@utoronto.ca (S.L. Pereira).

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and mitochondrial DNA sequences (Pereira et al., 2002) and osteological characters (Silveira, 2003). Although there is incongruence between the molecular and osteological hypotheses, all genera seem to be monophyletic (Silveira, 2003), and a new focus at lower taxonomic categories raise as an important line of research.

The piping guans (*Aburria* and *Pipile*) are a group that deserves further study to check the validity of their current status as separate genera. All *Pipile* species represent a morphologically well-defined group. Despite Vuilleumier's (1965) proposal, which merged this genus in *Penelope*, most authors (e.g., del Hoyo et al., 1994; Sick, 1993; Strahl and Schmitz, 1997; Vaurie, 1968) prefer to keep this genus as distinct amongst the Cracidae. Another notable exception is found in Delacour and Amadon (1973) which, based on characters of plumage and bare parts, merged *Pipile* into *Aburria*. Nuclear and mitochondrial DNA sequences indicate a very close relationship between *Pipile* and *Aburria* and no evidence to merge them with *Penelope* (Pereira et al., 2002), and osteological data corroborate the suggestion of Delacour and Amadon (Silveira, 2003). The divergence time estimated for *Aburria* and *Pipile* based on nuclear and mitochondrial DNA sequence data was around 3.8 MYA (million years ago), well below the range of 8–10 MYA among genera of curassows or 10–18 MYA among other genera of guans (Pereira et al., 2002). Also, this divergence time is within the range estimated for species of curassows within *Crax* and within *Mitu* based on mitochondrial DNA sequences (Pereira and Baker, 2004a).

The genus *Aburria*, as currently accepted, is monotypic (*Aburria aburri*). This species is found in W Venezuela and N Colombia, E and W Ecuador and S and SC Peru, occurring primarily in wet mountain forests, in altitudes from 500 to 2500 m above sea-level (del Hoyo et al., 1994; Ridgely and Greenfield, 2001). Contrastingly, all six currently accepted forms of *Pipile* (*sensu* del Hoyo et al., 1994) are mostly found in forests east of the Andes all over South America (and Trinidad) except in Chile and Uruguay (Fig. 1).

The taxonomy of the species of *Pipile* has been controversial and problematic, and there is a persistent disagreement among authors regarding the number of species, which varies from two (Delacour and Amadon, 1973; Sick, 1993; Vuilleumier, 1965) to four (del Hoyo et al., 1994; Strahl and Schmitz, 1997). There is also a disagreement regarding the existence and the number of subspecies. It is clear that we have a long way to go to have a better understanding of the diversity of the Amazonian piping guans, but in this paper we followed del Hoyo et al. (1994) who recognized four species of *Pipile*: *Pipile cujubi*, *Pipile cumanensis*, *Pipile jacutinga*, and *Pipile pipile*.

Amongst the species of *Pipile* considered here, only *P. jacutinga* and *P. pipile* are monotypic. *P. pipile* (Trinidad Piping-guan) is restricted to Trinidad, while *P. jacutinga* (Black-fronted Piping-guan) is found in the forests of E and S Brazil, NE Argentina, and SE Paraguay. The Blue-throated Piping-guan (*P. cumanensis*) has two subspecies recognized: *Pipile cumanensis*

