

TABLE S1 | Table of locations of the D-loop, ATPase, and COI haplotypes analyzed in the present study, including sampling site acronyms, geographic coordinates, assigned haplogroups (HG number), and relationships among haplotypes from different loci. *N*: number of specimens; D-loop haplotype (H^D number): haplotypes identified at each site, with the number of specimens bearing each haplotype shown in parentheses; ATPase haplotype (H^A number): haplotypes identified at each site, with the corresponding D-loop haplotype (respective D-loop = D number) for the same specimen indicated in subscript parentheses; COI haplotype (H^C number): haplotypes identified at each site, with the corresponding D-loop haplotype (respective D-loop = D number) for the same specimen indicated in subscript parentheses. Accession numbers of haplotypes obtained from GenBank and BOLD (COI data) for *Hypostomus ancistroides* and other outgroup species are presented in this table, whereas GenBank accession numbers for sequences generated in the present study are provided in Tabs. **S2** (D-loop), **S3** (ATPase) and **S4** (COI).

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype –
				H^D number	H^A number (respective D-loop = D number)	H^C number (respective D-loop = D number)
Laranjinha River main channel	L1	24°13'24.56"S 50°11'57.88"W	31	HG3: H^D10 (28), H^D63 (1), H^D66 (1), H^D67 (1)	HG3:	HG3:
					H^A53 ($D10$, $D67$)	H^C03 ($D63$, $D67$)
					H^A60 ($D63$)	H^C04 ($D10$, $D66$)
	L2	24°01'36.60"S 50°11'21.60"W	30	HG3: H^D10 (28), H^D67 (1), H^D68 (1)	HG3:	HG3:
					H^A53 ($D10$, $D67$, $D68$)	H^C03 ($D67$)
						H^C04 ($D10$)
	L3	23°51'34.73"S 50°22'43.04"W	29	HG3: H^D10 (24), H^D13 (1), H^D68 (2), H^D69 (1), H^D70 (1)		H^C07 ($D68$)
					HG3:	HG3:
					H^A51 ($D13$)	H^C03 ($D13$)
	L4	23°43'31.88"S 50°26'34.42"W	30	HG1: H^D06 (1)	H^A53 ($D10$, $D68$)	H^C04 ($D10$, $D69$, $D70$)
					H^A60 ($D69$, $D70$)	H^C07 ($D68$)
					HG1:	HG1:
	L5	23°24'53.06"S 50°27'08.60"W	39	HG3: H^D09 (1), H^D10 (29), H^D67 (3), H^D68 (5), H^D71 (1)	H^A09 ($D06$)	H^C01 ($D06$)
					HG3:	HG3:
					H^A53 ($D10$, $D67$)	H^C03 ($D67$)
	L6	23°17'49.95"S / 50°28'43.27"W	24	HG1: H^D03 (1), H^D19 (1)	H^A65 ($D66$)	H^C04 ($D10$, $D66$)
					HG3:	HG3:
					H^A52 ($D09$)	H^C03 ($D67$)
	L7	23°01'53.90"S 50°26'51.90"W	33	HG3: H^D09 (3), H^D10 (6), H^D30 (1), H^D63 (11), H^D68 (1)	H^A53 ($D10$, $D68$)	H^C04 ($D09$, $D10$, $D71$)
					H^A60 ($D71$)	H^C07 ($D68$)
					HG1:	HG1:
	L7	23°01'53.90"S 50°26'51.90"W	33	HG1: H^D06 (1), H^D19 (1), H^D73 (1)	H^A09 ($D19$)	H^C01 ($D03$, $D19$)
					HG3:	HG3:
					H^A51 ($D30$)	H^C03 ($D63$)
	L7	23°01'53.90"S 50°26'51.90"W	33	HG3: H^D09 (4); H^D10 (13), H^D30 (6), H^D31 (2), H^D33 (1), H^D63 (3)	H^A52 ($D09$)	H^C04 ($D09$, $D10$, $D30$)
					H^A53 ($D10$, $D68$)	H^C07 ($D68$)
					H^A60 ($D63$)	
	L7	23°01'53.90"S 50°26'51.90"W	33	HG6: H^D72 (1)	HG1:	HG1:
					H^A09 ($D06$, $D19$, $D73$)	H^C01 ($D06$, $D19$, $D73$)
					HG3:	HG3:
	L7	23°01'53.90"S 50°26'51.90"W	33	HG6: H^D72 (1)	H^A51 ($D30$, $D33$)	H^C03 ($D63$, $D31$)
					H^A52 ($D09$)	H^C04 ($D09$, $D10$, $D30$, $D33$)
					H^A53 ($D10$)	HG6:
	L7	23°01'53.90"S 50°26'51.90"W	33	HG6: H^D72 (1)	H^A59 ($D31$)	H^C06 ($D72$)
					H^A60 ($D63$)	
					HG6:	
	L7	23°01'53.90"S 50°26'51.90"W	33	HG6: H^D72 (1)	H^A61 ($D72$)	

TABLE S1 | (Continued)

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype –
				H ^D number	H ^A number (respective D-loop – D number)	H ^C number (respective D-loop – D number)
Água Grande Stream	S1 ^{LA}	Upper: 23°53'58.76"S 50°13'28.75"W	32	HG3: H ^D 10 (2), H ^D 67 (2), H ^D 69 (28)	HG3: H ^A 53 (D10, D67) H ^A 60 (D69)	HG3: H ^C 03 (D67) H ^C 04 (D10, D69)
		Middle: 23°54'47.61"S 50°14'36.47"W	30	HG3: H ^D 10 (2), H ^D 63 (1), H ^D 67 (1), H ^D 69 (26)	HG3: H ^A 53 (D10, D67) H ^A 60 (D63, D69)	HG3: H ^C 03 (D63, D67) H ^C 04 (D10, D69)
		Lower: 23°57'09.85"S 50°15'35.66"W	30	HG3: H ^D 10 (3), H ^D 66 (2), H ^D 67 (2), H ^D 69 (23)	HG3: H ^A 53 (D10, D67) H ^A 60 (D69) H ^A 65 (D66)	HG3: H ^C 03 (D67) H ^C 04 (D10, D66, D69)
Alecrim Stream	S2 ^{LA}	Upper: 24°01'14.31"S 50°21'36.44"W	30	HG3: H ^D 10 (29), H ^D 74 (1)	HG3: H ^A 53 (D10) H ^A 60 (D74)	HG3: H ^C 04 (D10) H ^C 03 (D74)
		Middle: 23°59'11.63"S 50°20'16.61"W	30	HG3: H ^D 10 (30)	HG3: H ^A 53 (D10)	HG3: H ^C 04 (D10)
		Lower: 23°57'01.54"S 50°18'41.78"W	25	HG3: H ^D 10 (22), H ^D 68 (1), H ^D 69 (2)	HG3: H ^A 53 (D10, D68) H ^A 60 (D69)	HG3: H ^C 04 (D10, D69) H ^C 07 (D68)
São Francisco Stream	S3 ^{LA}	Upper: 23°57'33.05"S 50°26'55.72"W	30	HG3: H ^D 10 (25), HG7: H ^D 75(5)	HG3: H ^A 53 (D10) H ^A 66 (D75)	HG3: H ^C 03 (D75) H ^C 04 (D10)
		Middle: 23°56'10.26"S 50°24'34.12"W	24	HG3: H ^D 10 (21), HG7: H ^D 75(3)	HG3: H ^A 53 (D10) H ^A 66 (D75)	HG3: H ^C 03 (D75) H ^C 04 (D10)
		Lower: 23°52'12.23"S 50°23'16.24"W	25	HG3: H ^D 10 (22), H ^D 66 (1), H ^D 69 (1), H ^D 70 (1)	HG3: H ^A 53 (D10) H ^A 60 (D69, D70) H ^A 65 (D66)	HG3: H ^C 04 (D10, D66, D69, D70)
Figueirinha Stream	S4 ^{LA}	23°54'40.40"S 50°25'4.34"W	5	HG3: H ^D 10 (5)	HG3: H ^A 53 (D10)	HG3: H ^C 04 (D10)
Barreiro Stream	S5 ^{LA}	Upper: 23°47'38.62"S 50°17'58.80"W	29	HG3: H ^D 10 (21), H ^D 65 (1), H ^D 66 (2), H ^D 67 (4), H ^D 76 (1)	HG3: H ^A 53 (D10, D67) H ^A 51 (D65) H ^A 60 (D76) H ^A 65 (D66)	HG3: H ^C 03 (D65, D67, D76) H ^C 04 (D10, D66)
		Middle: 23°43'02.07"S 50°20'41.84"W	30	HG3: H ^D 10 (30)	HG3: H ^A 53 (D10)	HG3: H ^C 04 (D10)
		Lower: 23°38'44.97"S 50°23'32.32"W	30	HG3: H ^D 10 (24), H ^D 67 (6)	HG3: H ^A 53 (D10, D67)	HG3: H ^C 03 (D67) H ^C 04 (D10)
Braúna Stream	S6 ^{LA}	23°10'20.26"S 50°32'52.57"W	3	HG3: H ^D 07 (1), H ^D 09 (1), H ^D 10 (1)	HG3: H ^A 50 (D07) H ^A 52 (D09) H ^A 53 (D10)	HG3: H ^C 03 (D07) H ^C 04 (D09, D10)



TABLE S1 | (Continued)

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype –
				H ^D number	H ^A number (respective D-loop = D number)	H ^C number (respective D-loop = D number)
Araras Stream	S7 ^{LA}	Upper: 23°09'30.59"S 50°35'28.47"W	18	HG3: H ^D 10 (10), H ^D 65 (8)	HG3: H ^A 51 (D65) H ^A 53 (D10)	HG3: H ^C 03 (D65) H ^C 04 (D10)
				HG1: H ^D 19 (1)	HG1: H ^A 09 (D19)	HG1: H ^C 01 (D19)
				HG3: H ^D 10 (12), H ^D 30 (1), H ^D 63 (4), H ^D 65 (10)	HG3: H ^A 53 (D10) H ^A 51 (D30, 65) H ^A 60 (D63)	HG3: H ^C 03 (D63, D65) H ^C 04 (D10, D30)
		Middle 23°09'37.03"S 50°31'24.56"W	28	HG1: H ^D 06 (1)	HG1: H ^A 09 (D06)	HG1: H ^C 01 (D06)
				HG3: H ^D 10 (11), H ^D 30 (1), H ^D 31 (1), H ^D 33 (2), H ^D 63 (9), H ^D 65 (5)	HG3: H ^A 53 (D10) H ^A 51 (D30, D33, D65) H ^A 59 (D31)	HG3: H ^C 03 (D33, D65) H ^C 04 (D10, D30, D31)
				HG3: H ^D 10 (3), H ^D 63 (27), H ^D 69 (1), H ^D 77 (1)	HG3: H ^A 51 (D13, D77) H ^A 60 (D63, D69)	HG3: H ^C 03 (D13, D63, D77) H ^C 04 (D69)
				HG3: H ^D 10 (3), H ^D 63 (21), H ^D 65 (2), H ^D 78 (2), H ^D 79 (1)	HG3: H ^A 51 (D65, D78, D79) H ^A 53 (D10) H ^A 60 (D63)	HG3: H ^C 03 (D63, D65, D78) H ^C 04 (D10) H ^C 09 (D79)
				HG3: H ^D 10 (5), H ^D 13 (1), H ^D 63 (12), H ^D 65 (1), H ^D 78 (7)	HG3: H ^A 51 (D13, D65, D78) H ^A 53 (D10) H ^A 60 (D63)	HG3: H ^C 03 (D13, D63, D65, D78) H ^C 04 (D10)
		Lower: 23°09'59.04"S 50°28'57.34"W	30	HG3: H ^D 10 (3), H ^D 17 (7), H ^D 23 (1), H ^D 24 (1), H ^D 81 (1)	HG3: H ^A 53 (D10) H ^A 57 (D17, D23, D24, D81)	HG3: H ^C 04 (D10, D17, D23, D24, D81)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
				HG3: H ^D 13 (1), H ^D 25 (2), H ^D 26 (1), H ^D 33 (1)	HG3: H ^A 51 (D25, D26, D33) H ^A 52 (D09)	HG3: H ^C 03 (D33) H ^C 04 (D09, D25, D26)
		23°32'5.71"S 50°5'11.48"W	18	HG3: H ^D 09 (2), H ^D 25 (10), H ^D 26 (3), H ^D 34 (1), H ^D 39 (1), H ^D 82 (1)	HG3: H ^A 51 (D25, D26, D33, D34, D39) H ^A 52 (D09)	HG3: H ^C 03 (D33) H ^C 04 (D09, D25, D26, D34, D39, D82)
Grande Stream	S8 ^{LA}	Upper: 23°19'53.93"S 50°20'44.34"W	32	HG3: H ^D 10 (3), H ^D 63 (21), H ^D 65 (2), H ^D 78 (2), H ^D 79 (1)	HG3: H ^A 51 (D65, D78, D79) H ^A 53 (D10) H ^A 60 (D63)	HG3: H ^C 03 (D63, D65, D78) H ^C 04 (D10) H ^C 09 (D79)
				HG3: H ^D 10 (5), H ^D 13 (1), H ^D 63 (12), H ^D 65 (1), H ^D 78 (7)	HG3: H ^A 51 (D13, D65, D78) H ^A 53 (D10) H ^A 60 (D63)	HG3: H ^C 03 (D13, D63, D65, D78) H ^C 04 (D10)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
		Middle: 23°17'24.19"S 50°24'30.87"W	29	HG3: H ^D 10 (3), H ^D 63 (21), H ^D 65 (2), H ^D 78 (2), H ^D 79 (1)	HG3: H ^A 51 (D65, D78, D79) H ^A 53 (D10) H ^A 60 (D63)	HG3: H ^C 03 (D63, D65, D78) H ^C 04 (D10) H ^C 09 (D79)
				HG3: H ^D 10 (5), H ^D 13 (1), H ^D 63 (12), H ^D 65 (1), H ^D 78 (7)	HG3: H ^A 51 (D13, D65, D78) H ^A 53 (D10) H ^A 60 (D63)	HG3: H ^C 03 (D13, D63, D65, D78) H ^C 04 (D10)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
		Lower: 23°16'00.70"S 50°27'58.06"W	26	HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
		24° 0'40.24"S 49°58'20.78"W	13	HG3: H ^D 10 (3), H ^D 17 (7), H ^D 23 (1), H ^D 24 (1), H ^D 81 (1)	HG3: H ^A 53 (D10) H ^A 57 (D17, D23, D24, D81)	HG3: H ^C 04 (D10, D17, D23, D24, D81)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
Cinzas River main channel	C1	24° 0'40.24"S 49°58'20.78"W	13	HG3: H ^D 10 (3), H ^D 17 (7), H ^D 23 (1), H ^D 24 (1), H ^D 81 (1)	HG3: H ^A 53 (D10) H ^A 57 (D17, D23, D24, D81)	HG3: H ^C 04 (D10, D17, D23, D24, D81)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
		23°51'13.42"S 49°56'47.36"W	33	HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
		23°43'15.09"S 49°58'49.83"W	5	HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
		23°32'5.71"S 50°5'11.48"W	18	HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)
				HG3: H ^D 10 (3), H ^D 16 (3), H ^D 17 (20), H ^D 21 (2), H ^D 22 (3), H ^D 23 (1), H ^D 24 (1)	HG3: H ^A 51 (D21) H ^A 53 (D10) H ^A 56 (D16) H ^A 57 (D17, D22, D23, D24)	HG3: H ^C 04 (D10, D16, D17, D21, D22, D23, D24)



TABLE S1 | (Continued)

