



# Model-based riverscape genetics: disentangling the roles of local and connectivity factors in shaping spatial genetic patterns of two Amazonian turtles with different dispersal abilities

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## Abstract

Genetic patterns are shaped by the interaction of different factors such as distance, barriers, landscape resistance and local environment. The relative importance of these processes may vary for species with different ecological traits. Here we compared two related Amazonian riverine turtle species (*Podocnemis erythrocephala* and *Podocnemis sextuberculata*) with distinct dispersal abilities to assess how differently local and connectivity variables influence their genetic patterns. We used a total of 609 genetic samples to estimate mitochondrial (mtDNA) genetic diversity and differentiation for each locality. We applied model selection on models associating genetic diversity to local variables representing hypotheses of climate and primary productivity, water level variation, hunting pressure and downstream increase in genetic diversity. We modeled the relationship of genetic differentiation with connectivity variables representing hypotheses of isolation by distance (IBD), isolation by resistance (IBR) and isolation by barrier (IBB). Model selection for genetic diversity was only important (excluded the null model) for the high-dispersal species (*P. sextuberculata*), with best models including hypotheses of productivity and hunting pressure. Genetic diversity was higher in more productive sites and in sites with higher concentration of villages (opposed to expected). Although a variable importance testing showed low importance for connectivity models, IBB (Amazon River) and IBR (resistance by current and past climatic suitability and river color) models explained more genetic differentiation turnover than IBD (riverway distance). Models explained a higher percentage of genetic differentiation for the low-dispersal species (*P. erythrocephala*), with Amazon River as main predictor. We show that, although local variables are often overlooked in riverscape genetics studies, they can influence intrapopulacional genetic diversity of aquatic species, even those with high dispersal ability. By applying a resistance-model framework and by using riverscape genetics factors relevant in basin-wide context, we provide a novel approach to investigate genetic patterns of other aquatic vertebrates in fluvial systems.

**Keywords** Amazon basin · Genetic differentiation · Genetic diversity · Landscape genetics · *Podocnemis* · Resistance model

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## Introduction

Associations between landscape factors and ecological processes such as dispersal, reproduction and survival of organisms can ultimately affect evolutionary processes such as gene flow, drift and selection (Sork and Waits 2010). Understanding these associations and their effects is essential for species conservation because factors that negatively impact the genetic diversity and connectivity of populations can eventually drive species extinction (Spielman et al. 2004). Landscape genetics emerged as a research field that combines population genetics, landscape ecology, and spatial analyses to explicitly quantify the effects of landscape composition, configuration, and matrix quality on evolutionary processes (Balkenhol et al. 2016). Since the term was coined (Manel et al. 2003), the field evolved from descriptive approaches to explicit hypothesis testing framework and modeling of genetic responses in response to predictive landscape variables (Storfer et al. 2010). Although only 15% of landscape genetic studies were conducted in freshwater habitats (Storfer et al. 2010), there is mounting evidence for complex spatial genetic structure in these habitats (e.g., Hughes et al. 2009; Ozerov et al. 2012; Hand et al. 2015). However, in freshwater environments, especially in fluvial systems, the process of isolation by distance-IBD (Wright 1943) can often overwhelm the importance of other processes that might shape genetic patterns (Selkoe et al. 2016). As such, in river systems, it is especially necessary to implement approaches that are able to disentangle the confounding effects of geographical distance and other environmental factors.

While IBD is responsible for part of the population genetic structure in several taxa (Jenkins et al. 2010), landscape environmental heterogeneity can affect synchronization of migration and mating processes among populations, modifying gene flow patterns and increasing genetic differentiation (Wang and Bradburd 2014). Riverscape genetics studies usually test for discrete barriers (isolation by barrier, IBB) such as waterfalls and dams (Kanno et al. 2011; Wofford et al. 2005). Nevertheless, less conspicuous factors, such as stream slope gradient and water physical–chemical dissimilarities may also act as barriers to gene flow and cause detectable differentiation (Beheregaray et al. 2015; Cook et al. 2011). These environmental dissimilarities are rarely—if at all—assessed in terms of resistance to migration between populations, resulting in a lack of empirical studies with riverscape resistance models (Davis et al. 2018). For terrestrial species, least-cost paths (LCPs) and resistance surfaces have been shown to better predict gene flow patterns among localities than direct measures of dissimilarity or distances (McRae 2006; Wang et al. 2013). Also, integration of climatic suitability models into LCP analyses can improve our understanding on landscape connectivity, potential routes of dispersal and distribution of suitable habitats for the species (Wang et al. 2008).

Connectivity variables alone often do not explain observed spatial genetic structure of freshwater populations, and local processes may also influence neutral genetic patterns (Murphy et al. 2010; Kovach et al. 2015). Local factors can affect effective population sizes ( $N_e$ ) and, through genetic drift, leave a strong signal in genetic diversity (Frankham 1996; Wagner and Fortin 2013; Wright 1931). Regardless of the importance of genetic diversity on maintaining population fitness and reducing extinction risk, very few landscape genetics studies consider the effects of site-based, local variables on intrapopulation genetic diversity (DiLeo and Wagner 2016). In river networks, for example, a broad variety of taxa show a pattern of downstream local accumulation of genetic diversity due to biased gene flow (Downstream Increase in Intraspecific Genetic Diversity—DIGD; Paz-Vinas et al. 2015). Additionally, local population persistence can be negatively affected by intense

human harvesting and inter-annual water level fluctuations, and positively affected by at-site suitable climate and productivity conditions (Allendorf et al. 2008; Kovach et al. 2015; Murphy et al. 2010; Ouellet-Cauchon et al. 2014).

Comparative studies are essential to elucidate how intrinsic ecological differences among species can generate distinct effects of local and connectivity landscape factors on genetic patterns (Reid et al. 2017). For instance, differences in dispersal ability among closely related species correlate with distinct genetic patterns (Steele et al. 2009). Low-dispersal species, compared to species with high-dispersal capacities, often exhibit higher genetic divergence, lower genetic diversity and more pronounced spatial genetic structure (Gomez-Uchida et al. 2009; Richardson 2012; Steele et al. 2009). This may lead to stronger genetic response to local and connectivity factors for poor-dispersers due to increased drift and lower gene flow among localities (Gomez-Uchida et al. 2009). These comparisons are particularly useful in guiding management strategies for threatened organisms inhabiting heterogeneous landscapes (Reid et al. 2017), as it is the case of aquatic Amazonian vertebrates.

The Amazon basin is the largest hydrographic basin in the world, composed of an environmentally heterogeneous system formed by rivers, streams and floodplain forests with varying geomorphology, flood pulse dynamism and physical–chemical water properties (Sioli 1984). This complexity influences the movement, mating and survival of organisms, shaping population genetic patterns of several aquatic vertebrates (Beheregaray et al. 2015; De Thoisy et al. 2006; Gravena et al. 2015; Pearse et al. 2006). However, to our knowledge, no study has attempted to use a spatially explicit model-based framework to test which Amazon basin riverscape factors may be behind the observed genetic patterns. Not only for Amazon basin, this type of approach is necessary to help develop a robust riverscape genetics framework, which will improve our understanding of the relationships between freshwater organisms and their environment (Davis et al. 2018). Therefore, we assessed the importance of local and connectivity variables in shaping the spatial genetic variation of two Amazon River turtle species differing in their dispersal abilities and habitat preferences. Because river turtles live in the land–water interface and have variable life history traits influenced by landscape factors, they are appropriate models to understand broader patterns and processes taking place at the Amazon basin.

Here we tested the hypotheses that (1) connectivity factors that reduce gene flow are related to genetic differentiation for a low-dispersal species (*Podocnemis erythrocephala* Spix, 1824), but not for a high-dispersal species (*Podocnemis sextuberculata* Cornalia, 1849); and (2) local factors are related to intraspecific genetic diversity of both species, but leave a stronger effect on the diversity of the low-dispersal species (*P. erythrocephala*). For this, we used biologically meaningful local variables representing hypotheses of climate and productivity, instability of inter-annual water levels, hunting pressure and downstream increase in intraspecific genetic diversity (Table 1). These local variables are hypothesized to reduce or increase effective population sizes ( $N_e$ ), consequently affecting the rate of genetic drift and diversity of populations. The connectivity variables we used represent hypotheses of isolation by distance (IBD), isolation by resistance (IBR) and isolation by barrier (IBB) (Table 1). The IBR models include resistance offered by different river types, by climatically unsuitable habitats (current and historical) and by slope. These connectivity variables are hypothesized to restrict dispersal and mating patterns among localities, reducing gene flow and increasing genetic differentiation among populations.

