Received: 16 September 2020 | Revised: 28 October 2020 | Accepted: 29 October 2020

DOI: 10.1111/jfd.13303

SHORT COMMUNICATION

Journal of Fish Diseases WILEY

Mixed Chromatophoroma (benign irido-melanocytoma) in a male Siamese fighting fish, *Betta splendens*, Regan

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Keywords: benign irido-melanocytoma, betta splendens, mixed chromatophoroma, pigment cell neoplasm, siamese fighting fish

1 | INTRODUCTION

The colour of fish skin derives largely from the presence of pigment cells (chromatophores), which produce membrane-bound organelles to elicit colour and absorb or reflect light (Fujii, 1993). Light absorbing chromatophores include melanophores (black pigment), erythrophores (red), xanthophores (yellow) and cyanophores (blue), while reflecting pigment cells include iridophores and leucophores (Bagnara & Matsumoto, 2007). *Betta splendens* have melanocytes, erythrophores, xanthophores, iridophores and leucophores (Khoo et al., 2012).

Pigment cell neoplasms have been reported to occur in fish both in natural and man-made habitats (Masahito et al., 1989). The pigment cells that give rise to these tumours originate in the neural crest; they are generated from nerve-associated stem cells in the post-embryonic phase of life (Parichy & Spiewak, 2015). Generally referred to as chromatophoromas, pigment cell-derived tumours may contain more than one cell type or pigment type (Schartl et al., 2016). The following is a description of a mixed chromatophoroma composed of two cell types (benign, irido-melanocytoma), which is the first definitive description of this type of neoplasm in *Betta splendens*, and only the third chromatophoroma described from this species (Ciambrone et al., 2019; Rahmati-Holasoo et al., 2019). Melanophores will be referred to as 'melanocytes' throughout the text, in concordance with recent recommendations by the European Society for Pigment Cell Research (Schartl et al., 2016).

2 | CASE PRESENTATION

In 2017, a captive male *Betta splendens* was referred for necropsy and histopathologic examination of a mass just cranio-dorsal to its caudal fin. The fish lived in a one-gallon unfiltered aquarium with glass beads and a bamboo plant. The owner performed a partial water change approximately once a week. The fish was fed a commercially available pelleted *Betta* fish diet. The fish was acquired approximately 2 years prior from a pet store. The owner first noticed the tumour approximately 6 months prior to death, with gradual tumour growth (Figures S1,S2). The fish did not exhibit noticeable swimming deficits until it acutely developed an erratic swim behaviour. It was found dead several days later. The owner placed the fish in a refrigerator overnight and submitted it for necropsy the following day.

When examined, a 3- to 6-mm-wide, verrucous, pale grey, firm sessile mass was present at the base of the caudal peduncle (Figure 1a). The fish was dissected and all tissues were fixed in neutral buffered formalin for about 24 hr, then decalcified in Kristensen's solution for about 12 hr, serially sectioned, embedded in paraffin wax, and processed for light microscopy slides. Tissues were stained with haematoxylin and eosin (H&E) or bleached with hydrogen peroxide and counterstained with H&E (melanin removal) prior to mounting. Subsequently, a 2-mm-wide block of formalin-fixed tissue was processed for ultramicroscopy using standard procedures; paraffin was removed, tissues were post-fixed in osmium tetroxide, washed, embedded in resin, and sectioned for transmission electron microscopy. A JEOL 1230 120 kV electron microscope was used to view stained grids and capture images.

The mass was a densely cellular expansile mesenchymal neoplasm composed of heavily pigmented cells that replaced skin, soft tissue, and muscle (Figure 1b,c). Two distinct cell types were arranged in haphazard densely packed fascicles, thoroughly admixed. Approximately half the cells contained abundant fine black granular cytoplasmic pigment that obscured the nuclei (melanocytes). The second population contained cytoplasmic pale grey/tan crystalline

