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Gene flow patterns reinforce the ecological plasticity of *Tropidurus hispidus* (Squamata: Tropiduridae)

Fernanda Ito, Danielle J. Gama-Maia, Diego M. A. Brito, Rodrigo A. Torres*

LAGEA – Laboratório de Genômica Evolutiva & Ambiental, Departamento de Zoologia, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Brazil
*Corresponding author: rodrigotorres@ufpe.br

Abstract. The analysis of gene flow patterns can provide important insights into population dynamics in the context of landscape ecology. In lizards, this approach has been used to evaluate patterns related to climate change, habitat fragmentation, and taxonomic uncertainties. *Tropidurus hispidus* is an ecologically plastic species, which presents some evidence of population structuring. In the present study, we investigated the potential structuring of *T. hispidus* populations across a gradient of tropical biomes, including the Amazon and Atlantic rainforests, the Caatinga dry forest, the Caatinga-Atlantic Forest transition zone (Agreste), coastal Restinga, and urban environments. Nuclear ISSR markers were obtained by PCR/electrophoresis, and a number of population parameters were estimated and analyzed. Despite the extreme environmental discontinuities found across the vast study area, the results revealed a high degree of genetic connectivity among the different demes. This pattern indicates that the species can be considered to be a single evolutionary taxon with gene flow among all populations, despite the marked environmental discontinuities. *Tropidurus hispidus* clearly has a marked capacity for dispersal, which may be favored by its intrinsic genetic diversity.

Keywords: *Tropidurus hispidus*, ISSRs, gene flow, dispersal capacity, population connectivity.

INTRODUCTION

Gene flow is one of the most important components in population structure because it can determine how much populations have evolved independently (Slatkin 2018). Therefore gene flow patterns can also provide important insights for studies on population ecology and also on population genetics based on a landscape ecology approach. The genetic admixture resulting from gene flow may contribute to a short-term increase in population fitness (Facon *et al.* 2005) and adaptive potential (Verhoeven *et al.* 2011). However, the approach usually focuses on micro-evolutionary phenomena and processes that lead to intraspecific discontinuities (Holderegger and Wagner 2006).

Genetic studies, especially in the Neotropical region, and in particular for reptile species, have been more frequent in the last years focusing on questions related to climate change, habitat fragmentation, and taxonomic uncertainties (*e.g.* Ricketts 2001; Stow *et al.* 2001; Berry *et al.* 2005; Driscoll and Hardy 2005; Sumner 2005; Hoehn *et al.* 2007; O'Neill *et al.* 2008; Tolley *et al.* 2009; Freedman *et al.* 2010; Levy *et al.* 2010; Werneck *et al.* 2015; Menezes *et al.* 2016; Fazolato *et al.* 2017; Cacciali and Köhler 2018; Oliveira *et al.* 2018). However studies focused on landscape genetics and on the gene flow patterns are still scarce in Neotropical region.

Tropidurus hispidus is one of the largest species of the genus, reaching a rostrum-caudal length (RCL) of 114 mm (Kolodiuk *et al.* 2010). It is found on a variety of substrates such as sand, tree trunks, and rocky outcrops, but it is primarily saxicolous, given that rocks provide space for foraging, shelter, nesting, and thermoregulation (Pelegrin *et al.* 2017). Also, this species is commonly found in urban areas, foraging and thermoregulating on walls and fences (Rodrigues 1987; Abreu *et al.* 2002; Pelegrin *et al.* 2017). The ecological tolerance of *T. hispidus* allows this species to occupy a number of distinct morphoclimatic domains, such as the Brazilian Atlantic Forest, coastal shrubby vegetation (Restinga), transition areas between the Caatinga scrub (in Portuguese Agreste), the Atlantic Forest, Cerrado savanna, and rocky outcrops in the Amazon basin (Vanzolini *et al.* 1980; Vitt 1995; Abreu *et al.* 2002; Carvalho 2013). The species is a habitat generalist, able to colonize a wide range of microhabitats (Rodrigues 1987; Vitt 1995; Vitt and Carvalho 1995; Vitt *et al.* 1997; Pelegrin *et al.* 2017).

