

**AUSTROLEBIAS QUIROGAI (ACTINOPTERYGII: CYPRINODONTIFORMES: RIVULIDAE)  
IN BRAZIL: OCCURRENCE, POPULATION PARAMETERS,  
HABITAT CHARACTERISTICS, AND CONSERVATION STATUS**

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**Background.** The Neotropical genus *Austrolebias* is the most diverse of the Rivulidae family, with 22 species recorded in Brazil, with 21 found in the state of Rio Grande do Sul. *Austrolebias quirogai* Loureiro, Duarte et Zarucki 2011 is an annual fish previously only found in Uruguay and there is currently no information about their ecology and life history. Here, we recorded *A. quirogai* for the first time in temporary wetlands in Brazil. Furthermore, we provide information about population parameters, life history traits, conservation status, and habitat characteristics.

**Materials and methods.** We captured the fish with a D-shaped hand net in a sampling survey carried out in October 2012 in the Jaguarão-Chico River, Aceguá municipality, Brazil. We analysed density (CPUA), body size, length–weight relation (LWR), condition factor (*K*), and sex ratio.

**Results.** We recorded a total of three populations of *Austrolebias quirogai* in the Jaguarão-Chico River. The CPUA was 0.33 fish · m<sup>-2</sup>. The sex ratio relation did not differ significantly and was 1.5 : 1 (M : F). The mean body size between sexes was not statistically different. The LWR of *A. quirogai* was  $W = 0.011TL^{3.23}$ . The value of parameter *b* was significantly different from 3.0 for males and females, both sexes presenting positive allometric growth. The *K* for *A. quirogai* differed between sexes. Females had higher values than males. All populations of *A. quirogai* were found in ponds smaller than 1000 m<sup>2</sup> and were degraded by the activity of rice and soybean cultivation and overgrazing. We consider *A. quirogai* an endangered species in Brazil because of: its reduced area of occupancy, populations becoming severely fragmented, and suffering a continuous decrease in their area of occupancy and the quality of habitat.

**Conclusion.** The observed results may be used to assist the development of management strategies and conservation of the species and its habitat. Moreover, they provide basic information for the evaluation of the conservation status of the species according to IUCN criteria and for the definition of priority areas for conservation in southern Brazil. Further studies should focus on the distribution, life history and ecology of the species to aid in their conservation.

**Keywords:** Length–weight relation, condition factor, temporary wetland, freshwater fishes, killifish

## INTRODUCTION

The Neotropical genus *Austrolebias* Costa, 1998 includes 40 small killifishes (Loureiro et al. 2011, García et al. 2012). The majority of them (65%) have been described in the last 25 years and are distributed in south-

ern Brazil, southern Bolivia, Paraguay, Uruguay, and north-eastern Argentina (Costa 2010). In the Rio Grande do Sul State, southern Brazil, the highest diversity of *Austrolebias* is recorded, with 21 species present (Costa 2006, Ferrer et al. 2008, Cheffe et al. 2010, Volcan et al. 2011b,

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Lanés et al. 2013). Despite the species richness of *Austrolebias* in southern Brazil, the group is considered the most endangered of all fishes in the country (Rosa and Lima 2008). The causes vary according to the region and are mostly related to human population density and economic activity. The main cause of habitat loss for killifishes in the Rio Grande do Sul is rice farming, which usually occurs in floodplains precisely where there is a greater concentration rivulid habitats (Reis et al. 2003, Rosa and Lima 2008, Volcan et al. 2011a).

All *Austrolebias* species in Brazil present restricted distribution ranges (Nogueira et al. 2010). In addition, the majority of the species are endangered (Reis et al. 2003, Rosa and Lima 2008) and little is known about their biology and ecology (Errea and Danulat 2001, Laufer et al. 2009, Volcan et al. 2011b, 2013a, 2013b). As a result, the National Action Plan for the Conservation of Rivulidae Fish (Anonymous 2012) was recently launched, whose main objective is to establish mechanisms to protect and prevent extinction of Rivulidae species and loss of their habitats in Brazil.

