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Microsatellite loci development for three catfish species from northwestern South America

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The Neotropical catfish species *Ageneiosus pardalis*, *Pimelodus grosskopfii*, and *Sorubim cuspicaudus* are important fishery resources in Colombia that show historical declines in their capture. This study used next-generation sequencing with 454 FLX technology (Roche Applied Science) and bioinformatics analysis to develop between 18 and 24 microsatellite loci for these species. The novel microsatellite loci showed high values of polymorphic information content -PIC (*A. pardalis*: 0.601–0.903, *P. grosskopfii*: 0.748–0.946 and *S. cuspicaudus*: 0.383–0.876), and the average number of alleles/locus ranged from 7–15 for *A. pardalis*, 9–30 for *P. grosskopfii* and 5–14 for *S. cuspicaudus*. The average observed and expected heterozygosities were respectively, 0.757 ± 0.035 and 0.834 ± 0.015 for *A. pardalis*; 0.596 ± 0.040 and 0.881 ± 0.009 for *P. grosskopfii*; and 0.747 ± 0.031 and 0.757 ± 0.025 for *S. cuspicaudus*. For future studies, these loci can be useful to estimate the genetic diversity and population structure in these three Neotropical catfishes.

Keywords: Freshwater fish, Molecular markers, Next-generation sequencing, Siluriformes

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Las especies de bagres neotropicales *Ageneiosus pardalis*, *Pimelodus grosskopfii* y *Sorubim cuspicaudus*, son importantes recursos pesqueros en Colombia y han mostrado disminuciones históricas en sus capturas. En este estudio se empleó la secuenciación genómica de próxima generación y análisis bioinformático para desarrollar entre 18 y 24 loci microsatélites para estas especies. Los loci microsatélites mostraron altos valores del contenido de información polimórfica CIP (*A. pardalis*: 0.601–0.903, *P. grosskopfii*: 0.748–0.946 and *S. cuspicaudus*: 0.383–0.876) y el número promedio de alelos/locus mostró un rango de 7–15 para *A. pardalis*, 9–30 para *P. grosskopfii* y 5–14 para *S. cuspicaudus*. Los valores promedio de heterocigosidad observada y esperada fueron respectivamente 0.757 ± 0.035 y 0.834 ± 0.015 para *A. pardalis*; 0.596 ± 0.040 y 0.881 ± 0.009 para P. *grosskopfii*; y 0.747 ± 0.031y 0.757 ± 0.025 para *S. cuspicaudus*. Los loci microsatélites desarrollados en este trabajo pueden ser útiles para estimar la diversidad genética y la estructura poblacional de estos tres bagres neotropicales en estudios futuros.

Palabras clave: Peces de agua dulce, Marcadores Moleculares, Secuenciación de próxima generación, Siluriformes.

INTRODUCTION

Genetic population studies are crucial in the generation of valuable information for different programs of management, conservation, and the genetic-diversity monitoring of several species (Schwartz *et al*., 2007; Allendorf *et al*., 2010; Frankham, 2010); particularly, those affected by different anthropogenic activities (Frankham, 2010). Among the different molecular markers utilized in genetic population studies, microsatellite loci are one of the most informative and widely used (Hamilton *et al*., 1999; Guichoux *et al*., 2011). However, the first approaches for microsatellite loci development in non-model species were expensive, complex (Hamilton *et al*., 1999; Castoe *et al*., 2010; Fernandez-Silva *et al*., 2013), and produced a low number of useful markers obtained for population studies (Zalapa *et al*., 2012). Fortunately, next-generation sequencing technologies allowed the fast development of different useful molecular markers to generate population and evolutionary information of species at lower costs (Ekblom, Galindo, 2011; Guichoux *et al*., 2011; Fernandez-Silva *et al*., 2013; Miller *et al*., 2013), although for the vast majority of fish species these markers are still limited or absent (Kumar, Kocour, 2017).

In Siluriformes, one of the richest taxonomic order of freshwater fishes in the Neotropics (Pereira *et al*., 2013; Reis *et al*., 2016), microsatellite loci have been developed only for 19 of 2,315 Neotropical valid species. Pimelodidae, the most studied family, includes 12 species belonging to the genera *Brachyplatystoma* (Rodrigues *et al*., 2009; Batista *et al*., 2010); *Conorhynchos* (Carvalho, Beheregaray, 2011), *Phractocephalus* (Souza *et al*., 2012), *Pimelodella* (Moeser, Bermingham, 2005), *Pimelodus* (Paiva, Kalapothakis, 2008; see Agostini *et al*., 2011), *Pseudoplatystoma* (Revaldaves *et al*., 2005; Saulo-

Machado *et al*., 2011; Prado *et al*., 2014), *Steindachneridion* (see Ojeda *et al*., 2016), and *Zungaro* (Carrillo-Ávila *et al*., 2009). Remaining studies includes three species of Loricariidae (Telles *et al*., 2010; Pereira *et al*., 2012; Galindo *et al*., 2015), two species of Trichomycteridae (Zamudio *et al*., 2009; Muñoz-Rojas *et al*., 2012), one species of Heptapteridae (Rodrigues *et al*., 2015) and one species of Pseudopimelodidae (Souza-Shibatta *et al*., 2013).

