UNIVERSIDADE ESTADUAL PAULISTA

"Júlio de Mesquita Filho"

Centro de Aquicultura da Unesp- CAUNESP

PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA

Estudo de associação genômica ampla e estimativas de parâmetros genéticos para resistência ao *Ichthyophthirius multifiliis* em tambaqui (*Colossoma macropomum*)

LIESCHEN VALERIA GUERRA LIRA

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LIESCHEN VALERIA GUERRA LIRA

Orientador: Dr. Diogo Teruo Hashimoto

Tese apresentada ao Programa de Pós-graduação em Aquicultura do Centro de Aquicultura da UNESP – CAUNESP, como parte dos requisitos para obtenção do título de Doutor.

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Centro de Aqüicultura da Unesp - CAUNESP

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TÍTULO DA TESE: Estudo de associação genômica ampla e estimativas de parâmetros genéticos para resistência ao *Ichthyophthirius multifiliis* em tambaqui (*Colossoma macropomum*)

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SUMÁRIO

Dedicatória

A meus pais Jorge Guerra e Mary Lira. A minhas irmãs Lissney, Sayuri e Jeyli. A minhas avós Alfonsa e Arlinda (In memoriam). A todas as pessoas que estão lutando pelos seus sonhos.

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RESUMO

O Tambaqui (*Colossoma macropomum*) é a principal espécie nativa produzida na América latina, contudo sua produção é afetada pela infestação do parasita *Ichthyophthirius multifiliis,* o que causa surtos de mortalidade resultando em importantes perdas econômicas. Os tratamentos atuais têm sido ineficazes para o controle desta doença e muitas vezes resultam poluentes para o meio ambiente. Diante desta perspectiva, a seleção genética para resistência ao *I. multifiliis* é uma proposta sustentável, pois resultaria numa prática com menor contaminação ao meio ambiente e muito mais eficiente a longo prazo na produção do tambaqui. O objetivo do presente trabalho foi estimar parâmetros genéticos da resistência ao *I. multifiliis,* além da integração de análises genômicas para a genotipagem de polimorfismos de nucleotídeo único (SNPs) correlacionados com esta característica. Para isto, foram formadas 8 famílias de tambaqui, as quais foram desafiadas por um experimento de coabitação com o parasita *I. multifiliis.* As variáveis analisadas foram a sobrevivência (SS), o tempo de morte (TD) e a carga parasitária (PL). Obteve-se variação fenotípica significativa entre as famílias para o SS (16 a 100%) e o TD (217 a 254 horas pós coabitação). Além do mais, foram estimados altos valores de herdabilidade para SS e TD (0,46 ± 0,09 e 0,60 ± 0,18, respectivamente). No entanto, não tivemos valores significativos para PL. Após várias etapas de filtragem, a genotipagem do SNP por dupla digestão do DNA associado ao sítio de restrição (ddRAD-seq) revelou um total de 7.717 SNPs em 119 indivíduos, que foram usados para GWAS pelo GBLUP de etapa única (ssGBLUP) e método de Bonferroni. As análises genômicas resultaram em quatro SNPs sugestivos detectados (três para TD e um para PL) em quatro grupos de ligação (2, 9, 11 e 20) que cruzaram o limiar de Bonferroni em todo o cromossomo.

Esses SNPs foram aplicados para mapear QTLs, encontrando 11 genes candidatos (*abcf3, znf830, ccr9, gli3, ackr4, tbata, ndr2, tgfbr3, nhej1, znf644b, cldn10a*) que provavelmente estão envolvidos na resistência à infestação por *I. multifiliis*. Os resultados apresentados neste estudo sugerem que a resistência a *I. multifiliis* em tambaqui pode ser melhorada através de programas de melhoramento genético e esta característica é considerada poligênica com várias regiões genômicas de menor efeito afetando a variância genética total.

ABSTRACT

Tambaqui (*Colossoma macropomum*) is the principal native species produced in Latin America, however, its production is affected by the infestation of the parasite *Ichthyophthirius multifiliis*, which causes outbreaks of mortality that result in significant economic losses. Current treatments have been ineffective in controlling this disease and often result in pollutants for the environment. Due to this perspective, genetic selection for resistance to *I. multifiliis* is a sustainable approach and efficient in long term to the production of tambaqui, as it results in less damage to the environment. The aim of this investigation was: 1) to estimate the genetic parameters of resistance to *I. multifiliis* in tambaqui, by a controlled experimental challenge; 2) to analyze the genetic architecture of the resistance to *I. multifiliis* in tambaqui, by genome wide association study (GWAS) using single nucleotide polymorphisms (SNPs). Eight full-sib families of tambaqui were experimentally analyzed by a cohabitation challenge with the parasite *I. multifiliis*. The resistant traits analyzed were survival status (SS), time of death (TD) and parasite load (PL). A significant phenotypic variation was obtained between the families for SS (16 to 100%) and TD (217 to 254 hours after cohabitation). High heritability values were estimated for SS and TD (0.46 ± 0.09 and 0.60 ± 0.18, respectively). However, we did not have significant heritability values for PL. After several filtering steps, the SNP genotyping by double-digest restriction-site associated DNA (ddRAD-seq) revealed a total of 7.717 SNPs in 119 individuals, which were used for GWAS by the single-step GBLUP (ssGBLUP) and Bonferroni method. The genomics analyzes resulted in four suggestive SNPs detected (three for TD and one for PL) in four linkage groups (2, 9, 11 and 20) that crossed the chromosome-wide Bonferroni threshold. These SNPs were applied to map QTLs, finding 11 candidate genes

(*abcf3, znf830, ccr9, gli3, ackr4, tbata, ndr2, tgfbr3, nhej1, znf644b, cldn10a*) that are likely to be involved in resistance to infestation by I. multifiliis. The results presented in this study suggest that resistance to *I. multifiliis* in tambaqui can be improved through genetic improvement programs and this trait is considered polygenic with several genomic regions of minor effect affecting the total genetic variance.

INTRODUÇÃO GERAL

Aspectos gerais

Com o crescimento da população e a consciência pelo consumo de alimentos saudáveis, a demanda global de produtos derivados de pescado tem sido prioridade nas últimas décadas (Bacher, 2015; Thilsted *et al*., 2016). Atualmente, os peixes constituem uma fonte essencial de alimentos nutritivos e fornecem aproximadamente 17% da proteína animal na dieta de grande parte da população mundial. O consumo mundial estimado de peixe continua aumentando, atingindo uma máxima histórica de 20,5 kg/capita/ano, em 2018 aproximadamente 87% da produção pesqueira mundial (156 milhões de toneladas) foi destinada para o consumo humano (FAO, 2020). Isto foi possível devido ao aumento da aquicultura com suprimento relativamente estável da pesca de captura.

Em 2018, o aporte da pesca continuou sendo maior que à aquicultura (54% e 46%, respectivamente), isto devido à captura de organismos marinhos (87,55%) (FAO, 2020). Contudo, este setor teve uma estagnação na produção, já que os ecossistemas aquáticos estão sendo fortemente afetados pela poluição e degradação ambiental, além da sobre-exploração dos recursos em níveis biologicamente insustentáveis. Nestas circunstancias, para poder abastecer a demanda do mercado, a produção de alimentos aquáticos deixou de ser baseada principalmente na captura para enfocar-se na criação de um número crescente de espécies cultivadas (FAO, 2020). Em consequência, a aquicultura está sendo o setor produtor de proteína animal que mais cresce no mundo (Olesen *et al*., 2011) e chegou a superar a produção de carne bovina em 2011 (Larsen e Roney, 2013).

A produção mundial da aquicultura atingiu 82,3 milhões de toneladas em 2018, sendo que 30,8 milhões de toneladas foram produtos da aquicultura marinha (37,5%) e 51,3 milhões de toneladas da aquicultura continental (62,5%). O principal produto da aquicultura continental são peixes, os quais representam 91,6% do total deste tipo de produção (47 milhões de toneladas) (FAO, 2020). Isto demostra a importância significativa da criação e comercialização de peixes da aquicultura continental para a produção global (Thilsted *et al*., 2016). A China lidera o ranking na piscicultura continental, com uma produção de 25,4 milhões de toneladas em 2018. Neste cenário, Brasil ocupou o oitavo lugar em nível mundial, com uma produção de 529,6 mil toneladas de peixes em 2019, observando-se um crescimento de 1,7% em relação ao ano anterior (IBGE, 2020).

O Brasil é o principal produtor de peixes continentais da América Latina (Valladão *et al*., 2018; Saint-Paul, 2017); esta produção representa 67,5% do total da aquicultura nacional (IBGE, 2020). Isto devido a que possui mais de dois milhões de hectares de pântanos, reservatórios e estuários adequados para a aquicultura (Saint-Paul, 2017), ainda dispõe de uma rica biodiversidade de peixes, do qual aproximadamente 40 espécies nativas são utilizadas para aquicultura nacional (Godinho, 2007). O Brasil é, portanto, o único país latino-americano com uma parcela significativa de espécies nativas em sua indústria de aquicultura (Pincinato e Asche, 2016). Entretanto, a participação destas espécies nativas fica abaixo dos 40% (Saint-Paul, 2017; Sidra, 2020), sendo que a maioria dos cultivos são realizadas com espécies exóticas, destacando-se a tilápia (*Oreochromis* sp.), com 61.1 % da produção total nacional (IBGE, 2020).

A tilápia domina o mercado nacional porque possui pesquisas relacionadas à pacotes tecnológicos para melhorar sua produção, destacando-se as técnicas de

melhoramento genético como a principal ferramenta para cumprir este objetivo, garantindo assim o contínuo crescimento e a viabilidade de sua cadeia produtiva (Gjedrem *et al*., 2012). Por exemplo, a tilápia GIFT (*Genétically Improved Farmed Tilapia*), vem ganhando cada vez mais espaço no mercado, pois após cinco gerações melhorou seu desempenho de crescimento em 88% (Bentsen *et al*., 2017). Este panorama revela a necessidade de programas de melhoramento e inovação genética em espécies nativas do Brasil, como o tambaqui (*Colossoma macropomum)* para que também possam suprir a demanda de pescado com qualidade.

Caracterização da espécie *Colossoma macropomum*

A espécie *C. macropomum* (Cuvier, 1818) (Figura 1), pertence à ordem Characiformes e a família Serrasalmidae, é um peixe conhecido popularmente como “tambaqui” (Brasil), “gamitana” (Perú), “cachama” ou “morocoto” (Venezuela) e “cachama negra” (Colombia) (Campos, 2015). É nativo das bacias dos rios Amazonas e Orinoco (Gomes *et al*., 2010). Na natureza pode chegar a medir 1 m e pesar 30 kg (Goulding & Carvalho, 1982), portanto, é considerado o segundo maior peixe de escamas das águas continentais amazônicas (Gomes *et al*., 2010). São onívoros com preferência frugívora (Gomes *et al*., 2010). Os exemplares desta espécie caracterizam-se por apresentar corpo robusto com formato arredondado, dorso alto e região das costelas ampla. A maturidade sexual ocorre aos 3-4 anos, apresentam desova total, sendo caracterizado por um período de reprodução que atinge o seu ápice nos meses de outubro a janeiro, épocas de temperaturas mais altas e maior incidência de chuvas (Campos, 2015). É considerado um peixe reofílico, realiza migrações sazonais dos lagos de várzea para a alimentação e

reprodução (Godinho *et al*., 2010). Devido a seu comportamento migratório, esta espécie pode formar uma população panmítica na bacia Amazônica (Santos *et al*., 2007), por isso, as populações de tambaqui poderão ser afetadas negativamente pela instalação e construção de hidrelétricas na bacia Amazônica (Godinho *et al*., 2010).

Figura 1. Imagem representativa da espécie *C. macropomum* (Tambaqui).

O tambaqui é um dos principais recursos explorados pela pesca extrativa na região Norte do Brasil, e os estoques desse peixe já são considerados sobre-explotados ou ameaçados de sobre-explotação e, em consequência foi proibido, anualmente, a pesca desta espécie desde outubro até março de acordo a Instrução Normativa do Ministério do Meio Ambiente (MMA, nº 05/2004 e MMA, n°35/2005, respectivamente). Assim, o cultivo e melhoramento desta espécie é uma alternativa

para suprir a demanda do mercado consumidor e uma estratégia para auxiliar na redução da pressão de pesca sobre as populações naturais.

O cultivo de tambaqui torna-se favorável, pois apresenta fácil aceitação às rações artificiais com pouco ou nenhum ingrediente de origem animal (Valladão *et al*., 2018). Adicionalmente, apresenta alta rusticidade, crescimento rápido, alta produtividade e é bem apreciado na culinária local e internacional (Campos, 2015; Valladão *et al*., 2018). Estas características zootécnicas fazem que esta espécie seja alvo na produção da América Latina, sendo cultivada em países como Bolivia, Brasil, Colombia, Peru, Equador e Venezuela (Campos, 2015; Valladão *et al*., 2018). Ademais, estão começando a produção em outros países como Republica Dominicana, Guiana, Panamá e Suriname (Woynárovich e Van Anrooy, 2019)

Em termos de produção na aquicultura brasileira, nas últimas décadas, o tambaqui tornou-se a principal espécie nativa e a segunda espécie mais produzida em nível nacional (IBGE, 2020). Em 2019, a produção de tambaqui alcançou o valor de 101,1 mil toneladas, o que representou 19,1 % da produção total nacional (IBGE, 2020). A maior produção se concentra na região do norte e nordeste (Garcia *et al*., 2008, IBGE, 2020), devido à temperatura ideal de cultivo: 25 a 30 °C (Zaniboni-Filho e Meurer, 1997). Já nas regiões do centro – oeste, sul e sudeste, onde as variações de temperatura durante as mudanças da estação são frequentes, o tambaqui é utilizado para cruzamentos com outras espécies de Serrasalmidae (*Piaractus mesopotamicus* e *Piaractus brachypomus*), resultando em híbridos interespecíficos (Hashimoto *et al*., 2012; Fernandes *et al*., 2018). Em 2019, os híbridos tambatinga (*C. macropomum x P. brachypomus*) e tambacu (*C. macropomum x P. mesopotamicus*) tiveram uma produção de 40,1 mil toneladas, o que representou o segundo maior valor para peixes nativos brasileiros (Sidra,

2020). Portanto, o tambaqui e seus híbridos têm contribuído com 26,7% da produção da aquicultura nacional. No entanto, os híbridos interespecíficos podem ser férteis e causar sérios riscos biológicos para populações naturais e cultivadas (Hashimoto *et al*., 2012).

A principal limitação para a produção de tambaqui no sul do Brasil é a baixa tolerância à oscilação de temperatura durante as mudanças de estação (Zaniboni-Filho e Meurer, 1997; Aguiar *et al*., 2018), que geralmente resulta em surtos de doenças, como a doença dos pontos brancos causada pelo protozoário ciliado *Ichthyophthirius multifiliis* (Martins *et al*., 2002; Matthews, 2005).

Caracterização do parasita *Ichthyophthirius multifiliis*

Ichthyophthirius multifiliis, popularmente chamado de “ictio” é um protozoário ciliado cosmopolita, que causa a parasitose chamada ictiofitiríase ou conhecida comumente como “doença dos pontos brancos” (Dickerson e Clark, 1998). O sinal clínico desta doença é caracterizado pela presença de pontos brancos ao longo do corpo (pele, nadadeiras, córnea, cabeça) e também nas brânquias dos peixes (Mattheus, 2005; Francis-Floyd *et al*., 2016) (Figura 2).

O ciclo de vida do *I. multifiliis* é composto por várias fases (Mattheus, 2005; Francis-Floyd *et al*., 2016; Zaila *et al*., 2016). O teronte (forma infectante) é caracterizada por um protozoário de vida livre (30 x 50 µm) que inicia a infestação, pois penetra no tecido cutâneo e nas brânquias dos peixes. Após penetrar no hospedeiro, este torna-se um trofonte (forma parasitária) o qual se alimenta e desenvolve (800 a 1000 µm) nestes tecidos, causando os efeitos nocivos. O parasito maduro deixa o hospedeiro (Figura 2C) e se diferencia em tomonte (forma

reprodutiva assexuada), o qual irá secretar um cisto de proteção. Os tomontes encistados se mantêm na água ou se fixam em substratos, até que as condições ambientais sejam favoráveis, momento no qual ocorrem divisões dentro deles formando de 500 a 1000 células-filhas, chamadas de tomitos. Os tomitos, por sua vez, são liberados e irão se diferenciar em terontes infectantes, os quais nadarão em busca de um novo hospedeiro susceptível para completar um novo ciclo (Buchmann *et al*., 2001; Ling *et al*., 2010).

