

Second observation of the reproductive biology of *Blythia reticulata* (Blyth, 1854) (Reptilia, Squamata, Colubridae)

Lal Biakzuala¹, Vanlal Hruaia², Lal Biakhlui³, Hmar Tlawmte Lalremsanga¹

¹ Developmental Biology and Herpetology Laboratory, Department of Zoology, Mizoram University, Aizawl, Mizoram, 796004, India

² Durtlang Mualveng, Aizawl, Mizoram, 796014, India

³ Department of Public Administration, Mizoram University, Aizawl, Mizoram, 796004, India

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Corresponding author: Hmar Tlawmte Lalremsanga (htlrsa@yahoo.co.in)

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Abstract

A second observation on the reproduction of *Blythia reticulata* was based on three eggs found on a forest path among leaf litter and successfully incubated. Given the limited knowledge on the natural history of the species, including its breeding biology, we provide information on egg measurements ($n = 3$; length = 25.54 ± 1.05 mm; width = 11.79 ± 0.37 mm; weight = 1.94 ± 0.24 g) and the first data on hatchling biometrics ($n = 3$; snout-vent length = 106 ± 1.73 mm; tail length = 14 ± 1.00 mm; weight = 1.13 ± 0.09 g) from Mizoram State, northeastern India.

Key Words

breeding, eggs, India, Iridescent Snake, Mizoram, natural history, reproduction

The Iridescent Snake *Blythia reticulata* (Blyth, 1854) is a poorly known semi-fossorial colubrid snake recorded from India, Bangladesh, China, and Myanmar at elevations between 949–1280 m asl. (see Das 2008, Purkayastha 2013, Das and Das 2017, Vogel et al. 2017, Biakzuala et al. 2020), listed as ‘Data Deficient’ in the IUCN Red List (Wogan and Vogel 2012). Among the only information on the species’ reproductive biology concerns its egg size. In addition, its fecundity of eight eggs, multiple mating and egg laying within a single reproductive season were reported by Biakzuala et al. (2020). Here, we provide additional data from Mizoram State, north-east India, on the reproductive habits of this little known snake.

We discovered three snake eggs (soft leathery texture, whitish in colour, and oblong shape) on a forest path among leaf litter near Selesih locality, Aizawl District, Mizoram, India (23.7961°N , 92.7315°E , WGS 84; ca. 1200 m asl.) on 9 September 2020 (Fig. 1). The collection site was a hilly terrain of secondary forest dominated by *Schima wallichii*, *Albizia* sp., *Cerasus cerasoides*,

Litsea sp., and *Zingiber* sp.; a small intermittent stream was located ca. 50 m from the oviposition site. The average annual rainfall in the district based on the previous past 30 years was 2593.4 mm (Guhathakurta et al. 2020), and the climate type is moist tropical to moist sub-tropical with temperatures between $18\text{--}29^{\circ}\text{C}$ and $11\text{--}24^{\circ}\text{C}$ in summer and winter, respectively. We relocated the eggs to one of the Herpetoculture rooms of the Developmental Biology and Herpetology Laboratory, Mizoram University located at Durtlang (23.783°N , 92.725°E , WGS 84; ca. 1210 m asl.). On the same day, we measured the three eggs using dial callipers (Mitutoyo, Model 506–671; ± 0.01 mm) and weighed them using an electronic balance (Gem20 High Precision Digital Milligram Scale, Smart Weigh; ± 0.001 g). We preserved the egg shells in 70% ethanol after the snakes hatched and deposited them in the Departmental Museum of Zoology, Mizoram University (MZMU; accession #s MZMU E2, E3, and E4).

The eggs were carefully maintained in a perforated plastic container on top of 4 cm bedding of coarse sand.



Figure 1. Eggs of *Blythia reticulata* in their natural oviposition site at a forest path, Selesih locality, Mizoram, India (Photo by H. Vanlalhmangaihi). Inset showing habitat of the oviposition site.

During the eggs' incubation period, the room's temperature and humidity were recorded three times per day using HTC-1 LCD Digital Hygrometer Thermometer (temperature accuracy of $\pm 1^\circ\text{C}$, humidity accuracy of $\pm 5\%$). The temperature and relative humidity fluctuated between 20–25 $^\circ\text{C}$ and 73–84%, respectively. The herpetoculture room conditions were comparable to the external environment conditions of the oviposition site located ca. 1.5 km aerial distance northward at similar elevation (ca. 1200 m asl.). We monitored the eggs by visual inspection during the day and by video coverage using a digital camera (Canon PowerShot, SX430 IS) at night. Five days after collection, on 14 September, we found a slit in the first egg at ca. 14:45 h. The neonate completely emerged out from the egg at ca. 15:05 h. We measured snout-vent length, tail length, and weight using the same tools we used for egg measuring on the next day (MZMU N1; Fig. 2A; Table 1). Both other eggs began hatching around the same time at ca. 09:10 h on 15 September (Fig. 2B). The second neonate (MZMU N2) voided the egg at ca. 09:50 h, and the last one completely hatched out at ca. 12:45 h (MZMU N3; Fig. 2C). Hatchlings had smooth glossy black dorsum with faintly visible three broken black parallel lines along the body and tail and dark ventrals with light coloured rims. But, they are without the yellowish-white collar, a character found

Table 1. Neonate biometric data (size in mm; weight in g) of *Blythia reticulata*. Meristics were taken on the left and right side.