Microsatellite loci are absent for catfishes from the west of the Eastern Cordillera of the Andes excepting *Pimelodus grosskopfii* (Hernandez-Escobar *et al*., 2011 in Agostini *et al*., 2011), limiting the population genetic studies for these species. Some authors have used microsatellite loci developed for close phylogenetically related species (heterologous loci); however, in some cases their use seems to be related to failures in the amplification, low levels of polymorphism, size homoplasy, null-alleles, and the amplification of non-orthologous loci (Primmer *et al*., 2005; Barbará *et al*., 2007; Castoe *et al*., 2010; Yue *et al*., 2010). This has stimulated the development of new molecular markers suitable for their population genetic studies.

Consequently, based on next-generation sequencing with the 454 GS-FLX technology (Roche Applied Science) and bioinformatics analysis, this study developed species-specific microsatellite loci for the non-model catfish species *Ageneiosus pardalis (Lütken*, 1874), *Pimelodus grosskopfii* (Steindachner, 1879), and *Sorubim cuspicaudus* (Littmann, Burr, Nass, 2000). These three carnivorous and migratory species are important for fisheries and many aspects of their basic biology and population genetics remain unknown, restraining the development of adequate management programs. This issue is important since population densities of these species have been decreased by anthropic activities in all Colombian watersheds (Galvis and Mojica, 2007; Usma-Oviedo *et al*., 2009; Mojica *et al*., 2012), which led to their classification as vulnerable in the red list of freshwater fish of Colombia (Mojica *et al*., 2012). Moreover, *P. grosskopfii* was also included as a critically endangered species in the Red List of Threatened Species of the International Union for the Conservation of Nature (IUCN; Villa-Navarro *et al*., 2016). These tools will allow for future population genetic studies that support different proposals focused on the sustainable management and conservation of these species.

MATERIAL AND METHODS

Samples were collected from 2011 to 2014 in the lower section of the Cauca River and supplied to the Laboratorio de Biología Molecular y Celular (Universidad Nacional de Colombia), through the scientific cooperation agreement CT-2013-002443; framed in the environmental license # 0155 of January 30, 2009 from Ministerio de Ambiente, Vivienda y Desarrollo Territorial. For each species, we took advantage of nuclear reads from pyrosequenced-genomic libraries of one individual collected in the lower section of the Cauca River (Restrepo-Escobar *et al*., 2016a,b). Identification of microsatellite loci, primer design, and electronic PCR to guarantee the correct alignment of primers were performed using the software and procedures used by Landínez-García, Márquez (2016). About 39 and 43 pairs from the list of primers validated by electronic PCR were selected to evaluate their consistent amplification and polymorphism under standard PCR conditions in 12 individuals from each species. Then, we selected pairs of primers

that fulfilled the conditions proposed by Landínez-García, Márquez (2016): (1) specific amplification in all individuals within the sizes that were designed (100 to 350 bp), (2) band resolution, (3) specificity, and (4) ability to detect heterozygotes. The forward primers of these pairs were directly fluorescently labeled or universal markers were added to their 5´-tail to produce their fluorescent label through the three primer PCR method (Blacket *et al*., 2012) and were further evaluated in 50 individuals of each species.

The PCR amplification was carried out under the conditions proposed by Landínez-García, Márquez (2016) for the directly labeled primers, and by Landínez-García, Marquez (2018) for the primers marked using the three primer method. In both cases, the PCR products were separated in an ABI 3130 automatic sequencer (Applied Biosystems, USA) using GeneScan-500 LIZ (Applied Biosystems, USA) as the size marker; the electropherograms obtained were reviewed using GeneMapper 4.0 (Applied Biosystems, USA). Before the statistical analysis, Micro-Checker 2.2.1 (Van Oosterhout *et al*., 2004) was used to detect possible genotyping errors.

For each species, the genetic diversity, the allelic frequencies, the observed (Ho) and expected (He) average heterozygosity and the average number of alleles per locus (Na) were determined with the GenAlEx 6.503 (Peakall, Smouse, 2012). Additionally, Arlequin 3.5.2.2 (Excoffier, Lischer, 2010) was used to determine the statistical significance of the allelic frequencies in Hardy-Weinberg and Linkage equilibria. In the case of multiple comparisons, the statistical significance was adjusted by sequential Bonferroni correction (Rice, 1989). Furthermore, the polymorphic information content (PIC) was determined for each loci with CERVUS 3.0.7 (Marshall *et al*., 1998).

