Quantitative genetic variation for resistance to the parasite *Ichthyophthirius multifiliis* in the Neotropical fish tambaqui (*Colossoma macropomum*)

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ABSTRACT

Tambaqui (*Colossoma macropomum*) is the main native fish produced in continental aquaculture from South America. However, its production has been negatively affected by significant economic losses due to frequent outbreaks caused by the parasite *Ichthyophthirius multifiliis*. Genetic selection for *I. multifiliis* resistance may represent a sustainable and effective alternative to reduce mortality and, therefore, improve production of tambaqui. The estimation of genetic parameters is needed to validate whether *I. multifiliis* resistance can be included in genetic improvement programs. The aim of this study was to estimate variance components and heritability for *I. multifiliis* resistance in tambaqui, through experimental challenge of 218 individuals from eight full-sib families. Survival status (SS), time of death (TD) and parasite load (PL) of fish presenting clinical signs of *I. multifiliis* infestation were recorded in the cohabitation experimental challenge. The total cumulative survival rate varied significantly among families (16 to 100%) and TD ranged from 217 to 254 hours post cohabitation, which indicates the presence of significant phenotypic variation related to resistance to *I. multifiliis* infestation. High values for heritability were estimated for SS and TD (0.46 ± 0.09 and 0.60 ± 0.18, respectively). However, differences among families and heritability value were not significant for PL. This study represents the first report on genetic parameters for disease resistance against the parasite *I. multifiliis* in a Neotropical fish species. The results presented here suggest that resistance to *I. multifiliis* in tambaqui can be improved through selective breeding.

1. Introduction

Tambaqui (*Colossoma macropomum*) belongs to the family Serrasalmidae and it is native to the Amazon and Orinoco basins. Differently from the well-known piranhas, which are serrasalmids of predatory behavior, tambaqui is considered an omnivore fish, with preference to frugivorous behavior (Gomes et al., 2010). Additionally, this species presents high rusticity, fast growth, acceptance of artificial feed, high productivity, and commercial value to international markets (Valladão et al., 2018). Therefore, tambaqui has desirable characteristics for its aquaculture development in Latin America, being farmed in countries such as Bolivia, Brazil, Colombia, Peru, Ecuador, and Venezuela (Valladão et al., 2018).

Tambaqui is the main native fish farmed in Brazil, with a major contribution to production from the Northern region of the country. The aquaculture production of tambaqui reached 136 thousand tons in 2016 (IBGE, 2017). In addition, tambaqui can be crossed with other

Serrasalmidae species (e.g. *Piaractus mesopotamicus* and *Piaractus brachypomus*), resulting in interspecific hybrids, which are widely used in the Midwest and Southeast regions of Brazil (Hashimoto et al., 2012). In 2016, the hybrids tambatinga (female of *C. macropomum* x male of *P. brachypomus*) and tambacu (female of *C. macropomum* x male of *P. mesopotamicus*) had a production of 44 thousand tons, which represented the second largest production for native fish in Brazil (IBGE, 2017). Otherwise, the reciprocal hybrids are not produced on large scale probably because they present lower performance, as demonstrated by Fernandes et al. (2018). Tambaqui and their hybrids have contributed with about 40% of the aquaculture production in Brazil.

One of the main reasons for the production of the hybrids in Southern Brazil is their higher tolerance to temperature variation when compared to the pure tambaqui species, which often occurs during seasonal changes (summer/fall and winter/spring) (Fernandes et al., 2018). However, interspecific hybrids may be fertile and cause serious biological risks to natural and cultivated populations (Hashimoto et al.,

2012). The key limitation for tambaqui production in Southern Brazil is the low tolerance to temperature oscillation during the season changes (Zaniboni-Filho and Meurer, 1997), which generally results in disease outbreaks, such as ich or white spot disease caused by the ciliate protozoan *Ichthyophthirius multifiliis* (Martins et al., 2002; Matthews, 2005). This parasite does not present host specificity and the disease is highly contagious (Matthews, 2005). Ich infestation frequently causes economic losses in fish farms worldwide in several species (Buchmann et al., 2001). In Brazil, an overall estimation of losses caused by diseases in tambaqui, including *I. multifiliis* infestation, ranged from 0.55 to 10 thousand tons, representing a value of approximately 0.28 million dollars (Tavares-Dias and Martins, 2017).

