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Integrating developmental biology and the fossil record of reptiles

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ABSTRACT **Numerous new discoveries and new research techniques have influenced our understanding of reptile development from a palaeontological perspective. They suggest for example that transition from mineralized to leathery eggshells and from oviparity to viviparity appeared much more often in the evolution of reptiles than was previously thought. Most marine reptiles evolved from viviparous terrestrial ancestors and had probably genetic sex determination. Fossil forms often display developmental traits absent or rare among modern ones such as polydactyly, hyperphalangy, the presence of ribcage armour, reduction of head ornamentation during ontogeny, extreme modifications of vertebral count or a wide range of feather-like structures. Thus, they provide an empirical background for many morphogenetic considerations.**

KEY WORDS: *evo-devo, palaeontology, embryology, development, ontogeny*

Introduction

Fossils are our main source of information about extinct organisms and ancestry of modern groups. Since the publication of Darwin's *On the Origin of Species*, people raised doubts about the utility of fossils in reconstruction of evolutionary processes (Darwin 1859). This was mainly due to the incompleteness of the fossil record. In fact, our picture of the history of life is far from being fully satisfactory. Many environments and taxonomic groups have poor preservation potential. Fossilization occurs mainly in hard, mineralized parts of the body. This is especially problematic for delicate embryos. Despite that, palaeontology and developmental biology have a long common past. As early as in nineteenth century, embryology had great influence on contemporary palaeontology. Palaeontology also influences developmental biology, because fossil record poses questions that can be tested in developmental studies (Hall 2002; Thewissen *et al.,* 2012). However, last decades showed that fossil record is much more informative then it was previously suspected. It is especially true for reptiles, where significant advances were made (e.g. Delfino and Sánchez-Villagra 2010). Our knowledge about evolution of their development increased in recent years thanks to the remarkable findings of embryos inside female skeletons (e.g. Ji *et al.,* 2010; Motani *et al.,* 2014) or within mineralized eggshells (e.g. Chiappe *et al.,* 2004; Kundrát *et al.,* 2008; Araújo *et al.,* 2013), sometimes even with preserved organic

remains (Reisz *et al.,* 2013), and development of new research techniques which allow us to investigate embryonic fossils which were previously inaccessible, like embryos *in ovo* (e.g. Balanoff *et al.,* 2008; Fernandez *et al.,* 2012).

It should be noted that distinguishing an embryo from the last meal might be very difficult. For example, a skeleton of a lizard preserved inside the ribcage of a theropod *Compsognathus* was first interpreted as an embryo of that dinosaur (see Delfino and Sánchez-Villagra 2010). Similarly, small skeletons enclosed within skeletons of adult *Coelophysis* theropods were sometimes regarded as embryos, subsequently assumed to be proof of cannibalism (cf. Coombs 1982) but some were recently reinterpreted as belonging to the basal crocodylomorph *Hesperosuchus* (Nesbitt *et al.,* 2006; but see Gay 2010). The lack of chemical and mechanical damage (occurring in digestion), association between adult and a smaller skeleton, as well as specific anatomical traits suggest that the smaller individual is an embryo (Sánchez 2012). Sometimes, taxonomic identification of an embryo may be relatively easy, when embryo has traits almost identical to adult individuals (Kear and Zammit 2014) but in some cases even embryos may already be sexually dimorphic (Xue *et al.,* in press), which may further hinder

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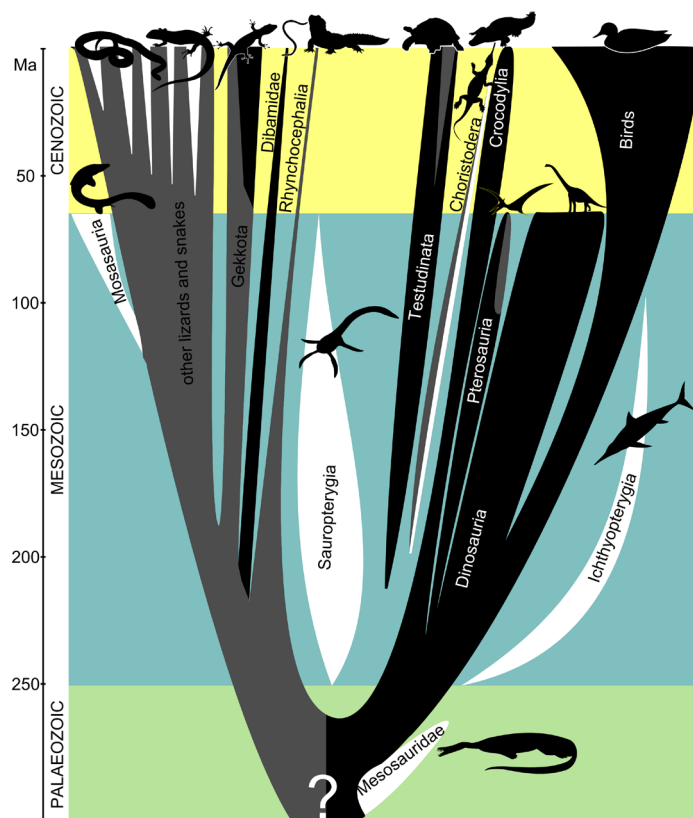
taxonomic identification. Unambiguous cases, such as fossil of a birth (e.g. Motani *et al.,* 2014), are exceptional.

We have also to admit that most of fossil embryos represent mainly large and/or aquatic forms. This bias is due to at least three reasons: 1) organisms living in water have greater preservation potential than terrestrial ones; 2) maternal body or mineralized eggshell provide a "shelter" for embryo remains; 3) large animals have relatively large and better ossified embryos. Thus, most of the research concentrate on dinosaurs and marine reptiles but have interesting implications for developmental biology of all amniotes – also the recent ones (see below). Fossilized embryos, juveniles and adults can test hypotheses concerning the antiquity of developmental processes (e.g. Motani *et al.,* 2014) and origin of morphological traits observed in living animals (e.g. Lyson *et al.,* 2013). They also provide information about the level of homology between similar structures (e.g. Buchwitz and Voigt 2012; Godefroit *et al.,* 2014) and answer how unique are features observed in modern taxa (Chen *et al.,* 2014). In this review we highlight some of the recent advances in the field of developmental palaeoherpetology.

