

SNP Discovery in Three South American Freshwater Characiformes Species by Deep Sequencing of Reduced Representation Libraries (RRL)

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Tambaqui (Colossoma macropomum) is the most important native aquaculture freshwater fish species raised in Brazil. A total of 180,000mt are produced annually, accounting to 43% of all finfish cultured domestically. Cross-species hybridization with two other Characiformes species, Pacu (Piaractus mesopotamicus) and Pirapitinga (Piaractus brachypomus), has been systematically used in the last two decades in terminal crosses to obtain individuals resistant to lower temperatures, diseases and faster growing destined to grow-out. Resulting hybrids are often phenotypically indistinguishable and fully fertile. Recent studies revealed hybrids in specimens sampled in farms as pure Tambagui. To prospect SNP markers with species-specific alleles, reduced representation libraries (RRL) with fragments between 400-500bp, 500-650 bp, and 650-850bp for each of the three species were produced using bulked DNA samples. Around 12% of the Tambagui, 11 % of Pacu and 9,5% of Pirapitinga genomes were sequenced using 2x160bp Illumina protocols with an average depth of >100x. Generated reads were aligned with the Tambaqui draft reference genome sequence using BWA and SNP discovery was performed using Freebayes. About 2.4 million SNPs were observed with read depths (RD) >50 and minor allele frequencies >0.05 in Tambaqui, 2.5 million in Pacu and 2 million in Pirapitinga. A total of 16,336 SNPs with species-specific alleles (Tambagui≠Pacu=Pirapitinga) helpful for development of tools for hybrid detection were observed (RD>50). This represents the first report of a large-scale effort to identify SNP markers for Pacu and Pirapitinga species, useful for prospecting species-specific SNPs for species-purity certification of commercial Tambagui broodstocks.

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