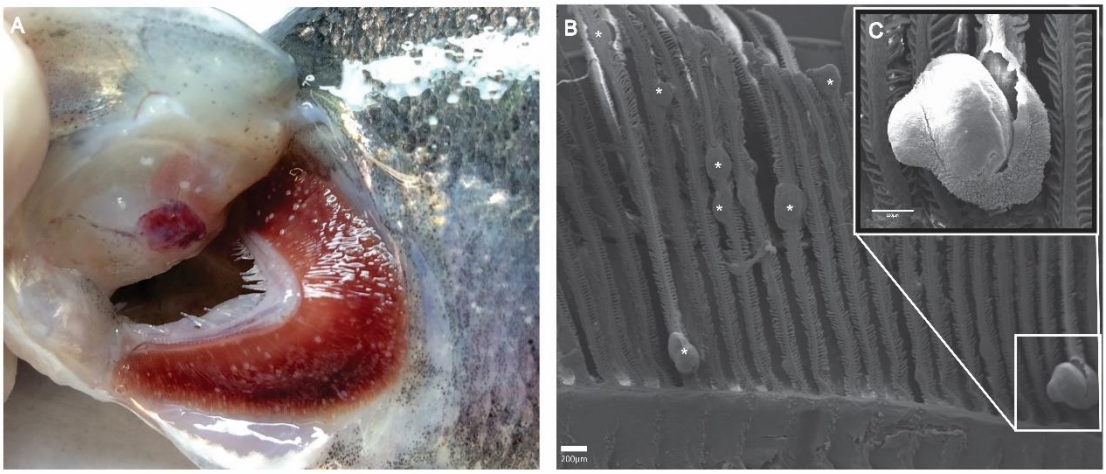
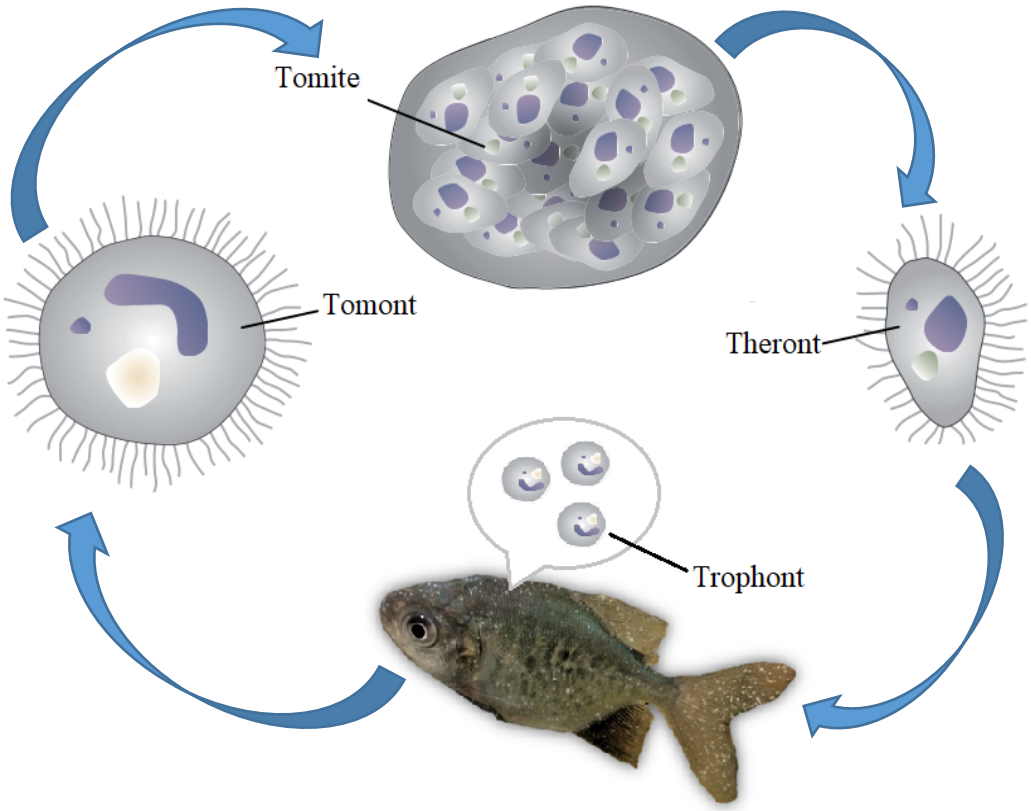


Figura 2. Branquias de peixes infectadas com o parasita *Ichthyophthirius multifiliis (*Pontos brancos) (A), Fotos em microscópio de varredura de brânquias infectadas com trofontes de *I. multifiliis* (*) (B), Trofonte saindo do tecido epitelial das brânquias (C).

O ciclo do *I. multifiliis* é direto ou monoxênico, ou seja, não depende da presença de hospedeiro intermediário para seu desenvolvimento direto (Figura 3) (Matthews, 2005; Francis-Floyd *et al*., 2016; Zaila *et al*., 2016). Além disso, é dependente da temperatura da água, observando-se um melhor desenvolvimento das fases nas temperaturas que variam entre 17 e 25 °C (Aihua e Buchmann, 2001; Matthews, 2005). Por isso, em lugares com climas tropicais, surtos de ictio surgem

frente a queda brusca de temperatura (Matthews, 2005), o que ocasiona estresse nos peixes, assim este parasita oportunista aproveita esta baixa na imunidade para se instalar no hospedeiro.

Figura 3. O ciclo de vida do *Ichthyophthirius multifiliis.* Adaptado de Coyne *et al.* (2012).



I. multifiliis causa mortalidade na produção de peixes de água doce em nível mundial (Osman *et al*. 2009), dado que se multiplica rapidamente (Buchmann *et al*., 2001), é altamente contagioso sendo transmitido por cohabitação (Xu, *et al*., 2007; Valladão *et al*., 2016) e não apresenta especificidade de hospedeiros, afetando espécies cultivadas e silvestres (Sigh *et al*., 2004; Santos *et al*., 2013; Zago *et al*., 2014; Valladão *et al*., 2016; Xu *et al.,* 2017).

Alguns dos principais tratamentos utilizados para combater este parasita são produtos químicos (Srivastava *et al*., 2004; Farmer *et al*., 2013) que estão sendo banidos pois causam efeitos indesejáveis no meio ambiente (Rico e Van den Brink, 2014). Na procura de alternativas mais sustentáveis (Olesen *et al*., 2010) encontra-se medicamentos fitoterápicos (Valladão *et al.,* 2016), que mesmo dando um resultado positivo ainda não são viáveis por não ser rentáveis numa produção extensiva; também encontra-se o uso de antígenos (Wang *et al*., 2002; Xu *et al*., 2009b), vacinas com terontes vivos (Xu *et al*., 2008; Moreira *et al*., 2017; Xu *et al*., 2017) e vacinas com trofontes inativos (Xu *et al*., 2009a), mas são técnicas que ainda não deram um resultado promissor, além do mais, não há provas que esta imunidade seja transmitida às próximas gerações aumentando o custo na produção em cada geração (Xiong *et al*., 2017).

Portanto, não existem estratégias eficazes atualmente disponíveis para controlar e prevenir a infecção por *I. multifiliis* em longo prazo, o que mostra a necessidade da utilização de indivíduos geneticamente superiores que apresentem desempenho superior em condições ambientais específicas. Assim, a reprodução seletiva dirigida a doenças proporciona uma alternativa eficiente para controlar parasitismos em produções intensivas onde o estresse é inevitável e a transferência de patógenos é iminente (Gjedrem, 2012), sendo considerada uma das principais medidas preventivas que podem ser usadas para melhorar a sobrevivência a longo prazo (Gjedrem, 2000; Hulata, 2001). Isto é de suma importância para aprimorar as condições de bem-estar dos animais e, simultaneamente, aumentar a produtividade das pisciculturas e diminuir os custos de produção (Ødegård *et al*., 2011; Olesen *et al*., 2011; Vieira e Oliveira, 2015).

Genética quantitativa na aquicultura - Foco na resistência a doença

O rápido desenvolvimento da aquicultura implicou na intensificação da produção, muitas vezes desordenada e sem controle sanitário, o que acrescentou a ocorrência de doenças transmitidas por bactérias, vírus, fungos e parasitas (Gjedrem, 2015). Em consequência, geram-se prejuízos econômicos devido aos custos dos tratamentos, as medidas de controle da doença e a perda da produção (Elaswad e Dunham, 2018). Por tanto, o sucesso dos sistemas de produção aquícola depende, em grande parte, do controle das doenças infecciosas (Yáñez and Martínez, 2010). A redução na ocorrência e gravidade de doenças melhoraria a produtividade, lucratividade, eficiência e bem-estar dos peixes (Elaswad e Dunham, 2018). Assim, é importante a realização de pesquisas tecnológicas e utilização de novas ferramentas genéticas para superar os entraves e assegurar uma produção continua e melhorada dos estoques.

Medicamentos e vacinas foram desenvolvidos para a prevenção e controle de doenças, no entanto, as mortalidades por doenças ainda é relativamente alta (Gjedrem, 2015). Além do mais, estes tratamentos são muitas vezes poluentes, caros e parcialmente eficazes, e o seu uso irrestrito pode gerar linhagens de patógenos resistentes (Houston, 2017). Frente a isto, os programas de melhoramento genético resultam como uma estratégia viável e potencialmente mais sustentável para o controle de surtos de doenças a longo prazo (Bishop e Woolliams, 2014), o que tem permitido melhorar a situação sanitária dos peixes.

Os investimentos em programas de melhoramento genético dirigidos à resistência a doenças bem planejados e gerenciados são únicos, pois fornecem

alguma medida de proteção para os estágios iniciais, especialmente os de alevinos (Gjedrem, 2015) mantendo uma alta taxa de sobrevivência (Ødegård *et al*., 2011). Os ganhos genéticos obtidos nesses programas são cumulativas e permanentes (Bishop e Woolliams, 2014; Gjedrem *et al*., 2012), posto que exploram a ocorrência natural da variação genética quantitativa para resistência a doenças (Yáñez and Martínez, 2010; Houston, 2017), a qual pode ser herdado pelas gerações futuras.

Nas últimas décadas foram realizados vários experimentos de desafio baseados em teste de sobrevivência a patógenos específicos para buscar estimativas confiáveis de seleção de famílias resistentes às doenças (Gjedrem, 2015). Os resultados dos testes de desafio são promissores e a herdabilidade da taxa de sobrevivência é geralmente alta (Gjedrem, 2000; Ødegard, *et al*., 2011; Yáñez and Martínez, 2010; Yáñez *et al*., 2014; Gjedrem e Rye, 2018). A maioria desses estudos abordam a resistência a bactérias e vírus (Barría *et al*., 2018; Bassini *et al*., 2019; Sukhavachana *et al*., 2019; Wang *et al*., 2019; Ariede et al., 2020; Jia *et al*., 2020; Mastrochirico-Filho *et al*., 2020). Em relação aos parasitas, o número de estudos é limitado e a maioria está dirigido a parasitas que afetam salmonídeos, como: *Caligus rogercresseyi* (Tsai *et al*., 2016; Correa *et al*., 2017; Robledo *et al*., 2019) e *Neoparamoeba perurans* (Taylor *et al*., 2009; Lillehammer *et al*., 2019)

As herdabilidades para características de resistência (sobrevivência, tempo de morte e carga parasitária) de tais estudos tem variado de baixa a moderada. Estudos envolvendo desafios com *Neoparamoeba perurans* mostraram valores de herdabilidade variando entre 0,07 e 0,49 (Taylor *et al*., 2009; Robledo *et al*., 2018; Bois *et al*., 2019; Lillehammer *et al*., 2019; Aslam *et al*., 2020). Estimativas semelhantes foram obtidas para resistência ao hospedeiro a *Caligus rogercresseyi*

~ 0,12 a 0,33 (Yáñez *et al*., 2014; Tsai *et al*. 2016; Correa *et al*., 2017; Robledo *et al*., 2018). Da mesma forma, pesquisas sobre resistência natural a outros parasitas como *Gyrodactylus salaris*, *Tetracapsuloides bryosalmonae* e *Ichthyophthirius multifiliis* também mostraram valores de herdabilidade que variam de moderados a altos (0,32 a 0,60) (Salte *et al*. 2010; Ahmad *et al*., 2018; Lira *et al*., 2020).

Aplicação da genômica na aquicultura - Foco na resistência a doença

A genômica está crescendo rapidamente à medida que a tecnologia de sequenciamento está melhorando. O uso das tecnologias de sequenciamento de nova geração (NGS) se tornou uma opção interessante, uma vez que permitem o sequenciamento de milhões de pares de base de qualquer organismos, inclusive de espécies não modelos, em curto período de tempo e a custos reduzidos (Miller *et al*., 2009; Kumar e Kocour, 2017).

Embora os custos de sequenciamento estejam caindo gradativamente, o sequenciamento, análise e a comparação de genomas inteiros ainda são custosas, pela enorme complexidade e demanda computacional exigida. Desta forma, estratégias que permitam análises e comparações representativas, são de amplo interesse. O RAD-seq (*Restriction-site associated DNA sequencing*) é uma estratégia de sequenciamento de genoma fracionário que utiliza enzimas de restrição e sequenciamento das regiões adjacentes aos cortes destas enzimas (Baird *et al*., 2008), reduzindo a necessidade de altas coberturas de sequenciamento. A vantagem desta técnica é que é possível formar um pool de uma grande quantidade de amostras a qual pode ser sequenciada de uma vez só (Andrews *et al*., 2016).

Uma variação para o RAD-seq é o ddRAD (*double digestion Restriction Associated DNA sequencing*), que é caracterizada pelo uso de duas enzimas de restrição que permite que os tamanhos dos fragmentos genômicos fiquem mais precisos, além do mais diminui os custo na elaboração de bibliotecas (Peterson *et al*., 2012). Este método é usado para a descoberta e genotipagem de SNPs (*Single Nucleotide Polymorphisms*) (Kumar e Kocour, 2017). Os SNPs são polimorfismos, presentes em no mínimo 1% da população, que afetam somente uma base na sequência do genoma. São os polimorfismos mais abundantes e amplamente distribuídos nos genomas, podendo estar presentes em praticamente todos os loci gênicos (Perkel, 2008). Também revelam variações ocultas não detectadas com outros marcadores (Vignal *et al.*, 2008). Por todas estas características, os SNPs são ideais para a busca de características quantitativas (QTLs) e facilitar a identificação de genes responsáveis por características de desempenho superiores.

Uma das principais estratégias para a descoberta dos QTLs é o estudo de associação genômica ampla (GWAS). O GWAS identifica as variações no genoma (SNPs) e as associa com o características quantitativas de interesse (QTLs) com base no desequilíbrio de ligação (Cantor *et al*., 2010). Os resultados obtidos do mapeamento QTL e GWAS podem ser usados para programas de melhoramento seletivo através da seleção assistida por marcadores (MAS) ou seleção genômica (Yáñez e Martínez, 2010; Yáñez *et al*., 2014; Kumar e Kocour, 2017).

Estudos de MAS e seleção genômica são particularmente úteis para serem direcionados a características que são difíceis ou impossível de medir diretamente sobre os candidatos de seleção (Yáñez *et al*., 2014; Ødegård *et al.,* 2014). Um dos exemplos bem-sucedido de análises de QTL é o caso de resistência à infecção da

necrose pancreática no salmão do Atlântico, a qual foi realizada por dos estudos independentes na Escócia e na Noruega, onde identificaram que um único QTL explicava mais de 80% da variação genética da resistência (Houston *et al*., 2008; Moen *et al*., 2009), o que permitiu diminuir as altas taxas de mortalidade (mais de 90%) para perto de zero (Houston *et al*., 2020). Esta descoberta representa um exemplo bem-sucedido de controle da doença, e mostra a importância das ferramentas genômicas para o desenvolvimento da aquicultura mundial. O GWAS tem sido utilizado para várias características economicamente importantes para a aquicultura, incluindo características de crescimento (Gutierrez *et al*., 2015; Li *et al*., 2018; Yoshida *et al*., 2019; Zhou *et al*., 2019; Ali *et al.,* 2020; Yang *et al*., 2020), determinação sexual (Bao *et al*., 2019; Cáceres *et al*., 2019; Gabián *et al*., 2019; Lin *et al*., 2020), resistência a doenças (Aslam *et al*., 2020; Jia *et al*., 2020; Mastrochirico *et al*., 2020; Yang *et al*., 2021), entre outros.

É necessário que estas práticas de uso de SNPs para associação com características de interesse zootécnico sejam aplicadas nas espécies nativas do Brasil, em especial no tambaqui, pois permitirá acelerar a produção aquícola nacional, aumentando a produtividade de uma maneira mais sustentável. Além do mais, permitirão identificar regiões genômicas de interesse comercial, por exemplo, relacionadas à resistência a doenças, com o objetivo de direcionar programas de melhoramento genético por seleção assistida por marcadores ou seleção genômica, a fim de evitar problemas decorrentes na produção.

Objetivos:

Geral

O objetivo do presente estudo foi explorar a arquitetura genética da resistência do tambaqui contra *I. multifillis* e investigar o potencial da seleção genômica para possivelmente acelerar o melhoramento genético.

Específicos

Estimar os componentes de variância e herdabilidade para resistência a *I. multifillis* em oito familias de tambaqui, por médio de um desafio experimental de coabitação.

Mapear QTL (loci de características quantitativas) associados à resistência a *I. multifiliis* em tambaqui por GWAS (estudo de associação genômica ampla).

