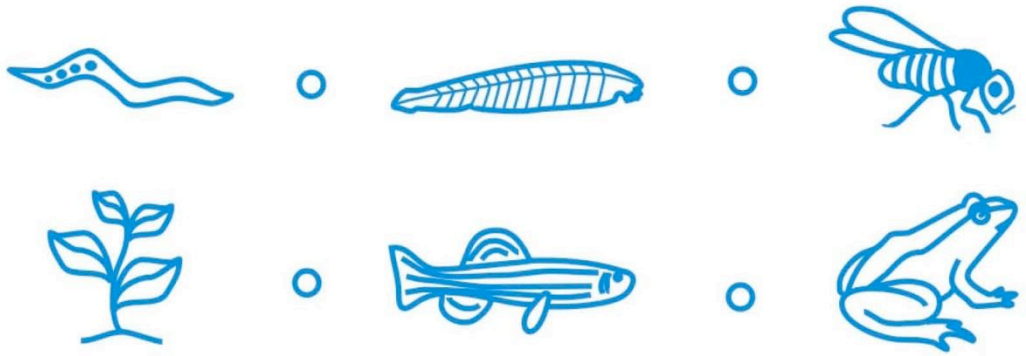
Model Organisms and
Innovative Approaches
In Developmental Biology



The early development of Gastrotheca riobambae and Colostethus machalilla, frogs with terrestrial reproductive modes

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Sections:

1. Introduction
2. The biology of *Gastrotheca riobambae*
3. The biology of *Colostethus machalilla*
4. Comparison of Brachyury (Bra) protein expression

Section 1: Introduction

Why do we study frogs that differ from *Xenopus laevis*?

- To discover possible variations in development.
- To advance the understanding of Ecuador's biodiversity.

Desirable features of alternative model organisms:

- Distinctive biology
- Ease of collection
- Simple maintenance

We work with two frogs:

1. The marsupial frog *Gastrotheca riobambae* (Hemiphractidae).

2. The dendrobatid *Colostethus machalilla* = *Epipedobates machalilla* (Dendrobatidae).

Section 2: Biology of the marsupial frog *Gastrotheca riobambae*

•Females of marsupial frogs brood their embryos inside a dorsal pouch. The outlines of embryos can be detected on her back.

•The pouch of the mother and the bell gills of embryos are specialized structures that allow exchanges with the mother and incubation of embryos.

Ocurrence of the frog: Highlands of northern Ecuador. Other species occur from Panama to northern Argentina and Brasil's South East.

Refs: del Pino EM. Sci . Am. 260:110-118 (1989);
Duellman W ,Trueb L. Biology of Amphibians. New York. McGraw-Hill Book Co (1986).

Maintenance of *G. riobambae*


Photo: E M del Pino

Advantages:

1.This frog reproduces in captivity (at high altitudes).

2.Accepts live prey and meat.

3.Egg laying can be induced by HCG administration.

Disadvantages for maintenance in captivity:

This frog requires the conditions of light & atmospheric pressure found at high altitudes. It needs:

- Cool nights & warm days.
- 12 hours light cycle with intense light.
- Low atmospheric pressure.

Ref: Elinson et al. Biol Bull 179: 163-177(1990).

The male and female *G. riobambae*

Male

Snout-vent length: about 4 cm

Color variation: Green and brown

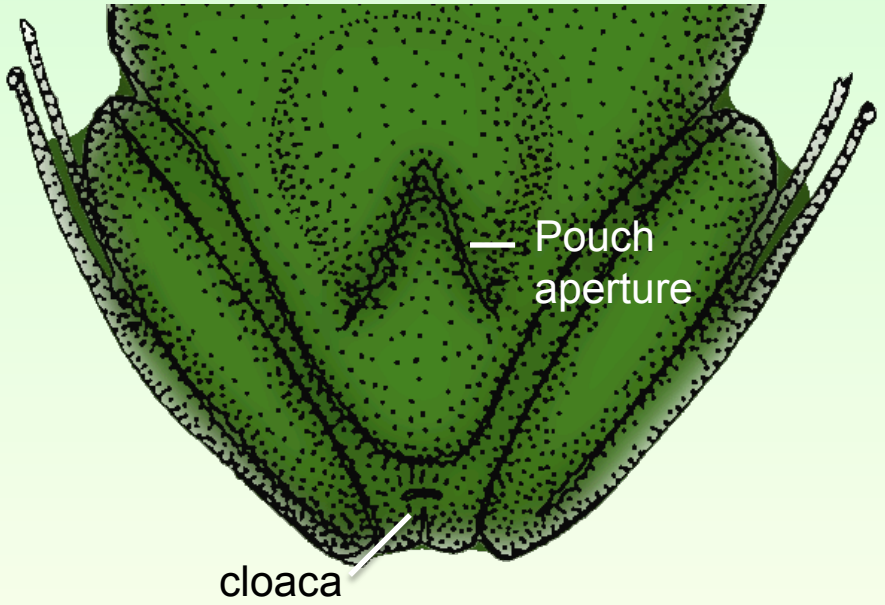
Non-brooding female

Snout-vent length: about 6 cm

Photos: JD Santillana Ortiz

Features of the pouch:

The pouch of a non-brooding female



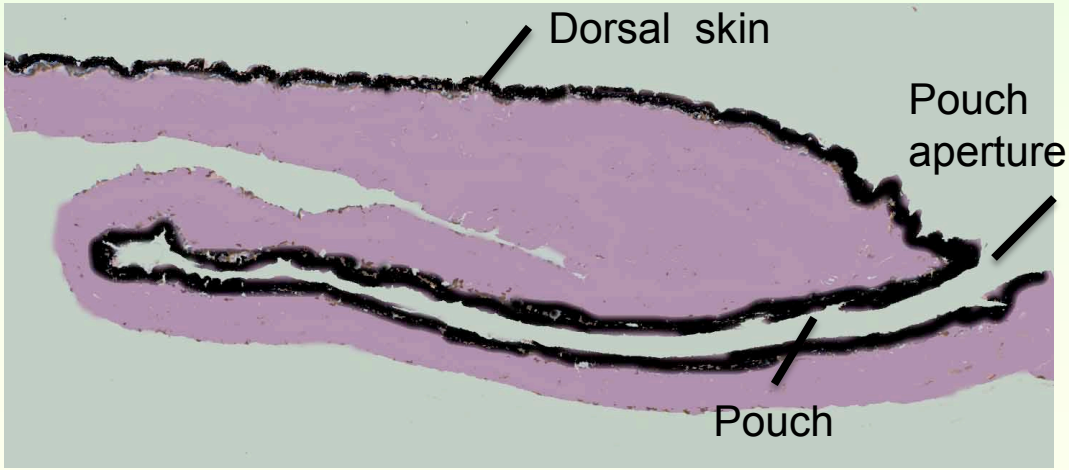
Modified from del Pino, EM., J. Exp. Zool. 227: 159-163 (1983).

Refs: del Pino EM et al. Biol Bull 149: 480-491 (1975); del Pino & Escobar J. Morphol. 167:277-295 (1981); del Pino EM. J Exp Zool 227: 159-163 (1983).

- The pouch derives from the skin.
- It is under hormonal control.
- It becomes vascularized during embryonic incubation.
- It forms an embryonic chamber for each embryo.
- Females brood 120 embryos on average.
- Embryonic incubation lasts 4 months.

The pouch is a derivative of the skin

Outline of a sagittal section through the pouch



The pouch derives from the dorsal skin & appears as an infolding of skin.

Its formation in juvenile females can be induced by estradiol.

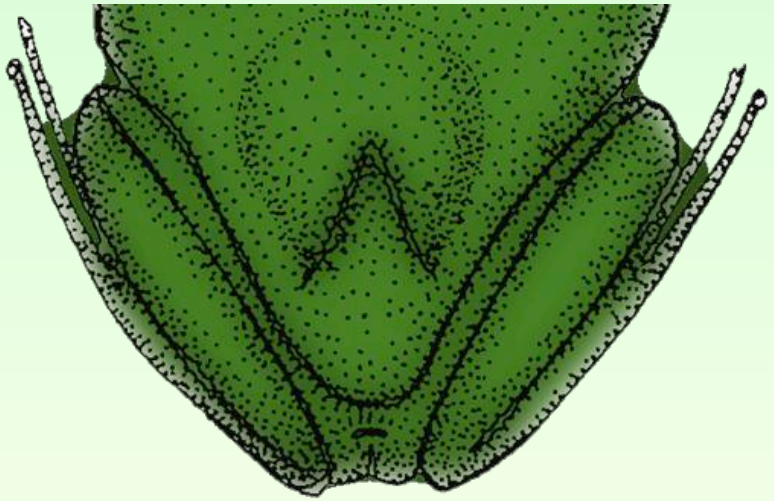
The non-incubating pouch resembles skin. It differs from skin, as it lacks poison glands.

Pouch histology changes during incubation of embryos.

Ref: del Pino EM. J. Exp. Zool. 227: 159 -163 (1983); Jones RE. J. Exp. Zool. 184: 177- 184 (1973).

