

The evolution of amphibian metamorphosis: insights based on the transformation of the aortic arches of *Pelobates fuscus* (Anura)

Hana Kolesová¹, Alois Lametschwandtner² and Zbyněk Roček³

¹*Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czech Republic*

²*Department of Organismic Biology, Vascular and Muscle Research Unit, University of Salzburg, Austria*

³*Laboratory of Palaeobiology, Geological Institute, Academy of Sciences, Prague, Czech Republic, and Department of Zoology, Charles University, Prague, Czech Republic*

Abstract

In order to gain insights into how the aortic arches changed during the transition of vertebrates to land, transformations of the aortic arches during the metamorphosis of *Pelobates fuscus* were investigated and compared with data from the early development of a recent ganoid fish *Amia calva* and a primitive caudate amphibian *Salamandrella keyserlingi*. Although in larval *Pelobates*, as in other non-pipid anurans, the gill arches serve partly as a filter-feeding device, their aortic arches maintain the original piscine-like arrangement, except for the mandibular and hyoid aortic arches which were lost. As important pre-adaptations for breathing of atmospheric oxygen occur in larval *Pelobates* (which have well-developed, though non-respiratory lungs and pulmonary artery), transformation of aortic arches during metamorphosis is fast. The transformation involves disappearance of the ductus Botalli, which results in a complete shunting of blood into the lungs and skin, disappearance of the ductus caroticus, which results in shunting of blood into the head through the arteria carotis interna, and disappearance of arch V, which results in shunting blood to the body through arch IV (systemic arch). It is supposed that the branching pattern of the aortic arches of permanently water-dwelling piscine ancestors, of intermediate forms which occasionally left the water and of primitive tetrapods capable of spending longer periods of time on land had been the same as in the premetamorphic anuran larvae or in some metamorphosed caudates in which the ductus caroticus and ductus Botalli were not interrupted, and arch V was still complete.

Key words Anura; circulatory system; development; evolution; metamorphosis.

Introduction

The transition of vertebrates from an aquatic environment to dry land in the Devonian, some 360 million years ago, was associated with profound changes in their physiology and anatomy. In piscine ancestors of tetrapods, oxygen was taken from the water and brought to the blood and tissues through the gills located on the surface of gill arches. In water-dwelling gnathostomes, each of these arches includes skeletal components

and the branchial muscles and nerves. In addition, each arch is traversed by the arterial blood vessels collectively termed the aortic arch, and is supported by endoskeletal elements called the branchials. The ensemble of branchials that form the skeleton of each gill segment is called the skeletal gill arch. In early gnathostomes it typically consisted of two pharyngobranchials that connected the entire skeletal arch to the neurocranium, and (dorsally to ventrally) the epibranchial, ceratobranchial and hypobranchial elements of each gill arch; skeletal arches of the left and right sides were interconnected by median ventral structures, the basibranchials (Bjerring, 1977). As early as in the early Devonian gnathostomes, however, some branchials either fused with surrounding structures, became reduced or were entirely lost (Moy-Thomas & Miles, 1971; Jarvik, 1980).

Correspondence

Hana Kolesová, Institute of Anatomy, First Faculty of Medicine, Charles University, U Nemocnice 3, CZ-128 00 Prague 2.
E: hana.kolesova@lf1.cuni.cz

Accepted for publication 18 January 2007

Tetrapods typically are air-breathing vertebrates in which gills were replaced by lungs (originally the swim bladder of their Devonian crossopterygian ancestors). Most gill slits became obliterated (except for the spiraculum, a vestigial slit between the mandibular and hyoid arches) and, consequently, the skeletal arches lost their original function and became reduced. They persist as the hyoid, which is a skeletal support of the muscles in the bottom of the mouth cavity, and the columella auris. The transformation of the skeletal gill arches into the hyoid was associated with reduction of their dorsal elements so that the hyoid lost its firm connection with the neurocranium. Although the transition of the vertebrates to land was associated with arrested ossification that also affected the gill arches (so the arches remain cartilaginous to various extent), transformations of at least some parts (e.g. columella) may be inferred from fossils (Clack, 1994, 1998; Clack et al. 2003; Brazeau & Ahlberg, 2006). However, the transformation of soft parts, including the aortic arches, may be inferred only from extant amphibians.

Some of the principal anatomical changes that occurred during the transition of vertebrates to land are reflected in the metamorphosis of contemporary amphibians with bi-phasic ontogeny. Their water-dwelling larvae take oxygen from the water, whereas terrestrial adults use lungs for gas exchanges. Metamorphosis involves not only the transformation of the gill-supporting skeleton, but also changes in the associated aortic arches. These processes are reflected in caudates better than in anurans because their skeletal arches, both in larvae and in metamorphosed adults, retain to a larger extent the original segmentation. The same holds true also for caecilians (see Wake, 2003). However, although caecilians have long been considered significant to understanding relationships among extinct and extant amphibians, very little is known about development of their branchial and aortic arches (Ramaswami, 1944; for reviews of the literature, see also Hafferl, 1933; Stadtmüller, 1936; Exbrayat, 2000; Wake, 2003). In anurans, on the other hand, the hyoid lost its original segmentation, supposedly because of modification of the larval branchial skeleton into a filter-feeding device in which the epibranchials disappeared whereas all remaining branchials fused together (for a review of the literature, see Roček, 2003). It is therefore interesting to explore several questions: (1) how the aortic arches of anuran larvae are transformed into the adult pattern; (2) to what degree adults retain larval

arrangement; and (3) whether anuran metamorphosis may provide evidence to infer the evolutionary transformations of aortic arches during the transition of vertebrates from water to land.

Information on the aortic arches in adult anurans is based mainly on *Xenopus laevis* (e.g. Grobbelaar, 1924a,b; Nikitin, 1925; Millard, 1941, 1942, 1945; Paterson, 1942; Graaf, 1957) although this differs remarkably from the anatomy of non-pipid anurans, for instance *Leiopelma hochstetteri* (Szarski, 1951), *L. archei* (Stephenson, 1951), *Rana esculenta* (Gaupp, 1899), *Pelobates fuscus*, *Bombina bombina* and *Bufo bufo* (Szarski, 1937, 1948).

In addition, development of the aortic arches is best known in *Xenopus laevis* (Nikitin, 1925; Paterson, 1942; Millard, 1945; Weisz, 1945; Nieuwkoop & Faber, 1967; Viertel & Richter, 1999), whereas only a small number of papers deal with aortic arch development or larval anatomy in non-pipid anurans (e.g. Schulze, 1889, 1892 in *Pelobates fuscus*; Schmalhausen, 1953a in *Bombina bombina*; Strawinski, 1956 in *Rana esculenta*; Schmalhausen, 1953a and Lanot, 1962 in *Rana temporaria*; De Saint-Aubain, 1985 in *Rana catesbeiana* and *Bufo bufo*; Magnin, 1959 in *Alytes obstetricans*; Witschi, 1956 in *Rana* sp.).

Materials and methods

We chose *Pelobates fuscus* because of the comparatively primitive phylogenetic position of the genus *Pelobates*. The large size of *P. fuscus* tadpoles makes them suitable for vascular corrosion casting and scanning electron microscopical analyses. The 34 individuals examined in this study include specimens from the advanced larval period to the end of metamorphosis (stages 50–66; staging after Nieuwkoop & Faber, 1967). Tadpoles were raised in the laboratory from a clutch collected near the town of Přebor, Czech Republic. The total length of tadpoles ranged from 6.2 to 9.2 cm (stage 58), and their snout–vent length at the end of metamorphosis (stage 66) ranged from 2.4 to 3.2 cm.

The vascular system was prepared by injecting a polymerizing resin, Mercox-Cl-2B (Ladd Research Inc., Burlington, VT, USA), into the blood vessels (Aichhorn & Lametschwandtner, 1996; Bartel & Lametschwandtner, 2000). First, tadpoles were narcotized in an aqueous solution of tricaine methansulfonate (MS 222, Sandoz, Basle, Switzerland). Then the heart and the conus arteriosus were exposed, a glass cannula was introduced

