Molecular phylogeography and the evolution and conservation of Amazonian mammals

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Abstract

The phylogeographic structure of 15 genera of Amazonian marsupials and rodents is summarized based on comparative sequence of the mitochondrial cytochrome *b* gene. The data are limited in geographical coverage, with samples widely scattered throughout Amazonia from the base of the Andes in Peru to the Guianan coast and eastern Brazil. We use this approach to define species boundaries, based minimally on the principle of reciprocal monophyly, in conjunction with morphological or other genetic discontinuities. The taxa so defined are older than previously appreciated, with many lineages dating from 1 to more than 3 Myr, and thus apparently predating the early Pleistocene. We relate patterns of concordant geographical shifts with underlying tectonic history and to current positions of major rivers. Finally, we provide comments on the utility of these data and patterns to conservation, articulating a need to incorporate phylogeographic information as part of the rationale in establishing conservation priorities at the organismal and geographical area levels.

Keywords: Amazonia, cytochrome *b*, mammals, mtDNA, phylogeography

Introduction

The mammalian fauna of the lowland neotropical forests is perhaps the richest in the world, yet remains poorly known. Gaps in our knowledge are substantial, and include the basic elements of species boundaries, geographical and habitat ranges, phylogenetic relationships, and population ecologies (see, for example, Voss & Emmons 1996). As a consequence, mammals have not figured importantly in any debate concerning Amazonian evolutionary diversification processes, with the exception of Alfred Russel Wallace's (1852) emphasis on riverine barriers and primate distribution patterns.

Here we examine the value of molecular phylogeographic studies for understanding the diversification of Amazonian mammals. Following Avise *et al.* (1987), we view 'phylogeography' as the geographical distribution of genealogical lineages, especially including those at the intraspecific level. Despite increased recent focus on Amazonian floral and faunal diversity, and general interest

in tropical forest conservation, very few phylogeographic studies are available for any Amazonian organism, plant or animal. We use our own work on terrestrial and arboreal marsupials and rodents to detail the implications of phylogeographic studies to each of four conceptual areas: (i) the definition of species boundaries; (ii) the determination of lineage ages; (iii) the delineation of regional areas of reciprocally monophyletic clades within Amazonia, and, consequently, the evaluation of processes of diversification; and (iv) the implications of such data for current and proposed conservation priorities. Aside from the intellectual and academic interests of understanding the world in which we live, phylogeographic patterns are especially useful to but uncommonly applied in conservation practices.

In amassing the data summarized, however, our goals have been both frustrated and complicated by the paucity of samples available for analysis from throughout the 6.5×10^6 km^2 of Amazonia, and by the primitive state of our taxonomic knowledge of the organisms in question. We hope the comments below provide a stimulus for continued research into the basic systematics and distribution of Amazonian mammals, especially for increased local

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and regional surveys, and thus ultimately in the preservation of this extraordinarily diverse fauna.

Materials and methods

All analyses are based on sequence variation in the mitochondrial cytochrome *b* gene (cyt-*b*). Data are available for 664 individuals from 15 genera of small, nonvolant mammals, including five genera of marsupials and 10 of rodents (Table 1). These encompass about 35 separate species. Some data have been published previously (see, for example, da Silva & Patton 1993; da Silva 1995; Mustrangi & Patton 1997; Patton & da Silva 1995, 1997a; Patton *et al*. 1994, 1996b). Methodology for DNA extraction, amplification, and sequencing, including primer sequences, can be found in these studies or in Lara *et al*. (1996). Additional data, based on more recent fieldwork, were generated using cycle-sequencing reactions and an ABI™ 377 automatic sequencer, following recommended ABI™ protocols. The database available ranges from the initial 399–801 bp of the cyt-*b* gene, depending upon the taxon (Table 1). Two to 15 individuals have been examined for each taxon from any single locality. All sequence divergence values reported are Kimura 2-parameter distances, generated using the MEGA program, version 1.02 (Kumar *et al*. 1993). All phylogenetic analyses summarized are from strict consensus trees generated by maximum parsimony using PAUP, version 3.1.1 (Swofford 1993), using 100 random orders of taxa to uncover any distinct island sets of trees (following Maddison 1991). Bases were treated as unordered character states, and no weights were applied as there was no evidence of saturation effects for any given taxon. Confidence of given nodes is based on the bootstrap (Felsenstein 1985), with 100 replicates. Specimen vouchers used are, or will be, catalogued in the collections of the Instituto Nacional de Pesquisas da Amazônia (Manaus), Museu Paraense Emílio Goeldi (Belém), Museu Nacional (Rio de Janeiro), Museu de Zoologia (São Paulo), Museo de Histplicates. Specimen vouchers used are, or will be, catalogued in the collections of the Instituto Nacional de Pesquisas da Amazônia (Manaus), Museu Paraense Emílio Goeldi (Belém), Museu Nacional (Rio de Janeiro), Museu de Zoologia (São Paulo), Museo de História Natural (Lima), Museum of Vertebrate Zoology (Berkeley), United States National Museum (Washington, DC), Museum of Natural History, University of Kansas (Lawrence), or Royal Ontario Museum (Toronto).

