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Original

Parvitaenia cochlearii (Cestoda: Gryporhynchidae) in a cultivation of Pacific fat sleeper *Dormitator latifrons* from Ecuador

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ABSTRACT

Objective. To identify and determine the infection parameters of the metacestode *Parvitaenia* in different culture stages of *Dormitator latifrons* and to evaluate the histological damage caused by this parasite. **Materials and methods.** Forty-five specimens were examined, 15 in each phase: pre-breeding, pre-fattening, and fattening, to identify the metacestode and tissue damage caused using conventional parasitology and histology techniques. Prevalence, intensity, and median intensity were calculated. A Mood's median test evaluated the difference between intensity per culture phase. Total length, weight, condition factor, and hepatosomatic index were correlated with intensity by linear correlation analysis. Liver fragments were evaluated to identify the tissue damage. **Results.** A total of 29,151 cestodes were found and were identified as *Parvitaenia cochlearii*. Cestode prevalence was 100% in each culture phase. The median intensity ranged from 22 to 625 individuals and showed significant differences between culture phases ($X^2 = 29.391$; $p<0.0001$). Intensity showed moderate positive correlation with total fish length ($r^2 = 0.45$; $p<0.05$) and low correlation with weight ($r^2 = 0.38$; $p<0.05$). In less parasitized livers, fibrosis, and congestion around the cysts were observed, while in livers with high levels of infection, parenchymal reduction, fibrosis, and increase in melanomacrophage centers were observed. **Conclusions.** This is the first report of *P. cochlearii* on *D. latifrons* in aquaculture. We suggest that there is a tendency for cestode accumulation during fish growth, which could have negative implications for trade.

Keywords: Eleotridae; histopathology; parasites; cestodes; *Parvitaenia cochlearii*; pisciculture (*Source: CAB Thesaurus*).

RESUMEN

Objetivo. Identificar y determinar los parámetros de infección del metacestodo *Parvitaenia* en diferentes fases de cultivo de *Dormitator latifrons* y evaluar los daños histológicos. **Materiales y métodos.** Se revisaron 45 ejemplares, 15 en cada fase (pre-cría, pre-engorda y engorda) para

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identificar el metacestodo y los daños tisulares provocados. Se calculó la prevalencia, intensidad e intensidad mediana. Se evaluó la diferencia de la intensidad mediana entre fase de cultivo mediante la prueba de medianas de Mood. La longitud total, peso, factor de condición de Fulton e índice hepatosomático se correlacionó con la intensidad mediante una correlación lineal. Los daños tisulares fueron evaluados en fragmentos de hígado. **Resultados.** Se encontró un total de 29151 cestodos, identificados como *Parvitaenia cochlearii*. La prevalencia del cestodo fue de 100%, la intensidad mediana varió de 22 a 625 individuos y mostró diferencias significativas entre fases de cultivo (X^2= 29.391; p<0.0001). La intensidad mostró una correlación positiva moderada con la longitud total de los peces (r^2= 0.45; p<0.05) y una correlación baja con el peso (r^2= 0.38; p<0.05). En hígados poco parasitados se observó fibrosis y congestión alrededor de los quistes. En hígados con altos niveles de infección se observó reducción del parénquima, fibrosis e incremento de centros melanomacrófagos. **Conclusiones.** Este es el primer reporte de *P. cochlearii* en *D. latifrons* de cultivo. Nuestros resultados sugieren que existe una tendencia de acumulación de cestodos durante el crecimiento de los peces, lo cual podría tener implicaciones negativas para el comercio.

Palabras clave: Eleotridae; histopatología; parásitos; cestodos; *Parvitaenia cochlearii*; piscicultura (*Fuente: CAB Thesaurus*).

