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Short Communication

Different embryonic origin of the basipterygoid process in two species of *Lacerta* (Squamata: Lacertidae)

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Abstract: The basipterygoid (or basitrabecular) process in lizards arises in ontogeny as an outgrowth from the posterior portion of the trabecle. The separate origin of the basipterygoid process has been currently recorded only in one lizard, the chamaeleonid *Bradypodion pumilum*. Herein, we describe the ontogeny of the basipterygoid process in two species of *Lacerta*. Although the basipterygoid process in *L. viridis* develops as in most other lizards, the *L. agilis* basipterygoid process arises as an independent nodule of cartilage and it fuses with the trabecle only later in ontogeny.

Key words: Squamata; skull; development; basipterygoid process; anatomy; ontogeny

Introduction

The basipterygoid (or basitrabecular) process of tetrapods participates in basicranial articulation, which involves the articulation of the basisphenoid portion of the braincase and the palatoquadrate (de Beer 1937; Romer & Parsons 1977). Generally, the basipterygoid process arises in ontogeny as an outgrowth from the posteroventral portion of the trabecle. However, in some archosaurs the basipterygoid process arises in ontogeny as a separate element (Klembara 1993). The same may also be true in snakes, as discussed in Bellairs & Kamal (1981).

The basipterygoid process is a distinct structure in the fully-formed chondrocrania of most, if not all, lizards (summary provided in Bellairs & Kamal 1981). In *Lacerta*, development of the chondrocranium of *L. agilis* was first studied by Parker (1879) and later by Gaupp (1900) and de Beer (1930), but the embryonic origin of the basipterygoid process cartilage in *L. viridis* has not been documented.

The main aims of this paper are to: (1) describe the embryonic origin of the basipterygoid process of *Lacerta viridis* and *L. agilis*; and (2) demonstrate the different patterns of ontogeny of the basipterygoid process in these two species.

Material and methods

The specimens of *Lacerta agilis* L., 1758 and *L. viridis* (Laurenti, 1768) were collected in spring 2008 before these species became protected in Ukraine. The specimens are deposited in the histological collections of the I. I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine. The specimens were caught in their natural habitats in Ukraine: *L. agilis* in the Zaporizhska region on the West coast of the "Molochnyi" estuary of the Azov Sea; and *L. viridis* in the Odeska region on the South-West coast of the Dniester estuary. Specimens were placed in terrariums, where they laid eggs; all adult specimens were then returned to their natural habitats. The eggs were incubated on moist vermiculite at 21 ℃. Each 24 hours after laying, eggs were dissected with scissors, and embryos were extracted from the eggshell and fixed in 3% formaldehyde solution.

The developmental stages of the lizard's embryos were determined according to the tables of normal development for *Zootoca vivipara* (Lichtenstein, 1823) (Dufaure & Hubert 1961). In total, 7 embryos of *L. viridis* (stages 32–34) and 17 embryos *L. agilis* (stages 32–35) were studied. To simplify the comparison of the individual developmental stages, we designated the studied developmental stages as Stage 1 to Stage 4. Stage 1 of both species corresponds to the stage 32; stage 2 of *L. viridis* and stage 2a of *L. agilis* correspond to the stage 33; stage 3 of *L. viridis* corresponds to the stage 34 and stages 2b and 3 of *L. agilis* correspond to the stages 34 and 35, respectively (Table 1).

Standard protocols were employed to embed the embryos in a wax-paraffin compound and they were then sectioned in the transverse plane in 5–7 μm and 10–12 μm thickness. The serial sections were stained with hematoxylin-eosin and alcian blue (8gx) according to Steedman (1950). The histological sections were captured as digital images under a Zeiss Axio Imager M1 microscope with Zeiss Axio Vision v.4.63 software. Finally, 3D models were generated using Amira v.5 software.

Table 1. Studied stages of two species of *Lacerta*.

Stages after DH	32	33	34	35
Lacerta viridis	Stage 1	Stage 2	Stage 3	–
Lacerta agilis	Stage 1	Stage 2	Stage 2a	Stage 3

Explanations: DH – Stages according to the staging system after Dufaure & Hubert (1961).

