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SACRUM AND PELVIC GIRDLE DEVELOPMENT IN LACERTIDAE

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Sacrum and pelvis structure and development in four species of Lacertidae currently assigned to different genera are studied: *Lacerta agilis*, *L. vivipara*, *L. saxicola*, *Podarcis muralis*. Size, shape, and position relationships between the cartilaginous elements as well as patterns and sequence of chondrification and ossification are given. Two sacral segments are characterized by a number of markers such as the branching of iliac arteries, development of the paired lymphatic hearts, and degeneration of sacral myomeres beginning from the dividing sacral myoseptum. Lizard species differ one from another by the number of presacral vertebrae and the extent of ossification. Degeneration of sacral myomeres and formation of the sacral gap in myomeres play a crucial role in development of sacral ribs and in the direction of their growth. Sacral vertebrae are defined as vertebrae, which ribs or transverse processes develop at the place of the sacral gap. There are ribs vs. diapophyses in Lacertidae and most probably in other lizards. Caudal ribs are not found, but prerequisites for their development take place, so they might be expected as a rare anomaly in Lacertidae. There is a strict order of maturation of skeletal elements: vertebral centra and neural arches, ilium, pubis, ischium and sacral ribs, which has its reflection in the adult phenotype. Additional elements of the pelvic girdle, namely, epipubis, epiischium, and hypoischium develop along the axial fibrous ligament and undergo chondrification and calcification separately. A hypothesis that the overall shape of the pelvic girdle is formed under the influence of the visceral cavity is partly supported. Cause-and-effect explanations of sacrum and pelvis morphogenesis are given. Certain anomalies of sacral structure are described and discussed in developmental terms.

Key words: Sacrum, pelvic girdle, ribs, development, Lacertidae, anomalies, morphogenesis.

The aim of this study is to examine in detail sacrum and pelvis development in lacertid lizards. This is a part of a larger project devoted to development and evolution of the sacro-pelvic skeletal complex in Amniota and papers on mammals and birds will appear in future. As we have already described the early events in the morphogenesis of the sacroiliac complex (Borkhvardt and Malashichev, 2000) much attention is drawn in the present paper to the later stages when patterns of chondrification and ossification develop. Size, shape and position relationships between the cartilaginous elements of the pelvic girdle and the axial skeleton, and surrounding organs and tissues are of my main interest. I also aimed to infer cause-and-effect explanations of the morphologically detectable changes in the shape and size of skeletal elements during lizards' ontogenesis. I had no particular interest to the development of vertebral centra and the skeleton of free limbs.

MATERIAL AND METHODS

Embryos of different age and adults (Table 1) of four species of lizards were studied: *Lacerta agilis* L., *Lacerta vivipara* Jacquin, *Lacerta saxicola* Eversmann, and *Podarcis muralis* (Laurenti).

Pregnant females of *L. agilis* were caught in the wild in Belgorod area (Russia) in spring 1998 and were released into the natural habitat after they laid eggs. The eggs were incubated in wet sand in the automatic climate cameras with programmed temperature and insulation regimes (Braun and Ghoryshin, 1978) at 16 h-long day at temperature $t = +26.79 \pm 0.05$°C ($t_{\min} = 23$°C; $t_{\max} = 29$°C), and humidity of 96%. Temperature control was made six times a day. Eggs were dissected on different days of incubation and embryos were sacrificed in Bouin's fixative. Embryos collected in the same region in 1974 – 1975 by V. G. Borkhvardt and stored in 4% formaldehyde solution was used as well.

Embryos of *L. vivipara* were collected in 1975 by Borkhvardt, and stored in 4% formaldehyde. Em-

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bryos of *P. muralis* were collected in the vicinities of Florence, Italy in the 20th ***(??: in 1920?)*** and preserved in a mixture solution of sublimate and acetic acid. Embryos were staged (Dufaure and Hubert, 1961), transferred through a series of alcohol solutions of increased percentage, chloroform, and embedded into paraffin. Sections of 7 – 10 μm thickness were made, and stained with hematoxylin-eosin. I reexamined histological slides through the embryos of *L. agilis* and *L. vivipara* stained with hematoxylin-eosin or azan made by Borkhvardt (1977) and G. O. Cherepanov. Whole-mount alizarin and alcian preparations (Hardaway and Williams, 1975) were used to study late embryos and adults.

Nomenclature for nerves was used according to Raynaud et al. (1975) and Akita (1992), for muscles — according to Raynaud et al. (1975), for blood vessels — according to Ghurtovoj et al. (1978), for lymphatic system — according to Cligny — 1899).

RESULTS

1. Sacrum and Pelvis Development in *Lacerta agilis*

1.1. Stage 29

In embryos of 29 stage an easily distinguishable marker of the first sacral segment is already exists — the branching of the paired allantoic artery from the dorsal aorta. In the embryos wholly cut in the sagittal plain this branching is found (at later stages as well, Fig. 1*a*) in the 30th (or in 29th) segment. At stage 29 the allantoic artery is subdivided and will be referred to as *arteria iliaca interna*. In the next segment, just behind the branching of *a. iliaca interna* (i.e., in the 31st or 30th segment) the *aorta dorsalis* becomes much narrower being the arteria caudalis — Fig. 1*a*). Just under this place the gut is widen to the cloaca and the allantoic duct leaves it — Fig. 1*c*). These two succeeding segments are correspondingly the first and the second sacral segments. Spinal ganglia are differentiated. The spinal nerves do not even reach hind limb bud, whereas arteries have already entered it.

TABLE 1. Number of Embryos Examined

Stage	*Lacerta agilis* L.	*Lacerta vivipara* Jacquin	*Podarcis muralis* (Laurenti)
29	9		
30	8		
31	9	3	
32	8		
33	5		1
34	8	1	
35	8		3
36	10	4	3
37	2		
38	6		
39	4	3	
40	1		
Juveniles	1 + 1*	1*	
Immmatures	3*	1*	
Adults	2*	2* + 1* (*L. saxicola*)	
Total:	86	14 + 1 (*L. saxicola*)	7

* Whole-mount alizarin-alcian preparations.

1.2. Stage 30

Hind limb bud increases in size and nerves enter it. In embryos of the transitional stage 30/31, hind limb bud increases in length, which is twice as much as the width. The central mesenchymal condensation of the hind limb bud, as well as mesenchymal condensations of neural arches and vertebral centra are firstly noted.

1.3. Stage 31

Dense mesenchyme of the neural arches is seen as preseptal condensations [according to classification by Borkhvardt (1982); Fig. 1*a*]. The transitional stage 31/32 is critical in the development of the sacroiliac complex in lizards (Borkhvardt, 1995; Malashichev and Borkhvardt, 1999; Borkhvardt and Malashichev, 2000). Degeneration of the hypaxial parts of sacral myomeres takes place at the stage. It begins from the first sacral myoseptum, which divides the first and the second sacral myomeres. It later spreads into adjacent myomeres leading to the formation of the sacral gap (see Borkhvardt and Malashichev, 2000 for more details). Therefore, at stage 31/32 an additional marker of the sacral region can be easily detected — the gap in myomeres (Figs. 1*b*, 2, 5*A*).

1.4. Stage 32

A. iliaca interna becomes relatively narrower if compared with the size of the segment (Fig. 1*c*). Beginning in the cranial part of the first sacral segment it goes backward, laterally, and passes under the preseptal condensation of the first sacral segment and under the sacral gap. This artery, colon entrance to the cloaca and allantoic duct exit, and the place of the future ischiadic symphysis are in the first sacral segment (Fig. 1*c*).

Myomeres are formed by myotubes, which are oriented near to parallel to the main body axis. Sacral myomeres are greatly degenerated in their hypaxial parts, especially the second one, forming the sacral gap filled in with undifferentiated mesenchymal cells (see Borkhvardt and Malashichev, 2000). This mesenchyme is clearly defined from surrounding tissues.

