

## CAPTIVE BREEDING AND REPRODUCTIVE BIOLOGY OF THE *Protobothrops cornutus* AND *Triceratolepidophis sieversorum*

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New data on the reproductive biology as well as results of the first captive breeding of very rare Asian pitvipers from Truongson Mountains: *Protobothrops cornutus* and *Triceratolepidophis sieversorum* are given. Characteristics of the reproductive biology of *Protobothrops cornutus* and *Triceratolepidophis sieversorum*, which are sympatric in Truongson Mountains (karst regions of Quang Binh Province) are discussed in the present paper. The data on copulation, gestation and incubation, clutches, size and weight of newborn snakes, temperatures during hibernation and the activity periods, temperature of incubation, a diet during different seasons for different age groups are given in the result of a successful breeding in the laboratory. The descriptions of size of eggs and structures of a shell are provided.

**Keywords:** rare snakes, *Protobothrops*, *Triceratolepidophis*, Annamite Mountains, reproductive biology, captive breeding, Vietnam.

### INTRODUCTION

The interest to taxonomy and various aspects of biology of Asian pitvipers is now increasing. During the last decade many new species from the Southeast Asia were described, the composition and status of genera were specified and elements of biology were studied (David et al., 2001; Grismer et al., 2006; Herrmann et al., 2002; Malhotra and Thorpe, 2004; Orlov et al., 2001, 2002, 2003, 2004; Vogel, 1991; Ziegler and Herrmann, 2002). Some elements of the reproductive biology of snakes are very complicated or even impossible for observing in the wild. It is connected with a secretive way of life, low density of a population or a great rarity of the species. Characteristics of the reproductive biology of rare snakes *Protobothrops cornutus* and *Triceratolepidophis sieversorum*, which are sympatric in Truongson Mountains (Annamit Mountains, karst regions of Quang Binh Province) are discussed in the present paper. The

data on mating, gestation and incubation, clutches, size and weight of newborn snakes, temperatures during hibernation and activity periods, temperature of incubation, a diet during different seasons for different age groups are given in the result of a successful breeding in the laboratory. The description of size of eggs and structure of a shell are provided.

### MATERIAL AND METHODS

Different pitvipers including 3 adult males (L 913 – 998 mm, Lcd 193 – 232 mm, weight of 200 – 230 g) and 1 adult female (L 1190 mm and Lcd 213 mm) of *Triceratolepidophis sieversorum* as well as 4 adult males (L 520 – 610 mm, Lcd 142 – 151 mm, weight of 49 – 65 g) and 4 adult females of *Protobothrops cornutus* (L 534 – 610 mm, Lcd 113 – 141 mm, weight of 62.5 – 103 g) have been brought in 2003 – 2004 from karst areas in Quang Binh Province, Vietnam (at the elevation 200 – 600 m) to Tula Exotarium.

All individuals of 7! *sieversorum* and *P. cornutus* were kept separately in plastic boxes (= terrariums) with the sizes of 600 x 350 x 350 mm, except for the female of *T. sieversorum* which was kept in a terrarium with the sizes 1000 x 700 x 700 mm. The boxes were equipped with humid shelters, water dishes, crossbeams for com-

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Fig. 1. Adult male of *Triceratolepidophis sieversorum* in primary rainforest in Phong Nha - Ke Bang National Park, Quang Binh Province, Central Vietnam.

fortable sitting, bottom heating in the back part. The laboratory was equipped with the adjustment of the photoperiod, and thermoregulation devices providing presence of a daily and seasonal temperature gradient. Repeated puttings together of sexual partners were made only during the reproduction season each time for several hours. In the boxes for *P. cornutus*, taking into consideration their habitat associated with karst, calcareous stones about 250 mm in diameter have been placed.