REFERÊNCIAS

Aguiar, J. da P., Gomes, P.F.F., Hamoy, I.G., Santos, S.E.B. dos, Schneider, H., Sampaio, I., 2018. Loss of genetic variability in the captive stocks of tambaqui, *Colossoma macropomum* (Cuvier, 1818), at breeding centres in Brazil, and their divergence from wild populations. Aquac. Res. 49, 1914–1925. https://doi.org/10.1111/are.13647

Ahmad, F., Debes, P. V., Palomar, G., Vasemägi, A., 2018. Association mapping reveals candidate loci for resistance and anaemic response to an emerging temperature-driven parasitic disease in a wild salmonid fish. Mol. Ecol. 27, 1385–1401. https://doi.org/10.1111/mec.14509

Aihua, B.L., Buchmann, K., 2001. Temperature and salinity dependent development of a Nordic strain of *Ichthyophthirius multifiliis* from rainbow trout. J. Appl. Ichthyol. 17, 273–276. https://doi.org/10.1046/j.1439-0426.2001.00279.x

Ali, A., Al-Tobasei, R., Lourenco, D., Leeds, T., Kenney, B., Salem, M., 2020. Genome-wide identification of loci associated with growth in rainbow trout. BMC Genomics 21, 1–16. https://doi.org/10.1186/s12864-020-6617-x

Andrews, K.R., Good, J.M., Miller, M.R., Luikart, G., Hohenlohe, P.A., 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. Nat. Rev. Genet. 17, 81–92. https://doi.org/10.1038/nrg.2015.28

Ariede, R.B., Freitas, M. V., Agudelo, J.F.G., Borges, C.H.S., Lira, L.V.G., Yoshida, G.M., Pilarski, F., Yáñez, J.M., Hashimoto, D.T., 2020. Genetic (co)variation between resistance to *Aeromonas hydrophila* and growth in tambaqui (*Colossoma macropomum*). Aquaculture 523, 735225. https://doi.org/10.1016/j.aquaculture.2020.735225

Aslam, M.L., Boison, S.A., Lillehammer, M., Norris, A., Gjerde, B., 2020. Genome-wide association mapping and accuracy of predictions for amoebic gill disease in Atlantic salmon (*Salmo salar*). Sci. Rep. 10, 1–9. https://doi.org/10.1038/s41598-020-63423-8

Bacher, K., 2015. Perceptions and misconceptions of aquaculture. Globefish Res. Program. 120, 35 p. https://doi.org/10.13140/RG.2.1.1399.3840

Baird, N.A., Etter, P.D., Atwood T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., Johnson, E.A., 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3, 1–7. https://doi.org/10.1371/journal.pone.0003376

Bao, L., Tian, C., Liu, S., Zhang, Y., Elaswad, A., Yuan, Z., Khalil, K., Sun, F., Yang, Y., Zhou, T., Li, N., Tan, S., Zeng, Q., Liu, Y., Li, Yueru, Li, Yun, Gao, D.,

Dunham, R., Davis, K., Waldbieser, G., Liu, Z., 2019. The Y chromosome sequence of the channel catfish suggests novel sex determination mechanisms in teleost fish. BMC Biol. 17, 1–16. https://doi.org/10.1186/s12915-019-0627-7

Barría, A., Christensen, K.A., Yoshida, G.M., Correa, K., Jedlicki, A., Lhorente, J.P., Davidson, W.S., Yáñez, J.M., 2018. Genomic predictions and genome-wide association study of resistance against Piscirickettsia salmonis in coho salmon (*Oncorhynchus kisutch*) using ddRAD sequencing. G3 Genes, Genomes, Genet. 8, 1183–1194. https://doi.org/10.1534/g3.118.200053

Bassini, L.N., Lhorente, J.P., Oyarzún, M., Bangera, R., Yáñez, J.M., Neira, R., 2019. Genetic parameters for *Piscirickettsia salmonis* resistance, sea lice (*Caligus rogercresseyi*) susceptibility and harvest weight in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 510, 276–282. https://doi.org/10.1016/j.aquaculture.2019.05.008

Bentsen, H.B., Gjerde, B., Eknath, A.E., de Vera, M.S.P., Velasco, R.R., Danting, J.C., Dionisio, E.E., Longalong, F.M., Reyes, R.A., Abella, T.A., Tayamen, M.M., Ponzoni, R.W., 2017. Genetic improvement of farmed tilapias: Response to five generations of selection for increased body weight at harvest in Oreochromis niloticus and the further impact of the project. Aquaculture 468, 206–217. https://doi.org/10.1016/j.aquaculture.2016.10.018

Bishop, S.C., Woolliams, J.A., 2014. Genomics and disease resistance studies in livestock. Livest. Sci. 166, 190–198. https://doi.org/10.1016/j.livsci.2014.04.034

Bois, S.A., Gjerde, B., Hillestad, B., Makvandi-Nejad, S., Moghadam, H.K., Boison, S.A., 2019. Genomic and transcriptomic analysis of amoebisc gill disease

resistansce in Atlantic salmon (S*almo salar L*.). Front. Genet. 10. https://doi.org/10.3389/fgene.2019.00068

Buchmann, K., Sigh, J., Nielsen, C.V., Dalgaard, M., 2001. Host responses against the fish parasitizing ciliate *Ichthyophthirius multifiliis*. Vet. Parasitol. 100, 105–116. https://doi.org/10.1016/S0304-4017(01)00487-3

Cáceres, G., López, M.E., Cádiz, M.I., Yoshida, G.M., Jedlicki, A., Palma-Véjares, R., Travisany, D., Díaz-Domínguez, D., Maass, A., Lhorente, J.P., Soto, J., Salas, D., Yáñez, J.M., 2019. Fine Mapping Using Whole-Genome Sequencing Confirms Anti-Müllerian Hormone as a Major Gene for Sex Determination in Farmed Nile Tilapia (*Oreochromis niloticus* L.). G3 Genes, Genomes, Genet. 9, 3213–3223. https://doi.org/10.1534/g3.119.400297

Cantor, R.M., Lange, K., Sinsheimer, J.S., 2010. Prioritizing GWAS Results: A Review of Statistical Methods and Recommendations for Their Application. Am. J. Hum. Genet. 86, 6–22. https://doi.org/10.1016/j.ajhg.2009.11.017

Campos, B.L., 2015. El cultivo de la gamitana en Latinoamérica. UNAP. 50 pp. ISBN:978-9972-667-95-4.

Correa, K., Lhorente, J.P., Bassini, L., López, M.E., Di Genova, A., Maass, A., Davidson, W.S., Yáñez, J.M., 2017. Genome wide association study for resistance to *Caligus rogercresseyi* in Atlantic salmon (*Salmo salar L*.) using a 50K SNP genotyping array. Aquaculture 472, 61–65. https://doi.org/10.1016/j.aquaculture.2016.04.008

Dickerson, H., Clark, T., 1998. *Ichthyophthirius multifiliis*: A model of cutaneous infection and immunity in fishes. Immunol. Rev. 166, 377–384. https://doi.org/10.1111/j.1600-065X.1998.tb01277.x

Elaswad, A., Dunham, R., 2018. Disease reduction in aquaculture with genetic and genomic technology: current and future approaches. Rev. Aquaculture. 10, 876–898. https://doi.org/10.1111/raq.12205

FAO. 2020. Organização das Nações Unidas para a Agricultura e Alimentação. The state of world fisheries and aquaculture: Rome

Farmer, B.D., Fuller, S.A., Mitchell, A.J., Straus, D.L., Bullard, S.A., 2013. Efficacy of bath treatments of formalin and copper sulfate on cultured white bass, Morone multifiliis. J. World Aquac. Soc. 44, 305–310. https://doi.org/10.1111/jwas.12027

Fernandes, E.M., de Almeida, L.C.F., Hashimoto, D.T., Lattanzi, G.R., Gervaz, W.R., Leonardo, A.F., Neto, R.V.R., 2018. Survival of purebred and hybrid Serrasalmidae under low water temperature conditions. Aquaculture 497, 97–102. https://doi.org/10.1016/j.aquaculture.2018.07.030

Francis-Floyd, R., Yanong, R., Pouder, D. *Ichthyophthirius multifiliis* (white spot) infections in fish. https://edis.ifas.ufl.edu/pdffiles/FA/FA00600.pdf (accessed 10 april 2019).

Gabián, M., Morán, P., Fernández, A.I., Villanueva, B., Chtioui, A., Kent, M.P., Covelo-Soto, L., Fernández, A., Saura, M., 2019. Identification of genomic regions regulating sex determination in Atlantic salmon using high density SNP data. BMC Genomics 20, 1–12. https://doi.org/10.1186/s12864-019-6104-4

Gjedrem, T., 2000. Genetic improvement of cold-water fish species. Aquaculture Res. 31, 25–33. https://doi.org/10.1046/j.1365-2109.2000.00389.x

Gjedrem, T., Robinson, N., Rye, M., 2012. The importance of selective breeding in aquaculture to meet future demands for animal protein: A review. Aquaculture 350–353, 117–129. https://doi.org/10.1016/j.aquaculture.2012.04.008

Gjedrem, T., 2015. Disease resistant fish and shellfish are within reach: A review. J. Mar. Sci. Eng. 3, 146–153. https://doi.org/10.3390/jmse3010146

Gjedrem, T., Rye, M., 2018. Selection response in fish and shellfish: a review. Rev. Aquaculture 10, 168–179. https://doi.org/10.1111/raq.12154

Garcia, L.D.O., Copatti, C.E., Wachholz, F., Pereira Filho, W., Baldisserotto, B., 2008. Freshwater temperature in the state of Rio Grande do Sul, Southern Brazil, and its implication for fish culture. Neotrop. Ichthyol. 6, 275–281. https://doi.org/10.1590/s1679-62252008000200016

Godinho, H.P., 2007. Estratégias reprodutivas de peixes aplicadas à aqüicultura: bases para o desenvolvimento de tecnologias de produção. Rev Bras Reprod Anim 31, 351–360.

Godinho, A.L., Lamas, I.R., Godinho, H.P., 2010. Reproductive ecology of Brazilian freshwater fishes. Environ. Biol. Fishes 87, 143–162. https://doi.org/10.1007/s10641-009-9574-4

Goulding, M., Carvalho, M.L.,1982. Life history and management of the tambaqui (*Colossoma macropomum*, characidae); an important amazonian food fish. Rev. Bras. Biol. 1, 193–221.

Gomes, L.C., Simões, L.N., Araujo-Lima, C.A.R.M., 2010. Tambaqui (*Colossoma macropomum*), in: Baldisserotto, B., Gomes, L.C. (Eds.), Espécies Nativas Para Piscicultura No Brasil. EditoraUFSM, Santa Maria, pp. 175–204.

Gutierrez, A.P., Yáñ Ez, J.M., Fukui, S., Swift, B., Davidson, W.S., 2015. Genome-Wide association study (GWAS) for growth rate and age at sexual maturation in atlantic salmon (*Salmo salar*). PLoS One 10, 1–15. https://doi.org/10.1371/journal.pone.0119730

Hashimoto, D.T., Senhorini, J.A., Foresti, F., Porto-Foresti, F., 2012. Interspecific fish hybrids in Brazil: Management of genetic resources for sustainable use. Rev. Aquaculture 4, 108–118. https://doi.org/10.1111/j.1753-5131.2012.01067.x

Hulata, G., 2001. Genetic manipulations in aquaculture: A review of stock improvement by classical and modern technologies. Genetica 111, 155–173. https://doi.org/10.1023/A:1013776931796

Houston, R.D., Haley, C.S., Hamilton, A., Guy, D.R., Tinch, A.E., Taggart, J.B., McAndrew, B.J., Bishop, S.C., 2008. Major quantitative trait loci affect resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*). Genetics 178, 1109–1115. https://doi.org/10.1534/genetics.107.082974

Houston, R.D., 2017. Future directions in breeding for disease resistance in aquaculture species. Rev. Bras. Zootec. 46, 545–551. https://doi.org/10.1590/S1806-92902017000600010

Houston, R.D., Jin, Y.H., Jenkins, T.L., Selly, S.L.C., Martin, S.A.M., Stevens, J.R., Santos, E.M., 2020. Harnessing genomics to fast-track genetic improvement in aquaculture. Nat. Rev. Genet. 21. https://doi.org/10.1038/s41576-020-0227-y

IBGE, 2020. Produção da pecuária municipal 2019. Inst. Bras. Geogr. Estat. (doi:ISSN 0101-4234).

Jia, Z., Chen, L., Ge, Y., Li, S., Peng, W., Li, C., Zhang, Y., Hu, X., Zhou, Z., Shi, L., Xu, P., 2020. Genetic mapping of Koi herpesvirus resistance (KHVR) in Mirror carp (*Cyprinus carpio*) revealed genes and molecular mechanisms of disease resistance. Aquaculture 519, 734850. https://doi.org/10.1016/j.aquaculture.2019.734850

Kumar, G., Kocour, M., 2017. Applications of next-generation sequencing in fisheries research: A review. Fish. Res. 186, 11–22. https://doi.org/10.1016/j.fishres.2016.07.021

Larsen, J., Roney, J.M., 2013. Farmed Fish Production Overtakes Beef. Earth Policy Institute, Washington, DC. Available at URL: http://www.earth-policy.org/plan_b_updates/2013/ update114. Accessed January 14, 2020.

Li, N., Zhou, T., Geng, X., Jin, Y., Wang, X., Liu, S., Xu, X., Gao, D., Li, Q., Liu, Z., 2018. Identification of novel genes significantly affecting growth in catfish through GWAS analysis. Mol. Genet. Genomics 293, 587–599. https://doi.org/10.1007/s00438-017-1406-1

Lillehammer, M., Boison, S.A., Norris, A., Løvoll, M., Bakke, H., Gjerde, B., 2019. Genetic parameters of resistance to amoebic gill disease in two Norwegian Atlantic salmon populations. Aquaculture 508, 83–89. https://doi.org/10.1016/j.aquaculture.2019.04.072

Lin, H., Zhou, Z., Zhao, J., Zhou, T., Bai, H., Ke, Q., Pu, F., Zheng, W., Xu, P., 2020. Genome-Wide Association Study Identifies Genomic Loci of Sex Determination and Gonadosomatic Index Traits in Large Yellow Croaker (*Larimichthys crocea*). Mar. Biotechnol. 127–139. https://doi.org/10.1007/s10126-020-10007-2

Ling, F., Wang, J.G., Liu, Q.F., Li, M., Ye, L.T., Gong, X.N., 2010. Prevention of *Ichthyophthirius multifiliis* infestation in goldfish (*Carassius auratus*) by potassium ferrate(VI) treatment. Vet. Parasitol. 168, 212–216. https://doi.org/10.1016/j.vetpar.2009.11.009

Lira, L.V.G., Ariede, R.B., Freitas, M. V, Mastrochirico-filho, V.A., Agudelo, J.F.G., Barría, A., Yáñez, J.M., Hashimoto, D.T., 2020. Quantitative genetic variation for resistance to the parasite *Ichthyophthirius multifiliis* in the Neotropical fi sh tambaqui (*Colossoma macropomum*). Aquac. Reports 17, 100338. https://doi.org/10.1016/j.aqrep.2020.100338

Martins, M.L., Onaka, E.M., Moraes, F.R., Bozzo, F.R., Paiva, A.M.F.C., Gonçalves, A., 2002. Recent studies on parasitic infections of freshwater cultivated fish in the State of São Paulo, Brazil. Acta Sci. 24, 981–985

Mastrochirico-Filho, V., Borges, C., Freitas, M., Ariede, R., Pilarski, F., Utsunomia, R., Carvalheiro, R., Gutierrez, A., Peñaloza, C., Yáñez, J., Houston, R., Hashimoto, D.T., 2020. Development of a SNP linkage map and genome-wide association study for resistance to *Aeromonas hydrophila* in pacu (*Piaractus mesopotamicus*). https://doi.org/10.21203/rs.3.rs-26811/v1

Matthews, R.A., 2005. *Ichthyophthirius multifiliis* fouquet and ichthyophthiriosis in freshwater teleosts. Adv. Parasitol. 59, 159–241. https://doi.org/10.1016/S0065-308X(05)59003-1

Miller, M.R., Dunham, J.P., Amores, A., Cresko, W.A., Johnson, E.A., 2007. Rapid and cost-effective polymorphism identification and genotyping using

restriction site associated DNA (RAD) markers. Genome Res. 240–248. https://doi.org/10.1101/gr.5681207.high-throughput

Moreira, G.S.A., Shoemaker, C.A., Zhang, D., Xu, D.H., 2017. Expression of immune genes in skin of channel catfish immunized with live theronts of *Ichthyophthirius multifiliis.* Parasite Immunol. 39, 1–9. https://doi.org/10.1111/pim.12397

Moen, T., Baranski, M., Sonesson, A.K., Kjøglum, S., 2009. Confirmation and fine-mapping of a major QTL for resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*): Population-level associations between markers and trait. BMC Genomics 10, 1–14. https://doi.org/10.1186/1471-2164-10-368

Ødegård, J., Baranski, M., Gjerde, B., Gjedrem, T., 2011. Methodology for genetic evaluation of disease resistance in aquaculture species: Challenges and future prospects. Aquac. Res. 42, 103–114. https://doi.org/10.1111/j.1365-2109.2010.02669.x

Ødegård, J., Moen, T., Santi, N., Korsvoll, S.A., Kjøglum, S., Meuwisse, T.H.E., 2014. Genomic prediction in an admixed population of Atlantic salmon (Salmo salar). Front. Genet. https://doi.org/10.3389/fgene.2014.00402

Olesen, I., Myhr, A.I., Rosendal, G.K., 2011. Sustainable aquaculture: Are we getting there? Ethical perspectives on salmon farming. J. Agric. Environ. Ethics 24, 381–408. https://doi.org/10.1007/s10806-010-9269-z

Osman, H.A.M., Monier, M. M., El Ghany, O.A.A., Ibrahim, T.B., Ismail,M.M., 2009. Protection of Goldfish (*Carassius auratus*) Against *Ichthyophthirius multifiliis* by Immunization with Live Theronts , Trophonts and Sonicated Trophonts. Glob. Vet. 3, 329–334.3, 329–334.

