DEVELOPMENT OF THE PECTORAL GIRDLE IN LARVAE OF SIBERIAN SALAMANDER *Salamandrella keyserlingii* (AMPHIBIA: HYNOBIIDAE)

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Cartilaginous pectoral girdle of *Salamandrella keyserlingii* arises as a single vertical column, which contains both scapular and coracoid parts. Procoracoid part arises later as outgrowth of the preglenoid portion of the girdle. A three radial form of adult pectoral girdle is a result of irregular growth of the originally simple anlage. It is possible that certain collagen bundles stimulate expansion of the cartilaginous parts. There is only one center of ossification in the girdle.

Key words: Caudata, *Salamandrella*, pectoral girdle, development.

INTRODUCTION

The pectoral girdle in adult urodeles consists of the two contralateral plates, which overlap each other midventrally. The plates are mainly cartilaginous, and they ossify in the glenoid region; only amphiuma and siren have two ossifications (Parker, 1868). Each plate consists of three regions — scapular (dorsal), coracoid (ventral) and procoracoid (anterior to the glenoid cavity) ones. These regions are partly subdivided by deep notches at the front (cranial) border of the plate, but there are no absolute boundaries between them.

The absence of the real boundaries between girdle parts was a source of much controversy in their designation (Gegenbaur, 1865; Parker, 1868; Wiedersheim, 1875; Goette, 1877; Engler, 1929; Howell, 1935; Hoffman, 1936). The homology of procoracoid was probably the most disputable question. It was considered as a part of coracoid (Gegenbaur, 1865), scapula (Goette, 1877; Howell, 1935), of both (Engler, 1929), and as an autonomous element (Detwiler, 1918; Makinouchi, 1932; Chen, 1935). Many authors believed that each part of the pectoral girdle is an autonomous element. The last opinion was supported by discovering three centers of chondrification (Detwiler, 1918; Engler, 1929; Makinouchi, 1932; Chen, 1935) and ossification (Engler, 1929) in the developing girdle.

The primary goal of our investigation was to examine the earliest developmental stages, first of all a mode of origin of the cartilaginous elements. We found only one center of chondrification in each half of the pectoral girdle in *Salamandrella*. Therefore, we switched our attention to the peculiarities of later development of the girdle, which could determine its distinctive three radial form. In addition, we describe some new details in the structure of the larval pectoral girdle.

MATERIAL AND METHODS

Salamandrella keyserlingii belonging to family Hynobiidae was studied. Eggs clutches were collected in vicinity of Ekaterinburg (Russia) in spring, 1999. Larvae were reared in laboratory using standard techniques. Larvae were sacrificed in Bouin's fixative and then were embedded in paraffin. Stages of larvae were determined according to the normal tables of *Salamandrella keyserlingii* (see Sytina et al., 1987). We used specimens from the limb bud stage till the end of the metamorphosis. Totally, 56 specimens were studied histologically. Moreover, pectoral girdle of one (two) adult(s) was (were) studied

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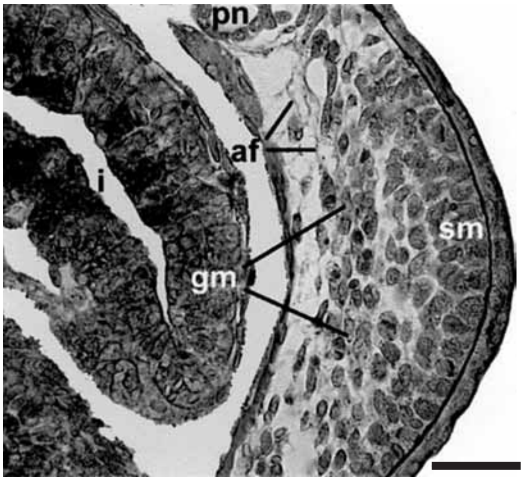

Fig. 1. Larva of *Salamandrella keyserlingii*, stage 32. Fragment of transverse section in the pectoral girdle region. *af*, Arched collagen fibers; *gm*, girdle mesenchyme; *i*, intestine; *pn*, pronephros; *sm*, subectodermal mesenchyme. Scale bar is 60 µm.

histologically (and totally). Serial paraffin histological sections of 10 µm thickness were made in three standard planes and the slides were stained by hematoxylin and eosin. Photographs were made with a "VideoTesT®-2200" digital video system.

