



BIOLOGICAL SCIENCES

DNA barcoding identifies three species of croakers (Pisces, Sciaenidae) in the ichthyoplankton of the High Paraná River

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Abstract: In the province of Misiones (Argentina), the filling of the Yacyretá Reservoir (Argentina-Paraguay) to its final dimensions in 2011 formed new aquatic ecosystem, e.g., Garupá Stream was converted into a subreservoir. Reports have been made in this stream of adult individuals and spawning of the Family Sciaenidae, excellent colonizers of modified environments. The larvae of this family are morphologically similar, particularly among *Pachyurus* and *Plagioscion* species, making taxonomic differentiation difficult. In the present work, sciaenidae larvae were characterized molecularly at the cytochrome c oxidase subunit I gene in order to determine which species use this environment for reproduction. Additionally, genetic distances, Barcode Index Number (BIN) and Automatic Barcode Gap Discovery method (ABGD) were estimated and phylogenetic trees were reconstructed. The results indicated the presence of *Plagioscion ternetzi* and *Pachyurus bonariensis* larvae, and for the first time in tributaries of the region, *Plagioscion squamosissimus*. The incorporation of *P. bonariensis* and *P. squamosissimus* to the faunistic assemblage of ichthyoplankton in the Garupá Stream supports better characterization of the species richness of this secondary watercourse modified by the Yacyretá Reservoir, and advancement in our understanding of use of this area for reproduction.

Key words: *Plagioscion ternetzi*, *Plagioscion squamosissimus*, *Pachyurus bonariensis*, Garupá Stream, reservoir, fish larvae.

INTRODUCTION

The identification of adult fishes relies on clearly defined criteria based on external morphological characters, making precise identification possible for everyone, from amateur anglers to professional taxonomists (Strauss & Bond 1990). However, these characters for specific identification cannot be applied to larval life-stages, at which point the morphological characters may not have yet developed. Furthermore, the morphology of a single species may change rapidly and significantly during ontogeny (Ko et al. 2013). The study of

ichthyoplankton is a developing field and includes the identification of larvae to the lowest possible taxonomic level, which then permits the analysis of fish population dynamics, detection of occurrence and intensity of reproductive activity, as well as an understanding of habitat use during reproduction and early ontogeny (Ko et al. 2013). This identification is possible through use of the DNA barcode (Hebert et al. 2003) based on the cytochrome c oxidase subunit I (COI) marker which has proven effective for species identification, even in cryptic or difficult to identify species (Carvalho et al. 2011, Cabrera et al. 2017, Cardoso et al. 2018, Pereira et al. 2011,

2013, Rosso et al. 2012), and also larvae and eggs (Almeida et al. 2017, Becker et al. 2015, Frantine-Silva et al. 2015).

Individuals of the Family Sciaenidae Cuvier, 1829, one of the most diverse families of the Order Perciformes, are commonly known as *corvinas*, or croakers, due to their propensity for producing sounds with the sonic muscles and swimbladder. They are fishes of commercial importance and are excellent colonizers of modified environments (Tower 1908, Ramcharitar et al. 2006). Larvae of the Family Sciaenidae are characterized as presenting 23 to 27 myomeres, and being relatively large, with a robust, somewhat bulbous head, a triangular stomach and a spiraled small intestine; at some life-stages, they present pigmentation in the head, nape, jaw, ventral area, and along the post-anal lateral line (Moser 1996, Taguti et al. 2015).

The Garupá Stream is a tributary of the Paraná River, having a watershed of 1,416 km². It is the sixth-largest waterway of Misiones Province (Argentina), and has a dendritic pattern with tributaries and sub-tributaries (Sistema Nacional de Información Hídrica 2017). Upon encountering the Yacyretá Reservoir's area of influence (Argentina-Paraguay), it undergoes substantial changes in its drainage regime, increasing its area of inundation, the period of time during which it is flooded, and decreasing the velocity of flow, which give it characteristics of a sub-reservoir (Fulco 2012, Meichtry de Zaburlín et al. 2010, 2013). These changes are reflected in the structural composition of its diverse aquatic communities, such as macrophytes, which provide refuge and reproductive habitat for some members of the fish community. The elevated biodiversity of fish in this stream has been cited by authors such as Flores et al. (2009) and Bogan et al. (2015) among others, reporting the presence of species such as a characin, locally known as *sabalito* *Cyphocharax spilodus*

(Vari 1987), cobi catfish *Pimelodella gracilis* (Valenciennes 1835), yellow catfish *Pimelodus maculatus* Lacèpede, 1803, bogas *Leporinus acutidens* (Valenciennes 1837), *L. obtusidens* (Valenciennes 1836), and the freshwater croakers *Plagioscion ternetzi* Boulenger 1895 and *Pachyurus bonariensis* Steindachner 1879.