Perkel, J., 2008. SNP genotyping: Six technologies that keyed a revolution. Nat. Methods 5, 447–453. https://doi.org/10.1038/nmeth0508-447

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7, e37135. https://doi.org/10.1371/journal.pone.0037135

Pincinato, R., Asche, F., 2016. The development of Brazilian aquaculture: Introduced and native species. Aquaculture Economics & Management, 20(3): 312-323. https://doi.org/10.1080/13657305.2016.1177862

Rico, A., Van den Brink, P.J., 2014. Probabilistic risk assessment of veterinary medicines applied to four major aquaculture species produced in Asia. Sci. Total Environ. 468–469, 630–641. https://doi.org/10.1016/j.scitotenv.2013.08.063

Robledo, D., Matika, O., Hamilton, A., Houston, R.D., 2018. Genome-Wide Association and Genomic Selection for Resistance to Amoebic Gill Disease in Atlantic Salmon 8, 1195–1203. https://doi.org/10.1534/g3.118.200075

Robledo, D., Gutiérrez, A.P., Barría, A., Lhorente, J.P., Houston, R.D., Yáñez, J.M., 2019. Discovery and functional annotation of quantitative trait loci affecting resistance to Sea lice in Atlantic salmon. Front. Genet. 10, 1–10. https://doi.org/10.3389/fgene.2019.00056

Salte, R., Bentsen, H.B., Moen, T., Tripathy, S., Bakke, T.A., Ødegård, J., Omholt, S., Hansen, L.P., 2010. Prospects for a genetic management strategy to control Gyrodactylus salaris infection in wild Atlantic salmon (Salmo salar) stocks. Can. J. Fish. Aquat. Sci. 67, 121–129. https://doi.org/10.1139/f09-168

Santos, M.C.F., Ruffino, M.L., Farias, I.P., 2007. High levels of genetic variability and panmixia of the tambaqui *Colossoma macropomum* (Cuvier, 1816) in the main channel of the Amazon River. J. Fish Biol. 71, 33–44. https://doi.org/10.1111/j.1095-8649.2007.01514.x

Sidra - Sistema IBGE de Recuperação Automática. Tabela 3940 - Produção da aquicultura, por tipo de produto. 2019. Available at URL: https://sidra.ibge.gov.br/tabela/3940. Accessed January 14, 2020.

Sukhavachana, S., Poompuang, S., Onming, S., Luengnaruemitchai, A., 2019. Heritability estimates and selection response for resistance to *Streptococcus agalactiae* in red tilapia *Oreochromis* spp. Aquaculture 502, 384–390. https://doi.org/10.1016/j.aquaculture.2018.12.075

Srivastava, S., Sinha, R., Roy, D., 2004. Toxicological effects of malachite green. Aquat. Toxicol. 66, 319–329. https://doi.org/10.1016/j.aquatox.2003.09.008

Santos, E.F., Tavares-Dias, M., Pinheiro, D.A., Neves, L.R., Barbosa, G., Kelly, M., Dias, R., 2013. *Colossoma macropomum* (Characidae) cultivado em tanque-rede no estado do Amapá , Amazônia oriental. Acta Amaz. 43, 105–112.

Saint-paul, U., 2017. Native fish species boosting Brazilian ' s aquaculture development. Acta Fish. Aquat. Res. 5(1), 1–9. https://doi.org/10.2312/ActaFish.2017.5.1.1-9

Sigh, J., Lindenstrom, T., Buchmann, K., 2004. The parasitic ciliate *Ichthyophthirius multifiliis* induces expression of immune relevant genes in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis. 27, 409–417. https://doi.org/10.1111/j.1365-2761.2004.00558.x

Taylor, R.S., Kube, P.D., Muller, W.J., Elliott, N.G., 2009. Genetic variation of gross gill pathology and survival of Atlantic salmon (*Salmo salar* L.) during natural amoebic gill disease challenge. Aquaculture 294, 172–179. https://doi.org/10.1016/j.aquaculture.2009.06.007

Thilsted, S.H., Thorne-Lyman, A., Webb, P., Bogard, J.R., Subasinghe, R., Phillips, M.J., Allison, E.H., 2016. Sustaining healthy diets: The role of capture fisheries and aquaculture for improving nutrition in the post-2015 era. Food Policy 61, 126–131. https://doi.org/10.1016/j.foodpol.2016.02.005

Tsai, H.Y., Hamilton, A., Tinch, A.E., Guy, D.R., Bron, J.E., Taggart, J.B., Gharbi, K., Stear, M., Matika, O., Pong-Wong, R., Bishop, S.C., Houston, R.D., 2016. Genomic prediction of host resistance to sea lice in farmed Atlantic salmon populations. Genet. Sel. Evol. 48, 1–11. https://doi.org/10.1186/s12711-016-0226-9

Valladão, G.M.R., Gallani, S.U., Ikefuti, C. V, da Cruz, C., Levy-Pereira, N., Rodrigues, M.V.N., Pilarski, F., 2016. Essential oils to control ichthyophthiriasis in pacu, *Piaractus mesopotamicus* (Holmberg): special emphasis on treatment with *Melaleuca alternifolia*. J. Fish Dis. 39, 1143–1152. https://doi.org/10.1111/jfd.12447

Valladão, G.M.R., Gallani, S.U., Pilarski, F., 2018. South American fish for continental aquaculture. Rev. Aquac. 10, 351–369. https://doi.org/10.1111/raq.12164

Vieira, M.B., Oliveira, P.M., 2015. Melhoramento de espécies aquícolas com foco na resistência a doenças. Embrapa, 32pp. ISSN 2318-1400

Wang, X., Dickerson, H.W., 2002. Surface immobilization antigen of the parasitic ciliate *Ichthyophthirius multifiliis* elicits protective immunity in channel

catfish (*Ictalurus punctatus*). Clin. Diagn. Lab. Immunol. 9, 176–81. https://doi.org/10.1128/cdli.9.1.176-181.2002

Vignal, A., Milan, D., SanCristobal, M., Eggen, A., 2008. A review on SNPs and other types of molecular markers. Genet. Sel. Evol. 40, 241–264. https://doi.org/10.1051/gse

Wang, W., Tan, S., Luo, J., Shi, H., Zhou, T., Yang, Y., Jin, Y., Wang, X., Niu, D., Yuan, Z., Gao, D., Dunham, R., Liu, Z., 2019. GWAS Analysis Indicated Importance of NF-κB Signaling Pathway in Host Resistance Against Motile Aeromonas Septicemia Disease in Catfish. Mar. Biotechnol. 2009, 335–347. https://doi.org/10.1007/s10126-019-09883-0

Woynárovich, A., Van Anrooy, R., 2019. Field guide to the culture of tambaqui (*Colossoma macropomum)*, FAO Fisheries and Aquaculture Technical Paper. No. 624. Rome, FAO.132 pp. ISSN 2070-7010

Xie, M., Ming, Y., Shao, F., Jian, J., Zhang, Y., Peng, Z., 2018. Restriction site-associated DNA sequencing for SNP discovery and high-density genetic map construction in southern catfish (Silurus meridionalis). R. Soc. Open Sci. 5. https://doi.org/10.1098/rsos.172054

Xiong, X.M., Chen, Y.L., Liu, L.F., Wang, W., Robinson, N.A., Gao, Z.X., 2017. Estimation of genetic parameters for resistance to *Aeromonas hydrophila* in blunt snout bream (*Megalobrama amblycephala*). Aquaculture 479, 768–773. https://doi.org/10.1016/j.aquaculture.2017.07.011

Xu, D.H., Klesius, P.H., Shoemaker, C.A., 2007. Evaluation of a cohabitation challenge model in immunization trials for channel catfish *Ictalurus punctatus* against *Ichthyophthirius multifiliis.* Dis. Aquat. Organ. 74, 49–55. https://doi.org/10.3354/dao074049

Xu, D.H., Klesius, P.H., Shoemaker, C.A., 2008. Protective immunity of Nile tilapia against *Ichthyophthirius multifiliis* post-immunization with live theronts and sonicated trophonts. Fish Shellfish Immunol. 25, 124–127. https://doi.org/10.1016/j.fsi.2008.03.012

Xu, D.H., Klesius, P.H., Shoemaker, C.A., 2009a. Effect of immunization of channel catfish with inactivated trophonts on serum and cutaneous antibody titers and survival against *Ichthyophthirius multifiliis*. Fish Shellfish Immunol. 26, 614–618. https://doi.org/10.1016/j.fsi.2008.09.015

Xu, D.H., Panangala, V.S., Van Santen, V.L., Dybvig, K., Abernathy, J.W., Klesius, P.H., Liu, Z., Russo, R., 2009b. Molecular characteristics of an immobilization antigen gene of the fish-parasitic protozoan *Ichthyophthirius multifiliis* strain ARS-6. Aquaculture Res. 40, 1884–1892. https://doi.org/10.1111/j.1365-2109.2009.02301.x

Xu, D.H., Moreira, G.S.A., Shoemaker, C.A., Zhang, D., Beck, B.H., 2017. Expression of immune genes in systemic and mucosal immune tissues of channel catfish vaccinated with live theronts of *Ichthyophthirius multifiliis*. Fish Shellfish Immunol. 66, 540–547. https://doi.org/10.1016/j.fsi.2017.05.051

Yang, Y., Wu, L., Wu, X., Li, B., Huang, W., Weng, Z., Lin, Z., Song, L., Guo, Y., Meng, Z., Liu, X., Xia, J., 2020. Identification of Candidate Growth-Related SNPs and Genes Using GWAS in Brown-Marbled Grouper (*Epinephelus fuscoguttatus*). Mar. Biotechnol. 22, 153–166. https://doi.org/10.1007/s10126-019-09940-8

Yang, M., Wang, Q., Chen, J., Wang, Y., Zhang, Y., Qin, Q., 2021. Identification of candidate SNPs and genes associated with anti-RGNNV using GWAS in the red-spotted grouper*, Epinephelus akaara*. Fish Shellfish Immunol. 112, 31–37. https://doi.org/10.1016/j.fsi.2021.02.010

Yáñez, J.M., Houston, R.D., Newman, S., 2014. Genetics and genomics of disease resistance in salmonid species. Front. Genet. 5, 1–13. https://doi.org/10.3389/fgene.2014.00415

Yáñez, J.M., Martínez, V., 2010. Factores genéticos que inciden en la resistencia a enfermedades infecciosas en salmónidos y su aplicación en programas de mejoramiento. Arch. Med. Vet. 42, 1–13. https://doi.org/10.4067/S0301-732X2010000200002

Yoshida, G.M., Lhorente, J.P., Correa, K., Soto, J., Salas, D., Yáñez, J.M., 2019. Genome-wide association study and cost-efficient genomic predictions for growth and fillet yield in Nile tilapia (*Oreochromis niloticus*). G3 Genes, Genomes, Genet. 9, 2597–2607. https://doi.org/10.1534/g3.119.400116

Zago, A.C., Franceschini, L., Garcia, F., Schalch, S.H.C., Gozi, K.S., Silva, R.J. da, 2014. Ectoparasites of Nile tilapia (*Oreochromis niloticus*) in cage farming in a hydroelectric reservoir in Brazil. Rev. Bras. Parasitol. Veterinária 23, 171–178. https://doi.org/10.1590/s1984-29612014041

Zaila, K., Cho, D., Chang, W.J., 2016. Interactions between parasitic ciliates and their host: *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* as examples, in: Witzany, G., Nowacki, M., 2016 (Eds.), Biocommunication of Ciliates. Springer, Switzerland, pp. 327-350

Zaniboni Filho, E., Meurer, S., 1997. Limitações e potencialidades do cultivo de tambaqui (*Colossoma macropomum* Cuvier, 1818) na região subtropical brasileira. B. Inst. Pesca. 24, 169-172

Zhou, Z., Han, K., Wu, Y., Bai, H., Ke, Q., Pu, F., Wang, Y., Xu, P., 2019. Genome-Wide Association Study of Growth and Body-Shape-Related Traits in

Large Yellow Croaker (*Larimichthys crocea*) Using ddRAD Sequencing. Mar. Biotechnol. 21, 655–670. https://doi.org/10.1007/s10126-019-09910-0

CAPITULO 1

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.

Keywords: breeding program; heritability; animal breeding; disease resistance.

1.1 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, 2016). 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, 2016). 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*.

1.2 Materials and methods

1.2.1 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.

1.2.2 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 keptin 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).

1.2.3 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 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.

1.2.4 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 2b, 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 parasite (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:

$$Pr(Y|ij) = \Phi\left(\mu + t_i + w_{ij} + a_j\right)$$

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:

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

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

1.3 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 (Fig. 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.

1.4 Discussion

1.4.1 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.

1.4.2 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; Gjedrem and Rye, 2018). 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 were 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 others 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 of 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.

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1.6 References

Aihua, B.L., Buchmann, K., 2001. Temperature and salinity dependent development of a Nordic strain of *Ichthyophthirius multifiliis* from rainbow trout. J. Appl. Ichthyol. 17, 273–276. https://doi.org/10.1046/j.1439-0426.2001.00279.x

Bassini, L.N., Lhorente, J.P., Oyarzún, M., Bangera, R., Yáñez, J.M., Neira, R., 2019. Genetic parameters for *Piscirickettsia salmonis* resistance, sea lice (*Caligus rogercresseyi*) susceptibility and harvest weight in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 510, 276–282. https://doi.org/10.1016/j.aquaculture.2019.05.008

Bois, S.A., Gjerde, B., Hillestad, B., Makvandi-Nejad, S., Moghadam, H.K., Boison, S.A., 2019. Genomic and transcriptomic analysis of amoebisc gill disease resistansce in Atlantic salmon (*Salmo salar L*.). Front. Genet. 10. https://doi.org/10.3389/fgene.2019.00068

Buchmann, K., Sigh, J., Nielsen, C.V., Dalgaard, M., 2001. Host responses against the fish parasitizing ciliate *Ichthyophthirius multifiliis*. Vet. Parasitol. 100, 105–116. https://doi.org/10.1016/S0304-4017(01)00487-3

Carneiro, P.C.F., Schorer, M., Mikos, J.D., 2005. Tratamentos terapêuticos convencionais no controle do ectoparasita *Ichthyophtirius multifiliis* em jundiá (*Rhamdia quelen*). Pesq. Agropec. Bras. 40, 99-102. http://dx.doi.org/10.1590/S0100-204X2005000100015

Correa, K., Lhorente, J.P., Bassini, L., López, M.E., Di Genova, A., Maass, A., Davidson, W.S., Yáñez, J.M., 2017a. Genome wide association study for resistance to *Caligus rogercresseyi* in Atlantic salmon (*Salmo salar L*.) using a 50K SNP genotyping array. Aquaculture 472, 61–65. https://doi.org/10.1016/j.aquaculture.2016.04.008

Correa, K., Bangera, R., Figueroa, R., Lhorente, J.P., Yáñez, J.M., 2017b. The use of genomic information increases the accuracy of breeding value predictions for sea louse (*Caligus rogercresseyi*) resistance in Atlantic salmon (*Salmo salar*). Genet. Sel. Evol. 49, 1–5. https://doi.org/10.1186/s12711-017-0291-8

Coyne, R.S., Hannick, L., Shanmugam, D., Hostetler, J.B., Brami, D., Joardar, V.S., Johnson, J., Radune, D., Singh, I., Badger, J.H., Kumar, U., Saier, M., Wang, Y., Cai, H., Gu, J., Mather, M.W., Vaidya, A.B., Wilkes, D.E., Rajagopalan, V., Asai, D.J., Pearson, C.G., Findly, R.C., Dickerson, H.W., Wu, M., Martens, C., Van de Peer, Y., Roos, D.S., Cassidy-Hanley, D.M., Clark, T.G., 2011. Comparative genomics of the pathogenic ciliate *Ichthyophthirius multifiliis*, its free-living relatives and a host species provide insights into adoption of a parasitic lifestyle and prospects for disease control. Genome Biol. 12, R100. https://doi.org/10.1186/gb-2011-12-10-r100

Dickerson, H.W., Findly, R.C., 2014. Immunity to ichthyophthirius infections in fish: A synopsis. Dev. Comp. Immunol. 43, 290–299. https://doi.org/10.1016/j.dci.2013.06.004

Dupont-Nivet, M., Vandeputte, M., Chevassus, B., 2002. Optimization of factorial mating designs for inference on heritability in fish species. Aquaculture 204, 361–370. https://doi.org/10.1016/S0044-8486(01)00839-0

Elaswad, A., Dunham, R., 2018. Disease reduction in aquaculture with genetic and genomic technology: current and future approaches. Rev. Aquaculture. 10, 876–898. https://doi.org/10.1111/raq.12205

Elaswad, A., Khalil, K., Ye, Z., Alsaqufi, A., Abdelrahman, H., Su, B., Perera, D.A., Dong, S., Abass, N., Dunham, R., 2019. Effects of Cecropin Transgenesis and Interspecific Hybridization on the Resistance to Ichthyophthirius multifiliis in Channel Catfish and Female Channel Catfish × Male Blue Catfish Hybrids. N. Am. J. Aquac. 81, 242–252. https://doi.org/10.1002/naaq.10096

Ewing, M., Black, M., Blazer, V., Kocan, K., 1994. Plasma chloride and gill epithelial response of channel catfish to infection with Ichthyophthirius multifiliis. J. Aquat. Anim. Health. 6,187– 196. https://doi.org/10.1577/1548-8667(1994)006<0187:PCAGER>2.3.CO;2

Farmer, B.D., Fuller, S.A., Mitchell, A.J., Straus, D.L., Bullard, S.A., 2013. Efficacy of bath treatments of formalin and copper sulfate on cultured white bass, Morone multifiliis. J. World Aquac. Soc. 44, 305–310. https://doi.org/10.1111/jwas.12027

Fernandes, E.M., de Almeida, L.C.F., Hashimoto, D.T., Lattanzi, G.R., Gervaz, W.R., Leonardo, A.F., Neto, R.V.R., 2018. Survival of purebred and hybrid Serrasalmidae under low water temperature conditions. Aquaculture 497, 97–102. https://doi.org/10.1016/j.aquaculture.2018.07.030

Francis-Floyd, R., Yanong, R., Pouder, D. *Ichthyophthirius multifiliis* (white spot) infections in fish. https://edis.ifas.ufl.edu/pdffiles/FA/FA00600.pdf (accessed 10 april 2019).

