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# Occurrence of two abdominal neurofibromas in a specimen of *Betta splendens*

## Ocurrencia de dos neurofibromas abdominales en un espécimen de *Betta splendens*

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**Key words**
Abdominal neurofibromas
*Betta splendens*
Electron microscopy
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Protein S-100

**ABSTRACT |** In this work we describe two neurofibromas in *Betta splendens* studied with optical microscopy, immunohistochemistry and electron microscopy. The specimen studied, that belonged to a private breeder, had marked abdominal distension, and was transferred for diagnosis to the Laboratory of Immunology and Pathology of Aquatic Organisms of the Universidade Federal do Rio Grande – FURG. It was diagnosed as neurofibroma, positive for the antibody to protein S-100. With electronic microscopy, it showed signs of degeneration in some cells and contained mast cells (MC) regularly distributed in the neoplasm mass. With immunohistochemistry we confirmed the histogenesis of the neoplasm, in which there were two neurofibromas, and with electron microscopy we confirmed that the granular cells within the neoplastic tissue were MCs.

**Palabras clave**
Neurofibromas abdominales
*Betta splendens*
Microscopía electrónica
Inmunohistoquimica
Neoplasias
Proteína S-100

**RESUMEN** | En este trabajo describimos dos neurofibromas en *Betta splendens* estudiados con microscopía óptica, inmunohistoquímica y microscopía electrónica. El espécimen estudiado, que pertenecía a un criador privado, presentaba marcada distensión abdominal, fue trasladado para diagnóstico al Laboratorio de Inmunología y Patología de Organismos Acuáticos de la Universidade Federal do Rio Grande – FURG. Fue diagnosticado como neurofibroma, positivo para el anticuerpo contra proteína S-100. Con microscopia electrónica, mostraba signos de degeneración en algunas células y contenía mastocitos distribuidos regularmente en la masa neoplásica. Con inmunohistoquímica confirmamos la histogénesis de la neoplasia, en la que existen dos neurofibromas, y con microscopía electrónica confirmamos que las células granulares que se encuentran entre el tejido neoplásico son MC.

There is a wide spectrum of peripheral nerve tumors, in which most arise from nerve sheath cells (Stoskopf, 1993). In humans, neurofibroma represents 0.2% of soft tissue tumors (Rosai, 2004). Among the 407 Mawdesley-Thomas tumors (1975) cited, only twelve neurofibromas were described, and in a recent review of neoplasms in fish, seven neurofibromas were found in the last twenty years (Romano and Pedrosa, 2020).

Neurofibromas were described in both humans and fish with the presence of mast cells (MCs), some degranulated (Schmale *et al.*, 2004; Staser *et al.*, 2010), wherein there is a difference between the solitary neurofibroma and isolated of the neurofibromatosis (multiples neurofibromas) that are observed in humans and fish (Schmale and Hensley, 1988; Korf, 2013; Farschtschi *et al.*, 2020).

In this work we describe two neurofibromas in *Betta splendens* studied with optical microscopy, immunohistochemistry, and electron microscopy.

A specimen of *Betta splendens* belonging to a private breeder with marked abdominal distention was transferred to the Laboratory of Immunology and Pathology of Aquatic Organisms of the Universidade Federal do Rio Grande – FURG. The fish, with a weight of 0.38 g, was euthanized with a bath in M-222 at 100 ppm (Western Chemical, USA) and subsequently dissected. In the abdominal cavity, two whitish nodular lesions were found on the flanks, one measuring 0.3 cm and the other measuring 0.4 cm (Fig. 1).

Fish tissues were fixed in 10% buffered formalin, and after fixation, the tissues were dehydrated through ascending concentrations of ethanol, diaphanized in xylol and embedded in paraffin. Then, the samples were cut in a microtome (Leica RM2245) and stained with hematoxylin and eosin and Giemsa for tissues (Luna, 1968).