RESULTS

A half of the girdle is used for description. The names of muscles, nerves and blood vessels are given on Francis (1934).

Stage 32 (Fig. 1). The front limb bud is disposed a little below the center of the body. One can distinguish two groups of mesenchymal cells in the pectoral region. The external cells form a dense subectodermal layer; cells adjacent to basal membrane stretch perpendicular it. The internal cells form vertical series, they stretch in dorsal-ventral line.

Stage 34 (Fig. 2). Cartilaginous girdle arises in the inner zone of pectoral region. Its anlage looks like a vertical column, which lies at the anterior border of general mesenchymal condensation. Humerus chondrificates at the same time or some later. The dense mesenchyme separates humerus from the girdle. The girdle anlage turns above into mesenchymal condensation, which fills in the narrow space between ectoderm and pronephros. The collagen "arched" fibers encircle the myomeres. Horizontal cranial-caudal fibers pass between the pectoral girdle and myomeres, and above and below the girdle too.

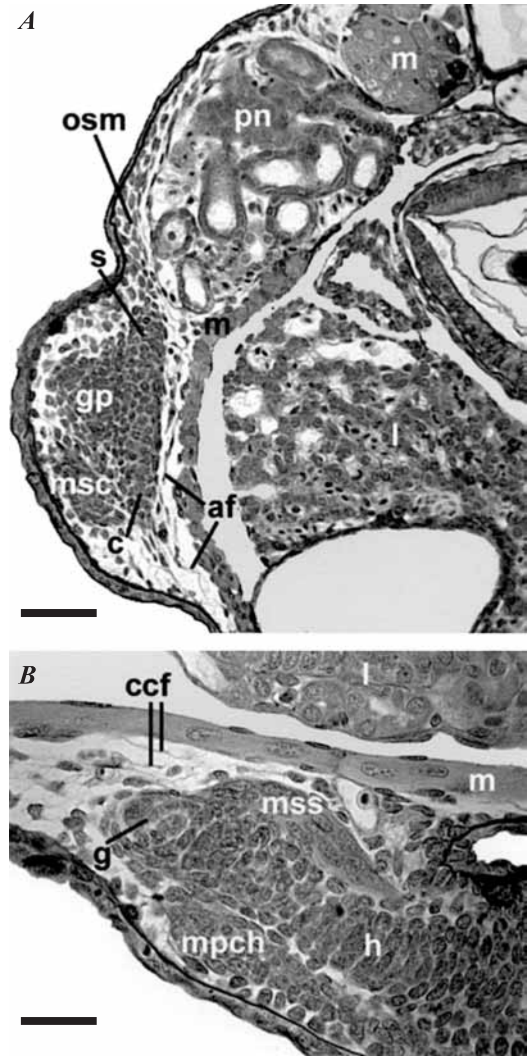

Fig. 2. Larvae of *Salamandrella keyserlingii*, stage 34. Fragments of transverse (*A*) and frontal (*B*) sections in the pectoral girdle region. *af*, Arched collagen fibers; *c*, coracoid part; *ccf*, cranial-caudal collagen fibers; *g*, pectoral girdle; *gp*, glenoid part; *h*, humerus; *l*, liver; *m*, trunk musculature; *mpch*, *m. procoracohumeralis*; *msc*, *m. supracoracoideus*; *mss*, *m. subscapuraris*; *osm*, overscapular mesenchyme; *pn*, pronephros; *s*, scapular part. Scale bars are 90 (*A*) and 60 (*B*) µm.

