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First report on the reproduction of captive Chinese many-toothed snake (*Sibynophis chinensis*) in Jeju Island, South Korea

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Abstract

The Chinese many-toothed snake (*Sibynophis chinensis*) is an endangered species in South Korea. To determine its reproductive activity, here we captured a gravid *S. chinensis* from Mulyeongari Oreum Wetland Conservation Area, Jeju Island, on May 27, 2018, and reared it at Animal Taxonomy and Morphology Laboratory, Jeju National University, South Korea. The snake oviposited a clutch of six eggs between June 12 and 20, 2018, with an average size of 24.86 ± 1.36 mm in length and 9.86 ± 0.49 mm in width, which were artificially incubated at a constant temperature of 27 °C. After incubating for 34–41 days, five of these six eggs successfully hatched. Average snout-vent length (SVL), tail length (TL), and body weight (BW) of hatchlings were 136.5 ± 5.17 mm, 47.76 ± 2.80 mm, and 1.66 ± 0.12 g, respectively. This study observed the reproductive activity of *S. chinensis* and characterized its eggs and hatchlings for the first time, providing valuable information for the long-term conservation plan of *S. chinensis* in South Korea.

Keywords: Clutch size, Egg characteristics, Egg-laying, Hatchling, *Sibynophis chinensis*

Background

The Chinese many-toothed (*Sibynophis chinensis*) is a non-venomous serpent native to Jeju Island in South Korea. Its ecology and biology have been poorly documented due to its small population size, limited distribution, and lack of study (NIBR 2011). *S. chinensis* was first documented by Gunther in 1889. It has been reported to be distributed in southern China, Taiwan, Vietnam, Laos, and Jeju Island of South Korea (Gunther 1889; IUCN 2014). In Korea, it is mainly found in lowlands with higher precipitation, very close to human activity (Kim and Oh 2005; Koo et al. 2018a; Koo et al. 2018b).

S. chinensis has a black head with a black stripe running up the dorsal neck to the back of the head. Its body scales are smooth with brighter red on its dorsal side and brilliant white on its ventral. Initially, it was described as *Sibynophis collaris* Paik (1982), but Kim and

Oh (2006) reviewed its taxonomy based on the number and shape of supralabial scales and the number of anterior temporal scales and finally reported it as *S. chinensis*. Several studies have reported its habitat and distribution (Kim and Oh 2005), evolution (Oh et al. 2015), breeding behavior (Koo et al. 2018a), and habitat prediction (Koo et al. 2018b); however, its reproductive biology remains unknown. We provide data on morphology of eggs, clutch size, incubation period, and hatchlings size of *S. chinensis* under captive condition. The present study will be the first contribution to the reproductive biology. It will help us maintain its captive populations that can ultimately raise the category of this endangered species in this country.

