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Molecular systematics, phylogeography, and population genetics of Xiphorhynchus (aves: Dendrocolaptidae) in the Amazon basin

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MOLECULAR SYSTEMATICS, PHYLOGEOGRAPHY, AND POPULATION GENETICS OF *XIPHORHYNCHUS* (AVES: DENDROCOLAPTIDAE) IN THE AMAZON BASIN

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ABSTRACT

Among those few hypotheses amenable to falsification by phylogenetic methods concerning the diversification of the Amazonian biota, three can be singled out because of their verifiable predictions: the riverine barrier, the gradient, and the basal trichotomy hypotheses. I used phylogenetic and population genetics methods to reconstruct the diversification history of the genus *Xiphorhynchus* (aves: Dendrocolaptidae) in Amazonia. First, I estimated the phylogeny of the entire genus *Xiphorhynchus* to test a key prediction of the gradient hypothesis; secondly, I documented phylogeographies of a superspecies associated with upland forest (*X. spixii / elegans*) and two species linked to floodplain forest (*X. kienerii* and *X. obsoletus*) to evaluate predictions of the riverine barrier and basal trichotomy hypotheses. The phylogeny estimated for the genus *Xiphorhynchus* falsified an anticipated sister relationship between floodplain and upland forest species, as predicted by the gradient hypothesis. Phylogeographic and population genetics analyses of the upland forest superspecies (*X. spixii / elegans*), and floodplain forest species (*X. kienerii* and *X. obsoletus*) indicated that predictions of the riverine barrier hypothesis hold only for populations of the upland forest superspecies separated by rivers located on the Brazilian shield; in contrast, rivers located in western Amazonia did not represent areas of primary divergence for populations of *X. spixii / elegans*. As expected, populations of the floodplain forest species showed high levels of gene flow and no geographic structure throughout the entire Amazon basin, a pattern consistent with their expected capacity to overcome riverine barriers. In agreement with predictions of the basal trichotomy hypothesis, populations of the *X. spixii / elegans* superspecies found on the Brazilian shield were basal in the phylogeny, exhibiting

some population genetics attributes typical of old populations having reached equilibrium. In contrast, populations found in western Amazonia were more recently derived and experienced a dramatic recent population expansion, probably colonizing the area from the geologically older Brazilian shield. The data presented herein supported important predictions of the basal trichotomy and riverine barrier hypotheses, indicating that they are not mutually exclusive, and may together account for the diversification of the genus *Xiphorhynchus* in Amazonia at different temporal and geographical scales.

CHAPTER 1. GENERAL INTRODUCTION

The bird fauna of the Amazon lowlands in South America is the richest in the world, with over 1,000 species, of which about 265 are endemic (Stotz et al. 1996, Nores 2000). This unparalleled ornithological diversity has intrigued naturalists since the early days of Amazonian exploration. As early as 1852, the British naturalist Alfred Russell Wallace put forward an evolutionary hypothesis to explain the history of diversification of Amazonian vertebrates (Wallace 1852), and since then, several alternative hypotheses have been proposed (Haffer 1969, 1993, Endler 1977, Colinvaux 1993, Bush 1994, Marroig and Cerqueira 1997). Few testable predictions, however, could be derived from these hypotheses of diversification (Patton and Silva 1998); the biggest hurdle is the lack of specific temporal and geographic contexts in the formulation of most of the proposed hypotheses, making them hard to falsify by phylogenetic methods (Patton and Silva 1998). For example, although the refuge hypothesis has been widely applied to tropical forest ecosystems around the globe (Haffer and Prance 2001), it is nearly impossible to be falsified in a phylogenetic context (Patton and Silva 1998).

To distinguish effectively among competing hypotheses of species diversification, several researchers have attempted to adopt a phylogeographic approach to study the diversification of the Amazonian biota (Patton and Silva 1998, Silva and Patton 1998, Lougheed et al. 1999, Moritz et al. 2000). The phylogeographic approach is the combined assessment of the phyletic (genealogical) and geographic components of allele distributions among populations and closely related species (Harrison 1991). These distributions can be contrasted with explicit expectations of geographical relationships among ancestral populations prior to divergence under the three

main models of species formation available: allopatric, parapatric, and sympatric (Harrison 1991). Furthermore, phylogeographies can be also used to infer the timing of speciation for groups with poor or no fossil data, as is the case for most terrestrial tropical vertebrates (Moritz et al. 2000).

Among those few hypotheses of Amazonian diversification amenable to falsification by phylogenetic methods, three can be singled out because of their generality and verifiable predictions: the riverine barrier, the gradient, and the recently developed basal trichotomy hypotheses (Moritz et al. 2000, Bates in press). Below, I introduce these three hypotheses and outline their main phylogeographic predictions. In spite of being hardly falsified by phylogenetic methods, I also discuss the refuge hypothesis and some of its predictions under a population genetics framework (Capparella 1991).

THE RIVERINE BARRIER HYPOTHESIS

Proposed originally by A. R. Wallace in the mid 19th century (Wallace 1852), this hypothesis states that major Amazonian rivers, because of their unmatched widths, significantly reduce or prevent gene flow between populations inhabiting opposite river banks, hence promoting speciation. That the ranges of several closely related vertebrate taxa abut along major Amazonian rivers has been interpreted as empirical support to this hypothesis (Hershkovitz 1977, Haffer 1992a, Avilla-Pires 1995).

In a phylogeographic framework, the main prediction of the riverine barrier hypothesis is that sister intraspecific clades and species will occur across major rivers rather than within major Amazonian interfluves; furthermore, phylogeographies will allow the distinction between

primary divergence across rivers (predicted by the riverine barrier hypothesis) versus secondary contact along rivers between non-sister taxa that diversified elsewhere (Moritz et al. 2000).

A second prediction of the riverine barrier hypothesis comes from the observation that the upper reaches of all major Amazonian rivers are narrower than the lower reaches; therefore, a gradual reduction of the "river-barrier effect" is expected to take place from the lower to the upper part of a river's course (Haffer 1992a). The expected outcome is a higher genetic similarity between populations from opposite banks in the headwaters than in the lower parts of rivers (Gascon et al. 2000).

A third prediction can be derived from the fact that a substantial portion of the Amazonian fauna thrives in habitats strongly influenced by major rivers, such as flooded *várzea* forests and river islands (Remsen and Parker 1983, Stotz et al. 1996). The riverine barrier hypothesis should not account for the diversification of *várzea* species because they are capable of colonizing river islands and crossing rivers, hence establishing populations on opposite banks (Capparella 1991, Matocq et al. 2000). Therefore, *várzea* specialist species should act as control groups when testing the riverine barrier hypothesis, inasmuch as its predictions are not expected to be fulfilled or verified to the same degree as for those species found exclusively in unflooded *terra-firme* forest, away from the influence of the Amazonian riverine system (Matocq et al. 2000, Moritz et al. 2000).

The main temporal prediction of the riverine barrier hypothesis is that sister taxa separated by rivers began to diverge about 5 million years ago (Late Miocene), when the last cycle of Cenozoic fluvio-lacustrine deposition ended in western Amazonia, and the Amazon river

system began to develop as a transcontinental drainage system (Hoorn et al. 1995, Campbell et al. 2001).

THE REFUGE HYPOTHESIS

The refuge hypothesis was first proposed to explain patterns of species richness in the Neotropics by Haffer (1969). The refuge hypothesis holds that climatic and vegetational changes promote cladogenesis in organisms by cyclically fragmenting and reuniting their ranges. Particularly in the case of upland (*terra-firme*) rainforest, dry climatic conditions triggered a contraction and disruption of the area covered by this habitat, which was reduced to a few isolated fragments, called refuges. Populations of *terra-firme* species isolated at different refuges started to diverge and eventually attained reproductive isolation (Haffer 1969). Subsequently, wet periods determined a re-expansion of the rainforest and associated populations of *terra-firme* species. When in secondary contact, sister lineages formerly isolated at different refuges interacted in two alternative ways: (1) did not introgress because they had become reproductively isolated (speciation was attained), or (2) introgressed across areas of secondary contact (different levels of intraspecific differentiation were attained; Haffer 1969).

Unfortunately, the main problem with deriving phylogenetic predictions from the refuge hypothesis is its ambiguity regarding the hierarchical temporal division of refuges as tracked by geographically concordant splitting events among independent lineages (Patton and Silva 1998). However, two important population genetics predictions can be derived from the refuge hypothesis: (1) episodes of population bottlenecks are expected during dry climatic periods of forest contraction and isolation, and (2) instances of demographic expansions are supposed to

follow the onset of wet periods of rainforest expansion (Capparella 1991). Obviously, episodes of population bottlenecks and demographic expansions are not exclusive predictions of the refuge hypothesis. Therefore, detection of population bottlenecks and demographic expansions do not provide irrefutable evidence confirming the refuge hypothesis, but are at least consistent with it.

The main temporal prediction of the refuge hypothesis is very broad: because dry and wet climatic cycles affecting the distribution of the rainforest are ultimately driven by astronomical Milankovitch cycles, splits between sister lineages happened during any period of earth's history (Haffer and Prance 2001). However, several paleoecological studies indicate significant climatic and vegetational changes throughout Amazonia during the last 60,000 years, with a return of wet conditions and associated rainforest expansion since the Last Glacial Maximum (LGM), about 20,000 years BP (see reviews in Haffer 1997a and Burnham and Grahan 1999). Therefore, population of *terra-firme* species are expected to have experienced a sudden population expansion during the last 20,000 years BP, after a population bottleneck.

A derivative of the refuge hypothesis known as the vanishing refuge hypothesis posits that rainforest species gradually adapted to drier conditions of a vanishing refuge and eventually "switched" habitats, abandoning the rainforest and colonizing open habitats (Vanzolini and Williams 1981). The main phylogenetic prediction of this derivative of the refuge hypothesis is that sister species will replace each other in dry and wet forest types found nowadays in Amazonia (Vanzolini and Williams 1981). Under a population genetics framework, population bottlenecks and range expansions are also expected to have affected populations of these species replacing each other in different dry and wet habitats.

THE GRADIENT HYPOTHESIS

The gradient hypothesis is derived from Endler's (1977, 1982) proposal that current habitat heterogeneity promotes cladogenesis in tropical habitats. Speciation is accomplished by strong divergent selection across sharp ecological gradients, even when gene flow is present. Therefore, genetic differentiation and subsequent speciation can be parapatric or sympatric, instead of allopatric (Endler 1977). Evidence and examples supporting this hypothesis were recently reviewed by Smith et al. (2001).

Under a phylogeographic framework, the main prediction of the gradient hypothesis is that sister species should occupy distinct but adjacent habitats (Moritz et al. 2000). This prediction is also shared with the vanishing refuge hypothesis, except that the latter hypothesis necessarily predicts severe population bottlenecks followed by range expansion (Vanzolini and Williams 1981).

No specific temporal prediction can be derived from the gradient hypothesis, except that if it accounted for the recent diversification of a lineage, then several sister species pairs replacing each other in distinct habitats should be observed in a phylogeny.

THE BASAL TRICHOTOMY HYPOTHESIS

The recently proposed basal trichotomy hypothesis is a derivative of the broader paleogeography hypothesis, which posits that speciation in Amazonia was caused by sea-level fluctuations and tectonic movements throughout the Tertiary and Quaternary periods (Emsley 1965, Bates in press). The basal trichotomy hypothesis is derived from a paleogeographic scenario of massive marine incursions into the Amazon during the Tertiary (Räsänen et al. 1995,

Webb 1995). These marine invasions created an epicontinental sea that inundated the Amazon lowlands and isolated three large, higher land blocks at different places of the basin: the Guianan shield in the north; the Brazilian shield in the south; and the base of the eastern slope of the Andes in the west (Fig. 1.1; Webb 1995). Ancestral populations isolated in these three land blocks began to diverge from each other and eventually speciated (Bates in press).

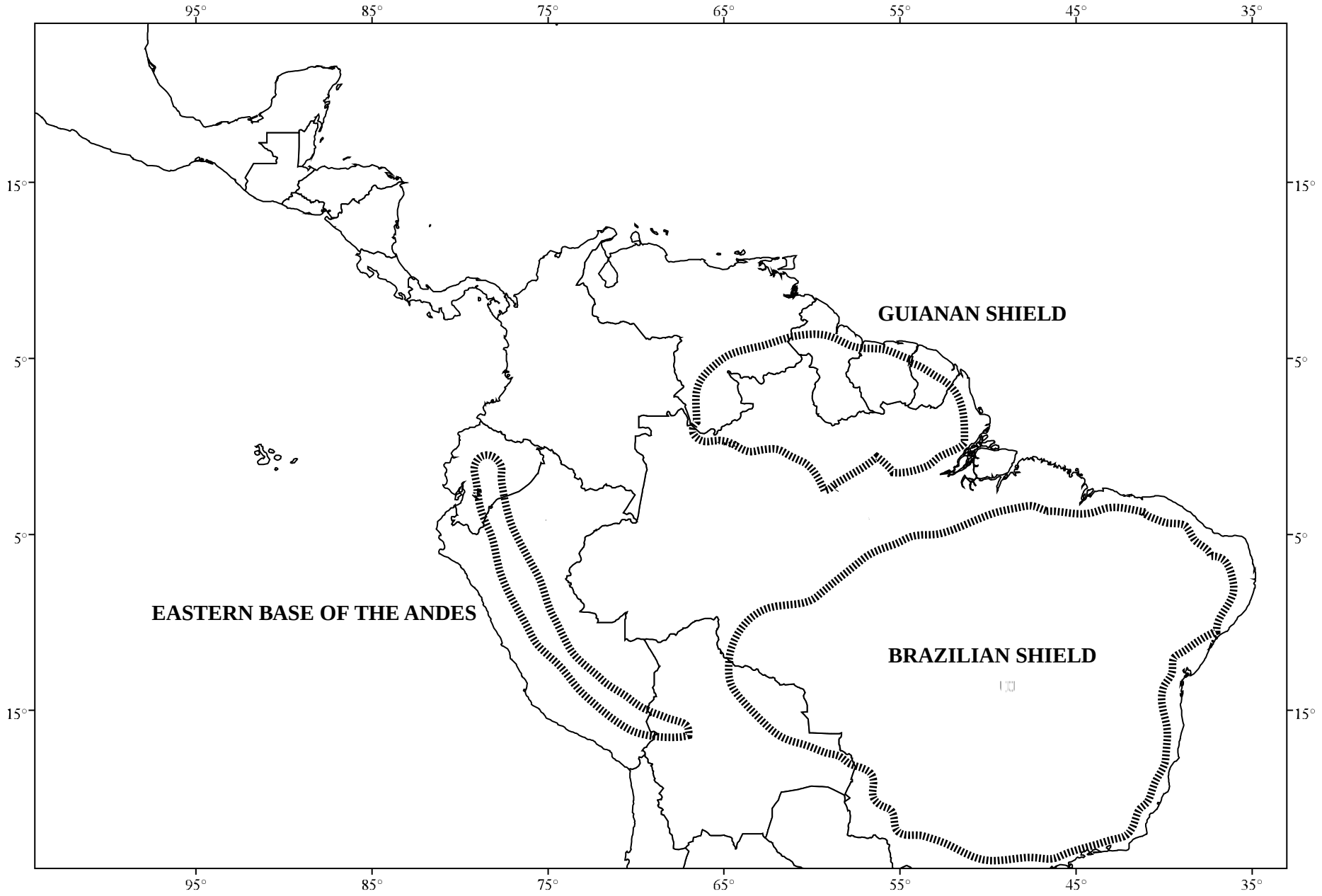
Area cladograms based on the distribution of Amazonian primates and birds (Silva and Oren 1996, Bates et al. 1998), and a few phylogenetic studies of some Amazonian birds (Cracraft and Prum 1988, Prum 1988, Hackett 1993) seem to support this hypothesis by placing one of the three areas thought to have escaped marine invasions as basal, and therefore inferred as the place of vicariance and origin for those lineages prior to their diversification and colonization of oth er parts of the Amazon. Miocene marine incursions into the Amazon have been invoked to explain the abundance of marine derived fishes and mammals occurring nowadays in Amazonian rivers as shown by several recent phylogenetic studies (Lovejoy et al. 1998, Lovejoy and Araujo 2000, Hamilton et al. 2000).

Bates (In press) derived three main predictions of area relationships between currently recognized centers of endemism for birds in the Amazon based on the basal trichotomy hypothesis (see Fig. 1.2 for location of areas of endemism):

(1) The Napo and Inambari areas (including the eastern Andean foothills) should harbor sister taxa relative to the Guianan (on the Guianan shield) and Belém / Pará (on the Brazilian shield) areas;

(2) The Pará / Belém areas should contain sister taxa relative to the Guianan and Napo / Inambari areas;

Figure 1.1. Areas inferred as being part of the basal trichotomy (according to Bates in press) and having escaped periods of massive marine incursions in Amazonia during the Tertiary. See text for detail.


95°
85°
75°
65°
55°
45°
35°
15°
5°
5°
15°
GUIANAN SHIELD
EASTERN BASE OF THE ANDES
BRAZILIAN SHIELD

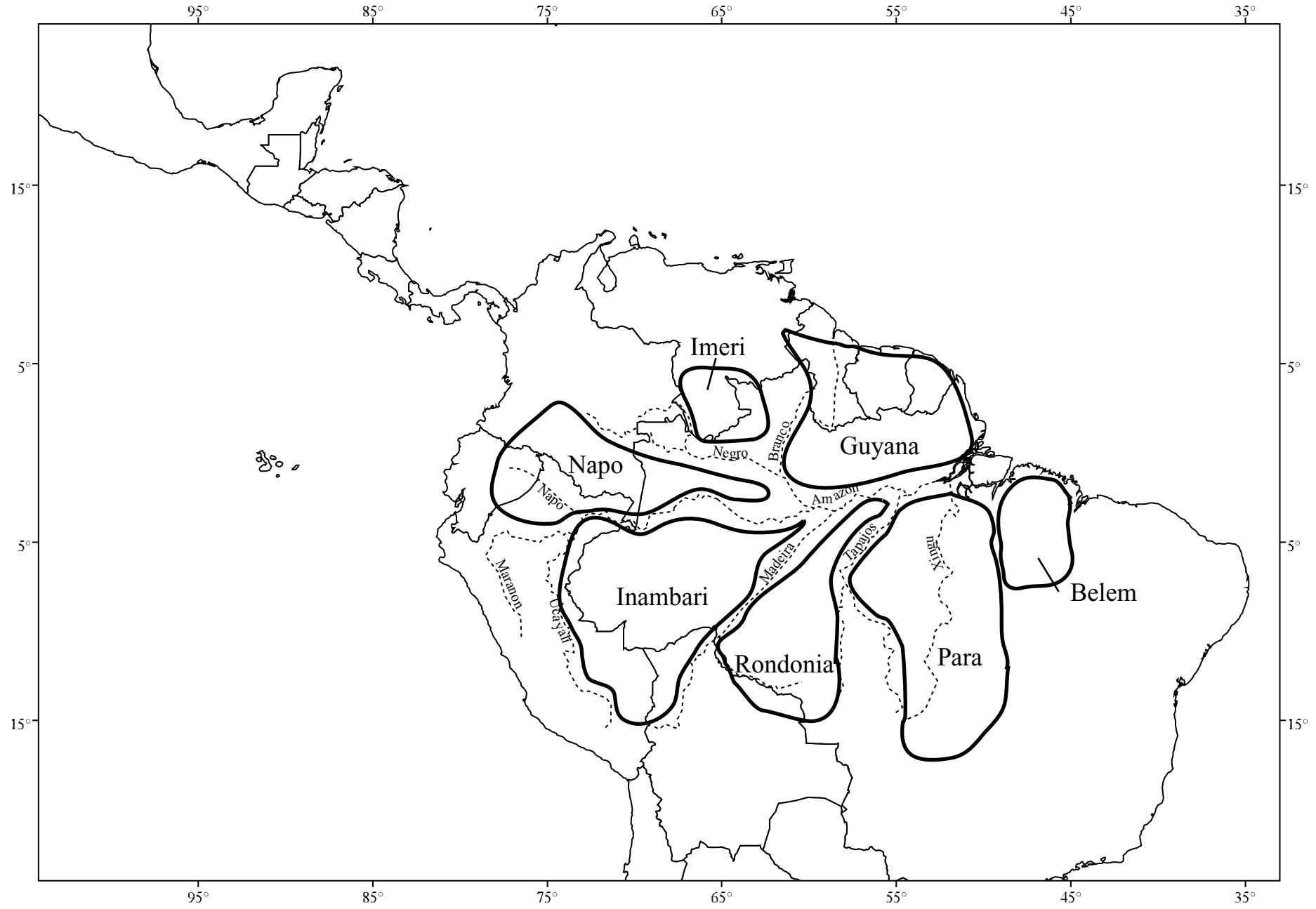
(3) The Guianan area should be basal relative to the Napo / Inambari and the Pará / Belém areas. Because splitting events leading to these sets of relationships could have happened in a relatively short period of time, area cladograms recovered could be ambiguous, exhibiting a trichotomy among the Guianan, Pará / Belém, and Napo / Inambari areas; Despite the well-documented existence of marine invasions in the Amazon, the timing of these incursions is still controversial (Hoorn 1996, Hoorn et al. 1995, Räsänen et al. 1995). The majority of researchers think that major marine introgressions in the Amazon occurred during the Early and Middle Miocene (Hoorn et al. 1995, Paxton et al. 1996), not in the late Miocene as advocated by Räsänen et al. (1995).

Whichever of those time frames is correct, the basal trichotomy hypothesis would be useful in explaining the early diversification of Amazonian avian genera rather than the splitting of sister species or populations (Bates in press). However, several global changes in sea level occurred since the Miocene (Hallam 1992), and a complete marine incursion in the Amazon may not be necessary to isolate forested areas in regions escaping flooding, hence promoting speciation among terrestrial organisms (Bates in press).

THE AVIAN GENUS *XIPHORHYNCHUS* AND ITS ROLE AS A MODEL TO STUDY DIVERSIFICATION IN AMAZONIAN ORGANISMS

Under the phylogeographic and population genetics frameworks, a test of the four hypotheses outlined above would require a group of organisms with three main characteristics: high species richness, high ecological diversity, and widespread distribution in the Amazon basin. High

Figure 1.2. Areas of endemism for birds in Amazonia according to Cracraft (1985). Areas were inferred based on the overlap in distribution of several endemic species and subspecies (Cracraft 1985).


95°
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35°
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5°
5°
15°
Imeri
Napo
Guyana
Inambari
Rondonia
Para
Belem
Negro
Branco
Amazon
Napo
Maranon
Ucayali
Madeira
Tapajos
Xingu

species richness is especially important to test the basal trichotomy hypothesis, because this hypothesis would account for the phyletic (long-term) evolution within a genus. High ecological diversity is key to test the main prediction of the gradient hypothesis, but also to test the riverine barrier hypothesis insofar as differences between floodplain (*várzea*) and upland (*terra-firme*) forest specialist species are expected to occur. Finally, a widespread distribution in the area of interest is essential to study the diversification of a lineage at a scale appropriate to allow generalizations for the entire Amazon basin.

The avian genus *Xiphorhynchus* (Dendrocolaptidae) fulfills all the requirements outlined above and provides an excellent model to investigate the historical diversification of Amazonian organisms. Two species (*X. kienerii* and *X. obsoletus*) occur solely in seasonally flooded forest types, whereas three other species (*X. ocellatus, X. spixii,* and *X. pardalotus*) are restricted to non-flooded forest (Stotz et al. 1996). Two other species (*X. picus* and *X. guttatus*) are found in a wide variety of habitats, including *várzea*, *terra-firme*, secondary forest, and forest edge (Stotz et al. 1996). Furthermore, species of *Xiphorhynchus* are among the commonest and most widespread woodcreepers in Amazonia, with up to five species occurring sympatrically in parts of western Amazonia (Terborgh et al. 1990, Ridgely and Tudor 1994, pers. obs.).

Here, I use the phylogeographic approach to reconstruct the history of diversification for the genus *Xiphorhynchus* in the Amazon basin. The main goal is to use phylogenies to evaluate predictions of the four diversification hypotheses discussed above. First, I generate a phylogenetic hypothesis for the entire genus *Xiphorhynchus* to test a key prediction of the gradient hypothesis. Second, I carry out phylogeographic and population genetics analyses of a superspecies of *Xiphorhynchus* associated with the *terra-firme* habitat (*X. spixii / elegans*), and

two species linked to *várzea* forest (*X. kienerii* and *X. obsoletus*) to evaluate predictions of the riverine barrier, refuge, and basal trichotomy hypotheses. Third, based on the data presented herein, I discuss the significance of the riverine barrier, refuge, gradient, and basal trichotomy hypotheses in the diversification of the Amazonian biota, proposing some refinements to these hypotheses.

CHAPTER 2. MOLECULAR SYSTEMATICS AND THE ROLE OF THE *VÁRZEA-TERRA-FIRME* ECOTONE IN THE DIVERSIFICATION OF *XIPHORHYNCHUS*

Avian species richness in the Neotropics has traditionally been explained by allopatric speciation models, such as the "refuge" (Haffer 1969), "river" (Snethlage 1913), and "Andean uplift" hypotheses (Chapman 1917). Alternative hypotheses involving sympatric and parapatric speciation scenarios have been largely dismissed as secondary in importance (Haffer 1992b), despite the scarcity of explicit tests evaluating their predictions under a phylogenetic framework (but see Bates and Zink 1994, Arctander and Fjeldså 1994). Endler (1982) argued that strong divergent selection across sharp ecological gradients can account for differentiation and speciation among tropical organisms. Evidence for such an important role played by ecotones as areas of population differentiation was found in studies on population genetics and morphometrics of two phylogenetically distinct central African bird species (Smith et al. 2001).

In the Amazon Basin, two distinct and adjacent forest types dominate the landscape: the *várzea* forest (which floods every year) and the *terra-firme* forest (which does not flood on a regular basis). About 15% of the terrestrial Amazonian avifauna is known to be restricted or nearly restricted to *várzea* forests (Remsen and Parker 1983). Little is known about the origin and evolution of this characteristic avifauna, in part because of the paucity of phylogenetic studies on Neotropical bird groups. One possible scenario, as suggested by the abrupt replacement of many congeneric avian species pairs across the *várzea* - *terra-firme* ecotone (Robinson and Terborgh 1997), is that this ecological gradient contributed directly to population differentiation and ultimately to speciation within these lineages. An important prediction of this

hypothesis is that congeneric species pairs replacing each other across the *várzea - terra-firme* ecotone ought to be recently derived sister taxa (Moritz et al. 2000).

With species restricted to both *várzea* and *terra-firme* forests, the avian genus *Xiphorhynchus* provides an excellent model for studying the history of habitat specialization and its role as a possible speciation mechanism among Amazonian organisms (Table 2.1). In the only phylogenetic hypothesis proposed so far for Dendrocolaptidae (sensu A.O.U 1998), relationships within *Xiphorhynchus* are largely unresolved, with most species making part of a polytomy that includes taxa grouped in other genera as well, such as *Campyloramphus, Dendrexetastes,* and *Lepidocolaptes* (Raikow 1994). Raikow (1994) suggested that the anatomical characters he studied could not distinguish species level differences in the genera *Hylexetastes*, *Xiphorhynchus*, and *Lepidocolaptes*, stating that "the solution…must await analysis of other types of data that show sufficient variation at the appropriate taxonomic level." More recently, García-Moreno and Silva (1997) found molecular evidence indicating that the Lesser Woodcreeper (*Lepidocolaptes fuscus*) is actually more closely related to *Xiphorhynchus* than to any of the six *Lepidocolaptes* species they sampled. Despite their findings, these authors suggested caution concerning the inclusion of *Lepidocolaptes fuscus* in *Xiphorhynchus* before a phylogeny of all *Xiphorhynchus* species is available. As yet, neither the monophyly nor the position of *Xiphorhynchus* within Dendrocolaptidae has been properly assessed. The situation at lower taxonomic levels is also poorly resolved: many polytypic *Xiphorhynchus* species have several well differentiated populations once considered separate species (Cory and Hellmayr 1925). In fact, even today there is no consensus regarding the taxonomic status of many

Table 2.1 - Common name, habitat preferences, and distribution of currently recognized species of *Xiphorhynchus* [a].