Stage 35 (Figs. 3, dark, and 4). Glenoid cavity lies noticeably beneath the middle of the body of larva. Scapular part of the cartilaginous girdle plate is oval in transverse section; it extends between ectoderm and pronephros above. Coracoid part lies in

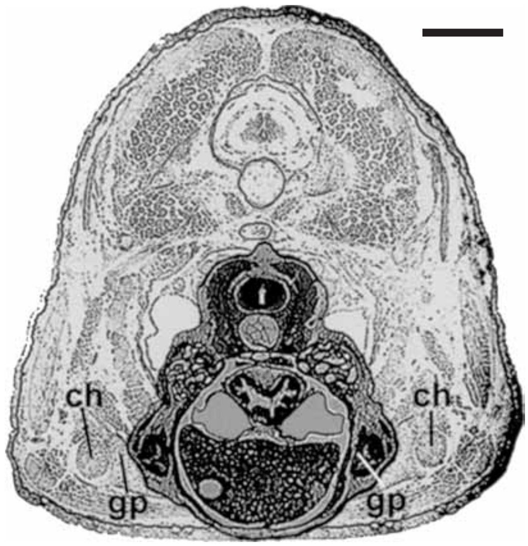

Fig. 3. Larvae of *Salamandrella keyserlingii*, stages 35 (dark) and 45 (light). Transverse sections in the pectoral girdle region. *ch*, Caput humeri; *gp*, glenoid part. Scale bar is 530 μm.

zone of arched and cranial-caudal fibers; it is broadened along the longitudinal body axis. Mesenchymal cells accumulate in front of glenoid region of the girdle. *Musculus procoracohumeralis* lies at the level of humerus, horizontal collagen fibers stretch from the muscle in anterior direction. *M. subscapularis* lies above; it is closely connected with cranial-caudal fibers passing between girdle and myomeres.

Stage 37 (Figs. 5 and 6). Narrow scapular part goes up above pronephros. Coracoid part finishes far from sagittal plane of the body. Procoracoid portion looks like a thin protuberance of the preglenoid part of cartilaginous girdle plate. *M. procoracohumeralis* attaches to its anterior edge. Horizontal collagen fibers stretch from this edge forwards. *M. subscapularis* lies mainly at the scapular level and have no close contact with procoracoid cartilage. The upper branch of arteria supracoracoidea penetrates the basis of procoracoid part. *Nervus supracoracoideus* and lower branch of supracoracoid artery pass through an open notch in the girdle plate.

Stage 39 (Fig. 7). Scapular part reaches the level of the upper border of notochord. In transverse section, it is broadened on top and is oval below. Cartilage of the scapular part hypertrophies at the level of *m. subscapularis*. Both upper and lower branches of supracoracoid artery pass within the closed canals.