The *Austrolebias quirogai* Loureiro, Duarte et Zarucki 2011 is distinguished from all other species of *Austrolebias* by the unique coloration and pigmentation pattern on the body and fins in males, which consist of a uniform bluish grey background colour on the body that lacks vertical bands and uniform pigmentation on the unpaired fins. *Austrolebias quirogai* is recorded in temporary wetlands from the middle to the upper and eastern tributaries of the Negro River (Uruguay River basin) and Jaguarão River basin (Patos-Mirim Lagoon system) in Uruguay.

In this study, we recorded *A. quirogai* for the first time in the temporary ponds Brazil, in the municipality of Aceguá, Jaguarão River basin. Additionally, we provide basic information about the population parameters, life history traits, conservation status, and habitat characteristics of this rare and threatened species.

## MATERIAL AND METHODS

**Study area.** As part of a research project conducted by the Ichthyology Laboratory of the Instituto Pró-Pampa to investigate the ecology, distribution, and conservation of annual fishes in the Pampa biome in southern Brazil (*Projeto Peixes Anuais do Pampa*) we sampled the municipally of Aceguá. The municipally is located near the northwest border of Brazil and Uruguay in the Pampa Biome. It has an area of 1550 km<sup>2</sup> and a population of 3859 inhabitants. The landscape is heavily degraded and is predominantly used for agricultural and livestock. Fishes were caught in temporary ponds in the floodplains of the Jaguarão-Chico River. This river is a tributary of the Jaguarão River, which drains to Mirim Lagoon.

The Aceguá municipality is inserted in the geomorphological unit of the Depressão Central, located in the central and southern half of the Rio Grande do Sul State. This region has a slightly undulating topography with wide floodplains. The average altitude is 100 m, while the maximum altitude is between 250 and 300 m. The Depressão

Central region is the warmest area in the state, and, according to the Köppen classification, it has a subtropical climate, with annual precipitation around 1600 mm.

**Fish surveys.** Fish were captured using the ‘kick sampling’ method by sweeping the net along the bottom using a D-shaped hand net (60 × 30 cm, 2 mm mesh). Twenty-five sweeps of 0.6 m<sup>2</sup> were carried out, corresponding to approx. 15 m<sup>2</sup> of sampled area per studied pond.

We identified species according to Loureiro et al. (2011). We measured total length (TL) to the nearest 1 mm and body weight (*W*) to the nearest 0.01 g. We sexed individuals using phenotypic criteria since the species exhibit marked external dimorphism. We deposited voucher specimens in the “Museu de Ciências e Tecnologia da PUCRS” (MCP 481245).

We used GPS to determined coordinates, altitude (meters above sea level), and wetland area (m<sup>2</sup>) of the sampling sites. We measured dissolved oxygen [mg · L<sup>-1</sup>], percentage of dissolved oxygen [mg · L<sup>-1</sup>], pH, salinity [ppt], conductivity [μS · cm<sup>-1</sup>], temperature [°C], total dissolved solids [g · L<sup>-1</sup>], and oxidation reduction potential [mV] with a multi-parameter Hanna HI 9828. We also determined transparency [cm], with a Secchi disk, and depth [cm].

We applied the categories and criteria of the International Union for the Conservation of Nature Red List of Threatened Species (Anonymous 2011) to determine the conservation status of the Brazilian populations of *A. quirogai*.

**Data analysis.** We assessed fish abundance using catch per unit area (CPUA: number of individuals per m<sup>2</sup>). To verify for possible difference between the mean TL of males and females, we performed a Student’s *t*-test. We used chi-square test ( $\chi^2$ ) to determine if the sex ratio deviated from the expected 1 : 1 ratio.

We estimated length–weight relation (LWR) independently for males and females. To estimate LWR, we fitted a least square linear model taking the base-10 logarithms of the allometric equation (Le Cren 1951, Froese 2006):

$$W = aTL^b$$

where: *W* is the total weight [g], TL is the total length [mm], *a* is intercept, and *b* is the slope. For each sex, we compared the slopes of length–weight regressions to three using a Student’s *t*-test to ascertain if the sex grew isometrically (Pauly 1984).

