

A Tale of a Tail: Variation during the Early Ontogeny of *Haddadus binotatus* (Brachycephaloidea: Craugastoridae) as Compared with Other Direct Developers

Author(s): Javier Goldberg and Florencia Vera Candiotti

Source: Journal of Herpetology, 49(3):479-484.

Published By: The Society for the Study of Amphibians and Reptiles

DOI: <http://dx.doi.org/10.1670/14-072>

URL: <http://www.bioone.org/doi/full/10.1670/14-072>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

A Tale of a Tail: Variation during the Early Ontogeny of *Haddadus binotatus* (Brachycephaloidea: Craugastoridae) as Compared with Other Direct Developers

JAVIER GOLDBERG^{1,3} AND FLORENCIA VERA CANDIOTTI²

¹Instituto de Bio y Geociencias (IBIGEO), Centro Científico Tecnológico-CONICET y Universidad Nacional de Salta, 9 de Julio 14, 4405, Rosario de Lerma, Salta, Argentina

²CONICET—Instituto de Herpetología, Fundación Miguel Lillo, Miguel Lillo 251, 4000, San Miguel de Tucumán, Argentina

ABSTRACT.—The New World direct-developing frogs (Brachycephaloidea = Terrarana) comprise nearly a thousand species that share direct development among other putative synapomorphies, yet embryonic development in this group has been thoroughly described in only about 20 species. Here we describe the early ontogeny of the craugastorid *Haddadus binotatus*, making special emphasis on tail structure and development, and its differences and similarities with that of other terraranans. The morphological changes during embryonic development of *H. binotatus* and those of other Neotropical direct-developing species are alike, with some variation including the absence of external gills, timing of limb differentiation, and tail configuration. The tail with a rotated core axis and lateral and asymmetric fins that cover the posterior half of the embryo represents an outstanding case of developmental repatterning. We present some interpretations of the evolution of the tail and its three major aspects, the rotation of the core axis, and the origin and extensions of the fins, and pinpoint that those mechanisms underlying fin development should be fairly plastic, allowing the ontogenetic and evolutionary variation within the Brachycephaloidea clade.

RESUMEN.—Las ranas de desarrollo directo del Nuevo Mundo (Brachycephaloidea = Terrarana) incluyen cerca de mil especies que comparten el desarrollo directo entre otras sinapomorfías putativas; sin embargo el desarrollo embrionario en este grupo ha sido descrito en no más de 20 especies. Aquí describimos la ontogenia temprana del craugastorido *Haddadus binotatus*, con especial énfasis en la estructura y el desarrollo de la cola y sus diferencias y similitudes con la de otros terraranos. Los cambios morfológicos durante el desarrollo embrionario de *H. binotatus* son similares a los de otras ranas neotropicales con desarrollo directo, con algunas variaciones que incluyen la ausencia de branquias externas, los tiempos de diferenciación de las extremidades y la configuración de la cola. La cola con su eje rotado y aletas laterales y asimétricas que cubren la mitad posterior del embrión representa un caso excepcional de cambio en los patrones del desarrollo. Aquí presentamos algunas interpretaciones sobre la evolución de la cola y sus tres aspectos principales: la rotación del eje y el origen y extensión de las aletas, y señalamos que los mecanismos que subyacen al desarrollo de la aleta parecen ser lo suficientemente plásticos como para permitir la variación ontogenética y evolutiva presente en el clado Brachycephaloidea.

Most studies of anuran embryogenesis involve the use of species that possess free-living larvae that metamorphose into terrestrial adults (Chipman et al., 2000; Mitgutsch et al., 2009; Romero-Carvajal et al., 2009; among others). In contrast, there have been relatively few published studies on the embryonic morphology of direct-developing taxa (e.g., Townsend and Stewart, 1985; Nokhbatolfoghahai et al., 2010; Anstis et al., 2011; Narayan et al., 2011; Goldberg et al., 2012). Causes of this scarcity of data come from the difficulty of finding recently laid eggs that allow the acquisition of complete embryonic series. In addition, *Eleutherodactylus coqui* has been extensively considered a model species for direct-developing frogs and therefore most studies have included this species (Townsend and Stewart, 1985; Elinson, 1990, 2001; Hanken et al., 1997a, b; Hanken, 1999; Callery et al., 2001; Kerney et al., 2010). As embryonic development in more species is studied, however, structural and temporal variations, including the evolution of novel traits, are described (Nokhbatolfoghahai et al., 2010; Narayan et al., 2011; Goldberg et al., 2012).

Direct development represents a widespread reproductive mode among anurans that has evolved independently at least 10 times, including more than once in some lineages (Hanken, 1999; Heinicke et al., 2009). The New World direct-developing frogs (Brachycephaloidea = Terrarana) are a monophyletic clade that comprises three families with nearly a thousand species that share direct development among other putative synapomorphies (Hedges et al., 2008; Heinicke et al., 2009; Pyron and Wiens, 2011; Taboada et al., 2013; Padial et al., 2014) but, despite

this enormous diversity, development in this group has been thoroughly described only in about 20 species (e.g., Sampson, 1904; Noble, 1925; Lynn, 1942; Gitlin, 1944; Lynn and Lutz, 1946, 1947; Jameson, 1950; Hughes, 1959; Adamson et al., 1960; Valett and Jameson, 1961; Townsend and Stewart, 1985; Elinson et al., 1990; Pombal, 1999; Nokhbatolfoghahai et al., 2010; Goldberg et al., 2012). Until the phylogeny of Heinicke et al. (2009), most of these species were assigned to the genus *Eleutherodactylus*, providing no basis for a phylogeny-based search beyond *E. coqui*. The current basal position of *Eleutherodactylus* within Brachycephaloidea, plus the reassignment of several described species to other families and genera within the clade (Padial et al., 2014), open new questions about morphological diversity in this group.