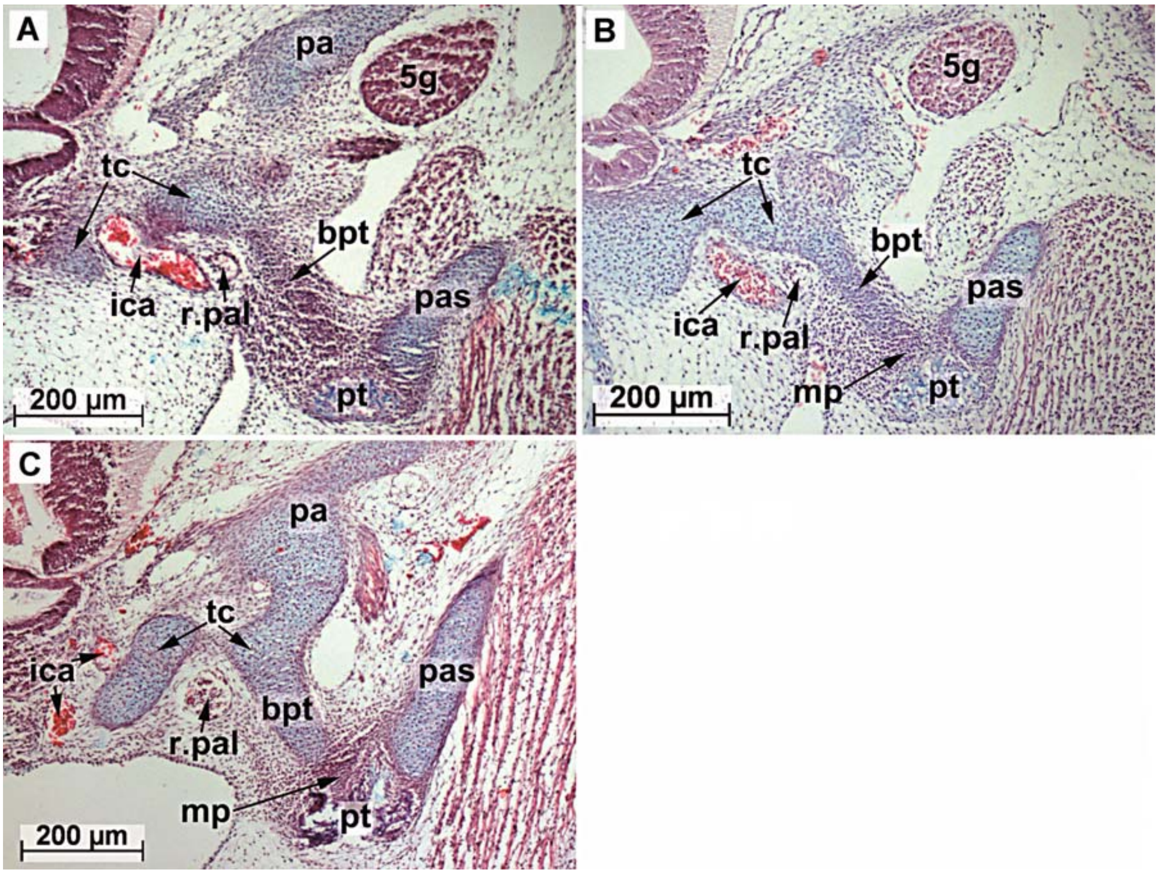

Fig. 1. *Lacerta viridis*: transverse histological sections through the region of the basipterygoid process (shown the left side of the head) in Stage 1 (A), Stage 2 (B) and Stage 3 (C). 5g – ganglion of trigeminal nerve; bpt – basipterygoid process; ica – internal carotid artery; mp – pterygoid meniscus; pa – antotic pila; pas – ascending process; pt – pterygoid; r.pal – palatine ramus of facial nerve; tc – trabecle.

Results

In this study, early development of the basipterygoid process is described in two species of *Lacerta*. This is documented on stages 1 to 3 of *L. viridis* and stages 1, 2A, 2B and 3 of *L. agilis*. Initially, the development of the basipterygoid process of *L. viridis* is described, documenting the embryonic origin of this structure as it occurs in most lizards. Then, the embryonic origin of the basipterygoid process is described in *L. agilis* showing the differences in embryonic origin of the basipterygoid process in this species of *Lacerta* relative to *L. viridis* and most lizards. The Stage 3 of *L. viridis* is presented to show the similarity in the fully formed condition of the basipterygoid processes of *L. agilis* and *L. viridis*.

Lacerta viridis (Fig. 1)

In Stage 1, the posterior portion of the trabecle is continuous with the mesenchymal ventrolateral outgrowth representing the future basipterygoid process (Fig. 1A). The internal carotid artery and the palatine ramus of the facial nerve lie ventral to the posterior portion of the trabecle. The distal end of the outgrowth joins the pterygoid bone and the basal portion of the ascending process, and the trabecle and ascending process are cartilaginous. The pterygoid meniscus is not present at this stage.

