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Embryonic Stages of *Gastrotheca riobambae* (Fowler) During Maternal Incubation and Comparison of Development With That of Other Egg-Brooding Hylid Frogs

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ABSTRACT A table of development (25 stages) for the period of incubation in the pouch was constructed for *Gastrotheca riobambae;* it can be used to stage embryos of other egg-brooding hylids. Analysis of embryonic weights during incubation shows that the mother does not contribute nutrients, but gases and other factors are probably exchanged between mother and embryos.

According to species, incubation on the back of the mother is carried to the froglet or to the tadpole stages. Development in these hylids is characterized by specialized gills, the bell gills derived from the branchial arches. In some species, the bell gills derive from the first branchial arch and cover less than 50% of the embryo, while in others, the bell gills come from both branchial arches I and II and cover from less than 50% to 100% of the embryo. The most complex bell gills derive from the fusion of the two branchial arches.

The majority of egg-brooding hylids live in tropical forests and carry development to the froglet stage. Tadpoles are produced by species of *Flectonotus, Fritziana,* and *Gastrotheca.* Tadpole-producing species of *Gastrotheca* have the most complex reproductive adaptations among egg-brooding hylids Acceleration and retardation in development seem to have played important roles in the evolution of these frogs. The evolutionary trend has been toward direct development, i.e., disappearance of the free-living larval stages through maternal incubation, and later to a recovery of the free-living tadpole stages in species of *Gastrotheca* with the most complex reproductive adaptations.

There are 56 described species of egg-brooding hylid frogs that are widely distributed throughout South America (Duellman, '77, '80; Duellman and Pyles, '80; Ruiz-Carranza and Hernandez, '76; Trueb and Duellman, '78). These frogs are found from Panama through eastern and western South America to northern Argentina. They are found also in the islands of Trinidad and Tobago and in southeastern Brazil, according to Duellman ('77).

Some species of egg-brooding hylids give birth to tadpoles, while others give birth to froglets (direct development). *Cryptobatrachus, Hemiphractus,* and *Stefania* do not have pouches and incubate the embryos directly on the female's back, while *Amphignathodon, Gastrotheca,* and *Flectonotus* have a pouch (Trueb, '74). Hylid frogs with pouches are called marsupial frogs (Duellman and Maness, '80). Frogs of the genus *Fritziana* have an incomplete pouch and the embryos are exposed during incubation, while in other marsupial frogs the pouch closes at incubation (del Pino, '80a).

Egg-brooding hylids produce few and large eggs that contain considerable reserves of yolk (del Pino and Humphries, '78). Development is modified by the yolk reserves and by the presence of embryonic adaptations such as the specialized bell gills (Noble, '31), which, in some species, envelop the embryo and form an extra-embryonic sac that becomes filled with fluid. Bell gills are highly vascularized and become separated from the equally vascularized tissues of the female's back by only a thin layer of egg jelly in frogs with a pouch or by the egg jelly and mucous secretions of the female's integument in frogs without a pouch (del Pino et al., '75; del Pino, '80a). A table of

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embryonic stages for the period of incubation has been prepared for *Gastrotheca riobambae;* it can also be used for other egg-brooding hylids. In addition, several characteristics of development are compared and possible evolutionary relationships are discussed.

MATERIALS AND METHODS

Embryonic development during incubation in the pouch was observed in embryos of about 60 females of *Gastrotheca riobambae*. The basic characteristics of the pouch and of embryonic development for this species have been described previously (del Pino et al., '75). Pregnant females were usually obtained from Machachi, about 50 km south of Quito. Frogs collected at other localities in the high inter-Andean valleys of the provinces of Pichincha, Cotopaxi, and Tungurahua were also studied. *Gastrotheca riobambae* can be collected alongside irrigation ditches in the morning or at night.

In the laboratory, the frogs were maintained in humid terraria at room temperature (average 17° C), and were provided with *Porcellio* sp. and *Drosophila* as prey items. Under these conditions, some frogs have been kept healthy for more than 2 years. Details of the maintenance in captivity have been explained by del Pino ('80b). In at least six instances, mating occurred spontaneously under laboratory conditions and in three cases ovulation and mating were induced by the intraperitoneal administration of human chorionic gonadotropin (Coriantin, Richter) to both male and female (200 and 800 I. U., respectively).

Two to four embryos were removed periodically from the pouch with a pair of blunt forceps, placed in amphibian Ringer's solution or modified Barth solution (according to Gurdon, '68), and observed under a microscope. Each time, one or two embryos were fixed in 10% neutralized formalin and kept for reference, while other embryos were fixed in Bouin's picro-formol and prepared as permanent whole-mounts as described by del Pino et al. ('75), or were embedded in Paraplast and cut into serial sections at 10 μm thickness. The sections were stained with Harris' hematoxylin and alcoholic eosin yellow. A total of 46 whole-mount permanent preparations were made, showing development from neurulation to the formation of bell gills (Figs. 11–18). Sixteen embryos showing stages of cleavage up to completion of the blastula were prepared as serial sections.

During early developmental stages (prior to the formation of embryonic blood), the removal of embryos from the pouch was easy, but as development proceeded, a close association was established between maternal pouch, egg jelly, and embryonic bell gills, and it became increasingly difficult to obtain uninjured embryos. The histology of the maternal-embryonic association was described by del Pino et al. ('75). Handling of the mother and removal of embryos resulted in injury to other embryos, which dried in the pouch and were released together with living tadpoles at the time of birth. The entire developmental sequence could not be followed in the brood of a single female, because the number of available embryos was reduced by embryos removed at previous occasions and by those that died in the pouch.

To correlate embryonic growth with development and to determine whether the maternal pouch serves a trophic function, embryos from 11 females were removed at intervals and weighed to the nearest milligram within their jelly capsules (wet weights); afterwards, they were dried in an oven at 55° C and were weighed again (dry weights). A total of 39 measurements of embryonic wet weights and of 53 measurements of the dry weights were made. Each measurement corresponds to the average weight of three embryos. The increase in total length during incubation was measured to the nearest millimeter in a total of 78 embryos from 18 females. Number and size of tadpoles at birth were determined from 146 newly born tadpoles from 25 females.

Living frogs of other species of egg brooding hylids were collected and brought to our laboratory by D.C. Cannatella, T.J. Berger, W.E. Duellman, J.D. Lynch, S.J. Maness, R.W. McDiarmid, and K. Miyata. Development of three species of *Gastrotheca* that give birth to tadpoles was studied in living embryos. In *G. cavia,* the developmental sequence was followed from the formation of the bell gills until birth in the broods of three females. Three whole-mount permanent preparations showing the fusion of branchial arches and the formation of bell gills were made. For *G. marsupiata,* and *G. monticola,* only the most advanced stages of development were available from the broods of two females of *G. marsupiata* and one female of *G. monticola.*

Development of *Flectonotus pygmaeus,* a species that gives birth to advanced tadpoles, was analyzed in the broods of 16 females provided by S.J. Maness. Sixteen embryos

from five brooding females were removed periodically and carefully preserved in 10% formalin or in Bouin's picro-formol by S.J. Maness and J. Cerda at the Estación Biológica de Rancho Grande, Maracay, Venezuela. Under captivity in Quito, four females became pregnant and development could be followed by the removal of embryos from the pouch. Three whole-mount permanent preparations showing the branchial arches and the formation of the bell gills were prepared. The broods of seven preserved specimens were also studied.

Living embryos of the following species with direct development were observed: *G. cornuta*-like, *G. excubitor,* and an undescribed species of *Gastrotheca* from Peru. For each species, the embryos of one brooding female were analyzed; while for *G. plumbea* and *G. excubitor,* four brooding females were studied. The earliest stage encountered was a gastrula of *G. plumbea;* two whole-mount permanent preparations were made showing the branchial arches and the formation of the bell gills. The brooding females of *G. excubitor* and of the undescribed species had advanced embryos and birth occurred in less than a week of captivity. For *G. walkeri,* only the newly born froglets were available. An excellent series of 43 advanced embryos from one female of *G. ovifera* was provided by S.J. Maness. For other species, only museum material was available (Table 2). In these cases one or two embryos were removed from the pouch, were observed, and afterwards replaced into the pouch or kept for reference. As development of the brood is almost synchronous, it was unnecessary to observe more than two embryos from a given specimen to obtain information on development. In the case of frogs without a pouch, the entire brood could be observed with the aid of a microscope. Information on the gill morphology and development of *Stefania goini* and *S. ginesi* was kindly provided by M.S. Hoogmoed. Egg diameter for different species was taken from previous work (del Pino and Humphries, '78) or was measured in two to three young embryos. Diameter of older embryos was not measured, since with development there is also increase in volume.