Species	Common name	Habitat [b]	Distribution
X. erythropygius	Spotted Woodcreeper	L, M	Central America and Chocó
X. flavigaster	Ivory-billed Woodcreeper	L, M, D, S, PO	Central America
X. guttatus	Buff-throated Woodcreeper	L, TF, V, S	Amazonia and eastern Brazil
X. kienerii [c]	Zimmer's Woodcreeper	V	Amazonia
X. lachrymosus	Black-striped Woodcreeper	L, MA	Central America and Chocó
X. obsoletus	Striped Woodcreeper	V	Amazonia
X. ocellatus	Ocellated Woodcreeper	TF, M [d]	Amazonia and eastern slope of the Andes
X. pardalotus	Chestnut-rumped Woodcreeper	TF, M [d]	Amazonia and Tepuis
X. picus	Straight-billed Woodcreeper	V, D, S, MA	Central America and Chocó
X. spixii	Spix's Woodcreeper	TF, M [d]	Amazonia and eastern slope of the Andes
X. susurrans	Cocoa Woodcreeper	L, D, S, MA	Central America and trans-Andean South America
X. triangularis	Olive-backed Woodcreeper	M	W. slope of the Andes

[a] Following the taxonomy of Zimmer (1934b), Peters (1951), and the A. O. U. (1998). The taxon *Xiphorhynchus striatigularis*, known only by its type specimen, is now regarded as an aberrant individual of *X. flavigaster* (Winker 1995).

[b] Based on Stotz et al. (1996) and complemented with personal observations. D - Tropical deciduous forest; L - Tropical lowland evergreen forest; M - Montane evergreen forest; MA - Mangrove forest; PO - Pine-oak forest; S - Secondary forest; TF - Amazonian *terra-firme* forest; V - Amazonian *várzea* forest.

[c] Formerly known as *X. necopinus*, a name now considered a junior synonym of *X. kienerii* (Aleixo and Whitney 2002.).

[d] Restricted to *terra-firme* forest in lowland Amazonia.

subspecies of *X. guttatus* and *X. spixii* (contrast Ridgely and Tudor 1994 with Stotz et al. 1996 and Haffer 1997b).

The current lack of resolution concerning the evolutionary history of *Xiphorhynchus* prevents its use as a model to study the role of habitat specialization as a possible diversification mechanism in the Neotropics. Here, I present a phylogenetic hypothesis for the genus *Xiphorhynchus* based on mitochondrial DNA (mtDNA) sequences to: (1) evaluate the monophyly of *Xiphorhynchus* and its relationship with other Dendrocolaptidae genera; (2) assess species limits within some polytypic *Xiphorhynchus* species; and (3) evaluate the prediction of a sister relationships between *várzea* and *terra-firme* species, as expected if the *várzea - terra-firme* ecotone played a decisive role in population differentiation and subsequent speciation within *Xiphorhynchus*.

METHODS

Taxa Sequenced. - In addition to all known *Xiphorhynchus* species, I sampled at least one species belonging to all extant woodcreeper genera except *Dendrocincla, Deconychura,* and *Drymornis* (Appendix 1). Studies based on anatomical characters indicate that the latter genera are not closely related to *Xiphorhynchus* (Feduccia 1973, Raikow 1994); instead, I sampled the genera *Lepidocolaptes* (Lineated Woodcreeper [*L. albolineatus*], Narrow-billed Woodcreeper [*L. angustirostris*], and *L. fuscus*) and *Campyloramphus* (Black-billed Scythebill [*C. falcularius*], Curve-billed Scythebill [*C. procurvoides*], and Red-billed Scythebill [*C. trochilirostris*]) more thoroughly because of their supposed closer relationship with *Xiphorhynchus* (Feduccia 1973, Raikow 1994, García-Moreno and Silva 1997). At the generic level, my goal was to assess the

monophyly of *Xiphorhynchus* and its relationships with other woodcreeper genera rather than to propose a phylogenetic hypothesis for the whole family Dendrocolaptidae. No genera from other families were included in the analysis because the monophyly of Dendrocolaptidae has been supported by studies based on DNA-DNA hybridization (Sibley and Ahlquist 1990) and morphological characters (Raikow 1994, Clench 1995). At lower taxonomic levels, I sampled subspecies of species whose limits have been controversial according to taxonomists working on Neotropical birds (Cory and Hellmayr 1925, Zimmer 1934b, Peters 1951, Pinto 1978, Ridgely and Tudor 1994, Haffer 1997b). Thus, taxa belonging to the following species were sampled: *brevirostris, chunchotambo*, *ocellatus*, and *weddellii* (*X. ocellatus*); *aequatorialis* and *insolitus (X. erythropygius); eytoni, dorbignyanus, guttatoides, guttatus, polystictus,* and *susurrans (X. gutattus*); *elegans, juruanus, ornatus,* and *spixii (X. spixii*); and finally *bangsi* and *intermedius (X. triangularis*). These taxa do not represent an exhaustive list of subspecies belonging to each polytypic species, but they cover major divisions within those species based primarily on plumage patterns (Cory and Hellmayr 1925, Zimmer 1934b). I also sampled subspecies belonging to species whose limits are not controversial to contrast their intraspecific level of genetic variation with those of the controversial polytypic species listed above. Thus, I sampled the following taxa: *eburneirostris* and *flavigaster* (*X. flavigaster);* and *altirostris, bahiae, phalara,* and *picus (X. picus*).

DNA Sequencing. - Total genomic DNA was extracted from tissue samples using a Qiagen tissue extraction kit or a standard phenol/chloroform method (Hillis et al. 1990). Samples from STRI were obtained as lyophilized DNA. Fragments of the mitochondrial genome were amplified using 11 primers spanning most of ctytochrome *b* (1,035 bp) and the entire NADH dehydrogenase

subunits 2 (ND2; 1,041 bp) and 3 (ND3; 354 bp) genes. Primers used for cytochrome *b* were: L14990 (Kocher et al. 1989), L15389 (Hackett 1996), H15710 (Helm-Bychowski and Cracraft 1993), HXIPH (CATTCTGGTTTGATGTGGGG; designed specifically for this project), L15505 (CTAACCTTCCTACACGAAACC; designed specifically for this project), L15656 (Helm-Bychowski and Cracraft 1993), and H16065 (Hackett 1996). Primers used for ND2 were: L5215 (Hackett 1996), H5578 (Hackett 1996), L5758X (modified from primer published by Johnson and Sorenson [1998; GGATGAGCRGGYCTAAAYCARAC]), and H6313 (Johnson and Sorenson 1998). For ND3, I used primers L10755 and H11151 (Chesser 1999). All primer numbers refer to the 3' base of the published chicken mtDNA sequence (Desjardins and Morais 1990). Fragments were PCR amplified 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 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, Norwalk, Connecticut), 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. I aligned and reconciled sequences from both strands within and between species using Sequencher 3.1.1 (Genecodes, Madison, Wisconsin). The following measures outlined by Sorenson and Quinn (1998) and Bates et al. (1999) were taken to ensure that the DNA fragments amplified were accurate and of mitochondrial origin (not pseudogenes): (1) most sequences were amplified

in large fragments (> 1,000 bp); (2) both DNA strands were sequenced; (3) sequences were aligned with the chicken complete mtDNA sequence, and inspected for insertions, deletions, and stop codons that would result in a nonfunctional protein; (4) sequences were expected to exhibit high transition to transversion substitution ratios characteristic of mitochondrial, not nuclear substitution patterns; and (5) a partition homogeneity test was performed to evaluate if the phylogenetic signal of the three different gene sequences was similar. Pseudogenes do not necessarily yield the same phylogenetic signal as mitochondrial genes. I could not detect any evidence of pseudogenes in the sequences used for this study. After these procedures, sequences were submitted to GenBank (AY089790 – AY089918; Appendix 1).

Phylogenetic Analyses. - I performed a partition homogeneity test as implemented in PAUP* 4.0b7 (Swofford 1998) with 100 replicates to determine if the different mitochondrial genes sequenced could be combined for phylogenetic analysis (Farris et al. 1995). Another partition homogeneity test compared third with first and second codon positions to evaluate if third positions gave a different phylogenetic signal due to saturation at deeper divergence levels. Maximum parsimony and maximum likelihood heuristic searches (referred to as MP and ML, respectively, throughout this paper) were conducted with PAUP* 4.0b7. MP analyses were based on unweighted sequence data. I used the likelihood ratio test as implemented in MODELTEST (Posada and Crandall 1998) to select the best and simplest model of molecular evolution fitting my dataset, which was then used in all ML analyses. I used 100 nonparametric bootstrap replications (referred to as BP throughout the paper) to evaluate confidence levels of nodes for all phylogenies obtained with MP and ML (Felsenstein 1985). Because of computer limitations, only one addition-sequence replicate was performed for each bootstrap replicate in

the likelihood analyses. To further explore the sensitivity of the data to methods of analysis, I also performed a Bayesian inference of phylogeny using the MrBayes software, version 1.11 (Huelsenbeck 2000). Bayesian analysis provides posterior probability values for different phylogenetic parameters, such as topology, branch lengths, and substitution patterns, producing essentially the same result as ML given the same model of nucleotide substitution (Huelsenbeck 2000). However, instead of estimating these parameters by maximizing their likelihoods on a single tree (like ML), the Bayesian approach samples multiple trees and parameter values from their near optimal position (i.e. near their global maximum). This produces a posterior probability distribution from which a consensus tree is generated. The interpretation of the result of a Bayesian estimate of phylogeny is straightforward: the posterior probability of any single clade in a given phylogeny is the percentage of time that the clade appeared in the sample of trees representing the posterior distribution. Because the posterior probabilities of all possible trees add up to 1, a given clade with a support of 1 or 100% occurred in all possible trees generated by MrBayes under a wide variety of substitution parameters, assuming a specific model of sequence evolution. In general, Bayesian analyses generate consensus trees with higher posterior probabilities than bootstrap proportions under a ML approach (Rannala and Yang 1996). I ran MrBayes 1.11 with the following specifications: (1) assuming a general time reversible model of nucleotide substitution with estimated base frequencies, proportion of invariable sites, and rates for variable sites following a gamma distribution (model GTR+G+I), as selected by MODELTEST; and (2) running the Markov chain for 500,000 generations, sampling 1 tree every 100 generations. Following recommendations outlined by Huelsenbeck and Hall (2001), I discarded trees obtained before the Markov chain reached convergent and stable likelihood values.

I used PAUP* 4.0b7 to compute a majority-rule consensus tree of the sampled trees. The proportion of times a given clade was sampled equal to its posterior probability of occurrence. Because the increase in computational time required for the completion of ML and Bayesian analyses grows with the number of taxa, I divided these analyses into two parts: (1) one containing only one individual each of the 25 sampled species (all the twelve *Xiphorhynchus* species plus thirteen outgroups) and (2) another containing 29 taxa belonging to 10 *Xiphorhynchus* species defined as monophyletic by the first analysis plus three outgroups. The purpose of the first analysis was to assess the monophyly of *Xiphorhynchus*, whereas the second analysis dealt with polytypic *Xiphorhynchus* species limits.

RESULTS

Informative Variation. - For most taxa, the dataset upon which phylogenetic analyses were inferred contained 2,430 characters, corresponding to positions 5241 to 6278 (ND2), 10776 to 11127 (ND3), and 15001 to 16035 (cyt *b*) of the mtDNA chicken sequence (Desjardins and Morais 1990). Parsimony informative sites were evenly distributed among the three genes: 330 ND2 (31.7% of total bases), 112 ND3 (31.6%), and 291 cty *b* (28.1%). A partition homogeneity test performed among the three genes did not detect significant differences in their phylogenetic content ($P = 0.3$). Another partition homogeneity test contrasting first and second with third codon positions also did not uncover significantly different phylogenetic signals among these data partitions ($P = 0.39$). Therefore, sequence data from all genes and codon positions were combined for phylogenetic analyses.

Sequence Divergence. - Uncorrected ("*p*") sequence divergence levels among all *Xiphorhynchus* taxa ranged from 0.08% (between two subspecies of *X. picus*) to 11.2 % (between *X. ocellatus* and *X. picus*; Table 2.2). When *X. picus* and *X. kienerii* are excluded, sequence divergence levels among the remaining monophyletic *Xiphorhynchus* taxa ranged from 0.37% (between two subspecies of *X. guttatus*) to almost 10% (between *X. obsoletus* and *X. ocellatus*; Table 2.2). Levels of sequence divergence between *Xiphorhynchus* (excluding *X. picus* and *X. kienerii*) and outgroups (excluding *Lepidocolaptes fuscus*) ranged from 9.2% (between *Lepidocolaptes angustirostris* and *X. spixii ornatus*) to almost 15% (between *X. guttatus dorbignyanus* and *Sittasomus griseicapillus* [Olivaceous Woodcreeper]; Table 2.2). When *X. picus* and *X. kienerii* were excluded, even third codon position substitutions accumulated linearly with overall genetic distance within and among *Xiphorhynchus* species (plot not shown), indicating that saturation does not seem to be a problem among these taxa. Levels of genetic differentiation among some subspecies of *X. guttatus*, *X. ocellatus*, and *X. spixii* reached or exceeded those found between undisputed sister biological species of *Xiphorhynchus*, such as *X. flavigaster* and *X. lachrymosus* (p = 4.2 - 4.4%; Table 2.2) or between *X. ocellatus* and *X. pardalotus* (p = 3.4 - 3.9%; Table 2.2). In contrast, subspecific genetic differentiation between subspecies of *X. erythropygius*, *X. flavigaster*, and *X. triangularis* averaged about 1% (Table 2.2).

MP Analyses. - Equally weighted MP analyses resulted in two most parsimonious trees (length 3,433; CI=0.35; RI=0.6). Figure 2.1 shows a strict consensus of these two most parsimonious trees and bootstrap confidence values for its nodes. All *Xiphorhynchus*, *Lepidocolaptes*, and *Campyloramphus* species were monophyletic at 97% BP support. The only difference between the topologies of the two most parsimonious trees pertained to the position of the sibling species

Table 2.2 - Uncorrected (*p*) distance among taxa.

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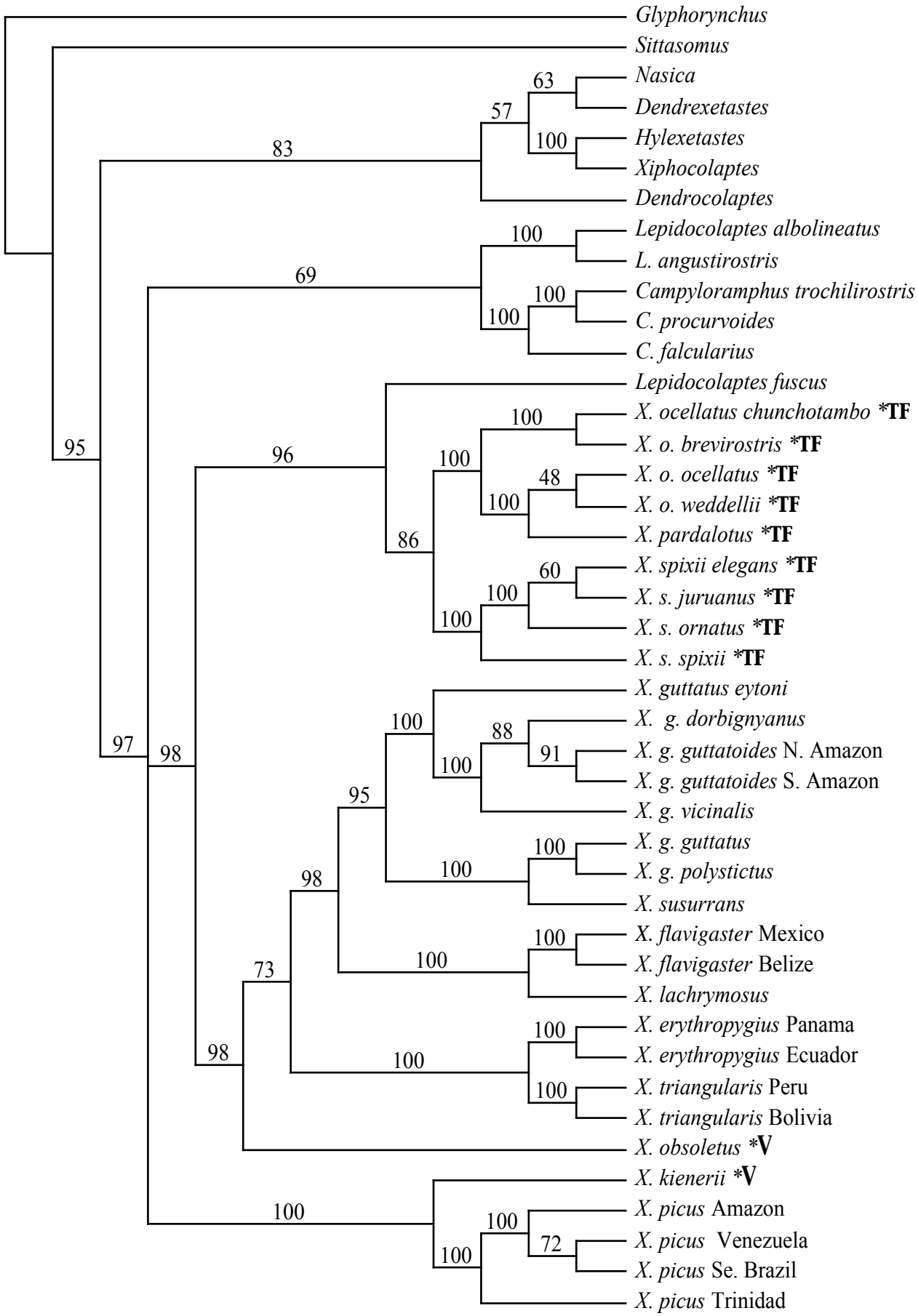
Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 *Glyphorynchus spirurus*																						
2 *Sittasomus griseicapillus*	0.156																					
3 *Nasica longirostris*	0.148	0.148																				
4 *Dendrocolaptes certhia*	0.144	0.141	0.112																			
5 *Lepidocolaptes albolineatus*	0.138	0.138	0.125	0.121																		
6 *L. angustirostris*	0.138	0.141	0.119	0.119	0.044																	
7 *L. fuscus*	0.137	0.136	0.124	0.115	0.099	0.093																
8 *Campyloramphus trochilirostris*	0.141	0.139	0.123	0.125	0.103	0.100	0.106															
9 *C. procurvoides*	0.140	0.133	0.123	0.123	0.104	0.102	0.102	0.041														
10 *C. falcularius*	0.142	0.136	0.125	0.123	0.099	0.099	0.100	0.073	0.073													
11 *Hylexetastes perrotii*	0.146	0.144	0.116	0.107	0.119	0.110	0.118	0.120	0.120	0.126												
12 *Xiphocolaptes promeropirhynchus*	0.139	0.142	0.109	0.106	0.117	0.115	0.112	0.124	0.123	0.121	0.090											
13 *Dendrexetastes rufigula*	0.139	0.141	0.105	0.102	0.120	0.115	0.114	0.117	0.115	0.116	0.112	0.106										
14 *Xiphorhynchus erythropygius* Panama	0.148	0.141	0.127	0.123	0.099	0.104	0.093	0.106	0.109	0.108	0.119	0.117	0.119									
15 *X. erythropygius* Ecuador	0.147	0.142	0.130	0.122	0.101	0.106	0.094	0.109	0.110	0.112	0.119	0.115	0.118	0.014								
16 *X. flavigaster* Mexico	0.132	0.141	0.128	0.117	0.105	0.104	0.088	0.104	0.104	0.106	0.121	0.122	0.117	0.077	0.077							
17 *X. flavigaster* Belize	0.131	0.139	0.126	0.117	0.102	0.104	0.083	0.103	0.106	0.106	0.119	0.120	0.120	0.073	0.074	0.017						
18 *X. guttattus guttatus*	0.140	0.146	0.127	0.115	0.102	0.104	0.090	0.106	0.109	0.110	0.121	0.124	0.120	0.080	0.083	0.062	0.063					
19 *X. g. dorbignyanus*	0.141	0.148	0.126	0.119	0.097	0.099	0.087	0.104	0.108	0.104	0.121	0.126	0.117	0.073	0.077	0.058	0.057	0.046				
20 *X. g. eytoni*	0.137	0.145	0.128	0.121	0.102	0.100	0.087	0.105	0.110	0.105	0.123	0.127	0.117	0.073	0.077	0.057	0.057	0.048	0.022			
21 *X. g. guttatoides* S. Amazon	0.141	0.149	0.127	0.120	0.099	0.101	0.087	0.107	0.112	0.107	0.122	0.124	0.118	0.074	0.078	0.061	0.059	0.046	0.006	0.023		
22 *X. g. guttatoides* N. Amazon	0.140	0.149	0.128	0.122	0.097	0.100	0.088	0.106	0.110	0.106	0.120	0.125	0.118	0.075	0.078	0.060	0.059	0.046	0.005	0.022	0.004	
23 *X. g. polystictus*	0.141	0.147	0.127	0.117	0.102	0.105	0.092	0.108	0.111	0.110	0.122	0.125	0.121	0.080	0.082	0.063	0.063	0.004	0.047	0.050	0.048	0.047
24 *X. g. vicinalis*	0.140	0.148	0.127	0.121	0.099	0.101	0.087	0.105	0.110	0.105	0.120	0.125	0.119	0.075	0.079	0.058	0.059	0.047	0.007	0.024	0.011	0.010
25 *X. kienerii*	0.149	0.137	0.124	0.123	0.105	0.099	0.104	0.105	0.108	0.107	0.116	0.129	0.115	0.106	0.106	0.111	0.109	0.107	0.107	0.107	0.107	0.107
26 *X. lachrymosus*	0.136	0.145	0.129	0.122	0.101	0.102	0.089	0.106	0.106	0.112	0.125	0.121	0.122	0.080	0.082	0.042	0.044	0.063	0.061	0.061	0.063	0.061
27 *X. obsoletus*	0.139	0.146	0.127	0.122	0.107	0.100	0.092	0.106	0.102	0.111	0.126	0.120	0.124	0.083	0.086	0.082	0.079	0.079	0.077	0.077	0.077	0.077
28 *X. ocellatus ocellatus*	0.137	0.134	0.115	0.115	0.101	0.098	0.079	0.103	0.098	0.100	0.119	0.118	0.110	0.094	0.094	0.091	0.089	0.095	0.094	0.092	0.096	0.095
29 *X. o. brevirostris*	0.140	0.137	0.116	0.114	0.105	0.102	0.077	0.111	0.107	0.100	0.119	0.117	0.115	0.095	0.095	0.089	0.085	0.091	0.092	0.090	0.093	0.093
30 *X. o. chunchotambo*	0.139	0.134	0.113	0.114	0.104	0.099	0.076	0.108	0.105	0.101	0.119	0.116	0.115	0.092	0.092	0.088	0.082	0.089	0.089	0.088	0.091	0.091
31 *X. o. napensis*	0.137	0.137	0.117	0.121	0.105	0.097	0.082	0.111	0.104	0.106	0.119	0.117	0.116	0.094	0.096	0.096	0.093	0.098	0.097	0.096	0.097	0.096
32 *X. pardalotus*	0.134	0.128	0.112	0.111	0.101	0.096	0.076	0.103	0.096	0.096	0.111	0.109	0.111	0.092	0.091	0.084	0.083	0.089	0.086	0.084	0.089	0.088
33 *X. picus* Venezuela	0.141	0.153	0.130	0.125	0.103	0.097	0.096	0.103	0.096	0.105	0.114	0.121	0.121	0.106	0.107	0.106	0.105	0.104	0.106	0.105	0.105	0.105
34 *X. picus* Trinidad	0.140	0.147	0.128	0.122	0.097	0.092	0.100	0.104	0.103	0.107	0.115	0.118	0.122	0.107	0.109	0.106	0.106	0.107	0.105	0.104	0.104	0.103
35 *X. picus* Amazon	0.141	0.152	0.130	0.126	0.103	0.097	0.096	0.104	0.098	0.106	0.115	0.121	0.121	0.107	0.108	0.105	0.105	0.104	0.106	0.105	0.105	0.104
36 *X. picus* Se. Brazil	0.142	0.153	0.129	0.126	0.103	0.097	0.096	0.103	0.095	0.105	0.113	0.120	0.121	0.107	0.108	0.106	0.106	0.105	0.107	0.106	0.106	0.105
37 *X. spixii spixii*	0.137	0.138	0.120	0.113	0.098	0.100	0.067	0.108	0.104	0.101	0.117	0.108	0.115	0.090	0.088	0.080	0.075	0.085	0.083	0.082	0.084	0.083
38 *X. s. ornatus*	0.139	0.129	0.114	0.109	0.090	0.092	0.067	0.100	0.099	0.098	0.110	0.108	0.112	0.083	0.085	0.084	0.077	0.080	0.084	0.083	0.084	0.084
39 *X. s. elegans*	0.140	0.137	0.116	0.114	0.095	0.097	0.069	0.104	0.104	0.103	0.114	0.112	0.114	0.082	0.083	0.086	0.081	0.086	0.083	0.083	0.083	0.083
40 *X. s. juruanus*	0.141	0.133	0.117	0.111	0.092	0.093	0.067	0.104	0.101	0.100	0.113	0.110	0.113	0.081	0.083	0.083	0.078	0.080	0.082	0.083	0.082	0.082
41 *X. susurrans*	0.143	0.146	0.126	0.115	0.104	0.104	0.093	0.105	0.108	0.108	0.120	0.125	0.121	0.080	0.081	0.062	0.064	0.035	0.054	0.053	0.053	0.052
42 *X. triangularis* Peru	0.140	0.140	0.126	0.117	0.093	0.097	0.091	0.102	0.105	0.107	0.109	0.107	0.113	0.049	0.046	0.077	0.076	0.081	0.074	0.074	0.074	0.074
43 *X. triangularis* Bolivia	0.141	0.140	0.127	0.117	0.094	0.097	0.093	0.105	0.106	0.107	0.108	0.107	0.115	0.051	0.049	0.079	0.078	0.080	0.075	0.075	0.076	0.076

Table 2.2. - Extended.

23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
0.049																			
0.107	0.109																		
0.063	0.063	0.102																	
0.081	0.077	0.108	0.083																
0.095	0.094	0.105	0.098	0.098															
0.092	0.092	0.104	0.092	0.099	0.050														
0.090	0.089	0.105	0.091	0.099	0.051	0.010													
0.098	0.095	0.110	0.099	0.100	0.039	0.058	0.056												
0.089	0.085	0.106	0.087	0.095	0.034	0.047	0.047	0.040											
0.106	0.106	0.078	0.097	0.108	0.111	0.107	0.105	0.109	0.108										
0.108	0.106	0.078	0.098	0.108	0.109	0.109	0.106	0.109	0.108	0.028									
0.106	0.105	0.078	0.097	0.108	0.112	0.108	0.106	0.109	0.109	0.002	0.029								
0.105	0.106	0.079	0.099	0.109	0.110	0.107	0.106	0.109	0.108	0.001	0.029	0.003							
0.086	0.083	0.104	0.085	0.090	0.067	0.061	0.063	0.066	0.061	0.106	0.106	0.105	0.106						
0.080	0.084	0.093	0.088	0.092	0.062	0.061	0.059	0.067	0.062	0.099	0.100	0.100	0.100	0.043					
0.087	0.085	0.097	0.089	0.092	0.065	0.066	0.063	0.066	0.063	0.101	0.103	0.101	0.102	0.043	0.019				
0.081	0.082	0.093	0.083	0.089	0.063	0.063	0.061	0.063	0.060	0.098	0.099	0.098	0.099	0.041	0.018	0.016			
0.035	0.054	0.107	0.065	0.082	0.093	0.095	0.091	0.100	0.094	0.102	0.104	0.102	0.102	0.089	0.081	0.084	0.078		
0.081	0.077	0.100	0.076	0.081	0.093	0.096	0.092	0.090	0.087	0.106	0.108	0.107	0.107	0.090	0.087	0.086	0.085	0.079	
0.080	0.079	0.101	0.079	0.083	0.094	0.098	0.094	0.090	0.087	0.108	0.110	0.109	0.109	0.091	0.088	0.087	0.086	0.080	0.004

Figure 2.1. Strict consensus of two most parsimonious trees (Length = 3,433, CI = 0.35, RI = 0.6) obtained with unweighted sequence data. Numbers above branches refer to bootstrap support based on 100 replicates. Note the monophyly of species restricted to *terra-firme* forest in lowland Amazonia (taxa indicated by an asterisk followed by TF) and the polyphyly of *várzea* specialist species (taxa indicated by an asterisk followed by V).