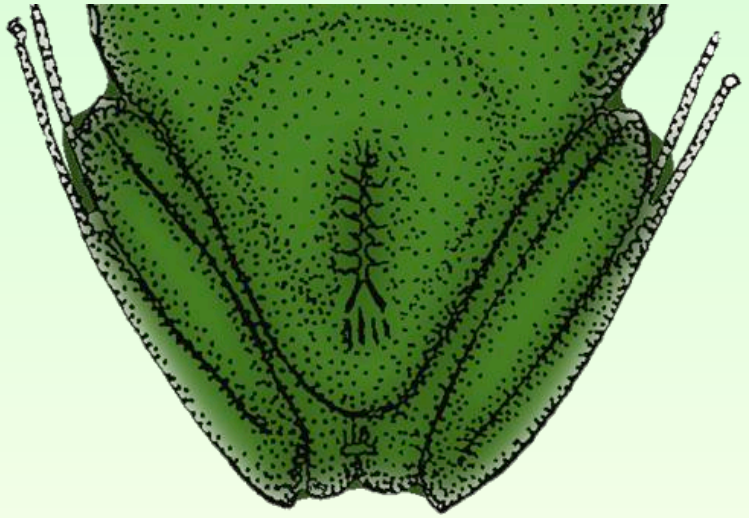
The pouch aperture in *G. riobambae*:

Open



Female with small ovaries

Closed

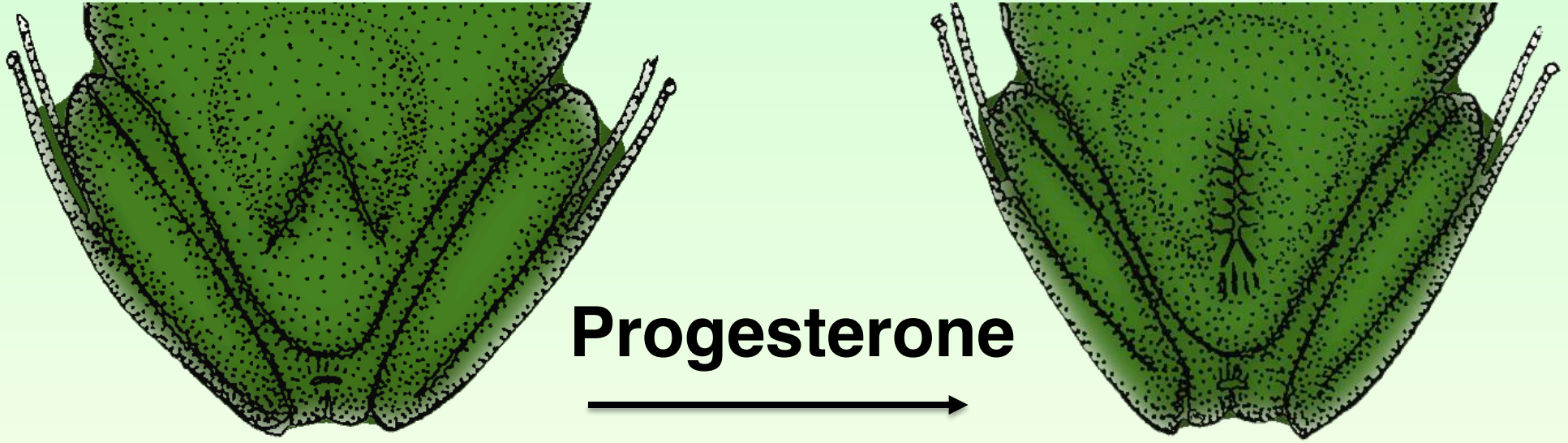


Female with large ovaries, & ready for reproduction

The borders of the pouch aperture come together when the pouch is closed. The pouch remains closed during embryonic incubation. Embryos, however, can be removed with forceps.

Modified from del Pino, EM., J. Exp. Zool. 227: 159-163 (1983).

Progesterone administration induces:



1. Pouch closure
2. Pouch vascularization &
3. Formation of embryonic chambers (around inert beads).

Modified from del Pino, EM., J. exp. Zool. 227: 159 -163 (1983).

Specialized gills: the bell gills

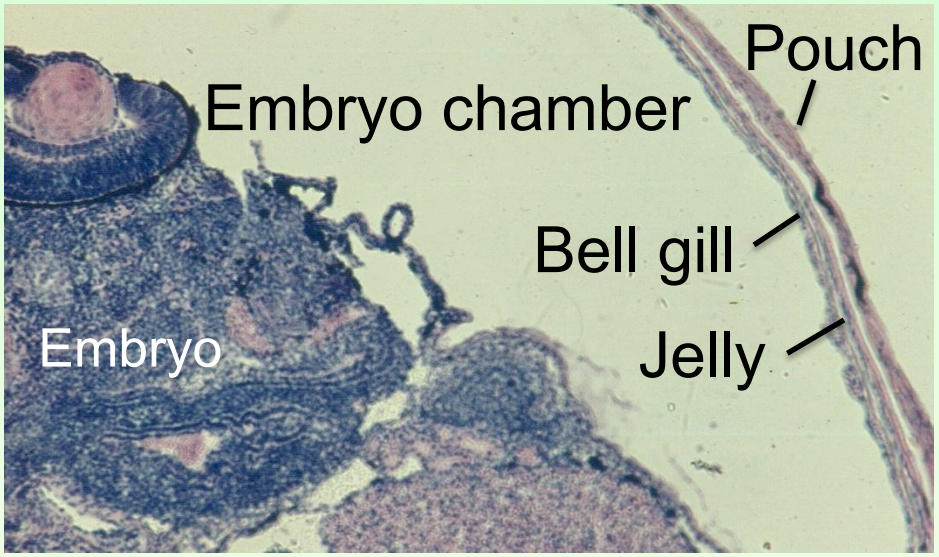
Bell gills form a vascularized sac that fully envelops each embryo.
Exchanges with the mother are mediated by the pouch & bell gills.

Photos: E M del Pino

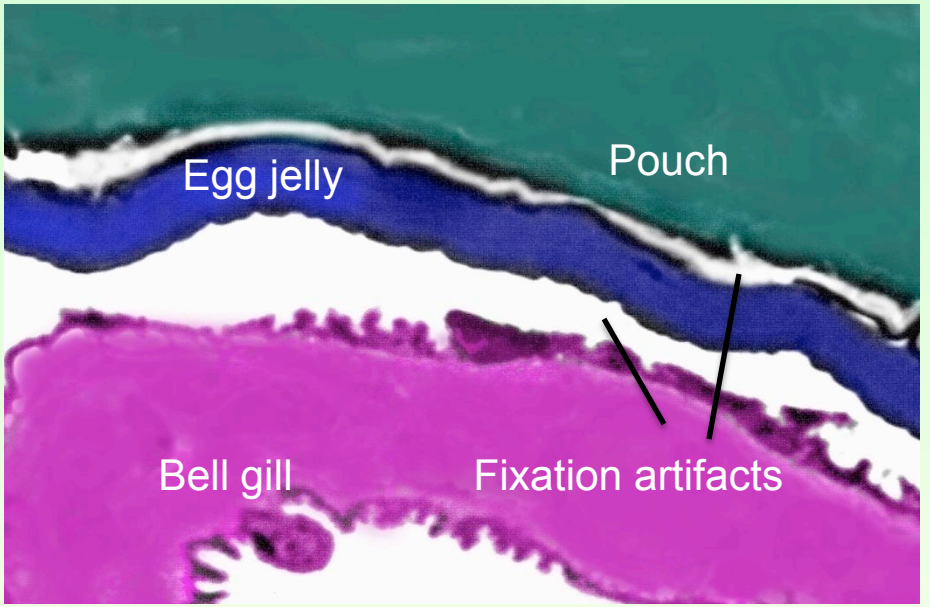
Ref: del Pino et al., Biol. Bull. 140: 480-491 (1975).

Pouch-bell gill association in *G. riobambae*

Section of embryo within the pouch



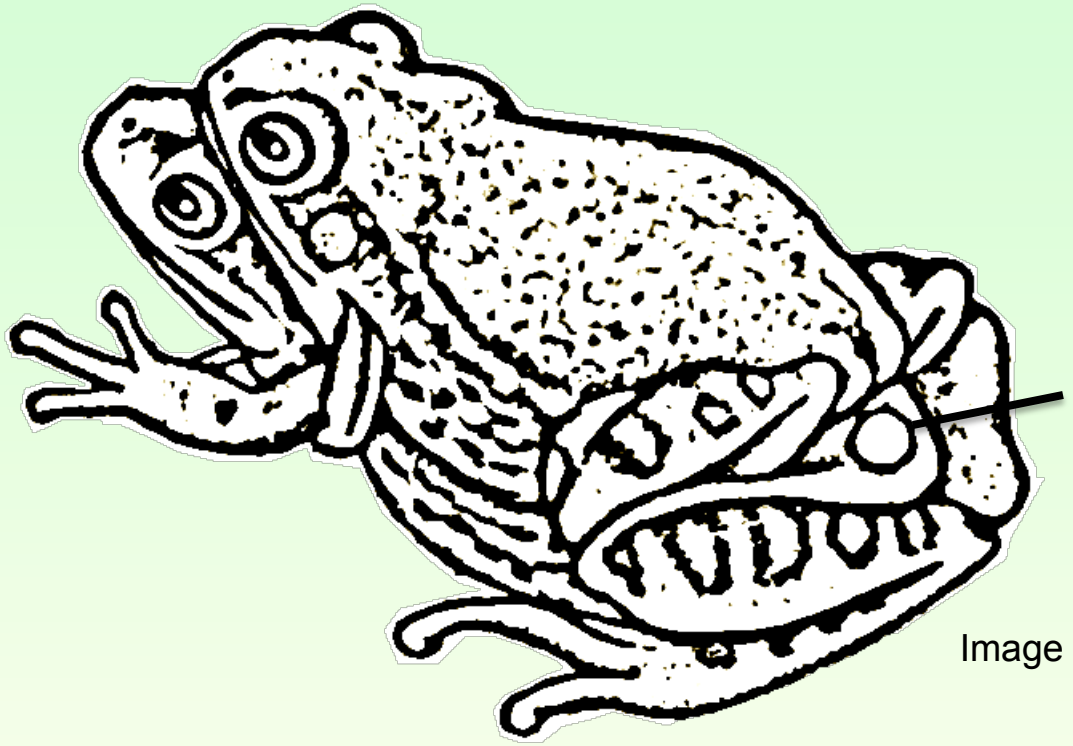
Outline of the pouch-bell gill association (from a histological section).



