



Confirmation of the occurrence of *Odontesthes argentinensis* (Valenciennes, 1835) (Atheriniformes, Atherinopsidae) in the Río Uruguay

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Abstract. In this work, the presence of *O. argentinensis* –a species considered marine or estuarine– is reported in fully freshwaters along four localities located in Río Uruguay, extending the habitat range of this species ca. 120 kilometers upstream from the mouth of this river.

Key words: Atherinomorpha, Silverside, Range extension.

Resumen: Confirmación de la presencia de *Odontesthes argentinensis* (Valenciennes, 1835) (Atheriniformes, Atherinopsidae) en el Río Uruguay. Este trabajo reporta la presencia de *O. argentinensis* –una especie considerada marina o estuarina– en aguas del Río Uruguay, extendiendo a la vez su distribución geográfica ca. 120 kilómetros río arriba desde su desembocadura.

Palabras clave: Atherinomorpha, pejerrey, ampliación del área de distribución.

The silversides of genus *Odontesthes* Evermann and Kendall, 1906 are a group of fish extensively distributed along tropical and temperate regions of South America, occupying freshwater, marine, and euryhaline coastal environments (Dyer 2006). *Odontesthes argentinensis*, also known as “pejerrey”, is commonly found along South Atlantic coasts, from Río de Janeiro in Brasil (21°S) to Chubut Province in Argentina (44°S) (Di Dario *et al.* 2014; Dyer 1988). It is reported that this fish inhabits shallow marine waters, as well as estuaries and coastal lagoons (Dyer 2006). Individuals of this species exhibit a noticeable phenotypic plasticity, allowing them to adapt to a high degree of heterogeneous environments (Bamber & Henderson 1988). This flexibility in life history traits permits to these silversides to live in a broad range of

environmental conditions, dealing with different temperatures and salinity water values. This possible local adaptation, associated to neutral process, could produce genetic differentiation among individuals sampled in different regions. As a possible example, *O. argentinensis* sampled along coastal lagoons in Brazil presented moderate levels of genetic differentiation, along with larvae and egg morphological distinctiveness respective to their oceanic counterparts (Beheregaray & Sunnucks, 2001 and references therein). In this work, we report the presence of *O. argentinensis* in fully freshwaters, in four localities along Río Uruguay, extending the currently known habitat range of this species in ca. 120 kilometers upstream Río Uruguay river.

Sampling area comprises four localities along Río Uruguay and its limit with Río de la Plata:

Nuevo Berlín (32°58'52"S, 58°04'06"W), Fray Bentos (33°06'35"S, 58°16'39"W), Las Cañas (33°09'48"S, 58°21'43"W) and Punta Gorda (33°54'53"S, 58°24'59"W); the last, located in the borderline between Río Uruguay and Río de la Plata (Fig. 1). Individuals of *O. argentinensis* were collected in each locality using eight standard Nordic gill nets (5.0, 6.25, 8.0, 10.0, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55 mm knot-to-knot, with 1.5 meters depth and 30 meters long); four of them were set parallel to the littoral zone and the remaining four were set parallel to each other in the pelagic area, from sunset to sunrise, (approximately 12 hours). Silversides were taxonomically classified following Dyer (2006) and morphometric and meristic data were obtained following Bemvenuti (2002) and Di Dario et al. (2014). Morphometric linear distances were obtained using a metallic ruler (0.50 mm) and a digital caliper (0.01 mm). Meristics counts were assessed under a Nikon SMZ445 stereomicroscope or by direct eye inspection. Linear distances and meristic counts differences were evaluated by means of Mann-Whitney U tests between individuals sampled in localities sited

upstream in Río Uruguay (i.e. Fray Bentos, Nuevo Berlín and Las Cañas) and individuals collected in Punta Gorda. We carried out this comparison because *O. argentinensis* is commonly found in Río de la Plata estuary (e.g. Beheregaray & Sunnucks, 2001; Thompson & Volpedo, 2015; Jaureguizar et al. 2016), and by hence its presence is expectable in the limits with Río Uruguay. With this comparison, we expect to find no differences between the two groups, supporting the hypothesis that individuals of this species also reach higher zones upstream Río Uruguay. After morphometric analyses, linear distances obtained were size corrected following Leonart et al. (2000). Subsequently, individuals were dissected and sexed by macroscopic inspection. Since this species does not exhibit sexual dimorphism, individuals from both sexes were pooled together in morphometric analyses (e.g. Di Dario et al. 2014).

