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Austrolebias queguay (Cyprinodontiformes, Rivulidae), a new species of annual killifish endemic to the lower Uruguay river basin

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Abstract

In this article we describe a new species of the annual fish genus *Austrolebias* from the lower Uruguay river basin. The fusion of the urogenital papilla to the first anal fin ray in males and the pigmentation pattern, indicates a close relationship with the clade formed by *A. bellottii*, *A. melanoorus*, and *A. univentripinnis*. The new species can be differentiated from those by the following combination of characters: presence of well-defined light bands contrasting with the sides of the body, the distal portion of the anal fin dark gray, pelvic fins dark bluish green and bases united at about 50–80% on their medial margins, pectoral fins with iridescent blue sub-marginal band, and general coloration of body bluish green. The new species can only be found in wetlands of the Queguay river, an area included in the Uruguayan protected areas system and represents so far the only annual fish species endemic to the lower Uruguay river basin.

Introduction

Austrolebias, one of the most specious annual fish genus in the family Rivulidae, is composed of 47 valid species and is distributed in Bolivia, Paraguay, southern Brazil, northeast Argentina, and Uruguay, in the La Plata, Patos-Merin, and southwestern Amazon basins (Alonso et al. 2018, Calviño et al. 2016, Costa, 2006, 2014, Costa et al. 2017, García et al. 2012, Loureiro et al. 2011, Nielsen and Pillet 2015, Volcan et al. 2014, 2017). In a systematic revision of the genus, Costa (2006) provided three exclusive synapomorphies and three synapomorphies independently arisen in other cynolebiatines.

Costa (2006) defined five species groups within the genus. One of them, the "*Austrolebias bellottii*" species group (clade A4; fig. 1 in Costa 2006), diagnosed by the presence of anteromedian rays of the anal fin elongated in females (triangular fin shape) is composed of two subclades: the "*Austrolebias adloffi*" species group (clade A5, fig. 1 in Costa 2006), diagnosed by chromatic characters; and an unnamed clade that lacks morphological diagnostic characters. This clade is composed of *A. bellottii* (Steindachner), *A. vandenbergi* (Huber), *A. apaii* Costa (all from La Plata basin), *A. melanoorus* (Amato) (from La Plata and Patos Merin basins), and *A. univentripinnis* Costa and Cheffe (from Patos Merin basin).

Afterwards, based on another morphological phylogeny, Costa (2010) added *A. patriciae* to the "*A. bellottii*" species group. In addition, Nielsen and Pillet (2015), described a species from the upper Mamoré river basin (Amazon basin), *A. accorsii*, morphologically very similar to *A. vandenbergi*. However, Alonso et al. (2016) questioned some of the diagnostic characters for *A. acorsii*.

In spite of the low statistical support of A5 and the unnamed clade in morphological analyses (Costa 2006, 2010), mitochondrial and total evidence phylogenetic reconstructions, support the monophyly of both clades (García et al. 2002, 2012, 2014, Loureiro and García 2008; Loureiro et al. 2018). However, these analyses differ in the relationships between and within them, and with other *Austrolebias* species groups. According to Garcia et al. (2014) and Loureiro et al. (2018), *A. patriciae* is not closely related to the "*A. bellottii*" species group, and according to Garcia et al. (2012), *A. apaii* should be considered a synonym of *A. bellottii*, a proposal that was followed by Calviño et al. (2016).

In this article we describe a new species of *Austrolebias* from wetlands of the Queguay river (lower Uruguay river basin), that shares similar morphological traits to the clade formed by *A. bellottii*, *A. melanoorus*, and *A. univentripinnis*.

Figure 1. *Austrolebias queguay* sp. n., ZVC-P 13576, 39.4 mm SL, holotype, male, Estancia La Beba (32°11'08"S, 57°26'08"W), wetlands of Río Queguay Grande, Paysandú Department, Uruguay.

Materials and methods

Specimens analyzed and comparative material are deposited in the Fish Collections of Facultad de Ciencias, Universidad de la República (ZVC-P), and Museo Nacional de Historia Natura (MHNM), Montevideo, Uruguay, Universidade Federal de Rio Grande do Sul (UFRGS), Rio Grande do Sul, Brazil, and The National Academy of Sciences, Philadelphia, USA (ANSP). Alcohol fixed individuals belong to the tissue collections of Genetica Evolutiva Section (GP) and Zoologia Vertebrados Laboratory (CAP) (Facultad de Ciencias, UdelaR), and Departamento de Zoologia (TEC) da Universidade Federal do Rio Grande do Sul. Measurements and meristic counts were taken under dissecting microscope, according to Costa (2006) and Loureiro and García (2008). Morphological variation was also assessed using a geometric morphometric analyses of landmark configurations using a thin plate spline approach (Bookstein 1991). Landmark positions were modified from D'Anatro and Loureiro (2005) (adding the lower insertion of pectoral fin as a new landmark), acquired from scanned fish images (MicroTek Scanner, Hsinchu, Taiwan), and digitized using the TPSdig program (Rohlf 2003). Weight matrices of partial warps were generated using MorphoJ 1.05f (Klingenberg 2011). Visualization of specimen groupings and the corresponding shape variation were obtained by canonical variate analyses. Cephalic neuromast series nomenclature follows Costa (2006). Cleared and double stained specimens (c&s) were prepared following Dingerkus and Uhler (1977).

Taxonomy

***Austrolebias queguay* sp. n.**

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Figs 1, 2A, B, G, 3A

Austrolebias sp. in Loureiro et al. (2018)

Holotype. ZVC-P 13576, male, 39.4 mm SL, Uruguay, Paysandú, wetlands of Río Queguay Grande, Estancia La Beba, 32°11'08"S, 57°26'08"W, M. Loureiro, A. Duarte, M. Zarucki, J. Bessonart and D. Hernández, Sep. 2011.

