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Historical diversification of floodplain forest specialist species in the Amazon: a case study with two species of the avian genus *Xiphorhynchus* (Aves: Dendrocolaptidae)

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Phylogeographical and population genetics methods are used to reconstruct the diversification history of two species of the genus *Xiphorhynchus* (Aves: Dendrocolaptidae) associated with seasonally flooded forest types in Amazonia. Sequences of the mitochondrial gene cytochrome *b* were assessed for 21 and 30 individuals, belonging to eight and ten populations, of *Xiphorhynchus kienerii* and *Xiphorhynchus obsoletus*, respectively. Uncorrected genetic distances among unique haplotypes recovered ranged only from 0.01% to 0.4% for both species. Over 90% of the genetic variation detected in both species was partitioned within populations, and therefore was not structured geographically. Mismatch distributions and values of Tajima's *D*-tests indicate that both *X. kienerii* and *X. obsoletus* have had small evolutionary effective population sizes, but experienced a recent demographic expansion. These demographic expansions are tentatively dated as occurring over the last 18 000 years BP, a time frame which coincides with the establishment of the early and mid-Holocene age floodplain forest in most of central and eastern Amazonia, following a period of increased river stages throughout the basin. Based on phylogenetic, phylogeographical, and populations genetics data obtained for *X. kienerii* and *X. obsoletus*, an evolutionary scenario is proposed to account for the historical diversification of floodplain specialist species in Amazonia. *Biological Journal of the Linnean Society*, 2006, **89**, 383–395.

ADDITIONAL KEYWORDS: Amazonian biogeography – evolution – phylogenetics – phylogeography – population genetics – seasonally flooded forest – upland (*terra-firme*) forest – vertebrates – *Xiphorhynchus kienerii* – *Xiphorhynchus obsoletus*.

INTRODUCTION

The first naturalists to travel throughout Amazonia attributed the replacement of closely-related species in different regions of the basin to wide rivers that posed barriers to dispersal (and consequently gene flow) of some species (Wallace, 1852). This so-called 'river effect' has been demonstrated for populations of birds inhabiting the interior of upland (*terra-firme*) forests, away from the influence of major Amazonian rivers (Capparella, 1987; Hackett, 1993; Cohn-Haft, 2000; Aleixo, 2004). By contrast, a substantial portion of the Amazonian avifauna lives in habitats affected by major rivers, such as floodplain forests (*várzea* and *igapó*; for a description of these two main types of seasonally flooded Amazonian forests, see Sioli, 1975), and river islands (Remsen & Parker, 1983; Stotz *et al*., 1996). The riverine barrier hypothesis of diversification (allopatric differentiation caused by restriction of gene flow across rivers; for a review, see Gascon *et al*., 2000) is not thought to apply to floodplain specialist species because they are capable of colonizing river islands and crossing rivers (Capparella, 1987; Patton & Silva, 1998). To date, most of the debate on Amazonian diversification has been centred around the more thoroughly studied upland forest species, with few studies focusing on the numerous lineages of floodplain forest species endemic to this region (Matocq, Patton & da Silva, 2000; Aleixo, 2002).

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The avian genus *Xiphorhynchus* (Passeriformes: Dendrocolaptidae) contains 15 species distributed in several forest types throughout the Neotropics, including Amazonian upland and floodplain forests (Marantz *et al*., 2003). Previous studies showed that upland and floodplain forests specialist species of *Xiphorhynchus* have been evolving separately for a long time, and that the upland specialist clade has experienced a much higher rate of recent speciation than the two independent and rather isolated lineages of floodplain specialist species (Aleixo, 2002). The causes of this apparent lower rate of differentiation among floodplain species should be further investigated with detailed phylogeographical and population genetics surveys similar to those carried out for an upland *Xiphorhynchus* lineage, the *Xiphorhynchus spixii*/*elegans* superspecies (Aleixo, 2004).

The present study investigated the phylogeography and population genetics structure of two *Xiphorhynchus* species endemic to the Amazon basin that are regarded as floodplain forest specialists (Ridgely & Tudor, 1994; Marantz *et al*., 2003): *Xiphorhynchus kienerii* and *Xiphorhynchus obsoletus*. The study aimed to answer the following questions concerning the current and historical diversification of these two floodplain specialist species: (1) what is the degree of population structure found among populations of *X. kienerii* and *X. obsoletus* throughout Amazonia; (2) how do the levels of phylogeographical and population differentiation observed for *X. kienerii* and *X. obsoletus* compare with those documented for some upland forest avian lineages, including the genus *Xiphorhynchus*; and (3) what possible historical scenarios could explain the pattern of phylogeographical and population differentiation documented for *X. kienerii* and *X. obsoletus* and other floodplain specialist species?

MATERIAL AND METHODS

Taxon and population sampling

A total of 21 individuals of *X. kienerii* and 30 individuals of *X. obsoletus*, collected throughout Amazonia, belonging to eight and ten different populations, respectively (for collecting localities, populations sample sizes, and specimens' voucher information, see Tables 1, 2) were sequenced. To carry out population genetics analyses (see below), sampled populations of *X. kienerii* and *X. obsoletus* were grouped into biogeographical units following different criteria. Because *X. kienerii* occurs only in seasonally flooded forest along some major Amazonian rivers, the different populations sampled were grouped by distance into four main areas or drainage systems (Fig. 1, Table 1): (1) lower Amazonia (from the Tapajós river eastward); (2) central Amazonia (lower portions of the Negro, Solimões, and Madeira rivers); (3) lower Japurá (lower portion of the Japurá river, situated approximately half-way between central Amazonia and upper Amazon); and (4) upper Amazon (upper course of the Amazonas/Solimões river). For *X. obsoletus*, grouping of the different populations sampled followed a different criterion because of this species' much wider distribution, which virtually encompasses the entire Amazon basin (Marantz *et al*., 2003). Therefore, the proposed areas of endemism for birds in Amazonia were used to cluster populations of *X. obsoletus* sampled in the present study. There are seven areas of endemism recognized for birds in Amazonia (Cracraft, 1985); each of those areas harbour an unique set of endemic taxa thought to be the result of vicariant mechanisms that promoted species diversification in this region (Haffer, 1985). Hence, at least one population of *X. obsoletus* was sampled from each Amazonian area of endemism, except the Imerí area, located in north-western Amazonia (Fig. 2).

