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Neotype designation and redescription of *Gloydius shedaoensis qianshanensis* Li, 1999 (Squamata, Serpentes, Viperidae)

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Abstract

The taxonomic status of *Gloydius shedaoensis qianshanensis* Li, 1999 has long been debated due to its narrow distribution range, low population, loss of type specimens, and lack of detailed descriptions. Neotypes were designated based on newly collected specimens in the Qianshan Mountain area, detailed comparisons between *G. s. shedaoensis*, and *G. s. qianshanensis* were recorded, while we redescribed *G. s. qianshanensis*. PCA results based on the external morphology of the two subspecies of *G. shedaoensis* demonstrate the inter-population distinction of *G. s. shedaoensis* and *G. s. qianshanensis*, including head length, and dorsoventral-postorbital stripe width. Additionally, the ecological data of *G. s. qianshanensis* were recorded as supplements while providing further conservation strategies.

Key Words

Gloydius shedaoensis, Liaodong Peninsula, Pit viper, Qianshan Mountain, Snake Island of China, subspecies

Introduction

Pallas (1776) first reported the Asian pit viper under the name *Coluber halys* Pallas, 1776. Later placed in the genus *Agkistrodon* Palisot de Beauvois, 1799. Hoge and Romano-Hoge (1978/1979 "1981") distinguished the Asian species of pit vipers from *Agkistrodon* on the basis of morphological characteristics including bones, and scales, and described the new genus *Gloydius* Hoge & Romano Hoge, 1978. The genus *Gloydius* contains 24 known species and is mainly distributed in Asia. Based on morphological characters, the genus *Gloydius* contains three clades: the *G. blomhoffii* group, *G. halys-intermedius* group, and *G. strauchi* group (Orlov and Barabanov 1999; Zhao 2006; Shi et al. 2017, 2018, 2021).

Zhao (1979, 1980) described *Agkistrodon shedaoensis* Zhao, 1979 based on the ecological and morphological characteristics of the pit vipers from Snake Island,

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Liaoning Province, China. This perspective was accepted by the following studies (Guo et al. 1999; Li 1999; Shi et al. 2016). Ji et al. (1987) first reported a pit viper species population distributed in the Qianshan Mountain Range of the Liaodong Peninsula with morphological similarities to *A. shedaoensis*, and placed it in the subspecific status of *A. saxatilis* (Emelianov, 1937) as *A. s. shedaoensis* Ji, 1987. With morphological comparisons and snake venom electrophoresis, Li (1999) considered the population from Qianshan Mountain as a subspecies of *Gloydius shedaoensis*, with the nomenclature *G. s. qianshanensis* Li, 1999. However, Li's taxonomic perspective was not accepted widely by peers. For example, the subspecific divergence of *G. shedaoensis* was not mentioned in Zhao (2006), but its geographical distribution was recorded as "The Snake Island near Lushun, Dalian; ? Qianshan Mountain, Anshan City". Shi et al. (2016) confirmed the validity of *G. s. qianshanensis* based on a sample from Wafangdian City, Liaodong Peninsula through morphological comparison and molecular systematics. Wen et al. (2021) considered the distribution of *G. shedaoensis* should not be limited to Snake Island, but the record of the *G. shedaoensis* population from the Liaodong Peninsula remains cryptic.

The taxonomic status of *Gloydius shedaoensis qianshanensis* is being debated due to the lack of detailed descriptions and image documents in the original description (Li 1999), and the whole type series are lost. Therefore, we collected new specimens of *G. s. qianshanensis* in Liaoyang City, Anshan City, and Wafangdian City of Liaodong Peninsula during field surveys. Based on the original description of *G. s. qianshanensis*, this study designates a neotype and re-describes *G. s. qianshanensis*.

Materials and methods

Sampling

Twenty-one specimens were collected in Liaoyang City, Anshan City, and Wafangdian City, Liaoning Province. After euthanasia, liver tissues were extracted from the specimens and preserved in 95% ethanol. All specimens were fixed in 75% ethanol and deposited in the Institute of Herpetology, Shenyang Normal University (SYNU).

Morphological analysis

Twenty samples of adult *Gloydius shedaoensis qianshanensis* were measured. The measurement includes a total of 16 morphological characteristics: Snout-vent length (SVL), tail length (TL), and total length (TTL) were measured to the nearest 1 mm. Other morphological measurements were examined with Vernier calipers to the nearest 0.1 mm: head length (HL, tip of snout to posterior margin of mandible), head width (HW, maximum head width), head depth (HD, maximum head depth), eye diameter (ED, measured as a horizontal distance), interorbital space (IOS, distance between the top margin of eyes), internasal space (INS, distance between nostrils), postorbital stripe width (PSW, postorbital stripe width on the largest temporal), temporal height (TH, distance between the upper and lower horn of the largest temporal) (Fig. 1). The numbers of supralabials (SPL), infralabials (IFL), dorsal scales (DS), ventral scales (V, including preventral scales), and subcaudal scales (SC) were counted (Table 1).