Gharbi, K., Bron, J., Tinch, A., Roberts, R., Stear, M., Matthews, L., 2015. The control of sea lice in Atlantic salmon by selective breeding. J. R. Soc. Interface 12, 20150574. https://doi.org/10.1098/rsif.2015.0574

Gjerde, B., Ødegård, J., Thorland, I., 2011. Estimates of genetic variation in the susceptibility of Atlantic salmon (*Salmo salar*) to the salmon louse *Lepeophtheirus salmonis*. Aquaculture 314, 66–72. https://doi.org/10.1016/j.aquaculture.2011.01.026

Gjedrem, T., 2000. Genetic improvement of cold-water fish species. Aquaculture Res. 31, 25–33. https://doi.org/10.1046/j.1365-2109.2000.00389.x

Gjedrem, T., Robinson, N., Rye, M., 2012. The importance of selective breeding in aquaculture to meet future demands for animal protein: A review. Aquaculture 350–353, 117–129. https://doi.org/10.1016/j.aquaculture.2012.04.008

Gjedrem, T., Rye, M., 2018. Selection response in fish and shellfish: a review. Rev. Aquaculture 10, 168–179. https://doi.org/10.1111/raq.12154

Glover, K.A., Aasmundstad, T., Nilsen, F., Storset, A., Skaala, 2005. Variation of Atlantic salmon families (*Salmo salar L*.) in susceptibility to the sea lice *Lepeophtheirus salmonis* and *Caligus elongatus*. Aquaculture 245, 19–30. https://doi.org/10.1016/j.aquaculture.2004.11.047

Glover, K.A., Hansen, T., Besnier, F., Solberg, M.F., Fjelldal, P.G., Eide, A.G.S., Dalvin, S., Nilsen, F., 2017. Cloned and outbred Atlantic salmon display equal parasite dispersion when infected with the salmon louse. Aquaculture 480, 83–88. https://doi.org/10.1016/j.aquaculture.2017.08.008

Gomes, L.C., Simões, L.N., Araujo-Lima, C.A.R.M., 2010. Tambaqui (*Colossoma macropomum*), in: Baldisserotto, B., Gomes, L.C. (Eds.), Espécies Nativas Para Piscicultura No Brasil. EditoraUFSM, Santa Maria, pp. 175–204.

Hashimoto, D.T., Senhorini, J.A., Foresti, F., Porto-Foresti, F., 2012. Interspecific fish hybrids in Brazil: Management of genetic resources for sustainable use. Rev. Aquaculture 4, 108–118. https://doi.org/10.1111/j.1753-5131.2012.01067.x

Hulata, G., 2001. Genetic manipulations in aquaculture: A review of stock improvement by classical and modern technologies. Genetica 111, 155–173. https://doi.org/10.1023/A:1013776931796

IBGE, 2020. Produção da pecuária municipal 2016. Inst. Bras. Geogr. Estat. (doi:ISSN 0101-4234).

Janssen, K., Chavanne, H., Berentsen, P., Komen, H., 2017. Impact of selective breeding on European aquaculture. Aquaculture 472, 8–16. https://doi.org/10.1016/j.aquaculture.2016.03.012

Kolstad, K., Heuch, P.A., Gjerde, B., Gjedrem, T., Salte, R., 2005. Genetic variation in resistance of Atlantic salmon (*Salmo salar*) to the salmon louse L*epeophtheirus salmonis*. Aquaculture 247, 145–151. https://doi.org/10.1016/j.aquaculture.2005.02.009

Kube, P.D., Taylor, R.S., Elliott, N.G., 2012. Genetic variation in parasite resistance of Atlantic salmon to amoebic gill disease over multiple infections. Aquaculture 364–365, 165–172. https://doi.org/10.1016/j.aquaculture.2012.08.026

Lhorente, J.P., Gallardo, J.A., Villanueva, B., Araya, A.M., Torrealba, D.A., Toledo, X.E., Neira, R., 2012. Quantitative genetic basis for resistance to *Caligus rogercresseyi* sea lice in a breeding population of Atlantic salmon (*Salmo salar*). Aquaculture 324–325, 55–59. https://doi.org/10.1016/j.aquaculture.2011.10.046

Lhorente, J.P., Araneda, M., Neira, R., Yáñez, J.M. 2019. Advances in genetic improvement for salmon and trout aquaculture: the Chilean situation and prospects. Rev. Aquac. 11, 340–353. https://doi.org/10.1111/raq.12335

Lillehammer, M., Boison, S.A., Norris, A., Løvoll, M., Bakke, H., Gjerde, B., 2019. Genetic parameters of resistance to amoebic gill disease in two Norwegian Atlantic salmon populations. Aquaculture 508, 83–89. https://doi.org/10.1016/j.aquaculture.2019.04.072

Martins, M.L., Onaka, E.M., Moraes, F.R., Bozzo, F.R., Paiva, A.M.F.C., Gonçalves, A., 2002. Recent studies on parasitic infections of freshwater cultivated fish in the State of São Paulo, Brazil. Acta Sci. 24, 981–985

Mastrochirico-Filho, V.A., Ariede, R.B., Freitas, M. V, Lira, L.V.G., Agudelo, J.F.G., Pilarski, F., Neto, V.R., Yáñez, J.M., Hashimoto, D.T., 2019. Genetic parameters for resistance to *Aeromonas hydrophila* in the Neotropical fish pacu (*Piaractus mesopotamicus*). Aquaculture 734442. https://doi.org/10.1016/j.aquaculture.2019.734442

Matthews, R.A., 2005. *Ichthyophthirius multifiliis* fouquet and ichthyophthiriosis in freshwater teleosts. Adv. Parasitol. 59, 159–241. https://doi.org/10.1016/S0065-308X(05)59003-1

Moreira, G.S.A., Shoemaker, C.A., Zhang, D., Xu, D.H., 2017. Expression of immune genes in skin of channel catfish immunized with live theronts of *Ichthyophthirius multifiliis.* Parasite Immunol. 39, 1–9. https://doi.org/10.1111/pim.12397

Ødegård, J., Baranski, M., Gjerde, B., Gjedrem, T., 2011. Methodology for genetic evaluation of disease resistance in aquaculture species: Challenges and

future prospects. Aquac. Res. 42, 103–114. https://doi.org/10.1111/j.1365-2109.2010.02669.x

Olesen, I., Myhr, A.I., Rosendal, G.K., 2011. Sustainable aquaculture: Are we getting there? Ethical perspectives on salmon farming. J. Agric. Environ. Ethics 24, 381–408. https://doi.org/10.1007/s10806-010-9269-z

Rico, A., Van den Brink, P.J., 2014. Probabilistic risk assessment of veterinary medicines applied to four major aquaculture species produced in Asia. Sci. Total Environ. 468–469, 630–641. https://doi.org/10.1016/j.scitotenv.2013.08.063

Rochus, C.M., Holborn, M.K., Ang, K.P., Elliott, J.A.K., Glebe, B.D., Leadbeater, S., Tosh, J.J., Boulding, E.G., 2018. Genome-wide association analysis of salmon lice (*Lepeophtheirus salmonis*) resistance in a North American Atlantic salmon population. Aquac. Res. 49, 1329–1338. https://doi.org/10.1111/are.13592

Rintamäki-Kinnunen, P., Rahkonen, M., Mannermaa-Keränen, A.L., Suomalainen, L.R., Mykrä, H., Valtonen, E.T., 2005. Treatment of ichthyophthiriasis after malachite green. I. Concrete tanks at salmonid farms. Dis. Aquat. Organ. 64, 69–76. https://doi.org/10.3354/dao064069

Salte, R., Bentsen, H.B., Moen, T., Tripathy, S., Bakke, T.A., Ødegård, J., Omholt, S., Hansen, L.P., 2010. Prospects for a genetic management strategy to control *Gyrodactylus salaris* infection in wild Atlantic salmon (*Salmo salar*) stocks. Can. J. Fish. Aquat. Sci. 67, 121–129. https://doi.org/10.1139/f09-168

Sigh, J., Lindenstrom, T., Buchmann, K., 2004. The parasitic ciliate *Ichthyophthirius multifiliis* induces expression of immune relevant genes in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis. 27, 409–417. https://doi.org/10.1111/j.1365-2761.2004.00558.x

Srivastava, S., Sinha, R., Roy, D., 2004. Toxicological effects of malachite green. Aquat. Toxicol. 66, 319–329. https://doi.org/10.1016/j.aquatox.2003.09.008

Srisapoome, P., Chatchaiphan, S., Bunnoy, A., Koonawootrittriron, S., Na-Nakorn, U., 2019. Heritability of immunity traits and disease resistance of bighead catfish, *Clarias macrocephalus* Günther, 1864. Fish Shellfish Immunol. 92, 209–215. https://doi.org/10.1016/j.fsi.2019.05.060

Tavares-Dias, M., Martins, M.L., 2017. An overall estimation of losses caused by diseases in the Brazilian fish farms. J Parasit Dis 41, 913–918. https://doi.org/10.1007/s12639-017-0938-y

Taylor, R.S., Kube, P.D., Muller, W.J., Elliott, N.G., 2009. Genetic variation of gross gill pathology and survival of Atlantic salmon (*Salmo salar* L.) during natural amoebic gill disease challenge. Aquaculture 294, 172–179. https://doi.org/10.1016/j.aquaculture.2009.06.007

Tsai, H.Y., Hamilton, A., Tinch, A.E., Guy, D.R., Bron, J.E., Taggart, J.B., Gharbi, K., Stear, M., Matika, O., Pong-Wong, R., Bishop, S.C., Houston, R.D., 2016. Genomic prediction of host resistance to sea lice in farmed Atlantic salmon populations. Genet. Sel. Evol. 48, 1–11. https://doi.org/10.1186/s12711-016-0226-9

Urbinati, E.C., Zanuzzo, F.S., Biller-Takahashi, J.D., 2014. Estresse e Sistema Imune em Peixes, in: Baldisserotto, B., Cyrino, J.E.P., Urbinati, E.C. (Eds.), Biologia e Fisiologia de Peixes Neotropicais de Água Doce. Funep, Jaboticabal, pp. 87–105

Valladão, G.M.R., Gallani, S.U., Ikefuti, C. V, da Cruz, C., Levy-Pereira, N., Rodrigues, M.V.N., Pilarski, F., 2016. Essential oils to control ichthyophthiriasis in

pacu, *Piaractus mesopotamicus* (Holmberg): special emphasis on treatment with *Melaleuca alternifolia*. J. Fish Dis. 39, 1143–1152. https://doi.org/10.1111/jfd.12447

Valladão, G.M.R., Gallani, S.U., Pilarski, F., 2018. South American fish for continental aquaculture. Rev. Aquac. 10, 351–369. https://doi.org/10.1111/raq.12164

Wang, X., Dickerson, H.W., 2002. Surface immobilization antigen of the parasitic ciliate *Ichthyophthirius multifiliis* elicits protective immunity in channel catfish (*Ictalurus punctatus*). Clin. Diagn. Lab. Immunol. 9, 176–81. https://doi.org/10.1128/cdli.9.1.176-181.2002

Xiong, X.M., Chen, Y.L., Liu, L.F., Wang, W., Robinson, N.A., Gao, Z.X., 2017. Estimation of genetic parameters for resistance to *Aeromonas hydrophila* in blunt snout bream (*Megalobrama amblycephala*). Aquaculture 479, 768–773. https://doi.org/10.1016/j.aquaculture.2017.07.011

Xu, D.H., Klesius, P.H., Shelby, R.A., 2004. Immune responses and host protection of channel catfish, *Ictalurus punctatus* (Rafinesque), against *Ichthyophthirius multifiliis* after immunization with live theronts and sonicated trophonts. J. Fish Dis. 27, 135–141. https://doi.org/10.1111/j.1365-2761.2004.00523.x

Xu, D.H., Klesius, P.H., Shoemaker, C.A., 2007. Evaluation of a cohabitation challenge model in immunization trials for channel catfish *Ictalurus punctatus* against *Ichthyophthirius multifiliis.* Dis. Aquat. Organ. 74, 49–55. https://doi.org/10.3354/dao074049

Xu, D.H., Klesius, P.H., Shoemaker, C.A., 2008. Protective immunity of Nile tilapia against *Ichthyophthirius multifiliis* post-immunization with live theronts and

sonicated trophonts. Fish Shellfish Immunol. 25, 124–127. https://doi.org/10.1016/j.fsi.2008.03.012

Xu, D.H., Klesius, P.H., Shoemaker, C.A., 2009a. Effect of immunization of channel catfish with inactivated trophonts on serum and cutaneous antibody titers and survival against *Ichthyophthirius multifiliis*. Fish Shellfish Immunol. 26, 614–618. https://doi.org/10.1016/j.fsi.2008.09.015

Xu, D.H., Panangala, V.S., Van Santen, V.L., Dybvig, K., Abernathy, J.W., Klesius, P.H., Liu, Z., Russo, R., 2009b. Molecular characteristics of an immobilization antigen gene of the fish-parasitic protozoan *Ichthyophthirius multifiliis* strain ARS-6. Aquaculture Res. 40, 1884–1892. https://doi.org/10.1111/j.1365-2109.2009.02301.x

Xu, D.H., Moreira, G.S.A., Shoemaker, C.A., Zhang, D., Beck, B.H., 2017. Expression of immune genes in systemic and mucosal immune tissues of channel catfish vaccinated with live theronts of *Ichthyophthirius multifiliis*. Fish Shellfish Immunol. 66, 540–547. https://doi.org/10.1016/j.fsi.2017.05.051

Yáñez, J.M., Bangera, R., Lhorente, J.P., Oyarzún, M., Neira, R., 2013. Quantitative genetic variation of resistance against *Piscirickettsia salmonis* in Atlantic salmon (*Salmo salar*). Aquaculture 414–415, 155–159. https://doi.org/10.1016/j.aquaculture.2013.08.009

Yáñez, J.M., Lhorente, J.P., Bassini, L.N., Oyarzún, M., Neira, R., Newman, S., 2014a. Genetic co-variation between resistance against both *Caligus rogercresseyi* and *Piscirickettsia salmonis*, and body weight in Atlantic salmon (*Salmo salar*). Aquaculture 433, 295-298. https://doi.org/10.1016/j.aquaculture.2014.06.026

Yáñez, J.M., Houston, R.D., Newman, S., 2014b. Genetics and genomics of disease resistance in salmonid species. Front. Genet. 5, 1–13. https://doi.org/10.3389/fgene.2014.00415

Zaila, K., Cho, D., Chang, W.J., 2016. Interactions between parasitic ciliates and their host: *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* as examples, in: Witzany, G., Nowacki, M., 2016 (Eds.), Biocommunication of Ciliates. Springer, Switzerland, pp. 327-350

Zaniboni Filho, E., Meurer, S., 1997. Limitações e potencialidades do cultivo de tambaqui (*Colossoma macropomum* Cuvier, 1818) na região subtropical brasileira. B. Inst. Pesca. 24, 169-172

Tables

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

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

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	$\boldsymbol{h^2}$
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)

Figure captions

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.

