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Observations on the Captive Reproduction of the Horned Marsupial Frog *Gastrotheca cornuta* (Boulenger 1898)

RONALD GAGLIARDO*
Atlanta Botanical Garden, 1345 Piedmont Avenue, Atlanta, Georgia 30309, USA

EDGARDO GRIFFITH
El Valle Amphibian Conservation Center, El Valle de Antón, Panama

ROBERT HILL
Atlanta Botanical Garden, 1345 Piedmont Avenue, Atlanta, Georgia 30309, USA

HEIDI ROSS
El Valle Amphibian Conservation Center, El Valle de Antón, Panama

JOSEPH R. MENDELSON III
Zoo Atlanta, 800 Cherokee Avenue SE, Atlanta, Georgia 30315, USA

ELIZABETH TIMPE
Department of Ecology and Evolutionary Biology, University of Connecticut
75 Eagleville Road, Unit #3043, Storrs, Connecticut 06269, USA

BRAD WILSON
Veterinary Clinic West, 763 Whitlock Avenue, Marietta, Georgia 30064, USA

**Corresponding author; present address: Amphibian Ark, Zoo Atlanta*
800 Cherokee Avenue SE, Atlanta, Georgia 30315, USA
e-mail: ron@amphibianark.org

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e-mail: ron@amphibianark.org

The marsupial frogs (Family Hemiphractidae) from Latin America are some of the most intriguing anuran species known. As their name implies, females of these frogs bear a dorsal pouch in which they carry eggs, tadpoles, and/or froglets. In this family, there are 93 species contained in five genera ranging from Costa Rica through South America to Brazil and northwestern Argentina (Frost 2009). The natural history and reproductive behavior of marsupial frogs is well documented in only a few taxa, mostly through wild-collected specimens brought into captivity at different stages. *Flectonotus pygmaeus*, *Gastrotheca riobambae*, and *G. argenteovirens* were observed in the field and laboratory (Duellman and Maness 1980). The Ecuadorian species *G. riobambae*, has been the most heavily studied by far. This species carries eggs until they develop into tadpoles (taking up to 120 days) that are released into a small body of water (usually a shallow depression or pond). Reproduction in captivity has been reported and has afforded several opportunities for documentation of breeding, gestation, and parturition (Auber-Thomay and Letellier 1986; Boonman 1985; Fitzgerald et al. 1979). Reproduction of *Gastrotheca plumbea* was also observed in the laboratory, including the positioning of eggs in the pouch of the female and birth of fully formed froglets (Auber-Thomay et al. 1986).

Differences in gestation times, fertility, and pouch morphology among taxa have been reviewed along with role of the male in assisting with inserting eggs into the pouch in *F. pygmaeus* and *G. riobambae* (Duellman and Maness 1980).

FIG. 1. Adult Horned Marsupial Frog *Gastrotheca cornuta*. Photo by Brad Wilson.

We recently began working with captive populations of the Horned Marsupial Frog, *Gastrotheca cornuta* (Boulenger 1898; Fig. 1). Historically, this species ranged from Costa Rica to Ecuador; however, in many localities, it has become increasingly rare during the past two decades.

In the field, *G. cornuta* is difficult to encounter. It is thought that this shy species stays high in the canopy of old growth forests, far from anthropogenic disturbance. Juveniles are hardly ever encountered and adults only rarely. In El Valle de Antón, Provincia de Coclé, Panama, we encountered two gravid females (eggs visible through the skin of the abdomen) and one female with eggs inside the pouch in primary forest in close proximity to the Río María ca. 1.5 m above the forest floor during June and July 2005. In June 2006, we observed an amplectant pair of *G. cornuta* ca. 10 m above the ground. Newly emerged froglets have not been found to date in this area. However, in early 2006, we encountered two juveniles ca. 2 m above the ground; these may be offspring born in late 2005.

