TABLE S1 | Information and Genbank accession number of specimens used for genetic studies. *New combination.

Species	Catalog number	Voucher	Locality	Basin	Accession number	Sequence provide by
Astyanax bimaculatus	DCC 01052	DCC01052	Lagoa da Olaria	São Francisco River	HM405053	Rossini *et al.* (2016)
Astyanax bimaculatus	DCC 38285	DCC38285	Rio Santo Antônio	São Francisco River	HM404950	Rossini *et al.* (2016)
Astyanax bimaculatus	LBP 29127	LBP 29127	Paraiba do Sul	Paraiba do Sul River	GU702110	Unpublished
Astyanax bimaculatus	LBP 29348	LBP 29348	Paraiba do Sul	Paraiba do Sul River	GU702109	Unpublished
Astyanax goyanensis	LBPV 68318	LBPV-68318	Rio São Miguel	Tocantins River	KY268091	Rossini *et al.* (2016)
Astyanax goyanensis	LBPV 68316	LBPV-68316	Rio São Miguel	Tocantins River	KY267248	Silva *et al.* (2025)
Astyanax jacuhiensis	UFRGS 18429	TEC3822A	Rio Maquiné	Tramandaí River	PQ341688	Silva *et al.* (2025)
Astyanax jacuhiensis	UFRGS 18429	TEC3822B	Rio Maquiné	Tramandaí River	PQ341689	Silva *et al.* (2025)
Astyanax lacustris	UFRGS 19055	TEC4030	Tramandaí	Tramandaí River	PX703210	This study
Astyanax lacustris	UFRGS 18513	TEC4102	Paranaguá	Paranaguá River	PX703211	This study
Astyanax lacustris	UFRGS 18732	TEC4160	Rio Putim	Paraíba do Sul River basin	PX703212	This study
Astyanax lacustris	UFRGS 18745	TEC4180	Rio Putim	Paraíba do Sul River basin	PX703213	This study
Astyanax lacustris	UFRGS 18910	TEC4263	Poço	Fluminense	PX703214	This study
Astyanax lacustris	UFRGS 18927	TEC4275	Córrego da Pratinha	Itabapoana	PX703215	This study
Astyanax lacustris	UFRGS 18957	TEC4772	Rio Santa Maria da Vitória	Santa Maria da Vitória	KY327440	Silva *et al.* (2017)
Astyanax maculisquamis	UFRGS 12815	TEC1525	Rio Guaporé	Guapore-Itenez	PX703216	Rossini *et al.* (2016)
Astyanax mexicanus	UFRGS 22645	TEC 7255B	Mexico	Mexico	MN119474	Wendt *et al.* (2019)
Astyanax obscurus	LBPV 21640	LBPV-21640	Rio Itapucu	Itapucu River	KY267890	Rossini *et al.* (2016)
Deuterodon burgerai	UFRGS 11636	TEC1154B	Rio Santa Cruz	East costal river dranaiges	PX703201	This study
Deuterodon giton	UFRGS 18952	TEC4767	Rio Santa Maria da Vitória	Santa Maria da Vitória	PX703203	This study
Deuterodon hastatus	UFRGS 19221	TEC4527	Poço	Fluminense	KY327464	Silva *et al.* (2025)
Deuterodon heterostomus	UFRGS 18758	TEC4184	Rio Paraíbuna	Paraiba do Sul River	KY327456	Silva *et al.* (2017)
Deuterodon iguape	UFRGS 20032	TEC4130	Ribeira do Iguape River basin	Ribeira do Iguape River	KY327421	Silva *et al.* (2017)
Deuterodon iguape	UFRGS 18525	TEC4138	Ribeira do Iguape River basin	Ribeira do Iguape River	KY327420	Silva *et al.* (2017)
Deuterodon intermedius	MZUFV 4458	CT2436	Doce River basin	Doce River	PX703204	This study
Deuterodon intermedius	MZUFV 4458	CT2801	Doce River basin	Doce River	KY327432	Silva *et al.* (2017)
Deuterodon intermedius	MZUFV 4459	CT3175	Doce River basin	Doce River	PX703205	This study
Deuterodon intermedius	MZUFV 4460	CT3205	Doce River basin	Doce River	PX703206	This study
Deuterodon intermedius	UFRGS 18739	TEC4158	Paraíba do Sul	Paraíba do Sul River	PX703207	This study
Deuterodon intermedius	UFRGS 18867	TEC4248	Rio São João	São João River	PX703208	This study
Deuterodon intermedius	UFRGS 18894	TEC4554	Rio São João	São João River	KY327433	Silva *et al.* (2017)
Deuterodon sp.	UFRGS 18913	TEC4271	Rio Imbé	Paraiba do Sul River	KY327427	Silva *et al.* (2017)
Deuterodon sp.	UFRGS 18913	TEC4271	Rio Imbé	Paraíba do Sul River	KY327427	Silva *et al.* (2017)
Deuterodon sp.	UFRGS 18913	TEC4272	Rio Imbé	Paraíba do Sul River	PX703209	This study
*Deuterodon keronolepis**	UFRGS 18773	TEC4192	Ubatumirim River basin	Ubatumirim River	MK012668	Silva *et al.* (2019)
*Deuterodon keronolepis**	UFRGS 18860	TEC4233	Rio Paraíso	Baia de Guanabara	MK012674	Silva *et al.* (2019)
Deuterodon langei	UFRGS 18508	TEC4103	Paranaguá River basin	Paranaguá River	KY327419	Silva *et al.* (2017)
Deuterodon luetkenii	UFRGS 18431	TEC3824A	Rio Maquiné	Tramandaí River	KY327458	Silva *et al.* (2017)