The pouch-egg jelly-bell gills adhere tightly in the living condition. Pouch & bell gills are highly vascularized, & have villi. They are separated by a thin layer of egg jelly, and vitelline membrane. Bell gills are connected to the body by gill stacks that contain afferent and efferent vessels. The nature of exchanges in the pouch are unknown.

Ref: del Pino et al., Biol. Bull. 140: 480-491 (1975).

Amplexus in *G. riobambae*



Egg emerging from the female's cloaca

Image modified from Sci. Am. 260: 110 (1989)

Mating takes place on land. As each egg emerges from the female's cloaca, the male moves it inside the pouch with his feet. Semen is deposited on the back of the female. Fertilization occurs as eggs are moved into the pouch.
Gametes of this frog die in the low salt concentrations used for the culture of *Xenopus* embryos, and survive in physiological saline solutions.

References: del Pino EM. Sci . Am. 260:110-118 (1989); Alcocer et al.Comp.Biochem.Physiol 101A: 229-231 (1992).

Tadpole birth:

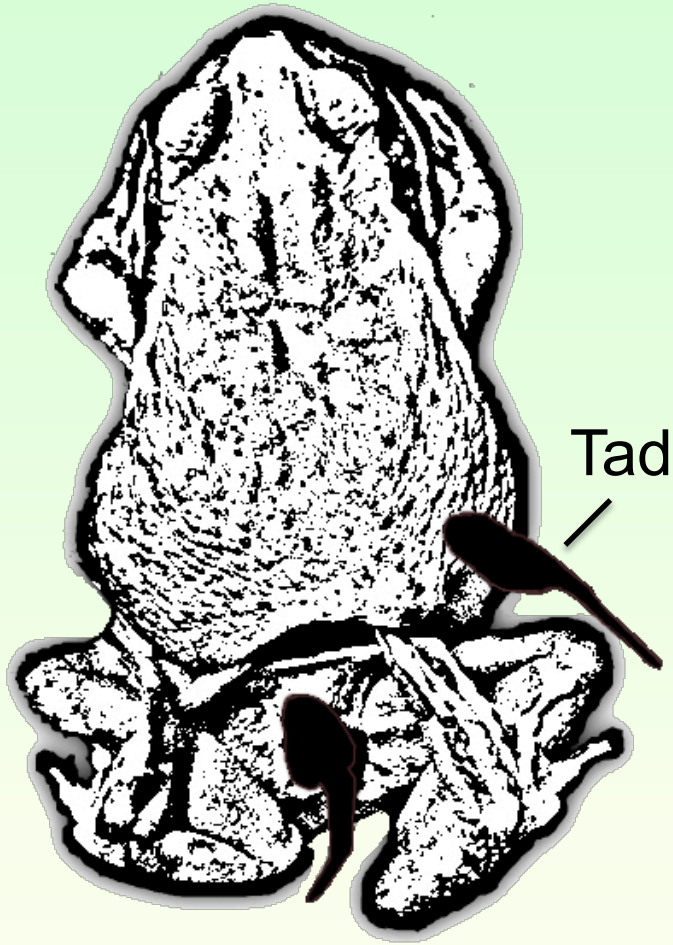


Image based on a photo by F. Koester

At the time of tadpole birth, the female inserts its toes into the pouch to distend the pouch aperture and aid in the emergence of tadpoles.

Free-living tadpoles metamorphose in times that vary from 60 days to one year.

The majority of marsupial frog species give birth to young frogs, and only few species release tadpoles.

Reference: del Pino EM., Sci. Am. 260:110-118 (1989).

Gastrulation in *G. riobambae*

Late gastrula

- Eggs are very large (3 mm diameter). Eggs measure up to 10 mm in diameter in other marsupial frogs.

- Development is slow (14 days from fertilization to the end of gastrulation).

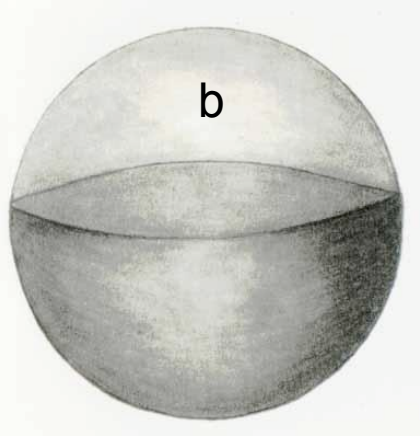
- An embryonic disk of small cells is formed around the closed blastopore. This frog has the most divergent mode of frog gastrulation.

d, embryonic disk.
Image reproduced from Developmental Biology (Dev Biol, 2007, 304: 467-478).

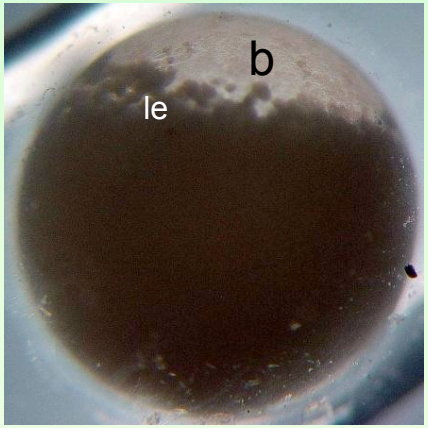
Ref: del Pino EM & Elinson RP. Nature 306: 589-591 (1983).

The early gastrula of *G. riobambae*

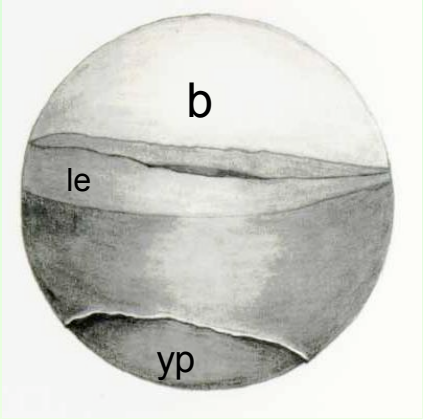
Blastula



Early gastrula



Early gastrula

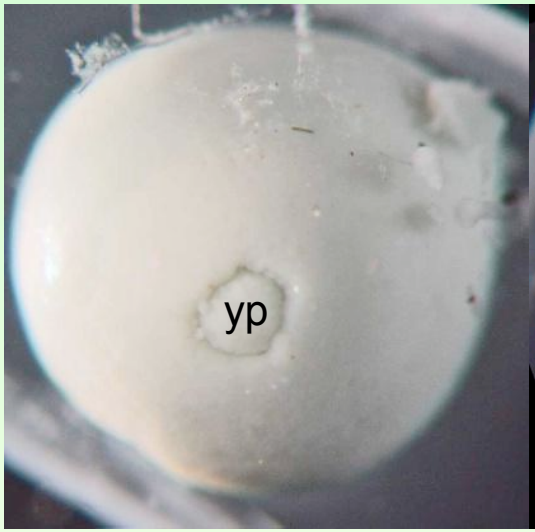


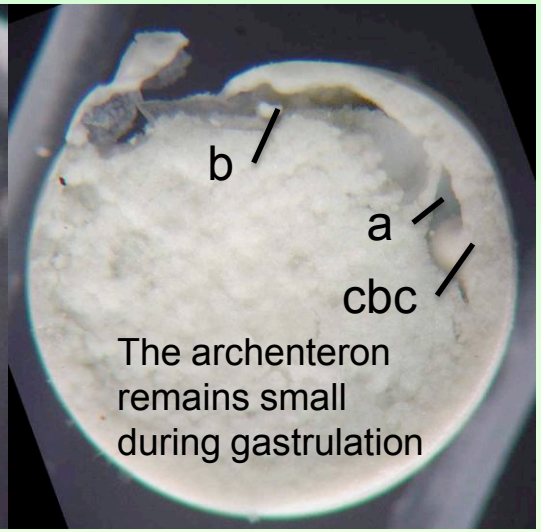
The blastocoel roof consists of a monolayer of cells. As it is transparent, the movements of the leading edge of the mesendoderm can be detected in living embryos, this is the first indication of the onset of gastrulation. During gastrulation, the gastrocoel roof will be completely covered by the mesendoderm. A blastopore is formed in the subequatorial region.

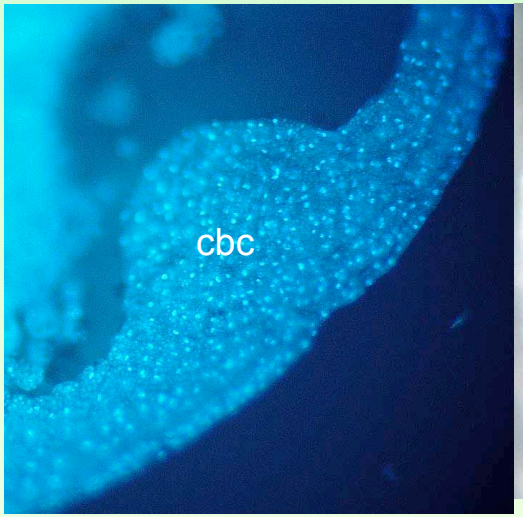
b, blastocoel, le, leading edge of mesendoderm; yp, yolk plug.

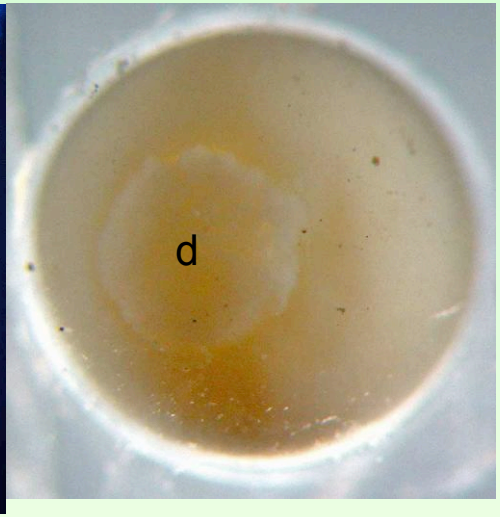
Refs: del Pino EM & Elinson RP. Nature 306: 589-591 (1983); Moya IM et al. Dev Biol 304: 467 -478. (2007).