The family Craugastoridae is the most diverse group among terraranan frogs, with 727 species; within this, the genus *Haddadus* includes three species (*H. aramunha*, *H. binotatus*, and *H. plicifer*) distributed along the Atlantic coastal forest of eastern and southern Brazil (Frost, 2014). The Clay Robber Frog *H. binotatus* is endemic to the Mata Atlántica and occurs from southern Bahia to Rio Grande do Sul and west into Mato Grosso do Sul and Paraná (Frost, 2014). Despite its wide distribution, little is known about the natural history of this species (Dias et al., 2012; Moura et al., 2012; Rebouças et al., 2013), and almost nothing about its early development (Costa and Carvalho e Silva, 2010). In this article we provide new data on the ontogeny of this species, and discuss some morphological aspects related to its developmental mode. We make particular emphasis on the tail, because recent observations (Nokhbatolfoghahai et al., 2010; Goldberg et al., 2012) have reported interesting structural

³Corresponding author; E-mail: jgoldberg@conicet.gov.ar
DOI: 10.1670/14-072

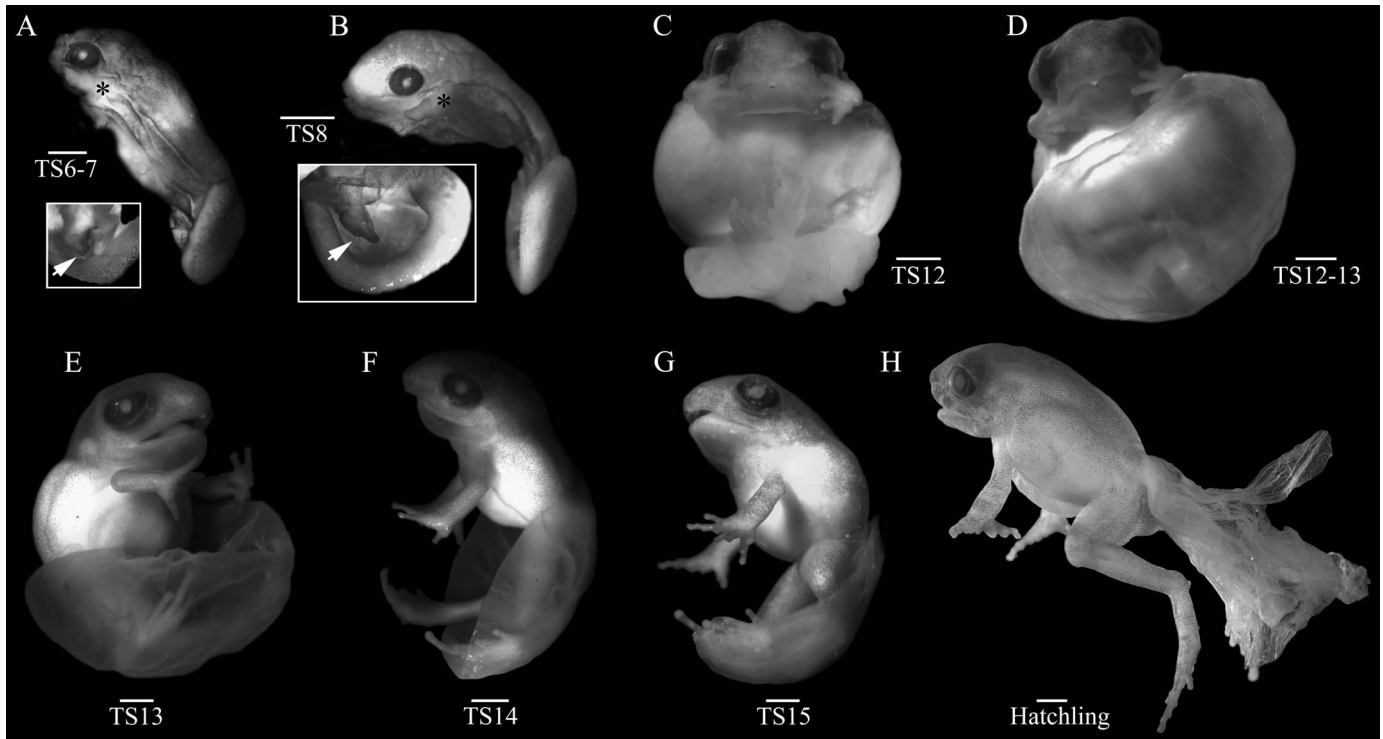


FIG. 1. Developmental series of *Haddadus binotatus*, from Townsend & Stewart stage 6–7 to hatching. The vitellum was removed in the two first specimens to photograph the hind limbs (insets with arrows), covered by the sac-like tail. Note the limb size difference at TS8. The dermal fold is indicated with an asterisk (*). Scale lines = 1 mm.

and heterochronic variations during its development in terraranans.