Paratypes. Uruguay: Paysandú: MHNM 3728, 2 males 29.9–33.6 mm SL, 2 females 27.4–33.3 mm SL, Río Queguay, 32°08'21"S, 57°26'19"W, M. Loureiro, A. Duarte, M. Zarucki, J. Bessonart and D. Hernández, Sep. 2011. MHNM 3729, 1 male 34.0 mm SL, 1 female 31.0 mm SL, same data of the holotype. ZVC-P 8657, 10 males 16.1–27.2 mm SL, 12 females 16.9–30.1 mm SL, Río Queguay, Rincón de Pérez, 32°08'23"S, 57°25'49"W, M. Loureiro and S. Clavijo, Aug. 2006. ZVC-P 11620, 37 males 22.8–39.4 mm SL (8 c&s 26.6–38.1 mm SL; 10 fixed in alcohol 95°, 21.9–28.4 mm SL, CAP 1193, GP 3353–3359 and GP 3364–3366), 60 females 24.2–40.1 mm SL (8 c&s 22.7-37.4 mm SL; 4 fixed in alcohol 95°, 22.7–28.7 mm SL, CAP 1193, GP 3360–3363), Río Queguay, 32°08'21"S, 57°26'19"W, M. Loureiro, A. Duarte, M. Zarucki, J. Bessonart and D. Hernández, Sep. 2011. ZVC-P 11621, 28 males 22.1–34.9 mm SL (5 fixed in alcohol 95°, 22.1–27.7 mm SL, CAP 1181, GP 3367–3371), 33 females 21.2–29.9 mm SL (5 fixed en alcohol 95°, 23.4–27.8 mm SL, CAP 1181, GP 3372–3376), Río Queguay, 32°07'26"S, 57°30'45"W, M. Loureiro, A. Duarte, M. Zarucki, J. Bessonart and D. Hernández, 8 Sep. 2011. ZVC-P 12460, 33 males 25.7–39.1 mm SL (2 c&s 32.4–39.1 mm SL; 4 fixed in alcohol 95°, 27.1–29.0 mm SL, CAP 1194, GP 3377–3380), 25 females 24.9–37.4 mm SL (2 c&s 27.2- 33.1 mm SL; 3 fixed in alcohol 95°, 24.9–26.5 mm SL, CAP 1194, GP 3381–3383), same data of the holotype.

Diagnosis. The new species differs from all the other species of the genus except *Austrolebias bellottii*, *A. univentripinnis* and *A. melanoorus*, by the presence of the urogenital papilla attached to the anal fin in males (vs. free from the anal fin). It differs from *A. bellottii* and *A. univentripinnis* by the presence of well-defined light blue bands contrasting with the sides of the body in adult males (vs. vertical rows of light blue dots) (Fig. 2); from *A. melanoorus*, by the presence of dark gray coloration of the distal portion of the anal fin in males (vs. distal portion of anal-fin black), pelvic-fins dark bluish green (observed in ventral view) and bases united at about 50–80% on their medial margins (vs. dark gray and united about 50% or less), pectoral-fins with iridescent blue sub-marginal band (vs. sub-marginal band absent), and general coloration of the body bluish green (vs. grayish sky blue).