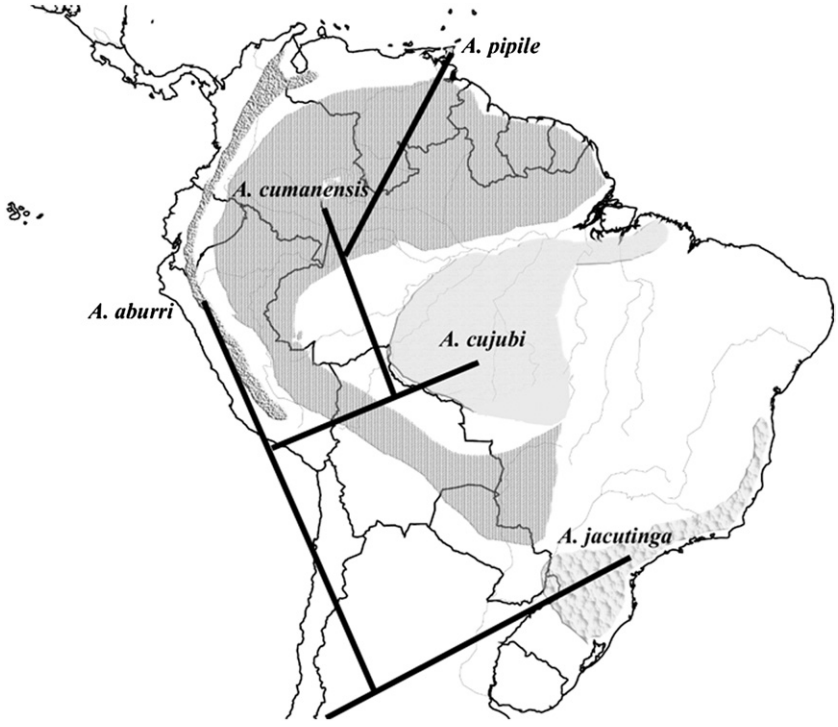

Fig. 1. Phylogeographic distribution of *Aburria* spp. Modified from Delacour and Amadon (1973) and phylogenetic relationships as estimated in the present study based on mitochondrial DNA sequences.

cumanensis largely distributed in French Guyana, Guyana, Suriname, Venezuela, NW Brazil and in the lowlands of C and E Colombia, E Ecuador and E Peru, and *Pipile cumanensis grayi* with a more limited distribution in SE Peru, NC Bolivia, SW Brazil, and NE Paraguay. This species is replaced in the Brazilian Amazon south of the Amazon river by the Red-throated Piping-guan (*P. cujubi*), also with two subspecies recognized: *Pipile cujubi cujubi*, which can be found from the lower Madeira river to N Pará state and *Pipile cujubi nattereri*, which inhabits the forests in W Brazil (del Hoyo et al., 1994).

Therefore, in this paper we investigated relationships among piping guans (*Aburria* and *Pipile* genera) based on mitochondrial DNA sequences and morphological characters to assess their status as distinct genera. We also estimated divergence time for this group and propose a phylogeographic hypothesis for their evolution.

2. Materials and Methods

2.1. Molecular data

2.1.1. Taxa sampling

Approximately 0.1 ml of blood was taken by venipuncture from seven Cracidae species (Table 1) and kept in 0.5 ml of absolute ethanol at room temperature. All sampled birds are kept in captivity by private conservationist or zoological gardens in Latin America. Samples are deposited at the Laboratório de Genética e Evolução Molecular de Aves, Instituto de Biociências, of the Universidade de São Paulo, Brazil.

2.1.2. DNA amplification sequencing, and alignment

Some cytochrome (cyt) *b* and subunit 2 of NADH dehydrogenase (ND2) sequences used here were obtained previously by Pereira et al. (2002) and are indicated in Table 1. For cyt *b*, ND2 and control region (CR) sequences obtained for this study, amplification, sequencing, and sequence alignment were performed as described in Grau et al. (2003). Primers used for PCR amplification are described in Grau et al. (2003) and Pereira and Baker (2004a).

2.1.3. Distances and saturation

Tamura–Nei distances assuming γ-distributed rates of DNA substitution (TN + G) was chosen as the best model of DNA substitution for the concatenated data set and the ND2 sequences according to a likelihood ratio test as implemented in MODELTEST 3.0 (Posada and Crandall, 1998). For control region and cyt *b*, the best model chosen was the Hasegawa-Kishino-Yano, also assuming γ distribution for rate variation across sites (HKY + G). For each gene and the concatenated data set, pairwise uncorrected (*p*-distances) and corrected distances were estimated among all seven species studied using Mega 2.0 (Kumar et al., 2001) and plotted against each other to check for saturation.