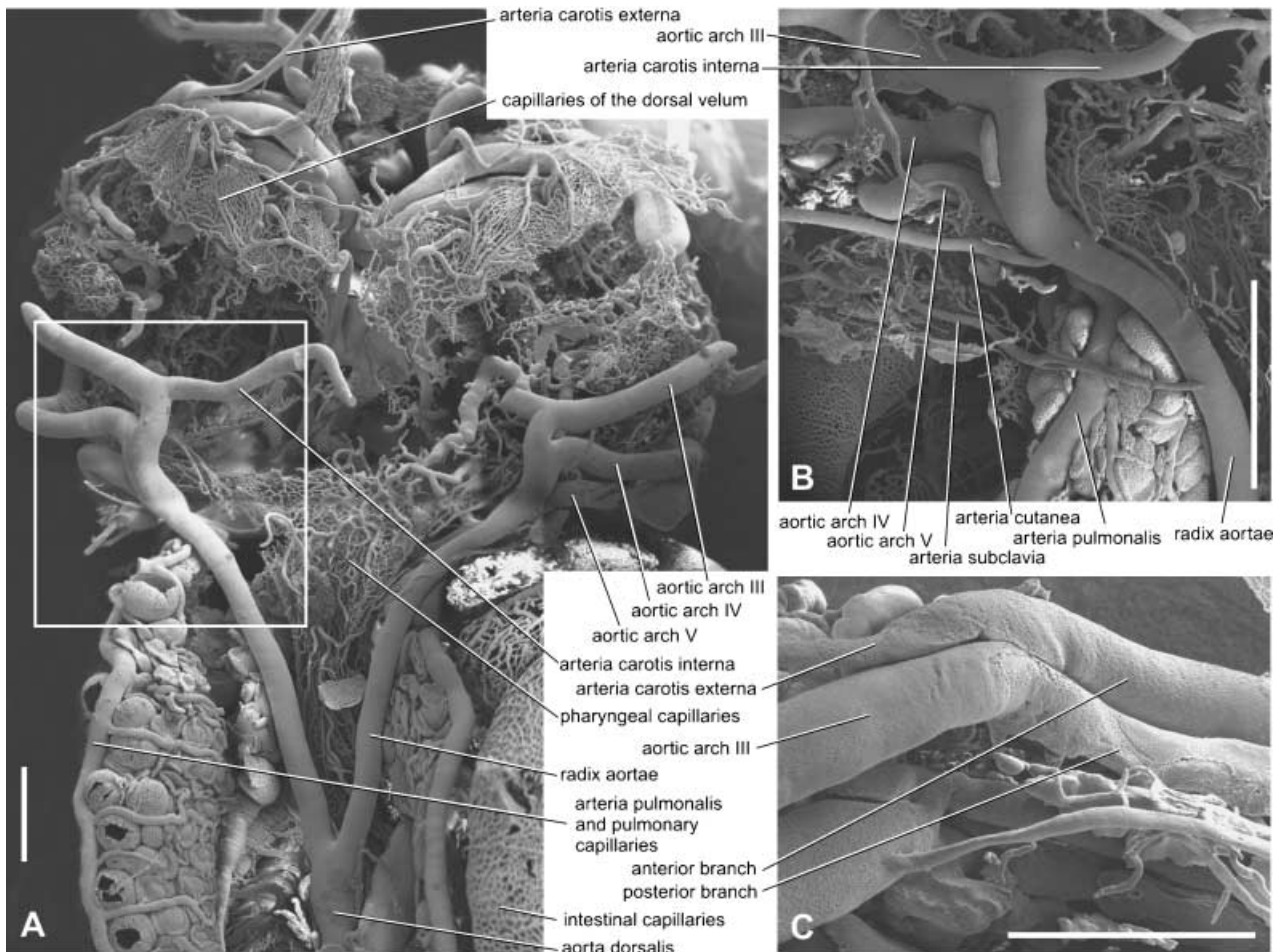


Fig. 1 (A) Larva of *Pelobates fuscus* at NF stage 50. Cast of the aortic arches and associated vessels, dorsal view. (B) Larva of *Pelobates fuscus* at NF stage 56. Dorsal view of the area corresponding to that marked by square in A. (C) Larva of *Pelobates fuscus* at NF stage 56. Dorsal view of the right arch III. Bars = 1 mm.

via the ventricle into the conus arteriosus, ligatured in place, and the vascular system was rinsed with Ringer solution. Injection of Mercox followed immediately after clear reflux of rinsing solution escaped the opened atria. Subsequent corrosion of the soft tissues was achieved in 7.5% KOH (24 h, 40 °C). Specimens were analysed with Cambridge 250 and Sigma 300 scanning electron microscopes at accelerating voltages of 5–10 kV. For further details on vascular casting, specimen processing and scanning electron microscopy analyses see Minnich et al. (2002).

In agreement with Taylor & Kollros (1946), we divided the examined developmental period into (1) the larval period, (2) prometamorphosis and (3) metamorphosis (= metamorphic climax). Numbering of the aortic arches was I–VI, which reflects also earlier larval stages in which all aortic arches, including I and II, were

developed. The nomenclature of vessels is based on Gaupp (1899).

Results

Larval period (stages 50, 52, 53)

Blood leaves the ventricle by way of the conus arteriosus, the interior of which is divided longitudinally by a spiral valve. The conus continues as a short unpaired truncus arteriosus (aorta ventralis), which gives rise to three aortic arches, called (anterior to posterior) the carotid (III), systemic (IV) and pulmo-cutaneous (common stem for V and VI) arches. Arches I and II are absent (Fig. 1A,B).

Arch III gives off the a. carotis externa, which turns backwards first and then immediately anteriorly

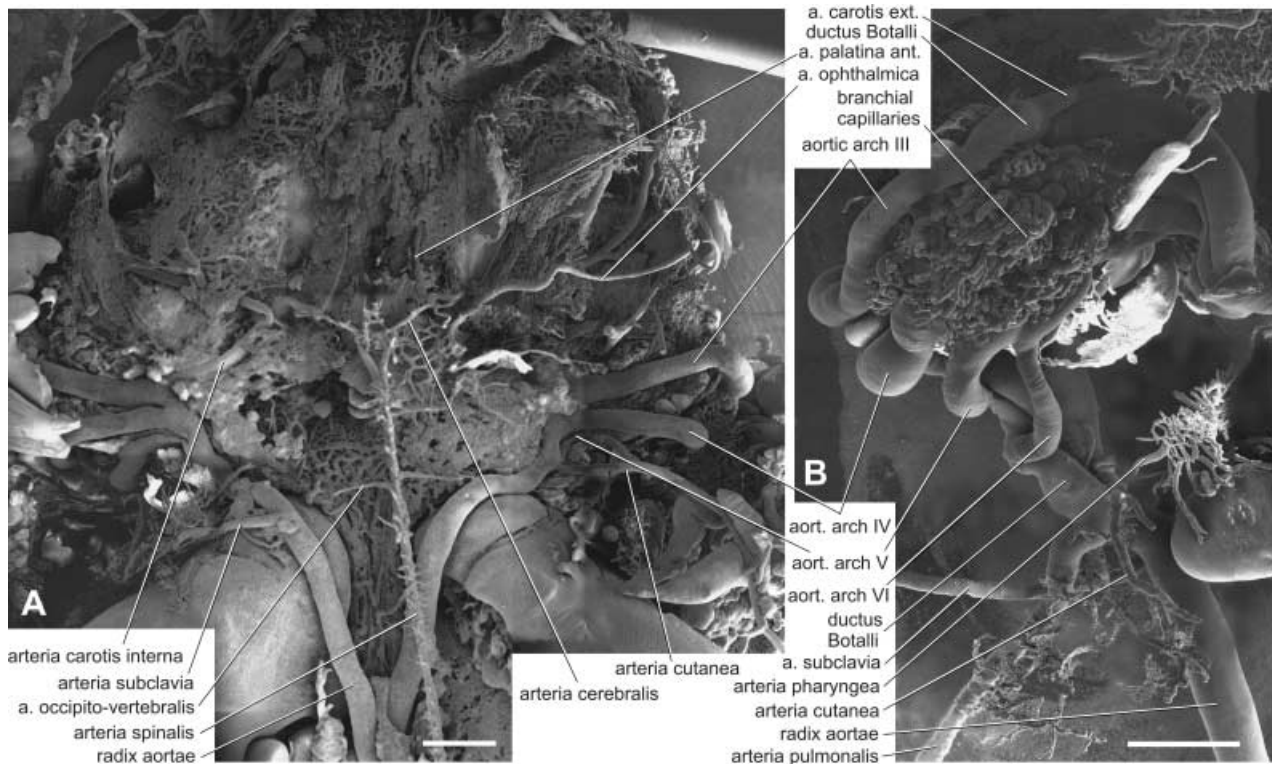


Fig. 2 (A) Larva of *Pelobates fuscus* at the beginning of metamorphosis (NF stage 62). Cast of the aortic arches and associated vessels, dorsal view. (B) Same stage. Aortic arches of the right side, ventral view. Bars = 1 mm.

before giving rise to branches to the *m. interhyoideus*. Then it continues anteriorly and branches in the bottom of the mouth cavity. At the level where the *a. carotis externa* splits off, the aortic arch is separated into an anterior and a posterior branch (Figs 1C and 6A). The posterior branch gives off the *aa. afferentes*, which supply the gills. The *aa. efferentes* join the anterior branch of the arch. The gills are located along the ventral side of the arch, whereas the filter apparatus (supplied by arch IV; see below) is situated dorsally. The anterior and posterior branches fuse and the resulting arch continues dorso-medially into the *radix aortae*.

The *a. carotis interna* arises from the point where arch III joins the root of the aorta (Fig. 1B). It runs medially and gives rise to the *a. palatina posterior*. The latter turns over the *a. carotis interna*, runs medially, then turns antero-laterally. It gives rise to a tiny artery and to the *a. hyoidea* (Fig. 3A), both supplying the posterior part of the roof of the branchial cavity and the *mm. levatores complex*. The *a. carotis interna*, after giving rise to the *a. palatina posterior*, turns abruptly anteriorly and continues as the *a. palatina anterior*. The *a. palatina anterior* ultimately divides into three branches that supply the roof of the mouth cavity.

At the point where the *a. carotis interna* turns anteriorly, it forms a short common stem for the *a. ophthalmica* and *a. cerebralis* ventrally (Fig. 3A). The *a. ophthalmica* runs laterally towards the eye. The *a. cerebralis* runs medially and then bifurcates into an anterior and a posterior branch (Fig. 3A,B), running rostrally and caudally, respectively. The posterior branches of the left and right sides are connected via a transverse anastomosis (*a. communicans retro-infundibularis*, or *a. communicans posterior*) and then gradually converge towards the midline where they merge to form the *a. spinalis ventralis* (= anterior), which runs along the ventral midline of the spinal cord. The *a. spinalis* passes through the vertebral canal posteriorly, sending small branches that supply the spinal cord. It is connected to the *radix aortae* via the *a. occipito-vertebralis*.

Arch IV splits off from the *truncus arteriosus* immediately behind arch III. Before entering the gill, it sends a dorsal artery to supply the filter apparatus of arches III and IV. The pattern of its branching is similar to that in arch III.