Several treatments and prevention strategies against ich have been proposed, including chemotherapeutic drugs (Srivastava et al., 2004; Rintamäki-Kinnunen et al., 2005; Farmer et al., 2013); herbal medicines (Valladão et al., 2016); vaccines, with live theronts or inactive trophonts (Xu et al., 2008, 2009a, 2017; Moreira et al., 2017); and i-antigens (Wang and Dickerson, 2002; Xu et al., 2009b). However, most of these treatments and strategies are still not viable for commercial aquaculture. For example, chemotherapeutic drugs are being banned because of their undesirable effects on the environment (Rico and Van den Brink, 2014); and vaccination is time-consuming, labor-intensive, and relatively expensive in large-scale production, since each fish needs to be vaccinated and there is no evidence that the immunity is passed on to the next generation (Xiong et al., 2017). Currently, there are no strategies available to control and prevent *I. multifiliis* in a sustainable and efficient way.

Selective breeding for disease resistance is one of the main preventive strategies that can be used to improve long-term survival (Gjedrem, 2000; Hulata, 2001). Genetic improvement for disease-resistant fish can provide an efficient alternative to control parasitism in commercial aquaculture (Gjedrem et al., 2012; Elaswad and Dunham, 2018), increasing the productivity and improving animal welfare (Ødegård et al., 2011; Olesen et al., 2011; Janssen et al., 2017; Lhorente et al., 2019). Understanding the level of additive genetic variation for *I. multifiliis* resistance in tambaqui is required before including that trait into the breeding goal. Nevertheless, there are no studies aimed at assessing genetic parameters for *I. multifiliis* resistance in this species. The study aimed to estimate the variance components and heritability for resistance against *I. multifiliis* in tambaqui, through an experimental cohabitation challenge in a pedigreed population from Brazil. The results presented here are mainly focused on understanding if genetic selection can be applied to obtain fish that are genetically resistant to *I. multifiliis*.

2. Materials and methods

2.1. Ethics Statement

This study was conducted in strict accordance with the recommendations of the National Council for Control of Animal Experimentation (CONCEA) (Brazilian Ministry for Science, Technology and Innovation) and was approved by the Ethics Committee on Animal Use (CEUA number 019006/17) of Faculdade de Ciências Agrárias e Veterinárias, UNESP, Campus Jaboticabal, SP, Brazil.

2.2. Experimental population

Data were obtained from 218 individuals of tambaqui belonging to eight full-sib families, generated by a hierarchical mating scheme using five dams and eight sires (Supplementary file 1). The breeders were obtained from four different commercial fish farming facilities from Brazil (São Paulo State) to obtain an appropriate representation of the genetic variation present in aquaculture stocks.

Induced spawning was performed using carp pituitary extract dissolved in saline solution (0.9% NaCl) and applied in two dosages, with a

12 h interval (first and second dosage of 0.5 and 5.5 mg/kg, respectively). For males, a single dosage was used, at the same time as the second dosage for females, equivalent to 2.5 mg/kg of carp pituitary extract. After hatching in conical fiberglass incubators of 20 l, the larvae were fed with artemia nauplii for 20 days. The artemia was gradually replaced by a 50% crude protein feed. In the fingerling stage, 1.2 mm pelleted feeds were used (40% of crude protein), being gradually replaced by 2 to 3 mm pelleted feeds (36% of crude protein) provided twice a day.