## RESULTS

### Captive breeding of *Triceratolepidophis sieversorum* Zigler, Herrmann, David, Orlov et Pauwels, 2000 (Fig. 1)

Immediately after putting of the snakes into the terrariums the males have started to eat small laboratory

mice with the weight of 12 - 20 g, the female — mice and rats with the weight up to 60 g. In the terrariums the snakes showed semiarboreal mode of life.

From 01/14/2004 until 03/20/2004 hibernation has been organized at an average temperature +15°C and duration of light day of 8 h. At the beginning of the hibernation (01/19/2004) a mating of the male No. 2 and the female No. 1 has been noted. Putting together of the partners have been made before switching-off of light in the laboratory. Copulation began at 17:30 and continued already in the darkness for 2 - 2.5 h, at temperature +15 - 16°C.

After the termination of the hibernation the female ate mice and small rats twice (03/24 and 04/02); after that it has ceased to eat and in the further showed signs of gestation (strong increase of follicles). However in 2004 oviposition has not occurred and the increased follicles were resorbed. In 2004 female has eaten for the third and the last time on 09/27. After that the colder hibernation has been offered for *T. sieversorum* from 10/30/2004 until 02/08/2005 at temperature +10 - 15°C. Copulations of snakes that time have not been noted. The female for the first time after receiving in the autumn of 2003 had shedding on 04/06/2005. In 2005 it refused to feed, that is why we 8 times used force-feeding with mice (from March until July). Hibernation of 2005 - 2006 was continued for the female from 11/17/2005 until 02/18/2006 under the photoperiod of 8 h.

Intensity of feeding of 3 adults males of *T. sieversorum* was very low during 3 years in the laboratory (2004 - 2006): the males fed from 3 up to 8 times per year ( $\bar{x} = 5.25$ ). Feeding activity was nocturnal, during every feeding snakes ate 1 - 4 laboratory mice with the weight 12 - 30 g each. Probably such intensity of alimentary activity is normal for males of *T. sieversorum*.

The beginning of feeding in spring is very late; it takes place not earlier than 1 - 2 months after the termination of hibernation and start of heating. In May - August the intensity of alimentary activity is maximal (more than 70% of feeding acts). The molt of adults males occurs once a year, as a rule, in the spring, in April - May (5 registered cases) or in first half of summer (2 registered cases). One of the males in 2004 did not slough as well as a the female.

The diurnal temperature was kept in summer of 2005 at a level +25 - 26°C and 30 - 32°C in a hot spot, at night -- +24 - 25°C. The light day was continued for 11 h; an everyday spraying humidity of air was 80 - 95%. In October there was a decrease of temperature and by November 9 the diurnal temperature was kept at a level +24 and +30°C in a hot spot. Nocturnal



Fig. 2. Female of *Triceratolepidophis sieversorum* on the clutch.



Fig. 3. Hatching of *Triceratolepidophis sieversorum* from the eggs.

temperature was +20 - 23°C, light day duration was reduced up to 9 h.

On 11/17 - 19/2005 the heating devices have been switched off. The values of diurnal and nocturnal temperatures were equalized. During the next 3 months hibernation temperature was changed within the limits of +7 - 18°C, on the average it has made 13.9°C (the ratio of total daily temperature to quantity of days of hibernation), light day was reduced up to 8 h.

The first display of sexual activity of male No. 3 was noted at night of 12/28/2005. Copulation of the male No. 3 and the female was registered during 2 h 20 min on 01/11/2006. Mating and copulation occurred on the floor of the terrarium, during the copulation the female was constantly holding its tail lifted vertically upwards. The temperature during copulation was +14.5 - 15°C.

Putting together of the males Nos. 1 - 3 with the female were carried out under a strong observation control. Copulation on January 11 was the only one.

The termination of the hibernation has occurred on 02/18/2006. In March - May at night the temperature was +18 - 22°C, in March in the daytime — +22 - 24°C, and in May — +23 - 26°C (up to +30° on the hot spot). Light day by the beginning of June has been increased up to 11 h and kept in this regimen until October.