2.1.4. Phylogenetic analysis

Phylogenetic reconstructions were performed with *P. cujubi*, *P. cumanensis*, *P. jacutinga*, *P. pipile*, and *A. aburri* as the ingroup and *Penelope obscura* and *Penelope superciliaris* as outgroup. Homogeneity of base composition using variable sites only was tested in TREEPUZZLE 5.0 (Strimmer and von Haeseler, 1996) prior to phylogenetic inference. PAUP 4.0 b10 (Swofford, 2001) was used for the phylogenetic reconstructions. A Maximum Parsimony (MP) tree was obtained with an exhaustive search. Maximum Likelihood (ML) tree was estimated using an exhaustive search under the model of DNA evolution selected by data analysis with MODELTEST 3.0 (Posada and Crandall, 1998). Branch support was estimated by bootstrapping of 100 replicates of 10 random sequence additions for both MP and ML analysis. Bayesian analysis (BA) with Markov chain Monte Carlo (MCMC) sampling was performed using partitioned likelihood (Lee and Hugall, 2003) in MrBayes 3.0 (Ronquist and Huelsenbeck, 2003). The substitution model chosen for ML analysis for each gene partition

Table 1
Species sequences used in the present study with their respective voucher number and GenBank accession numbers for the control region (CR), cytochrome *b* (cyt *b*) and NADH reductase (ND2)

	Voucher	Aviary	Origin	CR	ND2	Cyt *b*
Aburria aburri	C172/C173	Tropicus	Unknown	AF165430	AY140740*	AF165466*
Pipile cujubi cujubi	C156	Collected by R. Antonelli	Jacareacanga, Pará	AY145314	AY367098 AY367092	AY367104
Pipile cumanensis cumanensis	C164	Criadouro Conservacionista e Cultural Pocos de Caldas	Boa Vista, Roraima	AY145319	AY367099 AY367093	AY367105
Pipile jacutinga	C100	CESP-Paraibuna	Unknown	AF165431	AY140744*	AF165476*
Pipile pipile	C264	Granja La Siberia	Trinidad	AY145320	AY367100 AY367094	AY367106
Penelope obscura	C28/C150	CESP-Paraibuna	Paraibuna, São Paulo	AF165432	AY140742*	AF165474*
Penelope superciliaris	C084	CESP-Paraibuna	Paraibuna, São Paulo	AY145313	AY367096 AY367090	AY367102

Sequences previously published are indicated as * (Pereira et al., 2002).

was defined in the Bayesian analysis. We set up three independent runs for three million generations each, with one cold and three heated chains and a burn-in time determined by the time to convergence of the likelihood scores. One tree was sampled in every thousand.

2.1.5. Molecular dating of divergence time

Branch lengths for the ML tree were re-estimated while enforcing a molecular clock using PAUP 4.0 b10 (Swofford, 2001). Twice the difference between the likelihoods of ML and the clock ML trees were compared to a χ^2 distribution and estimates of divergence time were obtained. The program r8s version 1.6 (Sanderson, 2003) was used to estimate divergence times and confidence intervals. The approach chosen was a semi-parametric penalized likelihood (Sanderson, 2002) that allows rates of evolution to vary across lineages, but a penalty is applied to minimize changes of rates between a node and its descendants through a smoothing parameter. The smaller the smoothing parameter, the more rate variation is observed in the data set. A data-driven cross-validation test was applied to choose the best smoothing parameter (Sanderson, 2002). This procedure prunes lineages from the tree, estimates a smoothing parameter for the remaining lineages and repeatedly tries to predict the smoothing parameter for the pruned lineages until a best smoothing parameter is found (Sanderson, 2002). We assumed the outgroup (*Penelope*) to have split from the ingroup 10.5 million years ago (MYA; 95% credible interval 8.8–12.3 MYA), according to the estimates obtained by Pereira et al. (2002) using a large data set including mitochondrial and nuclear DNA sequences. Unfortunately, no non-molecular calibration point, such as a geological one used in Pereira and Baker (2004a), is available to check for the independence of our estimates. However, we are confident that we are using a good calibration point because the divergence dates and associated credibility intervals obtained within curassows (Pereira and Baker, 2004a) were very similar whether a molecular or a independent geological anchor point was used, providing some evidence that the estimates of Pereira et al. (2002) may be appropriate as secondary molecular anchor-points when no other data are available.

