Genetic Diversity and Population Structure of the Threatened Giant *Arapaima* in Southwestern Guyana: Implications for Their Conservation

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Information on population genetic structure and connectivity among populations is essential for the implementation of effective conservation and management strategies for threatened species. The giant *Arapaima* is one of the most heavily exploited and threatened freshwater fishes in Guyana. Using nuclear microsatellite markers and mitochondrial (mtDNA) sequences (partial 16S rRNA gene, trRNA-Leu gene, and ND1 gene), we evaluated the genetic structure of *Arapaima* from the Essequibo and Branco (i.e., Amazon) river basins in Guyana. Both markers showed low genetic diversity compared to previously reported studies of *Arapaima* from the Amazon. Only two mtDNA haplotypes were recovered in Guyana that differed in a single nucleotide position. One was novel and restricted to the Branco basin; the other had previously been reported from the Amazon but, in this study, was restricted to the Essequibo basin. Surprisingly, STRUCTURE analysis of microsatellite markers grouped *Arapaima* from Guyana into three distinct clusters; one was again restricted to the Branco basin, while the other two were sympatric at multiple sites in the Essequibo basin. This is the first time genetically distinct groups of *Arapaima* have been found in sympathy at multiple sites. Results have important implications for management of *Arapaima* and conservation of their genetic diversity.

Freshwater systems are among the most imperiled in the world (Dudgeon et al., 2006) with declines in freshwater biodiversity far exceeding that of the most affected terrestrial ecosystems (Sala et al., 2000). Freshwater fishes are particularly vulnerable because both fish and water are important resources for humans and often are subjected to unsustainable uses (Lévéque et al., 2008). The majority of freshwater fisheries are now either overexploited or close to being overexploited (Allan et al., 2005), and that situation is particularly serious for large-bodied species (Stone, 2007). Conservation of the world's freshwater fish diversity is especially challenging because much of it resides in large tropical river basins like the Amazon (Junk, 2007; Castello et al., 2013), Congo, and Mekong (Dudgeon et al., 2006) that cover enormous geographic areas with heterogeneous landscapes and great diversity of aquatic habitats. This complex distribution of variable habitats can result in genetic differentiation among populations despite inhabiting geographically interconnected drainage networks (e.g., Nielsen et al., 2009), highlighting the need to consider populations at multiple geographical scales for effective conservation of fish species in large tropical river drainages (Araripe et al., 2013). In a study of upstream genetics, Storfer et al. (2010) noted that genetic structure in aquatic systems was often explained by drainage structure, slope, elevation, and temperature, but they also found that studies on high-diversity, tropical systems were limited.

*Arapaima* are the largest scaled freshwater fishes in tropical South America (i.e., >3 m and 200 kg; Nelson, 1994). These obligate air-breathing giants were among the most important commercial fishes in the Amazon in the 1800s and early 1900s, but widespread overfishing has severely reduced abundances throughout their range (Queiroz and Sardinha, 1999; Castello and Stewart, 2010), with overall numbers in human population and that high genetic distance did not correlate at all with geographic distance. They concluded that Amazonian *Arapaima* form a panmictic population and that high *F_\text{ST}* values observed between some locations were very high. But genetic distance did not correlate at all with geographic distance. They concluded that Amazonian *Arapaima* form a panmictic population and that high *F_\text{ST}* values observed between some sites were the result of overexploitation and resulting bottlenecks. In a follow-up study with microsatellite markers, Hrbek et al. (2007) did detect strong correlations between genetic and geographic distances up to 2,500 km. More recently, Araripe et al. (2013) also used microsatellite markers to study genetic differentiation of Amazonian *Arapaima* at multiple scales and found little genetic differentiation at small distances (up to 25 km), low to moderate differentiation at intermediate distances (around 100 km), and strong genetic differentiation at long distances (1300–2300 km). Santos et al. (2014, using microsatellites) and Vitorino et al. (2015, using inter-simple sequence repeats or ISSRs) both found strong differentiation at intermediate geographic scales of about 100 km in relatively open systems. Geographic spacing of our samples is an order of magnitude lower than in Hrbek et al. (2005) and comparable to the small and intermediate scales mentioned above.

