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Early development of two species of *Phyllomedusa* (Anura: Phyllomedusinae)

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Abstract. We present a comparative study of the early development of *Phyllomedusa azurea* and *P. sauvagii*, in order to discuss morphological and ontogenetic variations of ecological and systematic interest. Similarities between the species include the large, yolk-rich eggs, the curled embryos at tail bud stage, the large external gills, and the sequential development of mouthparts. The main difference lies in the morphology and development of adhesive glands. Most of these characters can be related to leaf nest spawning and a long intracapsular period. The plasticity in the time of hatching (as observed in the related genus *Agalychnis*), and its relation to embryonic and larval morphology and ecology remain open questions in this genus.

Key words. Amphibia, Embryonic development, *Phyllomedusa azurea, Phyllomedusa sauvagii.*

Introduction

Historically, the early ontogeny of anurans has been approached from the perspectives of experimental embryology, biochemistry, and molecular biology. This implied that the vast majority of information concerned model organisms such as *Xenopus laevis*, and species of *Bufo* and *Rana* (e.g., Weisz 1945, Taylor & Kollros 1946, Nieuwkoop & Faber 1956, Limbaugh & Volpe 1957, Dickinson & Sive 2006). Recently, we are experiencing a renewed interest in comparative embryological studies, which emphasizes spatio-temporal variation during early developmental events, in several character systems and at different taxonomic levels (Chipman 2002, Chipman et al. 2000, Mitgutsch et al. 2009). Early ontogeny in anurans involves morphological characters that continue their development during larval stages (e.g., oral disc, lateral line system) and transient structures that appear at embryonic stages and disappear after eclosion (e.g., cement glands, external gills, body ciliation). Comparative studies on several of these characters are revealing a surprising extent of variation (Nokhbatolfoghahai & Downie 2005, 2007, 2008, Nokhbatolfoghahai et al. 2005, 2006).

The hylid subfamily Phyllomedusinae is distinctive because of several aspects regarding its reproductive biology. All species lay terrestrial clutches, and remarkable variation in behaviour prior to oviposition, oviposition site, clutch structure, larval development and morphology has been described (Faivovich et al. 2010). The most species-rich genus of this clade, *Phyllomedusa*, occurs from Panama to northern Argentina and Uruguay (Frost 2011). *Phyllomedusa* includes 30 species, most of which are assigned to four species groups, which in turn comprise two main clades. Oviposition occurs in leaves that the parents fold up during mating, and a clutch includes fertilized eggs and eggless jelly capsules. Early development takes place within the nest, and after hatching, larvae fall into the water where they continue their development until metamorphosis. Early ontogeny is known only from three species in this genus: *P. azurea* (Budgett 1899), *P. rohdei* (Lutz & Lutz 1939), and *P. trinitatis* (Kenny 1968).

In this paper, we present a comparative study of the early development of two species of *Phyllomedusa*, exemplary of the two main clades of the genus: *P. azurea* (*P. hypochondrialis* group) and *P. sauvagii* (sister species of the *P. burmeisteri* group) in order to discuss morphological and ontogenetic variations of ecological and systematic interest.

Materials and methods

Two clutches of *Phyllomedusa azurea* and *P. sauvagii* were collected in the field at Wichi (24°41'30.2" S, 61°25'48" W; Chaco, Argentina, March 2009; DB 8528) and Horco Molle (26°47'35" S, 65°19'00.2" W; Tucumán, Argentina, December 2010; FML 24189), respectively. The leaf nests were taken to the lab, and attached hanging on the walls of glass containers filled with tap water; the clutches were kept at ambient room temperature and exposed to a natural light cycle. An average of 6 eggs were carefully removed from

each nest every 8–12 hours, then euthanized with MS222 anaesthetic and preserved in 10% formalin. Embryos were staged according to the stages of early development described for *P. trinitatis* (Kenny 1968), and the generalized Gosner (1960) table for stages after spiracle development. The external morphology of the specimens was studied

