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DEVELOPMENT OF PTERYGOQUADRATE COMPLEX IN THE EMBRYOGENESIS OF SAND LIZARD, *LACERTA AGILIS* (REPTILIA, SQUAMATA)

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Development of Pterygoquadrate Complex in the Embryogenesis of the Sand Lizard, *Lacerta agilis* (Reptilia, Squamata). Yarygin A. N. — Serial sections of 15 *L. agilis* embryos on the successive developmental stages, stained with hematoxylin-eosin or alcian blue and hematoxylin-eosin, were examined. It was found that cartilaginous pterygoquadrate complex of *L. agilis* consists of four parts: quadrate cartilage, pterygoquadrate cartilage, pterygoid process, and processus ascendens. Pterygoquadrate cartilage and pterygoid process are reduced in prenatal ontogenesis, but not earlier than underlying pterygoid bone is developed. The processus ascendens becomes the first cartilaginous parts of this complex, and then the other elements undergo chondrification. Basipterygoid process is formed by two independent parts: aboral part of cranial trabeculas and basipterygoid cartilage. Meniscus pterygoideus is an independent structure, but not a rudiment of the pterygoid process. A previously unrecorded cartilage was revealed and a name basipterygoid cartilage is proposed for it.

Key words: cranial development, reptiles, pterygoquadrate complex, pterygoid bone, pterygoquadrate cartilage, processus ascendens, pterygoid process, quadrate.

Развитие крыловидно-квадратного комплекса в эмбриогенезе прыткой ящерицы, *Lacerta agilis* (Reptilia, Squamata). Ярыгин А. Н. — Общепринятыми гистологическими методами исследовано 15 эмбрионов последовательных стадий развития прыткой ящерицы. Установлено, что хрящевой крыловидно-квадратный комплекс прыткой ящерицы состоит из четырех частей: квадратного хряща, крыловидно-квадратного хряща и двух его отростков крыловидного и восходящего. В процессе развития крыловидно-квадратный хрящ и крыловидный отросток редуцируются, но не ранее чем разовьется подлежащая под ними крыловидная кость. Первым хрящевым элементом данного комплекса является восходящий отросток, после чего охрящевевают остальные элементы. Базиптеригоидный отросток формируется из двух независимых компонентов: аборальной части черепных трабекул и базиптеригоидного хряща. Meniscus pterygoideus — самостоятельная структура, которая не является рудиментом крыловидного отростка. Выявлена неописанная ранее структура, которую мы предлагаем назвать базиптеригоидным хрящом.

Ключевые слова: развитие черепа, рептилии, крыловидно-квадратный комплекс, крыловидная кость, крыловидно-квадратный хрящ, восходящий отросток, крыловидный отросток, квадрат.

The pterygoquadrate complex is defined in the cartilaginous skull of reptiles. It consists of quadrate cartilage, pterygoid process, processus ascendens and pterygoquadrate cartilage, which connects these processes with a quadrate cartilage (Bellairs, Kamal, 1981).

Little data are available on formation of pterygoquadrate complex in Lacertidae representatives. Moreover, the data are rather discrete: either cartilaginous stage (De Beer, 1930; Kamal, Abden, 1972) or bone stage (Rieppel, 1992, 1994) were described. Information about cartilaginous stage is also controversial. Particularly, there are contradictions in sequence of anlage appearance, and number of elements in pterygoquadrate complex. Also, there are no published works on development of cartilaginous and bone skull as coordinated system. Therefore, the main aim of our work was to analyze in detail the formation of pterygoquadrate complex in prenatal ontogenesis of sand lizard.

Material and methods

To obtain embryonic material, the pregnant females of *Lacerta agilis* were caught in their natural habitats in Zaporozhzhian region (Melitopol township, near Bohatyr village). After eggs were laid, lizards

were set free into their natural habitats. Eggs were incubated on moist substrate at 19–21°C. Then eggs were incubated for approximately 70 days.

In total, 15 *L. agilis* embryos on the successive developmental stages were studied. Special attention was paid to developmental stages with mainly qualitative changes (stages 32–36). The stages were determined according to the tables of normal development for *Zootoca vivipara* by J. Dufaure and J. Hubert (1961).

Embryos were fixed in 3% formaldehyde solution and serial sections (5–7 μm and 10–12 μm) were prepared. Sections were stained with hematoxylin-eosin or alcian blue — hematoxylin-eosin.

In this work, we use the terminology of G.R. De Beer (De Beer, 1930) and A.M. Bellairs and A.M. Kamal (Bellairs, Kamal, 1981).