In addition, TTL, HL, HW, HD, ED, IOS, INS, PSW, and TH of 15 *Gloydius shedaoensis qianshanensis*, 11 *G. intermedius*, and 14 *G. changdaoensis* were measured (Table 2), and the difference between them and *G. s. qianshanensis* was analyzed by principal component analysis (PCA). The PCA is performed in the "prcomp" package in R 4.2.2.

CT-scanning and three-dimensional reconstructions

The CT scanning was carried out with Nano-computerized tomography. Specimens were scanned using a GE v|tome|x m dual tube 300/ 180 kV system in IVPP, CAS. The specimens were scanned with an energy beam of 80 kV and a flux of 80× μA using a 360◦ rotation and then reconstructed into the 4096 × 4096 matrices of 1536 slices. The final CT reconstructed skull images were exported with a minimum resolution of 6.10 μm. The skull images were exported from the virtual 3D model which was reconstructed by Volume Graphics Studio 3.4 (Volume Graphics GmbH, 2017). The dataset of the 3D models included in this study is available online in the repository (ADMorph, Shi et al. 2016; Hou et al. 2020).

Results

Taxonomic account

Gloydius shedaoensis qianshanensis Li, 1999

Justification of neotype designation. Li (1999) described *Gloydius shedaoensis qianshanensis* based on specimens collected from Qianshan Mountain, Anshan City, and Longtanshan Mountain, Wafangdian City, Liaoning Province. The specimens were preserved in the Snake Island Natural Museum in Lushun. However, the type specimens were lost during the relocation and expansion of the museum, including the holotype SB980563 collected from Qianshan Mountain, Anshan City, and paratypes collected from Longtanshan Mountain, Wafangdian City (interview to Jian-Li Li who described briefly and named this subspecies). Additionally, the original description only listed some numbers of ventral and subcaudal scales, with data on the length and weight of pregnant and juvenile snakes, but lacked detailed morphological descriptions and photos of specimens, which led to disagreements among later authors on the validity of this subspecies.

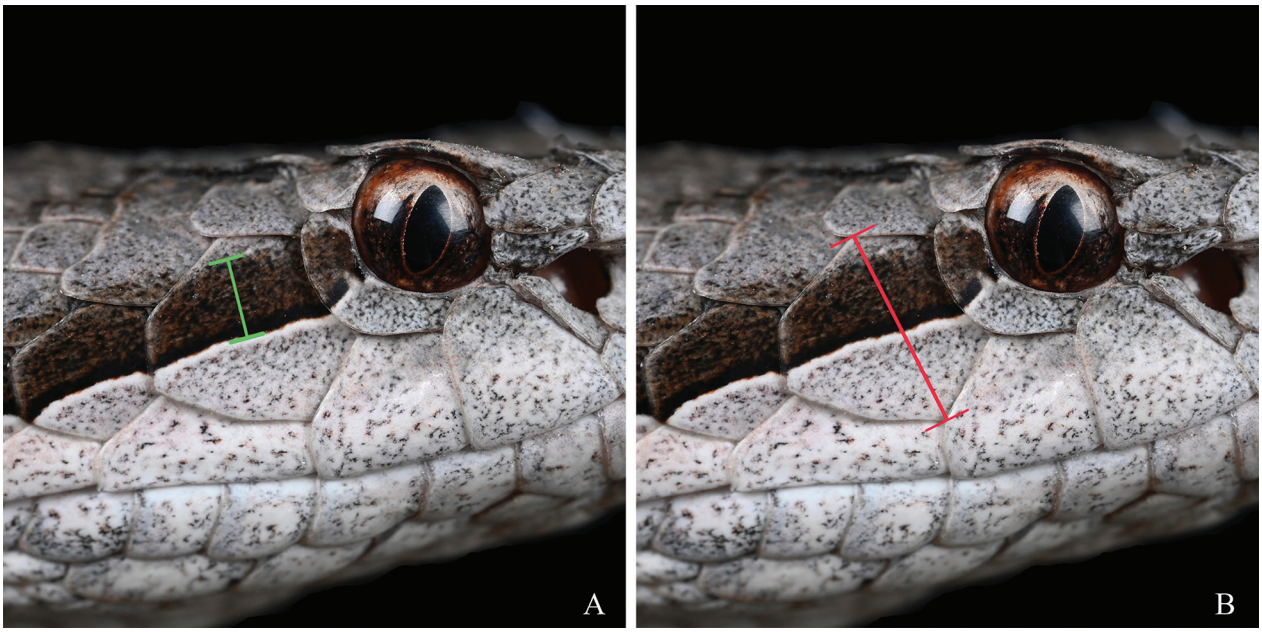

Figure 1. Illustration of measurement methods. **A.** Postorbital stripe width (PSW); **B.** temporal height (TH).