Arches V and VI arise from a common stem from the *truncus arteriosus*. When arches V and VI separate from each other, the latter is markedly thinner. The pattern of its branching is similar to that of arches III and IV.

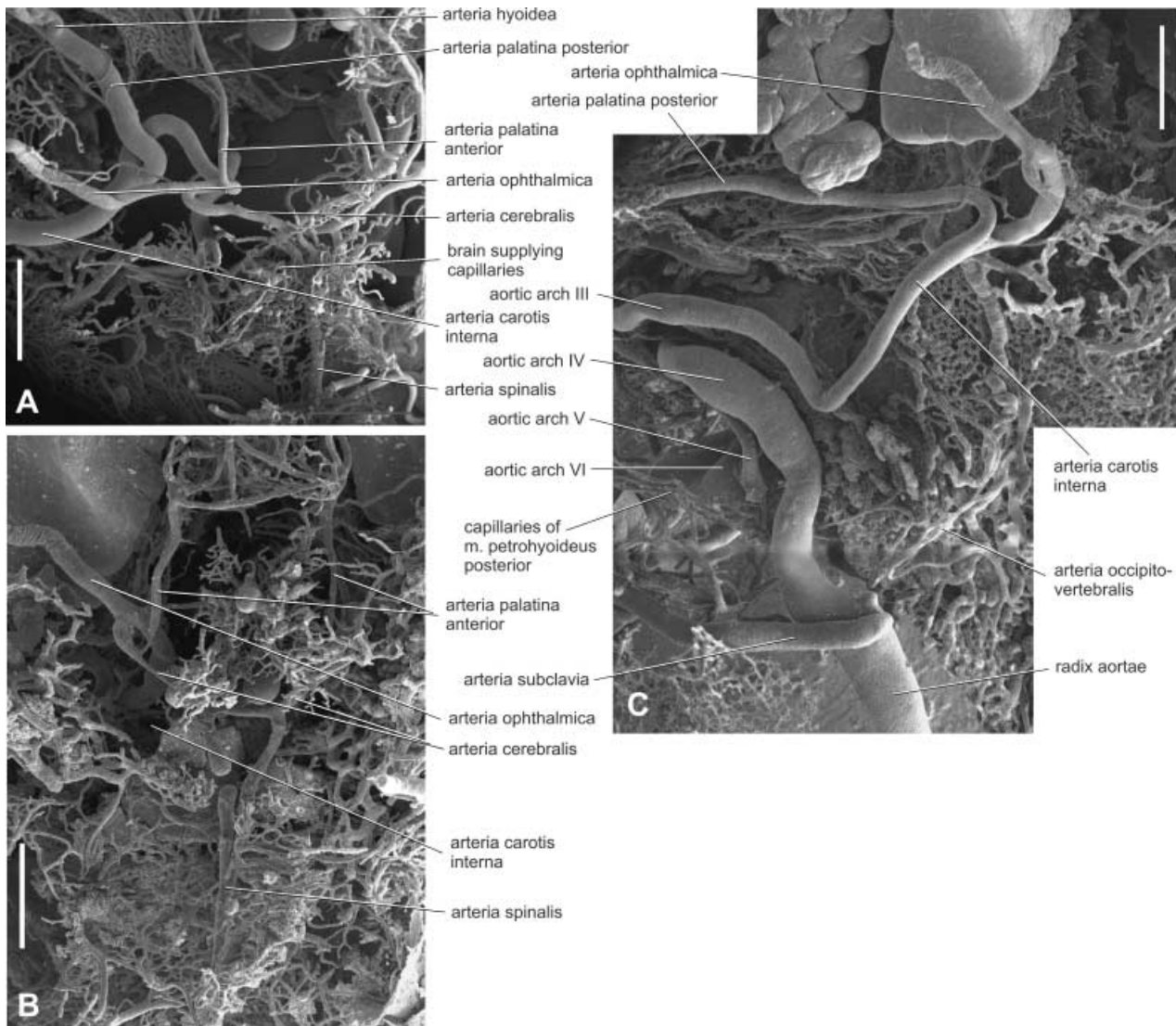


Fig. 3 (A) Larva of *Pelobates fuscus* at NF stage 56. Details of branching of the left arteria carotis interna, dorsal view. (B) Metamorphosing larva at NF stage 63. Details of branching of the left arteria carotis interna, dorsal view. Note triangular arrangement of the roots of the a. cerebialis. (C) Same stage as in B. Dorsal view of the aortic arches of the left side. Bars = 0.5 mm.

Arch V, however, is much shorter than arches III and IV, and re-joins arch VI just before the latter joins the radix aortae. The posterior branch of arch V sends an artery which supplies the filter plates of arches V and VI.

Arch VI is, in contrast to the other arches, very thin and runs posteriorly after its separation from the common stem with arch V. It does not divide into an anterior and a posterior branch. It produces only a small number of arteries that enter the gills; blood from the gills flows back into the arch. Therefore, its gill supply system is different from that of other arches. Before arch VI enters the radix aortae, it re-joins arch V. The a.

pulmonalis arises from the common stem of re-united arches V and VI. Consequently, the ductus Botalli, which is the vessel connecting the origin of the a. pulmonalis and the point where the common stem of arches V and VI joins the radix aortae, is very short (Fig. 4B). The a. cutanea, which runs laterally to supply the skin, also originates in the ductus Botalli. In two individuals the a. pulmonalis (and a. cutanea) originated from the radix aortae, which may suggest that their ductus Botalli was incorporated into the radix aortae.

The a. pulmonalis gives rise to the a. pharyngea, which turns medially and supplies the dorsal pharyngeal

wall (Fig. 1A). Another branch (not previously mentioned by other authors) originating from the a. pulmonalis passes along the ventral side of the branchial basket, then turns laterally to circumvent the branchial cavity, and terminates on its dorsal side to supply the dorsal velum. Branches of the a. pulmonalis enter the anterior ends of the lungs, but the arteries continue along the lateral sides of the lungs all the way up to their posterior ends, producing small branches that break up into the capillary nets around the alveoli.

The radix aortae, after accommodating the fused arches V and VI, runs postero-medially, joins its counterpart and gives rise to the aorta dorsalis. Each root sends the a. palato-nasalis which passes on the dorsal side of the head anteriorly up to the external naris. Another branch originating from the radix aortae is the a. subclavia (Fig. 1B), which is present even if the anterior limb is still rudimentary and not yet protruding from the body wall.

Prometamorphosis (stages 56, 57, 58)

At stage 56, the general pattern of the aortic arches is the same as in the premetamorphic larvae; we found only some individual variation in the branching of the a. palatina posterior and the a. carotis interna.

Later (stage 57), the posterior branch of arch III separates from the main stem of the arch and remains connected to the anterior branch only by means of the gill capillaries. In addition, the posterior branch of arch IV separates from the main stem; ventrally, it gives off two branches that pass anteriorly to supply the m. interhyoideus. The artery arising from the posterior branch and supplying the filter plates also sends branches to the roof of the branchial cavity (Fig. 6C). Arches V and VI arise by a common stem whereby arch V seems to be the direct prolongation of the stem, whereas arch VI seems to be only its side branch. The pattern of arch VI remains the same as in the premetamorphic larvae; however, in one individual we found that it was divided into an anterior and a posterior branch (Fig. 6B), as were the anterior arches. In addition, in some individuals, arch V only joins the ductus Botalli, so arches V and VI re-unite after the origin of the a. pulmonalis (Fig. 4A). Given that arch VI is much thinner than the a. pulmonalis, the main supply for the lungs is most probably via the ductus Botalli, not via arch VI.

The a. pharyngea retains its larval branching pattern, but sends some branches towards the dorsal surface of

the lungs. The lungs continue to grow posteriorly so as to reach the posterior end of the trunk at stage 58. The length of the ductus Botalli varies considerably among individuals. The a. cutanea has become thicker and splits into two branches that supply the skin of the ventral side of the body.

At stage 58, the a. ophthalmica begins to wind in its course, which seems to be associated with the tadpole's increasing ability to move the eyeball.

The radix aortae and the a. palato-nasalis display the same branching pattern as in the earlier stages. However, the palato-nasal artery sends one additional branch, which ends in the orbit (Fig. 5). In addition, the radix aortae produces an additional artery, the a. oesophagea, which supplies the dorsal surface of the digestive tube. The a. subclavia gives rise to several small branches, then passes beneath the a. cutanea and enters the developing anterior limb (Fig. 6B).

Metamorphosis (metamorphic climax; stages 59–66)

Stage 59. Afferent and efferent gill arteries begin to regress. The a. palatina anterior passes in the roof of the buccal cavity towards the anterior part of the orbit where it sends an artery which runs below the orbit into the roof of the branchial cavity. The a. palatina posterior passes laterally and then anteriorly along the surface of the head, and produces branches that supply the temporal region. The a. cutanea is now divided into the anterior and posterior branches, supplying the skin of the corresponding parts of the body (Fig. 6D).

Stage 61. Close to the area where the external carotid splits from the main stem of arch III and where the arch splits into anterior and posterior branches, the stem begins to thicken and produces a swelling. This swelling is an early rudiment of the glomus caroticum (Fig. 2B; see also Kusakabe, 1992). Arch IV remains unchanged, but its artery that supplied the filter plates has disappeared. Arch V is thinner than arch IV, which means that it has begun to regress; its basic pattern, however, remains unchanged. In contrast, arch VI increases in size and attains approximately the same diameter as arch V (Fig. 6B).