In the Garupá Stream in particular, there are records of large spawns of sciaenids, and the presence of adult *Plagioscion ternetzi* and *Pachyurus bonariensis* is periodically verified (Flores et al. 2009). However, these species present larval stages with very similar morphological characteristics, which makes it difficult to taxonomically differentiate between them at larval stages (Lakra et al. 2009, Taguti et al. 2015). On these grounds, in the present work, early stages of croakers obtained in the Garupá Stream (Misiones Province) were characterized at the molecular level, with the aim of recognizing the species which utilize this modified environment for reproduction.

MATERIALS AND METHODS

Ethics statement

In Argentina, fish handling during sampling was performed following guidelines of the ethical committee of the UFAW Handbook on the Care and Management of Laboratory Animals (<http://www.ufaw.org.uk>). Collection permits in Argentina were granted by Ministerio de Ecología y Recursos Naturales Renovables de Misiones (Disp. 013/16). These permits in Argentina are granted without a formal request concerning the protocol used for the humane killing of fish. Notwithstanding, we opted to kill the fish with an overdose of benzocaine, as recommended by the New South Wales Fisheries Animal Care and Ethics Committee (Barker et al. 2002).

Sampling methodology

The sampling of ichthyoplankton and adult specimens was carried out between December 2016 and January 2017 in the Garupá Stream (27°26'49.3"S, 55°48'15.8"W), Misiones Province, in the area of the current outlet resulting from the filling of the Yacyretá Reservoir to its final dimensions in 2011 (Fig. 1).

Samplings of adult specimens of *Plagioscion ternetzi* and *Pachyurus bonariensis* were carried out in diurnal and nocturnal hours making use of monofilament seine nets with the following mesh measurements: 20 cm, 16 cm, 14 cm, 12 cm, 8 cm, 7 cm, 6 cm, 5 cm, and 4 cm when stretched from knot to knot. Collected material was fixed with absolute ethanol, and in the laboratory species identity was corroborated according to the diagnostic characters provided by Casciotta et al. (2006). From the gathered material, one adult per species was selected for posterior

molecular characterization, and documented via digital photography (Fig. 2a, b). For their part, ichthyoplankton samples were taken in nocturnal hours in sub-superficial waters (up to 2 meters in depth). Cylindrical-conical nets with netting of 500 microns were used, these being equipped with mechanical flow meters which were operated in an active manner. Fixing of collected material was done in the field, using absolute ethanol. The larvae of the Family Sciaenidae were separated from the rest of the other animals, plants and drifting detritus, under a Leica MZ6 stereoscopic binocular microscope, and were classified, according to developmental stage according to the criteria of Nakatani et al. (2001), as being: yolk sac, pre-flexion, flexion or post-flexion. Then, 10 larvae representing different stages of development were selected for molecular characterization and documented via digital photography (Fig. 2c, l). The samples