This species is also an opportunistic sit-and-wait predator with a diverse trophic niche, feeding mainly on arthropods, in particular ants, but in some areas they may include plant material in their diet, especially flowers (Van Sluys *et al.* 2004; Ribeiro and Freire 2011; Pelegrin *et al.* 2017). Differences in the composition of the diet among biomes reinforce the ecological plasticity of the species (Pelegrin *et al.* 2017), but may also reflect distinct selective pressures on different populations.

In addition to these dietary differences, there is some evidence of genetic structuring among populations. Three distinct karyotypes have been found in six populations from different ecosystems in eastern Brazil (Kasahara *et al.* 1987; Kasahara *et al.* 1996). All karyotypes had 2n = 36 and XX/XY sex chromosomes, but three variants (prominent, mild or absent) were found in a secondary constriction of the second chromosome pair, which appeared to be typical of specific sites, suggesting genetic variation on an inter-population level. However, specimens from the six populations are morphologically indistinguishable (Kasahara *et al.* 1987; Kasahara *et al.* 1996). Also there is a clear evidence for cryptic diversity in *T. hispidus* as revealed by karyotype and DNA barcode sequences analyses (Matos *et al.* 2016).

Tropidurus hispidus is abundant across an extremely diverse ecological landscape (Carvalho 2013). From the coast of Pernambuco (north-eastern Brazil) to the Amazon basin there is a major shift in the geographical and ecological landscape, in which environmental variation may be reflected into distinct selective regimes, as previously suggested by the chromosomal and molecular evidences. Then, given previous ecological, distributional, karyotypical, and molecular evidence, we tested for the hypothesis of the existence of population-level divisions in *Tropidurus hispidus* along a highly diverse adaptive landscape in Brazil, using nuclear DNA markers adopting a gene flow approach.

MATERIALS AND METHODS

A total of 155 specimens of *Tropidurus hispidus* were captured at sites representing the distinct phytophysiognomic domains found across the landscape between the Pernambuco and Paraiba coasts in eastern Brazil, and the Amazon basin, in the north of the country (Table 1; Figure 1). The specimens were identified using the taxonomic key of Rodrigues (1987). Liver and muscle samples for DNA analyses were collected from each specimen. These samples were immersed in 96% ethanol and stored in a freezer at -20°C. Tissue was also obtained from three specimens of *Tropidurus torquatus* from Maricá, Rio de Janeiro, south-eastern Brazil, and one *Eurolophosaurus divaricatus* from Alagoado, Bahia, north-eastern Brazil, for inclusion in the study as outgroups.

DNA extraction and ISSR amplification

The extraction of DNA was conducted using the Sambrook and Russell (2001) procedure. The integrity of the DNA was checked by electrophoresis in agarose gel and the concentration was estimated by visual comparison with the intensity of the DNA of the Lambda phage. The DNA was then diluted to a standard concentration of 5 ng/ul for the PCR-ISSR reactions. Inter simple sequence repeats (ISSRs) are PCR-amplified nuclear genomic regions using primers anchored at microsatellite regions (SSRs) (Gupta *et al.* 1994; Zietkiewicz *et al.* 1994). These markers have been considered of low cost and highly reproducible (Sarwat 2012), and very effective

Table 1. Number (N) of *Tropidurus hispidus, T. torquatus*, and *Eurolophosaurus divaricatus* specimens captured in each site along the study area.