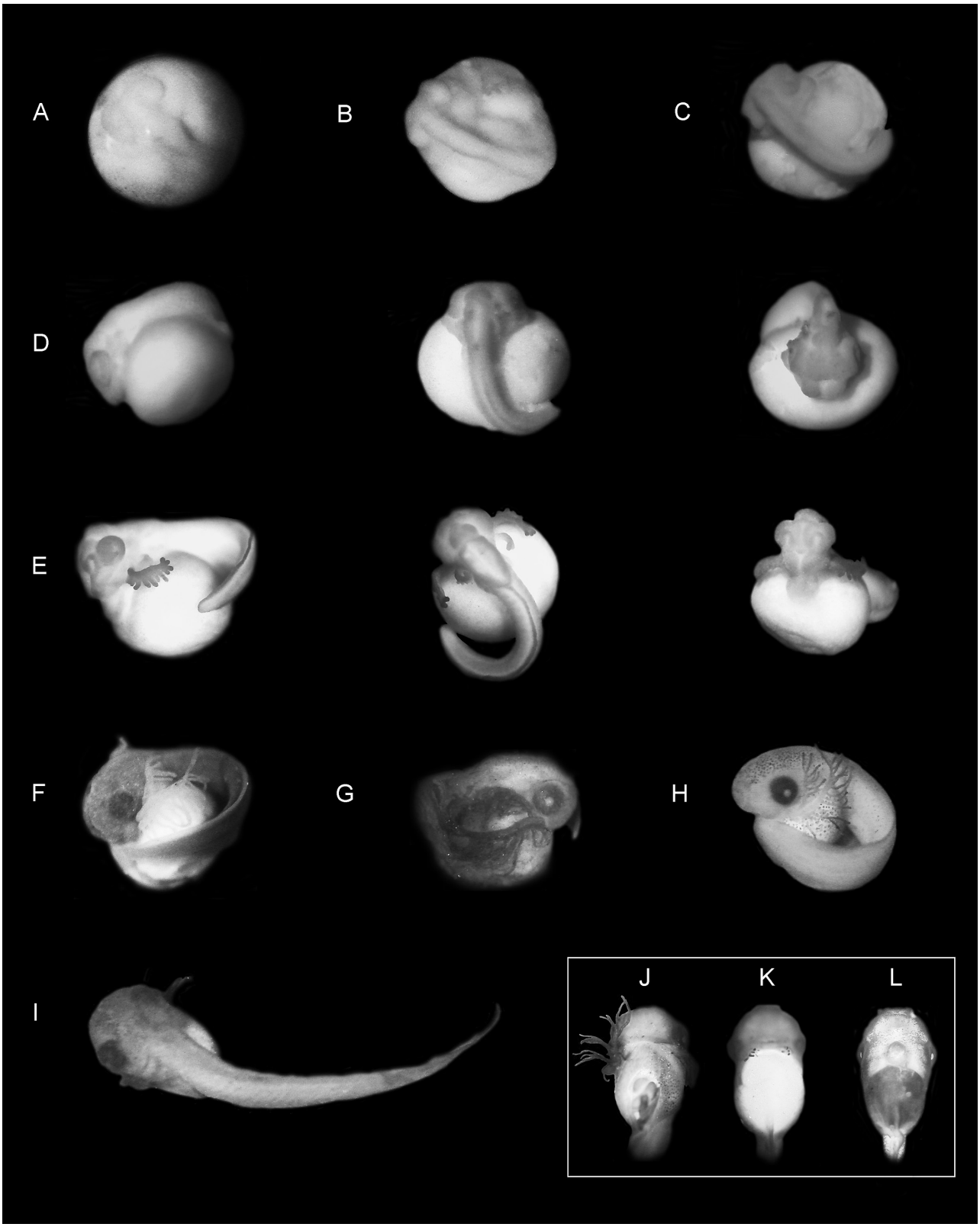

Figure 1. Developmental series of *Phyllomedusa sauvagii:* A. Stage 15; B. Stage 16; C. Stage 17; D. Stage 18; E. Stage 19; F. Stage 21; G. Stage 22; H. Stage 23; I. Stage 24; J–K. Stage 24; L. Stage 26.

with a stereomicroscope, staining was done with methylene blue for improved contrast, and scanning electron microscopy was used. In the descriptions, "total length" refers to the maximum length of the embryo. Terminology for oral morphology follows Altig (2007) and Van Dijk (1966).

Results

The early ontogeny in both species is very similar to that of *Phyllomedusa trinitatis* (Kenny 1968); the development of some characters is decoupled, for which reason traits employed to define stages are indicated with an asterisk.

Phyllomedusa sauvagii

The complete embryonic development lasted 7 days, which was when hatching of the last embryo occurred. Serially preserved specimens were assigned to 12 stages, starting with stage 12.

Stage 12. Diameter = 2.3 mm. Late gastrula: the yolk plug is still evident.

Stage 15 (Fig. 1A). Diameter = 2.4 mm. Egg heavily yolked and embryo outlined in the yolk mass. Neural folds thick anteriorly, and fused from optic vesicles beyond the embryo midlength*. Stomodeal invagination shallow.

Stage 16 (Fig. 1B). Diameter = 2.4 mm. Embryo has risen above the yolk mass. Neural folds fused excepting a short