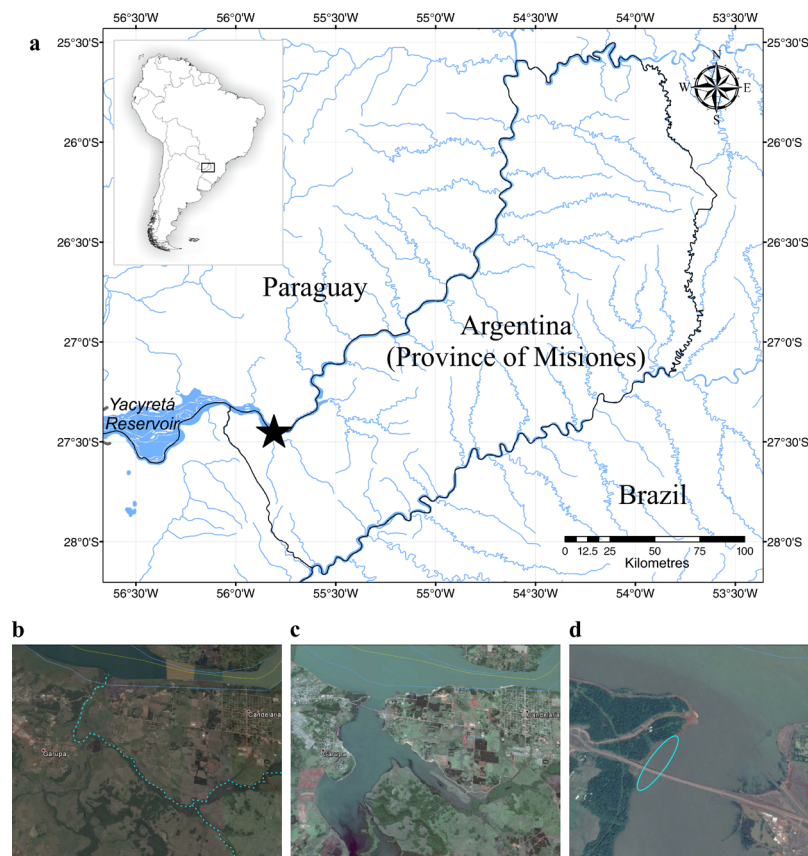


Figure 1. Location of the Garupá Stream and the sampling area. (a) The star indicates the location of the Garupá Stream in the province of Misiones. (b) and (c) Satellite images of the Garupá Stream in years 2008 and 2015, respectively; the dotted line in b indicates the course of the stream. (d) Satellite image of the mouth of the Garupá Stream in 2016; The oval indicates the zone sampling zone. Satellite images were taken from Google Earth (Version 7.1.7.2606).

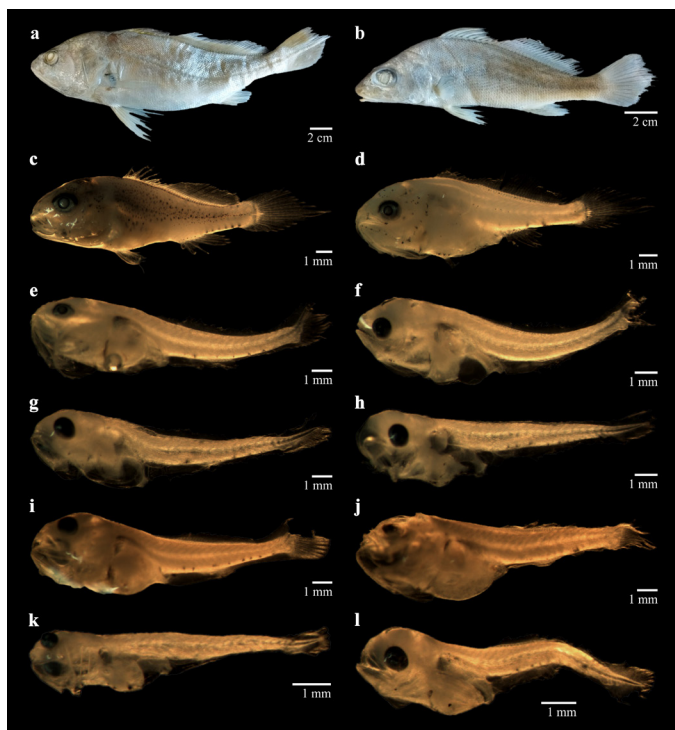


Figure 2. Photographs of adults and larvae of croakers used in this study. (a) Adult *Plagioscion ternetzi* (PGPA-11). (b) Adult *Pachyurus bonariensis* (PGPA-12). (c) Larva (PGPA-1). (d) Larva (PGPA-2). (e) Larva (PGPA-3). (f) Larva (PGPA-4). (g) Larva (PGPA-5). (h) Larva (PGPA-6). (i) Larva (PGPA-7). (j) Larva (PGPA-8). (k) Larva (PGPA-9). (l) Larva (PGPA-10). The codes in parentheses indicate collection numbers assigned within the “*Proyecto de Biología Pesquera Regional*”.

were deposited in the reference collection “*Proyecto de Biología Pesquera Regional*” (Instituto de Biología Subtropical Nodo Posadas, CONICET-Universidad Nacional de Misiones).