Glyphorynchus
Sittasomus
Nasica
Dendrexetastes
Hylexetastes
Xiphocolaptes
Dendrocolaptes
Lepidocolaptes albolineatus
L. angustirostris
Campyloramphus trochilirostris
C. procurvoides
C. falcularius
Lepidocolaptes fuscus
X. ocellatus chunchotambo *TF
X. o. brevirostris *TF
X. o. ocellatus *TF
X. o. weddellii *TF
X. pardalotus *TF
X. spixii elegans *TF
X. s. juruanus *TF
X. s. ornatus *TF
X. s. spixii *TF
X. guttatus eytoni
X. g. dorbignyanus
X. g. guttatoides N. Amazon
X. g. guttatoides S. Amazon
X. g. vicinalis
X. g. guttatus
X. g. polystictus
X. susurrans
X. flavigaster Mexico
X. flavigaster Belize
X. lachrymosus
X. erythropygius Panama
X. erythropygius Ecuador
X. triangularis Peru
X. triangularis Bolivia
X. obsoletus *V
X. kienerii *V
X. picus Amazon
X. picus Venezuela
X. picus Se. Brazil
X. picus Trinidad

pair *X. picus* and *X. kienerii*: one tree placed these species as basal to the entire *Lepidocolaptes* - *Campyloramphus* - *Xiphorhynchus* clade, whereas the other tree placed them as the sister group only to the *Campyloramphus* - *Lepidocolaptes* clade. Monophyly of *Lepidocolaptes fuscus* and all *Xiphorhynchus* species, except *X. picus* and *X. kienerii*, received 98% BP support.

When the two MP trees recovered are constrained (using software MacClade 4.0; Maddison and Maddison 2000), so that *X. picus* plus *X. kienerii* becomes the sister clade to all remaining *Xiphorhynchus* plus *Lepidocolaptes fuscus*, a cladogram with six additional steps is obtained. Within the *Xiphorhynchus – Lepidocolaptes fuscus* clade, two other, major well supported clades existed: (1) one containing all Amazonian *Xiphorhynchus* species specialized in *terra-firme* forest with the Atlantic forest endemic *Lepidocolaptes fuscus* as their sister taxon; and (2) another clade containing the remaining *Xiphorhynchus* species, found throughout the Neotropics. The strict MP consensus tree (Fig. 2.1) also had nodes with high BP values indicating the paraphyly of two *Xiphorhynchus* biological species: *X. guttatus* and *X. ocellatus*. The lowland Amazonian *X. o. ocellatus* and *X. o. weddellii* were sisters to the Guianan endemic *X. pardalotus*, whereas the two Andean foothill subspecies of *X. ocellatus* (*chunchotambo* and *brevirostris*) were basal to this clade. Lowland Amazonian subspecies of *X. guttatus* were also paraphyletic: *X. g. guttatus* from eastern Brazil and *X. g. polystictus* from coastal northeastern Amazonia were sisters to the Central American *X. susurrans*, to the exclusion of southern and western Amazonian subspecies of *X. guttatus*.

ML Analyses. - For both ML analyses performed, independent likelihood ratio tests as implemented in MODELTEST (Posada and Crandall 1998) selected a general time reversible model of nucleotide substitution with estimated base frequencies, proportion of invariable sites,

and rates for variable sites following a gamma distribution (Figs. 2.2 and 2.3). The first ML analysis produced a tree with all *Xiphorhynchus* species forming a well supported monophyletic group (BP = 95%) to the exclusion of *X. picus* and *X. kienerii* (Fig. 2.2). These latter species were placed as the sister clade to the genera *Campyloramphus* and *Lepidocolaptes*, as depicted in one of the two MP trees. However, in the ML analysis, the node linking *X. picus* and *X. kienerii* to *Campyloramphus* and *Lepidocolaptes* had a low BP (28%). As in MP analyses, within the clade containing all *Xiphorhynchus* species (excluding *X. picus* and *X. kienerii*), two clades supported by high BP values were found: (1) a "first" clade containing all *Xiphorhynchus* species restricted to *terra-firme* forest plus *Lepidocolaptes fuscus* as their sister taxon (BP = 95%), and (2) a "second" clade with the remaining *Xiphorhynchus* species (BP = 100%). The second ML analysis produced a tree depicting the same relationships among subspecies of polytypic *Xiphorhynchus* species as the MP trees but with higher bootstrap support for many nodes (Fig. 2.3). Both ML trees differed from the MP trees in their placement of *Xiphorhynchus obsoletus*: MP trees placed this species as the sister taxon to all the remaining species grouped in the "second" *Xiphorhynchus* clade defined above, whereas ML trees placed this species as sister only to the clade containing *X. flavigaster, X. guttatus, X. lachrymosus*, and *X. susurrans*. However, in both ML analyses, the node linking *X. obsoletus* with the latter species to the exclusion of *X. erythropygius* and *X. triangularis* was short and not well supported by bootstrap analyses (Figs. 2.2 and 2.3).

Bayesian Inference of Phylogeny. - Mirroring MP and ML trees, the first Bayesian inference of phylogeny depicting higher level relationships between *Xiphorhynchus* and other Dendrocolaptidae genera contained a clade with high probability of occurrence (99%) grouping all

Figure 2.2. Single most likely tree obtained with ML under the GTR+G+I model of molecular evolution (-ln likelihood = 15421.05). Estimated base frequencies were A = 0.33, C = 0.35, G = 0.09, T = 0.23; proportion of sites estimated to be invariant = 0.56; estimated value of gamma shape parameter = 1.68. Numbers above or under branches refer to bootstrap support of 50% or higher based on 100 replicates. Note the monophyly of species restricted to *terra-firme* forest in lowland Amazonia (taxa indicated by an asterisk followed by TF) and the polyphyly of *várzea* specialist species (taxa indicated by an asterisk followed by V).

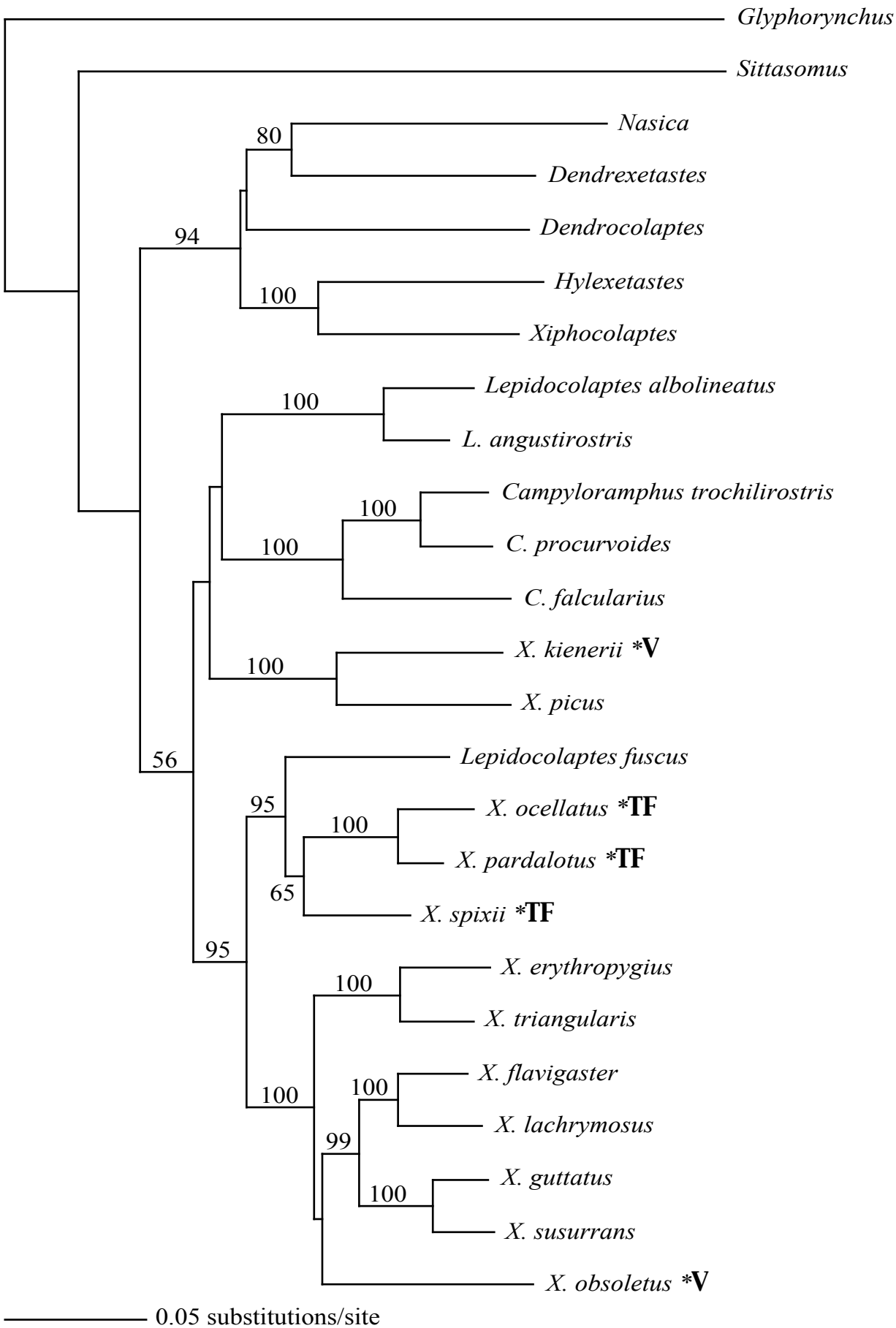
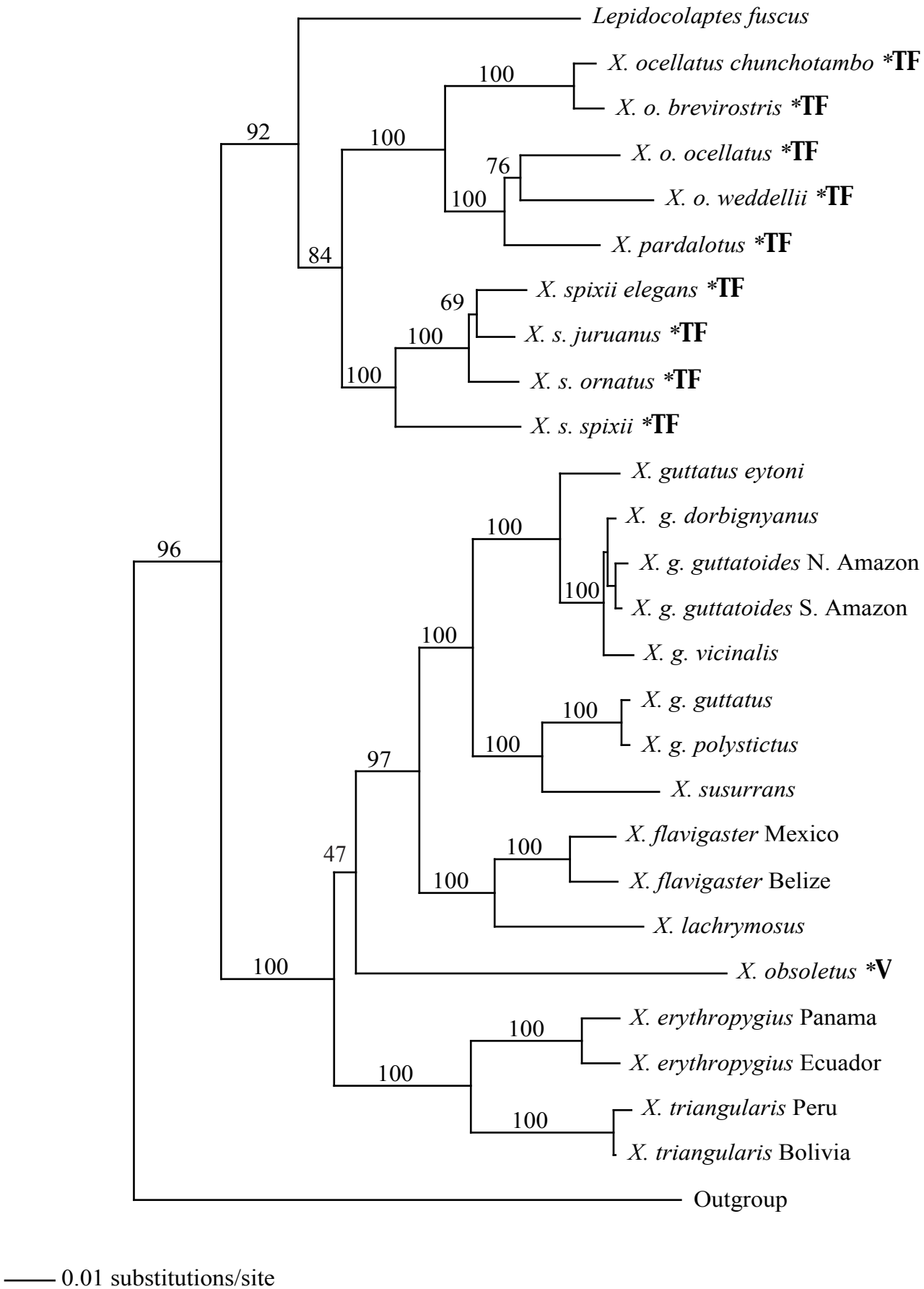

Glyphorynchus
Sittasomus
Nasica
Dendrexetastes
Dendrocolaptes
Hylexetastes
Xiphocolaptes
Lepidocolaptes albolineatus
L. angustirostris
Campyloramphus trochilirostris
C. procurvoides
C. falcularius
X. kienerii *V
X. picus
Lepidocolaptes fuscus
X. ocellatus *TF
X. pardalotus *TF
X. spixii *TF
X. erythropygius
X. triangularis
X. flavigaster
X. lachrymosus
X. guttatus
X. susurrans
X. obsoletus *V
80
94
100
100
100
100
100
100
56
95
100
65
95
100
100
99
100
0.05 substitutions/site

Figure 2.3. Results of ML analyses under the GTR+G+I model of molecular evolution (-ln likelihood = 11603.4). Estimated base frequencies were A = 0.31, C = 0.34, G = 0.10, T = 0.25; proportion of sites estimated to be invariant = 0.59; estimated value of gamma shape parameter = 1.86. Numbers above or next to branches refer to bootstrap support based on 100 replicates. Short branches without numbers received at least 82% support and are not shown here for sake of clarity. Taxa restricted to *terra-firme* and *várzea* forests in lowland Amazonia are indicated by asterisks followed by the codes TF and V, respectively.


Lepidocolaptes fuscus
X. ocellatus chunchotambo *TF
X. o. brevirostris *TF
X. o. ocellatus *TF
X. o. weddellii *TF
X. pardalotus *TF
X. spixii elegans *TF
X. s. juruanus *TF
X. s. ornatus *TF
X. s. spixii *TF
X. guttatus eytoni
X. g. dorbignyanus
X. g. guttatoides N. Amazon
X. g. guttatoides S. Amazon
X. g. vicinalis
X. g. guttatus
X. g. polystictus
X. susurrans
X. flavigaster Mexico
X. flavigaster Belize
X. lachrymosus
X. obsoletus *V
X. erythropygius Panama
X. erythropygius Ecuador
X. triangularis Peru
X. triangularis Bolivia
Outgroup
92
100
100
76
100
84
69
100
100
96
100
100
100
97
47
100
100
100
100
100
100
0.01 substitutions/site

Campyloramphus, *Lepidocolaptes*, and *Xiphorhynchus* species (Fig. 2.4). Within this clade, two subclades existed: (1) one with a posterior probability of 100%, containing *Lepidocolaptes fuscus* and all *Xiphorhynchus* species except *X. picus* and *X. kienerii*, and (2) a second clade with a posterior probability of 64% containing *X. picus*, *X. kienerii*, two *Lepidocolaptes* species, and *Campyloramphus* (Fig. 2.4). As in MP and ML analyses, *Xiphorhynchus* species specialized in *terra-firme* forest formed a monophyletic group sister to *Lepidocolaptes fuscus* with a posterior probability of 100% (Fig. 2.4). The second Bayesian inference of phylogeny yielded a majority-rule consensus tree depicting the same relationships among subspecies of polytypic *Xiphorhynchus* species as the MP and ML trees. However, the posterior probabilities of occurrence of clades tended to be higher than bootstrap values supporting those same clades in MP and ML trees (Fig. 2.5). Reflecting the conflicting position of *X. obsoletus* between MP and ML trees, the two Bayesian inferences of phylogeny obtained also differed in their placement of this species. The first Bayesian inference favors the arrangement found by MP analyses, whereas the second Bayesian inference agrees with ML analyses (Figs. 2.1-2.5). Consistently, in both Bayesian inferences of phylogeny, the lowest posterior probabilities of occurrence involved clades containing *X. obsoletus* or *X. erythropygius* plus *X. triangularis* as the sister group to the well supported *X. flavigaster* - *X. guttatus* - *X. lachrymosus* - *X. susurrans* clade (Figs. 2.4 and 2.5).

DISCUSSION

Monophyly of *Xiphorhynchus* and Its Relationship with Other Dendrocolaptidae Genera.- Two previous studies on dendrocolaptid systematics agreed in placing *Xiphorhynchus* in a

Figure 2.4. Majority-rule consensus of 4,000 trees obtained by a Bayesian inference of phylogeny under a variety of substitution parameters assuming the GTR+G+I model of molecular evolution. Numbers above branches refer to the posterior probability of occurrence of clades. Note the monophyly of species restricted to *terra-firme* forest in lowland Amazonia (taxa indicated by an asterisk followed by TF) and the polyphyly of *várzea* specialist species (taxa indicated by an asterisk followed by V).

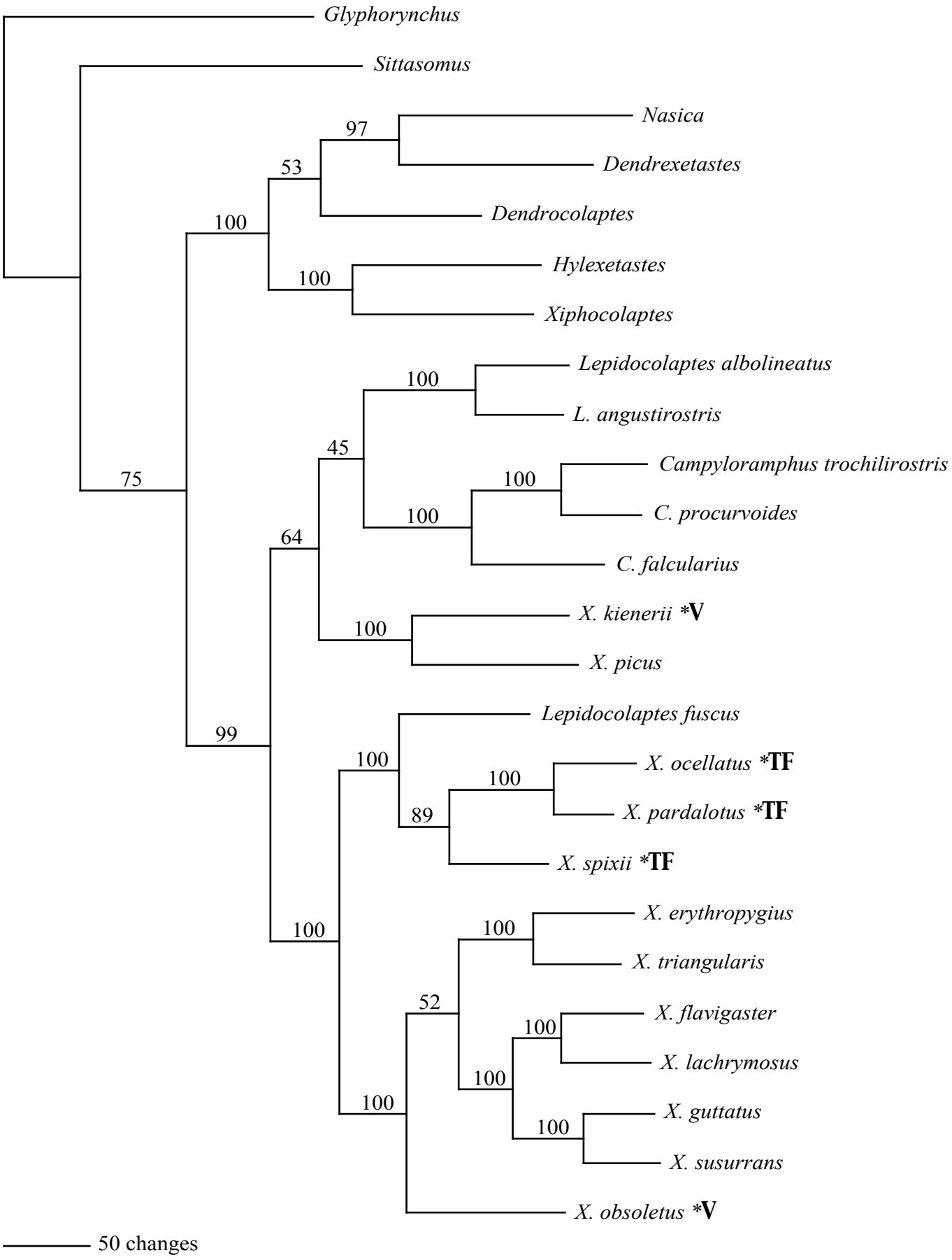
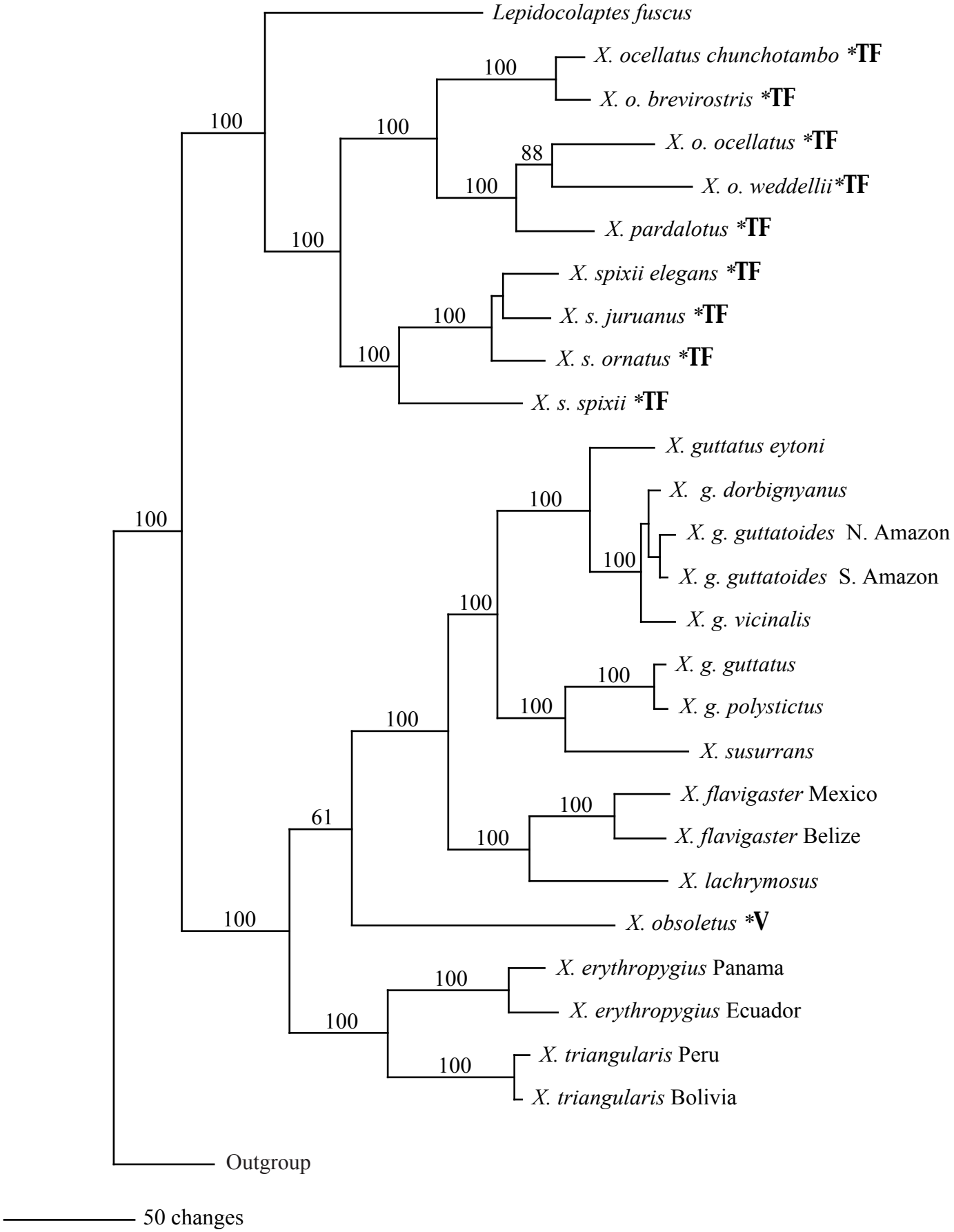

Glyphorynchus
Sittasomus
Nasica
Dendrexetastes
Dendrocolaptes
Hylexetastes
Xiphocolaptes
Lepidocolaptes albolineatus
L. angustirostris
Campyloramphus trochilirostris
C. procurvoides
C. falcularius
X. kienerii *V
X. picus
Lepidocolaptes fuscus
X. ocellatus *TF
X. pardalotus *TF
X. spixii *TF
X. erythropygius
X. triangularis
X. flavigaster
X. lachrymosus
X. guttatus
X. susurrans
X. obsoletus *V
97
53
100
100
100
45
75
100
100
64
100
99
100
100
89
100
52
100
100
100
100
50 changes

Figure 2.5. Majority-rule consensus of 4,000 trees obtained by a Bayesian inference of phylogeny under a variety of substitution parameters assuming the GTR+G+I model of molecular evolution. Numbers above branches refer to the posterior probability of occurrence of clades. Short branches without numbers had a posterior probability of occurrence of at least 87% and are not shown here for sake of clarity. Taxa restricted to *terra-firme* and *várzea* forests in lowland Amazonia are indicated by asterisks followed by the codes TF and V, respectively.


Lepidocolaptes fuscus
X. ocellatus chunchotambo *TF
X. o. brevirostris *TF
X. o. ocellatus *TF
X. o. weddellii*TF
X. pardalotus *TF
X. spixii elegans *TF
X. s. juruanus *TF
X. s. ornatus *TF
X. s. spixii *TF
X. guttatus eytoni
X. g. dorbignyanus
X. g. guttatoides N. Amazon
X. g. guttatoides S. Amazon
X. g. vicinalis
X. g. guttatus
X. g. polystictus
X. susurrans
X. flavigaster Mexico
X. flavigaster Belize
X. lachrymosus
X. obsoletus *V
X. erythropygius Panama
X. erythropygius Ecuador
X. triangularis Peru
X. triangularis Bolivia
Outgroup
100
100
100
100
88
100
100
100
100
100
100
100
100
100
100
100
61
100
100
100
100
100
50 changes

group (Feduccia 1973) or a clade (Raikow 1994) together with the following genera: *Campyloramphus, Dendrexetastes, Dendrocolaptes, Hylexetastes, Lepidocolaptes,* and *Xiphocolaptes*. These two studies differed only in their placement of the genera *Nasica* and *Drymornis*. Based primarily on osteological characters, Feduccia (1973) considered them as members of the "strong billed" woodcreeper assemblage, which included all the aforementioned genera and excluded the remaining, so called "intermediate" dendrocolaptid genera *Dendrocincla, Deconychura, Glyphorynchus,* and *Sittasomus*. Raikow's (1994) phylogeny was based primarily on myological characters and placed *Nasica* and *Drymornis* as sisters to all remaining "strong billed" and "intermediate" woodcreeper genera alike. In the present study, I sampled all "strong billed" genera except *Drymornis*, and two of the four existing "intermediate" genera as defined by Feduccia (1973). Phylogeny estimates obtained by the present study support Feduccia's (1973) placement of *Nasica* in the "strong billed" assemblage (Figs. 2.1, 2.2, and 2.4). In addition, the phylogenetic results presented here provide much better resolution of the non-controversial part of the "strong billed" clade consisting of *Campyloramphus, Dendrexetastes, Dendrocolaptes, Hylexetastes, Lepidocolaptes, Xiphocolaptes,* and *Xiphorhynchus* than the most complete phylogenetic hypothesis previously available for the Dendrocolaptidae (Raikow 1994). Within the "strong billed" clade, phylogenies reconstructed with three alternative criteria (MP, ML, and Bayesian inference of phylogeny) pointed to a clade grouping species of *Campyloramphus*, *Lepidocolaptes*, and *Xiphorhynchus*. Statistical support for this relationship was high in MP and Bayesian analyses but only modest in the ML tree (BP = 56%; Fig. 2.2). Unlike ML bootstrap analyses, Bayesian inference of phylogeny uses full models of DNA substitution and samples the entire available dataset to generate alternative tree topologies, thus providing a more robust

evaluation of the statistical support for the different nodes of a tree. When compared to posterior probabilities derived from a Bayesian inference of phylogeny, ML bootstrap proportions are likely to underestimate the probability of clades with inherent high probabilities of occurrence (Rannala and Yang 1996). Supporting this view, when the ML and the Bayesian majority-rule consensus trees obtained in this study were compared, despite their nearly identical topologies, bootstrap proportions for nodes of the ML tree were never higher than posterior probabilities of clades in the Bayesian tree (Figs. 2.2 and 2.4).

