Genetics and telemetry indicate unexpected movements among structured populations for *Brachyplatystoma platynemum* in the Amazon

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ABSTRACT

The genetic analysis of *Brachyplatystoma platynemum* individuals sampled from the lower Madeira River reinforces the existence of two structured populations in the Amazon Basin (Madeira and Amazon populations). However, the recapture of an individual from the Amazon population in the Solimões River, which was telemetry-tagged in the Madeira River after the damming, indicates that fish from the Amazon population move between the two river systems. This has not been observed, however, in the Madeira River population, which is currently divided and isolated in the lower and upper Madeira River by the construction of two dams.

KEYWORDS

Amazon Basin, *Brachyplatystoma platynemum*, genetic flow, migratory catfish, telemetry

The catfish *Brachyplatystoma platynemum* Boulenger 1898 undertakes a large-scale migration in the Amazon Basin, as do three other *Brachyplatystoma* Bleeker 1862 species: *Brachyplatystoma rousseauxii* (Castelnau 1855), *Brachyplatystoma juruense* (Boulenger 1898) and *Brachyplatystoma vaillantii* (Valenciennes 1840) (Barthem *et al.*, 2017). The general life-history patterns of these species are similar, with the spawning areas of *B. rousseauxii*, *B. vaillantii* and *B. juruense* in the western Amazon Basin and the nursery areas in the eastern Amazon Basin (Duponchelle *et al.*, 2016; Hermann *et al.*, 2016; Barthem *et al.*,

2017). However, the larval size distribution indicates that the spawning areas of *B. platynemum* are neither exclusively in the far western Amazon Basin nor are their nurseries restricted to or mostly in the eastern Amazon Basin (Barthem *et al.*, 2017). The peculiar life-history patterns of this catfish might be explained by the occurrence of more than one population, with each one presenting their own spawning and nursery areas.

Usually, migratory species exhibit low interpopulation genetic differentiation (Do Prado *et al.*, 2017), as observed for *B. rousseauxii* (Batista, 2010) and *B. vaillantii* (Formiga-Aquino, 2004). Although, genetic structure had been related strongly with water chemistry of different rivers in the Amazon Basin, that would represent a barrier to gene flow among populations inhabiting these rivers, as identified for another migratory catfish *Brachyplatystoma filamentosum* (Lichtenstein 1819) (Huergo, 2009). In contrast with the other large-scale migratory species that migrate in the same turbid waters, *B. platynemum* exhibits two structured populations in the Amazon Basin: one in the Purus, Solimões and Amazonas rivers (Amazon population); and one in the Madeira River (Madeira population), with no evident barrier between them (Ochoa *et al.*, 2015). The limits of these populations are not yet understood and the blockage of migratory routes by the construction of two dams recently completed (2012–2013) in the Madeira River may have affected their distribution, most likely that of the Madeira population. The focus of this study was to analyse the genetic structure of *B. platynemum* downriver of the recent dams constructed in the Madeira River and to understand the individual movement among the two structured populations between the Madeira and Amazonas rivers.

Large-scale fish telemetry (*i.e.*, radio and acoustic) studies are used worldwide to detect individual movements, but since 2011 have they been applied in the Amazon Basin, mostly focused on the evaluation of the effect of dams on catfish migration, such as in Xingu (Hahn *et al.*, 2015) and Madeira rivers. In the Madeira River, *B. rousseauxii*, *B. vaillantii*, *B.*

platynemum and *B. filamentosum* have been tagged with radio or combined acoustic and radio transmitters (CART) and marked with hydrostatic anchor tags (Hallprint, model standard TBA; www.halprint.com) following the procedures described by Hahn *et al.* (2011). Capture and tagging procedures were approved by the Brazilian Institute of the Environment (IBAMA; permit number 83/2012). Fish movements were detected by radio telemetry antennas deployed at the Santo Antônio dam structures (spillways, tailraces and in the fishway) and data are recorded on radio receivers and by acoustic dataloggers in the 100 km long reservoir. A total of 192 *B. platynemum* were tagged and released downstream of the Santo Antônio Dam (8° 46' 09" S, 63° 54' 43.12" W) from May 2012 to June 2017.

One of the individuals, tagged and released in the Madeira River on February 23, 2017 was captured by a fisher in the Solimões River (3° 22'15.51" S, 60° 42' 44.96" W), Manacapuru town (Figure 1) on May 18, 2017, 84 days after release. The total distance traveled was 1272 km (*c.*15 km day^{-1}). The recapture was reported *via* a call to the telephone number printed on the transmitter label (anchor tag was no longer attached to the fish's body). The recapture in the Solimões River of an individual of *B. platynemum* previously captured in the Madeira River was unexpected, based on the hypothesis of two independent structured population in these rivers by Ochoa *et al.* (2015).