Images of microsections were made using microscope Zeiss Axio Imager M1 and Zeiss Axio Vision v.4.63 software in the center of unique equipment at Schmalhausen Institute of zoology, NAS of Ukraine.

Results

At the beginning of stage 32, it was possible to reveal dense mesenchymal accumulation in head. The parts of future cartilaginous pterygoquadrate complex were clearly distinguished in general loose mass of mesenchymal cells.

Stage 32+

Anterolaterally from a cochlear part of the future auditory capsule, there is a dense accumulation of mesenchymal cells — that is the anlage of quadrate cartilage. *Vena capitis* is located dorso-medially from auditory process. Oral cavity is observed medially from anlage of quadrate cartilage. Future masticatory musculature is observed as the aggregation of myoblasts, located dorsally to the anlage and dorsolaterally to its anterior part. Ventral surface of anterior part of quadrate cartilage anlage borders with dorsal surface of Meckel's cartilage posterior part.

The anlage of pterygoquadrate cartilage goes aside dorsomedially from anterior part of dorso-medial margin of quadrate cartilage anlage, looking as a dense band of mesenchymal cells. In front of trifacial neuroganglion (pair V) pterygoquadrate cartilage gives rise to 2 processes — ascending and pterygoid.

Pterygoid process, that is mesenchymal one, goes anteriorly and rather laterally, and reaches the level of *crista sellaris*. Aforementioned *vena capitis* is located dorsally to the process. Oral cavity is located medially and ventrally to the process. Internal carotid artery and trabeculae are observed ventromedially to the pterygoid process.

Processus ascendens undergoes chondrification. It goes backwards dorsolaterally and rounds the mandibular branch of trifacial nerve. *Vena capitis* is located dorsomedially to the process (fig. 1, coloured inset I, p. 191).

To the end of stage 32, all parts of the complex are chondrified. The auditory process of quadrate cartilage grows backwards to the auditory capsule. The pterygoid process grows onward and almost reaches chiasm. Its anterior part bends in lateral direction, approximately at the level of the front edge of hypophysial fenestra.

Stage 33

Along the lateral surface of the whole body of pterygoid process and the anterior part of pterygoquadrate cartilage, a new structure appears, that is a dense strand of poorly differentiated cells. The lateral surface of its posterior part practically adjoins to the medial surface of posterior part of pterygoid process. And its anterior part lies rather distantly from the anterior part of pterygoid process. Apex of the strand goes behind the anterior end of pterygoid process and reaches the level of chiasm. Strand cells density decreases from its posterior to anterior end (fig. 2).

Stage 33+

Described in the previous stage, cells of the strand form an osteoid, which is rather dorso-ventrally flattened. Its thickness is approximately equal to that of pterygoid process. Thus, formation of the pterygoid bone begins (fig. 3).

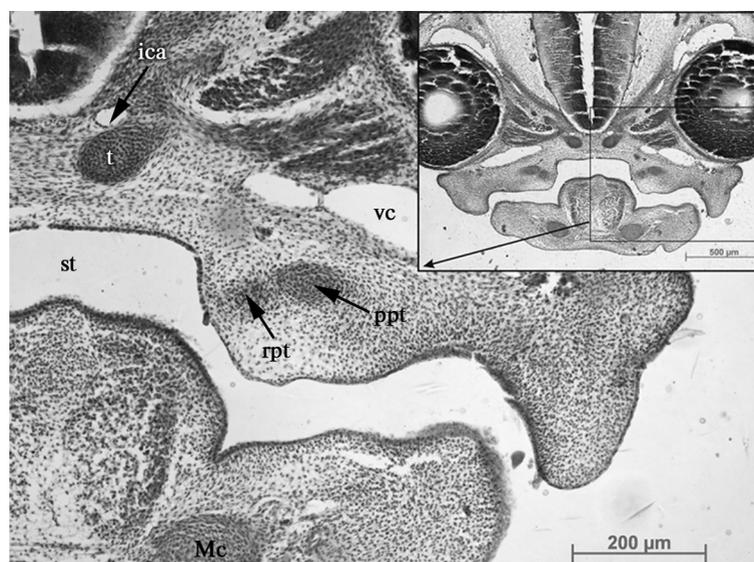


Fig. 2. Cross-section of *Lacerta agilis* embryos head Stage 33. ica — internal carotid artery, Mc — Meckel's cartilage, ppt — processus pterygoideum, rpt — rudiment of pterygoid bone, st — stomodeum, t — trabecula, vc — vena capitis

Рис. 2. Поперечный срез головы *Lacerta agilis*. Стадия 33. ica — внутренняя сонная артерия, Mc — меккелев хрящ, ppt — крыловидный отросток, rpt — закладка крыловидной кости, st — stomodeum, t — черепная трабекула, vc — vena capitis.