Table 1. Measurements (mm) and pholidosis data of adult *Gloydius shedaoensis qianshanensis*.

Voucher	Sex	SVL	TL	TTL	HL	HW	HD	ED	IOS	INS	PSW	TH	DS	V	SC	SPL	IFL	Location	Preserve
SYNU900701**	M	505	80	585	27.7	16.5	9.5	3.4	11.3	4.4	2.0	4.8	23-23-17	157	41	8/7	11/11	Liaoyang, Liaoning	SYNU
SYNU900702*	M	510	83	593	27.8	17.0	9.2	3.5	11.3	4.2	1.8	5.0	23-23-17	155	43	7/8	11/11	Liaoyang, Liaoning	SYNU
SYNU900703*	M	580	87	667	31.6	20.3	11.0	3.8	12.0	5.8	2.5	5.0	23-23-17	159	41	7/7	10/9	Liaoyang, Liaoning	SYNU
SYNU900704*	M	568	90	658	30.0	18.5	9.0	3.8	12.1	5.0	2.2	5.2	23-23-17	159	42	7/7	11/10	Liaoyang, Liaoning	SYNU
SYNU900705*	F	565	75	640	30.0	17.0	9.2	3.8	11.0	4.7	2.5	5.1	22-23-17	161	40	7/7	10/11	Liaoyang, Liaoning	SYNU
SYNU900706*	F	580	80	660	31.5	17.4	10.6	3.7	11.3	5.0	2.6	5.5	23-23-17	159	37	7/8	11/10	Liaoyang, Liaoning	SYNU
SYNU900707*	F	570	74	644	30.3	19.5	10.5	3.5	11.5	4.7	2.5	5.1	23-23-17	158	33	7/7	10/10	Liaoyang, Liaoning	SYNU
SYNU900708*	F	470	70	540	25.8	14.0	8.8	2.9	9.8	4.9	2.0	4.0	23-23-17	157	43	7/7	11/11	Liaoyang, Liaoning	SYNU
SYNU900709*	F	465	80	545	27.4	16.6	9.3	3.7	10.6	4.9	2.3	4.6	23-23-17	153	45	7/7	10/11	Liaoyang, Liaoning	SYNU
SYNU900710*	F	475	78	553	27.6	17.8	9.7	3.3	11.2	5.1	2.0	4.7	23-23-17	160	43	7/7	11/11	Liaoyang, Liaoning	SYNU
SYNU13090001*	M	591	89	680	30.8	19.6	11.1	3.7	12.3	5.6	2.6	5.5	23-23-17	159	43	7/8	11/11	Wafangdian, Liaoning	SYNU
SYNU900712*	F	514	89	603	29.1	16.7	10.5	3.6	11.3	5.2	2.4	4.5	23-23-17	158	48	7/7	10/11	Wafangdian, Liaoning	SYNU
SYNU900713*	M	558	96	654	29.1	16.3	10.1	3.1	10.6	4.7	2.6	4.9	23-23-17	154	45	8/7	12/12	Anshan, Liaoning	SYNU
220701	F	565	75	640	30.0	19.3	10.7	3.3	11.8	5.0	2.5	4.1	23-23-17	159	38	7/7	11/12	Liaoyang, Liaoning	/
220702	F	578	79	657	30.7	16.5	11.5	3.2	11.0	5.0	2.4	4.5	23-23-17	165	43	7/7	11/10	Wafangdian, Liaoning	/
220703	M	586	90	676	32.0	19.0	11.8	4.5	12.4	5.6	2.4	5.7	23-23-17	153	43	7/7	11/10	Liaoyang, Liaoning	/
220704	M	495	78	573	28.7	19.0	10.4	3.7	11.8	5.2	1.8	5.3	23-23-17	156	44	7/7	10/11	Liaoyang, Liaoning	/
220705	F	585	85	670	29.0	16.0	10.4	3.5	10.1	5.0	2.1	4.3	23-23-17	158	42	7/7	10/11	Liaoyang, Liaoning	/
220706	F	587	77	664	31.7	19.0	11.8	3.7	12.2	5.6	2.4	5.5	23-23-17	159	36	7/7	10/10	Liaoyang, Liaoning	/
220707	M	538	82	620	28.7	16.0	10.1	3.5	10.5	5.0	2.1	4.5	23-23-17	162	45	7/8	10/11	Wafangdian, Liaoning	/

Note: **, neotype; *, paraneotype; /, living body measured in the field.