Results and Discussion

Systematics and taxonomy

Species boundaries of Amazonian small mammals are poorly understood. For example, Emmons & Feer (1990),

in the first regional field guide published for neotropical mammals, necessarily limited the taxonomic treatment of most small-bodied taxa to the generic level, and the distribution maps provided for many species were acknowledged as tentative. Few modern revisionary studies for

Table 1 List of Amazonian mammals for which cytochrome *b* sequence data allow some appreciation of species identification and phylogeographic pattern. The number of probable species contained within the available samples is indicated, as is the number of localities, the number of individuals sampled per species, and the amount of sequence data available. Data are from da Silva & Patton (1993), da Silva (1995), Mustrangi & Patton (1997), Patton & da Silva (1995, 1997a), and Patton *et al*. (1994, 1996), supplemented by more recent collections. The listing is limited to those taxa within Amazonia; we exclude data for relatives sampled in the coastal Atlantic forest of Brazil, the Andean forests above 1000 meters, or the lowland tropics of Central America and northwestern South America

Genus	Number of species	Number of localities	Number of individuals	Base pairs of sequence
Didelphidae				
Didelphis	1	10	13	660
Philander	3[1]	16	23	660
Marmosops	4[2]	15	19	630
Metachirus	1	11	17	450
Micoureus	2	12	21	630
Echimyidae				
Echimys	1	3	9	798
Dactylomys	2[3]	6	7	798
Makalata	1	9	12	798
Mesomys	3[4]	23	109	399–798
Isothrix	3[5]	15	20	798
Proechimys	9[6]	41	107	399–798
Muridae				
Neacomys	4[7]	16	31	801
Oecomys	9[8]	31	76	801
Oryzomys	5[9]	15	190	401–801
Scolomys	2[10]	5	10	801

[1]See Patton & da Silva (1997a).
[2]See Mustrangi & Patton (1997).
[3]Following da Silva & Patton (1993).
[4]See da Silva & Patton (1993); Patton *et al*. (1994).
[5]See da Silva & Patton (1993); Vié *et al*. (1997).
[6]Representatives of each of the five Amazonian species groups recognized by Patton (1987) plus new taxa described by da Silva (1995).
[7]Unblished data suggests at least four species in the western Amazon.
[8]Includes data on nine of the provisional species listed by Musser & Carleton (1993).
[9]Sequence is available for the five Amazonian species recognized by Musser *et al*. (1997).
[10]See Patton & da Silva (1995).

most groups of Amazonian mammals exist, but those that do have made radical revisions to our understanding of both the number and geographical distributions of even the most common species present (as an excellent example, see Musser *et al.* 1997; a review of terrestrial rice rats, *Oryzomys*). For the moment, therefore, only limited confidence can be assigned to published statements regarding the number of species in many genera within Amazonia, especially the ubiquitous marsupials and rodents, no matter how easily those genera might be recognized.

Molecular sequences have the limitation that they identify gene trees, not those of taxa. Nonetheless, phylogeographic studies can be of substantial utility in documenting species diversity, regardless of the species concept employed (Brower *et al.* 1996), and mitochondrial DNA (mtDNA) is optimal for tracking species splits because of its fast sorting time (Moore 1995). In our view, species are natural entities representing independently evolving lineages. Where diagnosable entities are sympatric without evidence of gene exchange, species status is not questioned. But for allopatric forms, as an operational procedure we will recognize as species those reciprocally monophyletic clades of mtDNA haplotypes that have both regional coherence and diagnosability by characters other than the molecular ones that define them. These 'other' characters include those of morphology and chromosomes, products of the nuclear genome that offer corroboration of inferences derived from the single gene phylogenies obtained from mtDNA. By this approach, we have begun to recognize species boundaries for a number of complex and poorly understood groups (e.g. pouched four-eyed opossums, *Philander* (Patton & da Silva 1997a) and slender mouse opossums, *Marmosops* (Mustrangi & Patton 1997)); to uncover species previously hidden by inadequate sampling, lack of prior revisionary effort, or failure to distinguish geographical differentiation from individual variation (e.g. spiny tree rats, *Mesomys* (da Silva & Patton 1993) and terrestrial spiny rats, *Proechimys* (da Silva 1995)); and to signal probable increased species diversity in other taxa (e.g. bamboo rats, *Dactylomys* (da Silva & Patton 1993), terrestrial rice rats, *Oryzomys* (summarized in Musser *et al.* 1997)).

We detail two examples in which phylogeographic analyses may be used to delineate species boundaries and, thus, enhance our understanding of the mammalian species richness within Amazonia.

Case study 1: Proechimys *spp. (family Echimyidae).* The terrestrial spiny rats are usually the most common rodents in neotropical forests. The genus ranges from Honduras to Paraguay, and is present throughout Amazonia, often with four or five species sympatric at a single locality.

We provide data for the *Proechimys goeldii* group as an example of the types and degrees of divergence patterns emerging from our phylogeographic comparisons. The *goeldii* group is distributed throughout Amazonia, but with uncertain number (and range) of included species (Patton 1987). Data are available for 28 localities with up to five individuals sequenced per population (Fig. 1; see also da Silva 1995). Four regionally distributed and reciprocally monophyletic clades are definable, with nodes supported by bootstrap values of 99–100; three of these differ by more than 11%. By our operational definition given above, each of these three clades can be treated as a separate species: *amphichoricus* (north and north-western Amazon), *steerei* (south-western Amazon), and *goeldii* (south-eastern Amazon). Additionally, *steerei* is divisible into two monophyletic clades that differ by about 6.5%. One of these is distributed throughout the south-western Amazon south of the Rio Solimões; the other is limited to the small region, termed the Imerí (following Cracraft 1988), sandwiched between the Rio Solimões and lower Rio Negro. Samples of *steerei* and *amphichoricus* from opposite sides of the Rio Negro differ by an average of 10.6%. In each case, the degree of divergence within all regional monophyletic units is minimal, ranging from less than 1.9% (among the 15 samples of south-western *steerei*) to only 2.8% (among the three widely scattered samples of *amphichoricus*). These values are approximately five times less than the average amount of divergence between the three regional pairs of taxa.