Marsupial frogs exhibit multiple modes of parental care depending on species (Duellman 1970). In some species, such as *G. riobambae*, the female parent carries tadpoles in the pouch until they are released at late stages of growth into a water source. In other species, the female carries and delivers fully developed froglets, as in the case of *G. cornuta*. Considering all species of *Gastrotheca* and *Flectonotus*, there are six morphological pouch types based on size, position, and degree of coverage (del Pino 1980; Mendelson et al. 2007). The evolution of each of the differing types of pouch morphology and reproductive behavior bears further study for contributions to the science of natural history, evolution, and phylogenetics. Application of these findings to captive reproduction will have implications for *ex situ* conservation and potentially survival of threatened species.

The pouch develops from the skin of the dorsum and can extend nearly up to the back of the skull or even to the lateral lymph spaces. After eggs are inserted into the pouch, usually by the male, the interior skin of the pouch becomes vascularized and in some cases

FIG. 2. Enclosure for maintaining *Gastrotheca cornuta*. Photo by Robert Hill.

forms partitions between embryos (del Pino 1980). Species, such as *G. cornuta*, that undergo direct development of eggs to frogs within a pouch, exhibit an interesting example of a life history completely free of any significant body of water—a method far removed from the plesiomorphic and most common amphibian strategy involving the deposition of eggs directly into water (Wells 2008). Presumably this life history allows species to live under less than favorable environmental conditions with respect to availability of bodies of water, and may reduce exposure of developing offspring to water-borne threats such as predation or disease. Wells (2008) provided a thorough summary of the evolutionary and ecological aspects of this important question, and Todd (2007) proposed an important role of disease and parasites as selective agents in the evolution of alternative reproductive strategies in amphibians.

As the scope of the crisis of global amphibian extinctions becomes clear (Stuart et al. 2004) and the threat of emerging infectious diseases such as amphibian chytridiomycosis become more apparent (Daszak et al. 2003), a collaborative effort of the International Union for the Conservation of Nature (IUCN), Captive Breeding Specialist Group (CBSG), and World Association of Zoos and Aquariums (WAZA) has brought about the Amphibian Ark organization (www.amphibianark.org). The Amphibian Ark is dedicated to safeguarding the species that cannot currently be saved in nature; this is accomplished by managed breeding programs to safeguard species while threats can be further mitigated. In some cases, managed populations may serve as a stopgap for many species heading for extinction. The IUCN Amphibian Conservation Action Plan (Gascon et al. 2007) specifically calls for captive breeding and research programs for threatened amphibians. The use of captive breeding programs as a conservation tool is not new and summaries can be found in Zippel et al. (2002) and Gagliardo et al. (2008).

As chytridiomycosis entered the range of *G. cornuta* (Lips et al. 2006), adversely affecting this and other species (Gagliardo et al. 2008), a conservation breeding program was launched. In 2005, specimens of *G. cornuta* were legally exported from Panama to the Atlanta Botanical Garden (ABG), and in 2006 similar specimens were collected and moved to the El Valle Amphibian Conservation Center (EVACC) in El Valle de Antón, Panama. Here we present an overview of our program, some interesting behavioral observations (e.g., toe tapping, male and female vocalizations, egg fertilization and deposition), along with husbandry challenges for long-term care in captivity we have encountered.

Results at the El Valle Amphibian Conservation Center in Panama

Adults at EVACC were maintained in top-opening glass enclosures (60 cm × 30 cm × 40 cm). The substrate consisted of a "false bottom floor" constructed of plastic light diffuser material covered with soft fiberglass screen secured in place with plastic "zip" ties. Non-bleached paper towel was used as covering on the bottom of this false bottom. Potted plants including *Philodendron* and *Heliconia* were added along with pieces of cork bark or half-inch PVC for refuge and perching sites. Two 96-watt power compact fluorescent lights provided lighting and the temperature was maintained at 23–25°C. The frogs were misted automatically 10 times daily with filtered water. Males and females were housed separately until breeding attempts were made, at which time a male was introduced in late afternoon to the female's enclosure. The male vocalized occasionally during the day but mostly in the early evening and throughout the night. All adults were fed katydids (*Neoconocephalus saturatus*) every other day. Because of observed cannibalism, the males were kept individually until placed with a female. On several occasions we noted that the dominant male would attempt to consume another male. In one case, the whole front limb (all the flesh off the bones) and part of the back (partially) of the less dominant male were partially digested. During this process the less dominant male (attributed to its proclivity to hiding rather than perching in the open) was still alive despite being partially digested by its cage mate.

