Topography, more than land cover, explains genetic diversity in a Neotropical savanna tree frog

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Abstract
Aim: Effective conservation policies rely on information about population genetic structure and the connectivity of remnants of suitable habitats. The interaction between natural and anthropogenic discontinuities across landscapes can uncover the relative contributions of different barriers to gene flow, with direct consequences for decision-making in conservation. We aimed to quantify the relative roles of land cover and topographic variables on the population genetic differentiation and diversity of a stream-breeding savanna tree frog (Bokermannohyla ibitiguara) across its range.

Location: Serra da Canastra mountain range, Cerrado of Minas Gerais State, Brazil.

Methods: We collected samples and extracted DNA samples from 12 populations within and outside a strictly protected park, and used 17 microsatellite markers to assess genetic structure, among-population differentiation and within-population diversity measures. We incorporated landscape data derived from digital models and satellite images to create connectivity matrices to correlate with genetic differentiation using Mantel tests. We used generalized linear models and path analyses to assess the roles of each landscape variable in shaping genetic diversity in this species.

Results: Populations within and outside the park boundaries belonged to four genetic clusters. Most populations showed evidence of limited gene flow, with significant genetic differentiation, except for those within the park, which also had higher levels of allelic richness and heterozygosity. However, genetic differentiation among populations in this landscape was primarily explained by topographic complexity. Likewise, within-population measures of genetic diversity were best explained by models including elevation and topographic complexity, and not the amount of natural habitat or gallery forests.

Main conclusions: Our results underscore that topography may be a strong historical factor shaping genetic structure among amphibian populations. Therefore, effective conservation strategies for endangered amphibians should avoid focusing exclusively on habitat suitability, and incorporate topographic complexity, which seems to be a key factor for the fauna of the extremely threatened Brazilian savanna.

Correction added on 17th September 2020, after first online publication ‘Carlos Guilherme Becker’ has been corrected to read ‘C. Guilherme Becker’ in this version.
1 INTRODUCTION

Effective conservation actions rely on information about population genetic diversity, structure and connectivity (Allendorf & Luikart, 2007; Frankham, 2005; Moritz, 1994). Population genetic data provide estimates of individual migration across species’ ranges and the factors that have shaped the distribution of genetic diversity among populations; these data in turn can be used to mitigate potential negative effects that arise from isolation (Brauer, Unmack, Smith, Bernatchez, & Beheregaray, 2018; Habel et al., 2015). The higher the functional connectivity among populations, that is the degree to which individuals disperse throughout the landscape, the lower the chance that limited gene flow results in isolation and population inbreeding (Dixo, Metzger, Morgante, & Zamudio, 2009; Murphy, Dezanni, Pilliod, & Storfer, 2010; Spear & Storfer, 2008). For organisms that occupy heterogeneous landscapes, the biological integrity of populations relies on (a) the anthropogenically modified habitats in the landscape matrix and whether the remaining suitable habitats promote natural levels of dispersal and (b) the quality of the natural habitat itself (Angermeier & Karr, 1996; Coster, Babbitt, Cooper, & Kovach, 2015; Peterman, Rittenhouse, Earl, & Semlitsch, 2013).

Different historical and contemporary landscape features can facilitate or hinder animal dispersal, resulting in diversification of phylogeographic lineages and genetic differentiation even in pristine or non-fragmented habitats (Dixo et al., 2009; Funk et al., 2005; Titus, Bell, Becker, & Zamudio, 2014). Organisms are not homogeneously distributed throughout their geographic range, but rather live in patches of suitable habitat within a matrix (Levins, 1969; Smith & Green, 2005). Aside from geographic distance (Wright, 1943), other factors that can affect divergence in this heterogeneous matrix are historical factors such as topography, including elevation and degree of topographic relief, and contemporary factors such as land cover, including various types of vegetation and microhabitats that are selected by different species (Coster et al., 2015; Garroway, Bowman, Carr, & Wilson, 2008; Ovaskainen & Hanski, 2004; Peterman et al., 2013). For example, if a species’ preferred environment is found at higher elevations or mountain ranges, this may yield more divergent lineages and deeper phylogeographic structure (Guarnizo & Cannatella, 2013; Rodríguez et al., 2015; Zamudio, Bell, & Mason, 2016) than species selecting microhabitats that are more homogeneously distributed. The distribution of divergent lineages is secondarily affected by non-natural contemporary changes to the distribution of habitats over relatively short periods of time (Dixo et al., 2009; Laurence, 2010; Machado et al., 2004). If those changes affect the matrix, but still permit successful dispersal of individuals among selected habitat patches, populations will persist with sustained gene flow, resulting in outbred and genetically diverse populations (Galbusera, Githiru, Lens, & Matthysen, 2004). On the other hand, if anthropogenic changes are extreme, gene flow may be compromised, resulting in deleterious effects for isolated populations (Couvet, 2002; Dixo et al., 2009). These complex interactions between natural features, the distribution of preferred habitats and rapid anthropogenic change will likely dictate species persistence or extinction. Thus, landscape genetic research on organisms distributed in heterogeneous landscapes is key to examine the relative roles of historical and contemporary barriers to gene flow, how they are mediated in habitat specialist species, and their consequences for conservation (Chiucchi & Gibbs, 2010).

Landscape genetic studies in temperate ecosystems vastly outnumber those in the Neotropics (reviews in Manel & Holderegger, 2013; Torres-Florez et al., 2018). The Brazilian Cerrado is the second largest South American morphoclimatic domain, with an original area of approximately 2.5 million km² of highly heterogeneous landscapes (Silva, Farinas, Felfili, & Klink, 2006). It is the most species-rich savanna in the world and one of 35 worldwide biodiversity hotspots (Mittermeier et al., 2004). Before intense human modification over the past six decades (Myers, Mittermeier, Mittermeier, Fonseca, & Kent, 2000), the Cerrado included seasonal savannas, grasslands and gallery/riparian forests (Felfili, 1995; Meave, Kellman, Macdougall, & Rosles, 1991). The riparian forests occur alongside streams and provide suitable habitat for forest-dependent species (Johnson, Saraiva, & Coelho, 1999; Nali & Prado, 2012). In addition, the Cerrado is topographically diverse, including montane regions dominated by high-elevation plateaus (the chapadas or chapadões) separated by a network of low elevation savannas (Ab’Sáber, 1983; Cole, 1986). Understanding habitat connectivity in the Cerrado is imperative as almost half of its land area has been transformed into pasture or croplands (Weinietzel, Vačkář, & Medková, 2018) and only 2.2% of the original Cerrado is currently legally protected (Klink & Machado, 2005). The suitability of current conservation policies can be determined only by assessing the contemporary status of genetic differentiation across animal populations (Bergsten et al., 2012), providing information necessary to assign new protected areas to avoid genetic erosion.