Cytochrome *b* amplification and sequencing

Total genomic DNA was extracted from raw frozen tissues and dry skin samples of recently collected specimens (15 years old or younger) using a Qiagen tissue extraction kit or a standard phenol/chloroform method (Hillis *et al*., 1990). Several measures were taken to ensure that ancient DNA extracted from dry skin samples would not be contaminated by DNA from frozen tissue samples: (1) dry skin samples were extracted in a different building than were raw tissues; (2) separate Qiagen extraction kits and other consumable laboratory supplies were used to perform dry skin and raw tissue extractions; and (3) dry skin extractions were always performed with negative controls (which never showed signs of DNA contamination when run on an electrophoresis agarose gel). Most of the mitochondrial gene cytochrome *b* was amplified with the following primers: L14990 (Kocher *et al*., 1989), L15389 (Hackett, 1996), H15710 (Helm-Bychowski & Cracraft, 1993), HXIPH (CATTCTGGTTTGAT GTGGGG; designed specifically for this project), L15505 (CTAACCTTCCTACACGAAACC; designed specifically for this project), L15656 (Helm-Bychowski & Cracraft, 1993), and H16065 (Hackett, 1996). All primer numbers refer to the 3′ base of the published chicken mtDNA sequence (Desjardins & Morais, 1990). Fragments were amplified by the polymerase chain reaction (PCR) using standard conditions (available upon request): denaturation at 94 °C, annealing between 50 °C and 57 °C, and extension at 72 °C in a Hybaid OMN-E thermal cycler. A small aliquot of each amplification was electrophoresed on an agarose gel to check for the correct fragment size and to ensure that

Table 1. Collection locality, population number (as in Fig. 1), drainage of occurrence, voucher number, tissue or dry skin source, and GenBank accession number for specimens of *Xiphorhynchus kienerii* (Aves: Dendrocolaptidae) sequenced in this study

Collection locality	Population number	Drainage	Voucher number	Source*	GenBank accession number
Brazil: state of Pará: Lago do Maicá; 11.3 km SE Santarém; S bank of Río Amazonas; 02°28′S, 54°38′W	1	Lower Amazonia	B35627	LSUMNS	DQ157343
Brazil: state of Pará: Lago do Maicá; 11.3 km SE Santarém; S bank of Río Amazonas; 02°28′S, 54°38′W	1	Lower Amazonia	B35628	LSUMNS	DQ157344
Brazil: state of Pará: Lago do Maicá; 11.3 km SE Santarém; S bank of Río Amazonas; 02°28′S, 54°38′W	1	Lower Amazonia	B35630	LSUMNS	DQ157345
Brazil: state of Pará: Lago do Maicá; 11.3 km SE Santarém; S bank of Río Amazonas; 02°28′S, 54°38′W	1	Lower Amazonia	B35632	LSUMNS	DQ157346
Brazil: state of Amazonas: Río Amazonas, Careiro island, ~20 km E of Manaus	2	Central Amazonia	B25413	LSUMNS	AY089818
Brazil: state of Amazonas: Igarapé Panelão, 6 km W of Careiro da Castanha; 03°50′S, 60°30′W	3	Central Amazonia	B35658	LSUMNS	DQ157347
Brazil: state of Amazonas: Igarapé Panelão, 6 km W of Careiro da Castanha; 03°50′S, 60°30′W	3	Central Amazonia	B35659	LSUMNS	DQ157348
Brazil: state of Amazonas: Igarapé Panelão, 6 km W of Careiro da Castanha; 03°50′S, 60°30′W	3	Central Amazonia	B35662	LSUMNS	DQ157349
Brazil: state of Amazonas: Anavilhanas archipelago, Novo Airão	4	Central Amazonia	B20237	LSUMNS	DQ157350
Brazil: state of Amazonas: Jaú National Park, Caroçal island at the mouth of Río Jaú	5	Central Amazonia	B25477	LSUMNS	DQ157351
Brazil: state of Amazonas: Maraã, S Bank of Río Japurá	6	Lower Japurá	43117†	MPEG	DQ157352
Brazil: state of Amazonas: Maraã, S Bank of Río Japurá	6	Lower Japurá	43114†	MPEG	DQ157353
Brazil: state of Amazonas: Maraã, S Bank of Río Japurá	6	Lower Japurá	43115†	MPEG	DQ157354
Brazil: state of Amazonas: Maraã, S Bank of Río Japurá	6	Lower Japurá	43116†	MPEG	DQ157355
Brazil: state of Amazonas: N. bank of Río Amazonas, ~4.5 km NE São Paulo de Olivença; 03°25′S, 68°57′W	7	Upper Amazon	B35692	LSUMNS	DQ157356
Brazil: state of Amazonas: N. bank of Río Amazonas, ~4.5 km NE São Paulo de Olivença; 03°25′S, 68°57′W	7	Upper Amazon	B35693	LSUMNS	DQ157357
Brazil: state of Amazonas: N. bank of Río Amazonas, ~4.5 km NE São Paulo de Olivença; 03°25′S, 68°57′W	7	Upper Amazon	B35723	LSUMNS	DQ157358
Brazil: state of Amazonas: N. bank of Río Amazonas, ~4.5 km NE São Paulo de Olivença; 03°25′S, 68°57′W	7	Upper Amazon	B35724	LSUMNS	DQ157359
Peru: Department of Loreto: River island 8 km downstream from Iquitos in Río Amazonas, 03°41′S, 73°12′W	8	Upper Amazon	B29022	LSUMNS	DQ157360
Peru: Department of Loreto: River island 8 km downstream from Iquitos in Río Amazonas, 03°41′S, 73°12′W	8	Upper Amazon	B29023	LSUMNS	DQ157361
Peru: Department of Loreto: River island 8 km downstream from Iquitos in Río Amazonas, 03°41′S, 73°12′W	8	Upper Amazon	B29016	LSUMNS	DQ157362

