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### ORIGINAL ARTICLE

# Genetic evidence supports polygamous mating system in a wild population of *Prochilodus lineatus* (Characiformes: Prochilodontidae), a Neotropical shoal spawner fish

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## ABSTRACT

Behavioral observations made on fish have revealed remarkably diverse reproductive strategies, including polygamy by both sexes. Still, to date, most Neotropical species remain unstudied as to whether the observed reproductive behavior in natural populations correlates with their genetic mating systems. Here, we investigated the genetic mating system of a wild population of *Prochilodus lineatus* settled in the Middle Uruguay River basin. By using sibship reconstruction and parental inference methods based on microsatellites' genotypes, we inferred 45 females and 47 males as potential parents of the 87 larvae analyzed. We found evidence supporting polygamous mating in both sexes: while a high percentage of males (44.7%) fertilized the eggs of one female, 55.3% of the inferred males fertilized eggs of up to four females. Likewise, while 44.5% of the inferred females had their eggs fertilized by one only male, 55.5% of females were fertilized by multiple males. The estimated *proxy* of the effective population size ( $N_b$ ) was 126, exhibiting moderate to high levels of genetic diversity. The genetic evidence contributed in this study complements earlier behavioral observations of formation of spawning *nuclei* of aggregating breeders, which may be promoting a polygamous mating strategy in this long-distance migratory fish.

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**Keywords:** Genetic mating system; Microsatellites; Migratory fish; Parentage; Relatedness

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## RESUMO

Observações do comportamento de peixes neotropicais têm revelado estratégias reprodutivas marcadamente variáveis, incluindo poligamia nos dois sexos. Ainda assim, até então, a correlação entre comportamento reprodutivo observado em populações naturais e sistemas de acasalamento genético permanece pouco explorada para maioria de espécies Neotropicais. Neste estudo investigamos o sistema genético de acasalamento de *Prochilodus lineatus* em uma população natural estabelecida no Médio rio Uruguai. Utilizando métodos de reconstrução de grupos familiares e inferências parentais baseados em genótipos de microssatélites, inferimos 45 fêmeas e 47 machos como os possíveis parentais das 87 larvas amostradas. Encontramos evidência que permite apoiar a ocorrência de acasalamento poligâmico em ambos os sexos: enquanto uma percentagem alta de machos (44,7%) fertilizou somente uma fêmea, 55,3% dos machos inferidos fertilizaram mais de uma fêmea (até quatro por macho). Da mesma forma, enquanto que 44,5% das fêmeas inferidas tiveram seus ovos fertilizados por apenas um único macho, 55,5% das fêmeas tiveram ovos fertilizados por múltiplos machos. A estimativa do tamanho populacional efetivo ( $N_b$ ) foi 126, exibindo níveis entre moderados e altos de diversidade genética. A evidência genética que apresentamos nesse estudo complementa observações iniciais da formação de núcleos de desova que podem promover estratégias de acasalamento poligâmico nessa espécie migratória de longa distância.

**Palavras-Chave:** Microssatélites; Parentesco; Paternidade; Peixes migradores; Sistema genético de acasalamento

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## INTRODUCTION

The mating system of a given species (also known as breeding system; [Reynolds, 1996](#)) can be defined as a behavioral strategy for obtaining mates, and is influenced by many factors, such as life-history traits, ecology and behavior ([Klug, 2011](#)). This central biological trait of organisms can influence a number of other which, in turn, determine the effective population size,  $N_e$  ([Nunney, 1993](#); [Karageorge, Wilson, 2017](#)), and, thus, shape evolutionary processes. From a conservation standpoint, the mating system can accurately predict the extinction probability ([Legendre et al., 1999](#)). Mating systems can govern the population growth rate during and after periods of intense exploitation, and could be used as surrogates to assess the extinction risks and to settle conservation strategies, particularly in species exploited as economic resources ([Rowe, Hutchings, 2003](#)).

