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9	ORIGINAL ARTICLE / ARTÍCULO ORIGINAL
10	PARASITES OF THE WHITE MULLET, MUGIL CUREMA VALENCIENNES, 1836
11	(MUGILIFORMES: MUGILIDAE), FROM SANTA CATARINA STATE, SOUTHERN BRAZIL
12	PARÁSITOS DE LA LISA BLANCA, MUGIL CUREMA VALENCIENNES, 1836
13	(MUGILIFORMES: MUGILIDAE), DEL ESTADO DE SANTA CATARINA, SUR DE BRASIL
14	
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- 32 Running Head: Parasites of the White mullet
- 33
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- 41 ABSTRACT

This study investigates the parasitic fauna of Mugil curema Valenciennes, 1836 (Mugilidae) from 42 Laguna, southern Santa Catarina, Brazil, being the first parasitological survey of this species in the 43 region. A total of 52 specimens were examined for ecto- and endoparasites. The external examination 44 revealed Ergasilidae specimens and copepod eggs on the gills, while the monogenean Metamicrocotvla 45 46 macracantha (Alexander, 1954) was identified. Internal prospection showed the presence of the 47 acanthocephalan Neoechinorhynchus curemai Noronha, 1973 and one unidentified digenean from the family Haploporidae. Histopathological analysis revealed hepatic lesions and mild inflammation in 48 49 intestinal tissue. These findings underscore the importance of parasites in M. curema, contributing to 50 the ecological understanding of parasitism in marine fish and its potential implications for human 51 health and environmental monitoring.

- 52 Keywords: Environmental indicators Fish health Histopathology Parasitology Southern Brazil
- 53

54 **RESUMEN**

Este estudio investiga la fauna parasitaria de Mugil curema Valenciennes, 1836 (Mugilidae) de 55 56 Laguna, en el sur de Santa Catarina, Brasil, siendo el primer relevamiento parasitológico de esta 57 especie en la región. Se examinaron un total de 52 ejemplares en busca de ecto e endoparásitos. El 58 examen externo reveló la presencia de ejemplares de Ergasilidae y huevos de copépodos en las 59 branquias, mientras que se identificó el monogeneo Metamicrocotvla macracantha (Alexander, 1954). La prospección interna mostró la presencia de acanthocephala Neoechinorhynchus curemai Noronha, 60 61 1973 y de un digeneo no identificado de la familia Haploporidae. El análisis histopatológico reveló lesiones hepáticas e inflamación leve en el tejido intestinal. Estos hallazgos resaltan la importancia de 62 los parásitos en M. curema, contribuyendo a la comprensión ecológica del parasitismo en peces 63 64 marinos y sus posibles implicancias para la salud humana y el monitoreo ambiental.

65 Palabras clave: Indicadores ambientales – Salud de los peces – Histopatología – Parasitología – Sur
66 de Brasil

67

68 INTRODUCTION

The family Mugilidae Jarocki, 1822 consists of 20 genera and 78 valid species (Froese & Pauly, 2024), with a wide geographic distribution, occurring in tropical and subtropical regions worldwide, particularly along coastal areas and estuaries (Falkenberg *et al.*, 2021). In Brazil, the only genus of this family recorded is *Mugil* L., 1758, which includes six species: *Mugil brevirostris* Miranda-Ribeiro, 1915; *Mugil curema* Valenciennes, 1836; *Mugil curvidens* Valenciennes, 1836; *Mugil incilis* Hancock, 1830; *Mugil liza* Valenciennes, 1836; and *Mugil rubrioculus* Harrison, Nirchio, Oliveira, Ron & Gaviria, 2007 (Fischer *et al.*, 2011; Nascimento *et al.*, 2022).

According to the FAO (2022), mugilids are among the most produced fish in global coastal and marine aquaculture, ranking just behind two species: Atlantic salmon, *Salmo salar* L., 1758 (Salmoniformes: Salmonidae) and milkfish, *Chanos chanos* Forsskål, 1775 (Gonorynchiformes: Chanidae). The production of farmed mugilids reached 291.2 thousand tons in 2020, marking a 125%
increase over the previous five years (FAO, 2022). Brazil is recognized as one of the largest producers
of mullet worldwide (FAO, 2015; Santos *et al.*, 2021).