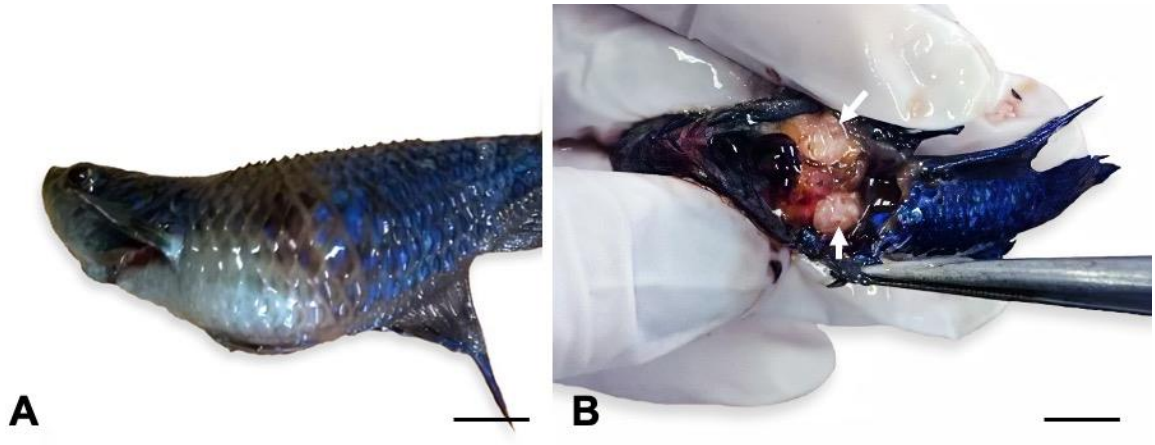


**Figure 1. A:** *Betta splendens* specimen with abdominal distention. Bar = 1 cm. **B:** Abdominal cavity of the same specimen where whitish nodules are observed (arrows). Bar = 1cm.

Histological sections were stained with immunohistochemical procedures, according to a modified avidin-biotinperoxidase complex technique (Hsu, 1981). Tissue slides were deparaffinized by rinsing with xylol and then rehydrated with alcohols of different concentrations (absolute ethanol, 90, 80, 70, 50%). The endogenous peroxidase activity was blocked by incubating the slides for 20 min in 0.3% $H_2O_2$ in a 5% methanol solution. After washing the slides in water and 0.05% Phosphate-Buffered Saline (PBS), they were subsequently incubated in 1/100 normal serum (Vectastain Universal Elite, BC Kit, Vector), in a 10% PBS solution of bovine serum albumin (BSA) at normal temperature for 30 min in a humid chamber. After incubation, the primary anti-protein S-100 antibody (Dako, Argentina) was applied, and the slides were incubated overnight at 41°C in a humid chamber. The slides were then rinsed in PBS and incubated for 7 min in a solution of 50 mL of 30.3-diaminobenzidine (DAB, Sigma- Aldrich) containing 1% PBS-BSA in 50 mL $H_2O_2$. Lastly, the counterstaining was performed with hematoxylin.

Small tissue fragments were cut into 1 mm blocks and immediately fixed in phosphate buffered glutaraldehyde (pH 6.9 at 4 °C), washed in Millonig's solution, and thereafter postfixed in 1% osmium tetroxide. The tissues blocks were then dehydrated in a graded ethanol-acetone series, immersed in propylene oxide, and embedded in Durcupan ACNI (Fluka Chemie A.G., Switzerland). Thin sections were cut with an LKB ultramicrotome and stained twice with uranyl acetate and lead citrate before examination with a Jeol JEM-8T electron microscope (Jeol, Tokyo, Japan).

With optical microscopy, it was possible to find a neoplasm formed by spindle cells, in fewer numbers, round or oval cells forming nests. Cellular components disperse in a flaccid, messy pattern, often in a lax myxoid stroma, elongated and serpentine cells predominate, with their thin spindle-shaped nuclei. The flaccid and disorderly architecture helps to differentiate these tumors from other spindle-shaped tumors such as fibromas. Both neoplasms showed a similar cellular pattern (Fig. 2 and 3).