Breeding systems of teleostean fishes vary widely and are extraordinarily diverse, from self-fertilization and social monogamy to promiscuous behavior and group spawning ([DeWoody, Avise, 2001](#); [Haddeland et al., 2015](#); [Ribolli et al., 2016](#); [Smith, Wootton, 2016](#)). All possible social mating systems can be found among these fishes: monogamy by both sexes, polygyny (male's polygamy), polyandry (females' polygamy), polygynandry (polygamy of both sexes) and promiscuity (both sexes mate with multiple partners) ([DeWoody, Avise, 2001](#); [Avise et al., 2002](#); [Haddeland et al., 2015](#); [Smith, Wootton, 2016](#)). Furthermore, studies have demonstrated that pheromones may regulate various aspects of the reproductive behavior of teleostean fishes, such as stimulation of mating ceremonies ([Liley, Stacey, 1983](#)), synchronization of reproduction ([Liley, Stacey, 1983](#); [Kobayashi et al., 2002](#)), spawning and increase of shoals ([Chung-Davidson et al., 2010](#)). However, in fishes, as it occurs in other vertebrates, such as birds ([Griffith et al., 2002](#)), the observed reproductive behavior may not correspond to the genetic mating system: for example, both males and females may take part in extra-pair spawnings ([Wootton, Smith, 2014](#)).

The Curimatá *Prochilodus lineatus* (Valenciennes, 1837) (= *Prochilodus scrofa* Steindachner, 1881) is commercially exploited, and represents one of the most important freshwater resources in South America ([Espinach Ros, Fuentes, 2000](#); [Avigliano et al., 2016](#)). Distributed in the Plata basin (Paraná, Paraguay, Uruguay, De La Plata River, among others), it is exploited by fisheries in Argentina, Brazil, Bolivia, Paraguay and Uruguay. Species of the genus *Prochilodus* Agassiz, 1829 are potamodromous, exhibit high fertility and lack of parental care ([Vazzoler, 1996](#); [Winemiller, 2005](#)). *Prochilodus lineatus* displays total spawning ([Lowe-McConnell, 1999](#); [Sato, Godinho, 1999](#)): females spawn over a short time-frame and no further eggs are shed in that breeding season ([Smith, Wootton, 2016](#)). The Curimatá also has external fertilization which takes place under continuous water flow ([Lowe-McConnell, 1999](#); [Sato, Godinho, 1999](#)). During the reproductive period, *P. lineatus* forms large fish aggregations, which are usually found downstream of waterfalls or dams ([Godoy, 1975](#)). As other Neotropical migratory species, the Curimatá performs upward reproductive migrations towards adequate spawning grounds; they release their eggs into running water, and eggs drift freely with the currents ([Harvey, Carolsfeld, 2003](#)). Initial reports of observations of numerous *P. lineatus* breeding events described large fish shoals, composed of hundreds of individuals, resembling a "mating dance", in which fish jump out of the water in an event that can last several hours ([Godoy, 1975](#)). Decades later, [Godinho et al. \(2017\)](#) described that males of a congeneric species *Prochilodus argenteus* Spix, Agassiz, 1829 perform characteristic spawning sounds, suggesting the occurrence of "lek choruses" (it is a call behavior performed by male, in chorus). However, despite the existence of some visual

reports made on wild populations of this migratory fish species, details of its spawning and breeding system remain unclear. Therefore, investigating the genetic mating system of *P. lineatus* can provide useful information to support conservation and management of this species.

