

TABLE S2 | Genetic divergences calculated for the *cox1* gene of the fish fauna from the São Francisco River, including data collected in this study and those available only in database. Also includes information on the orders and families to which each species belongs, as well as data on conservation status and distribution in different stretches of the river. LSF = lower São Francisco, MSF = middle São Francisco, SMSF = sub-middle São Francisco, USF = upper São Francisco. IUCN (<https://www.iucnredlist.org/>).

Order	Family	Species	IUCN	Sequence Length (bp)	Average Genetic Divergence	Maximum Genetic Divergence	Haplotypes	Basin stretch	BINS	Category
Characiformes	Acestrorhynchidae	<i>Acestrorhynchus lacustris</i>	Least Concern	605	0,000	0,000	1	USF, LSF	BOLD:ACE7854	Match
Characiformes	Acestrorhamphidae	<i>Astyanax bimaculatus</i> / <i>lacustris</i>	Least Concern	606	0,007	0,044	30	USF, MSF, SMSF, LSF	BOLD:ABY8634, BOLD:AAA7659, BOLD:ACK7589, BOLD:AAC5910	Split and Mixture
Characiformes	Acestrorhamphidae	<i>Astyanax</i> cf. <i>fasciatus</i>	Least Concern	634	0,002	0,010	3	USF	BOLD:ACJ1542	Match
Siluriformes	Pimelodidae	<i>Bergiaria westermanni</i>	Least Concern	654	0,000	0,000	1	USF	BOLD:ABZ5574	Match
Characiformes	Bryconidae	<i>Brycon orthotaenia</i>	Least Concern	630	0,001	0,003	3	USF	BOLD:AAE8064	Match
Characiformes	Iguanodectidae	<i>Bryconops affinis</i>	Least Concern	636	0,000	0,005	2	USF, LSF, SMSF	BOLD:AAF6579	Match
Siluriformes	Aspredinidae	<i>Bunocephalus</i> sp.	Data Deficient	652	0,002	0,003	3	USF	BOLD:AAI3810	Match
Siluriformes	Heptapteridae	<i>Cetopsorhamdia iheringi</i>	Least Concern	652	0,000	0,000	1	USF	BOLD:AAD2018	Match
Characiformes	Crenuchidae	<i>Characidium fasciatum</i>	Data Deficient	652	0,015	0,043	3	USF	BOLD:ADR1925, BOLD:ADR3920	Split
Characiformes	Crenuchidae	<i>Characidium zebra</i>	Least Concern	652	0,028	0,053	1	USF	BOLD:AAC6054, BOLD:ADR3188	Split
Cichliformes	Cichlidae	<i>Cichlasoma orientale</i>	Least Concern	584	0,000	0,000	1	SMSF	BOLD:AFF2485	Match
Cichliformes	Cichlidae	<i>Cichlasoma sanctifranciscense</i>	Least Concern	631	0,000	0,008	1	SMSF	BOLD:AFB1722	Match
Characiformes	Characidae	<i>Compsura heterura</i>	Least Concern	654	0,000	0,005	2	USF	BOLD:AAI9189	Match
Siluriformes	Pimelodidae	<i>Conorhynchos conirostris</i>	Endangered	611	0,003	0,007	4	USF	BOLD:AAD8771	Match
Cichliformes	Cichlidae	<i>Crenicichla lepidota</i>	Least Concern	652	0,001	0,002	2	USF	BOLD:AAD6380	Match
Characiformes	Curimatidae	<i>Curimatella lepidura</i>	Least Concern	569	0,000	0,005	8	USF, LSF	BOLD:AAD5595	Match
Characiformes	Curimatidae	<i>Cyphocharax gilbert</i>	Least Concern	652	0,004	0,005	3	USF	BOLD:AAE2114	Match
Gymnotiformes	Sternopygidae	<i>Eigenmannia virescens</i>	Unknown	652	0,032	0,093	3	USF	BOLD:AAB7525, BOLD:AAB7526	Split
Siluriformes	Doradidae	<i>Franciscodoras marmoratus</i>	Least Concern	611	0,001	0,002	2	USF, MSF	BOLD:AAL2828	Match
Cichliformes	Cichlidae	<i>Geophagus brasiliensis</i>	Least Concern	643	0,013	0,019	2	USF, LSF	BOLD:AAA8514	Match
Gymnotiformes	Gymnotidae	<i>Gymnotus carapo</i>	Least Concern	652	0,032	0,048	2	USF	BOLD:AAB6215, BOLD:AAB6216	Split
Siluriformes	Loricariidae	<i>Harttia</i> cf. <i>torrenicola</i>	Least Concern	612	0,001	0,002	2	USF	BOLD:AAE3235	Match
Siluriformes	Loricariidae	<i>Harttia leiopleura</i>	Least Concern	605	0,001	0,002	2	USF	BOLD:AAD3245	Match
Siluriformes	Loricariidae	<i>Harttia novalimensis</i>	Least Concern	584	0,000	0,000	1	USF	BOLD:AAE3279	Match
Siluriformes	Loricariidae	<i>Harttia</i> sp.	Data Deficient	643	0,000	0,000	1	USF	BOLD:AAI2036	Match
Characiformes	Acestrorhamphidae	<i>Hemigrammus marginatus</i>	Least Concern	652	0,035	0,111	4	USF, LSF	BOLD:AAC7039, BOLD:ABY2433*	Split
Siluriformes	Loricariidae	<i>Hemipsilichthys</i> sp.	Data Deficient	648	0,001	0,003	3	USF	BOLD:AAI2011	Match
Siluriformes	Loricariidae	<i>Hisonotus</i> sp.	Data Deficient	638	0,002	0,003	2	USF	BOLD:AAI1941	Match
Characiformes	Erythrinidae	<i>Hoplias intermedius</i>	Least Concern	654	0,000	0,001	2	USF, SMSF	BOLD:AAB1734	Match
Characiformes	Erythrinidae	<i>Hoplias malabaricus</i>	Least Concern	555	0,003	0,007	8	USF	BOLD:AAB1731	Match
Siluriformes	Callichthyidae	<i>Hoplosternum littorale</i>	Least Concern	652	0,000	0,002	2	USF, LSF, SMSF	BOLD:AAB5068	Match
Characiformes	Acestrorhamphidae	<i>Hypheobrycon santae</i>	Data Deficient	629	0,010	0,021	7	USF, SMSF	BOLD:AFE5680*, BOLD:AFE5679*, BOLD:AAE1434	Split
Siluriformes	Loricariidae	<i>Hypostomus alatus</i>	Least Concern	540	0,003	0,004	4	USF	BOLD:AAB9690	Match
Siluriformes	Loricariidae	<i>Hypostomus velhochico</i>	Least Concern	659	0,002	0,005	3	SMSF	BOLD:AEH0790	Match
Cyprinodontiformes	Rivulidae	<i>Hypsolebias flagellatus</i>	Least Concern	586	0,001	0,002	2	MSF	BOLD:ACC0713	Merge