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype –
				H ^p number	H ^A number (respective D-loop – D number)	H ^C number (respective D-loop – D number)
Cinzas River main channel	C5	23°8'6.30"S 50°14'28.68"W	22	HG1: H ^p 06 (1), H ^p 64 (1)	HG1: H ^A 09 (D06, D64)	HG1: H ^C 01 (D06, D64)
				HG3: H ^p 09 (1), H ^p 10 (3), H ^p 25 (2), H ^p 30 (3), H ^p 31 (6), H ^p 33 (1), H ^p 34 (1), H ^p 62 (1), H ^p 63 (1), H ^p 65 (1)	HG3: H ^A 24 (D62) H ^A 51 (D25, D30, D33, D34, D65) H ^A 52 (D09) H ^A 53 (D10) H ^A 59 (D31) H ^A 60 (D63)	HG3: H ^C 03 (D33, D63, D65) H ^A 04 (D09, D10, D25, D30, D31, D34, D62)
Tigrinho Stream	S1 ^{ci}	Upper: 24°10'10.75"S 49°49'16.59"W	29	HG3: H ^p 17 (20)	HG3: H ^A 57 (D17)	HG3: H ^A 04 (D17)
				HG5: H ^p 27 (6), H ^p 28 (1), H ^p 54 (2)	HG5: H ^A 58 (D27, D28, D54)	HG5: H ^A 05 (D27, D28, D54)
		Middle: 24° 9'43.96"S 49°52'34.04"W	26	HG3: H ^p 17 (1)	HG3: H ^A 57 (D17)	HG3: H ^A 04 (D17)
				HG5: H ^p 27 (24), H ^p 29 (1)	HG5: H ^A 58 (D27, D29)	HG5: H ^A 05 (D27, D29)
		Lower: 24°9'6.46"S 49°56'43.39"W	8	HG5: H ^p 27 (8)	HG5: H ^A 58 (D27)	HG5: H ^A 05 (D27)
Natureza Stream	S2 ^{ci}	23°52'41.17"S 49°53'47.52"W	5	HG3: H ^p 16 (2) H ^p 17 (3)	HG3: H ^A 56 (D16) H ^A 57 (D17)	HG5: H ^A 04 (D16, D17)
Iguape Stream	S3 ^{ci}	Upper: 23°46'2.66"S 50°10'9.44"W	27	HG3: H ^p 10 (2), H ^p 13 (3), H ^p 25 (21), H ^p 41 (1)	HG3: H ^A 51 (D13, D25) H ^A 53 (D10) H ^A 60 (D41)	HG3: H ^C 03 (D13) H ^C 04 (D10, D25, D41)
		Middle: 23°42'3.25"S 50° 8'12.42"W	31	HG3: H ^p 13 (3), H ^p 25 (27), H ^p 26 (1)	HG3: H ^A 51 (D13, D25, D26)	HG3: H ^C 03 (D13) H ^C 04 (D25, D26)
		Lower: 23°40'21.17"S 50° 4'58.78"W	18	HG3: H ^p 25 (16), H ^p 26 (2)	HG3: H ^A 51 (D25, D26)	HG3: H ^C 04 (D25, D26)
Peroba Stream	S4 ^{ci}	Upper: 23°36'2.84"S 49°55'12.06"W	31	HG3: H ^p 25 (16), H ^p 26 (14), H ^p 38 (1)	HG3: H ^A 51 (D25, D26, D38)	HG3: H ^C 04 (D25, D26, D38)
		Middle: 23°34'23.92"S 50° 0'19.53"W	30	HG3: H ^p 10 (1), H ^p 13 (3), H ^p 25 (16), H ^p 26 (8), H ^p 38 (1), H ^p 39 (1)	HG3: H ^A 51 (D13, D25, D26, D38, D39) H ^A 53 (D10)	HG3: H ^C 03 (D13) H ^C 04 (D10, D25, D26, D38, D39)
		Lower: 23°32'49.72"S 50° 3'48.30"W	30	HG3: H ^p 13 (5), H ^p 25 (14), H ^p 26 (7), H ^p 38 (2), H ^p 39 (1), H ^p 40 (1)	HG3: H ^A 51 (D13, D25, D26, D38, D39, D40)	HG3: H ^C 03 (D13) H ^C 04 (D25, D26, D38, D39, D40)
Bicas Stream	S5 ^{ci}	Upper: 23°18'18.41"S 50° 2'3.05"W	31	HG3: H ^p 09 (1), H ^p 13 (1), H ^p 25 (9), H ^p 26 (13), H ^p 35 (3), H ^p 36 (3), H ^p 37 (1)	HG3: H ^A 51 (D13, D25, D26, D35, D36, D37) H ^A 52 (D09)	HG3: H ^C 03 (D13, D36) H ^C 04 (D09, D25, D26, D35, D37)
		Middle: 23°19'40.92"S 50° 4'51.80"W	32	HG3: H ^p 09 (1), H ^p 25 (19), H ^p 26 (2), H ^p 36 (6), H ^p 37 (4)	HG3: H ^A 51 (D25, D26, D36, D37) H ^A 52 (D09)	HG3: H ^C 03 (D13, D36) H ^C 04 (D09, D25, D26, D35, D37)
		Lower: 23°20'19.37"S 50° 9'19.71"W	31	HG3: H ^p 09 (3), H ^p 10 (2), H ^p 13 (1), H ^p 25 (19), H ^p 26 (2), H ^p 30 (1), H ^p 36 (1), H ^p 37 (2)	HG3: H ^A 51 (D13, D25, D26, D30, D36, D37) H ^A 52 (D09) H ^A 53 (D10)	HG3: H ^C 03 (D13, D36) H ^C 04 (D09, D10, D25, D26, D30, D37)



TABLE S1 | (Continued)

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype –
				H ^D number	H ^A number (respective D-loop – D number)	H ^C number (respective D-loop – D number)
Antas Stream	S6 ^{CI}	Upper: 23°11'21.13"S 50°21'31.60"W	14	HG3: H ^D 10 (3), H ^D 30 (8), H ^D 31 (2), H ^D 34 (1)	HG3: H ^A 51 (D30, D34) H ^A 53 (D10) H ^A 59 (D31)	HG3: H ^C 04 (D10, D30, D31, D34)
				HG1: H ^D 06 (1)	HG1: H ^A 09 (D06)	HG1: H ^C 01 (D06)
				HG3: H ^D 09 (1), H ^D 10 (9), H ^D 13 (2), H ^D 30 (10), H ^D 31 (3), H ^D 32 (1), H ^D 33 (1)	HG3: H ^A 51 (D13, D30, D32, D33) H ^A 52 (D09) H ^A 53 (D10) H ^A 59 (D31)	HG3: H ^C 03 (D13, D33) H ^C 04 (D09, D10, D30, D31, D32)
		Middle: 23° 8'28.59"S 50°22'59.64"W	28	HG3: H ^D 09 (2), H ^D 10 (12), H ^D 30 (8), H ^D 31 (4), H ^D 33 (2), H ^D 34 (2)	HG3: H ^A 51 (D30, D33, D34) H ^A 52 (D09) H ^A 53 (D10) H ^A 59 (D31)	HG3: H ^C 03 (D33) H ^C 04 (D09, D10, D30, D31, D34)
				HG5: H ^D 51 (7), H ^D 52 (1), H ^D 53 (1), H ^D 54 (2), H ^D 55 (1), H ^D 56 (16)	HG5: H ^A 58 (D53, D54, D55, D56) H ^A 63 (D51, D52)	HG5: H ^C 05 (D51, D52, D53, D54, D55, D56)
				HG1: H ^D 06 (2), H ^D 19 (5), H ^D 45 (1)	HG1: H ^A 09 (D06, D19, D45)	HG1: H ^C 01 (D06, D19, D45)
		Lower: 23° 5'13.55"S 50°23'7.34"W	30	HG3: H ^D 25 (1)	HG3: H ^A 51 (D25)	HG3: H ^C 4 (D25)
				HG1: H ^D 01 (2), H ^D 02 (1), H ^D 03 (2)	HG1: H ^A 09 (D01, D02) H ^A 49 (D03)	HG1: H ^C 01 (D01, D02, D03)
				HG1: H ^D 01 (3), H ^D 04 (2)	HG1: H ^A 09 (D01, D04)	HG1: H ^C 01 (D01) H ^C 02 (D04)
Jaguariaíva River	JA	24°15'27.39"S 49°41'39.56"W	28	HG1: H ^D 05 (2), H ^D 06 (3)	HG1: H ^A 09 (D05, D06)	HG1: H ^C 01 (D06) H ^C 02 (D05)
Tibagi River	T1	23°30'2.28"S 50°56'55.03"W	9	HG1: H ^D 01 (1), H ^D 06 (2)	HG1: H ^A 09 (D01, D06)	HG1: H ^C 01 (D01, D06)
José Maria Stream	S1 ^{TB}	23°31'59.77"S 50°48'6.76"W	5	HG3: H ^D 07 (1)	HG3: H ^A 50 (D07)	HG3: H ^C 03 (D07)
Três Bocas Stream	S2 ^{TB}	23°23'56.08"S 51°15'14.25"W	5	HG1: H ^D 03 (2), H ^D 06 (11), H ^D 19 (3), H ^D 43 (1), H ^D 44 (1)	HG1: H ^A 50 (D03) H ^A 09 (D06, D19, D43, D44)	HG1: H ^C 01 (D03, D06, D19, D43, D44)
Água do Cerne Stream	S3 ^{TB}	22°56'45.69"S 51° 5'54.05"W	5	HG3: H ^D 42 (1)	HG3: H ^A 24 (D42)	HG3: H ^C 04 (D42)
Jacú Stream	S4 ^{TB}	22°53'26.22"S 51° 4'19.25"W	4	HG1: H ^D 01 (6), H ^D 04(3), H ^D 06 (4), H ^D 14 (3), H ^D 15 (1), H ^D 57 (12), H ^D 58 (3), H ^D 59 (1), H ^D 60 (1), H ^D 61 (2)	HG1: H ^A 09 (D01, D06) HG3: H ^A 50 (D07) HG1: H ^A 11 (D01, D59) H ^A 38 (D60) H ^A 64 (D58)	HG1: H ^C 01 (D01, D06) HG3: H ^C 03 (D07) HG1: H ^C 02 (D04, D57, D58, D59)
Congonhas River	CO	23°14'35.83"S 50°43'42.33"W	19	HG1: H ^D 01 (6), H ^D 04(3), H ^D 06 (4), H ^D 14 (3), H ^D 15 (1), H ^D 57 (12), H ^D 58 (3), H ^D 59 (1), H ^D 60 (1), H ^D 61 (2)	HG1: H ^A 09 (D01, D04, D06, D14, D15, D57, D61) HG3: H ^A 11 (D01, D59) H ^A 38 (D60) H ^A 64 (D58)	HG1: H ^C 01 (D01, D06, D14, D15, D60, D61) HG3: H ^C 02 (D04, D57, D58, D59)
Pirapó River	PI	22°54'35.88"S 52° 6'4.46"W	36	HG6: H ^D 49 (1), H ^D 50 (1)	HG6: H ^A 61 (D49) H ^A 62 (D50)	HG6: H ^C 06 (D49, D50)
Itapetininga River	IT	23°36'8.09"S 48°26'58.73"W	2			



TABLE S1 | (Continued)

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype -
				H ^p number	H ^A number (respective D-loop – D number)	H ^C number (respective D-loop – D number)
Paranapanema River (middle)	PP ^M	22°39'0.45″S 51°26'49.86″W	16	HG1: H ^p 01 (3), H ^p 03 (2), H ^p 06 (6), H ^p 18 (1), H ^p 19 (2), H ^p 20 (1), HG3: H ^p 10 (1)	HG1: H ^A 09 (D01, D04, D06, D18, D19, D20) H ^A 50 (D03) HG3: H ^A 53 (D10)	HG1: H ^C 01 (D01, D03, D06, D18, D19) H ^C 02 (D04, D20) HG3: H ^C 04 (D10)
				HG1: H ^p 06 (1) HG3: H ^p 09 (3), H ^p 13 (5)	HG1: H ^A 09 (D06) H ^A 52 (D09) H ^A 51 (D13)	HG1: H ^C 01 (D06) H ^C 03 (D13) H ^C 04 (D09)
				HG9: H ^p 11 (3); H ^p 12 (1)	HG9: H ^A 54 (D11) H ^A 55 (D12)	HG8: H ^C 12 (D11, D12)
				HG8: H ^p 83 (1) HG10: H ^p 92 (1)	HG8: H ^A 67 (D83) H ^A 02 (D92)	HG7: H ^C 14 (D92) HG8: H ^C 13 (D83)
Maitacas Stream	S1 ^{PP}	23° 0'33.72″S 50°43'13.44″W	9	HG1: H ^p 06 (1) HG3: H ^p 09 (3), H ^p 13 (5)	HG1: H ^A 09 (D06) H ^A 52 (D09) H ^A 51 (D13)	HG1: H ^C 01 (D06) H ^C 03 (D13) H ^C 04 (D09)
				HG9: H ^p 11 (3); H ^p 12 (1)	HG9: H ^A 54 (D11) H ^A 55 (D12)	HG8: H ^C 12 (D11, D12)
				HG8: H ^p 83 (1) HG10: H ^p 92 (1)	HG8: H ^A 67 (D83) H ^A 02 (D92)	HG7: H ^C 14 (D92) HG8: H ^C 13 (D83)
				HG4: H ^p 84 (1) H ^p 86 (1) HG8: H ^p 85 (1), H ^p 91 (1) HG11: H ^p 87 (1), H ^p 90 (1)	HG2: H ^A 45 (D87, D90) H ^A 47 (D84) H ^A 69 (D86) HG8: H ^A 68 (D85, D91)	HG2: H ^C 11 (D87, D90) H ^C 10 (D84, D86) HG8: H ^C 13 (D85) H ^C 12 (D91)
Ivaí River	IV	23°33'21.38″S 52°21'22.06″W	4	HG9: H ^p 11 (3); H ^p 12 (1)	HG9: H ^A 54 (D11) H ^A 55 (D12)	HG8: H ^C 12 (D11, D12)
				HG8: H ^p 83 (1) HG10: H ^p 92 (1)	HG8: H ^A 67 (D83) H ^A 02 (D92)	HG7: H ^C 14 (D92) HG8: H ^C 13 (D83)
				HG4: H ^p 84 (1) H ^p 86 (1) HG8: H ^p 85 (1), H ^p 91 (1) HG11: H ^p 87 (1), H ^p 90 (1)	HG2: H ^A 45 (D87, D90) H ^A 47 (D84) H ^A 69 (D86) HG8: H ^A 68 (D85, D91)	HG2: H ^C 11 (D87, D90) H ^C 10 (D84, D86) HG8: H ^C 13 (D85) H ^C 12 (D91)
				HG8: H ^p 83 (1) HG10: H ^p 92 (1)	HG8: H ^A 67 (D83) H ^A 02 (D92)	HG7: H ^C 14 (D92) HG8: H ^C 13 (D83)
Piquiri River (upper)	PQ ²	25° 1'49.13″S 52°51'16.59″W	2	HG8: H ^p 83 (1) HG10: H ^p 92 (1)	HG8: H ^A 67 (D83) H ^A 02 (D92)	HG7: H ^C 14 (D92) HG8: H ^C 13 (D83)
				HG4: H ^p 84 (1) H ^p 86 (1) HG8: H ^p 85 (1), H ^p 91 (1) HG11: H ^p 87 (1), H ^p 90 (1)	HG2: H ^A 45 (D87, D90) H ^A 47 (D84) H ^A 69 (D86) HG8: H ^A 68 (D85, D91)	HG2: H ^C 11 (D87, D90) H ^C 10 (D84, D86) HG8: H ^C 13 (D85) H ^C 12 (D91)
				HG8: H ^p 83 (1) HG10: H ^p 92 (1)	HG8: H ^A 67 (D83) H ^A 02 (D92)	HG7: H ^C 14 (D92) HG8: H ^C 13 (D83)
				HG4: H ^p 84 (1) H ^p 86 (1) HG8: H ^p 85 (1), H ^p 91 (1) HG11: H ^p 87 (1), H ^p 90 (1)	HG2: H ^A 45 (D87, D90) H ^A 47 (D84) H ^A 69 (D86) HG8: H ^A 68 (D85, D91)	HG2: H ^C 11 (D87, D90) H ^C 10 (D84, D86) HG8: H ^C 13 (D85) H ^C 12 (D91)
Piquiri River (lower)	PQ ¹	24°14'57.26″S 53°32'14.42″W	6	HG4: H ^p 84 (1) H ^p 86 (1) HG8: H ^p 85 (1), H ^p 91 (1) HG11: H ^p 87 (1), H ^p 90 (1)	HG2: H ^A 45 (D87, D90) H ^A 47 (D84) H ^A 69 (D86) HG8: H ^A 68 (D85, D91)	HG2: H ^C 11 (D87, D90) H ^C 10 (D84, D86) HG8: H ^C 13 (D85) H ^C 12 (D91)
				HG8: H ^p 83 (1) HG10: H ^p 92 (1)	HG8: H ^A 67 (D83) H ^A 02 (D92)	HG7: H ^C 14 (D92) HG8: H ^C 13 (D83)
				HG4: H ^p 84 (1) H ^p 86 (1) HG8: H ^p 85 (1), H ^p 91 (1) HG11: H ^p 87 (1), H ^p 90 (1)	HG2: H ^A 45 (D87, D90) H ^A 47 (D84) H ^A 69 (D86) HG8: H ^A 68 (D85, D91)	HG2: H ^C 11 (D87, D90) H ^C 10 (D84, D86) HG8: H ^C 13 (D85) H ^C 12 (D91)
				HG8: H ^p 83 (1) HG10: H ^p 92 (1)	HG8: H ^A 67 (D83) H ^A 02 (D92)	HG7: H ^C 14 (D92) HG8: H ^C 13 (D83)
Iguaçu River basin	IG	25°13'49.71″S 52°48'36.59″W	2	HG4: H ^p 84 (1) HG8: H ^p 83 (1)	HG2: H ^A 47 (D84) H ^A 67 (D83)	HG4: H ^C 10 (D84) HG8: H ^C 13 (D83)
				HG8: H ^p 83 (1) HG10: H ^p 92 (1)	HG8: H ^A 67 (D83) H ^A 02 (D92)	HG7: H ^C 14 (D92) HG8: H ^C 13 (D83)
				HG4: H ^p 84 (1) H ^p 86 (1) HG8: H ^p 85 (1), H ^p 91 (1) HG11: H ^p 87 (1), H ^p 90 (1)	HG2: H ^A 45 (D87, D90) H ^A 47 (D84) H ^A 69 (D86) HG8: H ^A 68 (D85, D91)	HG2: H ^C 11 (D87, D90) H ^C 10 (D84, D86) HG8: H ^C 13 (D85) H ^C 12 (D91)
				HG8: H ^p 83 (1) HG10: H ^p 92 (1)	HG8: H ^A 67 (D83) H ^A 02 (D92)	HG7: H ^C 14 (D92) HG8: H ^C 13 (D83)
Itaipu reservoir tributaries	IR ¹	25°13'39.51″S 54°12'6.02″W	8	HG8: H ^p 88 (2), H ^p 89 (2) HG10: H ^p 96 (1) HG11: H ^p 93 (1), H ^p 94 (1), H ^p 95 (1)	HG2: H ^A 45 (D93, D94, D95) H ^A 68 (D88, D89) H ^A 02 (D96)	HG2: H ^C 11 (D93, D94, D95) H ^C 14 (D96) H ^C 12 (D88, D89)
				HG12: H ^p 47 (1) GenBank: AJ318369	ATPase: No data	COI: No data
				HG7: H ^p 48 (1) GenBank: AJ412846	ATPase: No data	COI: No data
				HG2: H ^p 46 (2) GenBank: AJ412843 MT066232 H ^p 80 (1) GenBank: NC_052710 D-Loop: No data	HG2: H ^A 08 (3) l GenBank: AJ412843 MT066232 NC_052710	HG1: H ^C 15 (3) GenBank: AJ412843 MT066232 NC_052710
Grande River	GR	21°10'8.34″S 45° 7'22.93″W	1			
Ribeira de Iguape River	RG	23°48'00″S 46°55'00″W	1			
Tietê River	TI	23° 0'8.03″S 48° 0'17.64″W	3	HG2: H ^p 46 (2) GenBank: AJ412843 MT066232 H ^p 80 (1) GenBank: NC_052710	HG2: H ^A 08 (3) l GenBank: AJ412843 MT066232 NC_052710	HG1: H ^C 15 (3) GenBank: AJ412843 MT066232 NC_052710
				D-Loop: No data	HG4: H ^A 03 (2) GenBank: KT276011 (Ppn09) KT276122 (Ppn10)	COI: No data
Peixe River	PX	21°52'00″S 51°37'23″W	2			