Eggs

Eggshells are more common than embryos in the fossil record. They provide useful information about embryos size and number – the reproductive strategy of their parents (O'Connor *et al.,* 2014). Moreover, they show the environment in which these embryos

Fig. 1. A simplified reptile phylogeny showing major groups discussed in the article. *Groups in black colour lay eggs with rigid, well mineralized eggshell. Grey colour denotes groups with leathery, poorly or non-mineralized eggshell. Groups in white are viviparous. Note that some clades show variation in reproductive strategies.*

developed (Hou *et al.,* 2010).

The oldest known fossil eggs are much younger than the oldest known amniote fossil but, surprisingly, they are also significantly younger that the oldest known amniote embryos (Laurin *et al.,* 2000; Piñeiro *et al.,* 2012), despite the fact that first amniotes are generally thought to be oviparous (see Sander 2012 and discussion below). The oldest known eggs are no older than Jurassic in age (Reisz *et al.,* 2013), whereas amniote remains are currently known from rocks no older than about 314 million years (Reisz and Müller 2004; Benton and Donoghue 2007). The most common explanation of this paradox is that first amniotes could lay small eggs with soft, poorly or non-mineralized membranes (Laurin *et al.,* 2000). Such situation is observed in many modern and extinct groups of amniotes (e.g. Packard and DeMarco 1991; Lü *et al.,* 2011), so it seems reasonable that it is the primitive condition.

Attempts to reconstruct the evolution of the eggshell might be difficult because of uncertain phylogenetic position of many reptile groups and very sparse early fossil record (Fig. 1). Thus, it is difficult to say, for example, when and how many times mineralized eggshell evolved. Such membrane protects the embryo, serves calcium for skeletal growth and takes part in gas and chemical changes (Grellet-Tinner *et al.,* 2014). Well mineralized eggshell is known in birds, crocodylians, many turtles and some lizards among modern reptiles (Pike *et al.,* 2012; Sander 2012). Most probably it is primitive for archosaurs, as it is present in both recent birds and crocodylians (Zelenitsky 2006; Marzola *et al.,* 2015). Among archosaurs, only some pterosaurs from China (Fig. 2) are known to have non-mineralized eggshell (Unwin and Deeming 2008; Lü *et al.,* 2011). However, findings of pterosaur eggs from South America show thin calcified shell (Chiappe *et al.,* 2004; Grellet-Tinner *et al.,* 2014), as well as *Hamipterus* eggs from China (Wang *et al.,* 2014). Thus, condition in some Chinese forms was secondary and was probably a response to a different environmental factors and reproductive strategies. Similar variability is observed in modern gekkotans (see Grellet-Tinner *et al.,* 2014 and references therein).

Most molecular analyses suggest that turtles are more closely related to archosaurs than to lizards (see review in Lee 2013 and references therein). This gives some credit to hypothesis that eggshells of turtles and archosaurs may have some degree of homology, despite the microstructural differences (Packard and DeMarco 1991; Hou *et al.,* 2010). However, phylogenetic position of turtles remains controversial and does not allow us to make any definite statements. Similar situation is with the aquatic, extinct Choristodera. Like turtles, they are placed phylogenetically close to archosaurs, or lepidosaurs, or before the split of those two lineages. In contrast to most turtles, they produced leathery eggs, which makes all those considerations more complicated (Hou *et al.,* 2010).

Dibamidae and Gekkota – two most basal squamate clades, according to molecular data (e.g. Pyron and Burbrink 2014) – are groups whose most members have well calcified eggshells (Fig. 1). Despite that, ancestral condition for Gekkota is probably a parchment-shelled egg (Pike *et al.,* 2012). The closest relatives of lizards, rhynchocephalians, also lay soft-shelled eggs, which support the soft eggs as an ancestral lepidosaur condition (Fig. 1). Mineralized eggshell is probably ancestral for reptiles in general but it is yet unknown whether the leathery eggshell present in rhynchocephalians, choristoderes, most lizards and turtles is a secondary trait or a retained ancestral condition (Marzola *et al.,*

Fig. 2. A parchment-shelled egg of the pterosaur *Darwinopterus* *(specimen ZMNH M8802). Pterosaurs are the only known archosaurs to have leathery, poorly or non-mineralized eggshell. Image courtesy of David M. Unwin.*

2015; Fig. 1).

Alternatively, calcified eggs evolved multiple times independently. This is supported by microstructural differences between eggshells of squamates, archosaurs (composed mostly of calcite) and turtles (mostly aragonite) (Packard and DeMarco 1991). Variable structure of eggshell among gekkotans, pterosaurs and turtles also gives some credibility to that hypothesis.

Most of the research concerning fossil eggs is focused on dinosaurs because they had usually large eggs with rigid, well calcified shells. They exhibit great diversity of egg shapes and ornamentation. Linking each type to specific taxonomic group is still an ongoing work in palaeontology but much has already been done, especially concerning reptile-bird transition. Crocodylians have two functional oviducts and simultaneously ovulate all eggs. They produce hyper-ellipsoidal, symmetrical eggs (Zelenitsky 2006). This is probably primitive condition for all archosaurs. Advanced theropods, such as oviraptorosaurs, ovulated only one ovum per oviduct at a time. They already had asymmetrical eggs, resembling birds in this respect while retaining other primitive traits (Zelenitsky 2006). Functionality of the right oviduct was lost near the dinosaur-bird transition. Smaller clutches of relatively larger eggs evolved later in birds. This sequence was tested by discoveries of nests and eggs of advanced dinosaurs and by ovarian follicles and eggs of primitive birds. The first birds laid large number of relatively small paired eggs. It could be linked to their lower metabolic rates and slower yolk deposition compared to advanced birds (O'Connor *et al.,* 2014). They also did not move their eggs during incubation as modern forms do (Zelenitsky 2006).