Stage 33++

At this stage, mineralization of the osteoid process begins. Osteoid grows onward, its posterior part lies closely to the pterygoid process. Middle and anterior parts are still rather apart from the latter. Due to the lateral bend of pterygoid process, distance between it and a bone increases onward. Pterygoid bone is approximately 1.5 times longer than pterygoid process. Width of the back third of developing bone is equal to

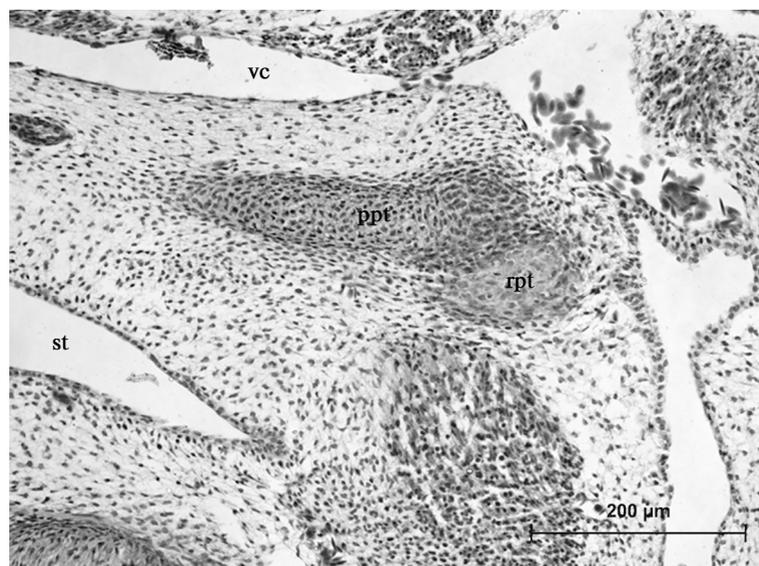


Fig. 3. Parasagittal section of *Lacerta agilis* embryos head. Stage 33+. ppt — processus pterygoideum, rpt — rudiment of pterygoid bone, st — stomodeum, vc — vena capitis.

Рис. 3. Парасагитальный срез головы *Lacerta agilis*. Стадия 33+. ppt — крыловидный отросток, rpt — закладка крыловидной кости, st — stomodeum, vc — vena capitis.

the width of pterygoid process, front two thirds of the bone are approximately 2 times wider than the process (fig. 4).

Stage 34

Pterygoid bone continues its growth. It grows backwards along pterygoquadrate cartilage, its posterior end does not reach the quadrate cartilage a little. Anterior end goes over the level of chiasm (fig. 5). Ventromedial surfaces of pterygoid process and pterygoquadrate cartilage lie in a groove formed by underlying pterygoid bone.

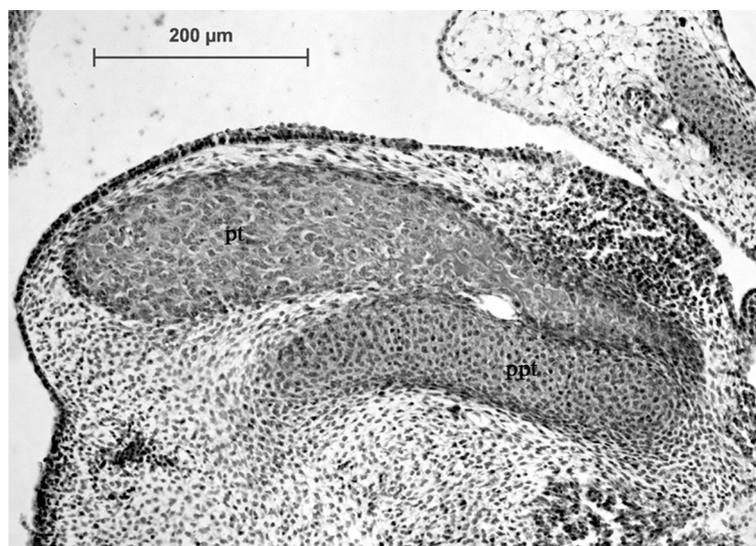


Fig. 4. Frontal section of *Lacerta agilis* embryos head. Stage 33++. ppl — processus pterygoideum, pt — pterygoid bone.