Higher level relationships within the *Campyloramphus* - *Lepidocolaptes* - *Xiphorhynchus* clade were conflicting and to some extent poorly supported. All phylogeny estimates obtained suggested a sister relationship between all *Campyloramphus* and two *Lepidocolaptes* species. This relationship received moderate support only in MP analyses and little support in ML and Bayesian analyses (Figs. 2.1, 2.2, and 2.4). All phylogeny estimates strongly supported the monophyly of the genus *Campyloramphus* and the paraphyly of the genus *Lepidocolaptes*. According to all trees, *Lepidocolaptes fuscus* was nested, with high support, within a clade containing only *Xiphorhynchus* species (Figs. 2.1, 2.2, and 2.4). These findings agree with two independent morphological and molecular datasets (Raikow 1994, García-Moreno and Silva 1997). Based on 36 anatomical characters, mostly myological, Raikow (1994) also found *Campyloramphus* to be monophyletic (he sampled two of the three species sampled in the present study plus the Brown-billed Scythebill [*C. pusillus*]). When Raikow's (1994) and the present study are viewed together, the only *Campyloramphus* species not sampled is the Greater Scythebill (*C. pucherani*), supporting the notion that at least 4 of the 5 extant species of *Campyloramphus* are monophyletic. Also in agreement with the present study, Raikow (1994)

found *Lepidocolaptes* to be paraphyletic, with *L. fuscus* lying outside a clade containing five *Lepidocolaptes* species (two of them sampled by the present study). García-Moreno and Silva (1997) sequenced fragments of the ND2 and cyt *b* mtDNA genes for all existing *Lepidocolaptes* species (following the taxonomy of Stotz et al. 1996), except the White-striped Woodcreeper (*L. leucogaster*); they also found that *Lepidocolaptes* is monophyletic to the exclusion of *L. fuscus*, which was found to be the sister taxon to one of their outgroups, namely *Xiphorhynchus spixii*. Raikow's (1994) and García-Moreno and Silva's (1997) studies can be regarded as complementary because together they sampled all species of *Lepidocolaptes*. Their findings and those of the present study strongly indicated that the genus *Lepidocolaptes* is not monophyletic because *Lepidocolaptes fuscus* is, in fact, a *Xiphorhynchus*.

All phylogeny estimates produced by the present study also show the genus *Xiphorhynchus* (sensu Peters 1951, Stotz et al. 1996) as paraphyletic. The sibling species pair *X. picus* and *X. kienerii* is never found as the sister group or within the highly supported clade containing all remaining *Xiphorhynchus* species plus *Lepidocolaptes fuscus*, regardless of the tree building method considered (Figs. 2.1, 2.2, and 2.4). However, the phylogenetic position of *X. picus* plus *X. kienerii* within the *Campyloramphus* - *Lepidocolaptes* - *Xiphorhynhcus* clade was either conflicting (according to MP analyses; Fig. 2.1) or poorly supported (according to a ML analysis; Fig. 2.2). Topologies of one of the two most parsimonious trees found by MP and those of ML and Bayesian consensus trees place *X. picus* plus *X. kienerii* as sister to a clade containing *Campyloramphus* plus *Lepidocolaptes*. Only the Bayesian estimate of phylogeny supported this relationship modestly (Fig. 2.4). The second MP tree (not shown) placed *X. picus* plus *X. kienerii* as the sister group to all members of the *Campyloramphus* - *Lepidocolaptes* -

Xiphorhynchus clade. Although no phylogeny recovered supported the monophyly of all *Xiphorhynchus* species, this relationship cannot be totally ruled out, given the low statistical support for the placement of *X. picus* and *X. kienerii* within the *Campyloramphus* - *Lepidocolaptes* - *Xiphorhynchus* clade. In any event, all phylogenetic hypotheses obtained strongly indicated that *X. picus* plus *X. kienerii* belong to a separate clade not nested within the genera *Campyloramphus*, *Lepidocolaptes*, or *Xiphorhynchus*. The distinctiveness of *X. picus* and *X. kienerii* was recognized by early taxonomists who grouped these species in a separate genus: *Dendroplex* (Cory and Hellmayr 1925, Zimmer 1934a). Without formal analysis, Todd (1948) transferred *kienerii* to *Xiphorhynchus* but kept *picus* in *Dendroplex*. Later, Peters (1951) lumped *Dendroplex* and *Xiphorhynchus* because the type of *Dendroplex* (consisting only of a published painting) is apparently a *Xiphorhynchus*, the name which has priority. In accordance with older taxonomy, phylogeny estimates of the present study supported the grouping of *X. picus* and *X. kienerii* in a separate genus.

Species Limits Within the *Xiphorhynchus triangularis / erythropygius* Superspecies.- Because they share a similar overall greenish plumage color, unique among dendrocolaptids, these two largely allopatric, montane taxa were previously regarded as conspecific (Cory and Hellmayr 1925). Eventually, *Xiphorhynchus triangularis* and *X. erythropygius* were recognized as separate species based primarily on differences in the extent of crown spotting and back streaking (Wetmore 1972). A recent anatomical phylogeny placed these two species in separate, distantly related clades (Raikow 1994). The present study however strongly supported the monophyly of the *X. triangularis / erythropygius* superspecies (Figs. 2.1, 2.3, and 2.5). Uncorrected sequence divergence between these two taxa averaged 4.8% (n = 4; Table 2.2), exceeding those observed

between undisputed, biological sister species of *Xiphorhynchus*: p = 3.4 - 4.4% (Table 2.2). Consistently, sequence divergence between subspecies of *X. triangularis* and *X. erythropygius* was much lower, ranging from 0.3% in *X. triangularis* to 1.4% in *X. erythropygius* (Table 2.2). The level of uncorrected mtDNA sequence divergence observed between *X. triangularis* and *X. erythropygius* was consistent with long term lineage sorting and reproductive isolation, a notion also supported by the lack of known hybrids between these species (A.O.U. 1998).

Species Limits Within the *Xiphorhynchus guttatus* Superspecies.- Trans-Andean populations of *X. guttatus* were split from their cis-Andean counterparts under the name *susurrans* based on song and size differences (Willis 1983), an arrangement followed by the A. O. U. (1998). The present study supported the distinctiveness of *X. susurrans* as a basal taxon sister to two cis-Andean taxa of *X. guttatus*: *X. g. guttatus* from eastern Brazil and *X. g. polystictus* from coastal northeastern Amazonia (Figs. 2.1, 2.3, 2.5 and Appendix 1). Uncorrected sequence divergence between *X. susurrans* and those taxa was 3.5%, thus within the range of values observed between some undisputed, biological sister species of *Xiphorhynchus* (3.4 - 4.4%; Table 2.2). However, in contrast with the traditional view, the major division within the *X. guttatus* superspecies was not between cis and trans-Andean populations (*susurrans* versus remaining taxa), but between the southern and western Amazonian taxa (*dorbignyanus, eytoni, guttatoides,* and *vicinalis*) and the trans-Andean, coastal Guianan, and eastern Brazilian taxa (*susurrans, polystictus,* and *guttatus*; Figs. 2.1, 2.3, and 2.5). Support for this relationship was high and uncorrected sequence divergence between these two clades ranged from 4.5 to 5.4%. This divergence was consistent with species level differences in *Xiphorhynchus* (Table 2.2). Within these two clades, uncorrected sequence divergence levels were lower than between clade comparisons (0.37 - 2.4% within the

southern - western Amazonian clade, and 0.37 - 3.5% within the trans-Andean - Guianan - eastern Brazilian clade). Comparatively lower levels of uncorrected sequence divergence found within the southern - western Amazonian clade were consistent with subspecific differentiation and intergradation, as inferred from plumage characters of specimens collected in contact zones between the neighboring parapatric taxa *dorbignyanus, eytoni,* and *guttatoides* (Zimmer 1934b). Thus, molecular data supported the traditional treatment of these taxa and *vicinalis* (Todd 1948) as conspecifics. The current analysis sampled all cis-Andean subspecies of *X. guttatus* except *X. g. connectens* (Todd 1948), found on the Guianan shield immediately north of the Amazon river. So far, *polystictus* appears to be restricted to coastal northeastern Brazilian Amazonia and the Guyanas, and the southern limit of its distribution and contact zone with *connectens*, if any, remain unknown (Peters 1951).

If trans-Andean *X. susurrans* is recognized as a valid species, then *X. guttatus* becomes a paraphyletic species (Figs. 2.1, 2.3, and 2.5). As mentioned before, some phenotypic characters in addition to the molecular evidence warranted the recognition of *X. susurrans* (Willis 1983) as a separate species. Unfortunately, no study so far has compared the variation in phenotypic characters among all taxa of the *X. guttatus* superspecies. In a study that provided an identification key for all cis-Andean taxa of *X. guttatus*, Pinto (1947) pointed to a close phenotypic similarity between nominate *guttatus* and *polystictus*, thus agreeing with the molecular data. The present study supported the recognition of at least three major evolutionary lineages in the *X. guttatus* superspecies: one including *dorbignyanus, eytoni, guttatoides,* and *vicinalis*, a second including *guttatus* and *polystictus*, and a third including trans-Andean populations. Relatively high levels of sequence divergence and reciprocal monophyly among

these three mostly allopatric clades suggest long term reproductive isolation and lack of recent widespread gene flow among them. Nevertheless, more samples from contact areas, coupled with analyses of morphological, vocal, and nuclear molecular characters are needed to better assess the existence or degree of gene flow between the three main lineages of *X. guttatus* detected in this study.

Species Limits Within the *Xiphorhynchus pardalotus / ocellatus* Superspecies.- This study strongly supported the inclusion of *X. pardalotus* in a clade containing four subspecies of *X. ocellatus* (Figs. 2.1, 2.3, and 2.5), thus contradicting earlier views that included *X. pardalotus* in the *X. spixii* superspecies (Cory and Hellmayr 1925 but see Zimmer 1934b). This study also indicated that the major division within the *X. pardalotus / ocellatus* superspecies is not between the Guianan (i.e. *X. pardalotus*) and non-Guianan Shield taxa, as implied by current taxonomy, but instead between Andean foothill (*X. o. chunchotambo* and *X. o brevirostris*) and lowland Amazonian taxa (*X. pardalotus*, *X. o . ocellatus* and *X. o. weddellii*), hence rendering *X. ocellatus* paraphyletic. Uncorrected levels of sequence divergence between these two clades ranged from 4.6 to 5.7% and were consistent with species level differences in *Xiphorhynchus* (Table 2.2). Sequence divergence between the two Andean foothill taxa (p = 1%) was within the range of those found between other subspecies of *Xiphorhynchus*, whereas that found between *X. o. ocellatus* and *X. o. weddellii* (3.8%) was slightly higher than that between *X. o. ocellatus* and *X. pardalotus* (3.4%), two taxa considered distinct biological species (Cory and Hellmayr 1925, Zimmer 1934b, Peters 1951).

The four divergent sequence types recovered for the *X. pardalotus* / *ocellatus* superspecies corresponded to taxa also diagnosable by discrete phenotypic characters.

Xiphorhynchus o. chunchotambo is such a distinctive taxon that it was treated as a separate species by Cory and Hellmayr (1925), but was subsequently merged with *X. ocellatus* based on putative intergradation with *X. o. napensis* (Zimmer 1934b). This intergradation was inferred from only two intermediate specimens (which I did not examine personally) collected in northeastern Peru, where the latter taxon and *X. o. chunchotambo* approach their ranges (Zimmer 1934b). Large series of specimens housed at the Louisiana State University Museum of Natural Sciences indicated that *X. o. chunchotambo* and *X. o. napensis* replace each other altitudinally in northeastern Peru, with the latter taxon restricted to the lowlands (pers. obs.); therefore, opportunities for interbreeding between *X. o. chunchotambo* and *X. o. napensis* are probably rare. *Xiphorhynchus o. weddellii* is morphologically distinct as well, but closer to nominate *ocellatus* (Zimmer 1934b), which also agreed with the molecular data. Finally, *X. pardalotus* has always been treated as a distinct species (Cory and Hellmayr 1925, Zimmer 1934b, Peters 1951). In further agreement with the molecular data, the low level of genetic differentiation found between *X. o. brevirostris* and *X. o. chunchotambo* was matched by their great phenotypic similarity (Zimmer 1934b). Missing from my sample were only two of the six *X. ocellatus* subspecies, *X. o. napensis* and *X. o. perplexus*, both found in lowland western Amazonia, and the second described taxon of *X. pardalotus* (*caurensis*). *Xiphorhynchus o. perplexus* and *X. pardalotus caurensis* are not much differentiated from their respective nominate forms (Cory and Hellmayr 1925, Zimmer 1934b, Todd 1948). However, *Xiphorhynchus o. napensis* is quite distinct and was considered either conspecific with *chunchotambo* (Cory and Hellmayr 1925) or with *ocellatus* (Zimmer 1934b). In addition to the paraphyly of *X. ocellatus* with respect to a traditionally undisputed biological species (*X. pardalotus*), the relatively high levels of sequence divergence found among

three of its taxa (*chunchotambo, ocellatus,* and *weddellii*) suggest long term reproductive isolation. Nevertheless, further studies with better sampling and nuclear molecular markers are needed to assess the extent of gene flow between lineages of the *X. pardalotus / ocellatus* superspecies, especially in areas where parapatric taxa approach their ranges.

Species Limits Within the *Xiphorhynchus spixii / elegans* Superspecies.- In contrast with the traditional classification that considered *X. spixii* and *X. elegans* conspecifics (Zimmer 1934b, Ridgely and Tudor 1994), Haffer (1997b) concluded, based on an analysis of plumage characters of large series of specimens, that *X. spixii* is a monotypic species. Except for nominate *spixii*, all remaining taxa of this superspecies (*buenavistae, elegans, insignis, juruanus,* and *ornatus*) were grouped under *X. elegans* because they intergraded with parapatric neighbors along localized contact zones (Haffer 1997b). This study corroborated Haffer's (1997b) classification by revealing two well supported clades: one containing only *X. spixii* and another with *X. s. elegans*, *X. s. juruanus*, and *X. s. ornatus* (Figs. 2.1, 2.3, and 2.5). Uncorrected sequence divergence between members of these two clades ranged from 4 to 4.3% and were consistent with species level divergences between other sister species pairs of *Xiphorhynchus* (Table 2.2), and reproductive isolation as inferred from the lack of phenotypically intermediate specimens in areas where *X. spixii* and *X. s. elegans* come near each other in central Brazil (Haffer 1997b). The range of uncorrected sequence divergence within the *X. elegans* clade (p = 1.6 to 1.8%) was within those observed among other subspecies of *Xiphorhynchus* (Table 2.2). The two subspecies of *X. spixii* missing from the molecular analyses (*buenavistae* and *insiginis*) are phenotypically weakly differentiated from *X. s. ornatus* (Zimmer 1934b, Haffer 1997b), and their inclusion in the molecular dataset would likely not change the topologies of the phylogenies obtained.

The Evolution of *Várzea* and *Terra-firme* Habitat Specialization in *Xiphorhynchus* .- This study strongly supported the monophyly of *Xiphorhynchus* species restricted to *terra-firme* forest in lowland Amazonia (taxa belonging to the *X. pardalotus / ocellatus* and *X. spixii / elegans* superspecies; Figs. 2.1-2.5). In contrast, the two *Xiphorhynchus* species restricted to *várzea* forest, *X. obsoletus* and *X. kienerii*, were found in two distantly related clades, more appropriately regarded as separate genera (Figs. 2.1-2.5). *Xiphorhynchus obsoletus* was nested in a clade containing *Xiphorhynchus* species found in a wide variety of habitats, from tropical lowland to pine-oak forests (Table 2.1). *Xiphorhynchus kienerii* was found in a clade with *X. picus*, a species also found in a variety of habitats (Table 2.1). Topologies of the molecular trees supported the hypothesis that *várzea* forest specialization in *Xiphorhynchus* evolved independently in two separate and highly ecologically diverse lineages.

That *várzea* and *terra-firme* specialist species of *Xiphorhynchus* appeared in separate clades falsifies the hypothetical sister relationship between *várzea* and *terra-firme* species, as expected if the *várzea - terra-firme* ecotone played a prominent role in the recent diversification of the genus *Xiphorhynchus*. The monophyly of all *terra-firme* specialist species and the basal position of *X. obsoletus* in a separate, ecologically diverse clade, suggest that the *várzea - terra-firme* habitat specialization evolved early on in the evolutionary history of *Xiphorhynchus*. Since then, the *terra-firme* clade has experienced a much higher rate of speciation leading to two superspecies composed of largely allopatric and genetically differentiated lineages. In contrast, as indicated by long branches separating *X. obsoletus* and *X. kienerii* from their closest relatives (Figs. 2.2 and 2.4), lineages containing *várzea* species have not diversified nearly as much as *terra-firme* ones. These findings support the notion that a significant part of the recent

diversification within *Xiphorhynchus* originated by allopatric speciation within the *terra-firme* forest habitat in lowland Amazonia.

Taxonomic Recommendations .- In spite of its sampling limitations, the current dataset provides new insights into the evolution and diversification of species in the genus *Xiphorhynchus*, which can be used to generate new hypotheses of classification. When proposing these hypotheses, I use the General Lineage Concept of Species (de Queiroz 1998) to draw species limits in the *X. guttatus, X. pardalotus / ocellatus*, and *X. spixii / elegans* superspecies. De Queiroz (1998) argued that most of the alternative species "concepts" in modern biology (including the Phylogenetic and Biological Species Concepts) are in fact different criteria of the same species concept, the General Lineage Concept of Species. Since the process of speciation is continuous, several sequencial events must take place for speciation to be completed; different species criteria determine species limits by arbitrarily emphasizing different events occurring during the speciation process (de Queiroz 1998). Critical to the completion of speciation is the achievement of reciprocal monophyly between sister lineages; the "monophyly criterion" is well suited to establish species limits in a phylogeny (de Queiroz 1998), which is now finally available for the entire genus *Xiphorhynchus* and many of its taxa. By using the monophyly criterion, I split paraphyletic genera (*Lepidocolaptes* and *Xiphorhynchus*) and species (*X. guttatus* and *X. ocellatus*), as depicted in the phylogenies obtained. Based on this rational, I make the following recommendations to the taxonomy of *Xiphorhynchus*:

(1) Exclusion of *X. picus* and *X. kienerii* from *Xiphorhynchus* and their provisional return to *Dendroplex* Swainson 1827. The diagnosis of *Dendroplex* unmistakably refers to *X. picus* (Cory and Hellmayr 1925), but its designated type specimen turns out to be the painting of a

bird presently classified as *X. ocellatus* (Peters 1951). A separate publication evaluating the nomenclatural validity of *Dendroplex* is under way (Aleixo in prep.).

(2) Removal of the Lesser Woodcreeper (*L. fuscus*) from the genus *Lepidocolaptes* and its inclusion in the genus *Xiphorhynchus*. In linear classifications, *Xiphorhynchus fuscus* should be placed right before the *X. pardalotus / ocellatus* and *X. spixii / elegans* superspecies.

(3) Recognition of three species in the *X. guttatus* superspecies: (1) Buff-throated Woodcreeper (*X. guttatus*), containing nominate *guttatus* and *polystictus* as subspecies; (2) Cocoa Woodcreeper (*X. susurrans*), containing all trans-Andean subspecies of former *X. guttatus* (A. O. U. 1998); and (3) Lafresnaye's Woodcreeper (*X. guttatoides*) (Lafresnaye) 1850, available name with priority, which would include the following Amazonian taxa: *dorbignyanus, eytoni, guttatoides,* and *vicinalis*. The taxon *connectens* should be kept in *X. guttatus* until mtDNA sequences allowing its precise placement in the *X. guttatus* superspecies become available.

(4) Recognition of three species in the *X. pardalotus / ocellatus* superspecies: (1) Chestnut-rumped Woodcreeper (*X. pardalotus*), including nominate *pardalotus* and *caurensis*; (2) Ocellated Woodcreeper (*X. ocellatus*), including nominate *ocellatus*, *perplexus*, and *weddellii*; and (3) Tschudi's Woodcreeper (*X. chunchotambo*) (Tschudi) 1844, including nominate *chunchotambo* and *brevirostris*. The taxon *napensis* should be kept in *X. ocellatus* until mtDNA data allowing its precise placement in the *X. pardalotus / ocellatus* superspecies become available.

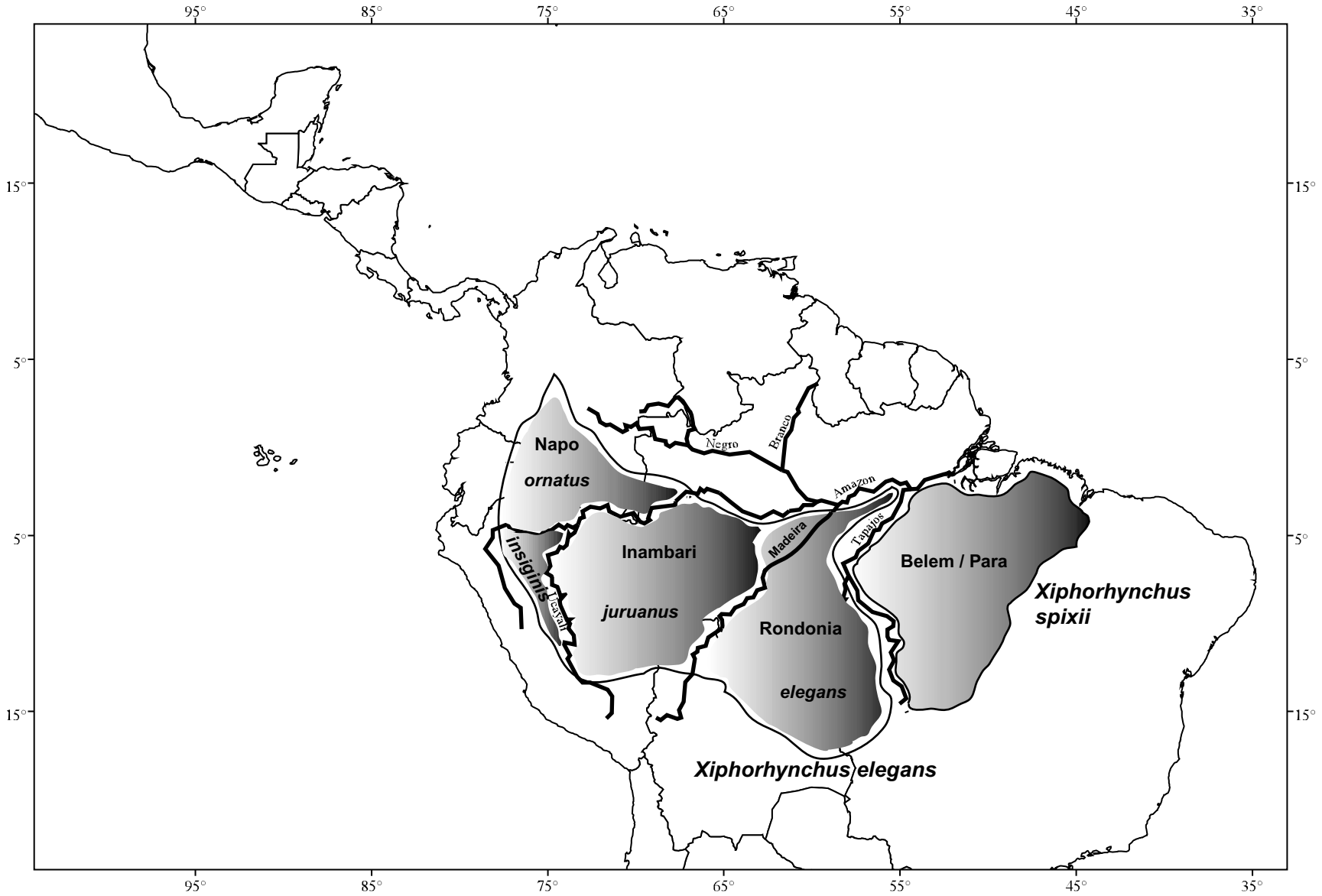
(5) Recognition of two species in the *X. spixii / elegans* superspecies: (1) monotypic Spix's Woodcreeper (*X. spixii*); and (2) Elegant Woodcreeper (*X. elegans*) (Pelzeln) 1868, including nominate *elegans, buenavistae, insignis, juruanus,* and *ornatus*.

CHAPTER 3. PHYLOGEOGRAPHY AND POPULATION GENETICS OF THE *XIPHORHYNCHUS SPIXII / ELEGANS* SUPERSPECIES

The study of Amazonian historical biogeography began with general analyses of species' distribution patterns (Wallace 1852, Snethlage 1913, Haffer 1969) and eventually evolved into phylogeographic studies with the use of molecular markers, focused at particular genera and species (Capparella 1987, Hackett 1993, Patton et al. 1994, Bates 2000, Gascon et al. 1998, Silva and Patton 1998). These more recent studies addressed specific predictions of some hypotheses proposed to explain the diversification of the Amazonian biota, sometimes reaching contradictory conclusions regarding their validity (e.g., Capparella 1987 versus Patton et al. 1994). However, the main contribution of phylogeographic studies was to point toward alternative hypotheses of diversification, not discussed before in the context of Amazonian biogeography (see Silva and Patton 1998), and to show that species with different ecologies and life-history attributes can differ in their response to historical events promoting speciation (Gascon et al. 1996, Lougheed et al. 1999, Matocq et al. 2000). Thus, it has become clear that the validity and generality of hypotheses concerning historical diversification proposed for Amazonia can only be properly evaluated with the accumulation of phylogeographic studies on several lineages of organisms (Bates et al. 1998, Moritz et al. 2000, Bates in press).

The *Xiphorhynchus spixii / elegans* (Aves: Dendrocolaptidae) superspecies is useful for phylogeographic studies of Amazonian organisms for the following reasons: (1) its distribution encompasses the entire Amazon basin, except the Guianan shield (Fig. 3.1); (2) the ranges of its taxa are usually bounded by major Amazonian tributaries, suggesting a strong response to rivers

Figure 3.1. Distribution of taxa of the *Xiphorhynchus spixii / elegans* superspecies. Names of areas of endemism (sensu Cracraft 1985) are shown in bold letters. Names of subspecies of *X. elegans* are shown in italics inside areas of endemism. Populations of *X. e. elegans* found on the western bank of the Madeira river belong to the Inambari area of endemism


95°
85°
75°
65°
55°
45°
35°
15°
5°
5°
15°
Napo
ornatus
Negro
Branco
Amazon
Madeira
Tapajos
Inambari
juruanus
insignis
Beni
Rondonia
elegans
Belem / Para
Xiphorhynchus spixii
Xiphorhynchus elegans

as barriers to dispersal, a pattern also shown by many other lineages of Amazonian vertebrates (Fig. 3.1; Haffer 1978), and (3) its taxa are fairly abundant, allowing the acquisition of large genetic samples. Once regarded as a single polytypic species, two biological species have been recognized more recently in the *X. spixii / elegans* superspecies (Haffer 1997b, Chapter 2): *X. spixii* (monotypic) and *X. elegans* (with four recognized subspecies). *Xiphorhynchus spixii* is endemic to the Belém and Pará areas of endemism on the eastern portion of the Brazilian shield (Fig. 3.1); *X. e. elegans* occurs in the Rondônia area of endemism, on the western part of the Brazillian shield, also reaching the easternmost part of the Inambari area of endemism, across the lower portion of the Madeira river; *X. e. juruanus* is found on the Inambari area of endemism in western Amazonia; *X. e. ornatus* is distributed in the Napo area of endemism; and *X. e. insiginis* occurs along the eastern base of the Andes (Fig. 3.1; Haffer 1997b).