Table 2. Measurements (mm) of *Gloydius shedaoensis shedaoensis*, *G. intermedius* and *G. changdaoensis* for PCA.

Voucher	Taxa	Sex	TTL	HL	HW	HD	ED	PSW	TH	IOS	INS	Location
/	*G. s. shedaoensis*	F	780	36.8	24.8	13.2	4.2	2.2	5.7	13.0	5.5	Snake Island, Liaoning
/	*G. s. shedaoensis*	F	743	36.9	24.0	12.3	4.4	2.1	4.7	14.2	6.5	Snake Island, Liaoning
/	*G. s. shedaoensis*	F	730	35.6	23.6	11.7	3.7	2.0	5.3	13.5	6.0	Snake Island, Liaoning
/	*G. s. shedaoensis*	M	745	35.0	21.4	12.0	4.2	1.8	6.0	14.3	6.0	Snake Island, Liaoning
/	*G. s. shedaoensis*	M	745	36.4	22.8	11.2	4.1	1.7	4.6	14.4	5.7	Snake Island, Liaoning
/	*G. s. shedaoensis*	F	700	35.6	20.0	11.2	3.8	1.9	5.5	12.9	5.5	Snake Island, Liaoning
/	*G. s. shedaoensis*	F	710	35.7	22.5	10.8	4.4	2.1	5.1	12.7	5.6	Snake Island, Liaoning
/	*G. s. shedaoensis*	F	745	36.4	25.5	11.7	4.1	2.0	5.8	14.3	6.1	Snake Island, Liaoning
/	*G. s. shedaoensis*	M	725	36.2	22.2	11.8	4.2	1.8	5.4	14.0	6.2	Snake Island, Liaoning
/	*G. s. shedaoensis*	F	730	35.9	23.9	11.5	4.4	2.2	5.5	13.4	5.6	Snake Island, Liaoning
/	*G. s. shedaoensis*	F	715	37.0	22.9	10.8	3.8	1.9	5.8	13.1	5.6	Snake Island, Liaoning
/	*G. s. shedaoensis*	F	730	33.3	22.5	12.5	3.7	1.8	5.3	13.1	5.2	Snake Island, Liaoning
/	*G. s. shedaoensis*	F	670	35.3	23.1	10.1	4.1	1.5	4.9	13.1	5.6	Snake Island, Liaoning
SYNU519001	*G. s. shedaoensis*	M	695	35.3	21.3	12.0	3.7	1.4	4.3	12.8	6.7	Snake Island, Liaoning
/	*G. s. shedaoensis*	M	815	40.4	24.6	11.0	4.3	2.2	5.2	14.4	6.0	Snake Island, Liaoning
/	*G. intermedius*	M	640	28.2	17.9	10.5	3.2	3.5	5.1	11.3	4.5	Changbai, Jilin
/	*G. intermedius*	F	770	35.9	22.1	12.6	3.6	3.9	4.9	13.2	6.1	Jilin
/	*G. intermedius*	M	720	33.1	18.9	11.3	3.5	3.7	5.4	6.0	6.0	Jilin
SYNU040271	*G. intermedius*	M	705	32.5	24.3	11.3	3.6	3.6	6.2	12.6	6.2	Kuandian, Liaoning
SYNU040272	*G. intermedius*	M	673	32.3	21.7	11.2	3.6	3.5	5.8	12.8	6.3	Kuandian, Liaoning
SYNU040273	*G. intermedius*	F	709	32.5	20.9	12.2	3.6	3.6	5.3	12.0	5.8	Kuandian, Liaoning
SYNU040274	*G. intermedius*	F	614	29.9	20.4	10.5	3.2	3.0	4.8	11.3	4.7	Kuandian, Liaoning
SYNU040275	*G. intermedius*	F	521	27.0	20.2	9.8	3.1	3.1	4.6	10.4	5.3	Kuandian, Liaoning
SYNU040276	*G. intermedius*	F	682	30.0	20.5	11.1	3.2	3.8	5.2	11.3	5.1	Kuandian, Liaoning
SYNU040277	*G. intermedius*	F	600	28.0	17.6	9.5	3.3	3.0	5.2	11.0	5.0	Kuandian, Liaoning
SYNU040278	*G. intermedius*	M	584	30.1	21.0	11.4	3.3	3.0	5.4	12.1	5.0	Kuandian, Liaoning
/	*G. changdaoensis*	F	617	32.5	20.8	13.0	3.9	3.4	6.0	11.4	5.4	Diaoyu Island, Shandong
/	*G. changdaoensis*	F	600	33.2	21.0	12.3	3.8	3.0	5.4	12.0	4.5	Diaoyu Island, Shandong
/	*G. changdaoensis*	F	645	34.2	19.8	11.8	4.0	3.6	5.4	12.5	5.7	Daheishan Island, Shandong
/	*G. changdaoensis*	F	610	31.3	21.0	11.6	3.4	3.5	5.5	11.3	4.8	Kunyu Mountain, Shandong
/	*G. changdaoensis*	F	558	35.2	21.2	11.1	4.2	3.1	5.3	12.2	5.3	Daheishan Island, Shandong
SYNU519002	*G. changdaoensis*	F	661	31.3	18.9	13.6	4.2	3.6	5.2	11.8	5.2	Rushan, Shandong
/	*G. changdaoensis*	M	680	33.7	23.3	12.5	4.2	3.9	7.4	12.4	6.3	Diaoyu Island, Shandong
/	*G. changdaoensis*	M	698	36.6	22.3	12.5	4.2	3.7	7.8	13.5	6.5	Diaoyu Island, Shandong
/	*G. changdaoensis*	M	670	35.7	24.8	12.5	4.0	3.3	6.5	12.6	6.0	Diaoyu Island, Shandong
/	*G. changdaoensis*	M	645	34.2	19.8	11.8	4.0	3.6	5.4	12.5	5.7	Daheishan Island, Shandong
/	*G. changdaoensis*	M	590	37.6	23.5	12.6	4.0	3.9	6.7	14.0	6.0	Daheishan Island, Shandong
/	*G. changdaoensis*	M	610	37.5	24.9	13.2	4.5	3.8	6.2	13.0	6.1	Daheishan Island, Shandong
/	*G. changdaoensis*	M	548	32.7	22.5	10.6	3.9	3.3	5.3	11.9	5.5	Daheishan Island, Shandong
/	*G. changdaoensis*	M	530	34.0	21.9	11.1	3.9	3.8	5.9	12.2	6.0	Daheishan Island, Shandong
/	*G. changdaoensis*	M	650	36.6	24.3	14.0	4.2	3.8	7.8	13.5	7.0	Daheishan Island, Shandong

