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

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The early development of frogs with terrestrial reproductive modes

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Why study a frog other than *Xenopus laevis*?

- To discover possible variations in development.
- To advance the knowledge of Ecuador’s biodiversity.
Features of alternative model organisms:

- Peculiar biology.
- Ease of collection.
- Maintenance in the lab should be easy.
Our Work is done with:

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

2. The dendrobatid *Colostethus machalilla* (Dendrobatidae).
Themes 1 & 2:

For Gastrotheca & Colostethus:

A. The biology of the frog.
B. Is this frog appropriate for lab. work?
C. Developmental features.

Theme 3. Comparison of Brachyury (Bra) expression
Theme 1: The biology of the marsupial frog

Gastrotheca riobambae
Amplexus
The female broods the embryos for 4 months

Photo: M. Fogden
Tadpole birth:

With her toes, the female helps in the emergence of tadpoles from the pouch.

Tadpoles metamorphose in times that vary from 60 days to aprox. 1 year.

Foto: Friedemann Koester
Features of the female:

Presence of a pouch to carry the embryos

The pouch derives from the skin.
The pouch aperture of *Gastrotheca riobambae*:

Open pouch  Closed pouch
Features of the pouch:

- The pouch derives from the skin.
- Vascularization of the pouch.
- Formation of embryonic chambers.
- Hormonal control of the pouch.
Progesterone induces:

1. Pouch closure
2. Pouch vascularization &
3. Formation of embryonic chambers
Embryos develop the bell gills

Exchanges with the mother are mediated by the bell gills and the chambers of the pouch
Maintenance in the laboratory:

- In terraria
- Accepts live prey and meat
- Cool nights
- 12 hours light
- Low atmospheric pressure

• Egg laying can be induced by HCG administration
Development of *G. riobambae*

- Marsupial frog
- Eggs of 3 mm diameter
- Slow development
- Modified gastrulation

Photo: F. Koester
Oocytes of 3 mm diameter

Xenopus  Gastrotheca
Slow development

14 days to the end of gastrulation
Modified gastrulation

- a, archenteron; b, blastopore

- No dorsal blastopore lip.
- Delayed archent. elongation and inflation
Modified gastrulation

Gastrotheca

Xenopus

A

B

bl, blastocoel
d, disk

a, archent.
Early embryos are flat

Neural crest

Somites
The appearance of the adult does not disclose the extraordinary development of *Gastrotheca*
Theme 2: Biology of *Colostethus machalilla*

- Tiny frog of 17 mm.
- Frequent reprod.
- nests of 15 eggs (1.6 mm diameter)
- Parental care
- 21 days until hatching

Photo: Juan Calles
Maintenance in the laboratory is possible

- Terraria.
- Eats only live prey
- Artificial nests
- Egg-culture method
- Metamorphosis in 5 months
- Frogs call after one year

Film box nest

Photo: J. Calles
Stages of development

First description of dev. in dendrobatid frogs

Artwork: Oscar D. Pérez
Delayed expansion of the blastocoel
Early Gastrula

The dorsal lip is subequatorial
C. machalilla: mid-gastrula

Thick blastopore lips and small archenteron
Late gastrula: Thick blastopore lips

Bc: Brachet’s cleft
A: archenteron
Y: yolk plug

Retardation of arch. elongation
Retardation of Neural Development

Neural fold formation and NCAM expression occur after blastopore closure
Retardation of Somite Development

Somite markers were detected after tailbud stage
Retardation of notochord elongation

In: C. machalilla & G. riobambae
Colostethus machalilla & other dendrobatids

Can be maintained in the laboratory & their development is retarded in comparison with X. laevis.

Photos: J. Calles
Theme 3. Comparison of Bra expression

Brachyury is expressed in the:
- Future mesod.
- (internal ring) &
- Notochord of *X. laevis*.

The notochord elongates in the mid gastrula.
Bra in the blastula

- Bra is expressed in a ring of surface cells in the blastula and early gastrula of both frogs.

*G. riobambae*

*C. machalilla*
Bra in the *Gastrotheca gastrula*

- Surface Bra-expression disappears in the late gastrula
- Deep expression is seen when the notochord elongates.
Bra in the *Colostethus* mid gastrula

- Superficial signal diminishes during gastrulation

*ty*, yolk plug; *cb*, bottle cells
Internal Bra signal in the *Colostethus* late gastrula

- **Surface**
- **Internal**
- **Section**

*ty*, yolk plug
Bra in the *Gastrotheca* neurula

Equivalent pattern in both frogs
Comparison of Bra expression

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A. *Xenopus laevis*

B. *Gastrotheca riobambae* y *Eleutherodactylus coqui*

C. *Colostethus machalilla*

Stippled = surface;
Black = deep expression
Thanks to my present and past collaborators!!