Case study 2: Micoureus *spp. (family Didelphidae).* The woolly mouse opossums are arboreal, omnivorous, and common members of the tropical forest marsupial assemblage. Widely distributed in the neotropical forests, the genus ranges from Belize to northern Argentina, and from the lowland Amazonian to mid-elevation elfin forests on both Andean slopes (Emmons & Feer 1990). While Gardner (1993) recognizes three species within Amazonia (*constantinae*, *demerarae* and *regina*), Emmons & Feer (1990) view the regional morphological variation present to signal a single, but very polymorphic single species, *M. demerarae*.

Sequence data for samples from western Amazonia clearly distinguish two sympatric taxa that are phylogenetically and morphologically (our unpublished data) distinct, which differ by more than 16% in cyt-*b* sequence (Fig. 2). These correspond to the western Amazonian *M. regina* and a more eastern species, *M. demerarae*. The latter species has a very broad distribution, ranging across Amazonia from near the Peruvian–Brazilian border to the Atlantic coast, and from northern Venezuela to the south-eastern Brazilian forests. Throughout Amazonia, this taxon is also divisi-
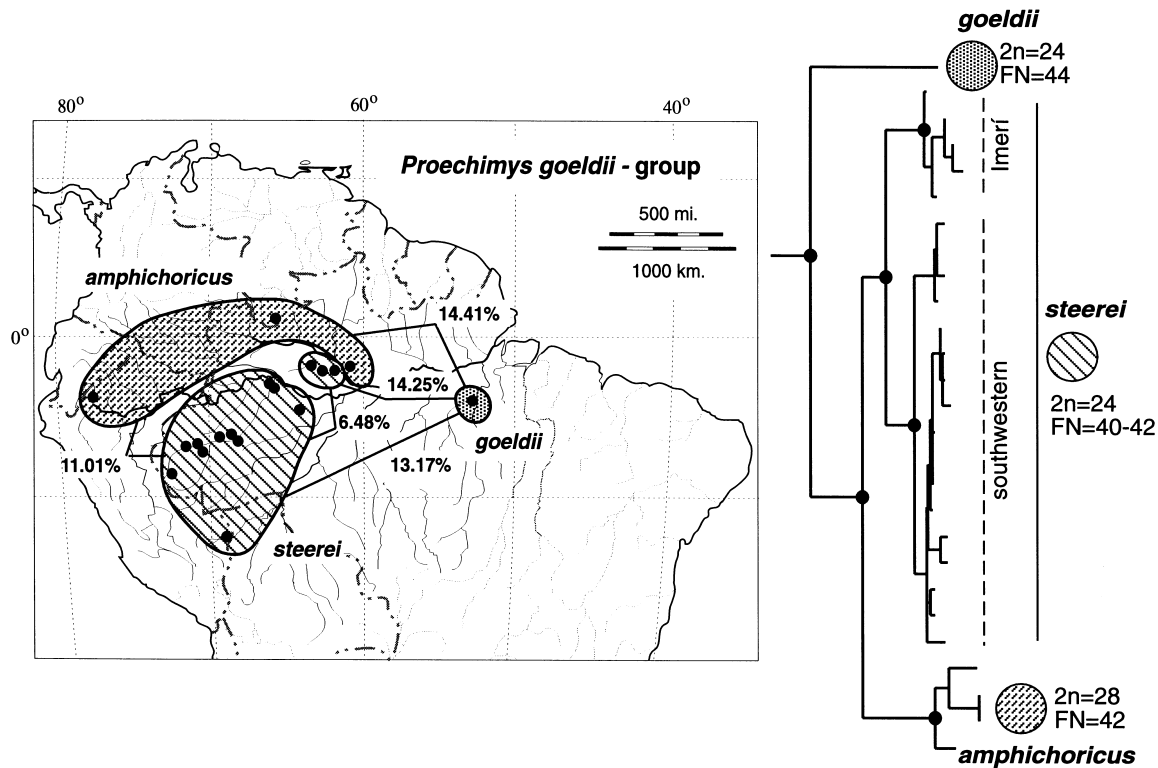
Fig. 1 Left: Phylogeographic position of reciprocally monophyletic cytochrome *b* haplotype clades of the terrestrial spiny rat *Proechimys goeldii* complex. Each of the three species we recognize in the complex, based on the species definition given in the text, are identified. Individual localities for the specimens that have been examined are indicated by solid symbols. Kimura 2-parameter distances between each pair of clades are indicated. Right: Strict consensus maximum parsimony tree of the 27 haplotypes recovered, based on 801 bp of sequence (tree length = 1002 steps, consistency index = 0.482). Solid circles identify nodes with bootstrap values of 99 or 100. Each of the three groups identified have unique chromosomal characteristics (see da Silva 1995).

ble into separate monophyletic haplotype clades, at divergence levels between about 5.2 and 8.7%, most of which are supported by bootstrap values above 94. These, in turn, differ collectively from samples of the coastal Brazilian forest (Mata Atlântica) by nearly 11% (data not shown in Fig. 2). As with geographical members of the *Proechimys goeldii* group, average divergence values within each regional Amazonian clade, where more than one population sample is available, are relatively low, ranging from about 4% within the northeastern clade to less than 1% within the Imerí region (Fig. 2). The presence of sympatry in western Amazonia between molecular clades that can also be recognized morphologically is prima facie evidence for the specific status of both *regina* and *demerarae*. Whether the regional reciprocal monophyletic clades of *M. demerarae* actually identify more than one species remains unresolved at the moment, but the molecular data do point to explicit geographical areas where samples would be needed to address this issue.

