

DEVELOPMENTAL PATTERN AND MORPHOLOGY OF *Salamandrella keyserlingii* LIMBS (AMPHIBIA, HYNOBIIDAE) INCLUDING SOME EVOLUTIONARY ASPECTS

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In this paper, we consider the pattern of skeletal development and morphology in the limbs of the hynobiid species, *Salamandrella keyserlingii*, with the overall aim of analyzing tetrapod limb evolution in context of the phylogeny. In comparison with anurans the urodeles demonstrate caenogenetic adaptation in distal structures of the limbs. As the most primitive family of Caudata hynobiids are of special interest since a study of early stages is likely to display some plesiomorphic features. In common with other larval salamanders *Salamandrella* has early development of digits 1 + 2. However, in contrast to them this species displays a number of distinctive characters, including larval adaptations and features which can be considered as phylogenetically primitive for caudates and sometimes for uniseriate sarcopterygians in general. The main peculiarities of *Salamandrella* can be summarized as follows:

- 1) early formation of the mesenchyme mass between the zeugopod bifurcation as the base for the proximodistal differentiation of preaxial, median and postaxial columns;
- 2) initial connection of the median column with postaxial one in the region of intermedium condensation;
- 3) early formation of the intermedium condensation in close contact with ulnar/fibular condensation;
- 4) dominance of the posterior or postaxial branch (ulnare/fibulare) in the early limb development;
- 5) comparatively late formation of the basale commune which arises primarily as a small distal 2 condensation at the base of the digit 2 and later fuses with distal 1 condensation;
- 6) fusion of the skeletal elements in longitudinal rather than in transverse direction; usually amalgamation of the median elements with postaxial than with preaxial ones;
- 7) frequent (in 75%) amalgamation of the intermedium with ulnare;
- 8) presence of two central elements in the standard morphology;
- 9) presence of a well developed, long temporary epidermal fin between first and second digits used in balance and locomotion of the larvae. This is caenogenetic adaptation.

The comparison of *Salamandrella* with other caudates and some fossil sarcopterygians led us to conclusion that many developmental pattern of its limb (1 – 4) can be considered as an ancestral features. It may be supposed that such characters of urodeles as the precocious appearance of anterior digits and basale commune, the gap between them and proximal (zeugopod/stylopod) portion, distal-to-proximal sequence in development of carpal and tarsal elements have a secondary character, probably correlated with their caenogenetic adaptations, and do not support the hypothesis of the polyphyly of tetrapods.

Key words: Caudata (Urodela), limb development, variation, evolution.

INTRODUCTION

For many years, the limbs of the Caudata (urodeles) have been used as a prototype of tetrapod limbs in general. At the same time studies of amphibian limb development led many workers to oppose anurans (and amniotes) to caudates on the basis of two

fundamentally different developmental patterns. On the basis of them, the hypothesis of the polyphyletic origin of tetrapods was proposed (Holmgren, 1933; Jarvik, 1980). In fact it was an attempt to define the “canon” of skeletal elements constituting each of the two “archetypes” (Hinchliffe and Griffiths, 1983). An alternative view was proposed by Schmalhausen (1915) who explained the differences between amphibian groups on a “morphophysiological” basis, i.e., on the species specificity of their adaptation and on the function of the developing limb for use. Thus

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the developmental changes in the limbs of free-living larvae of different Caudata were connected with the early use of the developing limbs for locomotion. Schmalhausen noted also that these caenogenetic adaptations of the larval limbs of salamanders are often distinguished even in quite closely related groups. In particular, he described (Schmalhausen, 1910, 1917) structural differences between genera of hynobiids (*Salamandrella*, *Ranodon*, *Onychodactylus*) whose limbs are characterized by the presence of claws or by epidermal fins with different locations.

Recently, there has been a consensus that the distance between anuran and caudate limb patterns is not so large and that both these groups have a monophyletic origin (Duellman and Trueb, 1986). There is a special interest in the salamanders with direct development whose limb development patterns are closer to the condition common to all amphibians and in particular to anuran specialities (Shubin, 1995). However metamorphosing caudates with aquatic larvae are regarded as a special case in tetrapod limb history, characterized by caenogenetic adaptations of their limbs and by different modes of limb development.

A recent upsurge of investigation has attempted analysis of limb development and adult morphology of the amphibians from the position of their biodiversity and in terms of modification of a common developmental “bauplane” (Shubin and Alberch, 1986; Shubin, 1991). In these process-based approaches (Hinchliffe, 1991), developmental changes in the position of segmentation of prechondrogenic condensations or their branching, or in the timing of these events are seen as the explanation of changes in adult morphology (Alberch and Gale, 1985; Blanco and Alberch, 1992; Shubin, Wake, and Crawford 1995). In addition some recent works on caudates have focused on intraspecific variation in limb skeletal patterns, reflecting internal constraints of variation (Shubin, Wake, and Crawford, 1995; Borkhvardt and Ivashintsova, 1993; Vorobyeva and Borkhvardt, 1994; Hanken, 1983; Blanco and Alberch, 1992). Observations of intraspecific and interspecific variation in limb patterns permit attempts to establish both phylogenetic relations and to discover the mechanisms of limb evolution.

In our paper, we discuss the developmental patterns and morphological variations of limbs in the “Siberian newt”, *Salamandrella keyserlingii*, from the position of its evolutionary importance. This spe-

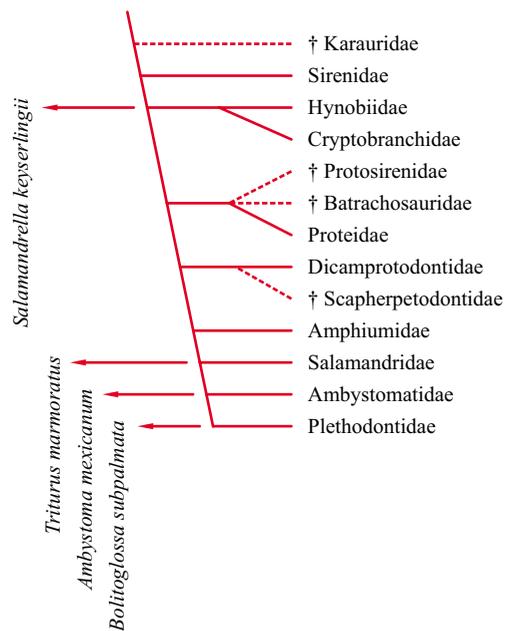


Fig. 1. Hypothetical phylogenetic relationships among the families of Caudata (fossil-dotted line), based on the non-paedomorphic features. After Duellman and Trueb (1986).

