
REVIEWS

Pregastrular Development of Amphibians: Ontogenetic Diversity and Eco-Devo

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Abstract—Comparative and ecological aspects of the reorganizations of early development in the class Amphibia are analyzed. We used data on the developmental diversity in a number of families belonging to the orders Anura and Caudata, in which many species had lost their connection with the aquatic environment. Model representatives of the class Amphibia (*Ambystoma mexicanum*, *Rana temporaria*, and *Xenopus laevis*) have small eggs (no more than 2.5 mm in diameter). In these species, the slowdown in the rate of cell divisions and the loss of synchrony occur at the midblastula stage. However, phylogenetically basal amphibian species (*Ascaphus truei*, *Cryptobranchus alleganiensis*) are characterized by the large (4–6 mm in diameter) yolky eggs and a short series of synchronous blastomere divisions (the synchrony is already lost at the 8-cell stage of cleavage). They do not have a “midblastula transition,” which is characteristic of the above model species. On the other hand, many evolutionarily advanced non-model species of caudate and anuran amphibians (for example, *Desmognathus fuscus*, *Gastrotheca riobambae*, *Phyloria sphagnicolus*), as well as the basal species, are characterized by the large, yolk-rich eggs and the early loss of cell division synchrony. Phylogenetic analysis suggests that the cleavage pattern of the most extensively studied amphibians, the Mexican axolotl (Caudata) and the African clawed frog (Anura), represents a homoplasy. The midblastula transition, which is characteristic of these two species, might have evolved convergently in these two orders of amphibians as an embryonic adaptation to development in lentic water.

Keywords: amphibians, cleavage division pattern, egg size, evolution of development, midblastula transition, reproductive strategies

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INTRODUCTION

Tailed amphibians *Ambystoma mexicanum*, *Lisso-triton vulgaris*, *Pleurodeles waltl*, anuran amphibians *Rana pipiens*, *Rana temporaria*, *Xenopus laevis* and several other species of European, Japanese and North American newts and frogs are model and thoroughly studied objects of developmental biology (Rugh, 1951; Dettlaff and Vassetzky, 1991; Nieuwkoop and Faber, 1994; Nieuwkoop, 1996; Dettlaff, 2001; etc.). In particular, the primary differentiation into animal and vegetal blastomeres occurs at the 8-cell stage (as a result of the third cleavage division, the furrows of which are latitudinal); in the animal hemisphere of the early embryo after fertilization, 10–12 rapid synchronous cleavage divisions occur. Then comes the so-called “mid-blastular transition,” when the rate of cell divisions slows down and the maternal control of early ontogenesis is replaced by zygotic control at the midblastula stage (Signoret and Lefresne, 1971; Newport and Kirschner, 1982; Collart et al., 2017; Jiang et al., 2017; Zhang et al., 2017; Vastenhouw et al., 2019).

However, the Mexican axolotl, the African clawed frog, and several species of frogs and newts from the temperate latitudes of the Northern Hemisphere do not represent all amphibians. The class Amphibia (as of September 12, 2022) includes 7500 Anura species (tailless amphibians), 774 Caudata species (tailed amphibians) and 215 Gymnophiona species (legless amphibians or caecilians) (AmphibiaWeb, 2022). Amphibians are characterized by high ecological and ontogenetic diversity (Duellman and Trueb, 1994; Elinson and del Pino, 2012; Gomez-Mestre et al., 2012; Pereira et al., 2017; Liedtke et al., 2022; etc.). Their reproductive strategy (reproductive mode) is defined as a combination of reproductive and developmental characteristics, including egg laying site, egg and clutch characteristics, rate and duration of development, stage and size of the organism at hatching, and type of parental care, if any (Duellman, 1985). Reproductive strategies are especially diverse in the order Anura, where they numbered about 70 according to the latest data (Nunes-de-Almeida et al., 2021).

Already about a hundred years ago, a significant number of species of anuran and tailed amphibians with large, yolk-rich eggs were known (Sampson, 1900, 1904; Eycleshymer, 1904; Hilton, 1904; Smith, 1906; Noble, 1927; etc.). The process of cleavage of their embryos differs significantly from the “standard” early development in such animals as, for example, the common newt, Mexican axolotl, clawed and grass frogs. Nevertheless, for many decades, amphibians with large eggs (more than 2.5–3 mm in diameter) almost did not attract the attention of embryologists. A certain revival of interest in studies of the early development of these animals appeared only about 30–40 years ago (del Pino and Escobar, 1981; Elinson and del Pino, 1985; del Pino, 1989; Elinson et al., 1990; Collazo and Marks, 1994; Wake and Hanken, 1996). However, until now, more attention has been paid to the comparative analysis of amphibian gastrulation (Keller and Shook, 2004; del Pino et al., 2007; Moya et al., 2007; Elinson and del Pino, 2012; Desnitskiy, 2020) than to the pregastrular stages of their embryogenesis.

This article is mainly devoted to the comparative and evolutionary-ecological aspects of the course of pregastrular development within the class Amphibia. In this case, one inevitably has to use also some data from the literature on the subsequent stages of ontogeny. We pay the greatest attention to representatives of the order Anura, the largest group of amphibians. Consideration of the pregastrular embryogenesis of two species of Neotropical frogs *Eleutherodactylus coqui* and *Gastrotheca riobambae*, the development of which has been studied in recent years, is taken as a starting point. Next, data on the early ontogeny of frogs from other families and/or from other parts of the world are included. Then, after considering the relevant literature data on the order Caudata, the data on the embryonic development of representatives of the order Gymnophiona are briefly discussed. Finally, the results of a phylogenetic analysis of the cleavage process in amphibian embryos are presented. The ancestral ontogenetic features for the class Amphibia are a large egg size (more than 2.5 mm in diameter), vertical furrows of the third cleavage division, and the loss of blastomere synchrony already at the early stages of cleavage (most often at the 8-cell stage), that suggests the absence of the midblastula transition in the development of the most primitive representatives of Anura and Caudata.