Рис. 4. Фронтальный срез головы *Lacerta agilis*. Стадия 33++. ppl — крыловидный отросток, pt — крыловидная кость.

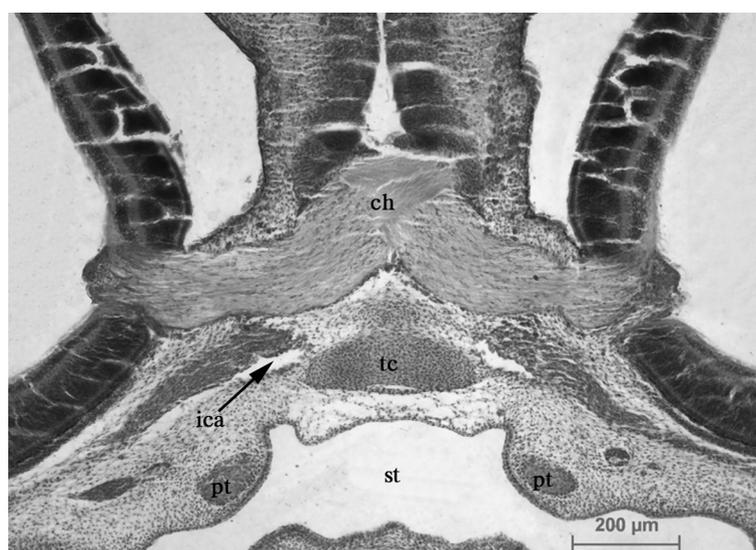


Fig. 5. Cross-section of *Lacerta agilis* embryos head. Stage 34. ica — internal carotid artery, ch — optic chiasm, pt — pterygoid bone, st — stomodeum, tc — trabecula communis.

Рис. 5. Поперечный срез головы *Lacerta agilis*. Стадия 34. ica — внутренняя сонная артерия, ch — хиазма зрительных нервов, pt — крыловидная кость, st — stomodeum, tc — трабекулярная пластинка.

Processus ascendens and pterygoid processes have the same base, which also lies in this bone groove.

Basitrabecular (basipterigoid) process goes ventrolaterally and onward from posterior part of trabeculas. Its end is directed to the base of processus ascendens. Cartilaginous meniscus pterigoideus is revealed near the dorso-medial surface of the base of processus ascendens. The dense mesenchymal “bridge” is observed between

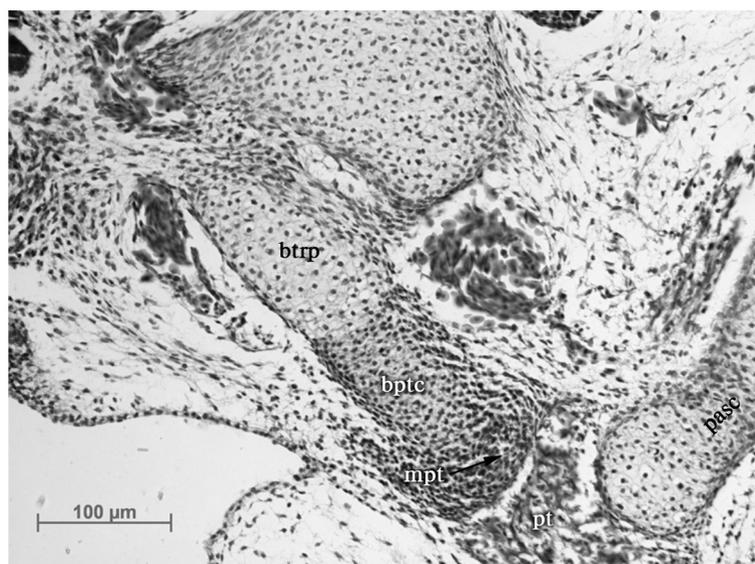


Fig. 7. Cross-section of *Lacerta agilis* embryos head. Stage 34+. bptc — basipterigoid cartilage, btrp — basitrabecular process, mpt — pterigoid meniscus, pasc — processus ascendens, pt — pterygoid bone.

Рис. 7. Поперечный срез головы *Lacerta agilis*. Стадия 34+. bptc — базиптеригойдный хрящ, btrp — базитрабекулярный отросток, mpt — птеригойдный мениск, pasc — восходящий отросток, pt — крыловидная.

