



Genetic variability in a population of *Astyanax scabripinnis*: recent bottleneck and the possible influence of individuals with B chromosomes

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ABSTRACT. Access the genetic variability of endangered and isolated populations has become an important conservation tool. *Astyanax scabripinnis* is a well-known fish model for genetic studies, forming very isolated populations in headwaters. Besides that, this species frequently presents supernumerary chromosomes, which elevates the interest on genetic studies. Genetic diversity of an *Astyanax scabripinnis* population from the Atlantic Forest (Serra da Mantiqueira region, Brazil) was assessed with microsatellite markers for the first time. Since microsatellite markers are not described for this species, we tested markers described for a related species for transferability to *A. scabripinnis*. Six polymorphic loci were sufficiently reliable for population genetic analysis. We found that this population passed through a recent bottleneck because of the presence of an excess of heterozygotes, low allelic diversity, heterozygosity excess, and small effective population size. Individuals with and without B chromosomes were previously identified in this population and our study found private alleles in the individuals without B chromosomes. Furthermore, when individuals without B chromosomes were removed from the analysis, the population did not present heterozygosity excess, suggesting that the bottleneck event was driven by individuals with B chromosomes. Our results provide an insight into the value of microsatellite markers as molecular tools and is the first genetic study using molecular data of *A. scabripinnis* from this area.

Keywords: allelic deficiency; heterozygosity excess; fixation index; microsatellites; supernumerary chromosomes.

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Introduction

The Neotropical freshwater fish tetra, *Astyanax scabripinnis* (Jenyns, 1842), has become an important research subject in conservation genetics because of its ability to maintain small isolated but stable populations and its capacity for inhabiting heavily anthropized streams (Matoso, Artoni, & Galetti, 2004; Abilhoa, 2007; Abilhoa, Braga, Bornatowski, & Vitule, 2011). However, such small isolated populations are susceptible to losing genetic diversity, which consequently reduces their ability to adapt to environmental variation (Westemeier et al., 1998). Inbreeding, another effect of reduction in population size, raises the probability of accumulation of deleterious alleles, causing deleterious phenotypic effects, such as mortality and reduced fitness (Galbusera, Lens, Schenck, Waiyaki, & Matthysen, 2000). However, populations that have experienced a reduction in size may maintain themselves over time through various genetic or ecological factors (Frankham, 1997), as can happen with *A. scabripinnis*. Molecular and chromosomal studies of other fish species from the same hydrographic system as that occupied by *A. scabripinnis* suggest gene flow between populations maintained through migration (Ferreira et al., 2016) or population differentiation through bottlenecks or selection pressures (Kavalco, Pazza, Brandão, Garcia, & Almeida-Toledo, 2011). It is likely that similar mechanisms may influence genetic diversity and population structure of *A. scabripinnis*.

A number of studies have shown karyomorphological variation, such as variable diploid number, chromosomal macrostructure, and karyotypic formulae, and the occurrence of supernumerary or B chromosomes in *A. scabripinnis* populations (Moreira-Filho & Bertollo, 1991; Souza & Moreira-Filho, 1995; Mizoguchi & Martins-Santos, 1997; Mestriner et al., 2000; Ferro, Néo, Moreira-Filho, & Bertollo, 2001; Ferro, Moreira-Filho, & Bertollo, 2003; Vicari, Noletto, Artoni, Moreira-Filho, & Bertollo, 2008). However,

population studies of *A. scabripinnis* using molecular markers are rare. Sofia et al. (2006), using RAPD genetic markers, found moderate population structure among three collection sites in the same stream. These findings reveal the possible occurrence of microscale genetic differentiation among populations of this species, which corroborates the proposal of a species complex (Moreira-Filho & Bertollo, 1991).

Among *A. scabripinnis* populations, those from the Serra da Mantiqueira region (the Atlantic Forest), between Minas Gerais and São Paulo States in Brazil, are some of the best studied cytogenetically and have been employed as evolutionary models in analyses involving genetic markers (Kavalco & Moreira-Filho, 2003; Moreira-Filho, Galetti, & Bertollo, 2004). This region contains the watersheds of two important hydrographic basins in Brazil, the Paraíba-do-Sul and Sapucaí-Grande-Paraná (Saadi, 1993), where *A. scabripinnis* is known to form small isolated populations in the tributaries of streams and rivers (Moreira-Filho et al., 2004).