Among the species found in the country, *M. curema* stands out due to its high demand, reflected not only in fishing statistics but also in its significance as a farmed species. This species displays pelagic behavior and is found in a variety of habitats, including sandy shores, coastal pools, brackish lagoons, estuaries, and muddy bottoms (Moutinho & Alves, 2014). In its natural environment, *M. curema* has a diverse diet, feeding on algae, particularly diatoms, and zooplankton.

Fish parasites represent an important component of aquatic biodiversity, as fish are directly affected by their environment or indirectly through their hosts (Mehana *et al.*, 2020). These parasites are notably diverse and abundant compared to other vertebrate classes, likely due to evolutionary processes that have fostered a close association with a wide range of invertebrates over time (Dezfuli & Scholz, 2022). Understanding the biological dynamics of marine populations and species is closely linked to the study of evolutionary processes that govern the history of biodiversity (Broglia & Kapel, 2011).

The family Mugilidae, with its broad distribution and diverse species, plays a significant role 94 in both natural ecosystems and global aquaculture. Additionally, the study of fish parasites provides 95 96 valuable insights into aquatic biodiversity, as evidenced by various studies on Mugil species, which have revealed a wide range of parasites. This diversity was summarized by Falkenberg et al. (2022), 97 98 who highlighted that the most extensively studied regions in Brazil are the Northeast and Southeast. 99 In this context, the present study aims to assess the occurrence of parasites in *M. curema*, representing 100 the first parasitological investigation of this species in Laguna, a municipality in southern Santa 101 Catarina, where fishing and fish consumption hold great economic and historical importance.

102

103 MATERIAL AND METHODS

104 Host specimens' collection

105 The study was conducted in the municipality of Laguna (28° 28' 57" S, 48° 46' 53" W), located 106 in the southern state of Santa Catarina (Figure 1). With a population of just over 50,000 inhabitants, 107 the region's main economic activities are fishing, commerce, and tourism. A total of 52 *M. curema* 108 specimens were studied, all commercially sold at the Tesoura Beach, a small stretch of beach 109 approximately 150 meters long, situated within the Barra Beach Mole.

These specimens, which had an average length of 40±55 cm and an average weight of 150±380 grams, were purchased from local fishermen between November 2019 and March 2020. After being kept refrigerated, the specimens were transported to the Zoology Laboratory at the State University of Santa Catarina (UDESC), located in Laguna Municipality.

114

115 Parasitological analyses

External and internal examinations were conducted at the Microbiology and Parasitology Laboratory at the Department of Fisheries Engineering and Biological Sciences, University of the State of Santa Catarina (UDESC). The inspection began with the oral, nasal, and opercular cavities. Skin mucus samples were then scraped using a microscope slide, and the contents were analyzed under a Nikon Eclipse optical microscope. While, the gills were removed through a lateral incision of the opercula and examined fresh under an Olympus SZ61 stereomicroscope.

In addition, a ventral incision was made from the operculum to near the anus to expose the abdominal cavity, muscle tissue fragments were placed in Petri dishes and examined under the same stereomicroscope for the detection of zoonotic nematodes. The intestinal content was also transferred to Petri dishes and inspected for acanthocephalans, nematodes, cestodes and trematodes.

126

127 **Preparation and identification of the helminthofauna**

128 Acanthocephalans and nematodes were fixed in AFA solution (70% ethanol, 5% formalin, and 2% glacial acetic acid), then preserved in 70% ethanol for later identification. Trematodes were stained 129 130 following the protocol by Amato et al. (1991), and their identification was based on internal parasite 131 structures, observed using a Nikon Eclipse optical microscope coupled with a Canon EOS R8 camera. 132 For species-level identification of the Monogenea specimen, we followed the studies of Hendrix (1994) and Kohn et al. (1994). The identification of Acanthocephala was based on the work of Martins 133 134 et al. (2000), while the classification of the Trematoda family followed the studies of Thatcher (1978) and Andres et al. (2018). All parasites are deposited in the didactic collection of the Laboratory of 135 136 Zoology and Parasitology at the Universidade do Planalto Catarinense (UNIPLAC), in Lages, Santa 137 Catarina, Brazil.