INTRODUCTION

The Pacific fat sleeper *Dormitator latifrons* (Richardson, 1844) is a freshwater species native to the central-eastern Pacific that has a high productive potential (1). In Ecuador, *D. latifrons* is cultivated on a small scale with productions ranging from 800 to 1000 Ton (2); however, cultivation of the species has been limited by the production of fingerlings (1,3). Aquaculturists capture juveniles from the natural environment for fattening (3), which can cause several problems such as the entry of pathogens (4,5) that could have an impact on public health (6) or cause economic losses in production (7). Despite the aquaculture importance of *D. latifrons* in Ecuador, there is no information on the pathogens that affect its culture systems there; however, in Mexico, 13 metazoan parasites have been recorded to infest *D. latifrons* organisms collected in natural environments and one crustacean parasite in an experimental culture system (1,5).

Recently, cestode infestation in the liver and subsequent mortality of *D. latifrons* kept in semi-intensive culture was observed in an aquaculture farm in Ecuador (*Empresario pers. comm.*). According to the producer, this infestation limited the entry of live *D. latifrons* into the international market due to sanitary restrictions at the border. *Parvitaenia cochlearii* is the only cestode species that has been reported to infest *D. latifrons*, and has been recorded in other fish species collected in natural environments in Mexico such as *Atherinella crystallina* (Jordan & Culver, 1895)*, Gobiomorus maculatus* (Günther, 1859)*, Agonostomus monticola* (Bancroft, 1834)*, Poeciliopsis gracilis* (Heckel, 1848)*, Chirostoma jordani* Woolman, 1894, and *Eleotris picta* Kner, 1863 (5,8,9,10,11,12,13). *Parvitaenia cochlearii* belongs to the family Gryporhynchidae, which are widely distributed parasites that infest several species of freshwater fish in Africa, Asia, Europe, and North America. In South America, several genera (*Glossocercus*, *Parvitaenia*, and *Valipora*) of this family have been recorded to infest fish collected from natural environments in Brazil (14,15,16,17); however, there are no reports of infestations in fish farming systems in the region.

Species of the family Gryporhynchidae of the genera *Valipora* and *Cyclustera* detected in culture systems of carp *Cyprinus carpio* (Linnaeus, 1758) in Mozambique cause a delay in the growth and weight of infested organisms, as well as pathological changes in the gallbladder, intestinal mucosa, and liver (18,19). Similarly, the presence of cestodes can cause marketing problems. The detection of these parasites has a negative effect on the aesthetics of the fish, giving it an unpleasant appearance and the prohibition of its commercial use by sanitary inspectors, which results in the rejection of the product in the market (20,21). Due to these negative effects, it is important to carry out constant monitoring in the culture system, identify the pathogenic agent, and evaluate the health status of the cultured organisms. Therefore, the objective of the present study was to identify and determine the parameters of cestode infection in the different culture stages of *D. latifrons*, as well as to evaluate the histological damage caused by the parasite.

MATERIALS AND METHODS

Collection of material. Forty-five specimens of *D. latifrons* were collected at different stages of culture, 15 pre-breeding, 15 pre-fattening, and 15 fattening organisms through simultaneous monitoring in an aquaculture farm located in the province of Guayas in February 2021. The water collected for the systems was a mixture of river and well water with an initial salinity of 7 ups. Once in the pools, the temperature was maintained between 25°C and 28°C, dissolved oxygen between 3 and 5 mg/L, pH between 7 and 8, and salinity between 3 and 4 ups. The water was changed twice per week for each pool. The fish were grown in earthen ponds with a stocking density of 12–20 fish/m^2 in pre-breeding (2000 m^2 ponds), while in pre-fattening the density was 3–5 fish/m^2, and in fattening it was 0.5–1 fish/m^2 (1 ha ponds). The captured organisms were checked in situ; weight in grams (g), total length (TL) in centimeters (cm), and liver weight in grams (g) were taken. The Fulton condition index (*K*) $K = 100\ (W/L^3)$ was calculated where W is the wet body weight and L is the total length (22); this index is considered an indicator of the nutritional status or health of the organisms, and it is expected that the higher the intensity of parasites, the lower the index. In addition, the hepatosomatic index HSI=(PH/PSV) x 100 was calculated, where PH is the weight of the liver and PSV is the weight of the eviscerated fish. A higher HSI in an organism implies a higher storage of energy reserves in the liver and therefore a better condition. Fish were euthanized by brain puncture following the American Veterinary Medical Association's guidelines for euthanasia of animals (23). The gall bladder, intestinal wall, kidney, and liver were checked for cestodes in all fish as was done in several previous studies (5,13,24). Fragments of the organs were fixed for histological analysis and the remainder of the organs were checked under a stereoscope. All cestodes observed were counted and preserved in eppendorf tubes with 70% alcohol until identification. Infection prevalence and intensity parameters were calculated according to the definition given by Bush et al (25), while the median intensity, which was not affected by the few highly infested hosts, was calculated according to Reiczigel et al (26).