MATERIALS AND METHODS

We worked with collection material, kindly provided by P. Costa, M. Almeida-Santos, and C. Siqueira (Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; MNRJ 86848). A terrestrial clutch (18 viable eggs) was collected in December 2009, in Ilha Grande, Angra dos Reis (Rio de Janeiro, Brazil), and then incubated at room temperature (20–25°C) in a plastic container with a lightly moistened, disaggregated substrate. This temperature is similar to the mean temperature reported for December at the collection site (Araujo and Oliveira, 1988). Eggs were fixed periodically (every 1–2 d) in 10% formalin. The species was identified from the embryos, which already show the diagnostic feature (i.e., finger II larger than the finger III; Hedges et al., 2008) before hatching. We staged embryos following the table for *Eleutherodactylus coqui* of Townsend and Stewart (1985) (TS from here) and differences were taken into account. Morphological features were observed with a stereomicroscope, and pictures were obtained with a digital camera. Measurements were made with dial calipers (0.02 mm) and are given in millimeters. For histological cross-sections of the tail, embryos of *H. binotatus* at TS6–7 and TS12, one embryo of *Oreobates barituensis* (TS5; MCN 1363—Herpetological Collection of the Museo de Ciencias Naturales, Universidad Nacional de Salta, Argentina), and one embryo housed as *Oreobates discoidalis* (but *O. sp.* from here, because three *Oreobates* species occur at the collection site; early TS5; FML 02342—Herpetological Collection of Fundación Miguel Lillo, Tucumán, Argentina) were dehydrated, embedded in paraffin, and sectioned at 6 μ m. Sections were stained with hematoxylin

and eosin following the protocol by Martoja and Martoja-Pierson (1970). We observed histological sections with a Nikon (Nikon Corp., Tokyo, Japan) E200 light microscope equipped with a digital camera.

RESULTS

Embryonic Development.—According to our data, development from fertilization to hatching lasted between 23 and 27 d in *H. binotatus*. Snout–vent length in our single hatched specimen was 8.90 mm. Examined embryos were at TS6–7 ($N = 1$), TS8 (1), TS 11 (1), TS12 (5), TS13 (2), TS14 (3), TS15 (4), and hatching (1).

Cephalic Region.—By TS6–8 (Fig. 1A,B), the head was completely differentiated from the rest of the body; the mouth had a subterminal position and the lower jaw recessed; the upper jaw showed a medial, pointed prominence where the egg tooth will develop. Embryos lacked external gills and adhesive glands. Eyes were fully developed, with a dark iris with coroid fissure, and a translucent pupil. The lower eyelid was outlined at early TS12 and fully differentiated and pigmented later in this period; the upper eyelid and the tympanic membrane developed at TS14. By stages 12–13 (Fig. 1C–E), the snout was rounded, and the upper jaw showed a bicuspid, keratinized egg tooth. The end of the lower jaw lagged far behind the upper jaw. The angle of the mouth progressed from before the middle of the eye in early TS12 specimens to a level surpassing the posterior edge of the eye at TS15. Nonprotruding nostrils were small, rounded, and deep. The lacrimal groove extended from each nostril to the eye and it was visible until TS15. At TS14 (Fig. 1F), the snout was sharpened and truncated, and at hatching the distal ends of the upper and the lower jaws were at the same level (Fig. 1H). A keratinized egg tooth was still present in the single hatched specimen.

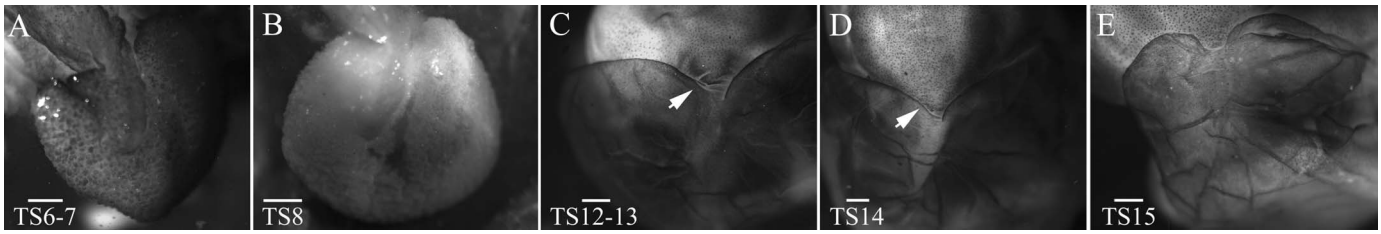


FIG. 2. Details of the tail, dorsal view. Note the origin of the left fin (arrows), slightly more rostral than the right fin, especially evident at TS12 and 14. Scale lines = 0.5 mm.

Limbs.—By TS6–7 (Fig. 1A) limb buds had two segments and foot paddles were evident; a dermal fold covered the base of the forelimbs. Hind limbs were not visible but concealed by the tail fin. Reaching TS8, incipient digits I to IV were differentiated in both limbs and the elongation of digit IV (primary axis) was noticeable. Hind limbs were slightly advanced in development compared with forelimbs, with foot indentations more evident, and their size was bigger, almost three times the length and width of the forelimbs. Before TS12, stylopodia, zeugopodia, and autopodia were delimited; limbs became progressively longer, with all toes (I–V) and fingers (II–V) individualized (Fig. 1C,D). By TS12, hind limbs were first typically crossed to each other. Finger II was characteristically larger than finger III. Inner metacarpal and metatarsal tubercles were present, and at the end of the period, the dermal fold was still evident at the forelimb base. Fingers and toes reached their maximum length at the next stage (Fig. 1E), and subarticular tubercles developed.