Describing the reproductive strategies of fishes in nature is often precluded by the complex biology of these organisms, which prevents direct behavioral observations of the individuals involved in reproductive encounters. In the wild, *P. lineatus* inhabits large and deep rivers, with high turbidity, and this precludes accurate observations of the spawning events and reproductive behavior of individuals ([Godinho et al., 2017](#)). In such situation, the mating system can be verified or inferred using DNA-based methods, mostly aimed at reconstructing kinship amongst individuals in an array, based on their *multilocus* genotypes ([Jones, Ardren, 2003](#)). When eggs are laid in masses so that the progeny may occur in family groups - as in some fishes - additional analytical power is gained by the kinship reconstruction analyses ([Flanagan, Jones, 2019](#)). This approach is therefore a keystone of molecular ecology, providing important insights into behavior, ecology and evolution ([Flanagan, Jones, 2019](#)), as well as surprising revelations about the behavior of species in their natural environments ([Griffith et al., 2002](#)), providing cues to broaden the knowledge on evolutionary aspects of such a fundamental biological trait. Indeed, during the last two decades, molecular markers have contributed to clarify the reproductive modes and unraveled the genetic mating systems of several fish species, such as, for example, some salmonids ([MacCrimmon, Gots, 1979](#); [Martinez et al., 2000](#); [Serbezov et al., 2010](#); [Weir et al., 2010](#); [de Mestral et al., 2012](#)), trout ([Fraser et al., 2005](#); [Serbezov et al., 2010](#); [Kanno et al., 2011](#)), and sturgeon ([Duong et al., 2013](#); [Jay et al., 2014](#)). To the extent of our knowledge, the molecular-parentage approach has only been applied to investigate the mating strategies of *Salminus brasiliensis* (Cuvier, 1816) ([Ribolli et al., 2016](#)). Thus, in contrast to the many investigations focusing on reproductive strategies and mating behavior of such commercially important organisms, the knowledge on Neotropical migratory fishes is still incipient.

The genetic mating system of fishes showing external fertilization in the freshwater courses, as the Curimbatá, can be approached by sibship reconstruction based on genotypic information. Here, we assessed aspects of the breeding system of wild *P. lineatus* by means of molecular markers and kinship reconstruction of larvae sampled at the Uruguay River, South Brazil. Our aims were to: i) estimate the number of breeding males and females in the population; ii) assess the most prevalent genetic mating system in both sexes; and iii) estimate the reproductive contribution of males or females during spawning.

## MATERIAL AND METHODS

**Field sampling and laboratory procedures.** We sampled *P. lineatus* eggs at a site located in the Middle Uruguay River basin (27°15'50.16"S; 54°03'16.97" W), located between Turvo State Park (Brazil) and Yabotí Biosphere Reserve (Argentina), which are both National protected areas. This area represents the largest dam-free stretch in the Middle Uruguay River (ca. over 800 km), and is of paramount importance for the reproduction of long-distance migratory fishes ([Reynalte-Tataje et al., 2012](#); [Ziober et al., 2015](#); [Reynalte-Tataje et al., 2017](#); [Ribolli et al., 2018](#)). Sampling was conducted in March 2014 (summer season in the southern hemisphere), at night time (around 9:00 PM), using conical-cylindrical plankton nets (0.5 mm mesh and mouth area of 0.11 m<sup>2</sup>) following methods described in [Hermes-Silva et al. \(2009\)](#). All eggs were sampled simultaneously and using the same plankton net, and were immediately incubated in plastic bags supplied with water flow and oxygen input. *Prochilodus lineatus* eggs were captured at the initial segmentation stage (about 8-10 hours after fertilization), thus confirming that all eggs derived from the same spawning event, and the larvae were cultivated in the laboratory until juvenile stage (3-month-old) according to the *live ichthyoplankton* methodology ([Reynalte-Tataje, Zaniboni-Filho, 2008](#)). A fin fragment of each 87 juveniles were cut using sterilized surgical scissors and preserved in ethanol 96% at -20 °C until processed. Voucher of *P. lineatus* from Uruguay River is deposited in the Museum of the State University of Londrina (MZUEL11729).

**DNA extraction and marker amplification.** Genomic DNA was isolated from fin samples following an universal salt extraction protocol ([Aljanabi, Martinez, 1997](#)). Samples were amplified at 11 microsatellite *loci*, using fluorescently-labeled primers (see details in Additional [File S1](#)). Polymerase Chain Reactions were carried out in 10 µL mixes containing: 50 ng of template DNA, 15 mM of MgCl<sub>2</sub>, 1U *Taq* DNA polymerase (Uniscience, Brasil), 1X PCR buffer, 2 mM of each *forward* and *reverse* primer, and 1.1 mM of dNTPs (Invitrogen, Brasil). Standard cycling parameters were: initial denaturation at 95 °C for 1 min, followed by 35 cycles of 95 °C for 30 sec, annealing temperature of each primer ([File S1](#)) for 45 s, extension at 72 °C for 30 sec, followed by a final elongation step at 72 °C for 20 min. Allele sizing was carried out by capillary electrophoresis using 1 µL of diluted PCR product in ultrapure water (2:15 µL), added to 0.25 µL of GS600 LIZ® and 8.75 µL of formamide HIDITM and run in an ABI 3500XL Sequencer (Applied Biosystems). Genotyping of each sample was carried out using automatic bins in Gene Mapper v.3.2 software (Applied Biosystems) and eye-checked.