Since the early 1990s, several studies have shown the occurrence of B chromosomes in *A. scabripinnis* populations from the Serra da Mantiqueira region (reviewed by Moreira-Filho et al., 2004). The B chromosomes vary in their frequencies between populations and between males and females and differ in size and chromatin constitution (Moreira-Filho et al., 2004). For example, in a population from the Lavrinha stream, located in the municipality of Guaratinguetá, São Paulo State, Ferro et al. (2001) found $2n = 50$ chromosomes, consisting of six metacentric, 22 submetacentric, 10 subtelocentric, and 12 acrocentric chromosomes, and one to two B chromosomes. In a subsequent study of the same population, they found that the B chromosomes were entirely heterochromatic and metacentric or submetacentric (Ferro et al., 2003).

Despite the large number of studies of *A. scabripinnis* from the Serra da Mantiqueira region that have employed cytogenetic markers, molecular studies, using microsatellites markers, of these populations are nonexistent. A quick and economic way to obtain molecular markers for a population genetic study is to test transferability of primers described for related species. A set of microsatellites primers have been described recently for a related neotropical fish, *Astyanax altiparanae* (Zaganini et al., 2012), and for the well-studied *A. mexicanus* (Strecker, 2003; Strecker, Bernatchez, & Wilkens, 2003; Panaram & Borowsky, 2005; Bradic, Beerli, León, Esquivel-Bobadilla, & Borowsky, 2012).

In order to understand better the genetic factors that may contribute to conservation of *A. scabripinnis* occurring as isolated populations and in anthropized environments in the Serra da Mantiqueira region, we aimed to: (1) test the transferability to *A. scabripinnis* of several microsatellite markers described in a related species; (2) perform a detailed study on the genetic variability of one population present in Serra da Mantiqueira; and (3) verify, using the microsatellite markers, whether genetic differentiation can be identified between individuals with and without B chromosomes.

Material and methods

Collection site and specimens

Astyanax scabripinnis specimens were collected in April 2012 from the Lavrinha stream (length, 1876 m; 22° 43' 09.6" S 45° 25' 38.5" W), a tributary in the Paraíba-do-Sul river basin, Serra da Mantiqueira region, in the municipality of Guaratinguetá, São Paulo, Brazil (Figure 1). Sampling was carried out in accordance with relevant guidelines and with permission from *Ministério do Meio Ambiente, Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, Instituto Chico Mendes de Conservação da Biodiversidade-ICMBio MMA/IBAMA/SISBIO*, Number 15115-1.

The population we sampled is known to have a high frequency of B chromosomes (Ferro et al., 2001). Therefore, we used DNA from 35 specimens previously analyzed by Cornelio et al. (2017) for reproductive biology. The authors identified the individuals with B chromosomes and those without. We used the cetyl trimethylammonium bromide method (CTAB; Boyce, Zwick, & Aquadro, 1989) for extraction of DNA from 0.5 cm³ of muscle tissue. We then verified the DNA concentration and adjusted it to 50 ng µL⁻¹. DNA extractions was carried out in 2012, immediately after the cytogenetics analyses.

Selection of markers

As there are no primers described for *A. scabripinnis* for the microsatellite markers, we tested those described for a related species, *A. altiparanae* Garutti & Britski, 2000 for Zaganini et al. (2012). First, we tested the transferability of 11 microsatellite markers described for this species to *A. scabripinnis*. For each pair of primers, the melting temperature cited in Zaganini et al. (2012) was used as the central temperature, with a gradient of four degrees above and below. The PCR conditions for this test were those described by the above authors.