The late gastrula of *G. riobambae*









The blastopore is formed in the subequatorial region of the embryo, as in *Xenopus*. The cells that involute during gastrulation remain in the blastopore lip and form a thick circumblastoporal collar (cbc). On the surface, a disk of small cells is formed in the region of the cbc. The body of the embryo will derive from the embryonic disk.

Refs: del Pino EM & Elinson RP. Nature 306: 589 -591 (1983); Moya IM et al. Dev Biol 304: 467 -478. (2007).

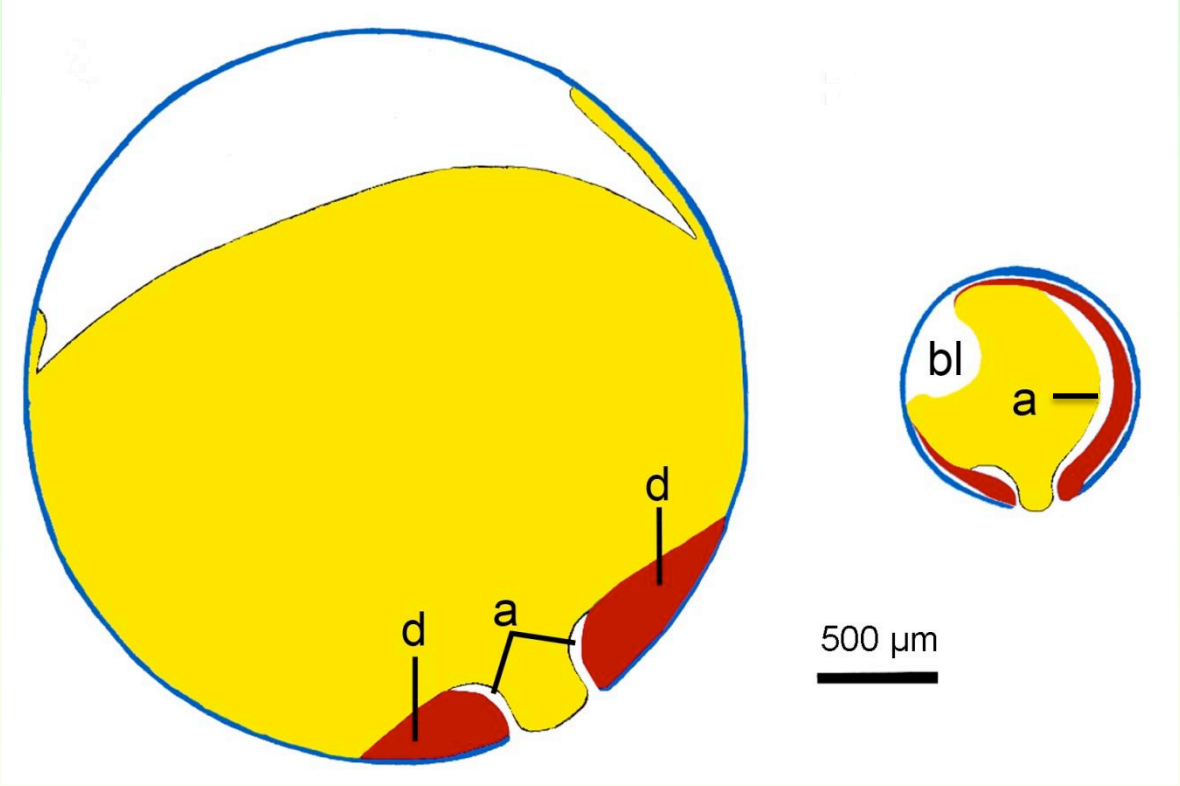
a, archenteron; b, blastocoel; cbc, circumblastoporal collar; d, embryonic disk; yp, yolk plug

Comparison of gastrulation

Gastrotheca

Xenopus

Formation of the embryonic disk is associated with a delay in elongation of the archenteron.



Archenteron elongation starts at the mid gastrula.

Abbreviations: a, archenteron; bl, blastocoel; d, embryonic disk.
Drawings are to scale.

Refs: del Pino EM & Elinson RP. Nature 306: 589 -591 (1983); del Pino EM. Development 107: 169 187 (1989).
Image modified from del Pino EM. Development 107: 169 187 (1989)

Conclusion:


Photo: E M del Pino

The external appearance of the adult does not disclose the extraordinary reproductive features of *Gastrotheca riobambae*

Section 3:
Biology of *Colostethus machalilla*

- Tiny frog of 17 mm in length.
- Reproduction at intervals of about 2 weeks.
- Terrestrial nests of 15 eggs (eggs of 1.6 mm in diameter).
- Parental care of the nest.
- 21 days until hatching.
- At hatching the father transports tadpoles on its back to a water pool.

Dendrobatid frogs are of interest for developmental work as they can be maintained in captivity, & their development deviates from *X. laevis.*

Ref: del Pino EM et al. Int. J. Dev. Biol 48:663 -670 (2004)

Maintenance of *C. machalilla*

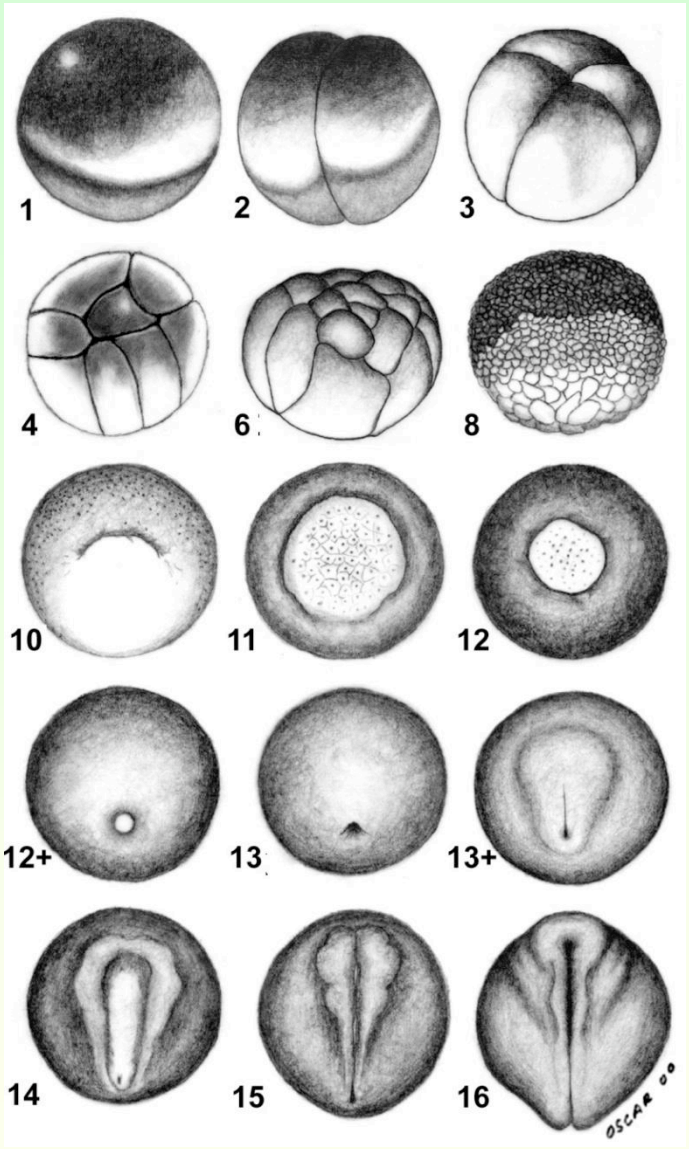
Disadvantage: Dendrobatid frogs are territorial, and under crowded conditions the nests are destroyed.

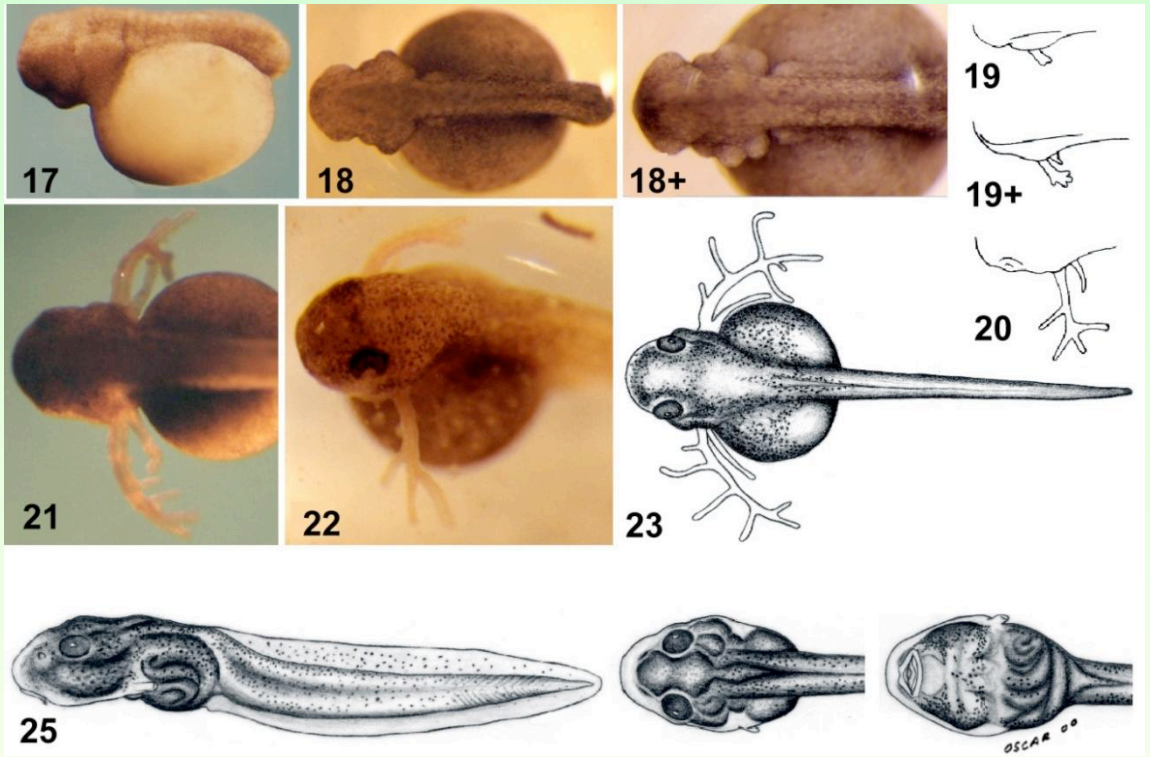
Advantages for developmental studies:

- Frogs can be kept in terraria.
- This frog eats *Drosophila* and other small insects.
- Frogs use artificial nests, such as plastic tubes.
- Eggs can be cultured in vitro.
- Metamorphosis occurs after 5 months.
- New frogs call one year after metamorphosis.
- Some dendrobatid frogs are available in pet shops.