Although the first reptiles were oviparous, the oldest known fossil eggs are significantly younger than the body fossils. First reptiles probably produced mineralized eggshell. Some lepidosaurs, choristoderes, pterosaurs and turtles had poorly mineralized, leathery eggshell but it is yet unknown if this is a retained primitive condition or a secondary loss. There can be some variation within a clade – for example, among pterosaurs there are known eggs with non-mineralized shell, as well as eggshells with calcareous layer, albeit very thin. Fossil eggs and their configuration within maternal body can give us important information about reproductive biology of extinct reptiles – it suggests that the functionality of the right oviduct was lost probably near the dinosaur-bird transition.
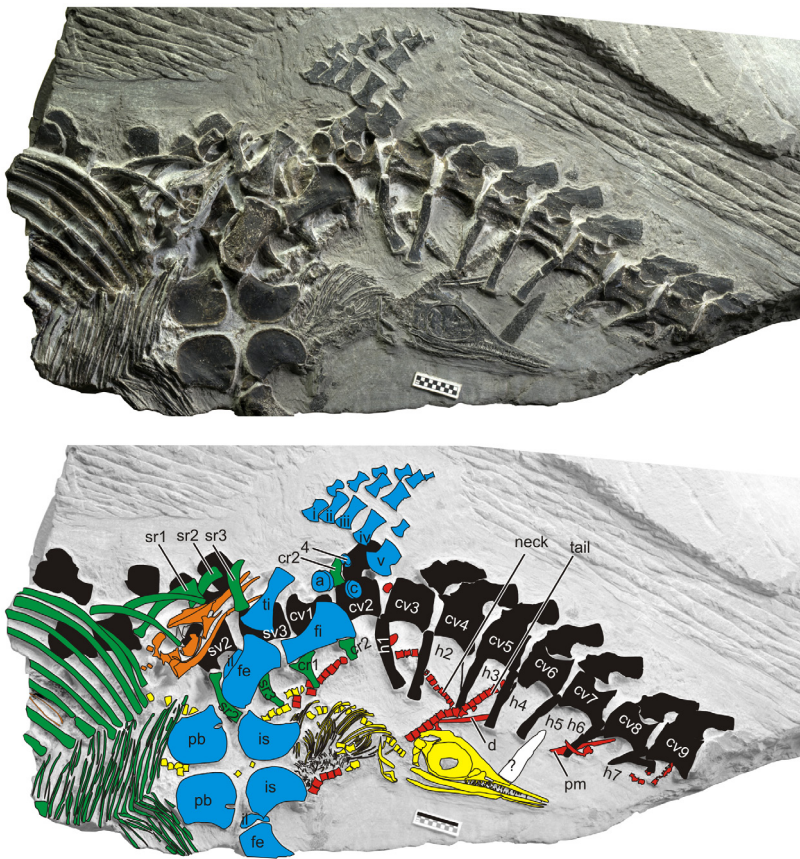
Viviparity

The egg is one of the key innovations in amniote evolution, however, in reptiles there were numerous transitions to viviparity – more than in other groups of amniotes combined. Viviparity is present in more than hundred different reptile lineages (e.g. Blackburn 2006; Pyron and Burbrink 2014), thus making reptiles an ideal group for studying the evolution of viviparity. What do fossils say on that subject?

Basically all palaeontologists agree that first reptiles were oviparous animals (Sander 2012). One of the explanations given for that hypothesis is that among extant lizards there are no known examples of transition from viviparity to oviparity (e.g. Sander 2012). However, recent phylogenetic studies on squamate reptiles suggest that parity mode is a more labile trait. Purported shifts from viviparity to oviparity are suggested at both interspecific (Surget-Groba *et al.,* 2006) and at higher taxonomic level (Pyron and Burbrink 2014). The latter one, however, is based on the assumption that transition from viviparity to oviparity is equally probable as a converse one, which is not the case (e.g. Griffith *et al.,* in press). Some cases of regaining oviparity from viviparous ancestors seem to be well supported (e.g. King and Lee, 2015), but they are exceptions rather than rule. Thus, there is no reason to suspect that the first reptiles were not egg-laying (Sander, 2012). Interestingly, the oldest known egg is much younger than the oldest known embryo of a live-bearing reptile (e.g. Sander 2012; see above). The early fossil record of reptile eggs and embryos is very scarce – the oldest known embryos (about 280 million years old) and the only found in Palaeozoic rocks belong to *Mesosaurus tenuidens*, a member of Mesosauridae, a group of basal, aquatic reptiles. Several mesosaur embryos are known – one isolated, one preserved within skeleton of adult individual and several perinatal specimens that show association with adults. Females probably carried only one or two embryos. These fossils suggests that mesosaurs were viviparous or laid eggs with well developed embryos (Piñeiro *et al.,* 2012). These two hypotheses are not necessarily mutually exclusive, as in extant reptiles there are at least few species that have both oviparous or viviparous populations (e.g. Surget-Groba *et al.,* 2006).

Fossil record of viviparous reptiles is much more rich in Mesozoic than in Palaeozoic but restricted mostly to marine animals. Viviparity has been described in several fossil groups: ichthyopterygians, sauropterygians, choristoderes and two lineages of lizards. Several common trends can be observed.

Viviparity probably evolved in terrestrial ancestors of aquatic groups. The embryo of an Early Triassic basal ichthyopterygian *Chaohusaurus* is preserved in a head-first birth posture (Fig. 3, 4) which is typical for land amniotes. In later ichthyosaurs the embryos were born tail-first (Fig. 4), which is an adaptation to fully marine lifestyle (Motani *et al.,* 2014). Similarly, in the Late Triassic basal sauropterygian *Keichousaurus*, the head of an embryo is caudally oriented, as in *Chaohusaurus* (Cheng *et al.,* 2004). The same is true for the Cretaceous freshwater choristodere *Hyphalosaurus* (Ji *et al.,* 2010). The embryos of basal mosasaur lizard *Carsosaurus* were first interpreted as born head-first (Caldwell and Lee 2001) but later it was suggested that such birth posture is unlikely (Motani *et al.,* 2014). Caudal orientation of the embryo skull at birth was proposed to be a reason of high mortality during parturition, as suggested for all these forms (Caldwell and Lee 2001; Cheng *et*

Fig. 3. Embryo of a basal ichthyopterygian *Chaohusaurus* preserved in a head-first birth posture, typical for land vertebrates *(from Motani* et al., *2014). Image available under Creative Commons Attribution 3.0 licence.*

al., 2004; Ji *et al.,* 2010; Motani *et al.,* 2014).

Curled up embryos are known in *Carsosaurus* (Caldwell and Lee 2001). In one *Hyphalosaurus* female, several embryos are curled up, probably still being in their soft-shelled eggs (Hou *et al.,* 2010), but two posterior-most embryos are straightened which is probably their birth posture (Ji *et al.,* 2010). Also embryos of basal sauropterygians *Neusticosaurus* and *Lariosaurus* are preserved with curled vertebrae (Sander 1988; Renesto *et al.,* 2003), in contrast to closely related *Keichousaurus* where there is no sign of curling of embryos (Cheng *et al.,* 2004) which suggests different reproductive strategies among basal sauropterygians. Ichthyosaur embryos were in straight posture except early stages of development (Maxwell and Caldwell 2003).

