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Phylogeography of Four Frog Species in Forest Fragments of Northeastern Brazil—A Preliminary Study[1]

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SYNOPSIS. I contrast mitochondrial DNA genealogies based on 612 bp of the cytochrome *b* gene across four co-distributed species of frogs in Northeastern Brazil. They are *Hyla albomarginata*, *Hyla branneri*, *Proceratophrys boiei*, and *Scinax nebulosus*. Samples were collected from the core or edge of six rainforest remnants in the states of Pernambuco and Alagoas. Three fragments are located within the humid Atlantic Forest morphoclimatic domain (municipalities of Cabo de Santo Agostinho, Ibateguara, and Jaqueira), two are located in the transition zone between the Atlantic Forest domain and the semi-arid *Caatinga* (Caruaru and Timbaúba), and one is found within the *Caatinga* (Brejo da Madre de Deus). Results show that local patterns and levels of genetic diversity are influenced by taxon-specific habitat requirements. Populations of the montane, closed-canopy species *P. boiei* show strong geographical structure, reflecting barriers to gene flow that predate human-driven habitat destruction. Species occurring along forest edges, such as *H. albomarginata* and *S. nebulosus*, show genetic patterns similar to those of *P. boiei*, but lower levels of genetic divergence. The more generalist *Hyla branneri* shows no geographic pattern. The data are in agreement with distribution and fossil data gathered for other groups of organisms, suggesting that mesic forests occupied the currently arid *Caatinga* in the recent past.

INTRODUCTION

The Atlantic Rainforest is an extraordinarily diverse and complex ecosystem found along the coast of Brazil. Home to approximately 21,361 species of plants and tetrapod vertebrates, of which *ca.* 8,567 occur nowhere else in the planet, it ranks among the top priority areas for conservation in the world (Myers *et al.*, 2000). Human-driven landscape changes have strongly impacted this area (Morellato and Haddad, 2000). Local deforestation began with the arrival of European colonizers in the sixteenth century and has increased dramatically ever since (Coimbra-Filho and Câmara, 1996). Today's remnants of this ecosystem barely add up to 8% of its original extent (Myers *et al.*, 2000). Causes of habitat destruction were many and synergistic, involving agricultural practices, cattle ranching, and urban growth (Câmara, 1992; Fundação SOS Mata Atlântica, 1992; Sales *et al.*, 1998).

To adequately preserve what is left of this biodiversity hotspot, it is imperative to deepen our knowledge about the history of the Atlantic Forest and investigate the consequences of habitat patchiness for local populations. Distribution patterns of extant taxa provide clues about local historical processes and inform conservation in this system (Vanzolini, 1981; Heyer and Maxson, 1983; Costa *et al.*, 2000). However, it is desirable to go beyond interspecific analyses and study patterns of genetic diversity within local taxa. Are remnant populations still managing to exchange genes? If not, how divergent are they? Are they genetically structured? If so, how? Ultimately, why?

The tools of phylogeography (Avise *et al.*, 1987) are central to conservation and can be informative for the preservation of the Atlantic Rainforest. This area of molecular biology investigates the spatial distribution of evolutionary lineages and their phylogenetic relationships (Bermingham and Moritz, 1998). Phylogeographic studies not only document the amount of current genetic diversity within and among geographical areas, but also assess the phylogenetic distinctiveness of populations, providing insight on how organisms have historically responded to local landscape changes (Moritz and Faith, 1998). Comparative phylogeographic analyses are yet more powerful. By contrasting gene trees of co-distributed species, these methods enable one to search for common historical events that might have affected multiple taxa in a given area (*e.g.*, Patton *et al.*, 1996; Schneider *et al.*, 1998; Bates, 2000, 2002; Stuart-Fox *et al.*, 2001; Schäuble and Mortiz, 2001). Phylogeographic studies involving taxa from the Brazilian Atlantic Forest are relatively few and recent, and most available data relate to species of mammals (Mustrangi and Patton, 1997; Ditchfield, 2000; Lara and Patton, 2000).

