

## Embryo retention in sarcopterygians, and the origin of the extra-embryonic membranes of the amniotic egg

Michel Laurin\*, Marc Girondot

*Équipe « formations squelettiques » UMR 8570, FR CNRS 1451,  
Collège de France, Muséum national d'histoire naturelle et université Paris-7, case 7077,  
2, place Jussieu, 75251 Paris cedex 05, France*

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**Abstract** – We reevaluate a recent suggestion that the extra-embryonic membranes of the amniotic egg do not constitute an adaptation to reproduction on land, but rather appeared in an embryo-retaining ancestor, in which they facilitated interactions between the embryo and the mother. On the basis of a revised data set on the distribution of extended embryo retention in sarcopterygians, we suggest that stem-amniotes probably did not retain their eggs in utero for a great proportion of the embryonic development. Therefore, we tentatively reject the hypothesis that the initial function of the extra-embryonic membranes was to facilitate embryo–mother interactions. However, current controversies on amniote phylogeny render our conclusions tentative. © 1999 Éditions scientifiques et médicales Elsevier SAS.

**embryo retention / Amniota / amniotic egg**

**Résumé** – **Rétention des embryons chez les sarcoptérygiens et origine des membranes extra-embryonnaires de l'œuf amniotique.** Nous évaluons une hypothèse récente selon laquelle les membranes extra-embryonnaires de l'œuf amniotique ne constituent pas une adaptation à la reproduction en milieu terrestre, mais qu'elles sont plutôt apparues chez un ancêtre qui pondait ses œufs à un stade de développement relativement tardif, chez qui elles auraient facilité des interactions entre l'embryon et sa mère. Une optimisation basée sur une nouvelle compilation de données sur la rétention d'embryons parmi les sarcoptérygiens suggère que les amniotes-souches pouvaient probablement leurs œufs à des stades précoces de développement. L'hypothèse selon laquelle le rôle initial des membranes extra-embryonnaires était de faciliter des interactions entre la mère et l'embryon est donc rejetée. Cependant, des controverses actuelles sur la phylogénie des amniotes rendent ces conclusions incertaines. © 1999 Éditions scientifiques et médicales Elsevier SAS.

**rétention d'embryons / Amniota / œuf amniotique**

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\* Correspondence and reprints  
laurin@ccr.jussieu.fr; mgi@ccr.jussieu.fr

## 1. INTRODUCTION

The origin of the amniotic egg is a fascinating evolutionary problem, but it is still poorly understood because the fossil record does not provide much evidence on this problem. The amniotic egg is present in all amniotes (as defined by Gauthier, Kluge and Rowe in 1988 [11]; the last common ancestor of mammals and reptiles<sup>1</sup>, and all its descendants), even (in a modified form) in viviparous amniotes, such as therian mammals and many squamates. This fact, and the fact that the amniotic egg is unique to amniotes, indicates that the amniotic egg must have appeared some time between the divergence of stem-amniotes and stem-amphibians (in the Lower Carboniferous or the Upper Devonian, about 360 Ma ago [17, 18]), and the divergence between synapsids and sauropsids (in the Upper Carboniferous, about 310 Ma ago [19]). Therefore, the appearance of the amniotic egg must have preceded the appearance of (crown) amniotes, but we have no idea of the timespan between these two evolutionary events. The absence of fossilized amniotic eggs before the Triassic [5] has long puzzled palaeontologists, although a hypothesis has recently been proposed to explain this gap in the fossil record of eggs; the earliest amniotic eggs were probably poorly mineralized [21]. If this hypothesis is correct, the fossil record is unlikely to yield additional data directly relevant to this problem.