TABLE S1 | (Continued)

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype –
				H ^D number	H ^A number (respective D-loop = D number)	H ^C number (respective D-loop = D number)
Paranapanema River (lower)	PP ^a	22°30'53"S 52°08'41"W	1	D-Loop: No data	HG1: H ^A 01 (1) GenBank: KT276009 (Ppn08)	COI: No data
				D-Loop: No data	HG10: H ^A 2 (2) GenBank: KT276010 (Pnb01) KT276032 (Pnb02)	COI: No data
Paranaíba River	PB	17°58'38"S 48°38'17"W	1	D-Loop: No data	HG10: H ^A 19 (1) GenBank: KT276045 (Pnb14)	COI: No data
				D-Loop: No data	HG2: H ^A 26 (1) GenBank: KT276075 (Pnb06) H ^A 42 (1) GenBank: KT276142 (Pnb09)	COI: No data
Paranaíba River	PB	17°56'24"S 47°29'08"W	2	D-Loop: No data	HG4: H ^A 3 (1) GenBank: KT276020 (GRd15)	COI: No data
				D-Loop: No data	HG4: H ^A 3 (9) GenBank: KT276039 (Grd08) KT276042 (Grd09) KT276047 (Grd02) KT276069 (Grd01) KT276149 (Grd05) KT276151 (Grd04) KT276168 (Grd10) KT276169 (Grd03) KT276174 (Grd11) H ^A 7 (1) GenBank: KT276016 (Grd06) H ^A 47 (2) GenBank: KT276178 (Grd12) KT276179(Grd07)	COI: No data
Grande River	GR	20°14'10.2"S 48°40'42.0"W	1	D-Loop: No data	HG2: H ^A 4 (4) GenBank: KT276023 (Grd31) KT276043 (Grd32) KT276158 (Grd25) KT276159 (Grd24)	COI: No data
				D-Loop: No data	HG2: H ^A 4 (3) GenBank: KT276154 (Grd28) KT276164 (Grd26) KT276170 (Grd27)	COI: No data
				D-Loop: No data	HG4: H ^A 6 (1) GenBank: KT276015 (Grd17)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (1) GenBank: KT276017 (Tie13)	COI: No data
				D-Loop: No data	HG2: H ^A 4 (4) GenBank: KT276023 (Grd31) KT276043 (Grd32) KT276158 (Grd25) KT276159 (Grd24)	COI: No data
				D-Loop: No data	HG2: H ^A 4 (3) GenBank: KT276154 (Grd28) KT276164 (Grd26) KT276170 (Grd27)	COI: No data
				D-Loop: No data	HG4: H ^A 6 (1) GenBank: KT276015 (Grd17)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (1) GenBank: KT276017 (Tie13)	COI: No data
				D-Loop: No data	HG2: H ^A 4 (4) GenBank: KT276023 (Grd31) KT276043 (Grd32) KT276158 (Grd25) KT276159 (Grd24)	COI: No data
				D-Loop: No data	HG2: H ^A 4 (3) GenBank: KT276154 (Grd28) KT276164 (Grd26) KT276170 (Grd27)	COI: No data
				D-Loop: No data	HG4: H ^A 6 (1) GenBank: KT276015 (Grd17)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (1) GenBank: KT276017 (Tie13)	COI: No data



TABLE S1 | (Continued)

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype –
				H ^D number	H ^A number (respective D-loop – D number)	H ^C number (respective D-loop – D number)
Grande River	GR	22°22'07"S 47°28'38"W	7	D-Loop: No data	HG2: H ^A 8 (3) GenBank: KT276079 (Grd21) KT276123 (Grd19) KT276157 (Grd16) H ^A 10 (1) GenBank: KT276022 (Grd22) H ^A 36 (1) GenBank: KT276124 (Grd20) H ^A 44 (1) GenBank: KT276153 (Grd23) H ^A 45 (1) GenBank: KT276165 (Grd18)	COI: No data
		21°19'37.1"S 47°14'19.6"W	4	D-Loop: No data	HG4: H ^A 14 (2) GenBank: KT276030 (Grd29) KT276040 (Grd30) H ^A 17 (1) GenBank: KT276038 (Grd13) H ^A 23 (1) GenBank: KT276059 (Grd14)	COI: No data
Paraná River Main Channel	PR	22° 37' 57.3" S -53° 03' 09.4" W	2	D-Loop: No data	HG4: H ^A 3 (1) GenBank: KT276098 (Prn02) HG10: H ^A 40 (1) GenBank: KT276132 (Prn03)	COI: No data
		22° 43' 03.2" S 53° 17' 27.6" W	1	D-Loop: No data	HG10: H ^A 5 (1) GenBank: KT276013(Prn06)	COI: No data
		20° 44' 43.7" S 49° 46' 45.3" W	4	D-Loop: No data	HG10: H ^A 5 (4) GenBank: KT276107 (Prn08) KT276109 (Prn11) KT276110 (Prn07) KT276161 (Prn09)	COI: No data
		22° 39' 01.2" S 53° 05' 29.9 W	1	D-Loop: No data	HG10: H ^A 25 (1) GenBank: KT276068 (Prn01)	COI: No data
		22° 38' 52.4" S 53° 04' 43.0" W	2	D-Loop: No data	HG10: H ^A 37 (2) GenBank: KT276126 (Prn04) KT276128 (Prn05)	COI: No data



TABLE S1 | (Continued)

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype –
				H ^D number	H ^A number (respective D-loop – D number)	H ^C number (respective D-loop – D number)
Ribeira de Iguape River	RG	23° 48' 00" S 46° 55' 00" W	20	D-Loop: No data	HG2: H ^A 4 (20) GenBank: KT276120 (Rib01) KT276155 (Rib02) KT276177 (Rib03) KT276130 (Rib04) KT276018 (Rib05) KT276012 (Rib06) KT276121 (Rib07) KT276037 (Rib08) KT276051 (Rib09) KT276048 (Rib10) KT276162 (Rib11) KT276140 (Rib12) KT276131 (Rib13) KT276052 (Rib14) KT276064 (Rib15) KT276014 (Rib16) KT276061 (Rib17) KT276024 (Rib18) KT276099 (Rib19) KT276125 (Rib20)	COI: No data
				D-Loop: No data	HG2: H ^A 4 (4) GenBank: KT276138 (Tie02) KT276074 (Tie03) KT276175 (Tie09) KT276055 (Tie11)	COI: No data
				D-Loop: No data	HG2: H ^A 4 (1) GenBank: KT276071 (Tie25) H ^A 32 (1) GenBank: KT276105 (Tie26)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (2) GenBank: KT276035 (Tie16) KT276160 (Tie24)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276114 (Tie15) KT276150 (Tie17) KT276115 (Tie18) KT276063 (Tie19)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
Tietê River	TI	23° 40' 50.29" S 46° 45' 25.70" W	6	D-Loop: No data	HG2: H ^A 4 (4) GenBank: KT276138 (Tie02) KT276074 (Tie03) KT276175 (Tie09) KT276055 (Tie11)	COI: No data
				D-Loop: No data	HG2: H ^A 4 (1) GenBank: KT276071 (Tie25) H ^A 32 (1) GenBank: KT276105 (Tie26)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (2) GenBank: KT276035 (Tie16) KT276160 (Tie24)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276114 (Tie15) KT276150 (Tie17) KT276115 (Tie18) KT276063 (Tie19)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (4) GenBank: KT276180 (Tie20) KT276076 (Tie21) KT276082 (Tie22) KT276163 (Tie23)	COI: No data

TABLE S1 | (Continued)

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype –
				H ^D number	H ^A number (respective D-loop – D number)	H ^C number (respective D-loop – D number)
Tietê River	TI	22° 21' 51" S 47° 30' 49" W	3	D-Loop: No data	HG2: H ^A 8 (3) GenBank: KT276086 (Tie12) KT276148 (Tie14) KT276147 (Tie27)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (3) GenBank: KT276094 (Tie04) KT276143 (Tie08) KT276145 (Tie07) H ^A 27 (1) GenBank: KT276085 (Tie06) H ^A 28 (1) GenBank: KT276087 (Tie05)	COI: No data
				D-Loop: No data	HG2: H ^A 15 (1) GenBank: KT276031 (Tie28) H ^A 22 (4) GenBank: KT276144 (Tie29) KT276100 (Tie30) KT276092 (Tie32) KT276058 (Tie33) H ^A 29 (1) GenBank: KT276089 (Tie31)	COI: No data
				D-Loop: No data	HG9: H ^A 16 (2) GenBank: KT276033 (Iva01) KT276156 (Iva02)	COI: No data
				D-Loop: No data	HG4: H ^A 39(1) GenBank: KT276129 (Iva03)	COI: No data
				D-Loop: No data	HG2: H ^A 4 (3) GenBank: KT276060 (Pnb10) KT276172 (Pnb11) KT276106 (Pnb13) H ^A 8 (6) GenBank: KT276067 (Pnb03) KT276176 (Pnb04) KT276078 (Pnb05) KT276021 (Pnb07) KT276056 (Pnb08) KT276080 (Pnb12)	COI: No data
				D-Loop: No data	HG4: H ^A 3(1) GenBank: KT276011 (Ppn09) KT276122 (Ppn10)	COI: No data
				D-Loop: No data	HG1: H ^A 46 (1) GenBank: KT276173 (Ppn40)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (3) GenBank: KT276086 (Tie12) KT276148 (Tie14) KT276147 (Tie27)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (3) GenBank: KT276094 (Tie04) KT276143 (Tie08) KT276145 (Tie07) H ^A 27 (1) GenBank: KT276085 (Tie06) H ^A 28 (1) GenBank: KT276087 (Tie05)	COI: No data
				D-Loop: No data	HG2: H ^A 15 (1) GenBank: KT276031 (Tie28) H ^A 22 (4) GenBank: KT276144 (Tie29) KT276100 (Tie30) KT276092 (Tie32) KT276058 (Tie33) H ^A 29 (1) GenBank: KT276089 (Tie31)	COI: No data
				D-Loop: No data	HG9: H ^A 16 (2) GenBank: KT276033 (Iva01) KT276156 (Iva02)	COI: No data
				D-Loop: No data	HG4: H ^A 39(1) GenBank: KT276129 (Iva03)	COI: No data
				D-Loop: No data	HG2: H ^A 4 (3) GenBank: KT276060 (Pnb10) KT276172 (Pnb11) KT276106 (Pnb13) H ^A 8 (6) GenBank: KT276067 (Pnb03) KT276176 (Pnb04) KT276078 (Pnb05) KT276021 (Pnb07) KT276056 (Pnb08) KT276080 (Pnb12)	COI: No data
				D-Loop: No data	HG4: H ^A 3(1) GenBank: KT276011 (Ppn09) KT276122 (Ppn10)	COI: No data
				D-Loop: No data	HG1: H ^A 46 (1) GenBank: KT276173 (Ppn40)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (3) GenBank: KT276086 (Tie12) KT276148 (Tie14) KT276147 (Tie27)	COI: No data
				D-Loop: No data	HG2: H ^A 8 (3) GenBank: KT276094 (Tie04) KT276143 (Tie08) KT276145 (Tie07) H ^A 27 (1) GenBank: KT276085 (Tie06) H ^A 28 (1) GenBank: KT276087 (Tie05)	COI: No data
				D-Loop: No data	HG2: H ^A 15 (1) GenBank: KT276031 (Tie28) H ^A 22 (4) GenBank: KT276144 (Tie29) KT276100 (Tie30) KT276092 (Tie32) KT276058 (Tie33) H ^A 29 (1) GenBank: KT276089 (Tie31)	COI: No data
Ivaí River	IV	24°04'24.60" S 52°17'28.02" W	3	D-Loop: No data	HG9: H ^A 16 (2) GenBank: KT276033 (Iva01) KT276156 (Iva02)	COI: No data
Paranaíba River	PB	17° 56' 24" S 47° 29' 08" W	9	D-Loop: No data	HG2: H ^A 4 (3) GenBank: KT276060 (Pnb10) KT276172 (Pnb11) KT276106 (Pnb13) H ^A 8 (6) GenBank: KT276067 (Pnb03) KT276176 (Pnb04) KT276078 (Pnb05) KT276021 (Pnb07) KT276056 (Pnb08) KT276080 (Pnb12)	COI: No data
Peixe River, tributary of Paraná River main channel	PX	21° 52' 00" S 51° 37' 23" W	1	D-Loop: No data	HG4: H ^A 3(1) GenBank: KT276011 (Ppn09) KT276122 (Ppn10)	COI: No data
Tibagi River	TB	22° 30' 38" S 52° 21' 46" W	1	D-Loop: No data	HG1: H ^A 46 (1) GenBank: KT276173 (Ppn40)	COI: No data



TABLE S1 | (Continued)