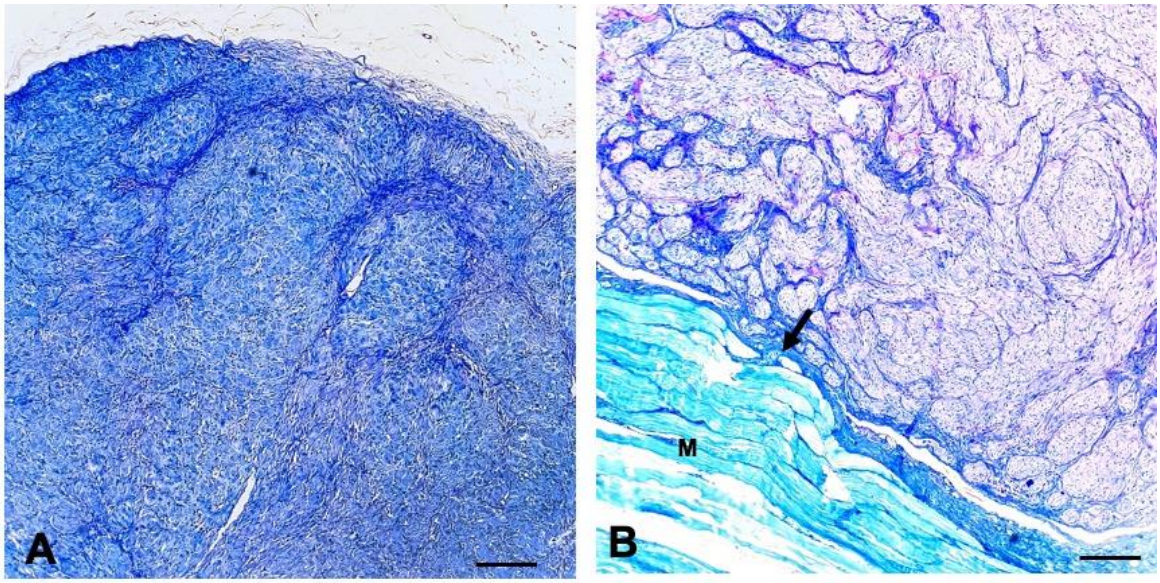


**Figure 2. A:** Panoramic view of the neoplasm lesion formed by spindle cells arranged in swirling H-E pattern. Bar = 200 µ. **B:** The proliferation of spindle cells with a myxoid stroma can be observed. A thin capsule separates it from the adjoining muscle and is not infiltrated (arrow). M = muscle. Giemsa. Bar = 200 µ.

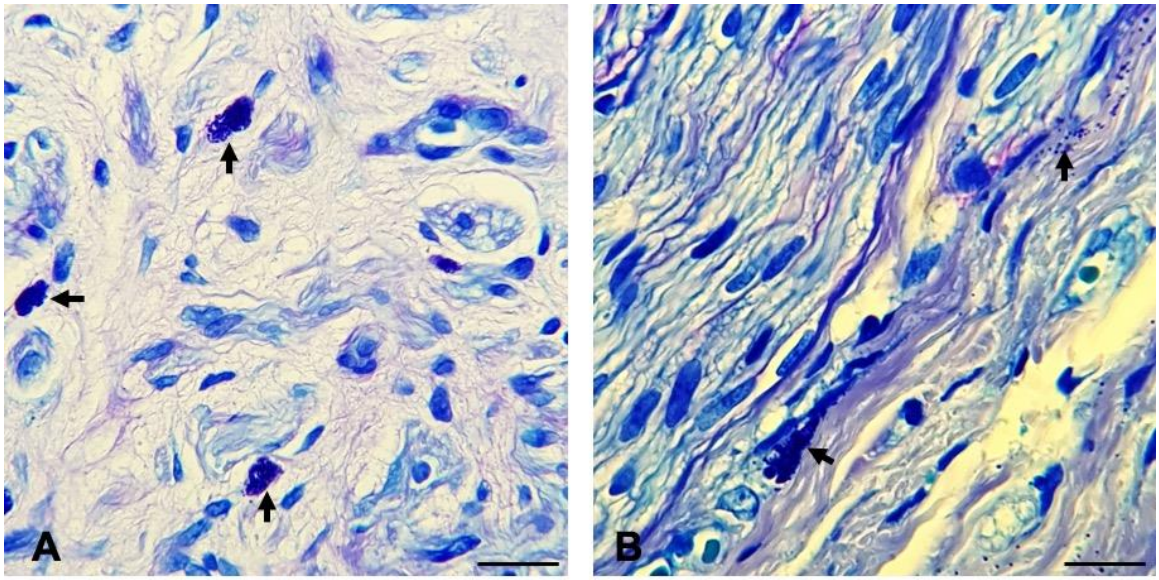


**Figure 3. A:** Spindle-shaped neoplasm cells with a myxoid stroma. Mast cells can be seen (arrow.) Giemsa. Bar = 100 µ. **B:** Spindle-shaped neoplastic cells with myxoid stroma showing degranulated mast cells (arrow). Giemsa. Bar = 20 µ.