The nature of the selective pressures and the biological context into which the extra-embryonic membranes unique to the amniotic egg appeared are still controversial. These membranes include the amnion, chorion, and allantois; the yolk sac is present in other groups, such as gymnophiones [2] and anurans [3]. The most widely accepted hypothesis is that these extra-embryonic membranes, along with the shell membrane of amniotes, are an adaptation to reproduction on dry land. Recently, a few authors [20, 21, 23] have suggested that these membranes appeared in an embryo-retaining ancestor, in which they facilitated embryo–mother interactions. Laurin and Reisz [20] argued that an optimization of a binary character (extended embryo retention present or absent) on a sarcopterygian phylogeny was ambiguous for stem-amniotes. On the basis of this optimization, they suggested that the hypothesis that stem-amniotes performed embryo retention was as parsimonious as the

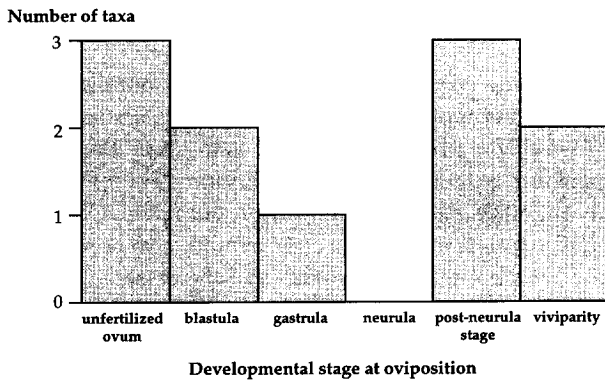
alternative (that it laid eggs at an early developmental stage). This thesis was recently attacked by Wilkinson and Nussbaum [36], who equated the state “extended embryo retention present” with viviparity. These authors showed that stem-amniotes were probably oviparous because gymnophiones were primitively oviparous (gymnophiones had been coded as possessing extended embryo retention by Laurin and Reisz [20]), but they did not show that extended embryo retention was absent. Laurin, Reisz and Girondot [21] concluded that not enough evidence was available to infer safely the ancestral state for the embryonic stage at oviposition of gymnophiones (beyond the inference that they were oviparous). Therefore, the possibility that stem-amniotes performed extended embryo retention could not be excluded [21]. However, an extensive literature search on this topic has revealed additional data that requires modification of this viewpoint.

## 2. METHODS

The developmental stage at which sarcopterygian eggs are laid can vary from an unfertilized egg (in lungfishes and most anurans, for instance) to viviparity (the egg is not laid at all, and all the pre-hatching development takes place in the uterus). The distribution of ontogenetic stages at oviposition appears to be bimodal (*figure 1*), and the two modes do not overlap (at least, in the taxa included in our survey). An appropriate way of defining the two states (if we decide to treat this character as binary) is to consider that taxa that lay eggs at a gastrula stage or earlier do not perform extended embryo retention. On the other hand, taxa that lay eggs in which the embryo already has somites (somites appear in the neurula stage, in squamates) perform embryo retention. This division works well because in our survey, we found no taxa that laid eggs between the gastrula stage, and a fairly advanced stage at which the embryo had at least nine somites (*table 1*). Applying this method to code the absence or presence of embryo retention leads us to modify our previous coding of this character [20] in the following ways:

1) Gymnophiones were coded as performing extended embryo retention [20], largely because information on the early development of gymnophiones is available mostly for viviparous species. Our extensive literature search yielded data on a single oviparous gymnophione species, out of the approximately 160 recognized species [8]. This gymnophione, *Hypogeophis rostratus*, appears to lay its eggs in

<sup>1</sup> Throughout this paper, the terms “reptiles” and “Reptilia” refer to the monophyletic group that includes the last common ancestor of all extant reptiles, and all its descendants [11]; as such, they include birds.



**Figure 1.** Distribution of the developmental stages at oviposition in sarcopterygians. The taxa included here are those found in *table 1*.

**Figure 1.** Distribution des stades de développement au moment de l'oviposition chez les sarcoptérygiens. Les taxons inclus dans cette compilation sont ceux figurant dans le *tableau 1*.

**Table I.** Developmental stage at oviposition in sarcopterygians. For each taxon, the developmental stage reported represents the inferred primitive condition. Variations reported within parentheses are probably derived within each taxon, with the possible exception of *Ascaphus*.

**Tableau I.** Stade de développement à l'oviposition chez les sarcoptérygiens. Pour chaque taxon, le stade de développement représente la condition primitive inférée. Les variations rapportées entre parenthèses sont probablement des variations dérivées à l'intérieur de chacun de ces taxons, avec l'exception possible d'*Ascaphus*.