**Statistical analyses.** The genotypic dataset was analyzed for the presence of null alleles, allelic dropout and stutter peaks using MICRO-CHECKER v2.2.3 ([van Oosterhout et al., 2004](#)). Departures from Linkage Equilibrium or Hardy-Weinberg Equilibrium (*HWE*) were investigated using GENEPOP 1.2 ([Raymond, Rousset, 1995](#)) and GenAIEx v6.4 ([Peakall, Smouse, 2006](#)), respectively. Mean number of alleles per locus, and mean observed (*H<sub>o</sub>*) and expected

( $H_e$ ) heterozygosity values were calculated using GenAlEx. Allelic richness ( $A_r$ ; [Leberg, 2002](#)), inbreeding coefficients ( $F_{IS}$ ; [Weir, Cockerham, 1984](#)) and tests for heterozygotes' excess ( $\rho_L$ ) or deficit ( $\rho_S$ ) were calculated using FSTAT 2.9.3.2 ([Goudet, 2001](#)).

Given that our aim was to maximize our ability to correctly reconstruct sibship arrays, in order to accurately infer the genetic mating system of *P. lineatus*, we first conducted an analysis in KinInfor v.1 ([Wang, 2006](#)), to determine the effect of the number of loci used on the power of relationship analysis ( $PWR$ ). For this, we simulated 1,000,000 genotypes of full-siblings as primary hypothesis and unrelated individuals as null hypothesis, used empirical allele frequencies, a genotypic error of 0.01 for each marker, and 0.05 alpha level.

We then used COLONY v2 ([Jones, Wang, 2010](#)) to infer potential parentage and full- and half-sibship arrays based on larvae genotypes, by running a full-likelihood method and assuming, separately: i) a polygamous mating system without inbreeding for both sexes, ii) a polygamous mating system for males and monogamy for females, iii) a polygamy for females and monogamy for males and iv) monogamy for both sexes. COLONY v2 allows for deviations from Hardy-Weinberg equilibrium. We run five replicate runs for each of the different mating system assumptions and only recorded the resulting full- and half-sibling assignments with a probability  $>0.80$  which were consistently recovered in all runs (*i.e.*, the five replicates of each of the four types of mating system). Finally, we used the Sibship Assignment method implemented in COLONY to estimate the effective number of breeders,  $N_b$  (and its corresponding 95% Confidence Interval) as *proxy* of the population effective size ( $N_e$ ), assuming an unknown number of parents, very high likelihood precision algorithm and polygamy in both-sexes.

## RESULTS

Population genetic parameters, larvae sibship arrays and inference on parentage patterns. The 11 microsatellites were polymorphic (total of 178 alleles, 4-33 allele/*locus*) ([Tab. 1](#)). Loci Pli34 and Plin139 deviated significantly from Linkage Equilibrium ( $P = 0.00$ ), therefore, locus Pli34 was excluded from sibship reconstruction analyses and estimates of  $N_b$ , which were then performed with a set of 10 unlinked microsatellites. Six *loci* deviated significantly from  $HWE$  ([Tab. 1](#)). The mean  $H_e$  ranged from 0.13 to 0.95 and  $H_o$  ranged from 0.13 to 0.93 ([Tab. 1](#)). The *multilocus*  $PWR$  to discriminate between full-siblings and unrelated individuals was 0.99. COLONY analyses recovered the number of sires producing each full-sib array with a high probability (minimum = 0.95, the value obtained for the two broods sired by four males, see below). Likewise, the number of mothers producing each progeny array was recovered with a high probability (minimum = 0.95).

