

TABLE S1 | Taxonomic identification, geographic data, and GenBank accession numbers of *Characidium* sequences used in this study. Reference sequences for *Characidium gomesi* are marked with an asterisk. Institutional abbreviations follow Fricke, Eschmeyer (2026), with the addition of MNLM (Laboratório de Pesquisa em Biodiversidade Molecular, Museu Nacional).

Genbank number	Species	Voucher specimens	Coordinates	River, basin	Authors
PV061185	<i>Characidium urucum</i>	NUP 25800	21°30'45"S 44°38'57"W	Rio Grande, upper Rio Paraná basin	This study
PX845974	<i>Characidium urucum</i>	CI-UFLA 4006	21°29'4.10"S 44°42'26.65"W	Rio Grande, upper Rio Paraná basin	This study
PX845975	<i>Characidium urucum</i>	CI-UFLA 4006	21°29'4.10"S 44°42'26.65"W	Rio Grande, upper Rio Paraná basin	This study
PX845976	<i>Characidium urucum</i>	CI-UFLA 4006	21°29'4.10"S 44°42'26.65"W	Rio Grande, upper Rio Paraná basin	This study
PX845977	<i>Characidium gomesi</i> *	CI-UFLA 3828	22°11'53"S 46°25'35"W	Rio Grande, upper Rio Paraná basin	This study
PX845978	<i>Characidium gomesi</i> *	CI-UFLA 3828	22°11'53"S 46°25'35"W	Rio Grande, upper Rio Paraná basin	This study
PX845979	<i>Characidium gomesi</i> *	CI-UFLA 3828	22°11'53"S 46°25'35"W	Rio Grande, upper Rio Paraná basin	This study
GU701422	<i>Characidium</i> aff. <i>gomesi</i> Paraná	LBP 33572	22°40'24"S 53°05'09"W	Rio Paranapanema, upper Rio Paraná basin	Pereira <i>et al.</i> (2013)
GU701424	<i>Characidium</i> aff. <i>gomesi</i> Paraná	LBP 33568	17°05'58"S 48°45'41"W	Rio Paranaíba, upper Rio Paraná basin	Pereira <i>et al.</i> (2013)
GU701425	<i>Characidium</i> aff. <i>gomesi</i> Paraná	LBP 33607	17°05'58"S 48°45'41"W	Rio Paranaíba, upper Rio Paraná basin	Pereira <i>et al.</i> (2013)
GU701426	<i>Characidium</i> aff. <i>gomesi</i> Paraná	LBP 33592	21°20'49"S 47°37'59"W	Rio Grande, upper Rio Paraná basin	Pereira <i>et al.</i> (2013)
GU701427	<i>Characidium</i> aff. <i>gomesi</i> Paraná	LBP 33569	17°05'58"S 48°45'41"W	Rio Paranaíba, upper Rio Paraná basin	Pereira <i>et al.</i> (2013)
GU701445	<i>Characidium oiticicai</i>	LBP 31218	23°00'46.1"S 47°59'51.7"W	Rio Tietê, upper Rio Paraná basin	Pereira <i>et al.</i> (2013)
GU701839	<i>Characidium</i> aff. <i>gomesi</i> Paraná	LBP 12126	22°59'25.4"S 48°25'37.9"W	Rio Paranapanema, upper Rio Paraná basin	Pereira <i>et al.</i> (2013)
GU702087	<i>Characidium alipioi</i>	LBP 40202	21°14'07.0"S 43°30'50.0"W	Rio Pomba, Rio Paraíba do Sul basin	Pereira <i>et al.</i> (2011)
GU702090	<i>Characidium alipioi</i>	LBP 40201	21°14'07.0"S 43°30'50.0"W	Rio Pomba, Rio Paraíba do Sul basin	Pereira <i>et al.</i> (2011)