Species	Municipality* or state**	Geographic coordinates	Acronyms	Biome	N
Tropidurus hispidus	Camaragibe*	8°02’31”S 35°06’17”W	AF	Atlantic Forest	24
	Canguaretama*	6°22’58”S 35°07’29”W	Rest	Restinga	2
	Gravatá*	8°16’02”S 35°27’35” W	TZ	Transition zone	25
	Manaus*	3°09’34”S 59°36’10”W	AM	Amazon Forest	19
	Petrolândia*	9°05’37”S 38°15’05”W	Ca1	Caatinga	26
	Recife*	8°10’43”S 34°42’46”W	UZ	Urban zone	25
	Serra Talhada*	7°59’7”S 38°17’34”W	Ca2	Caatinga	30
	Tamandaré*	8°45’28”S 35°06’18”W	Rest	Restinga	4
				Total	155
Tropidurus torquatus (og)	Rio de Janeiro**	22°22’28”S 42°57’01”W			3
Eurolophorus divaricatus (og)	Bahia**	13°07’07”S 38°28’50”W			1

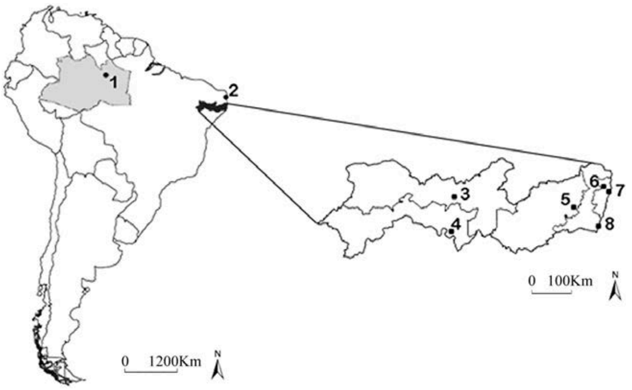

Figure 1. South America/Brazil map depicting capture sites of *T. hispidus*. In evidence are the different capture sites in Pernambuco (PE) Brazilian state. The biomes accessed are written here within parentheses as follows: 1. Manaus (Amazon Forest-AM), 2. Canguaretama-state of Paraíba (Restinga-Rest), 3. Serra Talhada (Caatinga-Ca2), 4. Petrolândia (Caatinga-Ca1), 5. Gravatá (Transition zone between Caatinga and Atlantic Forest-TZ), 6. Camaragibe (Atlantic Forest-AF), 7. Recife (Urban zone-UZ), 8. Tamandaré (Restinga-Rest).

in terms of studying the genetic variation and population cohesiveness in several biological groups (Gama-Maia and Torres 2016; Al Salameen *et al.* 2018; Hassaniem & Al Rashada 2019). The PCRs were carried out in a final volume of 20 μL in which consisted of 0.2 units of Taq DNA polymerase (New England/Biolabs), 1x buffer, 50 mM $MgCl_2$, 50 mM of primer, 0.2 mM dNTP and 20 ng of genomic DNA. The PCR reactions were run in a Biocycler thermocycler and comprised a cycle of 4 min at 94°C, 39 cycles of 40 s at 94°C, 40 s at the specific temperature of each primer (Table 2), and 120 s at 72°C, with a final annealing cycle of 7 minutes. All reactions were run with a negative control.

Horizontal electrophoresis was conducted in 1.8% agarose gel containing 0.5X TBE buffer diluted from an original 10X solution (0.89 M Tris, 0.89 M boric acid and EDTA, 0.01M, pH = 8.3) for 4 hours at 60 volts. In each well of the gel we placed a solution containing 10 μL of the PCR product in 1.5 mL of gel loading dye blue (6x) and 1.5 mL of gel green (0.5 ml 10,000x in H_20). To support the analysis of bands, we inserted 2 μL of 1 Kb DNA ladder marker with 1.5 mL of gel loading dye blue (6x) in one well. After the run, all gels were photographed using a transilluminator under an ultraviolet light source.