EARLY DEVELOPMENT OF SOME “NON-MODEL” ANURAN AMPHIBIANS

The vast majority of anuran amphibian species studied by embryologists live in the temperate latitudes of Europe, Asia, and North America. As a rule, these animals (for example, *Bufo bufo*, *Rana pipiens*, *R. temporaria*) are characterized by a biphasic life

cycle: they lay eggs in lentic water and have a larval stage of a swimming exotrophic tadpole, which then undergoes metamorphosis before exit from the water. The same biphasic cycle of development is characteristic of the African frogs *X. laevis* and *X. tropicalis* (family Pipidae), which have now become model objects in amphibian embryology (Dettlaff and Vasetzky, 1991; Nieuwkoop and Faber, 1994; Hirsch et al., 2002), as well as of some other tropical frogs and toads (Haddad and Prado, 2005).

However, a significant number of tropical species of Anura have direct development without the tadpole stage. Miniature froglets hatch from eggs laid on the ground, in underground burrows, or in tree nests (Lutz, 1947; Callery, 2006; Hedges et al., 2008; Padial et al., 2014; Liedtke et al., 2022). The tadpole stage is absent in more than 1800 species of anurans (more than 25% of all described species). Direct development has arisen independently within the order Anura more than 10 times and is derived compared to the ancestral biphasic life cycle, which includes the larval stage (Duellman, 1989; Elinson, 1990, 2013). Direct development in frogs is always associated with an increase in egg diameter, a significant decrease in the number of eggs in a clutch, and the presence of parental care for offspring (Gomez-Mestre et al., 2012).

Among anurans that do not have a tadpole stage, the most studied species is the leaf frog *E. coqui* (family Eleutherodactylidae), a typical representative of the fauna of the island of Puerto Rico (Elinson, 2021). The family Eleutherodactylidae includes 233 species of frogs (AmphibiaWeb, 2022) living in the tropical rainforests of Central and South America, as well as on the islands of the Caribbean. Almost all of these species seem to be characterized by direct development (Callery et al., 2001). The named family of frogs, together with other closely related Neotropical families Brachycephalidae (77 species), Ceuthomantidae (6 species), Craugastoridae (129 species) and Strabomantidae (775 species) are united in the huge Terrarana group (more than 1200 species over a vast territory from the south of the United States to the north Argentina). Typical characters for this group are reproduction on the ground and direct development, without the tadpole stage (Heinike et al., 2007, 2018; Hedges et al., 2008). The female *Eleutherodactylus* lays about 40 yolk-rich and almost unpigmented eggs, 3.5–4.0 mm in diameter (Elinson et al., 1990). Recall that a mature *X. laevis* egg has a diameter of 1.2–1.4 mm and, thus, its volume is about 20 times smaller than that of the *E. coqui* egg. Under laboratory conditions at a temperature of 25°C, after about 17 days of incubation, frogs hatch from *Eleutherodactylus* eggs.

More than 500 anuran species from several other families living in Africa, South and Southeast Asia, Australia, and Oceania are also characterized by the direct development (Duellman and Trueb, 1994; Callery et al., 2001; Desnitskiy, 2012; AmphibiaWeb,

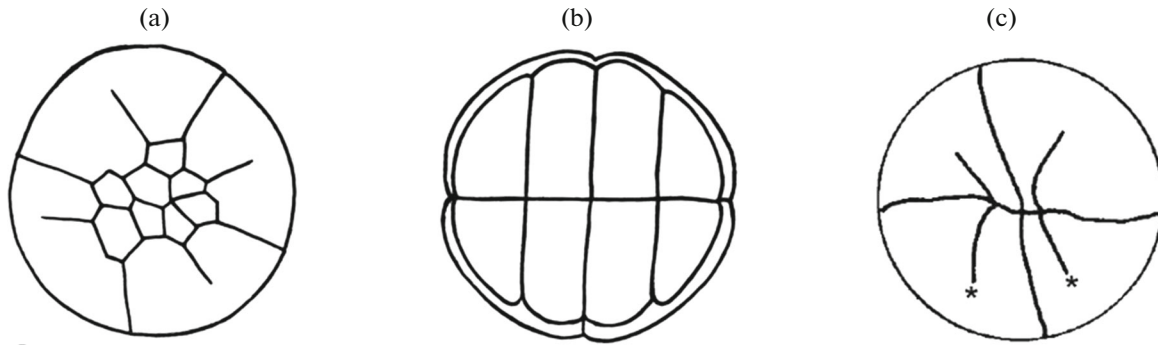


Fig. 1. Schematic representation of the early embryos of three frog species. View from the animal pole. (a) *Eleutherodactylus portoricensis* embryo at the stage of 16 blastomeres (simplified from Gitlin, 1944); (b) *Rana temporaria* embryo at the stage of 16 blastomeres (simplified from Morgan, 1897); (c) *Gastrotheca riobambae* embryo during the formation of the eight blastomeres stage (simplified from del Pino and Loor-Vela, 1990). Asterisks designate two vertical furrows of the third cleavage division of the *G. riobambae* embryo. The egg diameter in *E. portoricensis*, *R. temporaria*, and *G. riobambae* is 3–4 mm, about 2 mm, and about 3 mm, respectively.

2022). However, their early embryogenesis is practically not studied, and therefore we do not consider them now.