I contrast mitochondrial DNA genealogies based on 612 bp of the cytochrome *b* gene of four species of amphibians that co-occur in or along Atlantic Forest remnants in Northeastern Brazil. They are *Hyla albomarginata* Spix, *Hyla branneri* Cochran, *Proceratophrys boiei* (Wied-Neuwied), and *Scinax nebulosus* (Spix). These taxa differ in aspects of their natural history, such as habitat requirements and reproductive biology. Field data and literature records indicate that *Proceratophrys boiei* is a forest-associated species that occurs in closed-canopy areas, breeding in slow-moving waters such as side pools along streams (Cochran, 1955; Izecksohn *et al.*, 1979). *Hyla albomarginata* and

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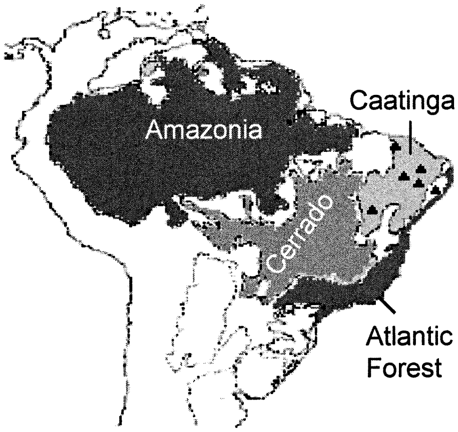

FIG. 1. South American morphoclimatic domains, modified from Ab'Saber, 1977. Triangles depict forest enclaves (*Brejos*) in the *Caatinga* and in the *Caatinga*-Atlantic Forest transition zone (based on Andrade-Lima, 1982).

Scinax nebulosus are commonly found along forest edges, breeding in standing water adjacent to forest borders (Cochran, 1955; Lutz, 1973; Arzabe *et al.*, 1998). *Hyla branneri* is reported to be more resistant to open areas, occurring along forest edges (Arzabe *et al.*, 1998) but also in drier, more remote zones of Northeastern Brazil (Andrade *et al.*, 2000).

By contrasting patterns of genetic diversity among these four co-distributed species of amphibians, I hope to improve our understanding of the history of the Atlantic Rainforest in Northeastern Brazil and inform conservation of its remnant populations. I specifically address the following questions: What do genetic patterns exhibited by multiple lineages suggest about the history of these forest remnants? Are populations found in or along fragments genetically structured? How? Does habitat selection influence levels and patterns of genetic diversity in the target taxa? If so, one would predict that genetic differentiation among populations should be higher in the most specialized, closed-canopy dependent *Proceratophrys boiei*, while intermediate among populations of forest-edge taxa such as *Hyla albomarginata* and *Scinax nebulosus*, and little or nonexistent among those of the more generalist *Hyla branneri*.

STUDY SYSTEM

Atlantic Rainforest fragments in Northeastern Brazil

Northeastern Brazil is composed of two main biomes or morphoclimatic domains, as defined by Ab'Saber (1977) (Fig. 1). The Atlantic Rainforest lies along the coast, occupying both lowland areas and mountain slopes that reach up to *ca.* 1,000 m. West of the Atlantic Forest is the *Caatinga*, occupying most of the Brazilian Northeast. This semi-arid zone is characterized by irregular rainfall patterns, and it is home to species well adapted to arid conditions (Brazão *et al.*, 1992).

Forest remnants persist not only in the humid coastal Atlantic domain, but also within the *Caatinga*, and in the transition zone between these two biomes. The term *Brejos de Altitude* or forest enclaves is used to denote such remnants of the Atlantic Forest found in the middle of the *Caatinga* and in transition areas under considerable *Caatinga* influence (Andrade-Lima, 1982) (Fig. 1). The *Brejos* occur on mountains of approximately 500–1,100 m of altitude that are exposed to humid winds, and it has been proposed that they represent extant refuges formally connected to Atlantic and Amazonian forests (Vanzolini, 1981; Andrade-Lima, 1982). The origin of the *Brejos* has been associated with humid paleoclimates established after the middle Tertiary, especially during interglacial phases of the Pleistocene (Bigarella *et al.*, 1975; Bigarella and Andrade-Lima, 1982). It is hypothesized that both the Atlantic Forest and *Amazonia* were able to expand and connect during such humid phases, extending their limits into what now is the semi-arid *Caatinga*. Today, lower temperatures and higher precipitation enable the maintenance of a forested environment in the *Brejos*, and they persist in slopes of the *Borborema*, *Araripe* and *Ibiapaba* plateaus (Andrade-Lima, 1982).