Due to characteristics such as high desiccation risks, limited mobility and site fidelity, amphibians make excellent study systems to determine mechanisms shaping fine-scale population divergences across heterogeneous landscapes (Blaustein, Wake, & Sousa, 1994; Cushman, 2006; Rothermel & Semlitsch, 2002; Smith & Green, 2005). The Neotropical region harbours the largest diversity of frogs, and ca.1,100 species occur in Brazil (Frost, 2020; Segalla et al., 2019). However, very few studies have investigated the processes leading to genetic differentiation of frogs living in Cerrado habitats (Eterovick, Sloss, Scalzo, & Alford, 2016; Nascimento, Chaves, Leite, Eterovick, & Santos, 2018). *Bokermannohyla ibitiguara* (Cardoso, 1983) is a stream-dwelling tree frog endemic to the Serra da Canastra mountain range in the Brazilian Cerrado (Frost, 2020; Haddad, Andrade,
streams across the range of B. ibitiguara in the Serra da Canastra mountain range: six located within and six located outside SCNP boundaries (Figure 1; Table S1.1 from Appendix S1). We collected 273 tissue samples from adults (liver, leg muscle or toe clip) and tadpoles (tail clip), and preserved them in absolute ethanol. We euthanized individuals with 10% lidocaine applied to the ventral region; specimens were then fixed in formalin 10% and preserved in ethanol 70% (McDiarmid, 1994). Tadpoles of B. ibitiguara do not form schools (Cardoso, 1983; R. C. Nali, pers. obs.), and thus, we randomly collected tadpoles of different body sizes throughout the streams, reducing the probability of collecting tadpoles from a single clutch. Voucher specimens and tissues were deposited at the Coleção de Anfíbios Célio F. B. Haddad, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil (Table S1.2 from Appendix S1). Field capture and collections were authorized by the Chico Mendes Institute for Biodiversity Conservation, Brazil (ICMBio/SISBIO permits # 23240 and # 33735). The animal procedures employed were ethically reviewed and approved by the Committee on Ethics for the Use of Animals (CEUA) at Universidade Estadual Paulista, Jaboticabal, São Paulo, Brazil (protocol # 016549/09).

2.3 | Laboratory protocols and microsatellite data

All individuals were first genotyped at 21 microsatellite loci previously developed and optimized for the focal species (Nali, Zamudio, & Prado, 2014). We extracted whole genomic DNA from tissue samples with DNeasy extraction columns (Qiagen), following manufacturer’s protocols. PCR profiles followed those in Nali et al. (2014). Each forward primer contained a 20 bp tag on the 5′ end to allow hybridization with the fluorescently tagged third universal primer (NED, PET, VIC or 6-FAM). After amplification, we combined 1 μl of individual PCR products from up to four different loci, diluted with 18.85 μl Hi-Di formamide and 0.15 μl GeneScan 500 LIZ, and ran the pooled samples on a 3730 ABI Prism Genetic Analyzer (Applied Biosystems) at the Genomics Facility of the Cornell Biotechnology Resource Center.

We used GENEMARKER v. 2.4.0 (SoftGenetics LLC) to analyse genotype profiles. We re-genotyped an average of 65 individuals per locus (ranging from 56 to 73) to calculate a genotyping error rate, defined as the percentage of the number of individuals genotyped differently for at least one allele, divided by the number of individuals re-genotyped. The remaining individuals were genotyped just once at all loci. We used MICROCHECKER v. 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004) to check for evidence of null alleles for each population. We tested for Hardy–Weinberg equilibrium for each population using GENEPOP 4.0.9 (Rousset, 2008), assessing statistical significance through Monte Carlo Markov Chains (MCMC) with 10,000 dememorization steps followed by 100 batches of 5,000 iterations each (Guo & Thompson, 1992). We also tested for linkage disequilibrium across individuals and all pairs of loci using GENEPOP.
Population genetic analyses

We calculated pairwise relatedness among individuals in GENALEX v. 6.5 (Peakall & Smouse, 2012) using the $r_{qg}$ coefficient (Queller & Goodnight, 1989). We permuted population genotypes 9,999 times and derived upper and lower 95% confidence intervals to obtain a range of $r_{qg}$ expected if mating was random across all populations. We bootstrapped confidence intervals 9,999 times to obtain

![STRUCTURE plot](image-url)
estimates of mean relatedness within populations. To confirm that our analyses of local population structure were not biased due to tadpole sampling (i.e., increased chance of sampling siblings from a single clutch; Table S1.1 from Appendix S1), we ran a Pearson correlation analysis between the $r_{bg}$ value and the percentage of tadpoles sampled per stream.

We used FSTAT 1.2 (Goudet, 1995) to calculate overall $F$-statistics (Wright, 1969): $F_{it}$ (inbreeding coefficient of an individual relative to the total population), $F_{is}$ (inbreeding coefficient of an individual relative to the sampling locality) and $F_{st}$ (effect of sampling locality compared with the total population). We also calculated $F_{is}$ per sampling locality. Pairwise $F_{st}$ [$(F_{st}/(1 - F_{st})]$ was calculated in GENALEX and used as a measure of genetic differentiation between sampling localities (Rousset, 1997); statistical significance of this measure was assessed by using 9,999 permutations. Following Narum (2006), we reported statistical significance for 66 comparisons using Bonferroni’s correction ($p = .00076$), and also the B-Y method ($p = .01037$), which is less conservative and more appropriate in conservation genetics (Benjamini & Yekutieli, 2001).