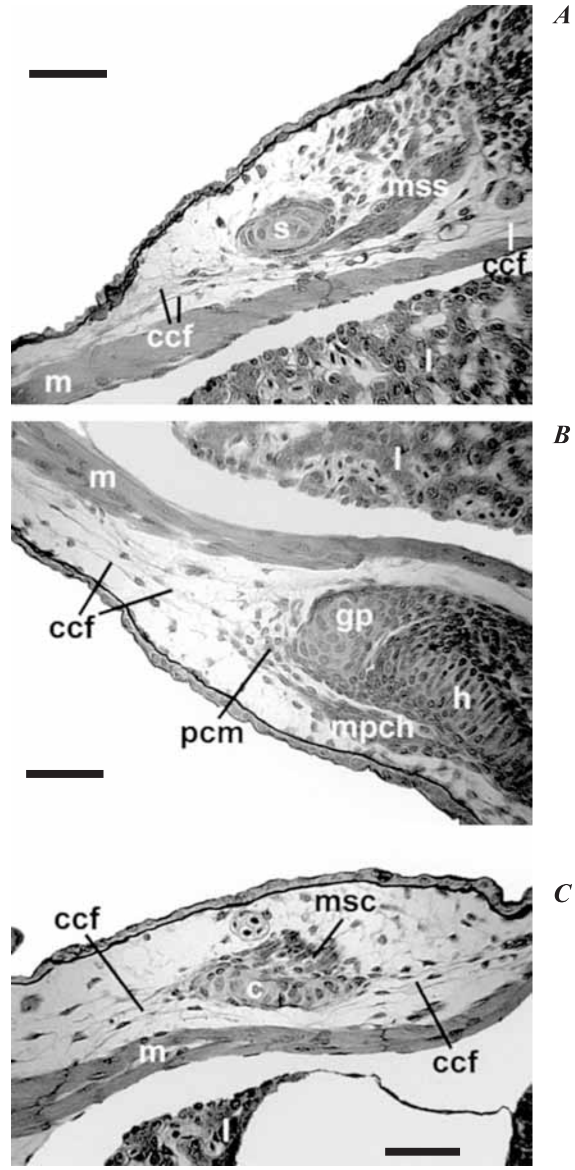

Fig. 4. Larva of *Salamandrella keyserlingii*, stage 35. Fragments of frontal sections at the level of scapular (*A*), glenoid (*B*), and coracoid parts (*C*). *c*, Coracoid part; *ccf*, cranial-caudal collagen fibers; *gp*, glenoid part; *h*, humerus; *l*, liver; *m*, trunk musculature; *mpch*, *m. procoracohumeralis*; *msc*, *m. supracoracoideus*; *mss*, *m. subscapularis*; *pcm*, procoracoid mesenchyme; *s*, scapular part. Scale bar is 90 μm.

The cartilaginous bridge, dividing these canals, is a part of the procoracoid basis. The lower edges of contralateral coracoid plates converge, they lie in the same horizontal plane. Small “muscle” foramina, which are filled in with undifferentiated cells, occur inside the coracoid cartilage. Arched collagen fibers pass medial to the girdle, occupy the *incisura coracoidea*, and join the edges of contralateral coracoid plates. Cranial-caudal fibers are also clearly visible in the inner area. More delicate fibers of the outer zone show different arrangement; some of them — "radial" fibers — regularly pass from ectoderm to the girdle and to the complex of inner fibers.

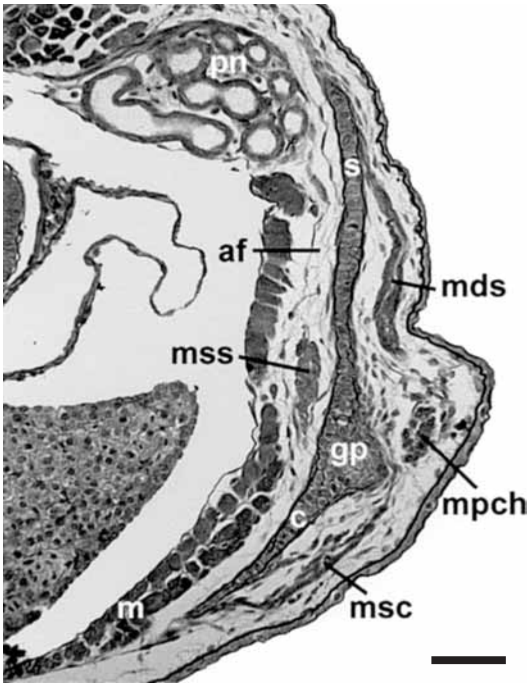

Fig. 5. Larva of *Salamandrella keyserlingii*, stage 37. Fragment of transverse section in the pectoral girdle region. *af*, Arched collagen fibers; *c*, coracoid part; *gp*, glenoid part; *m*, trunk musculature; *mds*, *m. dorsalis scapulae*; *mpch*, *m. procoracohumeralis*; *msc*, *m. supracoracoideus*; *mss*, *m. subscapuraris*; *pn*, pronephros; *s*, scapular part. Scale bar is 150 μm.