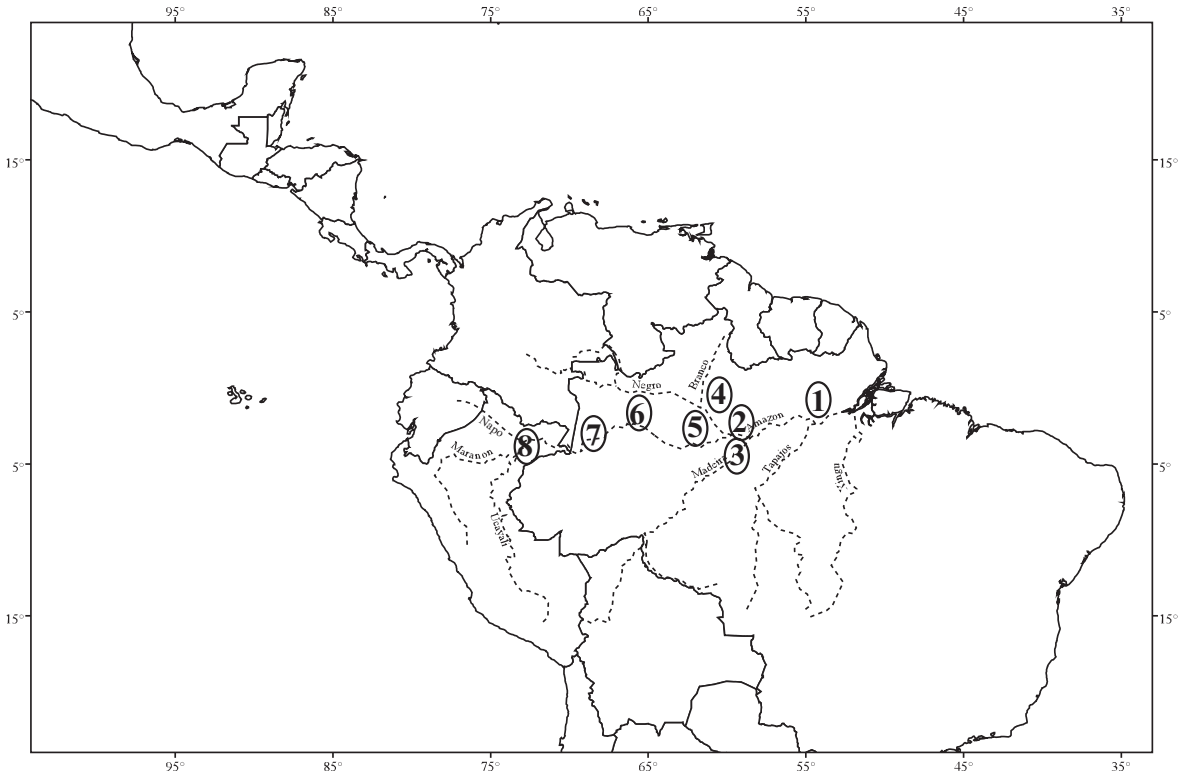
*Key to source abbreviations: LSUMNS, Louisiana State University Museum of Natural History, Baton Rouge, USA; MPEG, Museu Paraense Emílio Goeldi, Belém, Brazil.

†Dry skin samples.

Table 2. Collection locality, population number (as in Fig. 2), area of endemism, voucher number, tissue or dry skin source, and GenBank accession number for specimens of *Xiphorhynchus obsoletus* (Aves: Dendrocolaptidae) sequenced in this study

Collection locality	Population number	Voucher number	Source*	GenBank accession number
Brazil: state of Pará: Belém, Mata do Mocambo-EMBRAPA	1	B35670	LSUMNS	DQ157314
Brazil: state of Pará: 113 km SWW Santarém; Alto Río Arapiuns; 02°44′S, 55°41′W	2	B35585	LSUMNS	DQ157315
Brazil: state of Pará: 113 km SWW Santarém; Alto Río Arapiuns; 02°44′S, 55°41′W	2	B35620	LSUMNS	DQ157316
Brazil: state of Pará: 113 km SWW Santarém; Alto Río Arapiuns; 02°44′S, 55°41′W	2	B35592	LSUMNS	DQ157317
Brazil: state of Pará: 113 km SWW Santarém; Alto Río Arapiuns; 02°44′S, 55°41′W	2	B35593	LSUMNS	DQ157318
Brazil: state of Pará: 113 km SWW Santarém; Alto Río Arapiuns; 02°44′S, 55°41′W	2	B35595	LSUMNS	DQ157319
Brazil: state of Pará: Island on the Río Teles Pires, 6.1 km downriver from the mouth of Río São Benedito, 09°02′S, 57°05′W	3	B35388	LSUMNS	DQ157320
Brazil: state of Mato Grosso: W. bank of Río Teles Pires, 33 km NE of Alta Floresta	4	B35501	LSUMNS	DQ157321
Bolivia: Department of Santa Cruz: prov. Velasco; W. bank R'o Paucerna, 4 km upstream from Río Itenez	5	B12752	LSUMNS	DQ157322
Bolivia: Department of Santa Cruz: prov. Velasco; W. bank R'o Paucerna, 4 km upstream from Río Itenez	5	B12934	LSUMNS	DQ157323
Bolivia: Department of Santa Cruz: prov. Velasco; W. bank R'o Paucerna, 4 km upstream from Río Itenez	5	B12885	LSUMNS	DQ157324
Bolivia: Department of Santa Cruz: prov. Velasco; W. bank R'o Paucerna, 4 km upstream from Río Itenez	5	B12740	LSUMNS	DQ157325
Bolivia: Department of Santa Cruz: prov. Velasco; W. bank R'o Paucerna, 4 km upstream from Río Itenez	5	B12729	LSUMNS	DQ157326
Brazil: state of Amazonas: Igarapé Panelão, 6 km W Careiro; 03°50′S, 60°30′W	6	B35660	LSUMNS	DQ157327
Brazil: state of Amazonas: *c*. 4.5 km NE São Paulo de Olivença; 03°25′S, 68°57′W	7	B35682	LSUMNS	DQ157328
Brazil: state of Amazonas: *c*. 4.5 km NE São Paulo de Olivença; 03°25′S, 68°57′W	7	B35683	LSUMNS	DQ157329
Brazil: state of Amazonas: *c*. 4.5 km NE São Paulo de Olivença; 03°25′S, 68°57′W	7	B35690	LSUMNS	DQ157330
Brazil: state of Amazonas: *c*. 4.5 km NE São Paulo de Olivença; 03°25′S, 68°57′W	7	B35691	LSUMNS	DQ157331
Brazil: state of Amazonas: *c*. 4.5 km NE São Paulo de Olivença; 03°25′S, 68°57′W	7	B35732	LSUMNS	DQ157332
Ecuador: Sucumbíos; Imura Cocha	8	3231	ANSP	DQ157333
Ecuador: Sucumbíos; Imura Cocha	8	3174	ANSP	DQ157334
Ecuador: Sucumbíos; Imura Cocha	8	3183	ANSP	DQ157335
Peru: Department of Loreto: Lower Río Napo region, E. bank R'o Yanayacu, ~90 km N. Iquitos	9	B4396	LSUMNS	DQ157336
Peru: Department of Loreto: Lower Río Napo region, E. bank R'o Yanayacu, ~90 km N. Iquitos	9	B4361	LSUMNS	DQ157337
Peru: Department of Loreto: Lower Río Napo region, E. bank R'o Yanayacu, ~90 km N. Iquitos	9	B4192	LSUMNS	DQ157338
Guyana: Iwokrama Reserve, Kabocalli Landing, 04°17′N, 58°31′W	10	7965	ANSP	AY089823
Guyana: Iwokrama Reserve, Kabocalli Landing, 04°17′N, 58°31′W	10	8212	ANSP	DQ157339
Guyana: Iwokrama Reserve, Kabocalli Landing, 04°17′N, 58°31′W	10	8572	ANSP	DQ157340
Guyana: Iwokrama Reserve, Kabocalli Landing, 04°17′N, 58°31′W	10	8569	ANSP	DQ157341
Guyana: Iwokrama Reserve, Kabocalli Landing, 04°17′N, 58°31′W	10	8688	ANSP	DQ157342