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In our study area, fishing has always been an important subsistence and economic activity (Mistry et al., 2004), but before the 1960s, *Arapaima* were not fished commercially because they were regarded as the ‘Mother of all Fishes’ (Wetlands Partnership, 2008). In part because of that cultural norm, *Arapaima* were taken primarily on special occasions and for local consumption. Intense, unmanaged commercial harvests of Guyanese *Arapaima* apparently were instigated by immigrant fishermen about 1970 (pers. comm. to LCW, by several indigenous fishermen), and by 2001, the Essequibo basin population was reduced to only about 800 individuals above 1 m total length (Castello et al., 2002). Subsequently, the Guyanese government, local non-governmental organizations, and communities in the region have been facilitating recovery of populations of *Arapaima* through community-based management programs. However, information crucial for population management, such as population genetic structure, levels of genetic diversity, and gene flow among possible sub-populations remain unknown. Managing genetically distinct populations as a single entity, however, could result in overharvest of particular populations, leading to reduced genetic diversity and perhaps even extinction (Laikre et al., 2005; Allendorf et al., 2008; Dulvy and Reynolds, 2009; Castello et al., 2015).

The objectives of this study were to evaluate genetic structure among populations of *Arapaima* within the Essequibo and Pirara river basins in southwestern Guyana and to use that information to make management recommendations that maximize preservation of genetic and ecological diversity. We tested the null hypothesis that *Arapaima* in these river basins represent a panmictic population. Alternatively, *Arapaima* may show evidence of restricted gene flow among variously interconnected localities.

**MATERIALS AND METHODS**

**Sample collections.**—Five locations were sampled, one in the Branco River basin (Pirara = site a) and four in the Essequibo River basin (Karanambu = site b, Rewa = site c, Apoteri = site d, and Iwokrama = site e; Fig. 1); location of sites and number of samples analyzed are outlined in Table 1. The Pirara River in the Branco basin and one of the Essequibo basin sites (Karanambu) are located in savanna habitat with floodplains dominated by tall grasses, shrubs, and sparse, low trees. In contrast, floodplains of the remaining three Essequibo basin sites are covered with largely undisturbed, primary rain forest. The Essequibo River is a black water system and is the largest river in Guyana with an annual water-level fluctuation of about 5 m, while the Rupununi (Karanambu area) and Rewa rivers are primarily white water systems. The Pirara River is a black water system that originates in Lake Amaku, a large, shallow, seepage system with no permanent inflow streams; it is a headwater tributary of the Branco River basin, which drains to the Amazon via the Negro River.

Muscle tissues or anal-fin clips were collected by the authors and stored in 95% ethanol. Muscle tissues were taken from individuals that were collected for taxonomic studies and fin clips from individuals that were measured and released alive. Several voucher specimens of *Arapaima* were preserved in 10% formalin and deposited in the fish collection at the Centre for the Study of Biological Diversity at the University of Guyana, Georgetown; CSBD/UG catalogue numbers are provided below for vouchers corresponding to GenBank accessions. Sampling was done during low-water periods between October and December in 2007 and 2008 using a large seine (150 m × 7 m and 17 cm stretch mesh), gillnets (50 m × 3 m, with 8, 10, and 12 cm stretch meshes), hook and line, and for a few large individuals, indigenous field assistants used bow and arrow. Fish were not sampled twice in a given year because the sampling period was no greater than two months each year; missing scales (collected for age and growth studies) and fin clippings taken for genetic analyses would still be visible within that time frame. Fish sampled in 2007 were not re-sampled the following year either (i.e., 2008), because all fish that were released alive in 2007 were tagged with passive integrated transponders (PIT tags), and the presence of regenerated scales also could indicate a recaptured fish. Sampling of closely related individuals was avoided by taking fin or tissue samples from only one individual per school for young-of-year fish, which might have come from the same nest. Most of the fish that were sampled were at least one year old, in which case they no longer schooled under the protection of parent fish. They thus would have had one or more complete flood cycles to disperse away from siblings. Permission to collect samples was granted by the Guyana Environmental Protection Agency (Permit No. 060706 BR 055).

**Laboratory techniques for genetic analyses.**—DNA was extracted from approximately 25 mg of each muscle tissue or fin-clip sample using the DNeasy blood and tissue extraction kit (Qiagen, standard protocol).

