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Reproduction in the marsupial frog *Gastrotheca testudinea* (Anura: Hemiphractidae)

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Abstract. We provide the first report of reproduction in *Gastrotheca testudinea*. A brooding female from the Urubamba Valley in southern Peru gave birth to 59 froglets. This confirms the supposition that this species has direct development of eggs into froglets.

Keywords. Reproduction, *Gastrotheca testudinea,* Amphibia, Peru.

Female marsupial frogs of the genus *Gastrotheca* Fitzinger, 1843, are unique among living anurans in brooding eggs in dorsal pouches; in some species the eggs hatch as tadpoles that complete their development in ponds, whereas in other species the eggs are retained in the pouch and hatch as froglets that emerge from the pouch (Duellman, Maness 1980). For more than 150 years marsupial frogs were placed in Hylidae and more recently as the subfamily Hemiphractinae (e.g., Duellman 2001). Some molecular phylogenetic studies provided convincing evidence that hemiphractines were not hylids and possibly were most closely related to frogs that were recognized in Leptodactylidae (e.g., Faivovich et al. 2005; Wiens et al. 2005), and one study with poor taxon sampling placed the "hemiphractine" genera into three families (Frost et al. 2006). Subsequent investigations (e.g., Wiens et al. 2007; Guayasamin et al. 2008) recognized the marsupial frogs and their allied genera (*Cryptobatrachus, Flectonotus, Hemiphractus, Stefania*) as a monophyletic group, Hemiphractidae, and a rigorous analysis by Heinicke et al. (2009) clearly showed Hemiphractidae to be the sister taxon to the five families of direct-developing frogs recognized in the unranked taxon Terrarana by Hedges, Duellman, Heinicke (2008).

Determination of the developmental stage at hatching is based on observations of parturition in brooding females of several species. These include eight species that have been observed to produce free-swimming tadpoles *Gastrotheca aureomaculata* Cochran and Goin (1970), *G. chrysosticta* Laurent (Laurent, Lavilla, Teran 1986), *G. gracilis* Laurent (Laurent, Lavilla, Teran 1986), *G. lateonota* Duellman and Trueb (1988), *G. litonedis* Duellman and Hillis (1987), *G. piperata* Duellman and Köhler (2005), *G. riobambae* (Fowler) (del Pino, Escobar 1981), and *G. ruizi* Duellman and Burrowes, 1986). Tadpole production has been determined in nine other species by observing tadpoles in eggs or tadpoles metamorphosing; these include *G. espeletia* Duellman and Hillis, *G. lateonota* Duellman and Trueb, *G. marsupiata* (Duméril and Bibron), *G. monticola* Barbour and Noble, *G. peruana* (Boulenger), *G. phalarosa* Duellman and Venegas, *G. pseustes* Duellman and Hillis, *G. psychrophila* Duellman, and *G. trachyceps* Duellman.

The birth of froglets has been observed in 12 species—*G. christiani* Laurent (Barrio 1976), *G. cornuta* (Boulenger) (Gagliardo et al. 2010), *G. excubitor* Duellman and Fritts (Duellman, Maness 1980), *G. griswoldi* Shreve (W.E. Duellman, personal observation), *G. helenae* Dunn (Duellman, Ruiz-C. 1986), *G. nicefori* Gaige (Yustis 1978), *G. ochoai* Duellman and Fritts

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Figure 1. Brooding female *Gastrotheca testudinea*, CORBIDI 05385, 77.5 mm snout-vent length. Photo by G. Chávez.

(1972), *G. orophylax* Duellman and Pyles (1980), *G. ossilaginis* Duellman and Venegas (2005), *G. ovifera* (Lichtenstein and Weinland) (Mertens 1957; Duellman, Maness 1980), *G. plumbea* (Boulenger) (del Pino, Escobar, 1981), and *G. walkeri* Duellman (1980). Six other species also produce froglets; these are *G. abdita, G. andaquiensis, G. argenteovirens, G. galeata, G. guentheri,* and *G. williamsoni* (W.E. Duellman, personal observation).

The number of eggs brooded by females that produce tadpoles is much larger than those that hatch as froglets (Duellman, Lehr, Aguilar 2001). Distinct differences exist not only in the number of eggs but also in the size of eggs. Generally speaking, eggs in the brood pouches of species of *Gastrotheca* in which the eggs hatch as froglets are much larger and fewer in number than in those species in which the eggs hatch as tadpoles. Obviously, more yolk is required to reach metamorphosis than to hatch as a tadpole that after hatching obtains nutrients from the environment to development to metamorphosis; the former is the endotrophic mode and the latter the exotrophic mode, as defined by Altig and Johnson (1989).