Taxonomic identification. Metacestodes were flattened under light pressure, exposed to hot water at 70°C, and fixed in formalin. Subsequently, they were stained with Mayer's carmine, dehydrated in an ascending ethanol series, and mounted in Canada balsam. For visualization of the rostellar hooks, the scolexes of some specimens were flattened and mounted in a lactophenol solution. Morphometric determinations were performed with images obtained with an 18 MP AmScope® digital camera coupled with an Olympus CX41 optical microscope using ImageJ software. The identification of the metacestode was based on morphological descriptions by different authors (8,27,28). All measurements are given in microns.

Histological analysis. To assess tissue damage caused by metacestodes, liver fragments were fixed in 10% neutral formalin for 48–72 h. Tissues with cestodes present (white dots) were processed with paraffin-embedded histological techniques, stained with hematoxylin and eosin (H-E), and the slides were mounted with Entellan® resin (29). The slides were observed under an Olympus® BX53 optical microscope. The images were taken with an 18 MP AmScope® brand camera.

Statistical analysis. To determine possible differences in median metacestode intensity in the hosts evaluated at the culture stages, the Mood median test was performed according to Reiczigel et al (26) using the Coin package in the R program (30). The 95% confidence interval values for infection parameters were determined in QPweb. To determine possible correlations between metacestode infestation intensity and host size or weight and between metacestode infestation intensity and the Fulton and hepatosomatic indexes, Pearson's or Spearman's linear correlation analyses were performed according to the normality of the variables.

Ethical aspects. The animal bioethical procedures of this study had the permission of the Institutional Bioethics Committee of the Universidad Técnica de Manabí, established in volume 021-12 folio: 21-12-02.

RESULTS

The averages with standard deviation of total length, weight, Fulton index, and hepatosomatic index of fish according to culture stage are described in table 1.

Table 1. Biological variables recorded in the different phases of *Dormitator latifrons* culture. For each culture phase the average total length.

Cultivation phases	TL (cm)	W (g)	K	HSI
Pre-breeding	21.17 ±2.72	170.93 ±75.40	1.67 ±0.25	4.69 ±1.58
Pre-fattening	27.23 ±1.87	427.67 ±93.77	2.10 ±0.25	5.76 ±1.18
Fattening	30.13 ±1.08	535.27 ±89.92	1.97 ±0.36	5.15 ±1.96

(TL) ± standard deviation (S.D.), average weight (W) ± S.D., Fulton's index (K) ± S.D., and hepatosomatic index (HSI) ± S.D. are shown

Description of the parasite. (Based on 7 individuals). The metacestodes were found in the liver and presented a creamy white color (Figure 1A). The species was identified as *Parvitaenia cochlearii* Coil, 1955 (Figure 1B). Distinctive features were body with spherical scolex including rostellum and suckers (Figure 1C) and the neck and posterior part were oval (Figure 2A). The region posterior to the neck is fragile, so in most organisms, the scolexes became detached. The rostellum is composed of three types of hooks. The body is elongated (727–759 x 273–288 µm), divided into the spherical scolex (234 x 169 µm) and oval posterior parts (486 x 281 µm) and suckers (71 x 64 µm). They have large distal hooks (49–56 µm) (Figure 2B) and shorter proximal hooks (31–37 µm) (Figures 1D, 2C). All hooks are straight, except for the distal end of the blade which is clearly curved.