TABLE S2 | (Continued)

Order	Family	Species	IUCN	Sequence Length (bp)	Average Genetic Divergence	Maximum Genetic Divergence	Haplotypes	Basin stretch	BINS	Category
Cyprinodontiformes	Rivulidae	<i>Hypsolebias flavicaudatus</i>	Endangered	649	0,000	0,003	2	MSF	BOLD:ACC0504	Match
Cyprinodontiformes	Rivulidae	<i>Hypsolebias guanambi</i>	Vulnerable	650	0,005	0,009	1	MSF	BOLD:ACC0714	Match
Cyprinodontiformes	Rivulidae	<i>Hypsolebias janaubensis</i>	Critically Endangered	650	0,001	0,002	2	MSF	BOLD:ACC0713	Merge
Characiformes	Stevardiidae	<i>Hysteronotus megalostomus</i>	Least Concern	644	0,000	0,000	1	MSF	BOLD:ADR4989	Match
Siluriformes	Heptapteridae	<i>Imparfinis minutus</i>	Least Concern	631	0,001	0,002	2	USF	BOLD:AAC2104	Match
Characiformes	Stevardiidae	<i>Knodus moenkhausii</i>	Least Concern	634	0,000	0,000	1	USF	BOLD:AAM1485	Match
Characiformes	Anostomidae	<i>Leporinus piau</i>	Least Concern	630	0,004	0,019	5	USF, LSF, SMSF	BOLD:AAC5572	Match
Characiformes	Anostomidae	<i>Leporinus taeniatus</i>	Least Concern	589	0,000	0,000	1	USF, SMSF	BOLD:AAC2253	Match
Siluriformes	Pseudopimelodidae	<i>Lophiosilurus alexandri</i>	Vulnerable	621	0,004	0,008	4	USF, LSF, SMSF	BOLD:AAE4855	Match
Characiformes	Anostomidae	<i>Megaleporinus reinhardti</i>	Least Concern	600	0,001	0,002	2	USF	BOLD:AAD1729	Match
Siluriformes	Pseudopimelodidae	<i>Microglanis leptostriatus</i>	Least Concern	652	0,002	0,005	3	USF	BOLD:AAD6346	Match
Siluriformes	Loricariidae	<i>Microlepidogaster</i> sp.	Data Deficient	637	0,000	0,000	1	USF	BOLD:AAI1507	Match
Characiformes	Acestrorhamphidae	<i>Moenkhausia sanctaefilomenae</i>	Least Concern	652	0,005	0,009	2	USF, LSF	BOLD:AAB9506	Match
Characiformes	Serrasalminae	<i>Myelus micans</i>	Least Concern	582	0,003	0,005	7	USF, SMSF	BOLD:AAG7086	Match
Siluriformes	Loricariidae	<i>Neoplecostomus</i> sp.	Data Deficient	615	0,002	0,004	4	USF	BOLD:AAI3161	Match
Characiformes	Characidae	<i>Odontostilbe</i> sp.	Data Deficient	648	0,003	0,006	5	USF	BOLD:AAE1686	Match
Characiformes	Acestrorhamphidae	<i>Orthospinus franciscensis</i>	Least Concern	650	0,002	0,003	2	USF	BOLD:AAE5358	Match
Siluriformes	Loricariidae	<i>Otocinclus xakriaba</i>	Least Concern	651	0,000	0,000	1	USF	BOLD:AAI2030	Match
Cyprinodontiformes	Poeciliidae	<i>Pamphorichthys hollandi</i>	Least Concern	615	0,014	0,025	11	USF, SMSF	BOLD:AAC5535, BOLD:AFE8186*, BOLD:ADL3599, BOLD:AFE8199, BOLD:AFE9444	Mixture and Split