cies, considered as one of the most primitive among living urodeles belongs to the family Hynobiidae (Fig. 1). According to recent classification (Duellman and Trueb, 1986), this family is characterized by 20 primitive features from 27 analyzed non-paedomorphic characters, while in advanced Salamandridae only 10 and in Plethodontidae only 7 of these occur. Thus we can suppose that the development and possibly morphology of limbs in *Salamandrella* retain some features close to the ancestral ones. *Salamandrella* has been selected for discussion as the best known species of the hynobiid family (Schmalhausen, 1915; Borkhvardt et al., 1992, 1993, 1994; Vorobyeva and Borkhvardt, 1994), and because its life tables of normal development have been published (Lebedkina, 1964; Sytina et al., 1987). Variation in adult *Salamandrella* limb skeletons and some peculiarities of its development have been examined recently (Borkhvardt and Ivashintsova, 1993; Borkhvardt, 1994; Vorobyeva et al., 1995). Here we concentrate attention on the earlier stages of limb development and in particular on the pattern of mesenchyme pre-chondrogenic condensation, branching and segmentation. We can then compare this pattern with that in other caudates (Blanco and Alberch,

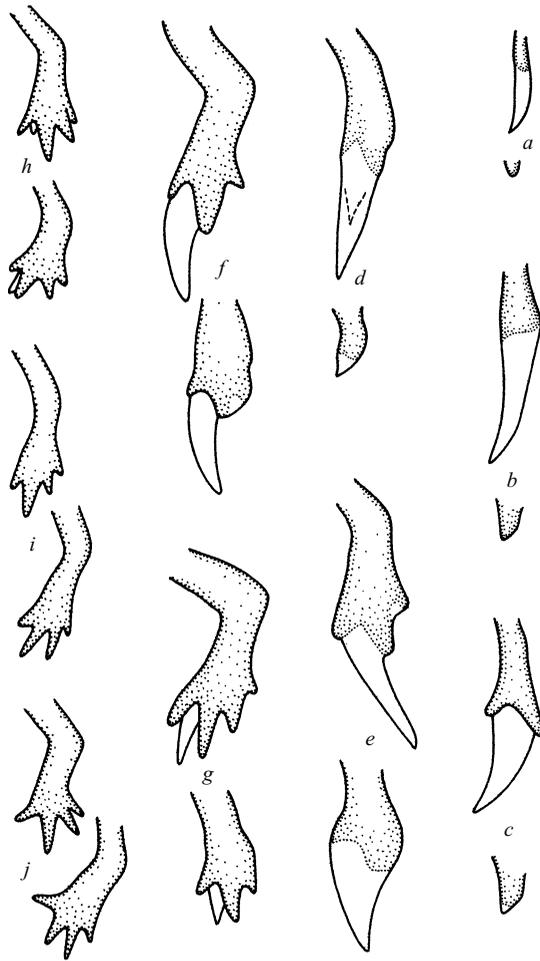


Fig. 2. The limb development of the *Salamandrella keyserlingii* at the different stages (after Sytina et al., 1987): upper) forelimb, below) hindlimb. a) Stage 34; b) stage 35; c) stage 36; d) stage 37; e) stage 38; f) stage 39; g) stage 40; h) stage 41; i) stage 43; j) stage 44; (34–39 larval stages, 40–44 premetamorphic stages).

1992; Shubin et al., 1995) and attempt both to draw phylogenetic conclusions, and to understand the process of limb evolution in tetrapods, particularly the dual roles of internal constraints and caenogenetic adaptation in larval salamanders.

MATERIAL AND METHODS

As the basis of this analysis we used the *Salamandrella* histological collection preserved in the Laboratory of Evolutionary Morphology, A. N. Severtsov Institute of Evolutionary Morphology and

Ecology of Animals, Russian Academy of Science, Moscow, Russia. In addition, we used eggs collected from the Ekaterinburg region (Talitsa station) in the spring of 1995 and maintained in an aquarium. From stage 36 onwards larval limbs were fixed either in Bouin's or acetic — alcohol, and were then blocked, sectioned and stained with haematoxylin and eosin. The main stages examined were 36–40 for the forelimb and 39–43 for the hindlimb, according to the staging system (Sytina et al., 1987). These stages cover the critical early stages of the development of the skeletal pattern in the limb.

In addition, with the intention of mapping more specifically stained prechondrogenic skeletal patterns we used the fluorescent tagged lectin PNA (peanut agglutinin). This is an effective method of mapping prechondrogenic element in anurans (*Xenopus*) and in amniotes (Hinchliffe and Vorobyeva, in preparation) but it is not effective in staining caudate tissue. We also attempted mapping using antibodies prepared against amniote skeleton extracellular matrix molecules such as chondroitin sulfate (gift of Prof. Charles Archer, Cardiff) but these were ineffective, presumably due to lack of cross-species reactivity.

In describing the adult limb skeleton and its variants, we used mostly the information on variation in *Salamandrella* given by Borkhvardt and Ivashintsova (1993) and also some other data (Schmalhausen, 1915; Vorobyeva and Borkhvardt, 1994).

RESULTS

1. External Changes

The changes in external form of the developing limbs have been used to determine the ontogenetic stages of *Salamandrella* (Schmalhausen, 1915; Lebedkina, 1964; Sytina et al., 1987). In this paper we use the tables of development according to latter work. The anterior limb buds first become visible at stage 22 (Sytina et al., 1987). Up to stage 32 the anterior limb bud has a conical shape and at stages 33–34 it develops an anterior epidermal web or filament at its distal end (Fig. 2). In the larval period (stages 35–39) (Fig. 2b–f) the main changes in limb formation take place: in the anterior limb at stage 36 and 37 two digits differentiate, at stage 38 the third digit, and at stage 40 the fourth digit appear. During the active feeding period beginning at stage 36 the web is large, but begins its regression at the