We calculated the allometric condition factor (*K*) for each sex using the equation:

$$K = (W \cdot TL^{-b}) \cdot 10^5$$

where: *W* is the total weight [g], TL is the total length [mm] and *b* is the slope of LWR. We used Student’s *t*-test to test differences of *K* between sexes. We performed all statistical analyses using BIOESTAT<sup>®</sup>5.0 and adopted a significance level of 5%.

**Ethical issues.** The present study has been carried with collect license of Instituto Chico Mendes de Conservação da Biodiversidade / Instituto Brasileiro de Meio Ambiente e dos Recursos Naturais Renováveis (authorization number: ICMBio/IBAMA 18334-2).

## RESULTS

### Distribution, abundance, and population parameters.

We recorded a total of three populations of *Austrolebias quirogai* (Fig. 1) in the Aceguá municipality, Rio Grande do Sul, Brazil. All populations were located in the Jaguarão-Chico River, Jaguarão River basin, Patos-Mirim Lagoon system (sites P1, P2, and P3, Fig. 2, Table 1).

We captured 15 specimens of *A. quirogai* (Table 1), six females and nine males. The CPOA was  $0.33 \text{ fish} \cdot \text{m}^{-2}$ . Although there was a tendency towards a greater number of males, a chi-squared test did not reveal significant differences in the sex ratio ( $\chi^2 = 0.60$ ,  $P = 0.44$ ), which was 1.5 : 1 (M : F).

The TL of *A. quirogai* ranged from 33 to 52 mm TL (mean  $\pm$  standard deviation:  $41.5 \pm 7.2$  mm) for males and 37 to 45 mm TL (mean  $\pm$  SD:  $40.3 \pm 4.2$  mm) for females. Weight ranged from 0.49 to 1.80 g (mean  $\pm$  SD:  $1.09 \pm 0.59$  g) and 0.81 to 1.52 g (mean  $\pm$  SD:  $1.09 \pm 0.38$  g) for males and females, respectively. The mean values of TL for males and females were not statistically different ( $t$ -test;  $P > 0.05$ ).

All LWR were highly significant ( $P < 0.001$ ; Table 2). The LWR of *A. quirogai* was  $W = 0.011 \text{ TL}^{3.23}$ . The value of parameter  $b$  was significantly different from 3.0 for males and females ( $t$ -test,  $P < 0.05$ ), characterizing a positive allometric growth ( $b = 3.23$ ). Therefore, the hypothesis of isometric growth for *A. quirogai* in the Jaguarão-Chico River was rejected.

The  $K$  for *A. quirogai* ranged from 0.54 to 0.64 (mean  $\pm$  SD =  $0.59 \pm 0.04$ ) and 0.67 to 0.70 (mean  $\pm$  SD =  $0.69 \pm 0.01$ ) for males and females, respectively. Females had higher values than males ( $t$ -test,  $P < 0.005$ ).

**Habitat characteristics.** The habitat of *A. quirogai* was essentially restricted to small (area less than 1000 m<sup>2</sup>) and shallow temporary ponds exposed to direct light and dense aquatic vegetation in grassland areas in the floodplains of the Jaguarão-Chico River (Fig. 3). The species occurred at elevations of 118 to 125 m and at a reduced area of occupancy, ranged from 25 to 900 m<sup>2</sup>.

Water samples revealed the parameters (mean  $\pm$  standard deviation): dissolved oxygen:  $1.37 \pm 1.97 \text{ mg} \cdot \text{L}^{-1}$ , percentage of dissolved oxygen:  $18.33 \pm 27.88 \text{ mg} \cdot \text{L}^{-1}$ , pH:  $5.94 \pm 0.35$ , salinity:  $0.06 \pm 0.05$  ppt, conductivity:  $0.136 \pm 0.10 \mu\text{S} \cdot \text{cm}^{-1}$ , temperature:  $27.57 \pm 8.00^\circ\text{C}$ , total dissolved solids:  $68 \pm 52.83 \text{ g} \cdot \text{L}^{-1}$ , oxidation reduction potential:  $-93.77 \pm 33.26 \text{ mV}$ , depth:  $17 \pm 8 \text{ cm}$ , and transparency:  $4 \pm 1.4 \text{ cm}$ . In co-occurrence to *A. quirogai*, we captured *Austrolebias juanlangi* Costa, Cheffe, Salvia et Litz in Costa 2006 at all sampling sites. There was a trend of an inverse relation between the abundance of the two species, always tending towards a higher abundance of *A. juanlangi* ( $n = 83$  vs.  $n = 15$  in *A. quirogai*) and CPOA ( $0.33$  in *A. quirogai* vs.  $1.84$  in *A. juanlangi*).