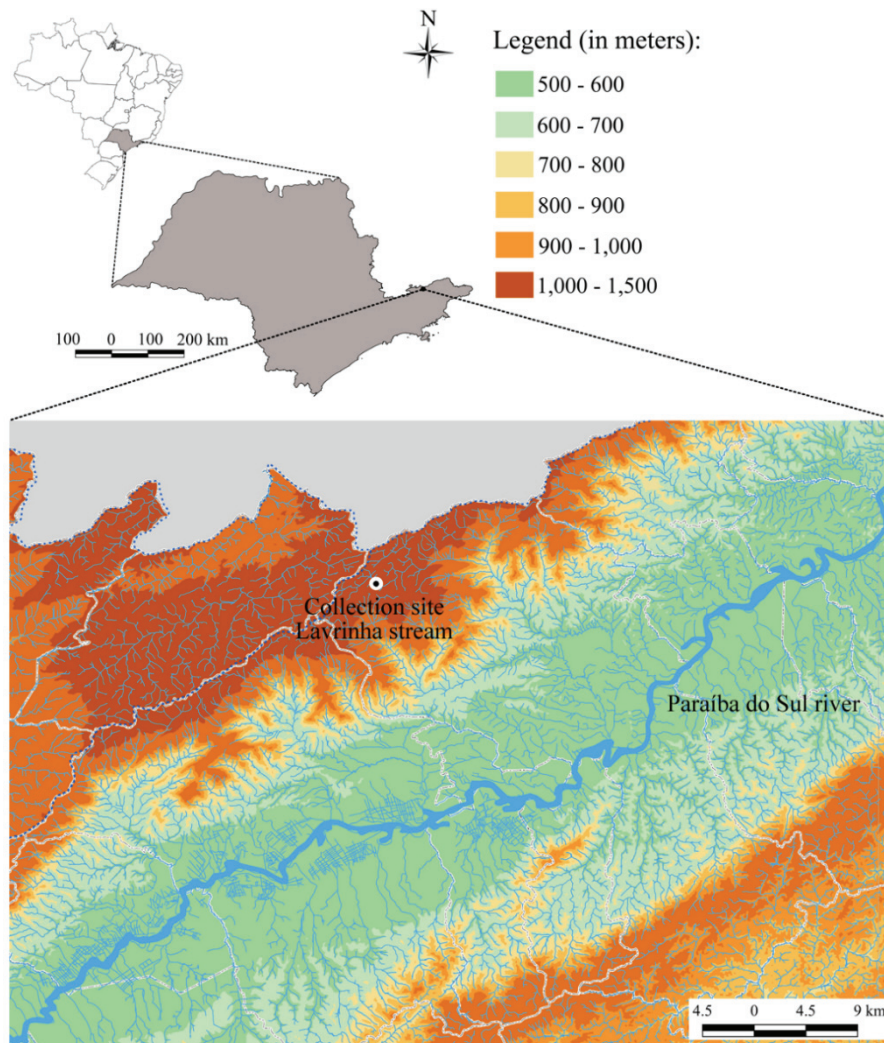


Figure 1. Hypsometric map showing the collection site of 35 individuals of *Astyanax scabripinnis*, Lavrinha stream, Paraíba-do-Sul river basin on Guaratinguetá municipality, São Paulo State, Brazil.

Amplification and genotyping

The amplification products were separated on a 2% agarose gel and stained with GelRed™ Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA), revealing the most appropriate melting temperature for the population studies. Based on this, six polymorphic microsatellite loci that resulted in good amplification for population analysis were selected as follows: Asty04, Asty12, Asty21, Asty23, Asty26, and Asty27. All amplifications were performed as described by Zaganini et al. (2012), with some minor adaptations. The PCR products were applied to a 6% polyacrylamide gel, stained with silver nitrate, and then photodocumented for genotyping.

Statistical analyses

The number of alleles, expected (H_e) and observed heterozygosity (H_o) were calculated using GenAlEx 6.501 software (Peakall & Smouse, 2012). The fixation index (F_{IS}) was calculated with Genepop 4.2 (Raymond & Rousset, 1995; Rousset, 2008; <http://genepop.curtin.edu.au/>), using two methods, Weir and Cockerham (1984) and Robertson and Hill (1984). Deviations from Hardy-Weinberg equilibrium were estimated with the F_{IS} statistic estimates, using Markov chain Monte Carlo (MCMC) runs for 100 batches, each of 1,000 iterations, with the first 1,000 iterations discarded before sampling. The MCMC method is the most appropriate when there are five alleles or more in at least one locus. For comparison purposes, deviations from Hardy-Weinberg equilibrium were also verified in GenAlEx 6.501. Whenever pertinent, the significance of P -values was adjusted following sequential Bonferroni corrections for multiple simultaneous statistical tests (Rice, 1989), with an initial alpha value of 0.05 k, where k was the number of tests.

