

**Table S3.** Collecting sites and GenBank accession numbers of Rivulidae sequences used in the present work.

Species	Collection Identification	Collecting site	Mitochondrial <i>cyt-b</i> Accession number
<i>Austrolebias adloffii</i> (Ahl, 1922)	GP304	Ponte do Gravataí, Rio Grande do Sul, Brazil	AY724374.1
<i>Austrolebias adloffii</i>	GP308	Ponte do Gravataí, Rio Grande do Sul, Brazil	AY724372.1
<i>Austrolebias affinis</i> (Amato, 1986)	GP470	Route 5, km 399.5, Tacuarembó Department, Uruguay	AF245464.1
<i>Austrolebias alexandri</i> (Castello & Lopez, 1974)	GP475	Salto City, Salto Department, Uruguay	AF245011.1
<i>Austrolebias arachan</i> (Loureiro, Azpelicueta & García, 2004)	GP657	Parque Municipal, Melo City, Cerro Largo Department, Uruguay	AF245466.1
<i>Austrolebias bellottii</i> (Steindachner, 1881)	GP400	Bañado Verocay, Salto Department, Uruguay	AF245007.1
<i>Austrolebias bellottii</i>	R156	Route 11, 21 km S from Río de Oro, Chaco Province, Argentina	FJ826891
<i>Austrolebias charrua</i> (Costa & Cheffe, 2001)	GP350	Route 19, km 7 (pond 33), Rocha Department, Uruguay	AY724390.1
<i>Austrolebias charrua</i>	GP362	Route 19, km 7 (pond 33), Rocha Department, Uruguay	AY724391.1
<i>Austrolebias cheradophilus</i> (Vaz-Ferreira <i>et al.</i> , 1964)	GP416	Los Sauces, Valizas, Rocha Department, Uruguay	AF245467.1
<i>Austrolebias cinereus</i> (Amato, 1986)	GP2018	F2 from Route 21, Carmelo City, Colonia Department, Uruguay	KF027476
<i>Austrolebias cinereus</i>	GP2541	Route 21, Carmelo City, Colonia Department, Uruguay	KF027477
<i>Austrolebias cinereus</i>	GP735	Route 21, Carmelo City, Colonia Department, Uruguay	AF245005.1
<i>Austrolebias duraznensis</i> (García, Scvortzoff & Hernández, 1995)	GP2396	Next to Mercedes, Soriano Departement, Uruguay	KF192624
<i>Austrolebias duraznensis</i>	GP2397	Next to Mercedes, Soriano Departement, Uruguay	KF192625
<i>Austrolebias duraznensis</i>	GP466	Estadio Rampla, Durazno City, Durazno Department, Uruguay	AF245012.1
<i>Austrolebias elongatus</i> (Steindachner, 1881)	GP2389	Villa Soriano Town, Soriano Department, Uruguay	KF027478
<i>Austrolebias elongatus</i>	GP2545	Villa Soriano Town, Soriano Department, Uruguay	KF027479
<i>Austrolebias elongatus</i>	R159	Azul City, Buenos Aires Province, Argentina	FJ826892
<i>Austrolebias elongatus</i>	R160	Azul City, Buenos Aires Province, Argentina	FJ826893