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype –
				H ^D number	H ^A number (respective D-loop = D number)	H ^C number (respective D-loop = D number)
Paranapanema River (lower)	PP ^L	22° 30' 53" S 52° 08' 41" W	1	D-Loop: No data	HG1: H ^A 01 (1) GenBank: KT276009 (Ppn08) H ^A 13 (1) GenBank: KT276028 (Ppn39)	COI: No data
Capivara River, Paranapanema River basin	CP	22° 36' 01" S 50° 40' 31" W	9	D-Loop: No data	HG1: H ^A 09 (3) GenBank: KT276084 (Ppn03) KT276057 (Ppn06) KT276097 (Ppn37) HG3: H ^A 21 (2) GenBank: KT276096 (Ppn04) KT276054 (Ppn07) H ^A 24 (1) GenBank: KT276102 (Ppn05) H ^A 34 (1) GenBank: KT276116 (Ppn38) H ^A 41 (1) GenBank: KT276137 (Ppn02)	COI: No data
	CP	22° 38' 51" S 50° 29' 51" W	1	D-Loop: No data	HG3: H ^A 24(1) GenBank: KT276066 (Ppn01)	COI: No data
Paranapanema River (lower)	PP ^L	23° 01' 27.4" S 48° 49' 41.0" W	4	D-Loop: No data	HG2: H ^A 8 (3) GenBank: KT276041 (Ppn43) KT276088 (Ppn44) KT276167 (Ppn45) H ^A 33 (1) GenBank: KT276113 (Ppn42)	COI: No data
Pardo River	PA	23° 29' 01.0" S 51° 04' 40.7" W	1	D-Loop: No data	HG1: H ^A 9 (1) GenBank: KT276019 (Ppn46)	COI: No data
Pirapó River	PI	23° 22' 28" S 51° 58' 09" W	16	D-Loop: No data	HG1: H ^A 9 (7) GenBank: KT276053 (Ppn13) KT276062 (Ppn15) KT276027 (Ppn18) KT276103 (Ppn24) KT276083 (Ppn27) KT276141 (Ppn28) KT276073 (Ppn34) H ^A 11 (5) GenBank: KT276046 (Ppn14) KT276025 (Ppn19) KT276081 (Ppn20) KT276091 (Ppn23)	COI: No data



TABLE S1 | (Continued)

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype –
				H ^p number	H ^a number (respective D-loop – D number)	H ^c number (respective D-loop – D number)
Pirapó River	PI	23° 22' 28" S 51° 58' 09" W	16	D-Loop: No data	KT276135 (Ppn26)	
					H ^a 18 (1)	
					GenBank: KT276044 (Ppn25)	
					H ^a 20(2)	
					GenBank: KT276049 (Ppn12)	
					KT276117 (Ppn22)	
Pirapó River	PI	23° 26' 34" S 51° 46' 11" W	4		H ^a 38 (1)	
					GenBank: KT276127 (Ppn17)	
				D-Loop: No data	HG1:	COI: No data
					H ^a 9 (3)	
					GenBank: KT276036 (Ppn35)	
					KT276077 (Ppn21)	
		23° 23' 00" S 51° 56' 30" W	2		KT276093 (Ppn36)	
					H ^a 11 (1)	
					GenBank: KT276065 (Ppn32)	
				D-Loop: No data	HG1:	COI: No data
					H ^a 9 (2)	
					GenBank: KT276050 (Ppn31)	
Paranapanema River (lower)	PP ^L	22°30' 53" S 52° 08' 41" W	1		KT276166 (Ppn33)	
				D-Loop: No data	HG1:	COI: No data
					H ^a 9 (3)	
					GenBank: KT276139 (Ppn29)	
					KT276111 (Ppn30)	
					KT276171 (Ppn41)	
Tibagi River	TI	23°40'54.90" S 51°18'54.78" W	3	D-Loop: No data	HG1:	COI: No data
					H ^a 13 (1)	
					GenBank: KT276028 (Ppn39)	
					H ^a 30 (1)	
					GenBank: KT276101 (Ppn49)	
					HG9:	
		23°39'23.16" S 51°14'33.72" W	1		H ^a 43 (1)	
					GenBank: KT276146 (Ppn47)	
					H ^a 48 (1)	
					GenBank: KT276181 (Ppn48)	
Itaipu reservoir tributaries	IR	24°53'10.49"S 54° 3'9.92"W	3	D-Loop: No data	HG1:	COI: No data
					H ^a 31 (1)	
					GenBank: KT276104 (Ppn50)	
				D-Loop: No data	ATPase: No data	HG9:
						H ^c 16 (2)
						GenBank: MN880037
						MN880038
						BOLD: GBMND27969-21
						GBMND27970-21
						H ^c 23 (1)
						GenBank: MN880039
						BOLD: GBMND27972-21

TABLE S1 | (Continued)

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype –
				H ^p number	H ^a number (respective D-loop – D number)	H ^c number (respective D-loop – D number)
Tietê River	TI	22°59'57.8"S 48°00'16.1"W	1	D-Loop: No data	ATPase: No data	HG1: GenBank: MT066232
		21°10'36.5"S 49°10'21.0"W	2	D-Loop: No data	ATPase: No data	HG9: H ^c 19 (1) GenBank: GU701727 BOLD: FUPR614-09 H ^c 20 (1) GenBank: GU701726 BOLD: FUPR616-09
		22°56'28.9"S 48°35'03.2"W	1	D-Loop: No data	ATPase: No data	HG9: H ^c 22 (1) GenBank: JX443491
		23°23'27.6"S 50°59'45.6"W	1	D-Loop: No data	ATPase: No data	HG1: H ^c 17 (1) GenBank: KM897277 BOLD: PDCAP214-14
Grande River	RG	21°10'8.34"S 45° 7'22.93"W	1	D-Loop: No data	ATPase: No data	HG9: H ^c 18 (1) GenBank: MK959857 BOLD: GBMND68750-21
Upper Tocantins River basin	TO	15°32'46.0"S 47°51'41.3"W	1	D-Loop: No data	ATPase: No data	HG5: H ^c 21 (1) GenBank: MK464111 BOLD: ANGBF56561-19
Ivinhema River basin	IN	22° 7'2.88"S 53°55'19.71"W	1	D-Loop: No data	ATPase: No data	HG7: H ^c 24 (1) GenBank: MN854458
Paraguay River	PY	not informed	1	<i>Hypostomus regani</i> GenBank: MN837762	ATPase: No data	COI: No data
Tibagi River	TB	23°18'44.19"S 50°59'48.98"W	1	<i>Hypostomus nigromaculatus</i> GenBank: AJ318355	ATPase: No data	COI: No data
Paraná River	PR	27°34'58.46"S 56°41'42.42"W	1	<i>Hypostomus microstomus</i> GenBank: JF290461	ATPase: No data	COI: No data
Paraguay River	PY	not informed	1	D-Loop: No data	<i>Hypostomus piratatu</i> GenBank: KT276136	COI: No data
Cuiabá River	CU	15°36'20.1"S 56°03'06.9"W	1	D-Loop: No data	<i>Hypostomus boulengeri</i> GenBank: KT276152	COI: No data
Paraíba do Sul River	PS	22°13'20.7"S 43°00'51.7"W	1	D-Loop: No data	<i>Hypostomus affinis</i> GenBank: KT276112	COI: No data
Ribeira de Iguape River	RG	24°14'06"S 47°13'43"W	1	D-Loop: No data	<i>Hypostomus interruptus</i> GenBank: KT276029	COI: No data
Jacuí River	JR	28°38'25"S 54°55'00"W	1	D-Loop: No data	<i>Hypostomus commersoni</i> GenBank: KT276182	COI: No data
Peixe River, Grande River basin	PE	21°34'28.02"S 46°19'22.03"W	1	D-Loop: No data	ATPase: No data	<i>Hypostomus microstomus</i> GenBank: GU701698
Claro Stream, Tietê River basin	CL	22°21'51.70"S 47°30'53.25"W	1	D-Loop: No data	ATPase: No data	<i>Hypostomus nigromaculatus</i> GenBank: GU701689
Araguari River, Paraíba River basin	AR	19°36'53.41"S 47°27'19.59"W	1	D-Loop: No data	ATPase: No data	<i>Hypostomus regani</i> GenBank: GU701964



TABLE S1 | (Continued)

Drainages	Code	Geographic Coordinates	N	D-loop haplotype –	ATPase haplotype –	COI haplotype –
				H ^D number	H ^A number (respective D-loop – D number)	H ^C number (respective D-loop – D number)
Guandu River	GU	22°48'42.6"S 43°37'42.8"W	1	<i>Hypostomus affinis</i> GenBank: KT239013	<i>Hypostomus affinis</i> GenBank: KT239013	<i>Hypostomus affinis</i> GenBank: KT239013
Lower La Plata basin	LP	33°55'25.78"S 58°28'51.23"W	1	<i>Hypostomus commersoni</i> GenBank: MG457240	<i>Hypostomus commersoni</i> GenBank: MG457240	<i>Hypostomus commersoni</i> GenBank: MG457240
Abaeté River	AB	18°36'22.40"S 45°49'41.67"W	1	<i>Hypostomus francisci</i> GenBank: NC045188	<i>Hypostomus francisci</i> GenBank: NC045188	<i>Hypostomus francisci</i> GenBank: NC045188
Essequibo River basin, Guianas	ES	Aquarium trade	1	<i>Hypostomus plecostomus</i> GenBank: NC025584	<i>Hypostomus plecostomus</i> GenBank: NC025584	<i>Hypostomus plecostomus</i> GenBank: NC025584

TABLE S2 | Table of 96 D-loop haplotypes analyzed for *Hypostomus ancistroides* (92 generated in the present study and four from GenBank), including the names of their respective ATPase and COI haplotypes. GenBank accession number (NCBI) of each D-loop haplotype obtained in the present study is shown in the second column of the table. Lines show the number of individuals per haplotype in each locality (columns). The number of records at each sampling site is presented first for the Laranjinha River main channel (LA¹-LA⁷) and its tributary streams (S1^{LA}-S8^{LA}), followed by the Cinzas River main channel (C1^C-C5) and its tributary streams (S1^{CL}-S6^{CL}), and other sub-basins of the Paranapanema River and upper Paraná River, including Tibagi River main channel (TB¹) and its tributaries (S1^{TB}-S4^{TB} and the Congonhas River - CO), Paranapanema River main channel (PP^M) and a tributary stream (S1^{PP}), tributary streams of the Itaipu Reservoir (IR), as well as the Pirapó (PI), Jaguariaíva (JA), Itapetininga (IT), Piquiri (PQ¹ - upper; PQ² - lower), Ivaí (IV), Iguaçu (IG), Ribeira de Iguape (RG), Grande (GR) and Tietê (TI) rivers. Streams sampled in three sections along the flow include abbreviations for the Upper (U), Middle (M), and Lower (L) sections.

D-Loop	GenBank accession number	Respective ATPase-COI	Laranjinha River and its tributary streams																											
		LA ¹	LA ²	LA ³	LA ⁴	LA ⁵	LA ⁶	LA ⁷	S1 ^{LA-U}	S1 ^{LA-M}	S1 ^{LA-L}	S2 ^{LA-U}	S2 ^{LA-M}	S2 ^{LA-L}	S3 ^{LA-U}	S3 ^{LA-M}	S3 ^{LA-L}	S4 ^{LA}	S5 ^{LA-U}	S5 ^{LA-M}	S5 ^{LA-L}	S6 ^{LA}	S7 ^{LA-U}	S7 ^{LA-M}	S7 ^{LA-L}	S8 ^{LA-U}	S8 ^{LA-M}	S8 ^{LA-L}		
H ^p 01	PQ553089	H ^A 09-H ^c 01																												
H ^p 02	PQ553090	H ^A 09-H ^c 01																												
H ^p 03	PQ553091	H ^A 50-H ^c 01						1																						
H ^p 04	PQ553092	H ^A 09-H ^c 02																												
H ^p 05	PQ553093	H ^A 09-H ^c 02																												
H ^p 06	PQ553094	H ^A 09-H ^c 01				1			1																	1				
H ^p 07	PQ553095	H ^A 50-H ^c 03																												
H ^p 08	PQ553096	H ^A 51-H ^c 04																				1								
H ^p 09	PQ553097	H ^A 52-H ^c 04					1	3	4													1								
H ^p 10	PQ553098	H ^A 53-H ^c 04	28	28	24	22	29	6	13	2	2	3	29	30	22	25	21	22	5	21	30	24	1	10	12	11		3	5	
H ^p 11	PQ553099	H ^A 54-H ^c 12																												
H ^p 12	PQ553100	H ^A 55-H ^c 12																												
H ^p 13	PQ553101	H ^A 51-H ^c 03																									3		1	
H ^p 14	PQ553102	H ^A 09-H ^c 01																												
H ^p 15	PQ553103	H ^A 09-H ^c 01																												
H ^p 16	PQ553104	H ^A 56-H ^c 04																												
H ^p 17	PQ553105	H ^A 57-H ^c 04																												
H ^p 18	PQ553106	H ^A 09-H ^c 01																												
H ^p 19	PQ553107	H ^A 09-H ^c 01						1	1																1					
H ^p 20	PQ553108	H ^A 09-H ^c 04																												
H ^p 21	PQ553109	H ^A 51-H ^c 04																												
H ^p 22	PQ553110	H ^A 57-H ^c 04																												
H ^p 23	PQ553111	H ^A 57-H ^c 04																												
H ^p 24	PQ553112	H ^A 57-H ^c 04																												
H ^p 25	PO553113	H ^A 51-H ^c 04																												

TABLE S2 | (Continued)

D-Loop	GenBank accession number	Respective ATPase-COI	Laranjinha River and its tributary streams																											
		haplotypes	LA ¹	LA ²	LA ³	LA ⁴	LA ⁵	LA ⁶	LA ⁷	S1 ^{LA-U}	S1 ^{LA-M}	S1 ^{LA-L}	S2 ^{LA-U}	S2 ^{LA-M}	S2 ^{LA-L}	S3 ^{LA-U}	S3 ^{LA-M}	S3 ^{LA-L}	S4 ^{LA}	S5 ^{LA-U}	S5 ^{LA-M}	S5 ^{LA-L}	S6 ^{LA}	S7 ^{LA-U}	S7 ^{LA-M}	S7 ^{LA-L}	S8 ^{LA-U}	S8 ^{LA-M}	S8 ^{LA-L}	
H ^p 26	PQ553114	H ^A 51-H ^C 04																												
H ^p 27	PQ553115	H ^A 58-H ^C 05																												
H ^p 28	PQ553116	H ^A 58-H ^C 05																												
H ^p 29	PQ553117	H ^A 58-H ^C 05																												
H ^p 30	PQ553118	H ^A 51-H ^C 04							1	6																1	1			
H ^p 31	PQ553119	H ^A 59-H ^C 03								2																	1			
H ^p 32	PQ553120	H ^A 51-H ^C 04																												
H ^p 33	PQ553121	H ^A 51-H ^C 03								1																	2			
H ^p 34	PQ553122	H ^A 51-H ^C 04																												
H ^p 35	PQ553123	H ^A 51-H ^C 04																												
H ^p 36	PQ553124	H ^A 51-H ^C 03																												
H ^p 37	PQ553125	H ^A 51-H ^C 04																												
H ^p 38	PQ553126	H ^A 51-H ^C 04																												
H ^p 39	PQ553127	H ^A 51-H ^C 04																												
H ^p 40	PQ553128	H ^A 51-H ^C 04																												
H ^p 41	PQ553129	H ^A 60-H ^C 04																												
H ^p 42	PQ553130	H ^A 24-H ^C 04																												
H ^p 43	PQ553131	H ^A 09-H ^C 01																												
H ^p 44	PQ553132	H ^A 09-H ^C 01																												
H ^p 45	PQ553133	H ^A 09-H ^C 01																												
H ^p 49	PQ553134	H ^A 61-H ^C 06																												
H ^p 50	PQ553135	H ^A 62-H ^C 06																												
H ^p 51	PQ553136	H ^A 63-H ^C 05																												
H ^p 52	PQ553137	H ^A 63-H ^C 05																												
H ^p 53	PQ553138	H ^A 58-H ^C 05																												
H ^p 54	PQ553139	H ^A 58-H ^C 05																												
H ^p 55	PQ553140	H ^A 58-H ^C 05																												
H ^p 56	PQ553141	H ^A 58-H ^C 05																												
H ^p 57	PQ553142	H ^A 09-H ^C 02																												
H ^p 58	PQ553143	H ^A 64-H ^C 02																												
H ^p 59	PQ553144	H ^A 11-H ^C 02																												
H ^p 60	PQ553145	H ^A 38-H ^C 01																												
H ^p 61	PQ553146	H ^A 09-H ^C 01																												
H ^p 62	PQ553147	H ^A 24-H ^C 04																												
H ^p 63	PQ553148	H ^A 60-H ^C 03	1						11	3		1														4	9	27	21	12
H ^p 64	PQ553149	H ^A 09-H ^C 01																												
H ^p 65	PQ553150	H ^A 51-H ^C 03																		1					8	10	5		2	1
H ^p 66	PQ553151	H ^A 65-H ^C 04	1			1						2						1		2										
H ^p 67	PQ553152	H ^A 53-H ^C 03	1	1		6	3				2	1	2							4		6								
H ^p 68	PQ553153	H ^A 53-H ^C 07		1	2		5	1							1															
H ^p 69	PQ553154	H ^A 60-H ^C 04			1						28	26	23		2			1										1		
H ^p 70	PQ553155	H ^A 60-H ^C 04			1													1												
H ^p 71	PQ553156	H ^A 60-H ^C 04					1												1											
H ^p 72	PQ553157	H ^A 61-H ^C 06								1																				
H ^p 73	PQ553158	H ^A 09-H ^C 01								1																				
H ^p 74	PQ553159	H ^A 60-H ^C 03											1																	
H ^p 75	PQ553160	H ^A 66-H ^C 03															5	3												
H ^p 76	PQ553161	H ^A 60-H ^C 03																		1										
H ^p 77	PQ553162	H ^A 51-H ^C 03																										1		