Stage 41 (Fig. 8). Scapular part reaches the level of upper border of the spinal cord. Contralateral coracoid parts overlap each other. Perichondral bone covers the hypertrophied cartilage of the lower region of scapular plate. Right and left cartilaginous anlagen of the sternum appear between the ventro-medial portions of myomeres and *mm. pectorales*; these anlagen are in close connection with arched fibers. *M. subscapularis* lies mainly above the procoracoid part, *m. procoracohumeralis* attaches to it widely.

Stage 45 (Fig. 3, light). Formation of the pectoral girdle is finished as a whole. Glenoid cavity takes very low position in the body. Coracoid part lies almost horizontally. Perichondral bone covers the lower portion of the scapular cartilage and comes up to the basis of the procoracoid plate. Cartilage in these parts is destroyed a little. Sternum is unpaired. Some "muscle" foramina within the coracoid plates contain fibers of *m. supracoracoideus* (see Fig. 9).

Stage 47. The cartilage is hardly destroyed under the perichondral bone. There are many "muscle" foramina in the coracoid plate. In zone of attachment of the supracoracoid muscle, most foramina contain its fibers. In the anterior (cranial) zone, where *m. supracoracoideus* is absent, "muscle" foramina contain undifferentiated cells.

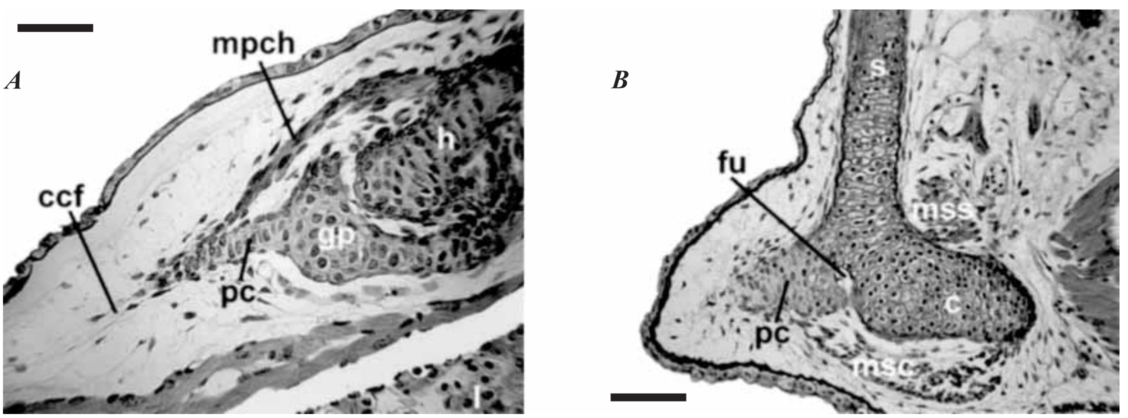

Fig. 6. Larvae of *Salamandrella keyserlingii*, stage 37. Fragments of frontal (*A*) and parasagittal (*B*) sections in the pectoral girdle region. *c*, Coracoid part; *ccf*, cranial-caudal collagen fibers; *fu*, upper supracoracoid foramen; *gp*, glenoid part; *h*, humerus; *l*, liver; *mpch*, *m. procoracohumeralis*; *msc*, *m. supracoracoideus*; *mss*, *m. subscapularis*; pc, procoracoid part; *s*, scapular part. Scale bars are 90 (*A*) and 150 (*B*) μm.

Stage 52, the end of metamorphosis (Fig. 9). Scapular cartilage is fully destroyed at the level of *m. subscapularis*. Perichondral bone reaches the lower supracoracoid foramen.

DISCUSSION

Development of Cartilaginous Girdle

The girdle anlage reveals itself already at mesenchymal stage (Fig. 1). That time, external cells of the pectoral region form dense subectodermal layer, those adjoining basal membrane are elongated perpendicular its surface. Later, subectodermal cells transform to muscles (see Borkhvardt, 1994). Internal cells — the prospective cells of the girdle — are prolonged dorsal-ventral and form the vertical rows.