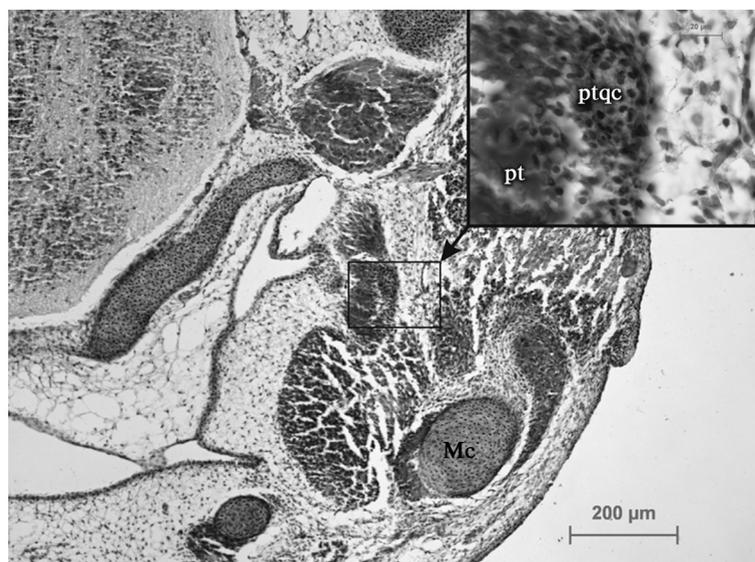


Fig. 8. Cross-section of *Lacerta agilis* embryos head. Stage 34 ++. Mc — Meckel's cartilage pt — pterygoid bone, ptqc — pterigoquadrate cartilage.

Рис. 8. Поперечный срез головы *Lacerta agilis*. Стадия 34 ++. Mc — меккелев хрящ, pt — крыловидная кость, ptqc — крыловидно-квадратный хрящ.

basitrabecular process and pterigoid meniscus. In this mesenchymal mass, it is possible to distinguish separate locus of chondrification, designated as “basipterigoid cartilage” (fig. 6).

Stage of 34+ (coloured inset, p. 191)

The main changes occur only in junction between an axial part of the skull and the pterigoquadrate complex. Mesenchymal “bridge”, described for the previous stage, is completely cartilaginous and fused with basitrabecular process. However, the border of this fusion is still well distinguished (fig. 7).

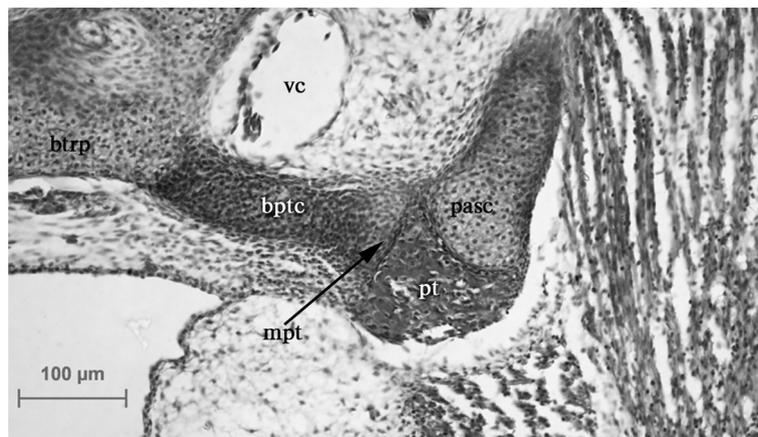


Fig. 9. Cross-section of *Lacerta agilis* embryos head. Stage 34 ++. bptc — basipterigoid cartilage, btrp — basitrabecular process, mpt — pterigoid meniscus, pasc — processus ascendens, pt — pterygoid bone, vc — vena capitis.

Рис. 9. Поперечный срез головы *Lacerta agilis*. Стадия 34 ++. бptc — базиптеригоидный хрящ, бtrp — базитрабекулярный отросток, mpt — птеригоидный мениск, pasc — восходящий отросток, pt — крыловидная кость, vc — vena capitis.