Eleven microsatellite loci (AgCAm4, AgCAm15, AgCAm18, AgCAm20, AgCAm26, AgCTm1, AgCTm3, AgCTm4, AgCTm5, AgCTm7, and AgCTm8) developed for *Arapaima* (Farias et al., 2003) were amplified using polymerase chain reaction (PCR). PCR was performed in total reaction volumes of 25 μl containing 1X MasterAmp premix E (Epipcentre Technologies), 0.06 μM of a marker-specific M13-labeled forward primer, 0.22 μM of the corresponding unlabeled reverse primer, 0.22 μM of fluorescently labeled M13 forward primer (labeled with 6-carboxyfluorescein [FAM]), 50 U/ml of Taq polymerase (Promega), and approximately 50 ng of DNA. Amplifications were carried out in a PTC 100 thermocycler (MJ Research, Inc.). PCR conditions were 94°C (3 min), 35 cycles of 30 s at 94°C, 30 s at 57°C, and 60 s at 72°C, followed by eight cycles of 30 s at 94°C, 30 s at 53°C, and 30 s at 72°C, and a final extension of 72°C for 10 min. Fragment analyses of diluted PCR products were performed on an Applied BioSystems 3730xl DNA Analyzer (Cornell University Life Sciences Core Laboratories Center, Ithaca, NY) using the GeneScan 500 LIZ size standard (Applied Biosystems). Fluorescent peaks corresponding to allele size were scored using Peak Scanner Software v1.0 (Applied Biosystems; https://www.lifetechnologies.com/order/catalog/product/4381867). The dataset was checked for null alleles, stuttering, allelic dropout, and scoring errors using the program MICRO-CHECKER v2.2.3 (van Oosterhout et al., 2004). We did not find evidence for any of the above errors in any of the 11 loci.

Following Hrbek et al. (2005), a mtDNA segment that included the 3’-end of the 16S rRNA gene, the RNA-1eu gene, and ND1 (or NADH dehydrogenase subunit 1) gene was amplified and sequenced for 21 individuals from the Essequibo basin and six individuals from the Branco basin. Unlike Hrbek et al. (2005), however, we did not analyze ATPase. PCR amplifications were performed in 50 μl reactions containing 1X MasterAmp premix E (Epipcentre Technologies), 0.22 μM forward primer, 0.22 μM reverse primer, 50 U/ml of Taq polymerase (Promega), and approximately 50 ng of DNA. Amplifications were carried out in a PTC 100
thermocycler (MJ Research, Inc.) and cycling conditions were 35 s at 94°C, 35 s at 50°C, and 90 s at 72°C for 35 cycles. PCR products were evaluated on a 1% agarose gel and then purified with Qiagen spin-columns (QIAquick PCR Purification Kit). Amplified mitochondrial segments were sequenced in both forward and reverse direction on an Applied Biosystems Automated 3730 DNA analyzer (Cornell University Life Sciences Core Laboratories Center, Ithaca, NY). The primers used for amplification and sequencing (following Hrbek et al., 2005) were L3079 (5′-ACGTGATCTGAGTTCA-
RESULTS

Genetic diversity.—A total of 28 distinct alleles were resolved at the 11 microsatellite loci, and the number of alleles at each locus ranged from one to eight with three loci being monomorphic. At polymorphic loci, the average allelic richness of each study site ranged from 1.526 at Iwokrama to 2.015 at Karanambu, and the average expected heterozygosity ranged from 0.083 (Iwokrama) to 0.200 (Karanambu; Table 2). Private alleles were observed in Rewa, Karanambu, and Pirara, and they ranged from one to three alleles per locality. The frequency of private alleles was relatively low in Rewa and Karanambu (0.015–0.020) but high in Pirara (0.833–1.0; see Appendix 1).

Two sites (Karanambu and Iwokrama) deviated significantly from Hardy-Weinberg expectations at \( \alpha = 0.05 \) (all representing heterozygote deficiencies, \( p < 0.02 \)) at locus AgCam15 before Bonferroni correction. After Bonferroni correction (\( \alpha = 0.002 \)), none of the sites exhibited heterozygote deficiency. Three populations (Rewa, Iwokrama, and Karanambu) exhibited linkage disequilibrium for locus pair AgCTm5 and AgCTm7. Tests for genetic bottlenecks revealed no evidence for such in any of the five sampling locations.

