

TABLE S1 | Sampling localities and their correspondent GenBank Accession numbers and catalogue number of Facultad de Ciencias Fish Collection (ZVCP) and Laboratorio de Genética Evolutiva (GE) of *Acantholebias*. LP = Laguna dos Patos ecoregion; LU = Lower Uruguay ecoregion.

ZVCP	GE	Locality Code	Ecoregion	Basin	Sub-basin	Taxon	Citochromes b GeneBank Accession number
14225	3402-3404, 3534-3537	Averías	LP	Merín	Cebollati	<i>A. luteoflammulatus</i>	PX713003, PX713002, PX713001, PX713000, PX713004, PX713005, PX713006
12465	3632	Olimar	LP	Merín	Cebollati	<i>A. luteoflammulatus</i>	PX712993
15720	3622, 3623	Cipacebollati	LP	Merín	Cebollati	<i>A. luteoflammulatus</i>	PX712995, PX712996
16452	3653	SurOlimar	LP	Merín	Cebollati	<i>A. luteoflammulatus</i>	PX712991, PX712992
16455	3519-3522, 3633	Gringo/ AveríasGringo	LP	Merín	Cebollati	<i>A. luteoflammulatus</i>	PX712997, PX712998, PX712999, PX712994,
16503	3572, 3573	R14469	LP	Merín	San Luis	<i>A. luteoflammulatus</i>	PX713007, PX713008
16504	3582, 3584	R9272LN	LP	Merín	Negra	<i>A. luteoflammulatus</i>	PX713011, PX713012
-	3663, 3664	Andreoni	LP	Merín	Negra	<i>A. luteoflammulatus</i>	PX713009, PX713010
16505	3612, 3613	R9272LC	LP	Atlántico	Castillos	<i>A. luteoflammulatus</i>	PX713013, PX713014
-	3552-3554	Barra	LP	Atlántico	Castillos	<i>A. luteoflammulatus</i>	PX713018, PX713019, PX713020
16456	3562-3564	Valizas	LP	Atlántico	Castillos	<i>A. luteoflammulatus</i>	PX712991, PX713016, PX713017
13467	3809-3814	Tacuaribajo	LP	Merín	Tacuarí	<i>A. luteoflammulatus</i>	PX712985, PX712986, PX712987, PX712988, PX712989, PX712990
15359	4476-4478	RochaW	LP	Atlántico	Rocha	<i>A. aff. luteoflammulatus</i> 1/ <i>A. tuireae</i>	PX712926, PX712927, PX712928
16458	3602-3603	R15	LP	Atlántico	Rocha	<i>A. aff. luteoflammulatus</i> 1/ <i>A. tuireae</i>	PX712944, PX712945
16457	3592-3594	Probides	LP	Atlántico	Rocha	<i>A. aff. luteoflammulatus</i> 1/ <i>A. tuireae</i>	PX712936, PX712937, PX712938,
16459	4469-4473	RochaE	LP	Atlántico	Rocha	<i>A. aff. luteoflammulatus</i> 1/ <i>A. tuireae</i>	PX712931, PX712932, PX712933, PX712934, PX712935
16506	3542-3546	Arachania	LP	Atlántico	Rocha	<i>A. aff. luteoflammulatus</i> 1/ <i>A. tuireae</i>	PX712939, PX712940, PX712941, PX712942, PX712943
15358	4474, 4475	Noques	LP	Atlántico	Rocha	<i>A. aff. luteoflammulatus</i> 1/ <i>A. tuireae</i>	PX712929, PX712930
13510	3452-3456	Fraile	LU	Negro	Fraile Muerto	<i>A. aff. luteoflammulatus</i> 2/ <i>A. quirogai</i>	PX713042, PX713043, PX713044, PX713045
16454	3398-3400, 3422	Melo	LP	Merín	Tacuarí	<i>A. aff. luteoflammulatus</i> 2/ <i>A. quirogai</i>	PX713053, PX713054, PX713055, PX713052
13490	3829-3834	Codo1	LP	Merín	Tacuarí	<i>A. aff. luteoflammulatus</i> 2/ <i>A. quirogai</i>	KF027488, PX713036, PX713037, PX713038, PX713039, PX713040, PX713041
13471	3840	Codo2	LP	Merín	Tacuarí	<i>A. aff. luteoflammulatus</i> 2/ <i>A. quirogai</i>	PX713035
9718	2536-2538, 3467, 3468, 3476, 3477	Quebracho	LU	Negro	Tupambaé	<i>A. aff. luteoflammulatus</i> 2/ <i>A. quirogai</i>	KF027488, KF027489, KF027490, PX712974, PX712975, PX712972, PX712973
9715	3423, 3424, 3426, 3427, 3430, 3431	Paraje 329	LU	Negro	Negro	<i>A. aff. luteoflammulatus</i> 2/ <i>A. quirogai</i>	PX713046, PX713047, PX713048, PX713049, PX713050, PX713051

TABLE S1 | (Continued)

ZVCP	GE	Locality Code	Ecoregion	Basin	Sub-basin	Taxon	Cytochrome b GeneBank Accession number
13505	3457-3459	Arriera	LU	Negro	Negro	<i>A. quirogai</i>	PX712976, PX712977, PX712978
9758	3972	Cordobés	LU	Negro	Cordobés	<i>A. quirogai</i>	PX712946
13462	3851, 3859-3867	Lechiguana	LU	Negro	Lechiguana	<i>A. quirogai</i>	PX712954, PX712955, PX712956, PX712948, PX712949, PX712950, PX712951, PX712952, PX712953, PX712947
13514	3413-3417	Palos	LU	Negro	Aceguá	<i>A. quirogai</i>	PX712979, PX712980, PX712980, PX712982, PX712983
16453	3401	Molles	LU	Negro	Aceguá	<i>A. quirogai</i>	PX712984
13486	3849, 3850	Carpinteria	LU	Negro	Carpinteria	<i>A. quirogai</i>	PX712957, PX712958
8731	3480, 3481, 3486-3488, 3490, 3491	Diego	LP	Merín	Yaguarón	<i>A. quirogai</i>	PX712970, PX712971, PX712965, PX712966, PX712967, PX712968, PX712969
13475	3819-3824	Paso de Melo	LP	Merín	Yaguarón	<i>A. quirogai</i>	PX712959, PX712960, PX712961, PX712962, PX712963, PX712964
15071	3388-3392	P9	LP	Merín	Cebollatí	<i>A. aff. quirogai/A. bertae</i>	PX713030, PX713031, PX713032, PX713033, PX713034
13502	3442, 3443, 3449-3451	P2	LP	Merín	Cebollatí	<i>A. aff. quirogai/A. bertae</i>	PX713025, PX713026, PX713027, PX713028, PX713029
14240	3506, 3507, 3512, 3513	P7	LP	Merín	Cebollatí	<i>A. aff. quirogai/A. bertae</i>	PX713024, PX713021, PX713022, PX713023



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