HM064986	<i>Characidium alipioi</i>	LBP 40203	21°14'07.0"S 43°30'50.0"W	Rio Pomba, Rio Paraíba do Sul basin	Pereira <i>et al.</i> (2011)
HM064987	<i>Characidium alipioi</i>	LBP 40204	21°14'07.0"S 43°30'50.0"W	Rio Pomba, Rio Paraíba do Sul basin	Pereira <i>et al.</i> (2011)
HM064988	<i>Characidium alipioi</i>	LBP 40205	21°14'07.1"S 43°31'26.0"W	Rio Pomba, Rio Paraíba do Sul basin	Pereira <i>et al.</i> (2011)
JN988779	<i>Characidium gomesi</i>	LBP 41989	18°34'08.0"S 52°05'08.2"W	Rio Paranaíba, upper Rio Paraná basin	Pereira <i>et al.</i> (2013)
JN988780	<i>Characidium gomesi</i>	LBP 41988	18°34'08.0"S 52°05'08.2"W	Rio Paranaíba, upper Rio Paraná basin	Pereira <i>et al.</i> (2013)
JN988781	<i>Characidium gomesi</i>	LBP 28408	21°44'50.3"S 46°28'01.2"W	Rio Grande, upper Rio Paraná basin	Pereira <i>et al.</i> (2013)
JN988782	<i>Characidium</i> aff. <i>gomesi</i> Paraná	LBP 10043	22°58'00.1"S 48°24'00.0"W	Rio Paranapanema, upper Rio Paraná basin	Pereira <i>et al.</i> (2013)
KF914694	<i>Characidium</i> aff. <i>gomesi</i> Paraná	LBP 31106	22°56'06.0"S 48°19'18.0"W	Rio Tietê, upper Rio Paraná basin	Pansonato-Alves <i>et al.</i> (2014)
KF914697	<i>Characidium lanei</i>	LBP 33505			Pansonato-Alves <i>et al.</i> (2014)
KF914699	<i>Characidium</i> aff. <i>gomesi</i> Paraná	LBP 33592	23°01'26.0"S 48°49'32.0"W	Rio Paranapanema, upper Rio Paraná basin	Pansonato-Alves <i>et al.</i> (2014)
KF914700	<i>Characidium pterostictum</i>	LBP 33636			Pansonato-Alves <i>et al.</i> (2014)
KF914701	<i>Characidium lauroi</i>	LBP 33722			Pansonato-Alves <i>et al.</i> (2014)



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Genbank number	Species	Voucher specimens	Coordinates	River, basin	Authors
KF914702	<i>Characidium lanei</i>	LBP 33730			Pansonato-Alves <i>et al.</i> (2014)
KF914706	<i>Characidium</i> aff. <i>gomesi</i> Paraná	LBP 9019-37652	14°35'25.0"S 57°42'35.0"W	Rio Sepotuba, Rio Paraguay basin	Pansonato-Alves <i>et al.</i> (2014)
KF914707	<i>Characidium gomesi</i>	LBP 38541	21°20'49"S 47°37'59"W	Rio Grande, upper Rio Paraná basin	Pansonato-Alves <i>et al.</i> (2014)
KF914710	<i>Characidium pterostictum</i>	LBP 33509			Pansonato-Alves <i>et al.</i> (2014)
KM229364	<i>Characidium serrano</i>	LBP 62715	28°08'01.8"S 55°13'56.9"W	Córrego Canoinha, Rio Uruguay basin	Scacchetti <i>et al.</i> (2015)
KM229365	<i>Characidium timbuiense</i>	LBP 69812	19°58'32.6"S 40°32'53.2"W	Rio Valsugana Velha, Rio Reis Magos basin	Scacchetti <i>et al.</i> (2015)
KM229366	<i>Characidium lauroi</i>	LBP 46417	23°23'14.2"S 45°07'17.0"W	Rio Grande, Rio Grande basin	Scacchetti <i>et al.</i> (2015)
MG825007	<i>Characidium vidali</i>	MNLM 3071	22°26'21.8"S 42°17'43.1"W	Stream affluent to Rio São João basin	Buckup <i>et al.</i> (Unpublished)