Tail.—The tail at TS6–7 had a short and curved muscular axial core; tail fins were flat, thick, lateral and caudal extensions, the whole structure having a sagittate shape (Fig. 2A). The tail represented 1/3 of the body length and at its entire margin it folded ventrally on itself, constituting a sac-like structure that envelops the hind limbs. Later, the tail acquired a circular shape (Fig. 2B) and grew laterally and ventrally, reaching half the body length. Histological sections through the tail at TS6–7 (Fig. 3) show the axial core with the notochord, the neural tube, muscles, and tail extensions. The core axis was slightly rotated to the left and the fins maintained their relationships with the dorsoventral axis: the dorsal fin originated dorsal to the neural tube, whereas the ventral fin grew almost ventral to the notochord. Fins exhibited a simple squamous epithelium with numerous folds surrounding capillaries. Beneath this folded epithelium lay an

embryonic connective tissue with globose fibroblasts, extracellular matrix, few collagen fibers, and blood vessels. At early TS12 the fins were more developed, covering the hind limbs up to the heels (Fig. 1C). At the base, the left fin originated slightly more rostral than the right fin (Fig. 2C,D). Later at this stage, the tail was highly vascularized and reached its full length, covering the posterior half of the embryo up to the forelimbs (Fig. 1D). Histologically, the axial core was slightly rotated to the left at the tail base, and the notochord appeared laterally compressed. Tail extensions were laterally disposed regarding the components of the axial core, and showed an asymmetric arrangement: the left-dorsal fin originated dorsolaterally through the whole tail length, whereas the right-ventral fin started dorsolateral at the tail base but became ventrolateral as the tail axis keeps rotating to the left (Fig. 4). Extensions were thick at the proximal portion (more pronounced at the base of the right fin), but next they became thinner and with a highly folded epithelium sustained by an embryonic connective axis. The tail started to regress at TS13, reaching half the abdomen; the tail fins were translucent and vascularized but began to wrinkle at this point. In the next stages (Fig. 1F,G) the tail shortened, wrinkled, and narrowed laterally, and at hatching (Fig. 1H) it remained as long as the hind limbs.

Histological sections through the tail of the two early embryos of *Oreobates* (early TS5 *O. sp.* and TS5 *O. barituensis*) showed surprising results: a dorsoventral arrangement of the fins was evident in the embryo of *O. sp.* (Fig. 5B), whereas the slightly advanced *O. barituensis* (Fig. 5C) had a pattern more similar to that of the TS6–7 *H. binotatus*.

Body Integument.—At TS6–8 the embryos were whitish, with the head and first half of the trunk showing scattered melanophores. Body pigmentation increased as development progressed, resulting in a dispersed pattern of scattered

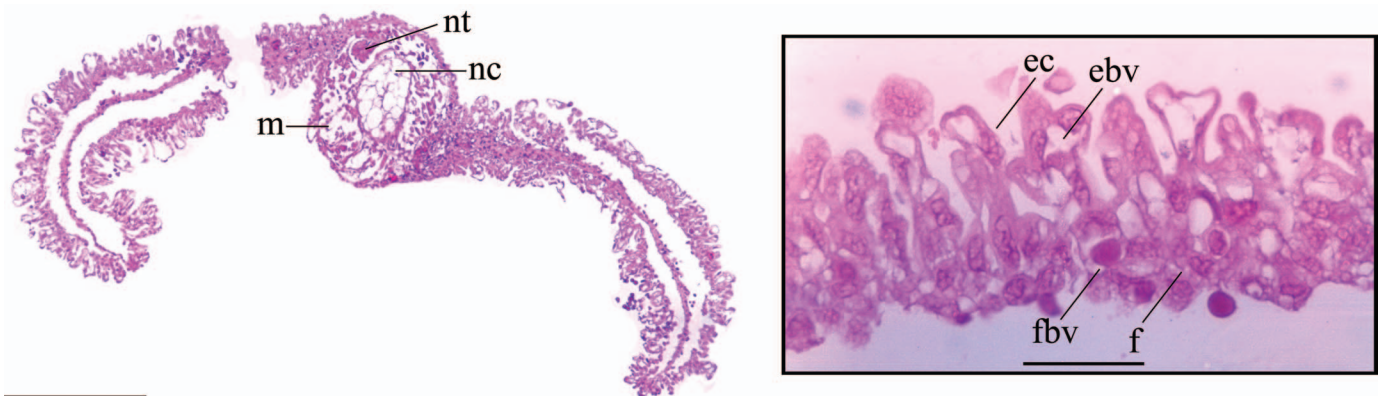


FIG. 3. Transverse section through the tail at TS6–7 and detail of the tail fin. Disregarding the odd, disaggregated appearance of the tail tissue (a fixation artifact), note the axis rotation, the pattern of fin disposition, and the fins formed of numerous epithelial folds that likely increase gas exchanging surface. ebv, empty blood vessel; ec, epithelial cell; f, fibroblast; fbv, full blood vessel; m, muscle anlagen; nc, notochord; nt, neural tube. Scale lines = 0.5 mm and 0.05 mm (inset).

