DISCOVERY OF THE EGGS OF **BOLITOGLOSSA PANDI** (CAUDATA: PLETHODONTIDAE) WITH OBSERVATIONS ON ITS REPRODUCTIVE BIOLOGY HALLAZGO DE LOS HUEVOS DE **BOLITOGLOSSA PANDI** (CAUDATA: PLETHODONTIDAE) CON OBSERVACIONES SOBRE SU BIOLOGÍA REPRODUCTIVA

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Resumen.– El hallazgo de dos posturas de huevos de la salamandra de Pandi (*Bolitoglossa pandi*) en un fragmento de bosque en los Andes orientales de Colombia, permitió el seguimiento in situ de estos durante cinco meses, hasta que las crías eclosionaron. Documentamos por primera vez los sitios de anidación, posturas, tasa de eclosión de los huevos y dimensiones de los neonatos para esta especie. Contribuimos al escaso conocimiento sobre la biología e historia natural de esta especie de anfibio con rango restringido para Colombia.

Palabras clave.– Amphibia, Colombia, crías, desarrollo directo, posturas de huevos, tasa de eclosión.

Abstract.– The discovery of two egg clutches of the Pandi's mushroom tongue salamander (*Bolitoglossa pandi*) in a forest fragment on the eastern Andes of Colombia, allowed in situ check-up fieldtrips for five consecutive months until the hatchlings emerged from the eggs. We here report the nesting sites, clutches, egg hatching rate and neonate measurements for the first time for this species, thus contributing to the current knowledge of the biology and natural history of this range-restricted amphibian of Colombia.

Keywords.- Amphibia, Colombia, development, egg clutches, hatchling, hatching rate.

The Pandi mushroom tongued salamander (Bolitoglossa pandi) was described by Brame & Wake (1963) based on a badly-preserved adult female (ZMH A00868, formerly ZSZMH 2858). The species was diagnosed as a medium size Bolitoglossa with a moderate number of maxillary teeth, with extensive webbing on hand and feet and by its coloration pattern being solid dark reddishgray dorsally with cream spots ventrally (Brame & Wake, 1963). For almost 20 years the species remained known only from the type locality: municipio de Pandi, Cundinamarca, Colombia, but various authors (Hanken & Wake, 1982; Acosta-Galvis & Rueda, 2004; Acosta-Galvis & Gutiérrez-Lamus 2012; Angarita-Sierra et al., 2020) have subsequently reported additional new localities for this species in the Eastern Andes of Colombia. Currently, B. pandi is categorized by IUCN as Endangered (EN), given that its total extent of occurrence (EOO) is thought to be 2,500 km², and its habitat is currently being degraded by human settlement, forest clearing and agriculture (IUCN SSC Amphibian Specialist Group, 2019).

As is the case for most species of salamanders, not much is known regarding the behavior, ecology and natural history of *B. pandi* aside from two publications. The first by Del Río-García et al. (2014) focused on the diet and microhabitat use from a population in Supatá, Cundinamarca (Fig. 1). The second publication is that of Angarita-Sierra et al. (2020), which expanded the distribution range of *B. pandi* along the eastern Andes of Colombia and commented on the population dynamics, activity patterns and color variation of the species. Given that we did not collect voucher specimens, we cannot ascertain the identity of the salamanders that we observed in Supatá, Cundinamarca but we consider them to be *B. pandi*.

During the course of a herpetological survey of the amphibian fauna of vereda San Marcos, Supatá, Cundinamarca (Fig. 1), we managed to find two egg clutches of *B. pandi* hidden amongst the leaf litter (Fig. 2) inside a forest fragment on the western versant of the Cordillera Oriental of Colombia in vereda San





Figura 1. Mapa del noroccidente de Sur America y Colombia, mostrando la ubicación geográfica del área de estudio, vereda San Marcos, Supatá, Cundinamarca; la ciudad de Bogotá está indicada en amarillo.

Figure 1. Map of northwestern South America and Colombia, showing the geographic location of the study site, vereda San Marcos, Supatá, Cundinamarca; the city of Bogotá is shown in yellow.



