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SHORT COMMUNICATION

Hybrids between *Pseudoplatystoma corruscans* and *P. reticulatum* (Siluriformes: Pimelodidae) previously reported in the Upper Paraná River are likely escapes from aquaculture farms: evidence from microsatellite markers

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ABSTRACT. The production of hybrids of the 'pintado', *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829) and 'cachara', *Pseudoplatystoma reticulatum* (Eigenmann & Eigenmann, 1889) in captivity has generated many concerns about the possibility of introduction of farmed hybrids into natural environments. In the last decade, hybrids between these species, known as 'pintachara' or 'cachapinta', were reported from different regions of the Upper Paraná River basin. Prospection of these hybrids is important in order to orient conservation programs for the species involved. Knowledge of the presence of these hybrids will direct conservation strategies towards prevention and/or mitigation of the effects of cross breeding in natural populations of *P. corruscans* (the native species of the genus) and farmed hybrids. In this study, surveyed the larval population using molecular tools to detect the presence and assess the origin (natural hybridization or escapes from fish farms) of hybrids in natural water bodies. Nine microsatellite markers were used to detect signals of hybridization and introgression of *P. reticulatum* in larvae and adults of *P. corruscans* in Upper Paraná River basin, between Itaipu Dam and Porto Primavera Dam. The specimens were sampled in the Upper Paraná channel and in tributaries where hybrids had been detected in the past, during two reproductive seasons. Despite of that, no sign of hybridization and introgression was found in the 171 larvae and 75 adults sampled, suggesting that the specimens detected in previous studies had originated from escapes of aquaculture farms.

KEY WORDS. Conservation genetics, fish larvae, interspecific hybrids, introgression, molecular markers.

Hybridization and introgression between different species is one of the greatest challenges faced by global biodiversity (Rhymer & Simberloff 1996, Allendorf et al. 2001, Olden et al. 2004). Hybrids are becoming more common in natural environments as a result of environmental degradation, introduction of foreign species, breeding of artificial interspecific hybrids for aquaculture, and shifts in the distribution of species associated with climate changes (Rhymer & Symberloff 1996, Allendorf et al. 2001, Scribner et al. 2001, Walther et al. 2009). Hybridization can homogenize distinct populations and species, and cause a reduction in local adaptations and genetic diversity (Olden et al. 2004).

The 'pintado', *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829), is the single native species of *Pseudoplatystoma* from the Upper Paraná River basin (Buitrago-Suárez & Burr 2007). This species is the largest catfish and one of the most widely commercialized fish in this region (Petrere-Jr et al. 2002). 'Pintado' populations have suffered negative effects from dams, fishery activities, and habitat loss (Welcomme 1985). In order to meet market demands in the face of decreasing populations, farming of the 'pintado' and its hybrid with the 'cachara', *Pseudoplatystoma reticulatum* (Eigenmann & Eigenmann, 1889), has increased in the region, (Carvalho et al. 2013). With that, concerns about the possibility of escapes from aquaculture farms have also increased, since farmed hybrids 'pintachara' (crossbreed between female 'pintado' and male 'cachara') and 'cachapinta' (crossbreed between female 'cachara' and male 'pintado') can potentially hybridize with natural populations of *P. corruscans* (Fernandes et al. 2003, Porto-Foresti et al. 2008, Prado et al. 2012a, Hashimoto et al. 2012). Even though

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the 'cachara' is native to the Paraguay River and Lower Paraná River basin (Buitrago-Suárez & Burr 2007), adult specimens have been reported in the Upper Paraná River basin, introduced by aquaculture (Vaini et al. 2014). Natural hybridization between the 'cachara' and *P. corruscans* is possible, since hybrids are fertile (Prado et al. 2012b), but highly undesirable for the maintenance of the genetic integrity of the natural local populations of *P. corruscans* (Rhymer & Simberloff 1996, Allendorf et al. 2001).