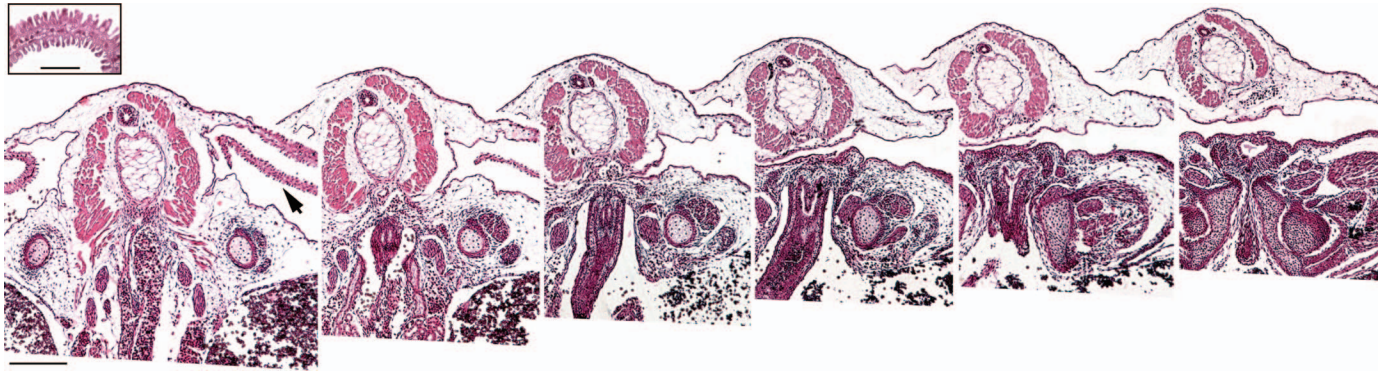


FIG. 4. Transverse sections through the tail from the base (left) to the tip (right) at TS12, showing the rotated axis and the origin of the fins. Note the base with a slightly rotated axis and fins originating dorsolaterally at the same level, and the right fin originating more ventrolaterally as the tail axis rotates toward the tip. The arrow points out the portion of the tail fin folding on itself; note also the lamellar-like epithelium in that region (inset). Scale lines = 0.3 mm and 0.1 mm (inset).

melanophores in the dorsum and lateral regions of the abdomen (TS12). Melanophores appeared in limbs at late TS12, and a bar-like pattern was evident in forelimbs at TS15. The proximal region of the forelimbs was covered by the dermal fold and not until close to hatching do forelimb skin and body tegument meet. The yolk mass started yellowish and subspherical, and then at TS12 became segmented caudally and later also laterally; in next stages it diminished substantially, but embryos hatched with vitellum remnants.

DISCUSSION

Our observations add new data to the limited information that exists so far on the ontogeny of species with direct development. *Haddadus binotatus* shows a larger hatching size compared with other species described: 8.90 vs. 6.1 mm in *E. coqui* (Townsend and Stewart, 1985); 6.3 mm in the brachycephalid *Ischnocnema guentheri* (Lynn and Lutz, 1946; as *Eleutherodactylus*); 5.5 mm in *Brachycephalus ephippium* (Pombal, 1999); 6.51 mm in *O. barituensis* (Goldberg et al., 2012). *Haddadus binotatus* froglets are still very small, so significant postmetamorphic growth is needed to reach the adult size of 30–35 mm (Carvalho and Martins, 2012; Coco et al., 2014).

Regarding the morphological changes along the embryonic period, the staging table of Townsend and Stewart (1985) for *E. coqui* has been extensively used in terraranan and nonterraranan species as well, and it represents a useful baseline for

comparative purposes. We staged embryos according to this table, but we noted some variation concerning gill presence, timing of limb differentiation, and tail configuration and development. The absence of external gills is common to all brachycephalids and craugastorids described to date (Lynn and Lutz, 1946; Valett and Jameson, 1961; Pombal, 1999; Goldberg et al., 2012). Conversely, presence and absence of these structures have been reported among eleutherodactylids (reviewed in Townsend and Stewart, 1985).

Limb development in *H. binotatus* follows the general pattern described for anuran species (Gosner, 1960). The species presents a heterochronic onset of limb bud development, with predisplaced hind limbs. Bininda-Emonds et al. (2007) proposed that the relative timing of fore- vs. hind-limb development in vertebrates shows a strong phylogenetic component where the plesiomorphic condition would be that of predisplaced forelimbs. They found variation in Lissamphibians, although they studied a small anuran sample (*Xenopus laevis* and *E. coqui*) that has predisplaced hind limbs. However, the three patterns—synchrony, predisplaced hind limbs, and predisplaced forelimbs—have been described in metamorphosing and direct-developing frogs (e.g., Thibaudeau and Altig, 1999; Fabrezi et al., 2009; Goldberg et al., 2012) and therefore the onset of limb buds is revealing more variability than Bininda-Emonds et al. (2007) considered.

Three aspects of terraranan tails are interesting to interpret: the rotation of the core axis, the origin of the fins, and the

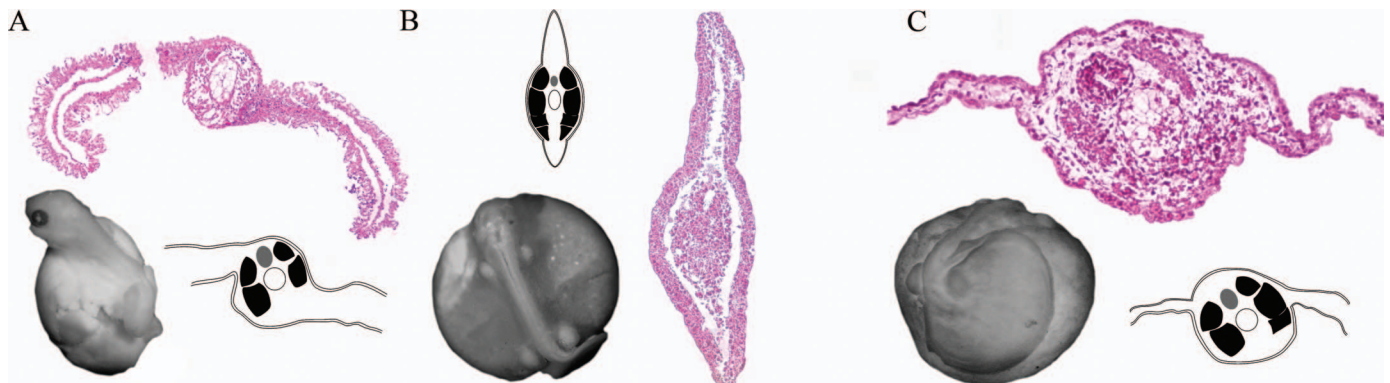


FIG. 5. Histological sections at the base of the tail in a TS6–7 embryo of *Haddadus binotatus* (A), and TS5 embryos of *Oreobates* sp. (B) and *O. barituensis* (C). The small line drawings highlight the arrangement of components of the tail: the notochord (white ellipse), neural tube (gray), and muscle anlagen (black).