Field work for this study was conducted in the northeastern states of Alagoas (AL) and Pernambuco (PE). Samples of the target species were collected from the core or edge of six forest remnants in the municipalities of Brejo da Madre de Deus (PE), Cabo de Santo Agostinho (PE), Caruaru (PE), Ibateguara (AL), Jaqueira (PE), and Timbaúba (PE) (Fig. 2). Three of the fragments are located within the Atlantic Rainforest morphoclimatic domain. They are Cabo de Santo Agostinho, Ibateguara, and Jaqueira. Brejo da Madre de Deus is located further inland in Northeastern Brazil, within the savanna-like, semi-arid *Caatinga*. Caruaru and Timbaúba are situated in the transition zone between the Atlantic Forest coastal domain and the dry *Caatinga* (Fundação Instituto Brasileiro de Geografia e Estatística, 1988). Forests in both Brejo da Madre de Deus and Caruaru are representatives of *Brejos* in the state of Pernambuco (Andrade-Lima, 1982; Sales *et al.*, 1998)

About the target species

Proceratophrys boiei (Fig. 3) is a medium-sized frog whose adults live under leaves and stones in forested mountains of up to approximately 1,000 m along the Brazilian coast (Cochran, 1955; Izecksohn *et al.*, 1979; Heyer *et al.*, 1990; Santos and Silva, 1998). This litter-dwelling amphibian is endemic to the Atlantic Rainforest, ranging in distribution from Pernambuco in the northeast to Santa Catarina in the south (Hoogmoed, 1990). In the breeding season, adults congregate near slow or moderately current water, where males call from well-hidden spots such as under roots, rocks, or foliage along the margins. Tadpoles develop in relatively shallow but current water (Izecksohn *et al.*, 1979), and it is common to encounter newly metamorphosed individuals moving throughout the forest floor on rainy days.

Hyla albomarginata and *Scinax nebulosus* (Fig. 3)

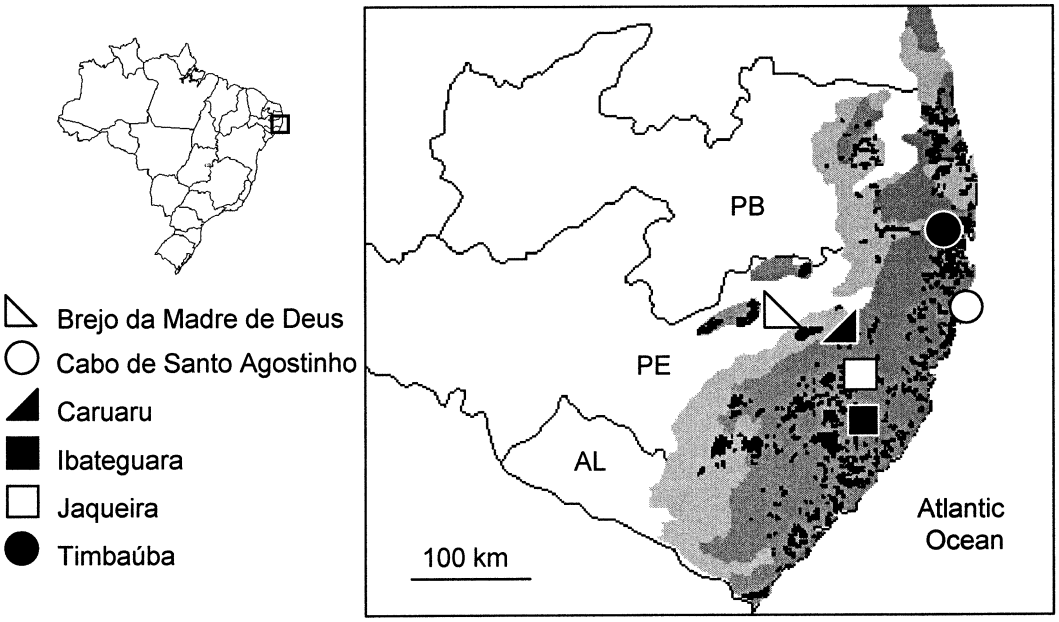

FIG. 2. Map of forest remnants along the easternmost range of the states of Paraíba (PB), Pernambuco (PE) and Alagoas (AL), Brazil, with sampling localities. Dark and light shades of gray depict the Atlantic Forest Domain and the *Caatinga*-Atlantic Forest transition zone, respectively. Areas occupied by the *Caatinga* in all three states are in shown in white. Forest remnants are pictured in black. Sampled fragments are denoted by symbols (circles, squares, and triangles in black or white), which always lie to the right of the fragment of interest. Modified from Fundação Instituto Brasileiro de Geografia e Estatística, 1988 and Fundação SOS Mata Atlântica and Instituto Nacional de Pesquisas Espaciais, 1993.