Hybrids between *P. corruscans* and *P. reticulatum* are often reported from the Aquidauana River (Paraguay River basin), the Mogi-Guaçu River and the Ivinheima River sub-basin (Upper Paraná River basin) (Prado et al. 2012a, Vaini et al. 2014). Presently, they have been reported from the Upper Paraná River basin only sporadically between the Itaipu Dam and Porto Primavera Dam (E.A. Rosa, pers. comm., A.A. Silva, pers. comm.). Genetic markers are important tools to detect the presence of hybrids (Sanz et al. 2009), and microsatellite markers have been used to detect hybrids between *P. corruscans* and *P. reticulatum* in the recent past (e.g., Prado et al. 2012a, Carvalho et al. 2013).

Thus, in this study, we apply microsatellite markers to detect evidence of hybridization in larvae and adults of *Pseudoplatystoma* spp. in the Upper Paraná River basin, between Itaipu Dam and Porto Primavera Dam. We consider that the inclusion of larvae in prospection of hybrids in the natural environment is more informative than studies exclusively with adults, especially in migratory species. Larvae allows us to assess both the presence and the origin of hybrids. Because larvae of migratory fishes are passively carried in the water flow (Nakatani et al. 2001), using larvae to detect hybrid individuals may help determining specific tributaries and areas were escapes from fish farms and/or reproductive areas of hybridization are located within a watershed. This information can contribute significantly with the development of conservation programs for specific rivers, maximizing their efficiency and minimizing costs.

Ichthyoplankton were collected from February 2012 to October 2015 (the reproductive season of *Pseudoplatystoma* spp. occurs between October and March, Godinho et al. 2007) in the Upper Paraná River basin (between Itaipu and Porto Primavera Dam) and its main tributaries (Ivaí, Ivinheima, Ivinheiminha, Iguatemi, Piquiri and Amambai rivers) (Appendix 1) using a conical-cylindrical plankton net (500 µm mesh) in the water surface for a period of 10 minutes, at night. Samples were fixed in 70% ethanol. The larvae were identified preliminarily by morphological methods using Nakatani et al. (2001) and molecular methods using the DNA barcode method (Hebert et al. 2003, see also Carvalho et al. 2012). A database of pure species was created with 75 adults of *P. corruscans* collected at the same sites than the larvae (Ivaí, Ivinheima, Iguatemi, Piquiri and Amambai rivers) and 20 individuals of *P. reticulatum* were obtained from the Aquidauana River (Paraguay River basin) (Appendix 1). Specimens of *P. corruscans* and *P. reticulatum* from the Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia) at the Universidade Estadual de Maringá, Maringá, Brazil (catalog number NUP 12798 and NUP 3587) were used as reference adult specimens. The larvae were photographed and their heads were deposited in the Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia) (accession numbers NUP17754 to NUP17757); the remaining larvae were used in genetic analysis.

Total DNA of the larvae were isolated with the DNeasy kit® (Qiagen), and the DNA of adult specimens were extracted with the EZ DNA® kit (Biological Industries). The amplification reaction of the mitochondrial gene cytochrome oxidase, subunit 1 (COI), was carried out in 25 µl PCR with 25x buffer, 3 mM of $MgCl_2$, 0.4 uM of dNTP, 1 pmol of each primer (FF2d and FR1d, Ivanova et al. 2007) and 20-40 ng of DNA. The PCR program included an initial denaturation at 94°C for 4 min, 35 cycles at 94°C for 4 min for denaturation, annealing at 58°C for 45 s, extension at 72°C for 1 min, and a cycle at 72°C for 5 min for final extension. Sequencing with the BigDye® kit (Applied Biosystems) followed the manufacturer's protocol. The sequencing products were purified with Sephadex G-50 (GE) and sequenced with an ABi 3130 automatic sequencer (Applied Biosystems). Barcode identification of each larva was performed in BoldSystems v3 (Ratnasingham & Hebert 2007).