extension of the fins. The tails of *Eleutherodactylus* species described so far are bent to the left or right (and even ventrally or dorsally) but exhibit their fins in a dorsoventral pattern, aligned with the dorsoventral axis (neural tube–notochord) of the axial core (Sampson, 1904; Lynn, 1942). Also, the ventral fin is usually deeper than the dorsal fin (e.g., Thibaudeau and Altig, 1999). While examining *Eleutherodactylus portoricensis*, Gitlin (1944) commented that after an initial stage where the tail flexure to the right or left is quite rigid, the tail grows in size and the flexure gradually disappears until the region acquires the flexibility of the rest of the tail. Accordingly, Hughes (1959) shows sectioned embryos of *Eleutherodactylus ricordii* (10 d before hatching) where the tail is twisted through a right angle, leaving the neural tube and notochord side by side; the fins, however, maintain their dorsoventral arrangement (last drawing of Hughes's [1959] text–fig. 1). Given the basal position of Eleutherodactylidae within Brachycephaloidea (Padial et al., 2014), this tadpole-like arrangement of the fins could be plesiomorphic for the group. Although no histological sections are shown at this level, the description of *Ischnocnema nasuta* embryos by Lynn and Lutz (1947, as *Eleutherodactylus*; Brachycephalidae) also suggests a tail with a rotated axis and fins in a dorsoventral pattern that appears lateral because of the rotation; the identity of the fins as dorsal and ventral is suspected by their different extent of development as well.

Within Craugastoridae, embryos of *Pristimantis urichi* and *O. barituensis*, with tails at full development (TS12), show a straight tail axis and fins originating laterally through the entire length of the axial core (Nokhbatolfighahai et al., 2010; Goldberg et al., 2012). The tails of *Craugastor augusti* (Valett and Jameson, 1961, as *Eleutherodactylus*) and *H. binotatus* are challenging, because they show a slightly rotated axis from the very base of the tail at this same stage and the fins have a lateral but asymmetric arrangement. These apparently species-specific features, however, need to be reinterpreted after examining the complete ontogeny of the tail in each species. Observations in early embryos of *Oreobates* and *Haddadus* suggest that, however puzzling, the rotation of the axis plus a shift in the arrangement of the fins could occur during a single ontogenetic trajectory that displays lateral fins as a final state. The rotation of the tail axis (as seen in all groups so far) appears to be just the result of a circumstantial array of the embryo within the confined space of the egg. Conversely, the change of fin disposition from dorsoventral to lateral would imply a rapid, unexpected rearrangement in a very short time lapse. Mechanisms underlying fin development, then, should be fairly plastic to allow this ontogenetic and evolutionary variation within the group, with several possible morphological outcomes (e.g., lateral symmetric fins, asymmetric fins, and fins with different extents of development).

Finally, a noteworthy observation about the extension of the fins: the tail fins of *Haddadus* (and also that of *Pristimantis*, as suggested in Nokhbatolfighahai et al., 2010 photographs) cover the posterior half of the embryo, but they fold to make a double membranous layer. The inner layer may reach the very base of the fin (Fig. 4), so its actual length could be almost twice what is seen. The evolutionary factors leading to develop a full enveloping tail such as that of *Craugastor* and *Oreobates* would make an already long tail extend and surround the embryo instead of folding on itself. The respiratory function of the tail in direct developers has been long hypothesized (e.g., Townsend & Stewart 1985; Nokhbatolfighahai et al. 2010), and the effective surface for gas exchange would have ecological and physiolog-

ical consequences. In this regard, the highly folded, gill-like epithelium of the tail in embryos of *H. binotatus* would also complement tail-fin extension. Considering all variations observed so far, this subject promises to reveal a wide range of possible morphologies as complete ontogenies of more species of this diverse group are examined in a comparative approach.

In the New World direct-developing frogs, a group of traits remains constant and they differentiate in a similar sequence in all species in which embryonic development has been described (e.g., mouth, eyes, eyelids, egg tooth), whereas others exhibit changes in developmental timing (e.g., synchrony/asynchrony of limb development), or a repatterning during embryonic development in which a spatial change could be involved. In this latter sense, in previous work we interpreted the tail lateral extensions of craugastorids as an evolutionary novelty resulting from heterotopic change of tail fins (Goldberg et al., 2012). Given that this spatial change likely occurs during an individual ontogeny instead of in an ancestor-descendent relationship, it could be discussed, beyond the fact it represents a developmental repatterning, if this kind of change should be still considered heterotopic. Nevertheless, analysis of direct development in an evolutionary context substantiates the prominent role of developmental processes in both constraining phenotypic variation and promoting phenotypic diversity (Wake and Hanken, 1996). The terraranan tail appears as an interesting example to study these processes and to assess the role of morphological changes in the extraordinary evolutionary success of this clade.