Information on specimens used in breeding event:

Female *Gastrotheca cornuta* (EVACC 001-3) was collected from Río María on 17 June 2006 (found in amplexus with a male) and male *G. cornuta* (EVACC 001-5) collected (also from Río María) on 20 June 2006.

Female history:

On 29 December 2006, the female (EVACC 001-3) was observed to be gravid. Different males (EVACC 001-8, 001-7, and 001-6) were placed with the female individually over the course of several weeks but removed when the male did not amplex the female. On 28 January 2007 amplexus was noted with male EVACC 001-6. On 4 February 2007 the female had eggs in her pouch. Fertiliza-

tion was not observed. On the morning (0800 h) of 20 April 2007, froglets (N = 14) were observed in the enclosure along with two incompletely formed young that were apparently aborted.

2008 Breeding event:

21 February: Male *G. cornuta* (EVACC 001-5) placed in tank with resident female (EVACC 001-3).

22 February: Male and female observed in axillary amplexus. Male had all hand digits in axillary position except one (unclear exactly which due to position and ambient light levels), which was on top of female's front limb.

23 February, 1429–41 h: Male and female observed in axillary amplexus on paper towel at bottom of terrarium. Male arched hind limbs to about a 50° angle while female started to rock (i.e., pushing movement) for about 10 seconds (s). Female maintained front limbs out in front of her, underneath her body (i.e., "praying" position). Male maintained the 50° position for about 5 s, and then settled in so that his legs were held tightly against his body, with his head resting perfectly on top of female's head.

1441–49 h: Male placed his feet on female's back and pushed hind limbs in the air in a 50–60° angle. The male inflated himself, and then raised his legs so that his heels were touching, and maintained position for about 10 s. Female remained motionless during this time, until 1449 h when she pushed against the ground with her back limbs, while the male moved his limbs slightly (15° angle).

1450 h: Male inflated and engaged in movement for 45 s.

1510 h: Female's cloaca reddened and pushed out with male's movement.

1512 h: Male inflated again and engaged in movement for 4 s.

1514 h: Appeared as if the male's movements were an attempt to help the female push out eggs. Female's cloaca tilted upward and aligned with male's swollen cloaca. No egg was released but male moved his hind leg as if to find an egg.

1519–24 h: Male inflated, then female moved her hind legs to 45°. Male inflated again, and then female's cloaca could be seen moving in a pulsing manner.

1525–29 h: Female lifted cloaca. Male touched female cloaca (raising hind limbs) with his left toes making sweeping motion on female's back. Male's feet (toes) were in the vicinity of female's pouch. Female then spread her hind limbs a bit farther apart and assumed a broader stance.

1530–34 h: Male inflated, then deflated, and a gel-like substance appeared. It was not clear if this originated from the cloaca or from the skin. It appeared that the male was glistening first and then after some time it was apparent that the female was coated with this substance as well. The male rubbed substance all over the lower back and pouch of the female with his toes.

1535–54 h: Male began cycles of inflation and deflation. Female moved forward and moved her hind legs; they still maintained a 45°angle but heels were now closer together. Male proceeded to rub the gel-like substance all over the female with his toes.

1555–1601 h: Female rearranged her feet, first right then left in a motion (position) similar to as if preparing to jump. Male and female cloacas aligned with each other. Male continued cycles of inflation and deflation, and continued to spread gel over female's body with his toes. The side of female glistened with the gel-like substance. Male's body was also covered by the substance, which did not appear to dry.

1602–05 h: Female's body was observed to contract. Female pushed forward while the male rose up and down (but did not inflate). Male moved his cloaca into a position directly dorsal to female's cloaca.

1607–14 h: Female arched body, raising her cloaca above the angle of her feet, and cycles of body contractions were observed. Male continues to inflate and deflate. Male then slid forward, pushing female downward and continued to spread the gel over her.