Larvae and the adults were genotyped for nine microsatellite loci (Pcor01, Pcor02, Pcor05, Pcor07, Pcor08, Pcor10, Pcor21, Pcor23, Pcor28) described by Revaldaves et al. (2005) and Pereira et al. (2009) to test for hybridization between the two species of *Pseudoplatystoma*. The 10 µl reactions contained 10x buffer, 1.5 mM of $MgCl_2$, 0.2 mM of dNTP, 0.05 pmol of each primer, 0.3 U of Taq and 5 ng of DNA. The PCR conditions included an initial denaturation at 95°C for 3 min, 35 cycles at 95°C for 30 s for denaturation, 55°C (50°C for Pcor10) for 1 min to annealing and 72°C for 1 min for extension, and a cycle at 72°C for 1 h for final extension. Genotyping was carried out in an ABi 3130 sequencer (Applied Biosystems).

We analyzed the presence of null alleles and scoring errors using the software Micro-Checker 2.2.3 (van Oosterhout et al. 2004). Hardy-Weinberg disequilibrium, linkage disequilibrium, diversity, and genetic differentiation analysis were done using the software Arlequin 3.5 (Excofier & Lischer 2010). Whenever necessary (multiple analyses), critical p-values were corrected using the B-Y correction (Narum 2006). Assignment tests were used to assign larvae to their respective pure species or hybrid cluster. Five runs of 5 million of generations (500 thousand of burn-in) and $1 < K < 5$ were performed in the software Structure 2.3.1 (Pritchard et al. 2000). The ad hoc method of Evanno et al. (2005), implemented on the online tool Structure Harvester (Earl & vonHoldt 2012), was used to assess the most likelihood value of K. Individuals with $0.1 < q < 0.9$ were considered hybrids (Vähä & Primmer 2006). A run of 5 million of generations (500 thousand of burn-in) in the software NewHybrids 1.1 (Anderson & Thompson 2002) was used to estimate the posterior probability of individuals belonging to the categories pure *P. corruscans*, pure *P. reticulatum*, hybrids F1 and F2, and both backcrosses (F1 with each pure population).

A total of 171 larvae of *Pseudoplatystoma* species, all identified as *P. corruscans* by morphological and DNA barcode methods (GenBank accession numbers KU220028-KU220190), were collected and genotyped for the 9 loci of microsatellites (Table 1). All larvae were in the pre-flexion or flexion stage.

The presence of null alleles, scoring errors, linkage, and Hardy-Weinberg disequilibrium were not recurrent between populations (larvae and adults of *P. corruscans* and *P. reticulatum*) and among loci. Adults of *P. corruscans* and *P. reticulatum* presented different values of genetic diversity (0.61 and 0.49, respectively – Table 2) and numbers of alleles (mean of 9.8 and 6.6, respectively). Adults of *P. corruscans* presented 88 alleles of which 69 are private. *Pseudoplatystoma reticulatum* presented 54 alleles, of which 35 are private. The 104 private alleles of these species compose 84.6% of the 123 alleles found. Although all loci presented private alleles, only the loci Pcor07, Pcor10, Pcor21 and Pcor23 did not present overlap in allele range (Table 2).

Tests of genetic differentiation support disjunction between the adults of species according to AMOVA, Fst, and Rst analyses. A total of 74% and 38% of the total genetic variation is due to differences between the species according with Rst (Rst = 0.74, p = 0.00) and Fst methods (Fst = 0.38, p = 0.00), respectively. The locus-by-locus AMOVA supports this distinction: significant Fst values varied between 0.11 and 0.74, and Rst values between 0.48 and 0.99, except for the Rst analysis with the Pcor02 locus (Rst = 0.00, p = 1.00).