In Stage 2, whereas the distal portion of the basipterygoid process still contains mostly mesenchymal cells, cartilage cells are well developed in the proximal portion (Fig. 1B). The pterygoid meniscus appears as a small condensation of cartilaginous cells.

In Stage 3, the basipterygoid process is completely cartilaginous (Fig. 1C). A stripe of tissue divides its distal end from the pterygoid and the pterygoid meniscus, which forms a small cartilaginous nodule, is firmly attached to the pterygoid.

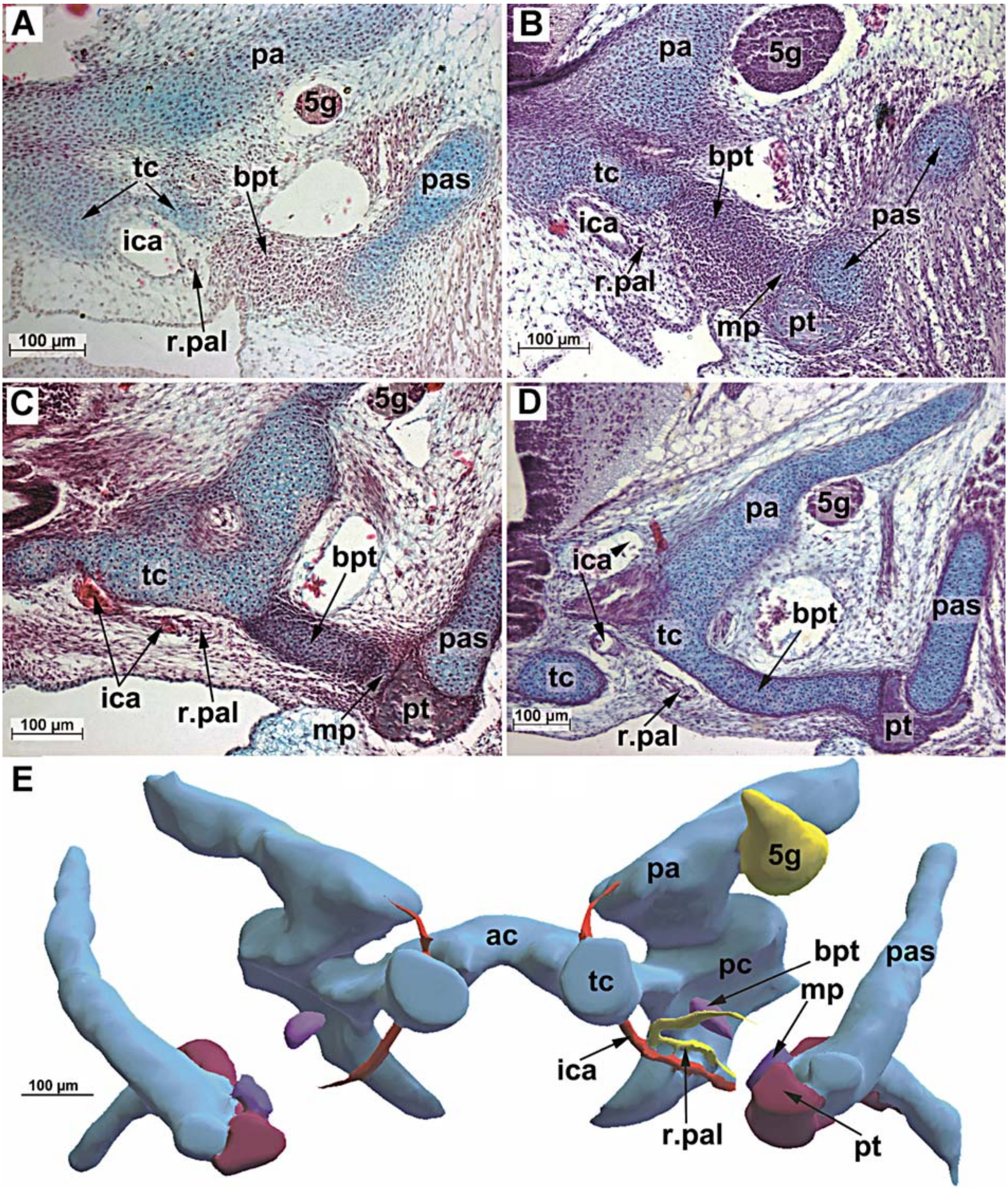

Fig. 2. *Lacerta agilis*: transverse histological sections through the region of the basipterygoid process (shown the left side of the head) region in Stage 1 (A), Stage 2A (B), Stage 2B (C) and Stage 3 (D). E – virtual model of the region of the basipterygoid articulation in Stage 2A in anterior view. 5g – ganglion of trigeminal nerve; ac – acrochordal cartilage; bpt – basipterygoid process; ica – internal carotid artery; mp – pterygoid meniscus; pa – antotic pila; pas – ascending process; pc – parachordal cartilage; pt – pterygoid; r.pal – palatine ramus of facial nerve; tc – trabecle.