Fourteen individuals were collected along the four sampling sites: Punta Gorda (n = 10) 31st May of 2017, Fray Bentos (n = 1) on 1st September of 2018, Nuevo Berlín (n = 1) on 30st August of 2018,

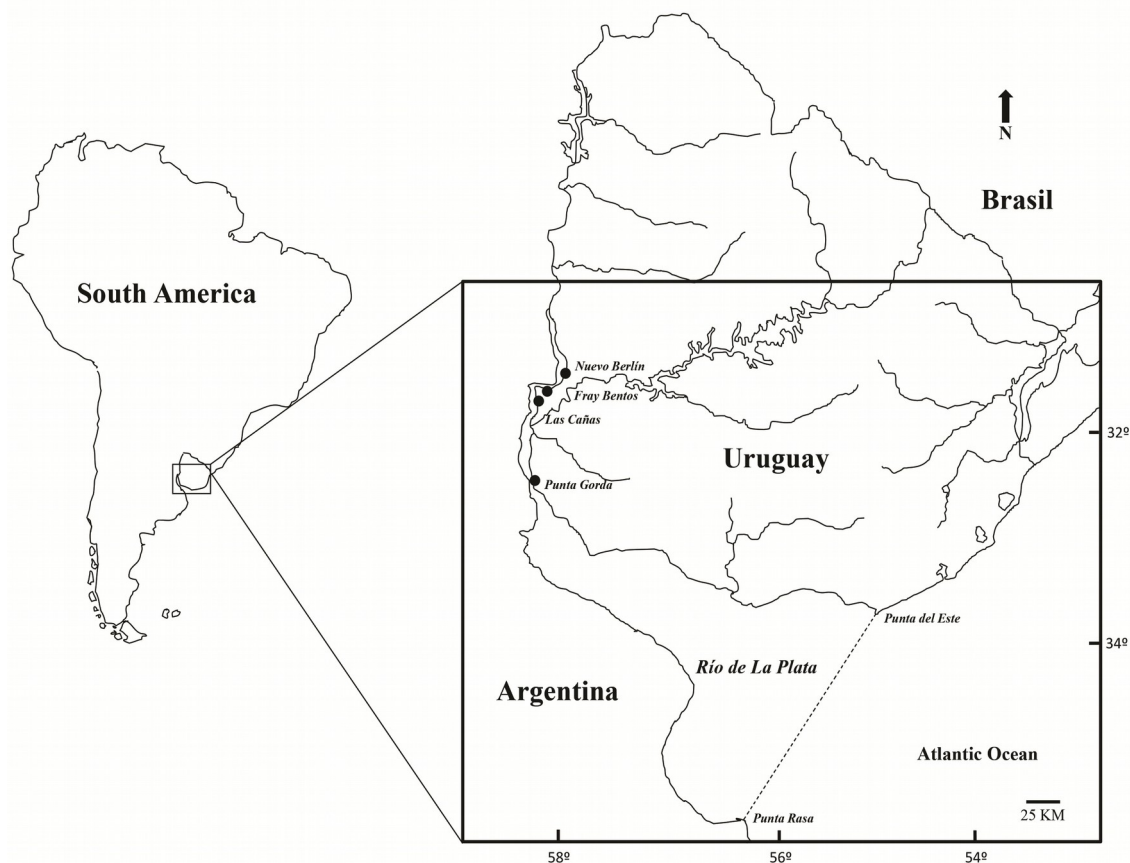


Figure 1. Map of the sampling sites considered in this study: Nuevo Berlín (n = 1), Fray Bentos (n = 1), Las Cañas (n = 2) and Punta Gorda (n = 10).