**Table 1** Local and connectivity variables hypothesized to influence genetic diversity (Hd and  $\pi$ ) and differentiation ( $\phi$ ST) for *Podocnemis erythrocephala* and *Podocnemis sextuberculata*

Response variables	Hypotheses	Predictor variables	Pred.	Mechanism	References
Genetic diversity	Energy availability	Net primary productivity	+	More productive locals provide more energy (resources, food) for populations, enhancing population persistence and population sizes	Wright (1983), Murphy et al. (2010). Data source: MODIS 17, NASA (2016)
	Current environmental stability	Current climatic suitability	+	Locals with current suitable climatic conditions favor higher survival and larger population sizes	Hand et al. (2015), Kovach et al. (2015). Data source: WorldClim v. 1.4.
	Historical environmental stability	Historical climatic suitability (average from last 120 kyrs)	+	Locals with historical climatic suitability sheltered higher survival and persistence of populations through time	Carnaval et al. (2009), Graham et al. (2006). Data source: WorldClim v. 1.4; Carnaval et al. (2014)
	Variability of high river flow (wet season)	Coefficient of variation of interannual high river flow	-	Annual variability on highest water levels decreases recruitment by nests inundation	Batisiella and Vogt (2008), Pantoja-Lima et al. (2009). Data source: Silva-Junior (2015)
	Variability of low river flow (dry season)	Coefficient of variation of interannual low river flow	-	Annual variability on lowest water levels decreases predictability of available nesting beaches for local populations, hampering nesting and reproductive success	Alho and Pádua (1982), Bermúdez-Romero et al. (2015), Onelle-Cauchon et al. (2014). Data source: Silva-Junior 2015
	Commercial hunting	Distance to nearest urban center	+	Populations distant from urban centers are less subjected to large removal of adult individuals for traffic and illegal consumption, sheltering larger population sizes	Alcántara et al. (2013), Schneider et al. (2011), Smith (1979). Data source: IBGE
	Subsistence hunting	Kernel density of riverine and rural human communities locations	-	Populations densely surrounded by human rural settlements are more exposed to predation of eggs (lower recruitment) and nesting females (smaller population sizes)	Bernardes et al. (2014), Schneider et al. (2011). Data source: IBGE
	Downstream increase in intraspecific genetic diversity (DIGD)	Distance from Amazon River mouth	-	Downstream locals have biased accumulation of alleles and immigrants	Paz-Vinas and Blanchet (2015), Paz-Vinas et al. (2015). Data source: Venticinque et al. (2016)

**Table 1** (continued)

Response variables	Hypotheses	Predictor variables	Pred.	Mechanism	References
Genetic differentiation	Isolation by distance (IBD)	Riverway distance	+	Reduced migration and mating between geographically distant populations	Wright (1943)
	Isolation by resistance (IBR)	Cost distance of resistance from river types	+	Reduced migration and mating between populations separated by costly water types	Beheregaray et al. (2015), De Thoisy et al. (2006). Data source: Venetique et al. (2016)
		Cost distance of resistance from slope	+	Reduced migration between populations separated by topographically complex pathways offering resistance from slope	Caldera and Bolnick (2008), Cook et al. (2011), Kanno et al. (2011). Data source: Domisch et al. (2015)
		Cost distance of resistance from current climatic suitability	+	Reduced migration and mating between populations separated by current climatically unsuitable pathways	Mitchell et al. (2015)
		Cost distance of resistance from historical climatic suitability (average from last 120 kyrs)	+	Reduced migration and mating through time between populations separated by climatically unsuitable historical pathways	Ortego et al. (2015)
	Isolation by barrier (IBB)	Presence of Amazon River (only for <i>P. erythrocephala</i> )	+	Reduced migration and mating between populations in opposite margins of Amazon River	Santos et al. (2016)

For each hypothesis, we reviewed the literature for evidences and potential biological mechanisms linking the variables to the predicted effects (“Pred.”) on genetic diversity or genetic differentiation. The predictor variables for genetic diversity describe variation within localities (local variables) while the variables for genetic differentiation describe environmental variation between localities (connectivity variables). References include both the literature reviewed and the data sources used to produce each predictor variable. Full references details on Supplementary Information

## Materials and methods

### Study species

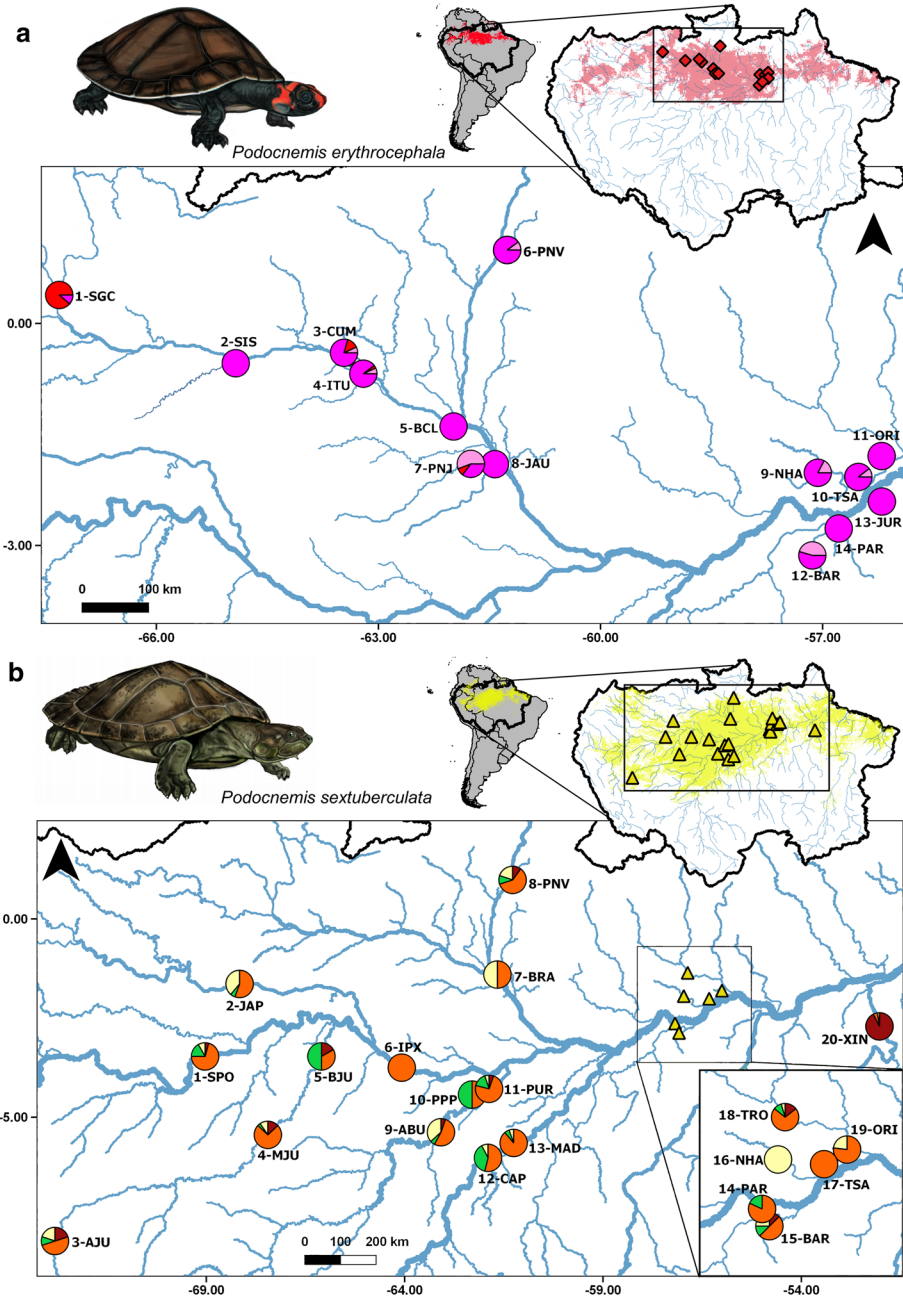
*Podocnemis erythrocephala* Spix, 1824 (Red-headed Amazon River Turtle) is the smallest *Podocnemis* species occurring in the Amazon basin, reaching 32.2 cm of carapace length. It is also the least broadly distributed, occurring in Brazil, Colombia and Venezuela, mainly in blackwater rivers and their tributaries, but also in clearwater lakes and rivers (Ferrara et al. 2017). The second smallest species, reaching 34 cm of carapace length, *Podocnemis sextuberculata* Cornalia, 1849 (Six-tubercled Amazon River Turtle), is broadly distributed in the Amazon River drainage in Peru, Colombia and Brazil, mainly in large whitewater and clearwater rivers (Ferrara et al. 2017). The geographic distribution of the two species overlap in a few regions in Amazon River tributaries. *Podocnemis sextuberculata* is a high-dispersal species whose females migrate long distances to nest in large groups in broad sandy beaches (Vogt 2008), with records of up to 60 km moved by a female in a year (Fachin-Terán et al. 2006). *Podocnemis erythrocephala* has lower dispersal potential, being commonly found in smaller streams and lakes instead of main river channels, and females nest alone or in small groups in sandy shrub lands or forests (Mittermeier et al. 2015).