Stage 62. Gills become shorter than in previous stages (stage 62 is a fully aquatic metamorphosing froglet with the tail still longer than body). The basic branching pattern of the a. carotis interna is preserved; there is only a larger number of tiny branches splitting from

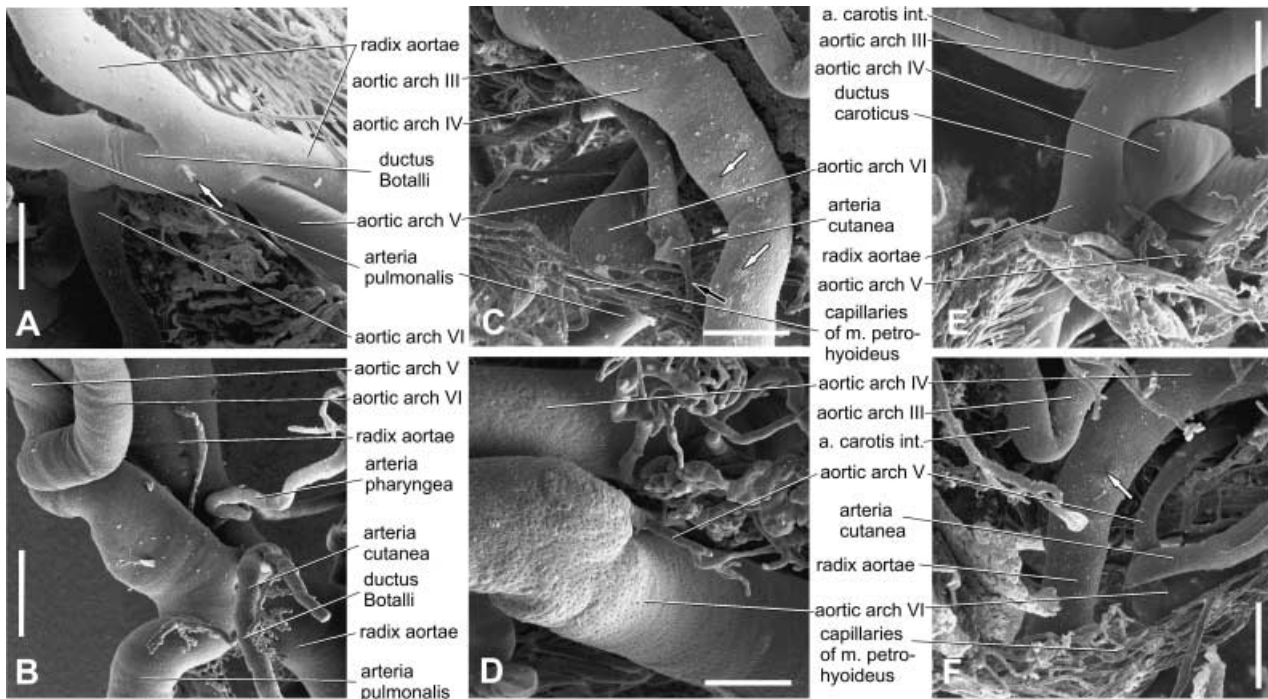


Fig. 4 (A) Larva of *Pelobates fuscus* at NF stage 61, lateral view of the right side (anterior is to the right). Connection of aortic arches V and VI to the radix aortae. Arrow marks position of the arteria cutanea (broken off on the cast). (B) Larva of *Pelobates fuscus* at NF stage 62, ventral view of the right side. Connection of the aortic arches V and VI to the radix aortae. (C) Metamorphosing larva of *Pelobates fuscus* at NF stage 63, dorsal view of the left side. Reduction of the arch V. Arteria cutanea is broken off. White arrows show scars indicating former fusion of the arches V and VI to the radix aortae. Black arrow marks reduced section of the arch V adjacent to the m. petrohyoideus. (D) Metamorphosing larva of *Pelobates fuscus* at NF stage 65, ventral view of the right side of the body. Note reduction of the arch V shortly before the end of metamorphosis. (E) Larva of *Pelobates fuscus* at NF stage 61, dorsal view of the right side. Ductus caroticus still present. (F) Larva of *Pelobates fuscus* at NF stage 63, same view as in E. Ductus caroticus already absent (scar is marked by an arrow). Note also that the a. cutanea takes its origin from the arch V, because of the larger extent of the m. petrohyoideus posterior. In all pictures except for A anterior is orientated to the top. Bars = 0.5 mm.

the main stems (Figs 2A, 3B and 6F). From this stage on, arch IV is the thickest arch (Fig. 2B). The pulmonary alveoli are already filled with air, indicating that pulmonary respiration has begun. Accordingly, the ductus Botalli is noticeably thinner than in the previous stage. It should be noted that inflation of lungs occurs as early as shortly after hatching in *Xenopus* (Wassersug, in litt.) and *Discoglossus pictus* (ZR, pers. obs.). Lung ventilation is a crucial phenomenon associated with anuran metamorphosis (Wassersug & Yamashita, 2000).

Stage 63. Gills on arch III disappeared completely; part of the arch that supplied them is noticeably shorter and without afferent and efferent arteries. This is an important observation because in the previous stage the gills were still well developed, though shorter. Only the anterior branch of arch III is preserved, whereas the

posterior one disappears. Moreover, arch III loses its connection with the posterior section of the radix aortae because the ductus caroticus, which is the section of the radix aortae interconnecting the dorsal parts of arches III and IV, disappeared (Figs 4E,F and 6G). Consequently, the a. carotis interna is the only continuation of aortic arch III. The a. carotis interna passes beneath the parasphenoid (one of the earliest ossifications) and the a. palatina posterior branches from it. This artery turns posteriorly and anastomoses with the a. cutanea. The a. palatina posterior then turns anteriorly and breaks into the capillary plexus of the orbit. The further course and the branching pattern of the a. carotis interna remains the same as in previous stages. Aortic arch IV is now the only arch which still retains its connection with the radix aortae. Consequently, it is now the main arterial vessel that supplies the trunk, i.e. the systemic arch (Fig. 3C). Arch V lost its

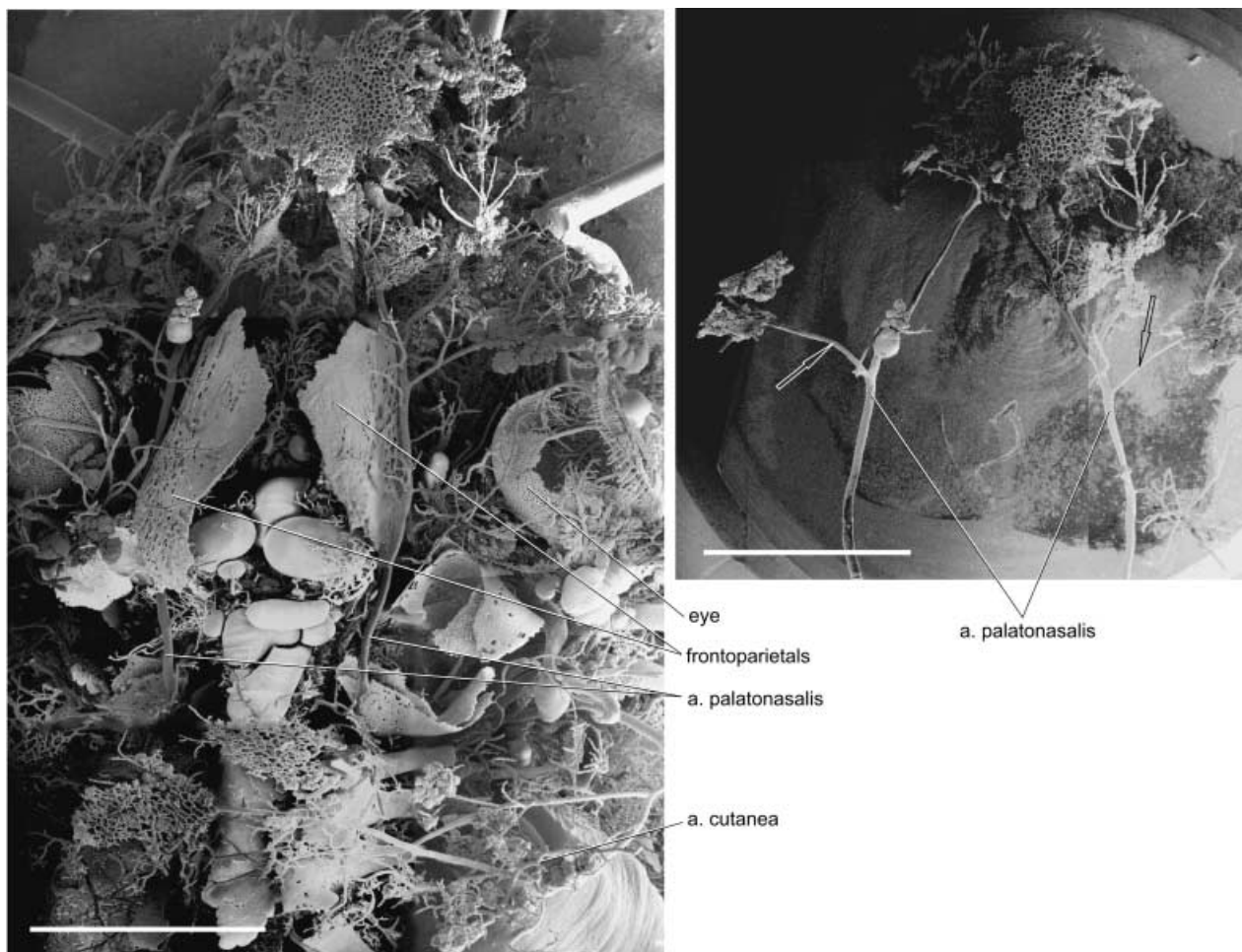


Fig. 5 (A) Larva of *Pelobates fuscus* at NF stage 59, cast of the vascular system of the head in dorsal view. The specimen was not decalcified, which is the reason why rudiments of the frontoparietals are preserved. (B) Same specimen after further preparation that revealed course of the arteria palatonasalis. Arrows mark branches to eyeballs. Bars = 1 mm.

connection with the root of the aorta and gradually regresses from that side. This regression correlates with the increase in size of the *m. petrohyoideus posterior*. In arch VI, the ductus Botalli has disappeared and, consequently, the arch has lost its connection with the radix aortae. Now, the arch is straight and laterally directed, not bent dorsally as in previous stages; this is associated with expansion of the lungs. Arch VI gives off the *a. cutanea* anteriorly and the *a. pulmonalis* posteriorly. The *a. cutanea* gives off a branch that supplies the dorsal skin. In its most anterior portion, the *a. cutanea* anastomoses with the *a. palatina* posterior. Then it turns posteriorly and gives off branches that supply the skin of the back and flanks. In one individual at this stage (of four investigated) the *a. cutanea* became, during regression of the ductus Botalli, a branch of aortic arch V. Points where arches III and V

connected with the radix aortae in earlier stages are indicated by scars (Fig. 4C).