Lacerta agilis (Fig. 2)
In Stage 1, a condensation of mesenchymal cells is observed where the future basipterygoid process will form (Fig. 2A). It is positioned between the posteroventral portion of the trabecle and the basal portion of the ascending process. Both these structures are cartilaginous at this stage; the pterygoid meniscus is absent. The internal carotid artery and the palatine ramus of the facial nerve lie immediately ventral to the trabecle and medial to the basipterygoid process anlage.

In Stage 2A, a dense cluster of chondrocytes is present in the mesenchymal cell condensation indicating the development of the future basipterygoid process. It is located nearby the posteroventral portion of the trabecle, but has no connection with the trabecle in any studied planes (Figs 2B, E). A dorsoventrally orientated stripe of chondrocytes of the pterygoid meniscus is now present ventrolaterally to the basipterygoid process. An emarginated pterygoid bone is present between the pterygoid meniscus medially and the basal portion of the ascending process laterally.

In Stage 2B, the basipterygoid process is fully cartilaginous, and easily distinguished from the surrounding tissue, with its medial end joining the ventrolateral portion of the trabecle (Fig. 2C). Although the pterygoid meniscus and the basipterygoid process are now at the same stage of development, both these structures are much less developed than the cartilage in the trabecle and ascending process.

In Stage 3, the basipterygoid process is completely fused with the trabecle with no visible boundary between these structures (Fig. 2D). The distal end of the basipterygoid process joins the medial wall of the pterygoid which is well developed, and the ascending process fits into the distinct pit in its dorsal wall. The pterygoid meniscus is a small, flattened cartilaginous element interposed between the basipterygoid process and the medial surface of the pterygoid (this is not illustrated herein).

Discussion

Previous studies have suggested that the basipterygoid process of lizards develops as an outgrowth from the posterolateral portion of the trabecle. The only recorded exception is the chamaeleonid *Brachypodion* (= *Pumilum*) *pumilum* (Gmelin, 1789), in which the basipterygoid process commences ontogenetic development as a separate mesenchymal blastema (Visser 1972).

Research into *Lacerta* skull development has a long history. According to Parker (1879), the basipterygoid process (his "basi-pterygoid") of *L. agilis* grows directly from the trabecle. Gaupp (1900) studied the fully formed chondrocranium of *L. agilis*. In this ontogenetic stage, the basipterygoid process is fully developed as a process extending lateroventrally from the posteroventral portion of the trabecle (Gaupp 1900). De Beer (1930) determined that the basipterygoid process of *L. agilis* appears as a small lateral projection from the hindmost region of each trabecle. More recently, Yarygin (2010) interpreted the basipterygoid process of *L. agilis* as a structure of dual origin, with: (i) the distal portion having an independent centre of chondrification, named the "basipterygoid cartilage"; and (ii) the proximal portion appearing as an outgrowth from the posterolateral portion of the trabecle.

Our results show that the basipterygoid process of *Lacerta viridis* develops as in most other lizards. However, contrary to most other studies, this is not the case in *L. agilis*. We found that the precursor of the basipterygoid process appears as an independent condensation of mesenchymal cells. Later, in our 2A and 2B developmental stages, the cartilaginous anlage of the basipterygoid process is still not fused with the trabecle. The cartilaginous basipterygoid process fuses with the trabecle only in the most advanced developmental stage. As previously mentioned, this similar *L. agilis* basipterygoid process embryonic origin was also described in the chamaeleonid *Bradypodion pumilum* (Visser 1972). Thus, there are only two cases of independent embryonic origin of the basispterygoid process known; and only one within the genus *Lacerta*. Because the basipterygoid process in all other lizards arises in ontogeny as an outgrowth of the trabecle, this condition has to be considered primitive, and independent ontogenetic origin of the basipterygoid process must be considered a derived state. Because *B. pumilum* and *Lacerta agilis* occupy different positions on the phylogenetic tree (e.g., Gauthier, 2012), it means that the origin of the basipterygoid process from the independent condensation of the mesenchymal tissue in ontogeny developed independently in the evolution of these two lizards.

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