Most fossil viviparous reptiles were giving birth to multiple progeny. If the embryos are located on both sides of the maternal body cavity, it suggests that both oviducts were functional. Such arrangement of embryos is present in ichthyopterygians (Maxwell and Caldwell 2003), *Keichousaurus* (Cheng *et al.,* 2004), *Hyphalosaurus* (Ji *et al.,* 2010), *Carsosaurus* (Caldwell and Lee 2001) and the Cretaceous terrestrial, though strongly related to aquatic habitats, lizard *Yabeinosaurus* (Wang and Evans 2011). An exception is the short-necked plesiosaur *Polycotylus* which probably gave birth to single but relatively very large progeny (Fig. 4). In other aquatic viviparous reptiles, at the time of birth the neonates attain no more than 30% of maternal body length, while in *Polycotylus* it is more than 40%. This suggests that in plesiosaurs a K-selected reproductive strategy

evolved, i.e. giving birth to large but few progeny (O'Keefe and Chiappe 2011).

Another group of marine reptiles are metriorhynchoid crocodyliforms. There is no direct evidence of their parity mode but there are reasons to suspect that they were also viviparous. In the most derived forms, such as *Cricosaurus*, the deltopectoral crest on a humerus was lost, as well as pisiform in the wrist, which resulted in the changes in forelimb musculature and acted as adaptations to exclusively marine lifestyle. These characters and the reduction of limb girdle size suggest that at least some metriorhynchoids could not walk on land – for example to lay eggs. Moreover, their pelvis had increased diameter (Young *et al.,* 2010), as in *Keichousaurus*, which may have been an adaptation to speeding up the birth process.

Viviparity seems to evolve almost concurrently with placentation in squamates – the clade to which all extant viviparous reptiles belong (Blackburn 2006). However, among known examples of fossil viviparous reptiles, only mosasaurs and *Yabeinosaurus* are squamates and sauropterygians are possibly lepidosauromorphs, while other groups are more distantly related. It is therefore unclear whether the model of evolution of viviparity in squamates can be applied to other groups. The fossil record does not give an answer to this problem because fossilized placentae are not yet known in extinct reptiles (although one example exists in the Devonian placoderm fish), even in well-preserved fossils due to the absence of relevant soft tissues (e.g. Motani *et al.,* 2014; see a more thorough review in Blackburn and Sidor 2015).

Does the fact that in the fossil record, viviparous taxa are more abundant in water and especially marine sediments suggest that viviparity among terrestrial reptiles is – in most cases – a relatively young phenomenon, as previously suggested? (e.g. Blackburn 2006). Not necessarily, because in water there is a higher fossilization potential. The bias would therefore be taphonomic. This is also one of the reasons why fossilized amphibian ontogenies are more common in the fossil record than reptile ontogenetic series (Fröbisch *et al.,* 2010).

Fossil evidence demonstrates that viviparity was present early in reptile evolution. Presently, all viviparous reptiles are squamates but in the Mesozoic giving birth to live young was more widespread. It was present mostly in aquatic clades such as ichthyosaurs, sauropterygians (Fig. 4) or choristoderes but at least one example of viviparous terrestrial lizard is known. This suggests a complex evolutionary history of viviparity, especially in the context of recent phylogenetic studies which suggest numerous transitions from oviparity to viviparity (and *vice versa?*) in squamates.

Sex determination

Sex determination is a crucial phenomenon in population biology and ecology and – in consequence – evolution. In reptiles, sex determination mechanisms are much more diverse than in other groups of amniotes – birds and mammals, where sex is determined only genetically. Except genetic (GSD), reptiles exhibit also temperature-dependent sex determination (TSD), where temperature during embryogenesis decides about the sex of an embryo. However, one should note that there is no solid barrier between GSD and TSD;

even in species with sex chromosomes, temperature may play some role in determining the sex of an embryo (e.g. Janes 2010). TSD and GSD should rather be seen as two ends of a continuum (e.g. Sarre *et al.,* 2004). Neither TSD, nor GSD are homogenous categories – in both of them, there are at least few major subtypes (such as homo- and heteromorphic sex chromosomes, three types of TSD; e.g. Janes 2010).

Despite the fact that sex-determining mechanisms do not fossilize, sex determination in fossil reptiles receive much attention. In extant reptiles, temperature-dependent sex determination occurs in all major lineages – squamates, turtles, crocodylians and sphenodontians; in the latter two, it is the only sex-determining mechanism (e.g. Janzen and Krenz 2004). Temperature-dependent sex determination probably appeared very early in reptile evolution and may be an ancestral trait for turtles, sphenodontians, crocodylians (Janzen and Krenz 2004) and the clade Archosauromorpha, which contains crocodylians and all reptiles more closely related to them than to lizards, i.e. archosaurs, their close extinct relatives and possibly turtles (Organ and Janes 2008). Quite commonly, there is intrafamilial or even intrageneric variation in sex-determining mechanism, which suggests that evolutionary transitions between GSD and TSD are relatively easy (Sarre *et al.,* 2004) but there are different possible explanations of that phenomenon – Janes *et al.,* (2010) point to the role of mutations, while Quinn *et al.,* (2011) suggest the effect of sex gene dosage and changes in thermosensitivity within population.
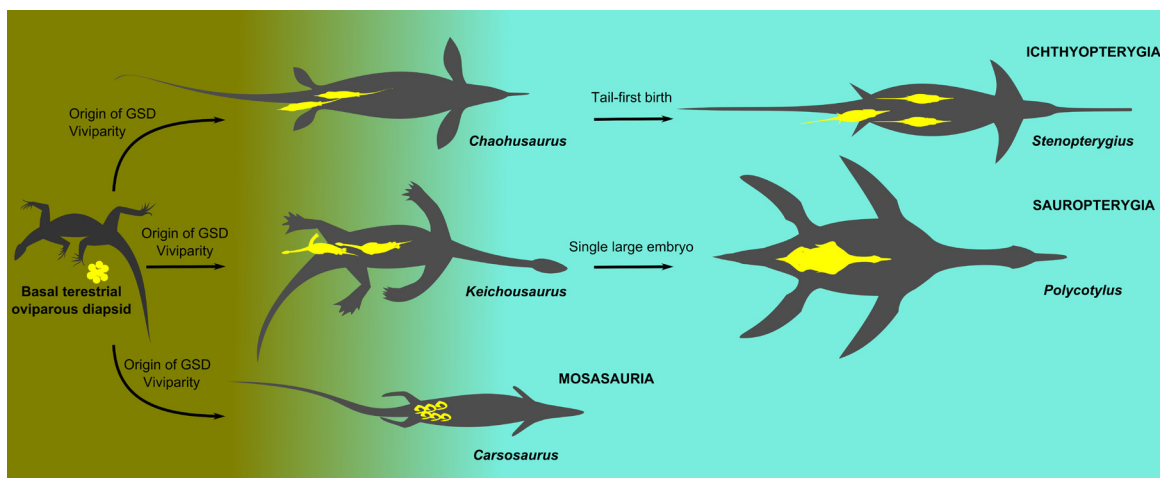
It has been suggested that sex determination in fossil taxa is impossible to infer (Rage 1998) but currently, based on anatomy and biology of extinct organisms, comparisons to related recent taxa and sophisticated statistical methods it is possible to infer probability of a given sex-determining mechanism in a given fossil taxon. Especially sex-determining mechanisms in non-avian dinosaurs attracted a lot of attention. Some authors suggested that dinosaurs exhibited TSD which led to their extinction at the end of the Cretaceous (e.g. Miller *et al.,* 2004). Extant Phylogenetic Bracketing does not give an answer to this problem because birds and crocodylians – the closest extant relatives of non-avian dinosaurs – have different types of sex-determining mechanisms (e.g. Silber *et al.,* 2011). However, discovery of neosauropod nesting site at hydrothermal environment, where dinosaurs nested repetitively (Grellet-Tinner and Fiorelli 2010), suggests that TSD is unlikely in these animals.