In 10 species known to have eggs that hatch as tadpoles, snout-vent length of 69 brooding females is 33.7–75.0 mm ($\bar{x}$ = 55.4 ±10.3), the number of eggs in the pouches is 20–197 ($\bar{x}$ = 95.4±21.6), and diameters of the ova are 2.3–6.3 mm ($\bar{x}$ = 4.8±1.0). In 17 species known to have eggs that hatch as froglets, snout-vent length of 131 brooding females is 29.7–88.0 mm ($\bar{x}$ = 49.0±16.2), the number of eggs in the pouches is 5–48 ($\bar{x}$ = 20.0±7.6), and the diameters of the ova are 4.0–13.2 mm ($\bar{x}$ = 7.38±2.1).

Duellman has examined many preserved specimens of *Gastrotheca testudinea,* a species known from elevations of 1100–2775 m on the eastern slopes of the Andes from Ecuador, Peru, and Bolivia. Of three brooding females, CAS-SU 5073 from Abitagua, Provincia Mera, Ecuador, having a snout-vent length of 72.7 mm contained 69 eggs with a mean diameter of 5.5 mm; LSUMZ 31971 from above Acamayo, Departamento Huánuco, Peru, having a snout-vent length of 59.4 mm contained 44 eggs with a mean diameter of 5.5 mm, and LSUMZ 31973 from Huaylaspampa, Departamento Huánuco, Peru, having a snout-vent length of 59.4 mm contained 30 eggs with a mean diameter of 4.1 mm. The number of eggs (69) in CAS-SU 5073 is 21 more than the highest number of eggs in 17 species of *Gastrotheca* known to have direct development and is within the range of 10 species known to have eggs that hatch as tadpoles.

Allozymic data showed *Gastrotheca testudinea* to be allied to *G. weinlandii* (Duellman, Hillis 1987).

One brooding female *G. weinlandii* (UMMZ 90248 from between Abitagua and the Río Pastaza, Provincia Pastaza, Ecuador) has a snout-vent length of 78.8 mm and contains 14 eggs with a mean diameter of 7.0 mm; all of these parameters are well within the ranges of variation in direct-developing *Gastrotheca.* Thus, *G. weinlandii* is associated with the *Gastrotheca ovifera* Group, as defined on immunological evidence by Duellman et al. (1988). Köhler (2000) considered *G. testudinea* to be a member of that group, all members of which have direct development, thereby implying that *G. testudinea* had direct development.

However, the reproductive mode of *Gastrotheca testudinea* remained questionable until 17 October 2009 when Chávez induced parturition in a brooding female having a snout-vent length of 77.5 mm (CORBIDI 05385; Fig. 1) by an injection of Lidocaine. This resulted in 59 hatchling froglets having snout-vent lengths of 9.4–11.75 mm ($\overline{x}$ = 10.7 mm, n = 59), plus three undeveloped ova (4.7, 4.9, 4.9 mm in diameter) and one embryo within the brood pouch. The froglets with snout-vent lengths of 11.0 mm or more jumped at the moment of the birth; apparently, they were ready for birth, but the smaller ones apparently were not ready for birth. The female was found on 15 October 2009 at the Ozonampiato Native Community (12° 39' 55.76'' S, 73° 09' 47.17'' W) at an elevation of 1800 m in the Río Urubamba Valley, Cusco Region, Peru. Two juveniles (CORBIDI 05999 and 06017) having snout-vent lengths of 22.3 mm were collected at the same locality on 23 January 2010.

Thus, the reproductive mode of producing froglets by *Gastrotheca testudinea* is confirmed. The large number of eggs in the brood pouches of two individuals (63 in CORBIDI 05385 and 69 in CAS-SU 5073) far exceeds the numbers in other *Gastrotheca* that have direct development. In other species the largest number of eggs is 37 found in brooding females of *G. nicefori* and *G. orophylax* (Duellman, personal observation). In contrast, the mean diameter of ova in the pouches of four *G. testudinea* is 4.7 mm, which is much smaller than the mean diameter (7.38 mm) of ova in pouches of other species of *Gastrotheca* that have direct development. Thus, although retaining direct development, productivity in *G. testudinea* is somewhat intermediate between that in tadpole-producing species (e,g., *G. marsupiata* and *G. riobambae*) and other direct-developing species (e.g., *G. cornuta* and *G. weinlandii).*

Codes for museum collections are: CAS-SU = California Academy of Sciences-Stanford University collection, San Francisco; CORBIDI = Centro de Ornitologia y Biodiversidad, Lima; LSUMZ = Natural Science Museum Louisiana State University, Baton Rouge; UMMZ = University of Michigan Museum of Zoology, Ann Arbor.

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