Cartilaginous anlage of the girdle is a single vertical column equally extending upwards and downwards from the prospective glenoid cavity that is it contains both scapular and coracoid parts (Fig. 2). Above, the anlage turns into mesenchymal condensation, which fills in a narrow space between the integument and pronephros. The scapular cartilage recruits these mesenchymal cells and grows quickly upwards. Mesenchymal cells are scanty under the coracoid part, and cartilage grows downwards not so fast (Fig. 5). Procoracoid part also has no autonomous anlage. It forms as outgrowth of the preglenoid part of the girdle in that site, where mesenchymal cells accumulate in front of the cartilaginous plate (Figs. 4*b* and 6). If one takes conventionally the glenoid cavity as a boundary between scapular and coracoid parts, one must consider the procoracoid process

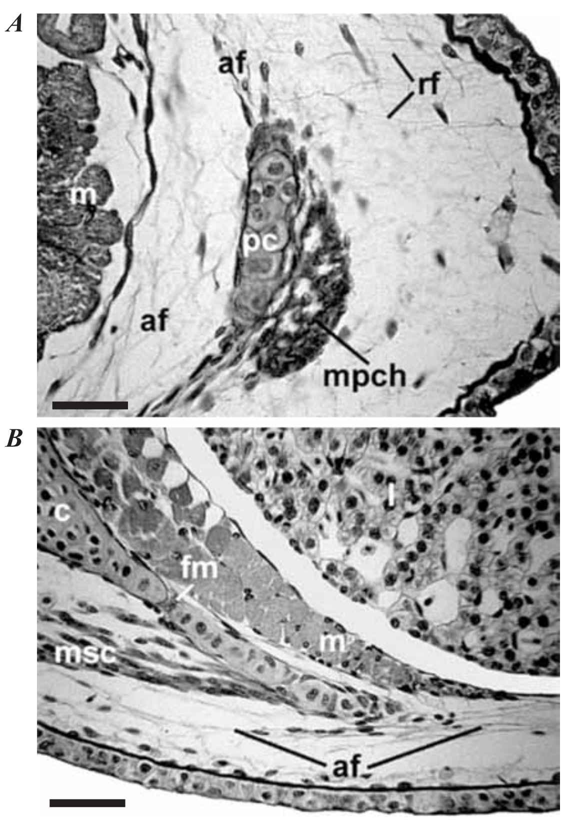

Fig. 7. Larvae of *Salamandrella keyserlingii*, stage 39. Fragments of transverse sections in the procoracoid (*A*) and coracoid (*B*) regions. *af*, Arched collagen fibers; *c*, coracoid part; *fm*, "muscle" foramen; *l*, liver; *m*, trunk musculature; *mpch*, *m. procoracohumeralis*; *msc*, *m. supracoracoideus*; *pc*, procoracoid part; *rf*, radial fibers; Scale bars are 60 (*A*) and 90 (*B*) μm.