Figura 2. Posturas de los huevos de *Bolitoglossa pandi* encontrados en Supatá, Cundinamarca, Colombia. Foto: Giovanni A. Chaves-Portilla. Figure 2. Egg clutches of *Bolitoglossa pandi* found in Supatá, Cundinamarca, Colombia. Photo: Giovanni A. Chaves-Portilla.



Marcos, municipality of Supatá, Cundinamarca, (5° 2' 45.9" N, 74° 14' 25.64" W, 2,000 m a.s.l.). The first clutch was discovered on 23 September 2015 and comprised 15 rounded eggs adhered to one another. Each egg was covered by a transparent gelatinous layer or capsule (also termed 'envelope' in the literature), with bits of substrate or dirt scattered over the eggs (Fig. 2). Underneath this gelatinous layer was a second transparent capsule covering the yolk, which was uniformly white. The diameter of the eggs ranged between 4.5-5.0 mm (measured with a dial caliper to the nearest 1 mm), these were grouped towards the stem of the clutch, grape-like in shape, mostly irregularly textured and held together by a transparent membrane. This clutch of eggs was found 7.5 cm below the leaf litter. The substrate on which the eggs were deposited had a temperature of 19.4°C and a relative humidity of 82 %. An attending adult female B. pandi with an SVL of 43.1 mm (Fig. 2) was found to the right of the eggs.

The second clutch of eggs was detected within the same forest fragment at a depth of 9 cm inside leaf litter; the substrate where the eggs laid had a temperature of 17.4°C and a relative humidity of 94 %. This clutch had 16 eggs of the same shape and size of those found in the first clutch but no attending adults were encountered in the vicinity of the eggs; one of the eggs was considered infertile because the transparent gelatinous capsule did not have a developing embryo (i.e., only yolk).

At the time when the second clutch of eggs was found, the eggs showed no sign of developmental activity, i.e., they were mainly yolk. The way in which the eggs were grouped together (grapelike), and the form of the structures (capsules) that surrounded the yolk resemble closely what McDiarmid & Worthington (1970) had previously reported for Central American species of Bolitoglossa and Valdivieso & Tamsitt (1965) for B. adspersa (Table 1) in South America. Following the discovery of these two clutches, we made monthly visits to the study site to record any changes that could be physically observed in the appearance of the eggs. The total number of eggs decreased in the ensuing months, probably due to attacks by unobserved predators. Nonetheless, an important observation was made: not all the eggs had been fertile, as some showed only the transparent gelatinous dome covering them but no signs of embryos. Sometime after the first month of monitoring, the adult female was no longer found near the eggs; we consider this to mark the end of parental care.

 Tabla 1. Compendio sobre los aspectos reproductivos y posturas de algunas especies de Bolitoglossa. La información fue tomada de referencias y fotografías.

 Table 1. Compendium of reproductive aspects and clutches of some species of Bolitoglossa. Information taken from the literature and from photographs.

Reproductive aspects/ Species	B. adspersa	B. nicefori	B. pandi	B. paraensis	B. platydactyla	B. rostrata	B. subpalmata	B. yucatana
Number of clusters	Unknown	2	2	Unknown	2	2	3-Jan	2
Shape of cluster	Oblong	Grape-like	Grape-li- ke	Unknown	Grape-like	Unknown	Grape-like	Unknown
Gelatinous capsules covering egg	0	2	2	Unknown	2	2	2	Unknown
Number of eggs per clutch	6-11	25-40	31	8-14	ca 30	22-54	13-38	Unknown
Maximum egg dia- meter	4.5 mm	2.5 mm	5 mm	Unknown	Unknown	3.5 mm	Unknown	Unknown
Parental care (attending adult)	Yes	Yes	Yes	Unknown	Yes	Unknown	Yes	Yes
Months of parental care	Less than 1	Unknown	Less than 1	Unknown	Unknown	Unknown	Unknown	Unknown
Annual reproductive activity	Continuous	Continuous	Seasonal	Seasonal	Unknown	Seasonal	Continuous	Unknown
Source of information	V & T 1965	0 et al 2009	This study	N-0 et al 2011	MD & W 1975	Houck 1977	MD & W 1975	MD & W 1975

 Tabla 2. Resumen de los datos registrados durante cinco meses de monitoreo a las dos posturas de huevos de Bolitoglossa pandi.