### Study region and genetic sampling

We used a total of 609 samples from 14 localities for *P. erythrocephala* and 20 localities for *P. sextuberculata* (Fig. 1; Appendix S1 in Supplementary Information), covering a large portion of their ranges. We used the mtDNA control region (CR) as molecular marker for both species due to the high polymorphism reported for the genus (Pearse et al. 2006; Santos et al. 2016; Viana et al. 2017), the broad sampling scale and the potential historical effects of predictor variables. For *P. erythrocephala*, we used 273 sequences (503 bp), from which 246 were from the work of Santos et al. (2016; GenBank KY702009–KY702254). We sequenced 27 additional samples (5-BCL, 8-JAU, 13-JUR and 14-PAR; Fig. 1a; Appendix S1; GenBank KY713319–KY713345) following the same procedures. For *P. sextuberculata*, we used 336 sequences (605 bp), from which 319 were from the recent work of Viana et al. (2017; GenBank KY702255–KY702573). We sequenced an additional 17 samples (6-IPX, 10-PPP and 12-CAP; Fig. 1b; Appendix S1; GenBank KY713302–KY713318) following the same procedures. The additional samples were collected under collecting permits 44832-1 and 5119-1 issued by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio).

### Genetic diversity and differentiation

To describe the genealogical relationships among localities we constructed a haplotype network for each species using HAPLOVIEWER (Salzburger et al. 2011) using maximum likelihood phylogenetic trees estimated in RAxML (Stamatakis 2006) with GTRGAMMA model. To assess patterns of population structure at the broad scale for each species we inferred the most probable number of genetic clusters (K) and individual's assignment to each cluster with a Bayesian analysis of population admixture implemented in BAPS v. 6.0 (Corander et al. 2006). For the mixture analysis we ran five independent simulations for each value of K ranging from 1 to the maximum number of localities of each species. We then ran an admixture analysis with 100 interactions, 100 individuals of reference and 10



**Fig. 1** Sampling localities for *Podocnemis erythrocephala* (a) and *Podocnemis sextuberculata* (b). Pie charts represent percentage of individuals belonging to biological clusters identified by Bayesian analysis of population structure in BAPS. BAPS graphs are depicted in Appendix S5 Figures S5.17 and S5.18. Background masks in maps correspond to potential geographic distribution of each species, as estimated by Fagundes et al. (2015). Turtle illustrations: Karl Mokross

interactions to evaluate the individuals. To characterize intraspecific genetic differentiation among localities we performed an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) using pairwise  $\phi_{ST}$  between sampling sites using the software ARLEQUIN v. 3.5.2.2 (Excoffier and Lischer 2010), testing for significance by randomization with 1000 permutations.

We calculated for each sampled locality two intraspecific genetic diversity indices (response variables): haplotypic diversity (Hd; Nei 1987) and nucleotide diversity ( $\pi$ ; Nei 1987), in DNASP v.5.10.1 (Librado and Rozas 2009). We also estimated pairwise  $\phi_{ST}$  between sampling sites using the software ARLEQUIN v. 3.5.2.2 (Excoffier and Lischer 2010), testing for significance by randomization with 1000 permutations. We used the diversity metrics as response variable for node-level analysis and the pairwise  $\phi_{ST}$  for the link-level analysis.

## Landscape data

We describe the hypotheses and mechanisms linking the local (nodes) and connectivity (links) factors to the expected effects on, respectively, diversity and differentiation indices of populations in Table 1. We collected several landscape metrics for each analytical approach (nodes and links) in order to represent non-mutually exclusive hypotheses that may explain diversity and differentiation patterns for the species.

## Node-level local variables

To test the *energy availability* hypothesis, we used mean Net Primary Productivity (NPP 2000–2015; Appendix S2 Figure S2.1) as a proxy of available resources in each locality sampled. For each locality, we obtained the mean NPP in a buffer of 5 km of radius for *P. erythrocephala* and of 12 km of radius for *P. sextuberculata*. We selected these buffer radii based on mark-and-recapture and movement studies describing mean linear distances for individuals of each species (Bernhard 2010; Fachin-Terán et al. 2006).

To test the *environmental stability* hypotheses, we used Ecological Niche Modeling (ENM) to predict the climatic suitability for each species. We used a total of 158 occurrence records for *P. erythrocephala* and 329 for *P. sextuberculata*. These occurrences were compiled by Fagundes et al. (2015) and include data from literature review, Brazilian scientific collections and museum specimens, and unpublished data from turtle specialists. We built models using the maximum entropy algorithm, MAXENT, implemented in the R package ‘dismo’ (Hijmans et al. 2015). To construct the models we used seven bioclimatic variables (BIO1, BIO4, BIO10, BIO11, BIO 12, BIO15, BIO16 AND BIO17) from the WorldClim database (<http://www.worldclim.org>) interpolated to 1 km resolution (Hijmans et al. 2005), removing highly correlated variables ( $r > 0.8$ ). We produced 20 replicate model runs to statistically evaluate the models, using 75% of the records for training and 25% for testing. We evaluated model performance using the area under the curve (AUC) of the receiver operating characteristic (ROC) plot, which ranges from 0.5 (random prediction) to 1 (maximum prediction). The output raster layers have an index of suitability for each cell ranging from 0 to 1, being low values indicative of unsuitable conditions for species occurrence and high values indicative of suitable conditions. We used the projection to present conditions (Appendix S2 Figure S2.2a, b) as the variable for *current environmental stability* hypothesis. In addition, to enable a continuous view of historical climatic suitability, we projected the models to 62 climatic reconstructions covering the last 120



thousand years (kyr) at small time intervals (1–4 kyr) using the Hadley Centre Climate model (HadCM3; Carnaval et al. 2014). We calculated the mean value of suitability for the 62 layers of time and used the resulting mean raster layer (Appendix S2 Figure S2.2c,d) as the variable of *historical environmental stability* hypothesis.

To test the *high variability of extreme water levels* hypotheses, we used two raster maps created by Silva-Junior (2015) representing extremes of river flows (Appendix S2 Figures S2.3–S2.4). The rasters were generated from the coefficient of variation of high (CVmax—*Variability of high river flow* hypothesis) and low (CVmin—*Variability of low river flow* hypothesis) river flows for 5 thousand points in Amazon basin for the period of 1998–2009.

We divided hunting pressure in two hypotheses: subsistence and commercial hunting pressures. To test for the subsistence consumption of turtles by rural/riverine human villages, we used the Heatmap plugin in QGIS to create a kernel-density map of villages in the sampling region from a shapefile with geographic points of villages and rural/riverine settlements. This generated a raster (heatmap) in which the “hot” spots have a high density of villages and the “cold” spots have low density of settlements (Appendix S2 Figure S2.5). We used the kernel values of each sampling locality as a surrogate for *subsistence hunting pressure* hypothesis. In addition, because urban centers are the final destination for illegally caught turtles (Pantoja-Lima et al. 2014), we measured for each sampling locality the distance (by riverway) to the closest urban center (Appendix S2 Figure S2.6) as a surrogate for *commercial hunting pressure* hypothesis.

Finally, to test for the hypothesis of *Downstream Increase in Intraspecific Genetic Diversity* (DIGD), we defined the mouth of Amazon River as the ultimate downstream point and extracted for each locality its riverway distance to Amazon River mouth (Appendix S2 Figure S2.7).

