Zoology 118 (2015) 255–280

Contents lists available at ScienceDirect

Zoology

journal homepage: www.elsevier.com/locate/zool

Bony skull development in the Argus monitor (Squamata, Varanidae, *Varanus panoptes*) with comments on developmental timing and adult anatomy

Ingmar Werneburg[a,b,*], Katja M. Polachowski[c], Mark N. Hutchinson[d,e]

[a] *Museum für Naturkunde, Leibniz-Institut für Evolutions- & Biodiversitätsforschung, Humboldt-Universität zu Berlin, Invalidenstraße 43, D-10115 Berlin, Germany*
[b] *Institut für Biologie, Humboldt-Universität zu Berlin, Philippstraße 13, D-10115 Berlin, Germany*
[c] *Paläontologisches Institut und Museum der Universität Zürich, Karl-Schmid-Strasse 4, CH-8006 Zürich, Switzerland*
[d] *South Australian Museum, North Terrace, Adelaide, SA 5000, Australia*
[e] *School of Earth and Environmental Sciences, University of Adelaide, Adelaide, SA 5005, Australia*

ARTICLE INFO

Article history:
Received 13 October 2014
Received in revised form 12 February 2015
Accepted 12 February 2015
Available online 19 May 2015

Keywords:
Lizards
Heterochrony
Ossification
Skeletogenesis
Embryogenesis

ABSTRACT

Varanids represent one of the most charismatic squamate clades and include the largest living lizards; however, little is known about their embryonic development and what it might reveal about the origin of their derived anatomy. In the present study, we describe external organogenesis and skull formation of *Varanus panoptes* in great detail. We compared timing of ossification with the patterns seen in other squamates, using three major hypotheses of squamate interrelationship as phylogenetic templates, and were able to detect heterochronic patterns in ossification that are associated with adult anatomy in each phylogeny. However, we refrain from preferring one topology given the current lack of congruence between molecular and morphological data sets. The rule of thumb that early appearance of developmental characters is correlated to larger prominence in adults is critically discussed and we conclude that such simple correlations are the exception rather than the rule. The entanglement of developmental processes detected herein highlights the non-independent formation of adult characters that are usually treated as independent in phylogenetic studies, which may bias the output of such studies. Our comprehensive descriptions of embryonic development may serve as a resource for future studies integrating the complex processes of embryogenesis into broad-scale phylogenetic analyses that are likely to show that change in embryonic timing is one of the major sources of morphological diversification.

1. Introduction

The growing interest in studying non-model organisms and the development of quantitative approaches to study ontogenetic data have led to more and more descriptive studies of diverse vertebrate species in recent years. Among the squamate reptiles (lizards and snakes), several recent works have expanded the comparative bases of skeletogenesis and external morphology (e.g., Maisano, 2002a,b; Sanger et al., 2008; Wise et al., 2009). For some squamate lineages sufficient developmental series have enabled their use in comparative studies of developmental timing in evolution (e.g., Maisano, 2002c; Hugi et al., 2012).

The taxon Varanidae (monitor lizards, goannas) includes strikingly large, predatory lizards that have received intensive interest regarding their anatomy and ecology as an important taxon for understanding the evolution of squamates (Fig. 1) (Werneburg and Sánchez-Villagra, 2015). In particular, there is evidence from a variety of sources that varanids and related, mostly extinct clades such as mosasaurs may have a close phylogenetic link to the origin of snakes, one of the major unresolved problems in understanding the evolutionary history of the Squamata. However, this idea remains controversial because different studies have arrived at conflicting conclusions regarding the phylogenetic position of varanids and snakes (Fig. 1). One hypothesis places the varanids as the closest living relatives of snakes (e.g., McDowell and Bogert, 1954; Estes et al., 1988; Forstner et al., 1995; Lee, 1998, 2005a, 2009). Alternative hypotheses place Varanidae in more distantly related positions relative to the Serpentes (crown snakes), all with burrowing and at least partly limbless clades identified as potential sisters of Serpentes, namely dibamids (Dibamidae), amphisbaenians (Amphisbaenia), and skinks (Scincidae) (summarised by Conrad, 2008; Gauthier et al., 2012). It is noteworthy

* Corresponding author.
E-mail address: i.werneburg@gmail.com (I. Werneburg).