RESULTS

Genomic sequencing for *A. pardalis* generated 176,196 reads, 75,442 (43%) contained microsatellite loci, 19,940 (11%) potentially amplifiable loci (PAL) and 8,906 (5%) were validated by electronic PCR. For *P. grosskopfii*, the genomic sequencing generated 138,830 reads, 47,438 (34%) containing microsatellite loci, 23,937 (17%) PAL and 12,542 (9%) were validated by electronic PCR. Finally, the genomic sequencing for *S. cuspicaudus* generated a total of 123,158 reads, 43,302 (35%) contained microsatellite loci; 16,428 (13%) PAL and 8,840 (7%) were validated by electronic PCR.

The three studied species showed higher diversity of microsatellite loci with 2-mer motifs (57%–69%), followed by the 4-mer (15%–23%), 3-mer (10%–12%), 5-mer (4%–5%) and a small portion of 6-mer motifs (2%–3%; Fig. 1). Additionally, the three species showed similar patterns in the abundance of specific repeat motifs. For 2-mer motifs, the most common recurrence pattern was AC, and the less common was CG. Among the 3-mer motifs, the most common was ATT, and the least common was TCG for the three species. Lastly, among the 4-mer, the five most common motifs were AAAT, ATCT, TCTG, AGTG, and AATG.

More than 68% of the evaluated loci generated fragments in the expected size range (100–350 bp) and were polymorphic in *A. pardalis* (27 loci), *P. grosskopfii* (27 loci), and *S. cuspicaudus* (37 loci). The PIC values for the microsatellite loci developed were 0.601–0.903 for *A. pardalis*, 0.748–0.946 for *P. grosskopfii*, and 0.383–0.876 for *S. cuspicaudus* (Tabs. 1–3). The average number of alleles per locus ranged from 7–15 alleles/locus

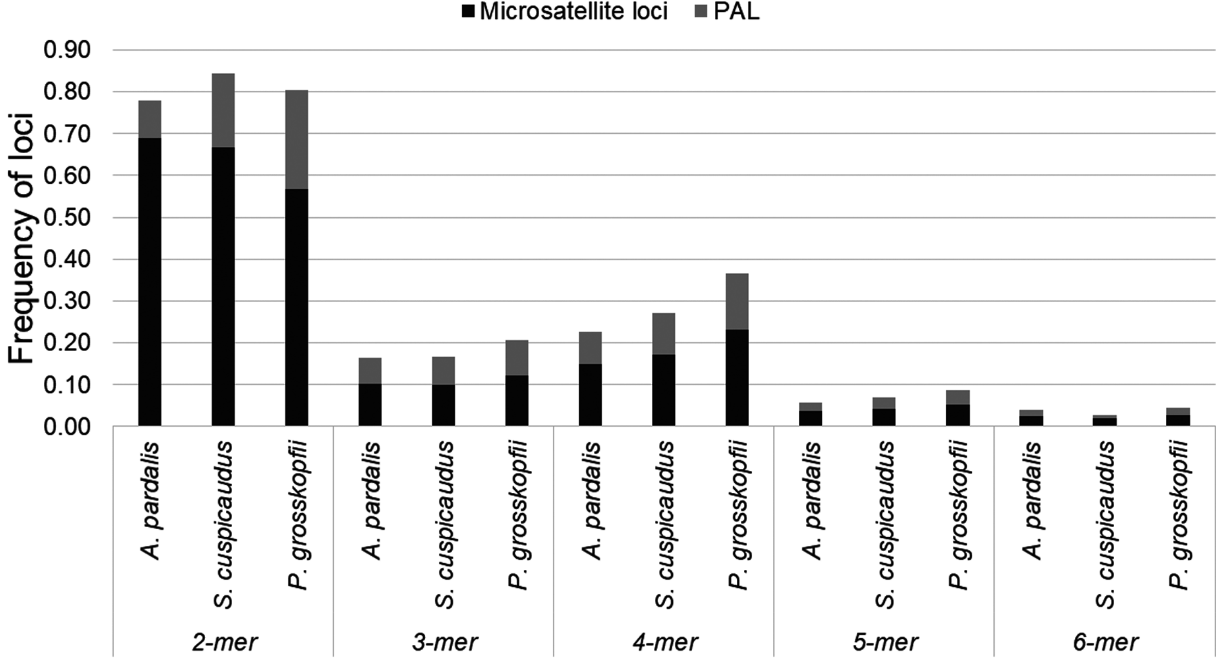

FIGURE 1 | Frequency of identified microsatellite loci and potentially amplifiable loci (PAL) obtained from 454 FLX sequencing for *Ageneiosus pardalis*, P*imelodus grosskopfii*, and *Sorubim cuspicaudus*.