TABLE S2 | (Continued)

D-Loop	GenBank accession number	Respective ATPase-COI	Laranjinha River and its tributary streams																											
		haplotypes	LA ¹	LA ²	LA ³	LA ⁴	LA ⁵	LA ⁶	LA ⁷	S1 ^{LA-U}	S1 ^{LA-M}	S1 ^{LA-L}	S2 ^{LA-U}	S2 ^{LA-M}	S2 ^{LA-L}	S3 ^{LA-U}	S3 ^{LA-M}	S3 ^{LA-L}	S4 ^{LA}	S5 ^{LA-U}	S5 ^{LA-M}	S5 ^{LA-L}	S6 ^{LA}	S7 ^{LA-U}	S7 ^{LA-M}	S7 ^{LA-L}	S8 ^{LA-U}	S8 ^{LA-M}	S8 ^{LA-L}	
H ^p 78	PQ553163	H ^A 51-H ^C 03																											2	7
H ^p 79	PQ553164	H ^A 51-H ^C 09																											1	
H ^p 81	PQ553165	H ^A 57-H ^C 04																												
H ^p 82	PQ553166	H ^A 51-H ^C 04																												
H ^p 83	PQ553167	H ^A 67-H ^C 13																												
H ^p 84	PQ553168	H ^A 47-H ^C 10																												
H ^p 85	PQ553169	H ^A 68-H ^C 13																												
H ^p 86	PQ553170	H ^A 69-H ^C 10																												
H ^p 87	PQ553171	H ^A 45-H ^C 11																												
H ^p 88	PQ553172	H ^A 68-H ^C 12																												
H ^p 89	PQ553173	H ^A 68-H ^C 12																												
H ^p 90	PQ553174	H ^A 45-H ^C 11																												
H ^p 91	PQ553175	H ^A 68-H ^C 12																												
H ^p 92	PQ553176	H ^A 02-H ^C 14																												
H ^p 93	PQ553177	H ^A 45-H ^C 11																												
H ^p 94	PQ553178	H ^A 45-H ^C 11																												
H ^p 95	PQ553179	H ^A 45-H ^C 11																												
H ^p 96	PQ553180	H ^A 02-H ^C 14																												

D-Loop	GenBank accession number	Respective ATPase-COI	Cinzas River and its tributary streams																				
		haplotypes	CI ¹	CI ²	CI ³	CI ⁴	CI ⁵	S1 ^{CI-U}	S1 ^{CI-M}	S1 ^{CI-L}	S2 ^{CI}	S3 ^{CI-U}	S3 ^{CI-M}	S3 ^{CI-L}	S4 ^{CI-U}	S4 ^{CI-M}	S4 ^{CI-L}	S5 ^{CI-U}	S5 ^{CI-M}	S5 ^{CI-L}	S6 ^{CI-U}	S6 ^{CI-M}	S6 ^{CI-L}
H ^p 01	PQ553089	H ^A 09-H ^C 01																					
H ^p 02	PQ553090	H ^A 09-H ^C 01																					
H ^p 03	PQ553091	H ^A 50-H ^C 01																					
H ^p 04	PQ553092	H ^A 09-H ^C 02																					
H ^p 05	PQ553093	H ^A 09-H ^C 02																					
H ^p 06	PQ553094	H ^A 09-H ^C 01						1														1	
H ^p 07	PQ553095	H ^A 50-H ^C 03																					
H ^p 08	PQ553096	H ^A 51-H ^C 04																					
H ^p 09	PQ553097	H ^A 52-H ^C 04				2	1											1	1	3		1	2
H ^p 10	PQ553098	H ^A 53-H ^C 04	3	3			3					2				1				2	3	9	12
H ^p 11	PQ553099	H ^A 54-H ^C 12																					
H ^p 12	PQ553100	H ^A 55-H ^C 12																					
H ^p 13	PQ553101	H ^A 51-H ^C 03			1							3	3			3	5	1		1		2	
H ^p 14	PQ553102	H ^A 09-H ^C 01																					
H ^p 15	PQ553103	H ^A 09-H ^C 01																					
H ^p 16	PQ553104	H ^A 56-H ^C 04		3							2												
H ^p 17	PQ553105	H ^A 57-H ^C 04	7	20				20	1		3												
H ^p 18	PQ553106	H ^A 09-H ^C 01																					
H ^p 19	PQ553107	H ^A 09-H ^C 01																					
H ^p 20	PQ553108	H ^A 09-H ^C 04																					
H ^p 21	PQ553109	H ^A 51-H ^C 04		2																			
H ^p 22	PQ553110	H ^A 57-H ^C 04		3																			
H ^p 23	PQ553111	H ^A 57-H ^C 04	1	1																			
H ^p 24	PQ553112	H ^A 57-H ^C 04	1	1																			
H ^p 25	PQ553113	H ^A 51-H ^C 04			2	10	2					21	27	16	16	16	14	9	19	19			
H ^p 26	PQ553114	H ^A 51-H ^C 04			1	3								1	2	14	8	7	13	2	2		



TABLE S2 | (Continued)

D-Loop	GenBank accession number	Respective ATPase-COI	Cinzas River and its tributary streams																					
		haplotypes	CI ^I	CI ^{II}	CI ^{III}	CI ^{IV}	CI ^V	S1 ^U	S1 ^C M	S1 ^a L	S2 ^U	S3 ^a U	S3 ^C M	S3 ^a L	S4 ^a U	S4 ^C M	S4 ^a L	S5 ^a U	S5 ^C M	S5 ^a L	S6 ^a U	S6 ^C M	S6 ^a L	
H ^p 27	PQ553115	H ^A 58-H ^C 05						6	24	8														
H ^p 28	PQ553116	H ^A 58-H ^C 05						1																
H ^p 29	PQ553117	H ^A 58-H ^C 05							1															
H ^p 30	PQ553118	H ^A 51-H ^C 04					3													1	8	10	8	
H ^p 31	PQ553119	H ^A 59-H ^C 03					6														2	3	4	
H ^p 32	PQ553120	H ^A 51-H ^C 04																				1		
H ^p 33	PQ553121	H ^A 51-H ^C 03			1		1															1	2	
H ^p 34	PQ553122	H ^A 51-H ^C 04				1	1														1		2	
H ^p 35	PQ553123	H ^A 51-H ^C 04																3						
H ^p 36	PQ553124	H ^A 51-H ^C 03																3	6	1				
H ^p 37	PQ553125	H ^A 51-H ^C 04																1	4	2				
H ^p 38	PQ553126	H ^A 51-H ^C 04													1	1	2							
H ^p 39	PQ553127	H ^A 51-H ^C 04				1										1	1							
H ^p 40	PQ553128	H ^A 51-H ^C 04															1							
H ^p 41	PQ553129	H ^A 60-H ^C 04									1													
H ^p 42	PQ553130	H ^A 24-H ^C 04																						
H ^p 43	PQ553131	H ^A 09-H ^C 01																						
H ^p 44	PQ553132	H ^A 09-H ^C 01																						
H ^p 45	PQ553133	H ^A 09-H ^C 01																						
H ^p 49	PQ553134	H ^A 61-H ^C 06																						
H ^p 50	PQ553135	H ^A 62-H ^C 06																						
H ^p 51	PQ553136	H ^A 63-H ^C 05																						
H ^p 52	PQ553137	H ^A 63-H ^C 05																						
H ^p 53	PQ553138	H ^A 58-H ^C 05																						
H ^p 54	PQ553139	H ^A 58-H ^C 05						2																
H ^p 55	PQ553140	H ^A 58-H ^C 05																						
H ^p 56	PQ553141	H ^A 58-H ^C 05																						
H ^p 57	PQ553142	H ^A 09-H ^C 02																						
H ^p 58	PQ553143	H ^A 64-H ^C 02																						
H ^p 59	PQ553144	H ^A 11-H ^C 02																						
H ^p 60	PQ553145	H ^A 38-H ^C 01																						
H ^p 61	PQ553146	H ^A 09-H ^C 01																						
H ^p 62	PQ553147	H ^A 24-H ^C 04					1																	
H ^p 63	PQ553148	H ^A 60-H ^C 03					1																	
H ^p 64	PQ553149	H ^A 09-H ^C 01					1																	
H ^p 65	PQ553150	H ^A 51-H ^C 03					1																	
H ^p 66	PQ553151	H ^A 65-H ^C 04																						
H ^p 67	PQ553152	H ^A 53-H ^C 03																						
H ^p 68	PQ553153	H ^A 53-H ^C 07																						
H ^p 69	PQ553154	H ^A 60-H ^C 04																						
H ^p 70	PQ553155	H ^A 60-H ^C 04																						
H ^p 71	PQ553156	H ^A 60-H ^C 04																						
H ^p 72	PQ553157	H ^A 61-H ^C 06																						
H ^p 73	PQ553158	H ^A 09-H ^C 01																						
H ^p 74	PQ553159	H ^A 60-H ^C 03																						
H ^p 75	PQ553160	H ^A 66-H ^C 03																						
H ^p 76	PQ553161	H ^A 60-H ^C 03																						
H ^p 77	PQ553162	H ^A 51-H ^C 03																						



TABLE S2 | (Continued)

D-Loop	GenBank accession number	Respective ATPase-COI	Cinzas River and its tributary streams																					
		haplotypes	CI ¹	CI ²	CI ³	CI ⁴	CI ⁵	S1 ^{CI-U}	S1 ^{CI-M}	S1 ^{CI-L}	S2 ^{CI}	S3 ^{CI-U}	S3 ^{CI-M}	S3 ^{CI-L}	S4 ^{CI-U}	S4 ^{CI-M}	S4 ^{CI-L}	S5 ^{CI-U}	S5 ^{CI-M}	S5 ^{CI-L}	S6 ^{CI-U}	S6 ^{CI-M}	S6 ^{CI-L}	
H ^p 78	PQ553163	H ^A 51-H ^C 03																						
H ^p 79	PQ553164	H ^A 51-H ^C 09																						
H ^p 81	PQ553165	H ^A 57-H ^C 04	1																					
H ^p 82	PQ553166	H ^A 51-H ^C 04				1																		
H ^p 83	PQ553167	H ^A 67-H ^C 13																						
H ^p 84	PQ553168	H ^A 47-H ^C 10																						
H ^p 85	PQ553169	H ^A 68-H ^C 13																						
H ^p 86	PQ553170	H ^A 69-H ^C 10																						
H ^p 87	PQ553171	H ^A 45-H ^C 11																						
H ^p 88	PQ553172	H ^A 68-H ^C 12																						
H ^p 89	PQ553173	H ^A 68-H ^C 12																						
H ^p 90	PQ553174	H ^A 45-H ^C 11																						
H ^p 91	PQ553175	H ^A 68-H ^C 12																						
H ^p 92	PQ553176	H ^A 02-H ^C 14																						
H ^p 93	PQ553177	H ^A 45-H ^C 11																						
H ^p 94	PQ553178	H ^A 45-H ^C 11																						
H ^p 95	PQ553179	H ^A 45-H ^C 11																						
H ^p 96	PQ553180	H ^A 02-H ^C 14																						

D-Loop	GenBank accession number	Respective ATPase-COI haplotypes	Other sub-basins of the Paranapanema River and Upper Paraná River																		
			JA	TB	S1 ^{TB}	S2 ^{TB}	S3 ^{TB}	S4 ^{TB}	CO	PI	IT	PP ^M	S1 ^{BP}	IV	PQ ¹	PQ ²	IG	IR	TI	GR	RG
H ^p 01	PQ553089	H ^A 09-H ^C 01			2	3		1		6		3									
H ^p 02	PQ553090	H ^A 09-H ^C 01			1																
H ^p 03	PQ553091	H ^A 50-H ^C 01			2				2			2									
H ^p 04	PQ553092	H ^A 09-H ^C 02				2				3											
H ^p 05	PQ553093	H ^A 09-H ^C 02					2														
H ^p 06	PQ553094	H ^A 09-H ^C 01		2			3	2	11	4		6	1								
H ^p 07	PQ553095	H ^A 50-H ^C 03						1													
H ^p 08	PQ553096	H ^A 51-H ^C 04																			
H ^p 09	PQ553097	H ^A 52-H ^C 04											3								
H ^p 10	PQ553098	H ^A 53-H ^C 04										1									
H ^p 11	PQ553099	H ^A 54-H ^C 12												3							
H ^p 12	PQ553100	H ^A 55-H ^C 12												1							
H ^p 13	PQ553101	H ^A 51-H ^C 03											5								
H ^p 14	PQ553102	H ^A 09-H ^C 01								3											
H ^p 15	PQ553103	H ^A 09-H ^C 01								1											
H ^p 16	PQ553104	H ^A 56-H ^C 04																			
H ^p 17	PQ553105	H ^A 57-H ^C 04																			
H ^p 18	PQ553106	H ^A 09-H ^C 01										1									
H ^p 19	PQ553107	H ^A 09-H ^C 01		5					3			2									
H ^p 20	PQ553108	H ^A 09-H ^C 04										1									
H ^p 21	PQ553109	H ^A 51-H ^C 04																			
H ^p 22	PQ553110	H ^A 57-H ^C 04																			
H ^p 23	PQ553111	H ^A 57-H ^C 04																			
H ^p 24	PQ553112	H ^A 57-H ^C 04																			
H ^p 25	PQ553113	H ^A 51-H ^C 04		1																	
H ^p 26	PQ553114	H ^A 51-H ^C 04																			
H ^p 27	PQ553115	H ^A 58-H ^C 05																			



TABLE S2 | (Continued)

D-Loop	GenBank accession number	Respective ATPase-COI	Other sub-basins of the Paranapanema River and Upper Paraná River																		
		haplotypes	JA	TB	S1 ^{TB}	S2 ^{TB}	S3 ^{TB}	S4 ^{TB}	CO	PI	IT	PP ^M	S1 ^{PP}	IV	PQ ^I	PQ ²	IG	IR	TI	GR	RG
H ^p 28	PQ553116	H ^A 58-H ^C 05																			
H ^p 29	PQ553117	H ^A 58-H ^C 05																			
H ^p 30	PQ553118	H ^A 51-H ^C 04																			
H ^p 31	PQ553119	H ^A 59-H ^C 03																			
H ^p 32	PQ553120	H ^A 51-H ^C 04																			
H ^p 33	PQ553121	H ^A 51-H ^C 03																			
H ^p 34	PQ553122	H ^A 51-H ^C 04																			
H ^p 35	PQ553123	H ^A 51-H ^C 04																			
H ^p 36	PQ553124	H ^A 51-H ^C 03																			
H ^p 37	PQ553125	H ^A 51-H ^C 04																			
H ^p 38	PQ553126	H ^A 51-H ^C 04																			
H ^p 39	PQ553127	H ^A 51-H ^C 04																			
H ^p 40	PQ553128	H ^A 51-H ^C 04																			
H ^p 41	PQ553129	H ^A 60-H ^C 04																			
H ^p 42	PQ553130	H ^A 24-H ^C 04							1												
H ^p 43	PQ553131	H ^A 09-H ^C 01							1												
H ^p 44	PQ553132	H ^A 09-H ^C 01							1												
H ^p 45	PQ553133	H ^A 09-H ^C 01		1																	
H ^p 46	AJ412843																	2			
	MT066232																				
H ^p 47	AJ318369																		1		
H ^p 48	AJ412846																			1	
H ^p 49	PQ553134	H ^A 61-H ^C 06								1											
H ^p 50	PQ553135	H ^A 62-H ^C 06								1											
H ^p 51	PQ553136	H ^A 63-H ^C 05	7																		
H ^p 52	PQ553137	H ^A 63-H ^C 05	1																		
H ^p 53	PQ553138	H ^A 58-H ^C 05	1																		
H ^p 54	PQ553139	H ^A 58-H ^C 05	2																		
H ^p 55	PQ553140	H ^A 58-H ^C 05	1																		
H ^p 56	PQ553141	H ^A 58-H ^C 05	16																		
H ^p 57	PQ553142	H ^A 09-H ^C 02							12												
H ^p 58	PQ553143	H ^A 64-H ^C 02							3												
H ^p 59	PQ553144	H ^A 11-H ^C 02							1												
H ^p 60	PQ553145	H ^A 38-H ^C 01							1												
H ^p 61	PQ553146	H ^A 09-H ^C 01							2												
H ^p 62	PQ553147	H ^A 24-H ^C 04																			
H ^p 63	PQ553148	H ^A 60-H ^C 03																			
H ^p 64	PQ553149	H ^A 09-H ^C 01																			
H ^p 65	PQ553150	H ^A 51-H ^C 03																			
H ^p 66	PQ553151	H ^A 65-H ^C 04																			
H ^p 67	PQ553152	H ^A 53-H ^C 03																			
H ^p 68	PQ553153	H ^A 53-H ^C 07																			
H ^p 69	PQ553154	H ^A 60-H ^C 04																			
H ^p 70	PQ553155	H ^A 60-H ^C 04																			
H ^p 71	PQ553156	H ^A 60-H ^C 04																			
H ^p 72	PQ553157	H ^A 61-H ^C 06																			
H ^p 73	PQ553158	H ^A 09-H ^C 01																			
H ^p 74	PQ553159	H ^A 60-H ^C 03																			
H ^p 75	PQ553160	H ^A 66-H ^C 03																			
H ^p 76	PQ553161	H ^A 60-H ^C 03																			