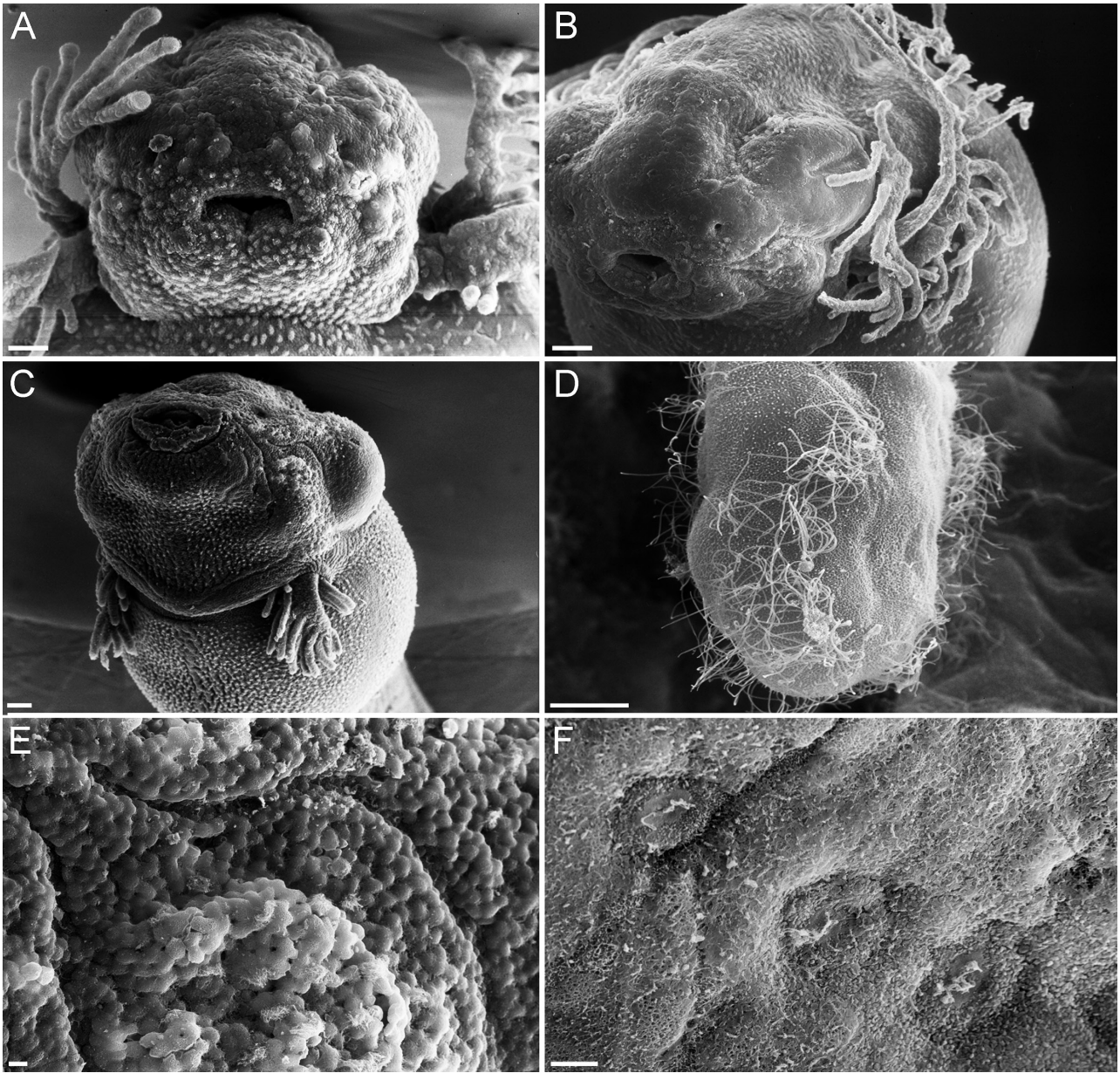

Figure 2. SEM micrographs of *Phyllomedusa sauvagii* embryos. A. Stage 21, frontal view, showing the stomodeum and the adhesive glands; B. Stage 22, anterolateral view, showing the developing oral disc, the adhesive glands and the primordial sensory ridges; C. Stage >24, ventrolateral view, showing the opercular fold, the reduced gills, and the sensory ridges; D. Detail of a gill secondary filament, showing the profuse ciliation; E. Detail of the sensory ridges, showing the absence of neuromasts; F. Stage 26, detail of the same lateral line, showing developed neuromasts. Scale bars in A–C = 100 μm; in D–E = 10 μm.

posterior gap*. Optic vesicles well-defined. Hyoid arches evident as pronounced ridges beneath the anterior region of the neural folds. Stomodeal invagination shallow, and proctodeal invagination with rudimentary rectal spout visible.

Stage 17 (Fig. 1C). Length =3.3 mm. Embryo elevated and twisted above the round yolk mass. Stomodeal invagination deeper. Visceral arches more defined. Optic vesicles well-defined with central indentation. Tail bud visible*.

Stage 18 (Fig. 1D). Length = 3.6 mm. Pronephros evident as two long ridges on the sides of the body. First pair of gills evident as two short bars behind the optic cups*. Tail longer (tail length / total length = 0.75) with rudimentary tail fin.

Stage 19 (Fig. 1E). Length = 4.7 mm. Olfactory pits connected with the stomodeum through a deep groove*. Stomodeum transversely elongate. Adhesive (cement) glands first visible as two rounded bumps ventral to the stomodeum. Two pairs of gills present: the first pair with long primary filament (gill major axis length / total length = 0.21) and short, bump-like secondary filaments: the second pair short, with very short secondary filaments*. Heart evident as a protuberance beneath the head. Rectal spout longer and conical. Tail longer (tail length / total length = 0.70) with well-marked myotomes and ventral fin about twice as deep as the dorsal fin; tail tip rounded.

Stage 21 (Fig. 1F). Length = 5.3 mm. Head with scattered melanophores. Olfactory pits not connected with the sto-