The timing of lineage diversification

There are surprisingly large degrees of haplotype differentiation among monophyletic clades identified for nearly all taxa that we have examined (Tables 2 and 3). Values often well above 10% for the cyt-*b* gene, with most above 6%, suggest deep ages of divergence. This is true regardless of whether the taxon is a didelphid marsupial or echimyd rodent, both groups of which had virtually their entire evolutionary histories within South America since the early Cenozoic. It is also true for the more recently arrived, but still autochthonously diversified murid rodents. We lack a proper understanding of the equivalency of rates of sequence evolution within and among these taxa for the cyt-*b* gene, and rate heterogeneity must exist, especially considering the short piece of sequence available. But large errors among taxa are unlikely; these are all small bodied (less than 1 kg in body mass) and endothermic, with short generation times. Even two-fold differences
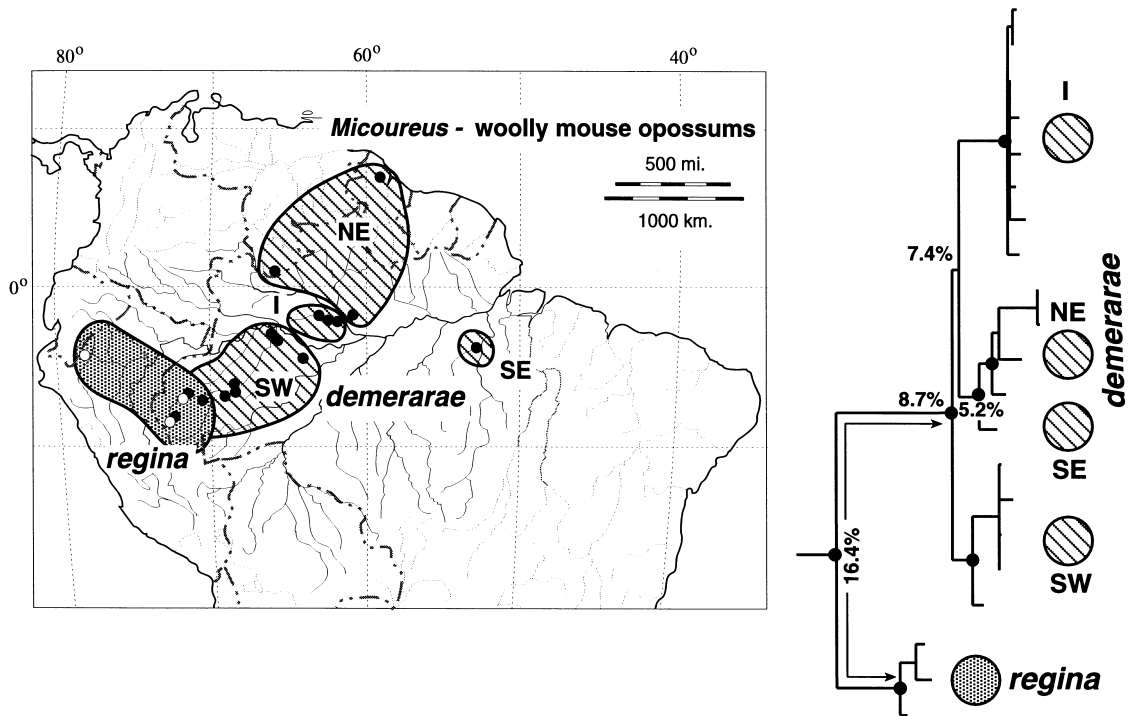
Fig. 2 Left: Phylogeographic position of reciprocally monophyletic cytochrome *b* haplotypes clades of the arboreal woolly mouse opossums *Micoureus demerarae* (closed circles) and *M. regina* (open circles) within Amazonia. Data available for *M. demerarae* from the coastal Brazilian Mata Atlântic are not included. Right: Strict consensus maximum parsimony tree of the 21 haplotypes, based on 630 bp of sequence (tree length = 376 steps; consistency index = 0.670). Average Kimura 2-parameter distances are given for internal nodes; solid circles identify nodes with bootstrap values of 94 or higher. The tree was rooted by sequence comparison to four other Amazonian didelphid marsupial genera (data from Patton *et al.* 1996b).

in rate will still require substantial time periods for the development of the divergence levels observed. Consequently, the molecular data are consistent with arguments that these lineages are old, some possibly dating to the Pliocene or even late Miocene (see discussions in da Silva & Patton 1993; Smith & Patton 1993). Certainly most or all lineages were well-defined before the usually highlighted Late and post-Pleistocene, the time period often invoked for Amazonian diversification.

Geographical patterns and processes

Phylogeographic analyses provide us with two very important windows into understanding the evolutionary past of organisms under study. For one, as estimates of phylogeny *per se*, we can test spatial models of speciation (see, for example, Harrison 1991; Lynch 1988; Patton & da Silva 1997b). Second, as reciprocally monophyletic groups probably result from long-term spatial barriers to gene flow, the concordant geographical placement of phylogeographic gaps for different taxa probably identifies bio-

geographic boundaries and common historical events (summarized in Avise 1994). In other words, phylogeography provides a basis for uncovering vicariant biogeographic histories, because shared distribution patterns among diverse clades of organisms are more parsimoniously explained by the occurrence of extrinsic geological or climatic events, which affected all groups in the same way, than by the intrinsic characteristics of each taxon independently (Platnick & Nelson 1978). Concordant distributions, or generalized tracks (Rosen 1975), may thus be used as evidence to infer the biogeographical history of a region. Below, we present three case studies that identify different patterns, and thus processes, in the diversification of Amazonian mammals.