TABLE S1 | (Continued)

Species	Catalog number	Voucher	Locality	Basin	Accession number	Sequence provide by
Deuterodon luetkenii	UFRGS 18431	TEC3824B	Rio Maquiné	Tramandaí River	PX168969	Unpublished
*Deuterodon michroschemos**	UFRGS 17542	CT1936	Doce River basin	Doce River	KY327428	Silva *et al.* (2017)
*Deuterodon michroschemos**	UFRGS17542	CT1940	Doce River basin	Doce River	KY327429	Silva *et al.* (2017)
Deuterodon pedri	MCP 47661	CT2521	Rio Santo Antônio	Doce River	KY327434	Silva *et al.* (2017)
Deuterodon pedri	UFRGS 17543	CT2529	Rio Santo Antônio	Doce River	KY327435	Silva *et al.* (2017)
Deuterodon ribeirae	UFRGS 18647	TEC3953	Guaratuba	Guaratuba	PX703217	This study
Deuterodon singularis	UFRGS 18518	TEC4087	Tubarão River basin	Tubarão River	KY327423	Silva *et al.* (2017)
Deuterodon stigmaturus	UFRGS 16519	TEC2350	Maquiné River basin	Tramandaí River	KY327424	Silva *et al.* (2017)
Deuterodon supparis	UFRGS 18495	TEC4651	Itajaí River basin	Itajaí River	KY327422	Silva *et al.* (2017)
Deuterodon taeniatus	UFRGS 19342	TEC4997	Córrego Aduelas	Macaé River	KY327460	Silva *et al.* (2017)
Deuterodon taeniatus	UFRGS 19342	TEC5000	Macaé River basin	Macaé River	KY327461	Silva *et al.* (2017)
*Psalidodon bagual**	UFRGS 17834	TEC3513	Rio Carreiro, a jusante da PCH Linha Emilia	Laguna dos Patos	MN119454	Wendt *et al.* (2019)
Psalidodon brachypterygium	UFRGS 21849	TEC6844	Upper Uruguai	Laguna dos Patos	MN119455	Wendt *et al.* (2019)
Psalidodon brachypterygium	UFRGS 21849	TEC6844	Rio Pelotas	Uruguai River	MN119455	Wendt *et al.* (2019)
Psalidodon cremnobates	UFRGS 18423	TEC3823A	Rio Maquiné	Tramandaí River	PQ341702	Silva *et al.* (2025)
Psalidodon cremnobates	UFRGS 18430	TEC3823B	Rio Maquiné	Tramandaí River	MN119456	Wendt *et al.* (2019)
Psalidodon cremnobates	UFRGS 18430	TEC3823D	Rio Maquiné	Tramandaí River	MN119457	Wendt *et al.* (2019)
Psalidodon cremnobates	UFRGS 18430	TEC 3823B	Rio Maquiné	Tramandaí River	MN119456	Wendt *et al.* (2019)
Psalidodon dissensus	UFRGS 16521	TEC2849	Rio Três Forquilhas	Tramandaí River	PX169266	This study
Psalidodon dissensus	UFRGS 17469	TEC3325C	Chanel between lagoa Fortaleza and Cerquinha	Tramandaí River	PQ341710	Silva *et al.* (2025)
Psalidodon dissensus	UFRGS 17469	TEC3325D	Chanel between lagoa Fortaleza and Cerquinha	Tramandaí River	PQ341711	Silva *et al.* (2025)
*Psalidodon douradilho**	UFRGS 18472	TEC3865	Rio Maquiné	Tramandaí River	PX169268	This study
Psalidodon eigenmanniorum	UFRGS 19221	TEC4916A	Lagoa Bacupari	Tramandaí River	PQ341714	Silva *et al.* (2025)
Psalidodon eigenmanniorum	UFRGS 19221	TEC4916B	Lagoa Bacupari	Tramandaí River	PQ341715	Silva *et al.* (2025)
Psalidodon fasciatus	UFRGS 19135	TEC4853A	South of lagoa dos Quadros	Tramandaí River	PX169261	Silva *et al.* (2025)
Psalidodon fasciatus	UFRGS 19135	TEC4853B	South of lagoa dos Quadros	Tramandaí River	PX169263	Silva *et al.* (2025)
Psalidodon fasciatus	UFRGS 19135	TEC4865A	Lagoa dos Quadros	Tramandaí River	PX169264	Silva *et al.* (2025)
Psalidodon fasciatus	UFRGS 19135	TEC4865B	Lagoa dos Quadros	Tramandaí River	PQ341707	Silva *et al.* (2025)
Psalidodon fasciatus	UFRGS 14913	TEC1056	Córrego Coqueiro	São Francisco River	PX703202	This study
Psalidodon gymnogenys	LBPV 69189	LBPV-69189	Rio Iguaçu	Paraná	KY268200	Rossini *et al.* (2016)
Psalidodon gymnogenys	LBPV 69188	LBPV-69188	Rio Iguaçu	Iguaçu River	KY267338	Silva *et al.* (2025)
Psalidodon henseli	UFRGS 19598	TEC5189A	Lagoa Emboabinha	Tramandaí River	MN119464	Wendt *et al.* (2019)
Psalidodon henseli	UFRGS 19598	TEC5189B	Lagoa Emboabinha	Tramandaí River	MN119465	Wendt *et al.* (2019)
Psalidodon laticeps	UFRGS 18428	TEC3821	Rio Maquiné	Tramandaí River	PQ341696	Silva *et al.* (2025)
Psalidodon laticeps	UFRGS 18428	TEC3825	Rio Maquiné	Tramandaí River	PQ341697	Silva *et al.* (2025)
Psalidodon laticeps	UFRGS 18503	TEC4113	Ribeirão Passagem	Ribeira de Iguape	KY327442	Silva *et al.* (2017)
Psalidodon laticeps	UFRGS 18503	TEC4115	Ribeirão Passagem	Ribeira de Iguape	KY3273	Silva *et al.* (2017)
Psalidodon laticeps	UFRGS 16514	TEC2842A	Três Forquilhas	Tramandaí River	MH029370	Bonato *et al.* (2018)
Psalidodon malabarborum	UFRGS 19746	TEC5291A	Ribeirão Tripuí	Doce River	KY327447	Silva *et al.* (2017)
Psalidodon malabarborum	UFRGS 19746	TEC5291E	Ribeirão Tripuí	Doce River	PX703199	This study