Animals used in the experiment were pit-tagged when their body weight reached a minimum of 5.0 g (SD = 1.0 g), to maintain the pedigree information known during the challenge experiments. After tagging, fish were kept in fiberglass tanks of 80 l during eight months at the Laboratory of Genetics in Aquaculture and Conservation (LaGeAC), at the Universidade Estadual Paulista (UNESP), Jaboticabal (São Paulo State, Brazil). The mean weight of animals before experimental challenge was 38.7 g (SD = 12.1 g).

2.3. Cohabitation challenge

The cohabitation challenge experiment was performed to test parasite infestation and proliferation based on cycles of temperature oscillation, which was the main stress factor that resulted in ich outbreaks in tambaqui (Zaniboni Filho and Meurer, 1997). In the experimental design, fish were distributed into three communal 100 l glass aquariums (three-replicate design), with water recirculation system, UV filter, and controlled temperature. The water temperature was adjusted using a thermal controller connected to a chiller (1 hp) and a heater (500 w). Averages of nine individuals from each family were randomly distributed into each aquarium. In total, 218 fish were used in the experiment, with about 73 fish per aquarium (Table 1). During the experiment, fish were fed with pelleted feeds (36% of crude protein) *ad libitum* once a day.

After an acclimation period of five days at 28 °C, 12 naturally *I. multifiliis* infested fish (called trojans) were incorporated into each aquarium (t_0). All the trojans were quantified and presented similar parasite load, classified as high infestation rates (> 200 trophonts/fish on the skin and gills) (Valladão et al., 2016), which guaranteed the same challenge dose across the three replicates. At t_0, the water temperature was maintained at 20 °C for two days. Posteriorly, two cycles of temperature oscillation (two days at 28 °C and two days at 20 °C) were performed to stimulate the ich infestation (Fig. 1). At 192 hours post-cohabitation hpc (t_1), all fish were analyzed for determining the degree of infestation (*i.e.*, the number of parasite trophonts). Then, the temperature was increased to 30 °C at 240 hpc (t_2) to decrease fish mortality because the ich life cycle cannot be completed at this temperature (Carneiro et al., 2005; Aihua and Buchmann, 2001). t_2 was determined in previous experiments and it corresponds when 50% of fish mortality occurs. The mortality was controlled after 254 hpc, when no mortality was detected.

The experimental design does not ensure that the operation of parasite counting could have influenced or not survival, *i.e.*, we did not use control aquariums with infected fish without the operation of parasite counting. However, based on results of one previous pilot

Table 1
Summary statistics for each replicated aquarium (1, 2, and 3) of the experimental challenge with *I. multifiliis* in tambaqui (standard deviation is in parenthesis).

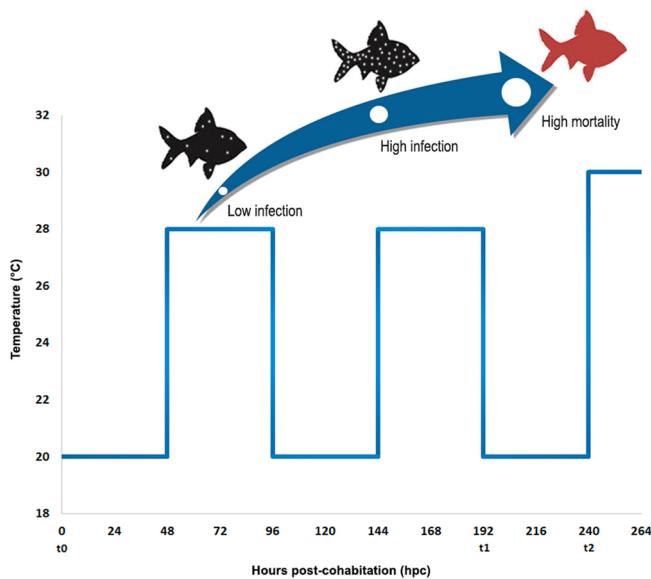| Aquarium	1	2	3
Total number of fish	73	73	72
Average number of fish per family	9.1 (1.2)	9.1 (1.1)	9.0 (1.3)
Average body weight (g)	37.7 (12.2)	39.8 (13.6)	38.5 (10.3)
Total number of dead fish	29	33	30
Final survival rate (%)	60.3	54.8	58.3