*Key to source abbreviations: LSUMNS, Louisiana State University Museum of Natural History, Baton Rouge, USA; ANSP, Academy of Natural Sciences, Philadelphia, USA.



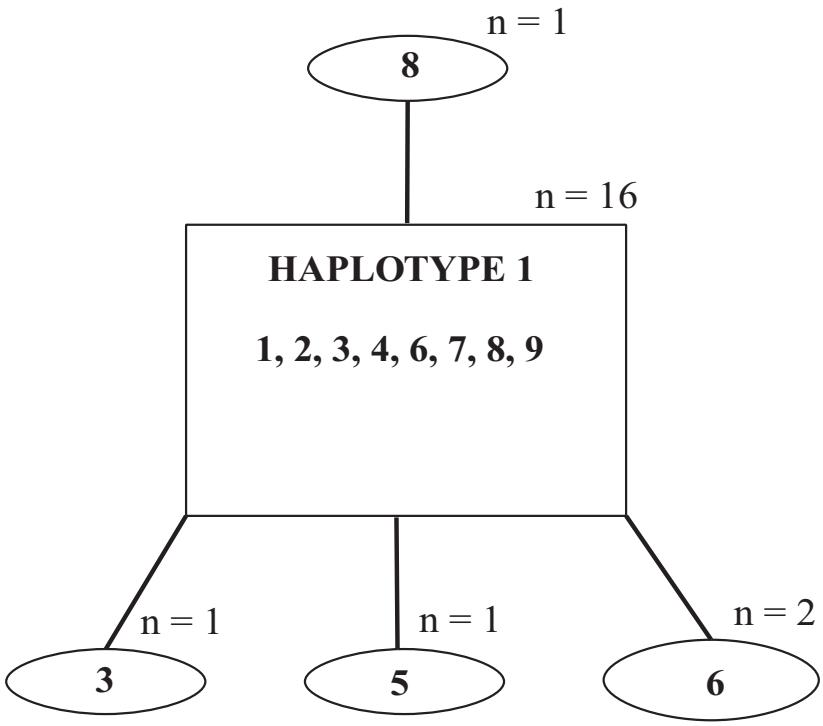
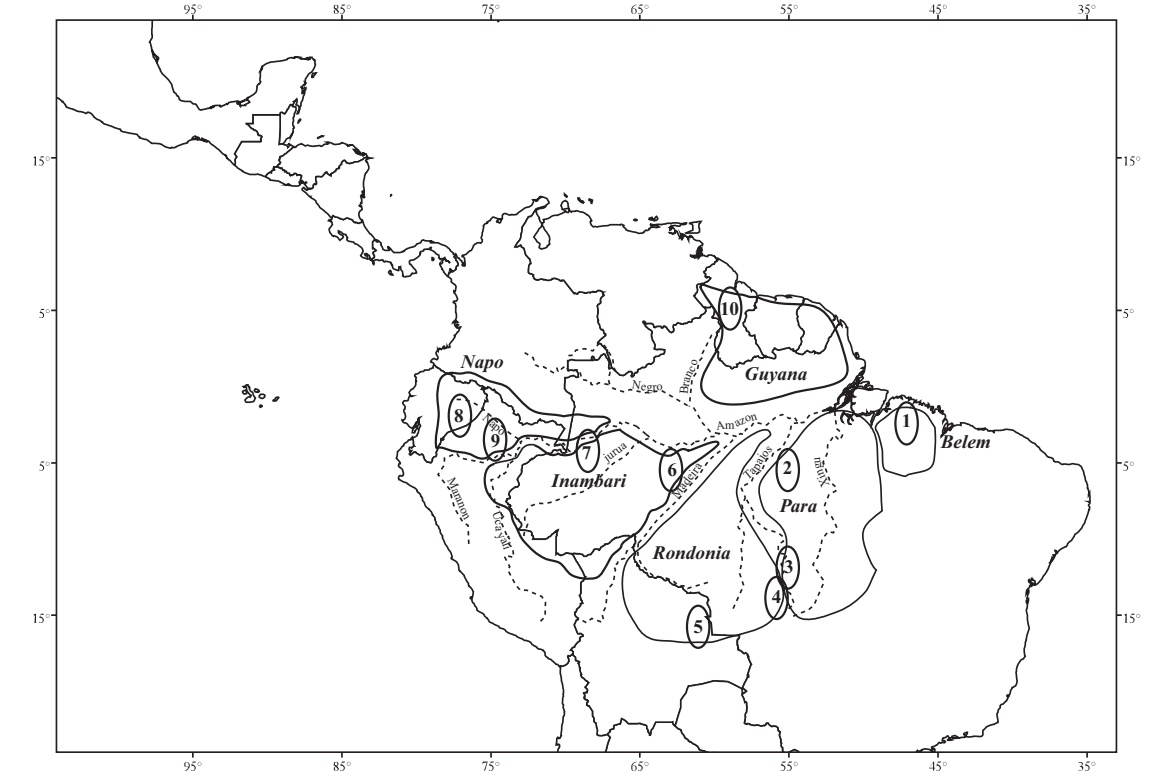


Figure 1. Map with location of populations sampled and statistical parsimony network estimated for *Xiphorhynchus kienerii* throughout Amazonia. The square and ellipses represent unique haplotypes, and their sizes correspond to frequencies of occurrence in all populations (also shown by numbers next to haplotype symbols). Each line connecting two haplotypes represents a single mutational step (substitution) separating them. Numbers within the square and ellipses indicate sampled populations (found on the map) where the haplotypes were recovered. For the exact location of sampled populations and voucher information, see Table 1.



