

## Ultra-structural characterization of *Diplostomum sp* metacercarian in *Orestias luteus* from Lake Titicaca, Peru

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### Abstract

**Montesinos, J.A.; Serrano, E.; Tantaleán, V.M.; Yañez, J.; Flores, R.: Ultra-structural characterization of *Diplostomum sp* metacercarian in *Orestias luteus* from Lake Titicaca, Peru. Rev. Vet. 33: 2, 215-219, 2022.** The objective of the present work was the ultra-structural characterization of metacercariae of *Diplostomum sp* in *Orestias luteus* from Lake Titicaca, Puno, Peru. 35 fish collected through non-probabilistic sampling were anesthetized with tricaine monosulfonate (Tricaine-s®) and necropsied to determine the number of *Diplostomum sp* present per fish. The extracted parasites were fixed and preserved for study by scanning electron microscope. The frequency of parasitosis was 48.6% (IC 95%: 32.0-65.1) with a mean parasitic intensity of infection of 3.53 (low). The metacercaria of *Diplostomum sp* are linguiform in shape with papillae unevenly distributed on the surface of the body, have a posterior border with a slight conical prominence, and an extreme anterior circle where the oral suction cup is located. There is also an ellipsoidal acetabulum located in the anterior and ventral third of the posterior segment of the body; behind that is the ovoid-shaped tri-bocytic organ. The morpho-anatomic characteristics of the parasite under study coincide with *Diplostomum mordax*. The prevalence of *Diplostomum sp* in Lake Titicaca is high compared to previous years. However, the average intensity is low; suggesting that, for the moment, *Diplostomum sp* is not a threat to the health of *O. luteus*.

**Key words:** *Orestias luteus*, *Diplostomum sp*, Lake Titicaca, parasites.

### Resumen

**Montesinos, J.A.; Serrano, E.; Tantaleán, V.M.; Yañez, J.; Flores, R.: Caracterización ultra-estructural de metacercarias de *Diplostomum sp* en *Orestias luteus* del Lago Titicaca, Perú. Rev. Vet. 33: 2, 215-219, 2022.** El objetivo del presente trabajo fue la caracterización ultraestructural de metacercarias de *Diplostomum sp* en *Orestias luteus* del lago Titicaca, Puno, Perú. Se recolectaron 35 peces mediante muestreo no probabilístico los cuales fueron anestesiados con tricaina mono sulfonato (Tricaine-s®) y se les realizó necropsia para determinar el número de *Diplostomum sp* presente por pez. Los parásitos extraídos se fijaron y conservaron para su estudio mediante microscopía electrónica de barrido. La frecuencia de parasitosis fue de 48,6% (IC95%: 32,0-65,1) con una intensidad de infección parasitaria media de 3,53 (baja). La metacercaria de *Diplostomum sp* son de forma linguiforme con papilas distribuidas de manera desigual en la superficie del cuerpo, tienen un borde posterior con una ligera prominencia cónica y un círculo anterior extremo donde se ubica la ventosa oral. También hay un acetábulo elipsoidal ubicado en el tercio anterior y ventral del segmento posterior del cuerpo; detrás de eso está el órgano tribocítico de forma ovoide.

Las características morfo-anatómicas del parásito en estudio coinciden con *Diplostomum mordax*. La prevalencia de *Diplostomum sp.* en el lago Titicaca es alto en comparación con años anteriores. Sin embargo, la intensidad media es baja; sugiriendo que, por el momento, *Diplostomum sp.* no es una amenaza para la salud de *O. luteus*.

**Palabras clave:** *Orestias luteus*, *Diplostomum sp.*, Lake Titicaca, parasites.

## INTRODUCTION

*Orestias luteus* along with 23 other *Orestias sp.* are endemic to Lake Titicaca and its tributaries, it is commonly known as “carachi amarillo” and is an important part of the economic activity of the inhabitants surrounding Lake Titicaca, generating direct and indirect jobs for the subsistence of rural families<sup>21</sup>.

However, *O. luteus* biomass has decreased its catch volume from 21,920 to 18,724 t<sup>11</sup>; which is related to over fishing and massive mortality<sup>2</sup>. The latter due to pathological factors that could influence the biological behavior of the species; seriously impacting the economy and food security<sup>17</sup>.

Current research is based on conservation and repopulation programs focused on food<sup>22, 27</sup>, production and reproduction<sup>17</sup>. The study of the health of these fish is being neglected, so much so that the only published works on *O. luteus* parasitosis are decades old<sup>8, 26</sup>. *Diplostomum sp.* are parasites whose metacercaria stage were recorded in more than 150 species of fish<sup>9</sup>, mainly parasitizing the cerebrospinal fluid of the brain and eye cavities<sup>4</sup>.

Heckmann<sup>8</sup> (1992) reported that infections of metacercariae of *Diplostomum mordax* in the cranial cavity of *O. agassi*, *O. olivaceus*, *O. luteus* and *Basilichthy sbonariensis* induced compression of the neural tissue and migration of the metacercariae to the brain, leading to hemorrhage, cell necrosis, inflammation fibrosis and the rupture of nerve fibers.