Methods

Snake capture and raising

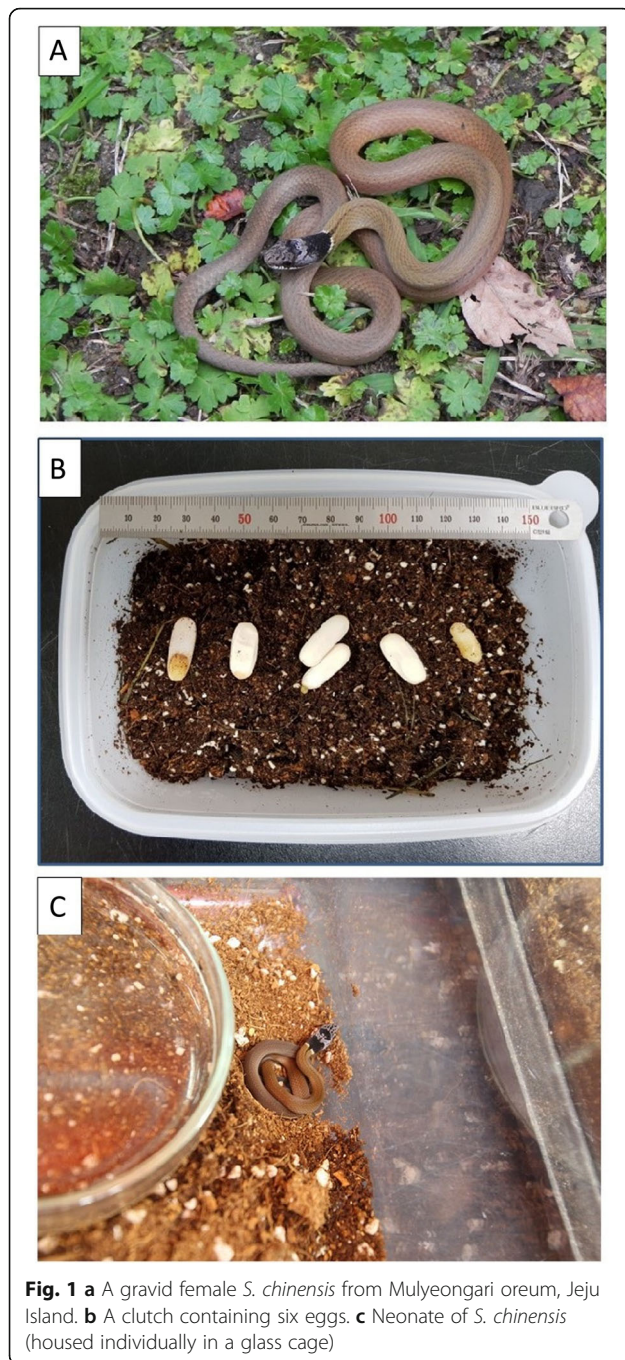
A gravid snake (Fig. 1a) was spotted accidentally and hand captured (using gloves) in Mulyeongari-oreum (“Oreum” = hills of Jeju Island) Wetland Conservation Area (33° 22′ 57″, 126° 42′ 42″) on May 27, 2018, located in Jeju Island, South Korea. It is a Ramsar site designated in 2006 (MOE 2009). The snake was measured to

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have a snout-vent length (SVL) of 460 mm, a tail length (TL) of 176 mm, a head length (HL) of 15.70 mm, and a body weight (BW) of 44.8 g. It was carried to the Animal Taxonomy and Morphology Laboratory of Jeju National University and kept inside a glasshouse ($45 \times 53 \times 45 \text{ cm}^3$). It was provided rocks and wooden logs. An ambient temperature was maintained using a heat lamp (CE 220–230 V, China) (temperature range 18 to 24 °C). Salamanders (*Hynobius spp.*) and grass lizard (*Takydromus wolteri*) (weight approx. 68–84 g) were offered as diet

once every 3–4 days. Servicing of the glasshouse consisted of removing fecal matter, providing water daily, and spraying with water to maintain humidity to be around 70–90%. The snake was observed to drink from water bowls of 8 cm in diameter filled to 5 cm in depth. In addition, rocks and wooden logs facilitated the shedding of its skin.

Morphology measurement

The gravid female was inspected regularly until oviposition. Shortly after oviposition, we weighted the body mass and eggs using an electronic balance (Quintix 3102-1SKR, Germany) of 0.1 g precision and simultaneously measured the width and length of eggs using a digital caliper CD-15CPX (Mitutoyo, Monotoro, Japan) with 0.1 mm precision. Eggs were separated from the mother and incubated in an automatic incubator (Green30m, Seoul, Korea) with temperature maintained at 25–28 °C and humidity at 70–92%. Within 34–41 days of incubation, eggs hatched out. Hatchlings were kept separately in plastic boxes $29 \times 14 \times 23 \text{ cm}^3$ with a bottom heating. Each box had a small water bowl together with pebbles, leaf litters, and branches to provide a relatively natural environment. These maintenance conditions were similar to those of adult snakes. Mealworms (*Tenebrio molitor*) were offered once every 2–3 days which they refused to feed. The interval between hatching and the first molt of hatchlings was 7–9 days. After complete molting, external morphologies of hatchlings including snout-vent length (SVL), tail length (TL), head length (HL), head width (HW), and the body weight (BW) were measured (Table 1). No attempt was made for sex determination due to the lack of skilled person for sex detection at juvenile stage. Finally, on August 6, 2018, 11 days after hatching, all hatchlings and their mother were released to the place from where the mother was previously captured.