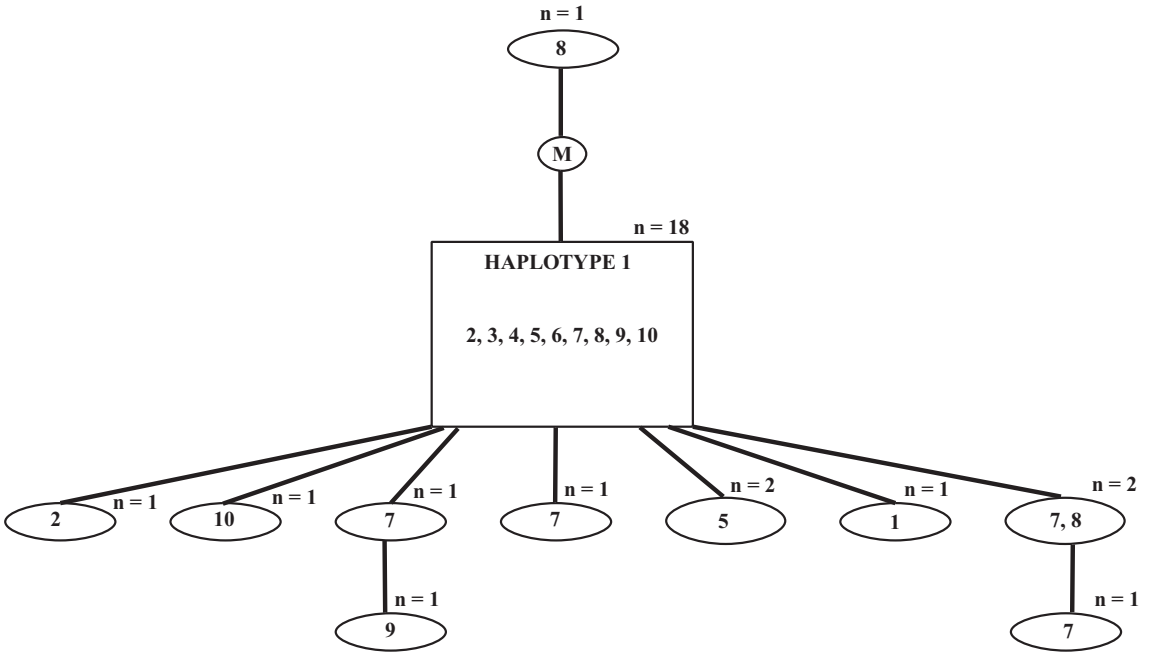


Figure 2. Map with location of populations sampled within recognized areas of avian endemism (Cracraft, 1985; the Imerí area, located between the Napo and Guyana areas in north-western Amazonia, is not shown), and statistical parsimony network estimated for *Xiphorhynchus obsoletus* throughout Amazonia. The square and ellipses represent unique haplotypes sampled, and their sizes correspond to frequencies of occurrence in all populations (also shown by numbers next to haplotype symbols). Each line connecting two haplotypes represents a single mutational step (substitution) separating them. A single missing haplotype inferred by statistical parsimony is represented by a circle filled with an 'M'. Numbers within the square and ellipses indicate sampled populations (found on the map) where the haplotypes were recovered. For the exact location of sampled populations and voucher information, see Table 2.

only a single amplification product was obtained. Amplification products were cleaned with a Qiagen PCR purification kit and cycle-sequenced using a Big Dye Terminator kit (Perkin Elmer), and all amplification primers listed above. Cycle sequencing reactions were NH_4OAC precipitated, dried, resuspended in a formamide EDTA, and run on an ABI 377 Automated DNA Sequencer. Sequences from both strands were aligned and reconciled using Sequencher 3.1.1 (Genecodes). The following measures outlined by Sorenson & Quinn (1998) and Bates, Hackett & Goerck.(1999) were taken to ensure that the DNA fragments amplified were accurate and of mitochondrial origin (not pseudogenes): (1) both DNA strands were sequenced; (2) sequences were aligned with the complete chicken mtDNA sequence, and then inspected for insertions, deletions, and stop codons that would result in a nonfunctional protein; and (3) sequences were expected to exhibit high transition to transversion substitution ratios characteristic of mitochondrial, not nuclear substitution patterns. No evidence was detected for the presence of pseudogenes in the sequences used in the present study (GenBank accession numbers AY089818, AY089823, and DQ157314-DQ157362).

Phylogeographical analysis

Due to the relatively shallow level of divergence among haplotypes sampled in this study, haplotype networks were estimated for *X. kienerii* and *X. obsoletus* using the software TCS 1.13 (Clement, Posada & Crandall, 2000). TCS uses the method known as statistical parsimony (Templeton, Crandall & Sing, 1992) to generate an unrooted cladogram based on a pairwise matrix of absolute differences among haplotypes (Clement *et al*., 2000). TCS 1.13 was run with the 95% limit of parsimony (Templeton *et al*., 1992). A likelihood ratio test (Yang, Goldman & Friday, 1995) was used to evaluate whether ingroup and outgroup cytochrome *b* sequences of *X. kienerii* and *X. obsoletus* were evolving in a clock-like manner. Therefore, the likelihood ratio test was first used as implemented in MODELTEST (Posada & Crandall, 1998) to select the best and simplest model of molecular evolution fitting the dataset, which was then used to construct maximum likelihood estimates of phylogeny for unique haplotypes of *X. kienerii* and *X. obsoletus* using the software PAUP*, version 4.0b10 (Swofford, 2002). Sequences of *Xiphorhynchus picus* and *Xiphorhynchus guttatus* were chosen as outgroups because these two species were found in the same clade as *X. kienerii* and *X. obsoletus*, respectively, according to a molecular phylogeny estimated for the entire genus *Xiphorhynchus* (Aleixo, 2002). For the rate heterogeneity test, scores of two maximum likelihood heuristic searches conducted in PAUP*, version 4.0b10 (under the model of nucleotide substitution selected by MODELTEST) were contrasted: one without enforcing a molecular clock and another assuming a clock-like rate of nucleotide substitution.

Population genetics analyses

Haplotype diversity (h), nucleotide diversity (π), and Tajima's (1989) D-test for departure of neutrality were calculated for grouped populations of *X. kienerii* and *X. obsoletus* using the software Arlequin 2.000 (Schneider, Roessli & Excoffier, 2000). Tajima's D was also calculated for all unique haplotypes recovered for both *X. kienerii* and *X. obsoletus*. An analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992) was performed for all populations of *X. kienerii* and *X. obsoletus* using Arlequin 2.000 (Schneider *et al*., 2000). AMOVA uses haplotype frequencies and the number of mutations between them to test the significance of the variance components associated with up to three hierarchical levels of genetic structure: within populations, among populations between groups, and among groups. Pairwise mismatch distributions (Rogers & Harpending, 1992) and parameters of Rogers's (1995) model of sudden population expansion were also calculated for all populations of *X. kienerii* and *X. obsoletus* using Arlequin 2.000 (Schneider *et al*., 2000).