Fig. 1. Temperature oscillations during the experimental challenge with *I. multifiliis* in tambaqui. t_0 represents the time of incorporation of trojans into the aquariums, t_1 is the time when all fish were analyzed for parasite load (PL), and t_2 is the time when the temperature was increased to 30 °C until the end of the experiment.

challenge experiment without parasite counting, we observed that time of death and survival was similar to the final experiment. In addition, we did not detect mortality within 24 hours after handling (mortality began 38 hours after handling); therefore, we supposed that the operation of parasite counting had little or none effect on animal survival.

Fish mortality was observed during all day (24 h) from the first mortality events (~230 hpc) until the plateau of mortality; and in intervals of 8 h in the remaining days of challenge. Clinical signs were recorded for all fish and six freshly dead fish (two per aquarium) were collected for routine microbiological analyses, to exclude secondary bacterial infection as cause of death.

2.4. Analysis of genetic parameters

Resistance was assessed as survival to the challenge test using the following trait definitions:

1 Survival status (SS), dead individuals presenting the clinical signs of ich infestation were recorded until 254 hpc (*plateau* of mortality). SS was scored as 1 if the fish died in the challenge test period and 0 if the fish survived at the end of the experiment. This trait was analyzed using a binary threshold (probit) model (THR) to account for the binary nature of the trait. Survival rate was plotted by aquarium and family across the experimental challenge period, using the Kaplan-Meier curve of the survival function (Kaplan and Meier,

1958) (Fig. 2a and b, respectively).

2 Time of Death (TD), which was scored in hours for each dead fish (susceptible), ranging from the moment of the first and last event of mortality. If fish survived to the end of the testing period, the time was recorded as 254 hpc, which was defined by the last event of mortality. This trait was analyzed using a linear model (LIN).

3 Parasite Load (PL), which was analyzed for all fish of the experiment at 192 hpc. This trait was analyzed using a linear model (LIN). The number of parasites (degree of infestation) was determined by counting the white spots (trophont) on the caudal fin under a stereomicroscopy (Leica EZ4). We previously selected the caudal fin because of the long time spent to the analysis of the parasite counting in the whole body (on average around 20 s for each caudal fin/fish) and to avoid risks of the operation of parasite counting affect survival. Moreover, we choose this body region in previous analysis due to two reasons: 1) caudal fin has the highest number of parasites than others fins or body regions; 2) there is a significant correlation of PL on the caudal fin in relation to the whole body (Pearson correlation coefficient $r = 0.81$, p-value < 0.01), which was the maximum value when compared to others fins or body regions (Supplementary file 2).

Data were analyzed with two different univariate animal models as defined below:

- LIN: A linear model was used to fit the continuous variables of TD and PL:

$$y_{ij} = \mu + t_i + w_{ij} + a_j + e_{ij}$$

Where, y_{ij} was the phenotype for the fish j, in aquarium i; μ was the fixed effect of the overall mean; t_i was the fixed effect of the aquarium i and w_{ij} the fixed effect of weight prior ich infestation for the fish j, in aquarium i as covariate; a_j was the random animal genetic effect of individual j; and e_{ij} was the random residual for the fish j in aquarium i.

- THR: A binary threshold (probit) model was used for analyzing SS:

$$Pr(Yij) = \Phi(\mu + t_i + w_{ij} + a_j)$$

Where, Y_{ij} was the phenotype (TS) for the fish j; $\Phi(\cdot)$ was the cumulative standard normal distribution and the other parameters as described above.