Genetic differentiation.—Analyses of the mean posterior probabilities from STRUCTURE using STRUCTURE HARVESTER resulted in an optimum K value of three, indicating the presence of three clusters (Figs. 1, 2). Fish in cluster one (C1) occurred only in the Pirara basin (site a), while fish in C2 and C3 co-occurred in the Essequibo basin in the proportions shown in Figure 1 (pie charts). Genetic differentiation (\( F_{ST} \)) between fish from different clusters was always significant, even where fish from clusters two and three co-occurred in the same sampling location (Table 3). There were no significant differences for the same cluster at different sites, however, except for Iwokrama versus Rewa (C3; Table 3). Overall, Arapaima from the Pirara basin were highly differentiated (\( F_{ST} = 0.678–0.816 \)) from those in the Essequibo basin. The AMOVA analysis showed that groupings by drainage basin maximized the percentage of variation (PhiST 3’-GGAGGACTARGAGTTTGA-3’).

Statistical analyses.—For microsatellite loci, genetic diversity within each location was measured as allelic richness (\( T_A \)), allelic richness corrected for differences in sample size (\( A_H \)) and heterozygosity using FSTAT (Goudet, 2001). Deviation of observed genotype frequencies from those expected under Hardy-Weinberg equilibrium was tested for each locus within each sampled location, and non-random association of alleles (linkage disequilibrium, LD) was tested for all possible pairs of loci using the Markov chain method with 1000 dememorizations, 1000 batches, and 1000 iterations per batch in GENEPOP 4.2 (Rousset, 2008). Bottleneck 1.2.02 (Cornuet and Luikart, 1996) was used to test for the presence of a population bottleneck within each of the sampling sites.

A Bayesian approach for identifying population clusters (K) was implemented using the software STRUCTURE (Pritchard et al., 2000). An admixture (describing the possibility of mixed ancestry in individuals) model with correlated allele frequencies was used. Values of K ranging from one to ten were tested using a burn-in period of 100,000 iterations and 100,000 Markov-chain Monte Carlo iterations after the burn-in period. Each value of K was run ten times to evaluate stability. The structure output files were then processed using STRUCTURE HARVESTER v0.3 (Earl and von Holdt, 2012) to determine the optimal number of clusters (Evanno et al., 2005). We then estimated the degree of differentiation between clusters and sites with the Weir and Cockerman (1984) estimator of genetic differentiation (\( F_{ST} \)) using GENALEX v6.1 (number of permutations set to 999). Bonferroni adjustment was used to correct for multiple comparisons (\( p \) value set to 0.05/28 comparisons = 0.0018).

An analysis of molecular variance (AMOVA; Excoffier et al., 1992) was conducted for the clusters identified based on the STRUCTURE analysis and by drainage basin using GENALEX. Significance of the test was based on 1000 permutations.

Mitochondrial DNA sequences were assembled and checked for accuracy of base determination using SEQUENCER 3.0 (Gene Codes Corp., Ann Arbor, MI; http://www.genecodes.com).

Table 1. Study areas with lakes sampled, geographic location, and number of samples analyzed. Low population numbers and logistical constraints (aquatic habitats were extensively covered with thick vegetation) prevented us from obtaining a larger sample size for the Pirara population.

<table>
<thead>
<tr>
<th>Study area</th>
<th>Lake</th>
<th>N Latitude</th>
<th>W Longitude</th>
<th>Microsatellites</th>
<th>mtDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Pirara</td>
<td>Diamond W</td>
<td>3°59.612’</td>
<td>59°32.040’</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Corial Pond</td>
<td>3°59.156’</td>
<td>59°31.110’</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>b) Karanambu</td>
<td>River Burst</td>
<td>3°45.402’</td>
<td>59°17.400’</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Crane Pond</td>
<td>3°43.505’</td>
<td>59°17.955’</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Mari Kupi</td>
<td>3°44.339’</td>
<td>59°20.216’</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Honey Pond 1</td>
<td>3°44.753’</td>
<td>59°19.242’</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>c) Rewa</td>
<td>Grass Pond</td>
<td>3°52.062’</td>
<td>58°46.007’</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Brantash</td>
<td>3°51.960’</td>
<td>58°46.302’</td>
<td>19</td>
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<tr>
<td></td>
<td>Makarapan</td>
<td>3°54.098’</td>
<td>58°47.806’</td>
<td>3</td>
<td></td>
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<tr>
<td></td>
<td>Banana Sucker</td>
<td>3°46.273’</td>
<td>58°45.532’</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>d) Apoteri</td>
<td>Grass Lake</td>
<td>4°1.740’</td>
<td>58°32.957’</td>
<td>5</td>
<td></td>
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<tr>
<td></td>
<td>Small Kumaka</td>
<td>3°55.484’</td>
<td>58°23.273’</td>
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</tr>
<tr>
<td></td>
<td>Inkapati Head Pond</td>
<td>4°7.027’</td>
<td>58°29.531’</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Yakat Lake</td>
<td>4°13.854’</td>
<td>58°51.073’</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>e) Iwokrama</td>
<td>Stanley Lake</td>
<td>4°46.164’</td>
<td>58°47.298’</td>
<td>15</td>
<td></td>
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<tr>
<td></td>
<td>Surama Lake</td>
<td>4°14.628’</td>
<td>59°03.927’</td>
<td>11</td>
<td></td>
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<tr>
<td>Totals</td>
<td></td>
<td></td>
<td></td>
<td>114</td>
<td>21</td>
</tr>
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</table>