The following museum specimens that belong to the collections of the American Museum of Natural History (AMNH), Museum of the Brigham Young University (BYU), the Museum of the California Academy of Sciences (CAS), the Museo de la Estación Biológica de Rancho Grande (EBRG), the Field Museum of Natural History (FMNH), the Museo del Instituto de Ciencias Naturales de la Universidad Nacional de Colombia (ICN), the Museo del Instituto de Desarrollo de los Recursos Naturales Renovables de Colombia (INDERENA), the Museum of Natural History of the University of Kansas (KU), the Museum of Zoology of the Louisiana State University (LSUMZ), the Museo de la Fundación Miguel Lillo (MFML), the Museu de Zoologia Universidade de São Paulo (MZUSP), the Museum of Natural History of the University of Southern California (USC), and the collection of W. C. A. Bockermann (WCAB) were studied: *Cryptobatrachus boulengeri:* ICN 02098, 02699, 03086, 03294, 03957, 04742: Colombia; *C. fuhrmanni:* ICN 03503, 03577: Colombia. *Flectonotus fissilis:* MZUSP 30A (Holotype), 30C (Paratype): Brasil: Rio de Janeiro: Serra de Macaé; MZUSP 9808: Brasil: Santa Catarina: Brusque; MZUSP 35461: Brasil: Santa Catarina: Novo Horizonte, 400–800 m; *F. fitzgeraldi:* AMNH 62888: Trinidad: Simla. *Fritziana goeldii:* WCAB 1607, 18716, 42496: Brasil. *Gastrotheca andaquiensis:* INDERENA 410 (Holotype): Colombia; *G. argenteovirens:* AMNH 38802; *G. aureomaculata:* KU 181196: Colombia: Cauca: Moscapan, 14.7 km W Leticia, 2,050 m; ICN 2515; *G. ceratophrys:* KU 77016: Panamá Darien: Laguna, 820 m; AMNH A-90984; *G. christiani:* MFML 00482 (Holotype): Argentina: Jujuy: Monumento Ruta Valle Grande-Calilegua; MFML 02117, 02117-3, 02117-5: Argentina: Jujuy: Abra de Cañas: Valle Grande; *G. cornuta:* KU 145063: Colombia: Cauca: La Costa, El Tambo, 1,000 m; *G. ernestoi* MZUSP 238 (Holotype): Brasil: Rio de Janeiro: Serra de Macaé; *G. galeata:* LSUMZ 32049; *G. gracilis:* MFML 01389 (Holotype): Argentina: Catamarca: La Banderita; *G. griswoldi:* KU 138223: Perú: Junin: Comas, 3,222 m; *G. longipes:* CAS 149429: Ecuador: Copahuary River; *G. marsupiata:* KU 138249: Perú: Huancavelica, 20 km W Pampas, 436 m; KU 138252: Perú: Cuzco, 10 km E Abra Huillque, 3,700 m; KU 138399: Perú: Huancavelica: Huancavelica, 3,780 m; KU 139161: Perú: Cuzco: San Jerónimo, 10 km ESE Cuzco, 3,150 m; *G. medemi:* FMNH 81367; *G. orophylax:* KU 14386: Colombia: Nariño: La Victoria, 2,700 m; *G. plumbea:* KU 132414: Ecuador: Cotopaxi: Pilaló, 2,460–2,580 m; *G. testudinea:* LSUMZ 31973; *G. viridis:* WCAB; *G. walkeri:* EBRG 48240: Venezuela: Estado de Aragua: Maracay: Estación Biológica de Rancho Grande; *G. weinlandii:* INDERENA. *Hemiphractus fasciatus:* BYU 19142: Panamá: Bocas del Toro: Upper Rio Changena Camp, 30

km W Almirante; BYU 23554; *H. johnsoni:* USC 716, 717: Perú; AMNH A-58631; *H. scutatus:* KU 129751: Ecuador: Zamora-Chinchipe: Macuma. *Stefania scalae:* KU 167239: Venezuela: Bolivar: El Dorado-Santa Elena de Uarién Road, km 112, 860 m.

RESULTS

Development of Gastrotheca riobambae

Development of *G. riobambae* occurs inside the maternal pouch until the embryo reaches a tadpole stage with limb buds (Fig. 26). At birth, the tadpoles are released from the pouch into standing water, where they complete metamorphosis. Mating and placement of eggs inside the pouch as well as birth have been described previously (see Duellman and Maness, '80, for references). Development inside the pouch corresponds to the prehatching period. Hatching, i.e., emergence from egg membranes, and birth occur simultaneously. Incubation has been divided into 25 stages recognizable by morphological characteristics. The length of the incubatory period was not determined for individual frogs because brooding females were never collected at the time of amplexus and fertilization and, therefore, for them the beginning of incubation was unknown. The few cases in which ovulation and mating occurred in the laboratory provided information on the duration of individual stages and the associated changes of development. Handling of these frogs and removal of embryos prevented the completion of the incubatory process. The length of the incubatory period was calculated for each female as the sum of the period actually observed plus the average duration of missing stages (Table 1). The duration of each stage was calculated as the average of two to nine measurements from the broods of 22 females; it is given in the figure legends. Based on these calculations, the incubatory period was estimated at 87 ± 9 (s.d.) days (Table 1).

Gastrotheca riobambae incubates an average of 128 ± 9 (S.E. of the mean) eggs, based on the broods of 21 females, and the tadpole measures 17.7 ± 0.2 (S.E. of the mean) mm at birth and hatching (based on 146 embryos from 21 broods). Development in the pouch is efficient; without handling of the mother and removal of embryos, only 0.6% of eggs do not develop (at birth, only 17 undeveloped embryos were found in a total of 2,710 embryos from 21 females).

TABLE 1. Duration of the incubatory period in Gastrotheca riobambae

Frog	Incubation observed		Average duration of missing stages (days)[1]	Total (days)
	Stages	Time (days)		
1	1–8	13	75	88
2	1–11	18	70	88
3	1–12	21	67	88
4	1–15	26	61	87
5	1–18	34	42	76
6	11–25	67	14	81
7	12–25	74	17	91
8	14–20	30	54	84
9	14–21	38	46	84
10	14–24	51	33	84
11	19–24	44	52	96
12	19–25	42	40	82
13	19–25	47	40	87
14	19–25	57	40	97
15	19–25	30	40	70
16	20–25	62	46	108
				$\bar{X}$ = 87 ± 9 (s.d.)

[1] The average duration for each stage is given in the figure legends.

The egg of *G. riobambae* is of uniform pale yellow color, it measures from about 2.7 to 3.6 mm in diameter and is spherical in shape. Presence of abundant yolk slows down and modifies embryonic development in *G. riobambae*. Eggs of anurans are frequently much smaller, in *Xenopus laevis*, the egg measures only 1.4 to 1.5 mm in diameter (Nieuwkoop and Faber, '67). Duellman ('78) lists the ovum diameters of 75 anuran species at Santa Cecilia in Amazonian Ecuador. Only nine species had egg diameters equal to or larger than 3 mm. The mean ovum diameter is 1.9 ± 0.7 mm (S.E. of the mean); calculations based on the data of Duellman ('78).

Rotation of the embryo after fertilization could not be easily observed, as the eggs are uniformly pale. However, after fertilization, there is slight difference in color at the animal pole which appears more whitish than that of the vegetal pole. The animal pole soon becomes flattened, representing stage 1 (Fig. 1). The second polar body and cleavage furrows form in this flattened area. The "gray crescent" is not present and, therefore, stages one and two of Gosner ('60) can be recognized as only one stage in *G. riobambae*.

The first cleavage furrow begins to form at the animal pole, dividing the egg into two blastomeres of equal size (stage 2, Fig. 2). The polar bodies were sometimes found inside the furrow. The second cleavage furrow is perpen-

dicular to the previous one, visible at first only near the animal pole (stage 3, Fig. 3), and proceding very slowly toward the yolky vegetal hemisphere. From here on, cleavage differs considerably from the pattern of anurans with aquatic reproduction, since the animal pole cleaves much more rapidly than the vegetal pole and synchrony is lost. At stage 4 (Fig. 4), two new cleavage furrows are formed perpendicular to the first one, giving rise to eight incomplete blastomeres. Often, the third cleavage furrows do not appear synchronously and intermediate situations with five or six blastomeres are found.

At stage 5 (Fig. 5), cleavage of the animal hemisphere produces numerous micromeres, while the vegetal hemisphere contains large and yolky macromeres. The 16- and 32-cell stages and mid-cleavage stage of Gosner ('60) correspond to just one stage (stage 5) in *G. riobambae,* as it becomes increasingly difficult to count the number of blastomeres because of the uniformly pale color of embryos. The entire embryo originally is opaque, but by stage 6 (Fig. 6), the animal hemisphere gradually becomes translucent due to the appearance of the blastocoele in the animal region and to the rapid cleaving of micromeres. In serial sections, the micromeres show little or no yolk and are organized into a monolayer forming the roof of the blastocoele. All of the yolk becomes partitioned into macromeres which occupy the vegetal hemisphere and form the floor of the blastocoele. In *G. riobambae,* the blastocoele is displaced toward the animal pole so much that it gives a translucent appearance to the roof of the blastula. This displacement corresponds to a modification imposed by the large reserves of yolk.

Gastrulation corresponds to stages 7–9 (Figs. 7–9). The translucent roof of the blastula allows the observation of some cell movement during gastrulation. A blastopore is formed as in other anurans (Gosner, '60; Nieuwkoop and Faber, '67; Shumway, '40); but at first, it is difficult to observe the dorsal lip of the blastopore because of the pale and even color of embryos. The translucent roof of the bastula gradually becomes invaded by yolky cells (stage 7, Fig. 7). By stage 8 (Fig. 8), a yolk plug is present at the vegetal pole; this can be found only with difficulty because of the uniformly light color of embryos. The roof of the blastocoele is invaded further by yolky cells; in addition, it seems that cells from the floor of the blastocoele move to the roof, which becomes somewhat opaque but is still translucent when compared with the fully opaque vegetal hemisphere. The movements of gastrulation displace the small yolk plug to one side, determining the anteroposterior axis of the embryo (stage 9, Fig. 9). The process of gastrulation is slow in *G. riombambae* (it takes about six days). The translucent roof becomes elongated anteroposteriorly. At stage 10 (Fig. 10), a neural plate develops in the translucent animal pole region as an elongated thickening in the midline. In this region, neural folds form by stage 11 (Fig. 11); these close in the middle, leaving an anterior and a posterior neuropore. By stage 12 (Fig. 12), the region of the anterior neuropore becomes enlarged to form the head, and lateral to the head the gill plates develop and become divided into prominent thickenings, the branchial arches (Rugh, '51). At the same time, the first pairs of somites are formed lateral to the neural tube. The morphogenetic processes affect mainly the animal pole region from stages 10 to 18, although the vegetal pole continues to cleave. It is possible to cut out the region of the animal pole and to prepare it as a permanent whole-mount, which facilitates observations of the process of neurulation and gill formation.