THR and LIN models were fitted using ASREML 4.0 package (Gilmour et al., 2009). For all the models, the random animal genetic effect was assumed to be $N(0, A\sigma_a^2)$, where **A** is the pedigree-based additive genetic kinship matrix among all the animals included in the population and σ_a^2 is the additive genetic variance. Residuals for LIN were assumed to be $N(0, I\sigma_e^2)$, where **I** is an identity matrix and σ_e^2 is the residual variance. For THR model, the residual variance on the underlying scale was set to 1. For both models, heritability was calculated as:
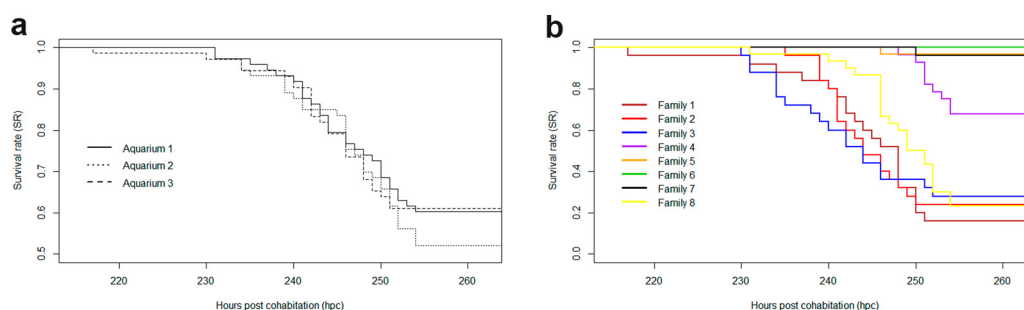

Fig. 2. Kaplan-Meier mortality curves for the three aquariums (**a**) and for the tambaqui families (**b**) of the experimental challenge with *I. multifiliis*.

Table 2

Variation of survival status (SS), time of death (TD) and parasite load (PL) between families of tambaqui after experimental infestation with *I. multifiliis*. Data are presented as the mean ± standard deviation; minimum (Min); maximum (Max); coefficient of variation (CV).

Trait	Mean	CV (%)	Min	Max
SS (%)	0.56 ± 0.37	66.04	0.16	1.00
TD (hour)	249.71 ± 4.02	1.61	217.00	254.00
PL (unity)	68.84 ± 40.09	58.24	5.00	193.00

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}$$

Where σ_a^2 was the additive genetic variance and σ_e^2 was the residual variance.

3. Results

Descriptive statistics for each replicate aquarium used in the *I. multifiliis* challenge is presented in Table 1. The average and variation of traits used to estimate ich resistance are presented in Table 2.

Fish belonging to family 8 had the highest body weight (BW) (50.0 g, SD = 19.1 g), while family 2 presented the lowest BW (30.8 g, SD = 7.8 g). Pearson correlation coefficients (*r*) were calculated to evaluate the occurrence of the phenotypic correlation between BW and ich resistance (SS, TD, and PL). According to the results, there was a weak correlation between BW and SS (r = 0.02, p-value > 0.05), BW and TD (r = 0.10, p-value > 0.05), and between BW and PL (r = 0.17, p-value > 0.05). Therefore, there was a low influence of body weight on the ich resistance.

First signals of ich disease (white spots in the body, fins, and gills) were observed at 72 hpc. The parasite load increased intensely from 72 hpc to 144 hpc (Fig. 1). At that time, all fish had a high number of white spots distributed in the whole body, characterizing high infestation rates (> 200 trophonts/fish), according to criteria of Valladão et al. (2016).

High mortality rate occurred between 230 hpc and 250 hpc (Figs. 1 and 2). Dead fish were considered free of bacterial infection (absence of clinical signs and negative for routine microbiological analysis of other pathogens, such as *Aeromonas hydrophila*, *Flavobacterium columnare* and *Streptococcus agalactiae*). Therefore, ich disease was confirmed as the cause of death, particularly because of the high proliferation of the parasite on the gill epithelium, resulting in the loss of the respiratory, excretory, and osmoregulatory functions of this organ, which leads to death of the host (Ewing et al., 1994).

