

Original paper

Histological observation of the external gills of a Mexican axolotl (*Ambystoma mexicanum*) with atypical blood vessels

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Abstract: The external gills of captive Mexican axolotl (*Ambystoma mexicanum*) sometimes develop atypical blood vessels, the cause of which is unknown. We observed the external gill filaments of an individual animal with dilated blood vessels that formed a semicircle within the filament tissue. The positioning of the swollen blood vessels compressed the adjacent capillaries and connective tissues. Normal external gill filaments in urodelans contain a blood-vessel system with afferent and efferent arterioles that connect to circumvent the outer gill periphery. We infer that the dilated blood vessels in the axolotl originated from these arterioles.

I. Introduction

The Mexican axolotl (*Ambystoma mexicanum*) is a tailed urodelan amphibian indigenous to Lake Xochimilco and Lake Chalco in Mexico (Zambrano et al. 2007). Over the past 50 years, it has been used as a model organism in disciplines such as evolution, embryology, and regeneration (Reiβ et al. 2015, Voss et al. 2009). Recently its genome has been sequenced to allow studies of comparative genomics, quantitative trait locus mapping, and gene expression analyses. In addition, gene-editing techniques have been used to develop genetically engineered strain of axolotls (Flowers et al. 2014, Keinath et al. 2015, Kuo et al. 2015, Nowoshilow et al. 2018, Page et al. 2013, Voss et al. 2015). Furthermore, the Mexican axolotl has gained widespread popularity as a pet (Lang 2013, Reiβ et al. 2015).

Amphibian larvae have either external or internal gills (Brunelli et al. 2009). Generally, urodele larvae have external gills on both sides of the neck until metamorphosis. However, the axolotl does not metamorphose at sexual maturity, and instead retains its external gills (Bishop 1994). In captivity, some axolotls exhibit atypical blood vessels that protrude from the gills (Fig. 1). This phenomenon is informally termed 'vascularization' among axolotl lovers, but there is limited information regarding the identification or

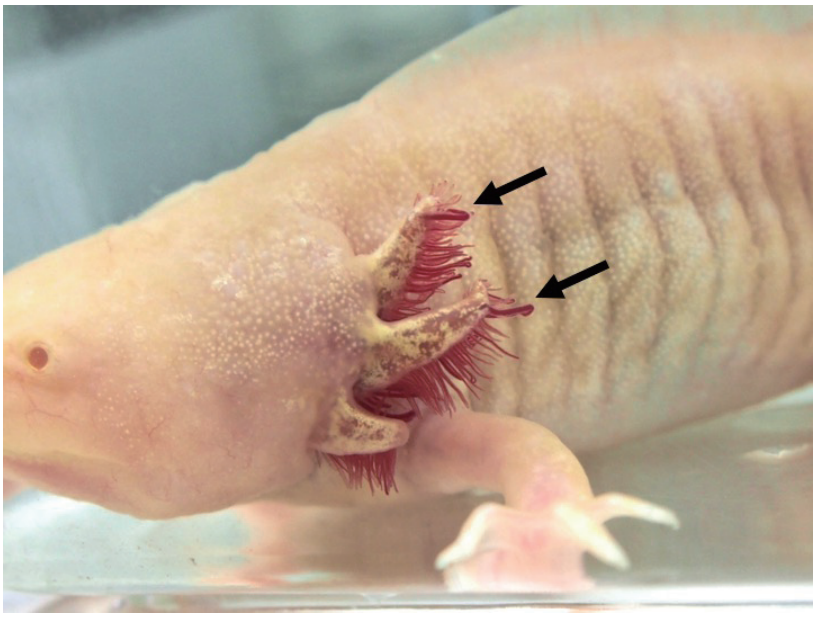

Fig. 1. The external gill of a Mexican axolotl. Arrows indicate atypical blood vessels.

