Patterns of ossification in the skeleton of *Liolaemus quilmes* (Iguania: Tropiduridae)

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Abstract. Observations of the cranial and postcranial development in *Liolaemus quilmes* were made. The general pattern of ossification in *L. quilmes* is similar to that of *L. scapularis*, but some sequences of ossification differ. In *L. quilmes*, ossification of the postcranium begins early in development relative to the skull.

Introduction

Anatomists have studied the skeleton and the ossification sequences, especially of the skull, for a long time (Parker, 1878, 1879; Howes and Swinnerton, 1901; Gaupp, 1908; Peyer, 1912; among others). Studies of cranial development in different groups of saurians became a major focus of research in the past decades (El Toubi and Kamal, 1959a, b, 1961a, b, c; Kamal, 1960; Kamal and Abdeen, 1972, etc.). The monumental synthesis on skull development in reptiles by Bellairs and Kamal (1980) summarizes much of this early work.

More recently, interest increased in the study of ossification sequences and patterns as a tool for the development of phylogenetic hypotheses, based on comparisons of different skeletal systems among different species and taxonomic groups (Burke and Alberch, 1985; Goodwin and Trainor, 1983; Rieppel, 1993a, b).

Studies of this nature are limited in South American saurians. Recently, Lobo et al. (1995) described chondrocranial structure and cranial and postcranial ossification sequences for the oviparous species *Liolaemus scapularis*. This paper presents the ossification sequence for another oviparous species, *Liolaemus quilmes*, and compares it with the sequences in *Liolaemus scapularis* (Lobo et al., 1995), *Lacerta vivipara* (Rieppel, 1992), *Lacerta agilis exigua* (Rieppel, 1994), and *Elgaria coerulea* (Good, 1995).

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Materials and methods

Embryos of *L. quilmes* at stages 33 (14 days after laying), 34 (20 days) and 35 (27, 36 and 42 days) according to the developmental table provided by Lemus (1967), and two recently hatched specimens of 50 days (fixed 5 days after hatching) were studied. The females of *L. quilmes* that provided the eggs were collected near Amaicha del Valle, Tucumán Province (provincial road 307), Argentina, and they are deposited in the Instituto de Herpetología collection of Fundación Miguel Lillo (FML 02940). The embryo numbers are FML 03478 (14 days, 2 embryos), FML 03479 (20 days), FML 03480 (27 days, 2 embryos), FML 03481 (36 days) and FML 3482 (42 days). The two newborn specimens have number FML 03483.

The eggs were incubated in plastic boxes with humid wood shavings, previously treated with potassium permanganate. Skeletal preparations were then made using the method of Alberch (1985) for differential staining of cartilage and bone.

It is important to note that when we say that a bone "appears" or "ossifies" at any stage or day we refer to the moment when we see the bone for the first time. It must be understood that the element really begins its ossification some time before our observation. The same idea should be kept in mind when we say "simultaneously": we mean that these elements all appeared in the same general time period.

Results

General developmental patterns do not differ significantly from those described for *Liolaemus scapularis* (Lobo et al., 1995). Therefore, only the most important observations in