By stage 13 (Fig. 13), the region immediately anterior to the head becomes thicker; in this zone the heart will originate. The branchial arches are more distinct but because of the modifications imposed by the yolk, these arches are spread out instead of being located on the sides of the head as in frog eggs with less yolk (compare with the generalized stage 18 of Gosner, '60; stage 18 of *Rana pipiens,* Shumway, '40; or with stage 25 of *Xenopus laevis,* Nieuwkoop and Faber, '67).

By stage 14 (Fig. 14), the heart is formed as a tube that becomes folded into a U shape; it does not beat, and there is no circulation, but blood cells begin to develop in the vegetal hemisphere. Branchial arches I and II enlarge gradually and begin to fuse with each other, giving rise to the single pair of bell gills. After fusion and the beginning of circulation, each bell gill receives blood from two aortic arches. The vessels that connect each gill to the body become enclosed into two gill stalks that are small and difficult to see at first, but during development they grow and become conspicuous. Each gill stalk consists of a membranous covering and connective tissue that envelop an afferent and an efferent vessel, derived from an aortic arch. The presence of one or two gill stalks per bell gill is important in

determining whether the bell gills come from one branchial arch or from the fusion of two. This criterion was used for the analysis of bell gill development in other species (Table 2).

Stage 15 (Fig. 15) is characterized by the beginning of the heart beat and by the absence of blood circulation, although more blood cells are being produced in the vegetal hemisphere. The bell gills appear as small, irregularly shaped structures on each side of the head. The otocyst and the lens of the eye are conspicuous. Vitelline vessels and other vessels are probably formed at about the same time that blood and the heart develop, but vessels

TABLE 2. Development in egg brooding hylid frogs

Species	No. of frogs	SVL[1] (mm)	CS[1]	OD[1] (mm)	OSF[1]	Development at birth 1 = tadpole 2 = froglet	Bell gills		
							No. of pairs	Stalks per gill	Area (%)
Frogs with pouch type one[2]									
Fritziana goeldii	3	36.9	16	4	1.7	1	2	1	< 25
Flectonotus fissilis	4	26.3	9	3	1.0	1	2	1	< 25
F. fitzgeraldi	1	18.0	3	3	0.5	1	1	1	< 25
F. pygmaeus	15	32.0	7	3	0.7	1	1	1	< 25
Frogs with pouch type two[2]									
Gastrotheca andaquiensis	1	73.7	~10	—	—	2	1	2	100
G. ceratophrys	2	74.0	9	10	1.2	2	1	2	100
G. cornuta	1	76.0	6	—	—	2	1	2	100
G. cornuta-like	1	79.8	12	~8	~1.2	2	1	2	100
G. longipes	1	95.0	17	8	1.4	2	—	—	—
G. viridis	1	66.2	5	9	0.7	2	1	2	100
G. walkeri	3	60.0	12	7	1.4	2	1	2	100
G. weinlandii	1	85.0	14	10	1.6	2	1	2	100
G. ovifera	4	79.8	32	8	3.2	2	1	2	100
Frogs with pouch types three and four[2]									
G. christiani	1	36.0	10	4	1.1	2	1	1	100
G. excubitor	1	41.1	10	6	1.5	2	1	2	100
G. galeata	1	55.0	20	5	1.8	2	1	1	100
G. griswoldi	1	41.0	12	8	2.3	2	1	1	100
G. testudinea	1	59.4	30	5	2.5	2	1	1	100
G. sp. (Peru)	1	40.0	17	6	2.6	2	1	2	100
Frogs with pouch types five and six[2]									
G. ernestoi	1	73.0	~33	6	~2.7	2	1	2	100
G. medemi	1	72.4	~30	~8	~3.3	2	1	2	100
G. orophylax	4	60.0	28	5	2.3	2	1	2	100
G. plumbea	3	69.0	28	4	1.6	2	1	2	100
G. sp. (FMNH-39889)	1	41.0	—	4	—	2	1	2	100
G. argenteovirens	1	42.0	~80	3	~5.7	1	1	2	100
G. aureomaculata	2	75.0	~70	~4	~4.2	1	1	2	100
G. cavia	2	63.0	~100	~3	~5.6	1	1	2	100
G. gracilis	1	40.0	~60	~3	~5.3	1	1	2	100
G. marsupiata	2	42.0	138	~2	~8.2	1	1	2	100
G. monticola	6	62.3	~80	3	~3.9	1	1	2	100
G. riobambae	21	53.9	128	3	7.1	1	1	2	100
Frogs without pouch									
Cryptobatrachus boulengeri	5	65.0	26	4	1.6	2	1	1	< 50
C. fuhrmanni	2	60.0	28	4	1.9	2	1	1	< 50
Hemiphractus fasciatus	1	68.7	13	7	1.3	2	1	2	100
H. johnsoni	1	77.2	17	—	—	2	2	1	100
H. scutatus	1	72.0	10	10	1.4	2	2	1	100
Stefania ginesi	—	—	3	—	—	2	2	1	100
S. goini	—	—	15	—	—	2	2	1	100
S. scalae	1	60.0	11	9	1.7	2	2	1	100

[1] SVL = snout-vent length; CS = clutch size; OD = ovum diameter; OSF = ovarian size factor (Duellman and Crump, '74).

[2] Pouch type according to del Pino ('80a).

cannot be easily observed in living embryos prior to the onset of circulation. The beginning of circulation characterizes stage 16 (Fig. 16). Vitelline circulation and circulation to the bell gills can be observed. The embryo has enlarged and has more pairs of somites. A tail fold has formed, separating the small tail from the segmented yolk. By stage 17 (Fig. 17), the head fold develops, bringing the heart to its ventral median position, and the bell gills have grown into disk-shaped structures that cover the slightly pigmented head.

The body is separated from the yolk by head, tail, and lateral folds by stage 18 (Fig. 18), and it becomes gradually pigmented, appearing as a very small tadpole on top of the yolk mass. The eyes are conspicuously pigmented; the mouth parts begin to develop but do not present a pigment pattern; the tail bears a transparent tail fin measuring about 3 mm in length. Internal organs are developing but the embryo does not have internal gills nor lungs and the intestine does not yet form coils. At the base of the tail are buds of the hind limbs. Following the criteria of Gosner ('60), the length of each bud (L) is smaller than one-half of its diameter (D), $L < 0.5D$, as in stage 26 of Gosner ('60). In *G. riobambae,* limb buds develop more precociously than in species with aquatic reproduction (Fig. 32). The bell gills have long gill stalks and have grown considerably, each gill covering one-half of the embryo. Both are joined together in the midline, forming in this way a sac that is filled with liquid in which the embryo floats. Sometimes, toward the end of incubation, the sac formed by the gills breaks in the area where the gills join each other. The bell gills are in close contact with the thin envelope formed by vitelline membrane and egg jelly which separate them from the vascularized pouch of the mother (see del Pino et al., '75, for a description of this association.)

Stages 19 and 20 are characterized by development of the operculum. The opercular fold has formed by stage 19 (Fig. 19), leaving in the midline a wide aperture. The gill stalks, which are large, come out of the opercular chamber on each side. The dorsal surface of the body, the tail, and part of the ventral surface become more pigmented; a conspicuous intestinal coil, lung buds, and internal gills are present. The length of the hind limb is equivalent to, or larger than, one-half of its diameter ($L \geq 0.5D$) as in stage 27 of Gosner ('60). At stage 20 (Fig. 20), the opercular fold closes from the right side to the midline, and therefore the opening is displaced from the midline to the left; gill stalks come out from this left spiracle and form several coils. The embryo has grown and it is more darkly pigmented; there is dark pigment even in the horny beaks and tooth rows of the mouth. The hind limbs appear as in stage 28 of Gosner ('60); that is, the length of the bud is equivalent to or larger than its diameter ($L \geq D$). At the same time, fore limbs are developing inside the opercular chamber. Development of the limbs from stages 21 to 25 (Figs. 21–25) is equivalent to stages 29 to 33 of Gosner ('60).

During these stages, the spiracle becomes smaller and it is gradually displaced to the left side; by stage 25 it is located in the posterior half of the body, on the ventral surface, toward the left (Fig. 26). The gill stalks are found mostly inside the opercular chamber, with only their more distal portions and the bell gills protruding from the spiracle. The internal gills and lungs become further developed.

During the incubatory period, the total size of an embryo increases from an egg of about 3 mm in diameter to a tadpole of 18 mm in length. Such increase in size is accompanied by a corresponding increase in the wet weight but by no noticeable increase in dry weights (Fig. 31). Although weight measurements included the weight of jelly capsules and capsular fluid, additional results (not shown) indicate that most of the increase is in the wet weight of the embryo itself. At birth, there is still yolk in the tadpole's intestine and feeding does not occur until 1 or 2 days after birth. This evidence suggests that during incubation there is no significant contribution of nutrients from the mother, although water, oxygen, and other factors needed for development may well be transferred from the mother to the embryos. Preliminary observations (of M. Campos) in this laboratory indicate that when the vital dye trypan blue is injected into the mother, there is slight incorporation of the dye into embryonic tissues. Therefore, it seems that exchanges between mother and embryos occur through the pouch. *Gastrotheca riobambae* can be considered to be at the limit between ovo-viviparity and viviparity. In addition, post-ovulatory follicles are present during early incubation (del Pino and Sánchez, '77). These follicles may aid in the maintenance of early incubation and associated changes of the pouch.