Due to its low specificity *Diplostomum sp.* affect both wild and cultivated fish species<sup>10</sup>, causing negative impacts that are still unknown and neglected in the local aquaculture of Lake Titicaca. Lymneid snails and teleost fish are the intermediate hosts for *Diplostomum sp.* and piscivorous birds are the definitive hosts<sup>18</sup>.

*Diplostomum sp.* are prevalent, cosmopolitan parasites in the birds<sup>5, 10</sup>. Severity of illness can be affected by the number of metacercariae present in the host<sup>19</sup>, and severe illness leads to increased predation by birds<sup>30</sup>. The effects caused by the metacercaria in fish range from unnoticeable<sup>16</sup> to severe cases causing blindness, deformation, weight loss<sup>5</sup> and even death of the fish.

Parasitosis in fish causes economic and health losses due to the deterioration of meat<sup>23</sup>, ultimately impacting the food industry and those who depend on it for survival<sup>29</sup>. Therefore, the aim of this study is the ultra-structural characterization of meta-cercariae of *Diplostomum sp.* in *O. luteus* of Lake Titicaca.

## MATERIAL AND METHODS

### Fish collection and sample preparation

Between February and May 2017, 35 *O. luteus* were collected from Peninsula of Capachica, Lake Titicaca - Puno, using chinchorros fishing gear<sup>6</sup>. Fish were sacrificed by asphyxiation with *Tricaine monosulfonate* (Tricaine-s®) and then packed in an expanded polystyrene box with ice for shipment to Lima.

### Necropsy and measurements

The necropsy and metacercaria collection of *Diplostomum sp.* was carried out in the Animal Parasitology Laboratory of the Faculty of Veterinary Medicine and Zootechnics of the Universidad Peruana Cayetano Heredia. At necropsy, the location of metacercariae in the fish was recorded and the frequency distribution and average parasitic intensity (total metacercariae / number of infected fish) of *Diplostomum* was determined<sup>13</sup>.

### Scanning electron microscopy

Scanning electron microscopy was performed in the specialized laboratory of the Faculty of Biological Sciences from the National University Greater than San Marcos, Lima - Peru.

### The extracted metacercariae

Were fixed in 4% glutaraldehyde and preserved in 70% ethyl alcohol. The samples were then dehydrated in 80%, 90% and 100% alcohol. The samples were desiccated to a critical point (Electron Microscopy Sciences, Model EMS 85), replacing water with carbon dioxide.

### The metallization with gold

Was done using spray-metallizer coating equipment (Sputter Coater SPI, 11430E-AX), similar

to the method used by <sup>12</sup>. Image registration was carried out using electronic microscope scanning with built-in camera (Fei Inspect S50).

## RESULTS

Of the 35 *O. luteus* analyzed, 17 (48.6%, IC 95%: 32.0-65.1) were infected by metacercariae of *Diplostomum* sp. In general, the mean parasitic intensity of infection was low 3.53 (1 to 5 metacercariae) in most fish; however, one fish had 23 parasites.

Metacercariae were found in the brain of the fish freely floating in the cerebrospinal fluid associated in the lobular in foldings, ventricular cavities and external meninges. Fish had parasites in the frontal lobes (58.8%), parietal lobes (11.8%), lateral lobes (17.6%), frontal and parietal lobes (5.9%) and in all three lobes (5.9%).

We did not observe a clear invasion of the cerebral parenchyma or severe inflammation in any fish. Also, the sampled fish did not show signs or symptoms of the disease (apparently healthy) and there was no evidence of metacercariae in other anatomical parts of the fish, as the target organ of the parasite is the brain.

Scanning electron microscopy analysis revealed that the parasites have papillae distributed irregularly on the surface of the body, both dorsal and ventral, and in greater numbers on the anterior end. The body is linguiform, 0.30 mm x 0.72 mm, slightly arched towards the ventral aspect.

The posterior border has a small conical prominence where the excretory pore is located, the anterior is slightly circular and a little ventral to this is the oral suction cup. The suction cup has a circular shape and its edges have roughness directed from the outside to the inside. On each side of the oral suction cup, a small pseudo-suction cup can be seen. The acetabulum has a transverse opening and the oval-shaped tribocytic organ is located posterior to it.

## DISCUSSION

The parasite observed in *Orestias luteus* from Lake Titicaca is likely *D. mordax* described by Tantaleán et al. <sup>26</sup>, Heckmann <sup>8</sup> and Siegmund et al. <sup>24</sup> who described hemorrhage, cell necrosis and nerve fiber rupture associated with the parasite.

*D. mordax* differs from *D. von Nordman* which parasitizes the brain and mesentery <sup>10</sup>. *D. mordax* also differs from *D. spathaceum* and *Diplostomum* sp, which parasitize *African Catfish* and are normally located in the eyes <sup>1</sup>.

The frequency of parasitoses found in the present study was notably higher (48.6%) than the 15% and 11% prevalence observed by Heckmann <sup>8</sup> and Sierralta et al. <sup>25</sup> in Lake Titicaca. But, slightly close than the prevalence of 60% and 7% to 100% reported in other fish species by Siegmund et al. <sup>24</sup> and Viozzi <sup>28</sup>, respectively.