Here, I present phylogeographic and population genetics analyses of the *Xiphorhynchus spixii / elegans* superspecies to address predictions of the riverine barrier and basal trichotomy hypotheses (see Chapter 1). Under the riverine barrier hypothesis, two main predictions can be made regarding the genetic differentiation of the *X. spixii / elegans* superspecies: (1) main Amazonian rivers separating populations of the *X. spixii / elegans* superspecies should act as areas of primary differentiation rather than secondary contact between lineages that diversified allopatrically (Moritz et al. 2000); and (2) populations inhabiting opposite banks of adjacent major Amazonian interfluvia should be more differentiated than populations found within the same interfluvia, even if separated by a greater distance than those populations separated by rivers.

When applied to the diversification of the *X. spixii / elegans* superspecies, the basal trichotomy hypothesis predicts that lineages found on the Brazilian shield (*X. spixii* and *X. e. elegans*) ought to derive from the sister group of those lineages found in western Amazonia, closer to the eastern slope of the Andes (*X. e. juruanus, X. e. insiginis,* and *X. e. ornatus*). Accordingly, ancestral populations of this superspecies would be found on the Brazilian shield and along the eastern base of the Andes, whereas more recently derived populations would be found in the western part of the Amazonian lowlands (Bates in press). If this is correct, then phylogeographies should have *X. spixii*, *X. e. elegans*, and *X. e. insiginis* as more basal taxa, whereas *X. e. juruanus* and *X. e. ornatus* would be the most recently derived taxa. In a population genetics framework, because of their older age, populations of *X. spixii, X. e. elegans*, and *X. e. insiginis* should be each in a mutation / drift equilibrium, show a multi-modal pattern of pairwise nucleotide differences among its individuals (mismatch distribution), have higher nucleotide diversity indices, and have most of their genetic diversity partitioned among, instead of within populations (Wright 1969, Zink 1997).

METHODS

Specimens. - I sequenced a total of 80 individuals of the *X. spixii / elegans* superspecies collected throughout Amazonia and the eastern Andean foothills (see Appendix 2 for collecting localities, populations sample sizes, and voucher information). Of these, 21 are specimens of *X. spixii*, and 59 are specimens of all valid subspecies of *X. elegans* (*X. e. elegans*, *X. e. insiginis, X. e. juruanus,* and *X. e. ornatus*; as pointed out by Haffer [1997b], *X. e. buenavistae* is barely distinguishable from *X. e. ornatus* and is thus better synonymized with it). In the phylogenetic analyses, I used

sequences of *Xiphorhynchus fuscus* and *Xiphorhynchus ocellatus* as outgroups because these two species are found in the same clade as the *X. spixii / elegans* superspecies according to a higher-level phylogeny estimated for the genus *Xiphorhynchus* (see Chapter 2).

DNA Sequencing. - Total genomic DNA was extracted from raw frozen tissue 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). I took the following measures to ensure that ancient DNA extracted out of dry skin samples would not be contaminated by DNA from frozen tissue samples: (1) dry skin samples were extracted in a different building than raw tissues; (2) separate Qiagen extraction kits and other disposable lab supplies were used to perform dry skin and raw tissue extractions, and (3) dry skin extractions were always performed with a negative controls, which never showed signs of DNA contamination when run on an electrophoresis agarose gel. I amplified most of the mitochondrial gene ctytochrome *b* (1,005 bp) with the following primers: L14990 (Kocher et al. 1989), L15389 (Hackett 1996), H15710 (Helm-Bychowski and Cracraft 1993), HXIPH (CATTCTGGTTTGATGTGGGG; designed specifically for this project), L15505 (CTAACCTTCCTACACGAAACC; designed specifically for this project), L15656 (Helm-Bychowski and Cracraft 1993), and H16065 (Hackett 1996). All primer numbers refer to the 3' base of the published chicken mtDNA sequence (Desjardins and Morais 1990). Fragments were PCR amplified 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 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, Norwalk, Connecticut), 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. I aligned and reconciled sequences from both strands using Sequencher 3.1.1 (Genecodes, Madison, Wisconsin). The following measures outlined by Sorenson and Quinn (1998) and Bates et al. (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 chicken complete mtDNA sequence, and 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. I could not detect any evidence of pseudogenes in the sequences used for this study.

Phylogenetic Analysis. – A maximum parsimony heuristic search (referred to as MP throughout this paper) was conducted on all unique haplotypes and outgroups with PAUP* 4.0b10 (Swofford 2002). The MP analysis was based on unweighted sequence data. I used 100 nonparametric bootstrap replications (referred to as BP throughout the paper) to evaluate confidence levels of nodes for the phylogeny obtained with MP (Felsenstein 1985). Only one random addition-sequence replicate was performed for each bootstrap replicate. I used the likelihood ratio test as implemented in MODELTEST (Posada and Crandall 1998) to select the best and simplest model of molecular evolution fitting my dataset, which was then used in a Bayesian inference of phylogeny with MrBayes software, version 2.01 (Huelsenbeck 2001). I

ran MrBayes 2.01 with the following specifications: (1) assuming a general time reversible model of nucleotide substitution with estimated base frequencies and site-specific rates for first, second, and third codon positions; and (2) running the Markov chain for 2,000,000 generations, sampling 1 tree every 1,000 generations. Following recommendations outlined by Huelsenbeck and Hall (2001), I discarded trees obtained before the Markov chain reached convergent and stable likelihood values. I used PAUP* 4.0b10 to compute a majority-rule consensus tree of the sampled trees. The proportion of times a given clade was sampled equal its posterior probability of occurrence. Only unique haplotypes were included in the Bayesian inference of phylogeny. I also used the likelihood ratio test to evaluate whether ingroup and outgroup cytochrome *b* sequences were evolving in a clock-like manner. To this end, scores of two maximum likelihood heuristic searches conducted in PAUP* 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. – As selected by MODELTEST, a Tamura and Nei model (Tamura and Nei 1993) with a gamma shape parameter ($\alpha = 0.15$) was used to estimate genetic distances among unique haplotypes. Haplotype diversity (h), nucleotide diversity (π) (Nei 1987, equations 8.5 and 10.5, respectively), and Tajima's (1989) D test for departure of neutrality were calculated for populations of each clade of the *X. spixii / elegans* and Amazonian area of endemism using the software Arlequin 2.000 (Schneider et al. 2000). Tajima's D was also computed for all unique haplotypes recovered for both *X. spixii* and *X. elegans* and outgroups. An analysis of molecular variance (AMOVA; Excoffier et al. 1992) for all clades and areas of

endemism was performed 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. Another AMOVA was performed for populations of individual clades of the *X. spixii / elegans* superspecies separated by the following Amazon river tributaries: Xingú, Madeira, Purús , Jurua, and Ucayali. Pairwise mismatch distributions (Rogers and Harpending 1992) and parameters of Rogers' (1995) model of sudden population expansion also were calculated for all clades and areas of endemism using Arlequin 2.000 (Schneider et al. 2000). To further test the "barrier-effect" detected for the Xingú river, I assessed the correlation between straight-line geographic distances and *Fst* values among populations of *X. spixii* using Mantel's (1967) test in Arlequin 2.000 (Schneider et al. 2000).

RESULTS

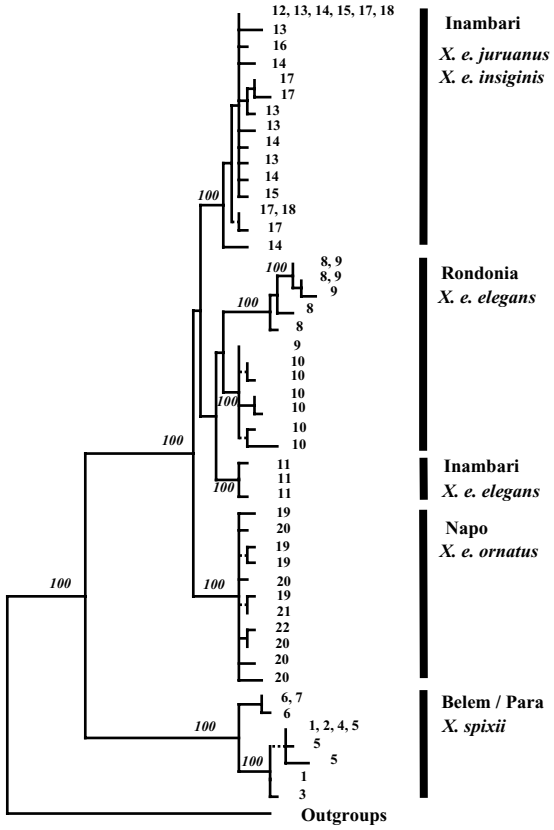
Description of mtDNA Sequences. – Cytochrome *b* sequences recovered were aligned unambiguously and showed expected codon biases and an overall deficit of guanines as reported for other avian cytochrome *b* gene sequences (Moore and DeFilippis 1997). A total of 7 and 41 unique haplotypes was recovered for *X. spixii* and *X. elegans*, respectively. These haplotypes varied in length from 940 to 1,005 bp, spanning positions 15030 to 16035 of the cytochrome *b* chicken sequence (Desjardins and Morais 1990). Nucleotide substitutions were observed at 161 sites (19%), 87 of which were phylogenetically informative. Tamura and Nei corrected distances (with a gamma shape parameter of $\alpha = 0.15$) among haplotypes ranged from 0.1% (between two

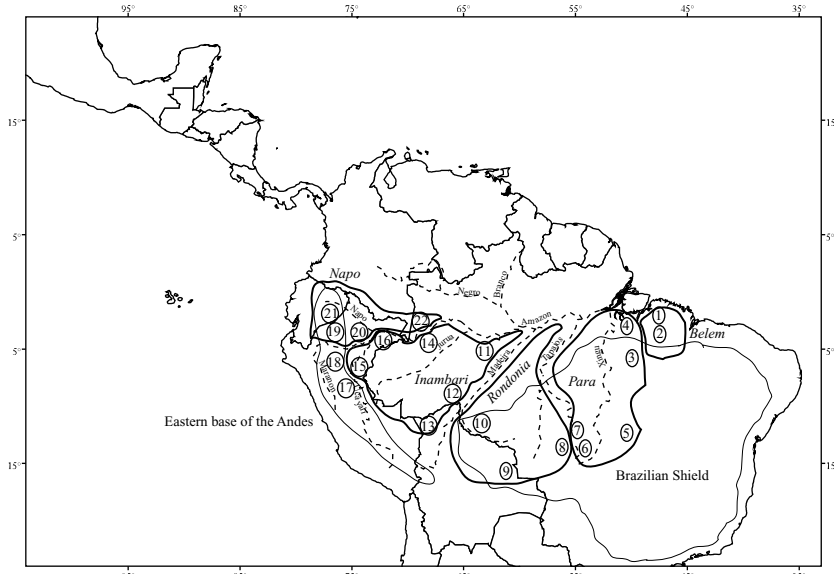
X. e. juruanus haplotypes) to 15% (between *X. fuscus* and *X. elegans*); the average distance between *X. spixii / elegans* haplotypes and their nearest common ancestor (*X. ocellatus*) was 12.4%. The average Tamura and Nei ($\alpha = 0.15$) corrected distance between *X. spixii* and *X. elegans* haplotypes was 7%, whereas that among haplotypes belonging to subspecies of *X. elegans* ranged from 1.6% (between *X. e. elegans* and *X. e. juruanus / insiginis*, and between *X. e. juruanus* and *X. e. ornatus*) to 1.9% (between *X. e. elegans* and *X. e. ornatus*). The result of a Tajima's test applied to all 48 unique haplotypes of the *X. spixii / elegans* and sequences of two outgroups (*X. fuscus* and *X. ocellatus*) showed no significant departure from neutrality ($D = -1.25$, $P > 0.10$). Likewise, the hypothesis of a clocklike rate of evolution for all cytochrome *b* sequences recovered for the *X. spixii / elegans* and outgroups could not be rejected (TrN+$G_{[clock]}$, lnL = -2655.1323, $\chi^2_{[48]} = 25.10795$, $P > 0.99$).

MP Analysis. – An equally weighted MP analysis of the 48 unique haplotypes of the *X. spixii / elegans* superspecies and two outgroups (*X. fuscus* and *X. ocellatus*) resulted in 108 most parsimonious trees (length 224; CI=0.75; RI=0.9). Figure 3.2a shows a 50% majority rule consensus of these 108 most parsimonious trees and bootstrap confidence values for its nodes. In the MP tree, the monophyly of the *X. spixii / elegans* superspecies was highly supported (BP = 100%), thus agreeing with a more extensive molecular dataset presented in chapter 2. Four main clades with various degrees of BP support can be recognized in the *X. spixii / elegans* superspecies according to the MP phylogeny: (1) *X. spixii* (BP = 100%); (2) *X. elegans elegans* (BP = 52%); (3) *X. e. ornatus* (BP = 100), and (4) *X. e. juruanus* / *insiginis* (BP = 78%). Resolution within the *X. spixii* clade was high, with the recognition of two well supported clades:

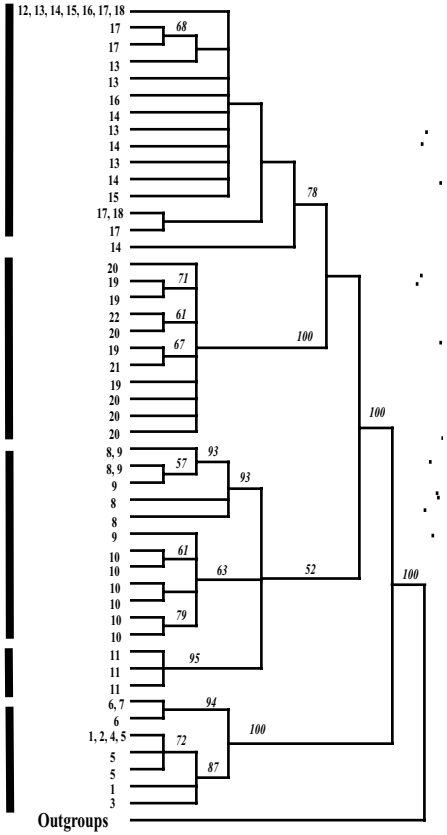
Figure 3.2. Map with location sampled and phylogenies estimated for 48 unique haplotypes recovered for the *Xiphorhynchus spixii / elegans* superspecies. Numbers at the tip of branches refer to localities (depicted inside circles on the map) where haplotypes were sampled. (A) Majority-rule consensus of 108 most parsimonious trees (length 224; CI=0.75; RI=0.9) obtained with unweighted sequence data. Numbers above branches refer to bootstrap support based on 100 replicates. Branches without numbers received less than 50% bootstrap support. (B) Majority-rule consensus of 1,800 trees obtained by a Bayesian inference of phylogeny under a variety of substitution parameters assuming the GTR model of molecular evolution with distinct rates for variable sites for first, second, and third codon positions. Numbers above branches refer to posterior probability of occurrence of clades. Dashed short branches also had a 100% posterior probability of occurrence

B. Bayesian inference





A. Parsimony



one occurring east of the Xingú river (BP = 87%), and another found west of the same river (BP = 94%). Resolution within the *X. elegans* clade was poorer: BP values for the basal position of *X. e. elegans*, and a sister relationship between *X. e. juruanus / insiginis* and *X. e. ornatus* were under 50% (Fig. 3.2a).

Bayesian Inference of Phylogeny. – The phylogeny estimated under the Bayesian approach was very similar to the MP consensus tree discussed above, with high posterior probabilities of occurrence supporting the monophyly of the *X. spixii / elegans* superspecies (100%), the reciprocal monophyly of *X. spixii* and *X. elegans* (100%), and the existence of four major clades in the *X. spixii* / *elegans* superspecies: (1) *X. spixii* (100%); (2) *X. elegans elegans* (probability of occurrence for the entire clade not significant, but 100% for each of the three recovered subclades); (3) *X. e. ornatus* (100%), and (4) *X. e. juruanus* / *insiginis* (100%). As in the MP consensus tree, higher-level relationships within the *X. elegans* clade were poorly supported, with several short internodes receiving very low posterior probabilities of occurrence (Fig. 3.2b).

Area Relationships. – Relationships among taxa within different areas of endemism recognized for Amazonia (Figs. 3.2a and 3.2b) inferred from the MP and Bayesian estimates of phylogeny for the *X. spixii / elegans* superspecies were essentially the same. The main split between *X. spixii* and *X. elegans* separates the Belém and Pará from the Rondônia, Inambari, and Napo areas of endemism. The Belém and Pará areas form a composite area of endemism (Belém / Pará), sharing most haplotypes recovered for *X. spixii* (Figs. 3.1 and 3.2). The two *X. spixii* clades observed did not exclude each other at different areas of endemism, but instead, across the Xingú river (Fig. 3.2). The Inambari area of endemism is inhabited by two distinct, non-sister, clades of the *X. spixii / elegans* superspecies: *X. e. juruanus* and *X. e. elegans*, the latter restricted to its

westernmost part (Figs. 3.1 and 3.2). The Napo area of endemism is entirely occupied by *X. e. ornatus*, a highly supported clade of the *X. spixii / elegans* superspecies according to MP and Bayesian phylogenies (Fig. 3.2). However, higher level relationships among taxa within the Rondônia, Inambari, and Napo areas of endemism were conflicting and statistically poorly supported according to the MP and Bayesian estimates of phylogeny. Contrary to a prediction of the basal trichotomy hypothesis, the two major clades of the *X. spixii / elegans* superspecies inhabiting the Brazilian shield are not sisters: populations of *X. e. elegans* from the western part of the Brazilian shield are closer to populations found in the western Amazonian lowlands than to populations of *X. spixii* found further to the east on the Brazilian shield.

Population Genetics Analyses. – Indices of haplotype diversity were high and similar among clades of *X. elegans*, but lower in *X. spixii* (Table 3.1). Populations of *X. spixii* and *X. e. elegans* occurring on the Brazilian shield (i.e., in the Belém / Pará and Rondônia areas of endemism, respectively) had higher nucleotide diversity indices than those populations occurring in the Inambari and Napo areas of endemism (*X. e. juruanus / X. insiginis* and *X. e. ornatus*, respectively) in western Amazonia. Results of Tajima's *D* tests indicate that populations of the *X. spixii / elegans* superspecies found on the Brazilian shield were in mutation / drift equilibrium, whereas those found in western Amazonia showed a statistically significant departure from neutrality, with strongly negative values consistent with a demographic expansion or population bottleneck (Rand 1996; Table 3.1). Analyses of Molecular Variance (AMOVA) showed that most genetic variation detected in populations of the *X. spixii / elegans* superspecies of the Brazilian shield (Belém / Pará and Rondônia areas of endemism) was partitioned geographically, among different populations (Table 3.1).

TABLE 3.1 - Geographically distributed genetic variation among members of the *X. spixii / elegans* superspecies in Amazonia. Includes number of individuals and populations sampled, haplotype diversity (*h*), nucleotide diversity *(π)*, results of Tajima's (1989) *D* test, and results from an analysis of molecular variance (AMOVA).

Area of endemism (Taxon)	**No. Individuals (Populations sampled)** [a]	**Haplotype diversity**	**Nucleotide diversity**	**Tajima's *D* test** [b]	**Variation among populations (%)**[c]	**Variation within populations (%)**
Belém / Pará (*X. spixii*)	21 (1 - 7)	0.79±0.06	$5.2\pm2.9 \bullet 10^{-3}$	0.48 N.S	85.5 **	14.4
Rondônia (*X. elegans elegans*) [d]	17 (8 - 10)	0.93±0.04	$8.9\pm4.8\bullet10^{-3}$	0.14 N.S.	57.1 **	42.8
Inambari (*X. e. juruanus* and *X. e. insiginis*)	24 (12 – 18)	0.89±0.05	$2.7\pm1.7\bullet10^{-3}$	- 2.05 **	6.7 *	93.2
Napo (*X. e. ornatus*)	13 (19 – 22)	0.96±0.04	$3.1\pm1.9\bullet10^{-3}$	- 1.73 *	13.7 *	86.3

[a] See Fig. 3.2 for geographic location of populations.
[b] Tajima's *D* test (1989); N.S., non-significant departure from neutrality (both $P > 0.60$); * significant departure from neutrality at $P = 0.03$; ** significant departure from neutrality at $P = 0.01$.
[c] * $P < 0.01$; ** $P < 0.001$.
[d] Excludes population 11, located west of the Madeira river in the westernmost part of the Inambari area of endemism.

In contrast, most genetic variation in populations of the western Amazonian lowlands (Napo and Inambari areas of endemism) was partitioned within populations (Table 3.1). Another AMOVA (Table 3.2) showed that among five main southern bank Amazon river tributaries separating the ranges of monophyletic populations of the *X. spixii / elegans* superspecies, only one (the Xingú river, located on the Brazilian shield) separated populations of *X. spixii* with most of their genetic variation partitioned between opposite river banks. Distance alone cannot explain this strong "river-effect" because correlation between straight-line geographic distances and pairwise *Fst* values among populations of *X. spixii* was positive but not statistically significant ($r = 0.88$; Mantel's test $P = 0.21$). Monophyletic populations of *X. elegans* separated by Amazonian tributaries located in western Amazonia (Madeira, Purús, Juruá, and Ucayali) had most of their genetic variation partitioned among populations located on the same river bank or within populations (Table 3.2), thus independently of the presence of the river. Nucleotide mismatch distributions for clades of the *X. spixii / elegans* superspecies occurring on the Brazilian shield were bimodal (*X. spixii*) or multimodal (*X. e. elegans*), but unimodal for clades occurring in western Amazonia (*X. e. juruanus / insiginis* and *X. e. ornatus* Figs. 3.3 and 3.4). Assuming a mitochondrial clocklike substitution rate of 2% per million years (Klicka and Zink 1997), unimodal mismatch distributions for *X. e. juruanus / insiginis* and *X. e. ornatus* were consistent with a recent population expansion, probably followed by a bottleneck, that took place between 6,000 and 60,000 years BP (Rogers 1995).

TABLE 3.2 – Results from an analysis of molecular variance (AMOVA) among monophyletic populations of the *Xiphorhynchus spixii / elegans* superspecies separated by five main southern bank Amazon river tributaries.

Taxon	**No. individuals (Populations)** [a]	**River**	**Among populations from opposite river banks (%)**	**Among populations from the same river bank (%)**[b]	**Within populations (%)**[b]
X. spixii	21 (1 – 7)	Xingú	87.6	1.5 **	10.8 **
X. elegans elegans	22 (8 – 11)	Madeira	20.1	48.6 **	31.2 **
X. e. juruanus	17 (12 – 16)	Purús and Juruá	2.9	- 0.5	97.6
X. e. juruanus and *X. e. insiginis*	18 (14 – 18)	Ucayali	13.6	- 2.5	88.9

[a] See Fig. 3.2 for geographic location of populations.
[b] * $P < 0.001$.

Figure 3.3. Pairwise nucleotide mismatch distributions for the *X. spixii* (A) and *X. elegans elegans* (B) clades of the *Xiphorhynchus spixii / elegans* superspecies found on the Brazilian shield.

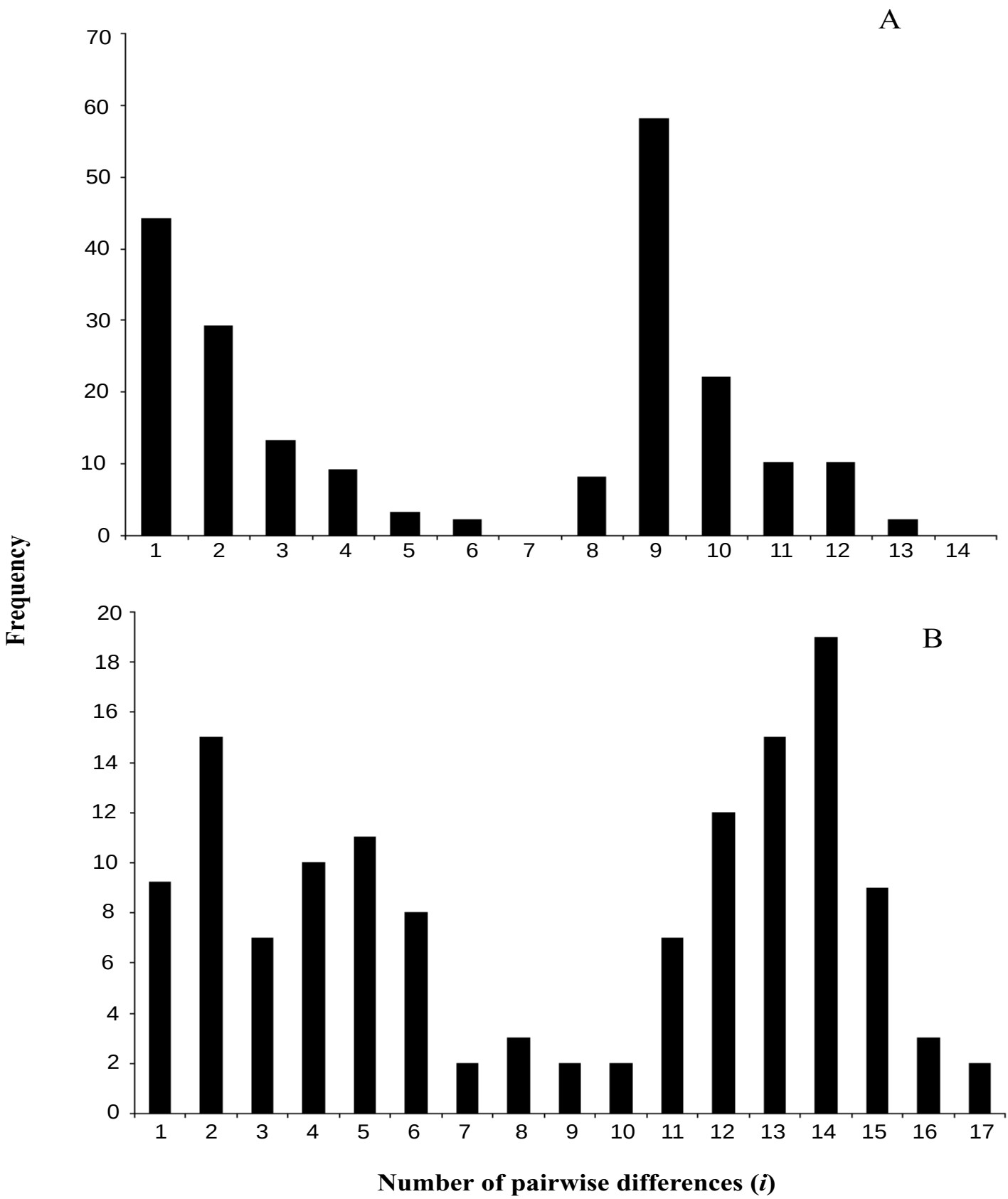
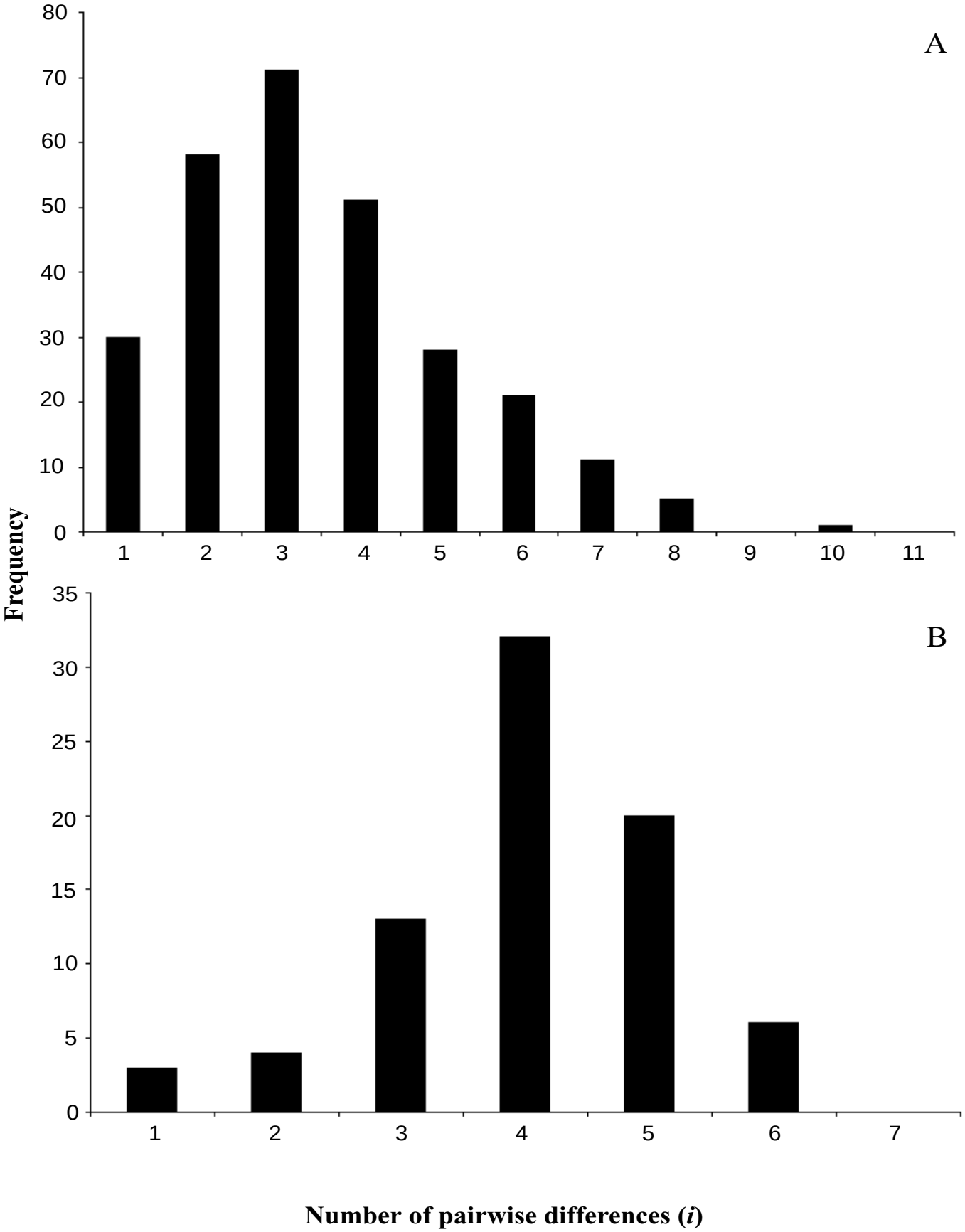

A
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Frequency
B
20
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
Number of pairwise differences (*i*)

Figure 3.4. Pairwise nucleotide mismatch distributions for the *X. elegans juruanus / insiginis* (A) and *X. elegans ornatus* (B) clades of the *Xiphorhynchus spixii / elegans* superspecies found in the western lowlands of Amazonia and eastern slope of the Andes.