Acknowledgments.—We are indebted to P. Costa, M. Almeida, and C. Siqueira for the loan of the specimens. M. Alcaide and V. Martínez kindly helped us with interpretation of histological details. This research was supported by Agencia Nacional de Promoción Científica y Tecnológica, PICT 2011 1524, PICT Bicentenario 0638, and PICT Bicentenario 616; Consejo Nacional de Investigaciones Científicas y Técnicas PIP 1112008010 2422, and Universidad Nacional de Tucumán CIUNT-G430.

LITERATURE CITED

- ADAMSON, L., R. G. HARRISON, AND I. BAYLEY. 1960. The development of the whistling frog, *Eleutherodactylus martinicensis* of Barbados. *Proceedings of the Zoological Society of London* 133:453–469.
- ANSTIS, M., F. PARKER, T. HAWKES, I. MORRIS, AND S. J. RICHARDS. 2011. Direct development in some Australopapuan microhylid frogs of the genera *Austrochaperina*, *Cophixalus* and *Oreophryne* (Anura: Microhylidae) from northern Australia and Papua New Guinea. *Zootaxa* 3052:1–50.
- ARAÚJO, D. S., AND R. OLIVEIRA. 1988. Reserva Biológica estadual da Praia do Sul (Ilha Grande, Estado do Rio de Janeiro): lista preliminar da flora. *Acta Botanica Brasílica* 1:83–94.
- BININDA-ÉMONDS, O., J. JEFFERY, M. SÁNCHEZ-VILLAGRA, J. HANKEN, M. COLBERT, C. PIEAU, L. SELWOOD, C. TEN CATE, A. RAYNAUD, C. OSABUTEY, ET AL. 2007. Forelimb–hind-limb developmental timing across tetrapods. *BMC Evolutionary Biology* 7:182.
- CALLERY, E. M., H. FANG, AND R. P. ELINSON. 2001. Frogs without polliwogs: evolution of anuran direct development. *BioEssays* 23: 233–241.
- CARVALHO, T. R., AND L. B. MARTINS. 2012. Advertisement call of *Haddadus binotatus* (Spix, 1824) (Anura: Terrarana: Craugastoridae) from three localities in the State of Rio de Janeiro, with comments on its bioacoustic variability. *Herpetology Notes* 5:419–422.
- CHIPMAN, A. D., A. HAAS, E. TCHERNOV, AND O. KHANER. 2000. Variation in anuran embryogenesis: differences in sequence and timing of early developmental events. *Journal of Experimental Zoology Part B Molecular and Developmental Evolution* 288:352–365.