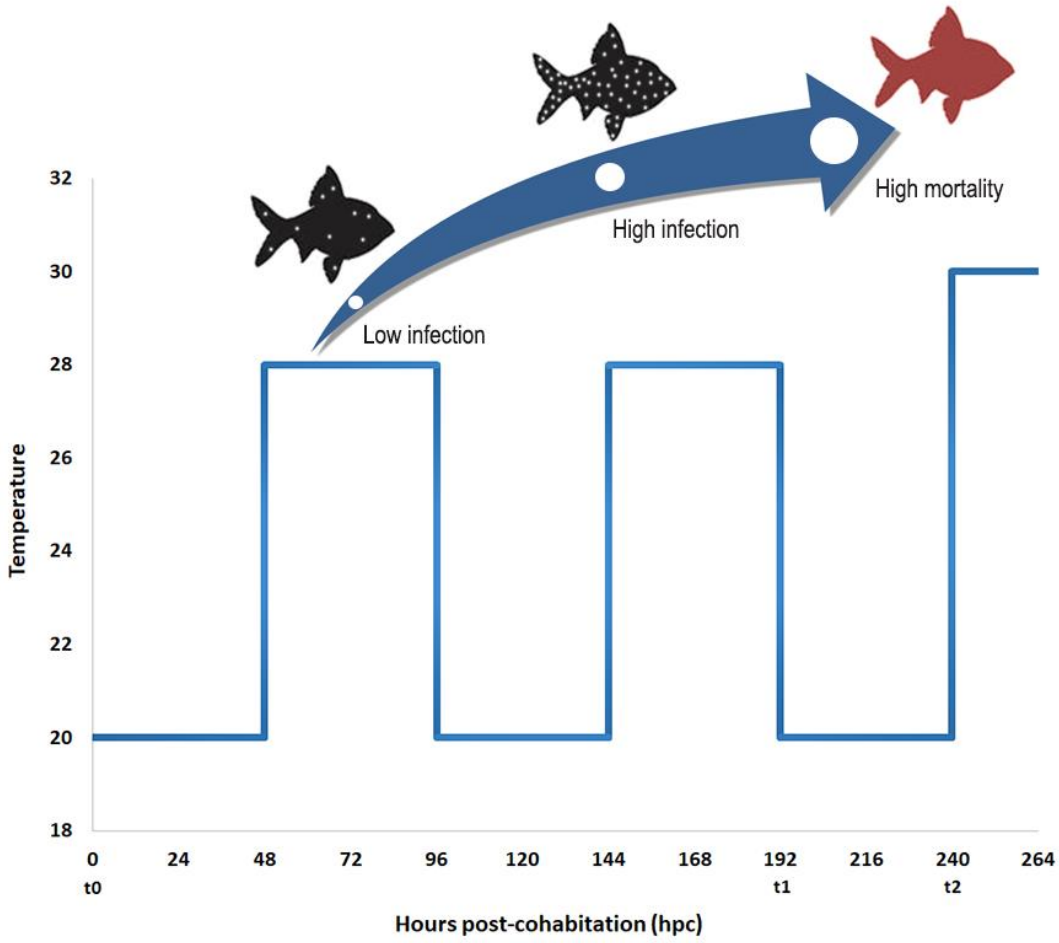
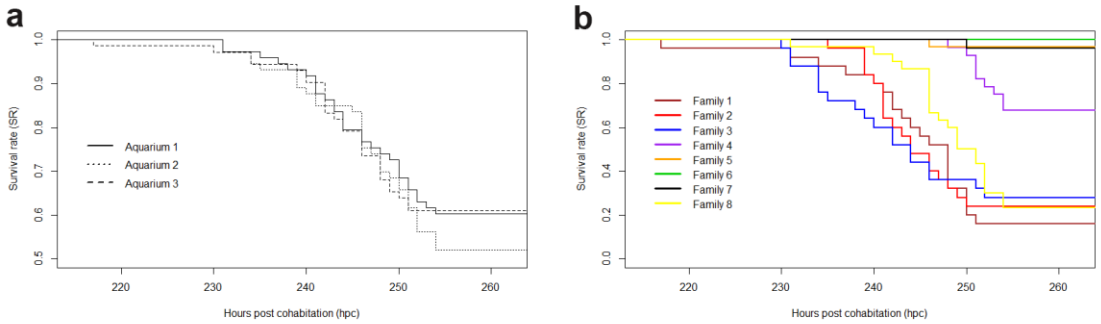


Fig. 2. Kaplan-Meier mortality curves for the three aquariums (**a**) and for the tambaqui families (**b**) of the experimental challenge with *I. multifiliis.*



CAPÍTULO 2

Genome-wide association study of host resistance to the ectoparasite *Ichthyophthirius multifiliis* in tambaqui (*Colossoma macropomum*)

Manuscrito nas normas do periódico “Aquaculture”

ABSTRACT

Tambaqui, *Colossoma macropomum*, is the most important farming native fish species in South America, particularly in Brazil, where its production is limited in the southern and southeastern regions due to the presence of the parasite *Ichthyophthirius multifiliis*. This pathogen induces infestations during season changes (particularly due to temperature variation), which limits the tambaqui production. Therefore, genome level analysis to understand the genetic architecture the host resistance against *I. multifiliis* is fundamental to improve this trait in tambaqui. The objective of the present study was to map QTL (quantitative trait loci) associated with resistance to *I. multifiliis* in tambaqui by GWAS (genome-wide association study). Individuals belonging to seven families, which were previously submitted to an experimental challenge to assess the natural resistance to the parasite *I. multifiliis*, were used for genomic analysis. A total of 7,717 SNPs were identified in this population, after ddRAD (double digest restriction site associated DNA) library construction, Illumina-sequencing and quality filtering. GWAS analysis revealed four SNPs significantly associated in the LGs (linkage groups) 2, 9, 11 and 20 for the traits time of death (TD) and parasite load (PL). The SNPs explained a low proportion of the variance to *I. multifiliis* resistance for TD and PL (about 0.622% and 0.375%, respectively). The associated SNPs were close to 11 genes related to the immune system: *abcf3, znf830, ccr9, gli3, ackr4, tbata, ndr2, tgfbr3, nhej1, znf644b,* and *cldn10a*. In conclusion, the resistance to *I. multifiliis* is probably under polygenic control in tambaqui, in which different QTLs of low variance can be involved in the immune responses against this ectoparasite.

2.1 Introduction

Tambaqui is a native fish from the Orinoco and Amazon basins (Goulding and Carvalho, 1982). This species is the main farmed fish of the continental aquaculture in several countries from South and Central America (Valladão *et al*., 2018; Woynárovich and Van Anrooy, 2019). Brazil leads the production of this species in the world (Woynárovich and Van Anrooy, 2019), with the largest production occurring in the North and Northeast (73.2 and 21.1 tons, respectively), nevertheless its production declines considerably in the South and Southeast regions (4 and 466 thousand kilograms, respectively) (IBGE, 2020). The temperature oscillations during the seasonal changes, particularly in autumn/winter, is one the limitation to the low production in South Brazil, as it facilitates infestations by the ectoparasite *Ichthyophthirius multifiliis* (Martins *et al*., 2002; Lira *et al*., 2020).

I. multifiliis is a worldwide disseminated ciliated protozoan, that acts as a fish parasite with low host specificity, and it is considered one of the most severe threats to the production of freshwater fish (Matthews, 2005; Dickerson and Findly, 2014; Tavares-Dias and Martins, 2017) through morbidity, mortality, or due to the high costs of treatments and prophylactic measures (Dickerson and Findly, 2017). Up to date, available treatments against this parasite are no cost-efficient, laborious, stressful to fish and can pollute the environment (Francis-Floyd *et al*, 2016). Currently, there are no profitable and sustainable strategies available to prevent or control infestation by *I. multifiliis* in long term (Dickerson and Findly, 2014; Wang *et al*., 2019).

In addition to traditional strategies of parasite outbreaks control, selective breeding can be considered as a complementary alternative to improve the natural resistance of fish against several diseases, particularly by the incorporation of

genomic tools (Yáñez *et al*., 2014; Elaswad and Dunham, 2018; Lhorente *et al*., 2019). Molecular markers based on single nucleotide polymorphism (SNPs) have been frequently used for the identification of quantitative trait loci (QTL) or major genes responsible for superior productive traits (Wenne *et al*., 2018). Genome-wide association study (GWAS) is one of the main methods to identify QTLs, which associates SNPs with QTLs based on linkage disequilibrium (Corradin *et al*., 2014).

GWAS has been applied to locate genome regions associated with natural resistance against parasites in fish only recently, such as *Tetracapsuloides bryosalmonae* (Ahmad *et al*., 2017), *Paramoeba perurans* (Robledo *et al*., 2018; Boison *et al*., 2019; Aslam *et al*., 2020b), *Caligus rogercresseyi* (Correa *et al*., 2017a; Robledo *et al*., 2019), *Sparicotyle chrysophrii* (Aslam *et al*., 2020a), and *Cryptocaryon irritans* (Kong *et al*., 2019; Zhao *et al*., 2021a). The unique investigation to detect genomic regions related with resistance against the parasite *Ichthyophthirius multifiliis* was carried out in rainbow trout (Jaafar *et al*., 2020), in which SNP markers were significantly associated on two specific chromosomes (16 and 17).

In a previous study, we estimated genetic parameters related to *I. multifiliis* resistance in tambaqui, which revealed a total cumulative survival rate (16 to 100%) and time to death (217 to 254 hours post cohabitation) varying significantly among families (Lira *et al.,* 2020). In the present study, GWAS was applied to map QTLs associated to resistance against *I. multifillis* in tambaqui, and to investigate the genetic architecture of the natural resistance to this disease, using survival status (SS), time of death (TD) and parasite load (PL) as trait definitions.

2.2 Material and methods

2.2.1 Ethics Statement

The present study was conducted in strict accordance with the recommendations of the Brazilian National Council for the Control of Animal Experimentation (CONCEA) (Brazilian Ministry of Science, Technology and Innovation) and was approved by the Ethics Committee on Animal Use (CEUA number 019006/17) of the Faculty of Agricultural and Veterinary Sciences, UNESP, Campus Jaboticabal, SP, Brazil.

2.2.2 Experimental population (fish families)

Eight full-sib families of tambaqui were generated by a hierarchical mating scheme using five dams and eight sires. The offspring 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 during eight months at the Laboratory of Genetics in Aquaculture and Conservation (LaGeAC), at the Universidade Estadual Paulista (UNESP), Jaboticabal (São Paulo State, Brazil. Specific details of the biological population, experimental conditions and challenge disease were previously described in Lira *et al*. (2020).

2.2.3 Parasite challenge

The experimental challenge against *I. multifillis* was carried out as described in detail by Lira *et al*. (2020). Briefly, 218 tambaqui juveniles from eight full-sib families were used in the experiment, which as conducted in three glass aquariums (100 l, three-replicate design), with water recirculation system, UV filter, and controlled temperature. After a five-day acclimatization period at 28°C, 12 fish

naturally infested by *I. multifiliis* (called trojans) were incorporated into each aquarium (t_0). Then, the water temperature was maintained at 20°C for two days. Subsequently, two cycles of temperature fluctuation (two days at 28°C and two days at 20°C) were performed to induce parasite infestation. At 192 hours post cohabitation hpc (t_1), all fish were removed from the aquaria and visually analyzed to determine the degree of infestation (measured as the number of white spots on the caudal fin). Later, at 240 hpc (t_2), the temperature was increased to 30°C.

Fish mortality was observed during all day from the first mortality event (~ 230 hpc) to the mortality plateau (254 hpc); and at 8-hour intervals on the remaining challenge days. Time of death and clinical signs were recorded for all fish.

2.2.4 ddADseq library preparation, DNA extraction and sequencing

ddRADseq libraries were prepared by multiplexing individuals following the protocol from Peterson *et al*. (2012). Fin fragments from 12 parents and 189 progenies were used for DNA extraction using the PureLink ™ Genomic DNA Kit according to the manufacter's protocol. The amount of extracted DNA (ng / µl) was measured with Qubit® 3.0 (Thermo Scientific™). The DNA from each individual was digested with two restriction enzymes: *MluCI* (5´...AATT...3´) and *SphI* (5´...GCATGC...3´). For each 2.50 µL of DNA (10 ng/µL), 0.10 µL of *MluCI*, 0.10 µL of *SphI* and 0.60 µL of the CutSmart 10X Buffer were used. The enzymatic digestion process was carried out at 37ºC for 60 min, followed by a 20 min inactivation period at 70ºC. The ligation reaction of the adapters was carried out using the enzyme T4 DNA ligase, incubated at 23°C for 120 min and subsequently at 65°C for 20 min.

To verify the efficacy of the digestion and ligation of the adapters a PCR was performed. PCR was prepared containing 7.90 µL of H2O; 1.25 µL of 10X PCR

Buffer; 0.50 µL of primer P1 (10.00 µM); 0.50 µL of primer P2 (10.00 µM); 0.50 µL dNTPs (10 mM); 0.75 µl MgCl2 (25 mM); 0.10 µl Platinum® Taq DNA Polymerase and 1.00 µL of digested DNA. Each sample was amplified using the following conditions: an initial cycle of 94ºC for 2 min, followed by 30 cycles (94ºC for 30 s, 60ºC for 30 s, 72ºC for 45 s) and cooling to 4ºC. The quality of the amplifications was verified through agarose gel electrophoresis.

After confirming the effectiveness of digestion, a cleaning process was carried out to remove the reagent residues, the short fragments resulting from the digestion of DNA and the connection of the adapters. The Agencourt AMPure XP cleanup step Kit (Beckman Coulter, DE) was used for this process, following the protocol proposed by the manufacturer. These beads were also used to concentrate the DNA and combine the sample pool in a single tube, for each family. This pool was used for fragment selection (approximately 350 bp) by E-Gel® Size Select 2% Agarose Gel (Invitrogen) for electrophoresis.

Then, the enrichment step was performed via PCR amplification with the Phusion® High-Fidelity PCR Kit. This step is necessary to incorporate the Illumina flowcell sequence and the regions of the Illumina primer sequences. To increase the number of DNA fragments, 20 independent PCR amplifications were performed, which combined 13.60 µL of H_2O; 0.40 µL 10 mM dNTPs; 4.00 µL 5X Phusion HF Buffer; 0.50 µL of primer P1 (5.00 µM); 0.5 µL of Primer index (5.00 µM); 0.2 µL Phusion DNA Polymerase and 0.80 µL of digested DNA. The amplification of the reactions occurred in an initial stage of 98 ºC for 30 s, followed by 12 cycles (98 ºC for 15 s, 60 ºC for 30 s, 72 ºC for 30s) and a final stage of 15 ºC. After completing this step, the PCR samples were grouped and purified by AMPure XP beads

(Beckman Coulter, DE), according to the manufacturer's protocol. The amplified sequence pool was analyzed using 2% agarose gel electrophoresis.

The concentration of the final library was verified by fluorometry on Qubit® 3.0 (Thermo Scientific ™). Next, an electrophoresis analysis was performed on an Agilent 2100 Bioanalyzer equipment (Agilent Technologies, USA). Finally, the libraries were sequenced on an Illumina Hiseq 2500 platform.

2.2.5 SNPs identification

The software Stacks v. 2.1 (Catchen *et al*., 2011; Catchen *et al*., 2013) was used to analyze the raw sequences resulting from the Illumina sequencing. The analysis was initiated with the "process_radtags" module, in which the barcodes were assigned to individuals, and low-quality adapters and sequences were excluded. To avoid duplicates problems, we used a loci catalog previously developed for tambaqui and validated by the plataform Axiom SerraSNP array (Affymetrix) (Mastrochirico-Filho, in press), which comprises about 20K polymorphic SNPs mostly discovered by the ddRADseq procedure herein used. Then, the sequences were aligned against the catalog using the Burrows-Wheeler Aligner software (BWA, v0.7.15) with the standard parameters (Li and Durbin, 2009). The mapped regions were used in the identification of genomic variants through the Samtools (Li. *et al*., 2009) and Vcftools (Danecek *et al*., 2011) software, where they were filtered considering the general quality score > 20 and minimum allele count ≥ 4. Posteriorly, the resulting variants were filtered to consider only one SNP per locus, particularly those already validated by the plataform Axiom SerraSNP array (Affymetrix) (Mastrochirico-Filho, in press). The filtered SNPs were present in at least 70% of the individuals of the population (call rate > 0.70).

2.2.6 GWAS

The resistance traits were evaluated using survival status (SS), time of death (TD) and parasite load (PL). The animal models adopted for this study were the same used for the estimation of genetic parameters in Lira *et al*. (2020), 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:

$$Pr(Y|ij) = \Phi\left(\mu + t_i + w_{ij} + a_j\right)$$

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.

All available information were considered for GWAS, including pedigree and phenotype records (n = 201 individuals), in addition to the genotypes. The variance components and genomic breeding value (GEBV) were estimated using ssGBLUP method (Wang *et al.,* 2012), by BLUPF90 family of programs. The kinship matrix used was H (Aguilar *et al.,* 2010), in which genotype and pedigree data are combined. The inverse of the matrix H is:

$$= \mathrm{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & \mathrm{G}^{-1} - \mathrm{A}_{22}^{-1} \end{bmatrix}$$

Where A^{-1} is the inverse numerator relationship matrix for all individuals, A_{22}^{-1} the inverse of a pedigree-based relationship matrix for genotyped individuals, and G^{-1} the inverse genomic relationship matrix.

TD and PL were analyzed as linear traits using AIREMLF90 and BLUPF90 programs, while SS was analyzed as a threshold trait using THRGIBBS1F90. SNPs were considered genome wide significant when exceeded the Bonferroni threshold for multiple testing (alpha = 0.05) of 0.05/*ts*, where *ts* =7,719 (total number of SNPs genome-wide); and chromosome-wide significant by the Bonferroni threshold for multiple testing (alpha = 0.05) 0.05/*nc* where *nc* = 285 (average number of SNPs per chromosome). The Manhattan plot (p-value of individual SNP effects) were designed using the q-qman software.

SNP that exceeds genome and/or chromosome wide Bonferroni threshold were considered as a suggestive QTL associated with resistance to I*. multifiliis* in tambaqui. Sequences containing SNPs associated with resistance were aligned against the tambaqui scaffolds genome (RefSeq GCF_904425465.1) using the BLASTn tool to investigate the potential genes with a role in the immune system within ±500 Kb window.

2.3 Results

2.3.1 ddRAD sequencing

Illumina's sequencing produced a total of 704,158,134 million reads, corresponding to an average of 100 million raw sequences per library (each library represents one family of tambaqui). The variation of reads number between the libraries was 85,871,048 and 119,885,536 (Table 1).

2.3.2 SNPs identification and filtering

After the initial quality control, a total of 432,440 reads were removed from the analysis. In addition, 91,146,660 ambiguous barcodes and 65,096,402 ambiguous RAD-Tags were detected. As a result, 75% of raw reads (528,485,032 reads) were retained for subsequent analysis. Libraries 1, 2 and 6 had the highest percentage of retained reads (90.4%, 88.8% and 87.7% respectively). More details on each library are shown in Table 1. The average reads count per individual was 1,846,660 (SD 906,175). Average read values per individual are seen in Table 2. After all the quality filters, 7,717 SNPs and 119 individuals were used for downstream analyzes.