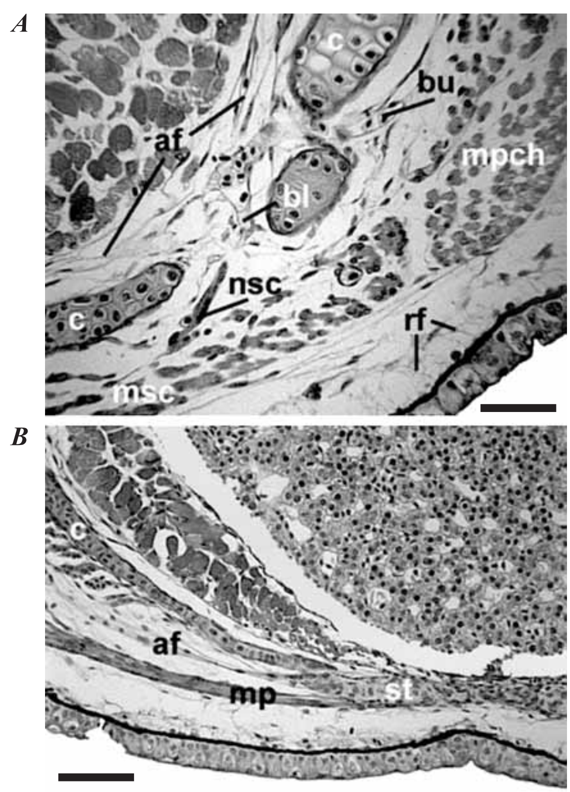

Fig. 8. Larva of *Salamandrella keyserlingii*, stage 41. Fragments of transverse sections in the region of supracoracoid foramina (*A*) and sternum (*B*). *af*, Arched collagen fibers; *bl* and *bu*, lower and upper branches of *a. supracoracoidea*; *c*, coracoid part; *mp*, *m. pectoralis*; *mpch*, *m. procoracohumeralis*; *msc*, *m. supracoracoideus*; *nsc*, *n. supracoracoideus*; *rf*, radial fibers; *st*, sternum. Scale bar is 90 μm.

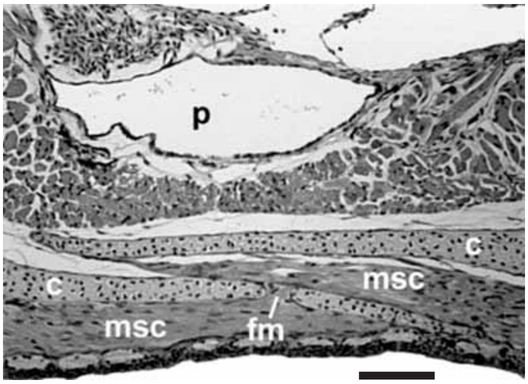

Fig. 9. Larva of *Salamandrella keyserlingii*, stage 52. Fragment of transverse section in the pectoral girdle region. *c*, Coracoid part; *fm*, "muscle" foramen; *msc*, *m. supracoracoideus*; *p*, pericardial cavity. Scale bar is 150 μm.

a derivative of both. Therefore, the strict name for it could be "preglenoid process."

Thus, the cartilaginous pectoral girdle arises as a single columnar piece. The presence of its three prominent parts (scapular, coracoid and preglenoid) is a result of an uneven growth of the originally simple anlage. Why is the growth irregular? Why are there deep notches above and below the preglenoid process?

At its early developmental stages, cartilage growths chiefly by means of recruiting adjacent mesenchymal cells. Hence, an uneven distribution of these cells around girdle anlage could be a cause of its irregular growth (see Borkhvardt, 1991). Really, an intensive outgrowth of scapular (upwards), preglenoid (forwards) and coracoid (in cranial and caudal directions) parts could be stimulated by accumulation of mesenchymal cells near their borders (Figs. 2*a*, 4*b*, 4*c*, and 6*a*). At the same time, there is a close connection between outgrowing skeletal parts and the certain collagen fibers.

Engler (1929) and Stäuble (1942) described near the larval pectoral girdle of different urodeles some complexes of collagen fibers and pointed at the close connection of these "membranes" with the cartilaginous plates. We distinguish two zones in the pectoral region of *Salamandrella* larvae, inner and outer ones, which differ in the mode of disposition of collagen fibers. Inner zone is occupied by cranial-caudal (Figs. 2*b*, 4, and 6*a*) and arched (Figs. 2*a*, 5, 7, and 8) fibers. In outer zone, the radial fibers stand out, which regularly link ectoderm with the girdle and the inner collagen complex (Figs. 7*a* and 8*a*).