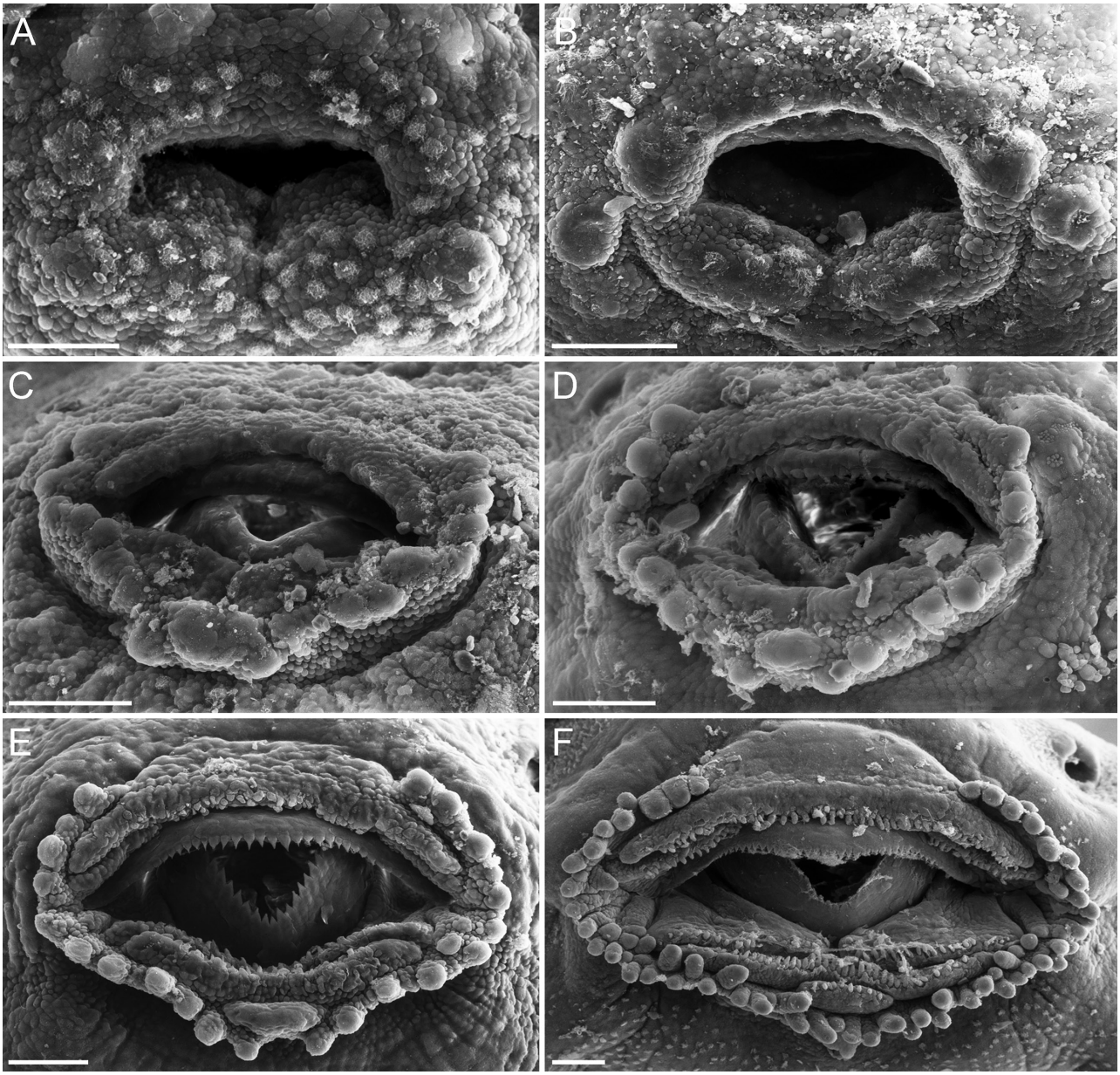

Figure 3. Oral disc development in *Phyllomedusa sauvagii* embryos, in frontal view. A. Stage 21, upper and lower labia; B. Stage 22, marginal papillae and lower tooth ridges developing; C. Stage 24, row P3 developing; D. Stage 24, row A2 developing; E. Stage 25, showing keratodonts developing on rows A1, P1 and P2; F. Stage 25, showing lower marginal papillae complete and keratodonts on all rows. Scale bar = 100 μm.

modeum. In the oral region, the upper and lower labia are outlined; the upper labium is narrow and arc-shaped, and the lower labium has a deep medial groove (Fig. 3A). Adhesive (cement) glands first visible as two separated small rounded structures posterior and lateral to the stomodeum (Fig. 2A, 2B and 3A). Eyes with dorsal half pigmented and choroid fissure; later, eyes with black pigment. Opercular fold first evident at the base of each gill. First and second pairs of gills longer (first gill major axis length / total length = 0.28), with very long and thin secondary filaments*; gill filaments covered with dense rows of very long cilia. Tail long (tail length / total length = 0.72), with translucent fins and pointed tip. Sensory ridges (primordia of lateral lines) first evident around the oral region (Fig. 2B).

Stage 22 (Fig. 1G). Length = 5.9 mm. Embryo still twisted around the yolk. Rostral region short and rounded in lateral view. Head and dorsal surface of the yolk mass with scattered melanophores. Pineal organ visible as an unpigmented spot anterior to the level of the eyes. Upper and lower jaw sheaths present in the oral disc; upper labium prominent, and lower labium thick with a medial groove (corresponding to the gap in row P1) and a transversal ridge outlined, divided into two halves (corresponding to row P2) (Fig. 3B). Adhesive glands rounded, at their maximum development (Fig. 3B). Eyes with black pigment and with choroid fissure. Opercular folds not medially fused*. Gills long (first gill major axis length / total length = 0.25). Lungs visible by transparency, behind the eyes. Tail long (tail length / total length = 0.73), curled around the embryo, with higher fins.

Stage 23 (Fig. 1H). Length = 8.7 mm. Embryo still twisted around the yolk. Rostral region longer, with protruding oral disc in lateral view. Melanophores scattered on the whole body, excepting the ventral surface of the yolk mass. In the oral disc, labia more prominent, elevated above the oral surface; medial groove in the lower labium deeper and row P2 well defined; later in this stage, commissural regions prominent, and large marginal papillae outlined. Adhesive glands regressing. Choroid fissure evident. Opercular fold medially fused*. Gills long (first gill major axis length / total length = 0.25). Tail long (tail length / total length = 0.70), with high fins.

Stage 24 (Fig. 1J–K). Length = 6.7 mm. Hatched embryos. Yolk reduced. Oral disc with serrated jaw sheaths and two marginal papillae at each end of the upper and lower labia; rows A1, P1 and P2 defined; later, more papillae appear in the upper and lower labia and a short row P3 differentiates in the medial region (Fig. 3C); row A2 appears as two short segments below the ends of row A1 (Fig. 3D). Choroid fissure still noticeable. Opercular fold covering the base of each gill; posterior margin of the opercular fold free and thickened (Fig. 1K–L and 2C). Gills reducing until being completely covered by the opercular fold*; gill ciliation still profuse (Fig. 2C and 2D). Tail long (tail length / total length = 0.70) not curled around the embryo, with high fins, Sensory ridges more defined: angular, oral, supraorbital, infraorbital, and presumably longitudinal oral (sensu Lannoo 1999) are identifiable (Fig. 2C and 2E).

Stage 25. Length = 10.2 mm. Oral disc with marginal papillae of the lower labium growing medially with a small ventral gap remaining, and keratodonts in rows A1, P1 and P2 (Fig. 3E and 4A); later in this stage, marginal papillae of the lower labium complete and keratodonts emerge on rows P3 and A2 (Fig. 3F, 4B and 4C). Medial spiracle formed*. Heart visible through the skin, anterior to the spiracle opening. Tail long (tail length / total length = 0.70).