(average: 11.286 ± 0.574) in *A. pardalis*; 9–30 alleles/locus (average: 14.944 ± 1.136) in *P. grosskopfii*; and 5–14 alleles/locus (average: 9.500 ± 0.552 alleles/locus) in *S. cuspicaudus*. In addition, the expected average heterozygosity ranged from 0.622 to 0.910 in *A. pardalis* (He: 0.834 ± 0.015), 0.771–0.948 in *P. grosskopfii* (He: 0.878 ± 0.010), and 0.419–0.887 in *S. cuspicaudus* (He: 0.757 ± 0.025). The observed average heterozygosity ranged from 0.320–0.920 in *A. pardalis* (Ho: 0.757 ± 0.035), 0.340–0.920 in *P. grosskopfii* (Ho: 0.612 ± 0.042), and 0.300–0.940 in *S. cuspicaudus* (Ho: 0.747 ± 0.031).

For *A. pardalis* (17 of 21, Tab. 1) and *S. cuspicaudus* (17 of 24, Tab. 3), all or most of the evaluated loci were shown to be in linkage and Hardy-Weinberg equilibria after the Bonferroni correction. In contrast, *P. grosskopfii* showed 13 loci with heterozygote deficit, allelic frequencies departures from Hardy-Weinberg equilibrium (Tab. 2), and significant linkage disequilibrium between the pairs of loci Pgrk01-Pgrk02 and Pgrk08-Pgrk20.

DISCUSSION

In this work, 63 microsatellite loci were designed for future studies of the genetic diversity of *A. pardalis* (21), *S. cuspicaudus* (24), and *P. grosskopfii* (18). The microsatellite loci of *A. pardalis* and *S. cuspicaudus* represent the first species-specific codominant markers for both Neotropical genera. Along with the above, the new 18 microsatellite loci for *P. grosskopfii* complement the currently available markers (Agostini *et al*., 2011). Pyrosequencing also

TABLE 1 | Primer sequences and characteristics of 21 polymorphic microsatellite loci identified in *Ageneiosus pardalis*. Ra: Allelic size range; Na: Average number of alleles per locus; Ho and He: observed and expected heterozygosity, respectively; P: Statistical significance for tests of departure of Hardy-Weinberg equilibrium; PIC: Polymorphic information content. Annealing temperature: 56.5 °C, for all primers.

Locus	Repeat motif	Primer sequence for forward (F) and reverse (R) (5'→3')	Ra	Na	Ho	He	P	PIC
Apar03	$(ATT)_n$	**F:** TTTAGAAGCAGCCTGGATGG **R:** CTTGACTTTGGGAAATGGGC	186-219	11	0.920	0.882	0.965	0.870
Apar25	$(TCTG)_n$	**F:** CCGGCTGTTCAATTTGTGG **R:** CACAGAGTAGAAGAGCTCACTTTGG	256-316	9	0.700	0.622	0.909	0.601
Apar11	$(ATGG)_n$	**F:** CACCAATGTATCCCCATCCC **R:** TTCAAGTACTGGCTACAAGCTGC	208-268	15	0.920	0.895	0.904	0.886
Apar36	$(AATAG)_n$	**F:** GAAGGAAATCACAGCCCTCG **R:** TCTGCTGGAAGGTGGAGAGG	230-280	8	0.800	0.825	0.832	0.801
Apar18	$(ATCT)_n$	**F:** GCATCTGCACCTCATATTTGC **R:** TGAGCTATTTCTGATTGGCTGG	206-266	13	0.860	0.871	0.816	0.858
Apar05	$(ATT)_n$	**F:** GGGAGGGAGAGGAGGATAGC **R:** GAAGGTAGGTGTTGGCAATGG	192-234	15	0.820	0.798	0.799	0.783
Apar35	$(AATAG)_n$	**F:** CCCTTTAAGCAAATTGTCTTCAGC **R:** CACCATCTGTCCTGTCCTGC	179-209	7	0.780	0.752	0.797	0.715
Apar22	$(ATCT)_n$	**F:** CAGAGGCTATTGTTCAGGCG **R:** TCATTGGGGTGGTGATATGC	235-271	10	0.800	0.850	0.612	0.833
Apar20	$(ATCT)_n$	**F:** TCCAACCATTACTGCATCCC R: GGTGATCGTTGGATGATTTGC	179-226	11	0.820	0.871	0.591	0.857
Apar21	$(ATCT)_n$	**F:** CACTGTGTGATCTCTCTGTGTTGG **R:** GAGATATTTCTTGAATTTCCTTCGG	173-225	13	0.920	0.891	0.494	0.881
Apar27	$(AATG)_n$	**F:** TCACCAAAGTGAAGCTGTTGC **R:** CAAACCAAGGACCTTCTCGC	146-178	9	0.900	0.827	0.416	0.806
Apar30	$(ATATC)_n$	**F:** GAACCTTGATTTTGCCCACG **R:** GCCAACATCAGGAAAGGGG	172-222	11	0.820	0.844	0.414	0.827
Apar04	$(ATC)_n$	**F:** CCTGCACTTCAACACTTCACC **R:** GAGAGAGTAAATGAGGGAAAGCG	256-298	10	0.880	0.844	0.401	0.826
Apar34	$(ATCAC)_n$	**F:** ACACTGCATCCCATCACACC **R:** TCCTCCTGGTCTTCTCACAGG	191-251	12	0.800	0.809	0.386	0.786
Apar23	$(ATCT)_n$	**F:** TCTTTGGTAACCCACCACCC **R:** ATGTGCAATGGGGATCTGC	241-269	8	0.860	0.814	0.295	0.791
Apar28	$(ATGG)_n$	F: AACTCCATGTCTGCTTCGGC **R:** CTGCTATTGCAGCCCATCC	273-301	8	0.600	0.698	0.179	0.666
Apar12	$(ATCT)_n$	**F:** GATTCCAGGATGCAGTTGAGG **R:** AGCGATTGCACACCATAACC	247-299	14	0.780	0.886	0.129	0.876
Apar31	$(AATAG)_n$	F: CTGGAGGGATGCAAACTGC R: GGGAATTCCGTTATTTTCAAGC	151-236	10	0.560	0.843	0.000	0.825
Apar14	$(ATCT)_n$	F: TTCCGGTATCGTGCATTTCC R: AACAATTGGGTGCCTAAGACG	165-221	15	0.500	0.910	0.000	0.903
Apar32	$(ATTAG)_n$	**F:** TTGCACTTCTGGTTGGATGC **R:** TCCAAAGCAAATGGTCATGG	151-216	13	0.540	0.900	0.000	0.892
Apar19	$(ATCT)_n$	**F:** TCAGCTAAGGCAAGTTGTTTGC **R:** GGGATTTCCTATATCGGCAGC	190-250	15	0.320	0.885	0.000	0.874
Across loci				11.286±0.574	0.757± 0.035	0.834± 0.015	0.000	0.817