A
B
Frequency
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
Number of pairwise differences (*i*)

DISCUSSION

Phylogenetic Relationships. – MP and Bayesian estimates of phylogeny for the *X. spixii / elegans* superspecies were in general well supported statistically (Fig. 3.2); the only exception was the set of higher-level relationships among the three clades recovered for *X. elegans*: *X. e. elegans*, *X. e. juruanus / insiginis,* and *X. e. ornatus*. Although MP placed *X. e. elegans* as basal in this clade, the Bayesian estimate of phylogeny placed *X. e. ornatus* in this same position, changing therefore the set of sister relationships in this three taxon clade. In the absence of a good statistical support for either alternative topology, independent evidence from the data shown here might provide better clues as to which alternative phylogeny is better supported.

Three of four clades of the *X. spixii / elegans* superspecies recovered by the MP and Bayesian phylogenies correspond to phenotypically diagnosable OTUs, formally recognized as distinct taxa (Haffer 1997b). The only discrepancy between phenotypic and genotypic data pertains to the position of *X. e. insiginis*, which is phenotypically very similar to *X. e. ornatus*, but genetically indistinguishable from *X. e. juruanus* (Fig. 3.2). This pattern can be explained by extensive gene flow between the *X. e. juruanus* and *X. e. ornatus* clades across the upper Maranon and Ucayali rivers, as documented by a clinal variation in some plumage characters of specimens collected in these areas (Zimmer 1934b, Haffer 1997b). In spite of several documented cases of extensive hybridization between non-sister lineages (Omland et al. 2000), I would tentatively interpret this extensive intergradation in genetic and plumage characters, added to an extreme similarity in song types (pers. obs.), as evidences of a sister relationship between the *X. e. juruanus / insiginis* and *X. e. ornatus* clades, therefore supporting the topology recovered by MP.

The Riverine Barrier Hypothesis. - In a phylogenetic and population genetics framework, the three main predictions of the riverine-barrier hypothesis are: (1) sister intraspecific clades and species will occur across major rivers rather than within major Amazonian interfluves (Moritz et al. 2000); (2) phylogeographic analyses should allow the distinction between primary divergence across rivers (predicted by the riverine barrier hypothesis) from secondary contact along rivers between non-sister lineages (Moritz et al. 2000); and (3) within a river basin, genetic similarity between populations separated by a river should be higher in the headwaters (where the river is narrower) than in its lower part (Gascon et al. 2000).

Predictions 1 and 2 hold only for two out of five southern bank Amazonian tributaries separating populations of the *X. spixii / elegans* superspecies: the Xingú and Tapajós rivers, both with their headwaters and most of their courses on the Brazilian shield, and for the upper Amazon river in western Amazonia (Figs. 3.1 and 3.2). The main split in the *X. spixii / elegans* superspecies (between the sister clades *X. spixii* and *X. elegans*, now regarded as separate biological species), coincides completely with the Tapajós river, making this river therefore the primary barrier causing an early divergence in the *X. spixii / elegans* superspecies (Figs. 3.1 and 3.2). Likewise, the Xingú river, farther to the east, coincides with the main split between the two sister clades of *X. spixii* (Fig. 3.2). Although a more thorough sampling of populations of *X. spixii* found west of the Xingú is necessary before strong conclusions can be drawn, two lines of evidence suggest that this main split across the Xingú is real and not a sampling artifact: (1) the lack of a significant correlation between straight-line geographic distances and pairwise *Fst* values among populations of *X. spixii*, and (2) the strong bimodality of the mismatch distribution among all haplotypes recovered for *X. spixii*, which is consistent with long-term isolation and lack of

recent gene flow between those two clades (Fig. 3.3a; Marjoram and Donnelly 1994). Also consistent with predictions of the river hypothesis is the replacement of the sister clades *X. e. juruanus / insiginis* and *X. e. ornatus* across the upper Amazon river in western Brazil and northeastern Peru (Fig. 3.2). However, as discussed earlier, gene flow between the *X. e. juruanus / insiginis* and *X. e. ornatus* clades occurs farther west, across the upper reaches of the Maranon river; therefore, the "barrier effect" of the upper Amazon river is local and ultimately insufficient to promote a complete isolation between those two lineages. On the other hand, no evidence of gene flow between sister clades of the *X. spixii / elegans* superspecies was found even at the headwaters of the Xingú and Tapajós rivers, where the width and associated "barrier effect" of these rivers are presumably smaller (Haffer 1992a).

Conversely, all predictions of the riverine barrier hypothesis outlined above were not fulfilled when the following white-water southern bank Amazonian tributaries are concerned: Madeira, Purús , Juruá, and Ucayali (Fig. 3.1). The Madeira river is by far the largest tributary of the Amazon river, accounting for many distributional limits in several lineages of vertebrates (Ayres and Clutton-Brock 1992, Haffer 1992a). According to predictions of the riverine barrier hypothesis, haplotypes found on the western bank of the Madeira should be closer to haplotypes found farther west than to haplotypes from the opposite (eastern) bank of the river. As the phylogenies recovered clearly indicated, this is not the case: haplotypes from the western bank of the lower Madeira group with haplotypes recovered across the river (*X. e. elegans* clade), rather than with haplotypes found in the same interfluvium (*X. juruanus / insiginis* clade), a pattern concordant with the phenotypic similarity between specimens collected in western bank of the lower Madeira and specimens of *X. e. elegans* found in the Madeira / Tapajós interfluvium

(Haffer 1997b, pers. obs). This set of relationships further falsifies the riverine barrier hypothesis because the "crossing" of the Madeira by *X. e. elegans* haplotypes took place in its lower course rather than its headwaters, where the "barrier effect" is expected to be weaker (Gascon et al. 2000). Farther upriver, the Madeira separates the *X. e. elegans* and *X. e. juruanus* clades, which, as discussed above, are not regarded as sisters. Finally, an AMOVA showed that most of the genetic variation found in populations in the *X. e. elegans* clade is not partitioned across the Madeira, but instead among populations from the Madeira / Tapajós interfluvium (Fig. 3.2 and Table 3.2).

Similarly, the Purús, Juruá, and Ucayali rivers do not separate sister lineages of the *X. e. juruanus / elegans* clade (Fig. 3.2), and two AMOVAs indicate that most genetic variation in this clade is partitioned within populations and is therefore independent from the locations of those three main Amazonian tributaries (Tables 3.1 and 3.2). Several phylogeographic studies on amphibians, reptiles, and mammals designed to test the riverine barrier hypothesis along the Juruá river overwhelmingly concluded that the Juruá does not represent a significant barrier promoting differentiation among lineages inhabiting its valley (Gascon et al. 1996, Peres et al. 1997, Silva and Patton 1998, Lougheed et al. 1999, Gascon et al. 2000). This pattern is not unexpected given the extensive meandering of the Juruá, and the small number of distinct avian taxa whose ranges abut along this river (Capparella 1987, Haffer 1997a). The same poor "barrier effect" detected along the Juruá was suggested to be extended to other meandering white-water Amazonian tributaries originating in the Andean slopes of western Amazonia (Gascon et al. 2000), a conclusion well supported by the present study.

When the historical diversification of the *X. spixii / elegans* superspecies is contrasted with predictions derived from the riverine barrier hypothesis, a dichotomous pattern emerges: Amazonian tributaries located on the Brazilian shield represent current and historical barriers restraining or suppressing gene flow between sister lineages, whereas white-water tributaries in the western Amazonian lowlands do not seem to play such a role. This lack of a "river-effect" in the western part of the Amazon, when compared to its central and eastern parts, is probably explained by the younger age of the western Amazonian lowlands. Although the Brazilian and Guianan shields have been geologically stable for the last 30-50 million years (Irion et al. 1995), the southwestern part of Amazonia began to take its current shape only in the last 2.5 million years or so (Late Pliocene), when the last cycle of Cenozoic fluvio-lacustrine deposition ended, and when the Amazon river system began to develop as a transcontinental drainage system (Hoorn et al. 1995, Campbell et al. 2001). Therefore, western Amazonian rivers are younger and less stable than rivers located on the Brazilian shield, and they experience frequent lateral channel migration responsible for across river transfer of large pieces of land (Salo et al. 1986). Furthermore, the impact of recurrent sea level changes on the Amazonian biota as recently as the Last Glacial Maximum (LGM, about 20,000 years BP; Irion et al. 2002) disproportionately influenced the poorly drained western lowlands compared to the higher Brazilian shield, where the main effect may have been the enlargement of the width of its main rivers such as the Tocantins, Xingú and Tapajós . The pattern of diversification revealed by the *X. spixii / elegans* superspecies leads to the conclusion that the most important variable responsible for the effectiveness of a river as a barrier to gene flow is not its width, but instead the geological history of its location (Ayres and Clutton-Brock 1992, Colwell 2000, Gascon et al. 2000).

The Basal Trichotomy Hypothesis. – The following predictions can be derived from the basal trichotomy hypothesis when applied to the diversification of the *X. spixii / elegans* superspecies (Bates in press; Chapter 1): (1) lineages found on the Brazilian shield (*X. spixii* and *X. e. elegans*) ought to be sisters to those lineages found in western Amazonia, closer to the eastern slope of the Andes (*X. e. juruanus, X. e. insiginis,* and *X. e. ornatus*); (2) phylogenetic estimates should have *X. spixii*, *X. e. elegans*, and *X. e. insiginis* as the more basal taxa, whereas *X. e. juruanus* and *X. e. ornatus* would be the most recently derived taxa; and (3) populations of *X. spixii, X. e. elegans*, and *X. e. insiginis* should be in mutation/drift equilibrium, show a multi-modal pattern of pairwise nucleotide differences among individuals (mismatch distribution), have higher nucleotide diversity indices, and have most genetic diversity partitioned geographically among populations.

Phylogenetic and population genetics analyses of the *Xiphorhynchus spixii / elegans* superspecies partially support some of the predictions outlined above. The first prediction is falsified because taxa found on the Brazilian shield are not monophyletic (Fig. 3.2). Instead, taxa endemic to the western Amazonian lowlands and eastern slope of the Andes (*X. e. juruanus*, *X. e. ornatus,* and *X. e. insiginis*) are derived from a taxon endemic to the western portion of the Brazilian shield corresponding to the Rondônia area of endemism (*X. e. elegans*). The second prediction is just partially fulfilled, because taxa endemic to the Brazilian shield (*X. spixii* and *X. e. elegans*) do occupy basal positions in their respective clades, but the taxon endemic to the eastern slope of the Andes (*X. e. insiginis*) does not: it is nested in a distal clade of *X. elegans*, together with *X. e. juruanus*. Thus, the eastern slope of the Andes cannot be regarded as an area of origin or primary differentiation for *Xiphorhynchus spixii / elegans* superspecies, as expected by the basal trichotomy hypothesis. This observation indicates either that: (1) the eastern slope

of the Andes never functioned as an "island" where isolated populations diversified, or (2) that different avian lineages diversified in different areas postulated to have escaped extensive Tertiary and Quaternary flooding (Guianan shield, Brazilian shield, and eastern slope of the Andes). Only future studies focused at other avian lineages will allow the distinction between these two alternative scenarios.

In the case of the *Xiphorhynchus spixii / elegans* superspecies, a combination of phylogenetic and population genetic analyses is consistent with an origin and early diversification on the Brazilian shield and a later colonization of the western Amazonian lowlands and eastern slope of the Andes (Figs. 3.2, 3.3, and 3.4; Table 3.1). Based on a mitochondrial clocklike substitution rate of 2% per million years (Klicka and Zink 1997), and using the correction for within phylogroup variation suggested by Avise and Walker (1998), the split between the western Brazilian shield taxon (*X. e. elegans*) and the western lowland Amazonian/Andean clade (*X. e. juruanus / insiginis* and *X. e. ornatus*) was completed about 500,000 years BP; therefore, colonization of the western Amazonian lowlands may have started earlier, sometime during the middle Pleistocene. Since then, populations of the *X. e. juruanus / insiginis* and *X. e. ornatus* clades expanded quite rapidly into western Amazonia and eastern slope of the Andes, probably after experiencing several population bottlenecks, which led to unimodal nucleotide mismatch distributions and a lack of phylogeographic structure (Table 3.1; Fig. 3.4). Sea level changes throughout the Quaternary affected western Amazonia by creating large floodplains, alternated by periods of extensive floodplain erosion (Irion et al. 1995). This dynamic scenario may have led to several population bottlenecks followed by rapid range expansions in the *X. e. juruanus /*

insiginis and *X. e. ornatus* clades, because these taxa tend to favor unflooded *terra-firme* forest as their main habitat type (Ridgely and Tudor 1994).

In contrast, the high degree of population structure and nucleotide diversity detected for the *X. e. elegans* and *X. spixii* clades on the Brazilian shield are consistent with long-term population stability, probably attained in a stable environment (Table 3.1). The split between the *X. elegans* and *X. spixii* clades was probably completed by the late Pliocene (*ca*. 3 million years BP), and could have been promoted by an extensive embayment of the Tapajós river in a period of high sea levels during the Pliocene (Haq et al. 1987, Marroig and Cerqueira 1997). Although populations of *X. e. elegans* on the western part of the Brazilian shield showed no signs of having experienced a recent population bottleneck (Table 3.1, Fig. 3.3b), the bimodal pattern of nucleotide mismatch distribution shown by *X. spixii* does not rule out two separate, recent bottlenecks in an already geographically structured population (Fig. 3.3a; Marjoram and Donnelly 1994). Estimated parameters of Rogers' (1995) model of sudden population expansion indicate that these two separate clades of *X. spixii* could have experienced population expansions between 5,000 and 70,000 years BP. In spite of its large confidence interval, the time frame encompassed by this estimate is consistent with major changes in the location of the *cerrado* - *terra-firme* ecotone in eastern Amazonia, when areas covered by forest probably experienced cycles of retraction and expansion caused by dry and humid periods, respectively (Van der Hammen and Hooghiemstra 2000). Thus, the two clades of *X. spixii* could have expanded their ranges after the return of humid climate conditions as recently as 10,000 years BP, which led to an expansion of humid forest types (Van der Hammen and Absy 1994). A more thorough sampling of the *X. spixii* clade is necessary to further address this hypothesis.

CHAPTER 4. PHYLOGEOGRAPHY AND POPULATION GENETICS OF TWO AMAZONIAN FLOODED FOREST BIRD SPECIALISTS: *XIPHORHYNCHUS KIENERII* AND *XIPHORHYNCHUS OBSOLETUS*

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 unflooded (*terra-firme*) forests, away from the influence of major Amazonian rivers (Capparella 1991, Hackett 1993; see Chapter 3). In contrast, a substantial portion of the Amazonian avifauna lives in habitats affected by major rivers, such as flooded (*várzea*) forests and river islands (Remsen and Parker 1983, Stotz et al. 1996). The riverine barrier hypothesis of diversification (allopatric differentiation caused by restriction of gene flow across rivers; see review in Gascon et al. 2000) is not thought to apply to *várzea* species because they are capable of colonizing river islands and crossing rivers (Capparella 1991). Some *terra-firme* and *várzea* rodent species were shown to differ in their population structure because distinct physical and ecological barriers affected gene flow among populations of these species (Matocq et al. 2000). *Várzea* forests are distributed linearly along rivers, a configuration which usually produces a narrow, linear gene flow pattern among neighboring populations, thus yielding undifferentiated populations within a single river basin (Patton et al. 2000); in contrast, this pattern of gene flow could potentially result in structured populations across river basin comparisons. If corroborated, this untested prediction has important conservation implications. Not only should protected sites encompass extensive areas

of *várzea* forest, but also continuous conservation units should be created along every major Amazonian tributary and interfluvium.

Here, I studied the phylogeography and population genetics structure of two bird species in the genus *Xiphorhynchus* (Dendrocolaptidae) found exclusively in flooded forest types throughout Amazonia: *X. kienerii* and *X. obsoletus*. My goal was to answer the following questions: (1) What is the degree of population structure found among these *várzea* species throughout Amazonia ?; (2) Are populations of *X. kienerii* and *X. obsoletus* from a single river basin more genetically similar to each other than populations from different river basins ?; and (3) If the riverine barrier hypothesis is not applicable to the diversification of *várzea* species, what possible alternative hypotheses can explain diversification in these lineages ?

METHODS

Specimens. - I sequenced a total of 21 individuals of *X. kienerii* and 30 individuals of *X. obsoletus* collected throughout Amazonia (see Appendix 3 for collecting localities, populations sample sizes, and specimens' voucher information).

DNA Sequencing. - Total genomic DNA was extracted from raw frozen tissue 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). I took the following measures 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 lab supplies were used to perform dry skin and raw tissue extractions; and (3) dry skin

extractions were always performed with a negative controls (which never showed signs of DNA contamination when run on an electrophoresis agarose gel). I amplified most of the mitochondrial gene cytochrome *b* with the following primers: L14990 (Kocher et al. 1989), L15389 (Hackett 1996), H15710 (Helm-Bychowski and Cracraft 1993), HXIPH (CATTCTGGTTTGATGTGGGG; designed specifically for this project), L15505 (CTAACCTTCCTACACGAAACC; designed specifically for this project), L15656 (Helm-Bychowski and Cracraft 1993), and H16065 (Hackett 1996). All primer numbers refer to the 3' base of the published chicken mtDNA sequence (Desjardins and Morais 1990). Fragments were PCR amplified 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 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, Norwalk, Connecticut), 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. I aligned and reconciled sequences from both strands using Sequencher 3.1.1 (Genecodes, Madison, Wisconsin). The following measures outlined by Sorenson and Quinn (1998) and Bates et al. (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. I could not detect any evidence of pseudogenes in the sequences used for this study.

Phylogenetic Analysis. – Due to the relatively shallow level of divergence among haplotypes sampled in this study, I estimated haplotype networks for *X. kienerii* and *X. obsoletus* using the software TCS 1.13 (Clement et al. 2000). TCS uses the method known as statistical parsimony (Templeton et al. 1992) to generate an unrooted cladogram based on a pairwise matrix of absolute differences among haplotypes (Clement et al. 2000). I ran TCS 1.13 with the 95% limit of parsimony (Templeton et al. 1992). I used the likelihood ratio test (Yang et al. 1995) to evaluate whether ingroup and outgroup cytochrome *b* sequences of *X. kienerii* and *X. obsoletus* were evolving in a clock-like manner. Therefore, I first used the likelihood ratio test as implemented in MODELTEST (Posada and Crandall 1998) to select the best and simplest model of molecular evolution fitting my 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 * 4.0 b10 (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 the phylogeny estimated for the genus *Xiphorhynchus* in chapter 2. For the rate heterogeneity test, scores of two maximum likelihood heuristic searches conducted in PAUP* 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) (Nei 1987, equations 8.5 and 10.5, respectively), and Tajima's (1989) D test for departure of neutrality were calculated for populations of *X. kienerii* and *X. obsoletus* using software Arlequin 2.000 (Schneider et al. 2000). Tajima's D was also computed for all unique haplotypes recovered for both *X. kienerii* and *X. obsoletus*. An analysis of molecular variance (AMOVA; Excoffier et al. 1992) was performed for all populations and areas of endemism 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 and Harpending 1992) and parameters of Rogers' (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

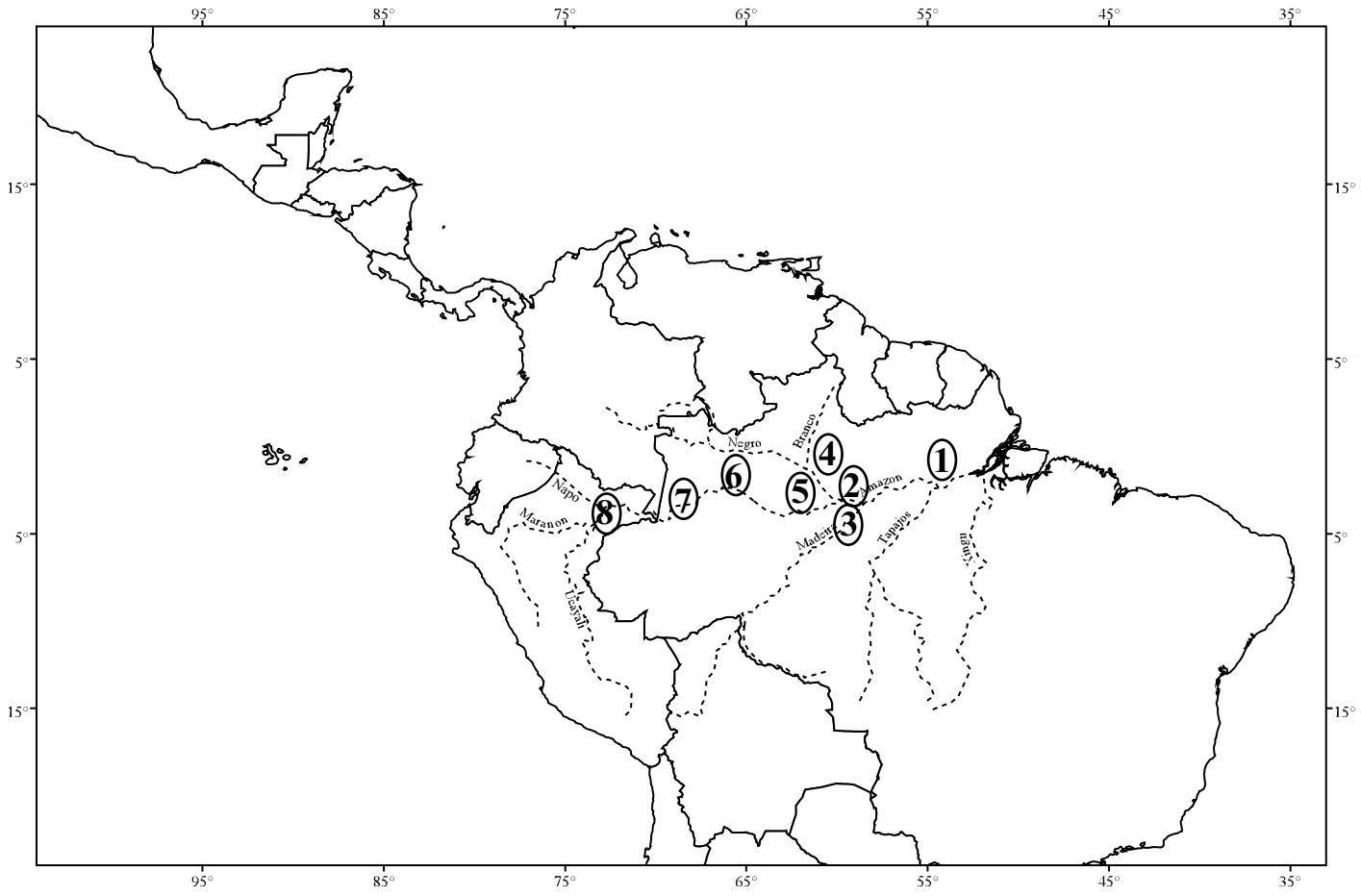
Description of mtDNA Sequences. – Cytochrome *b* sequences recovered were aligned unambiguously and showed expected codon biases and an overall deficit of guanines as reported for other avian cytochrome *b* gene sequences (Moore and DeFilippis 1997). Sequences obtained were trimmed to 908 and 919 bp for *X. kienerii* and *X. obsoletus*, respectively, spanning positions 15116 to 16035 of the cytochrome *b* chicken sequence (Desjardins and Morais 1990). A total of 5 and 11 unique haplotypes was recovered for *X. kienerii* and *X. obsoletus*, respectively. For *X. kienerii*, nucleotide substitutions were observed at 4 sites (0.4%), only one of which was potentially phylogenetically informative. For *X. obsoletus*, nucleotide substitutions

occurred at 11 sites (1.2%), 3 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 clocklike 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, $\chi^2_{[5]} = 2.55$, $P > 0.75$ for *X. kienerii* and $HKY_{[clock]}$, lnL = -1752.7817, $\chi^2_{[10]} = 4.62$, $P > 0.90$ for *X. obsoletus*).

Phylogeography. – A statistical parsimony network with 5 haplotypes was obtained for *X. kienerii* (Fig. 4.1). In this network, 4 haplotypes were separated from the most widespread haplotype (called haplotype 1) by just one mutational step each (Fig. 4.1). 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. 4.2). Most (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 3 other haplotypes were separated from haplotype 1 by two mutational steps (Fig. 4.2). Haplotype 1 for both *X. kienerii* and *X. obsoletus* had the highest frequency in most population sampled (Figs. 4.1 and 4.2). 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 phylogeographic structure for both species throughout Amazonia.

Population Genetics Analyses. – Indices of haplotype and nucleotide diversity were generally low for *X. kienerii* and *X. obsoletus* but varied considerably geographically, being higher for populations of both species in western Amazonia (Table 4.1).

Figure 4.1. 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. See Appendix 3 for exact location of sampled populations and voucher information.