When heterologous primers are used, as in this study, there may be increased probability of occurrence of null alleles, small modifications in allele size during amplification (stuttering), and different amplifications between small and big alleles (allelic dropout), thus generating errors in genotyping. The occurrence of allelic dropouts and stuttering was investigated using Micro-Checker 2.2.3 software (van Oosterhout, Hutchinson, Wills, & Shipley, 2004). The occurrence of null alleles was investigated in two ways. One method used Micro-Checker software, which compares Chakraborty, Andrade, Daiger, and Budowle (1992) and Brookfield (1996) algorithms with the algorithm developed for the program (van Oosterhout et al., 2004). The other method used FreeNA software (Chapuis & Estoup, 2007), which employs the Expectation Maximization (EM) algorithm of Dempster, Laird, and Rubin (1977), with 10,000 replicates. This software was also used for F_{ST} calculation, excluding and including null alleles.

From the genetic data, the effective population size (N_e) was calculated according to Pudovkin, Zaykin, and Hedgecock (1996) using heterozygote excess. This method uses a single sample but estimates the effective size of the population just one generation before sampling. This estimate was made using Neestimator v2 software (Do et al., 2014).

We also investigated a possible recent bottleneck in the population using Bottleneck 1.2.02 (Cornuet & Luikart, 1996). This software computes the probability of a bottleneck under three evolutionary models, the infinite allele model (IAM), stepwise mutation model (SMM), and two-phase model (TPM), comparing expected heterozygosity and the number of alleles. Populations that have experienced a recent bottleneck exhibit a reduction in allele numbers faster than the reduction in heterozygosity at polymorphic loci, i.e., they show heterozygosity excess determined in the sense of Nei's (1987) gene densities (Cornuet & Luikart, 1996). TPM appears to be the most appropriate model for microsatellites (Di Rienzo et al., 1994; Piry, Luikart, & Cornuet, 1999). Computations for all the tests were performed using 1,000 replicates. With TPM, we used 95% (Piry et al., 1999) and 70% (standard configuration) single-step mutations and a variance of 12 among multiple steps (Piry et al., 1999). This method has the advantage of not requiring prior knowledge of the population's pre-bottleneck genetic diversity.

In order to understand the effect of B chromosomes, we repeated all the statistical analyses for tests that included only the individuals with B chromosomes and for similar tests that included only the individuals without B chromosomes. We therefore assumed a subdivision of the population: 11 individuals with B chromosomes and 24 individuals without B chromosomes. All the individuals thus analyzed had been included in cytogenetic studies previously (Cornelio et al., 2017).

Results

All 11 loci demonstrated feasible transferability to *A. scabripinnis*. However, only six loci were used in the population studies, because they were shown to be more reliable for analyzing genetic variability in this species. The best melting temperatures for each marker determined by the gradient test are shown in Table 1.

The allele number varied from two to five and the effective allele number from 1.089 to 4.414. H_o was higher than H_e for almost all loci, with one exception Asty27. Thus, the mean value of F_{IS} (Weir & Cockerham, 1984) was negative, indicating a heterozygote excess.

Only two loci, Asty23 and Asty26, showed non-significant frequency deviations from Hardy-Weinberg equilibrium (Table 1). The null allele analysis revealed a possible presence only in the Asty27 locus, suggesting a heterozygote deficit for this locus. There was no evidence of stuttering or allelic dropouts.

Table 1. Population genetics analyses of *Astyanax scabripinnis* from Serra da Mantiqueira region, in the municipality of Guaratinguetá, São Paulo State (Lavrinha stream, Paraíba-do-Sul basin).