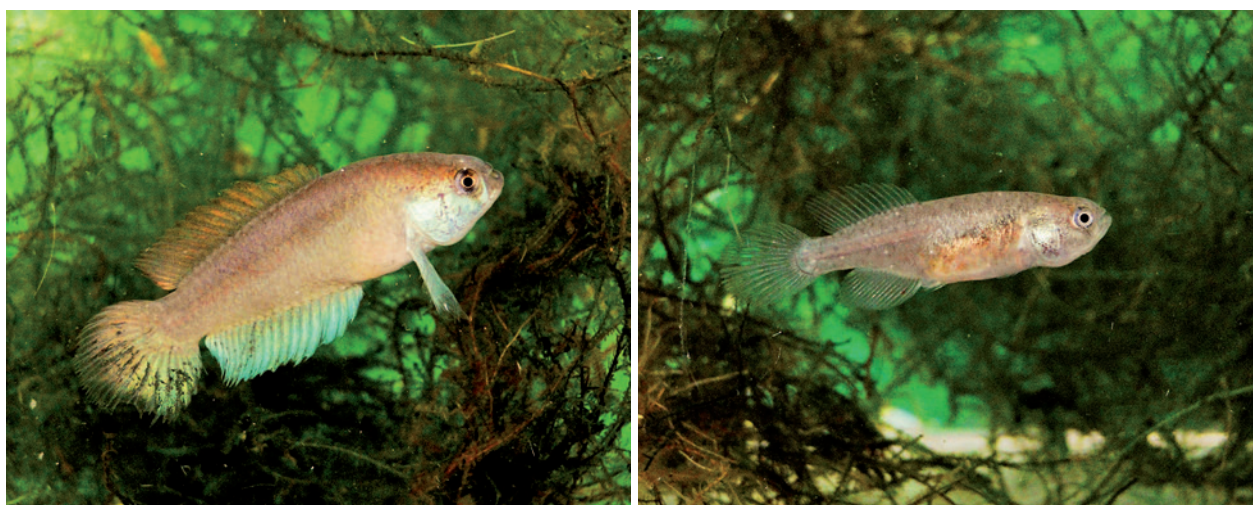
Furthermore, another four species of Characiformes were captured at low densities in the sampling sites: *Cheirodon ibicuiensis* Eigenmann, 1915 ( $n = 7$ ), *Pseudocorynopoma doriae* Perugia, 1891 ( $n = 5$ ), *Astyanax* cf. *eigenmanniorum* (Cope, 1894) ( $n = 1$ ), and *Oligosarcus robustus* Menezes, 1989 ( $n = 1$ ). The TL of these species did not exceed 50 mm.

**Conservation status.** In accordance with IUCN criteria, *Austrolebias quirogai* was considered as “Critically Endangered” species in Brazil, being included in the criteria CR B2ab (ii, iii). The species presents reduced area of occupancy (AOO less than 10 km<sup>2</sup>), populations severely fragmented (a) and continued decline (b) in area of occupancy (ii) and quality of habitat (iii).