In the lateral hypaxial part of the first caudal segment, a new marker of the sacral region can be easily found on hystological sections. It is an early anlage of the paired sacral lymphatic heart. The heart is connected with the posterior cardinal vein, intersegmental vein, which divide 31st and 32nd segments, and two lymphatic internal affluents ("affluent lymphatique interne") risen from the second sacral and the first caudal segments. The affluents connect the heart with the main lymphatic cavity — "cavité générale"), which passes along the longitudinal axis of the embryo under the notochord.

Pelvis anlage is seen in the basement of the limb bud as a dense field of mesenchyme bounded cranially and caudally by nerves of the lumbo-sacral plexus, which grow to the limb — *n. obturatorius* and *n. tibialis* (Fig. 1*b*). Laterally to the place shown, both nerves turn caudad to the limb bud and *n. obturatorius* appears in the close contact to the pubic region of this single mesenchymal field, being surrounded with mesenchymal cells. At the stage 32/33, separate condensations of three parts of the pelvic girdle can be observed. The most prominent is the ilium. *N. obturatorius* already penetrates the pubic mesenchymal condensation, i.e., the pubis arises around the *n. obturatorius*. At the stage, it is already possible to determine the sequential order of maturation of skeletal elements in the sacro-pelvic region: vertebral centra and neural arches, ilium, pubis, and ischium. The pelvic girdle is much less developed than the elements of the free limb.

1.5. Stage 33

Chondrification begins in the vertebral centra, and the basements of neural arches up to the mid-height of the spinal cord. Enlarged mesenchymal ilium reaches the level of the lower edge of the notochord and enters the sacral gap. It is clearly separated from the remaining pelvic anlagen with two rows of less differentiated cells. Pubis goes down to the upper level of the allantoic tube or lower. Ischium is not so well grown.

In embryos of 33/34 transitional stage, mesenchymal sacral ribs are separated from the neural arches (Fig. 2), the first rib being somewhat greater than the second one. Small separate mesenchymal anlagen are also seen in two succeeding caudal segments, but these are not traceable as distinct elements during the later cartilaginous stages. The chondrification of the pelvic girdle begins. Ilium is at the more

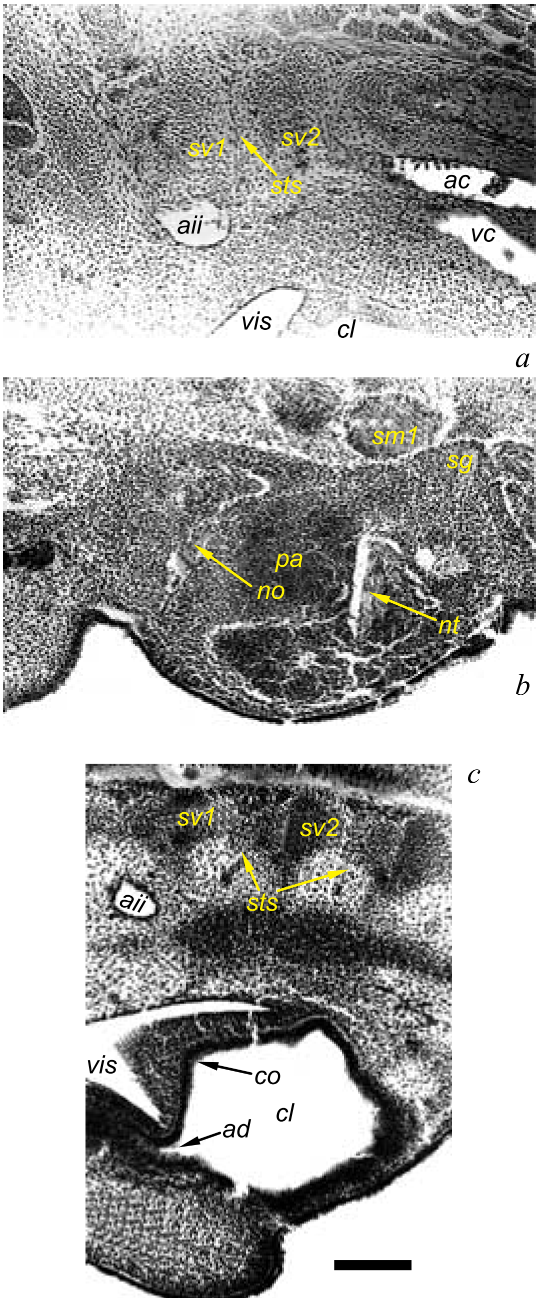

Fig. 1. Sagittal sections through the early embryos of *Lacerta agilis* showing the regional markers. Histological slides: *a*) No. 341-2 at stage 31; *b*) No. 2460-1 at stage 32; *c*) No. 1594-2 at stage 32/33. Head is on the left; *ac*) arteria caudalis; *ad*) allantoic duct; *aii*) *arteria iliaca interna*; *cl*) cloaca; *co*) colon entrance to the cloaca; *no*) *nervus obturatorius*; *nt*) *nervus tibialis*; *pa*) pelvic anlage; *sg*) sacral gap; *sm1*) the first sacral myomere; *sts*) the first sacral myoseptum, which divides the first and the second sacral segments; *sv1*, *sv2*) anlagen of the sacral vertebrae; *vc*) *vena caudalis*; *vis*) visceral cavity; asterisks show the place of the future ischiadic symphysis. Scale bar is 110 μm.

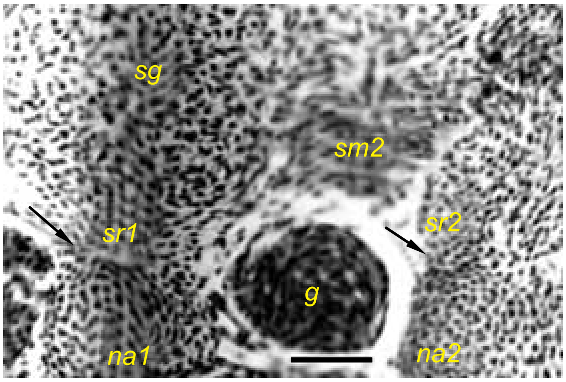

Fig. 2. Frontal section through the embryo of *Lacerta agilis* No. 2638-1 at stage 32. Early sacral rib (*sr1*, *sr2*) anlagen are seen in the deep zone of the sacral gap (*sg*). Arrows shows the borders between the mesenchymal neural arches (*na1*, *na2*). Head is on the left; *g*) spinal ganglion; *sm2*) the remains of the second sacral myomere. Scale bar is 45 μm.

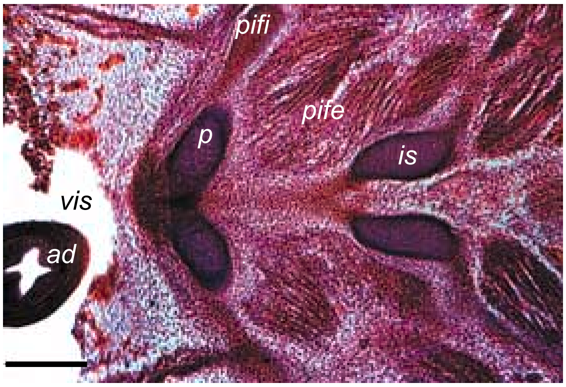

Fig. 3. Frontal section through the region of pelvic symphyses in the embryo of *Lacerta agilis* No. 1794-2 at stage 35. Azan staining shows that the cartilaginous pubic symphysis is not yet established, although the contralateral pubes (*p*) are in close contact. Head is on the left; *is*) ischium; *pife*) *musculus pubo-ischio-femoralis externus*; *pifi*) *m. pubo-ischio-femoralis internus*; *tp*) *m. transversus pubis*. For other designations see Figs. 1 – 2. Scale bar is 180 μm.

advanced stage of development, than the pubis, and pubis, in its turn — than the ischium. The tissue of sacral ribs is less differentiated than the tissue of neural arches and the ilium.