In 2 months after copulation, on 03/14/2006, the weight of the female was 460 g. At that moment it had the signs of pregnancy and did not start to eat. The force-feeding has been used; within 1 month 5 force-feedings with small rats and mice with the weight 5 - 10 g each (33 feeding items in the total) have been made. The date of the last force-feeding was 04/19/2006. On 05/06/2006 the female shed and in 15 days (05/21/2006) it laid 12 eggs. Duration of pregnancy was registered as 130 day. During the last days before

the oviposition (as it happens in many snakes) the female was actively moving in the terrarium.

After the egg-laying 11 eggs were situated inside of the body coils of the female protecting the clutch, and one egg lay beside (Fig. 2). The eggs are oblong, light gray, weakly pigmented, with semi-transparent membranes studded with calcium "stars" (Fig. 3).

After egg laying the female actively ate: she has taken a food 12 times from 05/24/2006 until the beginning of wintering (1 - 3 adult mice with the weight 20 - 30 g at one feeding). On 10/17/2006 the shedding was registered. The weight of the female was 580 g by January 15, 2007.

The size and weight of the eggs: 1) 34.7 x 22.6 mm, 10.2 g; 2) 34.5 x 22.2 mm, 10.0 g; 3) 35.8 x 22.2 mm, 10.1 g; 4) 38.4 x 21.7 mm, 10.6 g; 5) 34.4 x 22.3 mm, 10.0 g; 6) 35.0 x 22.5 mm, 10.15 g; 7) 36.5 x 22.0 mm, 10.35 g; 8) 37.2 x 21.7 mm, 9.95 g; 9) 35.3 x 23.0 mm, 10.5 g; 10) 38.9 x 21.5 mm, 10.65 g; 11) 40.2 x 22.1 mm, 11.35 g; 12) 39.8 x 21.8 mm, 11.0 g.

The incubation was conducted at diurnal temperature +25 - 28.5°C, and nocturnal +25 - 26.5°C on moist vermiculite. The first 2 newborns came through on 07/20/2006, the hatching process continued until 07/23/2006. The incubation lasted 60 - 63 days. 91.7% of the offspring were alive and one of the newborns cut the membrane but has not left the egg and has died. Sex ratio of the clutch is 4:8 (Table 1).

Young *T. sieversorum* nearly do not differ from adults in the background color and color pattern. Many of them have brighter and precisely expressed yellow border, limiting the color pattern of the dorsum (Figs. 4, 5). The processes of scales in the form of "horns" above eyes in adults stick out upwards whereas in the newborns they hang above eyes, being bent downwards



Fig. 4. Juvenile *Triceratolepidophis sieversorum* in the age of 4 months.



Fig. 6. Adult female of *Protobothrops cornutus* from Phong Nha - Ke Bang National Park, Quang Binh Province, Central Vietnam.



Fig. 5. Juvenile coloration of *Triceratolepidophis sieversorum*.

(Fig. 3). Sexual dimorphism in the coloration is not expressed.

The newborns are kept separately, in plastic boxes 24 x 12 x 8 cm with a bottom heating in the back part, with small branches and a drinking bowl. The conditions of the maintenance are similar to those for adult individuals.

The first molting of newborns was noted on 07/29 - 08/01, i.e., in 9 - 11 days after appearance from the eggs. After the molting all newborns demonstrated semiarboreal mode of life. First 3 individuals have eaten in a month after hatching, the other three — in 2 - 2.5 months after hatchlings. As a starting food for 4 newborns *T. sieversorum* have chosen small brown frogs (*R. temporaria*), for 2 individuals — juvenile sand lizards (*L. agilis*), all of them have eaten in the autumn of 2006 only 1 - 2 times. The remaining 5 individuals have not started to eat. From 11/10/2006 for all juvenile