## DISCUSSION

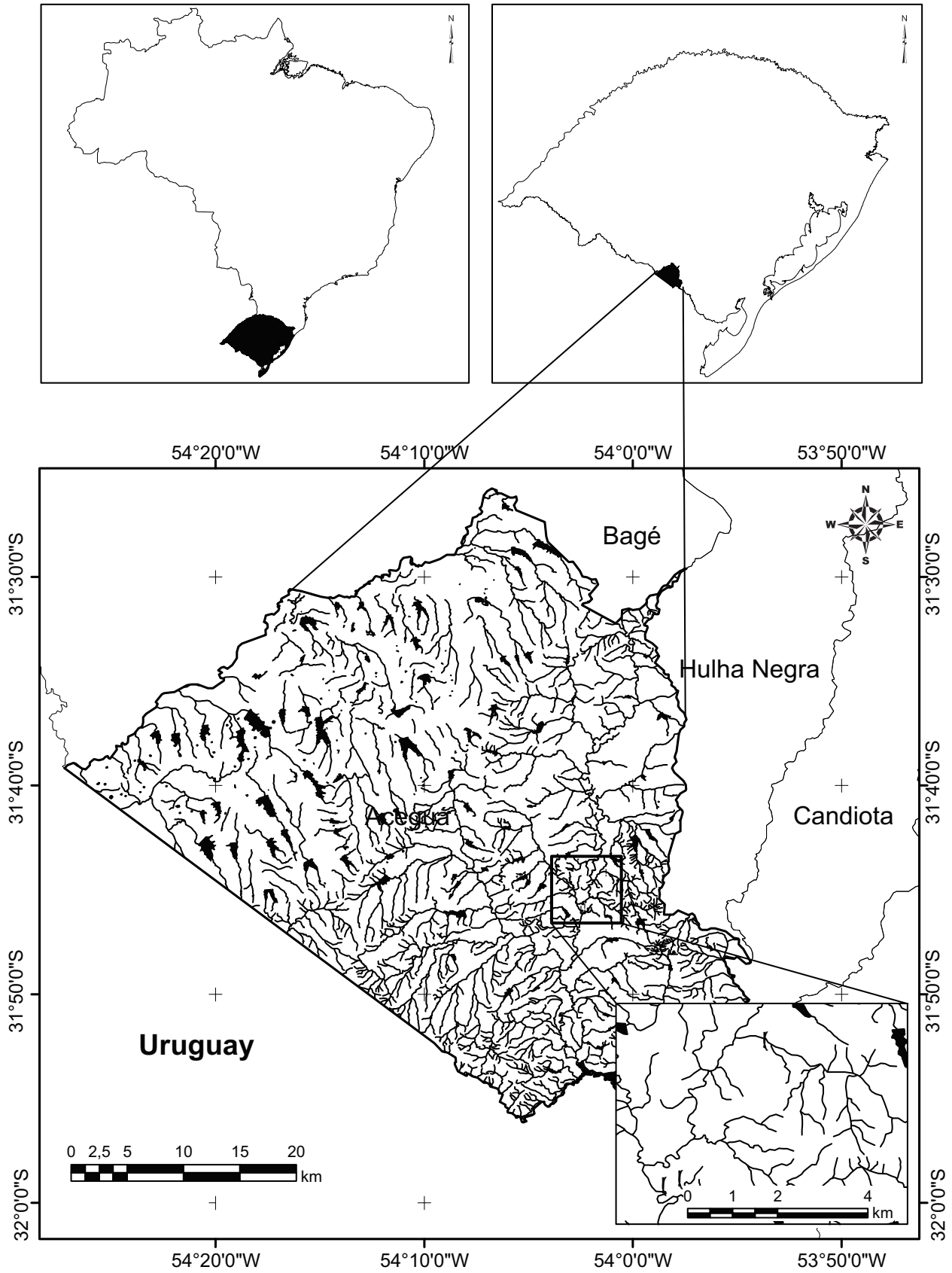
*Austrolebias quirogai* was found in shallow temporary freshwater ponds, exposed to direct sun light and dense aquatic vegetation. This is the typical habitat of killifishes in the Pampa Biome of southern Brazil (Costa 2002, Volcan et al. 2010, 2011a, 2011b, Lanés et al. 2013). Their habitat is highly vulnerable to anthropogenic changes that can compromise the viability of the populations.

