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A comparative table for staging anuran embryos from terrestrial clutches based on the Brilliant-thighed Poison Frog, *Allobates femoralis* (Anura: Dendrobatidae)

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Developmental stages in tadpoles are usually classified according to the scheme created by Gosner (Gosner, 1960). This staging table shows a generalised system that can be used to describe embryonal and larval development in anurans. This reference table, however, was initially developed for temperate zone anurans with aquatic oviposition, so the development of species that differ from this breeding mode might deviate from this description. Terrestrial oviposition has evolved independently in several anuran species (Vági et al., 2019; Furness et al., 2022), such as within the genus *Eleutherodactylus* (Townsend and Steward, 1994; Bourne, 1997), *Adenomera hylaedactyla* or *Amazophrynella minuta* (Lima and Magnusson, 2006), as well as poison frogs (Dendrobatidae *sensu* AmphibiaWeb, 2022).

In poison frogs, eggs are deposited on land, often directly in the leaf litter. After hatching, the terrestrial eggs turn into aquatic tadpoles, and therefore, in most dendrobatid species, one of the parents transports the tadpoles on its back to suitable water bodies (Ringler et al., 2013; Killius and Dugas, 2014; Frazão Luiz et al., 2015; Schulte and Mayer, 2017). It is still unknown which are the morphological changes that the newly hatched tadpoles go through before they can climb onto their parent's back to be transported.

The aim of this study was to provide a detailed description of the developmental stages of embryos and tadpoles of the Neotropical poison frog *Allobates femoralis* (Boulenger, 1884) from oviposition to hatching, with comparison to their respective Gosner stages. On one hand, this staging table will provide us with a reference table to assess the time elapsed since oviposition and the time until tadpole transport will occur for clutches under both field and lab conditions. On the other hand, it shall give insight into possible developmental

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differences between aquatic and terrestrial developing embryos, and thereby improve our knowledge of the embryonic morphological changes.

Allobates femoralis is a small diurnal leaf litter frog common throughout the Amazon basin and the Guiana Shield (Amezquita et al., 2009). During the reproductive season, males occupy territories of 64–417 m^2 (Ringler et al., 2011) from which they call to repel male competitors and attract females, while perched on elevated surfaces (Narins et al., 2003; Hödl et al., 2004). Males aggressively defend their territories against calling intruders (Narins et al., 2003; Hödl et al., 2004). Females display site fidelity and commute to male territories for courtship and mating (Fischer et al., 2020). The possession of a territory is of great importance for male reproductive success, as it is where pair formation, courtship, mating, and egg deposition occur (Montanarin et al., 2011; Ursprung et al., 2011; Stückler et al., 2019). Both sexes can mate multiple times with multiple partners (Ursprung et al., 2011). The courtship march in *A. femoralis* is one of the longest among poison frogs (Stückler et al., 2019). After the courtship march, eggs are laid in the leaf litter, where they develop for 15 to 20 days before hatching. Once hatched, tadpoles are transported by the males to medium sized natural pools located up to 200 m (on average 27.52 ± 30.90 m) away from their territory (Ringler et al., 2013, 2018). Males typically distribute the tadpoles across several water bodies (Erich et al., 2015). Occasionally, females take over tadpole transport, but only when males disappear (Ringler et al., 2015).

Herein, we provide a detailed description of the developmental changes that *A. femoralis* embryos and larvae undergo before tadpole transport. We recorded the morphological changes in tadpoles during development and related these stages to the ones proposed by Gosner (1960). Observations were conducted under controlled conditions in an *ex-situ* breeding population of *A. femoralis* at the University of Bern. We kept pairs of frogs in glass terraria (60 × 40 × 40 cm) with expanded clay pebbles on the floor. The back and side walls were covered with xaxim (tree fern stems) mats in the lower and cork in the upper half. We provided half a coconut shell, a small plant, and a branch in each tank. We provided autoclaved oak leaves as substrate for oviposition, and a small glass bowl of 10 cm diameter filled with water for tadpole deposition. We used an automatic rain, heating, and lighting system to ensure standardised climatic conditions in all terraria, similar to natural conditions in French Guiana. The temperature ranged from 21 °C at night to 28 °C during the day. Lights were on from 07:00 to 19:00h and humidity in the terraria was constantly at 100%.