Siluriformes	Loricariidae	<i>Pareiorhina</i> sp.	Data Deficient	652	0,000	0,000	1	USF	BOLD:AAI2657	Match
Characiformes	Parodontidae	<i>Parodon hilarii</i>	Least Concern	652	0,000	0,000	1	USF	BOLD:AAF8465	Match
Siluriformes	Loricariidae	<i>Parotocinclus</i> sp.	Data Deficient	624	0,000	0,000	1	USF	BOLD:AAI2429	Match
Cyprinodontiformes	Poeciliidae	<i>Phalloceros uai</i>	Least Concern	636	0,000	0,000	1	USF	BOLD:AAB7265	Match
Characiformes	Characidae	<i>Phenacogaster franciscoensis</i>	Least Concern	652	0,000	0,009	2	USF, SMSF	BOLD:AAL4972	Match
Characiformes	Stevardiidae	<i>Piabarchus stramineus</i>	Least Concern	652	0,005	0,006	3	USF	BOLD:AAD8849	Match
Characiformes	Stevardiidae	<i>Piabina argentea</i>	Least Concern	615	0,013	0,023	10	USF	BOLD:ABZ1563, BOLD:ABZ1565	Split
Siluriformes	Heptapteridae	<i>Pimelodella vittata</i>	Least Concern	652	0,049	0,093	4	USF	BOLD:AAD0881, BOLD:AAD0882	Split
Siluriformes	Pimelodidae	<i>Pimelodus fur</i>	Least Concern	652	0,000	0,000	1	USF	BOLD:AAF8120	Match
Siluriformes	Pimelodidae	<i>Pimelodus maculatus</i>	Least Concern	627	0,002	0,003	2	USF, SMSF	BOLD:AFE4407	Match
Siluriformes	Pimelodidae	<i>Pimelodus pohli</i>	Least Concern	652	0,005	0,009	3	USF	BOLD:AAI2970	Match
Characiformes	Stevardiidae	<i>Planaltina myersi</i>	Least Concern	648	0,003	0,009	3	USF	BOLD:AAE2970	Match
Cyprinodontiformes	Poeciliidae	<i>Poecilia vivipara</i>	Least Concern	643	0,005	0,008	2	SMSF, LSF	BOLD:AAC5535, BOLD:AAC0279	Mixture and Merge
Characiformes	Prochilodontidae	<i>Prochilodus argenteus</i>	Least Concern	613	0,007	0,027	9	USF, SMSF	BOLD:ADK5929, BOLD:ADJ8426	Split
Characiformes	Acestrorhamphidae	<i>Psalidodon fasciatus</i>	Unknown	586	0,016	0,037	10	USF, LSF	BOLD:AAC5910, BOLD:ACJ1542	Mixture and Match
Characiformes	Acestrorhamphidae	<i>Psalidodon rivularis</i>	Unknown	586	0,014	0,034	10	USF	BOLD:AAC5910	Mixture
Characiformes	Acestrorhamphidae	<i>Psellogrammus kennedyi</i>	Least Concern	632	0,000	0,005	2	SMSF	BOLD:AFE8745*	Match
Siluriformes	Pseudopimelodidae	<i>Pseudopimelodus charus</i>	Least Concern	587	0,002	0,003	3	USF	BOLD:AAH8247	Match
Siluriformes	Pimelodidae	<i>Pseudoplatystoma corruscans</i>	Near Threatened	591	0,001	0,002	2	USF	BOLD:AAD0242	Match
Siluriformes	Loricariidae	<i>Pterygoplichthys etentaculatus</i>	Least Concern	652	0,000	0,000	1	USF, LSF	BOLD:AAE8598	Match