Some information about the initial stages of embryogenesis of *E. coqui* is given in a number of articles (del Pino and Elinson, 2003; Elinson, 2009; Elinson et al., 2011; Schmid et al., 2012; Karadge and Elinson, 2013). The pattern in which the early cleavage furrows pass is different from that of *Xenopus*, *Rana*, and many other anuran amphibians. Thus, in most embryos of *E. coqui*, the morphological differentiation into animal and vegetal blastomeres occurs only at the 16-cell stage, i.e., after the fourth cleavage division. The eight small animal blastomeres that arise at this stage of cleavage make up only about 1% of the total volume of the egg of *E. coqui* and subsequently form a very thin, translucent blastocoel roof, consisting of a single layer of cells.

A similar pattern of the early cleavage is also characteristic of other members of the Eleutherodactylidae family: *Eleutherodactylus nubicola* (Lynn, 1942), *Eleutherodactylus portoricensis* (Gitlin, 1944), and *Adelophryne maranguapensis* (de Lima et al., 2016). However, they are less studied than *E. coqui*. Figures 1a, 1b show a comparison of the early embryos of *E. portoricensis* and one of the model frog species, *R. temporaria*.

In the embryos of *E. coqui*, the synchrony of divisions of the early cleavage seems to be maintained at least until the 32-cell stage (del Pino and Elinson, 2003; Schmid et al., 2012). The embryonic development is characterized by a rapid rate, but the duration of the intervals between first five synchronous cleavage divisions has not been precisely determined. It is very important to note that the initiation of zygotic transcription (similar to that during the midblastula transition in *X. laevis*) in *Eleutherodactylus* is associated not with the midblastula stage, but with the onset of gastrulation (Chatterjee and Elinson, 2014). There

is most likely no complete analogy with the midblastula transition of the model amphibian species, since in *A. mexicanum* and *X. laevis*, during the formation of the midblastula, not only the extensive activation of zygotic transcription occurs, but simultaneously also the loss of synchrony of cell divisions and rearrangement of the cell cycle. Thus, it can be seen that in some non-model Anura species, the activation of zygotic transcription and the loss of synchrony of blastomere divisions are not necessarily “linked” to each other and can occur at different stages of early development. Of course, a more detailed morphological and molecular genetic analysis of the pregastrular steps of embryogenesis in the Neotropical frog *E. coqui* is needed.

Among other Neotropical anurans with “non-standard” patterns of early ontogeny, only the marsupial frog *G. riobambae* (the family Hemiphractidae, which consists of 122 species) has been relatively well studied (del Pino and Escobar, 1981; Elinson and del Pino, 1985; del Pino and Elinson, 2003; del Pino, 1989, 2019, 2021). It lives in the mountain valleys of Ecuador at an altitude of 2500–3200 m above sea level. Frogs mate on the ground, and the clutch averages 130 yolk-rich and very weakly pigmented eggs about 3 mm in diameter. The embryos develop in the pouch on the mother’s back for approximately 3–3.5 months (temperature in the terrarium is 17–23°C), after which the formed tadpoles are released from the pouch into the water. Thus, this marsupial frog is characterized by the biphasic life cycle. Early embryogenesis of *G. riobambae* is characterized by a very slow rate: the duration of the first cell cycle in the cleavage period at 17°C is about 12 hours (however, the dependence of the rate of *Gastrotheca* development on temperature was not studied).

In the analysis of the characteristics of the initial stages of *G. riobambae* embryogenesis (del Pino and Loor-Vela, 1990), the classification of cleavage pat-

terns in the vertebrates proposed by Nelsen (1953, p. 283) was used. The furrows of the first two cleavages are meridional and gradually pass through the egg from the animal pole to the vegetal one. However, the furrows of the third cleavage are not meridional, but vertical (Fig. 1c). This is thought to be related to the large egg size (del Pino and Loor-Vela, 1990). The vertical furrows (as well as the meridional furrows of previous cleavage divisions) tend to run in the direction from the animal to the vegetal pole, but they do not dissect both poles of the egg and do not pass through its center. The loss of division synchrony, the appearance of nucleoli, and the associated activation of rRNA synthesis occur already at the stage of eight blastomeres (del Pino and Loor-Vela, 1990; del Pino, 2018). It is appropriate to recall that in *X. laevis*, the period of synchronous cleavage divisions includes about ten cell cycles, each of which lasts 45 min at 17°C (Dettlaff and Vassetzky, 1991).

According to a number of physiological features (very slow rate of cleavage, early loss of synchrony of cell divisions, activation of RNA synthesis at the initial stages of cleavage), *G. riobambae* embryogenesis, which occurs under the protection of the maternal organism, to some extent resembles the early development of mammals. The similarity with the embryos of mammals is also manifested in the fact that the embryos of this marsupial frog receive water from the maternal organism, and gas exchange is also carried out with the help of the mother (del Pino and Escobar, 1981; del Pino, 2018). However, the early development of marsupial frogs, of course, differs from the early development of mammals in terms of the large size of the eggs and the large supply of yolk.

The stages of midblastula and early gastrula in *G. riobambae* are reached approximately 4 and 7 days after fertilization, respectively (del Pino and Escobar, 1981; Elinson and del Pino, 1985; Elinson et al., 1990; del Pino, 2019). The blastula consists of small animal blastomeres, which form a single-layer, translucent roof of the blastocoel, and large, yolk-rich vegetal blastomeres.