To record morphological changes during the development, we checked the tanks for new clutches (Fig. 1) every day during the peak of the reproductive season and took daily pictures of the clutches from egg-laying to tadpole transport. We took daily photos of nine clutches (for a total of 120 eggs, mean = 13.33 ± 3.24 SD per clutch), using a Canon EOS 77D camera (Canon © Deutschland GMBH, Krefeld, Germany), equipped with a macro lens (Canon EF 100mm 1:2.8 USM). The clutches were placed in an open Petri dish, on white background, under an external artificial light source, to ensure that the light was homogeneously distributed and the image had a high contrast. All the clutches placed in Petri dishes were left in the parents' tank between daily inspections to prevent desiccation, as they were subjected to the automatic rain system, and to enable the father to transport

the clutch to the water bowl once the development completed. We show the pictures with reference to corresponding Gosner stages in Figure 2.

All 25 pre-feeding stages that Gosner (1960) described were also observed during *A. femoralis* embryonic development. Even if no stage is strictly related to day-to-day development, the three-week development period enabled us to successfully differentiate stages. As expected, we recorded small differences in the development of individual embryos even within the same clutch. Variability increased with time, most prominently with the start of muscular responses (stage 18; Fig. 2O). The first eight stages (fertilisation and cleavage processes) were finished within a day. First a lightening appeared on one hemisphere (Fig. 2A), then cell division began (Fig. 2B–G). Then, the gastrulation started and the blastopore became conspicuous (stages 11–12; Fig. 2H–J). Following this, neural folding usually began five days after oviposition (stages 13–15; Fig. 2K–L). The neural tube was formed quickly after, on the same or on the following day, and the embryo began developing a recognisable head (stage 16; Fig. 2M). The tail bud appeared between days five and six (stage 17; Fig. 2N), and noticeably elongated from then on, while the gill plate became less and less visible. Together with the development of the tail bud, the adhesive organ started developing (Fig. 2N). The organ is large, with soft edges, supposedly to enable the tadpoles to fix themselves on their father's back. Contrarily to most North American pelobatids, bufonids, hylids, and ranids, whose adhesive organs are initially united as a ridge before they become bifid (Gosner, 1960), in *A. femoralis* the mouth part developed as an invagination which later developed into the mouth part (stages 23–25). About a week post oviposition, we observed an initiation of spasmodic muscular responses, and the division of the gill plate into ridges (stage 18; Fig. 2O).

From this stage, the variability in developmental time increased. While a heartbeat was visible in most temperate zone anurans with aquatic oviposition at stage 19, we were not able to observe it in *A. femoralis*. The external gill filaments fully developed on stage 21, usually between 9 and 12 days post-oviposition (Fig. 2R). At this stage, the larvae transitioned to free-swimming tadpoles, the cornea became transparent and the eyes were clearly discernible. The fins became more transparent around day 12 (stage 22; Fig. 2S). After the first two weeks post-oviposition, the external gills disappeared, the oral disc and labial tooth developed and we observed a modification of the pigmentary patterns (stages 23–25; Fig. 2T). Only one clutch was successfully transported by the father. In that case, tadpole transport occurred on day 21 post-oviposition. While this is still within the range of what has been observed in the wild previously (Ringler et al., 2013), we noticed that males sat for a few days close to the Petri dish. Probably setting-up the clutch in a Petri dish disturbed the father and prevented him from reaching the tadpoles, or artificially increased the delay before transport.

Our results suggest that *A. femoralis* tadpoles develop a recognisable tadpole-like shape (i.e., with a discernible fin and head) between days 8 to 11. Most developmental modifications were similar to the ones described by Gosner (1960) in temperate zone anurans with aquatic oviposition, with exception of the development of the adhesive organ. This difference could be due to the physical requirements of holding on to the parent during tadpole transport, which is obligatory in all dendrobatid species. Future studies should

investigate the differences in the development of adhesive organs between species with and without tadpole transport. Additionally, comparative studies between species with lentic and lotic tadpoles might serve to identify functional similarities and differences in specific morphological traits (*cf*. Baldo et al., 2014; Candioti et al., 2016, 2020). Further studies are also required to understand when newly hatched tadpoles are physically able to climb onto their father's back to be transported, and how fathers know when each clutch needs to be transported. For instance, visual cues of the developmental stage of the clutch (e.g., the loss of external gills and the changes in pigmentation) might be a signal for parents to initiate tadpole pick up and transport. With this study, we provide a detailed staging table, related to the one proposed by Gosner (1960), describing embryonal and larval development in terrestrial breeding anurans. Discussed characteristics were easily traceable due to a long developmental time. The staging table we developed can serve for further laboratory experimentation, for example as a tool to reconstruct the day of oviposition in *A. femoralis*.