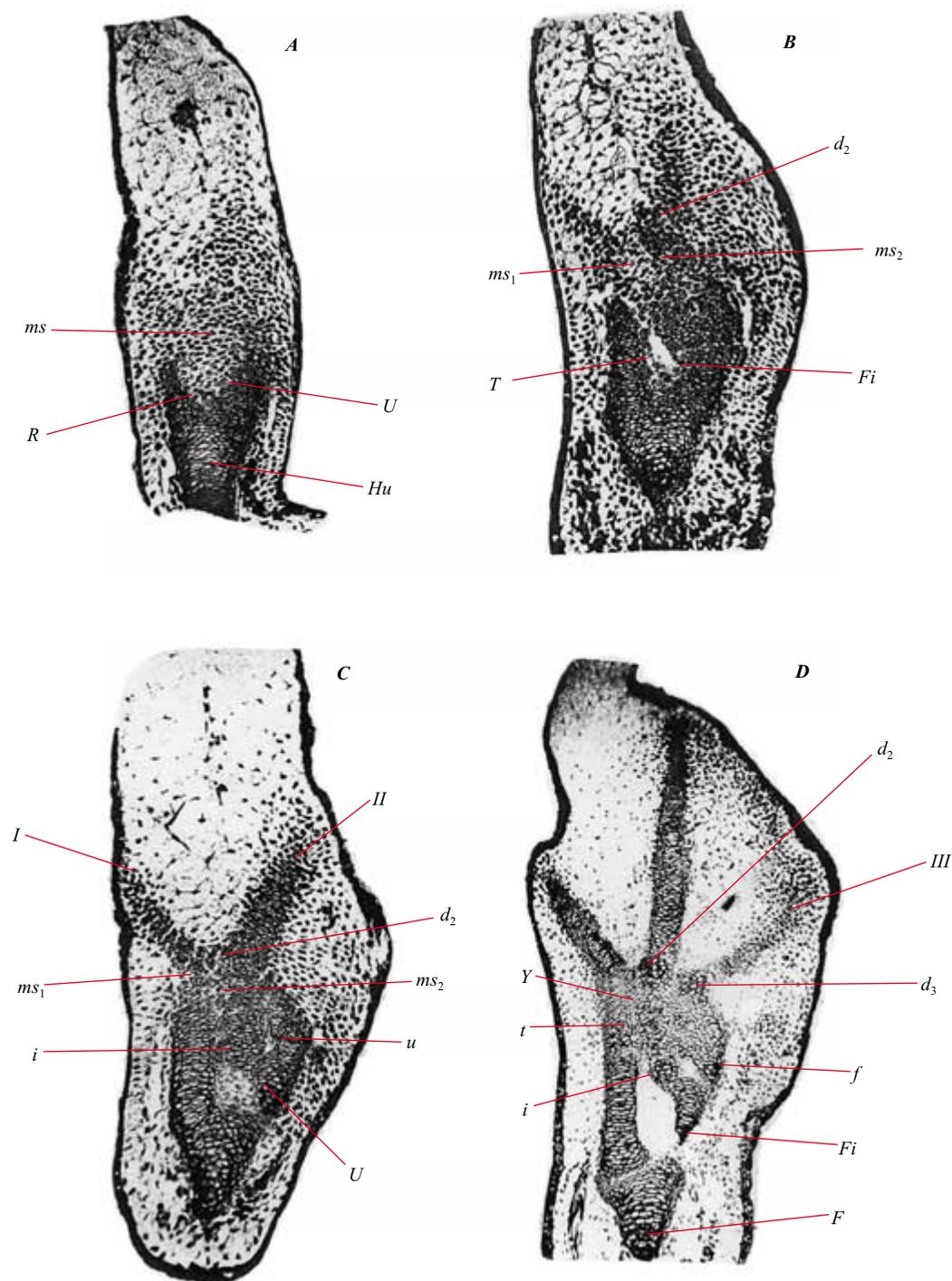


Fig. 3. Hystological sections along larval forelimb (A, B) and hindlimb (C, D) of *Salamandrella keyserlingii*. A) Stage 35; B) stage 36–37; C) stage 38; D) stage 39. From Schmalhausen (1915, plate VII). Abbreviations: *bc*) Basale commune; *c*(*c*₁, *c*₂) centralia; *d*₁₊₂) distal carpal/tarsal 1 + 2 (= *bc*); *d*₃₋₄) distal carpal/tarsal 3–4; *F*) femur; *f*) fibulare; *Fi*) fibula; *H*) humerus; *i*) intermedium; *I–V* or *3–5*) digits (metapods) *I–V* (3–5); *ms*) primary mesenchyme condensation between zeugopod; *ms*₁, *ms*₂) preaxial and postaxial mesenchyme condensations; *po*) postminimum; *pr*) prepollex/prehallux; *R*) radius; *r*) radiale; *st*) stylopod; *T*) tibia; *t*) tibiale; *U*) ulna; *u*) ulnare; *Y*) element “Y”; *z*) zeugopod.

premetamorphic stage 40. The web is considered to be a caenogenetic adaptation of the larvae (Schmalhausen, 1910) used in locomotion and balancing.

The hindlimb bud develops its web during stages 37–39, but this begins its regression at stage 40 (Fig. 2g). Two digits begin differentiation at stage 38 (Fig. 2e), the third at stage 39 (Fig. 2f), the fourth at stage 41 (Fig. 2h). By stage 43 (Fig. 2i) for both limbs the definitive adult morphology has been reached, and by stages 44–45 (Fig. 2j) the fore- and hind limbs are usually of equal size. Later, the hind limb becomes larger than the anterior. The long fingers have bent claws which disappear during casting the coat (stage 47) in metamorphosis. However, new claws appear at stage 50 and remain until stage 52 of the juvenile newt.

2. Internal Changes in Skeletal Development

Skeletal development follows the normal sequence of prechondrogenic condensation of mesenchyme, chondrogenesis and finally ossification with calcification. Endoskeleton development has a similar sequence and pattern in both anterior and posterior limbs, so that a single description of both can be given, based mostly on the forelimb.

Endoskeleton development begins with a proximal bar shape condensation with a short bifurcation distally, corresponding with stylopod (humerus/femur) and zeugopod (radius/tibia, ulna/fibula) (Figs. 3A, 4A). This condensation is typical not only for all amphibians but probably for all sarcopterygians (Vorobyeva and Hinchliffe, 1995). Between and distal to the zeugopod bifurcation at the stage 35 in the forelimb can be seen a diffuse mass of mesenchyme (ms), lacking individual condensations (Figs. 3A, 4B).

At the stages 36 (forelimb) and 38 (hindlimb) between the zeugopod bifurcation two mesenchyme condensed masses are distinguished (ms_1 , ms_2 , Figs. 3B, C; 4C). The narrower of them is connected with the preaxial (radius/tibia) branch of the zeugopod and includes the mesenchyme of the first digit. The second mass is larger and distally wide. It is connected with postaxial (ulna/fibula) branch of zeugopod and includes the mesenchyme of the second digit. At the stage 36 (forelimb) prechondral condensations of the first and second digits are well defined and chondrification of the proximal parts of the radius and ulna is beginning. Distal to the ulna in the

postaxial mesenchyme condensation can be seen two large prechondral condensations separated by thin mesenchyme (Fig. 3B). Both have the same size and develop in close contact with the cartilaginous ulna. One of them represents the ulnare, the other the intermedium (Fig. 4D). Distally to the intermedium the mass of mesenchyme condensation forms the median column which goes to the base of the second digit. At this base, there is a small and more compact condensation of mesenchyme cells which corresponds to the carpal distal 2 (d_2 , Figs. 3B–D; 4D) (later the major part of the basale commune; *bc*, Fig. 4E). The first digit in this stage is represented by solid condensing mesenchyme which continues distally the preaxial (radial) branch of mesenchyme and contacts at its base with second digit (metapod 2). Thus at this stage there can be distinguished three prechondral columns: 1) preaxial as a continuation of the radius to the first digit; 2) median arising on the median side of the distal part of ulna and continuing to the second digit; and 3) postaxial continuing the ulna and finishing in a large mesenchyme mass. These three prechondral columns can be distinguished for only a short time: the postaxial branch grows distally and fuses with the median column in a single prechondral mass. The preaxial column is more independent and later connects with the median column in the distal carpal region. These three columns are probably in limb development of all Caudata during a very short time (Schmalhausen, 1915). However the character of their differentiation is distinct in different groups and species.

