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Microvascular Pattern Formation in the Lungs of the Adult African Clawed Toad, *Xenopus laevis* Daudin as Revealed by Scanning Electron Microscopy of Vascular Corrosion Casts and Correlative Light Microscopy

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The amphibian respiratory tract has a complicated morphology with an intricate vascular system. Currently gross anatomy of the blood vascular system of larval and adult *Xenopus* is primarily known from detailed macroscopic and microscopic dissections [1]. With the exception of previous work on the larval [2] and adult lung [3] descriptions of the vasculature; however, end when vessels reach the organs parenchyma and little is known on the intrinsic microangioarchitecture of *Xenopus* tissues and organs. This work is aimed to demonstrate the microvascular anatomy of trachea, bronchi, and sac-lungs of the adult *Xenopus laevis* in qualitative and quantitative terms with particular stress on spatial aspects of vascular pattern formation.

Nineteen adult *Xenopus laevis* Daudin (body weights: 48 g to 92 g) were cast via the ventricle - conus arteriosus with the polymerizing resin Mercox-Cl-2B (Ladd Research Inc. Burlington, VT) diluted 4+1 (v + v) with monomeric methacrylic acid (20 ml of the monomer contained 1.50 grams of the accelerator paste MA) were injected. Injected animals were macerated (7.5% KOH; 40°C, 12-24h), decalcified (2% HCl), rinsed and frozen in distilled water, freeze-dried, mounted onto specimen stubs, evaporated with carbon and gold, and examined in an SEM (Phillips ; ESEM XL-30, FEI) at 10 kV. According to the necessity, specimens were repeatedly sectioned to expose interesting vascular territories [4]. For light microscopy (Olympus; BX51) animals were fixed with Bouin's solution by vascular perfusion, lungs were removed, immersed in fresh fixative, rinsed, dehydrated, and embedded in paraplast. Transverse sections (7µm) were stained with Goldner's trichrome stain. Micrographs were recorded using a digital camera (Color view III).

The trachea reveals a wide-meshed subepithelial capillary bed which is supplied via anteriorly directed branches of the pulmonary artery, i.e. the tracheal branch of the tracheobronchial artery. It drains into the pulmonary veins via the tracheal branch of the tracheobronchial vein. Between tracheal arterioles and tracheal venules capillaries lack. Left and right pulmonary veins unite shortly before they open into the left atrium (Fig. 1). The vasculature of the bronchus consists of a very dense two-dimensional network of subepithelial capillaries with the longitudinal, transverse and oblique bronchial cartilage impressions (Fig. 2). The whole lung reveals alveoli. They are fed by branches from the pulmonary artery, the circumferential arteries. These arteries run between alveoli and give off short branches, the radial arteries, to the lateral aspect of the alveoli. Alveoli drain via their medial aspects into branches of the pulmonary veins (Fig. 3). Short interalveolar communicating vessels interconnect adjacent alveoli (Fig 4). Tracheal lumen covers with a capillary network. Distinct tracheal cartilages support the trachea (Fig 5a). The bronchi have many bronchial cartilages covered with a dense capillary network (Fig 5b). Visceral pleura cover the entire lung. Septa divide the lung into three orders of alveoli with a double layer of capillaries (Fig 6).

Technically, the entire vascular bed of the respiratory tract was cast well. Although the same procedure was used for vascular casting the quality of filling varied from specimen to specimen.