The assignment analysis supports disjunction of these species (Fig. 1). The analysis in Structure using the most probable number of groups is 2 (K = 2), according to the method of Evanno et al. (2005), indicates that 99.7% of the genotype of adults of *P. corruscans* and 99.7% of the genotype of the *P. reticulatum*

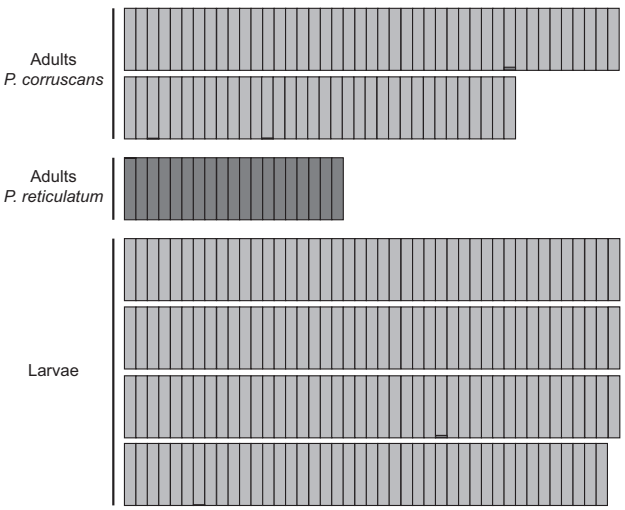

Figure 1. Genetic assigment of adults of *Pseudoplatystoma corruscans* and *P. reticulatum* and larvae sampled in the Upper Paraná River basin. Each column represent an individual and each color represent a species.

Table 1. Number total (N) and per reproductive period (October to March – N_1: 2012-2013, N_2: 2013-2014, N_3: 2014-2015, N_4:2015-2016) of larvae of *Pseudoplatystoma* spp. sampled, gene diversity (H), inbreeding coefficient (Fis), number of alleles of each 9 microsatellites loci. Bold numbers of Fis represent significant values ($p < 0.05$) and bold number of the number of alleles represent deviation of Hardy-Weinberg equilibrium ($p < 0.0177$ after B-Y correction).

Population	N_t	N_1	N_2	N_3	N_4	N_g	H	Fis	Pcor01	Pcor02	Pcor05	Pcor07	Pcor08	Pcor10	Pcor21	Pcor23	Pcor28	Mean
Ivinheima	47	24	23			47	0.60	0.03	5	9	10	3	**3**	17	14	13	**5**	8.8±5.1
Amambai	62		53		9	62	0.61	-0.09	**6**	**9**	**9**	2	4	16	14	15	4	8.8±5.2
Ivaí	57		9	48		57	0.58	0.01	8	8	9	2	5	17	15	15	5	9.4±5.4
Paraná	3		3			3	0.69	0.05	2	4	2	2	1	5	3	4	2	3.0±1.2
Ivinheiminha	2		2			2	0.61	-0.14	3	4	4	1	1	4	3	3	1	3.5±0.6
Overall	171	24	90	48	9	171	0.61	-0.01	**8**	12	12	3	6	25	18	19	5	12.0±7.4

Table 2. Number of adults of *P. corruscans* and *P. reticulatum* sampled (N), gene diversity (H), inbreeding coefficient (Fis), number of alleles (N_a), range of alleles (R_a) and number of private alleles (P_a) for each of nine microsatellites loci. Bold number of Fis represent significant values and bold number of N_a represent deviation of Hardy-Weinberg equilibrium.

	N	H	Fis	Pcor01			Pcor02			Pcor05			Pcor07		
				N_a	R_a	P_a	N_a	R_a	P_a	N_a	R_a	P_a	N_a	R_a	P_a
P. corruscans	75	0.61	0.02	6	86-110	4	11	191-215	4	11	138-176	4	2	208-210	2
P. reticulatum	20	0.48	0.04	6	104-120	4	11	185-217	4	11	136-158	4	5	246-260	5

	Pcor08			Pcor10			Pcor21			Pcor23			Pcor28		
	N_a	R_a	P_a	N_a	R_a	P_a	N_a	R_a	P_a	N_a	R_a	P_a	N_a	R_a	P_a
P. corruscans	5	163-177	3	**19**	174-266	19	15	114-162	15	15	99-142	15	4	95-105	3
P. reticulatum	11	167-195	9	3	146-168	3	3	103-107	3	3	89-93	3	1	99	0

individuals belonged to their own independent clusters. All individuals presented more than 95% of its genotype relative to its species cluster. Likewise, all adults of each species belong to its pure species with probabilities higher than 99.7%, as suggested by the NewHybrid analysis.