Note: /, living body measured in the field.

Neotype. SYNU900701, adult male (Figs 2, 3), collected at Qianshan Mountain Area, Liaoyang City, Liaoning Province, China (40°53'54"N, 123°17'42"E; 385 m a.s.l.). Collected by Xian-Chun Qiu on 6 July 2020.

Paraneotypes. Twelve adult and eight juvenile specimens. Males SYNU900702–900704, females SYNU900705–900710, and juvenile SYNU900712 were collected from the same locality as the neotype by Xian-Chun Qiu between July and October 2020. Male SYNU13090001, female SYNU900712, and juveniles 1510184, 1510184, SYNU13090003, SYNU13090004, SYNU13090005, SYNU13090024 and SYNU1309006, collected by Jing-Song Shi during summer and autumn (2013–2015) from Wafangdian City, Liaoning province (39°56'20"N, 122°15'28"E; 450 m a.s.l.). Male SYNU900713, collected by Xian-Chun Qiu in July 2014 from Qianshan Mountain, Anshan City, Liaoning Province, China (40°59'36"N, 123°7'36"E; 406 m a.s.l.).

Etymology. This subspecies is named after its type locality, i.e., Qianshan Mountain, Liaoning Province, China.

Diagnosis. The morphological distinctions between *Gloydius shedaoensis qianshanensis* and other congeneric species and subspecies are as follows: (1) medium size, TTL up to 730 mm. (2) three palatine teeth. (3) dorsum light gray and covered with dark gray irregular X-shaped pattern; (4) black postorbital stripe with white lower margin; (5) postorbital stripe width smaller than eye diameter but larger than half of the eye diameter; (6) head flat and slender, slightly triangular; (7) mid-body dorsal scales 23; (8) ventral scales 153–170; (9) subcaudals 33–49.