### Link-level connectivity variables

To test the hypothesis of *isolation by distance* (IBD) we measured the river distance between localities using the R package ‘gdistance’ (van Etten 2012) and a spatial-autocorrelation analysis was performed.

For the links analytical level we used resistance models (*isolation by resistance*, *IBR*), a novel approach for riverscape genetics that can increase our understanding of gene flow patterns as it tests specifically for migration complexity and resistance between populations. The least-cost paths (LCPs) are calculated by searching for the path that minimizes the total cumulative cost (or resistance) between two points (Wang et al. 2009). The riverscape genetics approach differs from landscape genetics (terrestrial habitats) in that for species using exclusively river ways to move, the only path possible is the river path. Therefore, the LCPs between two localities will always be the same regardless of the variable under consideration. However, the cost values of each pixel (and therefore the accumulated-cost of LCP) will be distinct for different variables. To characterize *isolation by resistance* (IBR) we used slope, river types (water types) and climatic suitability (Table 1).

We calculated LCPs of average upstream *slope* (Appendix S2 Figure S2.8; Domisch et al. 2015) between localities as a surrogate for the presence of topographic barriers (e.g., rapids or waterfalls) or increased topographic resistance to turtles’ movement.

The rivers in Amazon basin are classified in three types (black, white and clear waters; Appendix S2 Figure S2.9) based on different origins and physical–chemical properties of their waters (Sioli 1984). Since there is a lack of biological data on movement preference

related to water types, we used expert parameterization of resistance values (Zeller et al. 2012). We sent a questionnaire (Appendix S3) to six Amazon turtle experts, asking them to assign different costs to each water type representing how costly they are to the movement of each species. The cost values would range from 1 (low or no cost to animal movement) to 5 (high cost or barrier to movement). Because the responses varied among experts (Appendix S3), we used the mean cost value of their opinions to calculate the LCPs between localities.

To assess resistance to movement offered from present and past climatic unsuitable habitats we clipped the historical and current ENM maps generated (see *node-level local variables* section) to the river courses. We used the reverse of suitability values (1—suitability) to assign resistances to each pixel in the river network for each species, because places with lower suitability should represent higher resistance to the movement (Wang et al. 2013). The resulting raster maps (Appendix S2 Figures S2.10–S2.13) have resistance values ranging from 0 (no resistance to species movement) to 1 (complete resistance). We then calculated LCPs between localities for historical and current resistance imposed by unsuitable habitats for each species.

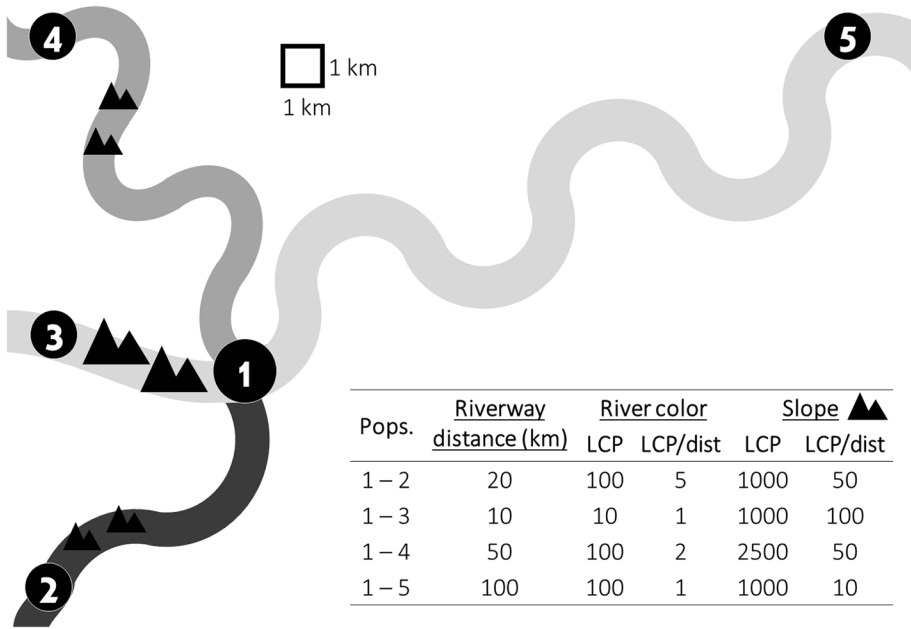
Additionally, the Amazon River was proposed as a potential barrier to the dispersal of *P. erythrocephala* (Santos et al. 2016) because of its large extension and whitewaters. Therefore, we attributed binary codes for localities from the same (0) or opposite (1) sides of Amazon River to test for *isolation by barrier* (IBB), only for *P. erythrocephala*.

## Landscape genetics analyses

Because the genetic metrics used here can be affected by sampling sizes (Goodall-Copestake et al. 2012), we only used localities for which we had at least 10 individuals sampled ( $N \geq 10$ ). This reduced our number of sites from 14 to 11 for *P. erythrocephala* and from 20 to 17 for *P. sextuberculata*.

For node-level analysis, we modeled the genetic response variables ( $H_d$  and  $\pi$ ) in relation to the predictor landscape variables using generalized linear models (GLMs). To avoid multicollinearity we only included non-correlated predictor variables in mixed models ( $r < 0.6$ ; Appendix S4 Table S4.3). We also tested for the presence of spatial autocorrelation in the response variables to ensure the relationships between genetics and landscape are not an artifact of spatial structure (Wagner and Fortin 2016). We built GLMs comprising all combinations of one to two predictors (except when they were collinear) and included a null model without predictors. To perform model selection, we calculated AIC corrected for small sample sizes (AICc) and Akaike's weight of evidence (wAICc) as the relative contribution of models (Burnham and Anderson 2003). We considered models with  $\Delta AIC$  (the difference between each model and the best model)  $\leq 2$  as equally plausible to explain the observed pattern. To run the AIC-based analyses, we used the R package 'AICcmodavg' (Mazerolle and Mazerolle 2016).

To assess the importance of each landscape factor in link-level analysis, we controlled for the geographic distance in the LCPs (IBR models) by dividing the accumulated-costs of LCPs by the riverway distance among pairs of localities. By doing so we are representing in each hypothesis solely the environmental dissimilarity of resistance among localities, despite longer or shorter geographic distances (Fig. 2). After this control, all correlations between predictor matrices (IBD, IBR/distance and IBB) were  $< 0.7$  (Appendix S4 Table S4.4) enabling the test of non-mutually exclusive hypotheses in multiple regression models (Wagner and Fortin 2016). To model genetic differentiation ( $\phi_{ST}$ ) in relation to



**Fig. 2** Hypothetical scenario illustrating how to represent more accurately the environmental dissimilarity among localities in isolation by resistance (IBR) models. Gray colors indicate resistance by river color (water types): light gray—low cost (1), medium gray—medium cost (2), and dark gray—high cost (5). Slope symbols indicate resistance by upstream slope between localities: large symbols—high slope (100), small symbols—medium slope (50), and absence of symbols—low slope (10). The hypothetical grid has cells with 1 km of resolution. Due to geographic distances being too large or too small, three paths would receive the same cost distance (LCP) for river color (1–2, 1–4 and 1–5) and for slope (1–2, 1–3, 1–5) despite the large environmental differences among them. By dividing by riverway distance (/dist), we are able to separate the effects of geography and environment. We can therefore obtain values (LCP/dist) that represent the environmental dissimilarity of pathways between pairs of localities and assess the sole effect of that variable in our response variable (genetic differentiation), despite geographical distance

geographic and environmental dissimilarities we applied a generalized dissimilarity modelling (GDM). GDM is a nonlinear extension of permutational matrix regression that models pairwise biological dissimilarity between sites (Ferrier et al. 2007). The two main advantages of using GDM in a landscape genetics approach are its particular suitability for genetic data (pairwise differentiation) and the possibility of using resistance/LCP models along with true measures of geographic distances (Fitzpatrick and Keller 2015). We therefore applied GDM, including six predictor variables for *P. erythrocephala* and five for *P. sextuberculata*, using the R package ‘gdm’ (Manion et al. 2016). We assessed the relationship among  $\phi_{ST}$  and each predictor by examining the response curves generated for variables for which I-spline basis functions could be calculated (i.e., presented non-zero coefficients). In these response curves, the maximum height represents the relative importance of variables retained in the model and the slopes indicate the rate of change in the response variable along the environmental gradient concerned (Ferrier et al. 2007). We also performed a test of variable importance using an iterative process that adds and removes variables to determine significance by computing the difference in deviance explained by a model with and a model without the variable concerned (Fitzpatrick et al. 2013). Although model selection would be the best approach to compare node and link-level analyses,

because the residuals of matrix regressions are not independent, information-theoretic indices commonly used for model selection (AIC, AICc or BIC) are not applicable to distance matrices (Wagner and Fortin 2016).