Data analyses

Initially, 17 different random ISSR primers were tested for their reproducibility and their degree of polymorphism. They were tested in five specimens from four sites using different PCR reagents from Fermentas (Thermo Fisher Scientific) and New England Biolabs Inc. (Table 2). The 10 most polymorphic primers were then selected for the amplification of the DNA of all the specimens (Table 2), with the objective of generating at least 60 polymorphic loci, as recommended by Telles *et al.* (2001) and Nelson and Anderson (2013). After photographic documentation, the gels were transformed into a binary matrix of presence and absence (0 = absent and 1= presence) of the DNA bands. In order to avoid the misinterpretation of valid markers, only clear and well-defined bands were assigned as markers. It is important to note that to increase sample size, the animals sampled in the localities of Caraguetama and Tamandaré were treated as a single sample in all analyzes, since both areas represent the same adaptive landscape (named Restin-

Table 2. ISSR primers tested in this study with sequence and annealing temperature. Selected primers are marked with (*).

Primer	Sequence (5' – 3')	Annealing temperature (ºC)
ISSR 1*	(AG)8T	50,4
ISSR 2*	(AG)8C	52,8
ISSR 3*	(GA)8T	50,4
ISSR 4	(GA)8C	52,8
ISSR 5*	(CT)8G	52,0
ISSR 6*	(AG)8YC	52,8
ISSR 7	(AG)8YA	54,0
ISSR 8*	(GA)8YT	52,8
ISSR 9*	(GA)8YC	52,8
ISSR 10	(GA)8YG	54,0
ISSR 11	(CT)8RA	50,0
ISSR 12	(AC)8YG	54,0
ISSR 13*	(GGAC)3A	51,0
ISSR 14	(GGAC)3C	51,0
ISSR 15	(GGAC)3T	51,0
ISSR 16*	(AACC)4	51,0
ISSR 17*	(GGAC)4	51,0

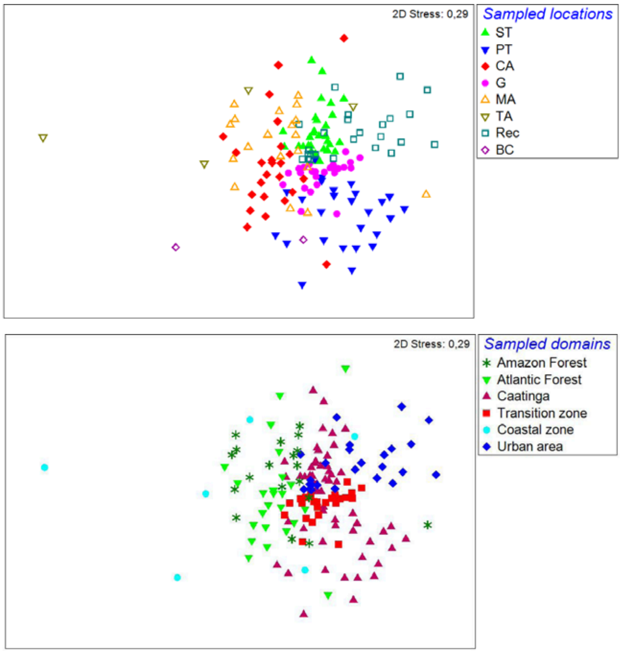

Figure 2. Multi-dimensional scaling plots of the genetic similarities (simple matching index) among the *Tropidurus hispidus* populations sampled in the present study. See Table 1 and text for acronyms. The graphs a/b show the different domains and localities respectively.

ga) (Table 1). The overall genetic variation was measured in percentage by the proportion of the polymorphic loci having the total number of observed loci as 100%.

To evaluate the existence of potential genetic and/or evolutionary groupings among biomes, multi-dimensional scaling (MDS) with neighbour-joining (NJ) genetic distances was applied on local and regional scales through the simple matching technique (Primer software) (Clarke and Gorley 2006). An additional NJ topology was also obtained by using PAUP* v.4.0b10 (Swofford 2000) in order to observe alternative groupings among sampled specimens. A Maximum parsimony (MP) method was also used in order to test for hidden evolutionary diversity in *T. hispidus* across those different adaptive landscapes (biomes) having *Eurolophosaurus divaricatus* and *Tropidurus torquatus* as outgroups given their phylogenetic proximity to the study species (Frost *et al.* 2001; Passoni *et al.* 2008). These analyses were run in PAUP* v.4.0b10 (Swofford 2000), in its graphic interface PaupUp v.1.0.3.1 (Calendini and Martin 2005). A maximum number of 100,000 random trees with 5000 replications were computed. The robustness of the branches was tested by the bootstrap method with 1000 random replicates.