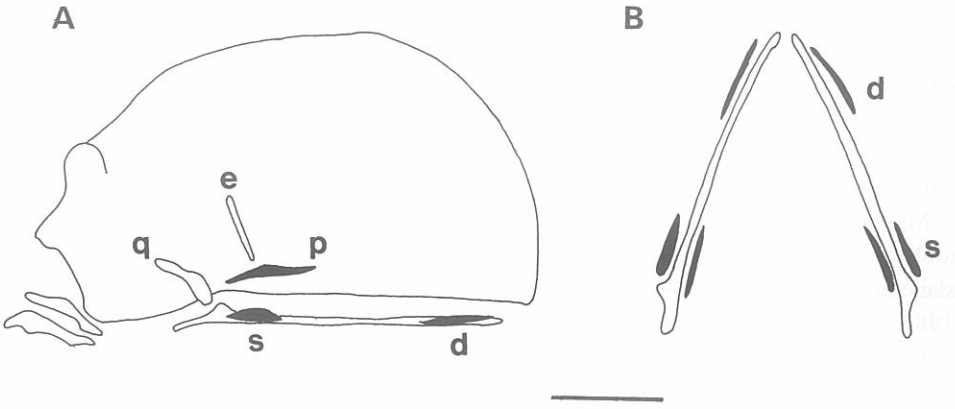

Figure 1. Initial ossification of elements in the skull of *Liolaemus quilmes* (in black). FML 03478 (14 day-embryo). A. Lateral view of the skull. B. Ventral view of the lower jaw. Scale bar: 1 mm. Abbreviations: d, dentary; e, epipterygoid; p, pterygoid; q, quadrate; s, surangular.

the development of the osteocranium and the postcranial skeleton of *Liolaemus quilmes* will be reported here.

14-day-embryo (stage 33). The pterygoids, dentary and surangular ossification centers appear in the skull (fig. 1). In the postcranium, the clavicles are visible and the sternum is paired and still cartilaginous (fig. 2). The pelvic girdle is joined at the pubis in the middle line, but the ischia remain separated. All long bone diaphyses have begun to ossify. The carpus presents seven cartilaginous and well defined elements, with only distal carpal I and the piciform present as barely visible condensations. The tarsus shows three cartilaginous elements. The metacarpals and metatarsals are ossified in mid-shaft. In the manus, the ossification is more advanced in metacarpals IV, III and II (fig. 2). In the metatarsals the beginning of ossification is just evident in I and V.

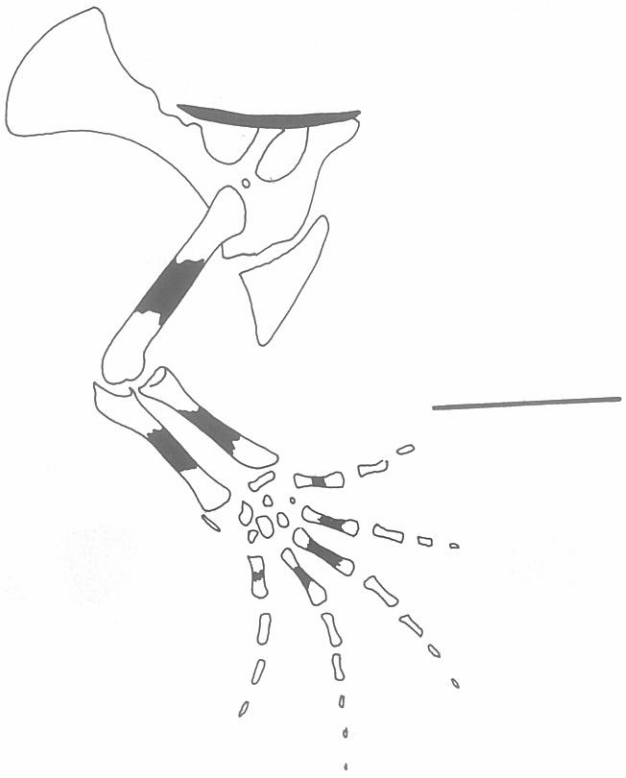

Figure 2. First ossifications in pectoral girdle and forelimbs of *Liolaemus quilmes*. FML 03478 (14 day-embryo). Scale bar: 1 mm.

20-day-embryo (*stage 34*). Almost all the dermal bones of the skull are present except the lacrimal, postfrontal and vomer. The quadrate and exoccipital are the only chondral elements that begin to ossify. All the dermal bones of the lower jaws are present and only the articular remains cartilaginous. In the postcranial skeleton, the coracoid, scapula and the three bones of the pelvic girdle present a scarcely evident ossification. The elements forming the sternum have united, as have those of the pelvic girdle.