## Results

### Genetic diversity and differentiation

Our data set for *P. erythrocephala* (N=273) included 38 polymorphic sites, resulting in 48 haplotypes of which 34 were observed only once. For *P. sextuberculata* (N=336), we found 42 polymorphic sites and 61 haplotypes of which 40 were observed only once. Both species have a high proportion of shared haplotypes among localities (Haplotype networks in Appendix S5). We recovered overall moderate haplotype diversity for both species (*P. erythrocephala*: Hd=0.627; *P. sextuberculata*: Hd=0.776; Appendix S1), with localities values ranging from 0.116 to 0.813 for *P. erythrocephala* and from 0.143 to 0.911 for *P. sextuberculata*. We found comparable nucleotide diversity (Appendix S1) in relation to other studies using control region of mtDNA for *Podocnemis* species (0.00006 for *P. lewyana*, Vargas-Ramírez et al. 2012; 0.00256 for *P. expansa*, Pearse et al. 2006), 0.00234 for *P. erythrocephala* (range of 0.00023–0.00316) and 0.00458 for *P. sextuberculata* (range of 0.00024 to 0.00581).

The analysis of population admixture implemented in BAPS recovered three genetic clusters (Ln likelihood = -713.0684) for *P. erythrocephala* and four genetic clusters (Ln likelihood = -1007.2423) for *P. sextuberculata* (Fig. 1; BAPS graphs in Appendix S5 Figs S5.17 and S5.18). The clustering of individuals for both species did not correspond to geographical locations, except for the clusters including mainly individuals from São Gabriel da Cachoeira (1—SGC) for *P. erythrocephala* and from Xingu River (20—XIN) for *P. sextuberculata*. The populations (i.e., localities) were significantly differentiated for both species (*P. erythrocephala*:  $\phi_{ST}$  = 0.34060,  $p < 0.0001$ ; *P. sextuberculata*:  $\phi_{ST}$  = 0.45353,  $p < 0.0001$ ; AMOVA Table S5.7 at Appendix S5), with pairwise  $\phi_{ST}$  between localities ranging from 0 to 0.898 for *P. erythrocephala* and from 0 to 0.937 for *P. sextuberculata* (full  $\phi_{ST}$  Tables S5.5 and S5.6 at Appendix S5).

Despite the fact that we added samples from four new localities for each species to the data set of previous studies (Santos et al. 2016; Viana et al. 2017), we recovered very similar results for measures of genetic diversity and differentiation as them.

### Landscape data

#### Ecological niche modelling (ENM)

The climatic variables included in the final ENMs for both species were: temperature seasonality (BIO4), mean temperature of warmest quarter (BIO10), annual precipitation (BIO12), precipitation seasonality (BIO15) and precipitation of wettest quarter (BIO16). The average training AUC for the replicate runs was high for both species (*P. erythrocephala*: 0.969, SD 0.004; *P. sextuberculata*: 0.933, SD 0.006), indicating high model fit. The estimated mean historical suitability (from 0 to 120 kya) for the species indicates that, in average, past climatic conditions were mostly unsuitable for species occurrence (Appendix S2 Figure S2.2).

**Table 2** Model selection for relationship of genetic diversity of *Podocnemis erythrocephala* and *Podocnemis sextuberculata* and local riverscape predictors. Best models selected based on  $\Delta AICc < 2$  are bolded

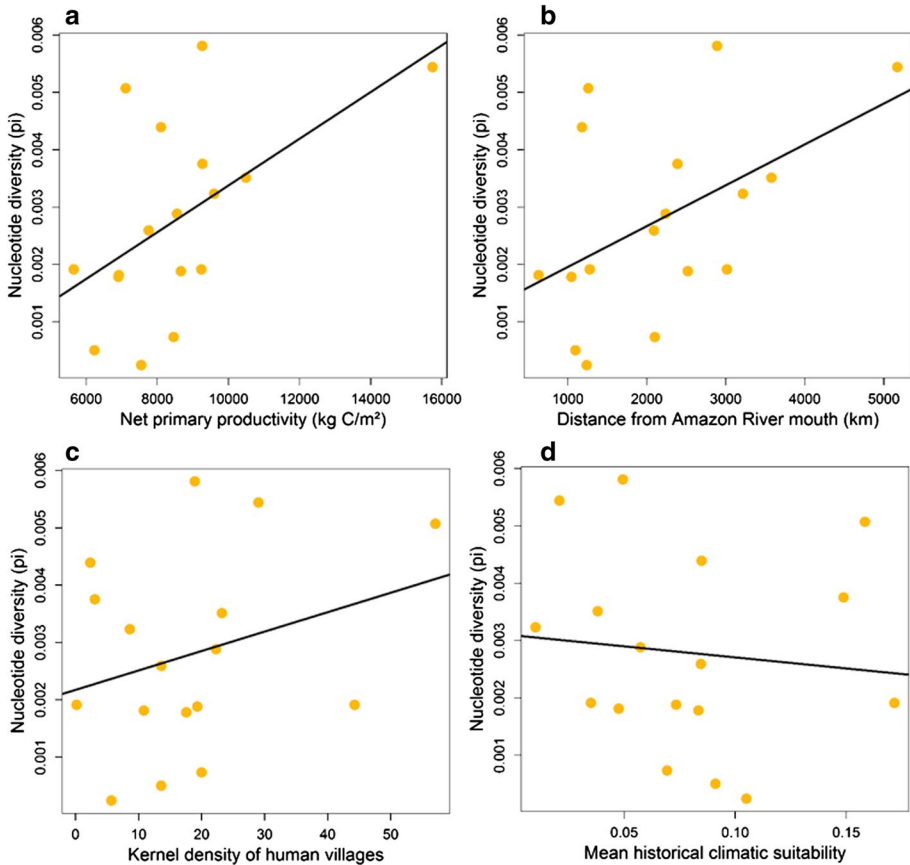
Species	Models	Hd			$\pi$		
		K	$\Delta AICc$	wAICc	K	$\Delta AICc$	wAICc
<i>P. erythrocephala</i>	CVmax + distCITY	4	<b>0.85</b>	<b>0.14</b>	4	<b>0</b>	<b>0.34</b>
	distCITY	3	<b>0.35</b>	<b>0.18</b>	3	<b>1.06</b>	<b>0.2</b>
	NULL	2	<b>0</b>	<b>0.22</b>	2	<b>1.96</b>	<b>0.13</b>
<i>P. sextuberculata</i>	NPP	3	<b>1.89</b>	<b>0.08</b>	3	<b>0</b>	<b>0.19</b>
	NPP + villages	4	2.44	0.06	4	<b>0.7</b>	<b>0.14</b>
	distMOUTH	3	<b>0</b>	<b>0.21</b>	3	<b>1.22</b>	<b>0.11</b>
	NPP + suit_past	4	4.49	0.02	4	<b>1.87</b>	<b>0.08</b>
	villages + distMOUTH	4	<b>0.64</b>	<b>0.15</b>	4	2.57	0.05
suit_past + distMOUTH	4	<b>1.79</b>	<b>0.09</b>	4	3.6	0.03	

Hd haplotype diversity;  $\pi$  nucleotide diversity;  $K$  number of parameters estimated for each model;  $\Delta AICc$  Akaike values corrected for small samples; wAICc Akaike's weight of evidence; CVmax coefficient of variation of interannual high river flow; distCITY distance to nearest urban center; NULL null model representing the absence of an effect; NPP net primary productivity; villages kernel density of human villages; distMOUTH distance from Amazon River mouth; suit\_past mean historical suitability