GACC–3’) and H4364 (5’–GGAGGACTARGAGTTTGA–3’).

No. of samples analyzed
supporting the distinctiveness of populations inhabiting the two basins.

**Sequence analysis.**—Two mtDNA haplotypes were recovered that differed by a single nucleotide position. One was novel and restricted to the Pirara basin (GenBank accession number KJ159903, and University of Guyana catalog number: CSBD/UG 1670); the other had previously been found in the Amazon basin by Hrbek et al. (2005). In Guyana, the latter haplotype was restricted to the Essequibo basin (GenBank accession numbers [and University of Guyana catalog numbers]: KJ200625 [CSBD/UG 1674], KJ200624 [CSBD/UG 1667], and KJ159902 [no preserved voucher specimen]). Although GenBank entries AY081868.1 and AY081884.1 for Amazonian Arapaima (submitted by Hrbek et al., 2005; without locality data) differ from each other by one nucleotide position, both appeared identical to our Essequibo haplotype because the change occurred at the very beginning, where our sequences had missing data. No haplotype was shared between the Pirara and Essequibo basins.

**DISCUSSION**

**Genetic diversity.**—Populations of Arapaima in Guyana undeniably exhibit low genetic diversity. Average allelic richness and expected heterozygosity for each locality (Table 2) were considerably lower than estimated for Arapaima in the Brazilian Amazon. Amazonian values for richness and heterozygosity were, respectively: 7.1, 0.6 (Araripe et al., 2013); 4.3, 0.62 (Farias et al., 2003); 8.0, 0.72 (Hamoy et al., 2008); and 4.04, 0.53 (Santos et al., 2014; average for two wild populations). Furthermore, DeWoody and Avise (2000) reviewed values for a wide diversity of freshwater fish populations and found, on average, 9.1 alleles and an expected heterozygosity of 0.54; these latter values are similar to those reported for Amazonian Arapaima. Interestingly, the lowest genetic diversity was found near Iwokrama Reserve (Fig. 1), which is probably largely a reflection of only one fish from that site being represented in STRUCTURE cluster 2. This site has not been well protected from illegal fishing. Furthermore, the Iwokrama Reserve area is separated from the upper Essequibo basin sites by ~100 km and a series of cataracts and low waterfalls (Fig. 1, arrows). Because Arapaima are relatively sedentary fishes that do not make long distance migrations (Castello, 2008a, 2008b; Núñez-Rodríguez et al., 2015), the Iwokrama Reserve site may have been somewhat isolated from the upper Essequibo basin.

**Genetic differentiation.**—The genetic results clearly indicate that the null hypothesis of panmixia for Guyanese populations of Arapaima should be rejected. Both mitochondrial and nuclear genetic markers indicated divergence between Pirara and Essequibo basin populations. The Pirara fishes were distinguished by very high $F_{ST}$ values versus Essequibo fishes (Table 3), three private alleles (Appendix 1), and a