Remarks. *Parvitaenia cochlearii* is distinguished by the presence of three rostellar hooks of different shape and size. The measurements of morphological structures in the specimens of the present study coincide with those reported for the species, such as elongate body 727–759 x 274–288 (670–800 x 336–344), scolex 234 x 169 (224–237 x 198–224), rostellum 54–57 x 57–58 (58–59 x 53–67), suckers 71–72 x 63–65 (71–85 x 53–63) (8), distal hooks 49–56 (55; 49–56; 49–55), and proximal hooks 31–37 (33–35; 32–37; 32–36) (8,31,32).

Infection parameters. The prevalence of *P. cochlearii* was 100% in all culture stages, while the median intensity varied between culture stages: in pre-breeding 22 (7–33); in pre-fattening 189 (162–218), and in fattening 625 (824–1721) (X^2 = 29.391; p<0.0001). The highest values were observed in the fattening phase.

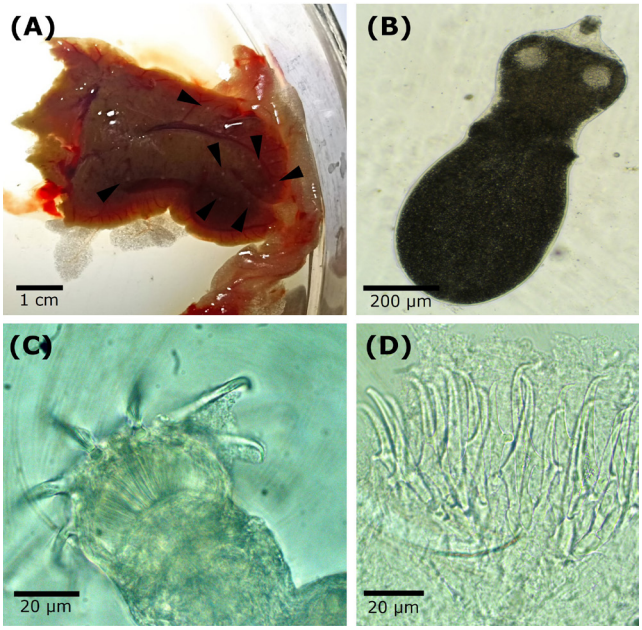

Figure 1 Metacestodes *Parvitaenia cochlearii*. A: Metacestodes (arrowheads) in a posterior liver fragment of *D. latifrons*; B: Fresh whole specimen (10x); C: Scolex (100x); D: Rostellar hooks (100x).

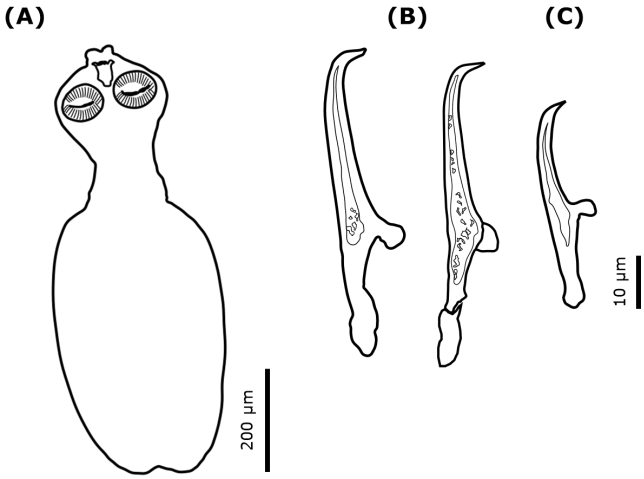

Figure 2. *Parvitaenia cochlearii*. A: Whole specimen (10x); B: Distal hooks; C: Proximal hook (100x).

Linear regression analysis showed a positive correlation between total fish length and cestode intensity (r^2 = 0.45; p<0.05), as did fish weight and cestode intensity (r^2 = 0. 38; p<0.05), while cestode intensity and Fulton index showed no correlation (r^2 = 0.01; p>0.05), as did intensity and hepatosomatic index (r^2 = 0.01; p>0.05).

Histological evaluation. Histological analysis of parasitized livers showed the presence of metacestodes among hepatocytes (Figure 3A-D). In livers with few parasites, the damage was focal. The parasites were isolated from the liver tissue by a thin layer of fibroblasts, and congestion of blood vessels and hepatic steatosis were observed around the cysts (Figure 3B–C). In livers with abundant metacestodes, a reduction of liver parenchyma was observed due to the presence of many parasites and an increase in the number and size of melanomacrophage centers (Figure 3D).