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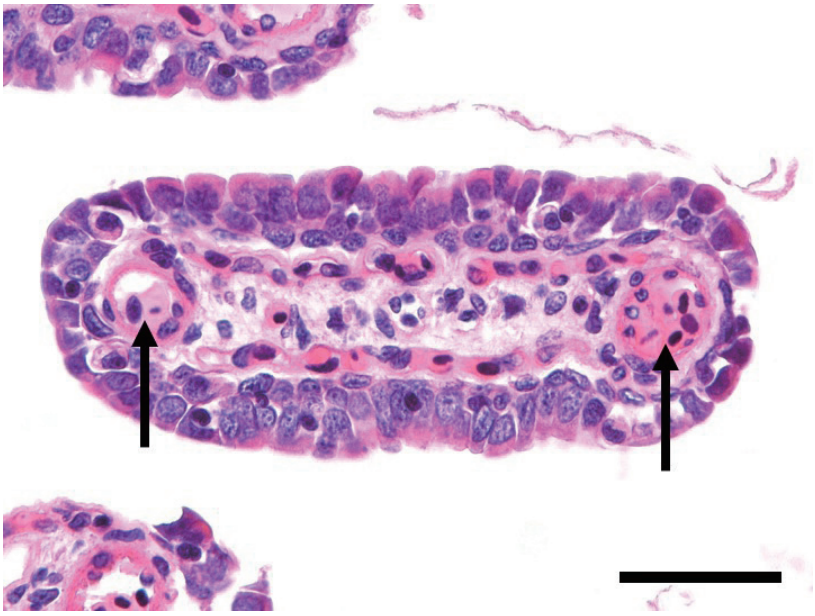

Fig. 2. Histological cross-section of normal external gill filament tissue stained with hematoxylin–eosin (HE). Large arterioles on both sides of the cross-section are indicated with arrows. Scale bar, 0.05 mm.

development of these formations. Therefore, we observed the histological structure of the external gills with atypical blood vessels to elucidate this phenomenon.

II. Materials and methods

The Mexican axolotls with normal external gill filaments (n = 3) and atypical external gill filaments (n = 1) were compared. The axolotls were purchased from private breeders as juveniles with outwardly normal gills. They were housed in tanks with running water at 17–25°C, and fed commercial fish pellets (Kyorin Co., Hyogo, Japan) twice a week for 6–10 months until atypical blood vessels developed in the gills. All animal care and use procedures were carried out according to high ethical and scientific standards.

The animals were euthanized with an overdose of isoflurane and dissected in 0.6% saline, after which the gills were isolated and studied under a stereoscopic microscope (SZX-ILLB100; Olympus Co., Tokyo, Japan). The gills were fixed overnight in Bouin's or Davidson's solution (Bell and Lightner, 1988), dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Microtome sections with a thickness of 4 μm were stained with hematoxylin–eosin (HE) and analyzed under an optical microscope (OPTIPHOTO-2; Nikon Co., Tokyo, Japan).

III. Results and Discussion

Cross-sections of normal external gill filaments revealed that they had an elliptical shape and a periphery covered with layers of epithelial cells (Fig. 2). Within the tissue, there were arterioles at each end of the cross-section. Minute blood vessel walls, presumably those of the capillaries, were also found interspersed throughout the connective tissue.

The external gills of the focal axolotl were composed of three lobes of different lengths, the back of which were irregularly covered with a dense growth of gill filaments (Figs. 3 and 4). Dilated blood vessels were observed on these irregular gill filaments (Fig. 5). Within the filament tissue was a single blood vessel with a semicircular configuration. The inside of this semicircle was coated with a membrane (Fig. 6).

Cross-sections of the atypical gill filament showed two larger arterioles than those observed in the controls, that appeared to compress the medial capillaries and connective tissues (Fig. 7). Serial cross-sections revealed similar dilating of the arterioles in the upper to lower parts of the external gill filament. Moreover, the two blood-vessel walls appeared to merge in the tip (Fig. 8), indicating that they were connected to form a semicircular single blood vessel, as observed in atypical gill filaments under a stereoscopic microscope (Fig. 5).

The epithelia of urodelan external gill filaments contain collagen fibers that fill the connective tissue, as well as Leydig, ciliated, and mitochondria-rich cells (Greven 1980, Jarial 1989, Brunelli et al. 2009). The normal gill filaments in this study likely contained these epithelial cells and collagen-filled connective tissue.

The atypical gill filaments also contained normal-looking epithelial cells. However, the arterioles were dilated and constricted the surrounding