Abbreviations

AN, anterior neuropore
AP, animal pole
B, blastopore
BG, bell gill
GS, gill stalk
H, heart
I, first branchial arch
II, second branchial arch
O, otocyst
S, somite
VP, vegetal pole

Fig. 1. Stage one of embryonic development in *Gastrotheca riobambae* at 17° C. The fertilized egg. The egg is evenly colored. Age 0 hours; diameter 3 mm. All bars represent 1 mm.

Fig. 2. Stage two, the two-cell stage. The first cleavage furrow appears at the animal pole. Approximate age 12 hours; diameter 3 mm.

Fig. 3. Stage three, the four-cell stage. Approximate age 33 hours; diameter 3 mm.

Fig. 4. Stage four, the eight-cell stage. Approximate age 2 days; diameter 3 mm.

Fig. 5. Stage five, cleavage. The animal pole region becomes segmented more rapidly than the vegetal pole. Approximate age 6 days; diameter 3.5 mm.

Fig. 6. Stage six, blastula. The animal pole region becomes completely translucent due to the rapid cleavage of micromeres. Approximate age 7 days; diameter 3.5 mm.

Fig.7. Stage seven, early gastrula. Due to the movements of gastrulation, the translucent region at the animal pole is invaded gradually by yolky cells. The blastopore is formed at the vegetal pole. Approximate age 9 days; diameter 3.5 mm.

Fig. 8. Stage eight, midgastrula. The translucent region at the animal pole has been invaded further by yolky cells. The blastopore and yolk plug are clearly visible at the vegetal pole region. Approximate age 10 days; diameter 3.5 mm.

Fig. 9. Stage nine, late gastrula. The translucent region at the animal pole is completely invaded by yolky cells and it becomes somewhat opaque. In this area, the body of the embryo will develop. The small blastopore has moved to a lateral position. Approximate age 13 days; diameter 3.5 mm.

Fig. 10. Stage ten, neural plate. The translucent region at the animal pole has become elongated anteroposteriorly. Cells accumulate in the midline, forming the neural plate. The blastopore is no longer visible. Approximate age 14 days; diameter 3.5 mm.

Fig. 11. Stage 11, neural folds. The neural plate originates neural folds which remain open at the anterior and at the posterior neuropores. Approximate age 17 days; diameter 4 mm.

Fig. 12. Stage 12, neural tube. The neural folds close to originate the neural tube. The embryo begins to develop a recognizable head. The gill plates have been formed and become divided into branchial arches. There are about six pairs of somites. Approximate age 18 days; diameter 4 mm.

Fig. 13. Stage 13, branchial arches. The branchial arches are clearly visible. The lens of the eye and the otocyst are developing. The region anterior to the head becomes thicker and it will originate the heart. Approximate age 21 days; diameter 4 mm.

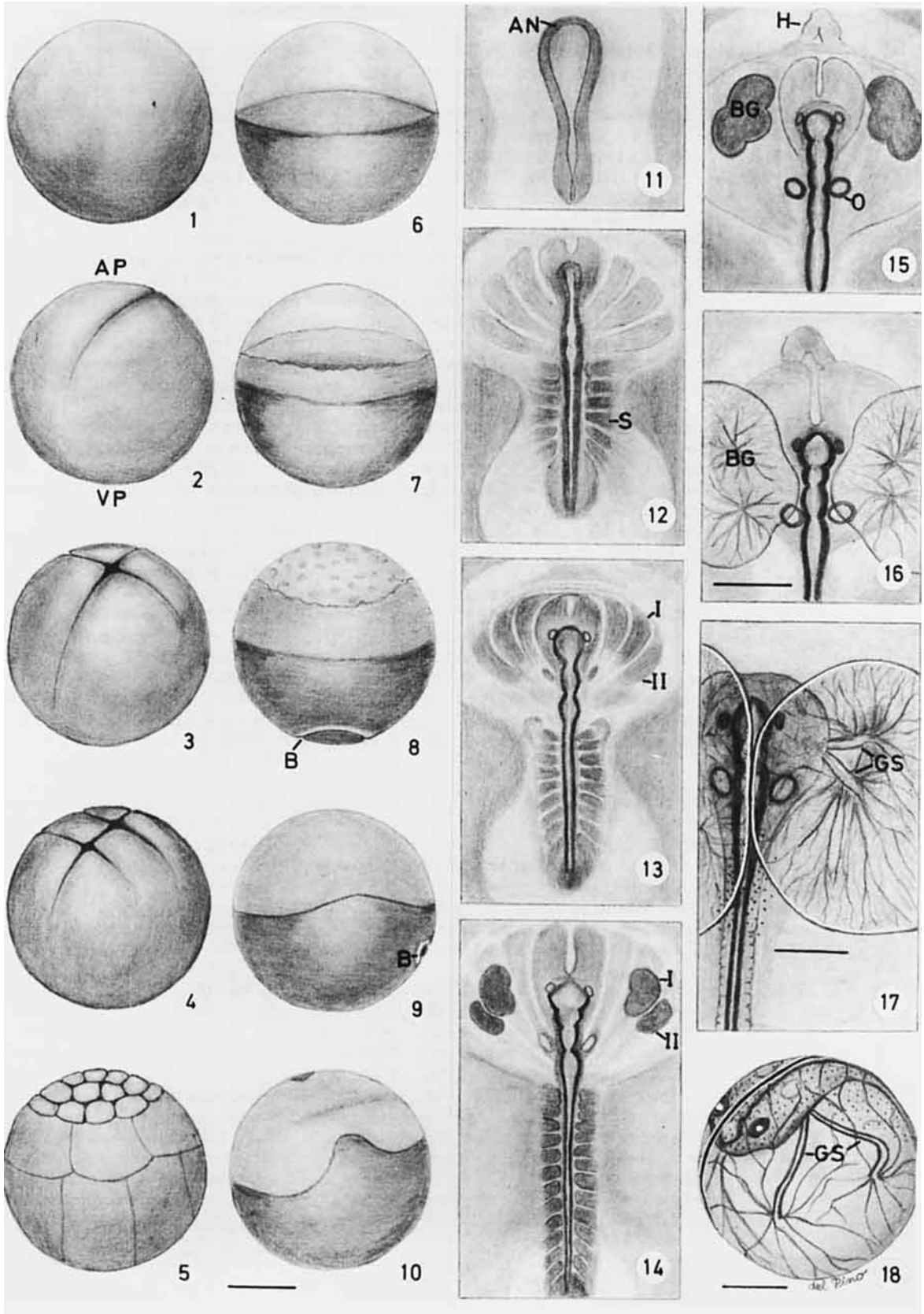
Fig. 14. Stage 14, fusion of branchial arches. The first and second branchial arches enlarge, become thicker, and begin to fuse with each other. Blood cells develop in the vegetal pole region, but circulation has not started. The heart is anterior to the head and does not beat. Approximate age 23 days; diameter 4 mm.

Fig. 15. Stage 15, heart beat. The heart appears as a U-shaped tube and it beats; blood circulation has not started yet. The branchial arches have fused to originate the single pair of bell gills which are small and irregular in shape. Approximate age 26 days; diameter 4 mm.

Fig. 16. Stage 16, circulation to the bell gills. The heart is anterior to the head; vitelline vessels bring blood to the heart and there is circulation to the bell gills. A tail fold has formed separating the small tail. Approximate age 27 days; size 4 mm.

Fig. 17. Stage 17, head fold. Development of the head fold brings the heart to its ventral median position. Bell gills have grown and cover the head region. Approximate age 31 days; size 5 mm.

Fig. 18. Stage 18, pigmented eyes. The embryo appears as a small and slightly pigmented tadpole on top of the yolk mass. The eyes are conspicuously pigmented and the tail has a transparent tail fin. The bell gills have grown to envelop the entire embryo and each one is connected to the embryo by two long gill stalks. Buds of the hind limbs are present and their length is smaller than their diameter, as in Gosner ('60) stage 26. Approximate age 40 days; size 6 mm.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At birth and hatching, stage 25 (Figs. 25–26), embryos are released from the pouch as previously described (del Pino et al., '75) and become active swimming tadpoles after 1 or 2 days, when feeding also starts. The vitelline envelope and jelly capsule rupture during birth and the tadpole straightens. Oral suckers are absent. Circulation through the bell gills ceases almost immediately after the embryo touches the water; but the gills protrude from the opercular aperture for about 1 day before being retracted into the opercular chamber, where they become smaller over about a week and finally disappear. The osmotic shock of contact with fresh water seems to start the circulatory changes that eventually contribute to the elimination of bell gills as respiratory organs, since, when tadpoles are kept in Ringer's or in 1.5 Ringer's solution after birth, circulation to the bell gills is maintained (del Pino et al., '75). After birth and the resorbtion of bell gills, development of the free-living tadpole is equivalent to that of other anurans.

Depending on the availablity of water and of food, free-living tadpoles can become large (snout-vent length 30 mm, total length 90 mm) before completing metamorphosis; the time needed varies depending on environmental factors. Hoogmoed ('67) observed metamorphosis 41 days after birth; however, in our laboratory, tadpoles fed on finely ground chicken feed reached metamorphosis from about 4 months to close to 1 year from the time of birth.

Development in other species of egg-brooding hylids

During incubation, embryos of egg-brooding hylids develop membranous and vascularized bell gills that in most species envelop the embryo completely (Table 2). Only in *Cryptobatrachus, Flectonotus,* and *Fritziana* (Table 2), the bell gills are small and do not cover the entire embryo.

Frogs without a pouch present several patterns of gill development (Table 2). In *H. fasciatus,* there is a single pair of gills, like those of *G. riobambae;* but in *H. johnsoni,* and *H. scutatus,* and in three species of *Stefania* (Table 2) there are two pairs of bell gills (Fig. 30), which cover the entire embryo. The four bell gills adhere to each other but are not fused; each one has a gill stalk that contains an afferent and an efferent vessel. Each gill, therefore, develops from one branchial arch. The anterior pair of bell gills covers the head and dorsal surface, while the posterior pair covers mainly the back and ventral surface of the embryo. In *S. scalae* the bell gills are thick and the first pair is conspicuously pigmented.