To analyse genetic discontinuities without considering subpopulations a priori, we ran a Bayesian analysis using STRUCTURE v. 2.3.4 to infer the number of genetic clusters ($K$) and genetic discontinuities among sampled populations. We assessed $K$ values from 1 to 10 using 25 MCMC runs with 200,000 steps discarded as burn-in followed by 1 million steps for each proposed $K$, assuming an admixture model, and correlated allele frequencies. We then compiled all results from $K$ runs in a single STRUCTURE HARVESTER analysis (Earl & vonHoldt, 2012) and inferred presence of genetic structure when the plotting of delta-$K$ provided evidence of a clear peak. The cluster data for the selected $K$ were permuted using CLUMPP (Jakobsson & Rosenberg, 2007).

2.5 | Landscape features and genetic differentiation

To determine the roles of geographic distance, topographic complexity and land cover on genetic differentiation across our 12 sampling localities, we calculated three connectivity indices: Euclidean distance (minimum straight-line distance between each pair of populations) and two surface resistance indices: topographic resistance and land cover resistance. For topography, we used the digital elevation model from the Shuttle Radar Topography Mission (SRTM; pixel resolution $= 30$ m) to generate a topographic complexity raster using the raster calculator feature in ArcGIS 9.3.1 (ESRI, 2009), where each elevation pixel was assigned the variance of the eight cardinal/intercardinal neighbouring pixels. This metric depicting terrain ruggedness is a proxy for microclimatic turnover and local habitat heterogeneity (Huaxing, 2008; Riley, DeGloria, & Elliot, 1999). For land cover resistance, we used the MapBiomas raster database for the Brazilian Cerrado (MAPBIOMAS, 2020; pixel resolution $= 30$ m). We chose the map from 2014, as this was the final year of our sampling. We then assigned resistance values for the seven land cover classes according to the current literature on habitat requirements for amphibians. Specifically, we followed the study by Titus et al. (2014), based on forest-dependent salamanders with aquatic breeding, which provided comparable measures with the classes from MapBiomas (see Table S1.3 from Appendix S1 for descriptions and classifications).

We used these two rasters as resistance layers in CIRCUITSCAPE v. 3.3 (McRae & Shah, 2009), in which pixels with high topographic complexity or high land cover resistance imposed higher dispersal costs throughout the landscape. We employed a cell connection scheme connecting each pixel (node) to the eight cardinal/intercardinal neighbouring pixels. Surface resistance weights all possible paths between pairs of our 12 sampling localities and produces a summary connectivity raster. As CIRCUITSCAPE will account for extreme differences among neighbouring pixels, geographic barriers will be automatically incorporated in the analysis. Finally, we used simple Mantel tests (Mantel, 1967) to correlate Euclidean distance and distances calculated from both surface resistance indices with the pairwise $F_{st}$ values using PASSAGE v. 2.0 (Rosenberg & Anderson, 2011). To avoid the likely bias of multicollinearity in partial Mantel tests (Cushman, Wasserman, Landguth, & Shirk, 2013; Guillot & Rousset, 2013), we used independent single Mantel tests for the three correlations.

2.6 | Landscape features and within-population genetic diversity

We also analysed the impact of landscape variables on the following within-population genetic parameters: allelic richness (AR), private allelic richness (PAR), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), number of alleles (NA), effective number of alleles (ENA) and inbreeding coefficient ($F_{is}$). We used AR and PAR in HP-RARE (Kaliniowski, 2005); NA, ENA, $H_o$ and $H_e$ in GENALEX; and $F_{is}$ in FSTAT. Land cover variables included in this analysis were topographic complexity, elevation, per cent of habitat loss and per cent of gallery forests. Topographic complexity was calculated within a radius of 700 m around each sampling site from the same topographic raster mentioned above. The elevation of each locality was averaged based on several GPS records of collected individuals. We used the national land cover database of natural versus disturbed habitat (1:50,000 scale shapefiles; CSR/IBAMA, 2014; Figure S1.2 from Appendix S1) and calculated per cent of habitat loss within a radius of 700 m around each sampling locality. We used high-resolution satellite images (CNES/Astrium satellites in 2013 or 2014; pixel resolution $= 0.35$ m) to manually quantify the gallery forests (%) within a radius of 500 m around each sampling locality using ArcGIS 9.3.1 (ESRI, 2009).

We then used a general linear model approach (GLM—standard least squares) running all possible models including explanatory landscape variables (topographic complexity, elevation, per cent of habitat loss and per cent of gallery forest), including one-level interactions, and genetic parameters as response variables in turn. We ranked models based on Akaike information criterion (AICc) and report the most parsimonious significant model for each run.
Comparisons among populations within versus outside SCNP

Even considering the likely role of geographic distance, we expected differences in connectivity and gene flow in populations within SCNP versus outside SCNP due to habitat protection within the park. Thus, we used a Kruskal–Wallis test with post hoc comparisons of Student–Neuman–Keuls to compare the average pairwise $F_{ST}$ values categorized in three groups: within/within SCNP, within/outside SCNP and outside/outside SCNP. The data were visualized using the ggplot2 package in R (R Core Team, 2019; Wickham, 2016). In addition, to test for statistical difference in pairwise $F_{ST}$ values from populations within versus those outside SCNP, we used a non-parametric analysis with 1,000 permutations implemented in FSTAT 1.2 (Goudet, 1995).

We compared genetic measures of diversity (AR, PAR, $H_{O}$, $H_{E}$, NA, ENA and $F_{IS}$) between localities within versus outside SCNP using one-way ANOVAs. We also conducted non-parametric permutation analyses implemented in FSTAT (see above) to compare these two groups for $H_{O}$, $H_{E}$ and AR.

Because of the configuration of our sampling populations, where protected ones are located within flat highlands (within SCNP) while the others are included in topographically more complex regions at lower elevations, we employed path analyses to provide information about the relative strength of direct and indirect effects of topographic complexity, elevation and habitat loss on genetic measures that differed within and outside the SCNP.

3 | RESULTS

3.1 | Microsatellite data

Our initial 21 markers were highly polymorphic (11–58 alleles; average 20.8 ± 2.4). All loci showed evidence of null alleles in MICROCHECKER when considering all individuals together. When divided by population, loci B11, B1122, B1521 and B3629 showed evidence of null alleles for more than seven populations, but not always in the same populations. Therefore, we used only the remaining 17 loci for all subsequent analysis. They remained highly polymorphic (11–58 alleles; average 20.8 ± 11.15) and yielded a final database with only 0.88% of missing data. Genotypes were deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.c2fqz 615x (Nali, Becker, Zamudio, & Prado, 2020). We had a genotyping error rate of average 3.3%, ranging from 0% to 7.3% across all loci.