Las Cañas (n=2) 2nd September of 2018 (Fig. 1). All of them were taxonomically classified as *O. argentinensis* following Dyer (2006) (see below), and deposited in the “Colección del Laboratorio de Evolución” of Facultad de Ciencias (Universidad de la República, Montevideo, Uruguay) under accession numbers: EVP140 - EVP153. Morphometric and meristic variables surveyed are depicted in Table I. All individuals captured in Fray Bentos, Nuevo Berlín, and Las Cañas were classified as females by gonadal macroscopic inspection; of those collected in Punta Gorda locality, 2 were classified as females and the remaining 8 as males. Mann-Whitney analyses did not show statistically significant differences between groups for any of the variables considered, after Bonferroni (1936) correction (Table I). All individuals reported here were classified as *O. argentinensis* following the taxonomical key depicted in Dyer (2006), but is important to note that the number of gill rakers of the individuals surveyed did not fall within the range proposed for this species by this author. However, other phenotypic differences between both species, – e.g. origin of the first dorsal fin respecting to the anus (Fig. 2A) and presence of pre-dorsal crenulate scales (Fig. 2B), points 7 and 10a in the taxonomical

key proposed by Dyer (2006), respectively–classified all of them unequivocally as *O. argentinensis*. In addition, not even the largest specimen reported in this work showed a prognathous lower jaw [point 11b in the taxonomical key of Dyer (2006)], and none of the specimens surveyed exhibited vomerine teeth as a single median patch (also point 11b), both phenotypic characteristics of *O. bonariensis*.

Furthermore, available taxonomical keys for these species are ambiguous for several characters, including gill rakers counts (reviewed in González-Castro et al., 2016). Gill rakers are cartilaginous or bony structures that project to the inside of the pharyngeal cavity and whose structure changes according to the feeding habit of fish (Almeida et al. 2013) and represent a heritable and ecologically important trait associated with diet preference (Häkli et al., 2018 and references therein). Several works analyzing the performance of different fish ecophenotypes found a noticeably correlation between gill rakers divergence and habitat use (e.g. Roesch et al., 2013), or due to the presence of coinhabiting species (e.g. Lindsey, 1981). Other studies also report ontogenetic changes in gill rakers number, length and spacing among them (e.g.

Table I. Morphometric and meristic data for the individuals analyzed. Std. Dev. = Standard Deviation; Min. = minimum value; Max. = maximum value. Mann-Whitney (M-W) p-value is considered statistically significant if $p < 0.0025$, after Bonferroni correction ($\alpha = 0.05$, two-sided test).

Measurements	N	Mean	Std. Dev.	Min.	Max.	M-W p-value
Standard Length (SL) in centimeters	14	27.7	3.28	23.5	35.5	0.525
Measurement in % of SL						
Pre-dorsal distance (1st dorsal fin)	14	62.2	3.70	58.6	73.2	0.016
Pre-dorsal distance (2nd dorsal fin)	14	73.3	3.55	62.0	76.8	0.777
Pre-anal distance	14	67.7	2.62	63.4	72.3	0.066
Pre-pectoral fin distance	14	27.3	0.66	25.8	28.0	0.832
Pre-pelvic fin distance	14	47.3	1.49	45.9	50.0	0.572
Head length (HL)	14	25.2	1.08	23.4	27.2	0.671
Snout length	14	6.1	0.89	3.8	7.2	0.258
Measurement in % of HL						
Eye diameter	14	15.5	0.98	14.0	17.5	0.777
Interorbital distance	14	29.9	1.81	27.2	33.6	0.005
Mouth width	14	25.9	1.73	23.3	29.0	> 0.999
Upper jaw length	14	25.5	1.34	23.5	28.4	0.572
Meristic Counts						
1st dorsal-fin rays	14	4.3	0.47	4	5	0.621
2nd dorsal fin (spine+rays)	14	11.8	0.80	10	13	> 0.999
Anal fin (spine+rays)	14	19.3	0.73	18	20	0.203
Pectoral-fin rays	14	14.6	0.65	14	16	0.120
Longitudinal row of scales	14	55.3	2.37	51	59	0.396
Caudal peduncle scales	14	19.9	1.33	17	22	0.396
Gill rakers on upper limb of 1st arch	13	10.2	0.83	9	11	0.487
Gill rakers on lower limb of 1st arch	13	34.4	2.14	30	37	0.621