1.6. Stage 34

Chondrified ilium rises up to the mid-height of neural arches. The first sacral rib, which has begun to chondrificate, is separated from the ilium with a streak of connective tissue rich with collagen fibers. Moreover, the ilium is surrounded with the perichondrium except of its distal end. Exfoliation of tissues along the ilium is seen as a usual artifact (also at later stages; Figs. 5*A*, *c*, 7*b*, 9). Pubis consists of young chondroblasts. They surround *n. obturatorius*, thus the obturator foramen exists. Contralateral pubes approach each other, turning under the visceral cavity. Ischia are much more widen cranio-caudally on their distal ends than the pubes. Acetabular region of the pelvis is mesenchymal.

1.7. Stage 35

Contralateral neural arches of hypertrophied cartilage are closed down upon the spinal cord. Spinal processes begin to form. The second sacral rib chondrificates. The first sacral rib is expanded along the upper part of the ilium. The cranial margin of the first sacral rib is bend down and the caudal margin distally overlay the second rib (Fig. 5*A*). Both ribs are connected by a “mesenchymal bridge” at their distal ends (Fig. 5*C*). Ilium reaches the upper caudal and lateral corner of the sacral gap growing along the body surface and repeating its curve. Lymphatic heart is situated under the ilium’ tip, but in the first caudal segment. Acetabulum is at an early chondrification stage. Contralateral pubes almost reach each other separated by approximately ten rows of mesenchymal cells (Fig. 3). Their symphysis is seen far cranially under the visceral cavity (see also Fig. 7*a*). A mesenchymal condensation rich with collagen fibers and organized in the shape of the vertical plate follows the pubes symphysis along the main body axis (Figs. 3, 6). It connects the visceral cavity and the integument. Contralateral ischia are in contact in their cranial edges via mesenchymal tips (Fig. 3). Distally, ischia change the direction of growth to a more vertical and are parallel to the surface of the visceral cavity (Fig. 4).

1.8. Stage 36

There is perichondral bone above and below the vertebral centra in their cranial parts. Bone covers internal surface of the neural arches at stage 36/37. Sacral ribs are distinguishable from the neural arches even at this progressive cartilaginous stage (Fig. 5*B*, *C*). The tip of the second sacral rib turns ventrally, not reaching the ilium. The lymphatic affluents penetrate the cartilaginous basement of the second sacral rib and the first caudal transverse process, making them slightly forked on the underside, and go to the sacral lymphatic heart.

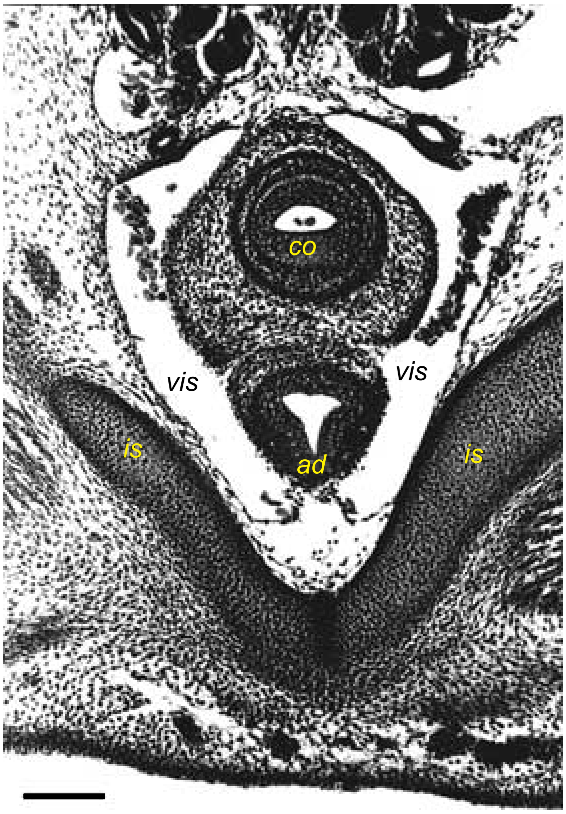

Fig. 4. Transverse section through the ischiadic symphysis in the embryo of *Lacerta agilis* No. 1962-5 at stage 35. Note the change in direction of ischia (*is*) by approaching the visceral cavity (*vis*). For other designations see Figs. 1 – 3. Scale bar is 80 μm.

The distal ends of the first and the second sacral ribs are close to each other. At stage 36/37 mesenchymal cells, which serve as a coupling agent of the two ribs, undergo chondrification. The cartilaginous sacral ribs are still, however, distinct (Fig. 5*C*). The second sacral rib is greatly inclined cranially and ventrally, occupying the zone of the sacral gap (well seen at later stages; Fig. 7*b*). Both ribs are expanded. Ilium is separated from the sacral ribs with five-eight rows of elongated cells that are oriented along its inner surface and can come off in a layer separate both from the ilium and from sacral ribs (Figs. 5*C*, 7*b*). The preacetabular process of the ilium is formed. The pubic symphysis consists of dense packed mesenchymal cells. There is an axial mesenchymal condensation of the hypoischium behind the ischiadic symphysis, which forms along the collagenous plate. Lamellar bone appears in the pelvis at stage 36/37 as thin plates in the ilium and pubis.

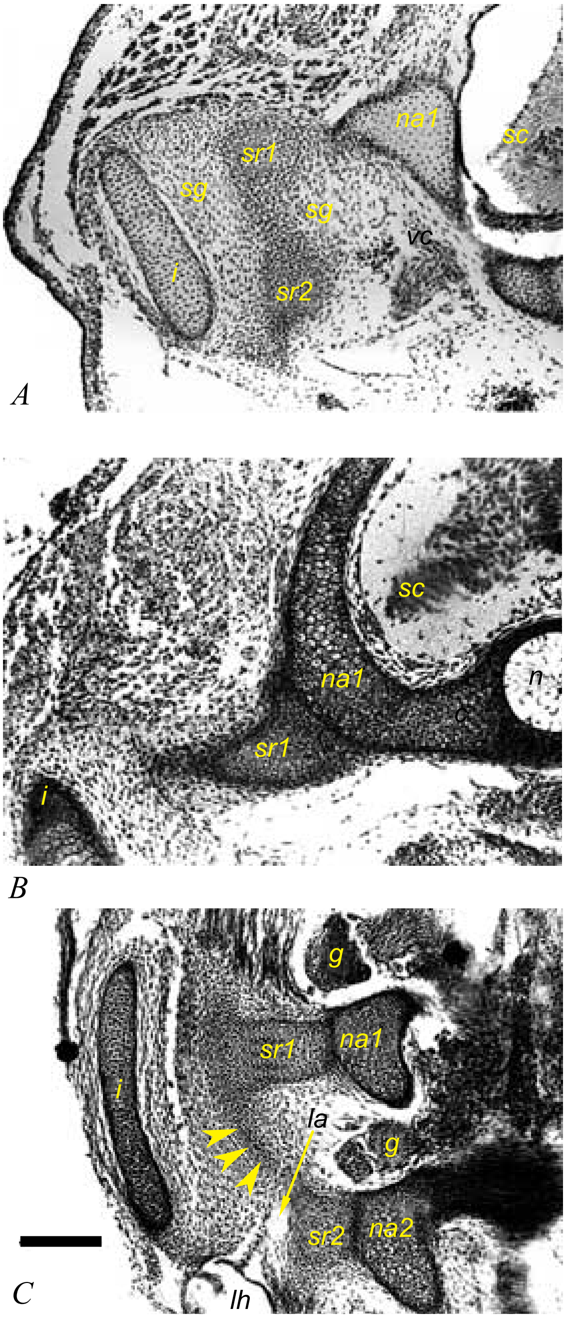

Fig. 5. Relationships between the sacral ribs (*sr1*, *sr2*), sacral gap (*sg*), neural arches (*na1*, *na2*), and the ilium (*i*) in *Lacerta agilis*. *A*) Transverse section through the embryo No. 1607-3 at stage 35/36 near the caudal border of the first sacral segment; *B*) transverse section through the embryo No. 2651-1 at stage 36; *C*) frontal section through the embryo No. 1960-1 at stage 36. Head is above. Mesenchymal “bridge” between the distal parts of the sacral ribs is shown with the arrowheads. *c*) Centrum; *la*) lymphatic affluent; *lh*) lymphatic heart; *n*) notochord; *sc*) spinal cord. For other designations see Figs. 1 – 4. Scale bar is 110 μm.