are frequently referred to as forest-edge species. Males of both taxa call from the vegetation surrounding permanent or temporary ponds adjacent to forest borders (Lutz, 1973; Arzabe *et al.*, 1998). Their tadpoles develop mostly in lentic water. Both occur in lowland forests. In Northeastern Brazil, they are commonly found at sites up to *ca.* 600 m of altitude (Lutz, 1973). *Hyla albomarginata* occurs throughout the entire range of the Atlantic Forest (Lutz, 1973; Hoogmoed and Gruber, 1983), whereas *Scinax nebulosus* occurs in Southeastern Venezuela, through the Guianas and the lower Amazon Basin, as well as in the Atlantic Forest of Northeastern Brazil (Hoogmoed and Gruber, 1983).

Compared to the other study taxa, *Hyla branneri* (Fig. 3) is a generalist. It is able to reproduce in accumulated water near forest margins, using the same breeding sites as *Hyla albomarginata* and *Scinax nebulosus* (Arzabe *et al.*, 1998), as well as large artificial ponds, lagoons, and marshlands (Lutz, 1973). It has also been reported to occur in drier regions, far away from forested areas, such as within the *Caatinga* (Andrade *et al.*, 2000). It occurs along the northeastern and southeastern ranges of the Atlantic Forest, being found from sea level to altitudes as high as 1,000 m (Lutz, 1973; Bastos and Pombal, 1996).

MATERIALS AND METHODS

A total of 86 tissue samples were collected from frogs in or next to six forest remnants (Table 1, Fig. 2). Frogs were located by visual or acoustical cues and collected during the day and at night. Tissue samples were harvested either post-mortem (in which case a portion of the liver was collected prior to fixation) or via toe clipping. They were maintained in salt-dimethyl sulfoxide (DMSO) solution (Seutin *et al.*, 1991) and kept at room temperature while in the field. Following transportation to the laboratory, tissues were stored at −80°C. Voucher specimens were anesthetized in 0.25% chloretone solution, fixed in 10% formaldehyde solution, transferred to and kept in 70% ethanol solution within seven days of fixation. They are deposited in the herpetological collection of Laboratório de Anfíbios e Répteis, Departamento de Zoologia, Universidade Federal do Rio de Janeiro, in Rio de Janeiro, Brazil.

Genomic DNA was extracted from tissue samples with Puregene Animal Tissue DNA Isolation Kit Protocol (Gentra Systems, Inc.). Amplification was obtained via polymerase chain reaction (PCR), including 0.5 μl of template, 14.75 μl of water, 2.5 μl of 10× PCR Buffer (Perkin-Elmer), 2.5 μl of dNTPs, 1.25 μl of each primer, 0.25 μl of Taq Polymerase (Roche Diagnostics Corporation), 1 μl of bovine serum albumine (10 mg/ml), and 1 μl of DMSO. PCR profiles comprised of a 2-min denaturation step at 94°C, five cycles of 20 sec at 94°C, 20 sec at 45°C and 40 sec at 72°C, 30 cycles of 20 sec at 94°C, 20 sec at 48–50°C and 40 sec at 72°C, and final 5-min extension step at 72°C. No single primer pair was found to easily amplify a portion of the cytochrome *b* gene in all four species, and a reverse primer had to be specifically designed for two taxa. Amplification in *Proceratophrys boiei* and *Hyla branneri* was obtained with frog primers CB1-L and CB3-H of Palumbi (1996). For *Hyla al-*

FIG. 3. Species included in the study. From top to bottom: *Proceratophrys boiei*, *Hyla albomarginata*, *Scinax nebulosus*, and *Hyla branneri*.

bomarginata and *Scinax nebulosus*, primers CB1-L of Palumbi (1996) and 5′-GTRACTAGWGGGTTAGC-3′ were used, the latter being designed from preliminary sequences based on re-amplified products. PCR products were electrophoresed in 1% low melt low EDTA agarose gel, from where the band of interest was cut and incubated with GELase (Epicentre Technologies) for five minutes at 50°C, followed by two hours at 45°C. Cycle sequencing reactions were performed with 2 μl of template, 3 μl of water, 1 μl of Big Dye Terminator ver. 2.0 (ABI Prism), 3 μl of Big Dye Buffer, and 1 μl of primer. Amplification profiles included 32 cycles of 30 sec at 96°C, 15 sec at 50°C, and 4 min at 60°C. Final products were precipitated in ethanol and resuspended in 10 μl of Hi-Di Formamide (ABI Prism), as per manufacturer's protocols. Sequencing reactions were run on an ABI PRISM 3100 Genetic Analyzer. Both strands were sequenced.