Species	Collection Identification	Collecting site	Mitochondrial <i>cyt-b</i> Accession number
<i>Austrolebias gymnoventris</i> (Amato, 1986)	GP2393	Salamanca Caves, Maldonado Department, Uruguay	KF027480
<i>Austrolebias gymnoventris</i>	GP2394	Salamanca Caves, Maldonado Department, Uruguay	KF027481
<i>Austrolebias gymnoventris</i>	GP490	Route 13, India muerta. Rocha Department, Uruguay	AF245463.1
<i>Austrolebias juanlangi</i> (Costa, Cheffe, Salvia & Litz, 2006)	R003	BR290, Arroio Don Marcos, Rio Grande do Sul, Brazil	AF245460.1
<i>Austrolebias luteoflammulatus</i> (Vaz-Ferreira, Sierra & Paulete, 1964)	GP2391	Route 9, Probides, Rocha Department, Uruguay	KF027482
<i>Austrolebias luteoflammulatus</i>	GP2392	Route 9, Probides, Rocha Department, Uruguay	KF027483
<i>Austrolebias luteoflammulatus</i>	GP2547	Route 9, Probides, Rocha Department, Uruguay	KF027484
<i>Austrolebias luteoflammulatus</i>	GP2549	Route 9, Probides, Rocha Department, Uruguay	KF027485
<i>Austrolebias luzardoii</i> (Perujo, Calviño, Salvia & Prieto, 2005)	R227	Artigas City, Artigas Department, Uruguay	FJ826894
<i>Austrolebias melanoorus</i> (Amato, 1986)	GP894	Route 5, km 399.5, Tacuarembó Department, Uruguay	AY724373
<i>Austrolebias nigripinnis</i> (Regan, 1912)	GP397	Bañado Verocay, Salto Department, Uruguay	AF245013.1
<i>Austrolebias nioni</i> (Berkenkamp, Reichert & Prieto, 1997)	GP677	Route 26, km 331, Tacuarembó Department, Uruguay	AF245457.1
<i>Austrolebias patriciae</i> (Huber, 1995)	R113	Typical locality, Paraguay	FJ826897
<i>Austrolebias periodicus</i> (Costa, 1999)	R261	Route BR 293, next to Don Pedrito, Rio Grande do Sul Estate, Brazil	FJ826898
<i>Austrolebias prognathus</i> (Amato 1986)	GP2378	Route 8, next to Jose Pedro Varela Town, Lavalleja Department, Uruguay	KF027486
<i>Austrolebias prognathus</i>	GP2379	Route 8, next to Jose Pedro Varela Town, Lavalleja Department, Uruguay	KF027487
<i>Austrolebias prognathus</i>	GP381	Route 19, km 6.5, Rocha Department, Uruguay	AF245458
<i>Austrolebias quirogai</i> (Loureiro, Duarte & Zarucki, 2011)	GP2536	Cerro de las cuentas Town, Cerro Largo Department, Uruguay	KF027488
<i>Austrolebias quirogai</i>	GP2537	Cerro de las cuentas Town, Cerro Largo Department, Uruguay	KF027489
<i>Austrolebias quirogai</i>	GP2538	Cerro de las cuentas Town, Cerro Largo Department, Uruguay	KF027490
<i>Austrolebias reicherti</i> (Loureiro & García 2005)	GP1085	Bañados del Este (pond 43), Treinta y Tres Department, Uruguay	AY724398.1
<i>Austrolebias reicherti</i>	GP1104	Bañados del Este (pond 42), Treinta y Tres Department, Uruguay	AY724401.1

Species	Collection Identification	Collecting site	Mitochondrial <i>cyt-b</i> Accession number
<i>Austrolebias robustus</i> (Günther, 1883)	R046	Route 63, Km 16.2, Dolores City, Buenos Aires Province, Argentina	FJ826899
<i>Austrolebias robustus</i>	R049	Vivoratá City, Buenos Aires Province, Argentina	FJ826900
<i>Austrolebias robustus</i>	R082	Next to 9 de Julio City, Buenos Aires Province, Argentina	FJ826896
<i>Austrolebias vandenbergi</i> (Huber, 1995)	R116	Typical locality, Paraguay	FJ826901
<i>Austrolebias vazferreiraia</i> (Berkenkamp, Etzel, Reichert & Salvia, 1994)	GP675	Melo City, Cerro Largo Department, Uruguay	AF245015
<i>Austrolebias vazferreiraia</i>	GP809	Melo City, Cerro Largo Department, Uruguay	
<i>Austrolebias viarius</i> (Vaz-Ferreira, Sierra de Soriano & Scaglia de Paulete 1964)	GP377	Intersection of the Routes 10 and 16, Rocha Department, Uruguay	AY724388.1
<i>Austrolebias viarius</i>	GP880	Route 16, km 2.5 (pond 11), Rocha Department, Uruguay	AY724383.1
<i>Austrolebias viarius</i>	GP881	Route 16, km 2.5 (pond 11), Rocha Department, Uruguay	AY724384.1
<i>Austrolebias wolterstorffi</i> (Ahl, 1924)	GP530	Route 15, km 137.6, Lascano City, Rocha Department, Uruguay	AF245014.1
<i>Austrolebias monstrosus</i> (Huber, 1995)	R197	Salta Province, Argentina	FJ826895
<i>Austrolebias patriciae</i> (Huber, 1995)	R113	Typical locality, Paraguay	FJ826897
<i>Austrolebias wichi</i> n.sp	R192	Salta, Argentina	-----
<i>Austrolebias cyaneus</i> (Amato, 1987)	R003	Arroio Don Marcos. BR 290, RS. BRASIL	AF245461
<i>Austrolebias minuano</i> Costa & Cheffe, 2001	-----		KJ475091
<i>Austrolebias nachtigalli</i> Costa & Cheffe, 2006	-----		KJ475098
<i>Austrolebias nigrofasciatus</i> Costa & Cheffe, 2001	-----		KJ475087
<i>Nematolebias whitei</i> (Myers, 1942)	-----		KF311332
<i>Neofundulus paraguayensis</i> (Eigenmann & Kennedy, 1903)	-----		AF002510