Population structuring was tested by the Bayesian approach using the Structure 2.3.3 software (Pritchard *et al.* 2000; Falush *et al.* 2003, 2007; Hubisz *et al.* 2009). In order to determine the number of populations (K) within the complete data set, ten independent runs for K= 1-10 and 100,000 MCMC (Markov Chains Monte Carlo) interactions after burn-in period were computed. The analysis was performed by using both the admixture model of population structure and allele frequencies correlated among populations. The number of populations (K) was estimated using the protocol described by Evanno *et al.* (2005).

In addition, we conducted an analysis of molecular variance (AMOVA) to check for patterns of genetic isolation within and among local populations (Excoffier *et al.* 1992) with Arlequin v.3.5.1.2 (Excoffier and Lischer 2010). This method also permits the calculation of the global fixation index (Φ_{ST}). Parameters of genetic differentiation among populations (G_{ST}) and the number of migrants per generation (Nm – gene flow) were calculated with PopGene 1.3.2 (Yeh *et al.* 1999).

RESULTS

Based on the 10 ISSR primers selected, a total of 283 loci were observed. Overall, 99.2% of the observed loci were polymorphic. Mean genetic neighbor-joinning distances among local populations varied from 0.12720 to 0.48763.

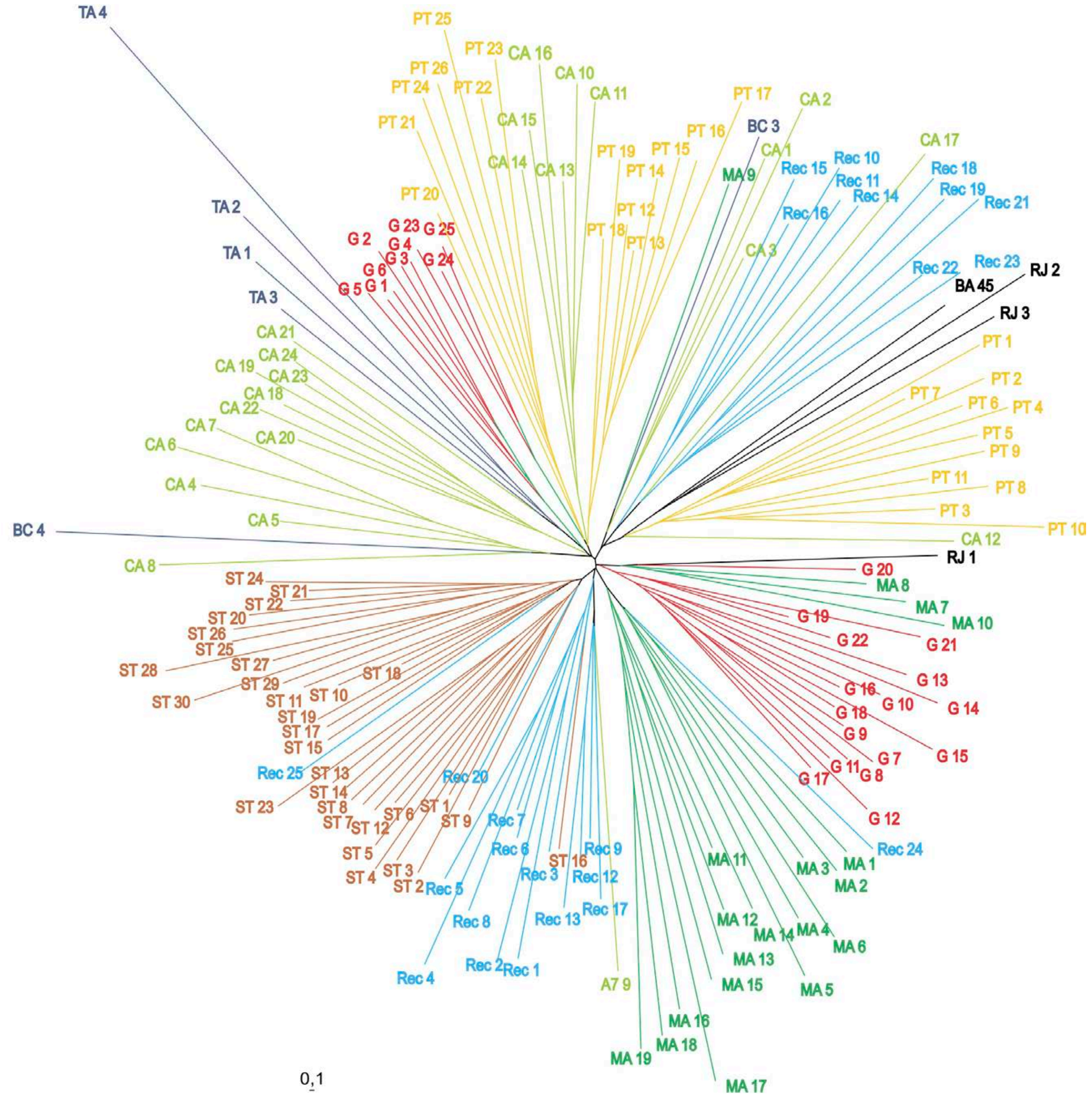

Figure 3. Neighbor-Joinning topology from *Tropidurus hispidus* specimens for ISSR markers. See Table 1 and text for acronyms.

The simple matching MDS analysis revealed a single grouping comprising all sampled populations on both regional and local scales (Figure 2a-b). The NJ topology showed also no particular genetic groupings among *T. hispidus* sampled from different biomes (Figure 3). The maximum parsimony (MP) analysis revealed 2 constant and 281 informative characters. The majority-rule consensus topology (Supplemental material) had a length (L) of 6236, a consistency index (Ci) of 0.045, and a retention index (R) of 0.310. This topology also failed to identify any evolutionary differentiation among the populations analysed.

