

# New Record of *Lycodon liuchengchaoi* in Anhui

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## ABSTRACT

One juvenile and one adult female wolf snake (Colubridae: *Lycodon*) were sampled at Yixian and Fuxi, Huangshan, Anhui, China in the summer of 2011 and 2012, respectively. The two specimens were identified as *Lycodon liuchengchaoi* based on external morphology and molecular data. This is a new reptile record in Anhui Province. In our laboratory, four eggs were laid and three neonates were hatched successfully. This is the first record of the laying and incubation of *L. liuchengchaoi* eggs. The five specimens were deposited at the Museum of Huangshan University (HUM20140001) and Guangdong Entomological Institute (HB-lcfsp12613, HB-lcfsp-ch1~3).

**Keywords:** Reptile; *Lycodon liuchengchaoi*; Incubation; New record

In our routine summer ecological investigations of amphibians and reptiles in Huangshan of Anhui Province, a juvenile wolf snake (Collection number: HUM20140001, Figure 1) was sampled at Yixian (E117°9.112', N29°9.248') on June 24, 2011, and a female adult wolf snake (Collection number: HB-lcfsp12613, Figure 2) was collected at Fuxi (E118°8.191', N30°5.439'; 700 m a.s.l.) on June 3, 2012. The two specimens were identified as *L. liuchengchaoi* (Zhang et al, 2011a). This is the first time that *L. liuchengchaoi* has been found in Anhui, China (Chen et al, 1991; Zhao et al, 1998; Zhao, 2006; Zhang et al, 2011a).



**Figure 1** Juvenile *Lycodon liuchengchaoi* (HUM20140001) sampled at Yixian, Huangshan

A: Dorsal; B: Ventral. Photo by Li-Fang PENG.



**Figure 2** Adult female *Lycodon liuchengchaoi* (HB-lcfsp12613) sampled at Fuxi, Huangshan

A: Dorsal; B: Ventral. Photo by Liang ZHANG.

## EXTERNAL MORPHOLOGY

External morphological examination of the two specimens showed elongated and subcylindrical bodies; head distinct from neck, distinctly flattened; snout projected beyond lower jaw; vertebral ridge poorly developed. When different from HS12088, the features of HB-lcfsp12613 follow in parentheses. HS12088 juvenile (adult female) with snout-vent length: 126 mm (316 mm), tail length: 36 mm (104 mm), tail length/total length: 0.222 (0.248); dorsal scale rows 17-17-15; dorsal scales small and feebly keeled in median several rows close with the ridge (invisible on neck); ventrals 190 (228), angulated weakly; anal entire; subcaudals 75.

Rostral large, triangular, about 1.5 times as broad as deep; nasal divided, nostril in the anterior nasal; internasals subtriangular and slightly broader than long; laterally in contact with nasal; loreals rectangular, contacting orbit; pupil elliptical; preocular 1/1, upward; postoculars 2/2; temporals 2+3/2+3; supralabials 8 (2+3+3)/8 (2+3+3), first supralabial in contact

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with rostral and nasal, second supralabial in contact with nasal and loreal, third, fourth and fifth entering eye, sixth in contact with postocular and anterior temporal, seventh in contact with lower anterior and lower posterior temporal, eighth in contact with posterior temporal; infralabial 8/8. Prefrontals not in contact with orbit but with loreal; prefrontal suture obviously longer than internasal suture; frontal subtriangular, long and wide slightly equal; parietals longer than broad, parietal suture obviously longer than frontal suture.

Body ground color black; crown largely black, except for a yellow crossband across the occipital, faint or incomplete along the midline; 25 (33) jagged-edged, brownish-yellow rings on the body, and 8 (12) on the tail. The width of all rings are 2 to 3 dorsal scales. The yellow rings extend across the belly as wide as 2 to 4 ventral plates. The above-mentioned data are listed in Table 1 and are compared with those of the type specimen (Zhang et al, 2011a) and Shaanxi specimen (Peng et al, 2014). They agreed in characteristics of scales and colored patches.

#### Uncorrected *P*-distance of partial cytochrome *b*

Total genomic DNA was extracted according to the phenol/chloroform extraction procedure (Sambrook et al, 1989). One mitochondrial DNA segment (cytochrome *b*, 832 bp) of the two specimens was obtained by polymerase chain reaction (PCR) and direct sequencing using the primers and methods described in Burbrink et al (2000). The two sequences shared one haplotype (GenBank accession number: KP898899). The uncorrected *P*-distance was 0.8% between this haplotype and *L. liuchengchaoi* (GenBank accession number: Kf732928; Lei et al, 2014). Genetically, they were identified as *L. liuchengchaoi*.