Taxon	Developmental stage at oviposition
<i>Latimeria</i>	Egg not laid; viviparity
Dipnoi	Unfertilized egg
Anura	Unfertilized egg (except in <i>Ascaphus</i> and a few other exceptions)
Caudata	Unfertilized egg (some have internal fertilization and a few species are viviparous)
Gymnophiona	Blastula (based on one or two species; many groups are viviparous)
Monotremata	Embryo with approximately 19 pairs of somites
Theria	Egg not laid; viviparity
<i>Sphenodon</i>	Blastula or gastrula
Squamata	Embryo with somites (in one species, blastula; in several others, viviparity)
Crocodylia	Embryo with nine to twenty pairs of somites
Aves	Blastula

the blastula stage [1]. Brauer [1] stated that Sarasin and Sarasin [31] had observed that *Ichthyophis glutinosus* also laid its eggs at the blastula stage, but when reading Sarasin and Sarasin, we were unable to find this statement in this publication [31]. A possible explanation for this discrepancy is that Brauer obtained this information by personal communication from Sarasin and Sarasin (they were all German scientists). If we assume that *Hypogeophis rostratus* (and perhaps, *Ichthyophis glutinosus*) is representative of most oviparous gymnophiones, and if we accept the relatively well-established hypothesis that gymnophiones were primitively oviparous [36], this group has to be coded as not performing extended embryo retention. This coding is justified because for optimization purposes, the coding should reflect the primitive condition of each terminal taxon (when it can be determined) rather than all variations present in each terminal taxon.

2) Squamates were previously coded as polymorphic for extended embryo retention [20], although they were primitively oviparous [22]. While at least one squamate, *Chamaeleo lateralis* (a species deeply nested within Squamata), lays its eggs at the blastula stage [16], most species lay eggs at a much more advanced developmental stage, when many somites are already present. Generally, squamates retain eggs in utero for about one half of the embryonic development [33]. Therefore, squamates are coded as having extended embryo retention (not polymorphic).

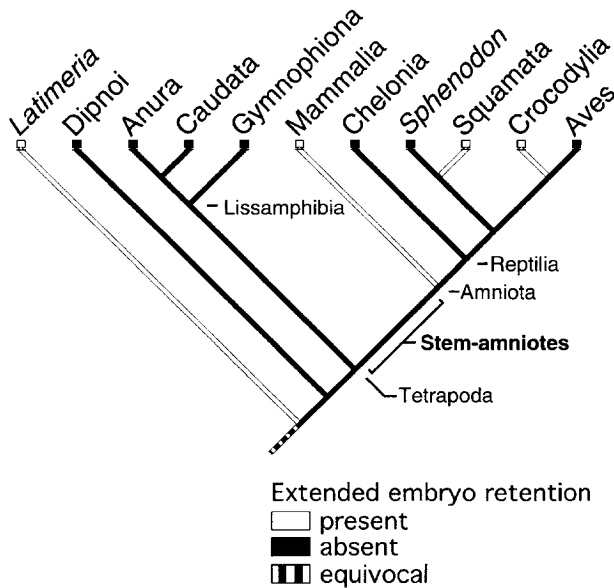
3) *Sphenodon* was not considered in the earlier study [20]. However, its widely accepted position as the sister-group of squamates [10] implies that this genus is important in any comparative study of amniotes. The exact stage at which *Sphenodon* lays its eggs is uncertain because contradictory information is found in the literature [29]. However, the few available data suggest that these eggs are laid at an early developmental stage, at the blastula or gastrula stage. Therefore, this taxon does not perform extended embryo retention.

4) Crocodylians were coded as not performing extended embryo retention [20], but our literature search revealed that crocodylian eggs are laid when the embryos have nine to twenty pairs of somites [34]. Therefore, we have recoded Crocodylia as performing extended embryo retention.

The new distribution of embryo retention was optimized on a classical (*figure 2*), "text-book" sarcopterygian phylogeny (i.e. the most widely accepted phylogeny, which is not necessarily the true or even the best supported one). Topological variations reflecting current debates on sarcopterygian phylogeny were examined to assess the impact of these phylogenetic uncertainties on the optimization. However, we have not tried to consider all published phylogenies. For instance, tetrapod phylogenies that were not based on a data matrix, or that have not been generally accepted by the scientific community, such as the one advocated by Løvtrup [24], are not considered below.