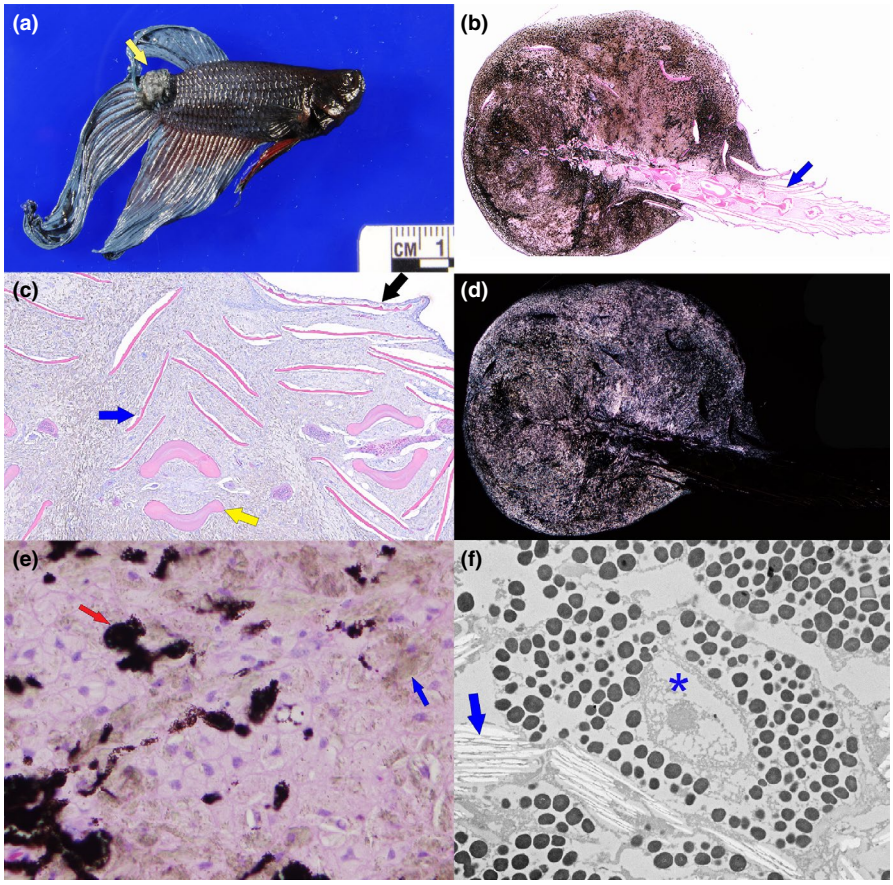

FIGURE 1 Mixed chromatophoroma in a Siamese fighting fish, *Betta splendens*. (a) Yellow arrow denotes a 6-mm-wide, verrucous neoplasm on the caudal peduncle, which is mottled grey/black. (b) Photomicrograph of the transverse cross section of the caudal peduncle. The blue arrow denotes the scales, muscles, and bone. The neoplasm is mottled black/grey/pink, is densely cellular and primarily expands the integument along the infiltrative margin. Large numbers of black pigmented cells are present throughout the mass. Haematoxylin and eosin, 20×, composite image. (c) Photomicrograph of the mixed chromatophoroma. A densely cellular mesenchymal neoplasm replaces muscle and soft tissues, with relative sparing of the scales (blue arrow) and hemitrichs (yellow arrow). The epidermis is intact (black arrow), with scales that have enlarged and mildly irregular circuli. Black pigment is not evident due to the use of a melanin bleach stain, 100×. (d) Photomicrograph of the transverse cross section of the caudal peduncle seen in panel B, viewed with polarized light. The iridophore reflecting platelets are clearly visible and outline a negative image of the non-neoplastic tissues and melanocytes. When compared with panel B, a complete mixture of cell types (melanocytes and iridophores) can be seen. Haematoxylin and eosin, 20×, composite image. (e) Photomicrograph of the mixed chromatophoroma. Cells are large and pleomorphic with distinct cell borders, ovoid nuclei, and abundant pale pink cytoplasm filled with pale grey/tan crystals (blue arrow- iridophores). Melanocyte (red arrow) cell features are obscured by abundant black pigment. Haematoxylin and eosin, 400×. (f) Ultramicrograph of the mixed chromatophoroma. The nucleus of a melanocyte is denoted by the asterisk, and is surrounded by many electron-dense mature melanosomes. The arrow denotes the many thin overlapping reflecting platelets of the iridophores. Note there is distinct separation of pigment cell types. Formalin-fixed, osmium tetroxide post-fixed, 8,000×

material (iridophore or leucophore), the abundance of which could be best appreciated under polarized light, where this pigment was birefringent (Figure 1d). The distinction between cell types (melanocyte and iridophore/leucophore) was marked; cells with black granular pigment were not birefringent, while cells with pale grey/tan crystalline were birefringent, without overlap. Both cell populations were otherwise similar, aside from pigment content; they were large and round to spindle with distinct cell borders, abundant fine cytoplasm (pale pink when unobscured by pigment) and intermediate to large ovoid nuclei with large central nucleoli (Figure 1e). Cell variation was minimal; however, pigment did vary considerably. Mitoses were not evident. Ultrastructurally, two distinct cell types were evident, each containing only one pigment type (Figure 1f). The melanocytes were filled with large spherical electron-dense cytoplasmic mature melanosomes. The iridophore/leucophore population interdigitated between melanocytes, containing an abundance of thin, stacked, non-staining crystal platelets. The character of these platelets is consistent with iridophores, and not leucophores (Lewis et al., 2019).