Specimen voucher (MZMU)	N1	N2	N3
Egg voucher (MZMU)	E4	E3	E2
Snout-vent length (at hatching)	108.00	105.00	105.00
Snout-vent length (ca. 15 days post-hatching)	111.00	107.00	109.00
Tail length (at hatching)	13.00	15.00	14.00
Tail length (ca. 15 days post-hatching)	13.00	15.00	14.00
Relative tail length (at hatching)	0.107	0.125	0.118
Relative tail length (ca. 15 days post-hatching)	0.105	0.123	0.114
Weight (at hatching)	1.23	1.10	1.06
Weight (ca. 15 days post-hatching)	1.20	1.08	1.04
Eye diameter	0.92	1.08	0.84
Eye-nostril distance	1.48	1.56	1.30
Interorbital distance	1.68	1.86	1.64
Internarial distance	1.21	1.48	1.13
Snout width	1.65	1.74	1.68
Snout length	1.89	2.01	1.73
Head length	5.23	5.65	5.18
Head width	3.23	3.41	3.23
Ventrals	140.00	127.00	128.00
Subcaudals	20.00	26.00	25.00
Dorsal scale rows		13:13:13	
Supralabials		6/6	
Supralabials touching eye		3 rd -4 th	
Infralabials		6/6	
Temporals		1+2/1+2	
Post-oculars		1/1	
Pre-oculars		-	
Anal shields		2	



Figure 2. Eggs of *Blythia reticulata* from Selesih, Mizoram, India. **A.** The first neonate hatching out; **B.** Hatching of the second and third eggs; **C.** The three neonates of *Blythia reticulata*.

in juveniles by Whitaker and Captain (2008). The hatchlings were monitored daily up to the completion of their first ecdysis. The beginning of shedding was indicated by a dull appearance of their skin; the complete shedding lasted for about one week. All hatchlings completed shedding approximately on 29 September 2020. They were typically gentle, timid, and most of the time hid burrowed in the sand although substrate from the oviposition site was provided for hiding. We provided a drinking bowl as well as soaked the hatchlings in water at regular intervals for their hydration, and occasionally sprayed the bedding with water to maintain proper humidity. As the species feeds on

earthworms (Whitaker and Captain 2008), the hatchlings were offered earthworms, termites and snails, but did not feed. Even so, we suggest using eggs and larvae of termites and ants, or perhaps slugs in future feeding attempts.

On 30 September 2020 we anaesthetized the animals using 250 mg/kg of 0.7% sodium bicarbonate buffered MS-222 (Tricaine Methanesulfonate) solution by intracoelomic injection, followed by intracoelomic injection of 0.1 ml unbuffered 50% (v/v) MS-222 solution (see Conroy et al. 2009). Specimens (MZMU N1–3) were fixed in 4% formalin, preserved in 70% ethanol, and then deposited in the Museum of Zoology Department,

Table 2. Eggs measurements (in mm) and weights (in g) of *Blythia reticulata* from this observation and Biakzuala et al. (2020). Specimens were measured by using the dial-calliper to the nearest 0.01 mm, except snout-vent length and tail length which were measured to the nearest of one millimetre with a measuring tape.

Egg voucher	This observation			Biakzuala et al. (2020)				
	Length	Width	Weight	Eggs of MZMU 1424 (n = 7)			Eggs of MZMU 941 (n = 7)	
				Length	Width	Weight (egg shell)	Length	Width
MZMU E2	25.81	11.92	1.87	11.10–20.70	8.2–10.7	0.38–0.48	13.80–16.80	6.60–8.20
MZMU E3	24.38	11.37	1.74					
MZMU E4	26.43	12.08	2.21					

Mizoram University, India. We identified the hatchlings as *B. reticulata* based on Blyth (1854) and Smith (1943). We followed Dowling (1951) for counting ventral scales, and measured 16 morphometric and 10 meristic characteristics on the dead specimens (Table 1). From hatching to after the first ecdysis (ca. 15 days post-hatching), the body length increased slightly, while the body weight decreased (Table 1). This body mass reduction may be attributed to the shedding of old skin as well as the lack of feeding for 15 consecutive days.

The onset of the species breeding season was reported to occur from May to July (Biakzuala et al. 2020). So, we suppose that the incubation period of the eggs lasts between two to four months based on the data from Biakzuala et al. (2020) based on the month of eggs hatching in this observation (mid–September). In this observation, the sizes of eggs recorded were larger than the two clutches of eggs reported previously (Biakzuala et al. 2020) (Table 2). We further speculate that the observation time (early July) in Biakzuala et al. (2020) possibly influenced the susceptibility of incubating eggs to fungal infection as it coincided with the humid and wettest part of the season (Guhathakurta et al. 2020). As decoding the reproductive biology of a species is crucial for understanding their general life history patterns and informing conservation management actions (Hollycross and Goldberg 2001), we encourage more studies on the reproductive biology of other poorly known snakes in the region, for example, the semi-aquatic *B. hmuifang* Vogel, Lalremsanga & Vanlalhrima, 2017, and members of the fossorial natricid snake genus *Trachischium* Günther, 1858.

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Autor(en)/Author(s): Biakzuala Lal, Hruaia Vanlal, Biakhlui Lal, Lalremsanga Hmar
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