2.3.3 Genome-wide association analysis and candidate genes

Heritability estimates obtained with genomic information were high and significant for SS and TD (0.51± 016 e 0.50 ± 0.16, respectively), however for PL were not significant (0.20 ± 0.12).

The genome-wide association study using 7,717 SNPs on 119 tambaqui individuals for resistance against *I. multifiliis* revealed no major QTL regions that reached the genome-wide significance threshold (Fig. 1). However, four suggestive QTLs were detected in four linkage groups (2, 9, 11 and 20) that crossed the chromosome-wide Bonferroni threshold (Fig. 1B and 1C): three corresponded to TD (AX-508796522, AX-508808623, AX-508808623) and one to PL (AX-508793455). The SNPs with the highest P-value for both traits explained 0.31% and 0.38% of the genetic variance for TD and PL, respectively. No significant SNP was found for SS (Fig. 1A).

For TD, the main three SNP were located on LGs 2 (AX-508795213), 11 (AX-508808623) and 20 (AX-508796522). The sum of these SNPs explained 0.62% of the genetic variance (Table 3 and Fig. 1B). For PL, only one SNP was found, which was located on LG 9 (AX-508793455), representing 0.38% of the genetic variance (Table 3 and Fig. 1C).

The search for candidate genes revealed 11 genes related to the immune response close to the significant SNPs (Table 3). For TD, three genes were located close to the SNP AX-508796522: *atypical chemokine receptor 4b* (*ackr4*; ~32 Kb downstream), *transcriptional activator GLI3* (*gli3*; ~365 Kb downstream) and *C-C chemokine receptor type 9* (*ccr9*; ~447 Kb downstream); two genes were identified associated to the SNP AX-508808623: *nodal-related 2* (*ndr2*; ~5 Kb downstream) and *TBATA* (*tbata*; ~433 Kb downstream); and four genes to the SNP AX-508795213: *non-homologous end-joining factor 1* (*nhej1*; ~198 Kb upstream), *transforming growth factor beta receptor type 3* (*tgfbr3*; ~379 Kb downstream), *claudin-10a* (*cldn10a*; ~393 Kb upstream) and *zinc finger protein 644* (*znf644b*; ~470 Kb downstream). For PL, two genes were close to the SNP AX-508793455: *ATP-binding cassette sub-family F member 3* (*abcf3*; ~462Kb upstream) and *zinc finger protein 830* (*znf830*; ~356Kb upstream).

2.4 Discussion

Our results of genomic heritability values confirmed the initial data registered for the same fish population using pedigree-based heritability (Lira *et al*., 2020). Both studies were also similar to that reported by Jaafar *et al.* (2020), where high heritability for survival trait was obtained in rainbow trout for resistance against *I. multifiliis* (0.58 ± 0.04). Therefore, the moderate to high heritability values obtained with both pedigree (Lira *et al*., 2020) and genomics data (present study) implies that

resistance to *I. multifiliis* in tambaqui can be increased through selective breeding, since the selection response is highly dependent on heritability (Ødegård *et al*., 2014).

The GWAS results of the present study suggest that resistance to *I. multifiliis* exhibits a polygenic architecture in tambaqui, similarly to the pattern also reported for other fish parasites (Tsai *et al*., 2016; Correa *et al*., 2017a; Robledo *et al*., 2018; Aslam *et al*., 2020a; Uchino *et al*., 2020). The absence of major QTLs indicates that marker-assisted selection is probably not the most efficient approach to improve genetic resistance against this parasite in tambaqui, unlike the results observed in trout by a similar GWAS analysis (Jaafar *et al*., 2020). Marker-assisted selection have been proposed as a viable approach for trout breeding due to the low number of QTLs (presented in two chromosomes) that explains a substantial part of genetic variance involved in *I. multifiliis* resistance (Jaafar *et al*., 2020). In the tambaqui case, genomic selection is a better alternative that might be tested to improve the accuracy of breeding values compared to traditional pedigree-based selection (Fraslin *et al*., 2020).

In general, the analysis of candidate genes associated to the significant SNPs demonstrated some genes of known function in the immune system, such as those already described in the involvement of the fish defense against parasitic infestation (Skugor *et al*., 2008; Ronza *et al*., 2016; Yang *et al*., 2017; Bai *et al*., 2020; Syahputra *et al*., 2020). The AX-508795213 was located in a region that contains genes associated with chemokines (*ackr4, ccr9* and *gli3*), which participates from leukocyte migration, and the differentiation of recruited cells under inflammatory conditions (Liu *et al.,* 2009; Ulvmar *et al*., 2011; Nibbs and Graham, 2013; Bird and Tafalla, 2015). *ackr4* (*Atypical chemokine receptor 4*) and *ccr9* (*G protein coupled*

chemokine receptor 9) are chemokine receptors (Liu *et al*., 2009; Bird and Tafalla, 2015) with known relevance in fish immune response against pathogens (Galindo-Villegas *et al*. 2013; Aquilino *et al.,* 2014; Azeredo *et al*., 2015; Qi *et al*., 2017; Tian *et al*., 2017; Yang *et al*., 2017; Fu *et al*., 2017; Choi *et al*., 2019). These proteins have a strong chemotactic capacity and a regulatory role on antigen presenting cells, while also attracting un-activated macrophages, and naïve B lymphocytes (Aquilino *et al.,* 2016; Tian *et al*., 2017). Meanwhile *gli3* is a transcriptional activator and though no current studies have assessed its role in fish immunity, recently it was corroborated that is a novel target of Toll-like receptors (TLRs) signaling pathway in mammals (Matissek and Elsawa, 2020; Matissek *et al*., 2021). TLRs are a member of pattern-recognition receptors (PRRs), they recognize pathogen-associated molecular patterns (PAMPs) of pathogens and initiate the innate immune responses (Ye *et al.*, 2020). These genes may play a role in resistance against *I. multifiliis* in tambaqui by mounting the first stages of innate and adaptive immunity through improved parasite recognition and cellular responses. In addition, chemokines have showed to have a lethal effect on *I. multifiliis* theronts, causing disruption of membranes and loss of cilia (Muñoz-Atienza *et al*., 2019). Higher chemokine responses from natural resistant fish could help to decrease parasite load, though this remains to be elucidated.

The gene *ndr2*, located at around 5 Kb distance from the SNP AX-508808623, is a cytoplasmic kinase that regulates morphological cellular changes, cellular proliferation and apoptosis. In mammals, it plays an important role in innate immunity including inflammation responses, particularly against viruses (Liu *et al.,* 2019; Ye *et al.,* 2020). The gene *tgfbr3* (near to the SNP AX-508795213) belongs to a superfamily that usually exerts pleiotropic actions and mainly acts morphogens

and cytokines (Naka and Hirao, 2017; Tamayo *et al*., 2018). *tgfbr3* is considered a co-receptor by binding several TGF-beta superfamily members (such as the *tgfb3*). GWAS studies for natural resistance to *Cryptocaryon irritans* in large yellow croaker *Larimichthys crocea* and *Edwardsiella ictaluri* in channel catfish also identified *tgfb3* as a candidate gene associated with host resistance (Zhou *et al*., 2017; Zhao *et al*., 2021b). Moreover, transcriptome analysis in rainbow trout gills infested by *I. multifiliis* showed down-regulation of the *tgfbr3* expression (Syahputra *et al*., 2020), confirming its participation in the immune response against this parasite.

In conclusion, our results have demonstrated that resistance to *I. multifiliis* is under strong genetic control (high heritability) and this trait has a polygenic architecture. In the present study, we have detected QTLs involved in *I. multifiliis* resistance contributing to a low proportion of the variance in tambaqui. Candidate genes have been also identified close to the associated SNPs, mainly chemokines (*ackr4* and *ccr9*) and *tgfb3* gene, which suggests they could be involved in the natural resistance of tambaqui against *I. multifiliis* infestation.

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2.6 References

Aguilar, I., Misztal, I., Johnson, D.L., Legarra, A., Tsuruta, S., Lawlor, T.J., 2010. Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. J. Dairy Sci. 93, 743–752. https://doi.org/10.3168/jds.2009-2730

Ahmad, F., Debes, P. V., Palomar, G., Vasemägi, A., 2018. Association mapping reveals candidate loci for resistance and anaemic response to an emerging temperature-driven parasitic disease in a wild salmonid fish. Mol. Ecol. 27, 1385–1401. https://doi.org/10.1111/mec.14509

Aquilino, C., Castro, R., Fischer, U., Tafalla, C., 2014. Transcriptomic responses in rainbow trout gills upon infection with viral hemorrhagic septicemia virus (VHSV). Dev. Comp. Immunol. 44, 12–20. https://doi.org/10.1016/j.dci.2013.11.006

Aquilino, C., Granja, A.G., Castro, R., Wang, T., Abos, B., Parra, D., Secombes, C.J., Tafalla, C., 2016. Rainbow trout CK9, a CCL25-like ancient chemokine that attracts and regulates B cells and macrophages, the main antigen presenting cells in fish. Oncotarget 7, 17547–17564. https://doi.org/10.18632/oncotarget.8163

Aslam, M.L., Carraro, R., Sonesson, A.K., Meuwissen, T., Tsigenopoulos, C.S., Rigos, G., Bargelloni, L., Tzokas, K., 2020a. Genetic Variation, GWAS and Accuracy of Prediction for Host Resistance to *Sparicotyle chrysophrii* in Farmed Gilthead Sea Bream (*Sparus aurata*). Front. Genet. 11, 1–14. https://doi.org/10.3389/fgene.2020.594770

Aslam, M.L., Boison, S.A., Lillehammer, M., Norris, A., Gjerde, B., 2020b. Genome-wide association mapping and accuracy of predictions for amoebic gill disease in Atlantic salmon (*Salmo salar*). Sci. Rep. 10, 1–9. https://doi.org/10.1038/s41598-020-63423-8

Azeredo, R., Pérez-Sánchez, J., Sitjà-Bobadilla, A., Fouz, B., Tort, L., Aragão, C., Oliva-Teles, A., Costas, B., 2015. European sea bass (*Dicentrarchus labrax*) immune status and disease resistance are impaired by Arginine dietary supplementation. PLoS One 10, 1–19. https://doi.org/10.1371/journal.pone.0139967

Bai, H., Zhou, T., Zhao, J., Chen, B., Pu, F., Bai, Y., Wu, Y., Chen, L., Shi, Y., Ke, Q., Yu, X., Xu, P., 2020. Transcriptome analysis reveals the temporal gene expression patterns in skin of large yellow croaker (*Larimichthys crocea*) in response to *Cryptocaryon irritans* infection. Fish Shellfish Immunol. 99, 462–472. https://doi.org/10.1016/j.fsi.2020.02.024

Bird, S., Tafalla, C., 2015. Teleost chemokines and their receptors. Biology (Basel). 4, 756–784. https://doi.org/10.3390/biology4040756

Boison, S.A., Gjerde, B., Hillestad, B., Makvandi-Nejad, S., Moghadam, H.K., 2019. Genomic and transcriptomic analysis of amoebic gill disease resistance in Atlantic salmon (*salmo salar L.*). Front. Genet. 10. https://doi.org/10.3389/fgene.2019.00068

Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W., Postlethwait, J.H., 2011. Stacks : Building and Genotyping Loci De Novo From Short-Read Sequences. Genes\ Genomes\Genetics 1, 171–182. https://doi.org/10.1534/g3.111.000240

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., 2013. Stacks : an analysis tool set for population genomics. Mol. Ecol. 3124–3140. https://doi.org/10.1111/mec.12354

Choi, K.M., Hwang, S.D., Joo, M.S., Hwang, J.Y., Kwon, M.G., Jeong, J.M., Seo, J.S., Jee, B.Y., Park, C. Il, 2019. The atypical chemokine receptor 4 in red sea bream (*Pagrus major*): Molecular characterization and gene expression analysis. Fish Shellfish Immunol. 93, 50–54. https://doi.org/10.1016/j.fsi.2019.07.004

Corradin, O., Saiakhova, A., Akhtar-Zaidi, B., Myeroff, L., Willis, J., Cowper-Sallari, R., Lupien, M., Markowitz, S., Scacheri, P.C., 2014. Combinatorial effects of multiple enhancer variants in linkage disequilibrium dictate levels of gene expression to confer susceptibility to common traits. Genome Res. 24, 1–13. https://doi.org/10.1101/gr.164079.113

Correa, K., Lhorente, J.P., Bassini, L., López, M.E., Di Genova, A., Maass, A., Davidson, W.S., Yáñez, J.M., 2017a. Genome wide association study for resistance to *Caligus rogercresseyi* in Atlantic salmon (*Salmo salar* L.) using a 50K SNP genotyping array. Aquaculture 472, 61–65. https://doi.org/10.1016/j.aquaculture.2016.04.008

Correa K, Bangera R, Figueroa R, Lhorente JP, Yáñez JM. 2017b. The use of genomic information increases the accuracy of breeding value predictions for sea louse (*Caligus rogercresseyi*) resistance in Atlantic salmon (*Salmo salar*). Genet Sel Evol. https://doi.org/10.1186/s12711-017-0291-8.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R.,

2011. The variant call format and VCFtools. Bioinformatics 27, 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Dickerson, H.W., Findly, R.C., 2014. Immunity to Ichthyophthirius infections in fish: A synopsis. Dev. Comp. Immunol. 43, 290–299. https://doi.org/10.1016/j.dci.2013.06.004

Dickerson, H.W., Findly, R.C., 2017. Vertebrate adaptive immunity-comparative insights from a teleost model. Front. Immunol. 8, 1–7. https://doi.org/10.3389/fimmu.2017.01379

Elaswad, A., Dunham, R., 2018. Disease reduction in aquaculture with genetic and genomic technology: current and future approaches. Rev. Aquac. 10, 876–898. https://doi.org/10.1111/raq.12205

Fraslin, C., Quillet, E., Rochat, T., Dechamp, N., Bernardet, J.F., Collet, B., Lallias, D., Boudinot, P., 2020. Combining Multiple Approaches and Models to Dissect the Genetic Architecture of Resistance to Infections in Fish. Front. Genet. 11. https://doi.org/10.3389/fgene.2020.00677

Francis-Floyd, R., Yanong, R., Pouder, D., 2016. *Ichthyophthirius multifiliis* (white spot) infections in fish. (accessed 10 april 2019). https://edis.ifas.ufl.edu/pdffiles/ FA00600.pdf.

Fu, Q., Yang, Y., Li, C., Zeng, Q., Zhou, T., Li, N., Liu, Y., Liu, S., Liu, Z., 2017. The CC and CXC chemokine receptors in channel catfish (*Ictalurus punctatus*) and their involvement in disease and hypoxia responses. Dev. Comp. Immunol. 77, 241–251. https://doi.org/10.1016/j.dci.2017.08.012

Galindo-Villegas, J., Mulero, I., García-Alcazar, A., Muñoz, I., Peñalver-Mellado, M., Streitenberger, S., Scapigliati, G., Meseguer, J., Mulero, V., 2013. Recombinant TNFα as oral vaccine adjuvant protects European sea bass against vibriosis: Insights into the role of the CCL25/CCR9 axis. Fish Shellfish Immunol. 35, 1260–1271. https://doi.org/10.1016/j.fsi.2013.07.046

Goulding, M., Carvalho, M.L.,1982. Life history and management of the tambaqui (*Colossoma macropomum*, characidae); an important amazonian food fish. Rev. Bras. Biol. 1, 193–221

IBGE, 2020. Produção da pecuária municipal 2017. Inst. Bras. Geogr. Estat. (doi:ISSN 0101-4234).

Jaafar, R., Ødegård, J., Mathiessen, H., Karami, A.M., Marana, M.H., von Gersdorff Jørgensen, L., Zuo, S., Nielsen, T., Kania, P.W., Buchmann, K., 2020. Quantitative trait loci (QTL) associated with resistance of rainbow trout *Oncorhynchus mykiss* against the parasitic ciliate *Ichthyophthirius multifiliis*. J. Fish Dis. 43, 1591–1602. https://doi.org/10.1111/jfd.13264

Kong, S., Ke, Q., Chen, L., Zhou, Z., Pu, F., Zhao, J., Bai, H., Peng, W., Xu, P., 2019. Constructing a High-Density Genetic Linkage Map for Large Yellow Croaker (*Larimichthys crocea*) and Mapping Resistance Trait Against Ciliate Parasite *Cryptocaryon irritans*. Mar. Biotechnol. 21, 262–275. https://doi.org/10.1007/s10126-019-09878-x

Lhorente, J.P., Araneda, M., Neira, R., Yáñez, J.M., 2019. Advances in genetic improvement for salmon and trout aquaculture: the Chilean situation and prospects. Rev. Aquac. 11, 340–353. https://doi.org/10.1111/raq.12335

Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Lira, L.V.G., Ariede, R.B., Freitas, M. V, Mastrochirico-filho, V.A., Agudelo, J.F.G., Barría, A., Yáñez, J.M., Hashimoto, D.T., 2020. Quantitative genetic variation for resistance to the parasite *Ichthyophthirius multifiliis* in the Neotropical fi sh tambaqui (*Colossoma macropomum*). Aquac. Reports 17, 100338. https://doi.org/10.1016/j.aqrep.2020.100338

Liu, Y., Chang, M.X., Wu, S.G., Nie, P., 2009. Characterization of C-C chemokine receptor subfamily in teleost fish. Mol. Immunol. 46, 498–504. https://doi.org/10.1016/j.molimm.2008.10.003

Liu, Z., Wu, C., Pan, Y., Liu, H., Wang, Xiumei, Yang, Y., Gu, M., Zhang, Y., Wang, Xiaojian, 2019. NDR2 promotes the antiviral immune response via facilitating TRIM25-mediated RIG-I activation in macrophages. Sci. Adv. 5, 1–14. https://doi.org/10.1126/sciadv.aav0163

Martins, M.L., Onaka, E.M., Moraes, F.R., Bozzo, F.R., Paiva, a. M.F.C., Gonçalves, a., 2002. Recent studies on parasitic infections of freshwater cultivated fish in the State of São Paulo, Brazil. Acta Sci. 24, 981–985.