Locus	T_m	n_a	n_e	H_o	H_e	$F_{IS(W\&C)}$	$F_{IS(R\&H)}$	
Asty04	56°C	5	4.414	1.000	0.773	-0.2796	-0.2390	*
Asty23	56°C	2	1.089	0.086	0.082	-0.0303	-0.0307	
Asty12	58°C	4	2.249	1.000	0.555	-0.7518	-0.2386	*
Asty26	58°C	2	1.298	0.265	0.230	-0.1379	-0.1397	
Asty27	58°C	2	1.998	0.000	0.500	1.000	1.0294	*
Asty21	57°C	4	3.690	0.875	0.729	-0.1850	-0.1226	*
Mean				0.538	0.478	-0.0641	0.0431	

T_m : melting temperature; n_a : number of alleles; n_e : effective number of alleles; H_o : observed heterozygosity; H_e : expected heterozygosity; $F_{IS(W\&C)}$: fixation index according Weir and Cockerham (1984). $F_{IS(R\&H)}$: fixation index according Robertson and Hill (1984); *Loci with significant deviation (at $p \leq 0.05$ level) from Hardy-Weinberg equilibrium after sequential Bonferroni adjustment.

In the analyses involving only the individuals without B chromosomes, the difference between H_o and H_e was smaller (0.533 and 0.479, respectively; Table 2) and F_{IS} increased near to 0, when compared to the analyses involving only the individuals with B chromosomes (0.55 and 0.464, respectively; Table 2), indicating a more moderate heterozygote excess. When these parameters were considered only for the individuals with B chromosomes, a striking heterozygote excess could be observed, indicating that these individuals contributed more to the heterozygote excess in the population (Table 2). The F_{ST} values between these two groups (with and without B chromosomes), including and excluding null alleles, were negative and, because of that, converted to 0.

These differences were due to the presence of a monomorphic locus in individuals with B chromosomes. In this subpopulation, the Asty23 locus had only one allele, while in individuals without B chromosomes there were two alleles. The latter also presented two additional private alleles in the Asty12 locus. Therefore, we found significant departures from Hardy-Weinberg equilibrium for the Asty27 locus in both subpopulations and for Asty12 only in the subpopulation without B chromosomes.

The effective population size (N_e) calculated using the heterozygosity excess in our sample of 35 specimens was four, which corresponds approximately 11% of the sample.

The investigation of a recent bottleneck under the three mutation models only showed a significant heterozygosity excess with the Wilcoxon test [according to Piry et al. (1999), the most appropriate test for a small number of loci] with 70% single-step and 30% multistep mutations under TPM ($p = 0.039$). The occurrence of a recent bottleneck may be assumed when there is a significant heterozygosity excess. Our study provides intriguing observations in this regard when considering subpopulations with or without B chromosomes. The subpopulation of individuals lacking B chromosomes did not show heterozygosity excess ($p = 0.218$, assuming TPM), therefore not implying a bottleneck. On the other hand, the subpopulation with B chromosomes, analyzed separately, showed heterozygosity excess ($p = 0.015$, assuming TPM), indicating a bottleneck.

Discussion

The very high genetic, chromosomal, and morphological diversity of the *A. scabripinnis* species complex, especially in populations in the Serra da Mantiqueira, has been investigated since the 1990s (e.g., Moreira-Filho & Bertollo, 1991; Moreira-Filho et al., 2004), and studies suggest that the number of species forming the complex easily exceeds 15 (Bertaco & Lucena, 2006; Vicari et al., 2008). Cytogenetic studies of the Lavrinha population showed variation in the karyotypic microstructure, as in the 18S rDNA an As51 satellite DNA, although the macrostructure is essentially constant (Barbosa et al., 2015). The diploid number is $2n = 50$ while the fundamental number is 88 (Ferro et al., 2001). Submetacentric and metacentric B chromosomes are present and are entirely heterochromatic in 55 % of the individuals (Ferro et al., 2003; Cornelio et al., 2017). However, the genetic diversity of these populations is not yet known at the molecular level, nor their genetic conservation status. In particular, studies on genetic variability of *Astyanax* populations using microsatellite data are scarce.

Table 2. Population genetics indices for two sympatric subpopulations (with and without B chromosome) of *Astyanax scabripinnis* from Serra da Mantiqueira region, in the municipality of Guaratinguetá, São Paulo State (Lavrinha stream, Paraíba-do-Sul basin).