DNA extraction, Polymerase chain reaction (PCR) and sequencing

The total genomic DNA was extracted from the abdominal portion of the adult specimens, as well as for each larvae, using the commercial *Puriprep-S Kit* (Inbio Highway, Tandil, Buenos Aires, Argentina), in accordance with the specifications of the manufacturer. The polymerase chain reaction (PCR) was utilized in order to amplify the mitochondrial *COI* gene. The primers used for the amplification of the *COI* gene were FishF1 TCAACCAACCACAAAGACATTGGCAC and FishR1 TAGACTTCTGGGTGGCCAAAGAATCA designed and described by Ward et al. (2005). Amplification was performed in a final volume of 50 μ l containing 1X Green GoTaq Reaction Buffer, 0.2mM dNTP mix, 0.5 μ M of each primer, 1.25U GoTaq DNA polymerase (Promega, Madison,

WI, USA) and 50-100ng of DNA template. The amplification was carried out in an OpenPCR thermocycler (Chai Biotechnologies Inc., Santa Clara, CA, USA), and the amplification protocol consisted of 95 $^{\circ}$ C for 2min; 35 cycles of 94 $^{\circ}$ C for 30sec, 52 $^{\circ}$ C for 30sec and 72 $^{\circ}$ C for 1min; with a final extension of 72 $^{\circ}$ C for 10min. PCR products were visualized on 1.5% agarose gels and then were purified in order to remove potential contaminants that could interfere with the sequencing process. The procedure was performed with the commercial AccuPrep[®] PCR Purification Kit (Bioneer, Daejeon, South Korea), following the recommendations of the manufacturer for products in solution. The purified products were sent to Macrogen, Inc. (Seoul, South Korea) and sequenced in both forward and reverse directions for each individual. Evaluation of the quality of the sequences was conducted with the QV scores provided by the sequencing service. In order to generate the consensus DNA sequence, the chromatograms obtained were analyzed

with Chromas Lite 2.6.4 (Technelysium Pty Ltd, Tewantin, Australia) and Clustal X 2.1 (Larkin et al. 2007). Once the consensus DNA sequences were obtained for both larvae and adults, the sequences were searched using the basic local-alignment search tool BLASTn against GenBank (Altschul et al. 1990) and in the Barcode Index Number (BIN) system in the Barcode of Life Data (BOLD) identification engine (Ratnasingham & Hebert 2013). In addition, we also explored species limits using the Automatic Barcode Gap Discovery method (ABGD) according to Puillandre et al. (2012).

Phylogenetic Analysis

Bioinformatic analysis of the partial regions of the *COI* gene was carried out individually for each of the individuals studied. Based on the sequences obtained in this study, a multiple alignment was carried out with Clustal X 2.1 to identify the presence of different haplotypes, which later were characterized for composition (i.e., number of each of the bases, relative frequency, content of G+C and A+T) using BioEdit 7.1.9 (Hall 1999). Then, a data matrix was generated with the consensus sequences obtained in this study, as well as reference sequences of phylogenetically close species, in the manner put forth by Lo et al. (2015): *Pachypops furcroi* (Monsch 1998), *Plagioscion surinamensis* (Bleeker 1873) and *Paralonchurus brasiliensis* (Steindachner 1875). This matrix likewise included the external group comprised of *Monotaxis grandoculis* (Forsskål 1775), *Sparus aurata* Linnaeus 1758 and *Dicentrarchus labrax* (Linnaeus 1758) (Table I). The data were subjected to estimation of genetic distance, Neighbor-Joining (NJ), and phylogenetic analyses using Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI). Genetic distances were calculated using MEGA 7.0.20 software (Kumar et al. 2016) through the

use of the number of differences (p -distance) and the Kimura 2 parameter substitution model (K2P). The analyses were performed with MEGA 7.0.20 (Kumar et al. 2016) for NJ and ML, and PAUP*4.0b10 (Swofford 2002) for MP. The BI analysis was carried out with Mr. Bayes 3.2 (Ronquist et al. 2012). In the distance method, the TN93 model (Tamura & Nei 1993) was used to calculate the divergence between the sequences. The MP analysis used of heuristic search, with characters equally weighted, tree bisection and reconnection branch-swapping, and 10 random stepwise additions. In the ML analysis, the optimal model of nucleotide substitution (HKY+G) was evaluated with a likelihood ratio test (LRT) and selected according to the corrected Akaike information criteria (AICc) with JModelTest 2.1.10 (Darriba et al. 2012). The topology of the ML tree was obtained in MEGA 7.0.20, following a heuristic search. The statistical support for the resulting phylogenies were assessed by bootstrapping with 1,000 (NJ, MP, ML) replicates (Felsenstein 1985). The BI analysis was carried out with two simultaneous runs of four Markov Monte Carlo chains, which were run for 10^6 generations, with sampling each 100 generations, using the HKY+G model in Mr. Bayes 3.2.6 (Ronquist et al. 2012), with a burnin of 1,001 generations, and the final consensus was based on 18,000 trees used to estimate posterior probabilities. The topologies of the trees obtained were evaluated using software FigTree 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

RESULTS

Drawing upon the consensus *COI* sequences for the individuals analyzed, the search results obtained with BLASTn showed high homologies and sequence identities equaling 100% with

Table I. Summary of the sequences and collection locations of the for specimens included in this study, as well as those of species assigned to the external group for the COI marker.