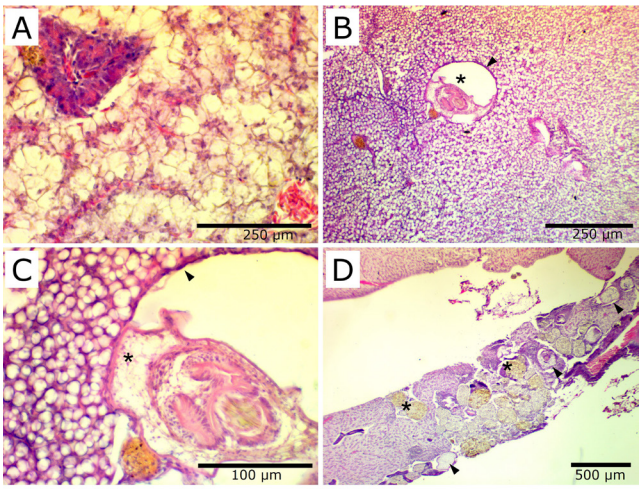

Figure 3. Histological sections of livers of *Dormitator latifrons* infested by metacestodes of *Parvitaenia cochlearii*. (A) Histological section of unaltered liver. (B) *Parvitaenia cochlearii* (*) encysted in liver, surrounded by a thin layer of fibroblasts (arrowheads). (C) Detail of image B showing the metacestode (*) surrounded by fibroblasts (arrowhead). Note the hepatic steatosis around the parasite. (D) Liver with abundant metacestodes (arrowhead) surrounded by melanomacrophage centers (*). H&E staining.

DISCUSSION

In this study we report the cestode *P. cochlearii* infesting *D. latifrons* at different stages of culture. *Parvitaenia cochlearii* corresponds to a larval stage (metacestodo), so *D. latifrons* is an intermediate host in the life cycle of the parasite. Several authors have recorded *P. cochlearii* infesting *D. latifrons* collected in natural environments in Mexico (5,8,13,27); however, this study is the first record of *P. cochlearii* in South America and the first in cultivated systems.

Parvitaenia cochlearii in this study showed the following combination of characters: body divided into spherical scolex and oval posterior part; scolex composed of four suckers and a rostellum with 20 hooks of three shapes, arranged in two concentric rows. These characters and the measurements of the body, scolex, suckers, and distal hooks (gd) and proximal hooks (gp) coincide with those reported by several authors (8,27,31,32). The genus *Parvitaenia* comprises more than 15 described species, of which only the larval stages of three are known, *P. cochlearii* Coil, 1955, *P. macropeos* (Wedl, 1855), and *P. samfyia* Mettrick, 1967 (8,33). However, *P. cochlearii* (gd: 49–56; gp: 31–37) differs from *P. macropeos* by the larger size of the rostellar hooks (gd: 43–46; gp: 26–30). *Parvitaenia samfyia* differs from *P. cochlearii* by having smaller distal hooks (gp: 27–30).

Our results showed the cestode infesting all the checked fish with a median intensity greater than 22 individuals, increasing from pre-breeding to fattening, and related to the increase in the size and weight of the fish. These values could be due to the life cycle of the parasite being completed in the culture system with the presence of its intermediate hosts (copepods and fish) and final hosts (ichthyophagous birds), thus accumulating the parasites. Poulin (34) studied the intraspecific relationship between fish length and infestation intensity of metazoan parasites and found that cestodes in particular show a correlation between fish length and infestation intensity. Experimentally, some studies have shown that planktonic copepods serve as the first intermediate host of cestodes of the family Gryporhynchidae. For example, Jarecka (35,36) showed that the copepod *Eudiaptomus graciloides* served as a host for the cestodes *Neogryporhynchus cheilancristrotus* and *Valipora campylancristrota*, and that the copepod *Mesocyclops oithonoides* was a host for *N. cheilancristrotus*. Subsequently, Sysolyatina-Andakulova (37) showed that *Arctodiaptomus salinus* is the first host of *V. campylancristrota*. On the other hand, *P. cochlearii* has been detected in birds of the family Ardeidae (31,32). Some species of this family have been reported in Ecuador, such as the agami heron *Agamia agami* (Gmelin, 1789), brown heron *Ardea cocoi* Linnaeus, 1766, and boot-billed heron *Cochlearius cochlearius* Linnaeus, 1766 (38,39,40,41).