Sequence alignment and editing were performed in *Sequencher* ver 4.1.2 (Gene Codes Corporation). All sequences were aligned to the complete mitochondrial DNA sequence of *Xenopus laevis* (Daudin) available in GenBank (http://www.ncbi.nih.gov/Genbank, accession number NC 001573) and translated to amino acids to confirm the reading frame. The use of different primer sets resulted in cytochrome *b* sequences of unequal lengths in different taxa. To make the data uniform, sequences were cropped to a fragment of 612 bp homologous to positions 16,369–16,980 in *Xenopus laevis*. This piece was used for all genetic analyses. Sequences are available in GenBank, under accession numbers AF549301–AF549386.

To investigate relationships among haplotypes of the study taxa, haplotype networks were generated for each species using *TCS* ver. 1.06 (Clement and Posada, 2000). Networks were chosen given that they can be more effective than trees to depict relationships among haplotypes at the intraspecific level, especially in cases where multiple haplotypes are derived from a single, ancestral sequence (Vilà *et al.*, 1999). Estimates of levels of sequence divergence across groups of populations were obtained in *PAUP** ver. 4.0 (Swofford, 1998) and correspond to mean uncorrected (''*P*'') values.

Hierarchical analyses of molecular variance (AMOVA, as per Excoffier *et al.*, 1992) were performed to test if genetic patterns were consistent across taxa. It became especially interesting to test if frogs found in Cabo de Santo Agostinho and Timbaúba were genetically differentiated from those collected elsewhere. To check if such geographic structure was shared by all taxa, a simplified AMOVA was performed for every species in *Arlequin* ver. 2.0 (Schneider *et al.*, 2000).

TABLE 1. *Geographic location of sampling sites and numbers of individuals sequenced per species.*

Municipality, state*	Collection site, latitude, longitude	*Proceratophrys boiei*	*Hyla albomarginata*	*Scinax nebulosus*	*Hyla branneri*
Brejo da Madre de Deus, PE	Sítio Bituri, 8°12′S, 36°24′W	10	—	—	2
Cabo de Santo Agostinho, PE	COMPESA-Gurjaú, 8°14′S, 35°02′W	—	4	3	3
Caruaru, PE	Brejo dos Cavalos, 8°22′S, 36°01′W	10	4	3	3
Ibateguara, AL	Usina Serra Grande, Coimbra, 8°59′S, 35°50′W	2	4	2	2
Jaqueira, PE	Usina Frei Caneça, 8°43′S, 35°50′W	5	4	2	2
Timbaúba, PE	Usina Cruangi, Água Azul, 7°36′S, 35°22′W	9	4	4	4
Total		36	20	14	16

*PE = State of Pernambuco; AL = State of Alagoas.

Haplotypes were assigned to one of two groups. Group 1, the ''northeastern group,'' was defined by lumping together haplotypes from Cabo de Santo Agostinho and Timbaúba. Group 2, the ''southwestern group,'' included combined haplotypes from Brejo da Madre de Deus, Caruaru, Ibateguara, and Jaqueira. The resulting AMOVA partitioned the total molecular variance in each species into a covariance component due to intra-group differences, and another due to intergroup differences. It was not set up to analyze variation among localities within each group because of the relatively small number of localities sampled (in the case of *Proceratophrys boiei*, for instance, no samples were obtained from Cabo de Santo Agostinho). An F_{ST} value was calculated for each species in *Arlequin* ver. 2.0 (Schneider *et al.*, 2000), reflecting the amount of differentiation among groups. Statistical significances of F_{ST} values were estimated with 16,000 permutations, in which haplotypes were randomly re-assigned to one of the two groups.