Result and discussion

Eggs of *S. chinensis* were white, elliptical, elongated, and non-adhesive, having soft flexible shell except the last laid egg which appeared infertile because of its atypical wet appearance and semi-transparent yellowish coloration (Fig. 1b). Average length, width, and weight of egg were $24.86 \pm 1.36 \text{ mm}$, $9.86 \pm 0.49 \text{ mm}$, and $1.43 \pm 0.15 \text{ g}$, respectively (Table 1). Due to the absence of peer-reviewed published data on the reproductive behavior of *S. chinensis*, we compared eggs and clutch size with species using similar habitats. *Oxyrhopus clathratus* and *Xylophis perroteti* laid larger eggs with length of $37 \pm 6.2 \text{ mm}$ to width of $19.5 \pm 2.5 \text{ mm}$ (Maia and Travaglia-Cardoso 2017) and length of $31 \pm 3.4 \text{ mm}$ and width of $12.6 \pm 1.3 \text{ mm}$ (Santoshkumar and Kannan 2016) compared to *S. chinensis*. These studies reported that *O.*

Table 1 Data on egg and newborns of *Sibynophis chinensis* biometrics

Individual ID	Traits of egg					Traits of newborn				
	EL (mm)	EW (mm)	WE (g)	IP (days)	HD (min)	SVL (mm)	TL (mm)	HL (mm)	HW (mm)	BW (g)
BBR01	28.16	9.12	1.6	41	240	122	46	9.42	4.97	1.7
BBR02	25.04	11.52	1.6	34	180	142	45.6	10.03	5.08	1.8
BBR03	27.36	10.27	1.6	34	180	152	58	10.02	4.14	2.03
BBR04	25.10	9.58	1.6	35	240	137.2	41.1	10.01	4.71	1.3
BBR05	24.86	10.60	1.6	36	270	129.3	48.1	9.8	4.91	1.5
BBR06*	18.69	8.07	0.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mean \pm S.E.**	24.86 \pm 1.36	9.86 \pm 0.49	1.43 \pm 0.15	36 \pm 1.3	222 \pm 18	136.5 \pm 5.17	47.76 \pm 2.80	9.85 \pm 0.12	4.76 \pm 0.18	1.66 \pm 0.12

EL egg length, EW egg width, WE weight of egg, IP incubation period from eggs laying to time when the neonates begin to slit the shell, HD hatching duration

*n.d. represents no data, this egg did not hatch (probably infertile egg)

**Mean and S.E. from only 5 individuals, except the first three columns

clathratus laid a clutch of 10–11 eggs and *X. perroteti* laid a clutch of 7–10 eggs, larger than the clutch size of *S. chinensis* (i.e., six). The clutch size of *S. chinensis* was similar to the clutch size of *Sibynophis subpunctatus* (Whitaker et al. 2004). The smaller clutch size and eggs size of *S. chinensis* could be due to its smaller body size compared to other species. The clutch size and egg size are known to depend on maternal characteristics such as body size and age (Roff 1992; Paterna 2017)

After 34 days of incubation, eggs began to hatch, although one egg remained unfertilized. The hatching process took an average of 222 ± 18 min to fully emergence from slitting eggshell. The body color of hatchling was brownish at the anterior region and grey at the posterior region (Fig. 1c). A distinct black-colored mid-dorsal line was present from the head to the tail region. The mid-dorsal line was dense at the anterior region and faint at the posterior region. The color morphology was similar to that of the mother, although hatchlings were brighter compared to their mother. The head was triangular with two rounded black eyes. The white ring was present at the lower part of the head.