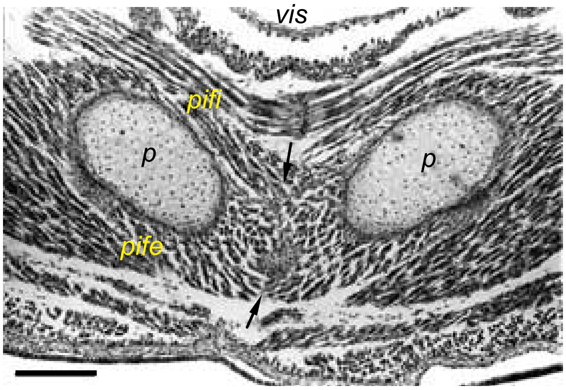

Fig. 6. Transverse section through the embryo of *Lacerta agilis* No. 1769-1 at stage 38 posterior to the pubic symphysis. Arrows show the connective tissue vertical plate, to which *m. pubo-ischio-femoralis externus* are attached. For other designations see Figs. 1 – 5. Scale bar is 110 µm.

Fig. 7. Contrasts of development between the pubis and the ischium (*a*; No. 2572-3 at stage 38/39), sacral ribs and the ilium (*b*; No. 2571-2 at stage 38/39) in *Lacerta agilis*. Pubis and ilium are covered with perichondral bone, whereas ischium and sacral ribs are not. *in*) Integument; *sm1*) the first sacral myomere. For other designations see Figs. 1 – 6. Scale bars are 180 (*a*) and 110 µm (*b*).

1.9. Stage 37

The cartilaginous ischia are elongated along the medial axis. The hypoischium became very long, as well. Endochondral ossification begins in the femur, but not in sacral vertebrae and pelvic girdle. The difference in the developmental extent between the pubis and the ischium becomes more obvious. Prepubic process of the pubis is formed.

1.10. Stage 38

A rod-like cartilage forms along the mid-axial mesenchymal-collagenous plate craniad to the ischiadic symphysis, reaching the level of the acetabulum. It is forked caudally in front of the symphysis. Vertebrae and distal parts of the pelvic elements consist of hypertrophied chondrocytes. Perichondral ossification in the pubis approaches the symphysial region. Lamellar bone is firstly noticeable at the surface of the ischium. It also spreads to the sacral ribs from the basement of the neural arches.

The distal end of the first sacral rib is half as much against the width of its proximal end and consists of young chondroblasts, surrounded with undifferentiated mesenchymal cells. The rib spreads up and down along almost vertically oriented ilium and joins smoothly with the second sacral rib distally. Both ribs are bounded from above by the horizontal myoseptum, which separates hypaxial and epaxial muscles. Endochondral ossification takes place within the vertebrae centra, neural arches, and the ilia. Blood vessels enter vertebral cartilage symmetrically from both sides of the notochord, beginning from the dorsal side of the vertebra. They enter neural arches cranially from the side of the spinal cord at their mid-height level. Blood vessels also penetrate the lamellar bone of the lower half of the ilium, under the level of sacral ribs. At stage 38/39, blood vessels enter pubis beginning from inner perimeter of the obturator foramen and endochondral ossification distributes distally. The cartilage appears to be destroyed in the segment between the obturator foramen and the pectineal process. At the time, the contrast of development between pubis and ischium is most noticeable (Fig. 7*a*): there is cartilage destruction in the pubis covered with the thick perichondral bone, whereas weak lamellar bone in the ischium is noted. Ischia and the hypoischium became longer and flatter.

1.11. Stage 39

The destruction of cartilage occurs in the basement of the neural arches. It is the time of starting endochondral ossification in the sacral ribs. The ilium is not fully a bony element at the time, as its most proximal — acetabular) end has no even the perichondral bone. The pectineal process of the pubis is covered with bone layer from sides, not at its top being cartilaginous inside. There is perichondral bone only in the center of the ischium.

The caudal parts of ischium near the ischiadic tubercle goes laterally as it follows the visceral cavity, which became wider approaching the anus. However, there is a free space, between the ischiadic symphysis and the cloaca, where hypoischium grows. The hypoischium is a single cartilaginous element, which is forked on its cranial end, connecting with contralateral ischia, and mace-shaped on its caudal end. Cranial tips go around a little cavity, which separates the hypoischium from the ischiadic symphysis.

At stage 39/40, endochondral ossification spreads along the ilium down to the acetabulum. The proximal parts of the sacral ribs are ossified, but young cartilage remains on their most distal ends. Endochondral ossification in the pubis appears between the acetabulum and obturator foramen (Fig. 8*a*). Ischium is ossified in a less extent, mostly in its center. The acetabulum became covered with a lamellar bone from its external side. Nevertheless, three pelvic elements are separated with chondroblasts, which are not covered with perichondral bone. The contribution by the ilium and the ischium to the acetabulum is near to equal, with that less by the pubis.

1.12. Stage 40 and juveniles

In the bony neural arches, the trabeculae of endochondral bone are formed. The shape of the distal ends of sacral ribs has finally formed. They consist of young cartilage and repeat the curve of the upper part of the ilium. Cartilage remains also on the tips of neural arches and the ilium, symphysial ends of contralateral pubes and ischia, and in the acetabulum between the composing it bony elements (Fig. 8*b*). One lizard approximately of the age of stage 40 was stained whole mount with alizarin. It is well seen that at least the first pair of sacral ribs has separate centers of calcification, which are clearly distinct from those of the neural arches (Fig. 9*a*).

1.13. Immatures and adults

Ossification of all the three pelvic bones is distinct and not united in the acetabular region. Cranially and caudally of the ischiadic symphysis, separate centers of calcification are observed: the epiischium and hypoischium. Both elements are clearly distinct from the ischia. Both are forked at their ends nearest to the ischia. Sacral ribs are on the 30th and 31st vertebrae in two exemplars; on the 29th and the 30th in one exemplar. The pelvic girdle ventral view of an adult is shown on Fig. 9*b*. There are 29 presacral vertebrae in the only exemplar studied.

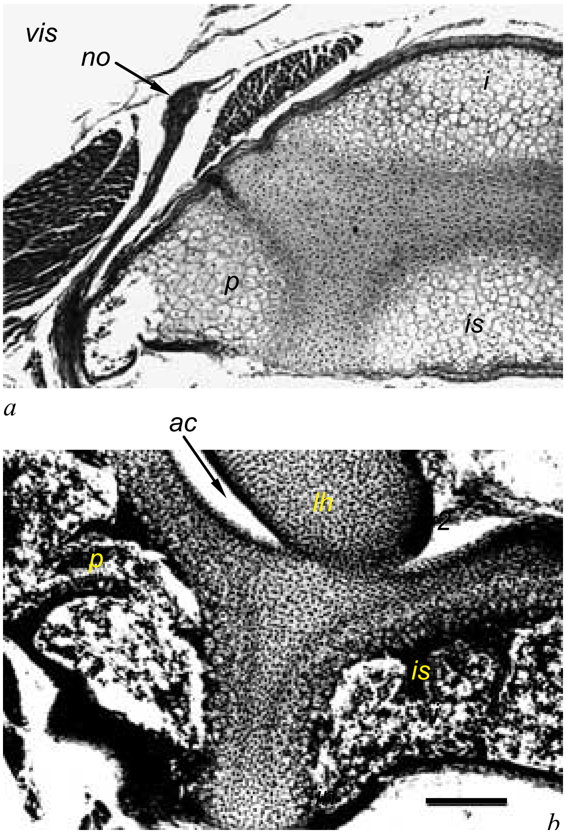

Fig. 8. Two developmental stages of the acetabular region of the pelvis in *Lacerta agilis*, embryo No. 2606-3 at stage 39 (*a*), embryo No. 1928-6-40 (*b*). Endochondral ossification starts in the proximal part of the pubis, beginning from obturator foramen (*a*). Note that acetabulum (*ac*) does not ossify at hatching; *fh*) femur head. For other designations see Figs. 1 – 7. Scale bar is 110 µm.