TABLE 2 | Primer sequences and characteristics of 18 polymorphic microsatellite loci identified in *Pimelodus grosskopfii*. Ra: Allelic size range; Na: Average number of alleles per locus; Ho and He: observed and expected heterozygosity, respectively; P: Statistical significance for tests of departure of Hardy-Weinberg equilibrium; PIC: Polymorphic information content.[1,2,3] Annealing temperatures: 56.5 °C, 59 °C, 60 °C, respectively.

Locus	Repeat motif	Primer sequence for forward (F) and reverse (R) (5'→3')	Ra	Na	Ho	He	P	PIC
[1]Pgrk40	$(ATCT)_n$	**F:** TTTGGTAACATTCAAGGTTTACTTGC **R:** AAAATAGCAACGTTCTAACTAGGGG	159-219	15	0.920	0.916	0.743	0.910
[1]Pgrk27	$(ATATT)_n$	**F:** CTTCCAAGCATGACAAGCCC **R:** CTCTCCACCACATCTTCCCC	264-359	16	0.920	0.866	0.517	0.852
[1]Pgrk15	$(ATCT)_n$	**F:** TGCCATCAGTGGTCTTCACC **R:** CCCCATCACCCTGTTCACC	268-328	14	0.760	0.852	0.225	0.840
[2]Pgrk14	$(ATCT)_n$	**F:** TCATGGCCTTGTACTTGTACCG **R:** CACCAGTTCATGCTTCTGCC	169-261	15	0.800	0.903	0.170	0.895
[2]Pgrk03	$(ATCT)_n$	**F:** GGATGAAAGAAAAGGATTTGGC **R:** GATAAGACGCGCTTACACTTGC	174-246	16	0.740	0.909	0.083	0.902
[2]Pgrk24	$(AAAT)_n$	**F:** ACACGCATGTCTCTTGCCC **R:** ACTTGTACTGCGGATGCGG	188-232	11	0.660	0.866	0.016	0.852
[2]Pgrk01	$(ATCT)_n$	**F:** GTCCCAGTCCTGCTTCACG **R:** GCTAATGGTACAACATCGCCC	156-292	30	0.760	0.948	0.002	0.946
[2]Pgrk19	$(AAAT)_n$	**F:** TAGTCGGTGCTAATTCGCCG **R:** ACTGACTGGAGACCACAGCG	201-237	10	0.520	0.810	0.000	0.787
[2]Pgrk12	$(ATCT)_n$	**F:** TGAGATTGGTTTATAACACAGACCG **R:** TGCAGCTGAAGAACTCAGGG	192-292	22	0.380	0.913	0.000	0.908
[1]Pgrk18	$(ATCT)_n$	**F:** GGATAAATATGGGTGGGTGGC **R:** GGAGCTGTGGAAGAGACATCG	210-282	15	0.580	0.907	0.000	0.900
[2]Pgrk07	$(ATCT)_n$	**F:** TGGCACATGAGTTGAAGACG **R:** AAACACATTGACTGATAGCCTTCC	197-265	17	0.400	0.907	0.000	0.899
[3]Pgrk06	$(ATCT)_n$	**F:** GCGGGAAAAGTACAGGAAGG **R:** CGACGCAGCTCAGAATAAAGC	180-264	13	0.520	0.899	0.000	0.890
[1]Pgrk31	$(ATT)_n$	**F:** TGGAATTGTGCATTTCTTTGC **R:** GGAGGTTGAATTCCTCTGTGG	154-208	15	0.580	0.888	0.000	0.878
[2]Pgrk02	$(ATCT)_n$	**F:** ATCTGTCTGGCCATCCACC **R:** CAGATAGACGGACGCACG	77-157	15	0.400	0.879	0.000	0.867
[1]Pgrk20	$(ATCT)_n$	**F:** CAAGCTGCCTCCTGAAAACC **R:** GCTCATCTGTGTGAGTGGTGC	124-216	14	0.600	0.868	0.000	0.855
[1]Pgrk10	$(ATCT)_n$	**F:** TGTTGCGTAATATTCCCAGGC **R:** GACACAAACTGGTGAAAATCTGC	230-282	11	0.480	0.864	0.000	0.849
[2]Pgrk28	$(AATAG)_n$	**F:** ACTGTCTTGTGGCTTTGTGC **R:** TACGGGAAAATAATCCTGGG	116-226	11	0.660	0.843	0.000	0.825
[2]Pgrk08	$(AAAT)_n$	**F:** TTGGCGTGCAAATGAATCC **R:** ATGGGACCACAATCAGAGGC	261-301	9	0.340	0.771	0.000	0.748
Across loci				14.944±1.136	0.612±0.042	0.878± 0.010	0.000	0.867