Case study 1: Andean orogeny and palaeobasin formation. Our studies have centred on detailed sampling along the Rio Juruá in western Brazil, where 17 species, or species pairs, of nonvolant mammals were surveyed along its 1000 km length. The original goal of this project was to assess the potential of the river as a barrier influencing differentiation. Our phylogeographic analyses,

Table 2 Cytochrome *b* sequence divergence levels (Kimura 2-parameter distances) for taxa with paired samples from opposite sides of the potential riverine barriers of the Solimões–Amazonas axis and lower Rio Negro. Only taxa for which clades on opposites sides are reciprocally monophyletic are included. Data are from opposite-bank, or near opposite-bank points for comparisons across the Rio Solimões (middle Amazon) and lower Rio Negro, but from more widespread localities in the comparisons of the lower Rio Amazonas (see text)

Taxon	Average sequence divergence
A. Rio Amazonas, Brazil (approximately Manaus to its mouth)	
Micoureus demerarae	5.18%
Philander opossum	1.46%
Dactylomys dactylinus	2.26%
Echimys chrysurus	4.21%
unweighted mean	3.278%
B. Rio Solimões, Brazil (up river of confluence with Rio Negro to Peruvian border)	
Metachirus nudicaudatus	8.26%
Micoureus demerarae	8.69%
Oryzomys megacephalus	15.03%
Oryzomys macconnelli	11.18%
Dactylomys dactylinus	3.23%
Isothrix bistriata	5.12%
Makalata didelphoides	8.40%
Mesomys hispidus	1.44%
Proechimys steerei	6.31%
Proechimys sp. 1	9.63%
unweighted mean	7.729%
C. Lower Rio Negro, Brazil	
Metachirus nudicaudatus	13.02%
Micoureus demerarae	7.52%
Philander andersoni vs. *opossum*	6.91%
Isothrix bistriata vs. *pagurus*	10.93%
Proechimys steerei vs. *amphichoricus*	10.21%
unweighted mean	9.718%

however, do not suggest such a role, with the sole exception of relatively recent divergence among saddle-back tamarins (*Saguinus fuscicollis*) for which opposite-bank subspecies are currently recognized in the taxonomic literature (Peres *et al.* 1997). A few taxa exhibited no divergence pattern (e. g. *Proechimys steerei* [see case study above] or the terrestrial rice rats *Oryzomys megacephalus* and *Oligoryzomys microtis*; Patton *et al.* 1996a). However, 11 of the 17 taxa examined were comprised of sharply defined reciprocally monophyletic haplotype clades with concordant phylogeographic breaks in the central part of the river (Fig. 3). The Rio Juruá is thus divided into up-river and down-river phyletic groups; it does not separate groups on opposite banks. Divergence levels between these clades are substantial, ranging from more than 4% to over 13%, suggestive of a deep history

to their respective times of differentiation (Patton & da Silva 1997b,c). Moreover, the concordant placement of clade boundaries in the central section of the river supports a common vicariant history, one that is not related to the distribution of current major habitats. The taxa sampled encompass terrestrial and arboreal forms as well as terra firme and várzea specialists, and localities within each clade occur within both dominant vegetation subtypes (e. g. *Floresta tropical aberta* and *Floresta tropical densa*) that exhibit a generally concordant transition through this same area (Projeto RADAMBRASIL 1977).

The geographical placement of clade boundaries is coincidental with an underlying geological structure, termed the Iquitos (or Jutaí) Arch, that separates the Acre and Central Amazon palaeobasins which were formed by subsidence initiated during the last episode of uplift of the central Andes to the west, which began in the late Miocene (reviewed in Räsänen *et al.* 1987, 1990, 1992). The location of sets of independent monophyletic clades coincidentally with an underlying geological feature suggests a causal linkage between tectonic activity and organismal diversification, either directly or through resulting differences in depositional histories, soils, and eventual vegetation types. This linkage is further

Table 3 Cytochrome *b* sequence divergence levels (Kimura 2-parameter distances) for comparisons of seven mammal taxa with representatives in both the Atlantic Forest of coastal Brazil and Amazonia. Values for the Atlantic Forest and the phylogenetically closest Amazonian area are given, as are those for the greatest distance between any two Amazonian areas. Comparisons may be between subspecies currently allocated to single species, or to related species

Taxon	Atlantic Forest vs. Amazonia	within Amazonia
Didelphis spp.	3.1%	2.31% (NE vs. SW)
Metachirus nudicaudatus	4.9% (SW)	13.02% (NE vs. NW)
Marmosops spp.*	17.9%	12.21% (SW vs. NW)
Micoureus demerarae	10.66%	8.69% (SW vs. others)
Philander spp.	13.94%	6.91% (NW vs. others)
Oryzomys megacephalus	13.11% (NE)	15.03% (NW + SW vs. NE + SE)
Proechimys (*sensu lato*)†	9.7%	–