The number of alleles of larvae was 12.0 and genetic diversity was 0.61, with no evidence of inbreeding (Table 1). The assignment test supported that 99.8% of the genotype of the sampled larvae belongs to *P. corruscans*; 98.2% of the total of larvae presented more than 99% of its genotype assigned as *P. corruscans*; and no larvae had more than 4% of its genotype associated to *P. reticulatum* (Fig. 1). The assignment test with NewHybrids supports these results and all larvae presented more than 99.7% of probability of belonging to *P. corruscans*.

Among the 75 adults of *P. corruscans* and the 171 larvae sampled in the Upper Paraná River basin, no individual presented mitochondrial DNA compatible with *P. reticulatum* nor evidence of hybridization and introgression in the nuclear DNA with this species.

Natural populations of *P. corruscans* and *P. reticulatum* occur in sympatry in some river basins (e.g., Paraguay River, Lower Paraná River, Uruguay River) but they present low level of natural hybridization (Carvalho et al. 2013). This may be a consequence of differences in growth and body size at sexual maturity (Resende et al. 1996, Godinho 2007), as well as fidelity to the reproductive area, as proposed for *P. corruscans* by Pereira et al. (2009) (Prado et al. 2012a). Alternatively, elevated proportions of hybrids were reported in regions with high density of fish farms that produce these hybrids, such as the Mogi Guaçu River (50% – Paraná River basin), the Ivinheima River (61% – Paraná River basin), and the Aquidauana River (30.75% – Paraguay River basin) (Prado et al. 2012a, Vaini et al. 2014). Most hybrids sampled by Vaini et al. (2014) in the Upper Paraná River basin and Paraguay basin correspond to "cachapinta", which is also the most traded hybrid in fish farms (Porto-Foresti et al. 2011, Prado et al. 2012a, Vaini et al. 2014). Furthermore, hybrids have already been collected in low frequency (3.6%) in an upper stretch of the Upper Paraná River, close to Ilha Solteira Dam, between 2003 and 2008 (Prado et al. 2012a). During the period of these studies, A.B. Silva (pers. comm.) reported that hybrids were frequently caught in professional fisheries in the stretch of Upper Paraná River between Itaipu reservoir and Porto Primavera dam.

Alternatively, our results indicate an absence of any sign of hybridization and introgression in the larvae and adults of the native population of *P. corruscans* from the Upper Parana River basin, including in the Ivinheima River population, the sub basin that presented hybrids in the study of Vaini et al. (2014). Supported also by the currently sporadic catch of hybrids in this region (E.A. Rosa, pers. comm., A.B. Silva, pers. comm., Vaini et al. 2014), we suggest that the hybrids captured there are most likely escapes from local fish farms, supporting the hypothesis of Bignotto et al. (2009) and Prado et al. (2012a).

To assess the possibility of future natural hybridization between *P. corruscans* and *P. reticulatum* in the Upper Paraná River basin, we recommend systematic surveys on larvae of *P. corruscans*, using molecular markers as part of a monitoring program. Continuous or sporadic escapes from aquaculture farms represent a risk of introduction of hybrid specimens into natural waters by increasing propagule pressure (see Simberloff 2009 for details). Thus, monitoring is fundamental to preserve the natural populations of the 'pintado'. Monitoring should primarily focus on larvae to control for the origin of hybrids detected in the Upper Paraná River (e.g., tributaries contributing to escapes or areas of hybridization). Specific control campaigns may be directed to fish farms located in those tributaries. This approach is promising and can be used to prospect hybrids of other species along Neotropical basins.

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LITERATURE CITED

Allendorf F, Leary R, Spruell P, Wenburg J (2001) The problems with hybrids: setting conservation guidelines. **Trends in Ecology and Evolution 16**: 613-622. doi: 10.1016/S0169-5347(01)02290-X

Anderson E, Thompson EA (2002) A model-based method for identifying species hybrids using multilocus genetic data. **Genetics 160**: 1217-1229.