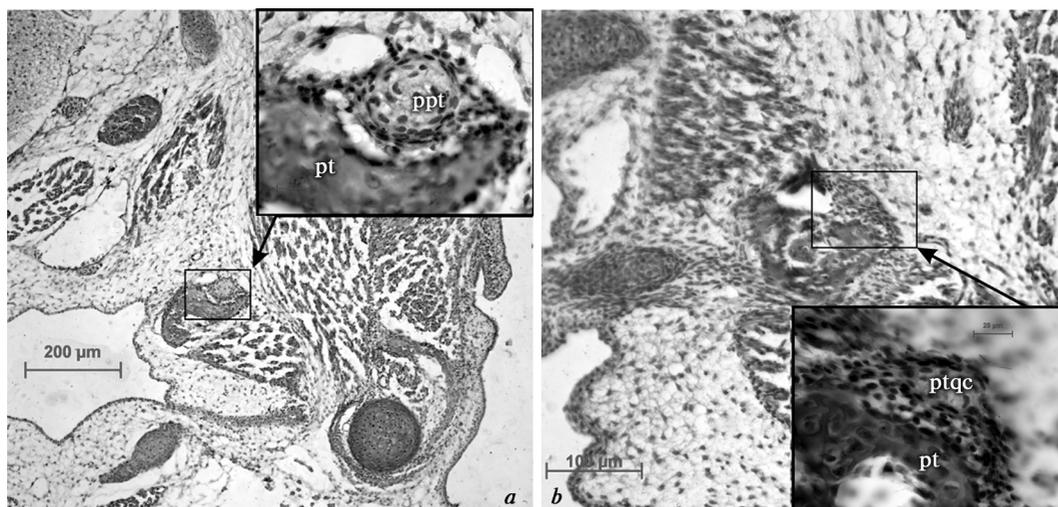


Fig. 10. Cross-sections of *Lacerta agilis* embryos head arranged in an anterior to posterior sequence (a, b). Stage 35. ppt — processus pterygoideum, pt — pterygoid bone, ptqc — pterygoquadrate cartilage.

Рис. 10. Поперечные срезы головы *Lacerta agilis* (a, b). Стадия 35. ppt — крыловидный отросток, pt — крыловидная кость, ptqc — крыловидно-квадратный хрящ.

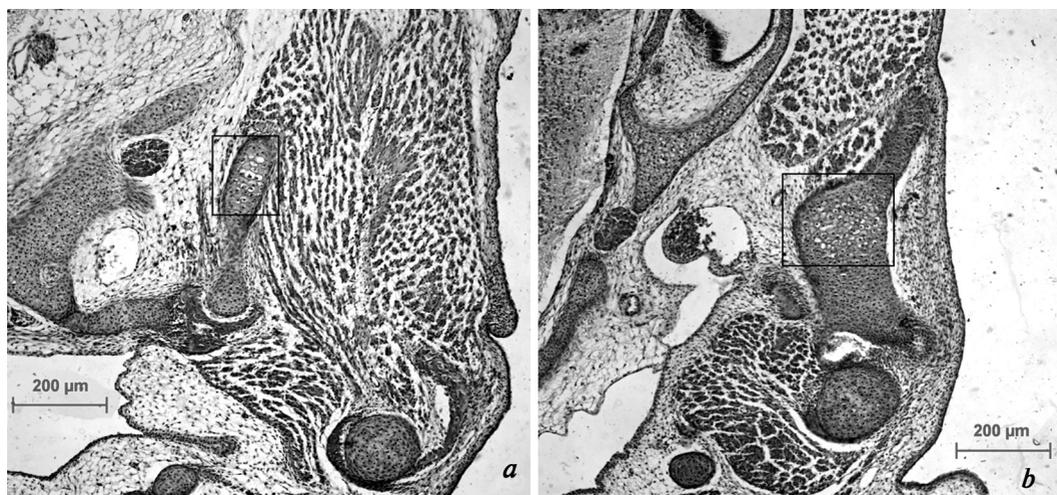


Fig. 11. Areas of hypertrophied cartilage (framed) in processus ascendens (a) and quadrate cartilage (b). Cross-sections of *Lacerta agilis* embryos head. Stage 35.

Рис. 11. Зона гипертрофированного хряща (выделено прямоугольником) в восходящем отростке (a) и квадратном хряще (b). Поперечные срезы головы *Lacerta agilis*. Стадия 35.

Stage 34 ++

The posterior end of pterygoid bone reaches the quadrate cartilage. Pterygoquadrate cartilage is thinned, its posterior end loses contact with quadrate cartilage (fig. 8). It is clearly visible that basitrabecular process consists of two cartilages (fig. 9).

Stage 35

Pterygoquadrate cartilage and pterygoid process are extremely thinned (fig. 10). The cells of processus ascendens and quadrate cartilage are hypertrophied (fig. 11).

Stage 36+

To investigate onset of ossification on a whole structure, the whole mounts of the embryos were studied at this stage.