In recent years, various species of *Austrolebias* have been recorded in southern Brazil. Only in the last ten years,



**Fig. 1.** Male (left) and female (right) of *Austrolebias quirogai* from Jaguarão-Chico River, Jaguarão River basin, southern Brazil; Photo by Matheus Volcan





**Fig. 2.** Occurrence of *Austrolebias quirogai* (black circles) in Jaguarão-Chico River, Aceguá municipality, Rio Grande do Sul State, Brazil; Source: Modified from Anonymous\*

\*Anonymous. 2005. Fundação Estadual de Proteção Ambiental Henrique Luiz Roessler, RS. [State Foundation of Environmental Protection Henrique Luiz Roessler] Biblioteca Digital. Arquivos digitais para uso em SIG. Base cartográfica Digital do RS. [Digital Library. Digital files for use in GIS. Digital cartographic base of RS] 1 : 250.000. Available at: [http://www.fepam.rs.gov.br/biblioteca/geo/bases\\_geo.asp](http://www.fepam.rs.gov.br/biblioteca/geo/bases_geo.asp). Accessed January 2014. [In Portuguese.]

four species previously known only in Uruguay, were recorded in border regions with Brazil: *A. prognathus* (Amato, 1986), *A. vazferreirai* (Berkenkamp, Etsel, Reichert et Salvia 1994), *A. melanoorus* (Amato, 1986), and *A. arachan* (Loureiro, Azpelicueta et García, 2004) (see Costa 2006, Cheffe et al. 2010, Volcan et al. 2010, 2011b, Lanés et al. 2013). Considering this record, among the 22 species of *Austrolebias* found in Rio Grande do Sul, six occur in the Jaguarão River Basin (Volcan et al. 2011b, Lanés et al. 2013), with this basin being one of the richest for Rivulidae species.

The present record of *A. quirogai* is the first in Brazil and is located about 25 km from the single occurrence area known for the Jaguarão River basin and about 150 km from the type locality in the Cordobés Stream, Negro River basin. *Austrolebias quirogai* is distributed both in the Negro River and in the Jaguarão River, Patos-Mirin lagoon system, in floodplains 100 m above sea level (Loureiro et al. 2011). This distribution pattern is similar to four other species of *Austrolebias*: *A. arachan*, *A. melanoorus*, *A. juanlangi*, and *A. vazferreirai* (see Costa 2006, Loureiro et al. 2004, 2011, Volcan et al. 2011b) and may be a result of the drainage rearrangement of the Negro River upstream tributaries (Uruguay River basin) and tributaries of the Patos-Mirim Lagoon system (Loureiro et al. 2011).

In the studied populations of *Austrolebias quirogai*, there was a tendency to greater proportion of males than females (M : F = 1.5 : 1), although no significant differences. These results differ from the common patterns among species of the genus *Austrolebias* that generally have a higher proportion of females (Laufer et al. 2009, Lanés et al. 2013). The relatively low abundance of males in temporary wetlands may be related to intra-specific

competition and predation (Lanés et al. 2013), but this does not explain the low representation of females in the samples, since there is no record of aggression among females of the same species in *Austrolebias* (see Belote and Costa 2004, Volcan et al. 2013a).

In this study, we provide new maximum sizes for males and females of *A. quirogai* compared to Fish Base (Froese and Pauly 2012) and Loureiro et al. (2011). The TL of *A. quirogai* does not differ between the sexes. This same pattern was observed for *A. wolterstorffi* (Ahl, 1924) maintained in captivity, where males and females showed similar average sizes (da Fonseca et al. 2013). The biggest length of males is considered a pattern of sexual dimorphism for many species of Rivulidae (see Costa 2003) and observed for many *Austrolebias* species (Costa 2006, Lanés et al. 2013, Volcan et al. 2013a). However, the small number of specimens captured hampers further conclusions.

The growth patterns are similar between males and females of *A. quirogai*. A *b* value close to three indicates that the fish grow isometrically, otherwise they are showing allometric growth (Froese 2006). Both sexes of *A. quirogai* showed allometric positive growth ( $b > 3$ ) with growth proportionally greater in weight than in length (Froese 2006). For specimens kept in captivity, Volcan et al. (2013a) and Fonseca et al. (2013) observed positive allometric growth for the LWR of males and females of *A. nigrofasciatus* Costa et Cheffe, 2001 and *A. wolterstorffi*, respectively. The same pattern was observed for both species collected in the natural environment (Volcan et al. 2013b). Lanés et al. (2013) observed which *A. arachan* showed a higher slope for males than females. On the other hand, Gonçalves et al. (2011) and Volcan et al. (2013b) found an inverse growth pattern when analysing the LWR of *Cynopoecilus melanotaenia* (Regan, 1912), which showed a higher slope for females than males.