RESULTS

Informative variation, levels of divergence, and rates of evolution

Cytochrome *b* sequences recovered were aligned unambiguously and showed expected codon biases and an overall deficit of guanines as reported for other avian cytochrome *b* gene sequences (Moore & DeFilippis, 1997). Sequences obtained were trimmed to 908 and 1004 bp for *X. kienerii* and *X. obsoletus*, respectively, spanning positions 15031–16035 of the cytochrome *b* chicken sequence (Desjardins & Morais, 1990). A total of five and 11 unique haplotypes was recovered for *X. kienerii* and *X. obsoletus*, respectively. For *X. kienerii*, nucleotide substitutions were observed at four sites (0.4%), only one of which was potentially phylogenetically informative. For *X. obsoletus*, nucleotide substitutions occurred at 11 sites (1.2%), three of which were potentially phylogenetically informative. Uncorrected p distances among haplotypes ranged from 0.01% to 0.2% for *X. kienerii* and between 0.1% and 0.4% for *X. obsoletus*. The hypothesis of a clock-like rate of evolution for all cytochrome *b* sequences recovered for *X. kienerii* and *X. obsoletus* and outgroups could not be rejected

[$TrN_{(clock)}$, lnL = –1664.9843, $\chi^2 = 2.55$, d.f. = 5, $P > 0.75$ for *X. kienerii*; $HKY_{(clock)}$, lnL = –1752.7817, $\chi^2 = 4.62$, d.f. = 10, $P > 0.90$ for *X. obsoletus*).

Phylogeographical and population genetics analyses

A statistical parsimony network with five haplotypes was obtained for *X. kienerii* (Fig. 1). In this network, four haplotypes were separated from the most widespread haplotype (called haplotype 1) by just one mutational step each (Fig. 1). For *X. obsoletus*, a statistical parsimony network with 12 haplotypes was recovered, 11 of which were directly sampled, whereas one was hypothetical and inferred as missing from my sample (Fig. 2). Most ($N = 7$) haplotypes recovered for *X. obsoletus* were separated from the commonest and most widespread haplotype (referred to as haplotype 1) by just one mutational step, whereas three other haplotypes were separated from haplotype 1 by two mutational steps (Fig. 2). Haplotype 1 for both *X. kienerii* and *X. obsoletus* had the highest frequency in most populations sampled (Figs 1, 2). This pattern, added to the shallow levels of divergence and few mutational steps separating haplotypes recovered for both *X. kienerii* and *X. obsoletus*, indicate a lack of phylogeographical structure for both species throughout Amazonia.

Indices of haplotype and nucleotide diversity were generally low for *X. kienerii* and *X. obsoletus* but varied considerably geographically. For populations of *X. kienerii*, the highest levels of haplotype and nucleotide diversity were detected in central Amazonia whereas, for *X. obsoletus*, western Amazonian populations inhabiting the Inambari and Napo areas of endemism were more diverse (Table 3). Results of Tajima's *D*-tests showed that most populations of *X. kienerii* and *X. obsoletus* had nonsignificant negative values (Table 3). Only one population of *X. obsoletus* (Napo area of endemism) showed a marginal departure of neutrality (Table 3). However, when Tajima's *D* was applied to all sampled populations of *X. kienerii* and *X. obsoletus*, significantly negative results were obtained ($D = –1.65$; $P < 0.05$ and $D = –2.08$; $P < 0.01$, respectively), and hence consistent with a recent demographic expansion or population bottleneck at a broad geographical scale (Rand, 1996). AMOVA showed that most of the genetic variation detected in populations of the *X. kienerii* (93.8%) and *X. obsoletus* (95.4%) was partitioned within populations, and therefore not structured geographically (Table 4). Nucleotide mismatch distributions for both *X. kienerii* and *X. obsoletus* were unimodal and could not reject the null hypothesis of a recent sudden population expansion, as formulated by Rogers (1995: Fig. 4) ($P > 0.20$ for *X. kienerii* and $P > 0.80$ for *X. obsoletus*). Assuming a mitochondrial clock-like substitution rate of 2% per million years (Klicka & Zink, 1997), unimodal mismatch distributions for *X. kienerii* and *X. obsoletus* were consistent with a recent population expansion, probably preceded by a bottleneck, that took place between 1500 and 15 500 years BP for

Table 3. Geographically distributed genetic variability in *Xiphorhynchus kienerii* and *Xiphorhynchus obsoletus* throughout Amazonia, including the number of individuals and populations sampled, haplotype diversity (h), nucleotide diversity (π), and the results of Tajima's (1989) *D*-test

Species/areas	Number of individuals [population(s) sampled]	Haplotype diversity $(h) \pm V(h)$	Nucleotide diversity $(\pi) \pm V(\pi)$	Tajima's *D*-test*
Xiphorhynchus kienerii				
Upper Amazonas	7 (7, 8)	0.28 ± 0.19	$2.8 \pm 3.9\ 10^{-4}$	–1.00 NS
Lower Japurá	4 (6)	0.83 ± 0.22	$4.9 \pm 6.1\ 10^{-4}$	–0.61 NS
Central Amazonia	6 (2–5)	0.73 ± 0.15	$8.6 \pm 8.0\ 10^{-4}$	–0.05 NS
Lower Amazonia	4 (1)	0	0	0
Xiphorhynchus obsoletus				
Guyana	5 (10)	0.40 ± 0.23	$4.0 \pm 5.0\ 10^{-4}$	–0.82 NS
Pará/Belém	8 (1–3)	0.46 ± 0.20	$5.0 \pm 5.3\ 10^{-4}$	–1.31 NS
Rondônia	5 (4, 5)	0.60 ± 0.17	$6.0 \pm 6.5\ 10^{-4}$	1.22 NS
Inambari	6 (6, 7)	0.93 ± 0.12	$1.5 \pm 1.2\ 10^{-3}$	–0.67 NS
Napo	6 (8, 9)	0.80 ± 0.17	$1.6 \pm 1.3\ 10^{-3}$	–1.34†

For geographical location of populations and areas of endemism, see Figs 1, 2.
*Tajima's (1989) *D*-test; NS, nonsignificant departure from neutrality (All $P > 0.12$); †marginally significant departure from neutrality at $P = 0.056$.