The two species of *Cryptobatrachus* examined (Table 2) have one pair of bell gills derived from one branchial arch. *Cryptobatrachus* does not have a pouch and the bell gills are thick and cover the dorsal surface of the embryo (less than 50% of the embryonic surface). It seems that the bell gills are predominantly oriented toward the exposed surface and therefore are separated from the outside environment just by the vitelline envelope and jelly capsule. This situation was encountered in all of the preserved specimens examined, and it suggests that little exchange should occur with the mother since the incubatory integument and the gills are separated. Histological evidence (del Pino, '80a) gives support to this hypothesis, as there is little vascularization of the incubatory integument. In addition, the maternal and embryonic tissues are separated by an additional layer of mucous secretion that cements the jelly capsule of the embryos to the incubatory integument of the mother.

Two pairs of bell gills (each one derived from one branchial arch) are present in *Flectonotus fissilis* and in *Fritziana goeldii* (Table 2)—marsupial frogs that have the simplest pouch (type one, del Pino, '80a). In these species, bell gills are small disk-shaped structures that partially cover the head region of the embryo (less than 25% of the embryonic surface). *Flectonotus fitzgeraldi* and *F. pygmaeus,* in contrast, have only one pair of small bell gills derived from the first branchial arch (Figs. 27–28), and the gills partially cover the head region of the embryo (less than 25% of the embryonic surface).

The embryos of *Gastrotheca* (Table 2) have one pair of membranous and vascularized bell gills derived from the fusion of two branchial arches. Each bell gill is connected to the embryonic body by two gill stalks, and each gill stalk has an afferent and an efferent vessel as in *G. riobambae.* However, in five species of *Gastrotheca* (Table 2), there is only one gill stalk per bell gill. In these cases, the single gill stalk had four vessels (two afferent and two efferent vessels), suggesting that there was fusion of the gill stalks; the bell gills developed from the fusion of two branchial arches as in other species of *Gastrotheca.* In all cases, the bell gills envelop the embryo completely during incubation and are in con-

tact with the outer vitelline envelope and thin jelly capsule. The vitelline envelope and jelly separate the vascularized bell gills from the maternal pouch as it happens in *G. riobambae* (del Pino et al., '75). In addition, the pouch undergoes vascularization and forms partitions between embryos as in *G. riobambae* (del Pino, '80a).

There are various levels of complexity in bell gill origin and maximal size among different species. One pair of small bell gills derived from the first branchial arch (*Cryptobatrachus, Flectonotus fitzgeraldi,* and *F. pygmaeus*) may be more primitive than two pairs of small bell gills (*Flectonotus fissilis* and *Fritziana goeldii).* A more complex condition corresponds to the presence of two pairs of large bell gills that envelop the embryo completely (*Stefania* and most species of *Hemiphractus*), and the most complex situation is the fusion of the two branchial arches to form a single pair of large bell gills (*Gastrotheca* and *H. fasciatus*). In embryos of *G. riobambae* cultured in vitro, the branchial arches sometimes did not fuse; the resultant two pairs of small bell gills covered only the head region of the embryo as in *F. fissilis.*

According to the incubatory mode and to bell gill structure and size, frogs without pouches of the genus *Cryptobatrachus* are the least complex; the two species examined (Table 2) give birth to froglets. All other egg-brooding hylid frogs without pouches give birth also to froglets (Table 2). Among frogs with pouches, *Flectonotus,* with a pouch that closes during incubation, and *Fritziana,* with a pouch that remains open, have the simplest pouch (type one, del Pino, '80a); these frogs correspond to the least elaborate forms of marsupial frogs. Bell gills in these frogs range in complexity from the single pair derived from one branchial arch to two pairs of small bell gills (Table 2). Frogs of both genera give birth to advanced tadpoles (limb development as in stage 39 of Gosner, '60).

The majority of species of *Gastrotheca* give birth to froglets (22 species of the 35 described). Some species of *Gastrotheca* have a simple pouch (type two, del Pino, '80a), while in others, the morphology of the pouch is increasingly more complex (pouch types three to six, del Pino, '80a). Species with the most complex pouch (type six, del Pino, '80a), give birth to tadpoles, while all species with pouch type two give birth to froglets (Table 2). It seems, therefore, that during evolution of *Gastrotheca* incubation has been modified from an unknown ancestor that gave birth to tadpoles, to direct development, and later again to the birth of tadpoles (del Pino, '80a). Frogs like *G. riobambae* that give birth to tadpoles are the most complex and advanced species of *Gastrotheca.*

Embryonic development is unknown for *Amphignathodon.* The single species of this genus, *A. guentheri,* has pouch type two (del Pino, '80a). The morphology of the pouch suggests that incubation should be carried to the froglet stage as it happens in other species with this kind of pouch (Table 2).

The spiracle develops differently in the tadpoles of *Flectonotus* and *Fritziana* (with pouch type one, del Pino, '80a), and in the tadpoles of species of *Gastrotheca* (pouch type six, del Pino, '80a). Tadpoles of *Flectonotus* and *Fritziana* have a midline spiracle, and swim little or not at all; while the tadpoles of *Gastrotheca* have a left spiracle and are good swimmers. Embryos of egg-brooding hylids with direct development do not form a spiracle during incubation. An opercular fold is formed but it remains widely open in the midline as in stage 19 of *G. riobambae* (Fig. 19). The opercular fold gradually becomes smaller until it disappears completely by the time of birth. The wide aperture of the operculum could be interpreted as partial development of the spiracle in frogs with direct development. Emergence of the fore limbs in these frogs differs from that of species with a true spiracle. The fore limbs emerge at metamorphosis by rupture of the peribranchial chamber in the free-living tadpoles, while in species with direct development the peribranchial chamber is incomplete and is later resorbed.

In *Flectonotus pygmaeus,* there is precocious development of the hind limbs; in an embryo otherwise comparable to stage 15 of *G. riobambae,* there are limb buds as in stage 18 (Fig. 32). The newly born tadpole has internal gills and lungs, it does not feed nor swim, and the hind limbs have developed to a stage comparable to Gosner's ('60) stage 39. It completes metamorphosis in about 30 days under laboratory conditions (del Pino and Humphries, '78). In nature, tadpoles of this species complete metamorphosis only in about 13 days (Duellman and Maness, '80). *Flectonotus fitzgeraldi* gives birth to advanced tadpoles, equivalent to those of *F. pygmaeus,* and metamorphosis is completed in about 5 days (Kenny, '69).

Development in the pouch in species of *Gastrotheca* that give birth to tadpoles (Table 2)

is equivalent to that of *G. riobambae;* however, the extent of limb development at birth differs slightly from one species to another and there are additional differences in regard to egg size, number of embryos per brood, and size of tadpole at birth.

Species of *Gastrotheca* with direct development (Table 2) differ considerably from *G. riobambae* in that the free-living tadpole stages have been omitted and the entire developmental sequence takes place inside the pouch. As in *G. riobambae,* eggs are devoid of dark pigment and early development up to stage 19 is similar to that of *G. riobambae.* Main differences begin to appear at about this stage in regard to the development of pigment, the operculum, the limbs, and the changes with metamorphosis. The opercular fold remains open in the midline during incubation, as in stage 19 of *G. riobambae,* and the gill stalks come out from the opercular chamber on each side. At birth, the opercular fold has been resorbed and the gill stalks come out directly from the body through small, rounded apertures of the skin at each side of the heart (Fig. 29).

During incubation, the embryo develops lungs and rudiments of the internal gills. The body appears as a small tadpole on top of the yolk mass; its dorsal surface becomes darkly pigmented, but pigment never covers the yolky ventral surface. Tadpole mouth parts are formed and acquire pigment. Later, the mouth undergoes the corresponding changes to produce the frog mouth as occurs during metamorphosis. Development of the limbs is completed inside the pouch. A small tail with a tail fin develops during incubation. The length of the tail varies according to species. Toward the end of incubation, the tail is resorbed and there are gradual changes in the pigment pattern and skin texture toward the adult condition. These changes occur in the pouch but are comparable to the process of metamorphosis in species with aquatic development. In egg-brooding hylids without pouch, the froglet develops as in species of *Gastrotheca* with direct development; but only a small tail is formed, and the tail fin is rudimentary or is absent.

Embryos of egg-brooding hylids can be staged by the combined usage of the developmental table of *G. riobambae,* and the generalized table of Gosner ('60). In tadpole-producing species, development in the pouch is similar to that of *G. riobambae,* and can be staged according to the table for this species. Differences in the origin and maximal size of bell gills should be noted as well as the characteristics of the spiracle, tail, limbs, and pigment pattern. When at birth there is more development of the limbs than in *G. riobambae,* the table of Gosner ('60) should be consulted to determine limb development. In species that give birth to froglets, development can be staged in a similar way. The final changes with metamorphosis are comparable to stages 41 to 46 of Gosner ('60), but in some species metamorphosis is accelerated and occurs simultaneously with limb development. Developmental differences, such as this, should be noted.

We have kept pregnant females of several species of egg-brooding hylids, and the length of incubation under laboratory conditions has been partially determined. *Gastrotheca cavia*—a species that gives birth to tadpoles—incubates embryos in the pouch for about 87 days, a period comparable to that of incubation of *G. riobambae* (Tables 1, 3). The length of incubation in species of *Gastrotheca* with direct development *(G. cornuta*-like, *G. orophylax,* and *G. plumbea)* appears slightly longer than that of *G. riobambae.*

Flectonotus pygmaeus requires only 29 days to reach an advanced tadpole stage (Table 3), which indicates that development in the pouch is accelerated when compared with that of other marsupial frogs for which the length of development is known. A species with a short incubatory period, such as *F. pygmaeus,* may have limited exchanges with the mother, and therefore, may not need well-developed bell gills; larval development is also accelerated in this species.