2.2. Morphological data

2.2.1. Taxa sampling

We used 28 skeletons belonging to all recognized species of *Pipile* and *A. aburri*, and 17 skeletons of two species of *Penelope* (*P. obscura* and *P. superciliaris*). These data were previously obtained for a larger study of the family Cracidae (Silveira, 2003). The list of all specimens is provided in Appendix A. The complete morphological study of the phylogenetic relationships of the Cracidae will be published elsewhere (Silveira and Höfling, in prep.). Our goal was to gather independent evidence to check the validation of *Aburria* and *Pipile* as separate genera and check the usefulness of these characters for inference of phylogeny at species level.

3. Results

3.1. Molecular data

3.1.1. DNA amplification and sequencing

We are confident that our sequences are mitochondrial in origin because (1) *A. aburri* sequences were obtained in another study using nested amplifications from a large amplification of 3 kb (Pereira et al., 2004), (2) only single PCR products were obtained and sequenced, and (3) the sequences for *Pipile* and *Penelope* species did not have unusual features typical of pseudogenes and were similar to those obtained for *A. aburri*. Moreover, chicken and other eukaryotic genomes sequenced to date do not seem to have a high number of mitochondrial pseudogenes larger than 1 kb inserted in the nuclear genome compared to human and plant genomes (Pereira and Baker, 2004b; Richly and Leister, 2004). Therefore, if this is a characteristic of avian genomes, mitochondrial pseudogenes will only rarely be amplified. Also, all sequences obtained had similarity to corresponding sequences of other cracid birds deposited in GenBank, and prediction of amino acid sequences for cyt *b* and ND2 was as expected and the CR had conserved blocks as found in other cracids (Pereira et al., 2004). The final concatenated data set consisted of 2727 bp (699 bp of cyt *b*, 895 bp of ND2, and the complete CR (1133 bp)). GenBank accession numbers are listed in Table 1.

3.1.2. Distances and saturation

Pairwise *p*- and TN + G distances among species studied are listed in Table 2. The sequences compared here did not show any evidence of saturation of DNA substitution (not shown). We then compared congeneric species to check the magnitude of the pairwise distances among genera. TN + G distances ranged from 0.7 to 2.9% with a mean of 2.2% for the *Pipile-Aburria* group. These estimates are in the range for accepted species of guans of the genus *Penelope* (4.4%; Table 2), and corresponding cyt *b* and CR fragments of curassows of the genera *Crax* and *Mitu* (1.3–3.0% estimated by Grau et al., 2003).

3.1.3. Phylogenetic analysis

Base composition among taxa is homogeneous (P from 0.87 to 0.97 for ingroup, and $P = 0.69$ for outgroup). The trees estimated by ML, MP, and BA resulted in the same topology, suggesting that *P. jacutinga* is a sister-group of a clade containing *A. aburri* plus the other *Pipile* species. Nodal support was high in all analyses, except for the sister relationship between *A. aburri* and the other *Pipile* species, in exclusion of *P. jacutinga* (Fig. 2). BA

Table 2
Pairwise uncorrected *p*-distances and TN + G distances, above and below diagonal, respectively