## Landscape genetics analyses

### Node-level analysis

For *P. erythrocephala*, the null model could not be rejected ( $\Delta AICc < 2$ ; Table 2). Although not differentiated from the null model, the two best models explaining haplotype and nucleotide diversity of *P. erythrocephala* are the distance to the nearest urban center (distCITY) and a combined effect of this distance and the coefficient of variation of high river flow (CVmax). The relationships among these predictor variables and response variables follow our expectations: increased genetic diversity on localities farther from cities (positive relationship with distCITY) and on localities with lower variability in maximum flows (negative relationship with CVmax). For *P. sextuberculata*, six competing models explained the two diversity metrics (Table 2): the site productivity (NPP) alone, the distance to Amazon River mouth (distMOUTH) alone, and the combined effects of each of these variables with density of rural human communities (NPP + villages and distMOUTH + villages) and historical climatic suitability (NPP + suit\_past and distMOUTH + suit\_past). The two most important variables, NPP and distMOUTH, are highly correlated ( $r = 0.93$ ;  $p < 0.001$ ), being difficult to determine which of the two influences genetic diversity. In addition, relationships between distMOUTH, villages and suit\_past with genetic diversity are opposed to the expected: increased genetic diversity on upstream localities (positive relationship with distMOUTH; Fig. 3b), on localities near higher density of human settlements (positive relationship with villages; Fig. 3c), and on localities with lower climatic suitability (negative relationship with suit\_past; Fig. 3d). The relationship for NPP was as expected: higher genetic diversity at more productive sites (higher NPP; Fig. 3a). The cumulative contribution (wAICc) of the models to the observed pattern was moderate, 0.53 for Hd and 0.52 for  $\pi$  (Table 2). Full tables of AIC models are available at Appendix S6 Tables S6.8–S6.11.



**Fig. 3** Relationship among nucleotide diversity ( $\pi$ ) of *Podocnemis sextuberculata* and local variables included in best models selected with AICc: NPP (**a**), distMOUTH (**b**), villages (**c**) and suit\_past (**d**). Relationships of genetic diversity with distMOUTH, villages and suit\_past are opposed to the expected. NPP: Net Primary Productivity; distMOUTH: distance (km) from Amazon River mouth; villages: Kernel density of riverine and rural human communities locations; suit\_past: historical climatic suitability

### Link-level analysis

The full GDM model explained 20.44% of the deviance in  $\phi$ ST turnover for *P. erythrocephala* and derived I-spline basis functions for four of the six variables (Table 3; Fig. 4). Summing the coefficients of I-spline basis functions as a measure of relative variable importance (i.e., height of each curve; Fitzpatrick and Keller 2015), the main predictor for genetic differentiation of *P. erythrocephala* was the Amazon River (0.387), followed by resistance from current climatic suitability (0.189), resistance from historical climatic suitability (0.136) and riverway distance (0.110). For *P. sextuberculata* the full GDM model explained 6.49% of the deviance in  $\phi$ ST turnover and derived I-splines for three of five variables (Table 3; Fig. 5). The most important variable to predict genetic differentiation of *P. sextuberculata* was the resistance from river color (0.953), followed by resistance from current climatic suitability (0.226) and riverway distance (0.187).

**Table 3** Model fit and relative importance of connectivity predictor variables (representing IBD (1), IBR (2, 3, 4, and 5) and IBB (6) hypotheses) for link-level GDM analyses of genetic differentiation of *Podocnemis erythrocephala* and *Podocnemis sextuberculata*

Best model	<i>P. erythrocephala</i>	<i>P. sextuberculata</i>
Model	1 + 4 + 5 + 6	1 + 2 + 4
Model deviance	20.12	57.07
Percent deviance explained	20.45	6.49
<i>p</i> Value	0.12	0.11
Variable importance	<i>P. erythrocephala</i>	<i>P. sextuberculata</i>
1. Distance	0.11	0187
2. River type resistance	–	0.953
3. Slope resistance	–	–
4. Current suitability resistance	0.189	0.226
5. Historical suitability resistance	0.136	–
6. Amazon River	0.387	NA

In “Model” the numbers are relative to the variables (below) included in that model. Variable importance is the sum of I-splines coefficients. Dashes indicate zero coefficients of I-splines. *NA* not assessed. No variable was significant after 1000 permutations

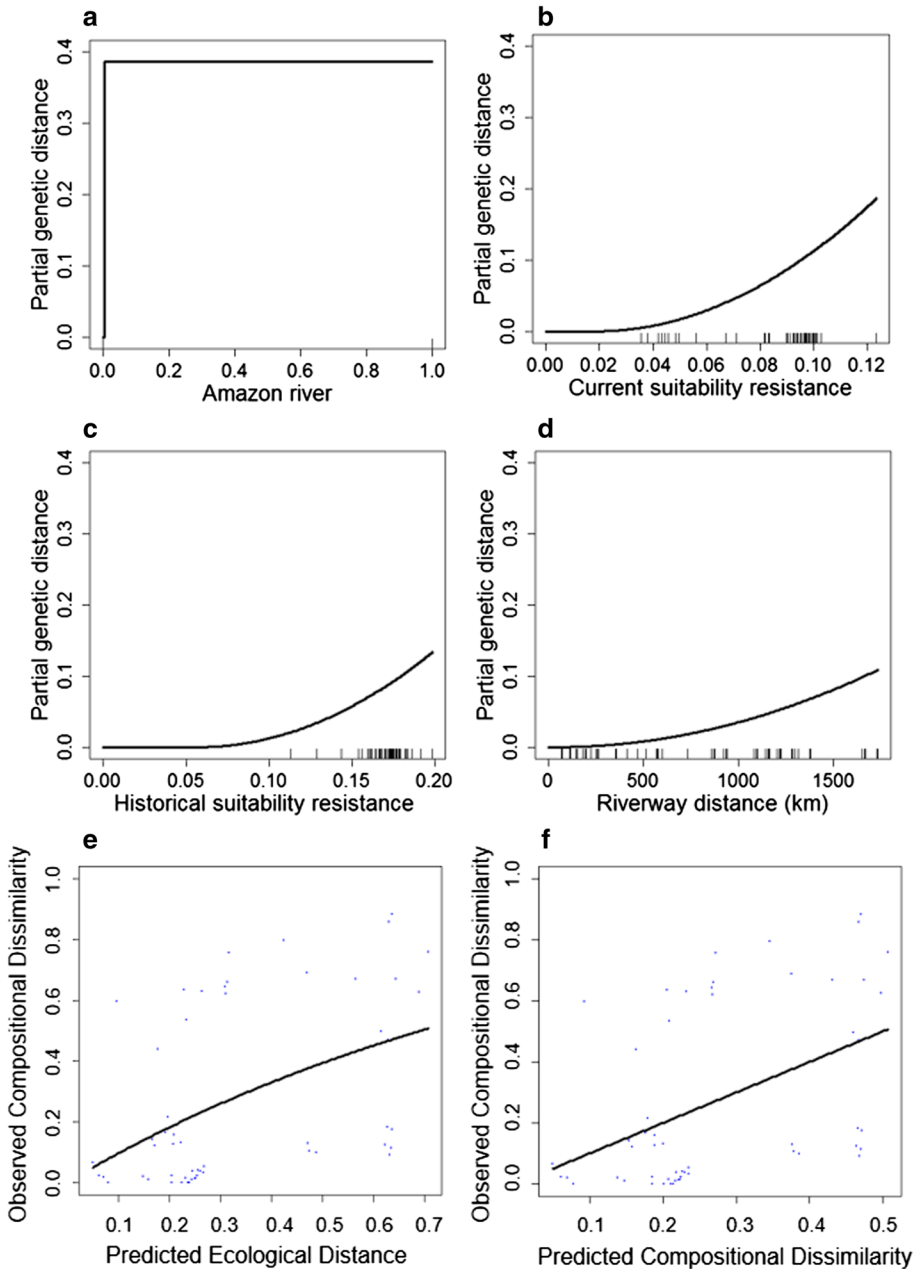
Although the response curves and I-splines coefficients can elucidate the most important variables to  $\phi$ ST turnover, we detected no significance for models or variables in terms of variable importance testing by permutations (Table 3). The correction of IBR models by geographic distance allowed us to disentangle the effects of riverway distance and environmental resistance, being IBD only retained as a potential predictor after this correction.

## Discussion

Here we investigated the influence of local and connectivity factors on genetic patterns of two closely related river turtle species with different dispersal abilities. We start discussing the potential drawbacks implied in using a single mtDNA locus. Taking that into consideration, we further discuss the distinct patterns found for each species within this comparative framework, highlighting ecological differences among them. We found relationships between spatial genetic patterns and environmental variables potentially relevant for other freshwater vertebrates in megadiverse Tropical river systems. Our results demonstrate the importance of assessing biologically meaningful variables in riverine systems, including at-site factors. We also show the usefulness of adding resistance factors to barrier and distance hypotheses often tested in riverscape genetics studies.