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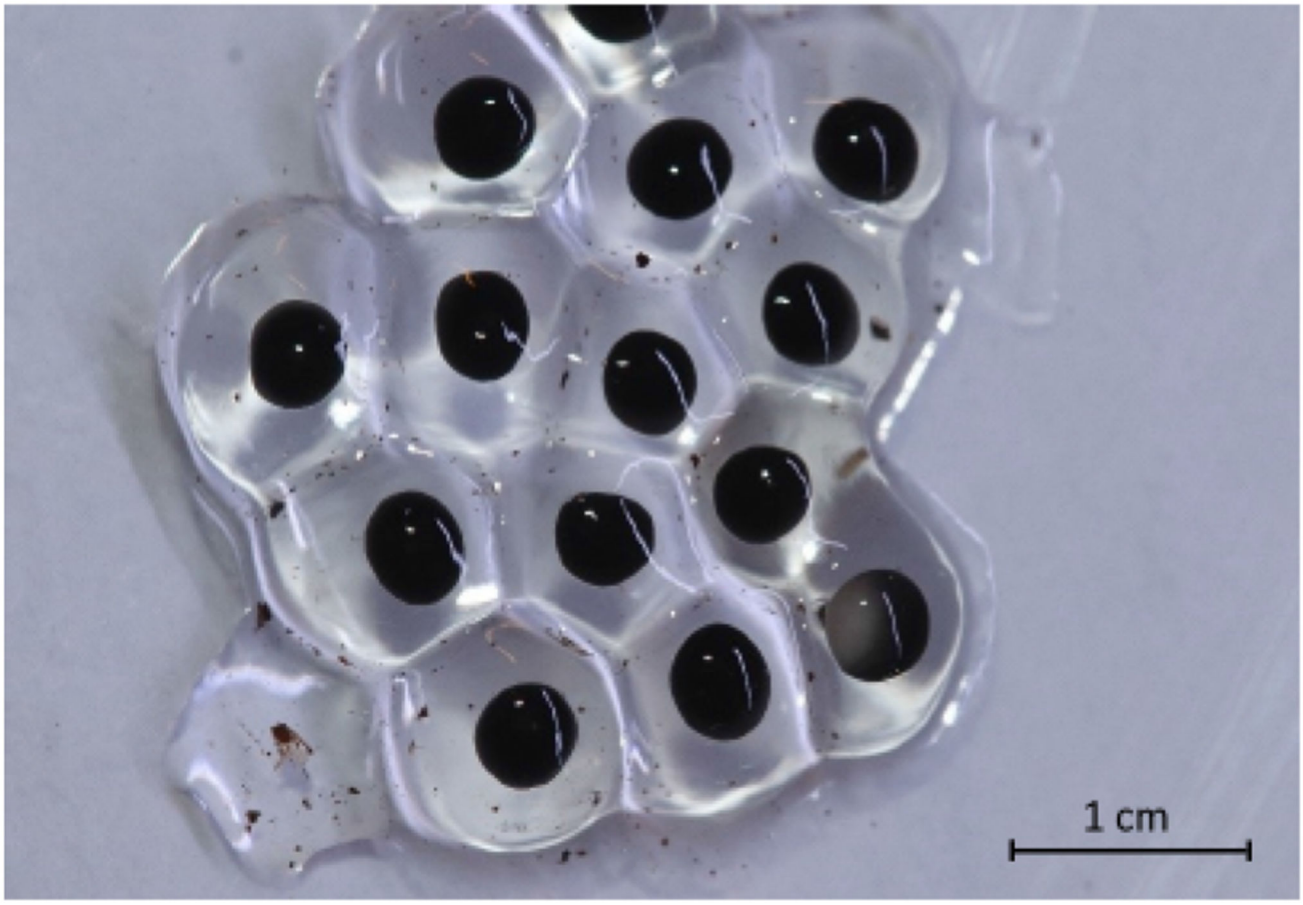

Figure 1.
Picture of a clutch oviposited and fertilised during the preceding night. Photo by Mélissa Peignier.