	Aburria aburri	*Pipile cujubi*	*Pipile cumanensis*	*Pipile jacutinga*	*Pipile pipile*	*Penelope obscura*	*Penelope superciliaris*
Aburria aburri	—	0.024	0.025	0.026	0.024	0.044	0.042
Pipile cujubi	0.027	—	0.012	0.023	0.012	0.043	0.045
Pipile cumanensis	0.028	0.013	—	0.022	0.007	0.044	0.045
Pipile jacutinga	0.029	0.025	0.025	—	0.022	0.043	0.041
Pipile pipile	0.027	0.013	0.007	0.024	—	0.046	0.045
Penelope obscura	0.053	0.053	0.054	0.052	0.056	—	0.037
Penelope superciliaris	0.050	0.055	0.054	0.049	0.055	0.044	—

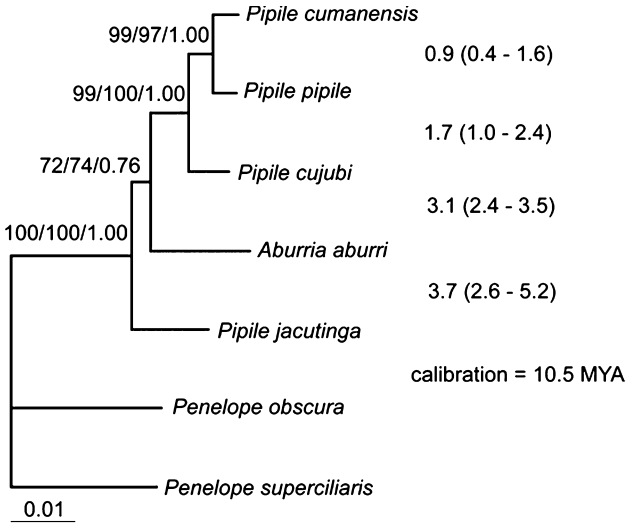

Fig. 2. Bayesian tree topology obtained from the combined analysis of CR, cyt *b*, and ND2 sequences. Bootstrap values obtained for MP and ML and posterior probabilities from BA for each node are indicated to the left of the tree. Estimates of divergence times (confidence intervals) based on a penalized likelihood approach are also shown to the far right of each node.

recovered only three alternative topologies: (1) one corresponding to our ML/MP/BA topology with posterior probability of 0.76; (2) a tree where *Aburria* was sister to all *Pipile* with posterior probability of 0.23; and (3) a tree where *Aburria* and *P. jacutinga* were sister species in exclusion to all other *Pipile* species, with posterior probability of 0.01. To further evaluate the three different topologies recovered in the BA, we performed the approximately unbiased (AU) test of Shimodaira (2002) as implemented in CONSEL version 0.1f (Shimodaira and Hasegawa, 2001). The *p* values for trees 1, 2, and 3 above were 0.710, 0.322, and 0.009, respectively. Posterior probabilities estimated in this program were 0.984 for our ML/MP/BA tree, 0.016 for *Pipile* being monophyletic, and virtually zero for the tree where *P. jacutinga* and *A. aburri* were a sister clade to the other species of *Pipile*.

3.1.4. Molecular dating of divergence time

Our results show that there is enough rate variation in DNA substitution among taxa to reject the assumption that these DNA sequences evolve according to a molecular clock (Ln non-clock: 5157.22691; Ln clock: 5710.82762; difference: 553.6007; $\chi^2 = 11.07$ at 0.05 level; df = 5). Therefore, we used a method that relaxes the clock assumption to estimate divergence times. The procedure chosen was the semi-parametric penalized likelihood (Sanderson, 2002), with a smoothing parameter set at 0.16 indicating substantial heterogeneity of rate of evolution among the DNA sequences used. Common ancestry of the *Aburria-Pipile* group dates to the Pliocene, and the most recent divergence of species occurred in the Pleistocene (Fig. 2). Divergence times and confidence intervals are indicated in Fig. 2.