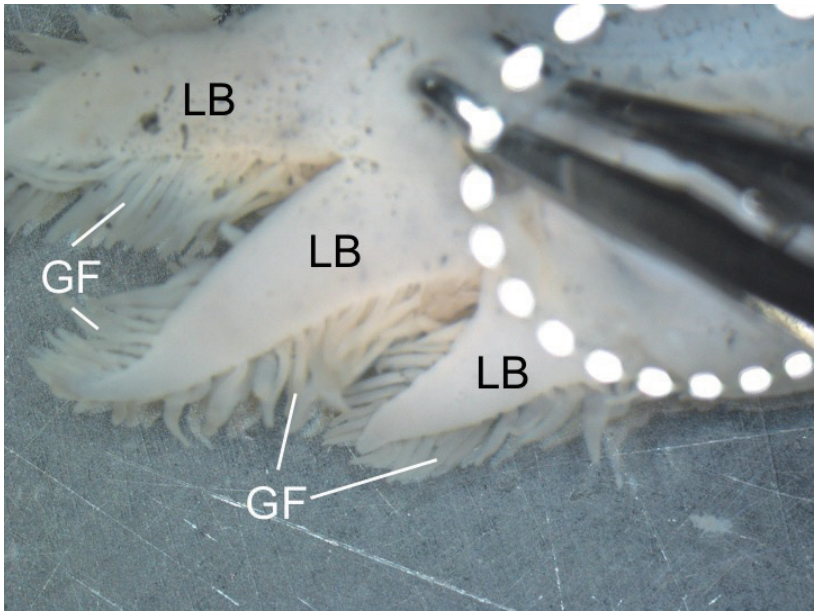

Fig. 3. A normal external gill of Mexican axolotl under a stereoscopic microscope. (LB) The external gill comprises three lobes, (GF) numerous external gill filaments.

Fig. 4. Normal external gill filaments of a Mexican axolotl under a stereoscopic microscope. The filaments are flat and narrow toward the tips. They grow irregularly and thicken along the medial side of the external gill.

connective tissue (Figs. 7 and 8). These blood vessels were positioned at end of the cross-sectional tissue, and potentially developed from efferent and afferent arterioles. In urodeles, these arterioles comprise a single blood vessel that runs along the inner periphery of the gill filament, distal to other capillaries and central venules (Fig. 9) (Kato and Kurihara 1989).

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Fig. 5. The external gill filaments under a stereoscopic microscope. Arrows indicate dilated blood vessels.

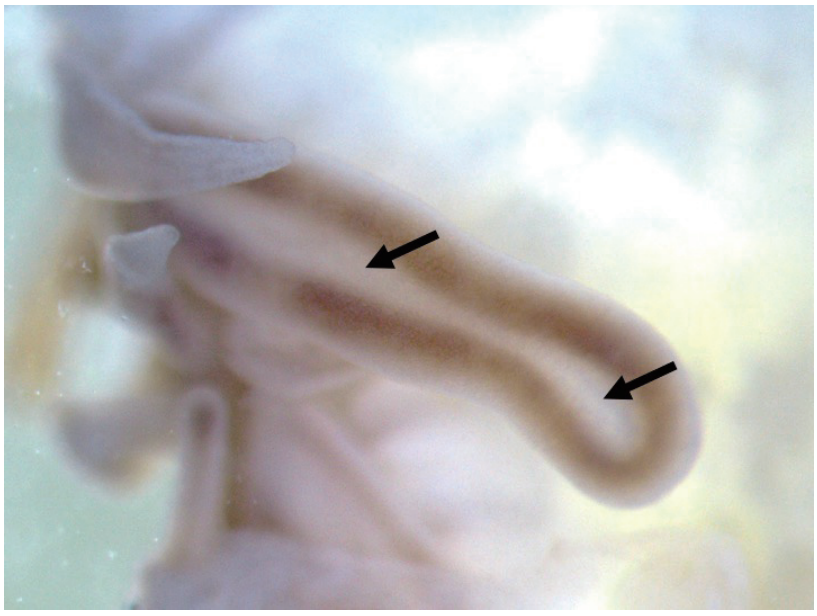

Fig. 6. External gill filament with dilated blood vessels magnified under a stereoscopic microscope. Arrows indicate a membrane visible inside the semicircular blood vessel.

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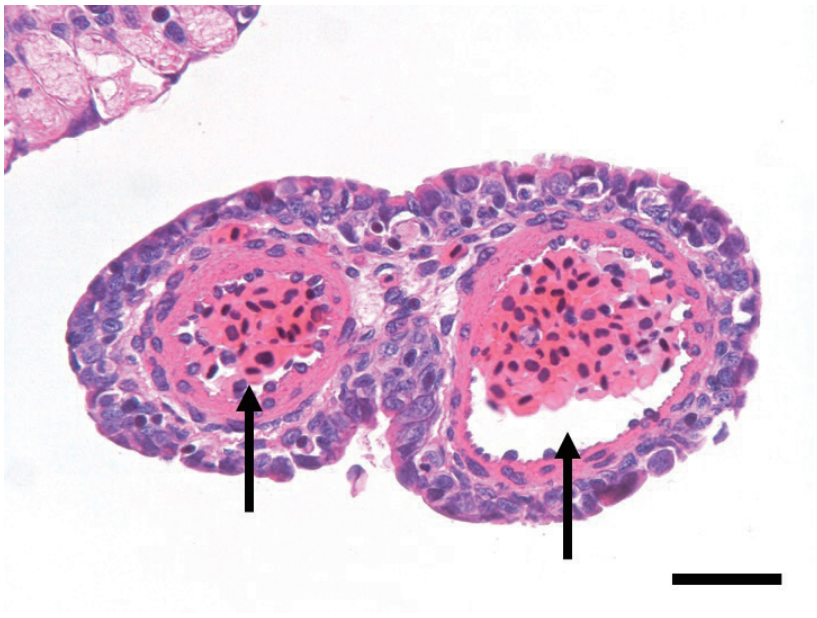