Stage 26 (Fig. 1M). Length = 23.6 mm. Keratodonts more numerous, with definitive morphology: long, narrow, straight keratodont with straight head with very short cusps. Spiracular tube longer. Tail long (tail length / total length = 0.67). Neuromasts developed in lateral lines (Fig. 2F).

Phyllomedusa azurea

Serially preserved specimens were assigned to 7 stages. We lacked stages earlier than stage 19.

Stage 19 (Fig. 5A). Length = 4.4 mm. Olfactory pits connected with the stomodeum through a deep groove*. Stomodeum diamond-shaped. Adhesive glands visible as two separated, large, oblong structures posterior and lateral to the stomodeum. Two pairs of gills present: the first pair with long primary filament and short, bump-like secondary filaments: the second pair very short, rod-like, without secondary filaments*. Heart evident as a protuberance beneath the head. Rectal spout longer and conical. Tail as long as the body (tail length / total length = 0.5) with well-marked myotomes and ventral fin deeper than the dorsal; tail tip rounded.

Stage 21 (Fig. 5B and 5C). Length = 5.5 mm. One specimen not twisted around the yolk mass. Embryo with voluminous, highly vascularized yolk mass. Without melanophores. Olfactory pits not connected to the stomodeum.

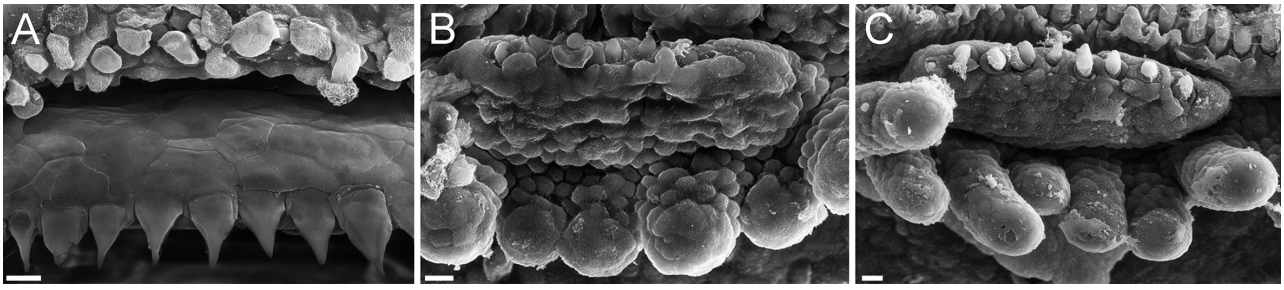

Figure 4. Details of the developing oral disc in a *Phyllomedusa sauvagii* embryo at Stage 25, frontal view. A. Upper jaw sheath serrations and emerging keratodonts of A1; B. Row P3 showing the first emerging teeth; C. Row P3 in a later embryo, showing more developed keratodonts. Scale bar = 10 μm.

The oral disc begins to form: the upper and lower labia are outlined and strongly ciliated; the upper labium is narrow and arc-shaped, and the lower labium has a deep medial groove; upper and lower jaw sheaths begin to differentiate. Eyes with diffuse coloration, concentrated on the dorsal half. Opercular fold first visible late during this stage. First and second pairs of gills longer (first gill major axis length / total length = 0.27), with very long and thin secondary fila-