Cestode intensity did not show an effect on fish condition factor *K* or HSI; this is consistent with most of the histological damage observed,

except in fish with high levels of infection, being focal and probably not sufficient to change health status immediately. Laboni et al. (42) evaluated the effect of infestation by cestodes of the family Lystocestidae and found that only heavily infested fish (> 10 individuals) presented a weight loss of 26.49%. Recent studies on the damage caused by metacestodes of the family Gryporhynchidae on their hosts are very scarce (43). Like our results, Bauer et al. (18) reported that metacestodes of the genus *Valipora* infesting the carp *Cyprinus carpio* in culture systems caused pathological changes in the gall bladder and other internal organs according to the degree of infection intensity. Fish infested with *Valipora* showed growth and weight retardation in contrast to uninfested fish or fish with low infection intensity. The chronic inflammatory reaction observed around the cyst of *P. cochlearii*, in the form of a fibrous capsule and congestion, is similar to that reported by Saraiva et al. (19), who found that larvae of the metacestode *P. samfyia* encysted in the lamina propria of the intestinal mucosa causes a chronic inflammatory reaction in the tissues of *C. carpio* carp, while *Cyclustera* larvae encysted in the connective tissue of the hepatic capsule are found surrounded by a thin fibrous layer and with necrosis and hyperemia in the adjacent tissue. However, only fish with high levels of infection showed lesions that could alter their health status, unlike what was recently reported by Scholz et al. (43) who evaluated the infestation by invasive larvae of *Amirthalingamia macracantha*, a cormorant parasite, in tilapia hybrids and mentioned that despite finding low values in infestation levels (prevalence 3% and average intensity 1–2 individuals), this species could represent a threat in tilapia farming systems. Our results provide the first information on the histological alterations caused by *P. cochlearii* in *D. latifrons*, but future studies correlating the degree of histological lesions with liver function and body condition will be necessary to determine the real effect of this parasite on infested organisms.

Prior to the development of the present study, an Ecuadorian producer of *D. latifrons* observed cestode infestation in the liver of the fish, which caused them to be rejected for export. *Dormitator latifrons* is an attractive species for the international market since it can be transported alive (44), with the main markets being the United States, Canada, and China (44). However, the export of this resource is a challenge due to restrictions imposed by the competent authority of the importing country. According to the World Organization for Animal Health (OIE), if the live aquatic animals to be exported are infested by parasites that could pose a risk to human health or to native wild populations, the producer may not export them without the prior consent of the importing country (45). It also mentions that the competent authority shall adopt the necessary measures to prevent the transport of aquatic animals showing clinical signs of any disease, and to prevent the introduction of possible vectors or pathogenic agents into the container. In this sense, it is recommended to maintain measures for the prevention of this parasitosis in cultures of *D. latifrons*. Scholz et al. (24) and Scholz, Davidovich, et al. (43) mention that the detection of gryporhynchids in culture systems should be a reason for constant monitoring to take preventive measures to avoid increasing infection levels.

In conclusion, this study constitutes an extension of the geographical distribution of *P. cochlearii*; it is the first report in culture and the first report of histological damage associated with *P. cochlearii* in *D. latifrons*. Although the results showed that the parasites do not influence the body condition of the fish, constant monitoring in culture systems is recommended to increase the sample size to determine if *P. cochlearii* constitutes a health risk to the fish. This monitoring could include non-destructive tests such as transaminase determination and thus evaluate liver function. Additionally, it is recommended that producers break the parasite's life cycle, probably by covering the pools with shade nets to avoid the final host of *P. cochlearii* (birds).

Conflict of interests

The authors declare that they have no competing interests.

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