Cumulative mortality in all families was 42.33%, with significant differences in survival rates between families (Fig. 2b). The values of mortality ranged from 0% to 84% among different families, which indicated a significant phenotypic variation related to ich resistance. TD ranged from 217 to 254 hpc, with an average of 245.60 ± 4.05 hpc. Family 7 had the highest TD (average of 250 hpc), while family 3 had a lower TD value (239.61 hpc) (Fig. 2b). PL varied widely between individuals (Table 2). Family 2 showed the lowest PL (45.16 ± 29.77), while family 3 exhibited the highest PL (87.48 ± 39.98).

Significant additive-genetic variation was observed for both SS and TD traits, but not for PL. Estimated heritabilities and variance components for the analyzed traits are presented in Table 3. The results showed high heritability values for ich resistance in tambaqui, which were estimated to be 0.46 (± 0.09) and 0.60 (± 0.18) for SS and TD, respectively. The estimated heritability for PL was not significant, and a high standard error was calculated.

Table 3

Estimates of additive genetic variance (σ_a^2), residual variance (σ_e^2), phenotypic variance (σ_p^2) and heritability (h^2) for *I. multifiliis* resistance in tambaqui, measured as survival rate (SS), time of death (TD) and parasite load (PL). The standard error is in parenthesis.

Trait	σ_a^2	σ_e^2	σ_p^2	h^2
TD	29.37	19.77	49.14	0.60 (0.18)
SS	0.86	1.00	1.86	0.46 (0.09)
PL	450.75	1137.25	1588.00	0.28 (0.18)

4. Discussion

4.1. Challenge test

In the past, assessment of disease resistance was considered challenging particularly in field conditions due to the several environmental variables that can influence fish mortality. Nowadays, genetic selection for disease resistance in fish is usually based on challenge testing, in which animals are exposed to the pathogen in controlled conditions, often through cohabitant fish or direct inoculation of the pathogen (Ødegård et al., 2011). In the present study, we described for the first time a controlled cohabitation challenge of the protozoan *I. multifiliis*, where temperature oscillations promoted disease transmission and high level of infestation in tambaqui. Moreover, this experimental protocol allowed for the first time to evaluate quantitative genetic parameters of resistance against ich parasitism in fish, which is a cosmopolitan protozoan that results in severe economic losses in different species worldwide (Matthews, 2005).

Although challenge by cohabitation might be better at reproducing the immune response to natural infestation, most of the challenges related to bacterial and virus in fish have adopted intraperitoneal injection of the pathogen directly into the host (Gjedrem, 2000; Ødegård et al., 2011; Yáñez et al., 2014b; Mastrochirico-Filho et al., 2019; Srisapoome et al., 2019). In the case of parasites, challenge tests have been performed mainly by cohabitation (Xu et al., 2007; Taylor et al., 2009; Salte et al., 2010; Gjerde et al., 2011; Glover et al., 2017), as it has been frequently reported for ich challenges because it closely mimics natural conditions of transmission (Buchmann et al., 2001; Sigh et al., 2004; Xu et al., 2004, 2007; Valladão et al., 2016). Experimental infestation by ich cohabitation may need to use environmental stressors to ensure disease transmission and proliferation in the host (Dickerson and Findly, 2014; Francis-Floyd et al., 2016). Here, we have performed cycles of temperature oscillation, which has been described to influence the immune responses against ich in fish (Aihua and Buchmann, 2001; Dickerson and Findly, 2014; Zaila et al., 2016). This environmental change was chosen to simulate the temperature fluctuations that occur in South and Southeastern Brazil during the season changes, when ich outbreaks are generally reported in tambaqui (Zaniboni Filho and Meurer, 1997; Martins et al., 2002).