MG825016	<i>Characidium lanei</i>	MNLM 4970	25°41'30.8"S 48°35'08.2"W	Rio Colonia Pereira, Rio Guaraguaçu basin	Buckup <i>et al.</i> (Unpublished)
MG936834	<i>Characidium marshi</i>	STRI 6730	08°37'33.2"N 77°49'01.2"W	Rio Membrillo, Rio Tuira basin	Birmingham <i>et al.</i> (Unpublished)
MG936836	<i>Characidium marshi</i>	STRI 4069	08°07'23.9"N 77°32'38.0"W	Rio Yape, Rio Tuira basin	Birmingham <i>et al.</i> (Unpublished)
MG936837	<i>Characidium marshi</i>	STRI 3611	08°58'45.8"N 78°30'20.2"W	Rio Itapeti, Rio Tuira basin	Birmingham <i>et al.</i> (Unpublished)
MH667860	<i>Characidium cricareense</i>	MNLM 5492	19°54'51.1"S 40°45'54.0"W	Rio Santa Maria do Rio Doce, Rio Doce basin	Malanski <i>et al.</i> (2019)
MH667861	<i>Characidium cricareense</i>	MNLM 8072	18°27'16.9"S 40°08'29.8"W	Córrego do Veado, Rio Itaúnas basin	Malanski <i>et al.</i> (2019)
MH667862	<i>Characidium cricareense</i>	MNLM 5398	19°50'04.9"S 40°41'12.1"W	Rio Santa Maria do Rio Doce, Rio Doce basin	Malanski <i>et al.</i> (2019)
MH667863	<i>Characidium cricareense</i>	MNLM 5397	19°50'04.9"S 40°41'12.1"W	Rio Santa Maria do Rio Doce, Rio Doce basin	Malanski <i>et al.</i> (2019)
MH667864	<i>Characidium cricareense</i>	MNLM 5316	19°50'04.9"S 40°41'12.1"W	Rio Santa Maria do Rio Doce, Rio Doce basin	Malanski <i>et al.</i> (2019)
MH667865	<i>Characidium cricareense</i>	MNLM 8172	18°00'29.9"S 40°23'51.0"W	Rio Itaúnas, Rio Itaúnas basin	Malanski <i>et al.</i> (2019)
MH667866	<i>Characidium cricareense</i>	MNLM 8176	18°17'48.1"S 40°35'16.1"W	Rio Cotaxe, Rio São Mateus basin	Malanski <i>et al.</i> (2019)
MH667867	<i>Characidium cricareense</i>	MNLM 5400	19°50'04.9"S 40°41'12.1"W	Rio Santa Maria do Rio Doce, Rio Doce basin	Malanski <i>et al.</i> (2019)
MH667868	<i>Characidium alipioi</i>	MNLM 5462	22°38'16.1"S 42°28'27.1"W	Rio Capivari, Rio São João basin	Malanski <i>et al.</i> (2019)
MH667869	<i>Characidium cricareense</i>	MNLM 8164	18°41'38.0"S 40°48'04.0"W	Rio Cricaré, Rio São Mateus basin	Malanski <i>et al.</i> (2019)
MH667871	<i>Characidium pterostictum</i>	MNLM 4901	29°35'01.0"S 50°16'50.9"W	Rio do Ouro, Rio Maquiné basin	Malanski <i>et al.</i> (2019)
MH667874	<i>Characidium alipioi</i>	MNLM 3007	22°17'15.0"S 42°00'36.0"W	Rio do Ouro, Rio Macaé basin	Malanski <i>et al.</i> (2019)
MH667875	<i>Characidium vidali</i>	MNLM 2043	22°29'49.9"S 42°59'38.0"W	Rio Soberbo, Rio Guapimirim basin	Malanski <i>et al.</i> (2019)
MH667892	<i>Characidium timbuiense</i>	MNLM 5403	19°58'31.1"S 40°32'52.1"W	Rio Valsugana Velha, Rio Reis Magos basin	Malanski <i>et al.</i> (2019)