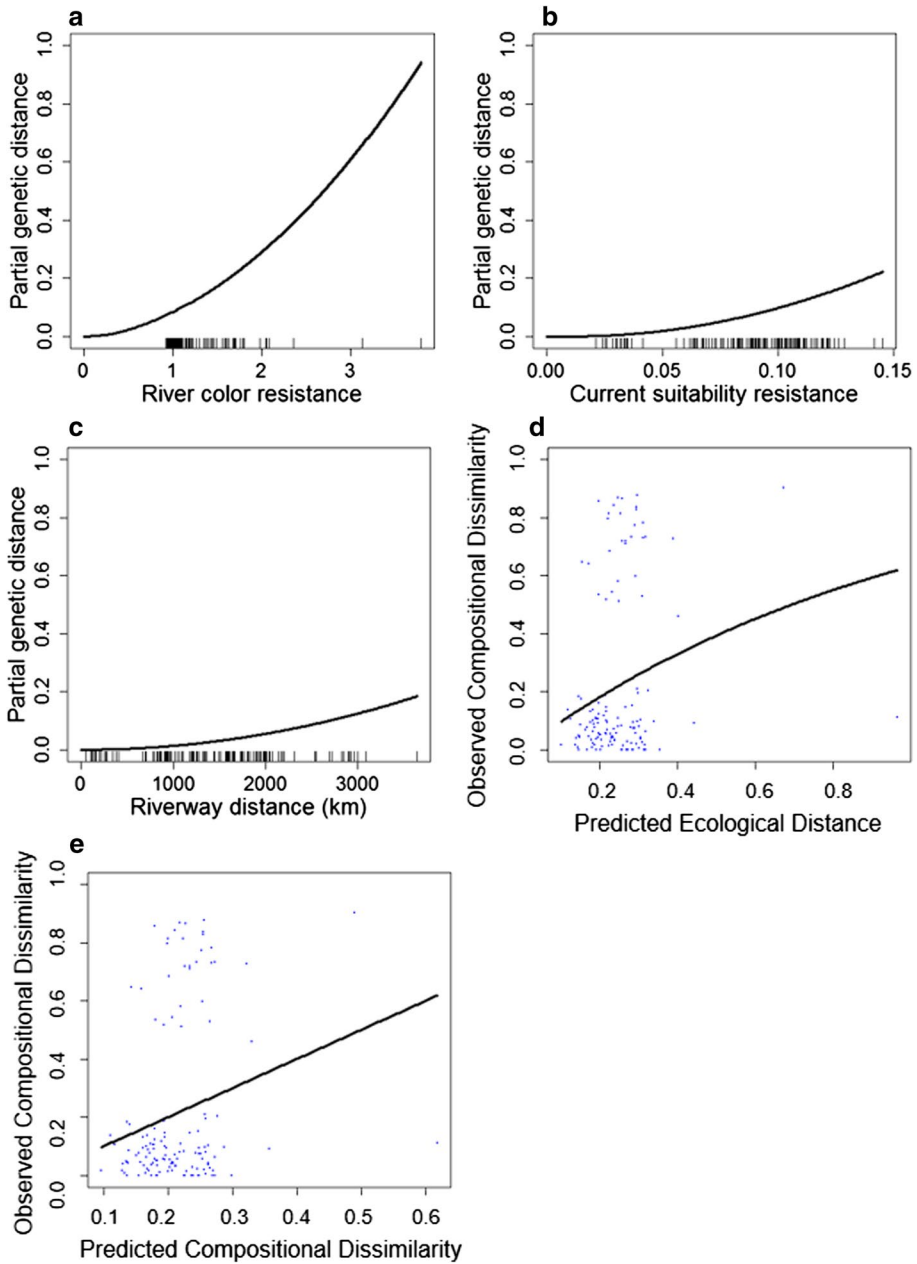
## Genetic marker caveat

The mitochondrial DNA (mtDNA) has been proven useful to identify influences of landscape features on the genetic patterns of co-distributed species, even when there are no visually apparent spatial genetic patterns (Liggins et al. 2016; Mitchell et al. 2015; Thomaz et al. 2015). This marker is particularly well suited to investigate population-level historical



**Fig. 4** Generalized dissimilarity model-fitted I-splines (panels **a–d**) for connectivity variables—for which non-zero splines coefficients could be calculated—associated to genetic differentiation ( $\phi_{ST}$ ) of *Podocnemis erythrocephala*. The variables are illustrated in descending order of their coefficient values (maximum height reached by each curve), indicating total amount of  $\phi_{ST}$  turnover associated with that variable. The shape of each function provides an indication of how the rate of  $\phi_{ST}$  turnover varies along the dissimilarity environmental gradient. *Rug plots* show actual dissimilarity values (LCP/riverway distance) between sampling locations. The final two panels illustrate the relationships between (**e**) observed pairwise  $\phi_{ST}$  in the dataset and the linear predictor of the GDM (“Predicted Ecological Distance”) and (**f**) observed versus predicted  $\phi_{ST}$





**Fig. 5** Generalized dissimilarity model-fitted I-splines (panels **a–c**) for connectivity variables—for which non-zero splines coefficients could be calculated—associated to genetic differentiation ( $\phi_{ST}$ ) of *Podocnemis sextuberculata*. The variables are illustrated in descending order of their coefficient values (maximum height reached by each curve), indicating total amount of  $\phi_{ST}$  turnover associated with that variable. The shape of each function provides an indication of how the rate of  $\phi_{ST}$  turnover varies along the dissimilarity environmental gradient. *Rug plots* show actual dissimilarity values (LCP/riverway distance) between sampling locations. The final two panels illustrate the relationships between (**d**) observed pairwise  $\phi_{ST}$  in the dataset and the linear predictor of the GDM (“Predicted Ecological Distance”) and (**e**) observed versus predicted  $\phi_{ST}$

effects of landscape factors across broad spatial scales (Murphy and Evans 2011), such as our study. In addition to these benefits, measures of gene flow from mtDNA represent migration rates of a single parent and should be interpreted as such. Although most markers can be used to measure genetic diversity and differentiation, multi-locus genotypic markers, for example Microsatellites ( $\mu$ sats) and Single Nucleotide Polymorphisms (SNPs), can measure fine temporal and spatial scales and have higher statistical power than less variable markers such as mtDNA (Storfer et al. 2010). These disadvantages of mtDNA might have limited the predictive power of our analyses because the available measures for response variables used here are rather contemporary and not necessarily reflect historical processes as we intended. Also, markers with higher mutation rates ( $\mu$ sats or SNPs) could have given more detailed insight. However, these markers remain to be developed and tested for *P. erythrocephala* and *P. sextuberculata*, especially in such a broad populational and geographic sampling.

### Influence of local factors on genetic diversity

Against our expectations we only find evidence for the influence of local factors on intrapopulational genetic diversity of the high-dispersal species (*Podocnemis sextuberculata*). For *P. erythrocephala*, the best models were not preferred over the null model. This might be explained by a higher proportion of shared haplotypes for *P. sextuberculata* than for *P. erythrocephala*. For *P. sextuberculata*, the density of rural human settlements (villages) was included in the second best models for haplotype and nucleotide diversity, combined with distance from the Amazon River mouth (distMOUTH) and primary productivity (NPP), respectively. However, the relationship is opposed to predicted, as intraspecific genetic diversity was higher in places with higher density of human communities. This is rather unexpected given historical use of turtles since the 18<sup>th</sup> century and high rates of consumption of *P. sextuberculata* by villagers reported along the Amazon basin (Pantoja-Lima et al. 2014; Smith 1979). Aside from direct consumption human settlements also often represent habitat loss for species (Turtle Conservation Fund 2002). Overall, human rural communities may pose a threat to *Podocnemis* species if exploitation occurs in an unsustainable manner, causing population declines (Bernardes et al. 2014; Conway-Gómez 2007) and ultimately affecting genetic patterns (Allendorf et al. 2008). Our results of higher genetic diversity where there is more human villages may be a consequence of human settlements often establishing in productive sites offering protein resources, where people hunt in proximities (Peres 2000). It may also be that density of villages is not a good proxy of turtle consumption, since feeding habits and consumption rates vary among places (Pezzuti et al. 2010). The model NPP + villages supports the hypothesis that human villages may be established in more productive sites, which in turn are expected to harbor larger population sizes of *P. sextuberculata*, therefore maintaining higher nucleotide diversity over time where productivity and number of villages are higher. Yet, we need to be cautious when interpreting effects of recent events on mtDNA genetic diversity (Wang 2010) because while population declines due to harvesting in turtles occur over years, genetic variation is lost over generations (Marsack and Swanson 2009). In addition to that, mtDNA has a bias towards registering historical events compared to other more polymorphic molecular markers (see above).