Stage 65. The glomus caroticum on arch III is surrounded by a capillary plexus. The *a. carotis externa* sends tiny branches to the *m. interhyoideus* and the ventral velum, which is considerably reduced. Arch IV remains the principal artery supplying the body, whereas arch V is preserved as a tiny vestigial artery that terminates after a short distance, seemingly branching from arch VI (Fig. 4D). The *a. pulmonalis* is well developed and much thicker than the *a. cutanea*; the lungs have become fully functional.

The *a. spinalis* passes beneath the brain and spinal cord. Below the brain, it splits into two branches that turn around each other and then fuse again in a single vessel (Fig. 6H).

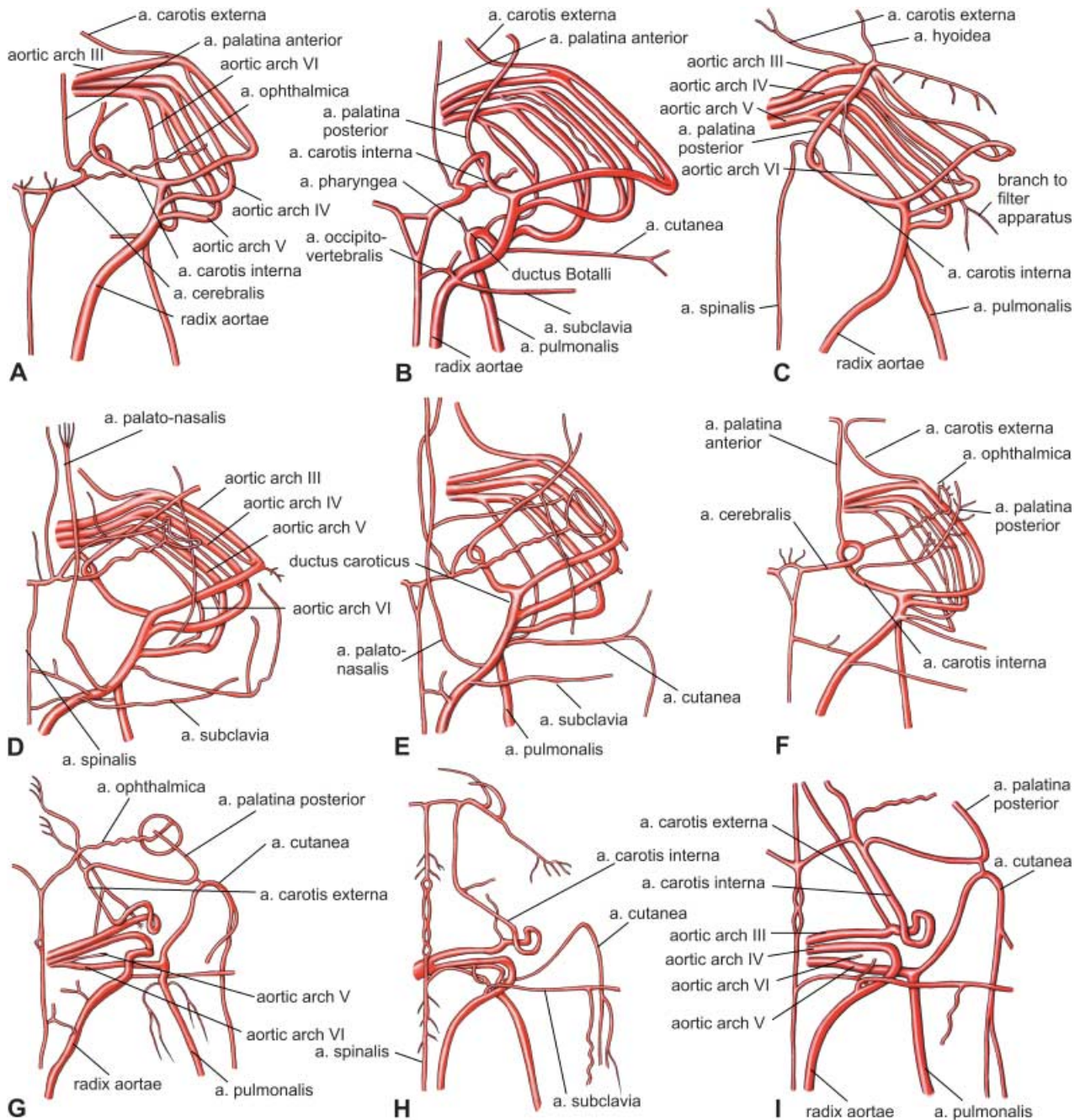


Fig. 6 Main developmental stages of the aortic arches of *Pelobates fuscus* in dorsal view (only right half is illustrated, semi-schematic). (A) Premetamorphic larva, stages 50–53. (B) Prometamorphosis, stage 56. (C) Prometamorphosis, stages 57–58. (D) Beginning of metamorphosis, stage 58. (E) Metamorphosis, stage 61. (F) Metamorphosis, stage 62. (G) Metamorphosis, stage 63. (H) Metamorphosis, stage 65. (I) End of metamorphosis, stage 66. Drawn after original specimens, not to scale.

Before entering the forelimb, the a. subclavia gives off the a. axillaris, which passes posteriorly along the flanks.

Stage 66. Metamorphosis of the aortic arch system is terminated by complete disappearance of aortic arch V. Other arteries remain the same as in the previous stage.

Discussion

Four aortic arches occur in premetamorphic larvae; they are homologues to the posterior four aortic arches in early gnathostomes. We did not observe arches I and II (mandibular and hyoid) in our stages of *Pelobates fuscus*. They would probably be early and transitory,

and therefore not observed in our material. The incomplete arches I and II were described only in early embryonic stages of *Xenopus laevis* (Millard, 1945; Nieuwkoop & Faber, 1967; Delsol & Flatin, 1972), and *Rana esculenta* and *R. temporaria* (Schmalhausen, 1953a). Weisz (1945) noted that the anterior two arches in *Xenopus laevis* were present up to hatching and several hours thereafter as antero-ventral lacunar spaces which soon disappear. However, because he referred to arch III as a small arch, which is never associated with gills, and which disappears in early embryonic development, most probably he referred to vestigial arch II. Nevertheless, it is obvious that both anterior arches disappeared early in development. There are some speculations as to whether the aa. palatinae may be their persisting vestiges but this cannot be supported by any substantial evidence.

In premetamorphic larvae the ventral aorta, through which blood is directed anteriorly from the heart, is extremely shortened. Blood leaves the heart by way of the conus arteriosus and the truncus arteriosus. Both are very short and the latter divides immediately into three trunks leading to the arches III and IV, and a common stem for arches V and VI. Therefore, blood is distributed to the aortic arches immediately in the periphery of the heart, and the truncus arteriosus may be considered to be fused ventral parts of the afferent branchial arteries.

Aortic arch III is the tract connecting the glomus caroticum and the radix aortae. It is, like other larval aortic arches, divided into an anterior and a posterior branch. Gills are always supplied by blood coming from the posterior branch through the arteriae afferentes, and blood is carried from the gills by the arteriae efferentes to the anterior branch. In accordance with this direction of blood flow, the proximal section of the posterior branch is thicker than the anterior, whereas the situation is reverse in its distal section. After both branches re-unite, the oxygenated blood is carried to the radix aortae. In *Pelobates fuscus*, there is no branch from arch III to the filter apparatus but such connection may occur in other anurans (e.g. in *Litoria*; McIndoe & Smith, 1984). According to Millard (1945), arch III in *Xenopus laevis* is, from stage 46 until metamorphosis, the main arch supplying the body with oxygenated blood ('larval aorta'). However, Weisz (1945) considered the third aortic arches to be very small, joined to the radices aortae, and not associated with external gills; he believed that arches III disappear during the second

day. Undoubtedly, he misidentified the vestigial second arch (see above). This is also supported by the fact that he considered the ductus caroticus as persisting.

Arches V and VI take a common origin from the truncus arteriosus, and both re-unite before joining the radix aortae. Arch V is fully developed and functional in *Pelobates fuscus* (in *Rana temporaria*, it consists of a single tube which lacks any gill-supplying capillaries and thus is not functional; Lanot, 1962). Arch VI is reduced, and although it has functional gills it does not divide into an anterior and a posterior branch. Besides, arch VI gives off the a. pulmonalis, which most probably vascularizes the lung tissue for growth and maintenance before the lung is functional (their pouches are still not yet fully inflated in premetamorphic larvae). Moreover, the proximal part of the a. pulmonalis is important for cutaneous respiration as evidenced by the well-developed a. cutanea. We note that in *Pelobates fuscus*, the a. pulmonalis develops as early as in the larval period whereas in *Rana* sp. it appears only before the end of metamorphosis (Delsol & Flatin, 1972).