Comparisons (Figs 4, 5): *Gloydius shedaoensis qianshanensis* belongs to the *G. halys-intermedius* group with 3 palatine teeth and 23-mid-body dorsal scale rows, which is different from the *G. strauchi* group with 21 rows of mid-body dorsal scales, including *G. strauchi* (Bedriaga, 1912), *G. himalayanus* (Günther, 1864), *G. qinlingensis* (Song & Chen, 1985), etc. It is different from the *G. blomhoffii*

group with 4 palatine teeth, including *G. blomhoffii* (Boie, 1826), *G. brevicaudus* (Stejneger, 1907), *G. ussuriensis* (Emelianov, 1929), etc. The external morphology of *G. s. qianshanensis* is very similar to some taxa of the *G. halys-intermedius* group, including *G. s. shedaoensis*, *G. intermedius*, and *G. changdaoensis* Li, 1999. The results of PCA indicate that significant differences in head morphology exist between *G. s. qianshanensis*, *G. s. shedaoensis*, *G. intermedius*, and *G. changdaoensis* (Fig. 6). HL and PSW are the main indexes to distinguish the above species in PCA (Table 3). Furthermore, *G. s. qianshanensis* can also be distinguished by the following characters (Table 4): (1) light or dark gray dorsum (vs. brownish red or dark brown in *G. intermedius* and *G. changdaoensis*); (2) dark gray X-shaped spots appear on the dorsum (vs. dark and light cross striations interspersed in *G. intermedius*, rounded spots with light central color and dark margins on the lateral body in *G. changdaoensis*); (3) black postorbital stripe (vs. dark brown or brown in *G. intermedius* and *G. changdaoensis*); (4) postorbital stripe width less than the eye diameter but more than half of the eye diameter (vs. less than or approximately equal to half of the eye diameter in *G. s. shedaoensis*, approximately equal to eye diameter in *G. intermedius* and *G. changdaoensis*); (5) wider stripes cover 3–21 or 4–20 rows of mid-body dorsal scales (vs. stripes cover 5–19 rows of mid-body dorsal scales in *G. s. shedaoensis*).

Figure 2. Neotype SYNU900701 of *Gloydius shedaoensis qianshanensis*.

Figure 3. Head of *Gloydius shedaoensis qianshanensis* (neotype SYNU900701). **A.** Lateral view; **B.** dorsal view; **C.** ventral view. Scale bar: 10 mm.

The skull of *Gloydius shedaoensis qianshanensis* is quite similar to *G. s. shedaoensis* but differs in the following characteristics: (1) the postorbital processes of *G. s. shedaoensis* is more anteroposteriorly elongated than it in *G. s. qianshanensis*; (2) the anterodorsal edge of the postorbital of *G. s. qianshanensis* is in contact with the caudolateral edge of the frontal, while the anterodorsal edge of the postorbital is not in contact with the caudolateral edge of the frontal (separated from the frontal by the postorbital process of the parietal); (3) *G. s. qianshanensis* have less pterygoid teeth (9–10, n = 6) than *G. s. shedaoensis* (11–12, n = 3); the dentary teeth of *G. s. shedaoensis* are longer and slenderer relative to *G. s. qianshanensis*;

Table 3. Variable loadings for principal components with eigenvalue.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
TTL	0.27	-0.37	-0.70	0.15	0.12	0.40	-0.15	0.22	-0.17
HL	0.41	-0.13	-0.03	0.00	-0.02	-0.17	-0.35	-0.11	0.81
HW	0.39	-0.01	0.08	-0.48	0.03	-0.03	-0.40	-0.49	-0.45
HD	0.35	0.23	-0.28	0.13	0.47	-0.35	0.55	-0.28	-0.02
ED	0.36	-0.12	0.39	0.67	0.01	-0.26	-0.23	0.19	-0.31
PSW	0.09	0.73	-0.18	-0.15	0.12	-0.12	-0.35	0.51	-0.04
TH	0.30	0.42	0.25	0.22	-0.07	0.74	0.14	-0.20	0.11
IOS	0.34	-0.28	0.39	-0.45	0.30	0.12	0.29	0.51	0.03
INS	0.36	0.05	-0.16	-0.11	-0.81	-0.18	0.33	0.14	-0.07
%Variance	0.57	0.18	0.07	0.04	0.04	0.04	0.03	0.02	0.01

Figure 4. Lateral heads of *G. halys-intermedius* group from the Liaodong Peninsula and the Shandong Peninsula. **A.** Paraneotype SYNU900707 of *Gloydius shedaoensis qianshanensis* from Qianshan Mountain Area, Liaoning; **B.** G. s. shedaoensis from Snake Island, Liaoning; **C.** *G. intermedius* from Changbai Mountain, Jilin; **D.** *G. changdaoensis* from Kunyu Mountain, Shandong.

Figure 5. Dorsolateral view of *Gloydius halys-intermedius* group from Liaodong Peninsula and Shandong Peninsula. **A.** Paraneotype SYNU900702 of *G. shedaoensis qianshanensis* from Qianshan Mountain Area, Liaoning; **B.** *G. s. shedaoensis* from Snake Island, Liaoning; **C.** *G. intermedius* from Ji'an, Jilin; **D.** *G. changdaoensis* from Kunyu Mountain, Shandong.

Table 4. Morphological comparison of *Gloydius shedaoensis qianshanensis*, *G. s. shedaoensis*, *G. intermedius*, and *G. changdaoensis*.