Bignotto TS, Prioli AJ, Prioli SMAP, Maniglia TC, Boni TA, Lucio LC, Gomes VN, Prioli RA, Oliveira AV, Julio-Junior HF, Prioli LM (2009) Genetic divergence between *Pseudoplatystoma corruscans* and *Pseudoplatystoma reticulatum* (Siluriformes: Pimelodidae) in the Paraná River Basin. **Brazilian Journal of Biology 69**: 681-689. doi: 10.1590/S1519-69842009000300022

Buitrago-Suárez UA, Burr BM (2007) Taxonomy of the catfish genus *Pseudoplatystoma* Blecker (Siluriformes: Pimelodidae) with recognition of eight species. **Zootaxa 1512**: 1-38.

Carvalho DC, Oliveira DAA, Beheregaray LB, Torres RA (2012) Hidden genetic diversity and distinct evolutionary significant units in an commercially important Neotropical apex predator, the catfish *Pseudoplatystoma corruscans*. **Conservation Genetics 13**: 1671-1675. doi: 10.1007/s10592-012-0402-6

Carvalho DC, Seerig AS, Brasil BSAF, Crepaldi DV, Oliveria DAA (2013) Molecular identification of the hybrid between the catfish species *Pseudoplatystoma corruscans* and *Pseudoplatystoma reticulatum* using a set of eight microsatellite markers. **Journal of Fish Biology 83**: 671-676. doi: 10.1111/jfb.12194

Earl DA, vonHoldt BM (2012) Structure Harvester: a website and program for visualizing Structure output and implementing the Evanno method. **Conservation Genetics Resources 4**: 359-361. doi: 10.1007/s12686-011-9548-7

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software Structure: a simulation study. **Molecular Ecology 14**: 2611-2620. doi: 10.1007/s12686-011-9548-7

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. **Molecular Ecology Resources 10**: 564-567. doi: 10.1111/j.1755-0998.2010.02847.x

Fernandes R, Gomes LC, Agostinho AA (2003) Pesque-pague: negócio ou fonte de dispersão de espécies exóticas? **Acta Scientiarum: Biological Sciences 25**: 115-120. doi: 10.4025/actascibiolsci.v25i1.2089

Godinho HP (2007) Estratégias reprodutivas de peixes aplicadas à aqüicultura: bases para o desenvolvimento de tecnologias de produção. **Revista Brasileira de Reprodução Animal 31**: 351-360.

Godinho AL, Kynard B, Godinho HP (2007) Migration and spawning of female surubim (*Pseudoplatystoma corruscans*, Pimelodidae) in the São Francisco river, Brazil. **Environmental Biology of Fishes 80**: 421-433. doi: 10.1007/s10641-006-9141-1

Hashimoto DT, Prado FD, Senhorini JA, Foresti F, Porto-Foresti F (2012) Detection of post-F1 fish hybrids in broodstock using molecular markers: approaches for genetic management in aquaculture. **Aquaculture Research 44**: 1-9. doi: 10.1111/j.1365-2109.2012.03092.x

Hebert PDN, Cywinska A, Ball SL, Dewaard JR (2003) Biological identifications through DNA barcodes. **Proceeding of the Royal Society of London 270**: 313-321. doi: 10.1098/rspb.2002.2218

Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. **Molecular Ecology Notes 7**: 544-548. doi: 10.1111/j.1471-8286.2007.01748.x

Nakatani K, Agostinho AA, Baumgartner G, Bialetzki A, Sanches PV, Makrakis MC, Pavanelli CS (2001) **Ovos e larvas de peixes de água doce: desenvolvimento e manual de identificação.** Maringá, EDUEM, XVIII+378p.