Let us turn to the consideration of the early development of some other frogs with the biphasic life cycle, in which ontogeny has features that differ from model anurans with the stage of an actively swimming exotrophic tadpole. Of particular interest are data (de Bavay, 1993) on the embryology of the Australian moss frog *Phyllorhina (Kyarranus) sphagnicolus* (family Myobatrachidae) living in the humid mountain subtropical forests of New South Wales. The clutch contains 30–90 non-pigmented and yolk-rich eggs, about 3.3 mm in diameter. Development occurs in a foam nest located in a small hole or depression in the wet moss. The synchrony of cleavage divisions is lost already at the 8-cell stage. This paper shows that “the plane of the third cleavage is vertical, as in many amphibians with yolk-rich eggs” (de Bavay, 1993,

p. 151). The rate of development is rather slow: at 18°C, gastrulation begins no earlier than 60 hours after fertilization. After hatching, *P. sphagnicolus* tadpoles remain in the nest (de Bavay, 1993). They are inactive and feed endotrophically, due to the large supply of yolk. Metamorphosis occurs in the nest and is completed approximately 55 days after fertilization.

Development in foamy and gelatinous nests is generally characteristic of several large families in tropical and subtropical frogs on different continents (Gomez-Mestre et al., 2012; Pereira et al., 2017; etc.). Such nests are a manifestation of care for offspring and are created on the basis of special allocations of one or both parent individuals (depending on the species). Nests can protect the laid eggs from predators, microbial contamination, desiccation, sun exposure, and temperature fluctuations. The presence of a foam nest does not necessarily correlate with large egg size, non-aqueous development, and endotrophic tadpole formation.

Returning to the Australian family Myobatrachidae, which includes 136 species, we note that the development of endotrophic sedentary tadpoles in the terrestrial foam or gelatinous nests (similar to *P. sphagnicolus*) is characteristic of about 10 species from the genera *Phyllorhina*, *Crinia*, and *Geocrinia* (Desnitskiy, 2010). It is also appropriate to recall 116 species of the subfamily Cophylinae (family Microhylidae) endemic to the tropical island of Madagascar, although their embryogenesis has not been studied. Narrowmouth frogs of this subfamily have large, yolk-rich eggs, from which the endotrophic sedentary tadpoles develop in foamy nests (on the ground, underground, or in hollows of trees) (Scherz et al., 2016; AmphibiaWeb, 2022). It is not our purpose now to enumerate all families of anuran amphibians with large eggs that develop in foamy or gelatinous nests out of water. It is difficult to name the exact number of such species of frogs and toads, but it is obvious that there are at least several hundred of them.

The family Ascaphidae, which consists of two species, deserves special attention. These very primitive (phylogenetically basal) frogs, living in the states of Idaho, Washington, California, Montana, Oregon (USA) and the province of British Columbia (Canada), lay large, yolk-rich and unpigmented eggs (about 4 mm in diameter) under stones or rocks at the bottom of mountain streams with cold, rapidly running water. Exotrophic swimming tadpoles begin metamorphosis no earlier than after one year of larval development (AmphibiaWeb, 2022). The study of the early embryogenesis of *Ascaphus truei* showed that furrows of the third cleavage division are vertical and the synchrony of blastomere divisions is lost at the 8-cell stage (Brown, 1975, 1989). Data on the features of the embryo cleavage pattern in anurans from a number of other families, as well as our proposed classification of

cleavage types in the class Amphibia, can be found in previous publications (Desnitskiy, 2014, 2019).

It is interesting to compare the data on pregastrular development and reproductive strategies in three species of frogs with large eggs, vertical orientation of the third cleavage furrows, and the very early loss of cell division synchrony. As noted above, reproductive strategies are quite different: embryonic development in *G. riobambae* takes place in the pouch on the back of the mother, in *P. sphagnicolus* in the terrestrial foam nest, and in *A. truei* in the rapidly running water. However, these reproductive strategies have very similar effects on the early cleavage pattern in different families of anurans from different parts of the world.

EARLY DEVELOPMENT OF SOME “NON-MODEL” TAILED AMPHIBIANS

Traditionally, the main model objects of experimental embryology among Caudata were the North American amphibians *Ambystoma maculatum*, *A. mexicanum*, the European *L. vulgaris*, *P. waltl*, and several other species from the families Ambystomatidae and Salamandridae (Rugh, 1962; Dettlaff and Vassetzky, 1991; Signoret and Collenot, 1991; Dettlaff, 2001). Their pregastrular ontogeny is similar (with respect to the cleavage pattern and the presence of the midblastula transition) to that of the anuran model species. Most non-model tailed amphibians live in North America, the herpetofauna of which is generally distinguished by the maximum diversity of Caudata (AmphibiaWeb, 2022). Representatives of nine out of ten families belonging to this order of amphibians live there; and five families live only on that continent.

At the beginning of the 20th century, North American tailed amphibians with large, yolk-rich eggs from the families Cryptobranchidae, Plethodontidae, and Proteidae repeatedly served as objects of experimental embryological studies (Eycleshymer, 1904; Goodale, 1911; Smith, 1922). However, later the interest of the developmental biologists in these amphibians faded, probably due to the achievements of the school of Hans Spemann (1938) and other well-known authors (for example, Briggs, 1972; Nieuwkoop, 1973; Toivonen et al., 1975), who worked with Caudata embryos from families Ambystomatidae and Salamandridae.

Let us start with the family Plethodontidae, which includes 495 species of lungless salamanders. This is more than 60% of the total number of species in the order of tailed amphibians. The family is characterized by significant ecological and ontogenetic diversity. Most species are characterized by the direct development occurring out of water: for example, in crevices between stones, under the bark of rotting logs, in tree crowns, etc. However, there are also various variants of the biphasic life cycle with aquatic larvae (Wake and

Hanken, 1996; AmphibiaWeb, 2022). The pregastrular development of the lungless salamander embryos has been studied in a number of works; egg diameter varied in the studied species from 2.2 to 6.9 mm (Hilton, 1904; Goodale, 1911; Humphrey, 1928; Collazo and Marks, 1994; Marks and Collazo, 1998; Collazo and Keller, 2010; etc.). Despite differences in reproductive strategies and egg sizes, the synchrony of cell divisions in early embryos of all species is lost already at the 8-cell stage (review: Desnitskiy, 2011). In most species of the Plethodontidae family, the furrows of the third cleavage division pass in the direction from the animal pole of the egg to the vegetal pole.