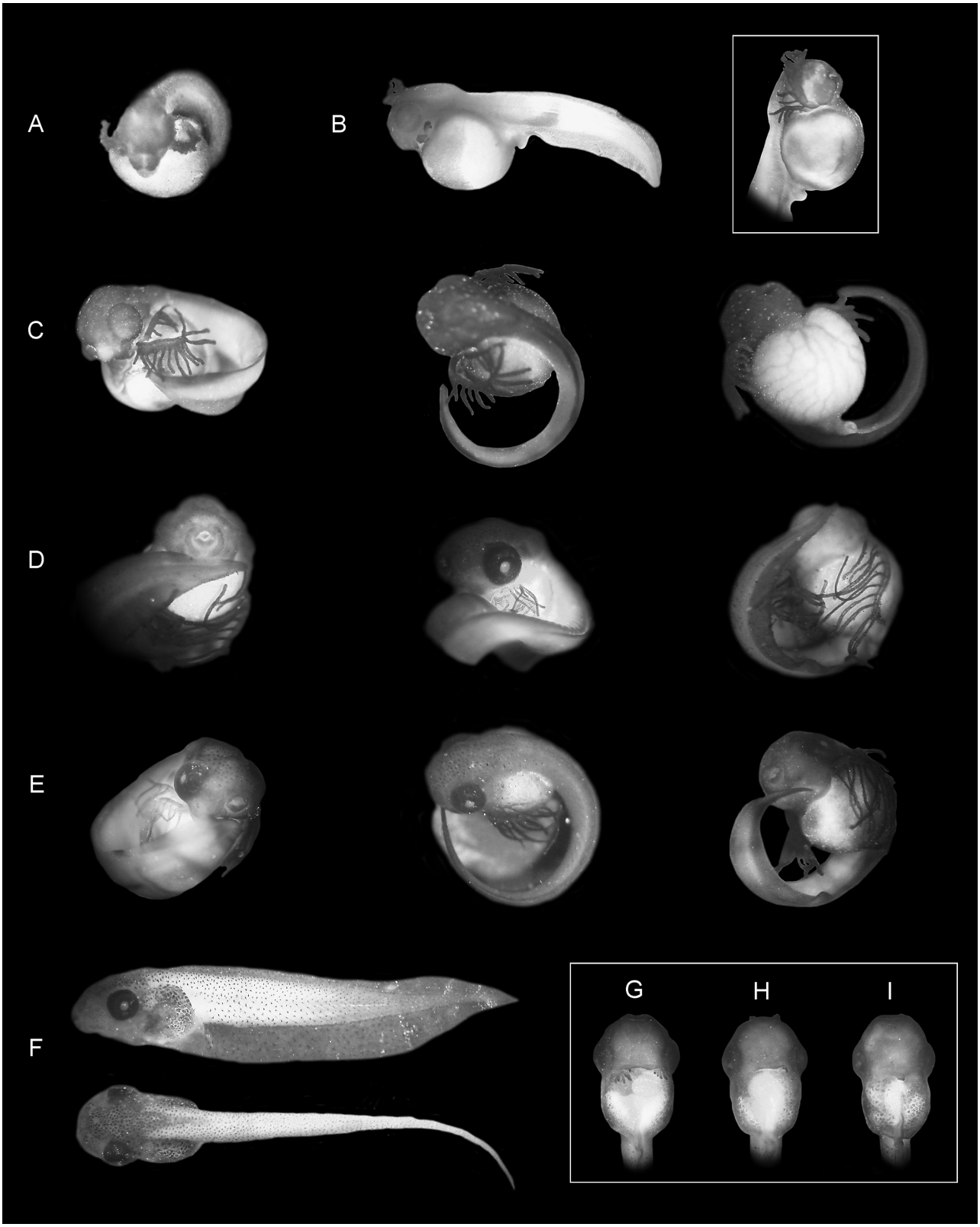

Figure 5. Developmental series of *Phyllomedusa azurea*. A. Stage 19; B. Stage 20, showing unequally developed gills in this specimen; C. Stage 21; D. Stage 22; E. Stage 23, hatched embryo; F–G. Stages 24–25, showing spiracle development.

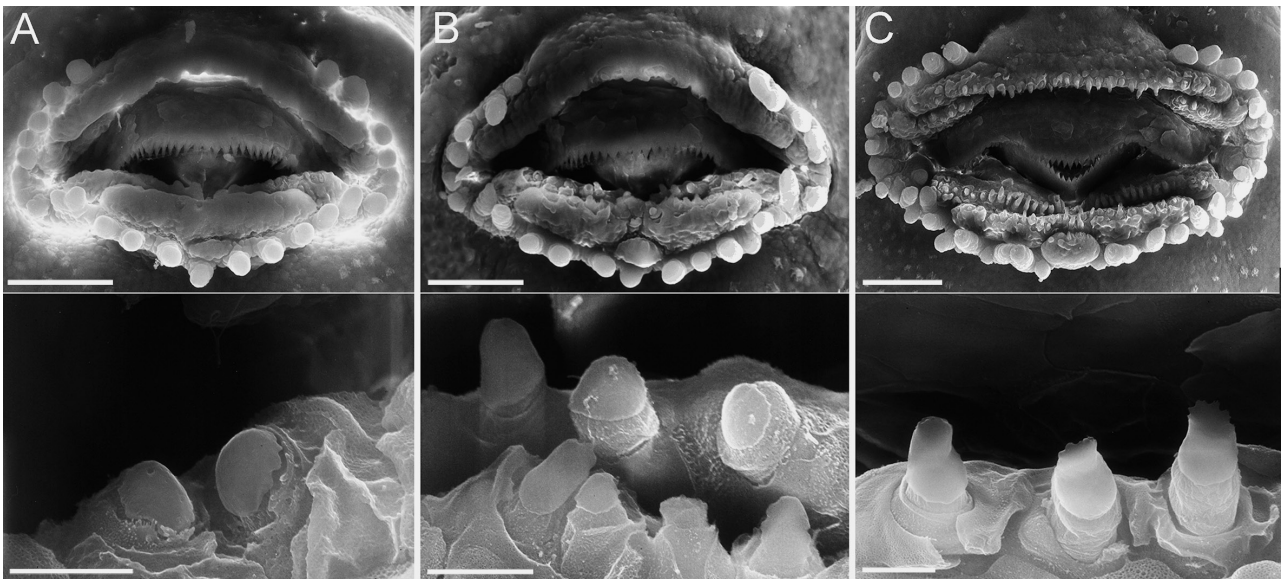

Figure 6. Oral disc development in *Phyllomedusa sauvagii* embryos after hatching; top, oral disc in frontal view; bottom, detail of keratodonts. A. Stage 24, keratodonts on row P1; B. Stage 24, keratodonts on rows P1 and P2; C. Stage 27, keratodonts on row P3. Scale bars = 100 μm (top) and 10 μm (down).

ments*; one specimen with unequal development of gills: the left gill very short, and the right one with a very large and ramified first gill and a shorter second gill. Tail long (tail length / total length = 0.72) with translucent fins, the ventral fin about twice as deep as the dorsal fin; pointed tail tip.

Stage 22 (Fig. 5D). Length = 7.6 mm. Head with scattered melanophores. Upper and lower jaw sheaths without serration; upper labium prominent, with two marginal papillae developing at each end, and lower labium thick with a shallow medial groove, corresponding to the gap in row P1. Adhesive glands at their maximum development, almost contiguous ventrally, forming a horseshoe-like structure around the oral disc. Eyes solid black and choroid fissure evident. Opercular folds not medially fused*. Gills longer (first gill major axis length / total length = 0.30). Tail long (tail length / total length = 0.72), curled around the embryo, concealing the oral disc.