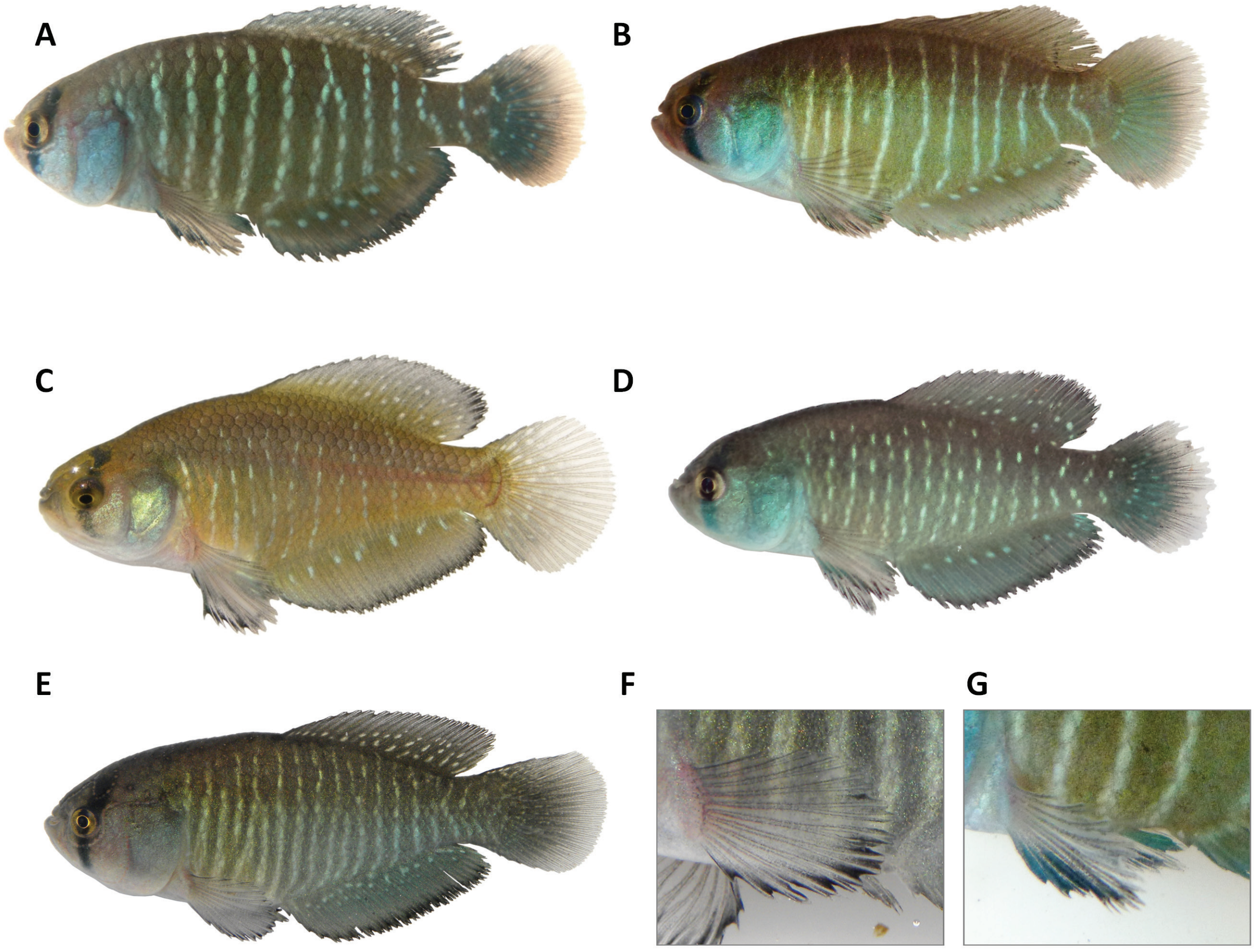

Figure 2. A. *Austrolebias queguay* sp. n. paratype male (ZVCP 11620); **B.** *A. queguay* non type male (32°07'26"S, 57°30'45"W), not preserved (right side, photo flipped); **C.** *A. bellottii* non preserved male; **D.** *A. univentripinnis* male (UFRGS 18064, right side photo flipped); **E.** *A. melanoorus* topotype male (ZVCP13651); **F.** Detail of pectoral and pelvic fins of *A. melanoorus*; **G.** Detail of pectoral and pelvic fins of *A. queguay*.

Figure 3. A. *Austrolebias queguay* sp. n. female: paratype ZVCP 11620; **B.** *A. bellottii* female (ZVCP 11560); **C.** *A. univentripinnis* female (UFRGS 18066); **D.** *A. melanoorus* topotype female (ZVCP 13651).

Description. Morphometric data in Table 1. Largest examined male 39.4 mm SL; largest examined female 40.1 mm SL. Body orbicular and compressed. Maximum body depth between pelvic-fin origin and anal-fin origin in both sexes. Dorsal profile of head straight to slightly concave. Dorsal profile of body convex between head and posterior insertion of dorsal fin. Ventral profile of body convex between anterior margin of mandible and the origin of anal fin; base of anal fin straight in males and straight to concave in females. Upper and inner margin of caudal peduncle usually straight. Snout short and rounded.

Posterior end of anal and dorsal fins rounded; presence of short filaments in distal margin of anal-fin in males. Anal fin in females triangular shaped (anteromedian rays prolonged forming anterior lobe). Caudal fin rounded. Pectoral fin elliptical, posterior margin on vertical between 2nd to 5th anal-fin ray bases in males, and between pelvic-fin origin and urogenital papilla in females. Pelvic fins medially united between 50–80%, with posterior tip reaching between urogenital papilla and base of 4rd anal-fin ray in both sexes. Urogenital papilla in males partially attached (only tip of papillae free) to anal fin. Base of dorsal-fin origin anterior to the anal-fin origin in males, between 8th to 11th vertebrae and 7th to 10th neural spine; in females usually vertical to posterior to the anal fin origin, between 12th to 14th vertebrae and 10th to 13th neural spine. Origin of anal fin between pleural ribs 8th to 9th and vertebrae 10th and 12th in males: between pleural ribs 9th and 12th and vertebrae 12th and 15th in females. Dorsal fin rays 22–25 in males and 17–20 in females; anal-fin rays 24–27 in males and 21–24 in females. Caudal fin rays 20–25; pectoral fin rays 11–13; pelvic fin rays 5.

Scales cycloid. Trunk and head scaled, except ventral surface of head. Longitudinal series of scales 28–33, regularly arranged; transversal series 11–16 (N=29 and one specimen with 21 scales); circumpeduncular series 13–20. Anal-fin base without scales; caudal fin with three rows of irregularly arranged scales. Contact organs present in all analyzed males, 1 to 8 contact organs per scale (usually 1 or 2); contact organs present in first 6 upper rays of pectoral fins; no contact organs on unpaired and pelvic fins.

Cephalic neuromasts: supraorbital 13–23, parietal 0–4, anterior rostral 1–2 (usually 1), posterior rostral 0–2 (usually 1), infraorbital 1–3 + 18–27, preorbital 2–4, otic 2–5, post-otic 1–5, supratemporal 1–3 (usually 1), median opercular 1–2 (usually 1), ventral opercular 1–3, preopercular 19–29, mandibular 11–15, lateral mandibular 3–7.

Basihyal cartilage anteriorly widened, about 50–60% of total length of basihyal; anterior margin of cartilage usually concave or with little commissures. Second pharyngobranchial with 3–8 teeth and 3rd with 17–37. First branchial arch with 3–4 epibranchial spines and 10–12 hypobranchial. Dermosphenotic ossifications present only in 5 % of specimens analyzed; proximal radials 3–5 (usually 4); ventral process posttemporal well-developed. Total vertebrae 27–30.

Color in life. Males (Fig. 2A–B). Ground color of body bluish green, darker in dorsal region, with 8–15 light sky blue vertical bands. Some specimens with dark green spots in the middle of the flank. Pectoral and ventral region whitish. Opercle and preopercle intense sky blue. Iris yellow; dark vertical band crossing the eye. Pectoral fins hyaline with black margin and iridescent blue sub-marginal band; pelvic fins blue to green. Dorsal and anal fins greenish blue with light sky blue dots on base; distal margin of anal fin darker. Caudal fin greenish blue with disperse light sky blue dots present or not, distal margin hyaline.

Table 1. Morphometric data of *Austrolebias queguay* sp. n., Standard length is expressed in mm; measurements numbered 2–13 are percentage of standard length; subunits of head measurements (numbered 14–18) are percentage of Head length. Ranges presented for males include the holotype.