2. Sacrum and Pelvis Development in Other Species

Generally, the development of the sacro-pelvic region of the lacertilian skeleton is very similar in all representatives of the family (see also Raynaud et al, 1975; Rieppel, 1992, 1994). I report only a few notes of particular interest here. The time of ontogenetic

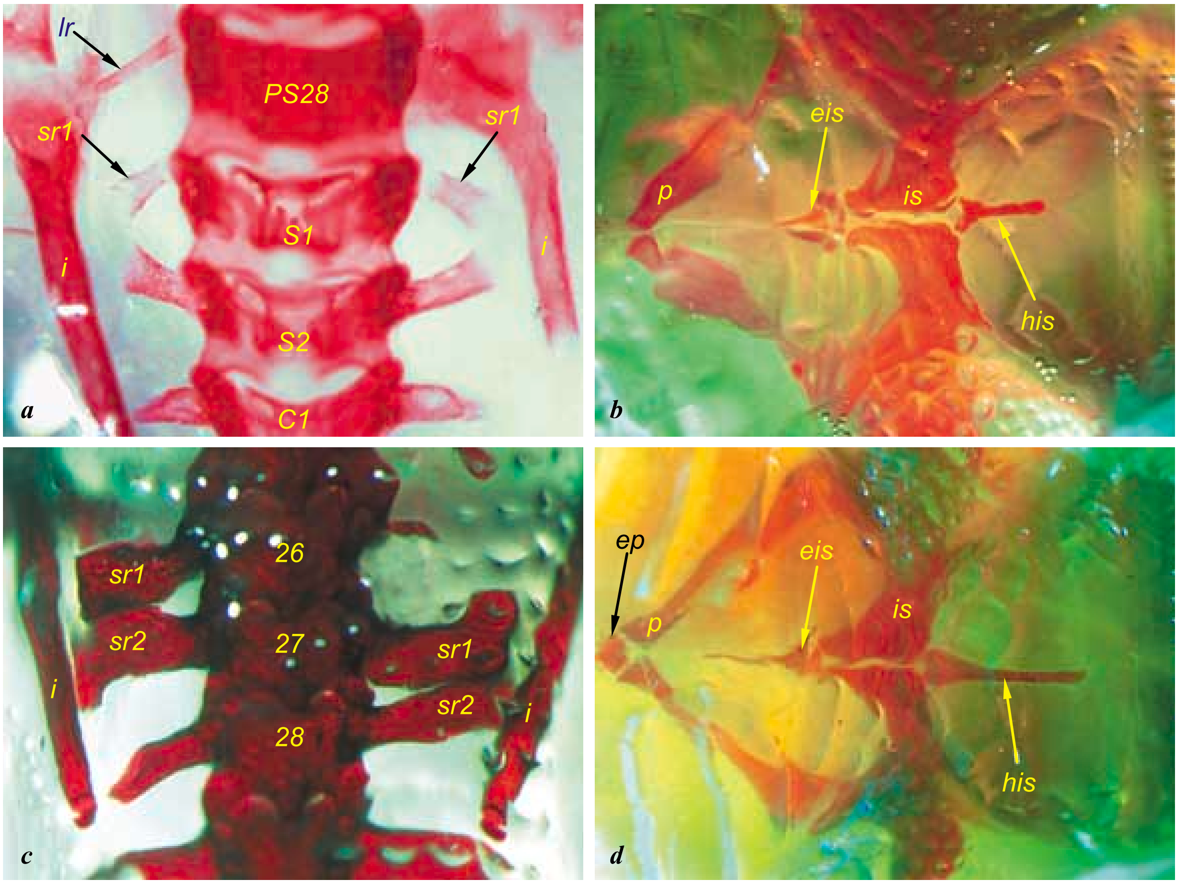

Fig. 9. Whole-mount alizarin preparations of a juvenile (*a*, *L. agilis* No. R49) and adults (*b*, *L. agilis* No. R48; *c*, *L. vivipara* No. R27; *d*, *L. saxicola* No. R34). *a*) Separate rib calcification centers (*sr1*) are clearly visible in the first sacral segment; *lr*) lumbar rib; *S1*, *S2*) sacral vertebra, *PS28*) the 28th presacral vertebra, *C1*) the first caudal vertebra. *b*, *d*) Ventral view of the pelves in two lizard species; *ep*) epipubis; *eis*) epiischium; *his*) hypoischium. *c*) Dorsal view of an anomalous specimen of *L. vivipara*. Sacral ribs of the right side are shifted one segment caudad; *26*, *27*, *28*) order numbers of subsequent vertebrae.

events in three lacertid species can be compared with that of *L. agilis* (Table 2).

2.1. *Lacerta vivipara* Jacquin

In *L. vivipara*, contralateral pubes form the symphysis only at stage 36. Distal ends of sacral ribs are still separated by a wide field of mesenchyme. There is no perichondral bone in the pubis at stage 36/37. Only at the stage ischia form the symphysis. At stage 39/40, endochondral ossification is not present in sacral ribs. In other words, the development of the skeleton in *L. vivipara* is slightly delayed. One of the specimens (*L. vivipara* No. 2577) at the stage shows an asymmetric anomaly of the sacrum (Fig. 10). Three instead of two sacral ribs are present on the left side of the vertebral column. *A. iliaca interna* passes in its usual place between the first and the second sacral ribs. The lymphatic heart on the left side is less in size than its right counterpart. However, it is supplied with two lymphatic affluents, which go from the third and the fourth segments beginning from the first sacral, instead of the second and the third. The lymphatic heart of the right side is of normal size, supplied with lymphatic affluents in the same manner. In other words, the sacral region in the specimen is expanded caudally for one additional segment, but not in all characteristic features. Both lymphatic hearts are situated in their usual first caudal, but sacralized segment.

The study of four whole mount preparations reveals some differences in the structure of the vertebral column of this species. Its seems that normally *L.*

TABLE 2. Timetable of Skeletal Development in the Sacrum and Pelvis of *Lacerta agilis*

	Vert. centra	Neural arches	Ilium	Pubis	Ischium	Sacral ribs
Mesenchymal anlage	30/31	30/31	32/33	32/33	32/33*	33/34
Start of chondrification	32/33	32/33	33/34	33/34	33/34*	34 — I 35 — II
Symphysis formation		35/36		35	36	36
Perichondral ossification	36	36/37	36/37	36/37	38	38
Endochondral ossification	37/38	38	38	38/39	39	39

* Mesenchymal anlagen appear and chondrification takes place a little bit later in ischium, but the time interval is less than one stage.

vivipara has 26 presacral vertebrae. The last one has no ribs. Obturator foramen is relatively smaller than in *L. agilis*. Two lesser specimens have no additional calcification centers in the pelvis. Two greater specimens have calcified epiischium, hypoischium, and epipubis. However, epiischium and hypoischium calcifications are shorter than those in *L. agilis* are. Both adult specimens are anomalous. Specimen No. R27 (Fig. 9*c*) has left sacral ribs on the 26th and 27th vertebrae, and right sacral ribs on the 27th and 28th vertebrae. The right ilium is shifted more caudally than the left one. The right 26th diapophysis looks like the last lumbar. The left 28th is slightly forked distally resembling the lymphapophysis. Specimen No. R36 has similar anomaly, but the sacral vertebrae are the 28th through 30th.