SPECIES	LOCATION	GENBANK	REFERENCE
<i>Plagioscion ternetzi</i>	Argentina, Misiones, Garupá Stream	MF289078	Present work
<i>Plagioscion ternetzi</i>	X	KP722764	Lo et al. (2015)
<i>Plagioscion surinamensis</i>	X	KP722763	Lo et al. (2015)
<i>Plagioscion squamosissimus</i>	Argentina, Misiones, Garupá Stream	MF289077	Present work
<i>Plagioscion squamosissimus</i>	Brazil, São Paulo, Upper Paraná	GU701891	Pereira et al. (2013)
<i>Plagioscion squamosissimus</i>	Brasil, São Paulo, Upper Paraná, Paranapanema River	KM897668	Frantine-Silva et al. (2015)
<i>Plagioscion squamosissimus</i>	Brasil, São Paulo, Upper Paraná, Paranapanema River	KM897618	Frantine-Silva et al. (2015)
<i>Plagioscion squamosissimus</i>	X	KP722762	Lo et al. (2015)
<i>Pachypops fourcroi</i>	X	KP722753	Lo et al. (2015)
<i>Pachyurus bonariensis</i>	Argentina, Misiones, Garupá Stream	MF289076	Present work
<i>Pachyurus bonariensis</i>	X	KP722754	Lo et al. (2015)
<i>Pachyurus bonariensis</i>	Argentina, Paraná Delta	KU288796	Díaz et al. (2016)
<i>Paralonchurus brasiliensis</i>	Brazil, Rio de Janeiro	JX124857	A.D.E.O. Ribeiro et al. (Unpublished data)*
<i>Paralonchurus brasiliensis</i>	Brazil, São Paulo	JQ365484	Ribeiro et al. (2012)
<i>Paralonchurus brasiliensis</i>	X	KP722756	Lo et al. (2015)
<i>Dicentrarchus labrax</i>	Turkey	KC500493	Keskin & Ataret al. (2013)
<i>Sparus aurata</i>	Turkey, Hatay	KY176642	M.B. Yokes (Unpublished data)*
<i>Monotaxis grandoculis</i>	Mozambique, Pomene	KF489648	D. Steinke et al. (Unpublished data)*

* Sequences published exclusively in GenBank. X refers to site not specifically mentioned in the respective reports.

reference sequences in GenBank. These results gave confirmation of the identity of the adults in accordance with the morphological identification (*Plagioscion ternetzi* –PGPA11– and *Pachyurus bonariensis* –PGPA12–) and provided evidence for the presence of not two, but rather three larval sciaenid species in the analyzed sample:

P. bonariensis (PGPA1, PGPA3, PGPA5, PGPA6, PGPA7 and PGPA10), *P. ternetzi* (PGPA2, PGPA4 and PGPA9) and *Plagioscion squamosissimus* (Heckel 1840) (PGPA8).

In addition, three BINs were recovered, ACG8729 for *P. bonariensis*, ACK2538 for *P. ternetzi* and AAC6616 for *P. squamosissimus*. The

analysis of the ABGD dataset using the default parameters resulted in clear partitions among three species. The sequence compositions of the different BINs were completely recovered by the groups proposed by the ABGD. Additionally, the sequences obtained were deposited in GenBank under the accession numbers MF289078, MF289077 and MF289076, for *Plagioscion ternetzi*, *P. squamosissimus* and *Pachyurus bonariensis*, respectively. As well as, the sequences and data of the specimens were also uploaded to BOLD in the project "SCIAE Sciaenids COI".

The interspecific distances obtained are presented in Table II. From the sequences obtained in this study and those available in Genbank for *Pachyurus bonariensis* and *Plagioscion ternetzi*, the intraspecific genetic distances were zero, while for *Plagioscion squamosissimus* there were variances of 0-1.7% and 0-1.8% for *p*-distance and K2P, respectively.