Ref: del Pino EM et al. Int J Dev Biol 48: 663-670 (2004).

Development of *C. machalilla*





Embryos have a typical frog-like external appearance. After 19 days, embryos hatch (st 25), & the father transports the tadpoles on his back to water.

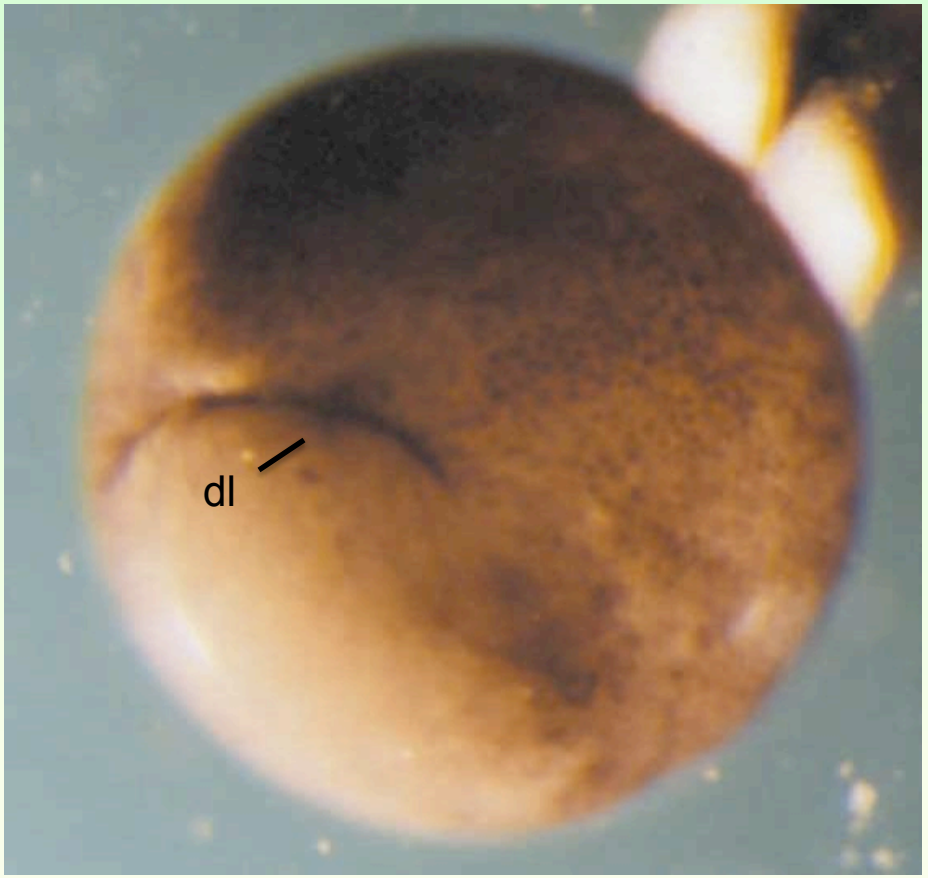
Reproduced with permission from The International Journal of Developmental Biology (Int. J. Dev. Biol. 2004, 48:663-670).
Ref: del Pino EM et al. Int J Dev Biol 48: 663-670 (2004).

Numbers indicate the developmental stage.

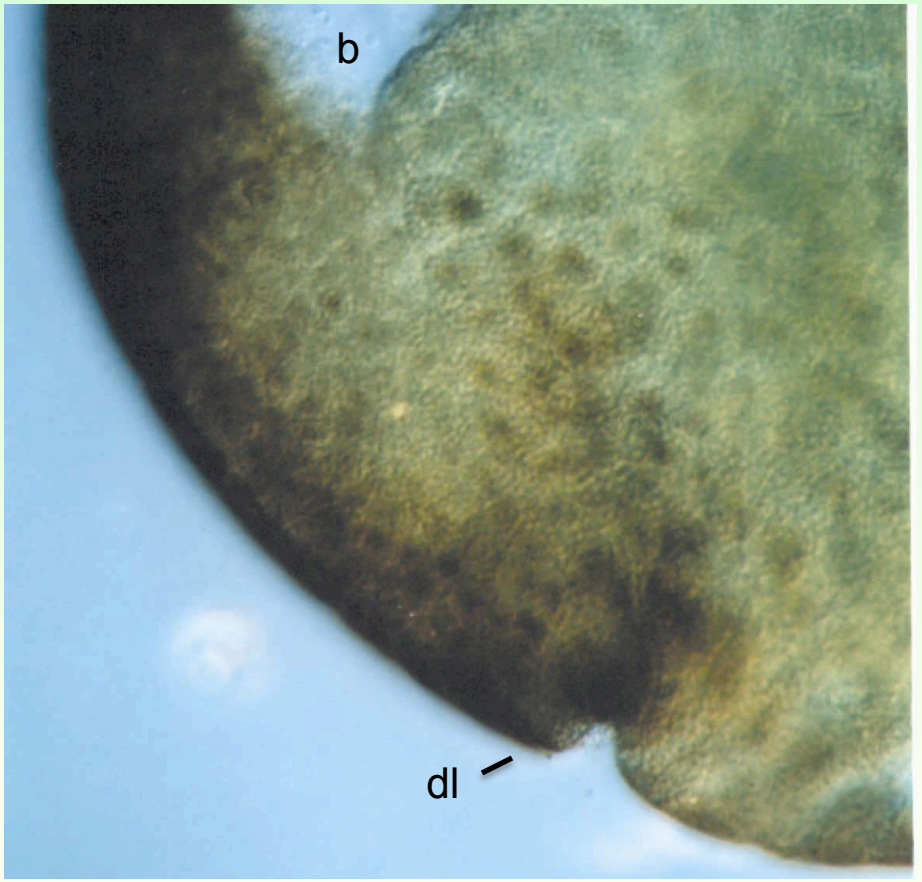
Artwork: O. D. Pérez

Early Gastrula of *C. machalilla*

External view



Sagittal section



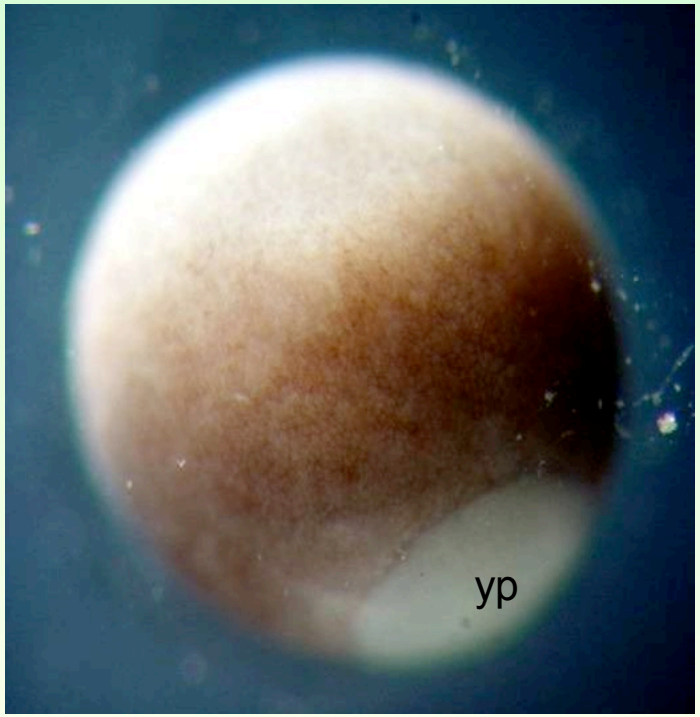
The dorsal blastopore lip is subequatorial. Embryos are unstained, the dark color observed is due to pigment.

b, blastocoel; dl, dorsal lip.