Representatives of the families Cryptobranchidae (5 species) and Proteidae (8 species) lay unpigmented and yolk-rich eggs, 5–7 mm in diameter, in water. In Cryptobranchidae, pregastrular ontogeny was studied using the American hellbender *Cryptobranchus alleganiensis* (Smith, 1906, 1922, 1926), as well as the Asian *Andrias davidianus* (Luo et al., 2007) and *Andrias japonicus* (*Megalobatrachus maximus*) (de Bussy, 1905). The descriptions of early cleavage in the embryos of these animals are in good agreement with the corresponding data obtained for Plethodontidae (the period of synchronous divisions is short and the differentiation into animal and vegetal blastomeres occurs only after the fourth cleavage division). In Proteidae, the initial stages of development have been studied only in the American mudpuppy *Necturus maculosus* (Eycleshymer, 1904; Eycleshymer and Wilson, 1910; Nelsen, 1953). The cleavage pattern is basically similar to that of Cryptobranchidae and Plethodontidae, but differs in greater variability in the passage of early cleavage furrows (even in embryos from the same clutch). The furrows of the first two cleavage divisions in *Necturus* can sometimes be vertical rather than meridional; furrows of the third cleavage are vertical (Nelsen, 1953).

Let us turn to the Asian family Hynobiidae (88 species). In most representatives of this family, the diameter of the pigmented egg laid in water is 1.5–3.2 mm. The furrows of the third cleavage are latitudinal and the synchrony of animal blastomere divisions is lost at the 16-celled stage in *Hynobius nebulosus* (Kunitomo, 1910) and *Salamandrella keyserlingii* (Sytina et al., 1987). However, the differences in the size of the smaller animal and larger vegetal blastomeres during the subsequent cleavage of both of these species are approximately of the same order as in the axolotl and newt embryos already mentioned. In general, there seems to be more similarity with the standard cleavage of Ambystomatidae and Salamandridae than with the non-standard cleavage of Plethodontidae and Cryptobranchidae. *Onychodactylus japonicus* (Iwasawa and Kera, 1980) deserves special attention. In this species, the eggs are large and unpigmented, about 5 mm in diameter, the furrows of the third cleavage are vertical (in contrast to the representatives of the family Hynobiidae mentioned above), and the cleavage process is

very similar to that in the embryos of the lungless salamanders, the American mudpuppy, and the hellbender. There are no data in the literature on the features of pregastrular ontogeny in four small families of tailed amphibians (Amphiumidae, Dicamptodontidae, Rhyacotritonidae, and Sirenidae) living in North America and including a total of 16 species.

Finally, let us return to the families Ambystomatiidae (32 species) and Salamandridae (130 species), which include several well-studied model species with the midblastula transition. Note that both of these families represent only slightly more than 20% of the total number of tailed amphibian species. However, there is a reason to believe that the Salamandridae family contains species with deviations from the standard course of the pregastrular ontogenesis. A significant number of European and Asian species of the Salamandridae family—*Calotriton asper*, *Echinotriton andersoni*, *Euproctus montanus*, *Mertensiella caucasica*, *Pachytriton brevipes*, *Pachytriton labiatus*, *Salamandra salamandra*, *Tylotriton taliangensis* and others—are characterized by large and yolk-rich eggs (Noble, 1927; Gasser, 1964; Tarkhnishvili and Serbinova, 1997; Buckley et al., 2007; AmphibiaWeb, 2022). The initial stages of development occur in *Echinotriton* on the ground under fallen leaves near a reservoir, in *Salamandra* in the oviducts of the mother, and in the other species mentioned in running water. In particular, data on cleavage morphology in the embryos of the viviparous *S. salamandra* (Grönroos, 1895) are more similar to the data obtained on species from the families Cryptobranchidae, Plethodontidae, and Proteidae than to those on the common, alpine, Spanish, or Japanese newts.

ABOUT EARLY ONTOGENY IN THE ORDER OF CAECILIAN AMPHIBIANS

Representatives of the order Gymnophiona (caecilians) live in the tropics of South and Central America, Africa, South and Southeast Asia. In species with aquatic larvae and species with direct development, the eggs are large and rich in yolk (diameter up to 8–10 and 3–6 mm, respectively); the clutch contains no more than a few dozen eggs (Exbrayat, 2006; Gomez et al., 2012). In viviparous species, the egg diameter is 1–2 mm, and the female usually bears only a few embryos (Wake, 2015). The first embryological studies of caecilian amphibians were carried out at the end of the 19th century (Sarasin, P. and Sarasin, F., 1887; Brauer, 1899). However, the data on early embryogenesis were preliminary, apparently due to difficulties in obtaining material. The problem of obtaining material for the analysis of the early stages of caecilian embryogenesis remained very relevant throughout the next century, since three tables of development published over the past 30 years did not include the stages of cleavage and gastrulation (Sammouri et al., 1990; Dünker et al., 2000; Pérez et al., 2009). Thus, today

there is not a single well-studied model species among Gymnophiona.

