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**Research Note**

**Pathological Alterations Induced by *Pozdnyakovia gibsoni* (Digenea, Didymozoidae), a Parasite of the Skipjack Tuna, *Katsuwonus pelamis* (Scombridae)**

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**ABSTRACT:** The pathological and histopathological alterations associated with parasitic infection induced by the didymozoid *Pozdnyakovia gibsoni* Justo and Kohn, 2012, in the stomach wall of the skipjack tuna *Katsuwonus pelamis* (Linnaeus, 1758) in the southwestern Atlantic Ocean off South America are presented. The parasites were encysted in the mucosal layer of the stomach and inserted into the connective tissue that forms the lamina propria. The parasite penetrates through the stomach wall, inducing white blood cell proliferation and a localized inflammatory reaction.

**KEY WORDS:** Fish parasite, Digenea, Didymozoidae, pathology.

The skipjack tuna (bonito-listrado), *Katsuwonus pelamis*, is a cosmopolitan epipelagic species of Scombridae widely distributed in tropical and subtropical waters. This fish occurs in superficial waters ranging between 14.7° and 30°C (Collette and Nauem, 1983). According to Food and Agriculture Organization of the United Nations (2014), skipjack tuna represents an important fishery resource, being the third marine species in the world in terms of number of captures. While the skipjack tuna is of great economic importance in Brazil due to the large canned-fish industry, little is known about its parasites in the southwest Atlantic Ocean.

*Pozdnyakovia gibsoni* was described by Justo and Kohn (2012) as parasitizing the wall of the stomach of *Katsuwonus pelamis* (Linnaeus, 1758) off the coast of Brazil in the southwest Atlantic Ocean. This digenean belongs to the subfamily Gonapodasmiinae Ishii, 1935, and, like other species in the subfamily, has a peculiar body shape. In short, parasites form globular cysts containing two completely hermaphroditic individuals whose posterior regions of their bodies fuse dorsally. The aim of this study is to determine the histopathological conditions associated

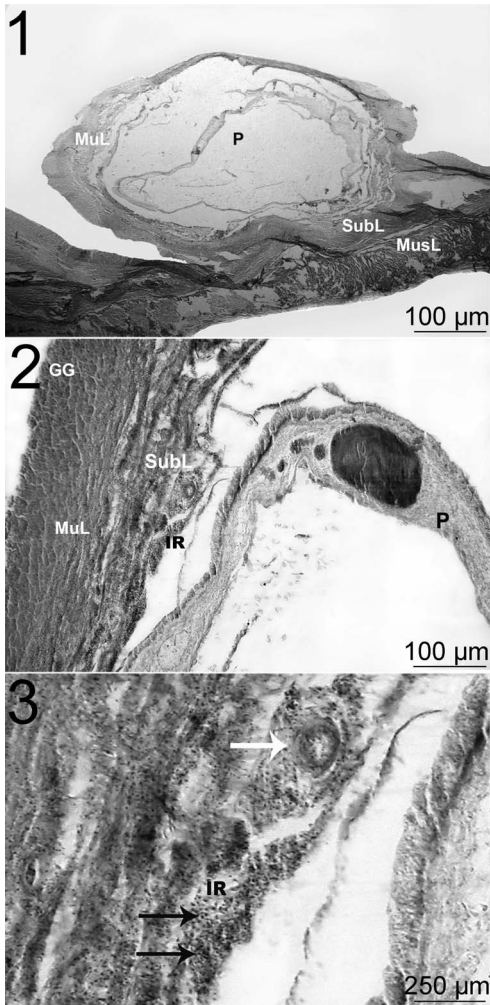
with the cysts of *P. gibsoni* in the stomach wall of *Ka. pelamis*.

Specimens of skipjack tuna were obtained by local fishermen from the coastal zone off the State of Rio de Janeiro, Cabo Frio, Brazil (22°52'46"S, 42°01'07"W). For histopathological analysis, the wall of the stomach of specimens was removed, fixed in 10% formalin, and prepared through routine dehydration in ethanol and impregnation, after which they were embedded in paraffin according to Gartner and Hiatt (2007). Sections (5–10 µm) were stained with hematoxylin and eosin (Michalany, 1980). Light micrographs were taken with a digital camera connected to a Nikon Eclipse E 800 microscope.