2.2. *Lacerta saxicola* Eversmann

In the only adult specimen of the species examined, sacral ribs are on the 29th and 30th vertebrae. Both hypoischium and epiischium elements are well ossified and very long; the epipubis is ossified as well (Fig. 9*d*).

2.3. *Podarcis muralis* (Laurenti)

At stage 33, large mesenchymal ilium and pubis are visible. Ilium is twice as great as pubis and reaches the sacral gap, which has already formed at the place of two subsequent sacral myomeres. Obturator nerve penetrates the pubis and turns to the limb bud. The lymphatic heart has started to develop in the first caudal segment. Contralateral pubes do not form the symphysis at stage 35, yet being separated by more than 20 rows of mesenchymal cells. The distal ends of ischia are very long cranio-caudally. Patterns and angles between pelvic muscles are evidently differed quantitatively from those in *Lacerta*. No perichondral bone is noted at stage 36 in vertebrae and pelvic girdle.

DISCUSSION

Morphological Markers of the Sacrum

The present study has shown that sacrum in three lacertilian species possesses characteristic features that allow simple determining of the first and the second sacral segments at any developmental stage. There are noticeable and stable markers of each segment in *Lacerta agilis*: branching of the *a. iliaca interna* from the dorsal aorta in the first sacral segment, reduction of hypaxial musculature of sacral segments beginning from the dividing myoseptum at stage 31/32, development of the paired lymphatic hearts within the first caudal segment at stage 32. The border between two sacral segments is lies approximately over the entrance of the colon to the cloaca, and the exit of the allantoic duct from the latter. It is worth of mentioning that just after the branching of *a. iliaca interna*, the *aorta dorsalis* become the *arteria caudalis*, which is twice as narrow as the *a. dorsalis*. It is well known that *Hox* genes play a crucial role in establishing the borders between the sections along the vertebral column including the sacral region (Davis and Capecchi, 1994; Fromental-Ramain et al., 1996; Gérard et al., 1996). On the other hand, genes of the *Hoxd* complex (Zákány and Duboule, 1999) and signaling by some morphogenetically active molecules (Smith and Tabin, 1999) are required to set up physiological constrictions along the gut mesoderm and formation of sphincters. These facts allow understanding most of described markers as fundamental and genetically determined features of the sacral zone of the vertebral column, which might be uniform for all vertebrates. These markers were very useful to follow the development of sacrum and pelvis in lizards and lead us to the following.

Sacrum and Pelvis Development in Different Species

It was found that lizard species differ one from another by the number of presacrals. Moreover, it appeared to be the feature of systematic value useful also for sex determination in some lizards (Arribas, 1999). Thus, the absolute segmental position of sacral markers may also differed. They showed that the sacrum is shifted cranially in *L. vivipara* to the 27th (28th) and 28th (29th) segments in comparison to *L. agilis* (30th or 29th and 31st or 30th). We did not count the number of presacrals in *Podarcis*, but it is known to be 26 in males and 27 in females, while *L. saxicola* has modal number of 27 presacrals in males and 28 in females (Arribas, 1999).

Most facts suggest some delay in pelvis and sacrum development in both *L. vivipara* and *P. muralis* (stage 0.5 – 1) in relation to *L. agilis*: later ossification and symphyses formation. Such an extended development of skeletal elements in these two species is probably due to longer incubation periods. While *L. agilis* hatches on the 40 – 50th day of incubation (Rieppel, 1994; our data), *L. vivipara* develop for about 71, and *P. muralis* for about 85 – 96 days (Saint-Girons, 1985). In contrast, the only specimen of *L. saxicola* showed advanced calcification of the pelvic girdle: longer hypoischium and epiischium elements, and well-calcified epipubis, which forms in older individuals (if ever) in other species examined. *L. saxicola* has 55 – 60 day incubation period, while *L. viridis*, in which only hypoischium was described (Raynaud et al., 1975) develops during 90 days (Saint-Girons, 1985). The right negative correlation is therefore observed between the time of incubation and the number and extent of calcification centers in lacertilian pelvis.

Although, all the four species examined are assigned currently to different genera (*L. vivipara* — to *Zootoca*, Mayer and Bischoff, 1996; *L. saxicola* — to *Darevskia*, Arribas, 1999) we have found few differences between them except the number of presacrals. The most prominent quantitative differences in musculature pattern were noted for *Podarcis muralis.* However, it might be the result of embryos fixation or their long-term storage.

Hypaxial Musculature and Sacrum Formation

Borkhvardt (1995) showed that the hypaxial musculature degeneration may be important for the formation of sacral diapophyses/ribs in a variety of tetrapods. It was suggested to take place due to reduction of innervation of the axial muscles in favor of limb muscles (Borkhvardt, 1995). Malashichev and Borkhvardt (1999) suggested that in serpentiform reptiles, hypaxial muscles of the sacral segments receive innervation that prevents their degeneration and formation of sacral gap in myomeres. We found also a positive correlation between the limb bud size, the extent of axial myomeres degeneration and the number of sacral vertebrae in a series of squamates with differently developed hind limbs (Borkhvardt and Malashichev, 2000). It was an argument for the "limb-bud hypothesis" proposed by Borkhvardt (1995). Independently, it was shown that in mice with down-regulated function of *Pax3* — gene responsible for the long range migration of myoblasts and formation of hypaxial musculature — sacral-like diapophyses arose in the lumbar vertebrae (Tremblay et al., 1998: Fig. 3*I*, *J*). Thus, the formation of the sacral gap in myomeres can indeed serve as an essential factor in development of sacral ribs. Here we can add that nerves of the sacral plexus enter limb bud at stage 30 — one stage and a half before the beginning of myomeres' degeneration and sacral gap formation. It is most probable that during this time neurons connect with their targets in the limb, while the axial muscles wait for innervation, but do not receive it and die.

Sacral gap formation leads not only to development of enlarged sacral ribs, but also allows outgrowth of ribs to meet each other. The myosepta are beveled in the embryos of lizards, thus the first sacral rib grows along the first sacral myoseptum slightly beveled caudally. The second sacral rib grows in the zone of the sacral gap, which is formed mostly at the place of the second sacral myomere craniad to the second sacral myoseptum. Thus the second sacral rib grows in latero-cranial direction and both ribs meet each other with their distal edges. The same direction of growth has the pygal pleurapophyses attached to the sacrum in some geckos (Holder, 1960).

Sacral Ribs vs. Diapophyses

In Lacertilia, the presence of sacral ribs has been for a long time the subject of discussion by zoologists (Moodie, 1907; El-Toubi, 1947; Kamel, 1951; 1952; Romer, 1956; Holder, 1960; Werner, 1971; Rieppel, 1992, 1993). To discuss this problem I shall accept the definition by Borkhvardt (1982), who apply the term "rib" to the distinct cartilaginous element.

Moodie (1907) stated that there are no sacral ribs in modern lizards. He did not found separate centers of ossification of the sacral ribs in cleared skeletons of several lizard species. Moreover, he found the ossification of the processes to be proceeding outward from the vertebra and concluded that "Lacertilia occupy an isolated place among all other known reptiles in not having any sacral ribs whatever" (Moodie, 1907: p. 88). El-Toubi (1947) found an anomalous specimen of *Agama stelio*, which seemed to support the opposite view. Later Kamel (1951, 1952) found in *Chalcides* (Scincidae) early mesenchymal anlagen of the first pair of sacral ribs. He believed that there were mesenchymal rudiments of the dorsal and ventral ribs, which later fuse together to form the sacral rib. This rib was attached to the distal end of the large transverse process of the first sacral vertebra and connected it to the transverse process of the second sacral vertebra. The latter was assumed by Kamel (1952) as not true sacral vertebra due to the absence of its own sacral rib. He referred to this vertebra as to "secondary sacral" or first caudal.