- COCO, L., V. N. T. BORGES JÚNIOR, L. A. FUSINATTO, M. C. KIEFER, J. C. F. OLIVEIRA, P. G. ARAUJO, B. M. COSTA, M. VAN SLUYS, AND C. F. D. ROCHA. 2014. Feeding habits of the leaf litter frog *Haddadus binotatus* (Anura, Craugastoridae) from two Atlantic Forest areas in southeastern Brazil. *Anais da Academia Brasileira de Ciências* 86:239–249.
- COSTA, P., AND S. CARVALHO E SILVA. 2010. *Haddadus binotatus* egg clutch. *Herpetological Review* 41:195–196.
- DIAS, I. R., R. L. MORAES, AND M. SOLÉ. 2012. Description of the advertisement call and morphometry of *Haddadus binotatus* (Spix, 1824) from a population from southern Bahia, Brazil. *North-Western Journal of Zoology* 8:107–111.
- ELINSON, R. P. 1990. Direct development in frogs: wiping the recapitulationist slate clean. *Seminars in Cell and Developmental Biology* 1: 263–270.
- . 2001. Direct development: an alternative way to make a frog. *Genesis* 29:91–95.
- ELINSON, R. P., E. M. DEL PINO, D. S. TOWNSEND, F. C. CUESTA, AND P. EICHHORN. 1990. A practical guide to the developmental biology of terrestrial breeding frogs. *Biological Bulletin* 179:163–177.
- FABREZI, M., S. QUINZIO, AND J. GOLDBERG. 2009. Giant tadpole and delayed metamorphosis of *Pseudis platensis* Gallardo, 1961 (Anura, Hylidae). *Journal of Herpetology* 43:228–243.
- FROST, D. R. 2014. Amphibian species of the world: an online reference. Version 6.0. Electronic database available from <http://research.amnh.org/vz/herpetology/amphibia/> American Museum of Natural History, New York, USA. Archived by WebCite at <http://www.webcitation.org/6PDsXDMhM>. Accessed 29 April 2014.
- GITLIN, D. 1944. The development of *Eleutherodactylus portoricensis*. *Copeia* 1944:91–98.
- GOLDBERG, J., F. VERA CANDIOTI, AND M. AKMENTINS. 2012. Direct-developing frogs: ontogeny of *Oreobates barituensis* (Anura: Terrarana) and the development of a novel trait. *Amphibia-Reptilia* 33:239–250.
- GOSNER, K. L. 1960. A simplified table for staging anurans embryos and larvae with notes on identification. *Herpetologica* 16:183–190.
- HANKEN, J. 1999. Larvae in amphibian development and evolution. Pp. 61–108 in B. K. Hall and M. H. Wake (eds.), *The Origin and Evolution of Larval Forms*. Academic Press, USA.
- HANKEN, J., D. H. JENNINGS, AND L. OLSSON. 1997a. Mechanistic basis of life-history evolution in anuran amphibians: direct development. *American Zoologist* 37:160–171.
- HANKEN, J., M. W. KLYMKOWSKY, K. E. ALLEY, AND D. H. JENNINGS. 1997b. Jaw muscle development as evidence for embryonic repatterning in direct-developing frogs. *Proceedings of the Royal Society of London Series B-Biological Sciences* 264:1349–1354.
- HEDGES, S. B., W. E. DUELLMAN, AND M. P. HEINICKE. 2008. New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation. *Zootaxa* 1737:1–182.
- HEINICKE, M. P., W. E. DUELLMAN, L. TRUEB, D. B. MEANS, R. D. MACCULLOCH, AND S. B. HEDGES. 2009. A new frog family (Anura: Terrarana) from South America and an expanded direct-developing clade revealed by molecular phylogeny. *Zootaxa* 2211:1–35.
- HUGHES, A. 1959. Studies in embryonic and larval development in amphibia. I. The embryology of *Eleutherodactylus ricordii*, with special reference to the spinal cord. *Journal of Embryology and Experimental Morphology* 7:22–38.
- JAMESON, D. L. 1950. The development of *Eleutherodactylus latrans*. *Copeia* 1950:44–46.
- KERNEY, R., J. B. GROSS, AND J. HANKEN. 2010. Early cranial patterning in the direct-developing frog *Eleutherodactylus coqui* revealed through gene expression. *Evolution and Development* 12:373–382.
- LYNN, W. G. 1942. The embryology of *Eleutherodactylus nubicola*. *Carnegie Institute of Washington Year Book* 190:27–62.
- LYNN, W. G., AND B. LUTZ. 1946. The development of *Eleutherodactylus guentheri*. *Boletim do Museu Nacional* 71:1–46.
- LYNN, W. G., AND B. LUTZ. 1947. The development of *Eleutherodactylus nasutus* Lutz. *Boletim do Museu Nacional* 79:1–30.
- MARTOJA, R., AND M. MARTOJA-PIERSON. 1970. *Técnicas de Histología Animal*. Toray-Masson S.A., Spain.
- MITGUTSCH, C., L. OLSSON, AND A. HAAS. 2009. Early embryogenesis in discoglossoid frogs: a study of heterochrony at different taxonomic levels. *Journal of Zoological Systematics and Evolutionary Research* 47:248–257.
- MOURA, M. R., J. V. A. LACERDA, AND R. N. FEIO. 2012. The advertisement call of *Haddadus binotatus* (Spix, 1824) (Anura; Craugastoridae). *Zootaxa* 3224:67–68.
- NARAYAN, E. J., J. M. HERO, K. S. CHRISTI, AND C. G. MORLEY. 2011. Early developmental biology of *Platymantis vitiana* including supportive evidence of structural specialization unique to the ceratobatrachidae. *Journal of Zoology* 284:68–75.
- NOBLE, G. K. 1925. An outline of the relation of ontogeny to phylogeny within the Amphibia. I. *American Museum Novitates* 165:1–45.
- NOKHBATOLFOGHAHAL, M., N. J. MITCHELL, AND J. R. DOWNIE. 2010. Surface ciliation and tail structure in direct-developing frog embryos: a comparison between *Myobatrachus gouldii* and *Pristimantis* (= *Eleutherodactylus*) *urichi*. *Herpetological Journal* 10:59–68.
- PADIAL, J. M., T. GRANT, AND D. R. FROST. 2014. Molecular systematics of terraranas (Anura: Brachycephaloidea) with an assessment of the effects of alignment and optimality criteria. *Zootaxa* 3825:1–132.
- POMBAL, J. P., JR. 1999. Oviposição e desenvolvimento de *Brachycephalus ephippium* (Spix) (Anura, Brachycephalidae). *Revista Brasileira de Zoologia* 16:967–976.
- PYRON, R. A., AND J. J. WIENS. 2011. A large-scale phylogeny of Amphibia with over 2,800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution* 61:543–583.
- REBOUÇAS, R., I. M. CASTRO, AND M. SOLÉ. 2013. Diet of *Haddadus binotatus* (Spix, 1824) (Anura: Craugastoridae) in Brazilian Atlantic Rainforest, Bahia state. *North-Western Journal of Zoology* 9:293–299.
- ROMERO-CARVAJAL, A., N. SÁENZ-PONCE, M. VENEGAS-FERRÍN, D. ALMEIDA-REINOSO, C. LEE, J. BOND, M. J. RYAN, J. B. WALLINGFORD, AND E. M. DEL PINO. 2009. Embryogenesis and laboratory maintenance of the foaming túngara frogs, genus *Engystomops* (= *Physalaemus*). *Developmental Dynamics* 238:1444–1454.
- SAMPSON, L. V. 1904. A contribution to the embryology of *Hylodes martinicensis*. *American Journal of Anatomy* 3:473–504.
- TABOADA, C., T. GRANT, J. D. LYNCH, AND J. FAIVOVICH. 2013. New morphological synapomorphies for the New World direct-developing frogs (Amphibia: Anura: Terrarana). *Herpetologica* 69:342–357.
- THIBAUDEAU, G., AND R. ALTIG. 1999. Endotrophic anurans: development and evolution. Pp. 170–188 in R. W. McDiarmid and R. Altig (eds.), *Tadpoles: The Biology of Anuran Larvae*. University of Chicago Press, USA.
- TOWNSEND, D. S., AND M. M. STEWART. 1985. Direct development in *Eleutherodactylus coqui* (Anura: Leptodactylidae): a staging table. *Copeia* 1985:423–436.
- VALETT, B. B., AND D. L. JAMESON. 1961. The embryology of *Eleutherodactylus augusti latrans*. *Copeia* 1961:103–109.
- WAKE, D. B., AND J. HANKEN. 1996. Direct development in the lungless salamanders: what are the consequences for developmental biology, evolution and phylogeny? *International Journal of Developmental Biology* 40:859–869.

Accepted: 2 October 2014.