TABLE S2 | (Continued)

D-Loop	GenBank accession number	Respective ATPase-COI	Other sub-basins of the Paranapanema River and Upper Paraná River																		
		haplotypes	JA	TB	S1 ^{TB}	S2 ^{TB}	S3 ^{TB}	S4 ^{TB}	CO	PI	IT	PP ^M	S1 ^{BP}	IV	PQ ¹	PQ ²	IG	IR	TI	GR	RG
H ^P 77	PQ553162	H ^A 51-H ^C 03																			
H ^P 78	PQ553163	H ^A 51-H ^C 03																			
H ^P 79	PQ553164	H ^A 51-H ^C 09																			
H ^P 80	NC052710																		1		
H ^P 81	PQ553165	H ^A 57-H ^C 04																			
H ^P 82	PQ553166	H ^A 51-H ^C 04																			
H ^P 83	PQ553167	H ^A 67-H ^C 13														1	1				
H ^P 84	PQ553168	H ^A 47-H ^C 10													1		1				
H ^P 85	PQ553169	H ^A 68-H ^C 13													1						
H ^P 86	PQ553170	H ^A 69-H ^C 10													1						
H ^P 87	PQ553171	H ^A 45-H ^C 11													1						
H ^P 88	PQ553172	H ^A 68-H ^C 12																2			
H ^P 89	PQ553173	H ^A 68-H ^C 12																2			
H ^P 90	PQ553174	H ^A 45-H ^C 11													1						
H ^P 91	PQ553175	H ^A 68-H ^C 12													1						
H ^P 92	PQ553176	H ^A 02-H ^C 14														1					
H ^P 93	PQ553177	H ^A 45-H ^C 11																1			
H ^P 94	PQ553178	H ^A 45-H ^C 11																1			
H ^P 95	PQ553179	H ^A 45-H ^C 11																1			
H ^P 96	PQ553180	H ^A 02-H ^C 14																1			

TABLE S3 | Table of GenBank accession numbers (NCBI) of the 27 ATPase haplotypes generated in the present study (H^A number), including the frequency recorded for each sampling site. Columns show the number of individuals per haplotype in each locality (Lines): Laranjinha River main channel (LA¹-LA⁷) and its tributary streams (S1^{LA}-S8^{LA}); Cinzas River main channel (C1^C-C5) and its tributary streams (S1^{CL}-S6^{CL}); Tibagi River main channel (TB^T) and its tributaries (S1^{TB}-S4^{TB} and the Congonhas River - CO); Paranapanema River main channel (PP^M) and a tributary stream (S1^{PP}); tributary streams of the Itaipu Reservoir (IR); as well as the Pirapó (PI), Jaguariaíva (JA), Itapetininga (IT), Piquiri (PQ¹ - upper; PQ² - lower), Ivaí (IV), and Iguaçu (IG) rivers. Streams sampled in three sections along the flow include abbreviations for the Upper (U), Middle (M), and Lower (L) sections. Accession numbers of ATPase haplotypes obtained from GenBank are provided in Tab. S1.

Site	GenBank	ATPase haplotypes																									
		H ^A 02	H ^A 09	H ^A 11	H ^A 24	H ^A 38	H ^A 47	H ^A 50	H ^A 51	H ^A 52	H ^A 53	H ^A 54	H ^A 55	H ^A 56	H ^A 57	H ^A 58	H ^A 59	H ^A 60	H ^A 61	H ^A 62	H ^A 63	H ^A 64	H ^A 65	H ^A 66	H ^A 67	H ^A 68	H ^A 69
		PV105475	PV105476	PV105477	PV105478	PV105479	PV105481	PV105482	PV105483	PV105484	PV105485	PV105486	PV105487	PV105488	PV105489	PV105490	PV105491	PV105492	PV105493	PV105494	PV105495	PV105496	PV105497	PV105498	PV105499	PV105500	PV105501
L ¹										29							1					1					
L ²										30																	
L ³										26							2										
L ⁴		1								28												1					
L ⁵									1	37							1										
L ⁶		1					1	1	3	7							11										
L ⁷		3						7	4	13						2	3	1									
S1 ^{LA} -U										4							28										
S1 ^{LA} -M										3							27										

TABLE S3 | (Continued)

Site	GenBank	ATPase haplotypes																									
		H ^A 02	H ^A 09	H ^A 11	H ^A 24	H ^A 38	H ^A 47	H ^A 50	H ^A 51	H ^A 52	H ^A 53	H ^A 54	H ^A 55	H ^A 56	H ^A 57	H ^A 58	H ^A 59	H ^A 60	H ^A 61	H ^A 62	H ^A 63	H ^A 64	H ^A 65	H ^A 66	H ^A 67	H ^A 68	H ^A 69
		PV105475	PV105476	PV105477	PV105478	PV105479	PV105481	PV105482	PV105483	PV105484	PV105485	PV105486	PV105487	PV105488	PV105489	PV105490	PV105491	PV105492	PV105493	PV105494	PV105495	PV105496	PV105497	PV105498	PV105499	PV105500	PV105501
S1 ^{LA} -L										5							23					2					
S2 ^{LA} -U										29							1										
S2 ^{LA} -M										30																	
S2 ^{LA} -L										23							2										
S3 ^{LA} -U										25														5			
S3 ^{LA} -M										21													3				
S3 ^{LA} -L										22							2					1					
S4 ^{LA}										5																	
S5 ^{LA} -U								1		25												2					
S5 ^{LA} -M										30																	
S5 ^{LA} -L										30																	
S6 ^{LA}									1	1	1																
S7 ^{LA} -U								8		10																	
S7 ^{LA} -M			1					11		12							4										
S7 ^{LA} -L			1					8		11						1	9										
S8 ^{LA} -U								4									28										
S8 ^{LA} -M								5		3							21										
S8 ^{LA} -L								9		5							12										
C ¹										3					10												
C ²								2		3				3	25												
C ³								5																			
C ⁴								16	2																		
C ⁵			2		1			8	1	3							6	1									
S1 ^{CI} -U															20	9											
S1 ^{CI} -M															1	25											
S1 ^{CI} -L																8											
S2 ^{CI}														2	3												
S3 ^{CI} -U								24		2								1									
S3 ^{CI} -M								31																			
S3 ^{CI} -L								18																			
S4 ^{CI} -U								31																			
S4 ^{CI} -M								29		1																	
S4 ^{CI} -L								30																			
S5 ^{CI} -U								30	1																		
S5 ^{CI} -M								31	1																		
S5 ^{CI} -L								26	3	2																	
S6 ^{CI} -U								9		3							2										
S6 ^{CI} -M			1					14	1	9							3										

TABLE S2 | (Continued)

Site	GenBank	ATPase haplotypes																									
		H ^A 02	H ^A 09	H ^A 11	H ^A 24	H ^A 38	H ^A 47	H ^A 50	H ^A 51	H ^A 52	H ^A 53	H ^A 54	H ^A 55	H ^A 56	H ^A 57	H ^A 58	H ^A 59	H ^A 60	H ^A 61	H ^A 62	H ^A 63	H ^A 64	H ^A 65	H ^A 66	H ^A 67	H ^A 68	H ^A 69
		PV105475	PV105476	PV105477	PV105478	PV105479	PV105481	PV105482	PV105483	PV105484	PV105485	PV105486	PV105487	PV105488	PV105489	PV105490	PV105491	PV105492	PV105493	PV105494	PV105495	PV105496	PV105497	PV105498	PV105499	PV105500	PV105501
S6 ^{CL} -L								12	2	12						4											
JA															20					8							
TB ¹			8					1																			
S1 ^{TB}			3					2																			
S2 ^{TB}			5																								
S3 ^{TB}			5																								
S4 ^{TB}			3					1																			
CO			16		1			2																			
PI			31	1		1																3					
IT																			1	1							
PP ^M			13					2			1																
S1 ^{PP}			1						5	3																	
IV												3	1														
PQ ¹							3																		2	1	
PQ ²		1																						1			
IG							1																	1			
IR		1					3																		4		

TABLE S4 | Table of GenBank accession numbers (NCBI) of the 13 COI haplotypes (H^c number) generated in the present study (H^c number), including the frequency recorded for each sampling site. Columns show the number of individuals per haplotype in each locality (Lines): Laranjinha River main channel (LA¹-LA⁷) and its tributary streams (S1^{LA}-S8^{LA}); Cinzas River main channel (CI¹-C5) and its tributary streams (S1^{CI}-S6^{CI}); Tibagi River main channel (TB¹) and its tributaries (S1^{TB}-S4^{TB}) and the Congonhas River - CO; Paranapanema River main channel (PP^M) and a tributary stream (S1^{PP}); tributary streams of the Itaipu Reservoir (IR); as well as the Pirapó (PI), Jaguariaíva (JA), Itapetininga (IT), Piquiri (PQ¹ - upper; PQ² - lower), Ivaí (IV), and Iguaçu (IG) rivers. Streams sampled in three sections along the flow include abbreviations for the Upper (U), Middle (M), and Lower (L) sections. Accession numbers of ATPase haplotypes obtained from GenBank are provided in Tab. S1.

Site	GenBank	COI haplotypes													
		H ^c 01	H ^c 02	H ^c 03	H ^c 04	H ^c 05	H ^c 06	H ^c 07	H ^c 09	H ^c 10	H ^c 11	H ^c 12	H ^c 13	H ^c 14	
		PV077306	PV077307	PV077308	PV077309	PV077310	PV077311	PV077312	PV077313	PV077314	PV077315	PV077316	PV077317	PV077318	
L1				2	29										
L2				1	28			1							
L3					26			2							
L4		1		6	23										
L5				3	31			5							
L6		2		11	10			1							
L7		3		6	23		1								
S1 ^{LA} -U				2	30										
S1 ^{LA} -M				2	28										
S1 ^{LA} -L				2	28										
S2 ^{LA} -U				1	29										
S2 ^{LA} -M					30										
S2 ^{LA} -L					24			1							
S3 ^{LA} -U				5	25										
S3 ^{LA} -M				3	21										
S3 ^{LA} -L					25										
S4 ^{LA}					5										
S5 ^{LA} -U				5	23										
S5 ^{LA} -M					30										
S5 ^{LA} -L				6	24										
S6 ^{LA}					3										
S7 ^{LA} -U				8	10										
S7 ^{LA} -M		1		14	13										
S7 ^{LA} -L		1		17	12										
S8 ^{LA} -U				31	1										
S8 ^{LA} -M				25	3				1						
S8 ^{LA} -L				21	5										
C1					13										
C2					33										



TABLE S4 | (Continued)

Site	GenBank	COI haplotypes													
		H ^c 01	H ^c 02	H ^c 03	H ^c 04	H ^c 05	H ^c 06	H ^c 07	H ^c 09	H ^c 10	H ^c 11	H ^c 12	H ^c 13	H ^c 14	
		PV077306	PV077307	PV077308	PV077309	PV077310	PV077311	PV077312	PV077313	PV077314	PV077315	PV077316	PV077317	PV077318	
C3			2	3											
C4				18											
C5		2		9	11										
S1 ^{CL-U}					20	9									
S1 ^{CL-M}					1	25									
S1 ^{CL-L}						8									
S2 ^{CL}					5										
S3 ^{CL-U}				3	24										
S3 ^{CL-M}				3	28										
S3 ^{CL-L}					18										
S4 ^{CL-U}					31										
S4 ^{CL-M}				3	27										
S4 ^{CL-L}				5	25										
S5 ^{CL-U}				4	27										
S5 ^{CL-M}				6	26										
S5 ^{CL-L}				2	29										
S6 ^{CL-U}				2	12										
S6 ^{CL-M}		1		6	21										
S6 ^{CL-L}				6	24										
JA						28									
TB		8			1										
S1 ^{TB}		5													
S2 ^{TB}		3	2												
S3 ^{TB}		3	2												
S4 ^{TB}		3		1											
CO		18			1										
PI		17	19												
IT							2								
PP ^M		14			2										
S ^P 1		1		5	3										
IV											4				
PQ ¹									2	2	1	1			
PQ ²												1	1		
IG									1			1			
IR										3	4		1		