http://dx.doi.org/10.1016/j.zool.2015.02.004
0944-2006/

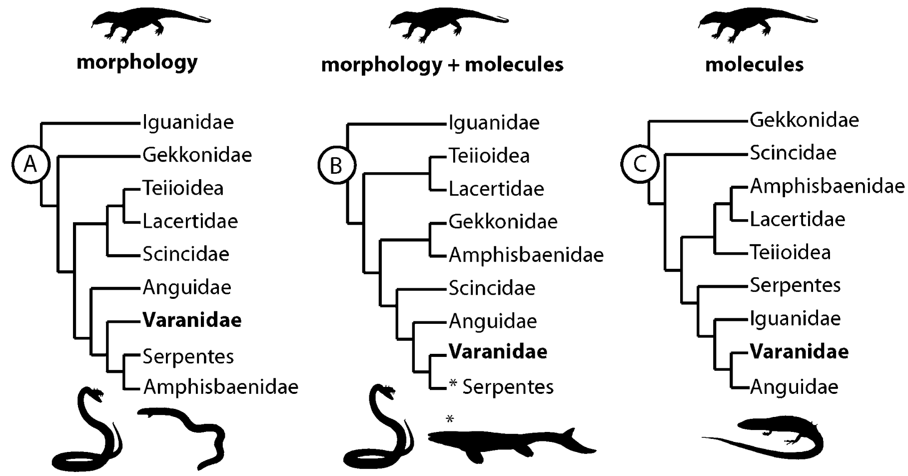

Fig. 1. The three major hypotheses for the position of Varanidae within Squamata following (A) Gauthier et al. (2012), (B) Lee (2005a), (C) Pyron et al. (2013). *In the study of Lee (2005a), snakes are situated within a paraphyletic clade of fossil marine anguimorphs to which the illustrated mosasaurid belongs.

that morphological and molecular studies differ in which of these conflicting hypotheses of relationships they propose; but the hypotheses also vary among morphological studies themselves. Arguments differ regarding character correlation, especially skeletal characters associated with limbless burrowing and feeding (Müller et al., 2011; Gauthier et al., 2012). In such cases of conflicting phylogeny, developmental data can contribute to the solution of the problem by addressing primary homology hypotheses (Freeman and Herron, 2007; Joyce et al., 2013; Werneburg et al., 2013a) or by generating 'characters' of relative timing that can be analysed in cladistic studies (Velhagen, 1997; Maxwell and Harrison, 2009; Werneburg and Sánchez-Villagra, 2015).

Studies on the development of Varanidae are, however, very scarce (Gregorovicova et al., 2012) and there is only a small amount of descriptive literature dealing with the skeletogenesis of the skull (Shrivastava, 1964a,b). The large body size and tropical distribution of varanids has made them less easy to obtain and maintain in laboratories. This has made it difficult for researchers to acquire series of eggs from which embryonic sequences could be obtained. We have been able to obtain two clutches of eggs of a large Australian species, the Argus monitor (yellow-spotted goanna) *Varanus panoptes* (Storr, 1980). This is a moderately large varanid, with the snout–vent length (SVL) of mature adults ranging from 310 to 670 mm (males grow larger than females) and hatchling SVL reported as about 105–106 mm (Shine, 1986). *V. panoptes* is a carnivore that mostly feeds on terrestrial vertebrate prey, mainly mammals and reptiles, but also on large invertebrates and aquatic prey such as fish and frogs (Shine, 1986).

The aim of the present paper is to provide new data on the external morphology and cranial bone formation during embryonic development in a member of the Varanidae. We characterise an embryonic series of *V. panoptes* by describing external morphology based on the Standard Event System (Werneburg, 2009). Micro-computed tomography (μCT) scanning was used in order to document the ossification sequence of the whole skeleton, and to provide data on which to base a detailed description of skull development. Currently there are only few comparable data on the developmental timing of external embryological characters in squamates. Only very few detailed descriptions of skeletogenesis in squamates exist for comparisons. Hence, we concentrate on the onset of the ossification sequence to provide a basis for broad-scale phylogenetic analyses. We compare the timing of these events to hypothesise potential correlations to adult anatomy and discuss the validity of the '*early equals important*' rule, which states that characters that appear earlier in ontogeny appear larger or are more complex in the adult (Mehnert, 1897, 1898; Huxley, 1932; Sánchez-Villagra et al., 2008; Maxwell, 2009; Maxwell and Larsson, 2009; Werneburg and Sánchez-Villagra, 2015).

2. Materials and methods

2.1. Specimens

The embryonic series of 15 specimens of *V. panoptes* were obtained with the help of the Australian Reptile Breeding Facility in Echunga, South Australia. The embryos stem from two different clutches produced by females held at the facility. Eggs were incubated at a constant temperature of 29.5 °C. At a series of intervals, starting at 9 days post-oviposition and concluding at day 186 (Table 1), eggs were removed and taken to the South Australian Museum, Adelaide (Australia), where the embryos were sacrificed with sodium pentobarbital, dissected from the eggs and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 24 h. After fixation they were preserved in 70% EtOH and are now housed in the laboratory collection of the Paläontologisches Institut der Universität Zürich (PIMUZ lab), Switzerland. To simplify matters, the specimens are named in the text as V1–V15 (Table 1 and Fig. 2). Specimen V6 shows some deformation, which may have been due to accidental damage or a developmental defect.

The weight of all embryos was measured with a precision balance. For weighing, the embryos were taken out of the 70% EtOH solution and placed on a tissue. They were superficially dried with the tissue until they did not appear glossy anymore and immediately placed on the balance. Because ethanol evaporates constantly out of the embryos, there was no constant value. To enable comparability, the first value was always recorded. It was measured to the nearest 0.01 g. Snout–vent length (SVL) was measured as the distance between the tip of the snout and the end of the cloaca. It was measured with a ruler along the ventral side of the body. Total length (TL) of all embryos was measured as the distance between tip of the snout and tail tip. It was measured, to the nearest 1 mm, with a ruler along the dorsal line of the body. Because the posture of the embryos was curved, they were rolled along the edge of the ruler during measurement.

2.2. External morphology

The description of the external morphology is based on the Standard Event System (SES) (Werneburg, 2009). This system provides a standardised protocol to document developmental characters in