Only populations MP3 and R25 were under Hardy–Weinberg equilibrium (Bonferroni’s $p$ = 0.0029). We found linkage disequilibrium for only four pairs out of 136 comparisons (Bonferroni’s $p$ = 0.00037) across all 17 loci and individuals (B194/B1397, B2313/B3370, B639/B3836 and B3003/B4144). We found no linkage disequilibrium among loci in each population individually.
differentiation yielded significant results for 54 out of our 66 pairs of populations (Table 1).

In our STRUCTURE analysis, delta-K showed a clear peak for four genetic clusters, with a high average coefficient of membership (percentage of individual assignment to the cluster) of 90.3% (Figure 1). Most populations within SCNP belonged to a single cluster, while the other populations belonged to the other three clusters; the only exception was CAL, which clustered together with other SCNP populations despite being located outside the park.

3.3 | Landscape features and genetic differentiation

Pairwise $F_{ST}$ values among sampling localities were positively correlated with Euclidean distances (Mantel: $R = .54$, $p < .001$). Reconstruction of surface resistance for topographic complexity showed high connectivity across some sampling localities, mainly within the SCNP (Figure 1). We found a highly significant effect of topographic resistance on $F_{ST}$ values across *B. ibitiguara* populations (Mantel: $R = .65$; $p < .01$). On the other hand, land cover resistance did not predict $F_{ST}$ values across our sampled populations (Mantel: $R = .26$, $p = .08$).

3.4 | Landscape features and within-population genetic diversity

Landscape variables significantly explained four of the within-population genetic parameters (AR, PAR, $H_E$ and ENA), with a marginally non-significant effect in $H_O$ (Table 2). The most parsimonious models consistently included topographic complexity and elevation as explanatory variables for AR, $H_E$, $H_O$ and ENA (Tables 2 and 3). Habitat loss and per cent gallery forests were not recovered as explanatory variables in any of the most parsimonious and significant models (le.). The results of all models are included in the Dryad Digital Repository.

### 3.5 Comparisons among populations within versus outside SCNP

We found significant differences among pairwise $F_{ST}$ for populations within SCNP, within versus outside SCNP and outside SCNP (Kruskal–Wallis; $H = 30.163; p < .0001$; Figure 4). Populations within SCNP showed the lowest levels of differentiation, populations outside SCNP showed the highest divergences, and within versus outside SCNP comparisons showed intermediate levels of differentiation (all with Student–Newman–Keuls $p < .05$; Figure 4). Likewise, the permutation analysis showed that $F_{ST}$ was significantly higher in populations outside the SCNP when compared to those within the park boundaries ($p = .01$). Populations outside SCNP showed significantly less AR, $H_O$, $H_E$ and ENA for at least one statistical test (Table 4).

Our path analysis confirmed that areas of high elevation harbour anuran populations with higher AR (Figure 3a). This coincides with our SCNP populations, which are located in a high-elevation plateau with more homogeneous topography (Figure 1). Lower elevation sites, on the other hand, show high topographic complexity, and this complexity predicted lower AR in our non-protected populations. Elevation was negatively associated with habitat loss, but the amount of natural vegetation cover did not significantly predict AR (Figure 3a). We observed higher ENA in populations at higher elevations, with no direct effect from habitat loss or topographic complexity (Figure 3b). The effects of landscape variables on higher elevations, with no direct effect from habitat loss or topographic complexity (Figure 3b). The effects of landscape variables on higher elevations, with no direct effect from habitat loss or topographic complexity (Figure 3b). The effects of landscape variables on higher elevations, with no direct effect from habitat loss or topographic complexity (Figure 3b). The effects of landscape variables on higher elevations, with no direct effect from habitat loss or topographic complexity (Figure 3b).

### Table 1 | Analysis of genetic differentiation (pairwise $F_{ST}$) among 12 populations of *Bokermannohyla ibitiguara* in the state of Minas Gerais, Brazil

| CAL | GLG | PRT | R1 | R2 | R3 | CM3 | MP3 | NSF | R25 | RC | RJ |
|-----|-----|-----|----|----|----|-----|-----|-----|-----|-----|----|-----|
| 0   | 0.027* | 0.035* | 0.122* | 0.095* | 0.073* | 0.014 | 0.034* | 0.008 | 0.019* | 0.017* | 0.016* | 0 |

Note: Two negative $F_{ST}$ values were converted to zero (CM3–R25: −0.003 and NSF–RC: −0.004). Comparisons among localities within SCNP (Serra da Canastra National Park) are in bold.

*Significant under Bonferroni’s $p = .00076$.
†Significant under B-Y method $p = .01037$. 

The results of all models are included in the Dryad Digital Repository.
4 | DISCUSSION

We characterized the genetic structure among populations of a Brazilian Cerrado tree frog and asked how different landscape variables (both historical and contemporary) might contribute to the observed pattern of genetic distribution in this highly threatened savanna. Our focal species provided insight into direct and indirect effects of habitat loss, distribution of suitable habitats, topography and geographic distance on spatial connectivity and genetic diversity of populations. We showed that frog populations distributed in patches of gallery forests embedded in a matrix of open environment are overall genetically differentiated, but the degree of differentiation and genetic diversity were associated with topography (historical factor), but not with land cover (contemporary factors). As predicted, populations were indeed less differentiated inside the SCNPNP, but contrary to our expectations, this pattern was not a direct result of land cover, but because of lower topographic complexity and lower geographic distances within the park, which likely facilitate dispersal.