Table 4. Results from an analysis of molecular variance among populations of *Xiphorhynchus kienerii* and *Xiphorhynchus obsoletus* distributed throughout Amazonia

Species	Number of populations	Variation among populations (%)	Variation within populations (%)
Xiphorhynchus kienerii	4	6.2*	93.8
Xiphorhynchus obsoletus	5	4.6†	95.4

For geographical location of populations and areas of endemism, see Figures 1, 2.
*$P > 0.10$; †$P > 0.08$.

X. kienerii and between the present and 18 000 years BP for *X. obsoletus* (Rogers, 1995).

DISCUSSION

Resolution of cytochrome *b* sequences

In comparison with some mtDNA markers such as the control region, the cytochrome *b* gene evolves at a slower rate, and has traditionally been employed in studies assessing phylogenetic relationships above the species level (Moore & DeFilippis, 1997; Prum *et al*., 2000; Irestedt *et al*., 2002). Given the fairly low divergence detected among cytochrome *b* haplotypes recovered for both *X. kienerri* and *X. obsoletus* (maximum uncorrected *p* distances of 0.2% and 0.4%, respectively), a major concern with the results presented here is that local population structure could simply not be detected for these species with cytochrome *b* sequences. If correct, this possibility would render the observed pattern of little phylogeographical structure detected throughout Amazonia for *X. kienerri* and *X. obsoletus* as an artefact caused by the use of an inappropriate molecular marker. However, the following main lines of evidence suggest that this is not the case: (1) the generally higher levels of molecular differentiation detected among lineages of tropical birds and other organisms, in comparison with those from the northern hemisphere, indicate that cytochrome *b* sequences might have a higher chance of recovering phylogeographic and population genetics structure among lineages of tropical rather than temperate birds (Hackett, 1993, 1996; Bates *et al*., 1999; Martin & McKay, 2004) and (2) recent studies focusing on Neotropical species belonging to the passerine suborder suboscines (which includes the genus *Xiphorhynchus*) show that cytochrome *b* sequences can also be useful in resolving intraspecific phylogenies (Bates *et al*., 1999; Marks, Hackett & Capparella, 2002; Cheviron, Hackett & Capparella, 2005). Particularly in the genus *Xiphorhynchus*, cytochrome *b* sequences uncovered significant variation among populations of a single species and between sister species belonging to the three main clades of this paraphyletic genus (Aleixo, 2002, 2004). That *X. kienerii* and *X. obsoletus* are not monophyletic and each belong to an ecologically diverse clade, where significant intraspecific molecular differentiation has been detected in cytochrome *b* sequences (Aleixo, 2002), supports the notion that the pattern of little phylogeographical structure documented in the present study for these floodplain specialist species is a real one and can be explained by: (1) life-history attributes such as high dispersal rates; (2) recent and strong demographic fluctuations; or (3) both alternatives.

Phylogeography of floodplain forest specialist species

Both *X. kienerii* and *X. obsoletus* exhibited the very similar pattern of virtually no phylogeographical structure throughout their ranges. This absence of phylogeographical structure fits the 'category IV phylogeographical pattern' described by Avise (2000), in which closely related lineages of a shallow gene tree are broadly sympatric. This phylogeographical pattern has been interpreted as resulting from high levels of gene flow among populations of species whose ranges were not fragmented by long-term vicariant barriers (Avise, 2000). Additionally, as can be inferred from the strongly unimodal shape of their mismatch distributions and significant negative values of Tajima's *D*-test (Table 3, Fig. 3), both *X. kienerii* and *X. obsoletus* probably have had small evolutionary effective population sizes but might have experienced a recent explosive demographic expansion (Rand, 1996; Avise, 2000).

This phylogeographical pattern contrasts markedly with that documented for Amazonian upland forest species of the genus *Xiphorhynchus* (*X. spixii*/*elegans* and *Xiphorhynchus pardalotus*/*ocellatus* species complexes), in which much deeper intraspecific cytochrome *b* gene trees were recovered, and major lineages were found to be largely parapatric or allopatric (Aleixo, 2002, 2004; fitting 'category I phylogeographical pattern' of Avise, 2000). Other floodplain terrestrial vertebrate species for which phylogeographical surveys are available include rodents in the genera *Mesomys* and *Proechimys* (Patton, Silva & Malcolm, 1994; Matocq *et al*., 2000). These studies have documented the same 'category IV phylogeo-

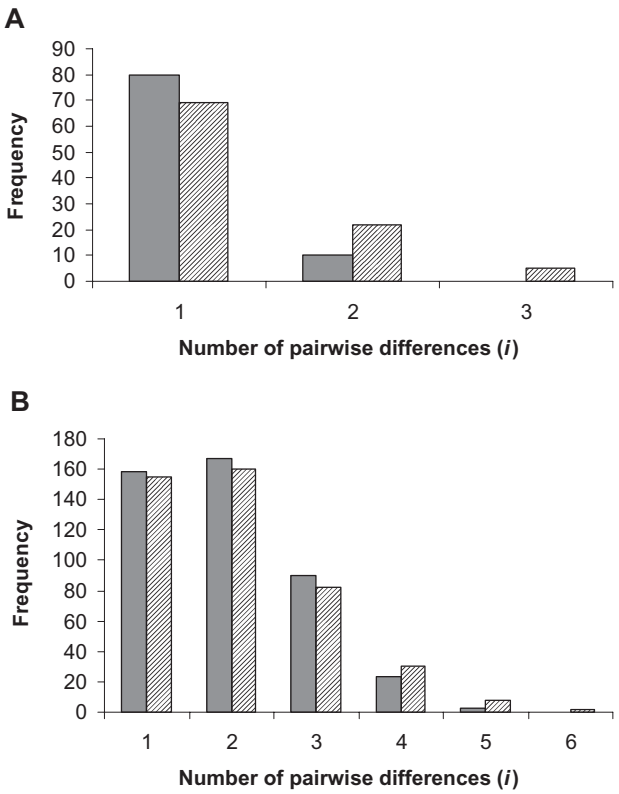


Figure 3. Pairwise nucleotide mismatch distributions for *Xiphorhynchus kienerii* (A) and *Xiphorhynchus obsoletus* (B). Solid histograms represent the observed differences, whereas barred histograms indicate the ideal distribution predicted by the model of sudden population expansion developed by Rogers (1995).

graphical pattern' (*sensu* Avise, 2000) for these floodplain species, with higher levels of gene flow and shallower gene trees than those recovered for other mammal upland forest species (Patton, Silva & Malcolm, 1996, 2000; Peres, Patton & da Silva, 1997). Therefore, this strongly dichotomous and apparently general pattern observed in birds and mammals suggests that populations of floodplain and upland forest species differ strikingly in their mode of diversification across Amazonia.