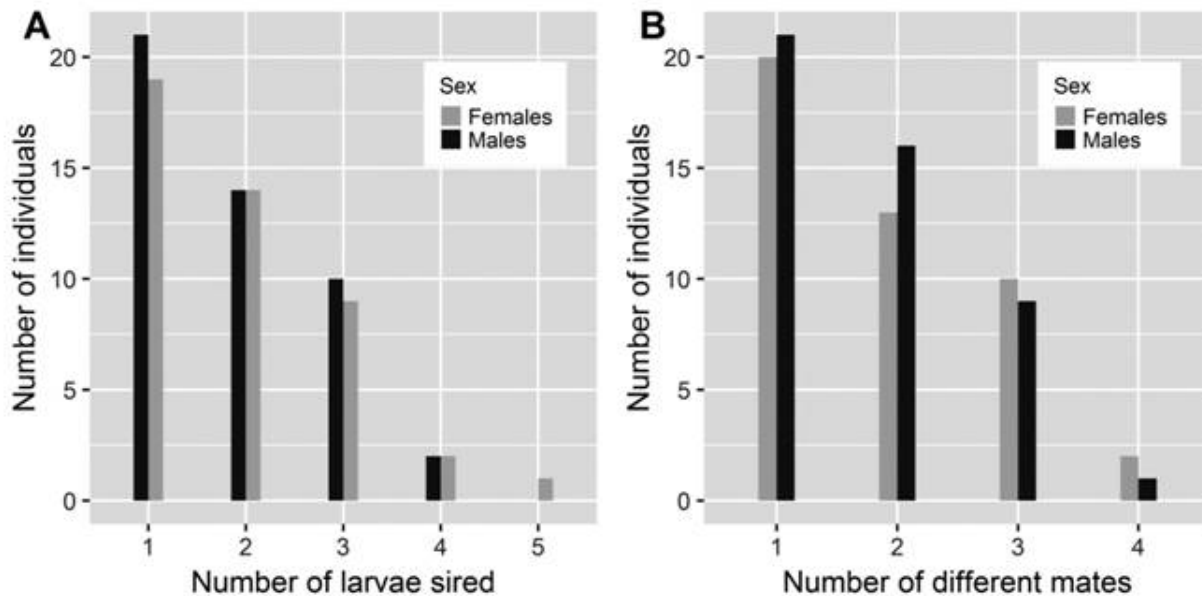
**TABLE 1** | Summary population genetic diversity for eleven microsatellite loci genotyped in *Prochilodus lineatus* larvae collected in the Middle Uruguay River basin, Southern Brazil. Number of individuals genotyped ( $N$ ), Number of alleles ( $A$ ), observed heterozygosity ( $H_o$ ), expected ( $H_e$ ) heterozygosity, inbreeding coefficient ( $F_{IS}$ ) and probabilities of tests for deviation from Hardy-Weinberg equilibrium ( $PHWE$ ) are shown (the asterisks denote significant values,  $P < 0.05$ ).

Primer	$N$	$A$	$H_o$	$H_e$	$F_{IS}$	$PHWE$
Plin14	79	16	0.76	0.81	0.16*	0.00*
Plin25	85	8	0.93	0.58	0.03	0.03
Pli34	71	12	0.78	0.80	0.02	0.25
Plin64	84	19	0.88	0.91	0.02	0.55
Plin119	87	33	0.67	0.95	0.34*	0.00*
Plin139	87	28	0.62	0.94	0.07	0.03
Plin190	44	16	0.82	0.84	0.23*	0.00*
Par12	86	23	0.70	0.92	0.16	0.00*
Par66	86	10	0.35	0.41	0.58*	0.00*
Par83	83	9	0.18	0.42	-0.04	1.00
Pcos03	83	4	0.13	0.13	-0.59*	0.00*