The final matrix utilized for the phylogenetic analyses consisted of 615 characters. The phylogenetic and distance reconstructions based on the partial sequence of the *COI* gene presented the same topology (Fig. 3). For NJ and ML a larger cluster was obtained containing the species of the Genus *Plagioscion* with a bootstrapping value of 100%. This group contained the sequences of the individuals identified as *P. ternetzi* and *P. squamosissimus* along with their reference sequences in GenBank. Likewise, the sequences obtained for the specimens of *P. bonariensis* formed a group together with their reference sequences with a maximum support value. For the BI analysis, the results were the same at those obtained in NJ and ML, with posterior probability values of 1 for each grouping. For the MP analysis, of the 615 characters of the matrix analyzed, 192 were informative for parsimony and 402 were constant. In the heuristic search,

Table II. Interspecific genetic distances (in percent) for the *COI* gene among the analyzed species.

		1	2	3	4	5	6
<i>Pachyurus bonariensis</i>	1	---	20%	19.8-21.1%	18.90%	19.30%	19.7-20.2%
<i>Plagioscion ternetzi</i>	2	17.10%	---	10.1-11%	7.40%	18.60%	20.9-21.4%
<i>Plagioscion squamosissimus</i>	3	17-17.9%	9.2-10%	---	9.7-10.3%	20.3-21.1%	20.1-22.1%
<i>Plagioscion surinamensis</i>	4	16.40%	6.90%	8.7-9.4%	---	17.80%	20.6-20.9%
<i>Pachypops fourcroi</i>	5	16.60%	16.20%	17.3-17.9%	15.60%	---	20.6-20.9%
<i>Paralonchurus brasiliensis</i>	6	17-17.3%	17.7-18.1%	17.1-18.5%	17.5-17.7%	17.5-17.7%	---

Beneath the diagonal line are *p*-distances, and above, K2P values. Information referring to the species mentioned is found in Table I.