The immunohistochemical examination of both neoplasms revealed that the cells were positive for the anti-protein S-100 antibody (Fig. 4).

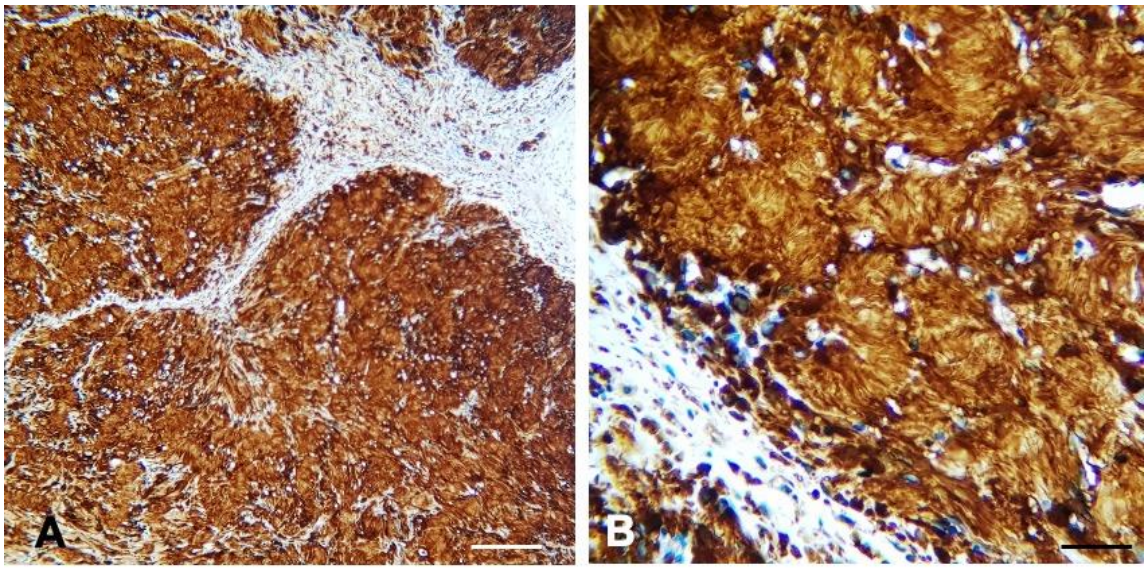


**Figure 4.** Neoplastic cells positive for the anti-S-100 protein antibody. **A:** More spindle cell area. **B:** More compact cell area. Bar = 20 µ.

With electron microscopy, spindle cells with nuclei of variable shape were identified. In the cytoplasm, were observed abundant organelles rough in the endoplasmic reticulum, Golgi apparatus and mitochondria. Degenerative processes of nerve fibers were observed in some cells, consisting of a roughly distended, disorganized, and focally fragmented endoplasmic reticulum. Isolated figures of myelin and dense osmophilic material could also be observed. The cells showed membranous elements and free ribosomes in the encircled area that extruded through a focal rupture of the basement membrane. In sectors, a cytoplasmic process could be observed with cellular degeneration marked by the disorganization of the rough endoplasmic reticulum and the loss of the ribosome (Fig. 5).