1617–22 h: Male continued inflating and deflating, and rubbing the gel over female with his feet. Then a round egg could be seen in the aperture of female's cloaca. Female rearranged her hind legs, and raised her cloaca while male inflated and deflated. Male moved to bring his cloaca in alignment with that of female.

1628 h: Male deflated. Egg inside of female's cloaca slightly visible as male pushed down. Male moved his toes toward female's pouch.

1630–53 h: Female's body continued with cycles of contractions, and male continued to inflate and deflate. The egg could be seen alternately appearing and disappearing at the aperture of females' cloaca.

1655 h: First egg emerged from female's cloaca, while her body appeared to be in a strong contraction. Male cradled the egg with his body, and maneuvered it into female's pouch with his hind toes. Male continued to inflate and deflate.

1701 h: Male pushed down on female. A second egg almost emerged from female's cloaca.

1706 h: The second egg emerged, and was pushed into the pouch by the male using his feet.

1711 h: Third egg emerged, and male trapped it with his cloaca and pushed it toward the pouch opening. Male used hind legs to position it in female's pouch.

1719 h: Fourth egg emerged, and male repeated behavior to insert it into female's pouch. It appeared as if the male's toes were inserted inside of the pouch, at least dextrally.

1724 h: Male engaged in head bobbing behavior.

1726 h: Fifth egg emerged, and male observed to insert toes into the pouch while inserting the egg.

1729–1834 h: Pair maintained amplexus, male continued to inflate and deflate and began to perform a "rocking" motion, but no additional eggs were produced. The female changed position in minor ways several times before disengaging amplexus.

Results at the Atlanta Botanical Garden

As part of a pilot study aimed at learning the logistics of an ex situ response to the rapid spread of the amphibian chytridiomycosis through pristine amphibian populations in Panama, several threatened amphibian taxa were exported to facilities at the ABG and Zoo Atlanta in 2005 (Gagliardo et al. 2008). The original breeding group of six male and two female *G. cornuta* was maintained at the ABG where several breeding events have occurred. Adults were maintained in either 60 × 30 × 60 cm or 60 × 60 × 90 cm front-opening glass enclosures for maintenance (smaller) or breeding (larger). The substrate consisted of a "false

bottom floor" constructed of plastic light diffuser material covered with soft fiberglass screen secured in place with plastic "zip" ties. Potted plants including *Philodendron*, *Heliconia,* and *Calathea* were added for hiding places. Pieces of driftwood or similar twigs were provided for perching sites (Fig. 2). Two 96-watt power compact fluorescent lights provided lighting and the temperature was maintained between 18°C and 27°C. The frogs were misted twice daily with filtered water either through an automated system or by hand sprayer bottle. A 15–20 cm diameter, 6-cm-deep shallow water dish containing smooth river stones was refreshed with clean water daily. The stones afforded a climb-out option for not only the frogs after soaking in water but also food items that happened to fall into the water. Males and females were housed separately until breeding attempts were made, at which time a male was introduced in late afternoon to the larger enclosure containing one female. Male vocalization, a very loud, single note similar to the sound of removing a cork from a bottle, was common in early evening.

The first breeding event occurred in April 2006, less than 48 h after introducing a male into a female's enclosure. Both individuals were exposed to an imposed "dry season" simulated by six weeks of slightly warmer temperatures and lower humidity achieved by less frequent misting of the enclosure and increasing ambient day time temperatures from 20–25°C. There were no signs of courting or amplexus before the female was discovered in the early morning (0700 h) on the second day after introduction of the male. Fourteen eggs appeared to have been inserted into the female's pouch (Fig. 3) and there were two infertile eggs found on the surface of a leaf in the tank. The recovered eggs were approximately 1.0 cm in diameter, not unexpected as this species is reported to produce the largest anuran egg known (Duellman and Trueb 1986). Immediately following this breeding event, the male was moved to separate enclosure to reduce stress on the gravid female.