TABLE 3 | Primer sequences and characteristics of 24 polymorphic microsatellite loci identified in *Sorubim cuspicaudus*. Ra: Allelic size range; Na: Average number of alleles per locus; Ho and He: observed and expected heterozygosity, respectively; P: Statistical significance for tests of departure of Hardy-Weinberg equilibrium; PIC: Polymorphic information content. Annealing temperature: 56.5 °C, for all primers.

Locus	Repeat motif	Primer sequence for forward (F) and reverse (R) (5'→3')	Ra	Na	Ho	He	P	PIC
Scus22	$(ATCT)_n$	**F:** CGCCACCTTAGGAACCTACC **R:** GTGGTATGGTGGTGTCGAGG	180-208	8	0.800	0.808	0.932	0.784
Scus15	$(TTC)_n$	**F:** GCCTTCATATGCTTGGACCC **R:** TGAAGGTCCCTTTCAATGGC	201-216	6	0.620	0.637	0.913	0.599
Scus41	$(ATTAC)_n$	**F:** CAGATAAAGGACCATGCTGAGG **R:** CTGGATGCTCTGTGTGAGGC	161-186	6	0.780	0.689	0.895	0.636
Scus24	$(ATCT)_n$	**F:** CCTTGAGATACCTGCAGCCC **R:** GTTCCCTCCCTTCGTCTTCC	257-289	9	0.820	0.831	0.847	0.809
Scus17	$(ATCT)_n$	**F:** TGATAGAGGCACAGAGTGAACG **R:** CCTGTGTGCCTTGATGTCG	181-229	12	0.900	0.862	0.809	0.847
Scus43	$(ATTAG)_n$	**F:** TGACTCTGTGACTCTAATTCCTGC **R:** TCAGTTTCCCTGAGGAACCC	141-166	6	0.720	0.629	0.809	0.568
Scus28	$(ATCT)_n$	**F:** TCGGATGCAAAGTTAGACGG **R:** AGGATTTGTTGCCTCATGCC	252-280	8	0.820	0.803	0.794	0.774
Scus25	$(ATCT)_n$	**F:** TAAAACTGCAAGCCCCTTCG **R:** CAGAGTCCAAACCCTGTGGG	173-237	14	0.920	0.867	0.746	0.853
Scus23	$(ATCT)_n$	**F:** GATCTTTGTTCAGTCCACGGC **R:** GACGTTGTTAGCCCAGATGC	275-309	11	0.780	0.838	0.523	0.818
Scus12	$(ATT)_n$	F: TCTACACAAGCAGAAATGCAGC R: TCACATTGCTTGACAGATGCC	204-258	11	0.680	0.695	0.514	0.670
Scus35	$(ATATT)_n$	F: GTCAAAACGCATTCTGTAAATGG R: GTAGTGCTCAGCTCCAGGGC	209-249	9	0.880	0.832	0.479	0.812
Scus40	$(ATCTT)_n$	F: TGGAAGTAGTTGCTGCTGTTGC R: CAGAAGGCATTTACTGTCTGGC	241-276	7	0.660	0.641	0.438	0.594
Scus20	$(ATCT)_n$	F: GTCACATCTGGGTGTGCTGG R: GCCTGAGCTTTGGATTGAGC	249-317	14	0.940	0.887	0.321	0.876
Scus39	$(ATCTT)_n$	**F:** GGGACACTATGATTTTCCTTCAGC **R:** CTGTCATCCCCAGCAGTAGC	128-168	9	0.820	0.812	0.199	0.785
Scus32	$(AGGGC)_n$	**F:** AACGAGCCATGCAAGAACG **R:** CCCAGAAGCAGTAAGGTGTGC	222-287	14	0.820	0.861	0.122	0.848
Scus21	$(ATCT)_n$	**F:** CTACCCAATCTAGCTGCCCC **R:** CGACCTGGTGGGTACGTAGG	117-221	11	0.900	0.820	0.080	0.798
Scus03	$(ATC)_n$	**F:** GGATCGTTGGTGTAAATCTCTGC **R:** TGACTCATGGTGTGTGGTGC	114-135	7	0.520	0.575	0.052	0.536
Scus11	$(ATT)_n$	**F:** CACTTCCACCTGAACCATGC **R:** GCATCAGGGTTAGCACAGGC	276-288	5	0.300	0.419	0.037	0.383
Scus16	$(AATG)_n$	**F:** ACGTCATCTGGTGTCGTTGG **R:** CCAGAAGATCTGTGTGAACCG	104-136	9	0.620	0.780	0.026	0.748
Scus13	$(ATT)_n$	**F:** AGACGAGAGGGGTGACATGC R: AAGGTGTTGGCAGGATGAGG	272-314	9	0.740	0.809	0.024	0.783
Scus10	$(ATT)_n$	**F:** CCTGAAGAAATCCAGCCACC **R:** ACCACAACTCCGACTCCAGG	168-213	13	0.820	0.865	0.020	0.851
Scus18	$(ATCT)_n$	**F:** CAAGCATCAAAGCAGAAAGCC **R:** ATCACCATCTTGCGTTCTATCC	257-305	12	0.640	0.867	0.005	0.853
Scus37	$(ATCAG)_n$	**F:** GATCTGGGCTCTGCTTTTGG **R:** GCAGCACAATGAGTACTGCG	265-330	9	0.540	0.597	0.002	0.564
Scus07	$(ATT)_n$	**F:** CAACATCTGCAACCAAATGC **R:** GATTTTGCACCAGGACAACG	146-176	9	0.880	0.753	0.000	0.713
Across loci				9.500±0.552	0.747±0.031	0.757±0.025	0.000	0.729