27-day-embryo (*stage 35*). The postfrontal, vomer and lacrimal develop. The epipterygoid and all the chondral elements of the neurocranium begin ossification. The supraoccipital ossifies by means of two centers at both sides of the middle of the tectum synoticum. The columella and prootic show a low degree of ossification. In the postcranium the palmar aponeurosis is visible as a plain cartilaginous condensation. The neural arches, vertebral centra, and the mid-shaft of the ribs begin ossification.

36-day-embryo (*stage 35*). The articular and orbitosphenoid are the last chondral bones in starting their ossification. All carpal elements have begun to ossify. A single ossification center also appears in the center of the proximal tarsal block (fig. 3A). The transverse processes of the vertebrae begin ossification.

42-day-embryo (*stage 35*). The carpal elements are all in process of ossification. The ulnare and distal carpal IV show higher degree of ossification than the other elements. Ossification centers appear in the calcaneum and distal tarsals III and IV (fig. 3B).

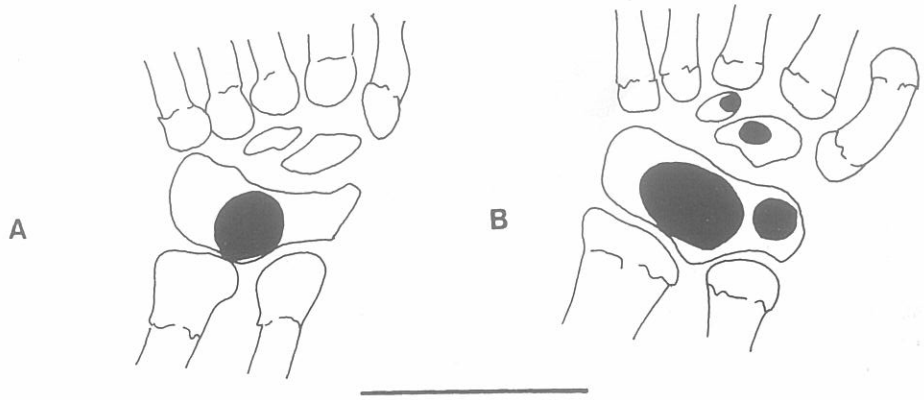

Figure 3. Ossification centers in the tarsus of *Liolaemus quilmes*. A. astragalus. FML 03481 (36 day-embryo). B. astragalus, calcaneus and distal tarsals III and IV. FML 03482 (42 day-embryo). Scale bar: 1 mm.

Discussion

The comparison of ossification sequences between *L. quilmes* and *L. scapularis* shows marked differences (table 1). The ossification of most elements in the skull of *L. quilmes* begins later than in the postcranium. The opposite condition was observed in *L. scapularis* (Lobo et al., 1995). The principal differences in the development of all bones of the skeleton between the species are presented in fig. 4. Differences with the ossification pattern present in *Lacerta vivipara*, *Lacerta agilis exigua* (Rieppel, 1992, 1994), and *Elgaria coerulea* (Good, 1995) are also discussed below.

The pterygoid is the first skull element to develop in both species of *Liolaemus*, a week before the other elements (on day 8 in *L. scapularis* and 14 in *L. quilmes*). The last dermal bones of the skull of *L. quilmes* to appear are the postfrontal, vomer and lacrimal, a week later (day 27) than all other elements. The nasal, postfrontal and vomer are the last elements to develop in *L. scapularis* (day 21). A similar condition was described for *Lacerta agilis exigua* where the first dermal bones to appear are the pterygoid, jugal and palatine, although the two latter present less ossification. In *Lacerta vivipara* the first elements to develop are bones related to the mandibular arch, with very advanced ossification of the pterygoids. The pterygoid is also the first element to ossify in *Podarcis sicula* (Rieppel, 1987) and *Elgaria coerulea*. In general, the last dermal elements to ossify are common in both *Liolaemus* and *Lacerta*. The principal differences are that the vomer is not among the last elements ossified in *Lacerta* and *Elgaria*, and the ectopterygoid is one of the last elements to ossify in *Lacerta vivipara* while nasal and lacrimal are the last ones in *Elgaria*.