Only three of all recent viviparous reptiles have TSD – all of them are skinks (Robert and Thompson 2010). Viviparity was also common in fossil reptiles (see above), especially in marine reptiles such as ichthyosaurs, sauropterygians and mosasaurs (Fig. 4). All recent fully pelagic, viviparous amniotes (i.e. sea snakes, sirenians and cetaceans) exhibit GSD so it seems probable that pelagic, viviparous fossil reptiles also had GSD. Organ *et al.,* (2009) discovered a correlated evolutionary change between genetic sex determination and viviparity in modern reptiles using a reversible-jump Markov-chain Monte Carlo algorithm to find a Bayesian posterior probability distribution of models of correlated change. The analysis strongly suggests that ichthyosaurs, sauropterygians and mosasaurs acquired GSD prior to the evolution of live birth (Fig. 4). Sex-determining mechanisms seem to be impossible to infer in fossil oviparous taxa, while in viviparous reptiles, a null hypothesis should probably be assuming that they had genetic sex determination (Organ *et al.,* 2009; Janes 2010).

Sex-determining mechanisms do not fossilize but they can be inferred even in some fossil reptiles, mostly based on data from living taxa. Statistical tests suggest that genetic sex determination predates origin of viviparity in a given lineage, which means that marine viviparous reptiles such as ichthyosaurs, sauropterygians and mosasaurs probably had genetic sex determination. Temperature-dependent sex determination probably appeared early in reptile evolution and may be ancestral for sphenodontians, turtles and archosauromorphs. Numerous transitions between TSD and GSD are known. Fossil evidence may also give clues about sex determination type of extinct reptiles. For example, repetitive nesting of some sauropod dinosaurs at hydrothermal site seems to argue against TSD in these animals.

Fossils, genes and development

Genes that underlie different developmental mechanisms do not fossilize. However, that does not mean that we cannot infer the degree of expression of a given gene based on fossilized phenotype and using an extant phylogenetic bracketing. Even fossils of adults can be informative, especially when there is an unambiguous correspondence between given phenotype and given developmental process (Sánchez 2012).

Fig. 4. Evolution of sex determination and parity mode in major groups of marine reptiles. *It seems that genetic sex determination evolved prior to viviparity in those clades (see text for details).*

Historically, morphology of the temporal region of the skull played an important role in reptile systematics. On the basis of a number and location of additional skull openings (fenestrae), reptiles were divided into several groups – anapsids, diapsids, synapsids and euryapsids. In turtles, which have anapsid skull (i.e. with no fenestrae) – during embryonic growth *Runx2* and *Msx2* genes are expressed in mesenchymal cells in the temporal region, what results in complete temporal roofing (Tokita *et al.,* 2013). It is possible that in other reptiles with anapsid skulls, like armoured pareiasaurs or procolophonids (all extinct), such pattern was present. However, this is far from certain due to the unstable position of turtles on the phylogenetic tree. Traditionally, turtles were considered to be the most basal living reptiles, while most molecular studies suggest that they are archosauromorphs which lost their temporal openings. It does not preclude the close relationship between turtles and other anapsids (Lee 2013). If those two groups are closely related, their similar morphologies suggest that their genes were also similar.

The turtle shell is a unique structure among amniotes. Given the uncertain phylogenetic position of turtles (e.g. Lee 2013), the evolutionary origin of this structure is contentious. However, evolutionary developmental studies shed some light on this subject. The shell is composed of dorsal part – the carapace – and ventral one – the plastron. Plastron is present in all known turtles, while the carapace is absent in the earliest known turtle, the Late Triassic *Odontochelys*. The carapacial ridge is a crucial structure in the development of the carapace; it is a longitudinal ridge in the flank, present only in turtle embryos. This suggests that in embryos of the earliest turtles like *Odontochelys*, the carapacial ridge formed only temporarily and incompletely. More advanced turtles, including early forms like the Late Triassic *Proganochelys*, probably had complete carapacial ridge (Kuratani *et al.,* 2011). *Eunotosaurus*, a Late Permian putative close relative of turtles, had no shell so probably also had no carapacial ridge. However, its ribs were broadened in a similar way to those of the turtles, probably as a result of outgrowing (sub)dermal bone from perichondrium (Lyson *et al.,* 2013). Some other reptiles also had broadened, plate-like ribs with limited movability, e.g. basal sauropterygians *Sinosaurosphargis* and *Henodus*. It was suggested that these taxa and turtles are closely related and all inherited genetic basis for carapace development. Similarly to turtles, ribs of *Sinosaurosphargis* are not derived from exoskeletal components (Hirasawa *et al.,* 2013). However, the phylogenetic position of both sauropterygians and turtles is highly unstable (e.g. Lee 2013), rendering the hypothesis of deep homology uncertain. Moreover, the armours of turtles and basal sauropterygians differ in their microanatomies, as shown by histological analyses (Scheyer *et al.,* 2013).