The common trunk of arches V and VI between the origin of the pulmonary artery and the fusion to the radix aortae (i.e. the ductus Botalli) is thick in the larvae and the main blood flow to the a. pulmonalis is thus most likely via the ductus Botalli. The unstable functional arrangement in the area of the ductus Botalli is expressed by the fact that in some premetamorphic larvae the a. cutanea, usually originating from the a. pulmonalis, may branch from the ductus Botalli, or that the a. pulmonalis splits from the radix aortae. In metamorphic stages (stage 63) we also found one individual in which the a. cutanea was a branch of arch V. This finding suggests that the ductus Botalli (and thus arch VI) does not connect to the radix aortae but rather joins the upper part of arch V.

Originally, the a. carotis interna was the anterior prolongation of the radix aortae. It begins at the point where arch III joins the radix. As in other anurans, it sends the a. palatina posterior which supplies the posterior part of the roof of the branchial cavity, the a. palatina anterior which supplies the roof of the buccal cavity, and the common stem of the a. ophthalmica which supplies the eyeball and the oculomotor muscles of the orbit, and the a. cerebralis which, together with its counterpart from the opposite side, gives rise to the a. spinalis ventralis (Millard, 1941). This pattern is basically the same in larvae and in adults, although there may be some variation in branching patterns and in anastomoses.

The climax of metamorphosis begins at stage 59 with the regression of the terminal branchial capillaries. In stage 61, arch V becomes thinner than the other arches and further regresses. The *m. petrohyoideus posterior*, the main breathing muscle of the adult frog, begins to develop between arches V and VI. It takes its origin on the otic capsule (more precisely, on the lower surface of the crista parotica) and inserts on the hyoid, therefore filling the space between the two arches (Lanot, 1962). The muscle is vascularized from the radix aortae via branches of the *a. occipito-vertebralis*. It is probably the increase in size of the muscle that causes arch V to regress. In stage 62, the gills are shorter, the branching pattern of the afferent and efferent arteries is markedly simplified, and their number is significantly reduced. The posterior branch lost its terminal connection to the arch; hence, the anterior branch connects directly to the radix aortae. This is undoubtedly a consequence of the shortening of the arch. Lungs are filled with air, which implies that they begin to take part in respiration. Simultaneously, the ductus Botalli becomes thinner.

The main metamorphic changes occurred in stage 63. Gills were completely reduced and aortic arches became short, continuous and sigmoid-shaped vessels. It may be inferred from their course that each arch is now represented by its persisting anterior branch. The ductus caroticus, which is still unchanged at stage 62, has entirely disappeared, leaving only small elevated scars at the former points of attachment. Consequently, the internal carotid artery and all its branches becomes the direct and sole continuation of arch III. Arch V lost its connection to the radix aortae and starts to regress from its distal end. Also the ductus Botalli is completely reduced, so arch VI only carries blood to the lungs and skin via the *a. pulmonalis* and *a. cutanea*. It is obvious that only arch IV is preserved as the main channel for the blood flowing from the heart to the body ('systemic arch'). It should be noted that in *Pipa pipa* the *a. cutanea* splits off from arch IV (Klinckowström, 1894) and not from arch VI or the pulmonary artery, as is the case in *Xenopus laevis* (Grobelaar, 1924b; Millard, 1941), *Pelobates fuscus* and other frogs.

The final phases of metamorphosis complete the processes that began in earlier stages, namely that (1) arch V entirely disappears in stage 66 and (2) the radix aortae becomes the direct and sole continuation of arch IV, or the systemic arch. The roots of the *a. carotis externa* and *interna* approach each other but remain separated at the end of metamorphosis. In *Rana*

esculenta, they fuse to form a common carotid artery (Gaupp, 1899).

The main changes associated with the transition of the metamorphosed froglets to dry land are (1) complete regression of the gills, (2) disappearance of the ductus Botalli, and (3) the disappearance of the carotid duct. Disappearance of the ductus Botalli, and a slightly delayed disappearance of arch V, result in a complete shunting of the deoxygenated blood into the lungs and skin, i.e. into the main respiratory organs of adult anurans. Disappearance of the carotid duct, which leads to an interruption of the radix aortae between the points where arches III and IV join the radix aortae, results in shunting the oxygenated blood into the head through the internal carotid artery and in shunting the oxygenated blood to the body through the systemic arch and radix aortae.

The loss of the ductus Botalli, arch V and the carotid duct (rarely, however, arch V and the ductus caroticus of both sides may persist in adult frogs too; Eales, 1949) are obviously primary adaptations to air breathing in adult anurans (and all amniotes). It may be argued that their disappearance had not occurred in the earliest tetrapods, such as *Ichthyostega* and *Acanthostega*. The pattern of their aortic arches could have been the same as in recent adult caudates, in which the piscine pattern persists (see below). This means that even if their blood was oxygenated in the lungs (and no doubt also in the skin), the blood supplying the head and trunk was only partly oxygenated because it was mixed with venous blood.

If we want to infer the situation in the Devonian piscine ancestors of the earliest tetrapods, we may examine larval stages of the extant ganoid fish *Amia calva* (Bjerring, 1967, 1973, 1977; Jarvik, 1980), which is one of the closest contemporary relatives. In larval *Amia calva* (Fig. 7A), five pairs of afferent arteries split off the truncus arteriosus, which is one that is vestigial to the hyoid skeletal arch and one to each of the four branchial units. The efferent branchial arteries of the branchial units empty into the radix aortae or directly into the dorsal aorta. Anterior to them, there are two more arteries. The posterior one is the *a. hyoidea efferens*, the anterior one is the *a. mandibularis efferens* (pseudobranchial artery *sensu* Jarvik, 1980). Although the hyoid aortic arch is, compared with those of the posterior branchial units, developed to a lesser degree and is apparently vestigial, it is still divided into two branches, an afferent dorsal branch which supplies the hyoid

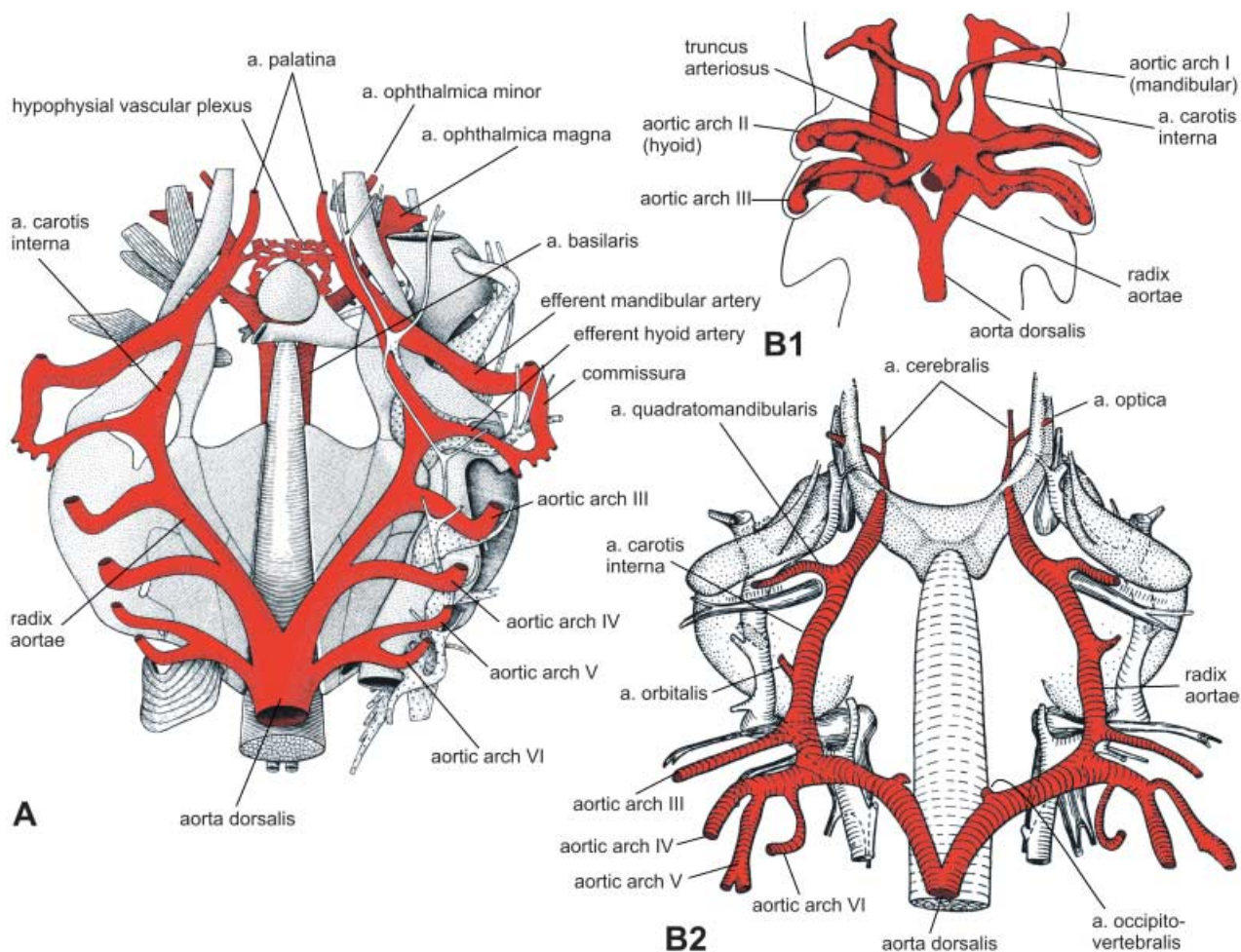


Fig. 7 (A) *Amia calva*, recent ganoid fish, embryo 8 mm. Dorsal part of the aortic arches in ventral view. (B1) *Salamandrella keyserlingi*, recent primitive caudate amphibian, embryo 8.5 mm. Arterial arches in ventral view. (B2) *Salamandrella keyserlingi*, 15 mm. Dorsal part of the aortic arches in ventral view. A, after Bjerring (1977), from Jarvik (1980); B, after Schmalhausen (1953a, 1968).

hemibranch, and an efferent ventral branch joining the radix aortae. However, as in elasmobranchs, there is another efferent artery which is an anastomosis between the hyoid and mandibular aortic arches, supplying the spiracular pseudobranch with arterial blood. Anteriorly, close to the efferent mandibular artery, the radix aortae (the internal carotid in later stages) sends the a. ophthalmica magna which enters the eye ball.