The only work in which the cleavage of the caecilian egg was analyzed in sufficient detail is the article by Svensson (1938) on the Asian species with larval development, *Ichthyophis glutinosus* (family Ichthyophiidae, including 57 species). The cleavage is holoblastic with very small animal blastomeres, and in general it resembles the cleavage in those representatives of Anura and Caudata that have large eggs with a high content of yolk. Therefore, it is difficult to agree with the opinion of O.M. Ivanova-Kazas (1995, p. 230) on the meroblastic nature of cleavage in Gymnophiona. All members of the class Amphibia studied so far have holoblastic embryonic cleavage (Elinson and del Pino, 2012; del Pino, 2018).

AN ATTEMPT AT A PHYLOGENETIC ANALYSIS OF PREGASTRULAR DEVELOPMENT

The pattern of pregastrular ontogeny described in textbooks (e.g., Signoret and Collenot, 1991; Barresi and Gilbert, 2020) is by no means characteristic of all amphibians. It seems that not much more than 50–60% of the species of the order Anura have the same (standard) early development pattern as, for example, *R. temporaria* or *X. laevis* and no more than 20% of the species of the order Caudata have the same developmental pattern as *A. mexicanum* or *P. waltil*. In the order Gymnophiona, there are apparently no species with the standard cleavage pattern (characteristic of the model species of Anura and Caudata) and subsequent midblastula transition.

The large size of the egg is very often associated with the transition to asynchronous divisions already at the early stages of embryo cleavage (Desnitskiy, 2018, 2019). These can be both phylogenetically basal species (the tailed amphibians *A. davidianus*, *A. japonicus*, *C. alleganiensis*, the frog *A. truei*) and evolutionarily advanced species (the lungless salamander *Ensatina eschscholtzii*, the frogs *G. riobambae*, *P. sphagnicolus*, *Rhacophorus arboreus*). The same course of cleavage is also characteristic of the lungfishes *Lepidosiren paradoxa* and *Neoceratodus forsteri* (Kemp, 1982; Kershaw et al., 2009; Desnitskiy, 2015), which are the closest living relatives of terrestrial vertebrates (Brinkmann et al., 2004; Liang et al., 2013).

It was suggested (Desnitskiy, 2014) that the pattern of pregastrular ontogeny with the loss of division synchrony as early as at the 8-cell stage and the absence of differentiation into animal and vegetal blastomeres during the third cleavage is ancestral for the class Amphibia, although many evolutionarily advanced species of anuran and tailed amphibians also have the same pattern of development. The standard pattern of early ontogeny (the extensive series of 10–12 rapid synchronous divisions of blastomeres, including lati-

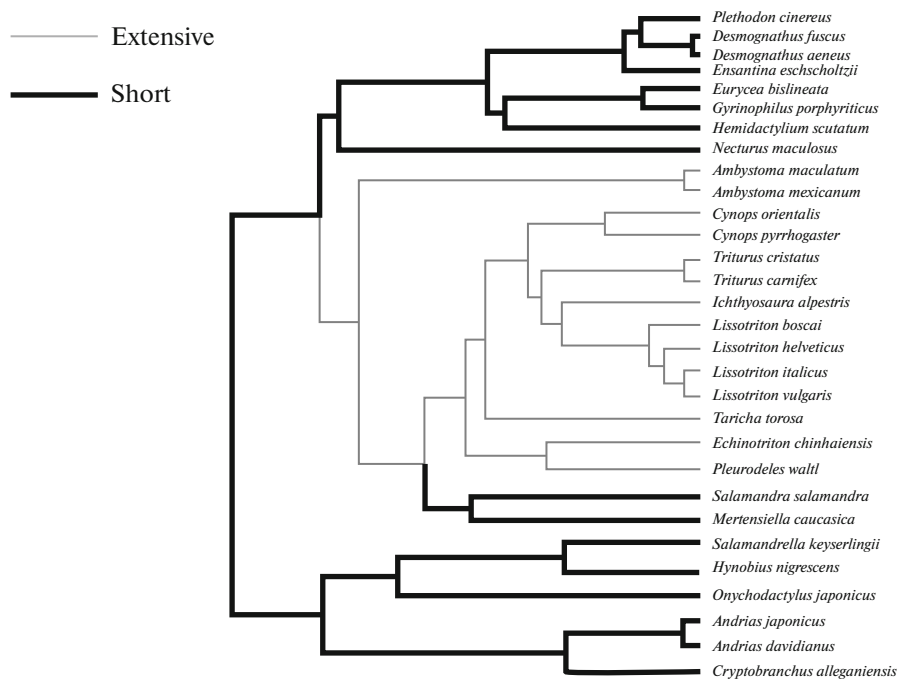


Fig. 2. Features of embryo cleavage in different Caudata species mapped onto the phylogenetic tree of this order (simplified from Desnitskiy and Litvinchuk, 2015). The magnitude of the period of synchronous cleavage divisions: extensive (up to 10–12 divisions) or short (up to 3–4 divisions).

tudinal furrows of the third cleavage) with characteristic model species *A. mexicanum* and *X. laevis* is probably derived, arising independently in the orders Anura and Caudata. In our opinion, in favor of the convergent occurrence of the midblastula transition is the fact of some differences in the cytophysiology of the blastula stage in these two species. In the axolotl, development is blocked at the midblastular transition by the transcriptional inhibitor α -amanitin (Signoret, 1980; Lefresne et al., 1998). On the contrary, in the clawed frog the process of cell desynchronization is not sensitive to α -amanitin (Newport and Kirschner, 1982). Finally, cells of the *X. laevis* embryo acquire individual motility immediately after the midblastula transition, whereas this was not observed in the *A. mexicanum* embryo (Andéol, 1994; Lefresne et al., 1998).

