Atlas of the Vasculature of Larval and Adult *Xenopus laevis*. Part: Digestive 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 the small intestine [2] 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 (i) to demonstrate the microvascular anatomy the digestive tract of larval and adult *Xenopus laevis*, (ii) to give insights into the vascular wiring of its tissues and organs, and (iii) to increase knowledge on qualities and quantities of microvascular communication routes between cells, tissues and organs in a vertebrate model organism.

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 water, 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 digestive tract was casted satisfactorily whereby the esophageal vasculature was often cast only fragmentarily both in tadpoles and in adults. In both cases, however, wide longitudinally arranged veins forming a prominent venous plexus was found (Figs. 1,2). The microvasculature of the stomach was well casted in many specimens whereby the glandular portion of the larval stomach was clearly outlined by the specific pattern of the blood vessels ensheathing the gastric glands (Figs. 1,3). In the small intestine the transition from a single layered two-dimensional vascular network (Fig. 4) to a three-dimensional vascular bed started around stage 60 to finally form densely packed longitudinally running zig-zag intestinal folds in the adult (Fig. 5). In contrast to the small intestine the large intestine vascular bed revealed no luminal folds and was less well developed with a dense subepithelial capillary bed in the adults (Fig. 6).

Technically, tadpoles from stage 48 onwards could be cast with Mercox-Cl-2B although casts displayed only parts of the circulatory system. 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. Nevertheless, the SEM analysis of good quality vascular casts of the digestive tract enabled to follow the maturation of the vascular beds and its means with a high temporal and spatial resolution.

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Figure 1. Vascular pattern of the esophageal (E) - stomach (ST) transition in a tadpole of *Xenopus laevis* at stage 57. External view. Vascular corrosion cast (VCC). Note the wide embryonic-like capillary plexus ensheathing the gastric glands. a artery, v vein. **Figure 2.** Vascular pattern of the esophageal (E) - stomach (ST) transition in adult *Xenopus*. Serosal view. VCC. Note the venous plexus (vp) of the esophagus and the dense vascular bed of the stomach (ST). **Figure 3.** Microvascular patterns of the larval stomach (ST) at stage 59. Ventral aspect. VCC. av abdominal vein, dv duodenal vein, vgv ventral gastric veins.



Figure 4. Two-dimensional network of the small intestine of a tadpole at stage 60. Luminal view. Detail. Note the immature wide capillaries. **Figure 5.** Microvasculature of the adult small intestine. Transverse section. L lumen, lf longitudinal intestinal fold. **Figure 6.** Microvasculature of the large intestine (LI) – rectum (RE) transition zone. Ventral aspect. VCC. Note the dense subepithelial capillary network (arrows). pma posterior mesenteric artery, phv posterior hemorhoidal vein.