95°
85°
75°
65°
55°
45°
35°
15°
5°
Negro
Branco
Amazon
Napo
Maranon
Ucayali
Tapajos
Madeira
1
2
3
4
5
6
7
8

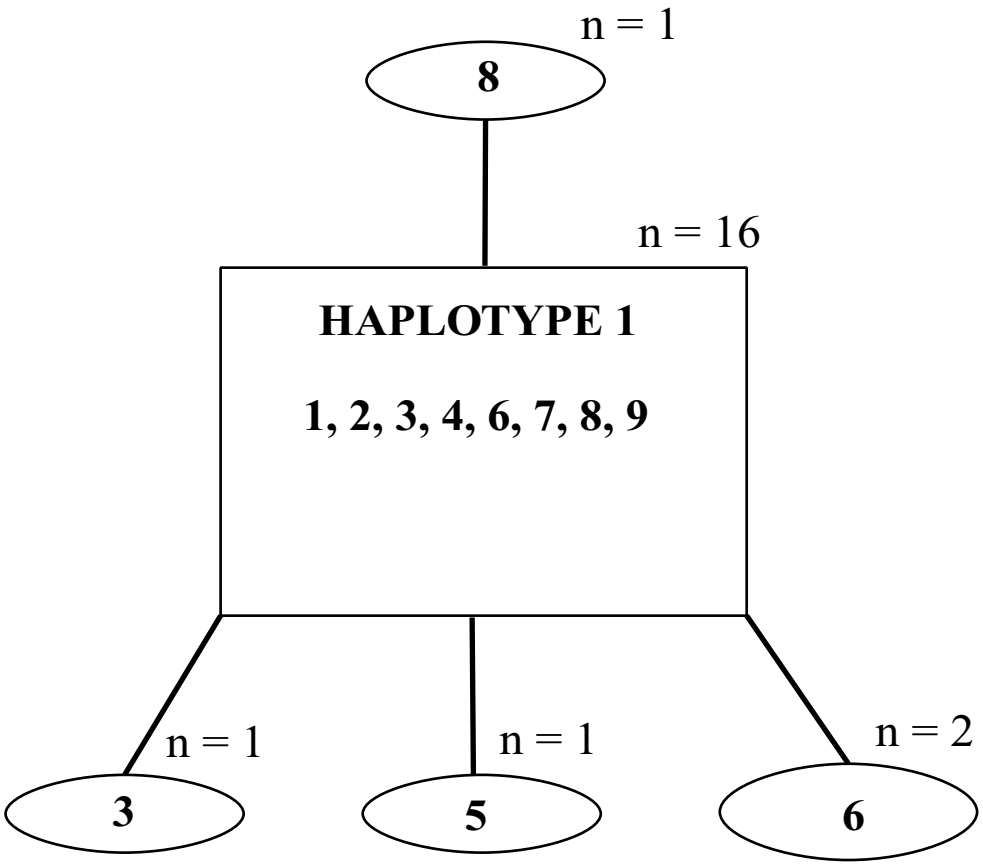
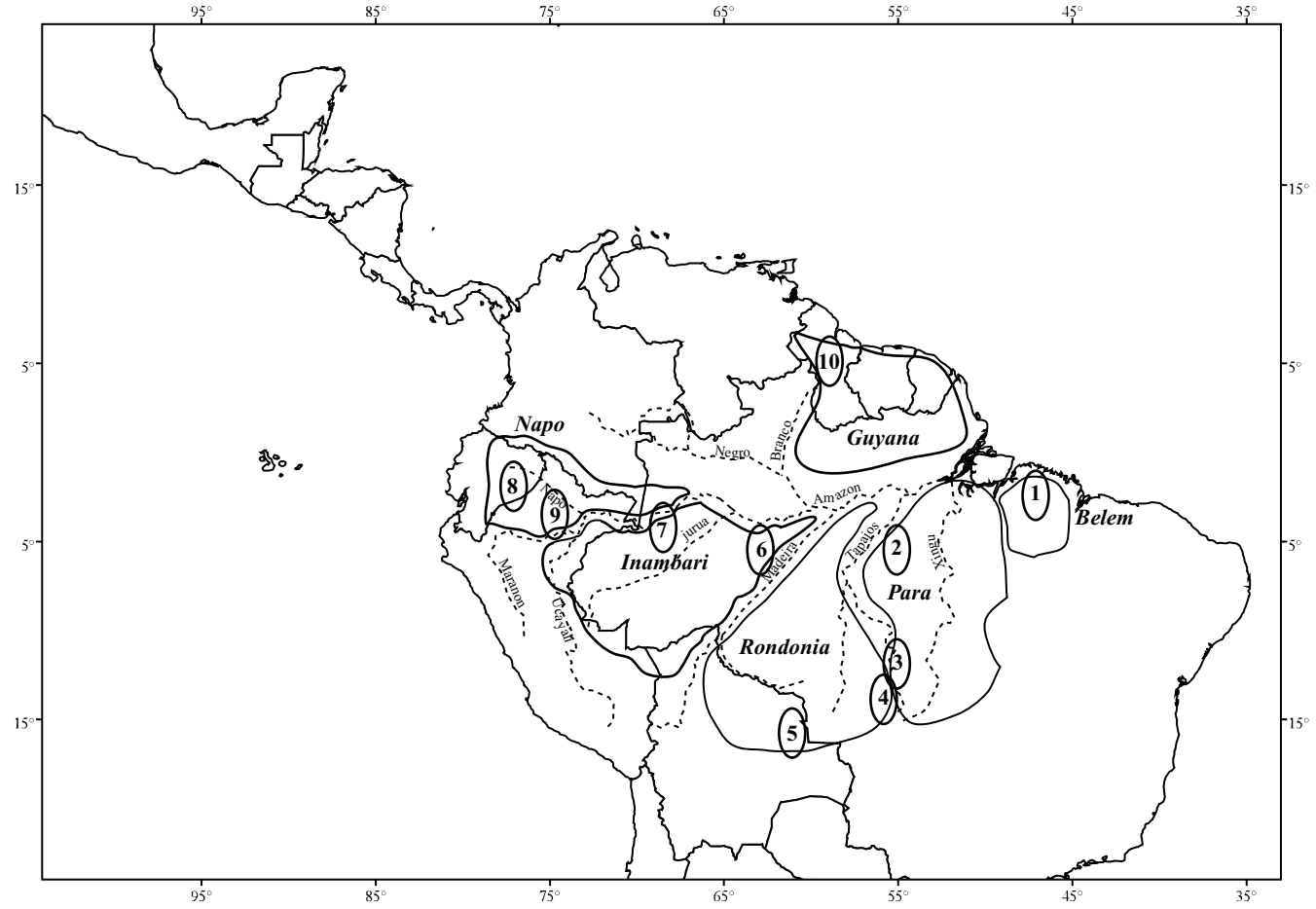

n = 1
8
n = 16
HAPLOTYPE 1
1, 2, 3, 4, 6, 7, 8, 9
n = 1
n = 1
n = 2
3
5
6

Figure 4.2. Map with location of populations sampled within recognized areas of avian endemism (Cracraft 1985), 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. See Appendix 3 for exact location of sampled populations and voucher information.


95°
85°
75°
65°
55°
45°
35°
15°
5°
Napo
Negro
Branco
Guyana
Amazon
Jurua
Inambari
Madeira
Tapajos
Xingu
Para
Belem
Rondonia
Maranon
Ucayali
1
2
3
4
5
6
7
8
9
10

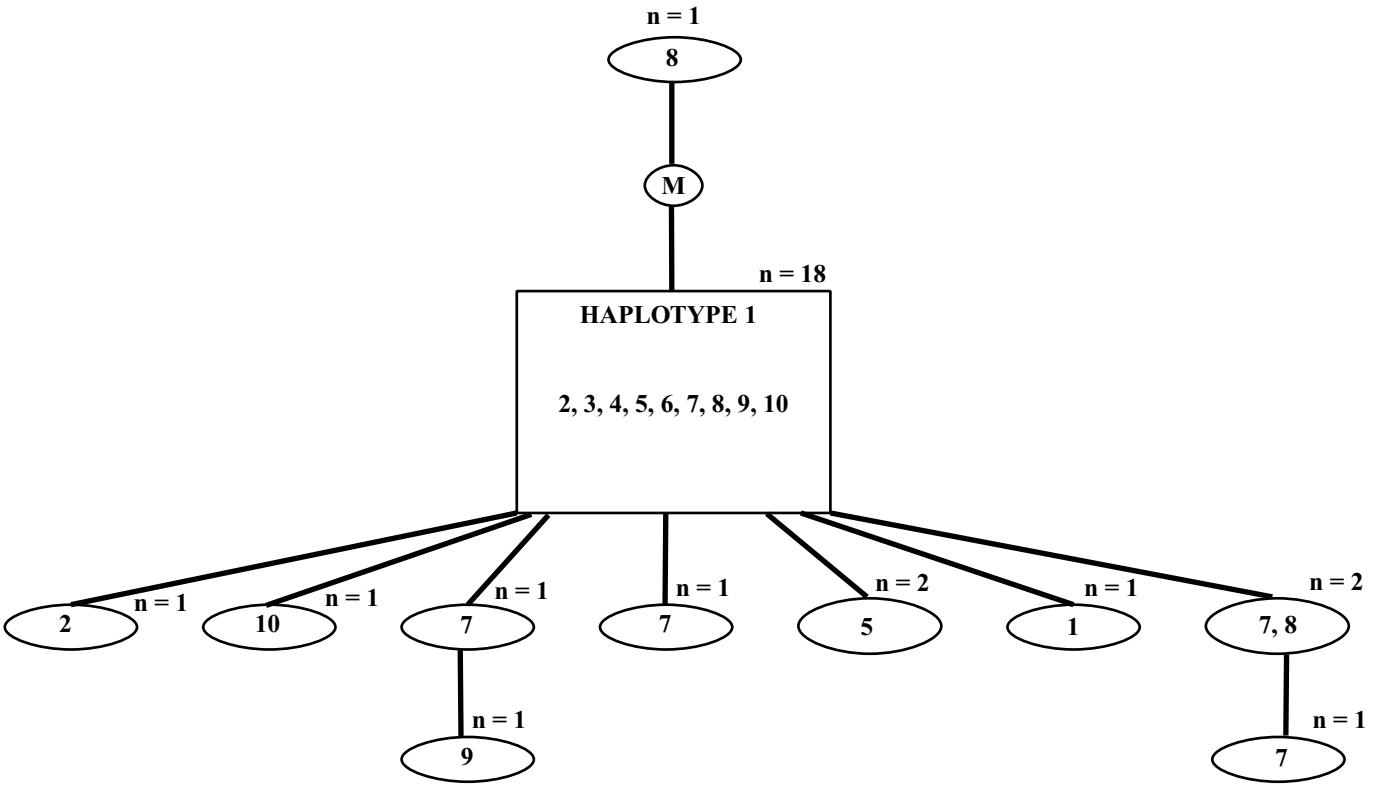

n = 1
8
M
n = 18
HAPLOTYPE 1
2, 3, 4, 5, 6, 7, 8, 9, 10
2
n = 1
10
n = 1
7
n = 1
9
n = 1
7
n = 1
5
n = 2
1
n = 1
7, 8
n = 2
7
n = 1

TABLE 4.1 - Geographically distributed genetic variability in *Xiphorhynchus kienerii* and *X. obsoletus* throughout Amazonia. Includes number of individuals and populations sampled, haplotype diversity (*h*), nucleotide diversity *(π)*, and results of Tajima's (1989) *D* test.

Species / Areas	**No. Individuals (Population [s] sampled)** [a]	**Haplotype diversity (h)±V(h)**	**Nucleotide diversity (π)±V(π)**	**Tajima's *D* test** [b]
X. kienerii				
Upper Amazonas	7 (7, 8)	0.28±0.19	$2.8\pm3.9 \bullet 10^{-4}$	- 1.00 N.S
Lower Japura	4 (6)	0.83±0.22	$4.9\pm6.1 \bullet 10^{-4}$	- 0.61 N.S.
Central Amazonia	6 (2 – 5)	0.73±0.15	$8.6\pm8.0 \bullet 10^{-4}$	- 0.05 N.S
Lower Amazonia	4 (1)	0	0	0
X. obsoletus				
Guyana	5 (10)	0.40±0.23	$4.0\pm5.0 \bullet 10^{-4}$	- 0.82 N.S
Pará / Belém	8 (1 – 3)	0.46±0.20	$5.0\pm5.3 \bullet 10^{-4}$	- 1.31 N.S
Rondônia	5 (4, 5)	0.60±0.17	$6.0\pm6.5 \bullet 10^{-4}$	1.22 N.S
Inambari	6 (6, 7)	0.93±0.12	$1.5\pm1.2 \bullet 10^{-3}$	- 0.67 N.S
Napo	6 (8, 9)	0.80±0.17	$1.6\pm1.3 \bullet 10^{-3}$	- 1.34 *

[a] See Figures 4.1 and 4.2 for geographic location of populations and areas of endemism.
[b] Tajima's *D* Neutrality test (1989); N.S., non-significant departure from neutrality (All $P > 0.12$); * marginally significant departure from neutrality at $P = 0.056$.

Results of Tajima's *D* tests showed that most populations of *X. kienerii* and *X. obsoletus* had non-significant negative values (Table 4.1). Only one population of *X. obsoletus* (Inambari) showed a marginal departure of neutrality (Table 4.1). 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 geographic scale (Rand 1996). Analyses of Molecular Variance (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 4.2).

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. 4.3; $P > 0.20$ for *X. kienerii* and $P > 0.80$ for *X. obsoletus*). Assuming a mitochondrial clocklike substitution rate of 2% per million years (Klicka and 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 1,500 and 15,500 years BP for *X. kienerii* and between the present and 18,000 years BP for *X. obsoletus* (Rogers 1995).

DISCUSSION

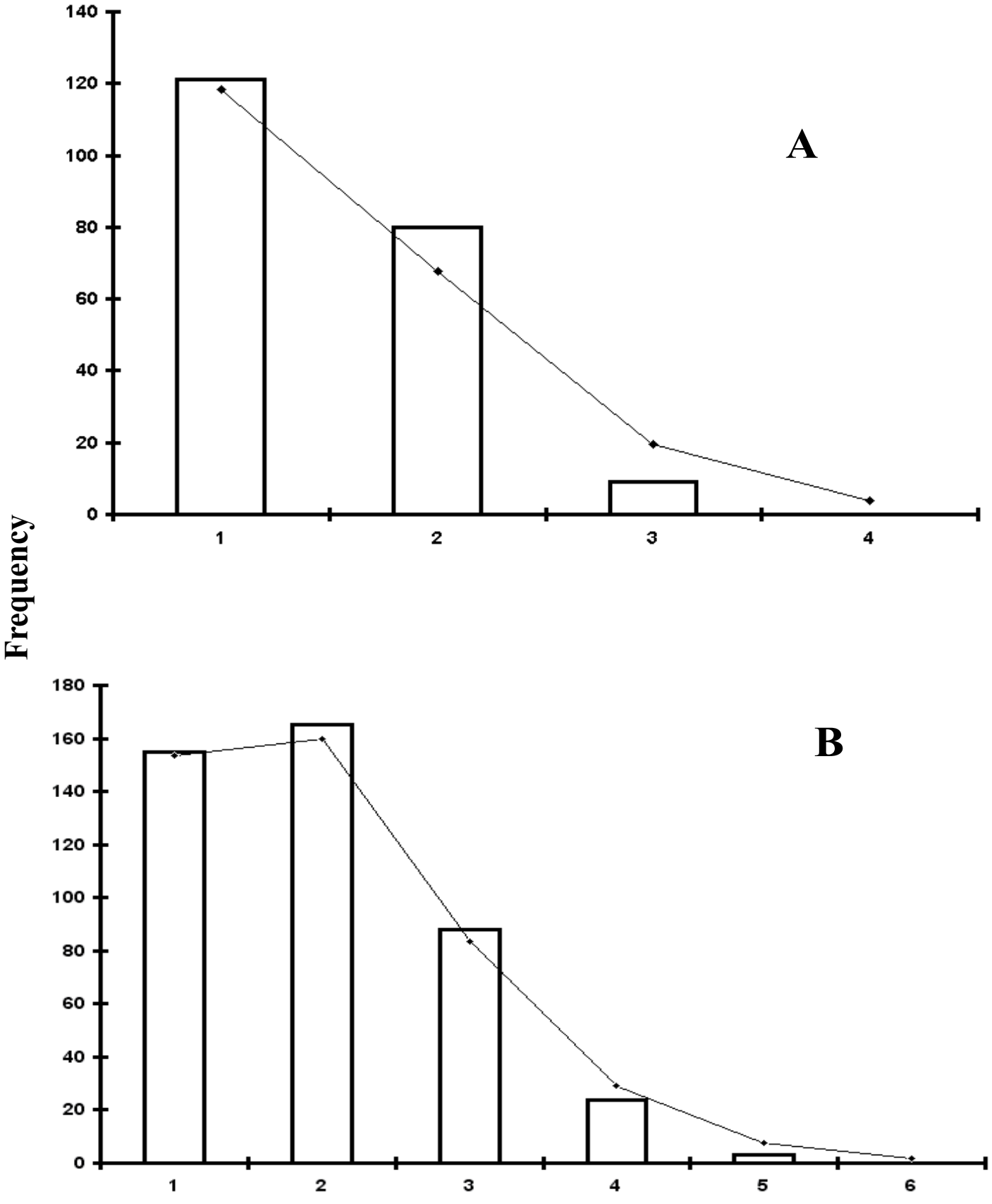
Population Structure of *Várzea* Species. – Both *X. kienerii* and *X. obsoletus* exhibited the very similar pattern of virtually no phylogeographic structure throughout their ranges.

TABLE 4.2 - Results from an analysis of molecular variance (AMOVA) among populations of *Xiphorhynchus kienerii* and *Xiphorhynchus obsoletus* distributed throughout Amazonia. See figures 1 and 2 for geographic locations of populations.

Species	No. of populations	Variation among populations (%) [a]	Variation within populations (%)
Xiphorhynchus kienerii	4	6.2 **	93.8
Xiphorhynchus obsoletus	5	4.6 *	95.4

[a] ** $P > 0.10$; * $P > 0.08$.

Figure 4.3. Pairwise nucleotide mismatch distributions for *X. kienerii* (A) and *X. obsoletus* (B). Histograms represent the observed differences, whereas thin lines indicate the ideal distribution predicted by the model of sudden population expansion developed by Rogers (1995).


A
B
Frequency
140
120
100
80
60
40
20
0
1
2
3
4
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
Number of pairwise differences (i)

This absence of phylogeographic structure fits the "category IV phylogeographic pattern" described by Avise (2000), in which closely related lineages of a shallow gene tree are broadly sympatric. This phylogeographic 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 4.1, Fig. 4.3), both *X. kienerii* and *X. obsoletus* probably have had small evolutionary effective population sizes but experienced a recent explosive demographic expansion (Rand 1996, Avise 2000). Other *várzea* vertebrate species for which phylogeographic surveys are available include rodents in the genera *Mesomys* and *Proechimys* (Patton et al. 1994, Matocq et al. 2000). These studies have documented the same "category IV phylogeographic pattern" (sensu Avise 2000) for these *várzea* species, with higher levels of gene flow and shallower gene trees than those recovered for other mammal *terra-firme* (unflooded) forest species (Peres et al. 1997, Patton et al. 1996, 2000). The same dichotomy in phylogeographic patterns detected for *várzea* and *terra-firme* species of mammals can be extended to birds in the genus *Xiphorhynchus*, because two of its *terra-firme* species (*X. spixii* and *X. elegans*) showed deep gene trees, with major lineages largely allopatric (Chapter 3).

Although populations of terrestrial mammals might be adversely affected by the annual floods occurring in *várzea* forests (Matocq et al. 2000), the same does not apply to arboreal bird species such as *X. kienerii* and *X. obsoletus*, which forage and roost in *várzea* forests even when flooded (pers. obs.). Therefore, high levels of gene flow among populations of *X. kienerii* and *X. obsoletus* can probably be explained by high dispersal capabilities associated with the narrow

shape and continuity of the *várzea* habitat in Amazonia. *Várzea* and other flooded forest types found in Amazonia occur only along rivers or their immediate influence and therefore are more limited in distribution than the more widespread *terra-firme* forest. Thus, when compared to *terra-firme* species, gene flow among populations of *várzea* specialist species can occur only through "corridors" of habitat paralleling the distribution of Amazonian rivers, which are ultimately all connected to the Amazon river. The continuity and "corridor" nature of the *várzea* habitat in Amazonia can thus explain why the expectation of finding genetically structured populations in different river basins was not fulfilled for *X. kienerii* or *X. obsoletus* (Figs. 4.1 and 4.2). As the haplotype networks showed (Figs. 4.1 and 4.2), 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 *várzea* forests found on both banks and on several islands of the Amazon river. This hypothesis can be tested in the future with faster genotypic markers such as microsatellites. Finally, two additional factors might promote admixture in populations of *várzea* species, especially between adjacent 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 *várzea* species (Patton et al. 2000).

The lack of population structure documented for *várzea* species in Amazonia (Patton et al. 1994, Matocq et al. 2000, this study), clearly indicates that populations of these species experienced historical and current high levels of gene flow probably associated with several

population bottlenecks, which may have cyclically erased genetic diversity. In the case of *X. kienerii* and *X. obsoletus*, mismatch distributions (Fig. 4.3) point to 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 since the Last Glacial Maximum (LGM), about 20,000 years BP (Irion et al. 2002). During the rapid sea-level rise in the early and mid-Holocene, the flow of the Amazon river system was gradually slowed down and dammed by the ocean. River stages increased significantly throughout Amazonia, causing stronger seasonal floods in a phenomenon called "damming back effect" (Irion et al. 2002). The "damming back effect" of the Amazon river system increased the area occupied by alluvial plains, therefore also causing an expansion of the *várzea* and other seasonally flooded forest types throughout Amazonia (Irion et al. 2002). Because sea-level changes have strongly affected the hydrology of the Amazon river system since the late Tertiary and early Quaternary, the extension of flooded forest habitats in Amazonia probably varied considerably and cyclically through time (Irion et al. 1995, 2002). Populations of *várzea* 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 population expansion in response to an increase in the area covered by alluvial plains (Irion et al. 2002). More studies on different lineages of *várzea* specialist species are needed to test the validity and generality of this hypothesis.

The western Amazonian lowlands could have been the source area for the last episode of population expansion inferred for *X. kienerii* and *X. obsoletus*. Recurrent periods of high sea-level stands affected the western Amazonian lowlands in a disproportionate way by creating larger

expanses of floodplains than in other, higher, parts of the basin such as the Brazilian and Guianan shields (Irion et al. 1995). Therefore, during periods of low sea-level stands, larger pockets of *várzea* forest might have remained in the poorly drained western lowlands than in other parts of Amazonia. Higher levels of haplotype and nucleotide diversity observed for *X. kienerii* and *X. obsoletus* in western Amazonia (Table 4.1) are consistent with this scenario. Ideally, this hypothesis could be tested with a better sampling and with more phylogenetically independent lineages.

Historical Diversification of *Várzea* Species – The low levels of phylogeographic differentiation found for *X. kienerii* and *X. obsoletus* pose a serious challenge to answering the question: what possible hypotheses can explain diversification in lineages of *várzea* species ? As expected, the riverine barrier hypothesis of Amazonian diversification (Gascon et al. 2000) cannot be invoked to explain diversification within lineages of *várzea* species. As noted in chapter 1, *várzea* species function as a control group when testing the riverine barrier hypothesis because these species inhabit riverine habitats along and on Amazonian rivers. The lack of phylogeographic structure observed for *X. kienerii, X. obsoletus*, and other *várzea* species (Patton et al. 1994, Matocq et al. 2000) is thus consistent with expectations of the riverine barrier hypothesis for these species.

In phylogenies estimated for the genus *Xiphorhynchus* (chapter 1), *X. kienerii* and *X. obsoletus* are found at the tip of long branches, and they are separated from their nearest relatives by large uncorrected sequence divergence values (*ca.* 8%), indicating a relatively older age compared to other species in the same genus. As also shown in chapter 1, cladogenesis in *terra-firme* lineages was far greater than in *várzea* lineages, which were found in ecologically diverse clades. Therefore, the phylogenetic positions of *X. kienerii* and *X. obsoletus* suggest that these

species occupied the *várzea* forest habitat early on during the first burst of diversification in the genus *Xiphorhynchus*. Since then, as suggested by low levels of population differentiation, historically high levels of gene flow associated with population bottlenecks prevented diversification and cladogenesis in *várzea* lineages of the genus *Xiphorhynchus*. Because the *várzea* and *terra-firme* habitats can be affected differently by the same mechanisms (e.g., changes in sea-level; see chapter 3), it is likely that *várzea* species will require a fundamentally different set of hypotheses than those proposed so far to explain the diversification of *terra-firme* species (e.g., riverine barrier, refuge, and basal trichotomy hypotheses).

CHAPTER 5. GENERAL DISCUSSION AND CONCLUSIONS

Phylogenetic and population genetics analyses of species in the genus *Xiphorhynchus* presented in the previous chapters provided important insight into the validity and generality of some hypotheses proposed to explain diversification among lineages of Amazonian organisms. Here, I discuss to what degree the predictions of these hypotheses were supported, and propose a few changes and additions aimed at improving them as models of diversification. Lastly, I discuss an integrated model to explain the diversification of both *várzea* and *terra-firme* species in Amazonia.

THE RIVERINE BARRIER HYPOTHESIS

The data presented in chapters 3 and 4 supported the predictions of the riverine barrier hypothesis only for: (1) *terra-firme* species and (2) populations inhabiting the Brazilian shield in the eastern part of Amazonia. As predicted (Capparella 1991, chapter 1), gene flow among populations of *várzea* species is not inhibited by rivers (or any other current physical barrier; chapter 4). *Terra-firme* species, on the other hand, can have gene flow among their populations inhibited by rivers, not only because of the width of many Amazonian rivers themselves, but also because of the lateral extension of the floodplain belt associated with them. The floodplain vegetation, from early successional scrub to tall flooded forest, is unsuitable habitat for *terra-firme* forest birds. Thus, rivers and its associated vegetation consist of physical and ecological barriers inhibiting gene flow between populations of *terra-firme* species from opposite river banks (Capparella 1991, Haffer 1992a, Chapter 3). Interestingly, data shown in chapter 3

indicated that patterns of gene flow in *terra-firme* species are not always affected by rivers and their associated floodplains. In the case of the *Xiphorhynchus spixii / elegans* superspecies, important western Amazonian rivers (Madeira, Purús, Juruá, and Ucayali) did not function as significant barriers inhibiting or impeding gene flow among populations from opposite river banks (Chapter 3). In contrast, rivers on the Brazilian shield did act as important barriers, coinciding with areas of primary divergence between sister lineages of *X. spixii* (Xingú river), and between the two main clades in the *X. spixii / elegans* superspecies: *X. spixii* and *X. elegans* (Tapajós river). No evidence of gene flow between these sister clades was found even at the headwaters of the Xingú and Tapajós rivers, where the width and associated barrier effect of these rivers are presumably smaller (Haffer 1992a). Furthermore, as anticipated by the riverine barrier hypothesis, the split between the *X. elegans* and *X. spixii* clades on the Brazilian shield was probably completed by 3 million years BP (Chapter 3), thus after the completion of the development of the Amazon river system as a transcontinental drainage in the late Miocene (Hoorn et al. 1995). As discussed in chapter 3, the younger age and more dynamic nature of the western Amazonian floodplains could be responsible for these contrasting patterns of barrier effect promoted by rivers in different parts of Amazonia (Ayres and Clutton-Brock 1992, Colwell 2000, Gascon et al. 2000).

The predictability of the riverine hypothesis can be improved if more phylogeographic studies show that white water Amazonian tributaries originating in the Andean slopes of western Amazonia do not act as important barriers restraining gene flow between populations of *terra-firme* species, and therefore are not important in promoting diversification within these lineages (see review in Gascon et al. 2000). As Colwell (2000) and data in chapter 3 implied, the

geological setting of a river's location may be the best predictor of its importance as a barrier to gene flow for populations of *terra-firme* species. Another prediction that can further refine the riverine barrier hypothesis is that rivers in geologically older and more stable parts of Amazonia (such as the Brazilian and Guianan shields) will function as important barriers restraining or impeding gene flow between sister lineages of *terra-firme* species, thus contributing to diversification in these lineages (Ayres and Clutton-Brock 1992, Haffer 1992a, Colwell 2000). An independent study on *Callithrix* marmosets also seemed to support an important role for rivers on the Brazilian shield in promoting population differentiation and speciation in lineages of *terra-firme* species (Roosmalen et al. 2000).