RESULTS

Haplotype networks indicate that frog populations in Northeastern Brazil are genetically structured (Fig. 4). The most extreme case is shown by the montane, closed-canopy species *Proceratophrys boiei*. In this taxon, the mean uncorrected sequence divergence between haplotypes from Timbaúba and those of Jaqueira, Ibateguara, Caruaru, and Brejo da Madre de Deus is *ca.* 2.7%. This level of divergence is so great that statistical parsimony was unable to confidently place a connection between these two groups of haplotypes (thus, the dashed line in Fig. 4).

The two forest-edge species, *Hyla albomarginata* and *Scinax nebulosus*, share similar genetic patterns. In both taxa, populations from Timbaúba are genetically distinct from those found in Jaqueira, Ibateguara, Caruaru, and Brejo da Madre de Deus. Furthermore, individuals collected in Cabo de Santo Agostinho are genetically similar to those from Timbaúba, suggesting historical connections or ongoing gene flow between these areas. Divergence levels between these northeastern (Timbaúba + Cabo de Santo Agostinho) and southwestern (Jaqueira + Ibateguara + Caruaru + Brejo da Madre de Deus) groups are nonetheless lower then those registered for *Proceratophrys boiei* (Fig. 4).

The more generalist *Hyla branneri* exhibits a much different genetic pattern, with no clear northeast-southwest split (Fig. 4). In addition, *H. branneri* haplotypes from Timbaúba are neither identical to nor closely related to those from Cabo de Santo Agostinho.

Hierarchical analyses of molecular variance performed between the northeastern (Timbaúba + Cabo de Santo Agostinho) and southwestern (Jaqueira + Ibateguara + Caruaru + Brejo da Madre de Deus) groups of haplotypes revealed that this genetic break is responsible for nearly 90% of the overall genetic diversity of *Proceratophrys boiei*, and for *ca.* 80% of that of *Hyla albomarginata* and *Scinax nebulosus* (Table 2). Conversely, in the open-area resistant species *Hyla branneri*, most (*ca.* 85%) of the genetic variation is found within groups. Only 15% of the overall diversity can be explained by a northeast-southwest break in this species. F_{ST} values are large and highly significant in all three forest-associated species ($F_{ST} = 0.89$, $P < 0.01$ in *P. boiei*; $F_{ST} = 0.80$, $P < 0.01$ in *H. albomarginata*; $F_{ST} = 0.79$, $P < 0.01$ in *S. nebulosus*), whereas lower and marginally significant in *H. branneri* ($F_{ST} = 0.15$, $P = 0.04$).

DISCUSSION

Atlantic Forest remnants in Northeastern Brazil are heterogeneous in many ways, varying in size, shape, elevation, topographical position, degree of connectivity, and isolation time (Andrade-Lima, 1957; Ab'Saber, 1982; Ranta *et al.*, 1998). In this study, patterns of genetic diversity of four species of frogs were screened in six remnants in the states of Pernambuco and Alagoas. Three of the fragments are part of the humid coastal forest domain (Cabo de Santo Agostinho, Ibateguara, and Jaqueira), two lie in the transition zone between the Atlantic domain and the *Caatinga* (Caruaru and Timbaúba), and one is found within the semi-arid *Caatinga* (Brejo da Madre de Deus). The results show that there is substantial genetic structure among some of these areas.

In the closed-canopy, montane species *Proceratophrys boiei*, haplotypes from the northeasternmost locality of Timbaúba show approximately 2.7% genetic divergence from those of other (southwestern) localities found *ca.* 100–200 km away. Assuming rates of change in cytochrome *b* of between 0.8 and 2.5% per Myr (Lougheed *et al.*, 1999), this level of intraspecific genetic variation may reflect a fragmentation event that occurred in the Atlantic Forest prior to or in the early Pleistocene (1.1–3.4 million years ago).