The high prevalence may be due to the decreased water quality of Lake Titicaca <sup>20</sup> and the increase in water temperature that occurs in the months of January, February, March and April. From January to April the average water temperature is 15.68°C <sup>3</sup>, which would encourage the growth of snails of the genus *Lymnaeidae* <sup>7</sup>. Also, Lyholt & Buchmann <sup>15</sup>, showed that cercariae of *D. spathaceum* have higher infectivity at 15°C.

The mean intensity of *Diplostomum* sp is similar to what Siegmund et al. <sup>24</sup> reported in Chilean silverside (*Basilichthys australis*), but lower than fish from tropical waters. Kembanya et al. <sup>14</sup>, who identified *Diplostomum* sp in *O. niloticus* from three areas bordering the county of Uasin Gishu-Kenya; attributes the higher parasite intensities to a greater population of piscivorous birds and to fish farms with a higher density of fish compared to the natural environment.

Machado et al. <sup>16</sup> agrees but also mentions that the degree of barium infestation is higher in tropical waters and mentions that native fish have a lower degree of infestation compared to introduced fish. This indicates that the lesions produced by these parasites are not detrimental to the health of the fish unless the intensity increases <sup>19</sup>.

The morpho anatomical details of the *Diplostomum* sp identified in this study coincide with that described by <sup>10</sup> with the exception of the acetabulum which the author describes as circular. Cavaleiro et al. <sup>4</sup> describe a tri lobed anterior end and the scanning electron microscopy described finger-like papillae, without apparent integument with a rounded base that ends in a kind of cilium.

Tantaleán et al. <sup>26</sup> describe a *Diplostomum* sp length between 0.860 to 0.890 mm and width from 0.470 to 0.490, which is slightly larger than that described in the present work. The size difference may have been caused by the procedures used for the measurement, since in the aforementioned study measurements were taken on flattened specimens for sheet preparation.

Additionally, mention that it is the first time that *Diplostomum* sp has been described at an ultra-structural level in the Peninsula of Capachica of Lake Titicaca, Puno. This supports the diagnosis of the disease in the various areas where *Orestia luteus* lives.

In the present study, the frequency of *Diplostomum* sp was 48.6% (IC 95%: 32.0 – 65.1) which is considered high. However the average parasitic intensity is low; suggesting that, at the moment, *Diplostomum* sp are not threatening the health of *O. luteus*. The location within the brain and the morpho anatomy of the parasite observed in this study coincide with *D. mordax*. Genotyping would be an essential tool to reach more specific conclusions.

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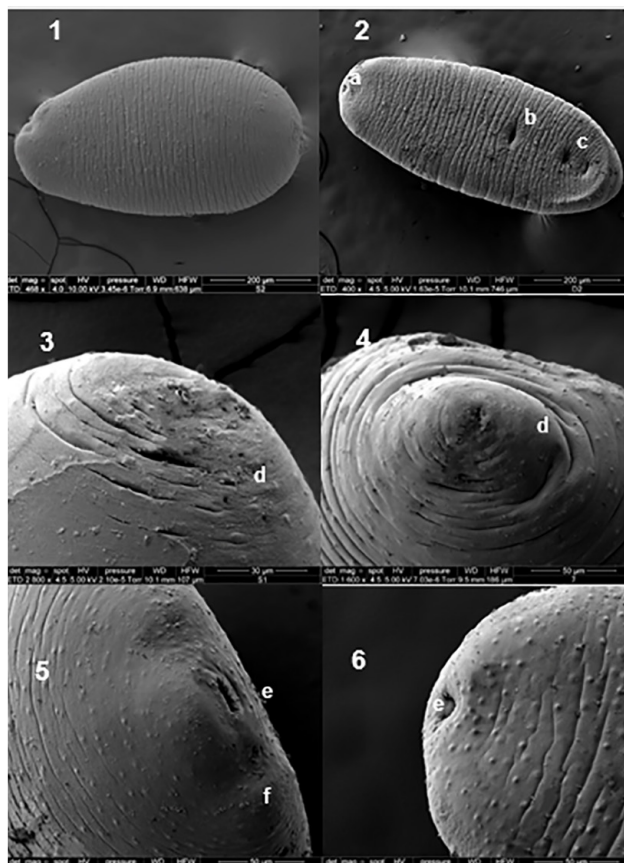
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### Annexed



**Figures 1-6.** Ultrastructural aspect of the metacercaria of *Diplostomum* sp. isolated from the brain of *Orestias luteus* from Lake Titicaca, Puno (Perú), revealed by electron microscopy. **1:** whole body, dorsal surface with irregularly distributed papillae. **2:** whole body, ventral surface, showing the oral suction cup (a), the acetabulum (b) and the tribocytic organ (c). **3 and 4:** posterior region with small conical prominence where the excretory pore is located (d). **5 and 6:** anterior region with oral suction (e) and pseudo-suction cup (f).