4.1 | Genetic differentiation among populations

Our first hypothesis was that populations would show significant genetic differentiation due to habitat specialization to gallery forests within a matrix of predominantly open habitat. This was partially corroborated: most of the studied population pairs showed significant pairwise \( F_{ST} \) differentiation (54 out of 66 pairs), although overall average \( F_{ST} \) was not particularly high (0.066; range = 0–0.14). The consistent deviations from a large, panmictic population (Figure 2) and high overall inbreeding coefficient \( F_{IS} = 0.115 \) explain the lack of Hardy–Weinberg equilibrium we found in many populations (Austin, Gorman, & Bishop, 2011; Nei, 1977). Amphibians show limited mobility, physiological constraints that confine adults to moist environments, and extreme site fidelity, so dispersal and gene flow are likely reduced in this group (Blaustein et al., 1994; Cushman, 2006; Peterman, Connette, Semlitsch, & Eggert, 2014). The Brazilian Cerrado is predominantly an open environment dominated by a mosaic of grasslands and shrubs, where streams with gallery forests are sparsely distributed (Meave et al., 1991; Silva et al., 2006; Figure 1c). In this landscape, forest-dependent animals such as B. ibitiguara may use those gallery forests for reproduction and refugia, instead of dispersing throughout grasslands (Johnson et al., 1999; Redford & Fonseca, 1986). In fact, phyllogeographic structure of frog lineages is more pronounced among forest-inhabiting species when compared to species that live and breed in open habitats (Rodríguez et al., 2015).

Specific reproductive characteristics and timing of reproduction can contribute to genetic differentiation in amphibians (Funk, Cannatella, & Ryan, 2009; Lourenço, Gonçalves, Carvalho, Wang, & Velo-Antón, 2019). In B. ibitiguara, tadpoles and juveniles potentially develop in the same streams and gallery forests that adults use as breeding sites. Thus, genetic differentiation among streams and the high relatedness values within populations may be caused by a higher philopatry in this species when compared to species that require migration from foraging areas to breeding sites, such as pond-breeding amphibians (Coster et al., 2015; Gamble, Mcgarigal, & Compton, 2007; Semlitsch, 2008). It is possible that tadpoles disperse downstream (Eterovich, Yazbeck, Dergam, & Kalapothakis, 2009; Lawson, 2013), but streams inhabited by B. ibitiguara are typically narrow, shallow and frequently partially obstructed (e.g., by fallen trees), which could hamper tadpole dispersal far enough to promote genetic differentiation among patches at a landscape scale. Although detailed quantification of amphibian larval dispersal is sorely needed (Lourenço, Antunes, Wang, & Velo-Antón, 2018; Wahbe & Bunnell, 2001), evidence suggests that migration/dispersal events of amphibians are primarily made by adults and/or juveniles instead of larvae (Cushman, 2006; Semlitsch, 2008). A similar pattern is also observed in stream fishes, which tend to show restricted movement during most of their lives, causing increased genetic differentiation (Comte & Olden, 2018; Rodríguez, 2002).

Another factor is that B. ibitiguara reproduces for over six months during the rainy season (Nali & Prado, 2012). Females spend energy to produce eggs to ensure maximum reproductive output, and likely deposit more than one clutch during a season (Nali & Prado, 2012; Wells, 2007; R. C. Nali, pers. obs.). Males, on the other hand, spend energy calling and defending oviposition territories to obtain females, which are the limiting resource, and engage in male–male vocal and physical duels (Nali & Prado, 2012, 2014). It is unlikely that the territorial males or mature females of this species would undertake major dispersal events while breeding. In the remaining non-reproductive months, the environment is much dryer and desiccation is likely a further deterrent to movement (Smith & Green, 2005; Titon & Gomes, 2015). As a result, genetic differentiation will accumulate with

**TABLE 2** Estimates of the most parsimonious model explaining each intra-population genetic index of *Bokermannohyla ibitiguara* from 12 localities within the state of Minas Gerais, Brazil

| Term                  | Beta  | \( R^2 \) | t    | p   |
|-----------------------|-------|----------|------|-----|
| Allelic richness      |       |          |      |     |
| Topographic complexity| -0.058| —        | -2.68| 0.025|
| Elevation             | 0.002 | —        | 3.06 | 0.014|
| Private allelic richness |    |          |      |     |
| Topographic complexity| -0.01 | .41      | -2.63| 0.025|
| Effective number of alleles | 0.002 | .47      | 2.96 | 0.014|
| Expected heterozygosity |      |          |      |     |
| Topographic complexity| -0.002| .49      | -3.12| 0.011|
| Observed heterozygosity | <0.0001 | .32      | 2.15 | 0.057|

Note: The most parsimonious models are in Table 3, and all models have been deposited in the Dryad Digital Repository. Beta = estimate from the regression analysis; \( R^2 \) = coefficient of explanation; t = size of the difference relative to the variation; p = probability value.

* Whole model: \( F = 17.68; p < .001; R^2 = .80. *
## TABLE 3  Generalized linear regression models of four landscape variables versus seven genetic measures for 12 populations of *Bokermannohyla ibitiguara* in south-eastern Brazil

| Variables                                      | AICc     | Δ AIC  |
|------------------------------------------------|----------|--------|
| **Effective number of alleles**                |          |        |
| Elevation                                      | 27.1504  | 0      |
| Topographic complexity                         | 28.6445  | 1.4941 |
| Topographic complexity, elevation              | 29.4275  | 2.2771 |
| Habitat loss, elevation                        | 29.8337  | 2.6833 |
| Gallery forest                                 | 30.8960  | 3.7456 |
| **Inbreeding coefficient**                     |          |        |
| Elevation                                      | -36.819  | 0      |
| Topographic complexity                         | -36.463  | 0.356  |
| Gallery forest                                 | -36.001  | 0.818  |
| Habitat loss                                   | -35.987  | 0.832  |
| Gallery forest, elevation                      | -33.593  | 3.226  |
| **Allelic richness**                           |          |        |
| Topographic complexity, elevation              | 24.4592  | 0      |
| Elevation                                      | 26.7665  | 2.3073 |
| Topographic complexity, elevation, topographic complexity × elevation | 27.5253  | 3.0661 |
| Topographic complexity                         | 28.3034  | 3.8442 |
| Habitat loss, elevation                        | 28.9756  | 4.5164 |
| **Private allele richness**                    |          |        |
| Topographic complexity                         | -15.676  | 0      |
| Habitat loss, elevation                        | -15.310  | 0.366  |
| Gallery forest, habitat loss                   | -13.575  | 2.101  |
| Gallery forest                                 | -12.207  | 3.469  |
| Habitat loss, elevation, habitat loss × elevation | -12.180  | 3.496  |
| **Expected heterozygosity**                    |          |        |
| Topographic complexity                         | -55.012  | 0      |
| Elevation                                      | -52.967  | 2.045  |
| Topographic complexity, elevation              | -52.713  | 2.299  |
| Habitat loss, elevation                        | -52.097  | 2.915  |
| Gallery forest, topographic complexity          | -50.577  | 4.435  |
| **Number of alleles**                          |          |        |
| Topographic complexity                         | 47.6695  | 0      |
| Elevation                                      | 48.8236  | 1.1541 |
| Gallery forest                                 | 49.9388  | 2.2693 |
| Habitat loss                                   | 51.0618  | 3.3923 |
| Topographic complexity, elevation              | 51.8753  | 4.2058 |
| **Observed heterozygosity**                    |          |        |
| Elevation                                      | -36.556  | 0      |
| Topographic complexity                         | -35.917  | 0.639  |
| Gallery forest, elevation                      | -34.086  | 2.470  |
| Habitat loss, elevation                        | -33.210  | 3.346  |
| Topographic complexity, elevation              | -33.189  | 3.367  |

Note: The five most parsimonious models for each variable are shown (complete models in the Dryad Digital Repository). Bold values = statistically equivalent models (Δ AIC < 2).