Narum SR (2006) Beyond Bonferroni: Less conservative analyses for conservation genetics. **Conservation Genetics 7**: 783-787. doi: 10.1007/s10592-005-9056-y

Olden JD, Poff NL, Douglas MR, Douglas ME, Fausch KD (2004) Ecological and evolutionary consequences of biotic homogenization. **Trends in Ecology and Evolution 19**: 18-24. doi: 10.1016/j.tree.2003.09.010

Pereira LHG, Foresti F, Oliveira C (2009) Genetic structure of the migratory catfish *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae) suggests homing behavior. **Ecology of Freshwater Fish 18**: 215-225. doi: 10.1111/j.1600-0633.2008.00338.x

Petrere Jr M, Agostinho AA, Okada EK, Julio Jr HF (2002) Review of the fisheries in the Brazilian portion of the Paraná/Pantanal basin, p. 123-143. In: Cowx IG (Ed.) **Management and Ecology of Lake and Reservoir Fisheries.** Oxford, Fishing New Books, XI+414p.

Porto-Foresti F, Hashimoto DT, Alves AL, Almeida RBC, Senhorini JA, Bortolozzi J, Foresti F (2008) Cytogenetic markers as diagnoses in the identification of the hybrid between Piauçu (*Leporinus macrocephalus*) and Piapara (*Leporinus elongatus*). **Genetics and Molecular Biology 31**: 195-202.

Porto-Foresti F, Hashimoto DT, Prado FD, Senhorini JA, Foresti F (2011) A hibridação interespecífica em peixes. **Panorama da Aquicultura 126**: 28-33.

Prado FD, Hashimoto DT, Senhorini JA, Foresti F, Porto-Foresti F (2012a) Detection of hybrids and genetic introgression in wild stocks of two catfish species (Siluriformes: Pimelodidae): The impact of hatcheries in Brazil. **Journal of Fisheries Research 125**: 300-305. doi: 10.1016/j.fishres.2012.02.030

Prado FD, Nunes TL, Senhorini JA, Bortolozzi J, Foresti F, Porto-Foresti F (2012b) Cytogenetic characterization of F1, F2 and backcross hybrids of Neotropical catfish species *Pseudoplatystoma corruscans* and *P. reticulatum* (Pimelodidae: Siluriformes). **Genetics and Molecular Biology 35**: 57-64. doi: 10.1590/S1415-47572012005000010

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. **Genetics 155**: 945-959.

Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). **Molecular Ecology Notes 7**: 355-364. doi: 10.1111/j.1471-8286.2006.01678.x

Resende EK, Catella AC, Nascimento FL, Palmeiras SS, Pereira RAC, Lima MS, Almeida VLL (1996) **Biologia do curimbatá (*Prochilodus lineatus*): pintado (*Pseudoplatystoma corruscans*) e cachara (*Pseudoplatystoma fasciatum*) na bacia hidrográfica do rio Miranda: Pantanal do Mato Grosso do Sul.** Corumbá, EMBRAPA – CPAP, Boletim de Pesquisa 2, 75p.

Revadalves E, Pereira LHG, Foresti F, Oliveira C (2005) Isolation and characterization of microsatellites loci in *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidadae) and cross-species amplification. **Molecular Ecology Notes 5**: 462-465. doi: 10.1111/j.1471-8286.2005.00883.x

Rhymer JM, Simberloff DS (1996) Extinction by hybridization and introgression. **Annual Review of Ecology and Systematics 27**: 83-109. doi: 10.1146/annurev.ecolsys.27.1.83

Sanz N, Araguas RM, Fernández R, Manuel V, García-Marín J-L (2009) Efficiency of markers and methods for detecting hybrids and introgression in stocked populations. **Conservation Genetics 10**: 225-236. doi: 10.1007/s10592-008-9550-0

Simberloff DS (2009) The Role of Propagule Pressure in Biological Invasions. **The Annual Review of Ecology, Evolution, and Systematics 40**: 81-102. doi: 10.1146/annurev.ecolsys.110308.120304

Scribner Kt, Page Ks, Bartron ML (2001) Hybridization in freshwater fishes: a review of case studies and cytonuclear methods of biological inference. **Reviews in Fish Biology and Fisheries 10**: 293-323. doi: 10.1023/A:1016642723238