At birth and hatching in egg-brooding hylids, the jelly capsule breaks, liberating the young. There is still some yolk in the intestine both in tadpoles and in froglets, which suggests that the mother does not contribute significantly with nutrients, although gases, water, and other factors are probably exchanged. In tadpole-producing species, blood circulation to the bell gills ceases soon after the tadpole is deposited in water. The bell gills are retracted into the peribranchial chamber and finally disappear as in *G. riobambae.* Newly born tadpoles of the following species have been observed: *Flectonotus pygmaeus, F. fissilis, G. cavia, G. marsupiata, G. monticola,* and *G. riobambae.*

In the newly born froglet, the fate of bell gills is different from that in tadpoles, as circulation to the bell gills ceases soon after birth and the bell gills are sloughed rather than resorbed. The body apertures for the gill

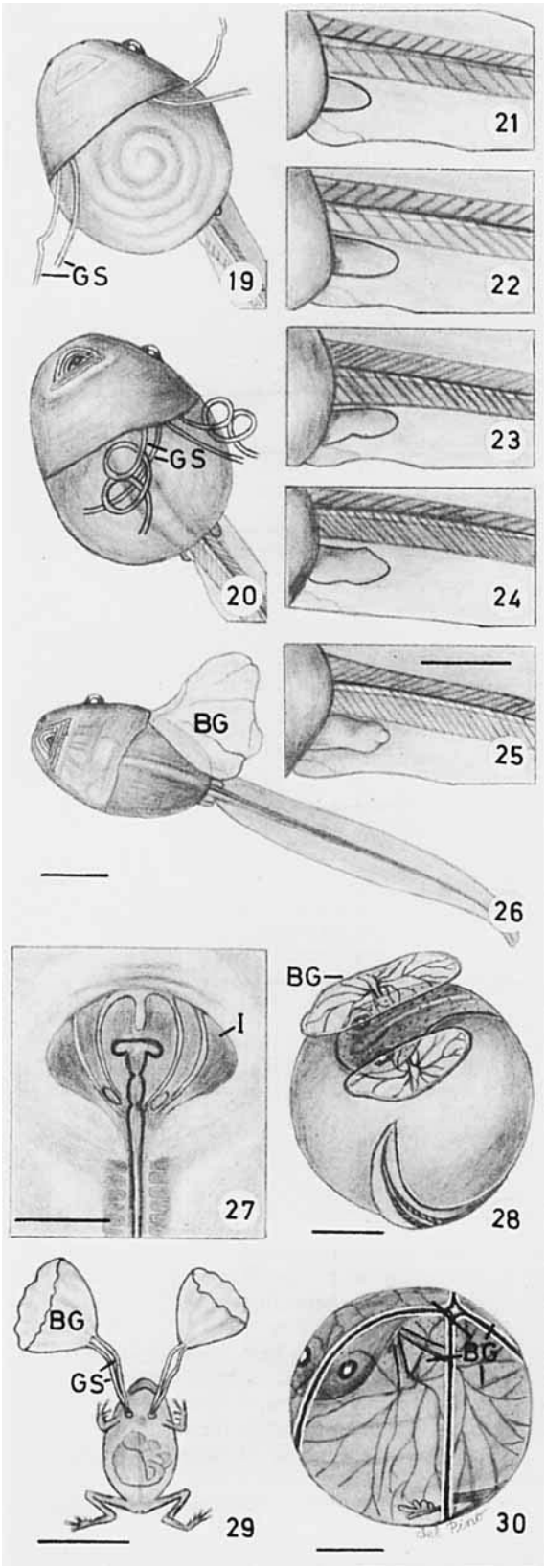

Fig. 19. Stage 19, opercular fold. The opercular fold has formed, leaving a wide aperture in the midline from where the gill stalks come out (the bell gills have been omitted in the figure). The mouth parts have developed but are unpigmented. The intestine forms several coils. The ventral surface does not have dark pigment. Buds of the hind limbs have a length that is equal to or larger than half of their diameter as in Gosner ('60) stage 27. Approximate age 46 days; size 8.5 mm.

Fig. 20. Stage 20, opercular closure. The opercular fold closes from the right side to the midline and the gill stalks that form several coils come out from this left aperture (the bell gills have been omitted in this figure). The body becomes more pigmented. The hind limbs appear as in stage 28 of Gosner ('60); the length is equivalent or larger than the diameter of the limb. Approximate age 49 days; size 12 mm.

Fig. 21. Stage 21, development of the buds of the hind limbs. The operculum continues to close until stage 25, when it is open through a small spiracle completely displaced to the left. The hind limbs are comparable to stage 29 of Gosner ('60); the length of the bud is equal to or larger than 1.5 times its diameter. Approximate age 55 days; size 13.5 mm.

Fig. 22. Stage 22, development of the buds of the hind limbs. Buds of the hind limbs have a length that is twice the diameter of the limb as in stage 30 of Gosner ('60). Approximate age 63 days; size 14 mm.

Fig. 23. Stage 23, toe development. The foot is paddle-shaped as in stage 31 of Gosner ('60). Approximate age 71 days; size 16 mm.

Fig. 24. Stage 24, toe development. Individual toes begin to form as in stage 32 of Gosner ('60). Approximate age 76 days; size 17 mm.

Fig. 25. Stage 25, hatching and birth. Development of the toes at hatching and birth is shown. Toe development is comparable to stage 33 of Gosner ('60). Approximate age 88 days; size 18 mm. Bar represents 1 mm (Figs. 19–25 are drawn to this magnification).

Fig. 26. The tadpole of *G. riobambae* at birth (stage 25). The spiracle is located on the ventral surface toward the left and the discs of the bell gills protrude from the spiracle. The bell gills are later resorbed into the opercular chamber. Approximate age 88 days; size 18 mm. Bar represents 2 mm.

Fig. 27. Embryo of *Flectonotus pygmaeus* at a stage comparable to stage 13 of *G. riobambae* (Fig. 13). Only the first branchial arch is being developed. Bar represents 1 mm.

Fig. 28. Embryo of *F. pygmaeus* at a stage comparable to stage 18 of *G. riobambae*. Bell gills are derived from the first branchial arch, are small, and just cover the head region of the embryo. Bar represents 1 mm.

Fig. 29. Froglet of *G. excubitor* at birth. The gill stalks come out through small apertures located laterally to the heart. These gills are eliminated soon after birth. Bar represents 10 mm.

Fig. 30. Embryo of *S. scalae*. There are two pairs of bell gills that envelop the embryo completely. Each gill is connected to the body by one gill stalk that contains one afferent and one efferent vessel. The tail is vestigial and there is a precocious development of the limbs. Bar represents 2 mm.

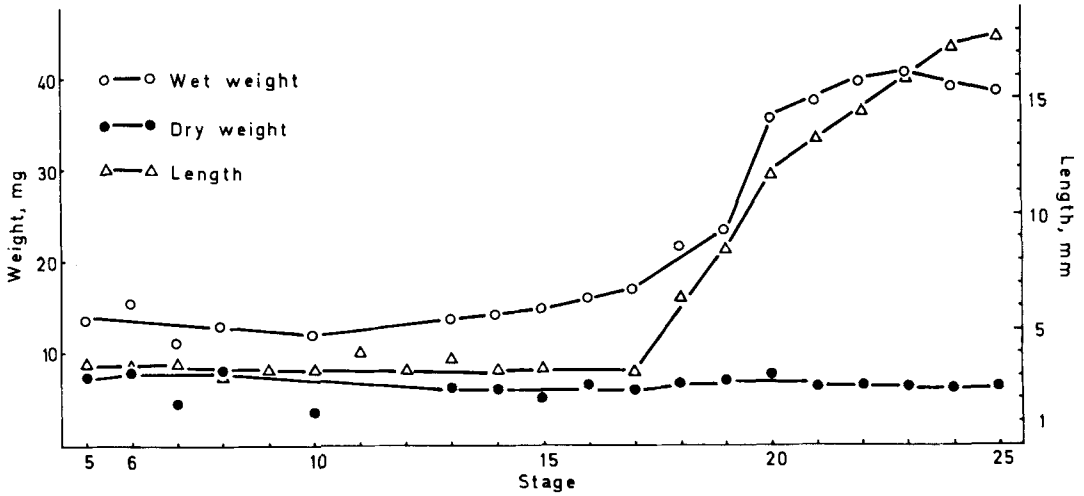

Fig. 31. Embryonic growth during the incubatory period in *G. riobambae*. Based on 39 measurements of wet weight and 59 of dry weights from embryos of 11 females. Each measurement corresponds to the average weight of three embryos.

