Atlas of the Vasculature of Larval and Adult *Xenopus laevis*: Part: The Spleen

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The spleen is a secondary lymphoid organ with important reticulo-endothelial functions. In the adult South African Clawed Toad, *Xenopus laevis* Daudin, development [1], histogenesis [2], innervations [3], histology [4], and gross arterial supply and venous drainage [5] are well described, but we neither know its intrinsic microangioarchitecture or the vascular relations between white and red pulp, nor exist any analyses of the microvascular patterns of the larval spleen and the changes the splenic vasculature undergoes during metamorphosis. Here we use scanning electron microscopy (SEM) of vascular corrosion casts [6], a superb technique to demonstrate the smallest blood vessels, their spatial arrangement, and their hierarchy in unsurpassed detail, and 3D-morphometry [7] to qualitatively and quantitatively analyze the intrinsic microvascular patterns of the larval and adult spleen, one of the compartments which in *Xenopus laevis* is also involved in the neuro-immune interactions [3].

280 tadpoles of *Xenopus laevis* Daudin (stages 48 to 66; body weight: 30mg to 599mg; staging according to [1]) and 11 adults (body weight: 48 grams to 92 grams) were killed by an overdose of MS 222. Then heart, conus arteriosus and arterial trunk were exposed and a glass cannula (tadpoles) or a blunt needle (adults) was inserted via the ventricle into the conus arteriosus, and the venous sinus was opened. After rinsing with Ringer solution a polymerizing resin (Mercox-Cl-2B, Ladd Res. Inc., Burlington,VT), diluted (v+v = 4:1) with monomeric methylmethacrylic acid (Fluka, Basle, CH) containing 1.5 grams of accelerator paste MA per 20 ml monomer, was injected with a flow rate of 3 ml/h (tadpoles) or 3-5 ml/min (adults). Specimens were then macerated (7.5 % KOH; 40 °C, up to 24 hours), rinsed in distilled water, decalcified (2% HCl; 20°C, 12-14 hours; adults only), rinsed, and cleaned (5 % HCOOH; 20°C, 5-15 min). After a final rinse in distilled water specimens were frozen in distilled water, freeze-dried (Lyovac, Leybold-Heraeus), mounted onto specimen stubs, evaporated with carbon and gold and analyzed in an environmental scanning electron microscope (ESEM XL-30; FEI-company, Eindhoven, NL) at an accelerating voltage of 5 kV. For 3D-morphometry (M^3, ComServ) [7] stereopaired images (tilt angle: 6°) were recorded.

Vascular corrosion casts of tadpoles of *Xenopus laevis* at stage 56 reveal a roundish spleen with an average diameter of 890 µm (Fig. 1), while those of adults have a length of 3.0 mm and a width of 3.4 mm (Fig. 2). In *Xenopus* the spleen is supplied by one splenic artery which arise from the haemorrhoidal artery. One to four splenic veins drain into the close by hepatic portal vein. Immediately after their origin from their parent vessel splenic arteries penetrate deeply into the parenchyma and branch off in a whorl-like manner at several levels radially directed central arteries (Fig. 4). Central arteries locate close to the centre of the white pulp and gives off side-branches to supply the sinuses of the adjacent red pulp (Fig. 4). In the larval spleen few true capillaries are present (Fig. 3) and splenic sinuses are much denser than in the adult spleen. Deep splenic sinuses drain into nearby deep splenic venules (Fig. 4), which run centrifugally, pierce the splenic capsule, and form the superficially located splenic venules which then drain the more peripheral splenic sinuses. Superficial splenic venules merge over up to 10 levels before they finally form a draining splenic vein.