Essentially, differentiation of those in *Salamandrella* has in general a proximodistal direction displaying both similarity and differences (in comparison with other known caudates) (Figs. 4, 6). In the preaxial column, radiale and “Y” element (Schmalhausens’ mediale 1) appear in sequence, first as condensations, which then chondrify (Fig. 4D–F). In the median column, the intermedium appears first, followed then by distal, carpal/tarsal 2 (Fig. 4D). This differentiates later than the intermedium, but earlier than centralia elements, which are represented by centralia 1 and 2 (Fig. 4F). In other caudates an element usually termed the basale commune as a single mesenchyme condensation lies at the base of metapods 1 and 2 (Fig. 4H). In *Salamandrella* the basale commune is a double element formed firstly (at stage 37) by distal carpal 2 (Fig. 4D). Later (stage 38–39) the anterior process extends from this element to the

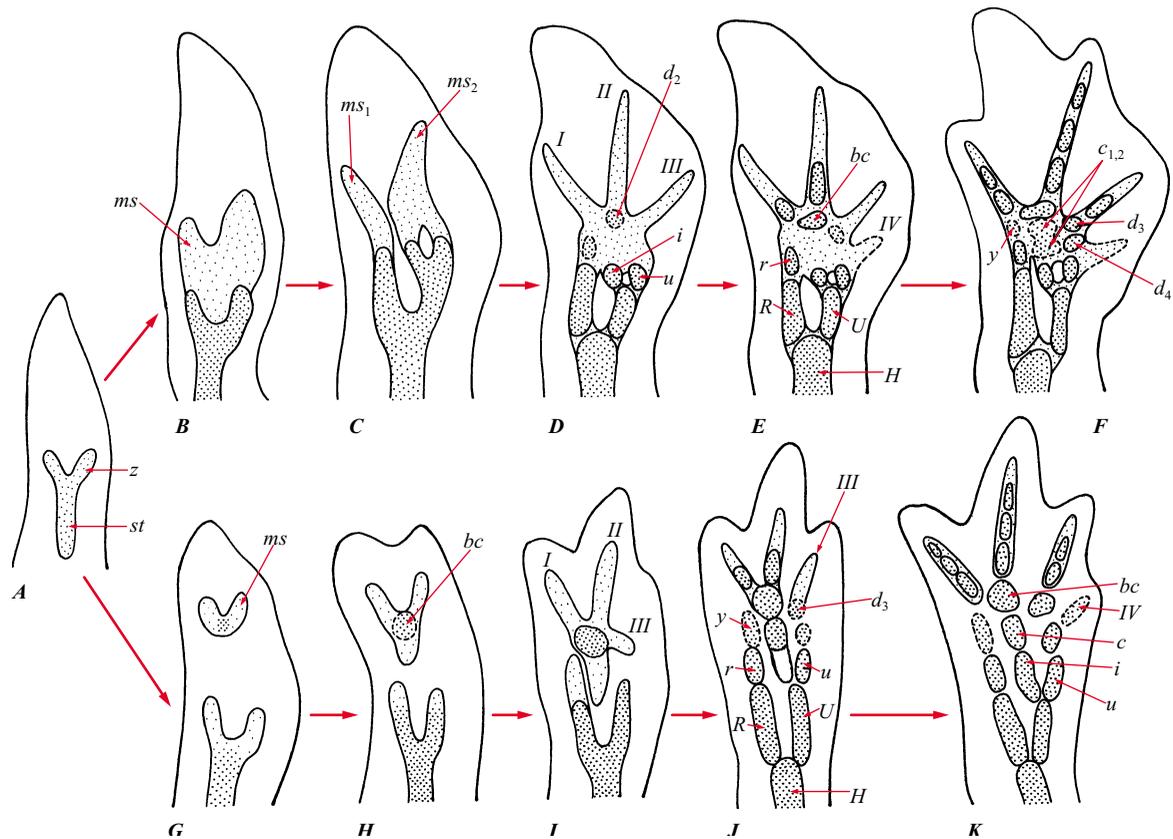


Fig. 4. The pattern of limb development of *Salamandrella keyserlingii* (A–F) in comparison with an advanced larval salamanders, such as *Triturus* (G–K). G–K) Modification from Blanko and Alberch (1992) and Shubin (1995). Abbreviations as in Fig. 3.

small condensation at the base of the metapod 1 which possibly is a second element of the basale commune corresponding to distal carpal 1. Thus a contact or bridge between the preaxial and postaxial mesenchymal masses is formed (Fig. 4E, F).

The basale commune has been a controversial element in phylogenetic interpretation (Holmgren, 1933) and it is important to emphasize in *Salamandrella* that it forms later than the intermedium (Fig. 4D), conforming with proximodistal developmental sequence along the median column. In the postaxial column, the ulnare/tarsale condensation forms first (about at the same time as the intermedium) followed by distal carpal/tarsal 3 (Fig. 3D) and then distal carpal/tarsal 4, emphasizing that the developmental sequence along the 2–4 metapods base is in an antero-posterior direction (Fig. 4F).

The first and second metapods for a long time remain prechondral. The chondrification of the mesopodium begins from intermedium and after that ex-

tends to the distal carpal 1. Later radiale and ulnare all chondrify at the same time. In the fingers, cartilage first appears in the second metapod then in first one. Next chondrification extends posteriorly to the third finger and to centrale 1 (mediale 2 after Schmalhausen, 1915). Soon after this, the distal chondrification centers become more compact: “Y” element and centrale 2, distal 3 and distal 4.

The calcification of mesopodial cartilages in *Salamandrella* limbs begins from the central region of carpus/tarsus and has a centrifugal character (Borkhardt et al., 1992). The ossification process displays some individual variation in the timing of this beginning and in different elements. However the calcification and ossification of the preaxial column proceed slowly as in the postaxial one. In some specimens preaxial endoskeleton in the posterior limbs can be unossified for about 6–9 years and only in rare cases show resorption of cartilage. It is connected probably with comparatively late differentiation and