The analysis identified a total of 283 loci and more than 90% were variable in terms of the proportion of polymorphic loci. This amount of molecular information satisfies the recommendation of Nelson and Ander-

Table 3. Results of the AMOVA for the *Tropidurus hispidus* populations in the study area. ($p < 0.01$).

Source of variation	Degrees of freedom	Sum of squares	Components of variance	% of variance
Among populations	6	729.782	3818.75 va	9.01
Within populations	148	5709.625	38,578.55 vb	90.09
Total	154	6439.407	42,397.30	100
Φ_{ST}	0.09007			

Table 4. Pairwise G_{ST} (above diagonal) and Nm (below diagonal) values recorded between *Tropidurus hispidus* populations. For acronyms, please refer to Table 1.

	Ca2	Ca1	TZ	AF	UZ	AM	Rest
Ca2	-	0.0420	0.0363	0.0363	0.0322	0.0360	0.0527
Ca1	11.4029	-	0.0457	0.0379	0.0368	0.0435	0.0511
TZ	13.2673	10.4325	-	0.0383	0.0388	0.0458	0.0605
AF	13.2673	12.7004	12.5522	-	0.0302	0.0341	0.0455
UZ	15.0321	13.0902	12.3747	16.0316	-	0.0341	0.0505
AM	13.3976	10.9953	10.4261	14.1587	14.1595	-	0.0492
Rest	8.9877	9.2855	7.7588	10.4786	9.4000	9.6556	-

son (2013) for the application of AMOVA and Bayesian structuring analyses. The AMOVA indicated that 90.99% of the total genetic variance was found within populations and only 9.01% among populations (Table 3). The Bayesian structuring analysis revealed the existence of two genetic populations (K= 2; Figure 4), and these genetic profiles were clearly distributed in all specimens throughout the geographic areas sampled. The global G_{ST} value was 0.07, while the Nm was 6.59. The pairwise analyses showed values ranging from 0.03 to 0.06 for G_{ST} and from 7.75 to 15.03 for Nm (Table 4).