Vähä JP, Primmer C (2006) Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. **Molecular Ecology 15**: 63-72. doi: 10.1111/j.1365-294X.2005.02773.x

Vaini JO, Grisolia AB, Prado FD, Porto-Foresti F (2014) Genetic identification of interspecific hybrid of Neotropical catfish species (*Pseudoplatystoma corruscans vs*. *Pseudoplatystoma reticulatum*) in rivers of Mato Grosso do Sul State: Brazil. **Neotropical Ichthyology 12**: 635-641. doi: 10.1590/1982-0224-20130169

van Oosterhout C, Hutchingson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. **Molecular Ecology Notes 4**: 535-538. doi: 10.1111/j.1471-8286.2004.00684.x

Walther GR, Roques A, Hulme PE, Sykes MT, Pysek P, Kuhn I, Zobel M, Bacher S, Botta-Dukát Z, Bugmann H, Czúcz B, Dauber J, Hickler T, Jarosík V, Kenis M, Klotz S, Minchin D, Moora M, Nentwig W, Ott J, Panov VE, Reineking B, Robinet C, Semenchenko V, Solarz W, Thuiller W, Vilà M, Vohland K, Settele J (2009) Alien species in a warmer world: risks and opportunities. **Trends in Ecology and Evolution 24**: 686-693. doi: 10.1016/j.tree.2009.06.008

Welcomme RL (1985) **River fisheries.** Roma, Food and Agriculture Organization of the United Nations, 330p.

Appendix 1. Sampling points, number of adults *Pseudoplatystoma corruscans* (Pc) and *P. reticulatum* (Pr), and larvae of *Pseudoplatystoma* spp. (LP) sampled.

Sampling point	Pc	Pr	LP	Coordinate
Ivinheima River	26	0	47	22°48′00″S / 53°32′00″W
				22°47′42″S / 53°32′42″ W
				22°47′40″S / 53°32′14″ W
				22°50′60″S / 53°34′30″ W
				22°49′22″S / 53°33′10″ W
				22°55′54″S / 53°39′11″ W
				22°56′46″S / 53°38′33″ W
				23°14′00″S / 53°43′24″ W
Ivinheiminha River	0	0	2	22°59′12″S / 53°38′56″ W
Amambai River	26	0	62	23°20′20″S / 53°51′24″ W
				23°21′28″S / 53°53′04″ W
				23°20′20″S / 53°51′29″ W
				23°15′01″S / 53°38′18″ W
				23°16′20″S / 53°37′58″ W
Ivaí River	5	0	57	23°17′17″S / 53°39′42″ W
				23°18′00″S / 53°41′32″ W
				23°15′05″S / 53°37′58″ W
Iguatemi River	10	0	0	23°55′38″S / 54°11′22″ W
				23°55′37″S / 54°10′45″ W
				23°55′27″S / 54°11′24″ W
				23°55′21″S / 54°11′15″ W
				23°55′38″S / 54°11′22″ W
				23°55′29″S / 54°11′39″ W
Piquiri River	8	0	0	24°01′47″S / 54°02′53″ W
				24°01′52″S / 54°04′33″ W
				24°01′51″S / 54°02′48″ W
Paraná River	0	0	3	23°40′18″S / 54°03′47″ W
				23°26′09″S / 53°58′00″ W
				23°38′51″S / 53°56′44″ W
				22°39′02″S / 53°05′26″ W
				22°45′39″S / 53°19′41″ W
				23°14′18″S / 53°43′04″ W
				22°53′41″S / 53°38′41″ W
				23°21′52″S / 53°52′48″ W
				23°55′28″S / 54°09′17″ W
				23°18′12″S / 53°41′54″ W
				24°01′24″S / 54°05′33″ W
				23°38′51″S / 53°56′44″ W
				24°01′06″S / 54°10′10″ W
				24°00′58″S / 54°10′37″ W
				23°48′50″S / 53°59′53″ W
Aquidauana River	0	20	0	20°28′53″S / 55°47′56″ W

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