Another notable trait of the sauropterygians is their variability in vertebral numbers. Vertebrate body axis consists of many repeated units – the vertebrae. Their development is driven by somitogenesis and *Hox* gene expression, so the morphology of the adult vertebrae can tell us about somitogenesis and rate of segmentation during embryonic growth. While mammals are highly conserved in the number of precaudal vertebrae, reptiles are very diverse in this respect. Reconstruction of ancestral vertebral counts suggests that first reptiles had six cervical and twenty one dorsal vertebrae. Several general trends in the evolution of vertebral counts can be observed: marine reptiles tend to have many more vertebrae than terrestrial (with some long-necked plesiosaurs having more than 70 cervical vertebrae) and armoured clades, such as pareiasaurs,

turtles or ankylosaurs, have relatively small vertebral counts. This gives us information on the rate of segmentation which is lower in some clades but much higher in others, like snakes which can have more than 300 precaudal vertebrae (Müller *et al.,* 2010). The increase in number of vertebrae in snakes was followed by reduction of regional differentiation, i.e. expansion of thoracic identity in axial skeleton, which occurred probably very early in snake evolution (Cohn and Tickle 1999; Zaher *et al.,* 2009). However, a recent morphometric study suggests that the snake skeleton did not lose its regionalization, but rather retained an ancestral amniote pattern of *Hox* gene expression (Head and Polly, 2015) – with relatively minor modifications (see Guerreiro and Duboule, 2015 and references therein) – which is supported by morphology of early reptiles such as Palaeozoic Captorhinus. High regionalization of skeletons of birds (and, to a lesser extent, crocodylians) and mammals – compared to snakes – seems to be acquired independently (Head and Polly, 2015). One of the most basal known snakes, *Najash*, still had sacrum – loss of sacral region must have then occurred in more advanced snakes. None of the known snakes, both extant and extinct, has forelimbs, which means that complete loss of their specification took place very early. However, well developed hindlimbs are known in some fossil snakes such as *Najash*, *Dinilysia*, *Eupodophis*, *Pachyrhachis*, *Pachyophis* or madtsoiids (e.g. Zaher *et al.,* 2009). These developmental changes are supposed to be followed by transformation of the entire axial skeleton towards thorax and finally to elimination of hindlimb specification in alethinophidians except booids and pythonoids (Cohn and Tickle 1999). However, phylogenetic position of the limbed taxa listed above is contentious – some of them may be nested within crown snakes (Zaher *et al.,* 2009), thus suggesting more complex pattern of evolution of developmental processes.

Even though birds are just a species-rich reptile lineage, traditionally they are not considered reptiles and they are not treated in this review. However, one of the traits historically considered to be unique to birds – feathers – could evolved in their non-avian ancestors. Feather-like structures have been discovered in many dinosaurs and even in pterosaurs, the probable sister group of dinosauromorphs, thus raising possibility that all those taxa were ancestrally feathered (Xu and Guo 2009). One specimen is of particular interest because it shows that both feathers and scales may be present on one individual. *Kulindadromeus*, a basal ornithischian dinosaur (a group that did not give rise to birds), had feather-like integuments around the head, thorax and proximal limbs and scales around the tail and distal hindlimbs. Especially the scales on the distal hindlimbs are interesting because of their similarity to scales on the birds' legs. In birds, these scales develop through inhibition of feather development, regulated by *sonic hedgehog* pathway. It is possible that similar process occurred in *Kulindadromeus* (Godefroit *et al.,* 2014). Elongated integuments are also known in the Triassic reptile *Longisquama* and some authors considered them to be very important in the evolution of feathers, despite highly uncertain phylogenetic position of that taxon among diapsid reptiles (e.g. Dzik *et al.,* 2010; Buchwitz and Voigt 2012). Development of these structures is quite similar to that of avian feathers, for example in differentiation along the proximo-distal axis. This suggests some degree of homology between those appendages. While there is no phenotypic evidence of feather-like integuments outside of Ornithodira, genes that control development of such structures might have been present much earlier,

possibly in the last common ancestor of *Longisquama* and birds (Buchwitz and Voigt 2012). This hypothesis is partly supported by finding that majority of feather regulatory elements originated before dinosaurs and were probably present in first archosaurs (Lowe *et al.,* 2015). The deep homology (i.e. homology of genes but not their phenotypic effects) has been hypothesized to play role also in the evolution of dinosaur integuments (Godefroit *et al.,* 2014). Studying morphogenesis of skin integuments of other reptiles may shed more light on that subject.

Another supposedly avian trait that appeared before the origin of birds is the digit configuration of the avian wing. Basal theropods had five digits in the manus, with the digits 4 and 5 strongly reduced. Birds and many advanced theropods have only three digits (e.g. Bever *et al.,* 2011 and references therein). Embryological studies showed that bird digits develop from digital positions 2-3-4. This conflict has been used as an argument against the dinosaurian origin of birds (e.g. Burke and Feduccia 1997). Wagner and Gauthier (1999) proposed a hypothesis explaining these differences, the "frame-shift hypothesis": it assumes that mesenchymal condensations that used to develop as digits 2-3-4, at some point of theropod evolution started to develop in positions of digits 1-2-3 (Wagner and Gauthier 1999). Although some experimental studies argue against this frame-shift (Towers *et al.,* 2011), most of them support this hypothesis (e.g. Tamura *et al.,* 2011; Salinas-Saavedra *et al.,* 2014). Currently, most authors tend to pose questions "how?" and "when?" rather than "whether?" the frame-shift occurred (e.g. Bever *et al.,* 2011; Young *et al.,* 2011; Seki *et al.,* 2012). The fossil record seemed to unambiguously suggest that advanced theropods (including birds) have digits 1-2-3 but that hypothesis was challenged by the discovery of a four-fingered basal ceratosaur *Limusaurus* with strongly reduced digit 1, which was interpreted as supporting a 2-3-4 hypothesis. The authors suggest that *Limusaurus* shows the intermediate stage of the frame-shift which was completed in more advanced theropods – the tetanurans (Xu *et al.,* 2009). However, this scenario has been questioned and the strong reduction of digit 1 was interpreted as a derived trait of the ceratosaurs, thus having nothing to do with the avian condition (Seki *et al.,* 2012). Moreover, recent embryological studies support digit identities in birds (and thus other advanced theropods) as 1-2-3 (e.g. Tamura *et al.,* 2011; Towers *et al.,* 2011; Salinas-Saavedra *et al.,* 2014). Discovery of more fossils of basal ceratosaurs and basal tetanurans would certainly help understand the digit homologies and time of the frame-shift in theropod evolution. Regardless of that, it seems that homeosis played an important role in the evolution of the theropod forelimb; it was proposed that also the "semilunate" carpal in the wrist of most theropods (including birds) underwent a homeotic transformation during evolution, as suggested by the positional shift of that structure (Xu *et al.,* 2014).