The present study shows no even slight indication of the dual origin of the sacral rib. Thus, the formation of "ventral" and "dorsal" ribs early in ontogenesis to form sacral rib (Kamel, 1951, 1952) is very questionable. In three species studied, we observed sacral ribs of two sacral vertebrae adjacent to, but clearly distinct from the basement of the neural arches beginning with the mesenchymal condensations (Fig. 2*b*) and at later cartilaginous stages (Fig. 5*B*, *C*). The sacral ribs grown into the zone of less differentiated mesenchyme (sacral gap) at the place of the former sacral myomeres (see also Borkhvardt and Malashichev, 2000). The distal zone of mesenchyme separates sacral ribs from the ilium and connects ribs to each other in a fashion, which I called "mesenchymal bridge." However, it does not form a separate (!) cartilaginous element (Figs. 5*C*, 7*b*), contrary to notes by Kamel (1951, 1952) and Werner (1971). While Kamel (1951, 1952) observed this element at mesenchymal stages and thought that it was the sacral rib, Werner (1971) wrote that it was a cartilaginous element and showed its different nature. We fully agree with Werner's suggestion that "transverse processes" themselves are sacral ribs. However, on figures presented by Werner (1971: Fig. 56), the distal group of cells does not leave the impression of additional **separate** element. I would characterize these mesenchyme, which undergo chondrification on later stages, as cells accumulated in the sacral gap at the distal ends of the sacral ribs (Borkhvardt and Malashichev, 2000), and served as the cambial zone of the latter. There are four well known ways of cartilaginous element growth (Hinchliffe and Johnson, 1983) of which appositional growth is characteristic for the neural arches and their processes (Wilting et al., 1994). It might be also characteristic for sacral ribs during embryonic development. Thus, the suggestion on the cambial zone seems to be reasonable.

In recent literature (Rieppel, 1992, 1993) the fact of the presence of sacral ribs in lizards was again the subject of some doubt, as there are no separate centers of perichondral ossification in sacral ribs in common lizards. Indeed, the perichondral ossification in sacral ribs is usually continuos with the perichondral bone of the neural arches. Nevertheless, we have found in a hatching embryo of *L. agilis* clear separate centers of perichondral ossification in the first pair of sacral ribs (Fig. 9*a*). Holder (1960) also observed sutures between the perichondral bone of the ribs and processes in two specimens of *Nephrurus* (Gekkonidae). Moreover, the endochondral ossification starts in the sacral ribs in a separate centers in some specimens — cranially, at about the middle of each rib length. As a delay in chondrification (and therefore ossification) of the sacral ribs occurs, we assume that their ossification may not always be separate as the perichondral bone of the neural arch stretches to the ribs earlier of the allotted time. The centers of ossification in the sacral ribs may therefore not develop or may develop in a very close contact with the perichondral bone grown off the neural arch, thus being rarely visualized. It might be the function of sacral rib size. Indeed, in *Alligator mississippiensis* (Rieppel, 1993) as well as large lizards such as *Uromastix* (Agamidae) or *Tropidurus* (Iguanidae) (Siebenrock, 1894, cited by Kamel, 1952) separate ossifications of the sacral ribs are usually found. Moreover, I observed the separate centers of ossification in the first pair of sacral ribs, which are greater, than the second pair. In other words, here is an example of a terminal modification of development of sacral ribs — true separate elements, and discussion and speculations about their presence or absence in lizards should be finally closed.

This discussion, however, brings up another point on the caudal ribs in Lacertilia, as well. Caudal ribs were first discovered by El-Toubi and Khalil (1950) in geckos. Although Romer (1956) wrote there are no caudal ribs in modern lizards, Holder (1960) in her careful paper on osteology of Australian Gekkonidae showed that there are caudal ribs on pygal (anterior caudal with no fracture plane) vertebrae in geckos. These ribs lack articulation with the transverse processes, forming a construction called "pleurapophysis," which consists of the transverse process and the distal rib element fused together. We have found early (stage 33) mesenchymal anlagen in at least two anterior pygal vertebrae in Lacertidae, which resembles the anlagen of the sacral ribs, but smaller in size. Thus, I suggest that not only in geckos, but also in some lacertid lizards it is possible to find caudal ribs in pygal vertebrae as a rare anomaly, as there are all the morphogenetic prerequisites to this event. These ribs may not necessary be sacral ones connected with the ilium.

Patterns and Sequence of Chondrification and Ossification

It was found in the present study, that the pattern of ossification in the sacro-pelvic region is generally correspondent to the pattern of chondrification. Repatterning in the sense of Rieppel (1994), i.e., not full correspondence of the number of centers of chondrification and ossification was not found here. The ossification centers appear in the oldest parts of the cartilaginous elements, even in the sacral ribs.

The first group of elements to chondrify and ossify consists of elements of free limb. The second group consists of vertebral centra and neural arches. The third group is the ilium and the pubis. The ischium and the sacral ribs are delayed in both chondrification and ossification processes (Table 2). The second pair of sacral ribs develops a little bit later than the first one due to more caudal position. This sequence remains constant from early chondrification through endochondral ossification.

The last to maturate are the group of additional elements of the pelvic girdle, namely: epipubis, hypoischium, and the element, which I called epiischium. As early as 1898, Wiedersheim depicted different structural variants of the region of the pubic and the ischiadic symphyses. For *Lacerta vivipara*, he showed the presence of the paired cartilaginous hypoischium, calcified cartilage of the epipubis and an unnamed piece of cartilage in front of the ischiadic symphysis (Wiedersheim, 1898: Fig. 113). The latter was connected with the epipubis by a ligament (fibröses Band). This structure was mentioned by Sabatier (1880), who referred it to as "epiischion." We found all the mentioned elements in the pelvic girdle of Lacertidae, although epiischium and the axial ligament are not shown in most works (El-Toubi, 1938; Raynaud et al., 1975; Ghurtovoj et al., 1978; Rieppel, 1992, 1994). Wellborn (1933) wrote that gecko *Gonatodes* shows a somewhat aberrant form if compared to others: the hypoischium has a rostral elongation. It should be emphasize, however, that in Lacertidae, and, perhaps, in other lizards, the cartilage craniad to the ischiadic symphysis (epiischium), as well as hypoischium behind are the cartilaginous elements distinct from the symphysis, and may undergo independent calcification. Therefore, we should postulate the presence of two separate additional elements in the pelvic girdle of lizards: hypoischium and epiischium. Together with the epipubis they form a row of elements developed in connection to the fibrous ligament (also noted by Stephenson, 1960). The ligament stretches along the entire body axis between the integument and the wall of the visceral cavity, dividing the myomeres of the contralateral sides (see also Anisimova, 1990). Contralateral *pubo-ischio-femoralis externus* muscles are attached to this ligament between the pubic and ischiadic symphyses (Figs. 3, 6).

The additional elements of the pelvic girdle develop in much the same way as the sternum (Borkhvardt, 1992). Their mesenchymal anlagen originate in the corners, between the contralateral pubes and ischia (coracoids in the case of sternum), where conditions advantageous for cell concentration are formed. For example, the presence of the ypsiloid cartilage in Urodela is dependent upon the extent of development of the ventral pelvis plate (Anisimova, 1990). Interesting, that Sabatier (1880) compared sternum with the ypsiloid cartilage of Urodela ("sternum abdominal") and the hypoischium of lizards ("sternum pelvien"). I fully agree to the conclusions by Sabatier (1880) and Borkhvardt (1992) on the structural and developmental similarity of the pectoral and pelvic girdles in this respect.

