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Short Communication

Invasion of melanoma to angiolipoma in a male Siamese fighting fish, *Betta splendens*, Regan

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Lipomas are mesenchymal and benign neoplasms arising of mature adipocytes. Lipomas have been reported from a number of fish species including bream, *Abramis brama* (L.) (Mawdesley-Thomas & Bucke 1968), largemouth bass, *Micropterus salmoides* (Lacepède), (Mawdesley-Thomas 1972), black crappie, *Pomoxis nigromaculatus* (Lesueur) (Harshbarger 1972), southern bluefin tuna, *Thunnus maccoyii* (Castelnau) (Lester & Kelly 1983; Johnston *et al.* 2008), channel catfish, *Ictalurus punctatus* (Rafinesque) (McCoy *et al.* 1985), European eel, *Anguilla anguilla* (L.) (Easa, Harshbarger & Hetrick 1989b; Easa *et al.* 1989a), striped mullet, *Mugil cephalus* (L.) (Easa *et al.* 1989a,b), common dab, *Limanda limanda* (L.) (Bruno, McVicar & Fraser 1991), striped seabream, *Lithognathus mormyrus* (L.) (Volpatti *et al.* 1998), northern bluefin tuna, *Thunnus thynnus* (L.) (Marino *et al.* 2006), European seabass, *Dicentrarchus labrax* (L.), (Marino *et al.* 2011) and molly, *Poecilia velifera* (Regan), (Stefano *et al.* 2012). Chromatophoromas or pigment cell tumours are common tumours in fish, and outbreaks have been reported in both marine and freshwater fish throughout the world (Okihiro *et al.* 1993; Ramos, Victor & Branco 2013). Melanoma is the most commonly reported pigment cell tumour in fishes living under natural conditions (Ghadially & Gordon 1957). Melanomas have been reported in zebrafish, *Danio rerio* (Hamilton) (Stolk 1953), hybrid Fish Lebistes × Mollienesia (Ghadially & Gordon 1957), *Astyanax jordani* (Hubbs & Innes) (Stolk 1958), Leopard corydoras, *Corydoras julii* Steindachner (Cohen 1965), Hawaiian butterflyfish, *Chaetodon miliaris* Quoy & Gaimard (Okihiro 1988), brown bullhead, *Ameiurus nebulosus* (Lesueur) (Sakamoto & White 2002), longhorned cowfish, *Lactoria cornuta* (L.) (Da Silva *et al.* 2010), nurse shark, *Ginglymostoma cirratum* (Bonnaterre) (Waldoch *et al.* 2010), coral trout, *Plectropomus leopardus* (Lacepède) (Sweet *et al.* 2012) and Hawaiian goldring surgeonfish, *Ctenochaetus strigosus* (Bennett) (Work & Aeby 2014). The Siamese fighting fish or Betta, *Betta splendens* Regan, an anabantid fish, is a popular fresh water ornamental fish worldwide due to its vibrant colours and because of the male's pugnacious nature and beauty, the representative fish is called *B. splendens* (the splendid battler) (Monvises *et al.* 2009; Lombardini, Law & Lewis 2010). Tumours in Siamese fighting fish, *B. splendens,* have been reported rarely. Nephroblastoma has been reported in two male Siamese fighting fish (Lombardini *et al.* 2010). This report is the first report

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of concurrence of melanoma and angiolipoma in Siamese fighting fish, *B. splendens,* and also the first report of angiolipoma in fish.

In July 2013, a male Siamese fighting fish, *Betta splendens*, with a mass in right side and base of the anal fin was referred to the Section of Fish Disease (Fig. 1a). Based on owner information, fish was 2 years old at the time of referral and the mass had started to grow from 3 months ago. Fish behaviour and appetite were normal. The mass was soft and greasy in appearance. Examination with stereomicroscope showed that base of the mass was black and white and centre of the mass was white to yellow with red spots (Fig. 1b). Fish was killed and necropsied for histopathological examination. Other organs exhibited normal appearance. The mass with surrounding tissues and internal organs were fixed in 10% neutral buffered formalin and routinely processed, dehydrated and embedded in paraffin wax, sectioned at 4 μm in thickness and stained with haematoxylin and eosin (H&E). The mass sections were also stained with Masson Fontana (Churukian 2002) and immunohistochemical staining for Melan A (Waldoch *et al.* 2010).