Character	Holotype	Males					Females				
		N	Low	High	Mean	SD	N	Low	High	Mean	SD
1. Standard length (mm)	39.4	54	22.8	39.4	31.0	–	88	21.2	40.1	29.2	–
2. Body depth	37.4	54	32.8	39.5	36.5	1.75	88	30.0	40.0	34.7	2.09
3. Head lenght	27.2	54	23.9	29.6	27.1	1.28	88	22.6	30.2	27.7	1.36
4. Caudal peduncle depth	14.6	54	11.6	15.6	13.7	0.77	88	11.3	15.8	13.1	0.83
5. Caudal peduncle length	11.5	54	6.5	17.3	11.5	2.16	88	12.1	26.4	16.0	2.25
6. Pre dorsal length	50.6	54	43.1	54.3	50.3	2.37	88	43.1	64.8	59.9	2.81
7. Dorsal-fin base length	42.3	54	34.8	47.7	40.6	2.79	88	22.4	32.6	27.3	2.42
8. Pre anal-fin length	50.5	54	46.7	60.2	51.9	2.39	88	52.6	67.1	60.4	2.65
9. Anal-fin base length	44.8	54	35.5	46.1	42.4	2.19	88	21.8	30.9	26.3	2.07
10. Pre pelvic-fin length	45.9	54	41.3	48.8	45.3	1.51	88	46.3	61.1	51.9	2.38
11. Pectoral fin length	20.2	54	18.8	27.7	23.5	2.10	88	20.2	30.4	24.4	1.74
12. Pelvic fin length	9.6	54	6.7	11.9	9.2	1.01	88	9.2	14.9	12.3	1.11
13. Caudal fin length	20.5	54	15.3	28.5	24.5	2.39	88	22.6	30.8	26.7	1.88
14. Head width	65.1	54	59.9	77.2	67.7	3.95	88	60.8	90.3	75.3	6.28
15. Head depth	112.3	54	92.1	125.7	110.3	6.70	88	88.5	133.7	104.7	7.33
16. Interorbital width	51.7	54	39.8	58.0	47.6	4.50	88	39.5	54.1	45.9	3.17
17. Eye diameter	30.9	54	25.0	37.7	30.3	2.83	88	25.7	35.7	30.9	2.12
18. Snout length	27.6	54	16.7	29.5	21.9	2.10	88	16.4	25.5	20.2	1.98

Females (Fig. 3A). Ground color light brown, darker in dorsal region; sides of abdominal area yellow; pectoral and ventral region whitish. Black spots in central area of flanks present or absent, when present usually horizontally aligned; rest of the body with diffuse brown spots or vertical bands. Iris yellow; diffuse gray vertical band crossing the eye. Opercle and preopercle with sky blue reflections. Paired fins hyaline; unpaired fins yellowish at base to hyaline on distal margin, sometimes with diffuse brown spots or bars in the space between rays.

Geometrical morphometric analyses. Canonical variate analyses discriminated *A. bellotti* specimens from the other species along Root 1 (84.7 and 83.7% of total variation in males and females respectively, Figs 4–5), and partially discriminated *A. queguay* specimens from the other species along Root 2 (10.2 and 11.8 % of total variation in males and females respectively, Figs 4–5). In both cases, but especially in females, shape changes along Root 1 were associated to a deeper body in *A. bellotti* in relation to the other species.

Etymology. The specific name, *queguay*, is in reference to Queguay river basin, the type locality of the new species, treated as a noun in apposition to the generic name.

Distribution. *Austrolebias queguay* is endemic to the wetlands of middle Queguay river basin (30 meters above sea level), Paysandú Department, Uruguay, which flows to the lower Uruguay river (Fig. 6).

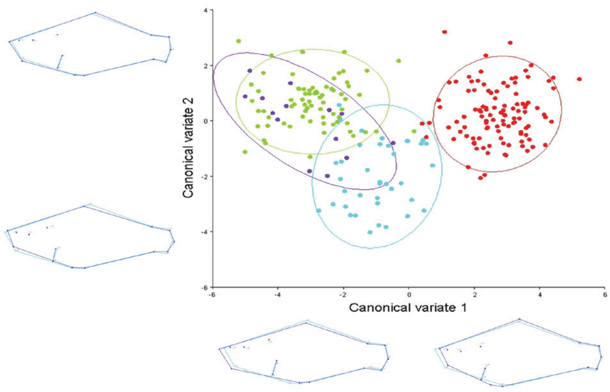

Figure 4. Canonical variate analysis of the morphometry of males. Red dots = *A. bellottii*, Purple dots *A. univentripinnis*, Green dots = *A. melanoorus*, Skyblue dots = *A. queguay*. Deformation (dark blue line) from consensus configuration (sky blue line) associated to each canonical axis.

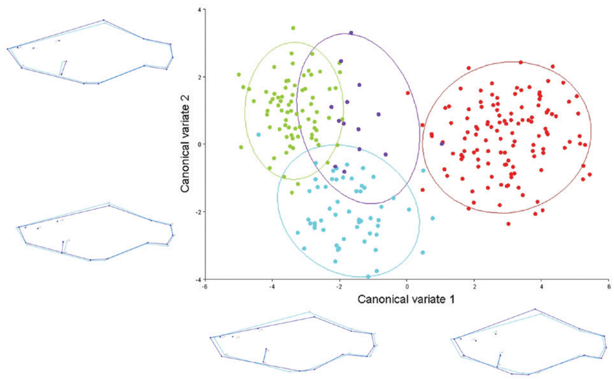

Figure 5. Canonical variate analysis of the morphometry of females. Red dots = *A. bellottii*, Purple dots *A. univentripinnis*, Green dots = *A. melanoorus*, Skyblue dots = *A. queguay*. Deformation (dark blue line) from consensus configuration (sky blue line) associated to each canonical axis.