Bone cuffs are clearly visible in the middle part of processus ascendens and the middle part of quadrate cartilage. They topographically correspond to hypertrophied cartilage areas observed at the previous stage (fig. 12, coloured inset II, p. 192).

Discussion

According to G. De Beer's data, quadrate cartilage is the first cartilaginous part of pterygoquadrate complex in cranial embryogenesis of *L. agilis*. Then processus ascendens develops, being not connected with any other cartilaginous structure. At the final stage of chondrogenesis, G. De Beer found an independent small cartilage — *meniscus pterygoideus* between basitrabecular process and basis of processus ascendens. The author did not find pterygoid process and pterygoquadrate cartilage at any stages examined. Ernst Gaup noted the presence of "dense tissue" between quadrate cartilage and processus ascendens, which later undergoes chondrification (pterygoquadrate cartilage) (Gaup, 1891, cited by De Beer, 1930). From these facts, G. De Beer (1930) suggested, that quadrate cartilage was not obviously represented as separate structure in embryogenesis of *L. agilis*.

According to A. M. Kamal and A. M. Abden's data on another representative of Lacertidae family (*Acanthodactylus boskiana*), all four parts of pterygoquadrate complex are formed simultaneously as a single cartilaginous structure. However, in evolution,

pterygoquadrate cartilage and pterygoid processes are exposed to “ontogenetic regression” (Kamal, Abden, 1972: 313). When formation of chondrocranium is completed, pterygoquadrate cartilage disappears, and pterygoquadrate complex consists of three separate elements: quadrate cartilage, processus ascendens and preserved back part of pterygoid process — *meniscus pterygoideus* (Kamal, Abden, 1972).

Our research showed that the pterygoquadrate complex of sand lizard was formed within a single mesenchymal mass. However, the first cartilaginous part of the complex is processus ascendens, which undergoes chondrification in the beginning of the 32nd stage. We did not succeed to find individual foci of chondrification in other parts of the complex. To the end of this stage, all parts of pterygoquadrate complex were fused into a single cartilaginous structure.

At the beginning of stage 34, we found *meniscus pterygoideus* in the base of processus ascendens, what corresponds to G. R. De Beer's data. Here we also revealed an individual cartilage (designated as basipterigoid cartilage) located between meniscus pterygoideus and basipterigoid process, which fused with basipterigoid process later in evolution. This cartilage was never been described earlier. Detailed study of serial sections provided the evidence that meniscus pterygoideus is an individual structure rather than a preserved part of pterygoid process, as it was considered earlier (Kamal, Abden, 1972).

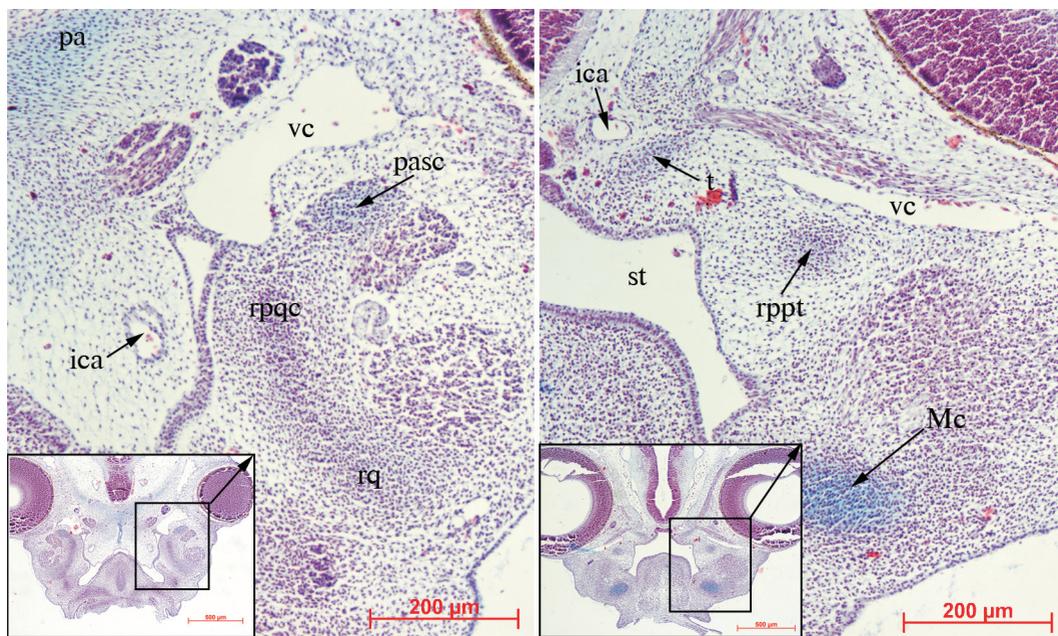
The pterygoid process and pterygoquadrate cartilage are really reduced what confirmed A. M. Kamal and A. M. Abden's data (1972). However, “ontogenetic regression” of cartilages began not earlier than the posterior end of the underlying pterygoid bone reached a quadrate cartilage, and the first lost connection was between quadrate and pterygoquadrate cartilages.