Figure 2. A) *Odontesthes argentinensis*, EVP 151, female, 350.0 mm of SL. **B)** Pre-dorsal crenulate scale of the same individual.

MacNeill & Brandt, 1990; Guinea & Fernandez, 1992). The high plasticity of these structures had led to some authors to reach the conclusion that gill rakers are an unreliable source of taxonomic characters, at least for suspension feeders (Liston 2013). Heras & Roldán, (2011) propose that genetic divergence between *O. argentinensis* and *O. bonariensis* fall within the range of intraspecific variation, in spite of some morphological (Dyer 2006), transcriptomic and possible reproductive differences; with the main dissimilarity between them consisting in the different environments they inhabit (Hughes *et al.*, 2017 and references therein). It has been proposed that *O. bonariensis* originated in lakes and lagoons of Province of Buenos Aires (Argentina) and also in Rio Grande do Sul (Brasil), with no records of native specimens in Uruguay (Dyer 2006). This allopatric distribution maybe better explained considering parallel divergence from marine origins along these areas, which is a common phenomenon in fish (e.g. Jones *et al.*, 2012). Moreover, this process may be dynamic, as it was suggested in González-Castro *et al.*, (2016), with new individuals of *O. argentinensis* from marine waters differentiating into other ecophenotypes in Río Uruguay. As was mentioned before, Beheregaray & Sunnucks (2001) already proposed a scenario of fine population structure and incipient estuarine differentiation in this species, supporting the ideas outlined above. Considering all the expressed before, we conclude that it is more accurate to taxonomically classify the individuals reported here as *O. argentinensis* than as *O. bonariensis*, as it could also be the case in other studies carried out in Río Uruguay (e.g. Serie *et al.*, 2016). Moreover, *O. argentinensis* found in Río Uruguay could constitute landlocked populations respecting their marine or estuarine counterparts, as was proposed for this species in “Salada de Pedro Luro” Lake in Argentina (Colautti *et al.* 2019) or for the anchovy *Lycengraulis grossidens* (Mai *et al.* 2017). In the other hand, this species could use all

these environments in a broad sense and *O. argentinensis* could be moving freely among these different aquatic systems (i.e. Atlantic Ocean, Río de la Plata and Río Uruguay) without settling differentiated populations. Following this idea, preliminary analysis of stable isotopes of C and N in these areas suggest that indeed, *L. grossidens* (López-Rodríguez *et al.* 2019) and *O. argentinensis* (González-Bergonzoni *et al.* unpublished data) populations in Río Uruguay represent a mixture of migratory and resident individuals. These findings reveal this silverside as a species adaptable to very heterogeneous environments, from oceanic to fully freshwaters, supporting the idea that some South American freshwater or estuarine silversides (e.g. the “*O. perugiae* complex”; Beheregaray *et al.* 2002) were derived from colonization and posterior divergence of marine individuals of *O. argentinensis*, with secondary contacts and genetic introgressions in some cases (Hughes *et al.* 2020). The presence of *O. argentinensis* in fully freshwater was already proposed by several authors, for example Berg (1895) mentioned that this species penetrate into rivers during winters, and the same phenomenon was alluded by Lahille (1929) for the Río Uruguay (Hirt-Chabbert, 1991). Further studies are needed in order to fully address the status of these putative populations of *O. argentinensis*, information that could be used in conservation strategies and management policies for this species.

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