**TABLE 1.** Size and Weight of Newborn *Triceratolepidophis sieversorum*

Sex	SVL, mm	Lcd, mm	SYL - Lcd, mm	SVL/Lcd	W, g
tiM	274	58.5	332.5	4.68	8.3
♂ 2	290	59	349	4.92	7.85
♂ 3	282	63	345	4.48	7.65
♂ 4	288	60	348	4.80	7.9
♀ 1	282	57	339	4.95	7.85
9 2 (dead)	270	52	322	5.19	7.0
9 3	289	53.5	342.5	5.40	8.05
9 4	288	57	345	5.05	7.8
9 5	275	52	327	5.29	8.1
9 6	286	55	341	5.20	7.8
♀ 7	282	59	341	4.78	7.8
9 8	287	54	341	5.31	8.5
min - max	♂ ♂ 274 - 290 ♀ ♀ 270 - 289	♂ ♂ 58.5 - 63 ♀ ♀ 52 - 59	322 - 349	♂ ♂ 4.48 - 4.92 ♀ ♀ 4.78 - 5.40	7.0 - 8.5
X	282.75	56.7	339.4	cfcf 4.72 995.15	7.88



Fig. 7. Head of adult *Protobothrops cornutus*.



Fig. 8. Mating of *Protobothrops cornutus*.

*T. sieversorum* hibernation has begun at temperature +10 – 18°C.

#### Captive Breeding of *Protobothrops cornutus* (Smith, 1930) (Figs. 6, 7)

*P. cornutus* in the laboratory have a semiarboreal mode of life. They spend a lot of time on the lime stones which are locally warmed up during the day up to temperature +28 - 32°C. Snakes usually seized their prey from above — from the crossbeams imitating the lower branches of a tree, or from a stone. The preferred food items were laboratory mice with the weight 10 - 15 g and nestlings of quail (*Coturnix coturnix*).

The males ate more rarely than the females — from 6 up to 16 times per year [according to our observations for 4 adults males during 3 years (2004 - 2006).  $x = 9.7$ ]. The breeding females ate from 12 up to 18 times per year ( $x = 15.4$ ). The peak of the alimentary activity of the males corresponds to the spring months (56% of the annual diet), for the summer — 30% of the annual diet, for autumn — 14%. For males a later beginning of the feeding activity (not earlier than March) is typical as well as an earlier stopping of the feeding activity (in September - October).

The females were eating more regularly, with a less expressed spring peak (43% of the annual diet), during the summer months they consume 26% of the annual diet, in the autumn months they consume 31%. The beginning of feeding is earlier than that of males, often since February. Females cease to eat later than males, at the end of September - November. Adult males and females shed 1 - 2 times per year.

In November - December, 2003 it was conducted a preparation for hibernation of 5 snakes (4 males and 1 female): decrease in temperature (especially night), reduction of light day (from 11 to 8 h from 11/14/2003

to 01/01/2004). The average temperature in the hibernation chamber was +15°C, air humidity 78 - 85%. In a week after switching-off of the heating devices (01/21/2004) the copulation of male No. 1 and female No. 1 has been noted. After a short courting, a copulation started at 18:40 was taking place during the dark time of the day at temperature +16°C. During the copulation the male has crept on a piece of limestone in the box, the female was lying constantly on the floor almost motionlessly. Duration of the copulation was 4 h 30 min. After the copulation the female has move to the crossbeams in the upper level of the box. This copulation of female No. 1 was the only copulation observed in 2003, and afterwards its fertility has proved to be true.

In 2004 the hibernation lasted a little more than 2 months (until 03/20/2004). On the third day after termination of the hibernation the gravid female has eaten two small mice with the weight 5 g each. On 04/30/2004 the female shed. In May it ate twice mice with the weight 20 g. The summer regimen (temperature, light day) have been introduced from the middle of May. Last time the female fed 69 days before the oviposition (05/19/2004): the last shedding took place 3 weeks before the oviposition (07/06/2004).