Taxa	Dorsum color	Dorsum stripe	Postorbital stripe color	Postorbital stripe width
G. s. qianshanensis	Light gray	Dark gray X-shaped pattern	Black	PSW is smaller than ED but greater than half of ED
G. s. shedaoensis	Light gray	Dark gray X-shaped pattern	Black	PSW smaller than or approximately equal to half of ED
G. intermedius	Brownish red or dark brown	Dark and light cross striations	Dark brown or brown	PSW approximately equal to ED
G. changdaoensis	Brownish red or dark brown	Round spots with a light middle color and dark edge color on the lateral body	Dark brown or brown	PSW approximately equal to ED

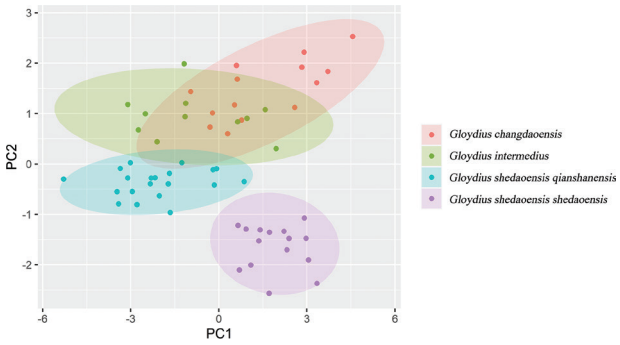

Figure 6. Plots of the first principal component (PC1) versus the second (PC2). *Gloydius changdaoensis* (red), *G. intermedius* (green), *G. s. qianshanensis* (blue), and *G. s. shedaoensis* (purple).

(4) *G. s. shedaoensis* has more curved palatine teeth than *G. s. qianshanensis*; (5) the dorsal edge of choanal process of the palatine in *G. s. qianshanensis* is significantly vaulted, rendering the palatine triangular shaped in lateral view, while the dorsal edge of choanal process of the palatine in *G. s. shedaoensis* is less vaulted and smooth (Figs 7, 8).

Description of neotype. SYNU900701, adult male, medium size, tail short (TTL 585 mm, TL 80 mm, TL/TTL 0.14). Head slightly triangular in dorsal view, slightly flat and slender, distinct from the neck (HL 27.7 mm, HW 16.5 mm, HD 9.5 mm, HW/HL 59.6%); snout blunt, slightly protruding from the lower jaw; eyes relatively small, pupil vertical (ED 3.4 mm, ED/HL 12.2%).

Scalation. Nine large scales are intact on the head. Rostral slightly trapezoidal, wide at bottom and narrow at top, the upper margin visible from dorsal view; nasal divided, anterior part larger, posterior margin of nostril tangent to the middle gap of nasal; two loreals, lower one forms the forward margin of pit; preoculars 3/3 (left/right), upper one turns up to dorsal head and extends to prefrontal, lower two slender and smaller, form the posterior margin of pit; postoculars 2/2, upper one small, lower one sickle-shaped and significantly longer, extending around the lower margin of eye to third supralabial; supralabials 8/7, first supralabial in contact with both parts of nasals, second supralabial smallest, third supralabial lagest and extending the bottom of orbit; temporals 2+3/2+3, largest temporal in contact with fourth and fifth supralabial on both sides; infralabials 11/11, first pair extends behind mental and connect, separate mental from chin shields; a pair of chin shields slightly rhombic, forming the mental groove in the middle; dorsal scales texture matte, keeled except for the row connecting with ventral