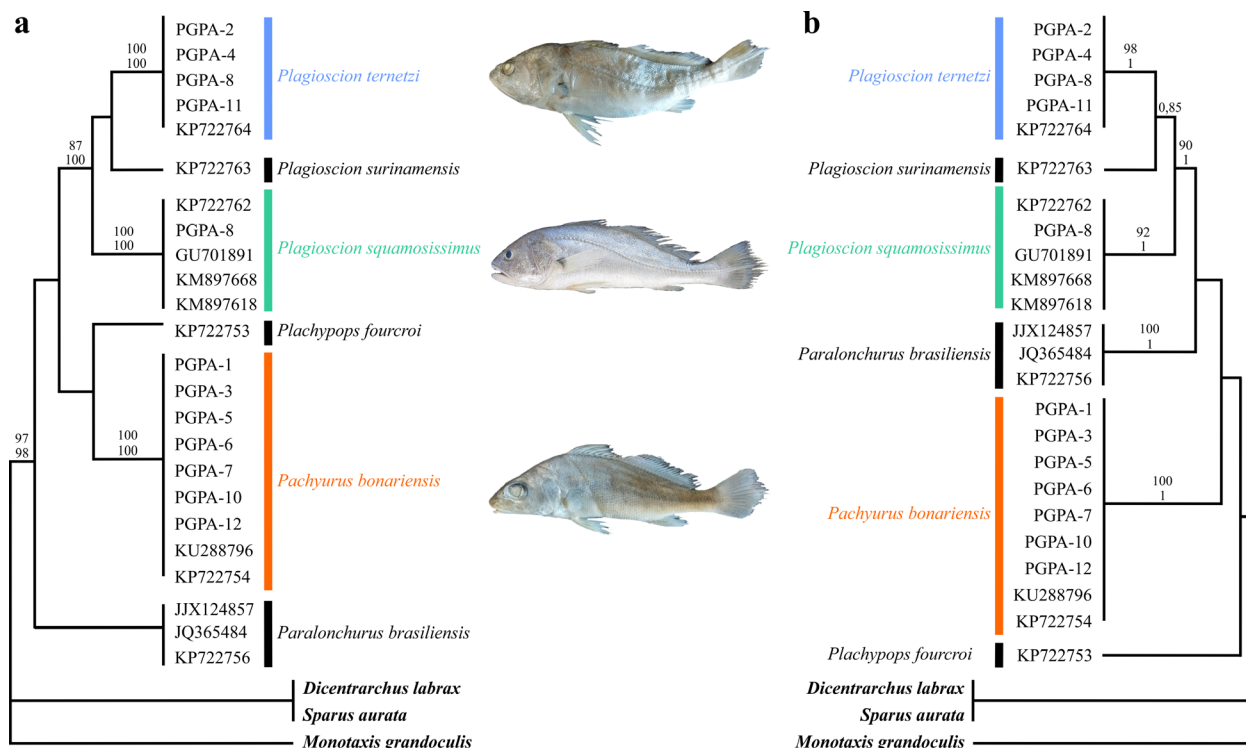


Figure 3. Phylogenetic and distance trees of species of the Family Sciaenidae based on 615 nucleotides of the mitochondrial gene *COI*. Tree of Neighbour-Joining, Maximum Parsimony, Maximum Likelihood and Bayesian Inference. Bootstrap values for: (a) NJ and MP; (b) ML and *a posteriori* probability values for BI, respectively, are shown above the branches. Values below 70/0.7 are not shown. References for the sequences correspond with the collection numbers assigned by the “Proyecto de Biología Pesquera Regional” for material from the Garupá Stream and access numbers from GenBank for the remaining sequences.

a total of 68,520 trees were evaluated and three equally parsimonious trees were captured, with 509 longitudinal steps.

DISCUSSION

The results of the present work show the presence of larvae with morphologically distinctive characteristics of the Family Sciaenidae in Garupá Stream. They might be differentiated from other freshwater species included in the Order Perciformes, being that they present a body form similar to those of adults, with a relatively large head, lower caudal peduncle proportionately small in relation to the head (giving the body the form of a water drop), small intestine being approximately triangular,

and spines in the cephalic region (Taguti et al. 2015). The larvae in question cannot be morphologically identified to the species level employing traditional methods of morphometric taxonomy, inasmuch as these species present similar morphological characteristics (Lakra et al. 2009). However, molecular identification using the *COI* gene allowed recognition of three species in the analyzed material based on the comparison to the totality of the DNA sequences available in GenBank and BOLD, i.e. *Plagioscion ternetzi*, *Pachyurus bonariensis* and *Plagioscion squamosissimus*.

With respect to the identification of larvae of *Plagioscion ternetzi* in the Garupá Stream, its presence was foreseeable, owing to other observations of the species in the area, it having

been catalogued as “constant” according to the Dajoz (1983) index, those species being regarded at constant which have an appearance rate greater than 50%. Nonetheless, there is novelty in the identification of *Plagioscion ternetzi* as being accompanied by other sciaenide larvae in this environment, and in the presence of *Pachyurus bonariensis* - for which there has been observation only of adult individuals in the area (Flores et al. 2009) - as well observation of *Plagioscion squamosissimus*, which constitutes the first record of the species within the area under influence of the Yacyretá Reservoir.

Pachyurus bonariensis is a fish of small size, with some commercial value (Casatti 2003), found frequently in a majority of tropical aquatic environments, notably those which undergo anthropogenic impact such as reservoirs (Benedito-Cecilio & Agostinho 1997). Its presence has been reported in watersheds of the Paraná, Paraguay, and Uruguay Rivers (Agostinho et al. 1993, Mantero & Fuentes 1997, Sverlij et al. 2008). In the Yacyretá Basin, there have been records of spawning of the species (Flores & Hirt 2002), although there had not been records of larvae up to the present work, which might owe to the difficulty of morphological identification. On the other hand, *Plagioscion squamosissimus*, commonly known as the Piauí croaker, is a species that originates in the Amazonas watershed (Parnaíba River) and was introduced in the reservoirs of northeast Brazil in the 1940s, with the objective of putting available lentic environments to use and improving the availability of high-quality fish in that region (Fontenele 1978, Carnelós & Benedito-Cecílio 2002, DNOCS 2002, Félix et al. 2007). Upon the beneficial results obtained in prior experience, the Companhia Energética de São Paulo decided to introduce the first juveniles in to its reservoirs in 1952 (Bialecki et al. 2004). The first spawning was obtained in the tanks of the Estação de