Our analyses inferred 47 males as sires of the larvae, 21 of which (44.7%) sired one larva, while 14 (29.8%) sired two larvae, 10 males (21.3%) sired three and two of males (4.2%) sired four larvae ([Fig. 1A](#)). Forty-five females were inferred as dams, 19 of which (42.2%) mothered only one larva, while 14 (31.1%) produced two, nine (20.0%) sired three larvae, two females (4.4%) produced four larvae, and one (2.3%) mothered five larvae ([Fig. 1A](#)). While 21 males fertilized the eggs from only one female, 16 males (34.0%) fertilized eggs of two females each, nine males (19.2%) fertilized eggs of three females each, and a single male (2.1%) fertilized eggs from up to four females ([Fig. 1B](#)). Likewise, while females (44.5% of the inferred females) had their eggs fertilized by a single male, 13 females (28.8%) had their eggs fertilized by two males, 10 females (22.2%) by three, and two (4.5%) by four males ([Fig. 1B](#)). From these figures, the number of males siring a brood varied from one to four and the population frequency of multiple paternity was 55.5% (sum of the sibship arrays sired by two or more males).

Under polygamy by both sexes, and assuming random mating, the  $N_b$  estimated in the studied population was 126 fishes (95% CI: 94-171).





**FIGURE 1** | Inferred parentage and mating patterns derived from analysis of 87 *Prochilodus lineatus* larvae sampled at the Middle Uruguay River in Brazil. A. Number of adults identified as parents of the sampled larvae; B. Number of different mates inferred for males and females.

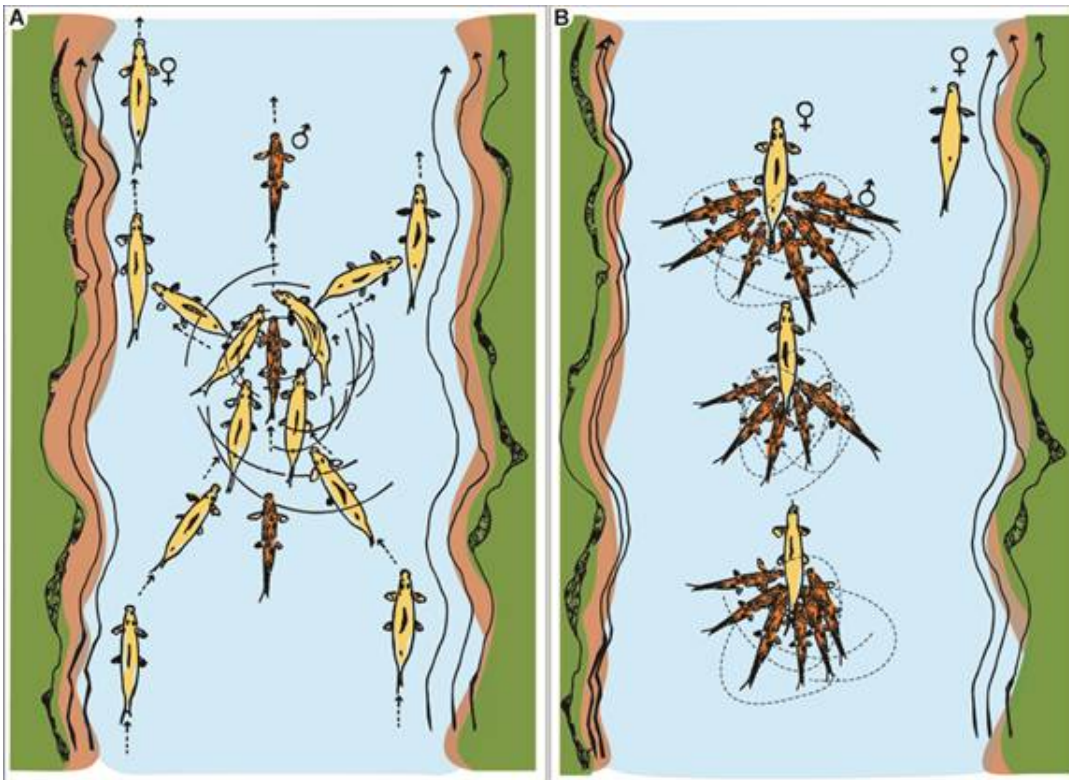
## DISCUSSION

We conducted kinship reconstruction in *Prochilodus lineatus* based on microsatellites' genotypes of larvae, and found genetic evidence suggesting that polygamy is the most prevalent mating system in both sexes, in agreement with previous field observations of shoals during *P. lineatus* spawning events in nature (Godoy, 1975). Kinship analyses showed that 45.98% of the 87 analyzed larvae were not attributed to any sibship array, *i.e.* they were unrelated to each other. This result was further supported by the fact that a high percentage of males (44.7%) and females (42.2%) contributed with only one descendant each. These results seem to suggest that the analyzed breeding population of *P. lineatus* exhibits a percentage of monogamous mating.