The mean of the condition factor (K) values of *A. quirogai* did not differ between males and females. The lack of studies on condition factor in *Austrolebias* species makes it difficult to compare our results. In addition, the low density of *A. quirogai* makes it difficult further conclusions. However, the values reported for *A. arachan* (see Lanés et al. 2013) and *A. nigrofasciatus* (see Volcan et al. 2013a) show similar results to those observed for *A. quirogai*.

**Table 1**

Characteristics of the sampling sites (Fig. 2) of *Austrolebias quirogai* in Brazil

Site	Coordinates	Altitude [m]	Area [m <sup>2</sup> ]	N
P1	31°44'10"S, 54°01'09"W	125	30	3
P2	31°45'49"S, 54°02'11"W	119	900	7
P3	31°45'54"S, 54°02'22"W	118	25	5

Altitude: above sea level, N = number of fish sampled.

**Table 2**

Regression parameters and 95% confidence interval for males (M), females (F), and both sexes pooled (M + F) of *Austrolebias quirogai* in Brazil

Sex	N	Regression parameters				
		<i>a</i>	<i>b</i>	<i>a</i> CI95%	<i>b</i> CI95%	<i>r</i> <sup>2</sup>
M	9	0.009	3.27	0.005 to 0.018	2.79 to 3.74	0.99
F	6	0.011	3.28	0.001 to 0.138	1.47 to 5.09	0.99
M + F	15	0.011	3.23	0.005 to 0.022	2.69 to 3.78	0.96

M = male, F = female, N = number of fish sampled, *a* = intercept of LWR, *b* = slope of LWR, aCI95% = 95% confidence interval of parameter *a*, bCI95% = 95% confidence interval of parameter *b*, *r*<sup>2</sup> = coefficient of determination.

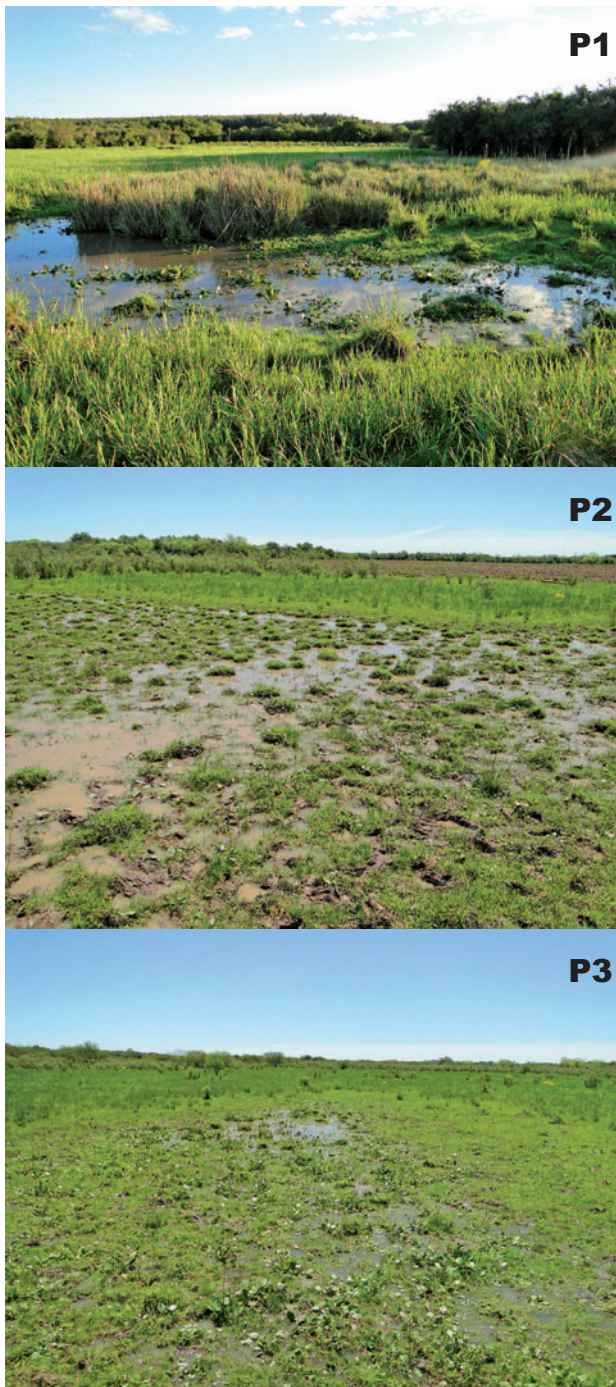
*Austrolebias quirogai* occurred at a low density and was syntopic with abundant populations of *A. juanlangi*. Volcan et al. (2011a) recorded *A. juanlangi* in the medium-low course of Jaguarão River, but no Rivulidae species were found in co-occurrence. Four other non-killifishes were collected. They are common in the Patos-Mirim Lagoon system and frequently recorded co-occurring with killifishes in temporary (Quintela et al. 2007, Laufer et al. 2009, Volcan et al. 2010, 2011b). Apparently, the abundance and richness of non-annual fishes did not influence