To optimize the challenge test against ich in tambaqui, the favorable temperature for each species (*I. multifiliis* and tambaqui) was used to determine the temperature oscillation of the experimental infestation. The natural habitat of tambaqui is preferential of warm water with temperatures at ≥ 25 °C (Gomes et al., 2010), while lower temperatures may result in homeostatic imbalance in farmed stocks and, consequently, affect fish immunity and physiology (Urbinati et al., 2014). Otherwise, proliferation of ich generally occurs between 11-21 °C, while higher temperatures (≥ 26 °C) interfere negatively in the life cycle of this parasite (Aihua and Buchmann, 2001; Zaila et al., 2016). Therefore, temperature oscillation from 20 to 28 °C was selected to stimulate homeostatic imbalance in tambaqui and to facilitate *I. multifiliis* proliferation.

Under the conditions of the present experimental challenge, the fish developed visible trophonts on the skin and fins at day three (72 hpc)

and most of the fish had high parasitic infestation at day six (144 hpc), characterized by more than 200 trophonts on the body and gills, according to results from previous studies (Xu et al., 2004, 2007; Gharbi et al., 2015; Valladão et al., 2016). These results are in accordance with the ich life cycle that is characterized by 4-7 days cycle (Coyne et al., 2011; Dickerson and Findly, 2014), depending on the water temperature, and represented by three stages: the tomont (asexual reproductive form) that can generate hundreds of infective theronts (infecting form) (Xu et al., 2007; Dickerson and Findly, 2014; Francis-Floyd et al., 2016); the theront that successfully invade the epithelial layer and differentiate into feeding trophont (parasitic form) (Zaila et al., 2016); the trophont that grow and exit the host (as tomonts) into the environment to complete the new life cycle.

4.2. Genetic parameters

Most of the studies to evaluate genetic parameters for disease resistance traits focused on bacterial and viral pathogens (Gjedrem, 2000; Ødegård et al., 2011; Yáñez et al., 2014b; Elaswad and Dunham, 2018; Ariede et al., 2020). In relation to parasites, few studies are available and they are associated mainly to three parasites that affects salmonid species, in special Atlantic Salmon *Salmo salar*: salmon louse (*Lepeophtheirus Salmonis)* (Glover et al., 2005; Kolstad et al., 2005; Gjerde et al., 2011; Rochus et al., 2018), sea lice *(Caligus rogercressegi)* (Lhorente et al., 2012; Tsai et al., 2016; Correa et al., 2017a, 2017b; Bassini et al., 2019) and amoebic gill disease (caused by *Neoparamoeba perurans*) (Taylor et al., 2009; Kube et al., 2012; Bois et al., 2019; Lillehammer et al., 2019). Therefore, the main result of this study was the evaluation of genetic parameters of resistance against ich, which is one of the main parasites affecting continental fish species (Matthews, 2005), representing a pioneer study that can be applied as framework to obtain fish genetically resistant to this parasite.

Most of the genetic studies carried out to evaluate genetic variation for resistance against parasites were based on measuring parasite count, which have reported moderate to high heritability values ranging from 0.28 to 0.48 (Taylor et al., 2009; Gjerde et al., 2011; Kube et al., 2012; Lhorente et al., 2012; Yáñez et al., 2014a; Tsai et al., 2016; Bois et al., 2019). In the present study, the heritability of PL was moderate but not significant (0.28 ± 0.18), with a relatively high value of standard error.

On the other hand, there are a few studies of resistance against parasites evaluating survival traits (e.g. SS and TD) (Taylor et al., 2009; Salte et al., 2010). In the present experiment, we have shown considerable phenotypic variation for these traits, *e.g.*, the most resistant and susceptible families showed 100% and 16% of cumulative survival rate, respectively, which suggests the possibility to improve tambaqui resistance against ich through selective breeding. Moreover, in relation to TD, substantial differences were found, ranging from 217-254 hpc between individuals. Increasing survival time is critical to obtain fish that are more tolerant and to control disease outbreaks, which can provide more time for adequate treatment of fish in the aquatic environment (Elaswad et al., 2019).