While reduction of some digits is a relatively common phenomenon among recent and fossil reptiles (e.g. theropods), the opposite trends – hyperphalangy and polydactyly – are very rare among extant amniotes but present in some fossil reptiles. It is difficult to reconstruct developmental processes based on osteology alone but some hypotheses can be put forward. For example, polydactyly in ichthyosaurs is supposed to evolve from fixed different expression of *Shh* or *Ihh* genes and isolated expression of *HoxA11-13* genes. Absence of fifth digit in mosasaur lizards may be result of prolonged expression of *HoxD* in the absence of *HoxA*. Modifications of time of gene expression or gene dosage effect might be
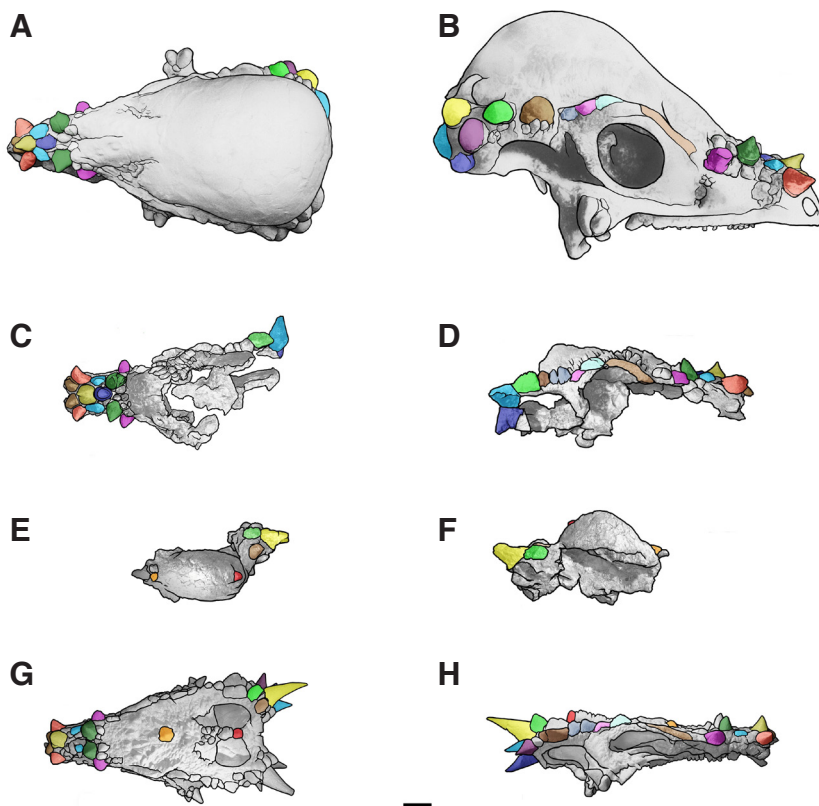
the causes of hyperphalangy (Caldwell 2002).

Clear correlations between a given developmental process and given phenotype allow us to reconstruct some aspects of development of fossil reptiles. It is especially interesting in groups with bizarre morphology. For example, highly modified anatomy of turtles seems to be a result of several changes during their development. These include expression of *Runx2* and *Msx2* in mesenchymal cells in the temporal region which results in closed skull and the origin of carapacial ridge which is crucial for the development of the carapace. It is yet unclear whether or to what extent we can extrapolate these processes on extinct reptiles with similar traits – anapsids and some sauropterygians. Data from fossils give us information about developmental plasticity of extinct reptiles, as exemplified by their high variation in vertebral numbers. Fossils suggest that limblessness, and thus developmental processes responsible for loss of limb specification, evolved several times within snake lineage. Homeosis probably played an important role in the evolution of the theropod forelimb – currently, most (but not all) authors agree that the frame-shift occurred and advanced theropods (including birds) have digits 1-2-3. The opposite phenomenon, increasing the number of digits, is very rare but was present in some extinct marine reptiles such as ichthyosaurs. While all modern reptiles have typical scaly skin, many extinct ones exhibited wide range of integumentary structures on their bodies. Homology between some of these integuments, like those of *Longisquama*, and others, such as bird feathers, is yet uncertain. Probably only future fossil discoveries can clarify their relations.

Indeterminate vs. determinate growth, age assessment and taxonomy

Age assessment is a crucial problem in developmental palaeobiology. It can give us important information about growth of the animal and processes responsible for its development. Ontogenetic data can also have serious implications for taxonomy.

It is commonly assumed that reptiles exhibit an indeterminate growth, i.e. they grow until death (see review in Congdon *et al.,* 2013). Fossil evidence show that this view is problematic and erroneous in many cases at least. Whether an animal grows indeterminately, can be checked through osteohistological analysis. Determinate growth is assumed when in the outermost bone cortex occurs an external fundamental system (ESF) – a bone microstructure which indicates cessation of bone growth. External fundamental system has been found in long bones of some individuals of not only highly derived modern alligators (Woodward *et al.,* 2011) and some Triassic crocodylian-line archosaurs which metabolism was higher than in recent crocodylians (Ricqlès *et al.,* 2003) but also in dyrosaurid crocodyliforms (Andrade and Sayão 2014), pterosaurs (Steel 2008), rhynchosaurs (Veiga *et al.,* in press), and even some lepidosaurs (Hugi and Sánchez-Villagra 2012), among others. This suggests that determinate growth is primitive for diapsids and may be "a rule rather than exception" (Woodward *et al.,* 2011). However, even in recent literature there are suggestions that indeterminate growth could play an important role in attaining large size by many dinosaurs (Delfino and Sánchez-Villagra 2010) but EFS is known in many dinosaur taxa (e.g. Curry 1999; Padian *et al.,* 2004), including sauropods such as *Apatosaurus* which was about 25 m in length (Curry 1999). Moreover, absence of an EFS does not necessarily imply indeterminate growth, it suggests rather that individual in