the abundance of *Austrolebias* species (Volcan et al. 2011a). The coexistence of different species in temporary wetlands may likely be explained by the connection of water bodies during the rainy season (Vaz-Ferreira et al. 1966, Errea and Danulat 2001). Possible explanations for the use of these habitats by small fish are that they offer shelter, protection from predators, and high availability of food resources (Gonçalves et al. 2011).

The exclusive survival in temporary environments and limited distribution areas, combined with the loss and degradation of wetlands, mean many Rivulidae species are under threat of extinction (Reis et al. 2003, Rosa and Lima 2008, Anonymous 2012). The loss and fragmentation of wetlands is the main threat to killifishes and the high risk of extinction of these species is related to agriculture, urbanization, drainage, and other human activities (Reis et al. 2003, Rosa and Lima 2008, Volcan et al. 2009, 2010, Lanés and Maltchik 2010, Anonymous 2012). The scenario is no different for *A. quirogai* that has all its Brazilian populations recorded in a completely altered and degraded landscape by rice and soybeans plantations. Furthermore, the biotopes are inserted in a large area of forest stands of *Acacia* and *Eucalyptus* trees, which may also compromise the quality of the environment. All points where the species was recorded are smaller than 1000 m<sup>2</sup> and their populations are greatly reduced. The conservation status of *A. quirogai* suggests that special care is required to avoid its extinction in Brazil. New developments in its area of occurrence, such as wind energy, exotic forestry, thermoelectricity and dam construction, may also increase the risk of extinction of the species if not properly licensed (Volcan et al. 2011a, 2011b, Lanés and Maltchik 2010, Lanés et al. 2013). The search for new populations, knowledge of life history traits and ecology of the species are key strategies for their conservation. Moreover, the full protection and recuperation of their remaining habitats is essential. The rarity of this species and its low density, coupled with the high degree of vulnerability of its biotopes, justify their inclusion in the next list of threatened fauna of Rio Grande do Sul and Brazil.

The justification for the inclusion of this species in future lists of endangered fauna is due to the fact that its area of occupancy in Brazil (and probably in the world) is less than 10 km<sup>2</sup> and their populations are severely fragmented and suffer continuous decreases in area of occupancy and quality of habitat. We consider *A. quirogai* as a “Critically Endangered” species in Brazil, which is included in the criteria CR B2ab (ii, iii) according to IUCN criteria.

Finally, this work presents the first record for Brazil of *A. quirogai*, an annual fish species considered threatened in Brazil. Additionally, we provide basic information about its population parameters and habitat. This information is important for defining priority areas for conservation, as well as future plans for killifish conservation in southern Brazil, where most wetlands have been degraded and lost, mainly due to agricultural activities, and those remaining are largely threatened.



**Fig. 3.** Sampling sites of *Austrolebias quirogai* in the municipality of Aceguá, southern Brazil, Jaguarão-Chico stream; Photos by Matheus Volcan



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