Our results about sequence of pterygoquadrate complex ossification events coincide with O. Rieppel's data (Rieppel, 1992, 1993).

Conclusion

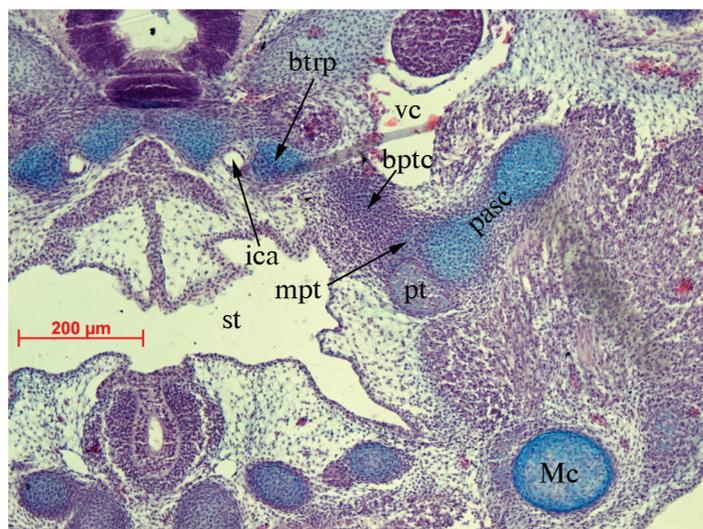
It was defined that cartilaginous pterygoquadrate complex of *L. agilis* consists of four parts: quadrate cartilage, pterygoquadrate cartilage, pterygoid process and processus ascendens. Pterygoquadrate cartilage and pterygoid process are reduced in prenatal ontogenesis, but not earlier than underlying pterygoid bone develops. The processus ascendens becomes the first cartilaginous part of this complex, and then other parts undergo chondrification. Basipterigoid process is formed by two independent components: aboral part of cranial trabeculas and basipterigoid cartilage. *Meniscus pterygoideus* is an independent structure, and it is not the rudiment of pterygoid process.

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Inset to the paper by A. N. Yarygin (p. 108). Fig. 1. Cross-sections of *Lacerta agilis* embryos head arranged in an anterior to posterior sequence (a, b). Stage 32+. ica — internal carotid artery, Mc — Meckel's cartilage, pa — pila antotica, pasc — processus ascendens, rppt — rudiment of processus pterygoideum, rpgc — mesenchymal rudiment of pterygoquadrate cartilage, rq — mesenchymal rudiment of quadrate cartilage, st — stomodeum, t — trabecula, vc — vena capitis.

К статье А. Н. Ярыгина (с. 108). Рис. 1. Поперечные срезы головы *Lacerta agilis* (a, b). Стадия 32+. ica — внутренняя сонная артерия, Mc — меккелев хрящ, pa — pila antotica, pasc — восходящий отросток, rppt — мезенхимная закладка крыловидного отростка, rpgc — мезенхимная закладка крыловидно-квадратного хряща, rq — мезенхимная закладка квадратного хряща, st — stomodeum, t — черепная трабекула, vc — vena capitis.



Inset to the paper by A. N. Yarygin (p. 112). Fig. 6. Cross-section of *Lacerta agilis* embryos head. Stage 34. bptc — basipterygoid cartilage, btrp — basitrabecular process, ica — internal carotid artery, Mc — Meckel's cartilage, mpt — pterigid meniscus, pasc — processus ascendens, pt — pterygoid bone, st — stomodeum, t — trabecula, vc — vena capitis.

К статье А. Н. Ярыгина (с. 112). Рис. 6. Поперечный срез головы *Lacerta agilis*. Стадия 34. bptc — базиптеригидный хрящ, btrp — базитрабекулярный отросток, ica — внутренняя сонная артерия, Mc — меккелев хрящ, mpt — птеригидный мениск, pasc — восходящий отросток, pt — крыловидная кость, st — stomodeum, t — черепная трабекула, vc — vena capitis.