#### Laying and incubation of eggs

All measurements and observations were conducted on live snakes, and the description of the eggs was taken at 90% humidity.

Wolf snakes are oviparous. The adult female snake (HB-1cfs12613) laid four eggs (Figure 3) in our laboratory on June 11, 2012. The eggs were white, smooth-shelled and non-adhesive. Egg weight was 0.77-0.84 g (average 0.805 g), long diameter was 24.8-25.0 mm (24.9 mm) and short diameter was 7.0-7.3 mm (7.2 mm). The ratio of long diameter to short diameter was 3.46.



Figure 3 Four eggs from female *Lycodon liuchengchaoi* (HB-1cfs12613) (Photo by Liang ZHANG)

The four eggs were incubated in our laboratory at room temperature. The temperature ranged from 24 °C to 35 °C in June and July, 2012, at Guangzhou. The eggs were buried in the substrate at near half the volume. Water was sprayed on the substrate once a day to maintain proper humidity. Figure 4 shows the four incubated eggs on the seventh day. On July 28, three neonate snakes were hatched successfully (Figure 5). The period of incubation lasted 48 days. On August 7, all three neonates sloughed their skin (intact) for the first time. Total length of the three neonates was 134.51-147.64 mm (average 141.65 mm), tail length was 32.44-35.94 mm (34.25 mm), tail length/total length was 0.242, and head length was 6.98-8.19 mm (7.68 mm).

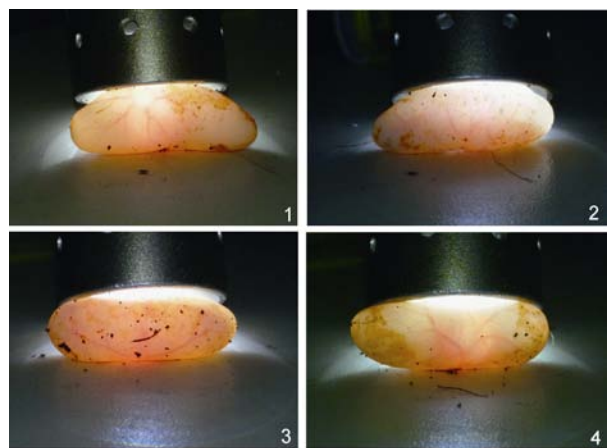


Figure 4 Four eggs on the seventh day of incubation (Photo by Liang ZHANG)

#### DISCUSSION

Huangshan lies in the southern part of Anhui Province. Seventy-eight percent of this region is forested and the level of snake diversity is highest. Among the 51 snake species found in Anhui, 49 species are found in Huangshan, including the newly discovered species described in this paper. (Chen et al, 1991; Chen et al, 2013; Huang, 1990; Huang et al, 2007; Peng & Huang, 2015; Zhao, 2006).

*Lycodon liuchengchaoi* was initially described based on three specimens from Tangjiahe and Monping (Hengduan Mountains) in Sichuan in 2011 (Zhang et al, 2011a). Peng et al (2014) found one female adult *L. liuchengchaoi* from Ningshan (Qinling Mountains) in Shaanxi. The new record sites (Huangshan Mountains, this paper) are nearly 2 000 km from the known distribution localities. There are many suitable habitats available between these sites, and this species might also occur in Henan (Funiushan Mountains) and Hubei (Dabieshan Mountains). As we only compared the cytochrome *b* fragments between our samples and the reported *L. liuchengchaoi*, and observed few differences, it might be desirable to collect more specimens of this species and perform phylogenetic analysis of related species within the same genus. It would also be appropriate to use other DNA markers with a high mutation rate to determine intraspecies genetic diversity, and reconstruct the potential migration route.



**Figure 5** Neonate emerging from its shell (1-6) (Photo by Liang ZHANG)

In recent years, a growing number of small-sized snakes have been found in the field (Chen et al, 2013; Li et al, 2012; Peng & Huang, 2015; Sun et al, 2013; Wang et al, 2015; Zhang et al, 2011b). One possible reason might be the decline of natural small snake predators. For example, some large-sized snakes, which naturally feed on small snakes, have been overhunted in recent years, e.g., *Elaphe carinata*, *Ptyas dhumnades*, *Orthriophis taeniurus*, *Naja atra* and *Bungarus multicinctus*.

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