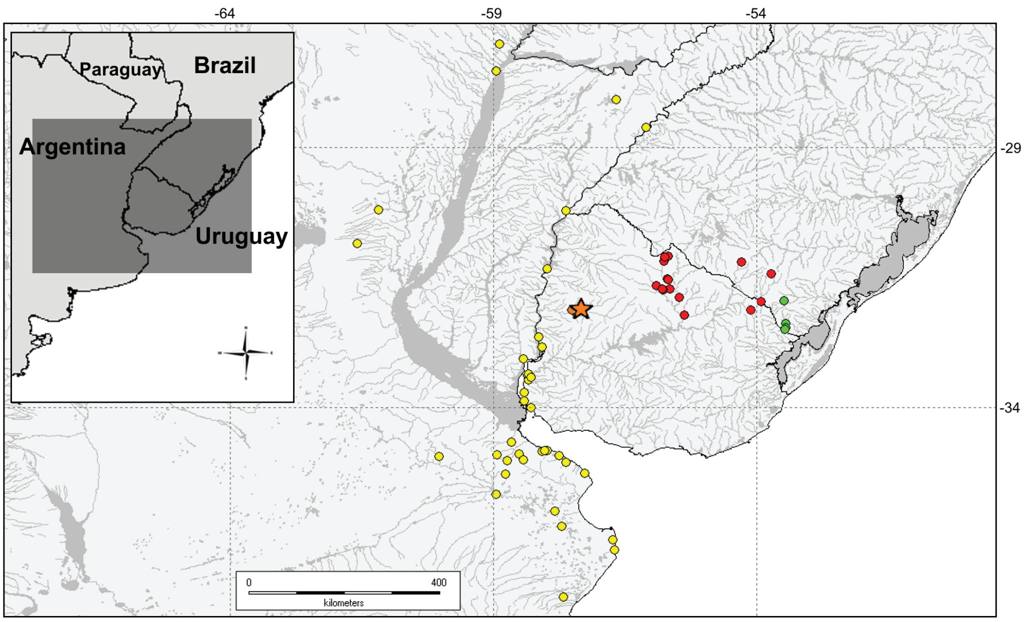

Figure 6. Geographic distribution of *Austrolebias queguay* sp. n. (orange dots, Uruguay river basin), *A. bellottii* (yellow dots, Paraná and Uruguay river basins), *A. melanoorus* (red dots, Negro and Yaguarón river basins), and *A. univentripinnis* (green dots, Yaguaron river basin). Orange star indicates type locality.

Conservation. All its known locations are within "Montes del Queguay", a permanent protection reserve under the control and regulation of the Uruguayan government, with an area of about 200 km2, but without a management plan (MVOTMA 2018; http://www.mvotma.gub.uy/portal/areas-protegidas/item/10006542-area-protegida-con-recursos-manejados-montes-del-queguay-paysandu.html). Furthermore, projected land-use changes for surrounding areas for the next decade may result in increases in forestry for cellulose production purpose (Brazeiro 2015), and wetlands are potentially vulnerable to hydric changes in the region. For these reasons, considering UICN criteria (IUCN 2012), and until a formal assessment is done, *A. queguay*, could be considered preliminarily as an 'Endangered Species'.

Ecology. As many species of the family Rivulidae, *A. queguay* presents an annual life cycle which includes drought resistant eggs and diapausing embryos. All species of *Austrolebias* are obligate annuals (Berois et al. 2016). In the Pampa biome there is not a defined dry season, so dried environments can be found between mid spring to early fall (depending on the year), when evaporation is higher than precipitations (Williams 2006; García et al. 2017). *Austrolebias* species can be found in small grassland ponds and seasonal floodplain wetlands; however the new species has been found so far only in the latter environments (Fig. 7).

Figure 7. Type locality of *Austrolebias queguay* sp. n., wetlands of middle Queguay river basin.

Discussion

The new species described in this article presents all diagnostic characters for the genus *Austrolebias* proposed by Costa (2006): absence of scales between corner of mouth and anterior portion of preopercular region and ventral portion of opercular region, deep urohyal, dark gray to black infraorbital bar and supraorbital spot, dorsal and anal fins rounded in males, long urogenital papilla in male, and reduced ventral process of angulo-articular. The partial fusion of the urogenital papilla to the first anal fin ray in males, places *A. queguay* in the clade composed by *A. bellottii*, *A. melanoorus*, and *A. univentripinnis*.

Differences with the other species of the clade concern pigmentation pattern in males. This kind of variation is common among species of other *Austrolebias* clades, such as the "*A. affinis*" species group, where *A. juanlangi* and *A. paucisquama* present well-defined clear bands (in different degree) over a darker background coloration, vs. vertical rows of light blue dots in the other species (Costa 2006; Ferrer et al. 2006); or in the "*A. adloffi*" species group, where background pigmentation can be concentrated enough to form black bars over a clear background (Costa 2006; Loureiro and García, 2008). Difference in pigmentation pattern among males in different species of animals has been hypothesized to have resulted from sexual selection and reinforcement (Panhuis et al. 2001). However, the allopatric distribution of species of the same clade in most *Austrolebias* (Loureiro et al. 2016), suggests that variation in these characters may be due to other forces, such as local adaptation, sensory drive (Boughman 2002), or just random variation caused by genetic drift.

Additionally, *A. queguay* differs from of *A. bellottii*, *A. melanoorus*, and *A. univentripinnis*, by the disposition of black spots on the central area of the flanks in females, when present horizontally aligned vs. when present, usually not aligned, from *A. bellottii* by dermosphenotic ossifications usually absent (95% of the *A. queguay* specimens analyzed) vs. usually present in *A. bellottii* (91% of specimens analyzed), and from *A. melanoorus* by the number of anterior rostral neuromasts, usually one pair (85 % of specimens analyzed) vs. usually two pairs in *A. melanoorus* (90% of specimens). Although these characters cannot be used as diagnostic, they support the idea of genetic isolation of the new species from the others, particularly from the nearby located populations of *A. bellottii*.

According to a recent morphological and molecular phylogenetic analysis the new species (*Austrolebias* sp; figs 9–12, Loureiro et al. 2018), is closely related to *A. bellottii*, and both represent the sister clade of *A. melanoorus* + *A. univentripinnis*. Interestingly, body shape of *A. queguay* is more similar to the phylogenetically and distributional distant species of the group, in the Negro and Yaguarón river basins (*A. melanoorus* and *A. univentripinnis*) than to *A. bellottii*, whose nearest localities are just 50 km away downstream and 25 five meters below the new species range.