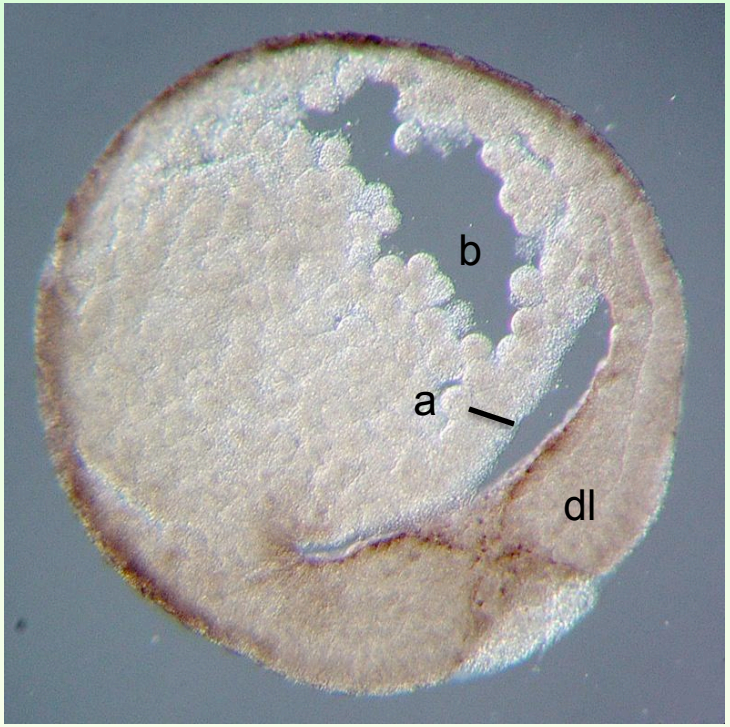
Ref: del Pino EM. et al Int J Dev Biol 48:663-670 (2004).

C. machalilla mid-gastrula

External view



Para-sagittal section

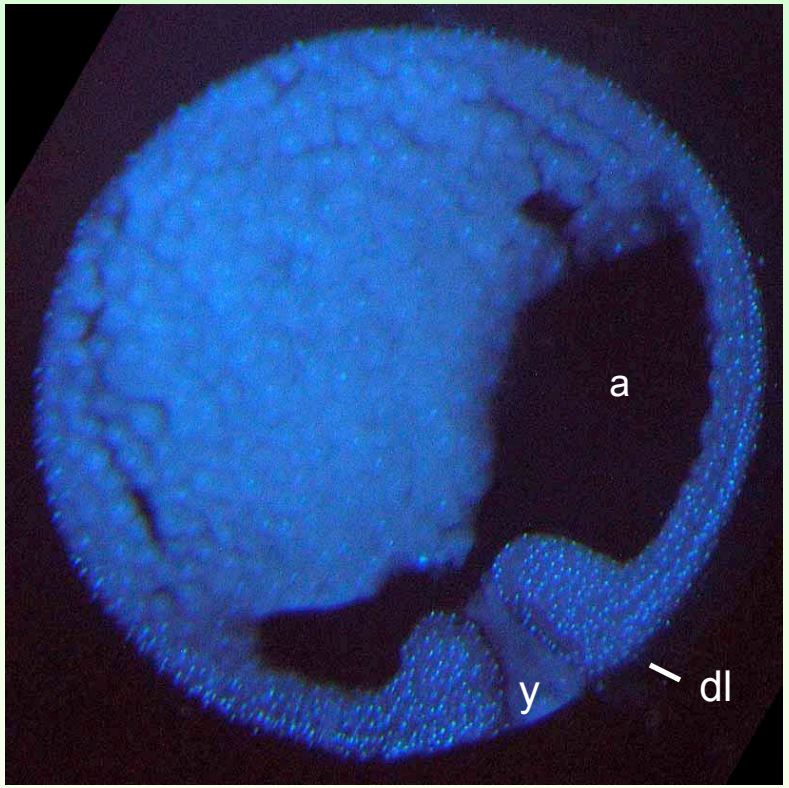


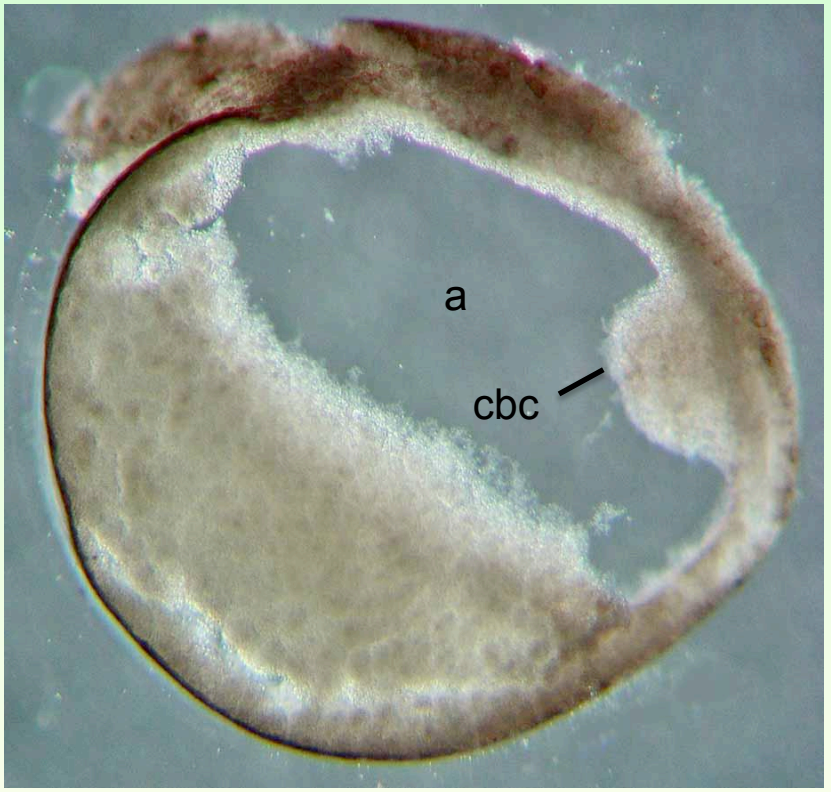
Although the external view of the gastrula is typical of frogs, the blastopore lip is thick, leading to the formation of a thick circumblastoporal collar. Moreover, the archenteron remains small during gastrulation, as in *G. riobambae*.

a, archenteron; b, blastocoel; dl, dorsal lip; yp, yolk plug.

Ref: del Pino EM. et al. Int J Dev Biol 48:663-670 (2004); Moya IM et al. Dev Biol 304: 467-478 (2007).

Late gastrula of *C. machalilla*





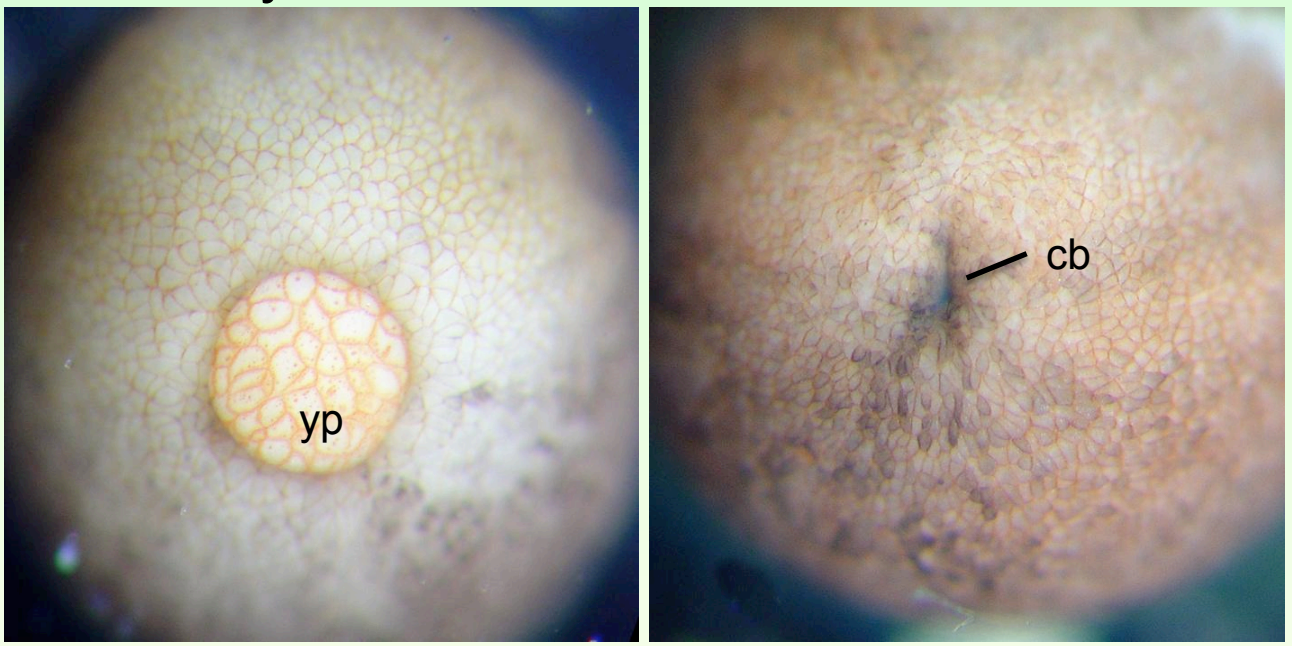
a, archenteron; dl, dorsal lip; cbc, circumblastoporal collar; y, yolk plug

The archenteron elongates in the late gastrula. Cells that involuted during gastrulation remain in the blastopore lip and form a thick circumblastoporal collar.

Ref: del Pino EM. et al. Int J Dev Biol 48:663-670 (2004); Moya IM et al. Dev Biol 304: 467-478 (2007).

Absence of an embryonic disk

Embryos were stained for cell borders



Although *C. machalilla* embryos form a thick circumblastoporal collar, an embryonic disk was not detected on the surface of embryos.

cb, closed blastopore; yp, yolk plug

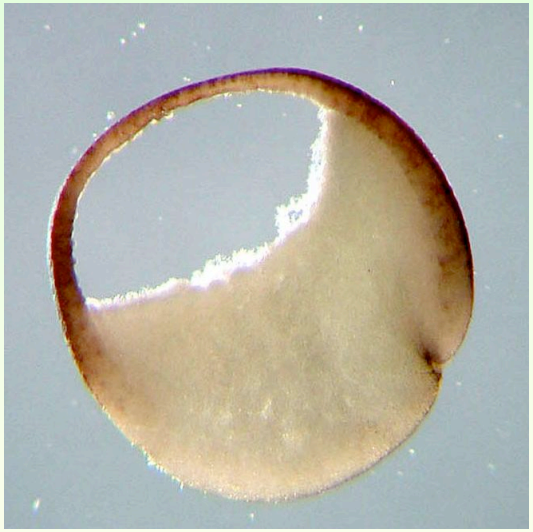
Reproduced from Developmental Biology (Dev Biol, 2007, 304: 467-478).