In *L. quilmes*, the dentary and surangular begin ossification one week before the rest of the lower jaw bones. This differs from *L. scapularis*, where all dermal bones of the lower jaws begin ossification synchronously. In both species the articular ossifies later, on the day 27 in *L. quilmes* in a synchronic way with the greater part of the chondral skull, and on the day 36 in *L. scapularis*, subsequent to the formation of the chondral skull elements. A similar condition to that of *L. scapularis* was described in *Lacerta vivipara* where all the mandibular elements are identified in the early specimen, but with the splenial and the angular poorly ossified. The retroarticular process starts ossification (perichondral) at the same time, but the rest of the articular stays cartilaginous and represents the last mandibular element to complete ossification (Rieppel, 1992, fig. 3).

Table 1. Percentage of bones ossified in four stages of development. C: cranium and lower jaw (21 dermal and 11 chondral bones); P: postcranium (28 elements from extremities, girdles, and vertebral column: vertebral centra, neural arch and ribs counted as separate elements).

	14 days	21 days	36 days	45 days
L. scapularis	C = 59% P = 17%	C = 96% P = 71%	C = 100% P = 82%	C = 100% P = 96%
L. quilmes	C = 10% P = 37%	C = 65% P = 66%	C = 100% P = 87%	C = 100% P = 96%

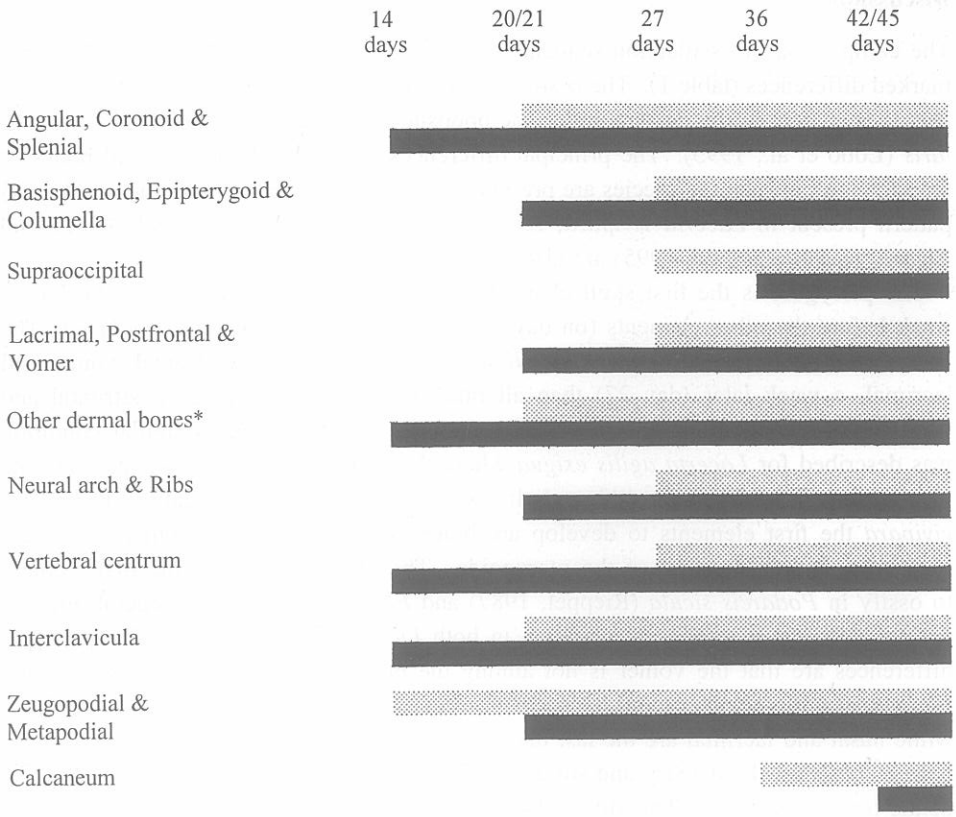

Figure 4. Onset of ossification in two species of *Liolaemus*. Days are since oviposition. * Ectopterygoid, Frontal, Jugal, Maxilla, Palatine, Parietal, Postorbital, Prefrontal, Premaxilla and Supratemporal.