Microscopic examination revealed the mass mainly composed of adipose cells and their cytoplasms showed a large vacuole (Fig. 2). The adipose cells were round or polyhedral in appearance and they had various sizes and had a flattened and curved nucleus that was pushed to the periphery of the cell. Many capillaries (Fig. 2), which were dilated and filled with erythrocytes, particularly at the periphery of the mass were observed densely packed among the adipose cells. Fibrin clots were occasionally observed in some of the capillaries. Based on histological findings, this was diagnosed as an angiolipoma (Fig. 2). Next to the angiolipoma, melanin pigments, both in the cells and in the stroma of the mass, were present in epidermis and dermis. Melanin pigments almost completely masked the histological detail (Fig. 3). Epithelioid cells were also seen in dermis (Fig. 4). These cells had abundant and eosinophilic cytoplasm and large pleomorphic nuclei with prominent nucleoli (Fig. 5). The mitotic activity was moderate. Blood vessels and haemorrhage were sparse throughout

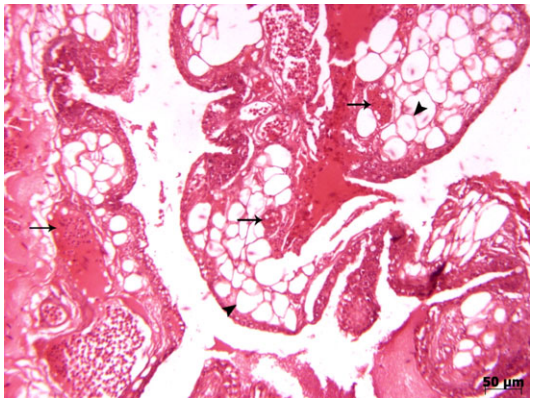

Figure 2 Histological section of the angiolipoma. Fat cells (arrowheads), small blood vessels (arrows) (H&E).

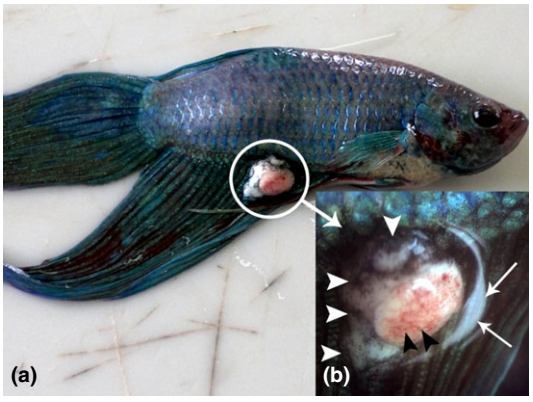

Figure 1 (a) Male Siamese fighting fish, *Betta splendens*, with a mass in right side and base of the anal fin. (b) Base of the mass is black and white (white arrowheads) and center of the mass is white to yellow with red streaks that were similar to vessels (black arrowheads), light reflection (arrows).

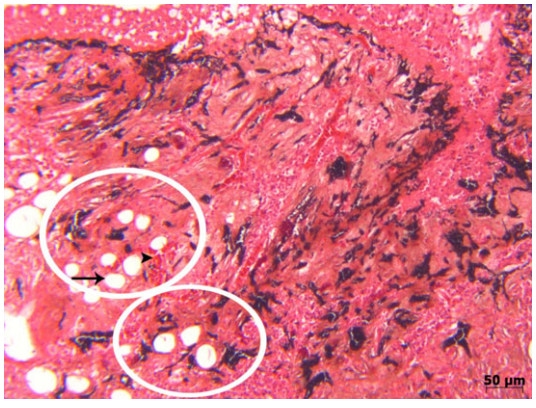

Figure 3 Histological section of angiolipoma (Lipocyte: arrow, blood vessel: arrowhead), melanoma and disorganization of structure of skin. Melanin pigments, both in the cells and in the stroma of the mass, in epidermis and dermis are seen. Local invasion of melanocytes into the angiolipoma (Circles) (H&E).

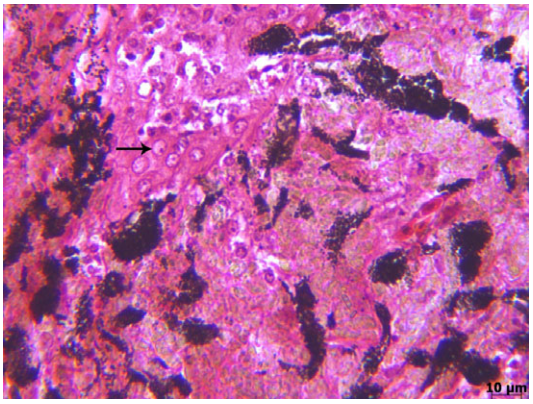

Figure 4 Epithelioid cell type melanoma. Epithelioid cells (arrow) (H&E).