Pop	Locus	n_a	n_e	a_r	H_o	H_e	$F_{IS(W\&C)}$	$F_{IS(R\&H)}$
With B	Asty04	5	4.481	4.996	1.000	0.777	-0.2429	-0.2125
	Asty23	1	1.000	1.000	0.000	0.000		
	Asty12	2	2.000	2.000	1.000	0.500	-1.0000	-1.0000
	Asty26	2	1.471	2.000	0.400	0.320	-0.2000	-0.2083
	Asty27	2	1.862	2.000	0.000	0.463	1.0000	1.1000 *
	Asty21	4	3.636	4.000	0.900	0.725	-0.1912	-0.0617
	M/S	16			0.550	0.464	-0.1268	-0.0765
	Asty04	5	4.347	4.801	1.000	0.770	-0.2793	-0.2337
Without B	Asty23	2	1.133	1.811	0.125	0.117	-0.0455	-0.0464
	Asty12	4	2.361	3.330	1.000	0.576	-0.6702	-0.2337 *
	Asty26	2	1.229	1.943	0.208	0.187	-0.0952	-0.0971
	Asty27	2	1.986	2.000	0.000	0.497	1.0000	1.0435 *
	Asty21	4	3.695	3.989	0.864	0.729	-0.1616	-0.1289
	M/S	19			0.533	0.479	-0.0420	0.0506

n_a : number of alleles; n_e : effective number of alleles; a_r : allelic richness; H_o : observed heterozygosity; H_e : expected heterozygosity; $F_{IS(W\&C)}$: fixation index according Weir and Cockerham (1984); $F_{IS(R\&H)}$: fixation index according Robertson and Hill (1984); M/S: mean or sum of the statistic. *Loci with significant deviation (at $p \leq 0.05$ level) from Hardy-Weinberg equilibrium after sequential Bonferroni adjustment.

The high variation in this species complex was also verified by morphometric ways. Castro et al. (2014) could distinguish four population of this species morphometrically, despite of the similar karyotypic macrostructure. Interestingly, the authors also found differences in parapatric populations and differentiation following isolation by distance. Similar results was also found by Barbosa et al. (2015) that compared Lavrinha population with two others.

In order to have a clearer idea of the genetic variability we obtained in this study, we reviewed several databases using the descriptors 'Astyanax' and 'microsatellites' and found only five investigations on genetic variability employing microsatellites for the genus (Table 3). Of these studies, one considered an *A. altiparanae* population (Zaganini et al., 2012) and the rest were on the well-studied *Astyanax mexicanus* De Filippi, 1853 populations. Comparison with congeneric populations reveals that the population we studied has relatively high heterozygosity levels.

Prior to our study, the only Brazilian *Astyanax* species for which microsatellite data were available was *A. altiparanae*. Zaganini et al. (2012) analyzed a population of this species in the Upper Paraná river basin (see Table 3). In contrast to the population we studied, all previous studies of *Astyanax* populations obtained values for expected heterozygosity in Hardy-Weinberg equilibrium usually higher than those for observed heterozygosity, therefore showing a deficit of heterozygotes.

Astyanax mexicanus and *A. altiparanae* populations presented much higher numbers of alleles, demonstrating greater allelic diversity, than the *A. scabripinnis* population we studied. The differences in alleles per locus between studies may be explained by the method of genotyping used, i.e., by polyacrylamide gel or by automated sequencer. The first method, which was used in this study, is less sensitive to variation in allele size and may underestimate the number of alleles.

Genetic variability of the *Astyanax* genus has also been assessed using other molecular markers, although these studies are scarce. Peres, Vasconcelos, and Renesto (2005) analyzed two *A. altiparanae* populations from the Paraná river basin using isozyme markers and obtained mean values for observed heterozygosity ranging from 0.073 to 0.075 and for expected heterozygosity ranging from 0.09 to 0.15, and 2.09 mean number of alleles for the polymorphic loci. In another study, using RAPD markers, of *A. scabripinnis* populations from three different sites from the same stream, Sofia et al. (2006) found expected heterozygosity values ranging from 0.24 to 0.25. Although this population has been identified as *A. scabripinnis*, it is an evolutionary unit different from the one studied here, but with a very similar natural history.