Stage 23 (Fig. 5E). Length = 8.2 mm. Embryo curled about the yolk mass; some embryos hatched. Head and dorsal surface of the yolk mass with scattered melanophores, and pineal organ visible as an unpigmented spot anterior to the eyes. Lower labium of the oral disc without ciliation; marginal papillae developing at each end, and a prominent transversal ridge corresponding to row P2; later, upper labium without ciliation and lower labial ridges P1 and P2 more defined. Adhesive glands starting to regress. Choroid fissure evident as a small notch. Opercular fold medially fused, with posterior margin somewhat thickened*. Gills long (first gill major axis length / total length = 0.20). Tail long (tail length / total length = 0.71) and curled around the embryo.

Stage 24 (Fig. 5F–H). Length = 9.9 mm. Hatched embryos. Yolk reduced. Melanophores scattered on the head, the dorsal surface of the yolk mass, and the tail. Oral disc more developed and wide: jaw sheaths with sharp serrations; upper labium with large marginal papillae at the ends and commissural regions, and row A2 present as two short segments below the ends of row A1; lower labium with marginal papillae complete and a very short row P3 appearing in the medial region (Fig. 6A); keratodonts first evident on row P1, and then P2 and A1; keratodonts emerge most frequently in a medial-to-lateral direction within each row, and keratodont morphology changes through development, with the first keratodonts to appear being simpler, very short and non-cusped (Fig. 6B). Adhesive glands reduced. Choroid fissure not evident. Opercular fold covering the base of each gill; posterior margin of the opercular fold free and thickened. Gills reducing until being completely covered by the opercular fold*. Tail long (tail length / total length = 0.74), not curled around the embryo; tail tip pointed.

Stage 25 (Fig. 5I). Length = 11.2 mm. Melanophores appear on the ventrolateral surface of the abdominal region. Adhesive glands greatly reduced but still evident. Medial spiracle formed*. Heart visible through the skin, anterior to the spiracle opening. Tail long (tail length / total length = 0.73).

Stage 27. Length = 13.9 mm. Keratodonts on rows A2 and P3, and submarginal papillae at the commissural region; keratodonts more numerous, with definitive morphology: short, wide-based, straight keratodont with short, straight head with very short cusps (Fig. 6C); later, marginal papillae become smaller and more numerous, and become arranged in an apparent double series. Tail long (tail length / total length = 0.71).

Discussion

The early ontogeny of the two *Phyllomedusa* species studied herein resembles that previously described for other phyllomedusines (Budgett 1899, Kenny 1968, Warkentin 1999, Vargas & Gutiérrez 2005). Several of the distinctive features of this type of development have been related with the leaf nest spawning, the long intracapsular period,

and plastic hatching (Kenny 1968, Warkentin 1999). The eggs in these species are relatively large (Rodrigues et al. 2007) and heavily yolked. Chipman et al. (1999) described a developmental pattern in yolk-rich embryos of *Hyperolius puncticulatus* that differs from that typical of anurans; these authors defined a pseudo-meroblastic cleavage that results in a large yolk mass that remains spherical until hatching and an embryo that curls across it, instead of showing the typical lengthening of other anurans. This difference is particularly evident at tail bud stage (Gosner stage 17), as depicted by these authors. Although we did not focus on a detailed study of gastrulation in *Phyllomedusa*, the resulting configuration of the embryos is almost identical to that described in *Hyperolius.* Large, yolk-rich eggs, and, when early development has been described, embryos twisted around the yolk mass are also seen in many other species, often in relation with oviposition in lotic environments (e.g., species of several genera of stream-breading hylids; Faivovich et al. 2006; Lang 1995), or oviposition outside the water and endotrophic development (e.g., *Myobatrachus gouldii*, *Philoria sphagnicola*, *Platymantis vitianus,* and several Terrarana; Anstis et al. 2007, De Bavay 1993, Narayan et al. 2011, Nokhbatolfoghahai et al. 2010, Townsend & Stewart 1985).

In general, pre-hatching development in the studied phyllomedusines lasts 6–9 days, after which embryos spontaneously wriggle out of their egg capsules and fall into the water (Budgett 1889, Gomez-Mestre et al. 2008, Kenny 1968, Pyburn 1980, Vargas & Gutiérrez 2005). Like the taxa studied herein, most species hatch at stages 23–24, with external gills still being well developed (Kenny 1968, Lescure et al. 1995, Pyburn 1980, Vargas & Gutiérrez 2005, Warkentin 1999). Studies in *A. callidryas* show that external gills remain large and functional in embryos that hatch late, even when other characters continue their normal development (Warkentin 1999). Gill regression begins once the tadpoles enter the water and can occur as fast as in two hours; loss of external gills in unhatched embryos can be induced by increasing the area of exposed egg surface, suggesting a relation with oxygen availability (Warkentin 2000, 2007). *Agalychnis lemur, Phasmahyla cruzi*, and *P. guttata* are described to hatch at stage 25, i.e., with a spiracle already formed (Costa & Carvalho-e-Silva 2008, Costa et al. 2010, Jungfer & Weygoldt 1994). Detailed studies on clutch structure, oxygen demand and early development of these species are needed to interpret differences in hatchling morphology.

Plasticity in the time of hatching has been described in species of the *Agalychnis callidryas* group, *A. dacnicolor*, and *Cruziohyla calcarifer* embryos, which can hatch as early as at 36% of the undisturbed modal hatching time in response to mechanical disturbance and flooding (reviewed in Warkentin 2007 and Gomez-Mestre et al. 2008). Considering the high reactivity of *Cruziohyla* to both mechanical cues and flooding, and its basal position within the subfamily, Gomez-Mestre et al. (2008) hypothesize that hatching plasticity represents a plesiomorphic character state within phyllomedusines. To conclude whether this capacity is a synapomorphy for phyllomedusines or for a more inclusive clade, will depend on whether hatching plasticity occurs in the few species groups of *Litoria* that lay eggs outside water, and the phylogenetic relationships of these species groups with the other species of *Litoria* (see below).