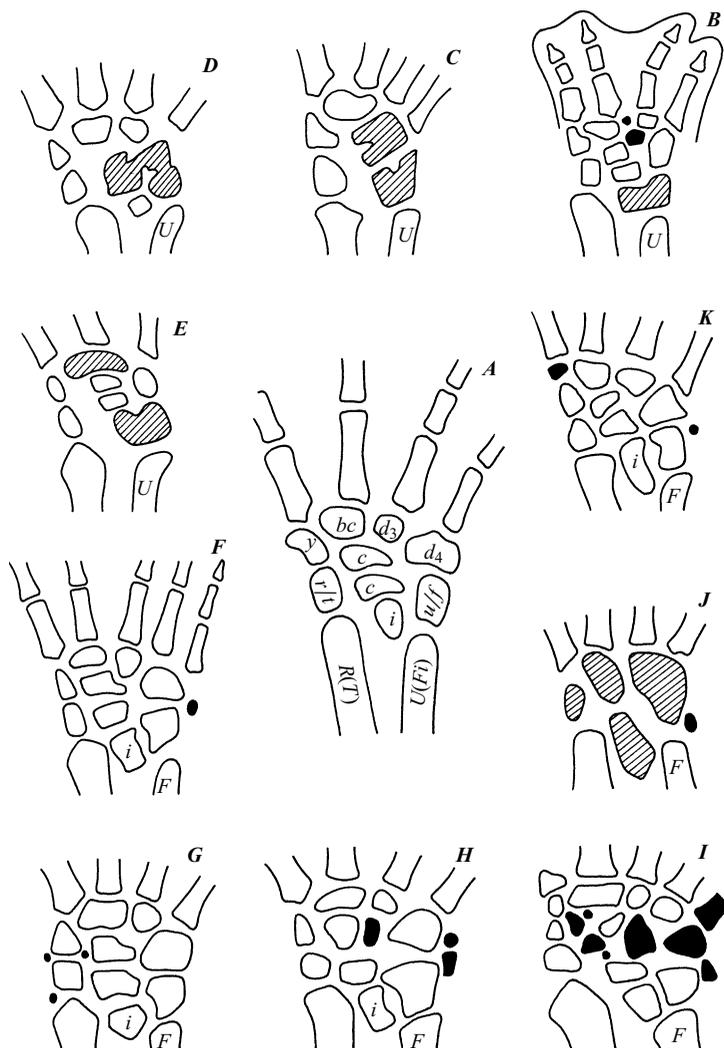


Fig. 5. The standard definitive morphology (A) and some variation of the limb skeleton (B–K) in *Salamandrella keyserlingii*. Amalgamations are parallel hatching, additional elements are black. Modified after Borkhvardt and Ivashintsova (1993). Abbreviations as in Fig. 3.

chondrification of the “Y” and tibiale in the ontogeny of *Salamandrella*.

3. Definitive Limb Structure and Variation

The standard morphology of both limbs is shown in Fig. 5A which also shows the possible variant patterns. Normally morphology of the mesopod is characterized by presence of two (proximal and distal) central elements, which are situated between intermedium and basale commune (distal 1 + 2). The first

of them (centrale 1) contacts the basale commune and distal 3, second the centrale 1 and distal 4, the last element contacts the ulnare/fibulare. The preaxial column is formed by two elements: radiale/tibiale and “Y” element (mediale 1, Schmalhausen, 1915), which contacts the metapod base of the first digit, basale commune and the centrale 1. Both limbs have usually four digits (hind limb sometimes with five digits) and their standard phalangeal formula is 2222 (Fig. 4E) for the fore and 2232 for the hind limb. Including variants, the number of the endoskeletal elements in the carpus ranges from 5 to 10, in the tarsus from 4 to 16. In typical cases there are 9 elements: including radiale/tibiale and “Y” element (mediale 1) in the preaxial column; ulnare/fibulare, distal 3 and distal 4 in the postaxial column; intermedium, centrale 1, centrale 2 and basale commune (distal 1 + 2) in the median column. For both limbs the amalgamation of the radiale/tibiale with “Y” is quite common (Fig. 5J). The fusion of preaxial elements with elements of median column never occurs. This supports the idea of the autonomous position of the preaxial branch (Schmalhausen, 1915) which is also supported by some distinguishing features of its elements in shape, exposition and calcifications, (Borkhvardt, 1992). The presence of tarsale postminimus (Fig. 5F, H, K) and of two or more additional centralia (Fig. 5B, H, I), as well as an additional distal and a prehallux (Fig. 5I) is found only as variants in posterior limbs.

The variants in adult morphology of the skeleton were studied in 140 specimens (Borkhvardt and Ivashintsova, 1993) from a single population in the Talitsa District. Standard morphology is founded in the hands and feet of less than 34% of individuals. Deviations from standard condition were founded in 90 tarsal and 123 carpal regions, which belong to 68 and 84 individuals, respectively.

Among variants were described 23 quantitative modifications in the carpal, 16 in tarsal regions together with 12 in the phalangeal formula of the forelimbs and 16 of the hindlimbs. In 15 cases there were five digits in the feet. The variations were connected with amalgamation (Fig. 5D) or with the appearance of some additional skeletal elements (Fig. 5B, F, H, I, K). It was established that for hands the fusion of elements is more typical, but for feet an increase in their number is more common. This is explained (Borkhvardt and Ivashintsova, 1993) as due to the larger size of the skeletogenous mesenchyme mass in developing hindlimbs.

It is known (Schmalhausen, 1915) that for aquatic Caudata the longitudinal amalgamations (along the preaxial, the median and the postaxial columns) are more common. In *Salamandrella* the longitudinal fusion of elements are common in the hindlimbs where their ratio to transverse ones is 22 to 6 (Borkhvardt and Ivashintsova, 1993). In forelimbs this ratio is reversed, and in this case this high number of transverse amalgamation (294 to 81) is explained with the very frequent fusion of ulnare with intermedium (66% of all variants). Among the longitudinal amalgamation the most frequent is centrale 1 with centrale 2 (8% of all variants in forelimb and 39% in hindlimb), then distal 3 with distal 4 (6% in forelimb and 25% in hindlimb). The distal 5, additional centrale and prehallux are quite common for the feet but as a rule fuse with neighboring cartilages belonging to the same column and form the processes on them (Schmalhausen, 1915; Borkhvardt and Ivashintsova, 1993).

DISCUSSION

Recent amphibians are classified into three orders: Anura (frogs), Caudata (urodeles or salamanders), and Gymnophiona (caecilians) (Duellman and Trueb, 1986). One of the main arguments used in the support of a polyphyletic origin of the amphibians (as in a tetrapods in general) has been the differences in development between anurans and urodeles (Fig. 6). Among these the peculiarities in the morphogenesis of the limb skeleton are most often considered (see discussions in Schmalhausen, 1915; Holmgren, 1933; Blanco and Alberch, 1992; Shubin, 1995). The anteroposterior formation of digits and distoproximal differentiation of mesopodials have emerged for a long time as typical urodele features which distin-

guished them from anurans. Recently the old idea is popular that the primitive amphibian tarsus (known in Palaeozoic temnospondyls) is similar to that of basal caudates (hynobiids, cryptobranchiids) (Borkhvardt et al., 1992; Shubin, 1995). At the same time basing on new data the authors came to conclusion that the urodele limbs have different developmental modes in different species (Blanco and Alberch, 1992; Shubin et al., 1995). The primitive condition of tetrapod limb development is seen at present in that of many anurans and amniotes (*An*, *Amn*, Fig. 6) and is characterized by the early connection between the postaxial (ulnar/fibular) branch and the digital arch and by the digit development in a postaxial-to-preaxial direction (Shubin et al., 1995). The salamanders with aquatic larvae (such as *Triturus*) (*Lar*, Fig. 6) are considered as a derived pattern where the digital arch develops precociously, from the basale commune (*bc*, Fig. 4H) distoproximally and from the second digit in a postaxial direction, and does not have an early connection with the ulnare/fibulare. The caudates with direct development (such as *Bolitoglossa subpalmata*) (*Bol*, Fig. 6) are similar to anuran — amniotes (*An*, *Amn*) in that an early connection develops between the postaxial branch and digital arch. The first digit to appear in these limbs is the third (Shubin, 1995).