MH667901	<i>Characidium alipioi</i>	MNLM 3004	22°17'15.0"S 42°00'36.0"W	Rio do Ouro, Rio Macaé basin	Malanski <i>et al.</i> (2019)
MH716095	<i>Characidium</i> aff. <i>alipioi</i> A	LBP 25740		Rio Paraíba do Sul basin	Serrano <i>et al.</i> (2019)
MH716097	<i>Characidium</i> aff. <i>alipioi</i> A	LBP 25740		Rio Paraíba do Sul basin	Serrano <i>et al.</i> (2019)



TABLE S1 | (Continued)

Genbank number	Species	Voucher specimens	Coordinates	River, basin	Authors
MH716119	<i>Characidium</i> aff. <i>alipioi</i> A	LBP 25740		Rio Paraíba do Sul basin	Serrano <i>et al.</i> (2019)
MH716122	<i>Characidium</i> aff. <i>alipioi</i> B	LBP 25740		Rio Paraíba do Sul basin	Serrano <i>et al.</i> (2019)
MH716123	<i>Characidium</i> aff. <i>alipioi</i> B	LBP 25740		Rio Paraíba do Sul basin	Serrano <i>et al.</i> (2019)
MH716124	<i>Characidium</i> aff. <i>alipioi</i> B	LBP 25740		Rio Paraíba do Sul basin	Serrano <i>et al.</i> (2019)
MH716127	<i>Characidium</i> aff. <i>alipioi</i> B	LBP 25740		Rio Paraíba do Sul basin	Serrano <i>et al.</i> (2019)
MH716128	<i>Characidium</i> aff. <i>alipioi</i> B	LBP 25740		Rio Paraíba do Sul basin	Serrano <i>et al.</i> (2019)
MK464031	<i>Characidium</i> cf. <i>fasciatum</i> Paracatu	JCB 107	15°50'59.5"S 47°23'13.3"W	Rio Paracatu, Rio São Francisco basin	Bagley <i>et al.</i> (2019)
MK464033	<i>Characidium</i> aff. <i>fasciatum</i> Paranaíba	JCB 27	15°54'54.1"S 47°54'40.5"W	Rio Paranaíba, upper Rio Paraná basin	Bagley <i>et al.</i> (2019)
MK464034	<i>Characidium</i> aff. <i>fasciatum</i> Paranaíba	JCB 28	15°54'54.1"S 47°54'40.5"W	Rio Paranaíba, upper Rio Paraná basin	Bagley <i>et al.</i> (2019)
MK464035	<i>Characidium</i> aff. <i>fasciatum</i> Paranaíba	JCB 29	15°54'54.1"S 47°54'40.5"W	Rio Paranaíba, upper Rio Paraná basin	Bagley <i>et al.</i> (2019)
MK464036	<i>Characidium</i> aff. <i>fasciatum</i> Paranaíba	JCB 33	15°54'54.1"S 47°54'40.5"W	Rio Paranaíba, upper Rio Paraná basin	Bagley <i>et al.</i> (2019)
MK464057	<i>Characidium gomesi</i>	CIUnB 913	15°44'07.8"S 47°55'06.7"W	Rio Paranaíba, upper Rio Paraná basin	Bagley <i>et al.</i> (2019)
MK464074	<i>Characidium gomesi</i>	CIUnB 951	15°44'27.1"S 47°39'51.7"W	Rio Paranaíba, upper Rio Paraná basin	Bagley <i>et al.</i> (2019)
MK464080	<i>Characidium gomesi</i>	CIUnB 960	15°41'57.4"S 47°42'07.6"W	Rio Paranaíba, upper Rio Paraná basin	Bagley <i>et al.</i> (2019)
MK464122	<i>Characidium</i> cf. <i>fasciatum</i> Paracatu	JCB 135	15°50'59.5"S 47°23'13.3"W	Rio Paracatu, Rio São Francisco basin	Bagley <i>et al.</i> (2019)
MK464123	<i>Characidium</i> cf. <i>fasciatum</i> São Francisco	JCB 136	15°47'13.4"S 47°27'00.6"W	Rio Paracatu, Rio São Francisco basin	Bagley <i>et al.</i> (2019)