The lower Uruguay and Paraná rivers have suffered the effect of sea level changes associated to the glacial cycles since the Pleistocene. The last transgression is hypothesized to have occurred around 6 thousand years ago where the sea level rose five meters above actual level (Bracco et al. 2011). During marine transgressions, wetlands and lowlands of the ancient lower Uruguay and Paraná rivers became estuarine or even marine environments (Martínez and Rojas 2011) and tributaries flowed directly into them, generating a barrier that may have isolated *A. queguay* and *A. bellottii* populations. The altitude of the inhabited wetlands and the fact that the highest sea level reached in the area in the last 100 thousand years has been only 5 meters above the current level (Lambeck et al. 2002; Bracco et al. 2011), may also suggest that its isolation from the other species of the group could have occurred before that.

Four species of *Austrolebias* had been recorded in the lower Uruguay river: *A. bellottii*, *A. nigripinnis*, *A. alexandri*, and *A. elongatus* (Costa 2006; Loureiro et al. 2016). The new species could be considered the only annual fish species endemic to this section of the basin, since the other *Austrolebias* species of the region present wider ranges: *A. bellottii* and *A. nigripinnis* in the lower Paraná and middle Uruguay river basins, *A. elongatus* in the lower Paraná basin, and *A. alexandri* in the middle Uruguay river basin. These species have been found living in syntopy in different combinations. Interestingly, *A. queguay* is the only *Austrolebias* inhabitant of the wetlands of the middle Queguay river. The extreme endemism of *Austrolebias queguay* urges National Authorities to elaborate management plans to secure the conservation of its populations. These plans need to address not only direct actions concerning the fish populations, but also oversight the productive activities in the surrounding basin.

Comparative material examined

Austrolebias bellottii: **ARGENTINA. Buenos Aires. MHNM 2425**, 13 males 18.6–41.6 mm SL, 52 females 17.5–40.1 mm SL, Santa Teresita, 36°32'S, 56°43'W, R. Taberner, 31 Oct. 1975. **MHNM 2801**, 5 males 27.8–35.8 mm SL, 18 females 30.1–39.8 mm SL, road between La Plata and Magdalena, 35°03'S, 57°37'W, J.R. Casciotta, Nov. 1986. **ZVC-P 517**, 1 male 38.3 mm SL, 1 female 37.0 mm SL, road between Villa Elisa and Punta Lara, 34°50'S, 58°01'W, R. Vaz-Ferreira, B. and J. Soriano, 2 Nov. 1962. **ZVC-P 707**, 9 males 30.7–48.6 mm SL, 2 females 38.9–39.4 mm SL, Camino de la Costa, 13km S of La Plata, 34°55'S, 57°45'W, R. López, R. Vaz-Ferreira, B. Sierra de Soriano and J. Soriano, 3 Nov. 1962. **ZVC-P 708**, 3 males 41.8–47.4 mm SL, 8 females 31.9–44.1 mm SL, Camino de la Costa, 13km S of La Plata, 34°55'S, 57°45'W, R. López, R. Vaz-Ferreira, B. Sierra de Soriano and J. Soriano, 3 Nov. 1962. **ZVC-P 711**, 42 males 30.0–48.4 mm SL, 51 females 25.8–49.3 mm SL, Camino de la Costa, 13km S of de La Plata, 34°55'S, 57°45'W, R. López, R. Vaz-Ferreira, B. Sierra de Soriano and J. Soriano, 3 Nov. 1962. **ZVC-P 714**, 1 female 33.1 mm SL, Punta Lara, 34°49'S, 57°57'W, R. Vaz-Ferreira, B. Sierra de Soriano and J. Soriano, 3 Nov. 1962. **ZVC-P 954**, 7 males 55.2–63.7 mm SL, 3 females 44.6–50.8 mm SL, road between Villa Elisa and Punta Lara, 34°50'S, 58°01'W, López-Grancelli, 21 Oct. 1963. **Chaco. MHNM 2566**, 4 males 23.1–36.0 mm SL, 40 females 27.1–39.9 mm SL, Puerto Vilelas, 27°30'S, 58°57'W, R. Taberner, 7 Apr. 1974. **ZVC-P 10448**, 2 males 34.7–34.8 mm SL, 20 km S of Río Oro, 27°00'S, 58°53'W, P. Calviño, 30 Jun. 2005. **Entre Ríos. ZVC-P 10451**, 15 males 14.3–32.7 mm SL, 2 females 19.5–21.5 mm SL, Club de Pesca, Gualeguaychú, 33°03'38''S, 58°25'32''W, 21 Sep. 2008. **Santa Fe. ZVC-P 6409**, 6 males 33.2–41.2 mm SL, 1 female 31.2 mm SL, Río Salado, 18 km N of San Cristobal, 30°11'S, 61°11'W, Lic. J.M. Gallardo, 17 Dec. 1966. **ZVC-P 10449**, 2 males 34.7–34.8 mm SL, 7 females 24.2–29.8 mm SL, Tacural, 30°50'S, 61°35'W, P. Calviño, 22 Aug. 2008. **URUGUAY. Artigas. ZVC-P 5346**, 12 males 24.5–48.2 mm SL (5 c&s 26.5–30.8 mm SL), 11 females 23.1–41.1 mm SL (5 c&s 23.1–27.7 mm SL), Franquia, Bella Unión, 30°13'09"S, 57°37'20"W, M. Loureiro, Jul. 2002. **Colonia. ZVC-P 876**, 17 males 24.6–47.2 mm SL, 11 females 21.6–44.7 mm SL, Radio of Carmelo, 34°00'03"S, 58°17'43"W, R. Vaz-Ferreira, 5 Nov. 1964. **ZVC-P 2086**, 6 males 38.2–48.2 mm SL, 11 females 32.5–43.2 mm SL, Radio of Carmelo, 34°00'03"S, 58°17'43"W, R. Vaz-Ferreira and G. Gannella, 23 Sep. 1973. **Río Negro. ZVC-P 11635**, 1 male 37.1 mm SL, Vizcaíno, 33°21'42"S, 58°20'34"W, D. García, D. Díaz, W.S. Serra and M. Loureiro, 11 Sep. 2012. **Salto. ZVC-P 5343**, 9 males 29.7–39.8 mm SL (4 c&s 29.7–34.0 mm SL), 6 females 26.2–34.4 mm SL (4 c&s 26.2–34.4 mm SL), City of Salto, 31°19'40"S, 57°58'28"W, M. Loureiro, F. Teixeira de Mello and E. Charbonier, May 2002. **Soriano. ZVC-P 7712**, 1 male 37.1 mm SL, 7 females 27.6–37.7 mm SL, W of Villa Soriano, 33°23'33"S, 58°20'41"W, M. Loureiro and S. Clavijo, 31 Jul. 2007. **ZVC-P 7826**, 4 males 44.6–49.6 mm SL, 7 females 37.2–46.4 mm SL, Estancia Curupí, 33°28'27"S, 58°20'03"W, M. Loureiro and S. Clavijo, 13 Jul. 2007. **ZVC-P 11560**, 4 males 29.0–39.4 mm SL, 5 females 27.9–37.9 mm SL, Agraciada, 33°42'42"S, 58°25'11"W, 8 Sep. 2010. **ZVC-P 12475**, 3 males 42.5–62.6 mm SL, 1 female 52.2