Piscicultura da Usina Hidrelétrica Limoeiro (State of São Paulo, Brazil). Nonetheless, the necessity of opening the reservoir's gates led to the liberation of juveniles into the Pardo River, and it later reached the Upper Paraná, most likely in 1972 (Bialecki et al. 2004). Up to the present date, the species has been found in the area influenced by the Binational Itaipú Reservoir (Brasil-Paraguay) (Hahn et al. 1997, Nakatani et al. 1997, Carnelós & Benedito-Cecílio 2002, Baumgartner et al. 2003, Barros et al. 2012), where, downstream, the High Paraná begins (Bonetto et al. 1986). López et al. (2005) reported the advance of *P. squamosissimus* in the High Paraná, but not in its tributaries, and indicated a possible threat to natural aquatic communities. The presence of the species in a tributary of the Paraná is now confirmed based upon the results of the present work, in accordance with various studies that have demonstrated the species' preference for areas of low water flow and its adaptation to reservoirs (Nomura 1973, Dourado 1981, Cruz et al. 1990, Okada et al. 1996). Piauí croaker is a fish with a great colonization success due to its dietary plasticity, which may suggest a high cost for the fauna of native fishes whose abundances could be altered (Barros et al. 2012, Hahn et al. 1997). This successful colonization is expected, as in most non-native species, mainly in situations where there is absence of predators, the abundance of preferential food resources and morphological traits providing a competitive advantage in early life-stages (Neves et al. 2015). Especially with use of our molecular methods, it is probable that there will be new records of *P. squamosissimus* made in the region owing to colonization opportunities associated with newly formed environments.

Croakers are of commercial importance for the region, relevant to both artisanal and sport fishing (Hirt et al. 2010, Araya et al. 2013). Studies carried out in the watersheds of the

Amazon (Oliveira & Ferreira 2008), including the Tocantins (Soares & Teixeira 2012), São Francisco (Melo & Severi 2010), Paraguay (Casatti 2005) and Upper Paraná basins (Bialetzki et al. 2005) show that larvae of the Family Sciaenidae are among the most abundant larvae of the pelagic zone. Their reproductive cycle is well known, notably increasing their reproductive activity in the warm months of spring and summer, such that in these months their capture is very likely. In this period, croakers prefer regions with slow current and environments with meanders (Vera et al. 2005), characteristics present in the Garupá Stream, which, nearing its outlet into the Paraná River, forms a fan of flooded terrains with slow current. This environment arose when the Yacyretá Reservoir reached its full water level. One example of the modifications associated with the change in water level in the fish communities of the reservoir's area of influence is reflected in the size structure (Agostinho et al. 2008, Hoeinghaus et al. 2009). Previously, when this stretch of the Paraná River was free of dams, it was much more likely to host fish species of larger size. Currently, with the changes produced by damming, the size structure is seen to be in a period of modification, where greatest success is achieved by those fish species of medium size, including the species currently under study, croakers. These results are comparable with those of Hoeinghaus et al. (2009) in the Upper Paraná, Brazil, before and after the construction of the Itaipú Dam.

In this context, the identification of the three species mentioned underlines the importance of the newly formed environments in reproductive events, such the Garupá Stream. All results obtained, in genetic distances, analysis of BINs, ABGD and phylogenetic trees reaffirm the presence of three species of sciaenid larvae in the stream. Also, they show the efficacy of the *COI* marker as a DNA

barcode for the identification of freshwater fish species, which was able to overcome a lack of differentiable morphological characteristics and ontogenetic variation for the identification of the sampled ichthyoplankton, allowing great taxonomic resolution which complements traditional morphological taxonomy. Owing to the difficulty in identification of ichthyoplankton, this approach provides an important taxonomic identification tool for regions that have barcode libraries at their disposal, such as that recently carried out in Argentina by Díaz et al. (2016). As such, the effort to identify these species is important, first of all, in order to characterize the fish community, and then also, to gain certitude in populational studies of the community composition and, in that basin to recommend measures for fishery management. In this sense, the incorporation of *Pachyurus bonariensis* and *Plagioscion squamosissimus* to the ichthyoplankton assemblage of the Garupá Stream, allows for better characterization of the species level richness of this secondary watercourse modified by the Yacyretá Reservoir. Furthermore, it permits an advancement in our understanding regarding the use of the area for reproduction and nursery, and provides evidence about spawning sites and recruitment for numerous fish populations, as these areas are of ecological importance and require management for the fishing resources of the region.

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Author contributions

Y.F.B and G.G.G designed the study. A.A and G.G.G provided tissues. Y.F.B performed laboratory work. Y.F.B and G.G.G analyzed the data. Y.F.B wrote the manuscript. All authors contributed to the final draft of the manuscript.



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