A result of the rigorous time sequence of the element maturation is the late formation of the ischiadic symphysis if compared to the pubic one. Effect that is much more important is that the degree of development of the ilium is greater than that of the sacral ribs at all embryonic stages. To the time, when sacral ribs rich the ilium, their distal ends consist of young cartilage, while the ilium is covered with the perichondrium or even with the bone layer, and soon undergo full endochondral ossification. Moreover, the second sacral rib does not even reach the ilium, being separated from the ilium with the less differentiated cells. The tissues of sacral ribs and the ilium at the place of their contact are mature in different extent and possess different physical properties in such a way that tissue splitting is often seen between these structures in histological preparations. This fact as well as possible moveability of the ilium about the sacral ribs might be the cause of why the sacral ribs do not fuse with the ilium. On the other hand, the difference in the tissue maturity of the first and the second sacral ribs is not great, so that the ribs can be fully fused in older or anomalous specimens (Holder, 1960; Hoffstetter and Gasc, 1969).

The difference in tissue physical properties leads to a common mode of definitive shape formation of a number of elements in the region of discussion. We have shown that sacral ribs overgrow ilium with their distal edges, forming a sort of "iliac bed," where the ilium is settled. In the same way finally form the second pair of sacral ribs, which overgrow lymphatic affluents, as well as hypoischium, which grows around a little cavity caudad to the ischiadic symphysis. In other words, the final shape of a distal cartilage of this or that skeletal element may be formed due to the overgrowing of an obstacle met in the direction of growth. In contrast, the obturator foramen forms when mesenchymal cells accumulate around, but not overgrow the *n. obturatorius*.

The Primary and Secondary Sacral Vertebrae

Kamel (1952) wrote that there is only one sacral vertebra in lizards, which is connected with the ilium — the primary one. The second sacral vertebra he called "secondary" as it showed more resemblance to the caudal vertebrae and carried transverse processes, but not sacral ribs. However, our data clearly shows that there are two pairs of vertebrae, which carry sacral ribs, although the vertebrae of the sacral series may differ one from the other in some characters. It is most often assumed that the sacral region is transitional between the trunk and the tail, so the succeeding vertebrae of the sacral series might possess gradient features. That is why it seems to be not so important to discuss which vertebra is the primary sacral one. Distal ends of sacral ribs of both sacral segments grow by involvement of cells from the sacral gap, which forms at the place of degenerating musculature. Both sacral vertebrae posses this common, unique and essential characteristics. That is why we could formulate a new definition of sacral vertebrae. Sacral vertebra is the vertebra, which transverse processes or ribs develop at the place of degenerating axial muscles. However, this definition is of no practical value in the work of, say, a herpetologist or a paleontologist. It differs from usually accepted understanding of the sacral vertebra in a way that a vertebra, which is not directly connected with the ilia through its diapophyses or ribs, can be, nevertheless, the sacral one. It might be acceptable in some cases to name such a vertebra as the "sacral-like" one (see for example: Tremblay et al., 1998).

Pelvis Development and the Visceral Cavity

In lizards, pelvic girdle develops in the large free space laterally and under the visceral cavity (Fig. 1*c*). Thus, the pubes and the ischia grow centrifugal off the acetabulum. At later stages they grow under the visceral cavity being limited neither in direction, nor in the length of their growth, as there were no any tissues or organs, which could play the obstacle role (Figs. 3, 7*a*). These facts allowed me proposing a hypothesis on the leading role of the relative volume and the shape of the visceral cavity in affecting the direction of pubic growth and the overall shape of the pelvic girdle (Malashichev, 1999). According to the hypothesis, the prepubic and opisthopubic pelves are developed in different tetrapods due to relatively small or large visceral cavity that cartilaginous pubes need to overgrow. I can not discuss this issue in details, however, it is worth to mention the recent discovery of a coelurosaur with well-preserved soft tissues (Dal Sasso and Signore, 1998). In this dinosaur with the prepubic pelvis, the intestine was shown relatively small being in position much more forward than usually thought, not reaching the pubes, that is in agreement with theory prediction (Malashichev, 1999) for carnivorous saurischians.

Sacrum Anomalies

A number of anomalies of the sacral region is described in the literature for different lizard species (El-Toubi, 1947; Holder, 1960; Hoffstetter and Gasc, 1969). Two anomalous specimens of *Lacerta vivipara* were found during the present investigation. An adult specimen showed a type of anomaly (Fig. 9*c*) usual for amphibians and called "asymmetric sacrum" (Kovalenko, 1992). The other specimen showed a more interesting variant of sacral structure (Fig. 10). The variant is characterized by shift in position of transverse lymphatic vessels one segment caudad, and appearance of an additional (third) sacral rib on the left side of the first caudal vertebra. However, the lymphatic hearts remain at their usual place in the third sacral (first caudal) segment, although the left one appear to be smaller. Such a position of lymphatic vessels occurs in *Chalcides*, in which two lymphatic vessels pass through the transverse processes of the first and the second caudal vertebrae, but not through the second pair of sacral ribs (Kamel, 1952).

One of the questions arisen in respect to this anomaly is why the enlarged third sacral rib is not forked, while overgrowing the lymphatic heart, as it takes place in the first caudal segment of *Anguis fragilis* (Borkhvardt and Malashichev, 2000). In-

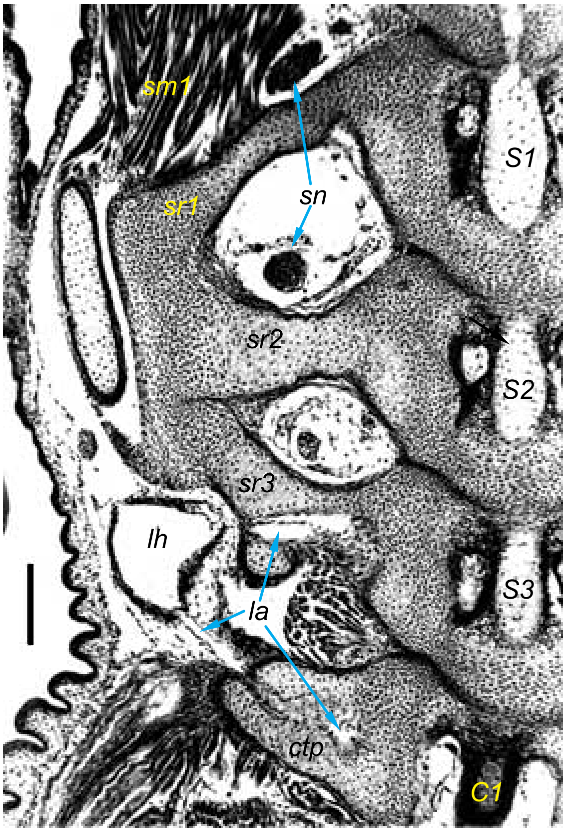

Fig. 10. Frontal section through the anomalous embryo of *L. vivipara* No. 2577-1 at stage 39/40. Left side is only shown. Additional sacral rib is observed. The central (second) sacral rib is the largest one. Lymphatic affluents are shifted one segment caudad. *sn*) spinal nerves. For other designations see Figs. 1 – 9. Scale bar is 125 μm.

stead, the lymphatic heart became smaller, giving the place to the rib! We have shown that the development of the sacral ribs is closely connected with the degeneration of sacral myomeres and accumulation of mesenchymal cells in the sacral gap. This degeneration takes place at least one stage before the formation of the earliest visible anlagen of the lateral sacral lymphatic hearts. It is possible to assume that in the discussed specimen an additional segment was involved in myomere degeneration beginning from the left second sacral myoseptum. Therefore, the formation of the third sacral rib began earlier than the formation of the lymphatic heart, which thus could be affected anyway. The anomaly resembles mutations of posterior *Hox*-genes, controlling the caudal border of the sacral region (Davis and Capecchi, 1994; Fromental-Ramain et al., 1996; Gérard et al., 1996). Otherwise, it can be a result of higher incubation temperature (Malashichev and Pawlowska-Indyk, 1997).

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