References
[1] N. Millard, *Trans Roy Soc S Africa*. 28 (1941) 387 - 439
[2] H. Bartel and A. Lametschwandtner, *Anat. Embryol*. 202 (2000) 155.
[3] D.G. Smith and L. Rapson, *Cell Tiss. Res*. 178 (1997) 1.
[4] A. Lametschwandtner et al., Scanning Microsc. 4 (1990) 941.
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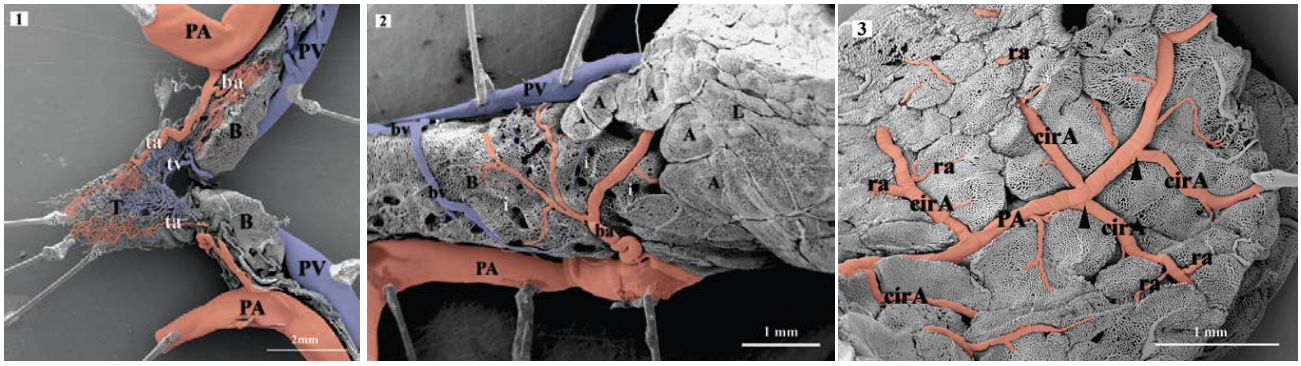

Figure 1. Trachea and bronchi of adult *Xenopus laevis*. Vascular corrosion cast. Dorsal view. B bronchus, T trachea, PA pulmonary artery, PV pulmonary vein, ba bronchial artery, ta tracheal artery, tv tracheal vein and * conductive bridge. **Figure 2.** Microvasculature of bronchus and lung. Dorsal aspect. A alveolus, B bronchus, L Lung, ba bronchial artery, bv bronchial vein and i impression of bronchial cartilage. **Figure 3.** Dorsal aspect of caudal lung, main arterial supply. PA pulmonary artery, cirA circumferential artery, ra radial artery and flow divider (arrowhead).

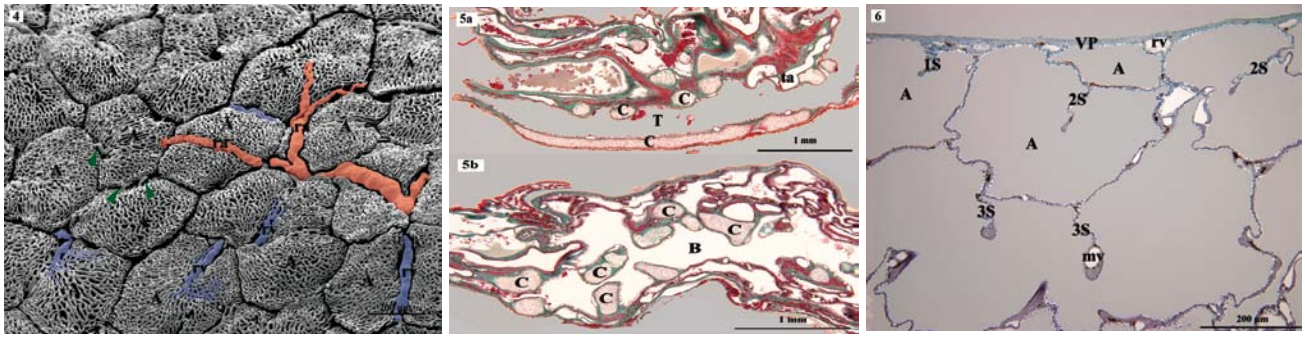

Figure 4. Surface view at alveoli. Vascular corrosion cast of the adult lung. A alveolus, ra radial artery, rv radial vein and interalveloar communicating vessel (arrowhead). **Figure 5a, 5b and 6**. Light microscopy. Paraffin embedding, 7µm, Goldner staining. **Figure 5a.** Longitudinal section of the trachea. T Trachea, C tracheal cartilage and ta tracheal artery. **Figure 5b**. Longitudinal sections of the bronchus. B Bronchus, C bronchial cartilage. **Figure 6**. Transverse section of the lung. A alveolus, 1S primary septum, 2S secondary septum, 3S tertiary septum, mv marginal vein, rv radial vein and VP visceral pleura.

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