Abbreviation: AICc, Akaike information criterion
* Models with p < .05.
** Models with 0.05 < p < .06 (marginally non-significant).
Although we did not detect contemporary barriers to gene flow, not all populations outside the SCNP, estimated from genotypes at 17 microsatellites, resulted in less genetic differentiation and clustering among habitat patches (Eterovick et al., 2009). Which historical and/or contemporary landscape features could then facilitate dispersal through this open environment, especially for a forest-dependent species?

One of the main drivers of genetic differentiation for every species is geographic distance, and our results corroborated distance as a relevant factor. However, other features also play a role in limiting dispersal events even in the presence of potential barriers, disrupting isolation by distance or resulting in a non-stationary pattern of IBD (Duforet-Frebourg & Blum, 2014; Marschalek & Berres, 2014; Murphy et al., 2010; Wright, Bishop, Matthee, & Heyden, 2015). Given that steeper terrain is potentially costly for dispersal, our second hypothesis was that gene flow would be facilitated by topographic homogeneity. Indeed, topographic complexity predicted limited gene flow more than any other variables. Conservation genetic studies have focused much more on habitat quality and distribution, rather than topography (e.g., Dixo et al., 2009; Miller, Bianchi, Mullins, & Haig, 2013; Telles et al., 2007; Titus et al., 2014). However, studies of different taxa have shown that topographic complexity is an important restrictor of gene flow among populations (e.g., Guarnizo et al., 2016; Pérez-Espona et al., 2008). And in fact, this historical factor has had a larger effect on genetic structure than any contemporary factors in B. ibitiguara.

When analysing contemporary barriers to gene flow, landscape genetic studies must take into account the spatial scale of sampling relative to habitat suitability and disturbance (Epps & Keyghobadi, 2015; Manel, Schwartz, Luikart, & Taberlet, 2003). Although we did not detect contemporary barriers to gene flow, studies with other amphibians that sampled at scales comparable to ours found genetic signatures of land cover disruption (Homola, Loftin, & Kinnison, 2019; Zancoli, Rödel, Steffen-Dewenter, & Storfer, 2014). Specifically, our sampled populations, which cover the entire species’ known range, showed enough variation in the seven land cover classes between population pairs (Figure 1c). Combined with the fact that genetic equilibrium is expected to occur rapidly with molecular markers with high mutation rates (e.g., microsatellites; Epps & Keyghobadi, 2015), this landscape allowed us to ask how land cover changes (Figure 1c) interact with historical factors such as topography and distance. At first look, some of our results (Figure 4, Table 4) could indicate a role of the protected status of populations within the SCNP in promoting less genetic differentiation and higher diversity, which supported our third hypothesis. However, those were related to topography, and not land cover variables. The SCNP is topographically homogeneous at higher elevations (>1,200 m) compared with the localities outside the park (<1,100 m; Figure 1a,b). Moreover, land cover was not directly associated with allelic measures in our path analyses, but topography was (Figure 3). A similar effect was found in another montane frog, for which differences in elevation explained heterozygosity and allelic richness (Funk et al., 2005). Our results show unequivocally—and for the first time for Neotropical frogs—that topography drives not only connectivity among habitats, but also the maintenance of genetic diversity within-habitat. Thus, topographic differences can play a role in limiting dispersal events even in the presence of pristine vegetation.

While topography was the most important variable in shaping genetic structure of B. ibitiguara, we did find inconsistencies in some populations. First, populations R1 and R2 + R3 separated into two genetic clusters, despite not being located within an area of particularly rough terrain (Figure 1). Second, population CAL belonged to the same cluster as the populations within SCNP, even though there is no obvious topographic connectivity with populations within the SCNP (Figure 1). Populations R1, R2 and R3 are found on the outer edge of the range, which normally have lower population sizes than core populations, decreased individual genetic diversity and increased isolation from other populations (Anderson & Danielson, 1997; Eckert, Samis, & Lougheed, 2008; Lawson, 2013; Marschalek & Berres, 2014; Marschalek & Berres, 2014; Telles et al., 2007; Titus et al., 2014). And in fact, this historical factor has had a larger effect on genetic structure than any contemporary factors in B. ibitiguara.

### Table 4: Comparisons of genetic measures between six populations of Bokermannohyla ibitiguara within the SCNP (Serra da Canastra National Park, state of Minas Gerais, Brazil) versus six populations outside the SCNP, estimated from genotypes at 17 microsatellites

| Genetic parameter | Within SCNP | Outside SCNP | One-way ANOVA p | Permutation test p |
|-------------------|-------------|--------------|----------------|------------------|
| Allelic richness  | 7.658       | 6.506        | .014*          | .009*            |
| Private allelic richness | 0.422 | 0.350        | .314           | —                |
| Observed heterozygosity | 0.719 | 0.657        | .062           | .037*            |
| Expected heterozygosity | 0.797 | 0.752        | .052           | .011*            |
| Inbreeding coefficient | 0.099 | 0.127        | .636           | —                |
| Number of alleles  | 9.627       | 8.775        | .337           | —                |
| Effective number of alleles | 5.654 | 4.842        | .048*          | —                |

*Significant values at p < .05.