All optimizations were performed using MacClade 3.06 [25]. All of the most parsimonious optimizations were

examined, not only those corresponding to DELTRAN (delayed transformation, that prefers parallelism over reversals, in all cases in which both optimizations are equally parsimonious) or ACCTRAN (accelerated transformation, that prefers reversals over parallelism, in all cases in which both optimizations are equally parsimonious).



**Figure 2.** Evolution of embryo retention based on a classical phylogeny. *Sphenodon* has been added, for the sake of completeness. The optimization shows that stem-amniotes did not perform extended embryo retention. This conclusion is valid when other topologies affecting *Latimeria*, Gymnophiona, and Chelonia are used, but it is invalid if *Sphenodon* and Chelonia are closer to archosaurs than to squamates. All uncontroversial clades (other than the terminal taxa) are labelled on the tree. The names of some tetrapod taxa do not correspond to those used by Laurin and Reisz [20] because we refer to crown-clades. Therefore, Chelonia, Caudata, and Gymnophiona have been used instead of Testudines, Urodela and Apoda [6].

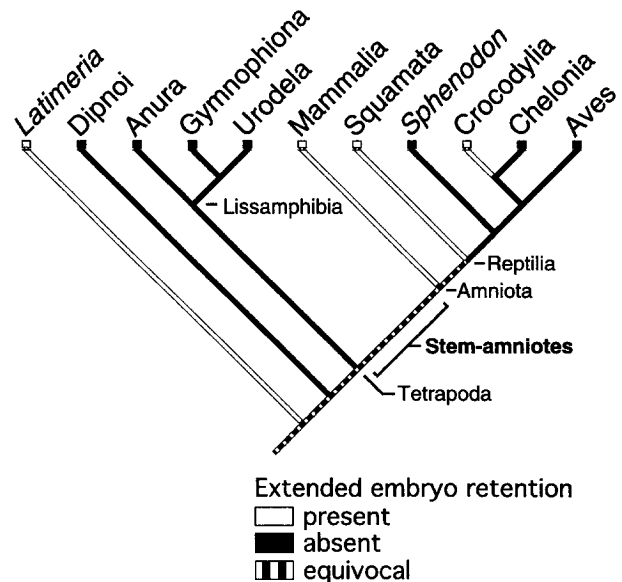
**Figure 2.** Évolution de la rétention d'embryons basée sur la distribution corrigée présentée ici. *Sphenodon* a été ajouté pour que tous les clades principaux de sarcoptérygiens soient représentés. L'optimisation suggère que les amniotes-souches poussaient des œufs à un stade précoce de développement. Cette conclusion demeure valable quand d'autres topologies affectant *Latimeria*, Gymnophiona et Chelonia sont utilisées, mais elle est invalidée si *Sphenodon* et Chelonia sont plus proches des archosaures que des squamates. Tous les clades dont la monophylie est universellement reconnue (en plus des taxons terminaux) sont identifiés sur l'arbre. Les noms de certains taxons ne correspondent pas à ceux utilisés par Laurin et Reisz [20] car nous utilisons les taxons qui correspondent à la cime des clades (*crown-groups* en anglais). Nous utilisons donc Chelonia, Caudata et Gymnophiona plutôt que Testudines, Urodela et Apoda [6].

### 3. RESULTS AND DISCUSSION

If these corrections are entered, the optimization of egg retention on a classical phylogeny is unambiguous (*figure 2*), and it indicates that stem-amniotes did not perform extended embryo retention. Therefore, the recent thesis that selective pressures to facilitate interactions between the embryo and the mother were responsible for the origin and evolution of the extra-embryonic membranes of the amniotic egg [20, 21, 23] is not supported on the basis of our revised data set and of a classical phylogeny.

Of course, our conclusions rest on an imperfectly known distribution of extended embryo retention on a traditional (but not necessarily correct) phylogeny of sarcopterygians. The position of many relevant taxa is currently controversial. The only clades that are strongly corroborated and that can be considered uncontroversial are Tetrapoda, Lissamphibia, Amniota, Reptilia and all the terminal taxa included in this analysis (*figures 2, 3*).