Fig. 7. Histological cross-section of atypical external gill filament tissue stained with hematoxylin–eosin (HE). Dilated arterioles b on both sides in the interior of the tissue are indicated with arrows. Scale bar, 0.05 mm.

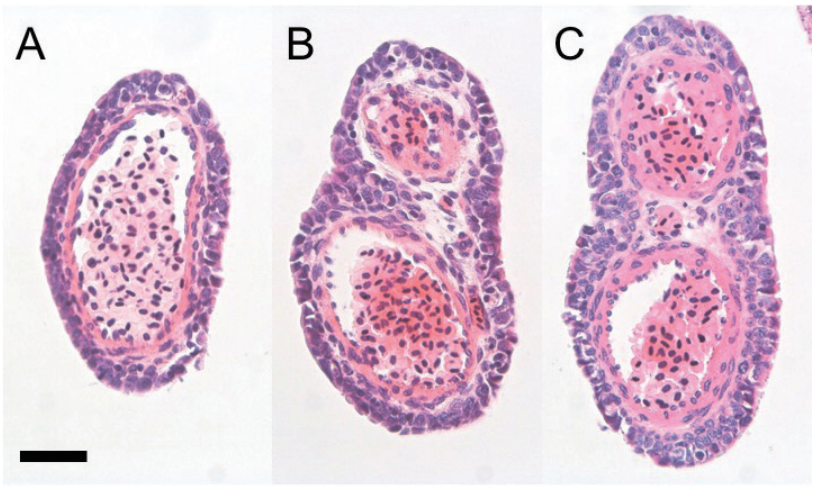

Fig. 8. Serial histological cross-section of an atypical external gill filament stained with Hematoxylin–eosin (HE) from the tip to the root section. (A) Tip of the filament, (B) medial filament section, (C) root section of the filament. Scale bar, 0.05 mm.

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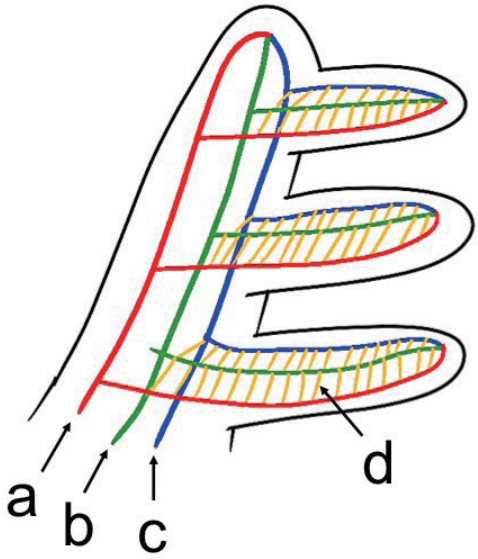

Fig. 9. Diagram of the blood vessels in external gill filaments, modified after Kato and Kurihara (1989). (a) Afferent arterioles, (b) efferent arterioles, (c) central venules, (d) capillaries.

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吉田咲貴・目加田和之：メキシコサラマンダー外鰓の異形血管に関する病理組織学的観察

摘要：メキシコサラマンダーを飼育していると，しばしば外鰓に太い血管が突出することがあるが，その詳細は不明である．この症状が生じた外鰓を実体顕微鏡下で観察したところ，半円形状の肥大化した血管が外鰓フィラメント組織内に認められた．それらの病理組織切片を観察したところ，肥大化した血管がフィラメント断面の周縁に位置しており，それらより毛細血管および結合組織領域が圧縮されたような像が認められた．有尾類の外鰓フィラメントには，外周を迂回するように接続する輸出および輸入細動脈血管が走っており，メキシコサラマンダーの外鰓で観察された肥大化した血管はこれらの細動脈に由来すると予想された．

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