Although we do not have data on the size of the population we studied, observations made during collections suggest that the population is small, highly isolated, and under anthropic pressure. The effective population size (N_e), inferred from the excess of heterozygotes, is an important datum that is in agreement with these observations. The $N_e:N$ ratio, inferred from the sample and extended to the population, approximates numerically to that obtained by Frankham (1995), who concluded, after an extensive review, that N_e corresponds to an average of 11% of the census size of threatened populations. According to the same author, the causes of a decrease of this magnitude in N_e in relation to census size are the cumulative effects of fluctuation in population size, variance in the size of families and, to a lesser extent, unequal sex ratio. In our case, fluctuations in population size seems to be the more likely cause.

Table 3. Mean observed (H_o) and expected (H_e) heterozygosities (above) and range (below), and number of alleles (n_a) in species of *Astyanax* (some values are rounded to two decimal points).

	H_o	H_e	n_a	
<i>Astyanax mexicanus</i>	0.43	0.80	20.33	Strecker (2003)
	0.27 - 0.56	0.61 - 0.89		
<i>Astyanax mexicanus</i>	0.45	0.49	6.23	Strecker et al. (2003)
	0.20 - 0.71	0.23 - 0.87		
<i>Astyanax mexicanus</i>	0.35	-	-	Panaram and Borowsky (2005)
	0 - 0.66	-	-	
<i>Astyanax mexicanus</i>	0.59	0.62	6.25	Bradic et al. (2012)
	0.39 - 0.82	0.40 - 0.85		
<i>Astyanax altiparanae</i>	0.65	0.80	7.5	Zaganini et al. (2012)

Astyanax mexicanus (the Mexican blind cavefish) populations can inhabit both surface waters and caves, forming small groups with low gene flow (Protas et al., 2006). Thus, this species has a similar natural history to that of *A. scabripinnis*. Using microsatellite data, Panaram and Borowsky (2005) found that the observed heterozygosity of small *A. mexicanus* populations ranged from 0 to 0.254, whereas the heterozygosity of large populations ranged from 0.139 to 0.656. Other studies with the same species also found populations with varying levels of observed heterozygosity (Table 3).

Reductions in effective population size are frequently associated with population bottlenecks. In this scenario, genetic diversity tends to decrease, leading to increased probability of inbreeding and decreased viability of the population (Spencer, Neigel, & Leberg, 2000). During this population event, allelic diversity tends to be affected more than heterozygosity, so that populations that have recently passed through a bottleneck typically exhibit relatively low allelic richness or low allele numbers (allelic deficiency) and relatively high heterozygosity values (heterozygosity excess; Cornuet & Luikart, 1996; Piry et al., 1999). In a comparison of our population with congeneric populations (Table 3), we note that our population has high values of heterozygosity, heterozygote excess and, mainly, low levels of allelic diversity (allele number, effective allele number, and allelic richness). All these factors, together with the small effective population (N_e), indicate a recent bottleneck.

However, as allelic richness decreases, heterozygosity is also expected to decrease. In small populations, such as the one analyzed in this study, a small number of alleles leads to a heterozygosity deficit (Cornuet & Luikart, 1996), which will increase F_{IS} values. In the *A. altiparanae* population studied by Zaganini et al. (2012), positive value for F_{IS} (0.177) were obtained, indicating heterozygosity deficit, and therefore inbreeding. The absence of inbreeding, even with the small size of our population, corroborates the hypothesis of a recent bottleneck.

In addition, we obtained statistical support for our hypothesis of a recent bottleneck. The TPM, assuming 70% single-step and 30% multistep mutations with Wilcoxon test, which, according to Piry et al. (1999), is the most appropriate test for a small number of loci, confirmed a heterozygosity excess in our population. As there is evidence of allelic deficiency and heterozygosity excess, our results suggest that this population either has passed a bottleneck very recently or is undergoing a bottleneck. Given that the heterozygosity excess observed during a genetic bottleneck is transient (Cornuet & Luikart, 1996), it is expected that after this population event, heterozygosity would also decrease.