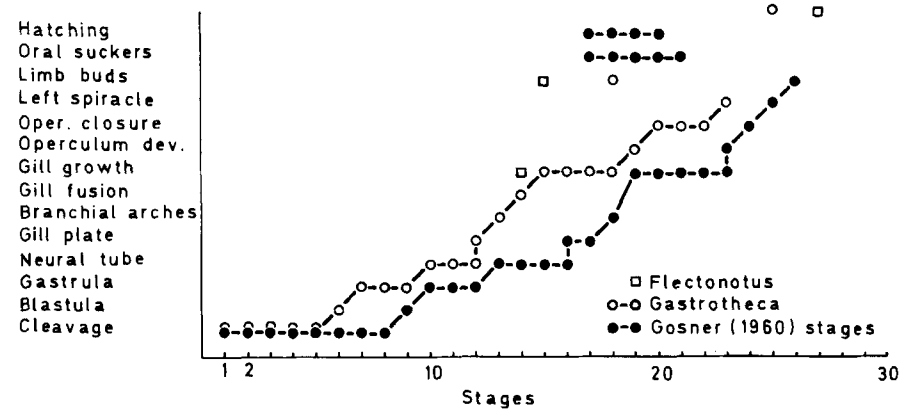

Fig. 32. Comparison of developmental characteristics in *G. riobambae* with those of frogs with aquatic reproduction (according to Gosner, '60) and with development in *Flectonotus pygmaeus*. In *F. pygmaeus*, development is comparable to that of *G. riobambae*, except for the gills, limb buds, and hatching.

stalks close and disappear in about 1 day. Absence of a peribranchial chamber lets the gills touch the substratum where birth takes place; this leads to rupture of the gill stalks. In some cases, the bell gills are sloughed at birth and are left inside the pouch. Newly born froglets of the following species were observed: *G. cornuta*-like, *G. excubitor*, *G. orophylax*, *G. plumbea*, and an undescribed species of *Gastrotheca* from Peru.

The events of birth in tadpole-producing species of *Gastrotheca* are equivalent to birth in *G. riobambae*. The female introduces the long toes of the hind limbs into the pouch for the removal of embryos as in *G. riobambae* (del Pino et al., '75). In *F. pygmaeus*, tadpoles emerge from the pouch unaided by the mother (Duellman and Maness, '80); that is, the mother does not introduce the hind limbs into the pouch for the removal of embryos. In marsu-

TABLE 3. Duration of the incubatory period in several species of Gastrotheca and in Flectonotus pygmaeus

Species	Development at birth		Incubation observed	
	1 = tadpole 2 = froglet	Stage[1]	Stages[1]	Time (days)
G. cavia	1	25	6–25	86
	1	25	13–25	48
G. cornuta-like	2	46	13–46	105
G. orophylax	2	44	22–44	88
G. plumbea	2	42	8–40	85
	2	42	13–42	118
F. pygmaeus	1	38	3–5	1
	1	38	8–36	28
	1	38	35–38	4

[1] Stages 1–25 according to the developmental stages of *G. riobambae;* followed by stages 33 to 46 according to Gosner ('60).

pial frogs with direct development, emergence of young is aided by the mother in some species, while in others, the froglets leave the pouch without the active help of the mother. Introduction of the hind limbs into the pouch for the removal of froglets was observed in *G. excubitor, G. plumbea,* and in an undescribed species of *Gastrotheca* from Peru. The females of *G. excubitor* and *G. plumbea* gave birth in water, while the female of the undescribed species was not provided with water. Froglets were observed to leave the pouch without the active participation of the mother in *G. cornuta*-like. There was a container with water in the terrarium, but the female gave birth on the ground. *Gastrotheca christiani* and *G. ovifera* give birth also on the ground and the froglets emerge from the pouch unaided by the mother (Barrio, '76; Duellman and Maness, '80). Introduction of the female's hind limbs into the pouch for the removal of embryos occurs in species of *Gastrotheca* that give birth to tadpoles and in those species of *Gastrotheca* with direct development that need water for the events of birth. Frogs that give birth on the ground seem to remain more passive during birth and do not use the hind limbs for the removal of froglets. Duellman and Maness ('80) noted that the pouch aperture is small in species in which the mother aids actively in the emergence of young and that the pouch aperture is long in species in which the female does not use the hind limbs during birth. Birth in frogs without pouches is unknown.

DISCUSSION

Twenty-seven of the 56 described species of egg-brooding hylids occur in Colombia and Ecuador (Duellman, '77, '79), and most live at elevations ranging from 300 to 2,000 m in the rain and cloud tropical forests. Information about the life history and ecology of these frogs is scarce, probably because most of them have been collected rarely. Equally little known are the aseasonal tropical environments in which these frogs live. Duellman ('78) studied the herpetofauna at Santa Cecilia and nearby localities in Amazonian Ecuador (340 m); he found that there are 81 species of anurans living sympatrically at Santa Cecilia. The interspecific competition for reproduction in the available water has favored the diversification of anuran reproductive modes in tropical environments (Duellman, '79). The anuran species at Santa Cecilia present 11 modes of reproduction; some frogs have adapted to the use of "unconventional" bodies of water such as the water collected in bromeliads, or the water-filled tree cavities for reproduction, instead of ponds and streams. Others, like some species of *Eleutherodactylus,* have evolved terrestrial reproduction and direct development in terrestrial nests (Lynn, '42). For a review of the adaptations to the terrestrial environment in anurans, see Lamotte and Lescure ('77).

Egg-brooding to the froglet stage presents the advantage of avoiding the free-living larval stages, where predation of eggs and young should be most heavy. In addition, parental care should insure greater reproductive success and the increment in size of the froglet at birth (up to 20 mm snout-vent length) increases the chance of survival. Therefore, the selective pressure in egg-brooding hylids with direct development has been toward the production of fewer, larger, and advanced froglets. This was accomplished by decreasing the number of eggs, increasing the egg size, and by modi-

fications in development (Table 2). The presence of multinucleate oogenesis in several of these species (del Pino and Humphries, '78) can be seen as an ovarian adaptation to produce fewer eggs endowed with nutrients and other factors necessary to carry development all the way to the froglet stage.

The relation of the number of eggs produced (clutch size, CS), and egg diameter (OD), relative to the adult snout-vent length of the female (SVL), is called the ovarian size factor: CS(OD)/SVL (Duellman and Crump, '74), and it gives an index of the fecundity of the species. Among anurans living at Santa Cecilia, Duellman ('78) found that the ovarian size factor was highest and more variable in species that deposit eggs in water and it was lowest (0.5–4.4) in species having terrestrial eggs undergoing direct development. For most species of egg-brooding hylids, the ovarian size factor (Table 2) is within the range given by Duellman ('78) for frogs with terrestrial eggs. Only some tadpole-producing species of *Gastrotheca* show larger values (3.9–8.2). The lowest values among egg-brooding hylids correspond to *Flectonotus* and *Fritziana,* frogs that give birth to advanced tadpoles and have pouch type one (del Pino, '80a). The highest values are those of the tadpole-producing species of *Gastrotheca* (Table 2).

Flectonotus and *Fritziana* live in cloud forests, while all species of *Gastrotheca* that give birth to tadpoles live at higher altitudes (from 1,500 to 4,600 m) (Duellman and Fritts, '72; Duellman, '74). In the highlands there are fewer anuran species than in the tropical forests (Duellman, '79). Our qualitative observations indicate that in the highlands of northern Ecuador, only the tadpoles of *G. riobambae* exploit both permanent and temporary bodies of standing water. Tadpoles of *Flectonotus pygmaeus,* in contrast, are born and live in the water collected in axils of bromeliads (S.J. Maness, personal communication).

The two groups of tadpole-producing species not only inhabit different environments, but have evolved different reproductive adaptations. All species of *Gastrotheca* that give birth to tadpoles have the regular mononucleate oogenesis, while multinucleate oogenesis is present in some species of *Flectonotus* (del Pino and Humphries, '78). Maximal egg size is comparable in both groups (approximately 3 mm in diameter), but the female size and clutch size are different. Species of *Gastrotheca* are bigger frogs, produce numerous eggs, and the ovarian size factor is larger (Table 2). The length of the incubatory period and of the free-living tadpole stages are also considerably larger in the tadpole-producing species of *Gastrotheca* (Tables 1, 3). *Flectonotus* and *Fritziana* develop small bell gills that cover less than 25% of the embryonic surface (Table 2), while in *Gastrotheca* the bell gills envelop the entire embryo. In addition, the bell gills of *Gastrotheca* develop from the fusion of branchial arches I and II, while in species of *Flectonotus* and *Fritziana* the bell gills develop either from the first or from the first and second branchial arches without fusion (Table 2).

The newly born tadpoles of *Gastrotheca* have small limb buds (stage 33 of Gosner, '60), are good swimmers, and present a left spiracle, while those of *Flectonotus* and *Fritziana* have advanced limb buds at birth (Gosner, '60, stage 39), do not swim or feed, and have a midline spiracle. Pouch morphology differs also among these frogs (Table 2). Species of *Gastrotheca* that give birth to tadpoles have the most complex pouch found among egg-brooding hylids (del Pino, '80a), while *Flectonotus* and *Fritziana* have a simple pouch (type one, del Pino, '80a). This pouch resembles the hypothetical lateral folds of the dorsal skin considered to be the precursors of the marsupial pouch (del Pino, '80a; Trueb, '74). The developmental differences found among *Flectonotus* and the tadpole-producing species of *Gastrotheca* as well as the differences in pouch morphology (del Pino, '80a) suggest that *Flectonotus* and *Gastrotheca* may have evolved independently, which is in agreement with the results of immunological comparisons of albumin (Scalan et al., '80).

In egg-brooding hylids without pouch, the ovarian size factor has values comparable to those of *Fritziana goeldii* (Table 2). The selective pressure in these species has been toward the production of fewer and advanced young, and toward the elimination of the aquatic larval period. Species of *Gastrotheca* with direct development that live in tropical forests have low values for the ovarian size factor (frogs with pouch type two, Table 2), while other species of *Gastrotheca* that have direct development present values that are close to the range of species of *Gastrotheca* with tadpoles (frogs with direct development and pouch types three to six, Table 2). These species live at higher altitudes (Duellman, '74, '79; Duellman and Fritts, '72). Species of *Gastrotheca* with pouch type two are large frogs

that incubate a low number of large eggs (Table 2). These species have the multinucleate type of oogenesis (del Pino, '80a), while species with more complex pouches are smaller frogs that produce smaller and more numerous eggs (Table 2) and present the regular mononucleate type of oogenesis (del Pino, '80a).