From this position the anteroposterior formation of digits and a distalproximal mesenchyme and cartilage differentiation of the carpal/tarsal elements are considered as a synapomorphy of the Caudata (Shubin et al., 1995).

At the same time the differences in the developmental patterns in urodele limbs, especially among larval salamanders, in comparison with anuran-amniote limbs and with direct developing salamander limbs are correlated with their larval (caenogenetic) adaptations.

1. Development and Evolution

Many Caudata with aquatic larval stages are characterized by precocious formation of the forelimbs, which are used for aquatic locomotion and support of the larva on the bottom substrate or on plants after hatching. Most these larvae use for these tasks the preaxial part of the autopod and usually their second and first digits develop earlier relatively to the other digits. These features are found in *Salamandrella*, which uses its precociously developed an-

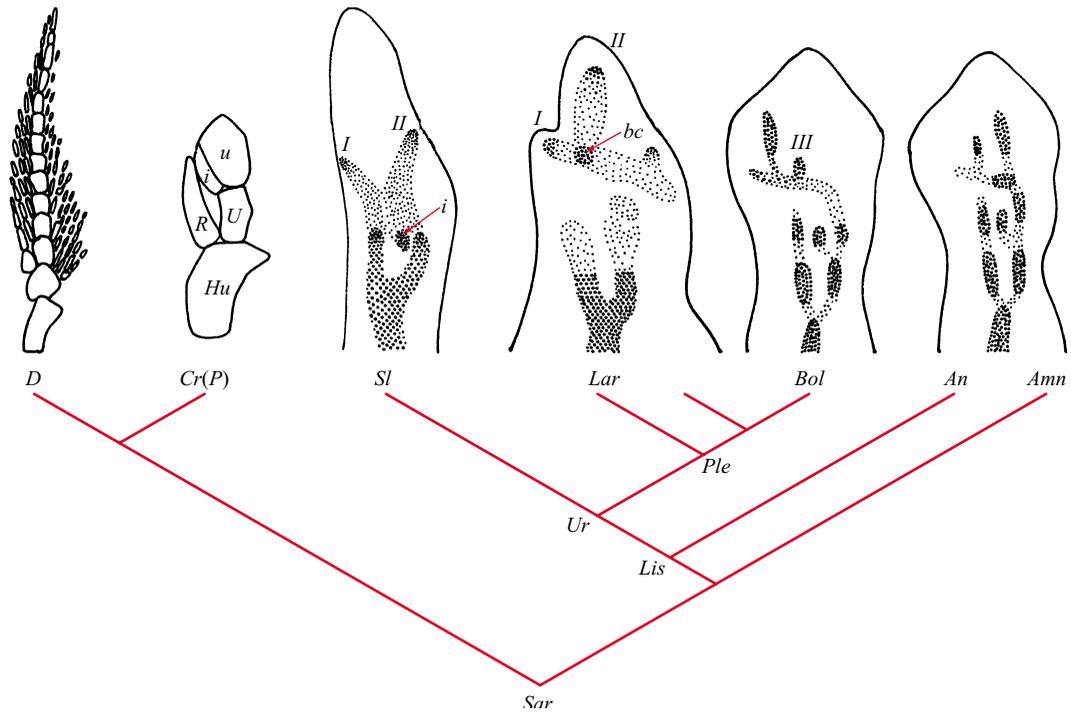


Fig. 6. Cladistic scheme of the limb development pattern: of recent amphibians and amniotes in comparison with skeletal pattern of sarcopterygian fins. Abbreviations of nodes: *Amn*) amniotes; *An*) anurans; *Bol*) *Bolitoglossa* (a direct developing salamander); *Cr(P)*) crossopterygians (*Panderichthys*); *D*) dipnoans; *Lar*) clades of salamanders with aquatic larval stages; *Lis*) lissamphibians; *Ple*) Plethodontidae; *T*) tetrapods; *Sl*) *Salamandrella*; *Sar*) sarcopterygians; *Ur*) urodeles. Modified after Shubin (1995).

terior limb very early for balancing when active feeding begins. As can be observed in the aquarium, *Salamandrella* at this stage takes up a vertical position in the water. In comparison with other hynobiids *Salamandrella* displays some specificity of its caenogenetic features. This is the presence of a long terminal fin-like mesenchyme filled epidermal filament on both limbs (Fig. 2). In another hynobiid such as *Ranodon sibiricus* an analogous filament is small and is positioned postaxially (Schmalhausen, 1917). A remarkable feature of *Ranodon* as in a closely related genera *Onychodactylus* is also the earlier presence of the small black claws on all toes of the larva which are used for clinging to plant in rapidly moving streams.

As in other larval caudates: *Axolotl*, *Siren*, *Thori-us*, *Triturus* (Schmalhausen 1915; Hanken, 1982; Blanco and Alberch 1992) the limb development of *Salamandrella* is characterized by: 1) initial proximal condensation of the stylopod and its first bifurcation (Fig. 4A); 2) dominance of the preaxial zeugopod elements (radius/tibia) in the early stages;

3) early appearance of first and second digits (postaxial, Fig. 4C, H); 4) strong anteroposterior digital differentiation (Fig. 4D – F, I – K); 5) presence of median column of condensation between the preaxial and postaxial branches of the mesopod; 6) proximodistal differentiation (mesenchyme condensation, chondrification and ossification) of the preaxial (radius/tibia) and postaxial (ulna/fibula) branches. Some of these characters (1, 2, 5, 6) may be considered as a plesiomorphic features of tetrapods probably in common with their fish ancestors (sarcopterygians). The features 3 and 4 are recognized as larval caudate characters. At the same time these larval salamanders in contrast to *Salamandrella* are characterized by: 1) precocious appearance of distal condensations (the basal commune connected with metapodials one and two) (Fig. 4G); 2) presence of a distance between distal (basal commune) and proximal (stylopod/zeugopod) mesenchyme condensations; 3) secondary connection of the digital arch to the postaxial branch of the mesopod (Fig. 4J); 4) dominance of the anterior or preaxial branch (radius/tibia)