allowed us to examine the richness of microsatellite motifs of the species and compare them with the information available for eukaryotic organisms (Meglécz *et al*., 2012), particularly bony fishes (Osteichthyes) of the order Siluriformes (Somridhivej *et al*., 2008; Mohindra *et al*., 2012; Zhang *et al*., 2014), Characiformes (Villanova *et al*., 2015; Yazbeck *et al*., 2018), Cypriniformes (Luo *et al*., 2012; Jorge *et al*., 2018), Perciformes (Saarinen, Austin, 2010), and Tetraodontiformes (Edwards *et al*., 1998).

Superficial sequencing on *A. pardalis*, *P. grosskopfii*, and *S. cuspicaudus*, showed a higher frequency of 2-mer motifs; a characteristic previously described for several eukaryotic organisms (Meglécz *et al*., 2012). Additionally, in this study, 4-mer are the second most frequent motif for the three species studied, a similar outcome to others fishes such as *Megaleporinus obtusidens* (=*Leporinus obtusidens* in Villanova *et al*., 2015); *Craterocephalus fluviatilis*, *Galaxias fuscus*, *Henicorhynchus lobatus*, *Henicorhynchus siamensis*, *Alticus arnoldorum*, *Amphiprion sandaracinos* and *Amphiprion mccullochi (*Meglécz *et al*., 2012). This result is in contrast however, to others species of fishes such as *Ictalurus punctatus* (Somridhivej *et al*., 2008), *Clarias batrachus* (Mohindra *et al*., 2012), *Tachysurus fulvidraco* (=*Pelteobagrus fulvidraco* in Zhang *et al*., 2014), *Brycon orbignyanus* (Yazbeck *et al*., 2018) and *Schizothorax biddulphi* (Luo *et al*., 2012), which exhibit 3-mer as the second most frequent motif.

The high frequency of the AC repeat motif is concordant with that described for all the Chordata phylum species, especially for the species of the Actinopterygii class (Meglécz *et al*., 2012). Similarly, the low frequency of the CG repeat motif found in this work is consistent with that described for most eukaryotic species (Meglécz *et al*., 2012). The most common repeat motifs found in this work, AC and ATT, have been described in bony fishes such as *Rhamdia* sp. (Rodrigues *et al*., 2015), *I. punctatus* (Somridhivej *et al*., 2008), *C. batrachus* (Mohindra *et al*., 2012), *T. fulvidraco* (Zhang *et al*., 2014), *M. obtusidens* (Villanova *et al*., 2015), *S. biddulphi* (Luo *et al*., 2012), and *Etheostoma okaloosae* (Saarinen, Austin, 2010). However, the frequency of the ATT motif differs from that found in *Piaractus brachypomus* (AGC, Jorge *et al*., 2018) and *Takifugu rubripes* (AGG; Edwards *et al*., 1998). The most frequent 4-mer motifs repeat found for the three studied catfish species (AAAT, ATCT, TCTG, AGTG, and AATG) are also among the most frequent for other catfishes, such as *I. punctatus* (Somridhivej *et al*., 2008), *C. batrachus* (Mohindra *et al*., 2012) and *T. fulvidraco* (Zhang *et al*., 2014), as well as other Neotropical fishes such as *P. brachypomus* (Jorge *et al*., 2018) and *M. obtusidens* (Villanova *et al*., 2015).