In adult *Amia calva*, the a. mandibularis efferens together with the a. ophthalmica are separated from the a. carotis interna, and connect with the latter only by a thin anastomosis. This separation is due to the occurrence of a new artery called the orbital artery (also termed the 'external carotid'), which supplies the eye muscles by its supraorbital and infraorbital branches.

In the Devonian fish *Eusthenopteron foordi* (Jarvik, 1980), the ventral aorta apparently produced afferent arteries to the branchial units as did *Amia calva*, and possibly there was an afferent hyoid artery too. The efferent arteries presumably started with two branches which soon merged into a single vessel. It is not clear whether the situation in *Eusthenopteron foordi* agreed with that in adult *Amia calva* or if there was an efferent mandibular artery as in larval *Amia calva*.

The hyoid hemibranchs have disappeared in all recent Amphibia (except, possibly, for some vestiges in the Gymnophiona; Brauer, 1897); however, their afferent and efferent arteries are preserved. In adult caudates, the most primitive condition was found in *Pleurodeles waltl* (Schmalhausen, 1953b) in which the internal carotid produces the hyoid artery and its branch, the orbital artery. In larval caudates (e.g.

Ranodon sibiricus, *Salamandrella keyserlingi*; Fig. 7B1, B2; see also Schmalhausen, 1954), the complete hyoid arch develops, although it disappears before metamorphosis (Schmalhausen, 1955). In anurans, only vestiges of the afferent hyoid arteries are preserved before metamorphosis (Schmalhausen, 1953b).

The piscine arrangement of the aortic arches is partly retained in anuran tadpoles, disregarding the anterior two that persist as mere vestiges in early development. The main difference from fish is that the ventral aorta was reduced and, consequently, the ventral sections of arches III–VI fused with each other to various degrees, thus giving rise to a short and partly bifurcated vessel called the truncus arteriosus. In adult *Pelobates fuscus*, its paired section is longer than in tadpoles, and its lumen is divided into parallel trunks (Szarski, 1948). As evidenced by the cast of a stage 50 tadpole, it is not yet divided in premetamorphic tadpoles. The a. carotis externa is generally believed to be a vestigial anterior end of the ventral aorta (Romer & Parsons, 1977; Balinsky, 1981). Noteworthy is the fact that it occurs only in tetrapods and lungfishes (Dipnoi). In contrast, a. carotis externa is absent in the early gnathostomes and teleost fishes. According to Goodrich (1958), in adult anurans the external carotids are represented by the lingual arteries. These arteries, together with branches of the a. carotis communis, supply the mucous membrane of the mouth cavity and probably also take part in gaseous exchange (Szarski, 1948). It should be noted that what is called the a. carotis externa in lungfishes develops from the a. carotis interna (Robertson, 1914) and consequently is not homologous with the like-named vessel in amphibians and amniotes.

In caudates, the piscine pattern of the aortic arches, disregarding external gills which are supplied by accessory capillary loops, persists into adulthood when the ductus Botalli and ductus caroticus may persist in reduced form in at least some taxa (e.g. *Triturus cristatus*, Boas, 1882; *Hynobius dunni*, Kato & Kurihara, 1989). Although it would seem logical to consider disappearance of the ductus Botalli to be associated with the beginning of pulmonary respiration, the persistent ductus Botalli in adult caudates, which use pulmonary respiration, seems to contradict this strict correlation (Baker, 1949). Nevertheless, the primitive pattern in caudates is structurally associated with their hyobranchial apparatus which largely maintains its segmentation. In anuran tadpoles, on the other hand, the hyobranchial skeleton is transformed into the branchial basket with filter-feeding function, and in

adult anurans it is transformed into the hyoid in which it is difficult to recognize the original segmentation.

We may speculate that in the Devonian crossopterygian fishes *Eusthenopteron*, *Panderichthys* and *Tiktaalik* (Jarvik, 1980; Vorobyeva, 1995; Daeschler et al. 2006), which represent successive evolutionary stages of piscine ancestors of tetrapods and which were permanently or predominantly aquatic, a limited degree of air breathing existed in water but because it was rather inefficient, pulmonary respiration was used only in cases of extreme necessity. In addition, dermal respiration could not have had great significance in fishes living in water because its effectiveness would not compare with the normal function of the specialized organs of branchial respiration (Schmalhausen, 1968). The general pattern of their aortic arches agrees with that in other aquatic gnathostomes (Jarvik, 1980). However, Romer (1937) reconstructed part of the arterial circulation in the Carboniferous crossopterygian fish *Megalichthys* and found some peculiarities that are normally not present in aquatic gnathostomes, e.g. the presence of the palatine artery, and that anticipate the condition typical for amphibians. We therefore suggest that these fishes could survive in moist environments using their dermal and pulmonary respiration for which some prerequisites in the circulatory system already existed, but they could also return to water and to branchial respiration. This intermediary condition is evidenced by vestigial preopercular and subopercular skeletal elements, which covered the branchial skeletal arches in the early tetrapods *Ichthyostega* and *Acanthostega* (Jarvik, 1952, 1995; Clack, 2000). In the larvae of early tetrapods the aortic arches probably remained unaffected.

Furthermore, we hypothesize that the basic branching pattern of the aortic arches of permanently water-dwelling piscine ancestors, of intermediate forms occasionally leaving water, and of primitive tetrapods capable of spending longer periods of time on land was the same as in the premetamorphic anuran larvae or in some metamorphosed caudates in which the ductus caroticus and ductus Botalli were not yet interrupted, and arch V was still complete. The process of a complete transformation of the aortic arches from piscine condition to that of a permanent land-dweller could last several million years (Carroll, 1995). The changes characterizing anuran metamorphosis may thus reflect later evolutionary transformations that occurred in more advanced amphibians.

Acknowledgements

We are indebted to David Fischer who provided us with a mating pair of *Pelobates fuscus*, and to Jan Kacvinský who prepared drawings for Fig. 6. Václav Seichert, Miloš Grim, and two anonymous reviewers provided useful comments. This research was made possible by grants GAUK 54/203 209 and MSM 0021620806 to H.K., and supported from grant AVOZ30130516 to the Geological Institute, Academy of Sciences of the Czech Republic.