In order to relate the tagged fish to the populations defined by Ochoa *et al.* (2015), genetic analysis was performed in the sample collected from adipose fin tissue of individuals captured and tagged in the same season in the Madeira River, downstream of the Santo Antônio dam. Samples were collected during the tagging process. Tissue samples were fixed and preserved in absolute ethanol at –20°C and deposited in the laboratory of ichthyology collection of the Universidade Federal do Rio Grande do Sul (vouchers UFRGS 23801–23820) and then processed in the laboratory of molecular biology of the zoology department of the same university. Genomic DNA was extracted using the cetyltrimethylammonium

bromide (CTAB) method (Boyce *et al.* 1989). In order to compare our results with those from Ochoa *et al.* (2015), the DNA was amplified by PCR (Sanger, 1977) for two genes: control region (*D-loop*) and cytochrome b (*cytb*), using primers from Palumbi *et al.* (1991) and Sivasundar *et al.* (2001). The PCR reactions were carried out (primers listed in the Table 1) in a total volume of 20 µl [13,5 µl of H20, 2 µl of 10× buffer (PlatinumTaq; Invitrogen; www.invitrogen.com), 0.6 µl of MgCl2 (50 mM), 2 µl of deoxynucleotide triphosphate (dNTP) (2 mM), 0.4 µl of each primer (2 µM), 0.2 µl (5 U) of Platinum Taq and 1 µl of genomic DNA]. *D-loop* PCR conditions were: 30s at 94°C for, 10 cycles of 1 min at 94°C, 1 min at 51°C , 1 min at 72°C ; 10 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min; 72°C for 10 min. *cytb* PCR conditions were: 95°C for 5 min, 2 cycles of: 95°C for 30s, 55°C for 45s, 72°C for 1 min; and 2 cycles of 95°C for 30s, 48°C for 45s, 72°C for 1 min; followed by 25 cycles of 95°C for 30s, 50°C for 45s, 72°C for 1 min; and 72°C for 5 min. PCR products were verified in 1% agarose gel and subsequently sent to ACTGene (www.actgene.com) for sequencing. Sequences were aligned using Clustal W in MEGA 6.0 (Tamura *et al.*, 2013) and visually inspected. Haplotypic diversity was estimated in DNAsp 5 (Librado & Rozas, 2009) and haplotype networks were constructed using Network 5.0 software.

The haplotype diversity analysis of 19 specimens collected downstream of Santo Antônio Dam identified 7 specimens belonging to the Amazon population and 12 specimens to the Madeira population. The specimen reported above, tagged in the Madeira River and captured in the Solimões River, belongs to the Amazon population (Table 1; UFRGS23818). We found 10 haplotypes from 17 *cytb* sequences (Table 1; Accession number: XXX) and 16 haplotypes from 16 *D-loop* sequences (Accession number: XXX). Of this total, 6 (*cytb*) and 8 (*D-loop*) haplotypes are new and exclusive from this work and the remaining are shared with Ochoa *et al.* (2015). The haplotype network for *cytb* and *D-loop* analysis is presented in the

Figure 2. Genetically, the 10 *cytb* haplotypes and 16 *D-loop* haplotypes were grouped in two populations as established by Ochoa *et al.* (2015). The haplotypes of the Madeira population were dominant, represented by 7 *cytb* and 11 *D-loop* and the haplotypes of the Amazon population were represented by 3 *cytb* and 5 *D-loop* (Figure 2). Ochoa *et al.* (2015) have discussed possible reasons for the high levels of haplotype diversity and the occurrence of two structured populations with high values for F_{ST} in *B. platynemum*, including climate changes, drainage isolation and differences in water chemistry. Dam construction, with the consequent blockage of migratory routes, may change the natural life cycle of fish species inhabiting the affected area, as well as the usual distribution of populations (Hegg *et al.*, 2015; Forsberg *et al.*, 2017). Our analyses indicate a dominance of *B. platynemum* specimens of Madeira population downriver of the recent dam constructed in the Madeira River and a movement of the Amazon population specimens to Madeira River. Ochoa *et al.* (2015) suggest that there is a minimal gene flow (given the sampling rarity) between populations of the Madeira and Amazon rivers and a preferential mating among the individuals of the Madeira population. It is not clear yet what is the main factor that limits the movement of the Amazon population in the Madeira River and vice-versa. The distance of the Madeira population spawning area from the Amazon River confluence (Barthem *et al.*, 2017) may be an important factor that reduce the interbreeding and reinforce the isolation of those two populations.