Note: Averages are reported for each category.
Peterman et al., 2013). Conversely, CAL is much closer to the core of the distribution, where higher genetic diversity is expected due to higher gene flow relative to peripheral populations and/or retained ancestral polymorphisms (Frankham, 1996; Zancolli et al., 2014). In addition, non-sampled populations may exist in streams between Chapadão da Babilônia and Chapadão da Canastra (S CNP), and wetlands/swamps could serve as stepping stones for dispersal using less complex pathways relative to topography (Coster et al., 2015).

Tropical species are underrepresented in the study of landscape genetics when compared to temperate ones (Manel & Holderegger, 2013), and topography is often not explicitly considered in the few Brazilian frogs studied (e.g., Eterovick et al., 2016; Prado, Haddad, & Zamudio, 2012; Telles et al., 2007). Zancolli et al. (2014) found that topographically complex regions in Africa did not represent barriers to gene flow in the stream-breeding frog Amietia wittei; the authors hypothesized that local environmental variables would act as cryptic factors driving differentiation instead of topography. Bokermannohyla ibitiguara occurs up to approximately 1,500 m, while A. wittei occurs from 1,700 to 3,500 m, with no high-altitude flat plateau. Thus, frogs with similar habitat specializations may still present different dispersal capabilities and adaptations depending on factors such as altitude range and configuration of highlands. Our study underscores that historical natural features that preclude individual dispersal may be more critical than recent events of anthropogenic habitat modification (e.g., González-Serna, Cordero, & Ortego, 2018). A general understanding of factors leading to diversification of stream-breeding frogs will only become feasible when we have more comparative landscape genetic studies in tropical habitats (Torres-Florez et al., 2018).

4.3 | Conservation implications

Amphibians are one of the most endangered vertebrate groups (Wake & Vredenburg, 2008) as evidenced by multiple species declines globally due to a combination of factors, including anthropogenic habitat modification (the Global Decline of Amphibians; Allentoft & O’Brien, 2010; Becker, Fonseca, Haddad, Batista, & Prado, 2007; Blaustein & Kiesecker, 2002; Cushman, 2006; Scheele et al., 2019). As a result, conservation genetic studies have focused mainly on vegetation loss and distribution of remaining suitable habitats, especially in the Neotropics (Dixo et al., 2009; Eterovick et al., 2016; Torres-Florez et al., 2018). By adding topography to the scenario, we bring novel information for amphibian conservation, especially for the highly threatened Brazilian Cerrado at both local and broad scales.

Brazilian law mandates that landowners maintain a percentage of undisturbed areas on their properties (Brasil, 2000, 2012). Because topographically complex regions are not adequate for cattle ranching and agriculture, these preserved areas are more likely to encompass topographically complex regions. Thus, our results reveal a conservation paradox, because safeguarding such complex areas may represent lower connectivity, instead of guaranteeing the desired maintenance of genetic diversity. At a broader scale, we draw attention to major Brazilian Cerrado formations with higher topographic complexity, and question whether the currently protected area of this domain (2% of Cerrado) is even close to sufficient. For instance, the Espinhaço mountain range, in south-eastern Brazil...
Brazil, is one of the top conservation priority regions in the Cerrado (Werneck, 2011). It is the only true Brazilian cordillera (Brazil, is one of the top conservation priority regions in the Cerrado, but topographic complexity could also be an important underlying mechanism.

Our results have practical implications for decision-making in conservation biology and indicate that safeguarding topographically homogeneous lands may prevent further genetic erosion of the remaining amphibian populations across their range. This is particularly critical for the Cerrado, where agriculture and cattle ranching expansion have accelerated throughout less complex terrains in the last three decades (Hunke, Müller, Schröder, & Zeilhofer, 2015). We recommend that future studies across taxa account for topography, in addition to land cover variables. This will help establish well-informed criteria for the assessment of conservation units that mitigate negative biodiversity impacts in already threatened ecosystems.

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PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are deposited as stated below:

- Microsatellite sequences: GenBank accession numbers KF977107–KF977119 and KF977121–KF977128 (Nali et al., 2014);
- Microsatellite genotypes and all generalized linear models: Dryad Digital Repository: https://doi.org/10.5061/dryad.c2fqz615x (Nali et al., 2020);
- Tissue samples, specimen vouchers and details on collection localities: published as Tables S1.1 and S1.2 from Appendix S1.

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REFERENCES

Ab’Saber, A. N. (1983). O domínio dos cerrados: Introdução ao conhecimento. Revista do Serviço Público, 111, 41–55.

Allendorf, F. W., & Luikart, G. H. (2007). Conservation and the genetics of populations. Oxford, UK: Blackwell.

Allentoft, M. E., & O’Brien, J. (2010). Global amphibian declines, loss of genetic diversity and fitness: A review. Diversity, 2, 47–71. https://doi.org/10.3390/d2010047

Anderson, G. S., & Danielson, B. J. (1997). The effect of landscape composition and physiognomy on metapopulation size: The role of corridors. Landscape Ecology, 12, 261–271.

Angermeier, P. L., & Karr, J. R. (1996). Biological integrity versus biological diversity as policy directives: Protecting biotic resources. In F. B. Samson, & F. L. Knopf (Eds.), Ecosystem management: Selected readings (pp. 264–275). New York, NY: Springer-Verlag.

Austin, J. D., Gorman, T. A., & Bishop, D. (2011). Assessing fine-scale genetic structure and relatedness in the micro-endemic Florida bog frog. Conservation Genetics, 12, 833–838. https://doi.org/10.1007/s10592-010-0176-7

Becker, C. G., Fonseca, C. R., Haddad, C. F. B., Batista, R. F., & Prado, P. I. (2007). Habitat split and the global decline of amphibians. Science, 318, 1775–1777. https://doi.org/10.1126/science.1149374

Benjamini, Y., & Yekutieli, D. (2001). The control of false discovery rate under dependence. Annals of Statistics, 29, 1165–1188.

Bergsten, J., Bilton, D. T., Fujisawa, T., Elliott, M., Monaghan, M. T., Balke, M., ... Vogler, A. P. (2012). The effect of geographic scale on sampling on DNA barcoding. Systematic Biology, 61, 851–869.