138

139 Histopathological analyses

Following standard histopathological procedures, liver and intestinal samples were collected and preserved in formalin (10%), prepared with destilated water, for these analyses. These organs were subjected to dehydration in a graded ethanol series, clarified in xylene, and embedded in paraffin at 60°C. Tissue sections were stained with Harris hematoxylin and eosin (HHE) for subsequent examination under a Nikon Eclipse optical microscope. All histopathological analysis was conducted at the Pathology Laboratory of the Department of Veterinary Medicine at the University of the State of Santa Catarina (UDESC), Lages, Santa Catarina, Brazil.

Ethis acpects: The fish were obtained directly from the fishermen at their beach sales. Ethics approval
Not applicable.

149 **RESULTS**

150 Parasites' identification

151 After completing all the processes described above, a total of 18 *M. curema* specimens were 152 found to be parasitized. Among these, copepod eggs were observed in the gills of nine individuals, along with two unidentified adult specimens of Ergasilidae (Copepoda: Cyclopoida). In another *M. curema* specimen, a digenetic trematode of the family Haploporidae Nicoll, 1914 (Platyhelminthes:
Trematoda) was found, a family commonly associated with fish of the genus *Mugil*. Additionally, in
the gills of one *M. curema* specimen, *Metamicrocotyla macracantha* (Alexander, 1954) (Monogenea:
Mazocraeidea: Microcotylidae) was identified (Figure 2). Furthermore, three specimens of *Neoechinorhynchus curemai* Noronha, 1973 (Acanthocephala: Neoechinorhynchidae) were recovered
from the intestines examined.

The species *M. macracantha* is characterized by an elongated, lanceolate body, measuring 15.2 160 161 mm in length and 4 mm in width, dorsoventrally flattened and tapering anteriorly. The apical glands 162 have ducts extending up to the level of the pharynx. The buccal opening is median, subterminal, and 163 ventral, located between the anterior extremity and the pharynx. It has two latero-median suckers (0.7 mm in length \times 0.6 mm in width), situated between the buccal opening and the pharynx. The intestinal 164 ceca are long. The haptor measures 1.8 mm in length and 1.3 mm in width, featuring 21 pairs of clamps 165 166 arranged in two longitudinal rows along the body. The clamps are composed of dark structures (sclerites). The genital opening is median and ventral, located at the level of the cecal bifurcation, and 167 armed with atrial spines (0.6 mm in length \times 0.1 mm in width), organized into two bilateral groups 168 arranged in two parallel rows (six anterior and eleven posterior). The testes are elongated and numerous 169 170 (3.3 mm in length \times 1.7 mm in width). The vitelline gland extends from the cecal bifurcation to the anterior end of the haptor. Eggs were not observed. While, the species N. curemai, which is host-171 specific, is characterized by a cylindrical trunk that is wider in the anterior third, a short proboscis 172 173 armed with two large hooks and three circles of smaller hooks, and elliptical eggs, consistent with the 174 specimens examined in this study.

175 Although the study involved the identification of several parasites, high-quality images are 176 available only for *M. macracantha*. Images of the other listed parasites were not obtained due to limitations in the collection process or viewing conditions. However, morphological descriptions and
bibliographic references were rigorously followed, ensuring the accuracy of the identification.

179

180 Histopathological analyses

The liver samples with light-colored spots and ulcerative lesions were analyzed and histopathologically revealed the presence of larval migration (Figure 3A) and areas of moderate to severe, diffuse hepatocellular degeneration. In the intestinal samples, the presence of parasites was observed in the submucosa, surrounded by a mild inflammatory reaction, with a predominance of macrophages (Figure 3B). Unfortunately, it was not possible to identify the causative agent of the lesions described above, requiring further studies.

187

188 **DISCUSSION**

Fish parasites, especially those in marine environments, can cause significant economic losses and pose public health concerns. Despite this, specific studies on parasite diversity, particularly nematodes in marine fish from the Americas, are scarce, with most research focusing on isolated cases of particular species (Pereira & González-Solís, 2022). In the present study, the absence of ectoparasites on the body surface may be attributed to the capture method, as the friction between the fish and the net could dislodge these parasites. This hypothesis is supported by the finding of parasites in the gills, an area protected from such mechanical effects.