References

- Aichhorn H, Lametschwandtner A** (1996) Vascular regression during the amphibian metamorphosis – a scanning electron microscope study of vascular corrosion cast of the ventral velum in tadpoles of *Xenopus laevis* Daudin. *Scanning* **18**, 447–455.
- Baker CL** (1949) The comparative anatomy of the aortic arches of the urodeles and their relation to respiration and degree of metamorphosis. *J Tennessee Acad Sci* **24**, 12–40.
- Balinsky BI** (1981) *An Introduction to Embryology*, 5th edn. Philadelphia: Saunders College Publishing.
- Bartel H, Lametschwandtner A** (2000) Intussusceptive microvasculature growth in the lung of larval *Xenopus laevis*. A light microscope, transmission electron microscope and SEM study of microvascular corrosion casts. *Anat Embryol* **202**, 55–66.
- Bjerring HC** (1967) Does a homology exist between the basicranial muscle and the polar cartilage? *Colloques int Cent Natn Rech Scient* **163**, 223–267.
- Bjerring HC** (1973) Relationships of coelacanthiforms. In *Interrelationships of Fishes* (eds Greenwood PH, Miles RS, Patterson C), pp. 179–205. London: Academic Press.
- Bjerring HC** (1977) A contribution to structural analysis of the head of craniate animals. *Zool Scr* **6**, 127–183.
- Boas JEV** (1882) Über den Conus arteriosus und die Arterienbogen der Amphibien. *Morph Jahrb* **7**, 488–568.
- Brauer A** (1897) Beitrag zur Kenntniss der Entwicklungsgeschichte und der Anatomie der Gymnophionen. *Zool Jahrb Abt Anat Ontog Tiere* **10**, 389–472.
- Brazeau MD, Ahlberg PE** (2006) Tetrapod-like middle ear architecture in a Devonian fish. *Nature* **439**, 318–321.
- Carroll R** (1995) Between fish and amphibian. *Nature* **373**, 389–390.
- Clack JA** (1994) The earliest known tetrapod braincase and the evolution of the stapes and fenestra ovalis. *Nature* **369**, 392–394.
- Clack JA** (1998) The neurocranium of *Acanthostega gunnari* and the evolution of the otic region in tetrapods. *Zool J Linn Soc* **122**, 61–97.
- Clack JA** (2000) The origin of tetrapods. In *Amphibian Biology, Vol. 4: Palaeontology* (eds Heatwole H, Carroll RL), pp. 979–1029. Chipping Norton: Surrey Beatty & Sons.
- Clack JA, Ahlberg PE, Finney SM, Dominguez Alonso P, Robinson J, Ketcham RA** (2003) A uniquely specialized ear in a very early tetrapod. *Nature* **425**, 65–69.
- Daeschler EB, Shubin NH, Jenkins FA Jr** (2006) A Devonian tetrapod-like fish and the evolution of the tetrapod body plan. *Nature* **440**, 757–763.
- De Saint-Aubain ML** (1985) Blood flow patterns of the respiratory system in larval and adult amphibian: functional morphology and phylogenetic significance. *Zool Syst Evol* **23**, 229–240.
- Delsol M, Flatin J** (1972) *Anatomie du système vasculaire des têtards de batraciens*. Paris: Librairie de la Faculté des Sciences.
- Eales N** (1949) Persistent fifth arterial arch in the frog. *Nature* **165**, 648.
- Exbrayat J-M** (2000) *Les Gymnophiones. Ces Curieux Amphibiens*. Paris: Societe nouvelle des editions Boubee.
- Gaupp E** (1899) *Anatomie des Frosches. Abt. 2. Lehre Vom Nerven- und Gefäßsystem*. Braunschweig: Vieweg u. Sohn.
- Goodrich ES** (1958) *Studies on the Structure and Development of Vertebrates*, Vol. 2. New York: Dover Publications Inc.
- Graaf AR** (1957) Investigations into the distribution of blood in the heart and aortic arches of *Xenopus laevis* (Daud.). *J Exp Biol* **34**, 143–172.
- Grobbelaar CS** (1924a) On the venous and arterial system of the 'Platanna' (*Xenopus laevis*, Daud.). *Z Anat Entwicklungs* **72**, 392–398.
- Grobbelaar CS** (1924b) Beiträge zu einer anatomischen Monographie von *Xenopus laevis* (Daud.). *Z Anat Entwicklungs* **72**, 131–168.
- Hafferl A** (1933) Das Arteriensystem. In *Handbuch der Vergleichenden Anatomie der Wirbeltiere, 6: Urogenital und Gefäßsystem* (eds Bolk L, Göppert E, Kallius E, Lubosch W), pp. 563–684. Berlin: Urban & Schwarzenberg.
- Jarvik E** (1952) On the fish-like tail in the ichthyostegid stegocephalians with descriptions of a new stegocephalian and a new crossopterygian from the Upper Devonian of East Greenland. *Meddr Grønland* **114**, 1–90.
- Jarvik E** (1980) *Basic Structure and Evolution of Vertebrates*. London: Academic Press.
- Jarvik E** (1995) The Devonian tetrapod *Ichthyostega*. *Fossils Strata* **40**, 1–213.
- Kato S, Kurihara K** (1989) The blood vascular architecture of the salamander external gill: a scanning microscopic study of corrosion cast. *Okajimas Folia Anat Japon* **66**, 171–194.
- Klinckowström AV** (1894) Zur Anatomie der *Pipa Americana*. 3. Gefäßsystem und subcutane Lymphsäcke. *Zool Jahrb Abt Anat Ontog Tiere* **7**, 647–666.
- Kusakabe T** (1992) Ultrastructural characteristic of glomus cells in the external carotid artery during the larval development and metamorphosis in bullfrogs, *Rana catesbeiana*. *Anat Rec* **233**, 461–466.
- Lanot R** (1962) Evolution des arcs artériels postérieurs au cours de la métamorphose chez la grenouille rousse (*Rana temporaria*). *B Biol Fr Belg* **96**, 703–722.
- Magnin E** (1959) Anatomie du têtard d'*Alytes obstetricans* Laur. *Acta Soc Linn Bordeaux* **98**, 1–60.
- McIndoe R, Smith DG** (1984) Functional anatomy of the internal gills of the tadpole *Litoria ewignii* (Anura: Hylidae). *Zoomorphology* **104**, 280–291.
- Millard N** (1941) The vascular anatomy of *Xenopus laevis* (Daudin). *Trans R Soc S Afr* **28**, 387–439.
- Millard N** (1942) Abnormalities and variations in the vascular system of *Xenopus laevis* (Daudin). *Trans R Soc S Afr* **29**, 9–28.

- Millard N** (1945) The development of the arterial system of *Xenopus laevis*, including experiments on the destruction of the larval aortic arches. *Trans R Soc S Afr* **30**, 217–234.
- Minnich B, Bartel H, Lametschwandtner A** (2002) How a highly complex three-dimensional network of blood vessels regresses: the gill blood vascular system of tadpoles of *Xenopus* during metamorphosis. A SEM study on microvascular corrosion casts. *Microvasc Res* **64**, 425–437.
- Moy-Thomas JA, Miles RS** (1971) *Palaeozoic Fishes*. London: Chapman & Hall.
- Nieuwkoop PD, Faber J** (1967) *Normal Table of Xenopus laevis (Daudin)*. Amsterdam: North-Holland Publishing Co.
- Nikitin B** (1925) Some particularities in the development of the vascular system of *Xenopus laevis*. *B Soc Nat Moscou* **34**, 286–308.
- Paterson NF** (1942) The anterior blood-vessels of *Xenopus laevis*. *S Afr J Sci* **38**, 279–291.
- Ramaswami LS** (1944) An account of the heart and associated vessels in some genera of Apoda (Amphibia). *Proc Zool Soc* **114**, 117–139.
- Robertson JI** (1914) The development of the heart and vascular system of *Lepidosiren paradoxa*. *Quart J Micr Sci* **59**, 53–132.
- Roček Z** (2003) Larval development and evolutionary origin of the anuran skull. In *Amphibian Biology, Vol. 5: Osteology* (eds Heatwole H, Davies M), pp. 1877–1995. Chipping Norton: Surrey Beatty & Sons.
- Romer AS** (1937) The braincase of the Carboniferous crossopterygian *Megalichthys nitidus*. *B Mus Comp Zool* **82**, 1–73.
- Romer AS, Parsons TS** (1977) *The Vertebrate Body*. Philadelphia: W.B. Saunders Company.
- Schmalhausen II** (1953a) The first arterial arches and the development of the carotid artery system in Amphibia. *Zool Zh* **32**, 937–954. [In Russian].
- Schmalhausen II** (1953b) Development of the arterial system in the head of tailed amphibians. *Zool Zh* **32**, 642–661. [In Russian].
- Schmalhausen II** (1954) Development of gills, their blood vessels, and musculature in Amphibia. *Zool Zh* **33**, 848–868. [In Russian].
- Schmalhausen II** (1955) Development of the visceral musculature in tailed amphibians. *Zool Zh* **34**, 162–174. [In Russian].
- Schmalhausen II** (1968) *The Origin of Terrestrial Vertebrates*. New York: Academic Press.
- Schulze FE** (1889) Über die inneren Kiemen der Batrachierlarven 1. Epithel der Lippen, der Mund-, Rachen- und Kiemenhöhle erwachsener Larven von *Pelobates fuscus*. *Abh König Akad Wiss* **1889**, 1–59.
- Schulze FE** (1892) Über die inneren Kiemen der Batrachierlarven 2. Skelet, Musculatur, Blutgefäße, Filterapparat, respiratorische Anhänge und Athmungsbewegungen erwachsener Larven von *Pelobates fuscus*. *Abh König Akad Wiss* **1892**, 1–66.
- Stadtmüller F** (1936) Kraniaum und Visceralskelett der Stegocephalen und Amphibien. In *Handbuch der Vergleichenden Anatomie der Wirbeltiere, 4: Skelettsystem* (eds Bolk L, Göppert E, Kallius E, Lubosch, W), pp. 501–698. Berlin: Urban & Schwarzenberg.
- Stephenson EM** (1951) The anatomy of the head of the New Zealand Frog, *Leiopelma*. *Trans Zool Soc Lond* **27**, 255–305.
- Strawinski S** (1956) Vascularization of respiratory surfaces in ontogeny of the edible frog, *Rana esculanta*. *Zool Pol* **7**, 327–365.
- Szarski H** (1937) The blood vessels of the thymus gland in some of the Salientia. *B Acad Pol Sci Lett B* **1937**, 139–149.
- Szarski H** (1948) On the blood-vascular system of the Salientia. *B Acad Pol Sci Lett B* **1948** (1947), 145–211.
- Szarski H** (1951) Remarks on the blood-vascular system of the frog *Leiopelma hochstetteri* Fitzinger. *Trans Roy Soc NZ* **79**, 140–147.
- Taylor C, Kollros JJ** (1946) Stages of the normal development of *Rana pipiens* larvae. *Anat Rec* **94**, 7–24.
- Viertel B, Richter S** (1999) Anatomy. Viscera and endocrines. In *Tadpoles. The Biology of the Anuran Larvae* (eds McDiarmid W, Altig R), pp. 92–148. Chicago: The University of Chicago Press.
- Vorobyeva E** (1995) The shoulder girdle of *Panderichthys rhombolepis* (Gross) (Crossopterygii), Upper Devonian, Latvia. *Geobios* **19**, 285–288.
- Wake M** (2003) The osteology of caecilians. In *Amphibian Biology, Vol. 5: Osteology* (eds Heatwole H, Davies M), pp. 1809–1875. Chipping Norton: Surrey Beatty & Sons.
- Wassersug RJ, Yamashita M** (2000) The mechanics of air-breathing in anuran larvae: implications to the development of amphibians in microgravity. *Adv Space Res* **25**, 2007–2013.
- Weisz PB** (1945) The development and morphology of the larva of the South African clawed toad, *Xenopus laevis*. *J Morph* **77**, 163–217.
- Witschi E** (1956) Integration of larval organs. In *Development of Vertebrates* (ed. Witschi E), pp. 115–137. Philadelphia: Saunders.