mm SL, camino de la Escuela N°1, Villa Soriano, 29 Aug. 2011. **ZVC-P 12480**, 7 males 34.5–62.2 mm SL, 6 females 31.1–50.6 mm SL, Route 96 km 8, M. Loureiro, A. Duarte and I. Berro, 29 Aug. 2011.

***Austrolebias melanoorus*: BRAZIL. Rio Grande do Sul**. **UFRGS 18050**, 16 males 22.0–36.8 mm SL (3 c&s 30.9–33.9 mm SL; 3 fixed in alcohol 95°, 26.4–31.5 mm SL, TEC 3693, GP 3777–3779), 27 females 22.4–30.7 mm SL (3 c&s 25.9–28.1 mm SL; 7 fixed in alcohol 95°, 22.4–26.5 mm SL, TEC 3693, GP 3780–3786), Bagé, temporary pool close to BR-293 highway, 31°12’32”S, 54°17’24”W), L. Malabarba and J. Ferrer, 8 Sep. 2013. **UFRGS 18067**, 20 males 19.3–44.2 mm SL (3 c&s 28.1–40.2 mm SL), 18 females 24.1–37.9 mm SL (3 c&s 24.1–36.2 mm SL), Candiota, Seival, 31°25’57”S, 53°43’05”W, L. Malabarba and J. Ferrer, 8 Sep. 2013. **URUGUAY. Cerro Largo. MHNM 3730**, 1 male 34.1 mm SL, 1 female 28.2 mm SL, Cañada de las Pajas, 32°07’10”S, 54°06’41”W, W.S. Serra, J. Bessonart and M. Loureiro, 2 Sep. 2015. **ZVC-P 7757**, 2 males 41.3–44.5 mm SL, 1 female 40.0 mm SL, Paso San Diego, 31°57’57”S, 53°54’52”W, M. Loureiro, M. Zarucki, S. Clavijo and F. Teixeira, 1 Sep. 2007. **ZVC-P 7780**, 1 female 36.4 mm SL, Paso San Diego, 31°57’57”S, 53°54’52”W, M. Loureiro, M. Zarucki, S. Clavijo and F. Teixeira, Sep. 2007**. ZVC-P 8732**, 28 males 23.6–33.5 mm SL (3 c&s 26.5–33.5 mm SL; 1 fixed in alcohol 95° 26.1 mm SL, CAP 339, GP 3318), 29 females 21.6–28.4 mm SL (3 c&s 22.8–26.1 mm SL), Paso San Diego, 31°57’57”S 53°54’52”W, M. Loureiro, M. Zarucki and A. Duarte, Sep. 2009. **ZVC-P 9721**, 7 males 20.1–32.4 mm SL (5 fixed in alcohol 95° 20.1–32.4 mm SL, CAP 282, GP 3318–3323), 5 females 20.4–28.0 mm SL (5 fixed in alcohol 95°, CAP 282, GP 3324–3329), Cañada de las Pajas, 32°07’10”S, 54°06’41”W, M. Loureiro, W.S. Serra, A. Duarte and J. Bessonart, 30 Sep. 2010. **ZVC-P 11622**, 19 males 21.4–42.4 mm SL (2 c&s 21.4–24.0 mm SL; 1 fixed in alcohol 95%, 42.4 mm SL, CAP 1188, GP 3340; 10 fixed in alcohol 95%, 21.7–30.0 mm SL, CAP 1192, GP 3341–3350), 13 females 20.9–40.0 mm SL (2 c&s 20.9- 22.8 mm SL; 2 fixed in alcohol 95%, 21.7–24.5 mm SL, CAP 1192, GP 3351–3352), Paso San Diego, 31°57’57”S, 53°54’52”W, W.S. Serra, A. Duarte and M. Loureiro, 19 Sep. 2012. **ZVC-P 11668**, 7 males 29.2–40.0 mm SL (2 c&s 29.2–37.5 mm SL), 3 females 28.0–29.8 mm SL (2 c&s 28.1–29.8 mm SL), Cañada de las Pajas, 32°07’10”S, 54°06’41”W, W.S. Serra, A. Duarte and M. Loureiro, 30 Jul. 2013. **ZVC-P 13577**, 10 males 25.6–34.5 mm SL, 6 females 22.9–32.2 mm SL, Cañada de las Pajas, 32°07’10”S 54°06’41”W, W.S. Serra, J. Bessonart and M. Loureiro, 2 Sep. 2015. **ZVC-P 13578**, male, 37.0 mm SL, Cañada de las Pajas, 32°07’10”S, 54°06’41”W, W.S. Serra, J. Bessonart and M. Loureiro, 2 Sep. 2015. **Rivera. MHNM 3676** (ex. CLT 1196), 13 males 16.7–33.9 mm SL, 18 females 18.4–30.0 mm SL, Paso Ataques, 31°05’42”S, 55°41’12”W, P. Laurino, T. Litz, E. Perujo, H. Salvia and J. Salvia, 25 Aug. 2004. **ZVC-P 8743**, 12 males 17.0–40.7 mm SL, 16 females 21.1–35.2 mm SL, Río Tacuarembó, Paso Manuel Díaz, 31°32’42”S, 55°40’17”W, M. Loureiro, A. Duarte and W.S. Serra, Oct. 2009. **Tacuarembó. MHNM 2545**, 1 male holotype**,** 35,7 mm SL, pond near Arroyo Tres Cruces, Route 5 km 399.5, 31°39’01”S, 55°54’01”W, L.H. Amato, 3 Nov. 1985. **MHNM 2546**, 21 males paratypes, 27.9–36.2 mm SL (2 c&s disjointed), collected with the holotype. **MHNM 2548**, 28 females paratypes, 24.8–39.5 mm SL (2 c&s 32.2–33.7 mm SL), collected with the holotype. **MHNM 3675** (ex. CTL 1213b), 8 males 20.3–29.1 mm SL, 4 females 23.7–25.3 mm SL, Route 26 y Río Tacuarembó, Pueblo Ansina, 31°52’28”S, 55°28’19”W, P. Laurino, T. Litz, E. Perujo, H. Salvia and J. Salvia, 25 Aug. 2004. **ZVC-P 4322**, 3 males 33.0–37.8 mm SL (1 c&s 37.8 mm SL), 10 females 23.7–36.1 mm SL, pond near Arroyo Tres Cruces, Route 5 km 399.5, 31°39’01”S, 55°54’01”W, M. Loureiro, G. Yemini and C. Hernández, Oct. 1999. **ZVC-P 4323**, 4 males 30.5–43.1 mm SL, 4 females 29.4–35.5 mm SL, Route 26 and Río Tacuarembó, Pueblo Ansina, 31°52’28”S 55°28’19”W, M. Loureiro, F. Teixeira de Mello, A. D’Anatro and L. Bocardi, 26 Sep. 2000. **ZVC-P 8729**, 5 males 36.0–41.5 mm SL (1 fixed in alcohol 95°, 36.0 mm SL, CAP 358, GP 3282), 14 females 26.1–34.2 mm SL (2 fixed in alcohol 95°, 30.1–31.9 mm SL, CAP 358, GP 3283–3284), pond near Arroyo Tres Cruces, Route 5 km 399.5, 31°39’01”S, 55°54’01”W, M. Loureiro, A. Duarte and W.S. Serra, 10 Oct. 2009. **ZVC-P 8753**, 19 males 18.2–45.6 mm SL (3 c&s 22.7–30.3 mm SL; 2 fixed in alcohol 95° 21.6–29.1 mm SL, CAP 342, GP 3285–3286), 29 females 22.4–41.3 mm SL (3 c&s 21.3–31.8 mm SL; 1 fixed in alcohol 95° 27.0 mm SL, CAP 342, GP 3287), Paso Rogerio, Río Tacuarembó, 31°43’32”S, 55°38’46”W, M. Loureiro, A. Duarte and W.S. Serra, 11 Oct. 2009. **ZVC-P 13579**, 20 males 19.4–39.9 mm SL (17 fixed in alcohol 95°, 19.4–28.9 mm SL, CAP 1274, GP 3953–3956), 8 females 18.6–28.0 mm SL (5 fixed in alcohol 95°, 18.6–24.4 mm SL, CAP 1274, GP 3957–3958), Paso de la Laguna, 32°13’27”S, 55°22’32”W, W.S. Serra, J. Bessonart and M. Loureiro, 3 Sep. 2015.