THE REFUGE HYPOTHESIS

Data presented in chapters 3 and 4 showed that populations of both *várzea* and *terra-firme* species of *Xiphorhynchus* probably experienced periods of population bottlenecks and expansion. According to the refuge hypothesis, under a population genetics framework, population bottlenecks are expected during periods of forest contraction (after the onset of dry climatic conditions), whereas population expansion is predicted during periods of forest expansion, following humid climatic conditions (Capparella 1991). The refuge hypothesis probably cannot be applied to *várzea* species, because gallery forests, bordering Amazonian rivers, probably remained intact even under dry climactic conditions (Haffer and Prance 2001), in a situation analogous to the one observed today in the *cerrados* of central Brazil and eastern Bolivia. Indeed, shallow haplotype divergences and a very low geographic population differentiation detected for both *várzea* species of *Xiphorhynchus* seem to indicate that these

species have not experienced historical impediments to gene flow at a large geographical scale (Chapter 4). Therefore, the population expansion estimated for *X. kienerii* and *X. obsoletus* to have taken place during the last 18,000 years BP was probably not influenced by a period of rainforest expansion, as envisioned by the refuge hypothesis (Haffer and Prance 2001). Instead, as discussed in chapter 4, these demographic expansions are more logically correlated with an increase in the area covered by floodplains in Amazonia, following a period of continuous sea level rise.

Terra-firme species, on the other hand, inhabit the forest away from the influence of major Amazonian rivers and can be affected by a reduction or replacement of rainforest by drier, more open forest types, as postulated by the refuge hypothesis (Haffer and Prance 2001). Population bottlenecks and a fairly recent range expansion were inferred for *X. spixii* (a *terra-firme* species) in eastern Amazonia (Chapter 3). The time frame of this demographic expansion (between 5,000 and 70,000 years BP) coincided with a period of at least four inferred episodes of forest regression and expansion in eastern Amazonia (Absy et al. 1991), followed by the return of humid climate conditions (starting about 10,000 years BP), which led to a re-expansion of humid forest types (Van der Hammen and Absy 1994). However, according to paleoecological data, rainforest may have also been disrupted by drier vegetation types in the western portion of the Brazilian shield (Van der Hammen and Absy 1994), where populations of *X. e. elegans* failed to exhibit signs of low genetic variability (expected during episodes of population bottleneck), or a recent sudden demographic expansion (Chapter 3), as expected under the refuge hypothesis. Therefore, the only way to reconcile this pattern with the refuge hypothesis is by postulating that climatic vegetational changes were much stronger in the eastern than in the western part of

the Brazilian shield, therefore substantially undermining the importance of the refuge hypothesis in accounting for the diversification of the entire Amazonian *terra-firme* forest biota. However, this regional difference in the impact of climatic vegetational changes is unlikely given the current available evidence for southern Amazonia as a whole (Van der Hammen and Absy 1994, Mayle et al. 2000).

Unfortunately, it is still very difficult to develop phylogeographic predictions based on the refuge hypothesis (Chapter 1), but population genetics studies can potentially address directly some of its key predictions. Hence, more population genetics studies are needed to document the existence, timing, and location of population bottlenecks and demographic expansions of *terra-firme* species in Amazonia. More paleoecological data are also needed to evaluate the impact of drier climates on the rainforest biota, and especially to clarify whether it was fragmented by open savanna or dry forest types (Haffer and Prance 2001). When these data become available, a better assessment of the validity and generality of the refuge hypothesis will be possible.

THE GRADIENT HYPOTHESIS

Phylogenies estimated for species in the genus *Xiphorhynchus* did not recover a single sister relationship between *várzea* and *terra-firme* species, as expected if the *várzea – terra-firme* ecotone contributed to the recent diversification of *Xiphorhynchus* (Chapter 2). Most phylogenetic studies testing the gradient hypothesis for tropical vertebrate taxa failed to verify its predictions (Mayr and O'Hara 1986, Cracraft and Prum 1988, Patton and Smith 1992, Arctander and Fjeldså 1994). However, recently the gradient hypothesis of parapatric speciation

was invoked to explain genetic and morphometric differentiation in several lineages of African and Australian vertebrates, even though direct evidence showing that sympatric speciation was responsible for the splitting within those lineages was lacking (Smith et al. 2001). Even if corroborated in some cases, the falsification of the gradient hypothesis (in its *várzea – terra-firme* version) by phylogenies of the genus *Xiphorhynchus* agrees with the overall consensus of failing to find support for this hypothesis in accounting for the diversification of tropical vertebrates (Haffer 1997a).

THE BASAL TRICHOTOMY HYPOTHESIS

Phylogenetic and population genetics analyses of the *Xiphorhynchus spixii / elegans* superspecies supported key predictions of the basal trichotomy hypothesis (Chapter 3). The most important one was that the Brazilian shield in central and eastern Amazonia represented the main area of population diversification and speciation for the *X. spixii / elegans* superspecies, in addition to being the source area for the colonization of the western Amazonian lowlands by populations of this superspecies (Chapter 3). This pattern is expected under the basal trichotomy hypotheses because the Brazilian shield is thought to have escaped the influence of marine invasions or a "damming back effect" of the Amazon river during periods of high sea level stands (Räsänen et al. 1995, Bates in press, Irion et al. 2002). The western Amazonian lowlands, on the other hand, because of their extremely low elevation and poor draining conditions, may have been affected disproportionately by periods of high sea levels, which led to the disruption of rainforest by a mangrove-like vegetation (in the case of a marine incursion; Hoorn 1994), or by seasonally flooded forest types (in the case of a "damming back effect" of the Amazon river system; Irion et

al. 2002). An estimate of the timing of the colonization of western Amazonia by populations of the *X. spixii / elegans* superspecies indicated that it started too late (Middle Pleistocene) to be explained by the end of a period of direct marine influence in western Amazonia (Chapter 3, Hoorn et al. 1995). Nevertheless, massive marine incursions into western Amazonia as early as the Early and Middle Miocene (Hoorn et al. 1995) could have separated sister superspecies of *Xiphorhynchus*, such as the *X. pardalotus / ocellatus* and *X. spixii / elegans* superspecies (Chapter 1). These two currently sympatric superspecies diverged by 12.4% in their cytochrome *b* sequences (Tamura and Nei distance with a gamma shape parameter of $\alpha = 0.15$; chapter 3), which translates to a split dating back to about 6 million years (assuming a clocklike rate of mtDNA substitution of 2% per million years; Klicka and Zink 1997). Phylogeographic and population genetics analyses of the *X. pardalotus / ocellatus* superspecies could potentially reveal whether populations of this superspecies from the Brazilian shield (inferred as the place of origin for its sister superspecies, *X. spixii / elegans*) colonized this area more recently, either from the Guianan shield or the eastern base of the Andes, two other centers of vicariance according to the basal trichotomy hypothesis (Bates in press). Distributional data indicate that populations of *X. ocellatus* from the Brazilian shield are scarce and locally distributed, whereas those in western Amazonia and Andean foothills are more common and widespread, the same also occurring with populations of *X. pardalotus* from the Guianan shield.

Therefore, I suggest the following two additions to the basal tricotomy hypothesis aimed at improving its predictability and generalization power. The first addition is to acknowledge that different lineages will differ in their areas of diversification and vicariance. Thus, as shown in

chapter 3, the Brazilian shield, Guianan shield, and eastern base of the Andes will be areas of primary differentiation for some lineages, but areas colonized more recently by younger populations of other lineages. This apparently simple suggestion was absent from the original formulation of the basal trichotomy hypothesis (Bates in press), probably because this hypothesis was based primarily on area cladistics, which treats dispersal as homoplasy, and therefore discards it from the inference of area cladograms. The second addition is to propose that mechanisms other than marine incursions can be also invoked as vicariant mechanisms behind the basal trichotomy hypothesis (Bates in press). For example, periods of high-sea level have been suggested to have strongly influenced the Amazon river system, promoting the so-called "damming back effect" and an associated back flooding of the Amazon river drainage (Irion et al. 2002). This "damming back effect" apparently affected the Amazon river valley and the western Amazonian lowlands more strongly than higher and hilly areas in Amazonia, such as the Brazilian and Guianan shields. I further suggest that although Early and Middle Miocene marine incursions could account for deeper splits between currently sympatric superspecies, periods of high sea level promoting the "damming back effect" in the Amazon river drainage could explain splits between closely related taxa within superspecies of Amazonian *terra-firme* birds.

AN INTEGRATED MODEL OF DIVERSIFICATION FOR *VÁRZEA* AND *TERRA-FIRME* SPECIES IN AMAZONIA

Of the four hypotheses of diversification addressed herein, three had some of their important predictions supported: the riverine barrier, the refuge, and the basal trichotomy hypotheses. Hence, these three hypotheses appear not to be mutually exclusive and may together account for

the diversification of the genus *Xiphorhynchus* in Amazonia at different temporal and geographical scales. As discussed earlier, these three hypotheses of diversification are readily applicable to *terra-firme* species but not to *várzea* species.

As suggested by phylogenetic and population genetics analyses of *várzea* species of *Xiphorhynchus* (chapter 4), I propose that the extensive floodplains of western Amazonia were refuges for *várzea* species during periods of low sea level and strong floodplain erosion throughout Amazonia (Irion et al. 2002). Thus, the western Amazonian lowlands were the source area for a recent colonization of the entire Amazon basin by *várzea* species, starting after the onset of periods of increasing high sea level during the LGM (20,000 years BP). This hypothesis can be tested with additional phylogeographic and population genetics assessments of different lineages of *várzea* species throughout Amazonia. The distribution and dynamics of the *várzea* and other flooded forest types in Amazonia is not conducive to population subdivision even at large time scales (chapter 4). Therefore, I suggest that extant lineages of *várzea* species will show little population subdivision and small rates of cladogenesis, being "relicts" of early radiations of widespread Neotropical lineages.

Although the extensive floodplains of western Amazonia might have functioned as refuges for *várzea* species, they constituted an inhospitable habitat to *terra-firme* species. Therefore, I predict that lineages of *terra-firme* species inhabiting western Amazonia will have colonized this area fairly recently (during the Middle and Late Pleistocene) from one of the three areas inferred as centers of vicariance by the basal trichotomy hypothesis: the Brazilian shield, the Guianan shield, or the eastern slopes of the Andes (Bates in press). Another expectation is that deeper splits in currently sympatric lineages of Amazonian *terra-firme* superspecies (predicted to date

back to the Late Miocene) will be consistent with expectations of the basal trichotomy hypothesis, in which sister superspecies will differ in their areas of origin and diversification. Main splits between allopatric taxa of *terra-firme* superspecies should date back to the Pliocene and are also expected to follow area relationships expected under the basal trichotomy hypothesis, or alternatively, to be consistent with the riverine barrier hypothesis in geologically older and more stable parts of Amazonia such as the Brazilian and Guianan shields. Finally, shallower levels of divergence in Amazonian *terra-firme* superspecies could be consistent with a multiple chain of events, including an invasion and rapid colonization of the western Amazonian lowlands by lineages experiencing cycles of population bottlenecks and demographic expansions (chapter 3), or with isolation by distance mechanisms, which can be enhanced by rivers or by climatic vegetational changes, as postulated by the refuge hypothesis.

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APPENDIX 1: VOUCHER INFORMATION FOR TISSUE SAMPLES USED IN CHAPTER 2

Taxon	Voucher institution [a]	Voucher number [b]	Tissue institution [a]	Collection Locality	GenBank accession numbers
Glyphorynchus spirurus	MPEG	JDW 445	LSUMZ	Bahia, Brazil	AY089806, AY089833, AY089890
Sittasomus griseicapillus	CBF	SWC 6769	LSUMZ	La Paz, Bolivia	AY089796, AY089834, AY089894
Nasica longirostris	LSUMZ	115014	LSUMZ	Loreto, Peru	AY089797, AY089835, AY089880
Dendrocolaptes certhia	MHNJP	DLD 133	LSUMZ	Loreto, Peru	AY089817, AY089856, AY089917
Lepidocolaptes albolineatus	LSUMZ	153311	LSUMZ	Santa Cruz, Bolivia	AY089825, AY089865, AY089876
Lepidocolaptes angustirostris	MHNNKM	MDC 363	LSUMZ	Santa Cruz, Bolivia	AY089811, AY089838, AY089881
Lepidocolaptes fuscus	MPEG	AA 568	LSUMZ	Bahia, Brazil	AY089819, AY089851, AY089904
Campyloramphus trochilirostris	LSUMZ	153671	LSUMZ	Santa Cruz, Bolivia	AY089822, AY089857, AY089906
Campyloramphus procurvoides	FMNH	DEW 2685	LSUMZ	Amazonas, Venezuela	AY089795, AY089836, AY089903
Campyloramphus falcularius	MZUSP	LFS 99/378	MZUSP	Bahia, Brazil	AY089810, AY089837, AY089905
Hylexetastes perrotii	LSUMZ	150674	LSUMZ	Santa Cruz, Bolivia	AY089809, AY089873, AY089916
Xiphocolaptes promeropirhynchus	LSUMZ	CCW 718	LSUMZ	Cajamarca, Peru	AY089798, AY089872, AY089907
Dendrexetastes rufigula	MHNJP	SWC 2358	LSUMZ	Loreto, Peru	AY089829, AY089839, AY089902
X. erythropygius aequatorialis	ANSP	FHS 85	LSUMZ	Pichincha, Ecuador	AY089832, AY089847, AY089879
X. e. insolitus [c]	LSUMZ	163547	LSUMZ	Chiriqui, Panama	AY089830, AY089858, AY089898
X. flavigaster eburneirostris	FMNH	DSW 2986	LSUMZ	Toledo district, Belize	AY089799, AY089871, AY089912
X. flavigaster flavigaster [c]	FMNH	394017	FMNH	Oaxaca, Mexico	AY089828, AY089849, AY089896
X. guttatus guttatus	MPEG	AA 570	LSUMZ	Bahia, Brazil	AY089808, AY089869, AY089908
X. g. eytoni	MPEG	MR-003	LSUMZ	Pará, Brazil	AY089794, AY089845, AY089884
X. g. dorbigignyanus	LSUMZ	153308	LSUMZ	Santa Cruz, Bolivia	AY089816, AY089840, AY089891
X. g. guttatoides	MPEG	AA 611	LSUMZ	Amazonas, Brazil	AY089792, AY089855, AY089892
X. g. guttatoides	MPEG	AA 695	LSUMZ	Amazonas, Brazil	AY089791, AY089866, AY089882
X. g. polystictus	MPEG	Ch202	FMNH	Amapá, Brazil	AY089814, AY089843, AY089887
X. g. vicinalis	MPEG	SML86-140	FMNH	Rondônia, Brazil	AY089803, AY089850, AY089888
X. kienerii	LSUMZ	165752	LSUMZ	Amazonas, Brazil	AY089818, AY089862, AY089911

X. lachrymosus	ANSP	185351	ANSP	Esmeraldas, Ecuador	AY089807, AY089870, AY089900
X. obsoletus obsoletus	ANSP	188595	ANSP	Iwokrama, Guyana	AY089823, AY089868, AY089913
X. ocellatus chunchotambo	LSUMZ	161705	LSUMZ	Loreto, Peru	AY089815, AY089844, AY089915
X. o. brevirostris	LSUMZ	101904	LSUMZ	La Paz, Bolivia	AY089793, AY089846, AY089885
X. o. ocellatus	MPEG	AA 581	LSUMZ	Pará, Brazil	AY089804, AY089861, AY089909
X. o. weddellii	LSUMZ	119520	LSUMZ	Loreto, Peru	AY089820, AY089859, AY089878
X. pardalotus	MPEG	AA 602	LSUMZ	Pará, Brazil	AY089831, AY089848, AY089910
X. picus picus	MPEG	MCH 362	LSUMZ	Amazonas, Brazil	AY089813, AY089867, AY089901
X. p. altirostris	___[d]	___[d]	STRI	Island of Trinidad	AY089790, AY089853, AY089877
X. p. bahiae	MPEG	AA 560	LSUMZ	Bahia, Brazil	AY089821, AY089860, AY089886
X. p. phalara[c]	___[d]	___[d]	STRI	Venezuela	AY089802, AY089854, AY089893
X. spixii elegans	MPEG	AA 290	LSUMZ	Rondônia, Brazil	AY089805, AY089852, AY089899
X. s. juruanus	MPEG	AA 236	LSUMZ	Rondônia, Brazil	AY089824, AY089874, AY089883
X. s. ornatus	LSUMZ	109706	LSUMZ	Loreto, Peru	AY089812, AY089841, AY089889
X. s. spixii	MPEG	MR-002	LSUMZ	Pará, Brazil	AY089801, AY089875, AY089897
X. susurrans	LSUMZ	163545	LSUMZ	Panamá, Panamá	AY089800, AY089863, AY089914
X. triangularis bangsi	LSUMZ	162637	LSUMZ	La Paz , Bolivia	AY089826, AY089864, AY089918
X. t. intermedius	LSUMZ	105872	LSUMZ	Pasco, Peru	AY089827, AY089842, AY089895

[a] ANSP = Academy of Natural Sciences, Philadelphia; CBF = Colección Boliviana de Fauna, Museo Nacional, La Paz, Bolivia; FMNH = Field Museum of Natural History, Chicago; LSUMZ = Louisiana State University Museum of Natural Science, Baton Rouge; MHNJP = Museo de Historia Natural Javier Prado, Lima, Peru; MHNNKM = Museo de Historia Natural Noel Kempff Mercado, Santa Cruz, Bolivia; MPEG = Museu Paraense Emílio Goeldi, Belém, Brazil; MZUSP = Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil; STRI = Smithsonian Tropical Research Institute, Balboa, Panamá.

[b] When specimens have not been catalogued, collector or field numbers are provided.

[c] Tentative subspecific identification because of incomplete locality data or questionable taxon diagnosis.

[d] No voucher specimen collected.

APPENDIX 2: COLLECTING LOCALITIES, SAMPLE SIZES, AND VOUCHER INFORMATION FOR POPULATIONS AND SPECIMENS OF *XIPHORHYNCHUS SPIXII* AND *XIPHORHYNCHUS ELEGANS*. NUMBERS ABOVE COLLECTING LOCALITIES REFER TO AREAS ON MAP IN FIGURE 3.2. SUBSPECIFIC IDENTIFICATIONS BASED ON VOUCHER'S PHENOTYPIC (MOSTLY PLUMAGE) CHARACTERS

Xiphorhynchus spixii

1
Brazil: Pará, 40 km NE Belém, 01º12´S, 48º14´W
(n = 3)
LSUMZ [a] 35542 [b]
LSUMZ 35541
MPEG 49271 [c]

2
Brazil: Pará, Ipixuna
(n = 1)
MPEG 51965 [c]

3
Brazil: Pará, Serra dos Carajás
(n = 1)
FMNH CA 088

4
Brazil: Pará, Estação Ecológica de Caxiuanã
(n = 2)
FMNH SA 047
FMNH SA 012

5
Brazil: Pará: Santana do Araguaia, Fazenda Fartura
(n = 4)
MPEG 48644 [c]
MPEG 48645 [c]
MPEG 48647 [c]
MPEG 48648 [c]

6
Brazil: Mato Grosso: S. bank of Rio Cristalino, 33 km NE of Alta Floresta, 09º37´S, 55º55´W (n = 5)
LSUMZ 35438
LSUMZ 35447
LSUMZ 35513
LSUMZ 35514
LSUMZ 35455

7
Brazil: Pará: 126 km NW of Alta Floresta, S. bank of Rio São Benedito, 09º06´S, 56º56´W (n = 5)
LSUMZ 35282
LSUMZ 35294
LSUMZ 35287
LSUMZ 35313
LSUMZ 35314

Xiphorhynchus elegans elegans

8
Brazil: Mato Grosso: W. bank of Rio Teles Pires, 32 km NE of Alta Floresta (n = 6)
LSUMZ 35347
LSUMZ 35472
LSUMZ 35490
LSUMZ 35495
LSUMZ 35496
LSUMZ 35516

9
Bolivia: depto. Santa Cruz: prov. Velasco: W. bank Río Paucerna, 4 km upstream from Río Itenez (n = 5)
LSUMZ 12785
LSUMZ 12962
LSUMZ 12825
LSUMZ 12713
LSUMZ 12685

10
Brazil: Rondônia: REBIO Ouro Preto, ca. 70 km E. Guajará-Mirim
(n = 6)
LSUMZ 31384
LSUMZ 36796
LSUMZ 36772
LSUMZ 36745
LSUMZ 36731
LSUMZ 36649

11
Brazil: Amazonas: Fazenda Toshiba, 8 km NE Careiro; 03º47´S, 60º17´W
(n = 5)
LSUMZ 35655
LSUMZ 35647
LSUMZ 35648
LSUMZ 35649
LSUMZ 35656

Xiphorhynchus elegans juruanus

12
Brazil: Rondônia: 50 km NW of Jaci Paraná, W. bank of Rio Madeira
(n = 1)
LSUMZ 31332

13
Bolivia: depto. Pando: prov. Nicolas Suarez, 12 km S. of Cobija, 8 km W on road to Mucden
(n = 5)
LSUMZ 9116
LSUMZ 9090
LSUMZ 9076
LSUMZ 8891
LSUMZ 8846

14
Brazil: Amazonas: Margem S Rio Solimões, 13.5 km E São Paulo de Olivença; 03º27´S, 68º49´W
(n = 5)
LSUMZ 35707
LSUMZ 35709
LSUMZ 35710
LSUMZ 35711
LSUMZ 35712

15
Peru: depto. Ucayali: W. bank of Río Shesha, 65 km ENE Pucallpa
(n = 5)
LSUMZ 10600
LSUMZ 10599
LSUMZ 10597
LSUMZ 10562
LSUMZ 10536

16
Peru: depto. Loreto: S. Río Amazonas, ca 10 km SSW mouth Río Napo
(n = 1)
LSUMZ 4607

Xiphorhynchus elegans insiginis

17
Peru: depto. Loreto: NE bank Río Cushbatay, 84 km WNW Contamana, 07º09´S, 75º44´W
(n = 5)
LSUMZ 27406
LSUMZ 27459
LSUMZ 27460
LSUMZ 27550
LSUMZ 27620

18
Peru: depto. Loreto: S. bank Maranon river along Samiria river, est. biol. Pithecia
(n = 2)
LSUMZ 103543
LSUMZ 103554

Xiphorhynchus elegans ornatus

19
Peru: depto. Loreto, ca. 54 km NNW mouth Río Morona on W bank, 140 m, 04º16´S, 77º14´W
(n = 4)
LSUMZ 42758
LSUMZ 42841
LSUMZ 42949
LSUMZ 42729

20
Peru: depto. Loreto: Lower Río Napo region, E. bank Río Yanayacu, ca. 90 km N. Iquitos
(n = 7)
LSUMZ 4171
LSUMZ 4198
LSUMZ 4212
LSUMZ 4165
LSUMZ 4193
LSUMZ 7179
LSUMZ 2750

21
Ecuador: Napo, Río Pacayacu, 6 km up from Río Aguarico
(n = 1)
ANSP 4783

22
Brazil: Amazonas: Margem N Rio Solimões, ca. 4.5 km NE São Paulo de Olivença; 03º25´S, 68º57´W
(n = 1)
LSUMZ 35681

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[a] Voucher and tissue institutions. ANSP = Academy of Natural Sciences, Philadelphia; FMNH = Field Museum of Natural History, Chicago; LSUMZ = Louisiana State University Museum of Natural Science, Baton Rouge; MPEG = Museu Paraense Emílio Goeldi, Belém, Pará, Brazil.
[b] Tissue number.
[c] Sequences obtained from dry skin samples.

APPENDIX 3: COLLECTING LOCALITIES, SAMPLE SIZES, AND VOUCHER INFORMATION FOR POPULATIONS AND SPECIMENS OF *XIPHORHYNCHUS KIENERII* AND *XIPHORHYNCHUS OBSOLETUS*. NUMBERS ABOVE COLLECTING LOCALITIES REFER TO AREAS ON MAPS IN FIGURES 4.1 AND 4.2

Xiphorhynchus kienerii

1
Brazil: Pará: Lago do Maicá; 11.3 km SE Santarém; Margem S Rio Amazonas; 02º28´S, 54º38´W
(n = 4)
LSUMZ [a] 35627 [b]
LSUMZ 35628
LSUMZ 35630
LSUMZ 35632

2
Brazil: Amazonas: Rio Amazonas, Ilha do Carreiro, ca. 20 km E Manaus
(n = 1)
LSUMZ 25413

3
Brazil: Amazonas: Igarapé Panelão, 6 km W Careiro; 03º50´S, 60º30´W
(n = 3)
LSUMZ 35658
LSUMZ 35659
LSUMZ 35662

4
Brazil: Amazonas: Novo Airão, Anavilhanas
(n = 1)
LSUMZ 20237

5
Brazil: Amazonas: Parque Nacional do Jaú, Ilha do Caroçal (island at the mouth of the Jaú river)
(n = 1)
LSUMZ 20237

6
Brazil: Amazonas: Maraã, S Bank of Rio Japurá
(n = 4)
MPEG [c] 43117
MPEG [c] 43114
MPEG [c] 43115
MPEG [c] 43116

7
Brazil: Amazonas: N. bank of the Amazon river, ca. 4.5 km NE São Paulo de Olivença; 03º25´S, 68º57´W
(n = 4)
LSUMZ 35692
LSUMZ 35693
LSUMZ 35723
LSUMZ 35724

8
Peru: Loreto Department: River island 8 km downstream from Iquitos in Río Amazonas, 03º41´S, 73º12´W
(n = 3)
LSUMZ 29022
LSUMZ 29023
LSUMZ 29016

Xiphorhynchus obsoletus

1
Brazil: Pará: Belém, Mata do Mocambo, EMBRAPA
(n = 1)
LSUMZ 35670

2
Brazil: Pará: 113 km SWW Santarém; Alto Rio Arapiuns; 02º44´S, 55º41´W
(n = 5)
LSUMZ 35585
LSUMZ 35620
LSUMZ 35592
LSUMZ 35593
LSUMZ 35595

3
Brazil: Pará: Island on the Rio Teles Pires, 6.1 km downriver from the mouth of Rio São Benedito, 09º02´S, 57º05´W
(n = 1)
LSUMZ 35388

4
Brazil: Mato Grosso: W. bank of Rio Teles Pires, 33 km NE of Alta Floresta
(n = 1)
LSUMZ 35501

5
Bolivia: depto. Santa Cruz: prov. Velasco; W. bank Río Paucerna, 4 km upstream from Río Itenez
(n = 5)
LSUMZ 12752
LSUMZ 12934
LSUMZ 12885
LSUMZ 12740
LSUMZ 12729

6
Brazil: Amazonas: Igarapé Panelão, 6 km W Careiro; 03º50´S, 60º30´W
(n = 1)
LSUMZ 35660

7
Brazil: Amazonas: ca. 4.5 km NE São Paulo de Olivença; 03º25´S, 68º57´W
(n = 5)
LSUMZ 35682
LSUMZ 35683
LSUMZ 35690
LSUMZ 35691
LSUMZ 35732

8
Ecuador: Sucumbíos; Imura Cocha
(n = 3)
ANSP 3231
ANSP 3174
ANSP 3183

9
Peru: depto. Loreto: Lower Río Napo region, E. bank Río Yanayacu, ca. 90 km N. Iquitos
(n = 3)
LSUMZ 4396
LSUMZ 4361
LSUMZ 4192

10
Guyana: Iwokrama Reserve, Kabocalli Landing, 04º17´N, 58º31´W
(n = 5)
ANSP 7965
ANSP 8212
ANSP 8572
ANSP 8569
ANSP 8688

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[a] Voucher and tissue institutions. ANSP = Academy of Natural Sciences, Philadelphia; LSUMZ = Louisiana State University Museum of Natural Science, Baton Rouge; MPEG = Museu Paraense Emílio Goeldi, Belém, Pará, Brazil.
[b] Tissue number.
[c] Sequences obtained from dry skin samples.

VITA

Alexandre Luis Padovan Aleixo was born in Ribeirão Preto, São Paulo State, Brazil, on August 27, 1973, to Luiz Manoel Aleixo and Odila Aparecida Padovan Aleixo. He attended elementary, middle, and high schools in Campinas (also in São Paulo State, Brazil), graduating from high school in December 1991. He got a bachelor's degree in biology from Universidade Estadual de Campinas (UNICAMP) in December 1995, and started a master's program in ecology at the same university on the following year. He defended his master's thesis on the impact of selective logging on bird communities in the Brazilian Atlantic forest in July 1997, and in August of the same year moved to the United States to begin his doctoral studies at Louisiana State University. He hopes to earn the degree of Doctor of Philosophy in the summer of 2002, defending a dissertation on the evolution of Amazonian birds of the genus *Xiphorhynchus*. He plans on returning to his native Brazil and to continue research on Neotropical avian molecular systematics and Amazonian biogeography.