3.2. Morphological data

3.2.1. Osteology

Although osteological data, as frequently observed, did not show any variation to establish the phylogenetic relationships of *A. aburri* and the four *Pipile* species, five unambiguous synapomorphies supported our assumption that *Aburria* and *Pipile* should be merged in one genus: the presence, in the skull, of a mediocaudal process in the nasolacrimal duct, a conspicuous crest caudally to the auditory chamber, associated with a pair of pits and a narrow choanal fossa of the palatines. In the ulnae, all species have a well-developed *impressio brachialis*.

4. Discussion

Our molecular and morphological data provided complementary data on the taxonomic status of *Pipile* and *Aburria*. Morphological data revealed the presence of five synapomorphies defining *Aburria* and *Pipile* as a distinct group within Cracidae, rejecting the inclusion of both genera in *Penelope* as suggested by Vuilleumier (1965). These morphological characters have been used to infer the phylogenetic relationships within Cracid genera (Silveira, 2003), and did not provide any further resolution for the phylogenetic relationships within piping-guans. Osteological data have proved to be non-informative below generic level in this clade. The DNA sequences indicated that *A. aburri* has a more recent common ancestry with (*P. cujubi*, (*P. cumanensis*, *P. pipile*)) but excluding *P. jacutinga*. Low nodal support

obtained from the phylogenetic analyses performed for the sister relationships of *A. aburri* with other *Pipile* species may reflect nearly simultaneous events of diversification, which prevented that enough DNA substitutions were accumulated to support this association. However, the AU test and posterior probabilities for sampled trees indicated that the tree where *A. aburri* is embedded within *Pipile* was the most likely one.

A complementary approach to our phylogenetic analysis is the comparison of genetic distances obtained for piping guans with other similar taxonomic ranks within Cracidae. Similar approaches have been used to check the taxonomic status of a number of genera and species (e.g., Aleixo, 2002; Grau et al., 2003; Zink and Blackwell-Rago, 2000). Although a general pattern has not been detected among different groups at the same taxonomic level, molecular data can be an important tool in these studies for closely related taxa. The percent sequence divergence between *A. aburri* and species of *Pipile* approximates that between non-disputed species of guans of the genus *Penelope* (Table 2), and curassows of the genera *Crax* and *Mitu* (Grau et al., 2003). DNA sequences for the CR, cyt *b*, and ND2 have diverged 1.3–3.0% for congeneric species of Cracidae whose taxonomic status is not questioned. Therefore, we recommend that *Aburria* and *Pipile* should be merged in one genus, as proposed two decades ago by Delacour and Amadon (1973) based on plumage, and bare part characters.

Following the rules of chronological priority (Article 23) of the International code of zoological nomenclature (1999, p. 24) the genus *Pipile* Bonaparte, 1856 should be considered a synonym of *Aburria* Reichenbach (1853), and all taxa currently allocated in the genus *Pipile* should then be transferred to the genus *Aburria*, in agreement with Delacour and Amadon (1973) and Silveira (2003). Thus, following the taxonomy adopted by del Hoyo et al. (1994), the genus *Aburria* Reichenbach (1853) includes the following species: *A. aburri* Lesson, 1828; *Aburria cujubi* Pelzeln, 1858; *Aburria cumanensis* Jacquin, 1784; *Aburria jacutinga* Spix, 1825 and *Aburria pipile* Jacquin, 1784. Hereafter, we use this classification.

4.1. Biogeography and divergence time

We estimated that intrageneric diversification in the *Aburria* clade occurred between 0.4 and 5.2 MYA based on confidence intervals. These dates, which correspond to the Pliocene and Pleistocene, postdate the formation of major topographic features of South America that have been evoked as vicariant agents for the diversification of birds, including cracids (Nores, 2004; Pereira and Baker, 2004a; Pereira et al., 2002). Geological events, such as the uplift of the northern Cordillera of the Andes, formation of the Brazilian and Guyana shields, and current river drainage systems, have already been established at 8 MYA (Clapperton, 1993; Lundberg et al., 1998). However, their influence in the formation of present-day South America extended into the following geological periods, and may also have influenced the diversification of flora and fauna, including the piping-guans. Here, we propose a biogeographic hypothesis for speciation of piping-guans based on known events of the geological and geographic history of South America that were consequences of these older changes, as well as other independent events of global influence.