While gill parasites typically exhibit low diversity and density, their interactions are often reported as isolated incidents. However, the coexistence of monogeneans on gills is well documented, with some studies, such as this one, reporting the presence of copepods alongside monogeneans (Baker *et al.*, 2005). For *M. curema*, the only record of coinfection by Monogenea and ergasilids on the gills of this species in Brazil comes from Falkenberg *et al.* (2021), who found evidence suggesting interactions between these parasites in this species in northeastern Brazil. In the present study, we identified ergasilid copepods and the monogenean species *M. macracantha* on the gills of *M. curema*.

203 Ergasilid copepods, which predominantly inhabit the gills, can cause inflammatory reactions, 204 increased mucus production, changes in gill filament anatomy, and cellular infiltration. In some cases, 205 these changes can lead to hyperplasia, potentially affecting host growth, fertility, and survival 206 (Cavalcanti et al., 2005; Mentz et al., 2016). Herein, only two complete copepod specimens were identified, with the remaining samples being eggs, making it difficult to observe any significant gill 207 alterations. Similar findings were reported in *M. curema* specimens from the northern region of Santa 208 209 Catarina, where ergasilid specimens were also found on the gills of M. curema specimens (Gueretz et 210 al., 2022).

211 Regarding to the monogenean species collected, we were able to identified up to species level 212 as *M. macracantha*, a monogenean from the family Metamicrocotylidae, which has been sparsely documented in Brazil, particularly in M. liza (Kohn et al., 1994; Cohen et al., 2004). Known to 213 parasitize the gills of marine fish, particularly those of the genus Mugil, this parasite can cause gill 214 lamella hyperplasia in cases of massive infection, thereby interfering with ion absorption and gas 215 exchange (Mentz et al., 2016). While this parasite has been previously reported in other Mugil species 216 in various regions (Vidal-Martínez et al., 2017), this study presents the first record of M. macracantha 217 218 in M. curema from southern Brazil.

Additionally, the present study identified *N. curemai* acanthocephalans in the intestines of three specimens. This species has also been reported in *M. curema* from the northern region of Santa Catarina, with a prevalence of 3.39%, further supporting the findings of this study (Gueretz *et al.*, 2022). *Mugil* species are catadromous, their migratory behavior could explain the distribution of *N. curemai* across coastal and estuarine environments (Cavalcanti *et al.*, 2012; Gueretz *et al.*, 2022). Acanthocephalans are significant in ecological studies because they can accumulate contaminants, making them valuable indicators of environmental quality (Sures, 2008; Roohi *et al.*, 2015). Fish, being highly susceptible to pollutants through diet or living in degraded ecosystems, often harbor high concentrations of metals in the tissues of acanthocephalans, suggesting the environmental conditions to which they are exposed (Abdallah *et al.*, 2019). The relationship between fish, parasites, and contaminants provides an effective means of assessing ecosystem health. As biological indicators, fish parasites can reflect the ecology of their hosts, including migration patterns, feeding habits, and population structures (Mehana *et al.*, 2020).

Digeneans from the family Haploporidae, which primarily parasitize herbivorous or 232 omnivorous fish, are common in marine ecosystems. In Brazil, these parasites have been recorded in 233 234 several Mugil species (Eiras et al., 2016; Andres et al., 2018). The present study adds to the knowledge of these trematodes, though taxonomic identification remains incomplete due to limited specimen 235 236 availability and morphological challenges. Similarly, the histological analysis of hepatic tissue 237 revealed larvae resembling Ascocotyle longa metacercariae, a zoonotic parasite. While precise 238 identification could not be made, previous studies in *M. curema* have also reported the presence of *A*. longa larvae in muscle tissue (Namba et al., 2012; Gueretz et al., 2022). Digenean trematodes may not 239 be immediately concerning due to their typically asymptomatic nature, due the fact that their potential 240 pathogenic effects are still debated and dependent on various factors as parasite type, host condition, 241 and the presence of intermediate hosts (Aly et al., 2020). However, these findings contribute to the 242 243 growing understanding of digenean trematodes and their potential implications for fish health, highlighting the need for further research to clarify the pathogenicity and ecological roles of these 244 245 parasites.