Ochoa *et al.* (2015) found that Teotônio Rapids (TRM; natural barrier) does not represent a physic barrier in gene flow for this species. Dams in the Madeira River, however, can impede *B. platynemum* specimens of the lower Madeira River migrating upriver and reaching the spawning area, which probably should strengthen the isolation of the Madeira population upstream of the dam from Amazon populations. However, larvae of *B. platynemum* has been sampled between the two dams in the Madeira River and downstream

of the lower dam, demonstrating that the species has somehow managed to reproduce upriver (Vasconcelos, 2017). Individuals of the Amazon population will return to the Amazon River after they reach the blocked path of the Madeira River, as was observed with the tagged specimen. However, the fate of the specimens of the Madeira population downriver of the dam is uncertain and it is possible that they form schools of non-breeding individuals that will never reach spawning areas if they cannot migrate in the Amazonas River, resulting in the suppression of the Madeira population of *B. platynemum* downriver of the dams. Such hypotheses can only be tested with the continuous analysis of the migration patterns and population structure of *B. platynemum* with telemetry and genetic analyses. The lower stretch of the Madeira River offers the rare opportunity to understand the complex processes that maintain two wild populations isolated (Madeira and Amazon populations) and to monitor the effect of damming the river on the genetic structure of two fish populations.

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References

Barthem, R. B., Goulding, M., Leite, R. G., Canas, C., Forsberg, B., Venticinque, E., Petry, P., Ribeiro, M. L., Chuctaya, J. & Mercado, A. (2017). Goliath catfish spawning in the far western Amazon confirmed by the distribution of mature adults, drifting larvae and migrating juveniles. *Sci Rep* **7**, 41784. doi: 10.1038/srep41784

Batista, J. S. (2010). Caracterização genética da dourada - *Brachyplatystoma rousseauxii*, Castelnau, 1855 (Siluriformes: Pimelodidae) na Amazônia por meio de marcadores moleculares mitocondriais e microssatélites: subsídios para conservação e manejo.

Ph.D. Thesis. Instituto Nacional de Pesquisas da Amazônia (INPA)/Universidade do Amazonas, Manaus, Brazil. http://bdtd.inpa.gov.br/handle/tede/721

Boyce, T.M., Zwick, M.E., Aquadro, C.F. (1989). Mitochondrial DNA in the bark weevils: size, structure and heteroplasmy. *Genetics* **123,** 825-836.

Do Prado, F. D., Fernandez-Cebrián, R., Hashimoto, D. T., Senhorini, J. A., Foresti, F., Martínez, P., & Porto-Foresti, F. (2017). Hybridization and genetic introgression patterns between two South American catfish along their sympatric distribution range. *Hydrobiologia* **788**, 319-343. doi: 10.1007/s10750-016-3010-5

Duponchelle, F., Pouilly, M., Pécheyran, C., Hauser, M., Renno, J. F., Panfili, J., Darnaude, A. M, García-Vasquez, A., Carvajal-Vallejos, F., García-Dávila, C., Doria, C., Bérail, S., Donard, A., Sondag, F., Santos, R. V., Nuñez, J., Point, D., Labonne, M., Baras, E. & Heino, J.. (2016). Trans-Amazonian natal homing in giant catfish. *Journal of Applied Ecology* **53**, 1511-1520. doi.org/10.1111/1365-2664.12665

Formiga-Aquino, K. (2004). Variação genética da piramutaba - *Brachyplatystoma vaillantii* (Valenciennes, 1840) (Siluriformes:Pimelodidae) no sistema Estuário-Amazonas-Solimões. Ms Thesis. Instituto Nacional de Pesquisas da Amazônia (INPA)/Universidade do Amazonas (UA), Manaus, Brazil.

Hahn, L., Agostinho, A.A., English, K.K., Carosfeld, J., Câmara, L. F. & Cooke, S.J. (2011). Use of radiotelemetry to track threatened dorados Salminus brasiliensis in the upper Uruguay River, Brazil. *Endangered Species Research* **15**(2), 103-114. doi: 10.3354/esr00363

Hahn, L., Nunes, L.D., Câmara, L.F., Machado, L.S., Abolis, D., Fries, L.C.C., Martins, E.G. & Garrone-Neto, D. (2015). Reporting on the recapture of electronic tagged fish by fishers in the Xingu River., Brazilian Amazon. *Braz. J. Biol* **15**, 19-101. doi: 10.1590/1519-6984.02214BM

Hermann, T. W., Stewart, D. J., Limburg, K. E. & Castello, L. (2016). Unravelling the life history of Amazonian fishes through otolith microchemistry. *Royal Society Open Science* **3**. doi.org/10.1098/rsos.160206