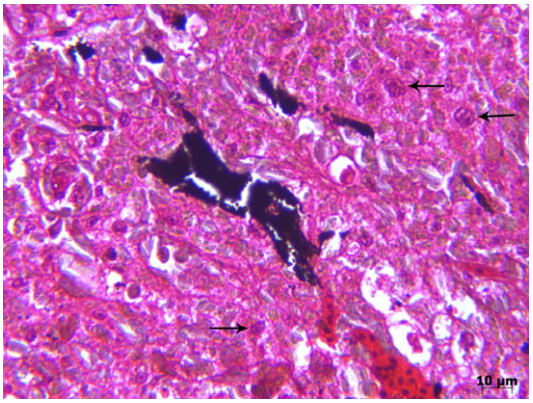

Figure 5 Epithelioid cell type melanoma. Tumor cells (Epithelioid cells) have abundant and eosinophilic cytoplasm and large pleomorphic nuclei with prominent nucleoli (arrows).

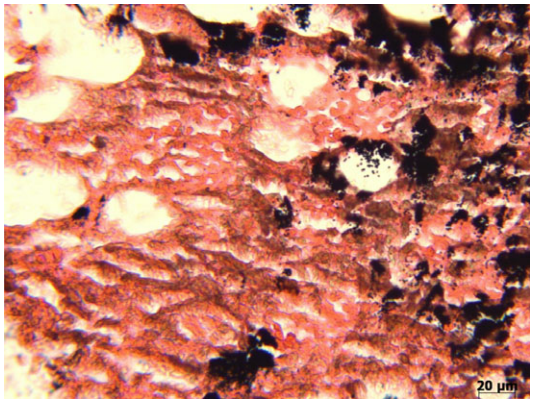

Figure 6 Positive staining for Masson-Fontana in the dermis of *Betta splendens.*

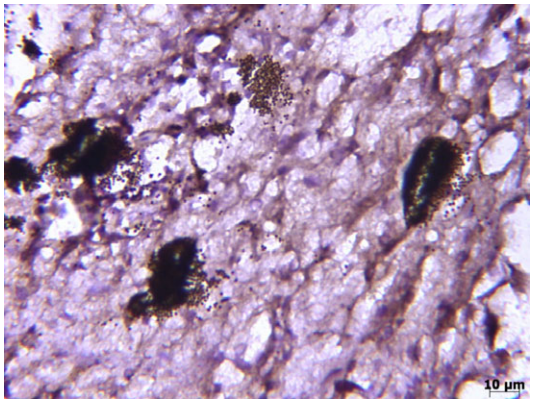

Figure 7 Positive immunohistochemical staining for Melan A.

the whole tumour. Melanocytes in the H&E-stained sections had positive staining reaction for the Masson-Fontana (Fig. 6) and immunohistochemical staining Melan A (Fig. 7), supporting the diagnosis of an epithelioid cell-type melanoma. Also, there was local invasion of melanocytes into the angiolipoma that was observed in H&E (Fig. 3) and confirmed by Masson-Fontana and Melan A staining (Fig. 6 & 7). Metastases were not observed in the internal organs.

Tumours arising from adipose tissue are classified as either benign (lipoma and angiolipoma) or malignant (liposarcoma) (Hendrick *et al.* 1998). Lipomas have been recorded as single or multiple well-circumscribed masses in subcutaneous tissue, liver and mesentery in a few fish species and can grow quite large up to 20 kg in bluefin tuna, *Thunnus thunnus* (Harshbarger 2001; Groff 2004; Roberts 2012). Lipomas of fish are soft and greasy in appearance and cut surfaces often feel greasy to the touch (Harshbarger 2001; Roberts 2012). Lipomas rarely may be associated with vascular (angiolipoma), fibrous (fibrolipoma) or bone (osteolipoma) tissue embedded with them (Harshbarger & Bane 1969; Goldschmidt & Hendrick 2002; Roberts 2012). Fibrolipoma in caudal peduncle of a rockfish, *Sebastes diploproa* (Gilbert) (Harshbarger & Bane 1969), mid-dorsal trunk of Pacific halibut, *Hippoglossus stenolepis* Schmidt (Wellings 1969), striped seabream, *L. mormyrus* (Volpatti *et al.* 1998), coho salmon, *Oncorhynchus kisutch* (Walbaum) (Harshbarger 2003), Lake trout, *Salvelinus namaycush* (Walbaum) (Heidel & Smith 2007), and osteolipoma in operculum of Pacific halibut, *H. stenolepis,* have been described (Wellings 1969). Capillaries were observed between the fat cells of hypodermal lipoma in a

striped mullet, *M. cephalus*, and multicentric lipoma in a molly, *P. velifera*, but vascularization were not prominent to be diagnosed as a angiolipoma (Easa *et al.* 1989b; Stefano *et al.* 2012). There is no report of angiolipoma in fish in literatures. In our case, number of capillaries was remarkable, and the tumour mass was solitary and showed capillary-like pattern. The aetiology of lipoma development is unknown, and errors in fat metabolism, endocrine or neurological disorders (Easa *et al.* 1989a, b) and dysraphic status of bone as a possible predisposing factor in fish (when lipomas arise close to the bone) (Marino *et al.* 2006) have been proposed as possible causes. Location of mass in our case was near to anal fin rays; so, suggested factor (Marino *et al.* 2006) can be a possible reason for our case. However, lipoma in channel catfish, *I. punctatus,* that has been exposed to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine were observed (Chen *et al.* 1996).