Our results demonstrated high and significant heritability values for SS (0.46 ± 0.09) and TD (0.60 ± 0.18). Although we used a low sample size (due to the low number of families) to calculate genetic parameters, the standard deviation of our heritability estimates was relatively low, which indicates that these values are statistically reliable for the analyzed population. According to Dupont-Nivet et al. (2002), several heritability accuracy scenarios were detected (based on the standard deviation of the heritability estimates) using stochastic simulations for different variables: total number of individuals (300 or 1000), mating design, number of families/family size and different level of heritability (0.1, 0.25 and 0.5). For example, for high heritability (0.5), whatever the sample size and the mating design, the best results were obtained for minimum family size (*i.e.*, three to five offspring in each full-sib family) (Dupont-Nivet et al., 2002). Therefore, higher

number of families/family size is still necessary to be considered in future studies to corroborate the high heritability values to ich resistance in tambaqui.

The heritability values calculated in the present study were similar to previous studies in other species for resistance against other parasites. For instance, values of moderate to high heritabilities for survival status and time to death have been found for Atlantic Salmon resistance against two parasites, *Gyrodactylus salaris* and *Neoparamoeba perurans*, with values ranging from 0.29-0.32 and 0.40-0.49, according to Salte et al. (2010) and Taylor et al. (2009), respectively.

In the present study, we adopted the last event of mortality (254 hpc) as phenotype for resistant fish to estimate heritability for TD, which would lead to the phenotypic data have a deviation from normality and causing a decrease of robustness in the linear model (LIN). In other studies, as alternative to linear models, proportional hazards frailty models have been suggested to estimate genetic parameters of TD (Yáñez et al., 2013). However, these methods account for data censoring (some fish still alive at the end of test), and the analyses may be biased if the bacteria appear to be non-lethal to part of the population (*i.e.*, resistant fish) (Ødegård et al., 2011). Moreover, in the study of Yáñez et al. (2013), when both time to death and censored data were taken into account using proportional hazard frailty models (Cox and Weibull), inconsistent heritability values were obtained in salmon, besides the accuracy of selection was very similar compared to linear models (without accounting for censored data). Therefore, due to the problems with others methods (such as proportional hazards frailty models) and assuming that survival times are usually non-normally distributed (independent of record as 254 hpc for survivors), we preferred to adopt linear model to estimate heritability for TD, similarly to others studies in fish (Yáñez et al., 2013; Li et al., 2019).

In conclusion, challenge experiments of ich showed significant genetic variation for SS and TD with high heritability values in a farmed tambaqui population. Nevertheless, heritability was not significant for PL. Therefore, we concluded that SS and TD could be incorporated as trait definitions for ich resistance into the breeding objective in tambaqui, which will result in animals of superior genotypes through selective breeding and assist to reduce production costs of this species. Consequently, this study represents a pioneering experiment to support the inclusion of ich resistance into genetic improvement programs for Neotropical species, which may help decrease the occurrence of parasite outbreaks in the aquaculture industry, supporting a sustainable and more efficient fish production.

CRediT authorship contribution statement

Lieschen V.G. Lira: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Raquel B. Ariede:** Investigation, Methodology, Writing - review & editing. **Milena V. Freitas:** Investigation, Methodology, Writing - review & editing. **Vito A. Mastrochirico-Filho:** Investigation, Methodology, Writing - review & editing. **John F.G. Agudelo:** Investigation, Methodology, Writing - review & editing. **Agustin Barría:** Formal analysis, Writing - review & editing. **José M. Yáñez:** Formal analysis, Writing - review & editing. **Diogo T. Hashimoto:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aqrep.2020.100338.

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