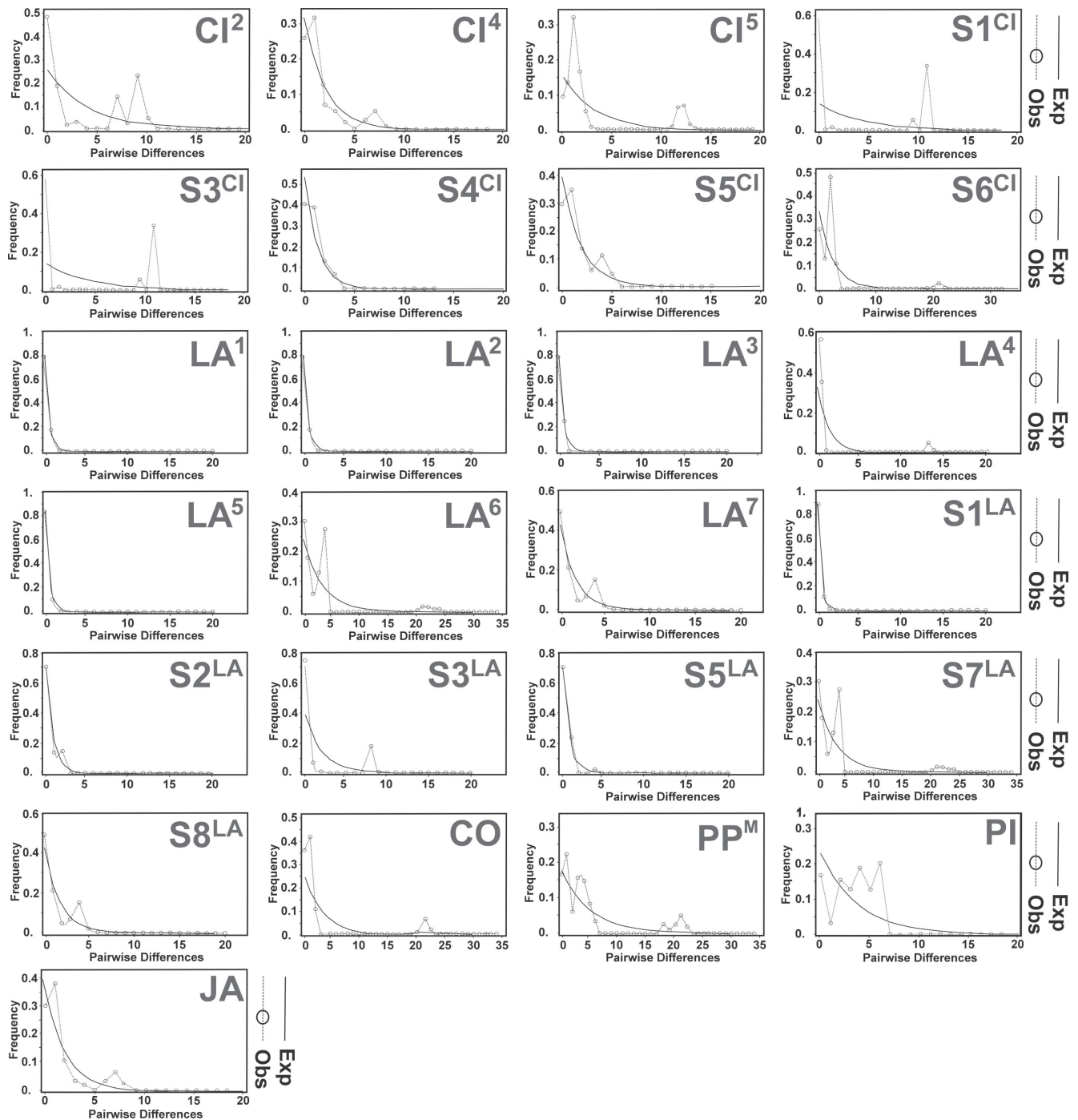
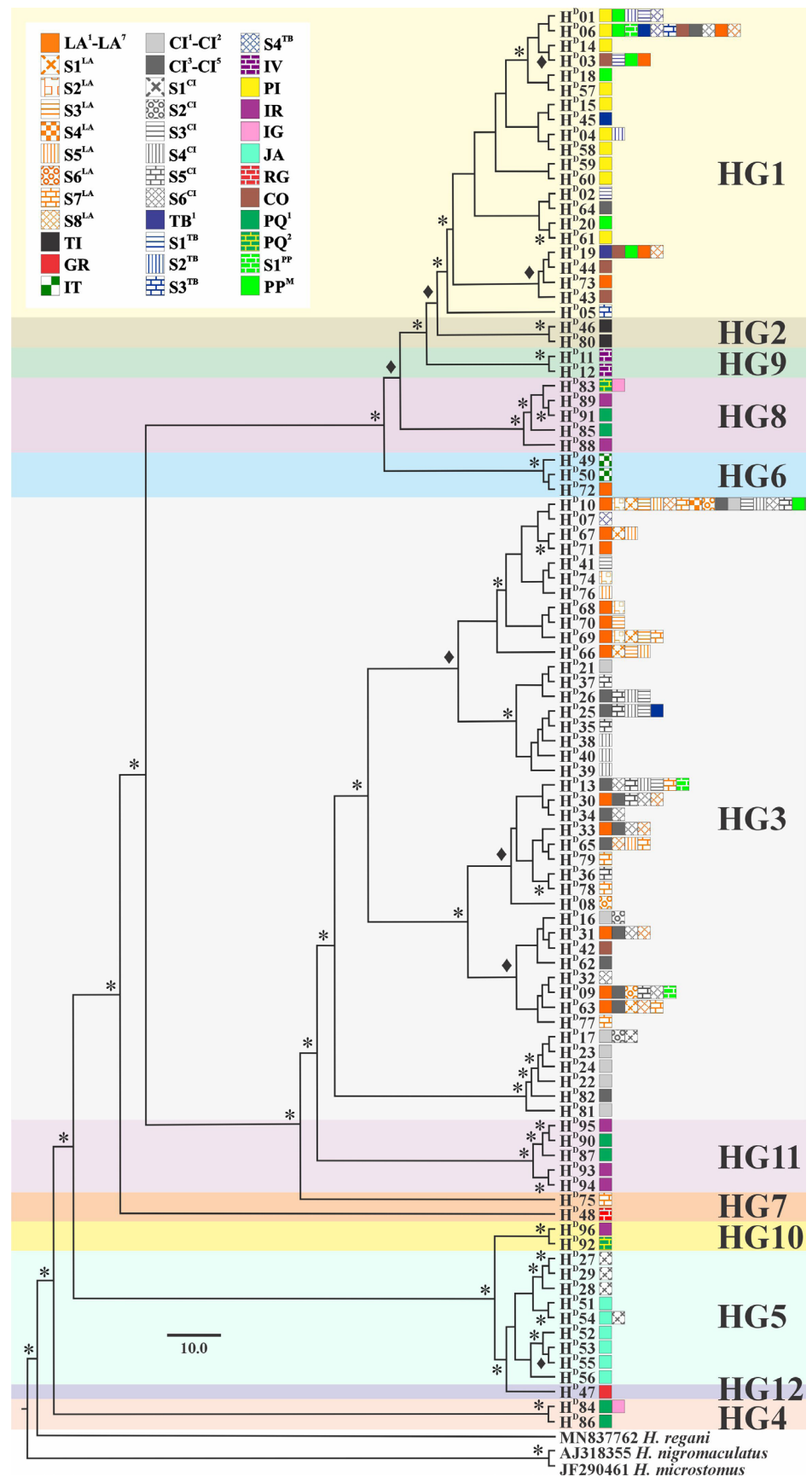


FIGURE S5 | Figure of mismatch distributions of D-loop haplotypes for *Hypostomus ancistroides* samples ($N > 15$) from sub-basins of the Paranapanema River and other locations in the upper Paraná River basin. Cinzas River main channel (CI^2 , CI^4 and CI^5) and its tributary streams ($S1^{CI}$, $S3^{CI}$ - $S6^{CI}$), Laranjinha River main channel (LA^1 - LA^7) and its tributary streams ($S1^{LA}$ - $S3^{LA}$, $S5^{LA}$, $S7^{LA}$, $S8^{LA}$), Congonhas River (CO), Paranapanema River main channel (PP^M), Pirapó River (PI), and Jaguariáiva River (JA).

FIGURE S6 | Figure of Bayesian phylogenetic tree based on D-loop data for *Hypostomus ancistroides* from sub-basins of the Paranapanema River and other locations in the upper Paraná River basin, including the occurrence sites of haplotypes and their assigned haplogroups. Dataset including: Laranjinha River main channel (LA¹-LA⁷) and its tributary streams (S1^{LA}-S8^{LA}); Cinzas River main channel (CI¹-CI⁵) and its tributary streams (S1^{CI}-S6^{CI}), Tibagi River main channel (TB¹) and its tributaries (S1^{TB}-S4^{TB} and the Congonhas River - CO); Paranapanema River main channel (PP^M) and a tributary stream (S1^{PP}); tributary streams of the Itaipu Reservoir (IR); as well as the Pirapó (PI), Jaguariaíva (JA), Itapetininga (IT), Piquiri (PQ¹ - upper; PQ² - lower), Ivaí (IV), Iguaçu (IG), Ribeira de Iguape (RG), Grande (GR) and Tietê (TI) rivers. Posterior probability support values are represented by symbols near the nodes: ♦ = from 50% to 70%; * = from 70% to 100%. Values lower than 50% are not displayed.



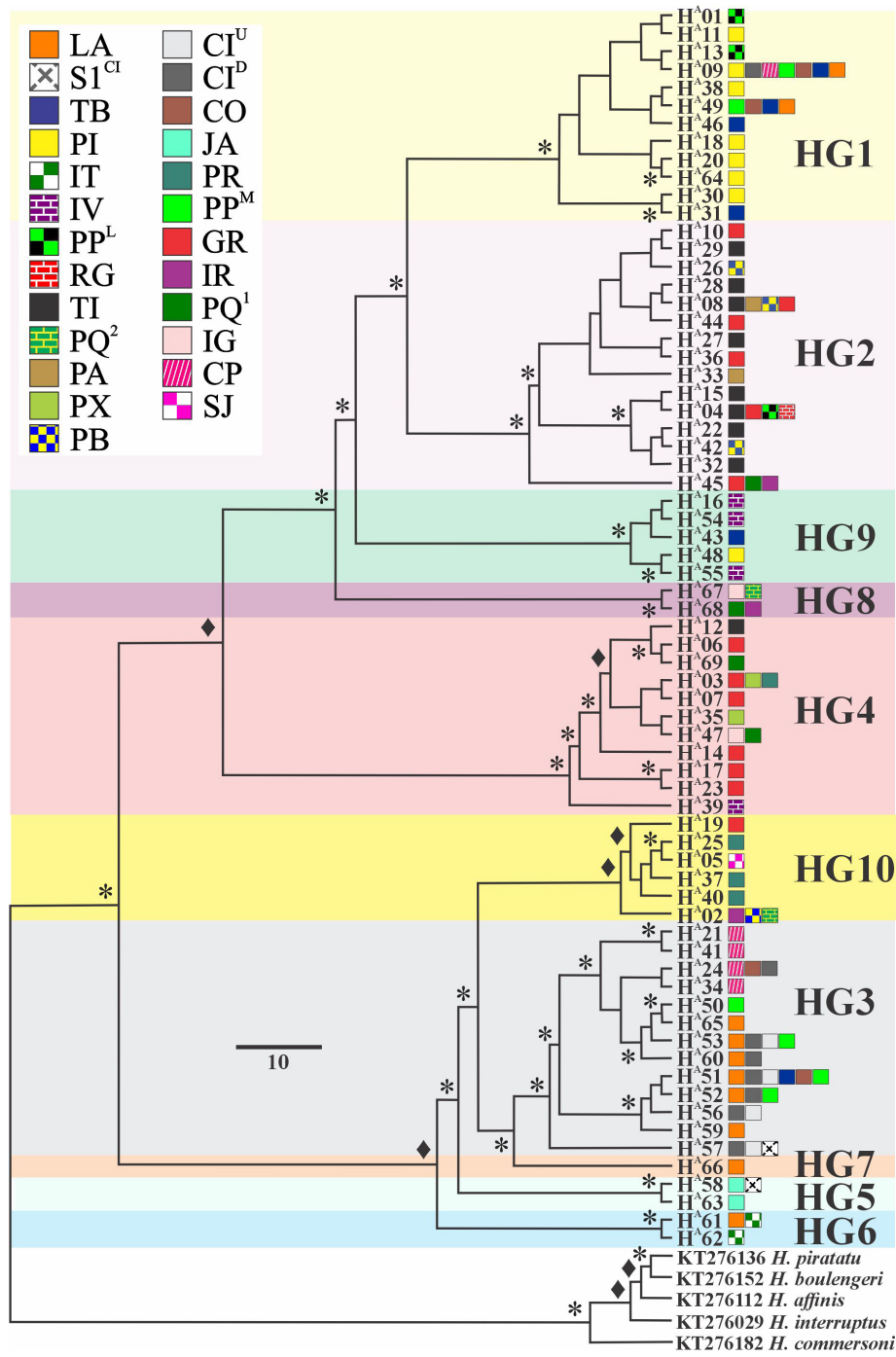


FIGURE S7 | Figure of Bayesian phylogenetic tree based on ATPase gene data for *Hypostomus ancistroides* from sub-basins of the Paranapanema River and other locations in the upper Paraná River basin, including the occurrence sites of haplotypes and their assigned haplogroups. In this analysis, the samples were organized as follows: Laranjinha River and its tributary streams (LA); Cinzas River and its tributary streams (CI^U and CI^D, upstream and downstream of Salto Cavalcante waterfall, respectively); Cachoeirinha Stream (S1^{CI}); Tibagi River and its tributary streams (TB); Congonhas River (CO); tributary streams of the Itaipu Reservoir (IR); Paranapanema River main channel (middle - PP^M, lower - PP^L); including also the Pirapó (PI), Jaguaiaíva (JA), Itapetininga (IT), Pardo (PA), Capivara (CP), Piquiri (PQ¹ - upper; PQ² - lower), Ivaí (IV), Iguaçu (IG), Ribeira de Iguape (RG), Grande (GR), São José dos Dourados (SJ), Peixe (PX), Tietê (TI), Paranaíba (PB) and Paraná (PR) rivers. Posterior probability support values are represented by symbols near the nodes: ◆ = from 50% to 70%; * = from 70% to 100%. Values lower than 50% are not displayed.

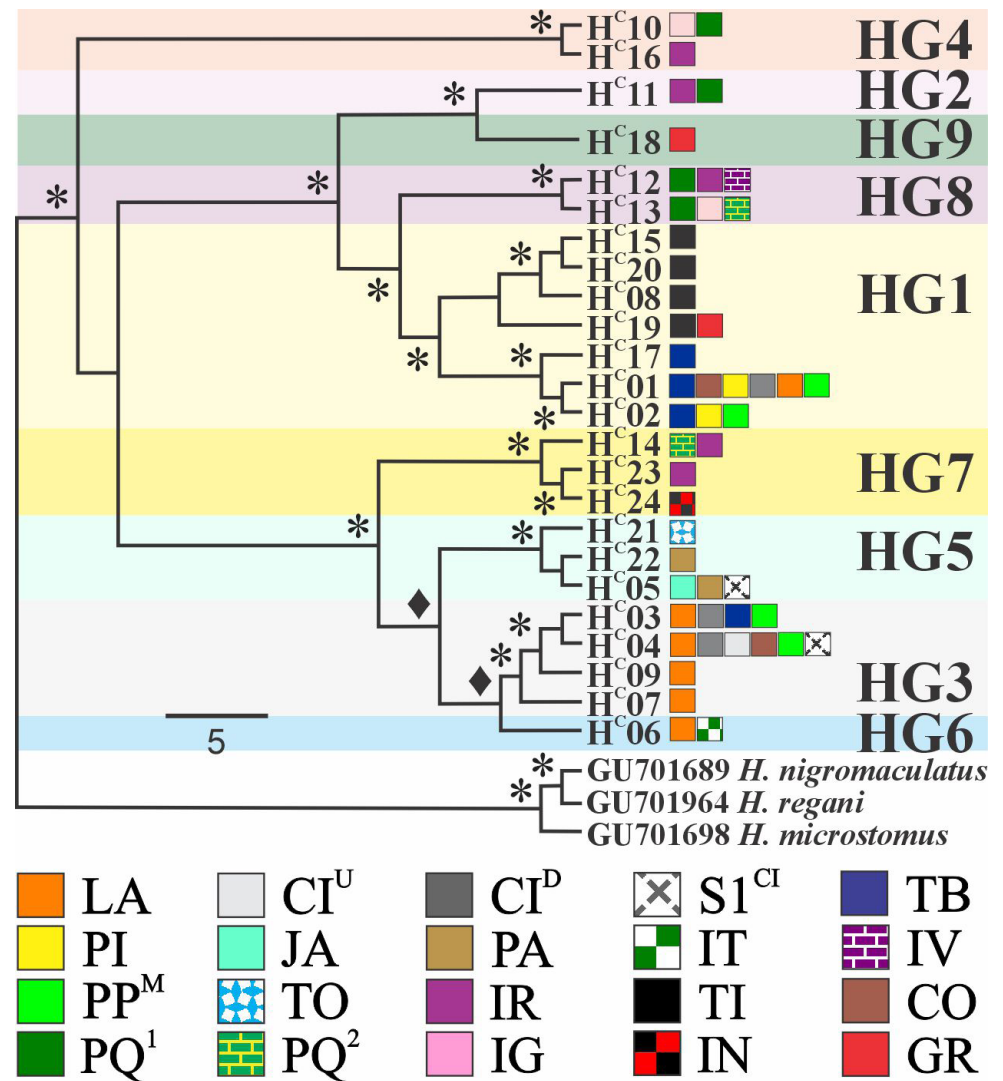


FIGURE S8 | Figure of Bayesian phylogenetic tree based on COI gene data for *Hypostomus ancistroides* from sub-basins of the Paranapanema River and other locations in the upper Paraná River basin, including the occurrence sites of haplotypes and their assigned haplogroups. In this analysis, the samples were organized as follows: Laranjinha River and its tributary streams (LA); Cinzas River and its tributary streams (CI^U and CI^D, upstream and downstream of Salto Cavalcante waterfall, respectively); Cachoeirinha Stream (S1^{CI}); Tibagi River and its tributary streams (TB); Congonhas River (CO); tributary streams of the Itaipu Reservoir (IR); Paranapanema River main channel (PP^M); including also the Pirapó (PI), Jaguariaíva (JA), Itapetininga (IT), Pardo (PA), Piquiri (PQ¹ - upper; PQ² - lower), Ivaí (IV), Iguaçu (IG), Grande (GR), Tietê (TI), Ivinhema (IN), and Tocantins (TO) rivers. Posterior probability support values are represented by symbols near the nodes: ◆ = from 50% to 70%; * = from 70% to 100%. Values lower than 50% are not displayed.