*Data from Mustrangi & Patton (1997), excluding *M. parvidens* which perhaps should be placed in a separate genus (see also Kirsch & Palma 1995).
†Data from Lara *et al.* (1996); Amazonian species of *Proechimys* (*sensu stricto*) are monophyletic relative to the Atlantic Forest *Proechimys* (*Trinomys*).
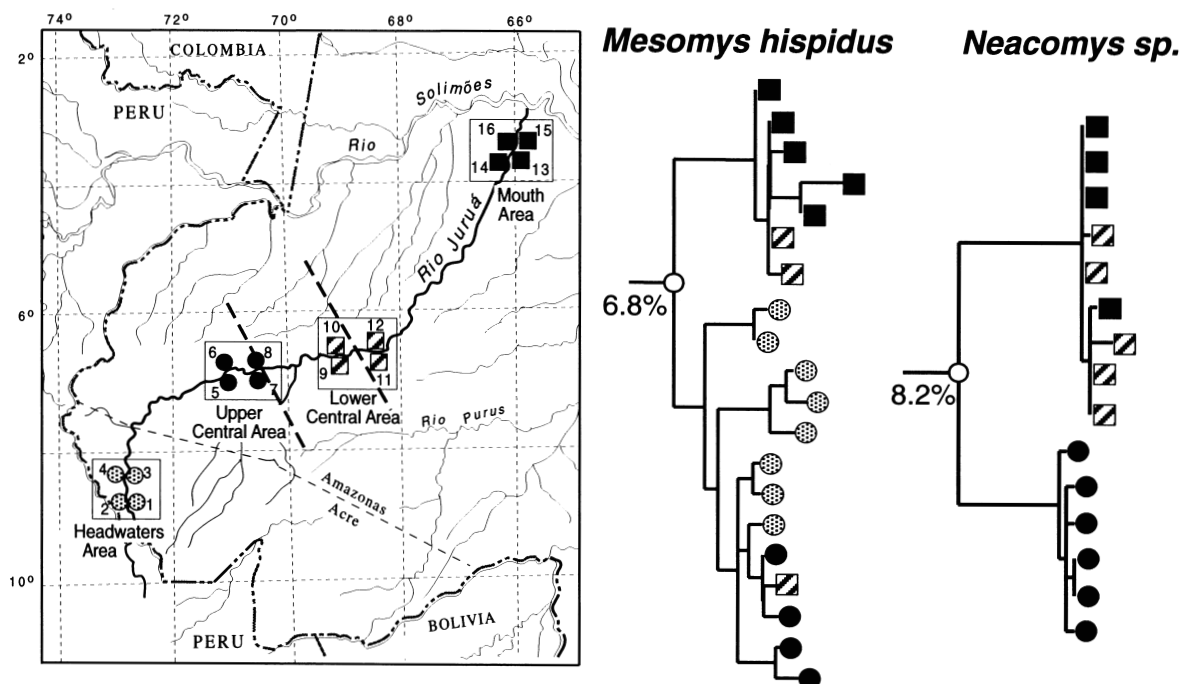
Fig. 3 Phylogeographic concordance of cytochrome *b* haplotype clades for rodents sampled along the Rio Juruá in western Brazil: the spiny tree rat *Mesomys hispidus*, and the terrestrial spiny mouse *Neacomys* sp. Consensus maximum parsimony trees of all uncovered haplotypes for both species are given to the right, with the percentage sequence divergence (Kimura 2-parameter distances) indicated at the basal node in each. Haplotypes are keyed by symbol to one of four regions sampled along the river, as in the map on the left. For each taxon, haplotype clades either break between the Upper Central and Lower Central sample areas, or within the latter area. The approximate position of the underlying Iquitos Arch as it dissects the Rio Juruá basin is indicated by the pair of heavy dashed lines.

strengthened by estimates for the timing of lineage splits (admittedly very approximate, and with large probable errors), which suggest divergences ranging from greater than 1 to at least 3 Myr (da Silva & Patton 1993; Patton & da Silva 1997b,c).

Tectonic activity within Amazonia has been little appreciated by most students of organismal diversification until quite recently. Its importance cannot be under-estimated, however, as summarized by Räsänen *et al.* (1990): 'The late Cenozoic foreland dynamics partitioning the western Amazon lowlands into more distinct intraforeland basins is the latest phase in the relief evolution which changed the Tertiary Neotropical lowlands into the present Andes… Instead of reflecting locations of isolated Pleistocene broad-leaved forests (climatic refuges), modern species distributions in the western Amazon may be a result of historical species dynamics controlled by landscape evolution. Owing to late Cenozoic palaeogeographical changes in relief evolution, the forest biota of the western Amazon have probably alternated between allopatry and sympatry, common-ness and rarity, and continuous distribution and frag-mentation.'

Despite the important, if underappreciated, influence of tectonic events on Amazonian history, geological pro-cesses associated with Andean uplift were probably stronger in the western parts of the basin, such as at points along the Rio Juruá coincidental with the Iquitos Arch. Although other structural ridges dissect the basin subsurface (see maps in Räsänen *et al.* 1987, 1990, 1992), tectonic influence should be lessened at successively greater distances east from the base of the Andes. Consequently, linkages other than Andean orogeny are likely to underlie divergence patterns in other parts of the basin.