It is worth noting that 47 larvae were produced by two or more females and two or more males (57.8% and 55.3%, respectively) with multiple breeders participating in the same reproductive event. Theoretically, reproductive aggregations in species that release pelagic eggs imply little opportunity to choose mates (Stockley et al., 1997). Despite the scarce information about spawning of Neotropical fishes in nature, parentage analyses conducted on *P. lineatus* in semi-natural conditions (reproduction in the laboratory) identified unequal contribution of parents to the progeny, suggesting reproductive advantage (Souza et al., 2018). Additionally, some gametes (or breeders) may have dominance during fertilization, as observed in pooled-milt fertilizations of *Rhamdia quelen* (Quoy, Gaimard, 1824) in laboratory conditions (Ribolli, Zaniboni-Filho, 2009). It is important to emphasize here that the observed pattern of skewed parental contribution, instead of suggesting male or female dominance, could be reflecting the sampling strategy. For example: considering that each *P. lineatus* female can produce on average 160,000 oocytes per kg of weight (Vazzoler, 1996), the 45 potential females inferred as participating in the spawning event would have produced at least 7,200,000 eggs, of which we randomly sampled 87. Therefore, the single-larvae arrays can be explained as the result of the mixture of eggs from distinct spawning nuclei (*i.e.*, sired by different parents) carried out by the river flow.

Another avenue of evidence which could support our findings comes from observations of a peculiar reproductive display in some *Prochilodus* species: in the spawning arenas, some males made 'calls' to attract partners (Godinho et al., 2017), similar to the spawning description by Godoy (1975). According to the last author, during the reproduction of *P. lineatus*, a group with thousands of individuals was observed, where males called females by characteristic sounds ("males concentrate in the center of the river and females remain on the margins, waiting for the moment to attack the males"; details in Fig. 2A; Godoy, 1975). Decades after Godoy's (1975) report, Godinho et al. (2017) reported a similar reproductive behavior for *P. argenteus* suggesting a "lek mating system", stimulated by male calls. On the other hand, direct observation of natural spawning of *P. lineatus* indicated that fishes aggregate in several nuclei composed by many shoals of fishes moving the water simultaneously. Likewise, in laboratory conditions, males touch female genitalia and compress the body of females, thus stimulating the release of oocytes (Evoy Zaniboni-Filho, 2019, pers. comm.). In these cases, several males simultaneously

stimulated a mature female, which, when exhausted, would move away, allowing males to start the same process with another mature female, and over again (for details see [Fig. 2B](#)). The sexual pheromones released by mature females would stimulate reproductive synchrony ([Stacey, 2003](#)), and could promote the reproductive aggregation described above. [Fonseca et al. \(2010\)](#) also report that artificially induced females of *Prochilodus* sp. do not spawn alone; they naturally spawn when stored in tanks together with males. The sexual pheromones released by mature females in this case would be responsible for attracting males and synchronizing the reproduction, as described above. In teleost fish, the pre-spawning release of sex pheromones causes a rapid increase of sexual activity and attraction and excitation of partners to mate ([Hoar et al., 1983](#); [Chung-Davidson et al., 2010](#)). Females' pheromones have been found to modulate males' reproductive behavior in several fish species, including *Salmo trutta* Linnaeus, 1758 ([Laberge, Hara, 2003](#)), *Carassius auratus* (Linnaeus, 1758) ([Kobayashi et al., 2002](#)) and *Misgurnus anguillicaudatus* (Cantor, 1842) ([Ogata et al., 1994](#)), but have not yet been reported in *P. lineatus*. The descriptions and hypotheses aforementioned of the spawning behavior of *P. lineatus* (Evoy Zaniboni-Filho, 2019, pers. comm.) could explain the polygamous mating by males and females inferred in our study, and complement our understanding of the reproductive behavior of *P. lineatus* in nature.