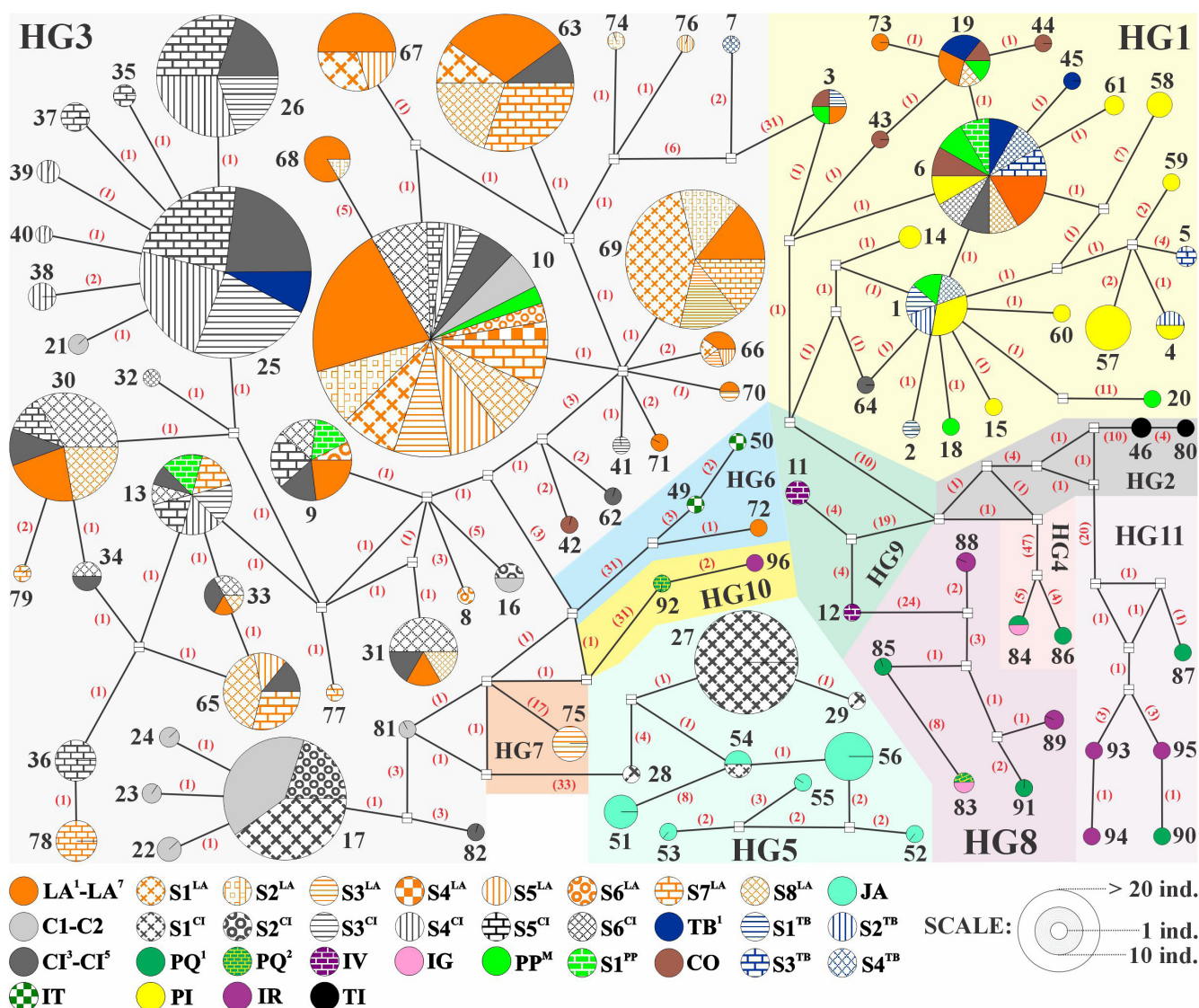


FIGURE S9 | Figure of haplotype network obtained from concatenated mitochondrial data (D-loop, ATPase and COI data) of *Hypostomus ancistroides* along sub-basins of the Paranapanema River and other locations in the upper Paraná River basin. Circle sizes are proportional to the haplotype frequency (scale: ind. = individuals), mutation steps are shown in parentheses (in red) and haplotype names are according to D-loop data. Concatenated mitochondrial dataset included: Laranjinha River main channel (LA¹-LA⁷) and its tributary streams (S1^{LA}-S8^{LA}); Cinzas River main channel (CI¹-CI⁵) and its tributary streams (S1^{CI}-S6^{CI}); Tibagi River main channel (TB¹) and its tributaries (S1^{TB}-S4^{TB}) and the Congonhas River - CO; Paranapanema River main channel (PP^M) and a tributary stream (S1^{PP}); tributary streams of the Itaipu Reservoir (IR); as well as the Pirapó (PI), Jaguaiaíva (JA), Itapetininga (IT), Piquiri (PQ¹ - upper; PQ² - lower), Ivaí (IV), Iguaçu (IG) and Tietê (TI) rivers.

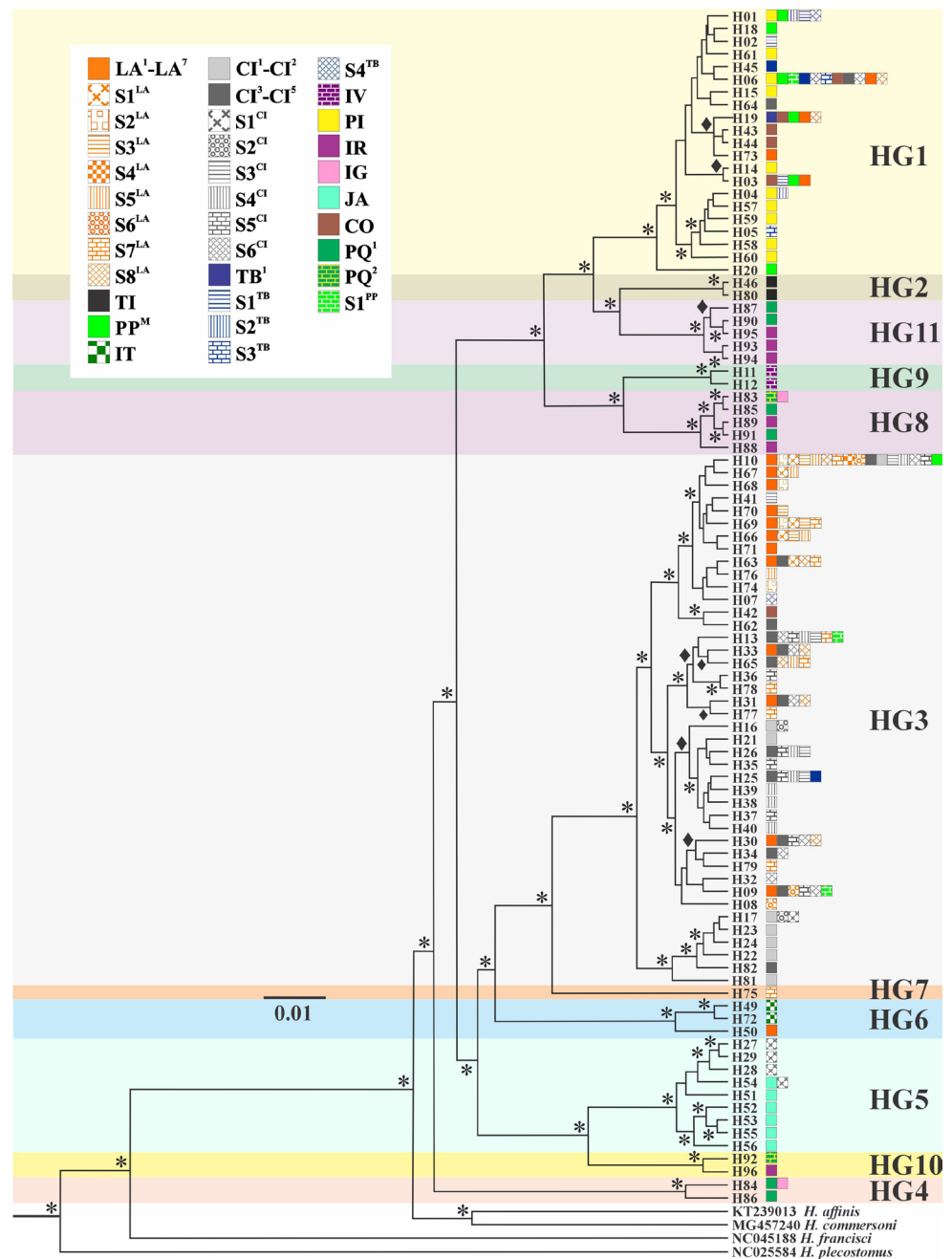


FIGURE S10 | Figure of Bayesian phylogenetic tree based on concatenated mitochondrial data (D-loop, ATPase and COI data) for *Hypostomus ancistroides* from sub-basins of the Paranapanema River and other locations in the upper Paraná River basin, including the occurrence sites of haplotypes and their assigned haplogroups. Concatenated mitochondrial dataset included: Laranjinha River main channel (LA¹-LA⁷) and its tributary streams (S1^{LA}-S8^{LA}); Cinzas River main channel (CI¹-C5) and its tributary streams (S1^{CI}-S6^{CI}); Tibagi River main channel (TB¹) and its tributaries (S1^{TB}-S4^{TB}) and the Congonhas River - CO; Paranapanema River main channel (PP^M) and a tributary stream (S1^{PP}); tributary streams of the Itaipu Reservoir (IR); as well as the Pirapó (PI), Jaguaiaíva (JA), Itapetininga (IT), Piquiri (PQ¹ - upper; PQ² - lower), Ivaí (IV), Iguaçu (IG) and Tietê (TI) rivers. Posterior probability support values are represented by symbols near the nodes: ♦ = from 50% to 70%; * = from 70% to 100%. Values lower than 50% are not displayed.

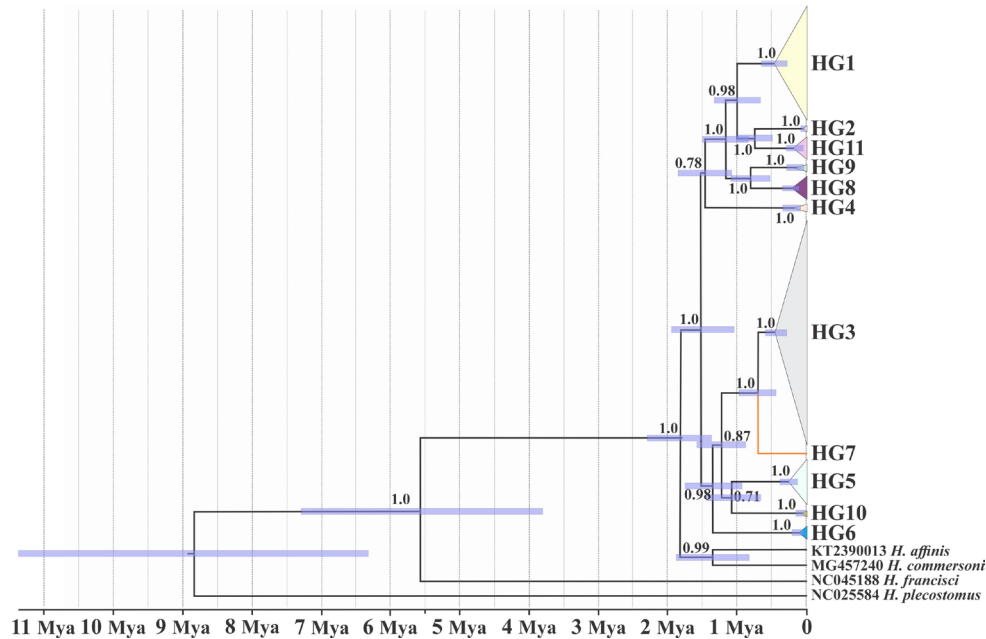


FIGURE S11 | Figure of divergence time (millions of years ago - Mya) among lineages (haplogroups) of *Hypostomus ancistroides* estimated from concatenated mitochondrial data (D-loop+ATPase+COI data) using the ATPase mutation rate (1.3% per million years), according to Bermingham *et al.* (1997). Branches within each haplogroup are collapsed. Haplogroups are numbered and colored as in Fig. 2. Posterior probability values are given next to the nodes. Blue bar indicates 95% credibility intervals.

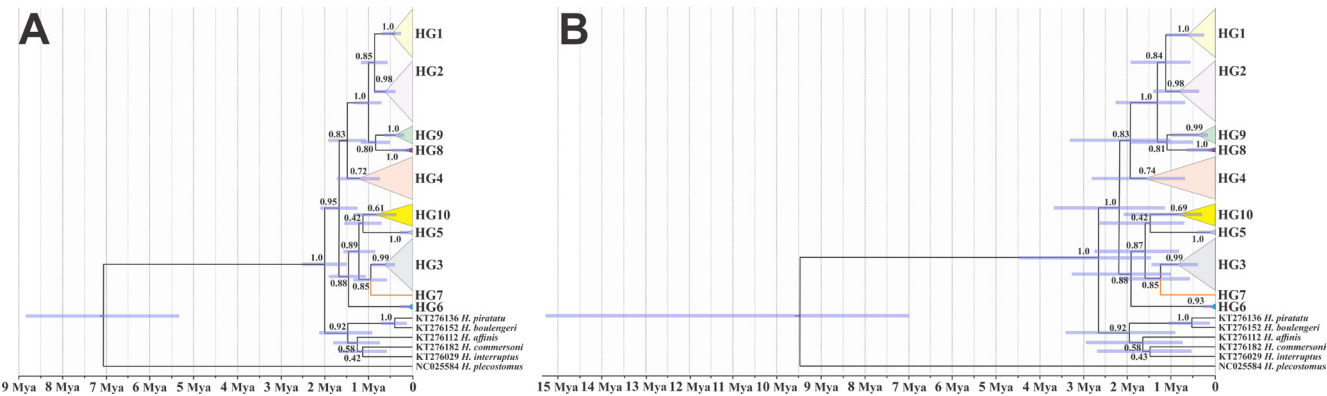


FIGURE S12 | Figure of divergence time (millions of years ago - Mya) among lineages (haplogroups) of *Hypostomus ancistroides* estimated from ATPase data. **A.** Approach using the Cardoso *et al.* (2021) time estimations for D2a clade. **B.** Approach using the ATPase mutation rate (1.3% per million years, Bermingham *et al.*, 1997). Branches within each haplogroup are collapsed. Haplogroups are numbered and colored as in Fig. 3A. Posterior probability values are given next to the nodes. Blue bar indicates 95% credibility intervals.

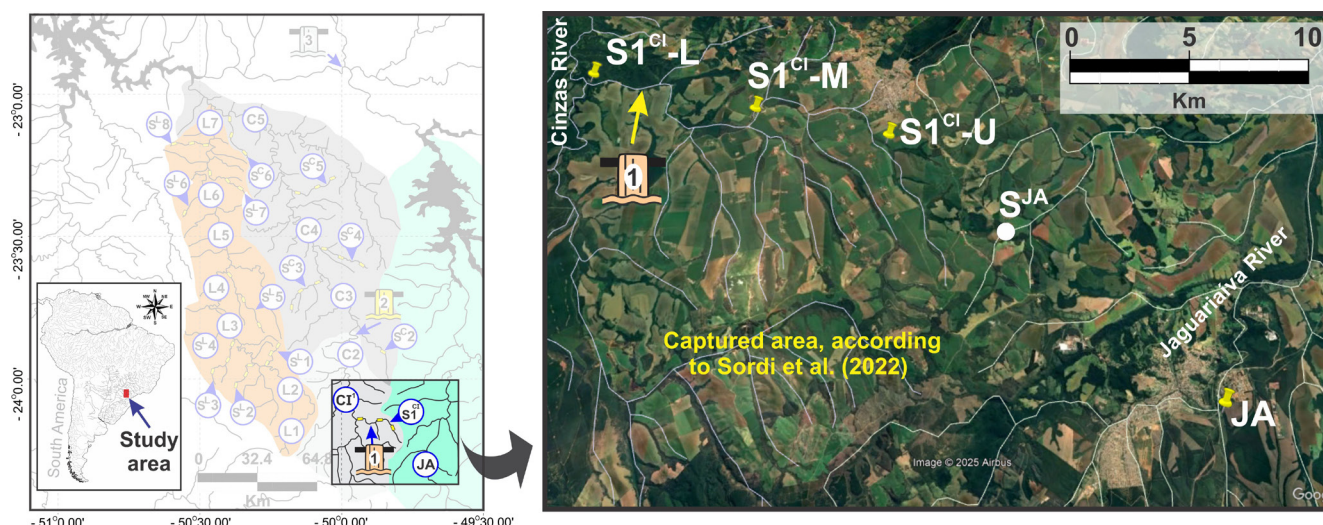


FIGURE S13 | Figure of the expansion of the possible headwater capture area between the Cinzas and Jaguariaíva rivers sub-basins, including sampling sites along the Cachoeirinha Stream (Upper: $S1^{Cl-U}$, Middle: $S1^{Cl-M}$, and Lower: $S1^{Cl-L}$) and the Jaguariaíva River (JA), location of the Cachoeirinha Falls (yellow arrow) and an example of a stream tributary of the Jaguariaíva River (SJA) near the headwaters of the Cachoeirinha Stream (modified from Google Earth, 2025).

Neotropical Ichthyology

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