Ref: del Pino EM. et al. Int J Dev Biol 48:663-670 (2004); Moya IM et al. Dev Biol 304: 467-478 (2007).

Time from fertilization to gastrulation:

14 hours

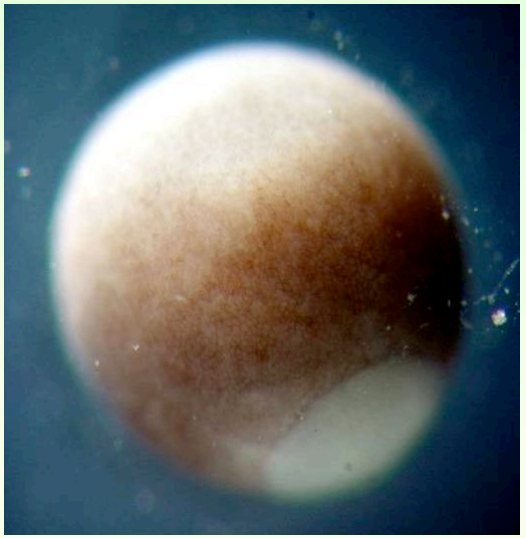
X. laevis



Diam: 1.3 mm

4 days

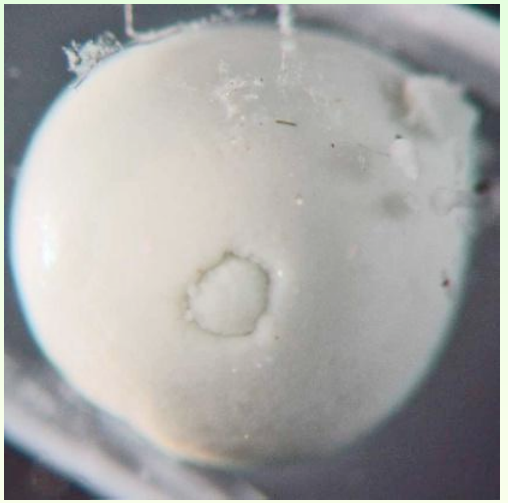
Dendrobatid frog
C. machalilla



Diam: 1.6 mm

14 days

Marsupial frog
G. riobambae



Diam: 3 mm

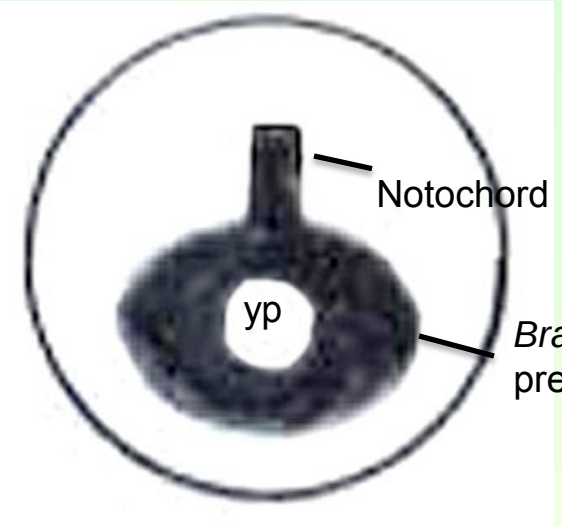
Refs: del Pino EM. Dev. Biol. 177: 64-72(1996); del Pino EM, et al. Int. J. Dev. Biol. 48:663 (2004); Nieuwkoop PD. Faber J. Normal table of *Xenopus laevis* (Daudin). New York, Garland Publishing (1994).

Not to scale.

Section 4
Comparison of *Brachyury (Bra)* expression

Bra expression in the *Xenopus* mid gastrula

Bra deep signal in black.
yp, yolk plug



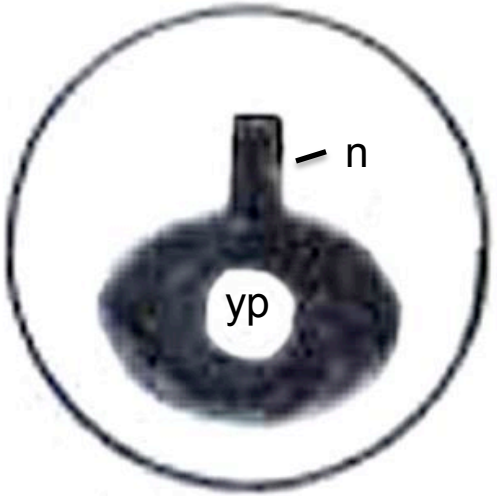
In *X.laevis, Bra* is expressed in the presumptive mesoderm, an internal ring around the blastopore. In addition, starting in the midgastrula, *Bra* is expressed in the notochord. *Brachyury* expression in the notochord results from the movements of dorsal convergence and extension (CE).

Ref: Smith, JC et al., Cell 67: 79-87 (1991).

Brachyury protein (Bra) expression

Xenopus

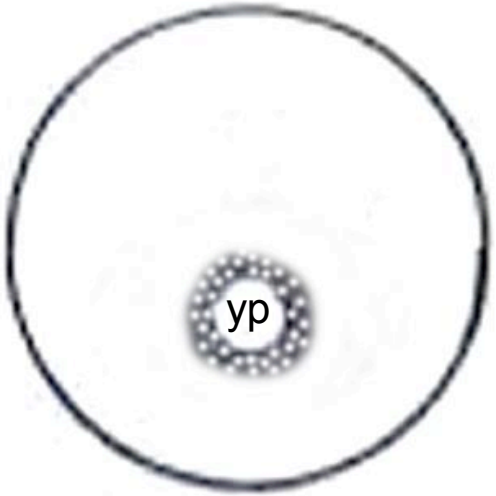
Mid gastrula



- Presumptive mesoderm,
- Notochord.

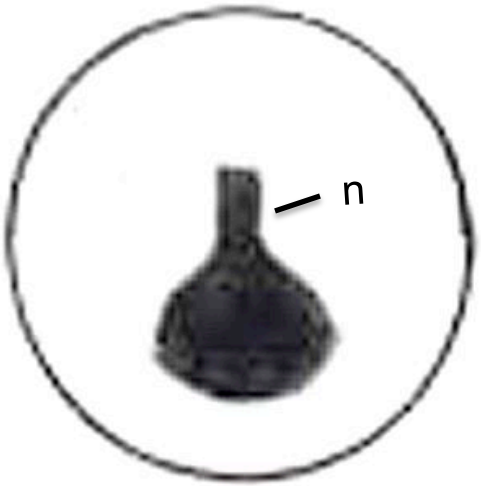
Gastrotheca/Colostethus

Mid gastrula



- Superficial ring around the blastopore (indicated by stippling).

Post-gastrula

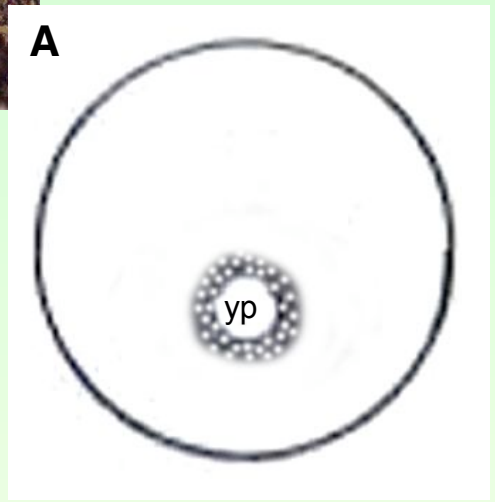


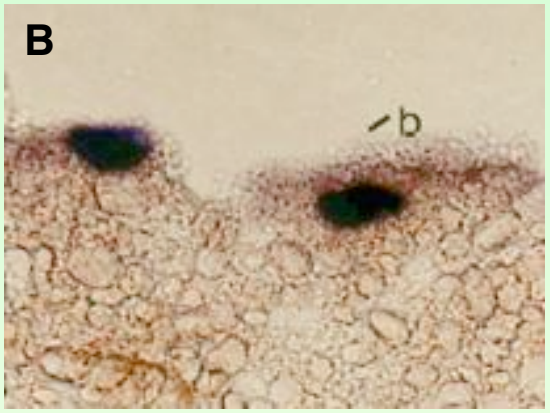
- Deep signal in the tail bud, likely the presumptive mesoderm,
- Notochord.

Bra signal in black; n, notochord: yp, yolk plug

Refs: del Pino EM. Dev. Biol. 177: 64-72 (1996);
Benítez MS & del Pino EM. Dev. Dyn, 225: 592-596 (2002);
Moya IM et al. Dev Biol 304: 467-478 (2007).

