

TABLE S1 | Information on the species included in the molecular analyses with their respective Genbank accession numbers. Freshwater ecoregions follow Abell *et al.* (2008).

Species	Voucher number	Accession number	Source authors	Ecoregion
1. <i>Ituglanis amazonicus</i>	LBPV 50532, LBP 11003	KY857936	Ochoa <i>et al.</i> (2017)	Madeira Brazilian Shield
2. <i>Ituglanis amazonicus</i>	SU 08-548	MZ051763	Papa <i>et al.</i> (2021)	Guianas
3. <i>Ituglanis amazonicus</i>	GWF 15-17	MZ051212	Papa <i>et al.</i> (2021)	Guianas
4. <i>Ituglanis amazonicus</i>	SU08-551	MZ051669	Papa <i>et al.</i> (2021)	Guianas
5. <i>Ituglanis amazonicus</i>	SU08-552	MZ051609	Papa <i>et al.</i> (2021)	Guianas
6. <i>Ituglanis amphipotamus</i>	UFRJ 12493	MW250305	Costa <i>et al.</i> (2021a)	Ribeira de Iguape
7. <i>Ituglanis australis</i>	UFRGS 19184, TEC 4887B	PX068399	Present study	Laguna dos Patos
8. <i>Ituglanis australis</i>	UFRGS 19184, TEC 4887A	PX068400	Present study	Laguna dos Patos
9. <i>Ituglanis boitata</i>	UFRGS 21916, TEC 6889A	PX068396	Present study	Tramandaí-Mampituba
10. <i>Ituglanis boitata</i>	UFRGS 17809, TEC 3490A	KR020517	Ferrer <i>et al.</i> (2015)	Tramandaí-Mampituba
11. <i>Ituglanis boitata</i>	UFRGS 19157, TEC 4874	KR020521	Ferrer <i>et al.</i> (2015)	Tramandaí-Mampituba
12. <i>Ituglanis boitata</i>	UFRJ 0582	MK123684	Katz <i>et al.</i> (2018)	Tramandaí-Mampituba
13. <i>Ituglanis boitata</i>	UFRGS 17219, TEC 3224	PX068397	Present study	Tramandaí-Mampituba
14. <i>Ituglanis boitata</i>	UFRGS 21916, TEC 6889B	PX068395	Present study	Tramandaí-Mampituba
15. <i>Ituglanis boitata</i>	UFRGS 18455, TEC 3848A	KR020519	Ferrer <i>et al.</i> (2015)	Tramandaí-Mampituba
16. <i>Ituglanis boitata</i>	UFRGS 17809, TEC 3490B	KR020518	Ferrer <i>et al.</i> (2015)	Tramandaí-Mampituba
17. <i>Ituglanis boitata</i>	UFRGS 22903, TEC 7531A	PX068394	Present study	Tramandaí-Mampituba
18. <i>Ituglanis boitata</i>	UFRGS 22903, TEC 7531B	PX068393	Present study	Tramandaí-Mampituba
19. <i>Ituglanis boitata</i>	UFRGS 19180, TEC 4883B	KR020520	Ferrer <i>et al.</i> (2015)	Tramandaí-Mampituba
20. <i>Ituglanis boitata</i>	UFRGS 18455, TEC 3848B	PX068398	Present study	Tramandaí-Mampituba
21. <i>Ituglanis boitata</i>	UFRGS 19180, TEC 4883A	KR020522	Ferrer <i>et al.</i> (2015)	Tramandaí-Mampituba
22. <i>Ituglanis cf. eichhorniarum</i>	LBP 10777, LBPV 49859	KY857938	Ochoa <i>et al.</i> (2017)	Paraguay
23. <i>Ituglanis cf. eichhorniarum</i>	LBP 7667, LBPV 36473	KY857940	Ochoa <i>et al.</i> (2017)	Paraguay
24. <i>Ituglanis eichhorniarum</i>	LBPV 24825, LBP 4686	KY857939	Ochoa <i>et al.</i> (2017)	Paraguay
25. <i>Ituglanis goya</i>	LBPV 68592, LBP 17131	KY857945	Ochoa <i>et al.</i> (2017)	Tocantins-Araguaia
26. <i>Ituglanis goya</i>	LBPV 68599, LBP 17137	KY857946	Ochoa <i>et al.</i> (2017)	Tocantins-Araguaia
27. <i>Ituglanis goya</i>	LBPV 77969, LBP 19465	KY858000	Ochoa <i>et al.</i> (2017)	Tocantins-Araguaia
28. <i>Ituglanis herberti</i>	LBPV 8028, LBP 676	KY857947	Ochoa <i>et al.</i> (2017)	Paraguay
29. <i>Ituglanis herberti</i>	LBPV 16211, LBP 2442	KY857935	Ochoa <i>et al.</i> (2017)	Tocantins-Araguaia
30. <i>Ituglanis inusitatus</i>	UFRGS 19311, TEC 4966B	MF975520	Ferrer, Donin (2017)	Lower Uruguay
31. <i>Ituglanis inusitatus</i>	UFRGS 20201, TEC 5052B	MF975519	Ferrer, Donin (2017)	Lower Uruguay
32. <i>Ituglanis inusitatus</i>	UFRGS 20201, TEC 5052A	MF975518	Ferrer, Donin (2017)	Lower Uruguay
33. <i>Ituglanis jaguarete</i>	UFRGS 29219, TEC 9987E	PX068388	Present study	Upper Uruguay
34. <i>Ituglanis jaguarete</i>	UFRGS 19179, TEC 4882B	PX068391	Present study	Upper Uruguay
35. <i>Ituglanis jaguarete</i>	UFRGS 29219, TEC 9987D	PX068389	Present study	Upper Uruguay
36. <i>Ituglanis jaguarete</i>	UFRGS 19179, TEC 4882A	PX068392	Present study	Upper Uruguay
37. <i>Ituglanis jaguarete</i>	UFRGS 21921, TEC 7923	PX068390	Present study	Lower Uruguay
38. <i>Ituglanis proops</i>	MCP 32509	PX068387	Present study	Ribeira de Iguape
39. <i>Cambeva zonata</i>	UFRGS 24538	MN995706	Donin <i>et al.</i> (2020)	Ribeira de Iguape
40. <i>Scleronema minutum</i>	LBPV 19841, LBP 3310	KY857957	Ochoa <i>et al.</i> (2017)	Laguna dos Patos

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