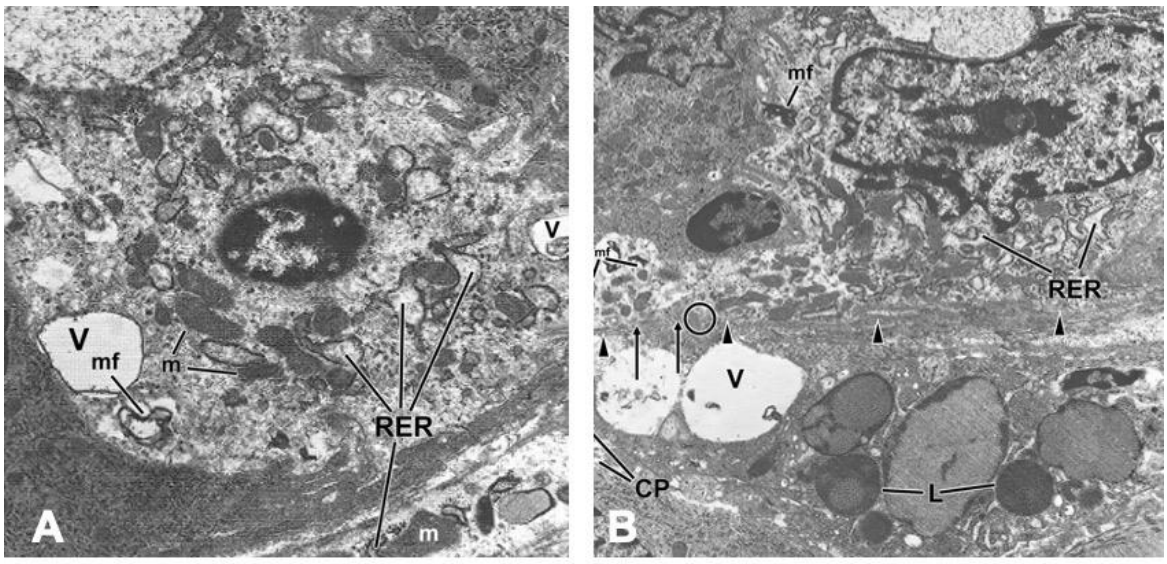


**Figure 5. A:** Degenerative cellular process of nerve fibers consisting of a distended, disorganized, and focally fragmented rough endoplasmic reticulum (RER), figures of myelin (mf) and polyribosomes, and relatively intact mitochondria (m) surrounded by what appears to be a rarefied cytoplasmic matrix with isolates and vacuoles (V). (x 15,750). **B:** Cells with membranous elements (mf) and free ribosomes in the surrounded area that are extruded through a focal rupture (arrows) of the basement membrane (arrowheads). Cellular material includes two large clear vacuoles (V), prominent lysosomes (L). A cytoplasmic process (CP) can be seen in the lower left corner. Prominent dilated Golgi (G), rough endoplasmic reticulum (RER) disorganization, and loss of attached ribosome are clearly demonstrated in this degenerating cell. (x 28,000).

Mast cells with granules exhibiting the typical intragranular formations were observed in isolation (Fig. 6).

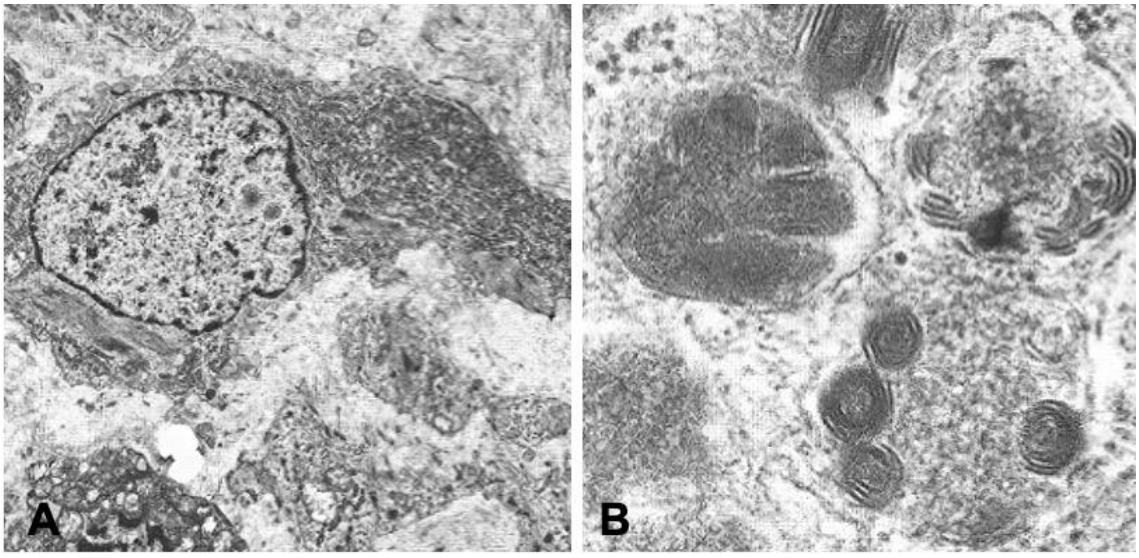


**Figure 6. A:** A cell is observed that contains a nucleus with scarce chromatin and filaments near the cytoplasmic margins. The cell appears to be bound together by collagen fibrils (F). In the lower left border, a cell with abundant mitochondria (M) is observed, possibly a fibroblast. (X 6000.). **B:** Mast cell with granules exhibiting shells cut in various planes as well as amorphous material. (x 39,000).