In *Agalychnis* and *Cruziohyla*, hatching competence is achieved after embryos reach stage 23 (Gosner 1960 and Kenny 1968, i.e., opercular fold medially fused; Gomez-Mestre et al. 2008). In *Phyllomedusa*, Kenny (1968) reported that *P. trinitatis* is able to hatch prematurely from stage 21, and embryos develop normally in the pond. Although we did not conduct any experiments to test the hatching plasticity in *P. sauvagii* and *P. azurea*, one observation is noteworthy: While preparing the developmental series of *P. sauvagii*, the leaf nest fell into the water, and half of the embryos hatched immediately (at about stage 23), and the rest of the embryos, once the nest was removed from the water, continued their development and hatched the next day. In terms of embryonic developmental period, this represents only an acceleration equivalent of up to 15% before normal hatching time, but whether induced hatching can be pushed to an even earlier point of time (as is the case in *Agalychnis* and *Cruziohyla*) remains an open question.

The role of the lateral line system in plastic hatching has not been studied, but Warkentin (1999) found no differences among the morphological configuration of pre- and post-hatching embryos of *Agalychnis callidryas*. Conversely, in *Phyllomedusa sauvagii,* the neuromasts develop at stage 26; whether this has implications on the plasticity of hatching in this species still has to be evaluated.

The mouthparts in *Phyllomedusa* (this study) and *Agalychnis* (Vargas & Gutiérrez 2005, Warkentin 1999) form sequentially, differing in some aspects from the scheme outlined for exotrophic larvae (Altig & McDiarmid 1999). In the lower labium, the first tooth ridge to develop is that corresponding to P1, and row P2 appears subsequently as a transversal prominent ridge. The development of keratodonts follows that of the tooth ridges, so that they first emerge on row P1. In *Phyllomedusa* and *A. spurrelli*, the oral disc completes its development after hatching, about stage 27. Furthermore, oral development is related to plastic hatching in embryos of *A. callidryas*, being slower within the egg than in the water (Warkentin 1999).

The extraordinary and precocious development of external gills could help to counteract the oxygen limitation caused by a confined environment and late hatching (Kenny 1968). At their maximum development, gills of *P. sauvagii* and *P. azurea* reach almost 30% of the total length, and Nokhbatolfoghahai & Downie (2008) estimated that the gill surface represents about 26% of the body surface area in *P. trinitatis*. Additionally, gill ciliation in these species is very dense, and ciliary movements plus motile gills can cope with the oxygen demand (Nokhbatolfoghahai & Downie 2008, Nokhbatolfoghahai et al. 2005, Warkentin 2000). As with the oral disc, the external gills of *A. callidryas* reduce faster in embryos that hatch prematurely than in those remaining within the nest (Warkentin 1999).

Previous reports concerning adhesive glands in phyllomedusines are inconclusive. In the first description of the development of a member of this family, Budgett (1899) reported the absence of glands in *Phyllomedusa azurea*. Kenny (1968) and Nokhbatolfoghahai & Downie

(2005) reported functional glands being absent in *P. trinitatis*. Lutz & Lutz (1939), Pyburn (1963) and Warkentin (1999) described well-developed glands in *P. rohdei* and *Agalychnis callidryas*. In the species we studied, two different morphogenetic patterns of adhesive glands are present. In *P. sauvagii*, glands are two separated small, conical structures like in the Type C pattern defined by Nokhbatolfoghahai & Downie (2005) for tadpoles of *Dendropsophus* (Hylidae). In *P. azurea*, two huge, oblong glands form a horseshoe-like structure around the oral disc, in a pattern similar to the one depicted for *Agalychnis callidryas* (Warkentin 1999). The taxonomic distribution, morphological variation, and relationship with hatching plasticity (as suggested by Faivovich et al. 2010) of adhesive glands in Phyllomedusinae will be addressed in a forthcoming paper.

Current phylogenetic hypotheses indicate that the sister taxon of Phyllomedusinae is the Australopapuan subfamily Pelodryadinae (Faivovich et al. 2005, Wiens et al. 2010). Many species groups in this latter group lay their eggs in streams (species in the *Litoria angiana, L. arfakiana, L. becki, L. dorsivena, L. eucnemis, L. leucova, L. nannotis,* and *L. napaea* groups, and also *L. aurifera, L. meiriana, rivicola, L. spartacus*, and species formerly included in the genus *Nyctimystes*, now unassigned to any species group in *Litoria*; Anstis et al. 2010, Davies 1989, Davies & Richards 1990, Günther & Richards 2005, Richards & Oliver 2006, Tyler & Davies 1978, 1979), and a few do it outside the water (the *Litoria iris* and *L. prora* groups, *L. longirostris*, and possibly *L. umarensis*; Anderson et al. 2010, Günther 2006, McDonald & Storch 1993, Menzies 1993). In both cases, eggs are usually large and yolk-rich (*L. aurifera* and *L. meiriana* are an exception in that their eggs are smaller; Anstis et al. 2010, Tyler & Davies 1978), and also, early ontogenetic stages apparently differ from those of pond species. At tail bud stage, embryos of *L. eucnemis* curl around the yolk mass (Davies 1989), in a striking resemblance to embryos of phyllomedusines. Hatching is usually late in these two environments; for instance, eggs of *L. iris*, which are laid on leaves overhanging water or around fern stems, may hatch as late as after 14 days (Tyler 1963), and Menzies (1993) depicted an unhatched, yolk-rich stage 22-embryo of *L. havina*. A phylogenetic analysis from densely sampled pelodryadines and phyllomedusines is still missing (Faivovich et al. 2010), and so it remains unknown whether these notable similarities can be considered homologous. Further studies on reproductive modes and embryonic development would be insightful for the understanding of morphological and ecological diversity in these related clades.

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