![Fig. 2. Mean posterior probability LnP(D) values (circles) per clusters (K) and delta K analysis (squares) of LnP(D); values were generated, respectively, by STRUCTURE (Pritchard et al., 2000) and by STRUCTURE HARVESTER (Earl and von Holdt, 2012).](image-url)
unique ND1 haplotype, which suggest they may represent a species distinct from *Arapaima* in the Essequibo drainage (a testable hypothesis, at least). This differentiation may be a result of the drainage divide within the Rupununi savanna acting as a barrier to gene flow. In exceptionally wet years, however, the savanna habitat spanning the drainage boundary may flood, forming a shallow, lentic corridor between the Pirara and Rupununi rivers (Fig. 1; Lowe-McConnell, 1964). During such events, *Arapaima* could potentially move between basins, but the genetic data do not show evidence for such movements. This does not rule out the possibility of movement but with reproductive isolation during secondary contact. The role of the Rupununi savanna as a barrier to gene flow also was supported by genetic evidence for some other fishes, including loricariid catfishes and *Cichla temensis*, but some other species were genetically similar on both sides of the divide (Lujan and Armbruster, 2011).

The genetic differentiation between *Arapaima* of the Pirara and Essequibo basins indicates the presence of two distinct lineages (species?) that, at least, can be considered as Evolutionarily Significant Units (ESUs). An ESU is defined as a unit that is reciprocally monophyletic for mitochondrial DNA and that shows significant divergence of allele frequencies at nuclear loci (Moritz, 1994). *Arapaima* from the Pirara and Essequibo basins did not share any haplotypes for the ND1 gene and were significantly different from each other at eight microsatellite loci.

Fish from the Essequibo basin separate into two genetic clusters in the STRUCTURE analysis, and in the STRUCTURE bar-graph (Fig. 1C), there were very few genetically intermediate or ambiguously classified individuals (i.e., <1%). Local residents in the Essequibo basin do not recognize two forms. We have observed two color morphs of juveniles, however, that tend to occupy blackwater versus whitewater habitats (ecotypic effect or differentiation along a pH gradient?). We also have found two configurations of nest holes that are exposed during the dry season. One form is cut sharply and relatively deep into the substrate with no vegetation clearing around the nest; the other is a broad, shallow basin with extensive clearing of vegetation for a 2–3 m diameter area. Further morphological analyses are in progress but are beyond the scope of this paper. Overall, there appears to be acline of decreasing proportion of C2 and increasing C3 going downstream (Fig. 1). That trend parallels the environmental transition from savanna habitats near Karanambu into rainforest habitats near Rewa and farther downstream. Genetic separation of clusters 2 and 3 was significant for every pair-wise comparison; $F_{ST}$ values ranged from 0.204 to 0.437 (Table 3), which could indicate sympatric species. Fish from those two clusters, however, were identical with respect to ND1 sequences. This sympatry of two genetic clusters (based on microsatellites) across at least three of the Essequibo basin localities differs from anything reported so far from the Amazon basin and, thus, begs further genetic and morphological analyses. Stewart (2013a) indicated that *Arapaima arapaima* (Valenciennes, in Cuvier and Valenciennes, 1847) was described from the Rupununi basin, and so that nominal taxon likely corresponds to one of these two genotypes.

It is interesting to note that Araripe et al. (2013) also used STRUCTURE analysis and found two “stocks” among their Amazonian samples located in lower versus middle Amazon areas, respectively, with some overlap. Contrary to our results, however, there were fish with mixed affinities in areas where stocks overlapped. Vitorino et al. (2015) observed three very distinct STRUCTURE clusters; one of those occurred at two sampling sites along the Araguaia River mainstream and the other two were found at one site each, with no spatial overlap among fish from the different clusters. In Guyana, relatively little genetic differentiation was detected between fish populations belonging to the same STRUCTURE cluster, which is in general agreement with the low differentiation observed by Araripe et al. (2013) at small scales. In contrast, the strong genetic differentiation observed here between fish populations belonging to different STRUCTURE clusters is strikingly similar to the findings of Vitorino et al. (2015; i.e., evidence does not support panmixia in the Tocantins basin).

**Implications for conservation and management of Guyanese Arapaima.**—Establishing a moratorium on fishing of *Arapaima* in Guyana in 2000 and subsequent development of a management plan contributed to population recoveries for Rewa and Apoteri, but poaching may be restraining recovery in other areas (Watson, 2011). The overall population recovery (from ~800 to ~3000 individuals over 1.0 m total length in about eight years) was the basis for a trial harvest of 100 adult individuals in 2009. At that time, no information existed on population genetic structure. Harvesting was done in relatively higher density areas within the Essequibo basin and implicitly assumed *Arapaima* in Guyana represented a panmictic population. Results from this study, however, indicate two ESUs, with one in the Essequibo basin and another in the Pirara River, and any future harvesting should be based on those two units. Further management for genetic diversity within the Essequibo basin would be desirable as well but is not feasible at this point as fish from STRUCTURE clusters two and three could not be differentiated in the field.