It was of interest to carry out a comparative phylogenetic analysis of the process of pregastrular development of amphibians in order to verify and refine our intuitive assumptions. This type of research has no analogues in the world literature. However, we had to restrict ourselves to the order Caudata, since the situation in the taxonomy and phylogeny of Anura is less stable than the situation in the taxonomy and phylogeny of tailed amphibians. Ideas about the classification and evolution of anurans are constantly refined or even revised (for example, Streicher et al., 2018). In addition, the number of extant Anura species is nearly ten times that of extant Caudata species (Amphibia-Web, 2022). Data on the features of embryo cleavage

are known for approximately 4.65% of the species of tailed amphibians. For anurans, relevant information is available in the literature for less than 1% of the species.

For our comparative and phylogenetic analysis of the cleavage process in embryos of the order Caudata (Desnitskiy and Litvinchuk, 2015), it was important to know: (1) the presence or absence of an extensive series of rapid synchronous cleavage divisions (up to 10–12 cell cycles) in the animal hemisphere of the early embryo and (2) the orientation of the furrow of the third cleavage division (latitudinal, vertical or variable). In species with the variable furrows of the third cleavage, they are either latitudinal or vertical in different embryos of the same clutch. Relevant data published in the embryological and herpetological literature were collected on the embryos of 36 species from six families: Ambystomatidae, Cryptobranchidae, Hynobiidae, Proteidae, Plethodontidae, and Salamandridae. Each character was mapped onto based on the results of recent evolutionary studies (San Mauro, 2010; Pyron and Wiens, 2011; Vieites et al., 2011; Zheng et al., 2012; Shen et al., 2013; etc.) phylogenetic tree of the order Caudata (Figs. 2 and 3). In addition, our analysis used the data on egg size, which are known for representatives of all ten families of the order of tailed amphibians, and so the character “egg size” was also mapped onto the phylogenetic tree (Fig. 4).

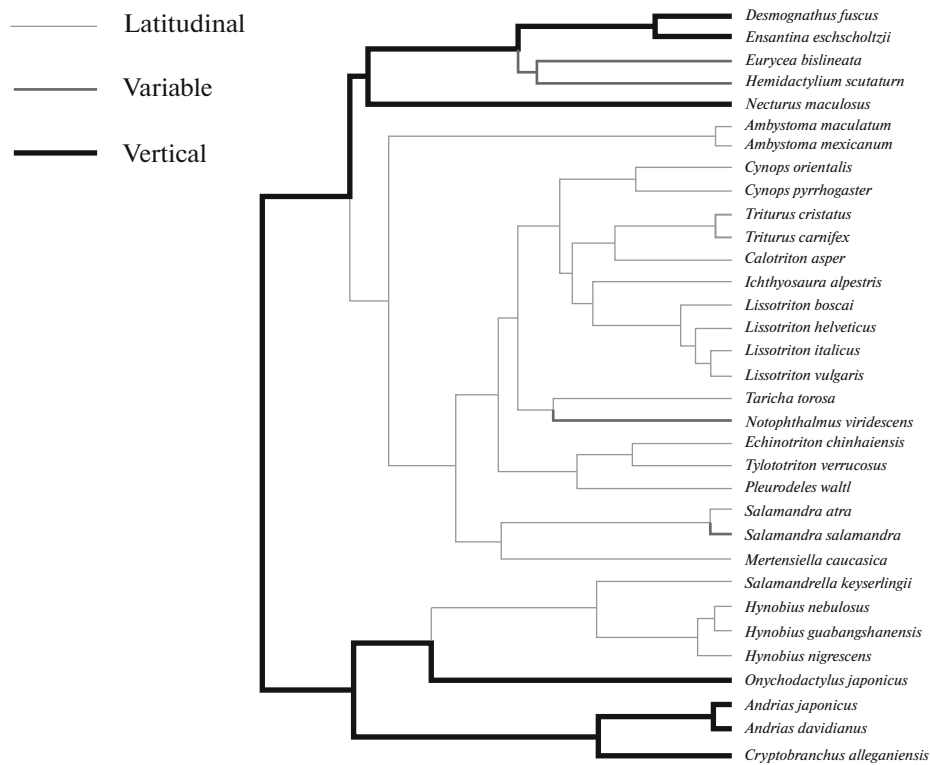


Fig. 3. Features of embryo cleavage in different Caudata species mapped onto the phylogenetic tree of this order (simplified from Desnitskiy and Litvinchuk, 2015). The orientation of the furrows of the third cleavage division: latitudinal, variable or vertical.

Our study showed that the pregastrular development of large, yolk-rich eggs with the short series of synchronous divisions and vertical furrows of the third cleavage represents an ancestral set of ontogenetic traits for the order Caudata. In contrast, the latitudinal furrows of the third cleavage and the period of 10–12 rapid synchronous divisions, accompanied by the midblastula transition, are derived features. The phylogenetic analysis of pregastrular ontogeny in tailed amphibian embryos (Desnitskiy and Litvinchuk, 2015) fully confirmed the idea of evolutionary relationships between cleavage types, which was put forward earlier (Desnitskiy, 2014).

Our analysis showed that the large egg size is among important ancestral traits of the order Caudata. In addition, tailed amphibians are characterized by an evolutionary transition from large egg size to small egg size, and then repeated reverse transitions from small egg size to large egg size in the Salamandridae family, for example, in *C. asper* and *S. salamandra* (Desnitskiy and Litvinchuk, 2015). It should be noted that a decrease in egg size in the families Ambystomatidae and Salamandridae was associated with the acquisition of the series of 10–12 rapid synchronous divisions, accompanied by the midblastula transition (*A. mexicanum*, *L. vulgaris*, *P. waltl*, and several other species).