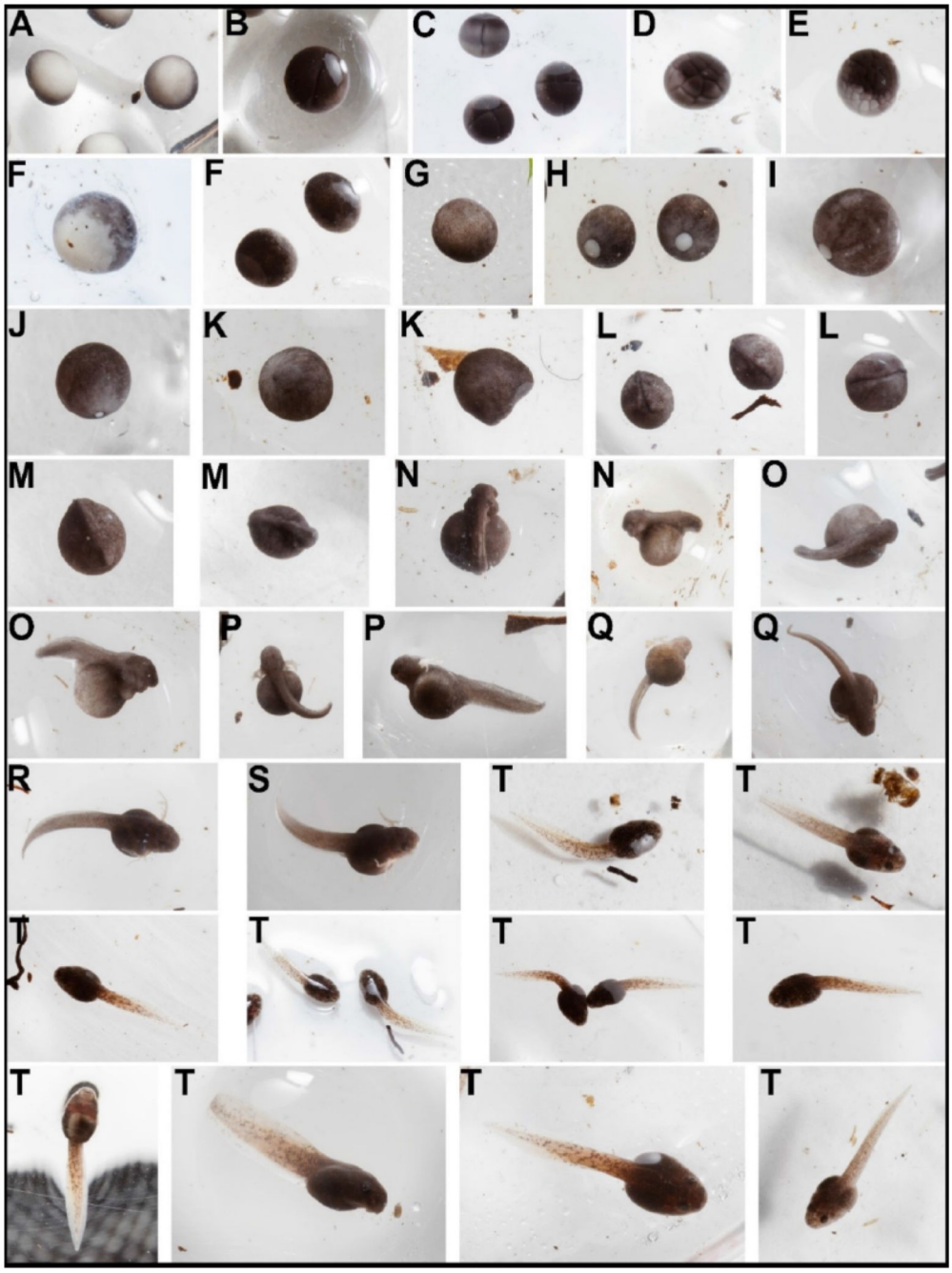

Figure 2.

Pictures of the embryonic development of *Allobates femoralis*. For each picture, the day of development, the principal embryonic changes and the embryonic stages equivalent to Gosner (1960) are given. A–G: days 1–2. fertilisation and cleavage (stages 1–9); H-I: days 2–3. mid-gastrula (stages 10–11); J: days 2–3. late gastrula (stage 12); K: days 3–5. neural plate (stage 13); L: days 4–5. neural folds (stage 14); M: days 4–5. neural tube (stage 16); N: days 5-–6. tail bud and mouth (stage 17); O: days 6–8. muscular response and ridges (stage 18); P–Q: days 7–13. external gills (stages 19–20); R-S: days 9–14. cornea and fins become

transparent, full development of external gills (stages 21-22); T: days 14–21. changes in pigmentation, disappearance of external gills, oral disc and labial tooth development (stages 23–25). Photos by Lauriane Bégué and Mélissa Peignier.

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