Table 3. Below diagonal: pairwise genetic differentiation index ($F_{ST}$) based on eight polymorphic microsatellite loci for *Arapaima* from five localities and three STRUCTURE clusters in southwestern Guyana; above diagonal: geographic distances between localities in km. Numbers in bold indicate significant values after Bonferroni correction for multiple comparisons ($P < 0.0018$). Iwokrama-C2 is omitted because only one individual from Iwokrama fell into cluster 2.

<table>
<thead>
<tr>
<th></th>
<th>Pirara-C1</th>
<th>Karanambu-C2</th>
<th>Rewa-C2</th>
<th>Apoteri-C2</th>
<th>Karanambu-C3</th>
<th>Rewa-C3</th>
<th>Apoteri-C3</th>
<th>Iwokrama-C3</th>
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<tbody>
<tr>
<td>Pirara-C1</td>
<td>–</td>
<td>45</td>
<td>120</td>
<td>145</td>
<td>45</td>
<td>120</td>
<td>145</td>
<td>220</td>
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<tr>
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<td>Apoteri-C2</td>
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<td>–</td>
<td>100</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Karanambu-C3</td>
<td>0.696</td>
<td>0.232</td>
<td>0.342</td>
<td>0.307</td>
<td>–</td>
<td>75</td>
<td>100</td>
<td>175</td>
</tr>
<tr>
<td>Rewa-C3</td>
<td>0.740</td>
<td>0.231</td>
<td>0.294</td>
<td>0.268</td>
<td>0.045</td>
<td>–</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Apoteri-C3</td>
<td>0.728</td>
<td>0.204</td>
<td>0.248</td>
<td>0.220</td>
<td>0.063</td>
<td>0.007</td>
<td>–</td>
<td>75</td>
</tr>
<tr>
<td>Iwokrama-C3</td>
<td>0.816</td>
<td>0.359</td>
<td>0.437</td>
<td>0.407</td>
<td>0.111</td>
<td>0.095</td>
<td>0.040</td>
<td>–</td>
</tr>
</tbody>
</table>
by bow and arrow fishermen. Based on the results from this study that indicate genetic mixing among sites for each of C2 and C3, we cautiously recommend that the Essequibo basin (or at least areas above the rapids) be treated as a single management unit. Still there is need for regional differences in conservation efforts, because areas with relatively easy access by motorized boats carried on trailers are experiencing excessive harvests (e.g., Karanambu and Iwokrama; Watson, 2011). Translocations and stocking of Arapaima from Brazil or between ESUs within Guyana should be avoided pending further genetic and morphological analyses to ensure genetic integrity of what could be endemic or locally adapted stocks. Translocations also could lead to problems like introgressive hybridization (if different species are involved; there are three species recognized for the Amazon basin; Stewart, 2013a, 2013b) or transfer of diseases (Costello, 2009; Santos et al., 2014).

To date, conservation efforts of Arapaima in Guyana have focused on the Essequibo basin, but additional efforts are urgently needed in the Pirara basin, where habitat availability is limited, and the ESU of that local population of Arapaima may be close to extirpation. Our preliminary observations made in the Pirara basin in 2008 indicated low abundances and ongoing poaching, which was facilitated by easy road access. That residual population is variously protected by a dense cover of aquatic macrophytes, but recovery from low densities will require engagement of local residents.

Implications for conservation of riverine biodiversity.—The Amazon and other large tropical river systems have a hierarchical organization from small headwater tributaries to larger tributaries, with a diversity of water chemistries, and ultimately, to low-gradient mainstream systems with floodplains (Junk, 2007). Within those divisions, we also may find high-gradient barriers and systems of lagoons that may be variously isolated from each other by features of the landscape. Based on these landscape features, we may expect to find distinct ESUs or fine-scale population structure as we did in Guyana and like the patterns found in the Tocantins drainage (Vitorino et al., 2015) and the central Amazon (Santos et al., 2014). Such spatial effects naturally will vary according to vagility of the organism being considered. Conservation of aquatic resources across such landscapes would be improved by focusing on entire sub-drainages, not just lowland forests (Castello et al., 2013; Carvajal-Vallejos et al., 2014).