Video surveillance of this first attempt did not record any breeding activity but did record evident toe tapping of the female who upon sight of a live, moving cricket (*Acheta domestica*), became keenly interested in the prey and began to tap and motion with toes of her hind feet. Pedal luring (Bertoluci 2002; Murphy 1976; Radcliffe et al. 1986) and providing a vibrational stimulus resulting in prey movement and ultimately prey detection (Sloggett and Zeilstra 2008) are two hypotheses for toe twitching and toe tapping in anurans. Although toe twitching and tapping are thought to be a common behavior among many frogs and toads (Sloggett and Zeilstra 2008), and have been reported in numerous anuran genera from several different families, including Batrachophrynidae (Radcliffe et al. 1986), Bufonidae (Hagman and Shine 2008; Radcliffe et al. 1986; Sloggett and Zeilstra 2008), Dendrobatidae and Hylidae (Bertoluci 2002), this is the first documentation of pedal luring in the Hemiphractidae.

FIG. 3. Female *Gastrotheca cornuta* with eggs in pouch. Photo by Brad Wilson.

FIG. 4. Newly emerged *Gastrotheca cornuta*. Photo by Heidi Ross and Edgardo Griffith.

An additional breeding event occurred in Atlanta in the spring of 2008. On 14 April a male was introduced to an enclosure containing a visibly gravid female. Within 24 h, amplexus was observed, followed overnight by eggs being visible in the pouch. These eggs incubated until 15 June 2008 when thirteen live froglets and two infertile eggs were discovered in the enclosure.

Gestation, Birth, and Handling of Offspring

Gestation periods ranged from 60–80 days over the course of several breeding events. In the final week before froglets emerged from the pouch, it was possible to see movement of the limbs of the embryos just beneath the skin of the pouch. At both ABG and EVACC, the newly born offspring were approximately 1.0 cm in length and averaged 400 mg in mass (Fig. 4). Upon their birth, offspring were separated into individual enclosures to avoid predation by the female and offered a variety of prey items including fruit flies, houseflies, and small (3–5 mm) crickets. Food items were dusted alternately with vitamins (Men's Health® multivitamin, once per week) and calcium supplements (RepCal® with Vitamin D3, twice per week).

Abortion of Developing Offspring

Case 1: On 20 June 2007, a gravid female ca. 50 days post-breeding aborted five developing offspring and seven non-developed eggs. There were no outward physical signs of any problems prior to this event. The pouch remained partially inverted for approximately 48 h before repositioning to its normal state (Fig. 5). The developing offspring displayed long, 2–4 cm filamentous gills attached through the skin under the throat (Fig. 6).

Case 2: An abortion of eggs occurred immediately following

FIG. 5. Inverted pouch of female *Gastrotheca cornuta* less than 24 h after emergence of froglets. Photo by Ron Gagliardo.

a July 2008 breeding event. Although our attempts to record the breeding event via night-vision video were unsuccessful (likely due to disturbance caused by shifting of blinds, camera tape changes, noise, etc.) and we did not capture the breeding event on film, we did observe vocalization by the female that consisted of a single soft "bop" periodically prior to amplexus. This could be an encounter call and although we found this unusual, encounter calls have been documented in other anurans (Quiguango-Ubillús and Coloma 2008). Five eggs were never inserted into the pouch and the seven eggs successfully moved into the pouch were aborted within 72 h. We noted that in contrast to the previous case, the lining of the pouch was not extruded possibly because the pouch never became vascularized. Attempts to artificially incubate five eggs on sterile paper toweling or sphagnum moss failed and the eggs disintegrated within 24 h.

Obstacles for Long-term Captive Management

Whereas the captive reproduction of *Gastrotheca cornuta* proved much less difficult than expected, raising offspring has been extremely challenging. Most losses occurred during the first 5–20 weeks after birth. Necropsy results indicated a range of issues including internal parasites (mostly rhabditiform nematodes), squamous metaplasia ("short-tongue syndrome" possibly indicative of Vitamin A deficiency), and signs of metabolic bone disease. Currently, there are 11 captive-born offspring in existence,

FIG. 6. Partially developed offspring of *Gastrotheca cornuta* that were aborted during the final weeks of development. The bell gills that characterize this group of frogs are clearly visible. Photo by Ron Holt.