The articular ossification in *Elgaria* starts after birth. As in *L. quilmes* the surangular is one of the first elements to ossify in *Lacerta agilis exigua* and *Elgaria coerulea*. In the first species the prearticular also appears in the same stage with the surangular.

The quadrate and exoccipital start ossification a week before other chondral skull elements in *L. quilmes*. It should be noted that at day 20, only the quadrate and the exoccipital bones could be observed in *L. quilmes*. On the other hand, almost all the chondral skull elements, except the supraoccipital, are ossified at day 21 in *L. scapularis*. In *L. quilmes* the orbitosphenoid is the last chondral bone in starting its ossification. In *Lacerta agilis exigua*, the basisphenoid, basioccipital, exoccipital, quadrate and epiptery-

goid ossify simultaneously. The last three bones are the first to appear in *Lacerta vivipara*. In *Elgaria*, basioccipital, exoccipital and epipterygoid are the first bones while the orbitosphenoid is the last to begin ossification.

In *L. quilmes* the vertebral centra, neural arches and ribs of cervical, dorsal and caudal vertebra start ossification at the same time (on day 27). In all others species the vertebral centra start to ossify before the neural arches. In *L. scapularis* the vertebral centra start ossification before the neural arches and the ribs (on day 14 and 21, respectively), and the transverse process start on day 36. Rieppel (1994) described a distinct antero-posterior gradient of vertebral column ossification in both species of *Lacerta*. In *Liolaemus quilmes* and *L. scapularis* all the vertebral centra began to ossify in the same stage.

In both species of *Liolaemus* the clavicles ossify before all the other pectoral girdle elements. The remaining pectoral girdle elements appear in a synchronic way on day 20-21 (except the interclavicle in *L. scapularis* which appears a week earlier). For both *Lacerta* species the first element to start ossification is the clavicle.

The sternum is present as a fused and single plate on day 20-21 in both species of *Liolaemus*. The pubis is fused anteriorly in the ventral middle line, slightly earlier in *L. scapularis* (on day 14) than in *L. quilmes* (on day 20).

The carpus in *L. quilmes* begins its ossification process around day 36. Ossification is just evident around day 45 in *L. scapularis*.

The metacarpals begin ossification simultaneously around day 14 in *L. quilmes*, while the metacarpal V remains cartilaginous for a longer time in *L. scapularis* (Lobo et al., 1995).

The tarsal elements begin ossification at the same time in both species: the astragalus on day 36 and the calcaneum and distal tarsals III and IV on day 42-45.

The ossification of the carpal and tarsal elements is also delayed in *Lacerta agilis exigua* (stage 39) and *Lacerta vivipara*, where these elements ossify in the newborn.

All the long bones of *L. quilmes* ossify in a synchronic way on day 14. In *L. scapularis* the humerus begins ossification in the middle part of the diaphysis on day 8, the femur on day 14, and all other long bones on day 21. Humerus and femur may also begin ossification earlier also in *L. quilmes*, but we do not have embryos earlier than day 14 so we cannot verify this assumption.

Conclusion

It is particularly interesting to note that differences in ossification sequences were observed between closely related species in both *Liolaemus* and *Lacerta*. However, a difference in the ossification sequences among the different regions of the skeleton is always evident. This observation supports Rieppel's idea (1994: 151-152) of skeletal "compartments": skull; axial skeleton; pectoral and pelvic girdles; stylo and zeugopodial elements of fore and hindlimbs and part of the autopodium (metacarpus and metatarsus) and finally carpus and tarsus. Developing the "compartments" idea further, we suggest

the existence of two skull compartments: the dermatocranium, in which osseous elements appear earlier and the chondral skeleton of the skull, which begins ossification after almost all the dermatocranium is present.

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