TABLE S2 | (Continued)

Order	Family	Species	IUCN	Sequence Length (bp)	Average Genetic Divergence	Maximum Genetic Divergence	Haplotypes	Basin stretch	BINS	Category
Characiformes	Serrasalminidae	<i>Pygocentrus piraya</i>	Least Concern	585	0,002	0,007	5	USF, MSF, SMSF	BOLD: AAB8750	Match
Siluriformes	Heptapteridae	<i>Rhamdia quelen</i>	Least Concern	652	0,016	0,055	4	USF, LSF	BOLD: ACF6906, BOLD: AAA6322	Split
Siluriformes	Loricariidae	<i>Rhinelepis aspera</i>	Data Deficient	593	0,000	0,003	2	USF, SMSF	BOLD: AAE3385	Match
Siluriformes	Loricariidae	<i>Rineloricaria</i> sp.	Data Deficient	652	0,000	0,000	1	USF	BOLD: AAG5713	Match
Characiformes	Characidae	<i>Roeboides xenodon</i>	Least Concern	652	0,002	0,003	2	USF	BOLD: AAE7710	Match
Characiformes	Bryconidae	<i>Salminus franciscanus</i>	Least Concern	624	0,001	0,003	4	USF, MSF, SMSF	BOLD: AAD1648	Match
Characiformes	Bryconidae	<i>Salminus hilarii</i>	Least Concern	599	0,009	0,020	10	USF	BOLD: ACF4958, BOLD: ABY7701	Split
Characiformes	Anostomidae	<i>Schizodon knerii</i>	Least Concern	630	0,000	0,002	2	USF, LSF	BOLD: AAD2983	Match
Characiformes	Characidae	<i>Serrapinnus piaba</i>	Least Concern	652	0,009	0,014	5	USF	BOLD: AAE1703	Match
Characiformes	Serrasalminidae	<i>Serrasalmus brandtii</i>	Least Concern	630	0,004	0,005	5	USF, LSF, MSF	BOLD: AAC6505	Match
Characiformes	Serrasalminidae	<i>Serrasalmus maculatus</i>	Least Concern	592	0,002	0,009	5	USF, LSF, SMSF	BOLD: AAE7443	Match
Characiformes	Curimatidae	<i>Steindachnerina elegans</i>	Least Concern	638	0,000	0,000	1	USF	BOLD: ABZ6262	Match
Gymnotiformes	Sternopygidae	<i>Sternopygus macrurus</i>	Least Concern	612	0,002	0,007	3	USF, LSF	BOLD: AAC0974	Match
Characiformes	Acestrorhamphidae	<i>Stygichthys typhlops</i>	Endangered	652	0,000	0,000	1	USF	BOLD: AAE2298	Match
Synbranchiformes	Synbranchidae	<i>Synbranchus marmoratus</i>	Least Concern	652	0,059	0,089	2	USF	BOLD: ACF0893, BOLD: AAB6829	Split
Characiformes	Characidae	<i>Tetragonopterus chalceus</i>	Least Concern	618	0,003	0,007	6	USF, LSF, SMSF	BOLD: AAI0694	Match
Siluriformes	Auchenipteridae	<i>Trachelyopterus galeatus</i>	Least Concern	652	0,008	0,017	4	USF, LSF	BOLD: ADZ8448	Match
Siluriformes	Trichomycteridae	<i>Trichomycterus brasiliensis</i>	Least Concern	571	0,007	0,011	2	USF	BOLD: AAD2591	Match
Siluriformes	Trichomycteridae	<i>Trichomycterus reinhardti</i>	Least Concern	601	0,003	0,007	2	USF	BOLD: AAE8617	Match
Characiformes	Triporthidae	<i>Triporthus nematurus</i>	Least Concern	618	0,000	0,000	1	SMSF	BOLD: AAD0064	Match

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