The female laid 1 fertile egg (37.6 x 18.2 mm and 6.65 g) and 3 unfertilized small eggs on 07/27/2004 into the shelter with moist vermiculite. The fertile egg was incubated in vermiculite at diurnal temperature -26 - 28°C, night temperature -24 - 25°C, and humidity of 90 - 95%. In August - October the female 9 times ate mice with the weight 15 - 20 g. The period of pregnancy lasted 118 days, 58 days of which corresponded to the period of the hibernation. Weight of an egg a week



Fig. 9. Female of *Protobothrops cornutus* on the clutch.



Fig. 11. Juvenile *Protobothrops cornutus* in the age of 3 months.



Fig. 10. Clutch of *Protobothrops cornutus* 06/11/2005.

before hatching was 7.8 g; the incubation period lasted 42 days. The newborn male (L 215 and Lcd 50 mm, weight 4.8 g) cut the egg membrane and has left the egg on 09/07/2004.

On 04/21/2004 5 additional females have been brought to the laboratory: 3 of them were gravid (females Nos. 3 - 5). In April - May they ate mice with the weight 10 - 15 g each 4 - 6 times, having stopped to eat 49 - 65 days prior to oviposition. Shedding before egg-laying was observed 21 - 25 days before oviposition.

Active movements of females were observed 10 - 15 days prior to oviposition. Egg-laying always took place at night in a specially prepared shelter with moist vermiculite and moss (Figs. 9, 10). The females always protected their clutches (Fig. 9). The temperature inside the shelter was kept at a level +26 - 30°C, at night — 24 - 25°C, humidity — 90 - 95% and higher. The dates of egg-layings of these three females are: 07/03 - 23/2004 (Table 2).

Young *P. cornutus* do not differ from adults in the color patterns. However many of them are much brighter than adults (Fig. 11). Scales in the form of "horns" above the eyes, sticking out upwards in adult individuals, in newborns hang above the eyes, being bent downwards. During the first weeks of a life after hatching the horns are gradually straightened and accepted the position, usual for the snakes of this species. The newborn snakes were kept separately, each in an individual plastic box 240 x 120 x 80 mm with the bottom heating in the back part of the box, with branches, a small flat stone located on the heating device, and with a drinking bowl.

The interval between hatching and the first molt of *P. cornutus* was 8 - 10 days ( $n = 10$ ). In 4 days after the first molt 5 snakes have already started to eat juvenile lizards (*Zootoca vivipara*) and frogs (*Rana temporaria*).

TABLE 2. Data on Clutches of *Protobothrops cornutus* (2004)

No. of female	Number of eggs in clutch	Total weight of clutch, g	Average weight of fertile egg, g	Size of egg, mm
1	1 + 3 non-fertile	—	6.65	37.6 x 18.2
3	4	26.67	$x = 6.67 (n = 4)$	37.6 x 17.0, 35.3 x 17.6, 39.2 x 16.7, 36.6 x 17.4
4	1 + 2 non-fertile	—	6.88	41.2 x 16.3
5	4	26.20	$x = 6.55 (n = 4)$	40 x 16.9, 37.6 x 17.1, 39 x 16.6, 35 x 16.9

3 other newborn snakes for the first time have eaten in 9 – 20 days after the molt, juvenile lizards (*Zootocavivipara* and *Lacerta agilis*) were used as starting food. The remaining 2 young snakes have not started to eat by themselves. After one month of force-feeding with newborn mice and lizards they shifted to a normal feeding.

Young *P. cornutus* have ceased to eat in the first half of November, having eaten by that time from 6 up to 10 times. The average interval between the feedings was 8 days.

At the age of 3 months the total body length of *P. cornutus* was 300 - 349 mm ( $x = 325$  mm,  $n = 10$ ), i.e., has increased on the average on 25%; the weight was 6.45 - 8.5 g ( $x = 7.68$ ,  $n = 10$ ), i.e., has increased on 56%. From 11/21/2004 till 02/08/2005 the hibernation has been conducted at +11 - 15°C. After termination of the hibernation the snakes have started to eat in March, basically by small frogs (*R. temporaria* and *R. arvalis*).