Inferred higher levels of gene flow among populations of floodplain species when compared to upland ones can probably be explained by higher dispersal capabilities of the former group in association with the narrow shape and continuity of the floodplain habitat in Amazonia. Floodplain forest types found in Amazonia occur only along rivers or their immediate influence and therefore are more limited in distribution than the more widespread upland forest. Thus, when compared to upland species, gene flow among populations of floodplain specialist species can occur only through 'corridors' of habitat paralleling the distribution of Amazonian rivers, which are ultimately all connected to the Amazon river. As the haplotype networks of *X. kienerii* and *X. obsoletus* showed (Figs 1, 2), populations from river basins as far apart as the Essequibo (Guyana), Tapajós (Brazil), and Napo (Ecuador) shared most of their haplotypes, indicating a connection that may have occurred along the extensive floodplain forests found on both banks and on several islands of the Amazon river. Finally, two additional factors might promote admixture in populations of floodplain species across Amazonian river basins: (1) the colonization of river islands with vegetation at a late successional stage, which may serve as a 'stepping stone' mechanism connecting populations from opposite river banks, and (2) the especially common phenomenon in western Amazonia of lateral river channel migration, which is responsible for across-river transfer of large pieces of land (Salo *et al*., 1986) and populations of floodplain species (Patton *et al*., 2000).

In addition to a postulated high dispersal rate (and associated high levels of gene flow), the lack of phylogeographical structure recovered for floodplain species in Amazonia is also consistent with past population bottlenecks, followed by sudden demographic expansions, which might have cyclically erased genetic diversity among floodplain specialist species (Matocq *et al*., 2000; the present study). In the case of *X. kienerii* and *X. obsoletus*, mismatch distributions (Fig. 3) indicate a fairly recent massive population expansion occurring during the last 18 000 years. This population expansion cannot be easily correlated with a single event but it coincides with a period of continuous sea level rise subsequent the Last Glacial Maximum (LGM), approximately 20 000 years BP (Irion *et al*., 1997; Behling, 2002). More details on the possible proximal causes of this fairly recent population expansion are discussed below.

Evolution of floodplain forest specialist species in Amazonia

Because floodplain and upland Amazonian forests are affected differently by the same mechanisms (e.g. historical and seasonal fluctuations in river stages), it is likely that floodplain species evolved in a fundamentally different scenario than that proposed so far to explain the diversification of species associated with the upland forest (Haffer, 1969; Bates, 2001; Aleixo, 2004; Cheviron *et al*., 2005).

Molecular phylogenies estimated for the genus *Xiphorhynchus* placed both *X. kienerii* and *X. obsoletus* at the tip of long branches, and they were separated from their nearest relatives by large uncorrected sequence divergence values (~8%), indicating a relatively older age compared to other species in the same

genus (Aleixo, 2002). In *Xiphorhynchus*, cladogenesis in upland forest lineages was far greater than in floodplain lineages, which in turn were nested within ecologically diverse clades (Aleixo, 2002, 2004). Therefore, the phylogenetic positions of *X. kienerii* and *X. obsoletus* suggest that these species occupied floodplain forests early on during the first burst of diversification of the genus *Xiphorhynchus* (Aleixo, 2002). Subsequently, as suggested by low levels of population differentiation, historically high levels of gene flow associated with population bottlenecks could have prevented diversification and cladogenesis in floodplain lineages of the genus *Xiphorhynchus*.

The distribution of floodplain forests in Amazonia has been directly affected by fluctuations in sea level during the Tertiary and Quaternary; because a significant part of the Amazon basin lies below 100 m, historical fluctuations in global sea levels are postulated to have promoted two alternate events in this region: (1) deep erosion and incision of the middle and lower Amazon river and its tributaries during cold glacial periods of low global sea levels, followed by (2) blockage of those rivers' outflow during warm interglacial periods of global high sea levels (Irion *et al*., 1997). These events caused the extension of floodplain forests in Amazonia to vary considerably and cyclically after the Tertiary (Irion *et al*., 1995, 1997; Lundberg *et al*., 1998; Behling, 2002). Theoretically, populations of floodplain species may have experienced population bottlenecks during periods of low sea level stands, which promoted a decrease in the area covered by alluvial plains in Amazonia. During periods of high sea level stands, population bottlenecks were replaced by episodes of rapid population expansion in response to an increase in the area covered by alluvial plains (Irion *et al*., 1997). Phylogeographical and population genetics data presented herein for *X. kienerii* and *X. obsoletus* are consistent with a recent population expansion coincident with the establishment of the Holocene floodplain forest in most of central and eastern Amazonia.

Because of their lower elevation and poorer draining conditions, the western Amazonian lowlands were more strongly flooded during periods of high sea levels than the neighbouring Brazilian and Guianan shields; this led to the formation of the so called *palaeo-várzea* floodplain forest in these lower areas, dating back to 120 000 years BP and thought to cover an area of approximately 1 million km^2 (Irion *et al*., 1997; Alvarenga & Guilherme, 2003). Under this scenario, the *palaeo-várzeas* of the western Amazonian lowlands could have been the source area for a recent colonization of a large portion of the Amazon basin by populations of floodplain species, beginning after the onset of a period of increasing sea levels since the LGM (20 000 years BP). Higher levels of haplotype and nucleotide diversity recovered for populations of *X. kienerii* and *X. obsoletus* in central and western Amazonia (Table 3) appear to support this notion; however, this hypothesis can be falsified temporally and spacially only with additional phylogeographical and population genetics assessments focusing on several lineages of floodplain specialist species throughout Amazonia. Ideally, these studies should be based on fast genotypic markers such as microsattelites.

In conclusion, the distribution and dynamics of floodplain forest types in Amazonia do not appear to be conducive to population subdivision at small and even at large time scales. Therefore, as inferred based on the pattern recovered for birds of the genus *Xiphorhynchus*, the following three main evolutionary characteristics are expected to be shared by lineages closely associated with Amazonian floodplain forests: (1) little population subdivision and phylogeographical structure throughout Amazonia; (2) smaller rates of cladogenesis when compared to upland forest lineages; and (3) no close phylogenetic affinities with speciose lineages of Amazonian organisms associated with upland forest; instead, lineages of floodplain forests specialist species should represent extant 'relicts' derived from early radiations of widespread lineages of Neotropical organisms.

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