MK464124	<i>Characidium</i> cf. <i>fasciatum</i> São Francisco	JCB 137	15°47'13.4"S 47°27'00.6"W	Rio Paracatu, Rio São Francisco basin	Bagley <i>et al.</i> (2019)
MK464127	<i>Characidium</i> cf. <i>fasciatum</i> São Francisco	JCB 235	15°50'59.5"S 47°23'13.3"W	Rio Paracatu, Rio São Francisco basin	Bagley <i>et al.</i> (2019)
MK464148	<i>Characidium</i> aff. <i>fasciatum</i> Paranaíba	JCB 341	15°35'08.5"S 47°30'26.6"W	Rio Paranaíba, upper Rio Paraná basin	Bagley <i>et al.</i> (2019)
MK464149	<i>Characidium gomesi</i>	JCB 342	16°01'08.3"S 47°48'10.6"W	Rio Paranaíba, upper Rio Paraná basin	Bagley <i>et al.</i> (2019)
MK464150	<i>Characidium</i> aff. <i>fasciatum</i> Paranaíba	JCB 343	16°01'08.3"S 47°48'10.6"W	Rio Paranaíba, upper Rio Paraná basin	Bagley <i>et al.</i> (2019)
MK464151	<i>Characidium</i> cf. <i>fasciatum</i> São Francisco	JCB 344	15°42'01.5"S 47°07'18.1"W	Rio Paracatu, Rio São Francisco basin	Bagley <i>et al.</i> (2019)
MT946184	<i>Characidium</i> cf. <i>purpuratum</i>	CHIFF 0009 - 1	01°01'50"N 76°32'12"W	Rio Caquetá, Rio Amazonas basin	Agudelo-Zamora <i>et al.</i> (2020)
MT946185	<i>Characidium</i> cf. <i>purpuratum</i>	CHIFF 0009 - 2	01°01'50"N 76°32'12"W	Rio Caquetá, Rio Amazonas basin	Agudelo-Zamora <i>et al.</i> (2020)
MT946186	<i>Characidium</i> cf. <i>purpuratum</i>	CHIFF 0009 - 3	01°01'50"N 76°32'12"W	Rio Caquetá, Rio Amazonas basin	Agudelo-Zamora <i>et al.</i> (2020)
MT946199	<i>Characidium tatama</i>	IMCN 8926	04°55'13.39"N 76°12'21.48"W	Rio Ingará, Rio San Juan basin	Agudelo-Zamora <i>et al.</i> (2020)
MT946200	<i>Characidium tatama</i>	IMCN 8926	04°55'13.39"N 76°12'21.48"W	Rio Ingará, Rio San Juan basin	Agudelo-Zamora <i>et al.</i> (2020)
PP725100	<i>Characidium cricareense</i>	LBP 52191	21°09'09.7"S 43°31'37.9"W	Rio Xopotó, Rio Doce basin	Oliveira-Silva <i>et al.</i> (2024)
PP725104	<i>Characidium</i> cf. <i>fasciatum</i> São Francisco	UFBA/PEI 2725	12°07'02.2"S 45°03'57.9"W	Rio Grande, Rio São Francisco basin	Oliveira-Silva <i>et al.</i> (2024)



TABLE S1 | (Continued)

Genbank number	Species	Voucher specimens	Coordinates	River, basin	Authors
PP725105	<i>Characidium cf. fasciatum</i> São Francisco	UFBA/PEI 2726	12°07'02.2"S 45°03'57.9"W	Rio Grande, Rio São Francisco basin	Oliveira-Silva <i>et al.</i> (2024)
PP725106	<i>Characidium cf. fasciatum</i> São Francisco	UFBA/PEI 2727	12°07'02.2"S 45°03'57.9"W	Rio Grande, Rio São Francisco basin	Oliveira-Silva <i>et al.</i> (2024)
PP725107	<i>Characidium cf. fasciatum</i> São Francisco	UFBA/PEI 2728	12°07'02.2"S 45°03'57.9"W	Rio Grande, Rio São Francisco basin	Oliveira-Silva <i>et al.</i> (2024)