DISCUSSION

The genetic evidence of this study indicates strong connectivity among local *T. hispidus* groups, despite the intense ecological distinctiveness of the landscapes seen in the study area. The MDS (Figure 2), the NJ topology (Figure 3), and the MP topology (Supplemental material) evidenced a lack of any genetic or evolutionary differentiation among *T. hispidus* groups, pointing to a high dispersive behaviour in this species.

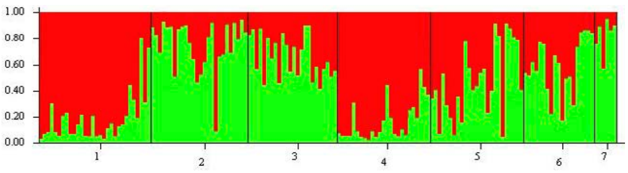

Figure 4. Bayesian structuring analysis. The Y axis indicates the probability-based assignments for the genetic composition of each specimen analyzed (vertical bars). Note (1) Ca2, (2) Ca1, (3) AF, (4) TZ, (5) UZ, (6) AM, (7) Rest (including specimens from Canguaretama and Tamandaré) . For a description of the sites, see Table 1.

Tropidurus hispidus is widely distributed in the Caatinga and can also be found along the Brazilian coast and in the Amazon Forest (Carvalho 2013). The extent of its distribution range would suggest a high probability of differentiation due to strong and diverse evolutionary pressures imposed to the populations (Kisel and Barraclough 2010). However, the clustering produced by the Bayesian analyses showed also no genetic structuring (Figure 4). Although there are two genetic populations, the analysis indicated a clear admixture of these two *T. hispidus* gene pools among the demes studied.

The lack of genetic structuring resulted from an intense gene flow among populations as indicated by the degree of migrants per generation (Table 4). The observed global Nm value (6.59), as well as the pairwise ones (7.75 – 16.03), support the hypothesis of strong evolutionary cohesion, since Nm ≥ 1 indicates a minimum amount of genetic migration capable of homogenizing demes within species (Mills and Allendorf 1996), including in lacertids (Levy *et al.* 2010). This feature of a highly cohesive species could be also explained by a recent irradiation phenomenon. However this hypothesis requires a robust phylogeographic study offering coalescence-dating analyses.

Considering all the results, it is possible to argument in favour to the hypothesis of panmixia in *T. hispidus*, despite the discontinuity and historical changes seen in the biomes studied. This is surprising since *T. hispidus* individuals are sit-and-wait predators, territorialists, and oviparous that would suggest a tendency for structurings (Prieto *et al.* 1976; Van Sluys *et al.* 2010; Ribeiro and Freire 2011). Besides not dispersing through long distances (Pontes *et al.* 2008), sit-and-wait predators are usually opportunists and can feed on a variety of food items according to the local availability (Rodrigues 1987, 1988; Vitt 1991; Bergallo and Rocha 1993; Vitt 1995; Vitt *et al.* 1997; Pontes *et al.* 2008) suggesting ecological plasticity. Ecological plasticity predicts high genetic diversity. Higher genetic diversity tends to favour a better adaptation at the population, community and ecosystem levels (Hughes *et al.* 2008). The feeding plasticity observed in *T. hispidus* (Pelegrin *et al.* 2017) is related to its high success in attempts to colonize new sites (Teixeira and Giovanelli 1999) and to its capacity of expanding towards new habitats (Levy *et al.* 2010; Breininger *et al.* 2012). This hypothesis is supported by the high degree of genetic diversity observed herein in *T. hispidus* and this feature might be favouring historically the species to a better adaptation to different biomes. These combined evidences suggest the stepping-stone model of range expansion as a probable explanation for wide distribution of *T. hispidus*.