Proceratophrys boiei haplotypes found in forest enclaves or *Brejos* (*i.e.*, in Brejo da Madre de Deus and Caruaru) are either shared with or closely related to those found along the humid coastal forest domain (Jaqueira and Ibateguara). Given the contemporary topography and forest distribution in Northeastern Brazil, it is highly unlikely that individuals of *P. boiei* are currently able to disperse between these localities and therefore allowed to exchange genes. Hence, the genetic relatedness of populations in Brejo da Madre de Deus, Caruaru, Ibateguara, and Jaqueira suggests the former existence of gene flow among these localities. This is in concordance with the idea that *Caatinga* enclaves and the Atlantic Forest were once connected, as suggested by Vanzolini (1970, 1981), Vivo (1997) and Sales *et al.* (1998) to explain species distribution patterns in the Neotropics. Pollen records and geological data from semi-arid sites in Northeastern Brazil indicate the occurrence of wetter local conditions during or near the Pleistocene-Holocene boundary (De Oliveira *et al.*, 1999; Auler and Smart, 2001). Based on fossil data, it has also been suggested that mesic forests occupied the presently dry *Caatinga* roughly 10,000 to 12,000 years ago (Cartelle and Hartwig,

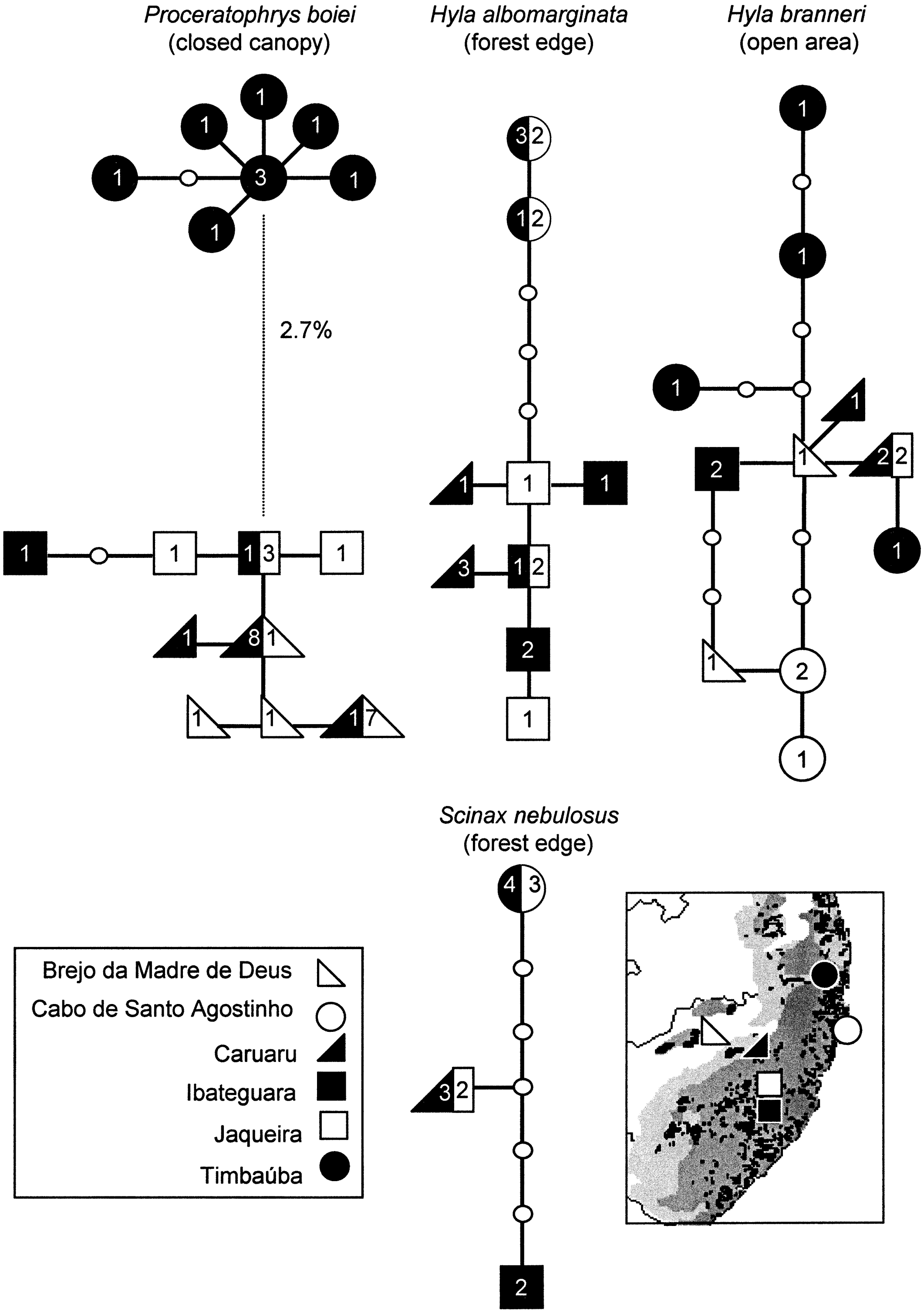

FIG. 4. Haplotype networks of *Proceratophrys boiei*, *Hyla albomarginata*, *Scinax nebulosus*, and *Hyla branneri* based on 612 bp of cytochrome *b*. Haplotypes are depicted by symbols (circles, squares, and triangles, in black or white), according to the geographical area where they occur. Small, empty circles represent hypothesized, unsampled haplotypes. Connecting bars denote single-base substitutions, and numbers indicate the frequency of each haplotype in the population of interest. The dashed line in *P. boiei* indicates an uncertain connection between two groups of haplotypes. The value next to it denotes the mean uncorrected genetic divergence between those haplotypes, measured in *PAUP** (Swofford, 1998).

TABLE 2. *AMOVA results**.

AMOVA results	*Proceratophrys boiei*	*Hyla albomarginata*	*Scinax nebulosus*	*Hyla branneri*
% variance among groups	89.4	79.7	79.2	15.4
% variance within groups	10.6	20.3	20.8	84.6
F_{ST} (P-value)	0.89 (0.00 ± 0.00)	0.80 (0.00 ± 0.00)	0.79 (0.00 ± 0.00)	0.15 (0.04 ± 0.00)

* Two groups were defined. Group 1 includes northeastern haplotypes (Cabo de Santo Agostinho + Timbaúba). Group 2 includes southwestern haplotypes (Jaqueira + Ibateguara + Caruaru + Brejo da Madre de Deus). Percentages of variation among and within groups are given, as well as F_{ST} values and their associated probabilities (P-values). P-values represent the probability of an F_{ST} value obtained via randomization being greater than or equal to the observed one.

1996; Hartwig and Cartelle, 1996; Vivo, 1997). Levels of genetic differentiation detected among populations of *P. boiei* are congruent with those observations, supporting the hypothesis that areas currently arid in Northeastern Brazil were forested in the recent past.