TABLE S1 | (Continued)

Species	Catalog number	Voucher	Locality	Basin	Accession number	Sequence provide by
Psalidodon paiva	LAGEEVO - 2614	LAGEEVO - 2614	Abaeté River	São Francisco River	MT428070	Rodrigues-Oliveira *et al.* (2025)
Psalidodon paranae		NC031380.1			NC031380	Silva *et al.* (2016)
Psalidodon paranae	LBPV 40215	LBPV-40215	Corrego Beijinho	Rio Grande/La Plata	KY267539	Silva *et al.* (2025)
*Psalidodon procerus**	UFRGS 19213	TEC4908A	Lagoa Fortaleza	Tramandaí River	PX169278	Unpublished
*Psalidodon procerus**	UFRGS 19213	TEC4908B	Lagoa Fortaleza	Tramandaí River	PX169279	Unpublished
Psalidodon rivularis	UFRGS 11375	TEC1213	Unaí	São Francisco River	PX703218	This study
Psalidodon rivularis	DCC 42098	DCC42098	Rio Das Velhas	São Francisco River	HM405009	Rossini *et al.* (2016)
Psalidodon scabripinnis	MZUFV 4456	CT2493	Doce River basin	Doce River	PX703219	This study
Psalidodon scabripinnis	MZUFV4456	CT2772	Doce River basin	Doce River	KY327444	Silva *et al.* (2017)
Psalidodon scabripinnis	MZUFV 4456	CT2773	Doce River basin	Doce River	KY327445	Silva *et al.* (2017)
Psalidodon troya	LGEP_780	LGEP_780	Argentina	Argentina	MK928378	Terán *et al.* (2020)
Psalidodon troya	LGEP_780_(2)	LGEP_780_(2)	Argentina	Argentina	MK928377	Terán *et al.* (2020)
Psalidodon varzeae	LBPV 9102	LBPV-9102	Rio dos Patos	Iguaçu River	KY267609	Rossini *et al.* (2016)
Psalidodon velhochico	LAGEEVO - 2030	LAGEEVO - 2030	Abaeté River	São Francisco River	MT428069	Rodrigues-Oliveira *et al.* (2025)
Psalidodon xiru	UFRGS 18438	TEC3831	Rio Maquiné	Tramandaí River	MN119472	Wendt *et al.* (2019)
Serrapinus heterodon	UFRGS 22004	TEC6956	Lagoa marginal do rio Doce	Doce River	KY327457	Silva *et al.* (2017)

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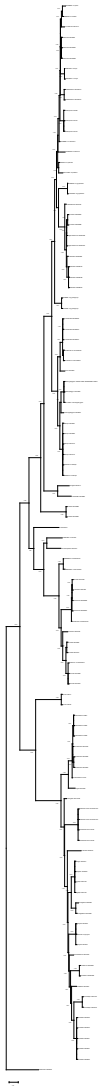

FIGURE S2 | Neighbor joining tree generated for *Astyanax*, *Deuterodon* and *Psalidodon* species. CLICK TO VIEW THE FULL IMAGE IN HIGH RESOLUTION.

TABLE S3 | P-distance between paralectotype sequences of *Psalidodon malabarborum*, UFRGS 19746, and the samples considered in this work. CLICK HERE TO VIEW THE FULL TABLE

Neotropical Ichthyology

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