Atlas of the Vasculature of Larval and Adult *Xenopus laevis.* Part: Respiratory Tract

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At present, 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] und adult lung [3] descriptions of the vasculature, however, end when vessels reach the organs parenchyma and little is known on the intrinsic microvasculature of *Xenopus* tissues and organs.

The aim of the present work is to demonstrate the microvascular anatomy of trachea, bronchi, and sac-lungs of larval and adult *Xenopus laevis* in qualitative and quantitative terms with particular emphasis on spatio-temporal aspects of vascular pattern formation.

280 tadpoles of *Xenopus laevis* Daudin from stages 48 to 66 [3], (body weights: 30mg to 599mg) and 19 adults (body weights: 48g to 92g) were used for vascular corrosion casting. Briefly, animals were killed by an overdose of an aqueous solution of tricaine-methane-sulfonate (MS 222, Sigma Chemicals, St. Louis, MI), the heart was exposed and the circulatory system was rinsed free of blood with Ringer solution via the arterial trunk with the venous sinus cut open to allow outflow of blood. When clear reflux escaped from the opened venous sinus 0.5 ml (tadpoles) or 10 ml (adults) of 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. After hardening of the injected resin, specimens were macerated (7.5% KOH; 40°C), decalcified (2% HCl; adults only), cleaned, and remaining vascular corrosion casts were frozen in distilled, and freeze-dried. Dry vascular casts were mounted onto specimen stubs, evaporated with carbon and gold, and examined in a scanning electron microscope (Stereoscan 250, Cambridge; ESEM XL-30, FEI) at an accelerating voltage of 10kv.

The circulatory bed of the respiratory tract was casted satisfactorily in a low number of tadpoles only, while in adults a higher percentage of cast animals displayed the vasculature of the respiratory tract well cast. In the tadpole lung alveolization proceeds from caudal towards rostral. While in the caudal lung portion alveoli are present (Figs. 1,2) with still ongoing intussusceptive microvascular growth in the center of alveoli (Fig. 3), in the rostral lung portion, bronchi and trachea still immature wide capillary-like vessels are present. They run circularly and have not yet clear arterial or venous endothelial cell nuclei imprint patterns making an identification of vessels only possible by following their origin from the laterally running pulmonary artery respectively from the medially located pulmonary vein which already can be clearly identified. In the adult the entire lung reveals alveoli (Fig. 4) which are fed by branches from the pulmonary artery and drain via the pulmonary veins (Fig. 5). Right and left pulmonary veins merge straight before they open into the left atrium (Fig. 6). Branches of the pulmonary artery run between alveoli and give off short branches to the

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lateral aspect of the alveoli which in turn drain via their medial aspects into branches of the pulmonary veins. The trachea reveals a wide-meshed subepithelial capillary bed which is supplied via anteriorly directed branches of the pulmonary artery and drains into the pulmonary veins (Fig. 6). In the adult the vasculature of the bronchi consists of a very dense two-dimensional network of subepithelial capillaries (Fig. 5).

Technically, tadpoles from stage 48 onwards could be cast with Mercox-Cl-2B but however, in a few tadpoles only, 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 in tadpoles as well as in adults.

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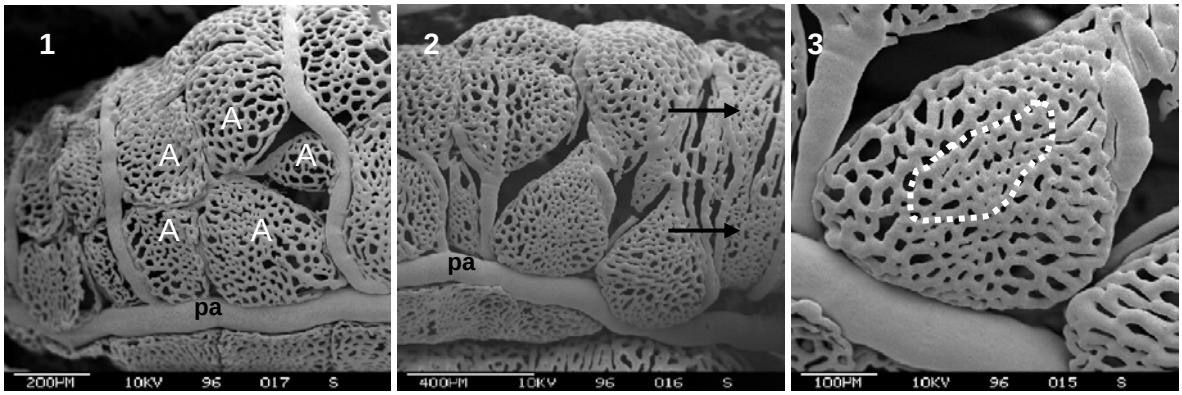

Figure 1. Vascular corrosion cast of the lung of a tadpole at stage 63. Lateral view. Caudal lung portion. A alveolus, pa pulmonary artery. **Figure 2:** Same as Figure 1, but transition zone into anterior portion with ongoing alveolization (arrows). pa pulmonary artery. **Figure 3.** Microvascular bed of an alveolus. Tadpole. Stage 63. External aspect. Note ongoing intusssuceptive microvascular growth (IMG) at the center of the alveolus (outlined area).

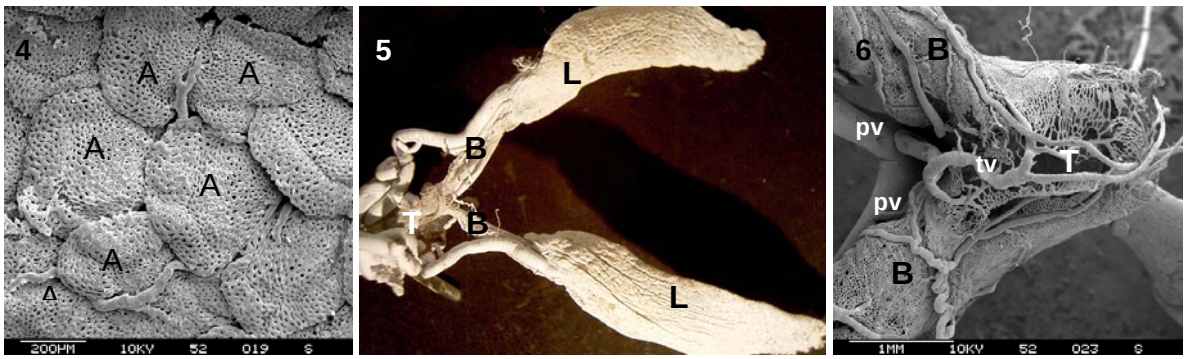

Figure 4. Vascular corrosion cast of the adult lung. External view at alveoli (A). **Figure 5.** Respiratory tract of adult *Xenopus laevis.* Vascular corrosion cast. Dorsal view. B bronchus, L lung, T trachea. **Figure 6.** Microvasculature of trachea (T) and bronchi (B). Vascular corrosion casts. Dorsal aspect. Tracheal vein (tv) drains into the left pulmonary vein (pv).

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