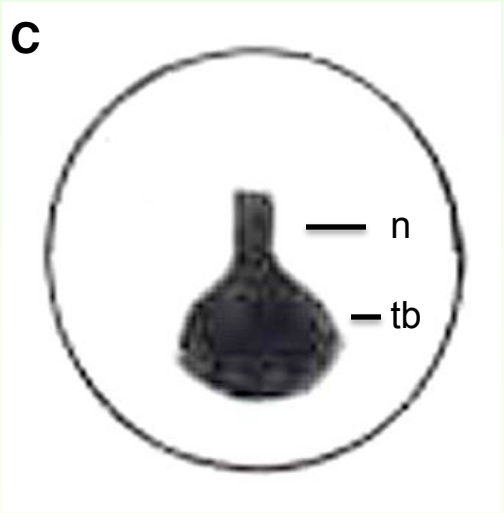
Brachyury protein expression in *G. riobambae*

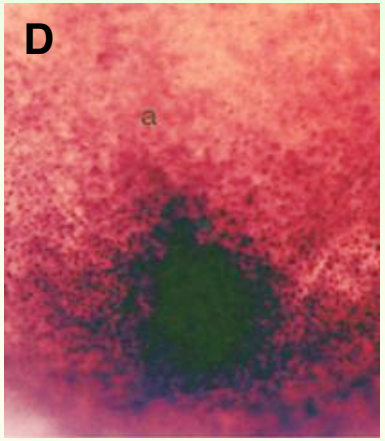


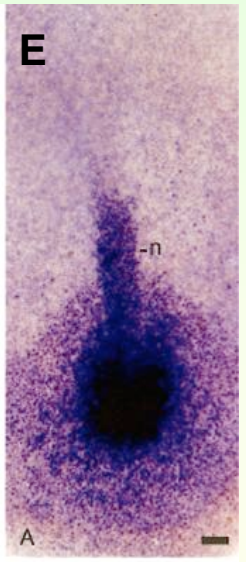


During gastrulation Bra is expressed exclusively in surface cells (Indicated by stippling in the drawing).

yp, yolk plug
n, notochord
tb, tail bud







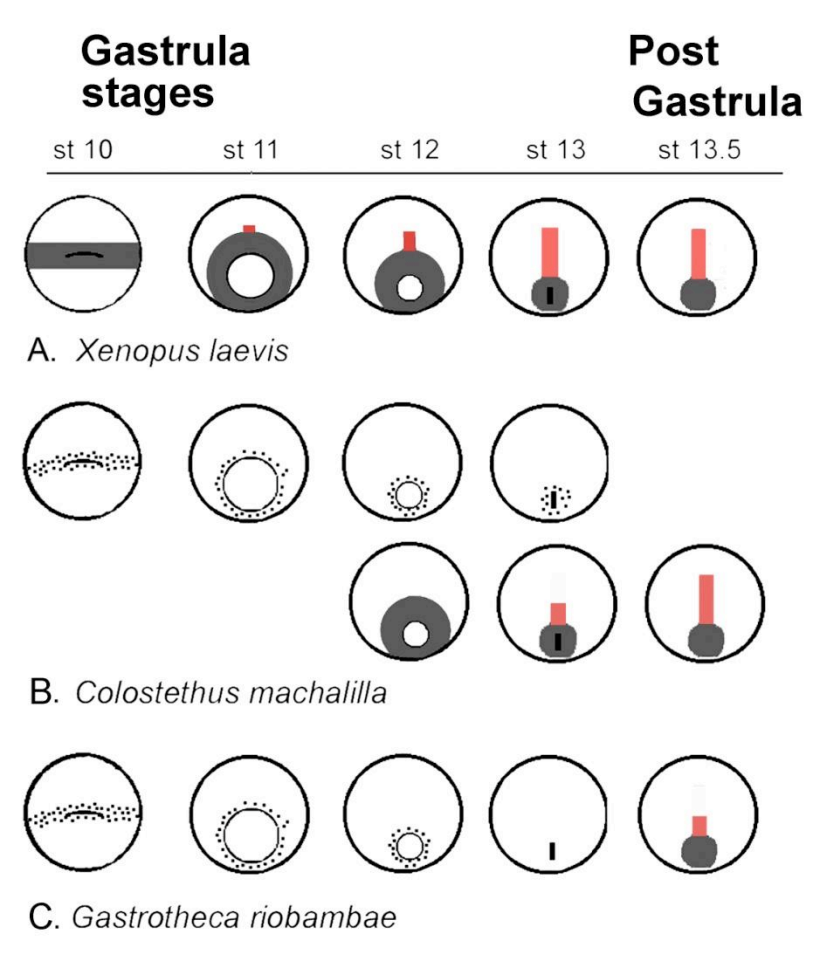
After blastopore closure, deep Bra expression occurs in the notochord & tailbud.

A similar pattern was detected in *C. machalilla.*

Figs. B,D,E reproduced from Developmental Biology (Dev. Biol.1996. 177: 64-72).

Ref: del Pino EM. Dev. Biol. 177: 64-72 (1996); Benítez MS & del Pino EM. Dev. Dyn, 225: 592-596 (2002).

Comparison of Bra protein expression



Stippled= surface signal; Black = deep signal; Red = notochord

Image modified from Benítez MS, del Pino EM. Revista de la Universidad Católica del Ecuador, 71: 7-14 (2003).

Ref: Venegas-Ferrin, M. et al. Int. J. Dev. Biol. 54: 195-202 (2010); Technau, U. BioEssays 23:788-794 (2001).

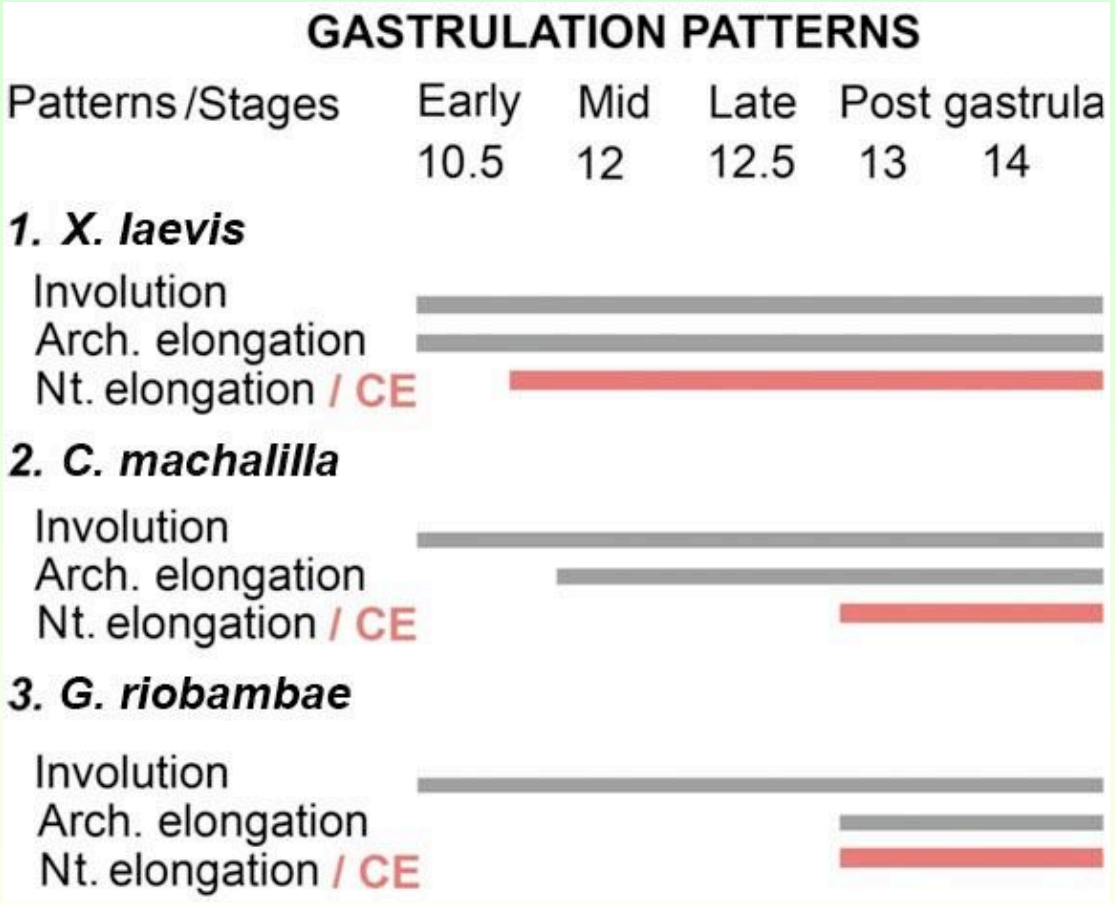
•Notochord elongation, & dorsal convergence and extension starts in the *X. laevis* mid gastrula.

• Elongation of the notochord occurs after gastrulation in *C. machalilla, & G. riobambae*, evidenced by Bra expression.

• It is unknown whether *X. laevis* embryos express *Bra* in surface cells around the blastopore.

• Surface *Bra* expression around the blastopore associates with blastopore closure in other organisms.

Implications of the analysis of Brachyury & gastrulation



1. Frog gastrulation is modular.
2. CE (body elongation) is not essential for gastrulation.

Arch, archenteron
Nt, notochord
CE, dorsal convergence & extension, evidenced by Bra expression in the notochord.

Image modified from del Pino et al. PNAS, USA 104:11882-11888 (2007).

Refs: Moya IM et al. Dev Biol 304: 467 (2007); del Pino et al. PNAS, USA 104:11882-11888 (2007).

Conclusions

Marsupial frog

Dendrobatid frog

The reproductive modes of these frogs is associated with:

- Slow development, in comparison with *X. laevis,*
- Separation of gastrulation from dorsal convergence and extension (CE), which implies a delay in body elongation.
- The comparison with *X. laevis* development indicates that CE is not essential for the movements of gastrulation.
- The description of the reproductive & developmental modes of these frogs enriches our knowledge of Ecuador's biodiversity.

Conclusions

Marsupial frog

Dendrobatid frog

Embryos of *X. laevis* quickly develop into tadpoles, and the simultaneous occurrence of gastrulation and dorsal convergence and extension (CE) may accelerate body elongation. In G. *riobambae,* and *C. machalilla,* CE occurs after gastrulation, suggesting a delay in body elongation in these slow developing frogs. However, separation of gastrulation from CE occurs in a rapidly developing frog, *Eleutherodactylus coqui,* indicating that there may be other reasons for the separation of these developmental processes in *E. coqui*. The modular nature of frog gastrulation allows for this interesting variation.

Many thanks to my present and past collaborators.