In mammals and humans, microscopically, there are several growth patterns in the same tumor, and they can cause confusion in interpretation. The main histopathological differential diagnosis of neurofibroma should be made with Benign Peripheral Nerve Sheath Tumor (BPNST), chromatophoroma, perivascular wall tumors (PWTs) including: schwannoma, neurofibroma, perineurioma, ganglioneuroma, fibroma, fibrolipoma, and traumatic neuroma. Schwannoma is the one that may cause the most confusion in the interpretation since both are positive when they are labeled with anti-protein-S-100 antibody. However, his classic histological pattern are the Antoni A and Antoni B patterns, in which the former consists of solid-looking nodules of compact bundles and whorls of Schwann cells whose nuclei are characteristically aligned in rows. The Antoni B pattern is commonly mixed with the Antoni A, but all neoplasms have classic separated Schwann cells and frequent lipid histiocytes can be seen. The other neoplasms mentioned are negative when immunolabelled with an anti-S-100 protein antibody. S-100 protein is extremely useful in this context, because it is strongly expressed by schwannomas and more variably expressed by neurofibromas. In addition, the presence of scattered neuro lament protein (NFP) positive axons is typical of neurofibroma, whereas axons are rare within schwannomas. The calretinin is typically diffusely present in schwannomas, whereas neurofibromas lack that marker or are labeled only weakly for it (Weiss *et al*., 1983; Weiss and Goldblum, 2011). An important component of this pattern is the accumulation of dilated blood vessels with thickened hyaline walls, perivascular hyaline deposits, and fibrinous mural thrombi (Haraida *et al.,* 1992; Zhang *et al.,* 2017).

Recent and old bleeding areas with hemosiderin-laden macrophages are common, while the presence of fine melanin deposits is very rare. It is interesting to find in neurofibromas mast cells, in some cases, abundant and degranulated. There are pleomorphic neurofibromas, with large mono and multinucleated hyperchromatic cells that are believed to indicate regressive changes and no malignant transformation (Meis-Kindblom and Enzinger (1994).

In our laboratory we have diagnosed several neoplasms in different species of fish, including multiple neurofibromas of the heart (Romano and Marozzi, 2004; Romano *et al.,* 2013; Romano *et al.,* 2014; Romano *et al.,* 2015). Some authors found MCs and EGCs similar and describe the latter in neurofibromas of some species of fish (Vicha and Schmale, 1994; Schmale *et al.,* 2004). The presence of MCs in various neoplasms is well known. In neurofibromas, especially, they are seen frequently (Dalton and Noelle, 2012; Derakhshani *et al.,* 2019).

The physiological relationship of the MCs involved in the development and progression of neurofibromas and other tumors in mammals is not clear. Some study results showed contradictions about

the role of MCs in neurofibromas and other tumors and factors affecting their distribution. Mast cell products were reported to stimulate the proliferation of some tumor cells both *in vivo* and *in vitro* (Roche, 1985; Roche, 1986; Yamazaki *et al.*, 2018). However, other study suggested that the presence of mast cells is an indicator of good prognosis (Donhuijsen *et al.*, 1992). It is possible that the activation mechanisms of MCs can release cytokines that produces a severe inflammatory response, that could control the neoplasm growth (Yamazaki *et al.*, 2018), but it is still necessary to determine the composition of MCs in the tumor environment. Based on current knowledge on the role of MCs in inducing inflammation, selective inhibition of angiogenesis and targeted immunosuppression by MCs and, on the other hand, the stimulation of their ability to produce cytotoxic cytokines, they can control the growth of neoplasms (Cimpean *et al.*, 2017; Yu *et al.*, 2018).

In summary, in this work we study two abdominal neoplasm lesions in a *Betta splendens* specimen that were diagnosed as neurofibroma, positive for the antibody to protein S-100 with an ultrastructure that showed signs of degeneration in some cells and contained mast cells regularly distributed in the neoplasm mass. The case presented in this work provides important information about the diagnosis of a fibrillar neoplasm, with characteristics of neurofibrillary origin, which may generate future discussions in the field of neoplasms in fish.

**Conflict of interest**

Authors declare no conflict of interest.

**Acknowledgements**

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