**FIGURE 2** | Alternative schematic representations of spawning in *Prochilodus lineatus*. A. Schematic drawing of spawning moment of *Prochilodus lineatus* (= *Prochilodus scrofa*), according to [Godoy \(1975\)](#), whom described that males, positioned in the center of the river channel, performed distinctive sounds, possibly attracting females (figure drawn by Josiane Ribolli and Dennis Fernando Moreno, inspired from the original by Godoy, M. P, 1975, fig. 153, p.684); B. Hypothetical schematic representation of reproduction and spawning of *P. lineatus* in the wild according to direct observations made by Evoy Zaniboni-Filho (personal communication). The exhausted mature female is marked with an asterisk. Figure drawn by Josiane Ribolli and Dennis Fernando Moreno.

Our genetic data inferring many males and females as sires of the collected eggs support previous evidence from experimental conditions and field observations in suggesting that reproduction of *P. lineatus* occur in different spawning nuclei. Although not investigated in the present study, the formation of such nuclei may result of orchestrated calls made by mature males or by attraction and stimulation mediated by sexual pheromones, or both factors acting complementarily. Yet, hormone-behavior studies were not carried out in *P. lineatus* to date, so that this hypothesis remains to be thoroughly investigated.

The studied larvae showed moderate to high levels of neutral genetic diversity, which could be indicative of a stable and large population, as observed in other Neotropical migratory fishes, such as, for example, *Prochilodus costatus* Valenciennes, 1850 ([Carvalho-Costa et al., 2008](#); [Braga-Silva, Galetti Jr., 2016](#)) and *P. lineatus* ([Rueda et al., 2013](#)). It is worth mentioning that the studied larvae were sampled at an area with low disturbance lying between two areas of high conservation concern, officially protected by Argentina and Brazilian laws. In such preserved habitats, it is possible that *P. lineatus* populations remain large and stable, thus maintaining moderate-high levels of genetic diversity. Retention of genetic diversity is important for the conservation of *P. lineatus* which is

vulnerable to habitat fragmentation, degradation, pollution, overexploitation, and cascade effects imposed on population dynamics by reduced dispersal, restricted by dams ([Smolders et al., 2002](#); [Agostinho et al., 2004](#); [Pessoa, Schulz, 2010](#); [Rueda et al., 2013](#); [Costa et al., 2018](#)). The analyzed population could be thus seen as a source of genetic diversity which can enable adaptation to new environmental pressures.

The set of polymorphic microsatellite markers was powerful enough to perform kinship reconstruction of family groups, and the significant deviations from Hardy-Weinberg equilibrium possibly reflect a non-closed population with migration. The  $N_e$  estimated for the studied population was higher than the number of potential inferred parents; such an increased  $N_e$  may be reflecting a balanced individual reproductive success during spawning, a low variation in family size, or the reproductive strategy of polygamy by both sexes. In addition, the dispersion of eggs by current-drifting allows the admixture of progeny from several families and different spawning *nuclei*, and might concurrently be contributing to the high genetic diversity of the examined sample, maximizing its effective size.

In sum, this study investigated, for the first time, the genetic mating system in of a wild population of *P. lineatus*, a spawner migratory fish which aggregates in shoals during reproduction. We contribute novel evidence suggesting that this species shows a monogamous genetic mating system in combination with polygamy by males and females, supporting earlier behavioral observations. We suggest that spawning happens with the formation of several *nuclei*, in which the eggs released by a female could be fertilized by several males, which in turn can accompany the spawning of other females and also take part in other fertilizations. The result of this reproductive behavior associated with the environmental characteristics of the site used for spawning (large river flow, which guarantees the dispersal and mixture of the eggs) has allowed the maintenance of a similar sexual proportion of the parents. This study expands the knowledge about the mating strategies and reproductive behavior of Neotropical migratory fishes in nature through the use of molecular approaches. Our results can also support the management and conservation of such an important fishery resource.

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## ADDITIONAL NOTES

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#### COMPETING INTERESTS

The authors declare no competing interests.

#### ETHICAL STATEMENT

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