The neoplasm was infiltrative along the integument, with progressive invasion and atrophy of muscle (Figure 1b). Interestingly, scales and hemitrichs were displaced but not resorbed; scales still associated with the epidermis exhibited mild irregular thickening of the circuli (Figure 1c). Several clusters of macrophages were

randomly present in the mass; cells had abundant foamy cytoplasm with mixed melanin and crystalline pigments (chromatomacrophage/melanomacrophage centres). Based on the presence of 2 distinct chromatophore populations, a lack of mitoses and cell atypia, as well as the ultrastructural features, a diagnosis of a benign mixed chromatophoroma (iridophore and melanocyte) was made.

3 | DISCUSSION AND CONCLUSIONS

Melanomas/melanophoromas have been commonly reported in fish, and the first report of a pigment cell-derived tumour in a Siamese fighting fish, *Betta splendens*, Regan, described a melanoma invading into a local angiolipoma (Rahmati-holasoo et al., 2015). A recent report of a mixed chromatophoroma on the head of a *Betta splendens* described a neoplasm similar to our case; however, the specific details of the reflecting pigment cell type were unresolved (Ciambrone et al., 2019). A more recent report of an iridophoroma in *Betta splendens* described a white neoplasm that was composed entirely of a population of iridophores (Rahmati-Holasoo et al., 2019). In addition, the iridophoroma was malignant, with moderate cell variability and metastasis. The iridophore component in that case was similar to the iridophore component in our case, including the grey/tan (greenish) tint to the pigment plates in the H&E sections. However in our case, approximately half the cells were melanocytes, and the other half were iridophores. The intimate admixture of cell types and the nature of the pigment seen by H&E were consistent with examination by electron microscopy. The ultrastructural features of the melanocytes and iridophores in the neoplasm of our case are similar to those described previously in *Betta splendens* (Amiri & Shaheen, 2012; Khoo et al., 2014). Interestingly, the close approximation of melanocytes and iridophores in this neoplasm mimics their natural affinity for one another in normal *Betta splendens* (Khoo et al., 2014). In this case report, we have shown evidence to support the presence of melanocytes and iridophores in the neoplasm by microscopic and ultrastructural analysis. Finally, our ultrastructural analysis did not find evidence for mosaic cells which have pigmented bodies of more than one chromatophore type; they have been seen in fish and reptile neoplasms (mosaic chromatophoromas) (Matsumoto et al., 1980; Ryan et al., 1981).

The aetiology of pigment cell derived tumours have not been well elucidated, but have been speculated to arise due to several different causes such as carcinogenic, hereditary, and ageing factors (Masahito et al., 1989). Epidemiological surveys suggest environmental contamination with chemical carcinogens (Black, 1983; Kimura et al., 1989; Okihiro, 1988) and the possibilities of sediment-borne or marine toxins have been proposed (Fabacher et al., 1991; Work & Aeby, 2014). In fact, experimental induction of pigment cell tumours has been accomplished (Fabacher et al., 1991; Kimura et al., 1984; Okihiro, 1988). Hereditary factors were shown to determine melanoma occurrence in platyfish/swordtail hybrids (Anders, 1967; Anders et al., 1984; Reed & Gordon, 1931). Okihiro (1988) made a persuasive case that oncogenic viruses should be considered as a potential aetiology. Spontaneous occurrences of chromatophoromas in a single fish have been described (Colorni, 1997; Schmidt-Posthaus et al., 2005) and Rahmati-holasoo et al. (2015) suggested that the melanoma described in their case report occurred due to male sex hormones or ultraviolet radiation.

The presence of melanocytes and iridophores in a neoplasm is not surprising, given their similar stem cell origin (Kelsh et al., 2017; Mort et al., 2015). Furthermore in *Betta splendens*, irido-melanophore units have been described in normal skin, which function to elicit all the many shades and colours seen (Khoo et al., 2014). This presents us with three likely explanations for the aetiology of this neoplasm: (a) the neoplasm arose from a bipotential stem cell (both cell types are neoplastic), (b) two separate neoplasms have developed (collision tumour-both cell types are neoplastic), or (c) only one chromatophore population is neoplastic, and the other is present by affinity and hyperplasia. Albeit presumptive, the authors consider the first option the most appealing, but without further research, none can be disproven.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Skip Jack for his assistance in the preparation of this manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Shivley JM, Brookshire WC, Baumgartner WA. Mixed Chromatophoroma (benign irido-melanocytoma) in a male Siamese fighting fish, *Betta splendens*, Regan. *J Fish Dis*. 2020;00:1–4. https://doi.org/10.1111/jfd.13303