While NPP is the most relevant variable explaining nucleotide diversity of *P. sextuberculata*, the best model determining haplotype diversity is distance from Amazon River mouth (distMOUTH). NPP and distMOUTH are highly correlated, probably due to a west–east

gradient of decreasing primary productivity (Malhi et al. 2004) and distance to Amazon River mouth. For distMOUTH we found a downstream decrease in genetic diversity of *P. sextuberculata*, as opposed to the expected pattern of Downstream Increase in Genetic Diversity (DIGD). This reverse pattern may occur because floodplains and wetlands, which serve as feeding and movement habitat for *P. sextuberculata* (Fachin-Terán and Vogt 2014), are more abundant in western compared to eastern portions of Amazon basin (Junk et al. 2011). Also, upstream sites (i.e., mostly western localities) are less affected by deforestation, urbanization, and other anthropogenic alterations of habitats widespread on eastern localities closer to Amazon River mouth. Hence, these conditions, along with productivity of upstream sites, could harbor larger effective population sizes and larger genetic diversity in *P. sextuberculata* across the basin. Contrary to our expectations, we find more influence of local variables on the genetic diversity of the high-dispersal species, *P. sextuberculata*, than on *P. erythrocephala* (low-dispersal ability). However, several life-history traits other than dispersal (e.g., generation time and habitat specialization) also influence intraspecific genetic diversity (Ellegren and Galtier 2016). We cannot discuss whether generation time would influence the genetic-landscape relationships, since this information is unknown for *Podocnemis* species. Considering that both species have similar body sizes, we assume they also have similar generation time and mutation rates (Martin and Palumbi 1993) and thus we believe this trait cannot explain the different responses showed by the two species. Nest site requirements is a trait that could also explain the differences. *P. sextuberculata* disperses large distances to nest, its nests are only found in high points of sandy beaches (Vogt 2008). On the other hand, *P. erythrocephala*, nests in a wider variety of substrates, including sandy beaches, shrub lands (known as *campinas* and *campinaranas*) and savannas (Vogt 2008). This wider variety of nesting substrates may therefore reduce the influence of local variables on recruitment and population sizes of *P. erythrocephala* and counterbalance its low-dispersal ability.

### Influence of connectivity factors on genetic differentiation

As expected, connectivity variables explain a higher percentage of genetic differentiation for the low-dispersal species (*P. erythrocephala*). The GDMs showed that 20% of  $\phi_{ST}$  turnover in *P. erythrocephala* is explained by connectivity variables, while for *P. sextuberculata* this value is only 6%. The percent deviance explained is used as a measure of model fit in GDM (Fitzpatrick and Keller 2015). However, the variable importance permutation test did not recover significance for any variable or model. This outcome could be a result of temporal mismatch between genetic and landscape variables or of long lifespan of turtles. First, temporal mismatch between landscape effects and genetic responses is a general criticism to landscape genetic studies, especially when using a historical marker to assess contemporary landscape changes (Epps and Keyghobadi 2015). Here we employed connectivity variables that likely represent the landscape configuration across several past decades and millennia. Accordingly, we believe their effect is historical and could have been reinforcing potential gene flow restrictions until recent times. Therefore, the lack of significance was probably not an artifact of temporal mismatch between our predictor and response variables. Second, turtles, as long-lived organisms with delayed maturation time, are expected to have longer time to manifest changes in genetic patterns (Kuo and Janzen 2004; Marsack and Swanson 2009). But overlapping generations and multiple paternity of highly harvested *Podocnemis* species (Fantin et al. 2010, 2015) may be buffering potential bottlenecks from past centuries (Escalona et al. 2009; Pearse et al. 2006). In addition,

studies with turtles and other long-lived organisms detected effects of landscape factors on genetic divergence within few generations (Epps et al. 2005; Moore et al. 2008; Reid et al. 2017). Thus, lack of significant relationship between genetic differentiation and connectivity factors may be an evidence of high gene flow, but also a consequence of several other factors affecting the rate at which neutral genetic differentiation reach equilibrium, such as effective population sizes and population dynamics (Epps and Keyghobadi 2015).

Despite potential limitations of mtDNA already mentioned, the lack of significance for relationships among connectivity and genetic differentiation is an indication that connectivity variables are less important in shaping genetic patterns of Amazon River turtles than local variables. Nevertheless, the height of spline curves can still represent a measure of the importance of a variable in influencing genetic differentiation in these species. Our GDM analyses fitted I-spline functions for four connectivity variables for *P. erythrocephala* and three for *P. sextuberculata*. The asymptotic shapes of these curves demonstrate the usefulness of GDM to model non-linear relationships commonly found in link-level landscape genetics analyses (Spear et al. 2016).

Amazon River was the most important variable explaining genetic differentiation of *P. erythrocephala*. The role of Amazon River as a potential barrier to dispersal of *P. erythrocephala* was suggested in a previous population genetics study (Santos et al. 2016). By adding samples from two localities on the right margin of Amazon River to their dataset and employing a riverscape genetics approach, we corroborate the idea that the river is the most important predictor of genetic differentiation, at least among the set of variables here tested. Surprisingly, resistance offered by different water types was not important in explaining the genetic differentiation of *P. erythrocephala*, which is restricted to black and clear waters. Therefore, we cannot accurately state whether Amazon River works as a barrier due to its large width (Hayes and Sewlal 2004), its white waters (Beheregaray et al. 2015) or a historical process of river dynamics. On the other hand, resistance from water type was the most important variable for *P. sextuberculata*, which can be found in all three types of water. This suggests that populations of *P. sextuberculata* are increasingly divergent along paths containing costlier water types, despite total distance to be travelled among sites. However, due to low percent of variation explained by GDM (6%) and lack of significance, this pattern remains only as a topic for future investigation.

For both species, IBR models explained more genetic differentiation turnover than IBD. In Amazon basin, riverway distance has often a minor or no role in explaining genetic differentiation of aquatic vertebrates, potentially because of high connectivity offered by flooded habitats (Cantanhede et al. 2005; Hrbek et al. 2005; Pearse et al. 2006). Our results emphasize the utility of adding resistance-based (IBR) models to classical IBD and IBB models when studying riverscape genetics. We also reinforce the usefulness of expert's opinion to parameterize LCPs in systems for which empirical resistance evidence is lacking (Zeller et al. 2012). In addition, dividing cost-weighted distances of each variable by riverway distance allowed us to assess the accumulative cost of traversing costly environments despite the total distance to be travelled. This control by distance allowed us to disentangle IBR models from IBD and test whether distance by itself or resistance by itself increased genetic differentiation. We suggest this approach when dealing with species that move exclusively through linear habitats (i.e., rivers), for which there is only one path possible between populations, but environmental dissimilarity may be more determinant to dispersal than distance.

The weak relationship among genetic diversity and climatic suitability and the lack of relationship among genetic differentiation and resistance by climatic unsuitability seen here corroborate the idea that climatic stability is overall less important in structuring genetic

variation in aquatic organisms than in terrestrial species, as suggested by Thomaz et al. (2015). Thomaz et al. (2015) found that palaeodrainages influence the genetic patterns of a freshwater fish dependent upon forest habitat, while habitat stability (as measured by climatic suitability) do not. Similarly, our results highlight that other aspects of the riverscape are more important to both genetic diversity and genetic differentiation patterns for river turtles.

## Conclusions and perspectives

Overall, our study shows that despite major attention is often given to connectivity variables, local variables can be important factors correlated to genetic diversity patterns, even when dealing with high-dispersal species without apparent discrete genetic structure. We assessed variables biologically relevant for other Amazonian riverine species in a basin-wide context and hope this work can stimulate further research in the region. Our study is the first to engage empirical model-based riverscape genetics in Amazon basin and to develop resistance models in a riverscape genetics context. Therefore, it should provide a framework to investigate spatial genetic patterns of other high-dispersal riverine species in drainage systems.

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