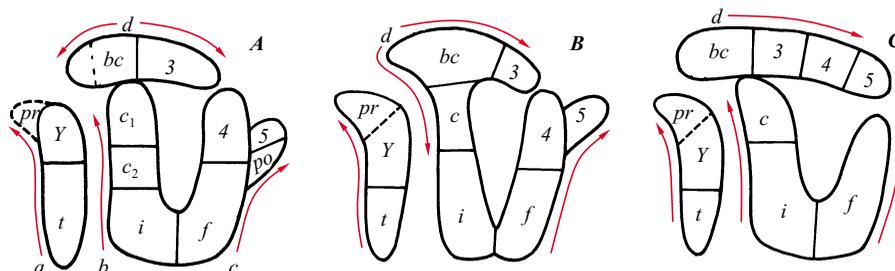


Fig. 7. Three different modes of limb development in the larval salamanders; *A*) *Salamandrella keyserlingii* (Hynobiidae); *B*) *Triturus marmoratus* (Salamandridae); *C*) *Ambystoma mexicanum* (Ambystomatidae). Arrows show the sequence (proximodistal, distoproximal, and anteroposterior axis) of mesenchyme differentiation in the elements in preaxial (*a*), median (*b*), and postaxial (*c*) columns, basale commune and distal carpal formation (*d*). *bc*) Modified after Shubin et al. (1995). Abbreviations as in Fig. 3.

in the early limb development (Fig. 4I); 5) distoproximal differentiation of the carpus/tarsus elements in the region of the median column (Fig. 4J); 6) comparatively late differentiation of the intermedium in comparison with basale commune; 7) late proximal connection of median column with the posterior (postaxial) column thus linking intermedium and ulna/fibula (Fig. 4K).

Thus in these pond salamanders (*Lar*, Fig. 6) the basal commune and metapods one and two are the first elements of the digital arch and their appearance is independent from zeugopod region (Fig. 4H). In *Triturus marmoratus* (Blanco and Alberch, 1992) the intermedium and centrale (Fig. 7B) are formed in distoproximal sequence within a proximal extension of basale commune towards the stylopod-zeugopod bifurcation. The digital arch develops in anterior to posterior direction and includes three anterior fingers.

However in some other larval Caudata such as *Ambystoma mexicanum* (Shubin and Alberch, 1986) and probably *Taricha granulosa* (Shubin et al., 1995) two major axes of development are distinguished (Fig. 7C): 1) proximodistal one includes intermedium and centrale and extends to basale commune and to third mesopodial element and 2) anteroposterior axes including five digital elements. These two axes converge in the region of the intermedium and ulnare/fibulare. In both these patterns the basale commune develops earlier as intermedium.

In contrast in *Salamandrella* the branching of the chondrogenic stylopod into two equal in size zeugopod condensations (Fig. 4A) is very early, when the anterior limb bud has a conical shape. After this at the time that these elements become chondrogenic, two separate mesenchymal masses appear distally

(Fig. 4B). One is preaxial in which the radiale/tibiale, element "Y" and the anterior (*I*) digit appear. The second larger mass differentiates into both 1) the central mesopod column in which the intermedium, distal carpal/tarsal 2 and then two centralia develop plus distally second digit (*II*) and 2) the postaxial mesopod column in which develop the ulnare/fibulare and the remaining distal tarsals/carpals plus distal to them, digits three and four (*III*, *IV*) (Fig. 4D, E).

Thus *Salamandrella* limb developmental pattern has some characters which separate it from all known larval salamanders. Such features include (Fig. 7a): 1) the early intermedium and ulnare/fibulare bifurcation at the distal end of the ulna and as a result the initial proximal linking of the median column condensation with postaxial one; 2) the proximo-to-distal differentiation of the three mesopod columns, together with relatively late (in comparison with intermedium and ulnare/fibulare) development of the distal carpal/tarsal 2 (as a main portion of the basale commune); 3) comparatively late formation of the radiale/tibiale.

The early separation of the preaxial (radial/tibial) mesenchyme column, initial connection of the median and postaxial columns as comparatively late connection of the digital arch with postaxial series through centralia elements and proximodistal differentiation of the mesopod element link *Salamandrella* with many anurans, amniotes and salamanders with direct development such as *Bolitoglossa* (*Bol*, Fig. 6). These characters may be considered as a homoplasy during tetrapod evolution based on their morphogenetic community. This similarity is particularly significant if it is remembered that *Salamandrella* is phylogenetically primitive among Caudata. We can suggest also the hypothesis that the limb develop-

mental patterns of more advanced larval caudates display features which have been secondary modified through caenogenetic changes during their evolution.

It is possible that some stabilization in development and morphological peculiarities of *Salamandrella* (as in some other amphibians) derived from sarcopterygian fish ancestors whose paired fin skeletal morphology demonstrated similarity with them. Those characters are seen in the Devonian *Panderichthys* (Cr, Fig. 6) regarded as a sister group of tetrapods (Vorobyeva and Schultze, 1991). The pectoral fin endoskeleton of this fish had a simple construction including a well developed tetrapod like humerus, long radius stout ulna and closely disposed intermedium and ulnare. Proceeding from this condition it is possible to believe that all more distal elements of mesopodial and metapodial regions in the tetrapod limb are neomorphic. In the developmental bauplane those are: radial and "Y" element in the preaxial column, centralia, distal carpal/tarsals in the postaxial and median columns and digits. All these skeletal elements display diversification in their developmental pattern and digits as is typical for neomorphic structures. In consequence they occur as homoplasy in different phylogenetic lines both in the Palaeozoic amphibians and in the recent urodeles. So, *Salamandrella* is characterized by presence of two or more (as in some variations) centralia. This number (two) is also a feature of most other hynobiids, for example *Hynobius damontanus* (Hasumi and Iwasawa, 1993). More derived caudates have usually a single centrale (e.g., *Taricha*) or the occurrence of two separate centralia is a low frequency variant described as atavistic (Shubin et al., 1995). The presence of two centralia is typical probably for many temnospondyl stegocephalians (such as *Trematops*, *Protogyrinus*, *Eryops*, *Greerpeton*). However the hindlimb of the oldest Devonian tetrapod — *Ichthyostega* had only a single centrale (Coates and Clack, 1990). In this case we prefer to consider the presence of two centralia in *Salamandrella* as a synapomorphy of hynobiids and plesiomorphic developmental feature of tetrapods.

The other developmental feature points to the same conclusion. The basale commune is formed in *Salamandrella* from two mesenchyme condensations. One of them is the distal carpal/tarsal 2 which is disposed at the base of the second digit and then extends anteriorly under the base of metapod 1 fusing with small distal carpal/tarsal 1 condensation, forming basale commune. In more advanced caudates the

basale commune is formed directly (and earlier as in *Salamandrella*) as a single element between and at the base of the first and second digits. The *Salamandrella* pattern is probably closer to the some temnospondyl one which is characterized by presence of two separate distal carpal/tarsal 1 and 2 elements.

Their is special interest from an evolutionary position in the relation between intermedium and ulnare/fibulare. The presence of the intermedium, is considered (Schultze, 1991) as an apomorphy of the uniseriate sarcopterygians including tetrapods. The loss of the independent intermedium would be an apomorphy of the biseriata sarcopterygian groups such as dipnoans (*D*, Fig. 6), porolepiform and actinistian crossopterygians (Chang, 1991). There is also the opinion (Rosen et al., 1981) that the intermedium and ulnare are sarcopterygian apomorphies and that the radiale is an apomorphy of the porolepiform – dipnoan – tetrapod clade.