Genetic structure of Arapaima in the Amazon basin showed significant differentiation of mtDNA haplotypes, with high FST values among several localities (Hrbek et al., 2005: Table 3, 20 of 28 pairwise comparisons significant). Hrbek et al. (2005) concluded, nevertheless, that Arapaima in the Amazon basin constitute one large, panmictic population. They hypothesized that observed genetic patterns resulted, in part, from genetic bottlenecks brought about by overexploitation.

Extending their earlier study with microsatellite analyses, Hrbek et al. (2007) recommended that three large protected areas be established along the length of the Amazonian várzea to conserve genetic diversity among the different areas. That idea is reasonable, but additional measures may be needed to conserve diversity in Arapaima. Hrbek et al. (2005, 2007) and Araripe et al. (2013) sampled one southern tributary, the Tocantins River basin, but other major tributaries were not sampled. The Negro River basin, for example, has endemic species for a variety of fish genera, including Cichla (Kullander and Ferreira, 2006; Willis et al., 2007) and Osteoglossum (Ferraris, 2003), and a suspected unrecognized species of Astronotus (Colatreli et al., 2012). The mtDNA sample from the Pirara River, a headwater of the Negro/Branco system, does not share its ND1 haplotype with any published sequence from the Amazon (e.g., Hrbek et al., 2005), suggesting restricted gene flow between the Pirara basin and the Amazon mainstream. Further studies that include samples from Brazilian areas of the Branco River are needed to determine if this haplotype is restricted to the Pirara River or if it also is present elsewhere in the Amazon basin.

Genetic differentiation also is expected on smaller geographic scales. In this study, we found ESUs separated by the drainage divide and significant differentiation above and below a series of rapids. Similarly, Arapaima from Mamirauá Reserve in the central Amazon showed significant differentiation between populations in two lagoon systems that were only about 100 km apart (Araripe et al., 2013). Santos et al. (2014) also found significant differentiation among Arapaima from those same two lagoon systems (i.e., based on 19 microsatellite loci, FST = 0.135, which they considered ‘high’ differentiation); their STRUCTURE analysis also indicated that those represented distinct populations. Most recently, Vitorino et al. (2015) observed three ISSR genotypes separated from each other by about 100 km in an open system (i.e., no physical barriers). Surprisingly, the most differentiated population (FST = 0.66–0.73) was at the downstream end of their study area, where one might expect Arapaima to migrate with ease.

It seems clear that understanding genetic patterns across multiple geographic scales in the Amazon will require more sampling effort on a spatial scale comparable to that used here, as well as broader-scale sampling that includes major tributaries. There is an emerging pattern where nuclear markers show strong differentiation at geographic scales from zero (e.g., sympatry in this study) to 100 km (Santos et al., 2014; Vitorino et al., 2015), yet some mtDNA markers (e.g., ND1 and COI, cytochrome c oxidase subunit I gene, at least) show only small or no differences (this study; Vitorino et al., 2015). Resolution of such contradictory results may require analyses of morphology (e.g., Stewart, 2013a, 2013b) and/or genetic markers that show higher levels of variation. Multidisciplinary studies involving genetics, morphology, and ecology could provide useful guidance for establishing community-based management units and protected areas and also could reveal if various areas harbor ESUs or even sympatric species that need focused conservation attention (e.g., Lundberg et al., 2000; Piorski et al., 2008; Colatreli et al., 2012; Stewart, 2013b). Such analyses applied across multiple geographic scales could lead to new perspectives on diversity of fishes and other aquatic organisms in all large rivers of the world.

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LITERATURE CITED


Kullander, S. O., and E. J. G. Ferreira. 2006. A review of the South American genus Cichla, with descriptions of nine...


### Appendix 1

| Private allele counts. Shown are allele number and relative frequency (in parentheses) for the three locations where they occurred. |
|---|---|---|
| Pirara | Karanambuu | Rewa |
| AgCam15 | 162 (0.020) | 156 (0.015) | 2 |
| AgCtm5 | 207 (0.020) | 1 |
| AgCtm3 | 170 (1.0) | 1 |
| AgCam20 | 292 (1.0) | 1 |
| AgCam26 | 239 (0.833) | 1 |
| Total | 3 | 2 | 1 | 6 |