6 at EVACC and 5 at ABG. The five frogs at ABG are over one year in age, weigh between 7.1–12.7 grams and have snout–vent lengths (SVL) of 42–55 mm. Specimens appear to be in overall good health, but have grown very slowly and show some slight rear limb deformities possibly attributable to improper vitamin and mineral supplementation, and/or inadequate exposure to UV-B. At EVACC, the offspring are 12–15 months of age and five of the six also exhibit problems consistent with metabolic bone disease and other skeletal deformities. One frog, (a single survivor from a clutch that emerged on 27 May 2008) has received exposure to UV-B radiation (45 minutes daily from an Eiko® 50-watt halogen bulb with lens removed and positioned atop enclosure) and has not developed any obvious skeletal deformities. The slightly deformed animal from the first clutch born in April 2007 actively hunts and is now calling (Fig. 7). Experiments with Vitamin A supplementation and UV-B exposure currently underway are aimed to mitigate these problems. It is crucial to the survival of these colonies to decipher the husbandry issues, raise offspring to adulthood, and produce subsequent generations.

FIG. 7. Juvenile captive born *Gastrotheca cornuta* exhibiting skeletal deformity. Photo by Brad Wilson.

Conclusions and Implications for Conservation

In comparison to other anuran families such as Dendrobatidae and Ranidae, relatively little is known about the natural or captive reproduction of hemiphractine frogs. Mating behavior in captive specimens of *Gastrotheca riobambae* by Matthews (1957), Deckert (1963), and Hoogmoed (1967), as summarized by Means et al. (2009), was similar to what we have described here, in the male producing a fluid that is rubbed over the posterior area of the female (from cloacal region extending to the anterior limit of the brood pouch) and using his hind legs to insert eggs into the pouch as they are extruded. We speculate that the clear fluid observed by Means et al. (2009) and by us in Panama might have been produced by the male. We should not rule out the possibility that such secretions from the male may contain hormones or other chemicals that stimulate observed contractions in the females. In addition, the vocalization of the female during amplexus remains a mystery. Clearly, these interesting observations should be subject to future investigation.

Along with the actual physical reproductive behavior in hemiphractine frogs such as *Flectonotus* and *Gastrotheca*, we should consider how the natural history affects the developmental physiology of these taxa. Some species are known to bask only while incubating embryos, thus exposing themselves to UV-B radiation (Auber-Thomay et al. 1990). Does this suggest that eggs or developing froglets need UVB or heat for proper development? There are also reports of infection of tadpoles by rhabditiform nematodes while incubating inside the pouch (Auber-Thomay et al. 1990).

What are the Implications of Such Parasitism in the Wild or Captivity?

Over half of the 57 known species of *Gastrotheca* are exhibiting population declines (www.iucnredlist.org). The IUCN Red list categorizes *G. cornuta* as Endangered, and a more recent prioritization by the Panamanian government and the Amphibian Ark placed it among the top Panamanian species in need of *ex situ* intervention (Amphibian Ark 2009; IUCN 2009). This highly threatened status warrants continued searches in Panama and elsewhere for additional founder specimens to increase genetic variability of managed colonies. Clearly, the phylogenetically, taxonomically, and physiologically unusual masterpieces that are *Gastrotheca* (Fig. 9) are worthy of conservation efforts. In cases where threats in nature cannot be mitigated in time, managed *ex situ* populations may be the only hope for safeguarding these species until such threats are reversed or until other methods for re-establishing these species in nature are developed. Learning more about the complex natural history and physiology will certainly be of great assistance in the future conservation of these and other endangered amphibian species. We also offer that our natural history and behavioral observations presented here are unlikely to have been documented in the wild, thus supporting the claim that captive programs provide real opportunities for basic research (Chiszar et al. 1993), in this case relating to natural history and behavior. As such, these programs function as a crucial component of the "multidisciplinary approaches to conservation" (Gans 1994).

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