Blaustein, A. R., & Kiesecker, J. M. (2002). Complexity in conservation: Lessons from the global decline of amphibian populations. Ecology Letters, 5, 597–608. https://doi.org/10.1046/j.1461-0248.2002.00352.x

Blaustein, A. R., Wake, D. B., & Sousa, W. P. (1994). Amphibian declines: Judging stability, persistence, and susceptibility of populations to local and global extinctions. Conservation Biology, 8, 60–71. https://doi.org/10.1046/j.1523-1739.1994.08010060.x

Brasil (2000). Sistema Nacional de Unidades de Conservação da Natureza. Lei nº 9.985 de 18 de julho de 2000.

Brasil (2012). Código Florestal. Lei nº 12.651 de 25 de maio de 2012.

Brauer, C., Unmack, P., Smith, S., Bernatchez, L., & Beheregaray, L. (2018). On the roles of landscape heterogeneity and environmental variation in determining population genomic structure in a dendritic system. Molecular Ecology, 27, 3484–3497. https://doi.org/10.1111/mec.14808

Cardoso, A. J. (1983). Descrição e biologia de uma nova espécie de Hyla Laurenti, 1768 (Amphibia, Anura, Hylidae). Iheringia Série Zoologia, 62, 37–45.

Chiucchi, J. E., & Gibbs, H. L. (2010). Similarity of contemporary and historical gene flow among highly fragmented populations of an endangered rattlesnake. Molecular Ecology, 19, 5345–5358. https://doi.org/10.1111/j.1365-294X.2010.04860.x

Cole, M. (1986). The savannas: Biogeography and geobotany. London, UK: Academic Press.

Comte, L., & Olden, J. D. (2018). Fish dispersal in flowing waters: A synthesis of movement- and genetic-based studies. Fish and Fisheries, 19, 1063–1077. https://doi.org/10.1111/faf.12312

Coster, S. S., Babbitt, K. J., Cooper, A., & Kovach, A. I. (2015). Limited historical gene flow among highly fragmented populations. Conservation Genetics, 16, 369–376. https://doi.org/10.1007/s10592-014-0587-5

Couvet, D. (2002). Deleterious effects of restricted gene flow in fragmented populations. Conservation Biology, 16, 369–376. https://doi.org/10.1046/j.1523-1739.2002.99518.x

CSR/IBAMA (2014). Projeto de Monitoramento do Desmatamento dos Biomas Brasileiros por Satélite – PMDBBS. Retrieved from: http://siscom.ibama.gov.br/monitorabios.
Rodríguez, A., Börner, M., Pabijan, M., Gehara, M., Haddad, C. F. B., & Vences, M. (2015). Genetic divergence in tropical anurans: Deeper phylogeographic structure in forest specialists and in topographically complex regions. *Evolutionary Ecology*, 29, 765–785. https://doi.org/10.1007/s10682-015-9774-7

Rodríguez, M. A. (2002). Restricted movement in stream fish: The paradigm is incomplete, not lost. *Ecology*, 83, 1–13.

Rosenberg, M. S., & Anderson, C. D. (2011). Passage: Pattern analysis, spatial statistics, and geographic exegesis. V. 2. *Methods in Ecology and Evolution*, 2, 229–232.

Rothermel, B. B., & Semlitsch, R. D. (2002). An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. *Conservation Biology*, 16, 1324–1332. https://doi.org/10.1046/j.1523-1739.2002.01085.x

Rousset, F. (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, 145, 1219–1228.

Rousset, F. (2008). GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, 8, 103–106. https://doi.org/10.1111/j.1471-2286.2007.01931.x

Scheele, B. C., Pasmins, F., Skerratt, L. F., Berger, L., Martel, A. N., Beukema, W., ... Canessa, S. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science*, 363, 1459–1463. https://doi.org/10.1126/science.aav0379

Segalla, M. V., Caramaschi, U., Cruz, C. A. G., Garcia, P. A., Grant, T., Haddad, C. F. B., ... Langone, J. A. (2019). Brazilian amphibians: List of species. *Herpetologia Brasileira*, 8, 65–96.

Semlitsch, R. D. (2008). Differentiating migration and dispersal processes for pond-breeding amphibians. *Journal of Wildlife Management*, 72, 260–267. https://doi.org/10.2193/2007-082

Silva, J. F., Farinas, M. R., Fellfi, J. M., & Klink, C. A. (2006). Spatial heterogeneity, land use and conservation in the Cerrado region of Brazil. *Journal of Biogeography*, 33, 536–548. https://doi.org/10.1111/j.1365-2699.2005.01422.x

Smith, M. A., & Green, D. M. (2005). Dispersal and the metapopulation paradigm in amphibian ecology and conservation: Are all amphibian populations metapopulations? *Ecography*, 28, 110–128.

Spear, S. F., & Storfer, A. (2008). Landscape genetic structure of coastal tailed frogs (*Ascaphus truei*) in protected vs. management forests. *Molecular Ecology*, 17, 4462–4465.

Telles, M. P. C., Diniz-Filho, J. A. F., Bastos, R. P., Soares, T. N., Guimarães, L. D., & Lima, L. P. (2007). Landscape genetics of *Physalaemus cuvieri* in Brazilian Cerrado: Correspondence between population structure and patterns of human occupation and habitat loss. *Biological Conservation*, 139, 37–46. https://doi.org/10.1016/j.biomolcon.2007.06.003

Titon, B. Jr, & Games, F. R. (2015). Relation between water balance and climatic variables associated with the geographical distribution of anurans. *PLoS One*, 10, e0140761. https://doi.org/10.1371/journal.pone.0140761

Titus, V. R., Bell, R. C., Becker, C. G., & Zamudio, K. R. (2014). Connectivity and gene flow among eastern tiger salamander (*Ambystoma tigrinum tigrinum*) populations in highly modified anthropogenic landscapes. *Conservation Genetics*, 15, 1447–1462.

Torres-Florez, J. P., Johnson, W. E., Nery, M. F., Eizirik, E., Oliveira-Miranda, M. A., & Galetti, P. M. (2018). The coming of age of conservation genetics in Latin America: What has been achieved and what needs to be done. *Conservation Genetics*, 19, 1–15. https://doi.org/10.1007/s10592-017-1006-y

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4, 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Wahbe, T. R., & Bunnell, F. L. (2001). Preliminary observations on movements of tailed frog tadpoles (*Ascaphus truei*) in streams through harvested and restored forests. *Northwest Science*, 75, 77–83.

Wake, D. B., & Vredenburg, V. T. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences*, 105, 11466–11473. https://doi.org/10.1073/pnas.0801921105