When the incubation period and hatchling size of *S. chinensis* were compared to those of related species, *Gonyosoma boulengeri* and *Ptyas korros* had longer incubation period of 52–57 days (Kane et al. 2017) and 55 days (Dieckmann et al. 2014) for their eggs compared to *S. chinensis* (34–41 days, this study). *G. boulengeri* and *P. korros* also had large hatchling sizes (7.75–10.5 g and 6–8 g, respectively) compared to hatchlings of *S. chinensis* (1.3–2.03 g in this study). The short incubation time of *S. chinensis* could be due to the relatively small weight of hatchlings compared to *G. boulengeri* and *P. korros*. In general, difference in habitation and environmental condition may induce different morphological features of different species (Seebacher et al. 2016). The *S. chinensis* found in Jeju Island inhabits in a subtropical region, which is one of the coldest areas of *Sibynophis* habitats. Due to the relatively long winter seasons, its annual

activation time is shorter than those of tropical snakes, which might have affected its morphological and ecological characteristics including hatchling size and incubation period.

Our observation revealed that the female of *S. chinensis* oviposits at mid-June and hatched out at the end of July (i.e., summer season). Marques and Pizzatto (2002), Lin et al. (2012), and SantoshKumar and Kannan (2016) have reported similar patterns of egg laying and hatching of *Oxyrhopus guibei*, *P. korros*, and *Xylophis perroteti*, respectively. This captive egg laying in summer might be the result of two mating seasons (i.e., autumn and spring). Koo et al. (2018a) have observed group mating between one female and three males in autumn. From this evidence, we hypothesized that the sperm received in autumn mating could be stored in the oviduct and remained viable during winter dormancy period. In early spring, sperms are expelled out from the oviduct to fertilize with oocyte shortly after subsequent spring mating. Many reports have explained that reptiles have two distinct mating seasons prior to ovulation, e.g., *Thamnophis sirtalis* in Michigan, *Bothrops neuwiedi* in Brazil, and *Crotalus atrox* in Arizona (Schwartz et al. 1989; Hartmann et al. 2004; Clark et al. 2014). Fertilization in spring ensures that eggs are laid in the middle of rainy seasons. Thus, more eggs will be incubated during warmer and wetter months. Despite of the previous observation of autumn mating in this species (Koo et al. 2018a), we could not completely exclude the possibility of spring mating at the moment.

Based on our observation, before egg laying, only one instance of feeding was observed for the mother. Decrease in feeding by gravid snakes is partly related to reduced locomotor speed and endurance, which might compromise their ability to catch a live prey (Seigel et al. 1987; Gregory 2009). After oviposition, the female displayed a more active feeding behavior to a maximum of 14 records until release into the wild. It is probably due to the accumulation of lipid reserves in fat bodies, which

is related to vitellogenesis in females (Girons 1982). Once this takes place, a new breeding cycle begins (Solorzano and Cerdas 1989).

To the best of our knowledge, this is the first study to report the reproductive biology of *S. chinensis* from egg laying to hatching. This study is limited by its sample size. To establish an accurate reproductive activity of *S. chinensis*, it is necessary to analyze large samples in the wild.

Abbreviations

BW: Body weight; HL: Head length; HW: Head width; SVL: Snout-vent length; TL: Tail length

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Authors' contributions

MB and YHJ participated in raising the specimen. MB and YKM drafted the manuscript. SHH and HSO edited the manuscript draft. BSK provided specimen along with technical support. All authors read and approved the final manuscript.

Authors' information

Not applicable

Availability of data and materials

The specimen during the current study is available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Authors receive permission from "Yeongsan Gang Regional Environmental Office" for handling and capturing the specimen, without harm.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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