Case study 2: Riverine barriers. Wallace (1852) observed that primate communities differed in species composition on opposite sides of the major rivers within Amazonia, and he divided the basin into four biogeographic regions bounded by the east–west Rio Solimões-Amazonas axis and the north–south Rio Negro and Rio Madeira. His observations were quantified by Ayres & Clutton-Brock (1992), who showed that opposite-bank similarity in pri-mate communities for a diverse set of Amazonian rivers exhibited a significant decline as a function of increasing
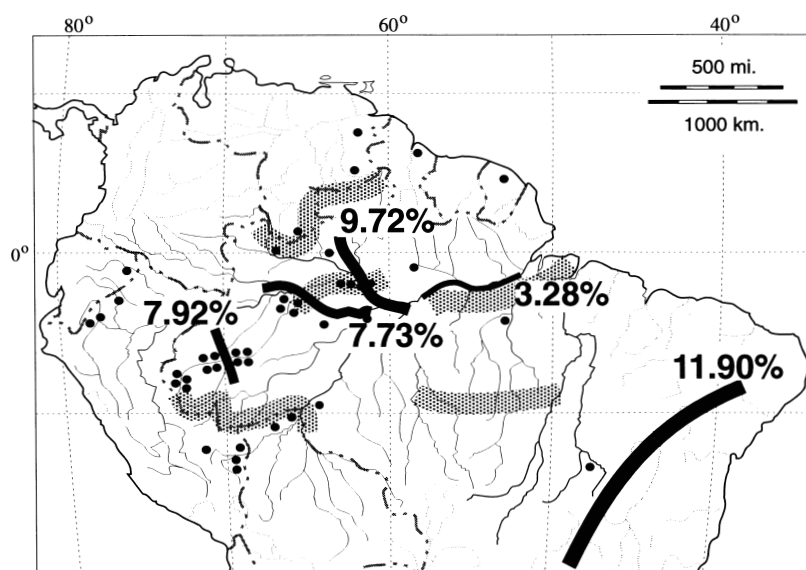
Fig. 4 Isoclines of average Kimura 2-parameter sequence divergence (solid bars) in the cytochrome *b* gene for geographically adjacent reciprocally monophyletic clades of Amazonian and Mata Atlântica small mammals (data from Tables 2 and 3 and Patton & da Silva 1997b,c). Solid dots indicate sample localities, although not each taxon was available from all. Stippled bars identify the location of five conservation corridors proposed under the PPR plan (see text and Ayres *et al*. 1996).

river size. There are many examples in the literature where organismal distributions (ranging from insects to birds and monkeys) show opposite-bank discontinuities, and rivers delineate points of genetic divergence for opposite-bank as opposed to same-bank populations of sedentary understorey birds (e.g. Capparella 1991). However, other examples exist where major rivers are not effective barriers (e.g. Patton *et al*. 1994; review by Haffer 1993). Amazonian rivers are extremely dynamic entities, with long and complex histories of lateral channel migrations that can radically effect across-river transfer, particularly of taxa localized in the bordering várzea (Peres *et al*. 1997).

Our samples are inadequate at the moment to paint more than the broadest view of phylogeographic pattern throughout Amazonia, and thus to examine the reality of Wallace's four-area division. However, we do have matched samples for several taxa on both sides of the Solimões-Amazonas axis as well as the Rio Negro. We lack any samples close to both banks of the Rio Madeira, so that comparisons south of the Amazon are probably compromised by a general isolation-by-distance phenomenon.

Figure 4 presents summary isoclines of sequence divergence, averaged for adjacent samples of each available taxon. Taxon pairs and their degrees of sequence divergence across potential barriers are given in Table 2. The positioning of substantial clade breaks in the central Rio Juruá is clearly evident, coincident with the position of the underlying Iquitos Arch (Fig. 3). The average sequence divergence between 10 paired taxon clades at this break point is 7.92% (Patton & da Silva 1997b,c). The only transition area with higher levels of

divergence is along the lower Rio Negro, where average divergence reaches nearly 10% for five pairs of taxa. Note, however, that divergence levels along the Solimões–Amazonas axis are less than those found along the Rio Negro, or through the middle Rio Juruá. Moreover, the degree of divergence is highest (above 7.7%, for eight taxon pairs) in the central portion of the Solimões–Amazonas river, rather than increasing in extent downriver as the river continues to broaden towards its mouth.

Part of the difference in the degree of riverine effect of the Rio Negro relative to that of the larger Solimões–Amazonas might reside in differences in channel dynamics. While both the Negro and Solimões are anastomosing rivers characterized by an interconnected network of channels separated by stable and vegetated floodplains and islands, the extent of lateral seasonal flooding and thus bordered várzea forest is much greater along the Solimões. Moreover, Amazonian rivers not only differ in current disposition, but also in their temporal histories. There are examples of tectonic activity in the western basin affecting 40 km shifts in river channels (Kalliola *et al*. 1992; Räsänen *et al*. 1992), and the western portion of the current Amazon River apparently drained north into the Caribbean in the late Miocene (Hoorn 1994), so that its current west-to-east course is relatively recent. Finally, and most importantly, even where rivers do delimit the boundaries of related taxa, they may merely be the convenient secondary meeting places of organisms that diverged elsewhere, rather than being the primary cause of diversification (Simpson & Haffer 1978). Phylogeographic analyses can potentially resolve these different

roles, as sister relationships would be expected between clades located on opposite banks if divergence were primary. Additional sampling for all taxa which we summarize here will be required before the role of Amazonian rivers in small-mammal diversification can be determined adequately.

Case study 3: The uniqueness of Amazonian clades. An important question to ask regarding the diversification of Amazonian organisms is the degree to which their evolution has been influenced by historical connections with the trans-Andean forests of north-western South America and Central America or with the Mata Atlântica of coastal Brazil. How much of the total diversification of Amazonian mammals has been autochthonous to that region, or impacted by periodic external connections to the south or north-west?

Our data are inadequate to address relationships between Amazonian and their trans-Andean relatives in north-western Colombia and Central America, as has been done recently for a number of avian taxa (Hackett 1993; Brumfield & Capparella 1996). However, we have comparative data for eight of the 15 genera or species groups of marsupials and rodents with both Amazonian and Atlantic forest representatives (Table 3; Fig. 4). For five of these (species of the marsupial genera *Marmosops*, *Philander*, *Micoureus*, and *Didelphis* as well as the echimyid rodent *Proechimys* [*sensu lato*]), the Mata Atlântica component is the area outgroup to a monophyletic assemblage of Amazonian areas, with samples available from all five Amazonian regions recognized by Cracraft (1988). There are two exceptions to this pattern. The brown four-eyed opossum, *Metachirus nudicaudatus*, exhibits a sister-area relationship between the Atlantic forest and the south-western Amazon. Also, members of the *Oryzomys megacephalus* group show a tie between coastal Brazil and south-eastern-north-eastern Amazonia. Results for an eighth group, the *Oryzomys nitidus* complex, are equivocal, with an unresolved area trichotomy between the Atlantic forest, eastern Amazonian, and western Amazonian regions. While data on the remaining taxa need to be obtained, for many groups diversification within Amazonia appears to have been largely unconstrained by historical connections to the outside, at least with the Mata Atlântica.