Matissek, S.J., Elsawa, S.F., 2020. GLI3: A mediator of genetic diseases, development and cancer. Cell Commun. Signal. 18, 1–20. https://doi.org/10.1186/s12964-020-00540-x

Matissek, S.J., Han , W., Hage, A., Karbalivand, M., Rajsbaum , R., Elsawa, S.F., 2021. A novel mechanism of regulation of the transcription factor GLI3 by toll-like receptor signaling. J. Chem. Inf. Model. 53, 21–25. https://doi.org/10.1101/2021.01.01.424866

Matthews, R.A., 2005. *Ichthyophthirius multifiliis* fouquet and ichthyophthiriosis in freshwater teleosts. Adv. Parasitol. 59, 159–241. https://doi.org/10.1016/S0065-308X(05)59003-1

Muñoz-Atienza, E., Aquilino, C., Syahputra, K., Al-Jubury, A., Araújo, C., Skov, J., Tafalla, C. (2019). CK11, a teleost chemokine with a potent antimicrobial activity. Journal of Immunology, 202, 857–870. https:// doi.org/10.4049/jimmu nol.1800568

Naka, K., Hirao, A., 2017. Regulation of hematopoiesis and hematological disease by TGF-β family signaling molecules. Cold Spring Harb. Perspect. Biol. 9, 25. https://doi.org/10.1101/cshperspect.a027987

Nibbs, R.J.B., Graham, G.J., 2013. Immune regulation by atypical chemokine receptors. Nat. Rev. Immunol. 13, 815–829. https://doi.org/10.1038/nri3544

Ødegård, J., Moen, T., Santi, N., Korsvoll, S.A., Kjøglum, S., Meuwisse, T.H.E., 2014. Genomic prediction in an admixed population of Atlantic salmon (Salmo salar). Front. Genet. https://doi.org/10.3389/fgene.2014.00402

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7, e37135. https://doi.org/10.1371/journal.pone.0037135

Qi, Z., Holland, J.W., Jiang, Y., Secombes, C.J., Nie, P., Wang, T., 2017. Molecular characterization and expression analysis of four fish-specific CC chemokine receptors CCR4La, CCR4Lc1, CCR4Lc2 and CCR11 in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 68, 411–427. https://doi.org/10.1016/j.fsi.2017.07.031

Robledo, D., Matika, O., Hamilton, A., Houston, R.D., 2018. Genome-Wide Association and Genomic Selection for Resistance to Amoebic Gill Disease in Atlantic Salmon 8, 1195–1203. https://doi.org/10.1534/g3.118.200075

Robledo, D., Gutiérrez, A.P., Barría, A., Lhorente, J.P., Houston, R.D., Yáñez, J.M., 2019. Discovery and functional annotation of quantitative trait loci affecting resistance to Sea lice in Atlantic salmon. Front. Genet. 10, 1–10. https://doi.org/10.3389/fgene.2019.00056

Ronza, P., Robledo, D., Bermúdez, R., Losada, A.P., Pardo, B.G., Sitjà-Bobadilla, A., Quiroga, M.I., Martínez, P., 2016. RNA-seq analysis of early enteromyxosis in turbot (*Scophthalmus maximus*): new insights into parasite invasion and immune evasion strategies. Int. J. Parasitol. 46, 507–517. https://doi.org/10.1016/j.ijpara.2016.03.007

Skugor, S., Glover, K.A., Nilsen, F., Krasnov, A., 2008. Local and systemic gene expression responses of Atlantic salmon (*Salmo salar* L.) to infection with the

salmon louse (*Lepeophtheirus salmonis*). BMC Genomics 9, 1–18. https://doi.org/10.1186/1471-2164-9-498

Syahputra, K., Kania, P.W., Al-Jubury, A., Marnis, H., Mathiessen, H., Dirks, R.P., Buchmann, K., 2020. Association between stress, metabolism, and growth in *Ichthyophthirius multifiliis* infected rainbow trout gills: Transcriptomic evidence. Aquaculture 526, 735384. https://doi.org/10.1016/j.aquaculture.2020.735384

Tamayo, E., Alvarez, P., Merino, R., 2018. TGFβ superfamily members as regulators of B cell development and function—implications for autoimmunity. Int. J. Mol. Sci. 19. https://doi.org/10.3390/ijms19123928

Tavares-dias, M., Laterça Martins, M., 2017. An overall estimation of losses caused by diseases in the Brazilian fish farms. J Parasit Dis 41, 913–918. https://doi.org/10.1007/s12639-017-0938-y

Tian, F., Tong, C., Feng, C., Wanghe, K., Zhao, K., 2017. Transcriptomic profiling of Tibetan highland fish (*Gymnocypris przewalskii*) in response to the infection of parasite ciliate *Ichthyophthirius multifiliis*. Fish Shellfish Immunol. 70, 524–535. https://doi.org/10.1016/j.fsi.2017.09.003

Tsai, H.Y., Hamilton, A., Tinch, A.E., Guy, D.R., Bron, J.E., Taggart, J.B., Gharbi, K., Stear, M., Matika, O., Pong-Wong, R., Bishop, S.C., Houston, R.D., 2016. Genomic prediction of host resistance to sea lice in farmed Atlantic salmon populations. Genet. Sel. Evol. 48, 1–11. https://doi.org/10.1186/s12711-016-0226-9

Uchino, T., Tabata, J., Yoshida, K., Suzuki, T., Noda, T., Fujinami, Y., Ozaki, A., 2020. Novel Benedenia disease resistance QTLs in five F1 families of yellowtail (*Seriola quinqueradiata*). Aquaculture 529, 735622. https://doi.org/10.1016/j.aquaculture.2020.735622

Ulvmar, M.H., Hub, E., Rot, A., 2011. Atypical chemokine receptors. Exp. Cell Res. 317, 556–568. https://doi.org/10.1016/j.yexcr.2011.01.012

Valladão, G.M.R., Gallani, S.U., Pilarski, F., 2018. South American fish for continental aquaculture. Rev. Aquac. 10, 351–369. https://doi.org/10.1111/raq.12164

Wang, H., Misztal, I., Aguilar, I., Legarra, A., Muir, W.M., 2012. Genome-wide association mapping including phenotypes from relatives without genotypes. Genet. Res. (Camb). 94, 73–83. https://doi.org/10.1017/S0016672312000274

Wang, Q., Yu, Y., Zhang, X., Xu, Z., 2019. Immune responses of fish to *Ichthyophthirius multifiliis* (Ich): A model for understanding immunity against protozoan parasites. Dev. Comp. Immunol. 93, 93–102. https://doi.org/10.1016/j.dci.2019.01.002

Wenne, R., 2018. Single nucleotide polymorphism markers with applications in aquaculture and assessment of its impact on natural populations. Aquat. Living Resour. 31. https://doi.org/10.1051/alr/2017043

Woynárovich, A., Van Anrooy, R., 2019. Field guide to the culture of tambaqui (*Colossoma macropomum)*, FAO Fisheries and Aquaculture Technical Paper. No. 624. Rome, FAO.132 pp. ISSN 2070-7010

Yáñez, J.M., Houston, R.D., Newman, S., 2014. Genetics and genomics of disease resistance in salmonid species. Front. Genet. 5, 1–13. https://doi.org/10.3389/fgene.2014.00415

Yang, M., Zhou, L., Wang, H.Q., Luo, X.C., Dan, X.M., Li, Y.W., 2017. Molecular cloning and expression analysis of CCL25 and its receptor CCR9s from *Epinephelus coioides* post *Cryptocaryon irritans* infection. Fish Shellfish Immunol. 67, 402–410. https://doi.org/10.1016/j.fsi.2017.06.039

Ye, X., Ong, N., An, H., Zheng, Y., 2020. The Emerging Roles of NDR1/2 in Infection and Inflammation. Front. Immunol. 11, 1–7. https://doi.org/10.3389/fimmu.2020.00534

Zhao, J., Zhou, T., Bai, H., Ke, Q., Li, B., Bai, M., Zhou, Z., Pu, F., Zheng, W., Xu, P., 2021a. Genome-Wide Association Analysis Reveals the Genetic Architecture of Parasite (*Cryptocaryon irritans*) Resistance in Large Yellow Croaker (*Larimichthys crocea*). Mar. Biotechnol. https://doi.org/10.1007/s10126-021-10019-6

Zhao, J., Bai, H., Ke, Q., Li, B., Zhou, Z., Wang, H., Chen, B., Pu, F., Zhou, T., Xu, P., 2021b. Genomic selection for parasitic ciliate *Cryptocaryon irritans* resistance in large yellow croaker. Aquaculture 531, 735786. https://doi.org/10.1016/j.aquaculture.2020.735786

Zhou, T., Liu, S., Geng, X., Jin, Y., Jiang, C., Bao, L., Yao, J., Zhang, Y., Zhang, J., Sun, L., Wang, X., Li, N., Tan, S., Liu, Z., 2017. GWAS analysis of QTL for enteric septicemia of catfish and their involved genes suggest evolutionary

conservation of a molecular mechanism of disease resistance. Mol. Genet. Genomics 292, 231–242. https://doi.org/10.1007/s00438-016-1269-x

Tables

Table 1. Number of reads in each library before and after quality control in *Process_radtags*

Library	*Total Paired-end Reads*	*Ambiguos Barcodes*	Low quality	*Ambiguos RAD-Tags*	*Remaining Paired-end Reads*	*Retained reads* (%)
1	102.622.108	125.180	78.087	7.194.855	92.778.787	90,4
2	102.081.308	126.984	74.569	8.392.645	90.606.122	88,8
3	103.739.498	22.061.804	43.032	15.672.556	62.696.441	60,4
4	85.871.048	19.369.712	56.093	5.331.939	58.325.888	67,9
5	119.885.536	27.416.368	65.822	9.655.177	79.809.296	66,6
6	99.613.032	159.946	69.910	9.916.000	87.329.606	87,7
7	90.345.604	21.886.666	44.927	8.933.230	56.938.892	63,0

Table 2. Values of mean and standard deviation (SD) of reads per individual of challenged tambaqui (*Colossoma macropomum*) families submitted to ddRAD-seq analyzes

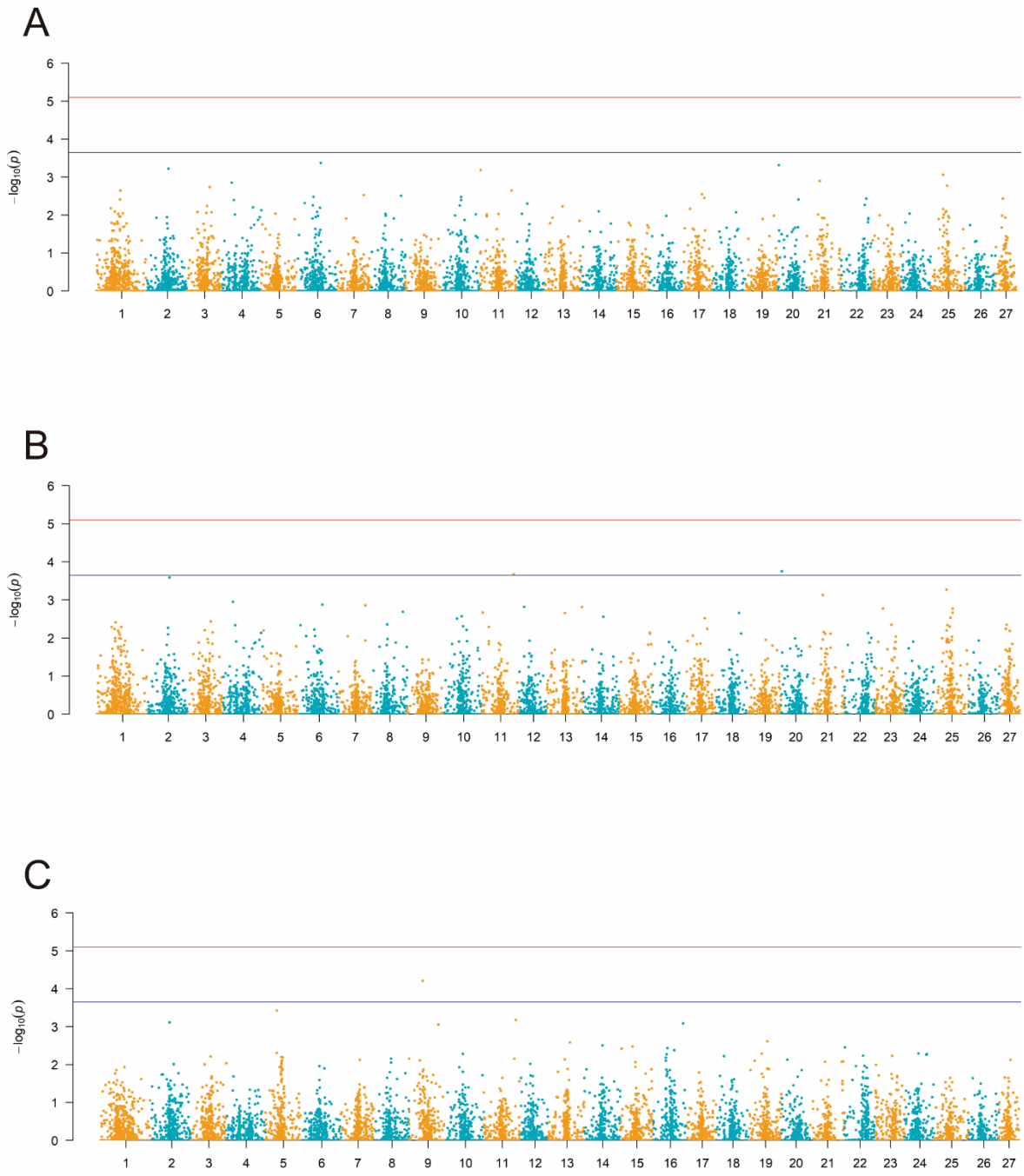
Library	*Reads* (average)	*Reads* (SD)
1	1832674	507327
2	1903672	353965
3	1828929	1001765
4	1630107	950319
5	2258740	1329766
6	1856023	486283
7	1617828	973627

Table 3. The single-nucleotide polymorphisms (SNPs) significantly associated with resistance to *I. multifillis* in tambaqui *Colossoma macropomum*.

SNP ID	Trait	Chr	Pos genoma	Ref	Alt	Var	P-value	Gene (~±500 Kb)
AX-508793455	PL	9	10296733	A	T	0.375	6.22e-05	*abcf3, znf830*
AX-508796522	TD	20	31330555	C	G	0.214	1.77e-04	*ccr9, gli3, ackr4*
AX-508808623	TD	11	3252160	C	G	0.099	2.14e-04	*tbata, ndr2*
AX-508795213	TD	2	20167272	A	T	0.309	2.59e-04	*tgfbr3, nhej1, znf644b, cldn10a*

Figure caption

Figure 1. Manhattan plot of GWAS with p-values distributed across different linkage groups for the traits of survival status (A), Time of death (B) and Parasite load (C). The red line represents the genome-wide while the blue line displays the chromosome-wide significance thresholds.



CONSIDERAÇÕES FINAIS

Ichthyophthirius multifiliis é um protozoário ciliado disseminado mundialmente, que atua como parasita de peixes com baixíssima especificidade de hospedeiro, sendo considerado uma das ameaças mais severas à produção de peixes de água doce. Os tratamentos disponíveis contra esse parasita são caros, laboriosos, estressantes para os peixes e podem poluir o meio ambiente. O melhoramento genético pode ser um método eficiente para controlar doenças infecciosas a longo prazo, devido aos ganhos permanentes e acumulativos. Assim sendo, este trabalho é pioneiro no desenvolvimento de ferramentas genômicas para determinar a resistência natural a *I. multifiliis* no peixe Neotropical tambaqui (*Colossoma macropomum*).

Após o experimento de coabitação, foi obtida variação fenotípica significativa relacionada à resistência à infestação de *I. multifiliis* nas famílias de tambaqui para as características de sobrevivência, tempo de morte e quantidade de parasita, além do mais, os altos valores de herdabilidade baseados no pedigree e nos dados genômico sugerem que o melhoramento seletivo para aumentar a resistência a *I. multifiliis* é plausível.

As associações entre o marcador e características fenotípicas (GWAS) para identificação de regiões genômicas (QTLs) envolvidas na resistência natural à infestação de *I. multifiliis* em tambaqui, evidenciaram a natureza poligênica desta característica, pois trata-se de uma característica tipicamente controlada por vários genes, o que sugere que a seleção genômica é a abordagem mais eficiente para melhorar a resistência genética contra esse parasita em tambaqui.