In June - July, 2005, only 3 males were eating mice with the weight 3 - 5 g. The other individuals shifted to the feeding on rodents only after the next hibernation, in April, 2006, at the age of about 1.5 years. They preferred at first *Mastomys coucha*, at the third feeding they ate laboratory mice. *P. cornutus* have reached the sizes of adult individuals at the age of 2 years; in the autumn 2006 the males weighed 40 - 70 g, the females – 94- 101 g.

It is interesting to note that the female No. 4 on 06/11/2005 has laid a clutch without a copulation in the year 2005 with 5 fertile eggs (the gravid female has been received on 04/21/2004 and laid eggs on 07/03/2004, Table 3). This fact indicates that females of this species are capable to a prolonged sperm storage. The female has refused to feed 63 day before oviposition and shed 17 day before egg-laying (05/25/2005). The sizes (32.2 - 36.2 x 18.1 - 19.3 mm) and weight (6.85 - 7.3 g,

TABLE 3. Size and Weight of Newborn *P. cornutus* (2004 - 2005)

No, female, and year of oviposition	Sex	SVL, mm	Lcd. mm	SVL-Lcd, mm	SVL/Lcd	W, g
No. 1, 2004	cf	215	50	265	4.30	4.80
No. 3, 2004	Cf	206	47.5	253.5	4.34	4.75
	cf	212	49	261	4.33	5.10
	♀	212	44	256	4.82	5.05
	9	211	42	253	5.02	4.85
No. 4, 2004	cf	221	50	271	4.42	5.10
No. 5, 2004	cf	215	48	263	4.48	4.95
	cf	210	47.5	257.5	4.42	4.55
	9	223	42.5	265.5	5.25	5.10
	♀	222	41.5	253.5	5.11	4.75
So. 4. 2 <sup>+</sup>	♂	218	51	269	4.27	5.05
	♂	212	47.5	259.5	4.46	4.95
	♂	208	47	255	4.43	4.75
	♂	218	49	267	4.45	4.80
	♀	217	47.5	264.5	4.57	5.05
min-max	♂♂	206 - 221	Cf ♂ 47 - 51	253 - 271	♂♂ 4.27 - 4.48	4.55 - 5.10
	♀♀	21 1-223	♀♀ 41.5 - 47.5		♀♀ 4.57 - 5.25	
$\bar{x}$		214.0 (n = 15)	46.93 (n = 15)	260.93 (n = 15)	Cf cf 4.39 (n = 10) ♂♂ 4.95 (n = 5)	4.91 (n = 15)

TABLE 4. Data on Clutches of *Protobothrops cornutus* (2004)

No. of female	Description of eggs in the clutch	Duration of incubation in days	Date of hatching
1	Egg shell is semitransparent, light-gray, with numerous dark-gray points and lines, with numerous calcium "stars"	41	09/06/2004
3	Egg shell is semitransparent, light-gray, with numerous dark-gray points and lines, with numerous calcium "stars"	40-41	08/16 - 17/2004
4	Egg shell white, without pigmentation and calcium "stars"	41	08/14/2004
5	Egg shell white, without pigmentation and calcium "stars"	40-41	09/1 - 2/2004

$x = 6.98$ ) of the eggs are very similar to those for clutches of 2004. At an average diurnal temperature +26°C and nocturnal temperature +25°C the incubation lasted 54 - 55 days. The hatching of the young snakes occurred on 08/4 - 5/2005 (Table 3). As a starting food juvenile frogs (*R. temporaria*) and lizards (*L. agilis*) were used, but transition to mice has been carried quickly out. In this case, at the age of one year the weight of snakes made 15 — 36.5 g ( $x = 25.8$ ,  $n = 5$ ).

It is necessary to note, that the weight of the adult females for three years of the maintenance has increased up to 95 - 140 g ( $x = 111$  g,  $n = 5$ ), the sizes and weight of the adult males have not changed.

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