

**TABLE S1** | Overview of sequences analyzed for molecular species delimitation. BOLD: Barcode of Life database; TICT-FAC: correspond to the Colección de Peces de Agua Dulce del Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva; CIUA: Colección de Ictiología de la Universidad de Antioquia, Medellín; MCP: Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre; MHNG: Muséum d'Histoire Naturelle, Geneve; MNRJ: Museu Nacional, Rio de Janeiro; NUP: Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Maringá.

Species	Sequence ID	Database	Hydrographic basin (River)	GPS Location	Voucher ID	References
<i>Parodon alfonsoi</i>	CIUA1580-24	BOLD	Magdalena basin (Tucuy River)	09°35'13.2"N 73°18'00"W	CIUA 8373	Direct submission
<i>Parodon apolinari</i>	FBCH026-21	BOLD	Orinoco basin (Upia River)	04°50'52.8"N 73°04'04.8"W	TICT-FCA-40	Direct submission
<i>Parodon apolinari</i>	FBCH059-21	BOLD	Orinoco basin (Upia River)	04°50'52.8"N 73°04'01.2"W	TICT-FCA-57	Direct submission
<i>Parodon apolinari</i>	FBCH165-21	BOLD	Orinoco basin (Guavio River)	04°42'03.6"N 73°04'58.8"W	TICT-FCA-281	Direct submission
<i>Parodon cf. buckleyi</i>	PV834389– PV834393	GenBank	Amazon basin (Branco River)	11°55'51.1"S 62°09'09.4"W	NUP 23214	Present study
<i>Parodon cf. buckleyi</i>	PV834394	GenBank	Amazon basin (Pindaituba River)	15°00'41"S 59°17'18"W	MCP 38672	Present study
<i>Parodon caliensis</i>	CIUA1496-24	BOLD	Magdalena basin (Cauca River)	03°11'56.4"N 76°29'49.2"W	CIUA 7967	Direct submission
<i>Parodon guyanensis</i>	GBOL1092- 16; GBOL1198-16	BOLD	Atlantic North Coast basin (Marouini River)	02°40'36.9"N 53°59'57.5"W	MHNG 2756.095; MHNG 2758.004	Direct submission
<i>Parodon guyanensis</i>	GBOL1320-16	BOLD	Atlantic North Coast basin (Marouini River)	02°51'30.9"N 53°58'38.3"W	-	Direct submission
<i>Parodon guyanensis</i>	GBOL2161- 17; GBOL2162-17	BOLD	Atlantic North Coast basin (Litany River)	02°45'01.4"N 54°14'08.9"W	-	Direct submission
<i>Parodon guyanensis</i>	GBOL2307- 17; GBOL2489-17	BOLD	Atlantic North Coast basin (Tampok River)	03°23'24.3"N 53°55'33.6"W	-	Direct submission
<i>Parodon guyanensis</i>	GBOL993-16	BOLD	Atlantic North Coast basin (Paloemeu River)	03°11'54"N 55°24'27"W	MHNG 2717.034	Direct submission
<i>Parodon guyanensis</i>	GBOL979-16	BOLD	Atlantic North Coast basin (Tapanahony River)	03°21'57.6"N 55°25'55.6"W	MHNG 2716.098	Direct submission
<i>Parodon hilarii</i>	HM405177; HM405178	GenBank	São Francisco basin (Olaria stream)	17°35'06"S 44°39'03.6"W	-	Carvalho <i>et al.</i> (2011)
<i>Parodon hilarii</i>	HM405179	GenBank	São Francisco basin (Curimatai River)	17°59'02.4"S 44°10'08.4"W	-	Carvalho <i>et al.</i> (2011)
<i>Parodon hilarii</i>	PV834395– PV834398	GenBank	São Francisco basin (Araras River)	20°16'15"S 45°55'39"W	NUP 25162; NUP 25163	Present study
<i>Parodon magdalenensis</i>	UDEA160-18	BOLD	Magdalena basin (Porce River)	06°50'16.2"N 75°10'34.3"W	CIUA 8041	Direct submission
<i>Parodon magdalenensis</i>	UDEA161-18	BOLD	Magdalena basin (Porce River)	06°56'32"N 75°08'05.6"W	CIUA 8060	Direct submission
<i>Parodon magdalenensis</i>	UDEA162-18	BOLD	Magdalena basin (Purnio River)	05°24'33.2"N 74°41'35.9"W	CIUA 8879	Direct submission
<i>Parodon nasus</i>	OL584320– OL584324	GenBank	La Plata basin (Paio Grande stream)	22°40'34"S 45°41'00"W	MNRJ 28131	Azambuja <i>et al.</i> (2022a)
<i>Parodon pongoensis</i>	PV834399– PV834406	GenBank	Tocantins/Araguaia (Taquaralzinho River)	15°53'28"S 52°14'56"W	NUP 12147	Present study
<i>Parodon sp.</i>	PV834407– PV834411	GenBank	Amazon basin (Peixe River)	08°39'15.5"S 55°09'24.3"W	NUP 22248; NUP 23216	Present study
<i>Parodon suborbitalis</i>	CIUA1568-24	BOLD	Caribbean Coast basin (Peralonso River)	07°55'15.6"N 72°36'00"W	CIUA 8300	Direct submission
<i>Leporinus piau</i>	HM405030	GenBank	São Francisco basin (Três Marias Reservoir)	18°13'40.8"S 45°14'52.8"W	-	Carvalho <i>et al.</i> (2011)