Fig. 5. Hypothesized cranial ontogeny of *Pachycephalosaurus,* showing inflation of the skull dome but also reduction of cranial ornamentation. *The oldest individual is shown in* **(A,B)** *while* **(G,H)** *are the youngest, originally described as* Dracorex. **(C,D)** *were originally described as* Stygimoloch. *Scale bar, 5 cm (from Horner and Goodwin 2009). Image available under Creative Commons Attribution 2.5 licence.*

with morphology and computer tomography suggest that two former taxa are only growth stages of the latter (Fig. 5). This is interesting because *Dracorex* and *Stygimoloch* had more pronounced skull ornamentation than *Pachycephalosaurus* (Fig. 5). This ontogenetic series would therefore be a rare example of reducing cranial ornamentation during ontogeny (Horner and Goodwin 2009). Another case for integrating developmental data in taxonomy is the debate about potential synonymy of the ceratopsian dinosaurs *Torosaurus* and *Triceratops* ("synonymy through ontogeny"), where the former may be mature form of the latter, as suggested by morphology and osteohistology (e.g. Scannella and Horner 2010). However, this case seems to be more controversial than in *Pachycephalosaurus* (e.g. Maiorino *et al.,* 2013).

Bone histology is an important technique in studying ontogeny of fossil animals. It may contribute to challenging some of the traditional concepts such as indeterminate growth in reptiles – in at least several clades of reptiles, both extant and extinct, an external fundamental system has been found which indicates cessation of growth. Osteohistological analyses may have serious implications for taxonomy, for example by showing that animals regarded as different taxa were in fact only ontogenetic stages of the same taxon (or conversely). Sometimes ontogenetic series recognized this way show rare phenomena, such as reduction of cranial ornamentation during ontogeny, as observed in pachycephalosaurs.

Conclusions

New remarkable findings and methodological advances give us new perspective on the evolution of development in reptiles. However, developmental biology of the earliest reptiles remains enigmatic. Reptile body fossils are known from the Carboniferous but the oldest known embryos come from the Permian while the oldest known eggs are from the Jurassic. This huge gap in the fossil record makes it very difficult to conclusively reconstruct ancestral reptilian condition. This is further hindered by uncertain phylogenetic placement of some major clades.

Viviparity has been demonstrated in several lineages of fossil reptiles, mostly aquatic ones. It seems that transition to viviparity preceded colonization of fully marine habitats, as suggested by caudal orientation of embryos within maternal body cavity of an early ichthyopterygian. Most of the viviparous fossil reptiles gave birth to multiple progeny but an example of K-selected reproductive strategy has been found among short-necked plesiosaurs. Strong correlations between evolution of genetic sex determination and viviparity suggests that sex of extinct marine reptiles was determined genetically. Some aspects of developmental genetics can be inferred in fossil animals, especially when there is a clear correspondence between developmental process and a specific phenotype.

Studying late ontogeny of some fossil reptiles brought other unexpected discoveries. Bone histological studies of fossil reptiles show that many of them were capable of ultimately cease growing, as indicated by presence of an external fundamental system. EFS

question died before reaching maximum size (e.g. Woodward *et al.,* 2011). This may be due to the high mortality and/or habitat differences – as a result, only some animals attain skeletal maturity (Myhrvold 2013). While EFS is usually not present in all specimens of a given taxon – sometimes in none of them (Myhrvold 2013) – its widespread presence on a phylogenetic tree supports hypothesis about early origin of capability to stop growing. In some cases, EFS is present in relatively small animals while absent in bigger ones (e.g. Woodward *et al.,* 2011) – this means that it is impossible to assess skeletal maturity based on size alone. Timing of suture closing is also problematic (e.g. Irmis 2007), meaning that it can be achieved only by histological analysis. This may have serious implications for taxonomy. Numerous pterosaur mandibular symphyses of different sizes from the Upper Cretaceous of Hungary were thought to represent a growth series of an azhdarchid *Bakonydraco galaczi*. However, the smallest histologically studied individual turned out to be an adult, not juvenile, as expected. This, combined with microanatomical differences between that specimen and other adult pterosaurs in the sample, suggests that at least two distinct species are present in that assemblage (Prondvai *et al.,* 2014).

Histological analyses can be a reason not only of splitting but also lumping taxa together. For example, *Dracorex*, *Stygimoloch* and *Pachycephalosaurus* were regarded as three distinct taxa of pachycephalosaurid dinosaurs but cranial osteohistology combined

has been discovered in bones of most major reptile groups which suggests that determinate growth may be ancestral for reptiles. It is thus possible that determinate growth will be discovered in greater number of extant reptiles. Major morphological changes have been discovered in late ontogeny of dinosaurs like pachycephalosaurs, where reduction of cranial ornamentation has been postulated – a very rare phenomenon among extant animals.

Many discoveries in reptile developmental biology would be impossible without incorporating palaeontological data and taking an interdisciplinary approach. It is important to study fossil taxa because they give us information about the origin of traits present in modern animals and developmental processes that underlie them. Moreover, they often exhibit morphologies that are significantly different from those of modern animals and document phenomena that are rare or even completely absent among extant organisms.

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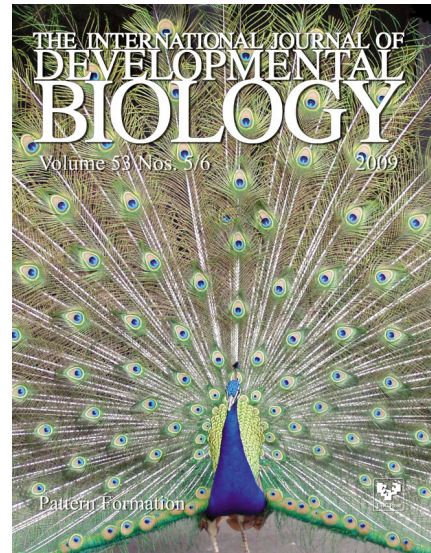**Reptile scale paradigm: Evo-Devo, pattern formation and regeneration**
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