Phylogeography and conservation

Conservation initiatives for Amazonia have been developed with only the broadest understanding of mammalian diversity at the local or regional levels, or of the evolutionary processes underlying that diversity. In part, this is due to a paucity of necessary information,

coupled with the large area involved and the speed with which management strategies have been needed. Only primates and large carnivores have played a substantive role, either as 'flagship' species or in support of a particular model of diversification (see Kinzey 1982, for example). Again, we generally lack adequate data on real levels of diversity and patterns of diversification (Voss & Emmons 1996).

A landmark in Amazon conservation planning was the Workshop 1990, held in Manaus, where scientists and policy-makers set conservation priorities and produced the first comprehensive map of the Basin's biodiversity. The final map identified 94 priority areas established on the basis of overall species richness and/or degree of endemism (Rylands 1990). These were evaluated on a three-point scale, with highest priority given to areas with the maximum overlap between the biodiversity estimates from different organismal groups (Conservation International 1990). A second conservation plan is under development at the moment by the Ministry of the Environment of Brazil (MMA) and the Pilot Program to Conserve the Brazilian Rain Forest, financed by the G-7 countries. This plan, The Neotropical Forests Corridors of the Parks and Reserves Project (PPR, Ayres *et al.* 1996), centres on the creation of biological corridors created basically by connecting existing conservation units (national and federal parks, national forests, and indigenous reserves). Five such corridors are identified for the Brazilian Amazon. In what way might studies of Amazonian phylogeography, such as we are developing for mammals, add positively to these proposals?

Our results define an increased level of local faunal diversity and uniqueness at three points within Amazonia: (i) the region south-west of the Rio Solimões to the Peruvian border; (ii) between the banks of the Rio Solimões; and (iii) between the banks of the lower Rio Negro (Fig. 4). In each area there are sharply defined and concordant lineage breaks for a number of taxa, with a substantial degree of divergence between them indicative of great time depth. As distinct as these three areas of lineage transition are, only that of the Rio Negro is contained fully within the PPR conservation proposal. Most of the Rio Juruá basin, for example, is rated as moderate priority, or less, at the regional level in the Workshop 1990 map. Our data, however, indicate that the basin of the Rio Juruá has one of the richest mammal faunas within Amazonia, and one that contains elements of two very old sets of organismal lineages. Moreover, both the geographical placement and degree of lineage division within the Juruá Basin were totally unexpected prior to our investigations, suggesting that many other surprises are to be found in the very large tracts of Amazonia that remain to be surveyed. This is especially true because so little of

Amazonia has been adequately surveyed from a phylogeographic perspective.

Our phylogeographic studies of small mammals are beginning to identify geographical areas of high diversity as well as those where concordant breaks among sharply defined lineages occur. The recognition and use of deeply divergent lineages, or evolutionary significant units, has only been recently recognized as a valuable criterion in conservation planning, equally important as species richness and endemism as major criteria (Moritz 1994). If this criterion is to serve an important role in conservation strategies for Amazonia, then the adequacy of either the priority areas identified by Workshop 1990 or the corridors of the PPR proposal in this respect must be evaluated. Phylogeographic studies of many different kinds of organisms, performed within the framework of an explicit geographical sampling program, are thus needed.

Conclusions and prospectus

This is a time of exceptional opportunity for understanding the evolutionary dynamics of the lowland Amazonian forest biota, due to conceptual and methodological breakthroughs. It is also a time for considerable apprehension, as the rate of development and exploitation of the Amazon rainforest is far greater than is that of our increasing knowledge of its biodiversity. As we hope to have demonstrated, the phylogeographic approach is proving exceedingly useful in overcoming our ignorance of taxonomic and distributional boundaries. It provides a mean to assess areas of enhanced diversity, to identify areas of endemism, and to document taxa or lineages that are evolutionarily unique. It also offers a means to test alternative models of diversification. The patterns documented, generated for mammals as well as for other organisms, must be available and understood if the conservation goal of preserving biodiversity is to be realized.

Extensive field surveys are badly needed in the northwestern Amazon (of Colombia and adjacent Brazil), in the region north of the Rio Amazonas in north-eastern Brazil, and across virtually all of Amazonia between the Rio Madeira and the Atlantic coast south of the Solimões–Amazonas axis. The impact of development has been both impressively high and depressingly extensive in recent years (Fearnside 1986). Nonetheless, the region is vast, and the promise that even small fragments can maintain faunal diversity, if only for the short-term (Turner & Corlett 1996), gives us hope that sufficient time remains for continued discoveries of this remarkable biota, as well as for developing the database necessary for critical and urgent management concerns. We urge our fellow fieldworkers to emphasize collecting efforts, including other groups of codistributed terrestrial vertebrates, and to make their findings available quickly. We entreat our museum colleagues to finalize their systematic studies, and include the kinds of broadened data provided by the phylogeographic approach so important to conceptual evolutionary issues and empirical conservation strategies. Finally, we recognize the compromises often required by individuals in positions of responsibility for conservation welfare, to juggle adequately the information available in decision making. Nonetheless, we encourage them to think in evolutionary terms in defining and establishing effective strategies to preserve Amazonian biodiversity.

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