The relationships between dipnoans, the coelacanth, and tetrapods can be considered unresolved [32]; the



**Figure 3.** Evolution of embryo retention based on the recently proposed phylogeny of Hedges and Poling [15]. The position of *Sphenodon* was not resolved, but this genus formed a clade with Chelonia, Crocodylia and Aves.

**Figure 3.** Évolution de la rétention d'embryons basée sur la phylogénie récemment proposée par Hedges et Poling [15]. La position de *Sphenodon* n'était pas résolue, mais ce genre formait un clade avec Chelonia, Crocodylia et Aves.

coelacanth may be closer to tetrapods than to lungfishes [38], or lungfishes and the coelacanth may form a clade that excludes tetrapods [37], although the prevailing view is that lungfishes are closer to tetrapods than to the coelacanth [13, 26]). However, the position of Dipnoi in the phylogeny does not affect the optimization.

Laurin and Reisz [20] considered gymnophiones to be the sister-group of Batrachia, a clade thought to unite Caudata and Anura [4]. This hypothesis of relationships is supported by several morphological studies [27, 28, 35], and (more weakly), by some molecular phylogenies based on rRNA sequences [12]. However, some recent molecular phylogenetic analyses based on rRNA sequences suggest that Gymnophiona and Caudata form a clade that is the sister-group of anurans [9, 14]. In this latter topology, even if gymnophiones are coded as performing extended embryo retention, the optimization of extended embryo retention on a sarcopterygian phylogeny indicates that stem-amniotes did not perform extended embryo retention. However, if we assume that gymnophiones did not perform extended embryo retention ancestrally (as we argued above), their position in the phylogeny does not affect the optimization.

The position of turtles in amniote phylogeny is controversial; they may be the sister-group of Sauria (the group that includes lepidosaurs and archosaurs, in the extant fauna) [19], they may be lepidosauromorphs (more closely related to lepidosaurs than to archosaurs) [7], archosauromorphs (more closely related to archosaurs than to lepidosaurs) [30], or even archosaurs (more closely related to crocodiles than to birds) [15]. In all these cases, if *Sphenodon* is the sister-group of squamates, the optimization is unambiguous and indicates that stem-amniotes did not perform extended embryo retention.

Hedges and Poling [15] recently suggested, on the basis of an analysis of sequences of eight nuclear, ribosomal and mitochondrial genes, that *Sphenodon* is more closely related to archosaurs and turtles than to squamates. If this conclusion is accepted, the optimization is ambiguous in most topologies (Hedges and Poling [15] could not resolve the relationships between Chelonia, *Sphenodon*, Crocodylia and Aves), and stem-amniotes may have performed extended embryo retention (figure 3). This optimization is obtained in all topologies in which turtles and *Sphenodon* are archosauromorphs, provided that at least one of these two taxa (Chelonia or *Sphenodon*) is outside Archosauria. If Chelonia and *Sphenodon* are both located in Archosauria and are more

closely related to Aves than to Crocodylia (but this topology has never been advocated, to our knowledge), the optimization becomes unambiguous and indicates that stem-amniotes performed extended embryo retention.

#### 4. CONCLUSION

The evidence discussed above suggests that extra-embryonic membranes of the amniotic egg may not have appeared in an embryo-retaining ancestor. This conclusion is reached when the most recently published phylogenies of sarcopterygians are used. Therefore, the widely accepted hypothesis that these membranes are part of an adaptation to reproduce on dry land cannot be rejected. However, our data cannot be used to confirm this hypothesis either; the fact that stem-amniotes laid eggs at an early developmental stage does not imply that the extra-embryonic membranes are an adaptation to reproduction on land (although this is a possibility). To confirm this hypothesis, other tests must be devised. Unfortunately, the low preservation potential of the extra-embryonic membranes and the single appearance of the amniotic egg will certainly hamper future studies in this area.

Current uncertainties about the position of Chelonia and *Sphenodon* show that our conclusions must remain tentative. Our work shows that new studies on the phylogeny of amniotes are badly needed, especially for *Sphenodon*.

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