## REFERENCES

- **Azambuja M, Marcondes DS, Nogaroto V, Moreira-Filho O, Vicari MR.** Population structuration and chromosomal features homogeneity in *Parodon nasus* (Characiformes: Parodontidae): a comparison between lower and upper Paraná River representatives. *Neotrop Ichthyol.* 2022a; 20(1):e210162. <https://doi.org/10.1590/1982-0224-2021-0162>
- **Carvalho DC, Oliveira DAA, Pompeu OS, Leal CG, Oliveira C, Hanner R.** Deep barcode divergence in Brazilian freshwater fishes: the case of the São Francisco River basin. *Mitochondrial DNA.* 2011; 22:80–86. <https://doi.org/10.3109/19401736.2011.588214>

**TABLE S2** | Estimates of evolutionary divergence over sequence pairs among groups and standard error in *Parodon* sample groups. Bold values on the main diagonal represent the intraspecific genetic distance. The number of base substitutions per site from averaging over all sequence pairs between groups are shown. Kimura-2-parameters genetic distance and standard error. Analyses were conducted using the Kimura 2-parameter model. The analysis involved 49 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 602 positions in the final dataset. The presence of n/c denotes cases in which it was not possible to estimate evolutionary distances.

	1	2	3	4	5	6	7	8	9	10	11
1. <i>Parodon alfonsoi</i>	n/c										
2. <i>Parodon apolinari</i>	0.1159 (0.0157)	<b>0.0000</b> ( <b>0.0000</b> )									
3. <i>Parodon cf. buckleyi</i>	0.0993 (0.0143)	0.0859 (0.0128)	<b>0.0046</b> ( <b>0.0019</b> )								
4. <i>Parodon caliensis</i>	0.1000 (0.0144)	0.1204 (0.0158)	0.1131 (0.0148)	n/c							
5. <i>Parodon guyanensis</i>	0.1517 (0.0180)	0.1553 (0.0174)	0.1741 (0.0187)	0.1685 (0.0191)	<b>0.0000</b> ( <b>0.0000</b> )						
6. <i>Parodon hilarii</i>	0.1300 (0.0160)	0.1495 (0.0181)	0.1400 (0.0175)	0.1430 (0.0175)	0.1844 (0.0204)	<b>0.0000</b> ( <b>0.0000</b> )					
7. <i>Parodon magdalenensis</i>	0.0000 (0.0000)	0.1160 (0.0157)	0.0997 (0.0144)	0.1004 (0.0145)	0.1555 (0.0181)	0.1278 (0.0157)	<b>0.0000</b> ( <b>0.0000</b> )				
8. <i>Parodon nasus</i>	0.1129 (0.0157)	0.0937 (0.0140)	0.0433 (0.0085)	0.1149 (0.0148)	0.1830 (0.0195)	0.1399 (0.0174)	0.1111 (0.0154)	<b>0.0010</b> ( <b>0.0010</b> )			
9. <i>Parodon pongoensis</i>	0.1393 (0.0167)	0.1564 (0.0182)	0.1577 (0.0181)	0.1581 (0.0187)	0.1662 (0.0190)	0.1614 (0.0188)	0.1409 (0.0166)	0.1661 (0.0189)	<b>0.0012</b> ( <b>0.0007</b> )		
10. <i>Parodon</i> sp.	0.1230 (0.0157)	0.0933 (0.0140)	0.0617 (0.0104)	0.1129 (0.0148)	0.1839 (0.0197)	0.1330 (0.0165)	0.1210 (0.0154)	0.0523 (0.0097)	0.1581 (0.0186)	<b>0.0040</b> ( <b>0.0019</b> )	
11. <i>Parodon suborbitalis</i>	0.0397 (0.0087)	0.0951 (0.0139)	0.1048 (0.0149)	0.0772 (0.0123)	0.1436 (0.0172)	0.1326 (0.0168)	0.0409 (0.0088)	0.1026 (0.0150)	0.1370 (0.0169)	0.1131 (0.0158)	n/c

## Neotropical Ichthyology



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