PP725108	<i>Characidium cf. interruptum</i>	UFBA/PEI 2485	16°58'22.0"S 39°11'44.3"W	Rio do Queimado, Rio do Queimado basin	Oliveira-Silva <i>et al.</i> (2024)
PP725109	<i>Characidium cf. interruptum</i>	UFBA/PEI 2486	16°58'22.0"S 39°11'44.3"W	Rio do Queimado, Rio do Queimado basin	Oliveira-Silva <i>et al.</i> (2024)
PP725110	<i>Characidium cf. interruptum</i>	UFBA/PEI 2490	16°58'54.3"S 39°22'10.9"W	Rio do Queimado, Rio do Queimado basin	Oliveira-Silva <i>et al.</i> (2024)
PP725113	<i>Characidium helmeri</i>	UFBA/PEI 2504	16°59'08.5"S 39°12'52.5"W	Rio Cahy, Rio do Queimado basin	Oliveira-Silva <i>et al.</i> (2024)
PP725114	<i>Characidium kamakan</i>	UFBA/PEI 2708	15°25'16"S 39°31'48"W	Rio Panelão, Rio Pardo basin	Oliveira-Silva <i>et al.</i> (2024)
PP725115	<i>Characidium kamakan</i>	UFBA/PEI 2709	15°25'16"S 39°31'48"W	Rio Panelão, Rio Pardo basin	Oliveira-Silva <i>et al.</i> (2024)
PP725116	<i>Characidium kamakan</i>	UFBA/PEI 2710	15°25'16"S 39°31'48"W	Rio Panelão, Rio Pardo basin	Oliveira-Silva <i>et al.</i> (2024)
MT946177	<i>Characidium dule</i>	IMCN8929	08°00'7.1"N 77°06'56.2"W	Rio Arquía, Rio Atrato basin	Agudelo-Zamora <i>et al.</i> (2020)
MT946178	<i>Characidium dule</i>	IMCN8929	08°00'7.1"N 77°06'56.2"W	Rio Arquía, Rio Atrato basin	Agudelo-Zamora <i>et al.</i> (2020)
MT946179	<i>Characidium dule</i>	CHIFF0012	05°44'39.7"N 76°31'54.2"W	Rio Tutunendo, Rio Atrato basin	Agudelo-Zamora <i>et al.</i> (2020)
MT946180	<i>Characidium dule</i>	IMCN8929	08°00'7.1"N 77°06'56.2"W	Rio Arquía, Rio Atrato basin	Agudelo-Zamora <i>et al.</i> (2020)
MT946181	<i>Characidium dule</i>	IMCN8929	08°00'7.1"N 77°06'56.2"W	Rio Arquía, Rio Atrato basin	Agudelo-Zamora <i>et al.</i> (2020)
PQ233871	<i>Characidium boavistae</i>	CIUA 8287-A	07°52'18.2"N 72°37'05.2"W	Quebrada Ocarena, Lago Maracaibo basin	Jimenez-Segura <i>et al.</i> (Unpublished)
PV962477	<i>Characidium cf. bolivianum</i>			Peru	De la Sota <i>et al.</i> (Unpublished)
MZ051887	<i>Melanocharacidium blennioides</i>	SU 08-228	03°11'53.9"N 55°24'27.0"W	Rio Paloemeu, Maroni River basin	Papa <i>et al.</i> (2021)
ON062375	<i>Crenuchus spilurus</i>	INPA-ICT 049574 - P29501			Ohara <i>et al.</i> (2023)
ON063044	<i>Poecilocharax weitzmani</i>	INPA-ICT 049036 - P29843			Ohara <i>et al.</i> (2023)

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Official Journal of the Sociedade Brasileira de Ictiologia

HOW TO CITE THIS ARTICLE

- **Uzeda PLC, Stabile BHM, Reis RB, Sartori LJ, Pavanelli CS, Margarido VP, Graça WJ.** Integrative taxonomy reveals a remarkably colored species of *Characidium* (Characiformes: Crenuchidae) from the upper rio Paraná basin, Brazil. *Neotrop Ichthyol.* 2026; 24(1):e250174. <https://doi.org/10.1590/1982-0224-2025-0174>