 Table 2. Summary of the data recorded during five months of monitoring the two egg
 clutches of Bolitoglossa pandi.

Clutch no.	Date	Fertile eggs	Infertile eggs	Preyed upon	Destroyed
1 (15 Eggs)	Sep-15	15	0	0	0
	Oct-15	15	0	0	0
	Nov-15	8	2	3	2
	Dec-15	2	3	5	5
	Jan-16	2	3	5	5
	Feb-16	2	3	5	5
2 (16 Eggs)	Sep-15	15	1	0	0
	Oct-15	15	1	0	0
	Nov-15	15	1	0	0
	Dec-15	13	2	1	0
	Jan-16	12	2	1	1
	Feb-16	10	2	3	1

During the second month of monitoring, the first signs of hatchlings became visible (Fig. 3), and by the fifth month, specifically 151 days since the egg clutches were first discovered, the first batch of fully developed *B. pandi* offspring were detected (Fig. 4). The hatching success, i.e., the percentage of hatched offspring was calculated as follows: percentage of hatching = (N ° total number of neonate salamanders * 100) / N ° total number of eggs recorded. Hatching success from the first group was only 13.3 %, while that of the second group was 66.6 % (Table 2). The neonates of *B. pandi* had a slender body and a mean SVL of 8.5 mm (8.1-8.8 mm; n = 10, SD = 0.26), a short tail with a mean length (TL) of 4.1 mm (3.9-4.4 mm; n = 10, SD = 0.15)

The hatching of the eggs of *B. pandi* occurred at the end of February 2016, which supports or coincides with the observations reported by Angarita-Sierra et al. (2020) "...During the second sampling occasion, a bias in the median SVL towards smaller individuals was observed, suggesting that the major recruitment peak occurs during March when the high rainy season start...". Angarita-Sierra et al. (2020) recorded a higher



Figura 3. Huevo de *Bolitoglossa pandi* con el embrión en desarrollo. Foto: Giovanni A. Chaves-Portilla.

Figure 3. Egg of *Bolitoglossa pandi* with developing embryo. Foto: Giovanni A. Chaves-Portilla.

number of neonates of *B. pandi* during March, and they regarded this month as the peak of recruitment, which coincides with the first prolonged, intense rainy season of the year in this region of Cundinamarca (IDEAM, 2017).

This discovery of the eggs of *B. pandi* in Supatá, Cundinamarca, on the contrary, coincides with the second prolonged rainy season of the year, which usually occurs during the months of September, October and November (IDEAM, 2017). Our data suggest that females of *B. pandi* lay eggs at the beginning of the second rainy season of the year (from September through November), and that the offspring hatch at the end of the first dry season of the year (from December through February) (IDEAM, 2017); contrary to females of *B. nicefori* - another congener from the western slopes of the Eastern Andes of Colombia - that lay eggs from December through March, i.e., throughout the first dry season of the year (Ortega et al., 2009)

The fact that *B. nicefori* and *B. pandi* (both restricted to the western slopes of the Eastern Andes) are faced with very similar climatic conditions of annual rainfall and humidity, yet have not only different but almost completely opposite cycles of reproductive activity throughout the year, suggest that this could be one of the reasons behind the "high diversity" achieved by the genus *Bolitoglossa* in this mountainous region of Colombia; but further ecological, taxonomic and phylogenetic research is needed to thoroughly answer this question.





Figura 4. Neonatos eclosionados de una de las nidadas halladas de *Bolitoglossa pandi*. Foto: Giovanni A. Chaves-Portilla. Figure 4. Neonates hatched from one of the clutches found of *Bolitoglossa pandi*. Photo: Giovanni A. Chaves-Portilla.

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