The main paleogeographic events during Pliocene–Pleistocene epochs are related to the Milankovitch cycles, resulting in glaciation and interglaciation periods (Haffer, 1974; Haffer and Prance, 2001; Marroig and Cerqueira, 1997; Nores, 1999). In this context two main hypotheses have been forwarded to explain the richness of species seen in the Neotropical region. One of them, the refuge hypothesis, states that Neotropical forest became fragmented during dry glacial periods and expanded during the humid interglacial climatic conditions. This would isolate populations in pockets of remnant forest, called refuges, and promote differentiation between them and consequently lead to allopatric speciation (Haffer, 1974; Haffer and Prance, 2001). An alternative mechanism of habitat fragmentation and species diversification has been proposed based on evidence of sea transgressions (Nores, 1999) and the formation of the Amazon Lagoon during interglacial epochs (Marroig and Cerqueira, 1997). The existence of an Amazon Lagoon has been suggested previously by several authors (e.g., Guerra, 1956; Haffer, 1974; Irion, 1984; Paula Couto, 1956; Sioli, 1957).

The warm interglacial periods of the Pliocene–Pleistocene have been claimed to cause the elevation of sea level to up to 180 m above present-day level at around 2.5 MYA (Klammer, 1984). In South America, ancient sea transgressions affected three main areas (Lundberg et al., 1998: (1) the lowlands of Argentina, Uruguay, Paraguay and southern and central Brazil affecting the basins of the La Plata and Paraná rivers, (2) Central Venezuela, Colombia and Northern Peru at the present Orinoco river basin, and (3) the lower basin of the Amazon river in northern South America. Also, for the last two areas, the simultaneous formation of a fresh water lagoon, termed the Amazon Lagoon, may have affected ecosystems in most of the Amazon Forest as the invading waters occupied all lowlands below the sea level. This lagoon resulted from an increased rate of rainfall and melting of Andean glaciers. The total water discharge has been considered as sufficient to prevent the marine transgression at the Amazon delta (Marroig and Cerqueira, 1997). The current distribution of *Aburria* species together with our estimates of their divergence times suggest that ancestral populations of these birds became isolated and speciated in the elevated areas of the Atlantic Forest in eastern South America (*A. jacutinga*), in the

slopes of the Andes (*A. aburri*), and the Brazilian and Guyana shields (*A. cujubi* and *A. cumanensis*, respectively) as a consequence of marine and fresh water invasions of lowlands. This hypothesis is consistent with the biogeographic hypothesis proposed for curassows (Pereira and Baker, 2004a), as Atlantic representatives of two curassow genera and *A. jacutinga* split from their Amazonian sister species around the same time. The split of *A. cujubi* and of *A. cumanensis* and *A. Pipile* around 2.4–1.0 MYA is in agreement with the divergence times estimated for the curassow species living in the same areas (Pereira and Baker, 2004a), and corresponds to the estimated time (2.5 MYA) for the maximum sea transgression of 180 m above sea level (Klammer, 1984).

We also hypothesize that *A. cumanensis*, during the water regression epochs, occupied the lowland territories and that the several glacial/interglacial periods led to the formation of its two subspecies *A. c. cumanensis* and *A. c. grayi*. A similar situation is also proposed for *A. cujubi*, with the ancestral population surviving on the Brazilian Shield, where it likely occupied the gallery forests because a considerable part of the shield has been historically occupied by cerrado (Brazilian savannah) and caatinga, both poor in arboreal covering. *A. cujubi* is today represented by *A. c. cujubi* and *A. c. nattereri* occurring preferentially alongside gallery forests. Moreover, the presence of *A. cumanensis grayi* and *A. cujubi* nattereri in the Pantanal, in Mato Grosso state in Brazil, indicate that these species expanded their range as this region is a confluence of different biomes (Amazon forest, cerrado, and marsh). However, *A. c. nattereri* occurs in the higher forest areas whereas *A. c. grayi* occurs in the cerradão/forest transition (Olmos, 1998).