Transferability of microsatellite primers or cross-amplification is an important approach used in population genetics studies. It can reduce costs and time when working on taxa with low microsatellite frequencies or from which microsatellites are difficult to isolate (Oliveira, Pádua, Zucchi, Vencovsky, & Vieira, 2006). This factor is helping scientists in many studies on conservation genetics of natural populations of fishes, where primers described for one species could be used on another of the same genus (Barroso et al., 2003; Revaldaves, Pereira, Foresti, & Oliveira, 2005; Sanches & Galetti, 2006). We have shown that the primers described by Zaganini et al. (2012) for *A. altiparanae* are also effective in population genetics studies of *A. scabripinnis* and were able to demonstrate the genetic conservation status of this population through their levels of genetic variability. Furthermore, these microsatellites loci can be important and very informative tools for population genetics studies of other *Astyanax* species.

Astyanax scabripinnis and *A. altiparanae* are genetically close species that probably underwent a recent process of divergence (Rossini et al., 2016). It is believed that in species whose evolutionary diversification is recent, cross-amplification of microsatellite primers occurs easily because there is a high degree of conservation of these regions (Goldstein & Pollock, 1997). This was the case when we tested cross-amplification of *A. altiparanae* primers with *A. scabripinnis*. All primers amplified, but not all of them gave good results for analysis. The occurrence of null alleles in only one locus also corroborates a high degree of conservation, especially in the flanking regions. When describing these primers, Zaganini et al. (2012) tested cross-amplification in six other species of *Astyanax* (*A. scabripinnis* was not tested), in which at least nine primers were effective and of these 67% demonstrated polymorphic loci.

Although it has not been possible to detect significant evidence of isolation between subpopulations with and those without B chromosomes, our data suggest some form of differentiation between them. This is demonstrated by the presence of private alleles in individuals without B chromosomes, by the presence of a monomorphic locus in the subpopulation with B chromosomes and by the marked heterozygosity excess in the same subpopulation. When the individuals with B chromosomes are removed from the analysis, the population does not present evidence of a bottleneck, thus the allelic deficiency and heterozygosity excess of this population appears to be driven by individuals with B chromosomes.

The occurrence of supernumerary chromosomes has been associated with several deleterious effects in animals and plants, leading to reduced fitness of individuals carrying them (Camacho, Sharbel, & Beukeboom, 2000). It is generally believed that B chromosomes represent additional, mostly parasitic, elements in the genome, which are maintained in populations by a balance between their rates of accumulation and transmission and their effects on the fitness of the individuals hosting them (Camacho et al., 2000).

However, in other cases, B chromosomes have been associated with increased genetic variability. Pantulu and Manga (1975) found that the *variation* in mean chiasma frequency of A chromosomes increased with increasing number of supernumerary chromosomes. They also found that when more than four supernumerary chromosomes were present, they had a deleterious effect on A chromosome behavior and plant fertility. Yao, Yang, Pan, and Rong (2007) identified a relationship between the number of B chromosomes and increase in heterozygosity in Chinese maize landraces. They found higher expected heterozygosity in individuals with two Bs, although the highest number of B chromosomes in the landraces was three. In our case, although the number of individuals without B chromosomes is higher than the individuals with Bs, there is evidence of heterozygosity excess among the subpopulation with B chromosomes.

Although most B chromosomes are heterochromatic and therefore thought to be genetically inert, there is increasing evidence that they carry transcriptionally active genes (Houben, Banaei-Moghaddam, Klemme, & Timmis, 2014; Makunin et al., 2014). However, the role of these genes is not fully understood. Some authors have suggested that the products of these genes may favor survival of the B chromosomes, thereby promoting their evolutionary success (Navarro-Domínguez et al., 2017).

Conclusion

Our study is the first to use molecular markers to address the genetic variability in a population of *Astyanax scabripinnis* from Serra da Mantiqueira region and the microsatellite primers used are good candidates as molecular tools in conservation genetics of these populations. We have uncovered important information, such as the satisfactory genetic variability, when compared with others populations, the reduced effective population size and evidence of a recent bottleneck. Interestingly, this event seems to have been driven largely by individuals with B chromosomes, since they demonstrated greater allelic deficiency and a heterozygosity excess compared to individuals without B chromosomes.

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