***Austrolebias univentripinnis*: BRAZIL. Rio Grande do Sul. UFRGS 18062**, 25 males 20.7–29.0 mm SL (5 c&s 20.7–26.9 mm SL), 31 females 18.6–23.3 mm SL (5 c&s 19.2–22–3 mm SL), pond near the road Jaguarão-Nossa Senhora da Glória, Jaguarão, 32°22’45”S, 53°26’35”W, L. Malabarba and J. Ferrer, 7 Sep. 2013. **UFRGS 18064**, 7 males 21.9–29.0 mm SL, 2 females 19.4–21.1 mm SL, pond near the road Herval-Pedras Altas, Herval, 31°56’29”S, 53°29’00”W, L. Malabarba and J. Ferrer, 8 Sep. 2013. **UFRGS 18066**, 30 males 15.6–31.6 mm SL (5 c&s 19.4–30.6 mm SL), 47 females 14.2–30.6 mm SL (5 c&s 18.5–29.8 mm SL), pond near the road Jaguarão-Pedras Brancas, Jaguarão, 32°27’49”S 53°26’43”W, L. Malabarba and J. Ferrer, 7 Sep. 2013.

***Austrolebias vandenbergi*: PARAGUAY. Boquerón. MHNM 2557,** 1 male 46.8 mm SL, 6 females 25.0–52.3 mm SL, 100 km al SW de Filadelfia, 12.6 km de la Estancia Heisseque del Dr. Durán, Servicio Forestal Nacional, 13 Jun. 1981. **ANSP 175282,** 5 males 36.7–48.1 mm SL, 11 females 28.4–39.7 mm SL (2 c&s 32.9–34.0 mm SL), pond along road from Filadelfia-Teniente Montania, 22°10’59”S, 60°04’05”W, D.W. Fromm and J. Barnett. **ANSP 175283,** 3 males 33.3–41.3 mm SL, long pool S of road, 21°49’60”S, 60°51’31” W, D.W. Fromm and J. Barnett. **ANSP 175286,** 9 males 35.1–47.4 mm SL (2 c&s 43.3–45.6 mm SL), 1 female 37.5 mm SL, roadside pool on W side of road N from Teniente Montania, 22°03’17”S, 59°57’13”W, D.W. Fromm and J. Barnett. **ANSP 175289**, 10 males 41.4–55.2 mm SL (2 c&s 46.4–46.6 mm SL), 10 females 30.0–38.4 mm SL (2 c&s 30.0–35.5 mm SL), roadside ditch along Filadelfia - Teniente Montania road, 22°10’59”S, 60°04’05”W, D.W. Fromm and J. Barnett. **ARGENTINA. Salta. MHNM 2575**, 1 male 69.1 mm SL, Hickmann, Sr. Pierroti, 20 Jan. 1974.

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