The recent diversification between *A. cumanensis* and *A. pipile* (restricted to Trinidad) was estimated between 0.4 and 1.6 MYA. This dating is inconsistent with the estimated age of Trinidad at around 11,000 years (Ffrench et al., 1991). However, as Trinidad is an island of continental origin only 10–15 km from Venezuela and surrounded by a continental platform less than 200 m below sea level, dispersal of ancestors of *A. pipile* from the mainland of South America to Trinidad is a plausible hypothesis. During glacial ages Pleistocene sea level regression as low as 180 m below present-day sea level has been reported (Salgado-Labouriau, 1994), which would make Trinidad continuous with the continental surroundings.

The present physiography of Trinidad is very peculiar: most of its area is constituted by lowlands and low hills. However, in the north, a prolongation of the coastal cordillera of Venezuela, where *A. pipile* can still be found, are high peaks over 1000 m and steep slopes without any plateau above 500 m. In the centre of the island there are low hills, all with less than 300 m with flanks whose height rarely reaches 200 m (Ffrench et al., 1991). During the large sea transgressions of the past, most of these areas were under water.

Similar dates of divergence time were estimated for congeneric species of curassows (Pereira and Baker, 2004a), monkeys (Collins and Dubach, 2000; Cortés-Ortiz et al., 2003) and vesper mice (Salazar-Bravo et al., 2001). These studies have shown a separation of Atlantic taxa from their sister-species occurring either in the Amazon or Pantanal/Chaco regions in the Pliocene. Sea transgressions together with the formation of the Amazon Lagoon and acquisition of present day river basins due to the rising of the Andes have been claimed as possible vicariant events leading to diversification in these groups. Thus, these palaeogeographic events might have contributed to Neotropical taxa diversification to a greater extent than previously suspected, unveiling a common temporal pattern of vicariance for organisms with similar geographic distribution in the Neotropics.

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Appendix A. Osteological material

Material is housed in the following institutions: American Museum of Natural History (AMNH), Louisiana State University, Museum of Zoology (LSUMZ), United States National Museum, Smithsonian Institution (USNM), British Museum, Natural History (Tring—BMNH), Muséum National d'Histoire Naturelle, Paris (MNHN), Naturhistorisches Museum Wien (NMW), Museu Paraense Emílio Goeldi (MPEG), Museu de História Natural de Taubaté (MHNT) and Coleção de Aves of the Departamento de Zoologia, Instituto de Biociências of the Universidade de São Paulo (AZ).

Aburria pipile: AMNH 12817; LSUMZ 60835; and LSUMZ 136473.

Aburria cumanensis: AMNH 2629; LSUMZ 86447; LSUMZ 148871; LSUMZ 148872; USNM 622219; BMNH 1854.3.29.4; BMNH 1858.5.26.21; BMNH 1869.9.12.22; BMNH 1952.2.; MNHN 1876.839; MPEG 591; USNM 345799; AZ 650; and LSUMZ 136697.

Aburria cujubi: MPEG 666; MPEG 755; MPEG 1323; MPEG 1557; and MPEG 3208.

Aburria jacutinga: BMNH 1959.19.2; NMW 179; and MHNT 1785.

Aburria aburri: AMNH 2625; LSMUZ 118170; and LSUMZ 112293.

Penelope superciliaris: AMNH 516; AMNH 516b; AMNH 3495; AMNH 8623; AMNH 13639; AMNH 13638; LSUMZ 169273; BMNH 1849.12.6.9; BMNH 1849.6.20.18; BMNH 1866.8.6.6; NMW 4970; and MPEG 569.

Penelope obscura: MNHN 1923.345; NMW 175; MHNT 202; MHNT 594; and MHNT 600.

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