Fish melanomas (or melanophoromas) are tumours of the pigmented skin cells, the melanophores, which have the black or dark brown melanin pigment and were best studied in the platy cross swordtail hybrids (Reavill 2010; Roberts 2012; Ramos *et al.* 2013). also melanomas have been reported from non-hybrid *Xiphophorus* fish (Schartl *et al.* 1995). Gimenez-Conti *et al.* (2001) proposed a classification scheme for *Xiphophorus* melanomas based on histopathologic analyses and mentioned that epithelioid cell-type melanoma is formed by cells resembling epithelial cells. They are polyhedral cells with large nuclei and prominent nucleoli. This tumour is predominantly exophytic and appears to be less invasive into the muscle bundles than are the melanocytic and spindle cell-type melanomas (Gimenez-Conti *et al.* 2001). Based on proposed classification scheme by Gimenez-Conti *et al.* (2001), our case is classified as an epithelioid cell-type melanoma. Amelanotic melanomas in interstrain hybrid of medaka after exposing to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine were observed (Hyodo-Taguchi & Matsudaira 1987). Melanomas can also be induced by exposure to ultraviolet (UV) light or carcinogenic chemicals (Masahito, Ishikawa & Sugano 1989). Malanomas have been induced in *Xiphophorus* fish by UV radiation (Sokkar, Mahmoud & Mahrous 2001; Wood *et al.* 2006). Melanomas were induced experimentally in platyfish–swordtail hybrids, southern platyfish, *Xiphophorus maculatus* (Günther) crossed with green swordtail, *Xiphophorus hellerii* Heckel (Gimenez-Conti *et al.* 2001) and swordtail fish using UV rays (Setlow, Woodhead & Grist 1989; Sokkar *et al.* 2001). Melanoma has been induced by chemical agents such as *N*-nitroso-*N*-methylurea in *Xiphophorus* hybrid fish (Kazianis *et al.* 2001). Also, melanoma has been observed in *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-treated inbred strain of medaka, *Oryzias latipes* (Hyodo-Taguchi & Matsudaira 1984). In *Xiphophorus* hybrids, tumours may be selectively induced by exposure to UV radiation (Setlow *et al.* 1989; Wang *et al.* 2001). In swordtail–platyfish hybrids, the melanophore colour gene is present, but the modifying genes may be missing, resulting in uncontrolled proliferation of melanophores (Roberts 2012). In some other hybrids, melanomas do not arise spontaneously but do so after exposure to UV light or carcinogenic chemicals, either of which could inactivate suppressor genes through mutations (Harshbarger 2001). Setlow *et al.* (1993) and Wang *et al.* (2001) mentioned that ultraviolet A (UVA) induce melanomas in *Xiphophorus* hybrid fish, but further study showed that ultraviolet A does not induce melanomas in a *Xiphophorus* hybrid fish model (Mitchell *et al.* 2010). Noonan *et al.* (2012) showed melanoma induction in mice by ultraviolet A (320–400 nm) requires the presence of melanin pigment and is associated with oxidative DNA damage within melanocytes. In contrast, ultraviolet B radiation (280–320 nm) initiates melanoma in a pigment-independent manner associated with direct ultraviolet B DNA damage (Noonan *et al.* 2012). Siciliano, Perlmutter & Clark (1971) mentioned that melanoma mostly occurred in male hybrids of *Xiphophorus* fish and suggested that male sex hormones probably increase melanotic process. Male sex hormones or ultraviolet A or B radiation maybe caused occurrence of melanoma in our case.

Alonso, Rodríguez-Peralto & Pérez-Espejo (2003) suggested that the peculiar high vascularity of angiolipomas along with the fibrin thrombi into the vessels might encourage intravascular adherence of tumour cells and could be important factors to explain the mechanism of metastasis of melanoma to angiolipoma. However, suggested reason by Alonso *et al.* (2003) can be a possible cause of invasion of melanoma to angiolipoma in our case. On the basis of the macroscopic and microscopic characteristics, cause of this condition remained unknown.

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