In species of *Gastrotheca* with direct development (Table 3), no distinction was found in the length of incubation between a species with pouch type two that lives in the cloud forest (*G. cornuta*-like), and two species with pouch type five that live in the sub-paramo (*G. plumbea* and *G. orophylax*). No differences were found either in the development and maximal size of bell gills (Table 2). However, there were differences in embryonic morphology; *G. cornuta*-like develops a small tail during incubation, and the embryo becomes a little pigmented. The embryos of *G. plumbea* and of *G. orophylax,* in contrast, appear as pigmented tadpoles with well-developed tails, tail fins, and mouth parts during incubation. The midline spiracle is partially developed as in other species with direct development.

Some embryos from the pouch of *G. plumbea* and *G. orophylax* were cultured in water (limbs as in stage 35 of Gosner, '60) to the completion of metamorphosis (about 60 days). Most embryos died after a few days of culture because the bell gills were not resorbed. Molds grew in the dead tissues of the gills and formed a plug that occluded the midline aperture of the opercular cavity. Some embryos became swollen and died. In others, the dead bell gills were removed with forceps; these embryos developed to the completion of metamorphosis. These tadpoles did not swim or feed, and some yolk was eliminated from the cloaca. As advanced embryos from the pouch can be cultured in water, and as these two species have a water requirement for birth, it is likely that these species can give birth either to froglets or to advanced tadpoles. A developmental situation such as this is suggestive of how the free-living tadpole condition could have been recovered by some species of *Gastrotheca*. Resorbtion of the bell gills into the peribranchial chamber (instead of sloughing), and the formation of a left spiracle, should have allowed the tadpole-producing species to use the resources of ponds and of temporary pools, which were unexploited by other anurans. Duellman and Fritts ('72) noted that in the Andes of Peru, when two species of *Gastrotheca* occur sympatrically, one of them produces tadpoles, while the other has direct development; therefore, there is no competition for the same aquatic resources.

Length of incubation for species of *Gastrotheca* with direct development that live in the highlands is unknown (Duellman and Fritts, '72). It would be interesting to compare the length of incubation in these species with that of a sympatric tadpole-producing species. Species with tadpoles probably have incubatory periods equivalent to those of species with direct development (Tables 1, 3), but development has been retarded as birth occurs at a less advanced stage.

The inter-Andean valleys of Ecuador have low iodine concentration (manifested in the high incidence of endemic goiter in human populations), but iodine is a factor needed for amphibian metamorphosis. It is likely, therefore, that the combination of low iodine in the environment and the presence of unused water were conditions that favored the return to the free-living larval stages. In the highlands, the limited amount and variety of prey items for adults was probably another factor that favored the utilization of water resources.

Among species of *Gastrotheca* with direct development there are, at least, two basic evolutionary trends. One is present in the inhabitants of the tropical forests and it has favored the production of few and advanced young (frogs with pouch type two, Table 2). The other is present in species that are distributed at higher altitudes; these frogs are smaller, present more complex pouches, and produce numerous froglets of smaller size (frogs with direct development and pouch types three to five, Table 2). Based on the values of the ovarian size factor, the characteristics of the pouch, oogenesis, development, and distribution, it is likely that some species of *Gastrotheca* with direct development, originally found in the tropical forests, became adapted to higher altitudes, which is in agreement with current ideas about the origin of the herpetofauna of the northern Andes (Duellman, '79). It is also likely that species of *Gastrotheca* that give birth to tadpoles were derived from an ancestor with direct development. Immunological comparisons of albumin in marsupial frogs (Scalan et al., '80) indicate that large arboreal species from the cloud forests are uniformly distant from small and terrestrial Andean species. The Andean marsupial frogs, in contrast, form clusters of related species that include both froglet and tadpole producers. These findings are in agree-

ment with the evolutionary implications of this analysis of reproduction and development.

Evolution of marsupial frogs gives excellent examples of the role of acceleration and retardation in development (as explained by Gould, '77). The comparison of development favors the trend proposed by del Pino ('80a): egg-brooding hylids evolved from an unknown ancestor with aquatic reproduction. Evolution has favored the formation of the pouch, and through acceleration, incubation to the froglet stage. The most complex species have recovered again the free-living tadpole stages through a process of retardation in development.

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LITERATURE CITED

Barrio, A. (1976) Observaciones sobre la reproducción de *Gastrotheca christiani* Laurent (Anura, Hylidae). Physis *36:*337–344.

del Pino, E.M. (1980a) Morphology of the pouch and incubatory integument in marsupial frogs (Hylidae). Copeia *1980:*10–17.

del Pino, E.M. (1980b) El mantenimiento y aspectos del comportamiento en cautiverio del sapo marsupial *Gastrotheca riobambae* (Hylidae). Revista de la Universidad Católica, Quito *8*(27):41–49.

del Pino, E.M., M.L. Galarza, C.M. de Albuja, and A.A. Humphries, Jr. (1975) The maternal pouch and development in the marsupial frog, *Gastrotheca riobambae* (Fowler). Biol. Bull. *149:*480–491.

del Pino, E.M., and A.A. Humphries, Jr. (1978) Multiple nuclei during early oogenesis in *Flectonotus pygmaeus* and other marsupial frogs. Biol. Bull. *154:*198–212.

del Pino, E.M., and G. Sánchez (1977) Ovarian structure of the marsupial frog *Gastrotheca riobambae* (Fowler) Anura, Hylidae, J. Morphol. *153:*153–162.

Duellman, W.E. (1974) A systematic review of the marsupial frogs (Hylidae: *Gastrotheca*) of the Andes of Ecuador. Occas. Papers. Mus. Nat. Hist. Univ. Kansas *22:*1–27.

Duellman, W.E. (1977) Liste der rezenten Amphibien und Reptilien. Hylidae, Centrolenidae, Pseudidae. In R. Mertens, W. Hennig, and H. Wermuth (eds): Das Tierreich. Lieferung 95. Berlin: Walter de Gruyter.

Duellman, W.E. (1978) The Biology of an Equatorial Herpetofauna in Amazonian Ecuador. Univ. Kansas Misc. Publ. No. 65. 352 p.

Duellman, W.E. (1979) The herpetofauna of the Andes: Patterns of distribution, origin, differentiation, and present communities. In W.E. Duellman (ed): The South American Herpetofauna: Its origin, evolution, and dispersal. Monograph Mus. Nat. Hist. Univ. Kansas, Vol. 7, pp. 371–459.

Duellman, W.E. (1980) A new species of marsupial frog (Hylidae: *Gastrotheca*) from Venezuela. Univ. Michigan Mus. Zool. Occas. Papers, *690:*1–7.

Duellman, W.E., and M.L. Crump (1974) Speciation in frogs of the *Hyla parviceps* group in the upper Amazon basin. Occas. Papers Mus. Nat. Hist. Univ. Kansas *23:*1–40.

Duellman, W.E., and T.H. Fritts (1972) A taxonomic review of the southern Andean marsupial frogs (Hylidae: *Gastrotheca*). Occas. Papers Mus. Nat. Hist. Univ. Kansas *9:*1–37.

Duellman, W.E., and S.J. Maness (1980) The reproductive behavior of some hylid marsupial frogs. J. Herpetol. *14:*213–222.

Duellman, W.E., and R.A. Pyles (1980) A new marsupial frog (Hylidae: *Gastrotheca*) from the Andes of Ecuador. Occas. Papers Mus. Nat. Hist. Univ. Kansas *84:*1–13.

Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica *16:*183–190.

Gould, S.J. (1977) Ontogeny and Phylogeny. Cambridge: Harvard University Press.

Gurdon, J.B. (1968) Changes in somatic cell nuclei inserted into growing and maturing amphibian oocytes. J. Embryol. Exp. Morphol. *20:*401–414.

Hoogmoed, M.S. (1967) Mating and early development of *Gastrotheca marsupiata* (Duméril and Bibron) in captivity (Hylidae, Anura, Amphibia). Br. J. Herpetol. *4:*1–7.

Kenny, J.G. (1969) The Amphibia of Trinidad. Study of the Fauna of Curaçao and Caribbean Islands. No. 29. 78 p.

Lamotte, M., and J. Lescure (1977) Tendances adaptatives à l'affranchissement du milieu aquatique chez les amphibiens anoures. La Terre et la Vie, Paris *31:*225–312.

Lynn, W.G. (1942) The embryology of *Eleutherodactylus nubicola,* an anuran which has no tadpole stage. Contrib. Embryol. Carnegie Inst. Washington *30:*27–62.

Nieuwkoop, P.D., and J. Faber (1967) Normal table of *Xenopus laevis* (Daudin). 2nd ed. Amsterdam: North Holland Publishing Co.

Noble, G.K. (1931) The Biology of the Amphibia. New York: McGraw-Hill Book Co.

Rugh, R. (1951) The Frog. Its Reproduction and Development. New York: McGraw-Hill Book Co.

Ruiz-Carranza, P.M. and J.I. Hernandez-Camacho (1976) *Gastrotheca andaquiensis,* nueva especie de la cordillera oriental de Columbia (Amphibia, Anura). Caldasia *11:*149–160.

Scalan, B.E., L.R. Maxon, and W.E. Duellman (1980) Albumin evolution in marsupial frogs (Hylidae: *Gastrotheca*). Evolution *34:*222–229.

Shumway, W. (1940) Stages in the normal development of *Rana pipiens.* I. External form. Anat. Rec. *78:*139–147.

Trueb, L. (1974) Systematic relationships of neotropical horned frogs, genus *Hemiphractus* (Anura, Hylidae). Occas. Papers Mus. Nat. Hist. Univ. Kansas *29:*1–60.

Trueb, L., and W.E. Duellman (1978) An extraordinary new casque-headed marsupial frog (Hylidae: *Gastrotheca*). Copeia *1978:*498–503.