All the microsatellite loci developed in this work (except Scus11) have PIC values that allow them to be considered highly informative according to the classification proposed by Botstein *et al.* (1980), and also are higher than those described for *Brachyplatystoma rousseauxii* (PIC: 0.207–0.910; Batista *et al*., 2010), *Microglanis cottoides* (PIC: 0.115–0.927, Souza-Shibatta *et al*., 2013), *Steindachneridion parahybae* (PIC: 0.429; Fonseca *et al*., 2016), *Hypostomus ancistroides* (PIC: 0.089–0.880; Galindo *et al*., 2015), and *Pterygoplichthys pardalis* (PIC: 0.294–0.880; Pereira *et al*., 2012). Moreover, the loci designed for *A. pardalis* and *S. cuspicaudus* showed evidence of linkage equilibrium and most of their allelic frequencies are in Hardy-Weinberg equilibrium, which make them highly informative for determining the diversity and structure of populations of these species. In contrast, most of the loci of *P. grosskopfii* showed allelic frequencies deviated from the Hardy-Weinberg equilibrium and two pairs of loci showed signals of linkage disequilibrium. It remains to be explored if these characteristics are technical

problems or a consequence of the high levels of exploitation of *P. grosskopfii*. The linkage disequilibrium has been described in some pairs of loci for the exploited species *B. rousseauxii* (Batista *et al*., 2010), *S. parahybae* (Fonseca *et al*., 2016), and *Pimelodus maculatus* (Paiva, Kalapothakis, 2008).

Despite the high frequency of the 2-mer motifs, the microsatellite loci selected in this work were preferentially perfect repeats of 3-mer, 4-mer, and 5-mer motifs; because these type of motifs (uninterrupted and longer monomer) are most recommended in practice, due to their ease in genotyping and classifying the alleles (Gusmão *et al*., 2006; Castoe *et al*., 2010, 2012; Guichoux *et al*., 2011). Contrary to the expectation for longer repeat motifs, the average number of alleles/locus found for *A. pardalis*, *P. grosskopfii*, and *S. cuspicaudus* is higher than those 2-mer microsatellite loci found in other Neotropical catfishes (Revaldaves *et al*., 2005; Moeser, Bermingham, 2005; Paiva, Kalapothakis, 2008; Rodrigues *et al*., 2009, 2015; Carrillo-Ávila *et al*., 2009; Zamudio *et al*., 2009; Batista *et al*., 2010; Telles *et al*., 2010; Agostini *et al*., 2011; Carvalho, Beheregaray, 2011; Saulo-Machado *et al*., 2011; Muñoz-Rojas *et al*., 2012; Pereira *et al*., 2012; Souza *et al*., 2012; Souza-Shibatta *et al*., 2013; Prado *et al*., 2014; Galindo *et al*., 2015; Ojeda *et al*., 2016).

Levels of observed and expected heterozygosity for *A. pardalis* and *S. cuspicaudus* are higher than those reported in microsatellite loci developed for other Neotropical catfishes (Moeser, Bermingham, 2005; Revaldaves *et al*., 2005; Paiva and Kalapothakis, 2008; Rodrigues *et al*., 2009, 2015; Carrillo-Ávila *et al*., 2009; Zamudio *et al*., 2009; Batista *et al*., 2010; Telles *et al*., 2010; Agostini *et al*., 2011; Carvalho, Beheregaray, 2011; Saulo-Machado *et al*., 2011; Souza *et al*., 2012; Muñoz-Rojas *et al*., 2012; Pereira *et al*., 2012; Souza-Shibatta *et al*., 2013; Prado *et al*., 2014; Ojeda *et al*., 2016). Additionally, the microsatellite loci identified in this work for *P. grosskopfii* showed average values of alleles/locus and expected heterozygosity greater than those previously designed for this species by Hernandez-Escobar *et al.* (2011). Thus, we recommend the loci developed in this work for future population genetic studies and monitoring of populations and stocks of *A. pardalis*, *P. grosskopfii*, and *S. cuspicaudus* required in different conservation measures for these species.

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AUTHOR CONTRIBUTIONS

Natalia Restrepo-Escobar: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing (original draft), Writing (review & editing).
Edna J. Márquez: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing (original draft), Writing (review & editing).

Neotropical Ichthyology

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COMPETING INTERESTS

Not applicable.

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