Hegg, J.C., Giarrizzo, T., Kennedy, B.P. (2015). Diverse early life-history strategies in migratory Amazonian catfish: implications for conservation and management. *PLoS ONE* **10**. doi:10.1371/journal.pone.0129697

Huergo, G.M. (2009). Estimativa da diversidade genética da piraíba (*Brachyplatystoma filamentosum* Lichtenstein, 1919) e da piraíba negra (*Brachyplatystoma capapretum* Lundberg & Akama, 2005), na Amazônia brasileira, inferidas através de DNA mitocondrial: subsídio para manejo e conservação. Tese de Doutorado, BADPI, INPA, Manaus, 114 pp. http://bdtd.inpa.gov.br/handle/tede/1484

Forsberg, B.R., Melack, J.M., Dunne, T., Barthem, R.B., Goulding, M., Paiva, R.C.D., Sorribas, M.V., Silva Jr., U.L. & Weisser, S. (2017). The potential impact of new Andean dams on Amazon fluvial ecosystems. *PLoS ONE* **12**, doi: 10.1371/journal.pone.0182254.

Librado, P. & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**(11). 1451-1452.

Ochoa, L.E., Pereira, L.H.G., Costa-Silva, G.J., Roxo, F.F., Batista, J.S., Formiga, K., Foresti, F. & Oliveira, C. (2015). Genetic structure and historical diversification of catfish *Brachyplatystoma platynemum* (Siluriformes: Pimelodidae) in the Amazon basin with implications for its conservation. *Ecology and evolution* **5**(10), 2005-2020. doi: 10.1002/ece3.1486

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G. (1991). *The Simple Fool's Guide to PCR*. Honolulu: University of Hawaii Press.

Sanger, F., Nicklen, S. & Coulson, A.R. (1977). DNA Sequencing with chain terminating inhibitors. *Proceedings of National Academy of Science* **74**, 5463-5467.

Sivasundar, A., Bermingham, E. & Ortí, G. (2001). Population structure and biogeography of migratory freshwater fishes (Prochilodus: Characiformes) in major South American rivers. *Molecular Ecology* **10**, 407-417.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725-2729.

Vasconcelos, L.P. (2017). Hydropower projects in the Amazon and migratory fish: dams and reservoirs as potential impacts to the reproductive success of these populations. Ph.D. Thesis, Universidade Estadual de Maringá, Maringá, Brazil. https://www.oceandocs.org/handle/1834/10515.

TABLE 1 List of specimens studied with catalog numbers, haplotypes, populations and accession numbers.

Catalogue number	Tag number	cytb Haplotypes	*D–loop* Haplotypes	Population
UFRGS23801	NT2396	H20	H67 = H59	Amazonas
UFRGS23819	NT2397	H22	H68	Madeira
UFRGS23820	NT2398	H20	–	Amazonas
UFRGS23802	NT2399	H6	H69	Madeira
UFRGS23803	NT2400	H23	H57	Madeira
UFRGS23816	NT2451	H7	H70 = H66	Madeira
UFRGS23804	NT2452	H20	–	Amazonas
UFRGS23805	NT2453	–	H71 = H58	Madeira
UFRGS23813	NT2454	H6	–	Madeira
UFRGS23814	NT2455	H20	H72	Amazonas
UFRGS23815	NT2456	H7	H64	Madeira
UFRGS23806	NT2457	H21	H73	Madeira
UFRGS23807	NT2458	H24	H74	Madeira
UFRGS23808	NT2459	H25	H49	Madeira
UFRGS23809	NT2460	H20	H75 = H30	Amazonas
UFRGS23811	NT2462	H6	H76	Madeira
UFRGS23817	NT2463	H26	H77	Amazonas
UFRGS23812	NT2464	–	H78	Madeira
UFRGS23818*	NT2465	H27	H79	Amazonas

* Tagged and recaptured specimen.

FIGURE 1 Movement of a tagged *Brachyplatystoma platynemum* in the Amazon Basin: (1) release site downstream of the Santo Antônio Dam on the Madeira River; (2) the recapture site on the Solimões River.

FIGURE 2 (**a**) Haplotype network of *cytb* and (**b**) *D-loop* for specimens of *Brachyplatystoma platynemum* from this study and Ochoa *et al*. (2015). Circle size is proportional to the number of specimens with a same haplotype. Two populations can be identified among the specimens collected at the Santo Antônio Dam on the Madeira River: one related to haplotypes of the Madeira River and another, smaller, more related to haplotypes of the Amazon River.