CONCLUDING REMARKS AND PERSPECTIVES

According to the molecular phylogenetic data (San Mauro, 2010), orders of tailed and anuran amphibians diverged about 290 million years ago (at the beginning of the Permian). Our studies suggest that the cleavage pattern in early embryos of the model species *A. mexicanum* (Caudata) and *X. laevis* (Anura) represents a homoplasy. This cleavage pattern is associated with the presence of numerous small eggs in clutches, as well as with the course of embryonic and larval development in lentic water bodies (where the opportunities for exogenous feeding of larvae are much better than in running water). In connection with the above, it is logical to assume that the midblastula transition, which accompanies an extensive series of synchronous egg divisions in the pregastrular ontogenesis of a significant number of model and non-model species of Anura and Caudata, arose convergently in these two orders of the class Amphibia as an embryonic adaptation to life and development in the lentic water.

For further comparative analysis of the pregastrular development, some non-model species of the order Anura are of particular interest. For example, a large and yolk-rich egg, vertical furrows of the third cleavage, and the absence of the midblastula transition are characteristic not only of the basal frog *A. truei*

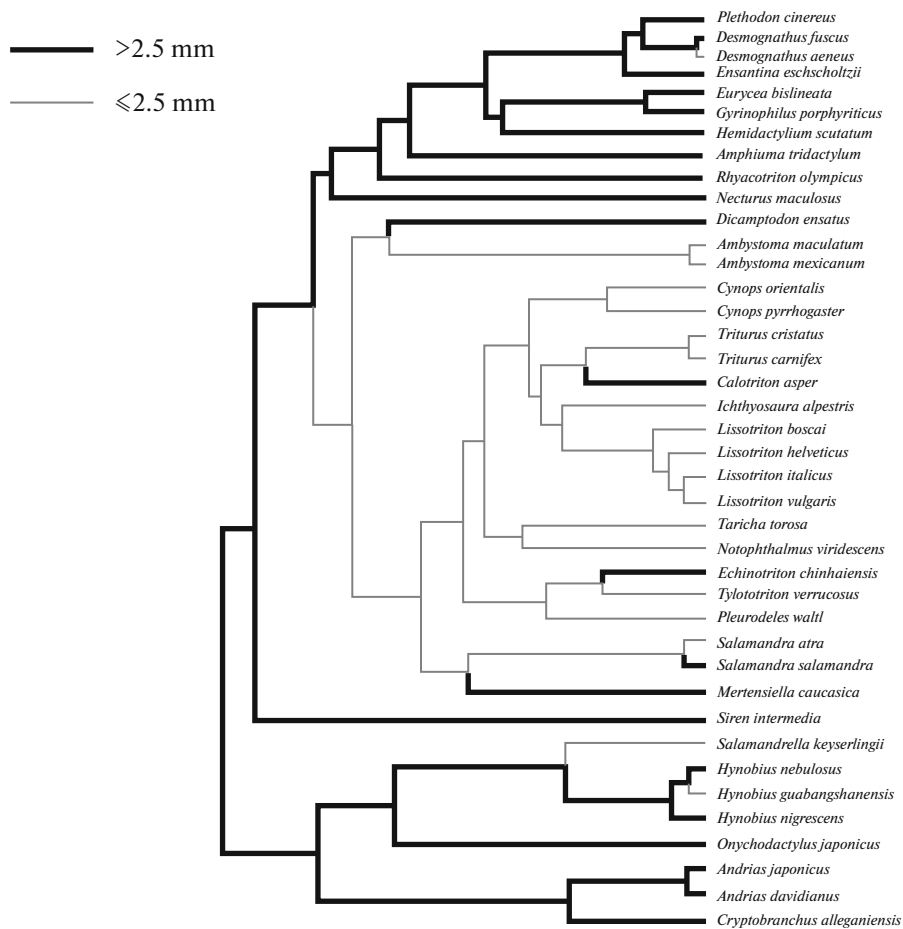


Fig. 4. Egg size in different Caudata species, mapped onto the phylogenetic tree of this order (simplified from Desnitskiy and Litvinchuk, 2015).

(Archaeobatrachia), but also of *E. coqui*, *G. riobambae*, and *P. spagnicolus*, representatives of various families of evolutionarily advanced frogs (Neobatrachia). Did the large size of the egg always remain in a few phylogenetic lineages of Neobatrachia, or did the loss and reacquisition of the mentioned character (and other accompanying features) occur sequentially? At present, there is no clear answer to this question.

Finally, in some non-model Anura species, the transition from maternal control of ontogeny to zygotic control occurs not at the midblastula stage, but at other stages of development: at the stage of early cleavage in the marsupial frog *G. riobambae* and at the beginning of gastrulation in *E. coqui*, which has the direct development. In this regard, a number of questions arise. When does the change in developmental control occur in other groups of frogs with direct development: from Australia, Asia, Africa or Oceania (since it had occurred independent of the direct development of Neotropical Terrarana)? When does the change in control of early development occur in the lungless salamanders (Plethodontidae) with direct development and with biphasic life cycle?

Concluding the review, we note that both in the case of the order Anura and in the case of the order Caudata, there is undoubtedly a rather flexible relationship between the reproductive strategy and the features of the pregastrular development. The ontogenetic diversity of amphibians is a reflection of their ecological diversity, and different reproductive strategies presumably can often have a very similar (although not necessarily identical) effect on the pattern of early development of the embryo. For further elaboration of this concept (and elucidation of causal relationships between ecological and ontogenetic traits), it would be important to attract additional information on the initial stages of development in a significant number of non-model amphibian species, which, however, have so far hardly attracted the attention of embryologists.

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predecessor of the Department of Embryology of Saint-Petersburg State University.

COMPLIANCE WITH ETHICAL STANDARDS

The author declares that he has no conflicts of interest.

In preparing this review, humans and animals were not used as objects of experimental research.

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