In conclusion, this study contributes important insights into the diversity of parasitic fauna in *M. curema* and highlights the ecological interactions between fish and their parasites. The findings not only expand the knowledge of parasitic species in Brazilian marine ecosystems but also emphasize the role of parasites as indicators of environmental health. Continued research is crucial to further

- understand these interactions and their implications for both fish health and human safety, particularly
- 251 in relation to zoonotic parasites in fish consumed by humans.
- 252

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- 262 **Data curation:** RMQ
- 263 Formal Analysis: RMQ, ABFR, PVC, RAC, RBS
- 264 Funding acquisition: RMQ
- 265 Investigation: RMQ, ABFR, PVC, RAC, RBS
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- 269 Software: RMQ, ABFR, JACJ, PVC, RAC, RBS
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- 271 Validation: RMQ, RBS
- 272 Visualization: RMQ, RBS
- 273 Writing original draft: RMQ, ABFR, JACJ, PVC, RAC, RBS
- 274 Writing review & editing: RMQ, RBS
- 275
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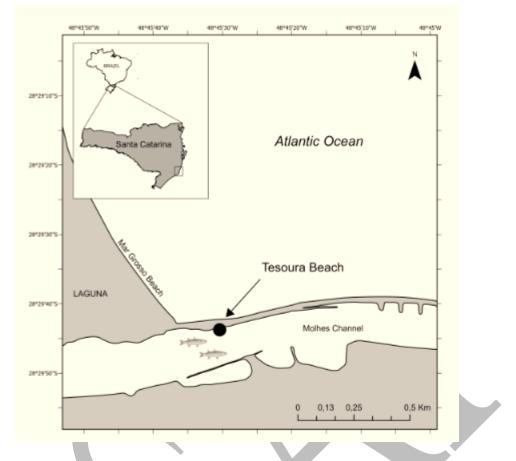
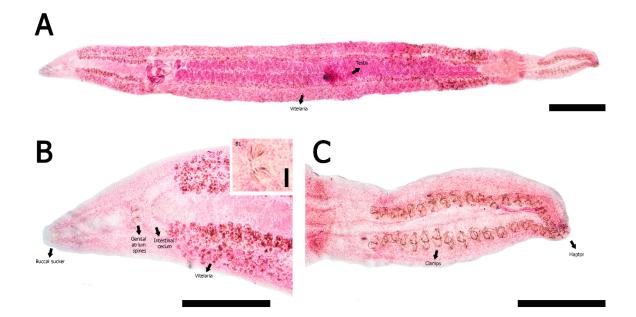
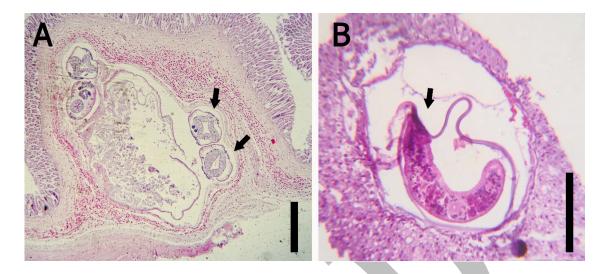


Figure 1. Location of Tesoura Beach, Laguna Municipality, Santa Catarina State, Brazil.



371 Figure 2. Microscope image of the *Metamicrocotyla macracantha* (Alexander, 1954) (Monogenea:

- 372 Mazocraeidea: Microcotylidae) collected from the gills of the white mullet, *M. curema*. A General
- 373 view of the endoparasite; B Close up of the anterior region; $B_1 Close$ up of the genital atrium; C Close up of the genital atrium
- 374 Close up of the posterior region. Scale bar: A 1.5 mm, B, B₁ and C 0.5 mm.



376 Figure 3. Histopathological images of the *M. curema*'s liver and intestine. A - Cross section of the

- 377 intestinal muscle. **B** Cross section of the liver parenchyma. Black arrows show the endoparasite. All
- 378 images in HE stains. Scale bar: 200 μm.