The independence of intermedium in *Salamandrella*'s limb is rare: in 75% of the anterior limbs this element is fused with ulnare. It may be explained by initial contact of the mesenchyme condensations corresponding to these elements and their almost simultaneous formation. Both these condensations extend from the ulna and their disposition is very similar with zeugopod bifurcation. It is possible to believe that the developmental pattern of the fusion between intermedium and ulnare/fibulare have the same developmental mechanism as between elements of zeugopods (radius/ulna) (Vorobyeva, 1991). The loss of the independent intermedium (as the presence of the biseriata endoskeleton) is more likely a homoplasy as an apomorphy in sarcopterygians (Vorobyeva and Hinchliffe, 1995). The amalgamation of intermedium with ulnare is known as variation in some other caudates (e.g., in *Triturus*) — (Blanco and Alberch, 1992). In case of *Salamandrella* it must be considered a species specificity feature as it represents the normal pattern.

2. Variations

Because of the attention paid to variation in the adult morphology of a number of caudates (Hanken, 1983; Reisnel and Wagner, 1992; Blanco and Alberch, 1992; Shubin et al., 1995) we consider this subject briefly here.

In comparison with other caudates *Salamandrella* show a very high level of variation patterns. Only

34% of its individuals are considered as having a standard morphology (Borkhvardt and Ivashintsova, 1993) (Fig. 5). As a result it is very difficult to speak of a reaction norm for this species. In comparison with *Salamandrella*, in the adult newts of *Taricha*, for example, the standard morphology is found in the hand and feet in more than 70% of individuals (Shubin et al., 1995). Possibly these differences can be explained by a high level of morphogenetic plasticity in *Salamandrella* in general (Vorobyeva, 1995). There are also many important differences in the arrangement of variations and their character. If in *Taricha* the tarsals express a variant pattern twice as frequently as the carpals (18.9 – 9.3%), the situation with *Salamandrella* is opposite. The fusion and appearance of additional elements in *Salamandrella* limbs occur as variant patterns in its hands more than four times as in feet. But if we exclude the cases of fusion between intermedium and ulnare (which are about 75% in forelimb), then the number of variations in the tarsus is very close to that in the carpus. The fusion of intermedium with ulnare in case of *Salamandrella* may be considered as a specific block for this species in analogy with “block system” of limb endoskeleton in relation to the Plethodontidae (Hanken, 1983; Hanken and Dinsmore, 1986). The presence of the usually single element composed of ulnare and intermedium in *Salamandrella* has been observed quite often in many species of *Triturus* (Blanco and Alberch, 1992). Borkhvardt and Ivashintsova (1994) draw attention to the relative rarity of transverse fusions between the preaxial mesopod column elements with those of the median and postaxial columns. This emphasizes the autonomous character of the preaxial column (Schmalhausen, 1915) which is characterized mostly by the fusions along its axis. Some of the variants may be described as “looking backwards” or atavisms, others as “looking forwards” to the more advanced Caudata (Shubin et al., 1995). An example of the first in *Salamandrella* is the additional 3 or 4 centralia variant, while the relatively common intermedium — ulnare fusion in the forelimb, and the occasional fusion of centralia 1 + 2 represent the second. These variants emphasize that “developmental constraints” limit the patterns of variation in Caudata limbs both at the intraspecific and interspecific level. They again emphasize the importance of developmental studies for understanding the transformation of limb structures of both phylo-

genetically close and far forms in the context of their evolution and adaptivity.

CONCLUSIONS

Relative to other Caudata the development and morphology of the *Salamandrella* limb endoskeleton are characterized by some features which can be linked with its primitiveness and with its species specificity and larval (caenogenetic) adaptations. Among them are:

- a) initial connection of the distal mesenchyme condensation with zeugopod and its early splitting into the preaxial and postaxial portions;
- b) comparatively early formation of the postaxial region of the mesopods in comparison with preaxial one;
- c) clear proximo-to-distal sequence in mesenchyme differentiation and chondrification of the mesopodial (carpus/tarsus) elements, including the preaxial, postaxial and median column where there is earlier formation and chondrification of the intermedium in comparison with distal 1 + 2 (basale commune);
- d) dual nature of the basale commune and its initial origin at the base of the second digit as distal 2;
- e) frequent fusion of intermedium with ulnare but comparatively rare fusion with fibulare;
- f) presence of two constant centrale elements;
- g) different pattern of variations in fore- and hindlimbs: the first is characterized by fusion of elements, the second by the formation of additional elements and (as a variation) of the development of one or two additional centrale elements posteriorly;
- h) predominance of the longitudinal (in three columns) fusions of carpal/tarsal elements relative to transversal;
- i) presence of a well developed filament or web between first and second fingers in forelimb beginning from early larval stages.

The presence of these peculiarities support the view on the diversification of limb developmental patterns including pond salamanders and caudates with direct development (Shubin et al., 1995)

Many of the *Salamandrella* features (close contact of the distal mesenchyme with zeugopod, initial joint contact between intermedium, ulna and ulnare and early intermedium condensation, unstable centrale region and presence of two or more centralia) probably are plesiomorphic and have been second-

arily lost by more advanced Caudata. The secondary modifications probably include 1) the gap between stylopod/zeugopod mesenchyme condensation and metapodial 1 + 2, and 2) early formation of the basale commune pattern exemplified by accelerated development at the base of metapod elements 1 + 2 supporting the second digit.

The comparison of *Salamandrella* with other caudates and with fossil sarcopterygians leads us to the following conclusions:

1. The unique (anteroposterior) sequence of the digital development in larval Salamanders, the independence of the digital arch from the ulnare/fibulare during early development (in such larval urodeles as *Ambystoma*, *Triturus*) and the distal-to-proximal sequence of development in the central part of carpal/tarsal region (*Triturus*) may be considered as a secondary condition in amphibian evolution.

2. The different patterns of limb development in larval salamanders and the variations of their larval limb morphology correlate with different caenogenetic adaptations and with phylogeny.

3. The pattern of *Salamandrella* limb development may be regarded as primitive among caudates.

Such characters of *Salamandrella* as an initial mesenchyme connection of the "digital arch" region with zeugopod (radius/ulna) condensation, the early development of the intermedium in the close contact with ulnare/fibulare and a proximal-to-distal differentiation of the central elements may be considered as a plesiomorphic for tetrapods. The constant presence of two centralia, dual origin of the basale commune (from distal I and distal II condensations), normally fusion of the intermedium with ulnare are homoplastic features common with different sarcopterygian groups. This agrees with the idea (Vorobyeva, 1992) of the morphogenetic community and great reserve of its capacity in sarcopterygians beginning from Palaeozoic time.

4. The similarity in adult limb morphology of *Salamandrella*; of some temnospondyls and of Caudata with different developmental patterns supports the opinion (Schmalhausen, 1915; Shubin, 1995) that morphogenetic deviations of Caudata from other tetrapods have an exclusively caenogenetic character and do not suggest polyphyly of tetrapods.

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