AMOVA results and haplotype networks of the two other forest-associated species, *Hyla albomarginata* and *Scinax nebulosus*, show a genetic pattern congruent with that of *Proceratophrys boiei*. Levels of divergence between northeastern and southwestern populations of the former taxa are nonetheless lower than that observed in *P. boiei*. *Hyla branneri* provides additional support that patterns of genetic diversity in these species are tracking and reflecting the natural history and habitat requirements of the study species. The genetic pattern observed within this more generalist frog differs considerably from those of the other taxa, as there is no clear genetic break between haplotypes from northeastern and southwestern populations.

The overall results of this paper are consistent with studies conducted for other groups of organisms, such as Knowlton *et al.*, 1993 (shrimp), Patton *et al.*, 1996 (mammals), and Schneider *et al.*, 1998 (Australian lizards and frogs), demonstrating that the establishment of a barrier to gene flow does not necessarily occur abruptly in time. Rather, it can be a gradual process, to which species respond according to their ecology.

CONCLUSIONS

Our knowledge of the amphibian fauna of the Atlantic Rainforest has been steadily increasing over the years, but additional phylogenetic studies are needed to clarify both the relationships among local taxa and how they relate to the fauna of other South American morphoclimatic domains (Duellman, 1999). Studies of levels and patterns of genetic divergence within Neotropical species of frogs only now are being reported (*e.g.*, Lougheed *et al.*, 1999). My preliminary study shows that there is considerable genetic structure among isolated populations of forest-dependent frogs in Northeastern Brazil. Both patterns and levels of genetic diversity in local species reflect taxon-specific habitat requirements. Levels of divergence between some populations are high, reflecting fragmentation events that predate human-driven habitat destruction, and must be taken into consideration in conservation planning. Genetic data indicate that populations found in *Brejos* or forest enclaves (Brejo da Madre de Deus and Caruaru) were able to exchange genes with those in the Atlantic Forest domain (Jaqueira and Ibateguara) in the recent past. This is in agreement with fossil data, pollen records and species distribution patterns gathered for other groups, suggesting that mesic forests once occupied the currently arid *Caatinga*. Further phylogeographic studies along the entire range of the Atlantic Rainforest and in other *Caatinga* forest enclaves (including *Brejos* located further away from the coast) are much needed and will likely provide relevant information about the history of this Neotropical region.

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