Molecular studies with species of *Tropidurus* have revealed different patterns of evolutionary cohesion among populations, depending on the species studied. For instance, in *Tropidurus semitaeniatus* and *T. hygomi*, populations tend to be highly structured, but due to different processes. In *T. semitaeniatus* the process of population structuring was mediated by the course of the River São Francisco (Northeastern Brazil; Werneck *et al.* 2015). In *T. hygomi*, the population structuring was associated to different marine transgression/regression events, which isolated or connected regions along the Brazilian coastal plains (Fazolato *et al.* 2017).

Tropidurus hispidus was expected to show the same pattern of genetically structured populations due to geographic isolation by different ecological pressures of morphoclimatic domains of humid forests and the Caatinga (González *et al.* 2011; Matos *et al.* 2016). The data supporting these conclusions were the karyotypic structure and COI gene sequences, respectively (Matos *et al.* 2016). However, our data failed to reinforce this idea and the analyses of the hypervariable regions of ISSR nuclear markers strongly pointed to panmixia. This occurred despite the geographical distances and the different selective pressures among studied biomes. A likely explanation for these contrasting evidences could be an intense dispersive behavior showed by *T. hispidus* males. Indeed, the use of bi-parental genetic markers has been recommended as a strategy to understand patterns of gene flow and demography (Goudet *et al.* 2002). The ISSRs markers analysed in this study agree with this recommendation and allow inferences about the gene flow among *T. hispidus* demes.

Cases of male-mediated dispersion in lizards have been documented in the literature in the last years (e.g. Johansson *et al.* 2008; Mouret *et al.* 2011; Ferchaud *et al.* 2015). When considering mitochondrial markers, of female inheritance, populations seem structured, (Matos *et al.* 2016) but when the male genetic pools is also analysed such structuring disappears, as seen here using the ISSRs markers. This supports the hypothesis that the expansion of *T. hispidus* distribution range, and therefore, new colonisations, would depend on a higher ecological ability of males to disperse farther than females. Mark-recapture studies of males and females could confirm the explanations given herein.

The occurrence of *T. hispidus* in urban areas, and its use of anthropogenic structures, (Carvalho 2013; Pelegrin *et al.* 2017) could lead to facilitated dispersion and extend its distribution range. Human-facilitated dispersion occurs in other lizard species, including exotic and invasive species (Vanzolini 1978; Mausfeld *et al.* 2002; Anjos and Rocha 2008). The *T. hispidus* population of Manaus (Amazon), which was recently invaded by individuals from Roraima (Northern Brazil), is an example of this phenomenon (Ávila-Pires 1995; Carvalho 2013). However, this is speculative since we lack genetic data from Roraima. On the other hand, the individuals from Manaus had the same genetic profiles as the populations from Pernambuco, and did not show any type of genetic structuring, corroborating the hypothesis of panmixia along our study area.

Our results revealed also that *Tropidurus hispidus* has a genetic variation above 90%. This points to an excellent conservation status along the studied area, considering that low genetic variation would decrease this species' ability to adapt to current and stochastic selective pressures (Frankham and Ralls 1998; Frankham *et al.* 2002; Allendorf and Lundquist 2003). Indeed, *T. hispidus* seems to have a high tolerance to habitat modifications (Rodrigues 1987; Ávilla-Pires 1995), and it is a generalist regarding its microenvironmental requirements (Vitt 1995; Mendonça and Moura 2011; Pelegrin *et al.* 2017). Therefore, our data reinforces this biological attribute (evolutionary potential), due to the high genetic variation observed.

To conclude, according to our results, the sharing of a high genetic variation among the several *T. hispidus* population demes from different morphoclimatic domains seems to explain its ecological plasticity/evolutionary potential. According to Vitt *et al.* (1997) and Ivkovich *et al.* (2010) this is common in species with wide distribution ranges. This is a testable hypothesis that could be further tested in other Neotropical lizard species that have distribution patterns similar to *T. hispidus*.

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