A close spatial connection exists between the arched (Fig. 7*b*) and cranial-caudal (Fig. 4*c*) fibers of inner zone and the coracoid plate, which grows along the collagen bundles. The procoracoid part (preglenoid process) also growths along the cranial-caudal fibers stretching from *m. procoracohumeralis* forwards (Figs. 4*b* and 6*a*). Sternum forms in connection with arched fibers (Fig. 8*b*). On the contrary, the lower portion of scapular cartilage lies entirely outside the fiber bundles (Fig. 4*a*). Just this portion expands the least, and a deep notch forms between scapular and procoracoid parts. Cranial-caudal fibers are also few inside *incisura coracoidea*, another deep notch of the pectoral girdle. It is possible that both arched and cranial-caudal collagen fibers are not only topographically connected with the cartilage, but they influence upon its growth and thus determine the structure of definitive girdle.

Ossification of the Girdle

Engler (1929) observed three centers of ossification in the pectoral girdle of *Salamandra atra*. In the newt (Erdmann, 1933) and the axolotl (Keller, 1946), bone arised in only one place, around the lower portion of the scapular plate, and expanded from there downwards. The same is in *Salamandrella*.

Most of authors use the name scapula only for ossified portion of the girdle. Then, the upper cartilaginous part of the girdle is designated as suprascapula. Goette (1877) used the name coracoid for subglenoid ossified portion of the girdle, and the cartilaginous subglenoid part he named epicoracoid. More often (Parker, 1868; Detwiler, 1918; Howell, 1935), a term epicoracoid is attributed to the very ventral part of the cartilaginous plate. In the last case, it is quite uncertain where a boundary-line to draw (see Borkhvardt, 1993). We prefer to regard the pectoral girdle of *Salamandrella* as a single three radial cartilaginous plate with one, paraglenoid, ossification. This ossification can be passed off a special name.

Position of Glenoid Cavity

During larval development, glenoid cavity changes significantly its position relatively the upper border of the larva body, but doesn't change it relatively the lower one (Fig. 3). Glenoid cavity is hardly able to move along the vertical line because humerus, which "penetrates" the body wall, stands in the way of this movement. It seems more probable that the glenoid cavity and the lower body border almost do not change their position to outside (abstract) observer. So, the change in position of glenoid cavity shows that the growth of larval body along the vertical line is not uniform, that is the body growths upwards much more intensive than downwards.

Supracoracoid Foramina

In most urodeles, supracoracoid artery and nerve penetrate the girdle through a single *foramen supracoracoideum* (Parker, 1868; Osawa, 1902; Okajima, 1908; Engler, 1929; Chen, 1935; Francis, 1934; Howell, 1935; Hoffman, 1936; Keller, 1946). Stäuble (1942) discovered two foramina in larvae of *Hynobius peropus* and *H. naevius* and named them *ff. diazonale and paradiazonale*; *n. supracoracoideus* penetrated *f. diazonale*. In adults, the author saw only one foramen. In larvae of *Salamandrella*, supracoracoid artery divides usually into two branches, the upper and lower ones, which penetrate accordingly the upper and lower foramina; supracoracoid nerve penetrates the lower one (Fig. 8*a*). This character varies something — we saw two foramina only on one side in some specimens. It was not simple to see the upper foramen in total skeletons of adult Siberian salamanders, because it passed slantwise through the plate. But the both foramina, which pierced an ossified part of the girdle, were clearly visible at the histological sections.

"Muscle" Foramina

Up to ten small foramina pierced the ventral portion of coracoid cartilage in all larvae starting from stage 39. At stages 39 – 43, these foramina were filled in with undifferentiated cells (Fig. 7*b*). Later, fibers of *m. supracoracoideus* appeared within foramina (Fig. 9), that is why we conventionally named them the "muscle" ones. Foramina outside zone of *m. supracoracoideus* never contained muscle fibers.

We suppose that undifferentiated cells of "muscle" foramina are in fact the cells, which are pre-determined for muscle differentiation already at the early stages. Therefore, the growing coracoid cartilage doesn't incorporate these cells but flows them round. As a result, the cartilaginous plate becomes perforated. Later, the cells of foramina differentiate into the muscle ones and form part of *m. supracoracoideus*. Interestingly, the "muscle" foramina never occured within scapular and procoracoid parts of the girdle.

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