Sixty-one specimens of skipjack tuna, *Ka. pelamis* (26–73 cm in total body length and ranging from 1 to 8 kg in weight), including 30 males (49.2%) and 31 females (50.8%), were examined for helminths. Among the 61 specimens examined, only 2 (3.28%) were found to be infected with 1 and 4 cysts of *P. gibsoni*, respectively, upon visual examination of the mucosa of the stomach wall of the host. From the 5 cysts, each containing 1 pair of adult specimens, 4 cysts were used for the previous morphological description of *P. gibsoni* (Justo and Kohn, 2012), and the other reserved for the histopathological analysis described herein.

Cysts of *P. gibsoni* were found in the mucosa layer of the stomach of *Ka. pelamis* and were inserted into the connective tissue that forms the lamina propria (Fig. 1). The presence of the didymozoid in this region induces the proliferation of white blood cells, a hallmark of an inflammatory reaction, around the parasite (Fig. 2). Significantly, the following was also observed to occur (data not shown): a thin layer of connective tissue surrounds the parasite and dilatation of the villi resulting in the raising of the epithelial

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**Figures 1–3.** Individual of *Pozdniakovia gibsoni* encysted in the stomach of a skipjack tuna, *Katsuwonus pelamis*. **1.** Histological sections showing: P—parasite, MuL—mucosa layer, SubL—submucosa layer, MusL—muscle layer. **2.** P—parasite, MuL—mucosa layer, SubL—submucosa layer, GG—gastric glands, IR—inflammatory reaction. **3.** IR—inflammatory reaction with dilated blood vessels (white arrow) and lymphocyte proliferation (black arrows).

tissue and a small portion of the lamina propria (connective tissue).

Underneath the parasite a thin layer of connective tissue of the mucosa was found with dilated blood vessels filled with erythrocytes and leukocytes (mainly lymphocytes), further indicating a localized inflammatory reaction (Fig. 3). Below this layer there was a thick layer of connective tissue that also showed moderate amounts of immune cells.

Studies have demonstrated that the presence of didymozoids in gill sites causes little or no pathological reaction, with damage being restricted only to the affected area (Lester, 1980; Eiras and Rego, 1987; Abdul-Salam and Sreelatha, 1992; Perera, 1992a, 1992b, 1994; Cruz-Lacierda et al. 2001). Mladineo (2006) proposed that the absence of a strong inflammatory response suggests that didymozoids do not elicit a significant cellular response. On the other hand, the same author observed that the presence of cysts of *Koellikerioides intestinalis* Yamaguti, 1970, from *Thunnus thynnus thynnus* in the submucosa of the intestine results in a strong proliferation of connective tissue around and near the cyst, as well as intensive cell infiltration, desquamation of columnar epithelium, and a loss of goblet cells.

Lester (1980), after studying didymozoids under a light microscope, observed that the defense response indicates a typical chronic inflammatory reaction characterized by cellular infiltration and extensive fibrosis. Abdul-Salam and Sreelatha (1992) observed that the tissue response contained active macrophages and mature fibroblasts in the zone between the capsule wall and the coiled worms, and leucocytes and collagen bundles between coils of the worms. Perera (1994) observed that didymozoid cysts live in a true capsule and did not verify an increase or decrease in the number of fibroblasts due to didymozoid infection. However, more lymphocytes were observed in the capsule dermis than in uninfected tissue. Cruz-Lacierda et al. (2001), studying didymozoid trematodes in *Epinephelus coioides*, observed that the response of the host to the parasite was confined to the infected filament only. These authors showed pathological alterations that included distortion in the shape of the filament, mild hyperplasia of the interlamellar epithelium, and an increase in the number of mucous cells.

Our results show that *P. gibsoni* is inserted into the connective tissue that forms the mucosa layer. Mladineo (2006) observed that in different didymozoid species, the range of induced tissue changes depends on the site of infection. Such damage is more accentuated in the submucosa of the intestine, as observed with *Ko. intestinalis* (see above). Mladineo (2006) did not report any damage in the stomach of *T. thynnus* caused by the presence of cysts of *Ko. internogastricus* Yamaguti, 1970. However, Justo et al. (2009) described major damage caused by *Ko. internogastricus*, which is highly pathogenic to its hosts. In this case, *Ko. internogastricus* destroys the stomach layers associated via an intense inflammatory

reaction that appears to interfere with food digestion, which may then lead to host death.

Based upon our current level of understanding it is clear that further research should be undertaken to increase our understanding of the pathological changes induced by the presence of didymozoids, in general, in their hosts.

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