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Located deeply inside the abdominal cavity - ventral to the kidneys and dorsal to the caudal margins of the liver lobes - the microangioarchitecture of the spleen of *Xenopus* best can be studied by SEM of vascular corrosion casts. The small sizes of spleens together with the dense network of splenic sinuses make dissection of casts and exposure of intrinsic vascular patterns prone to breakage of the delicate connections between terminal portions of supplying central arteries and splenic sinuses and splenic sinuses and their draining venules, but enables to analyze the distinct vascular routes blood cells have to pass on their way through the this lymphoid organ in *Xenopus.*

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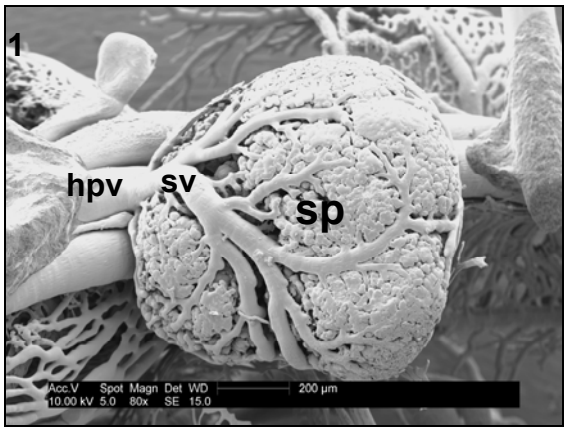


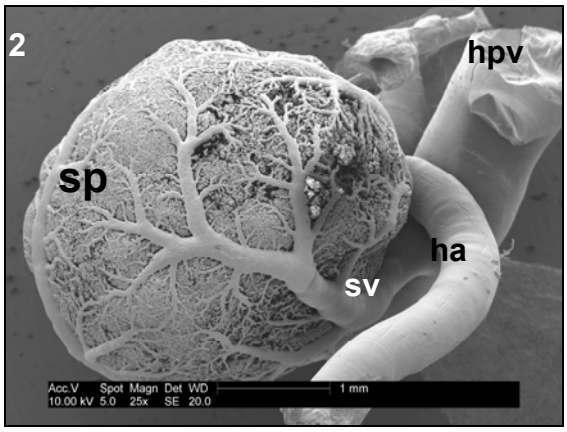
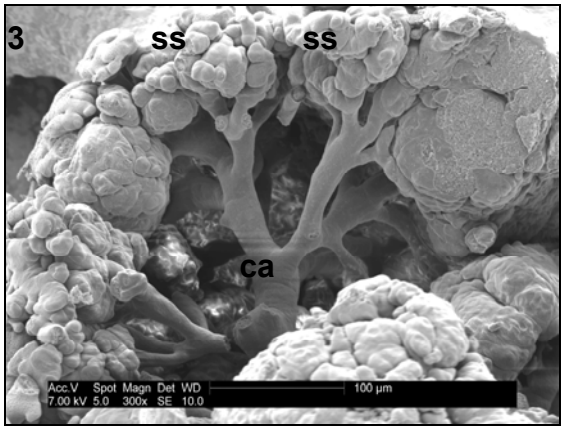


Figure 1. Vascular corrosion cast of a tadpole of *Xenopus laevis* at stage 56. Anterior is to the left. Note the spleen (sp) in the centre, the splenic vein (sv), and the hepatic portal vein (hpv). **Figure 2.** Vascular corrosion cast of the spleen (sp) of adult *Xenopus.* Anterior is to the right. ha hemorrhoidal artery, sv splenic vein, hpv hepatic portal vein.



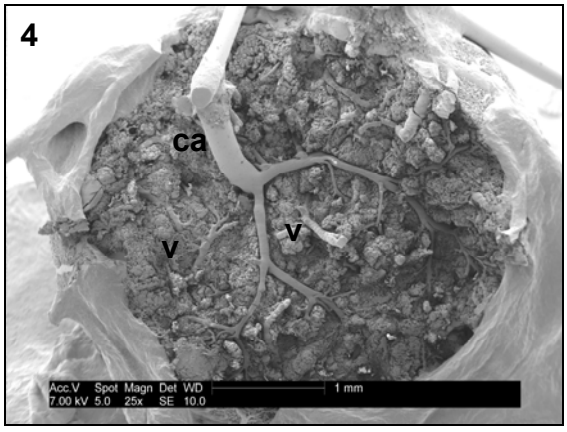


Figure 3. Spleen of a tadpole at stage 56. Vascular corrosion casts. Marginal zone is removed. ca central artery. ss splenic sinus. **Figure 4.** Spleen of adult *Xenopus*. Note the mature imposing central artery (ca) and the splenic veins (v).

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