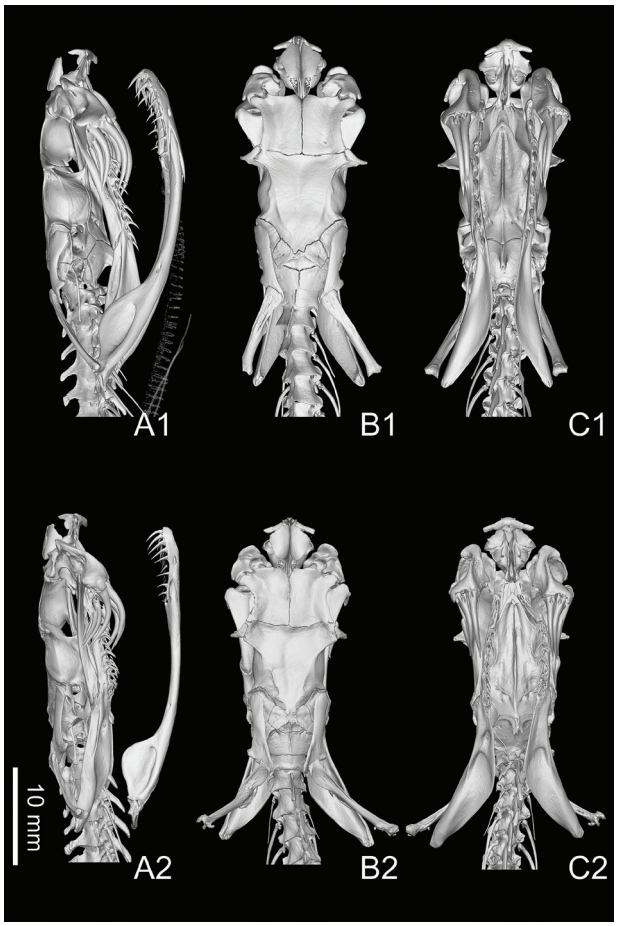

Figure 7. 3D reconstructed skull model of *Gloydius shedaoensis qianshanensis* (SYNU900701, A1–C1) and *G. s. shedaoensis* (SYNU519001, A2–C2). From left to right: lateral, dorsal, and ventral views.

scales; dorsal scale rows 23-23-17; ventral scales 157; anal plate single; subcaudals 41, in pairs.

Coloration. The background coloration of dorsal head is light gray, a pair of discontinuous black stripes on both sides extending backward from the outside of supraocular and parietal; eye rust red on the upper half while brown on the bottom half, pupil black with rust red margin; a black stripe appears behind eye and extends to the corner of mouth, black stripe slightly wider than half eye diameter, bottom of stripe is wavy with a very thin white margin; temporals, supralabials, and infralabials below black postorbital stripe are light gray and scattered with dark brown tiny blobs; a dark gray pincer shaped pattern with an opening toward the head appears on dorsal neck; dor-

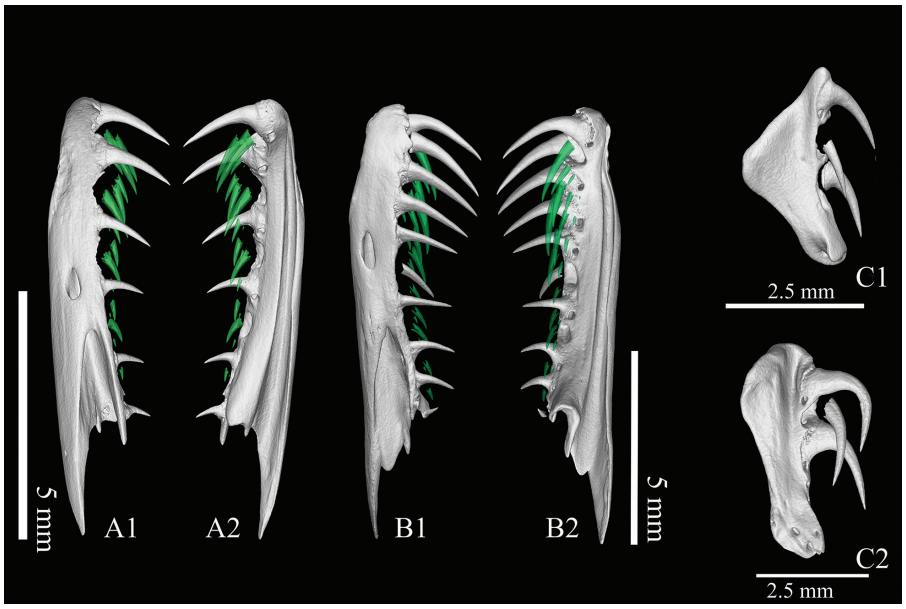

Figure 8. 3D reconstructed model of dentary and palatine of *Gloydius shedaoensis qianshanensis* and *G. s. shedaoensis*. **A1.** Labial view of the left dentary bone of *G. s. qianshanensis* (SYNU900701); **A2.** Lingual view of the left dentary bone of *G. s. qianshanensis* (SYNU900701); **B1.** Labial view of the left dentary bone of *G. s. shedaoensis* (SYNU519001); **B2.** Lingual view of the left dentary bone of *G. s. shedaoensis* (SYNU519001); **C1.** Labial view of the left palatine of *G. s. qianshanensis* (SYNU900701); **C2.** Labial view of the left palatine of *G. s. shedaoensis* (SYNU519001).

sum basically light gray with dark gray irregular X-shaped pattern, a light gray crossband composed of 1–2 scales is formed between two X-shaped pattern; a row of dark gray vaporous blobs separated from X-shaped pattern appear on lateral body; the end of tail is dark brown with grey crossbands; ventral surface of head grayish white, and the color gradually darkens from ventral neck to rear, the ventral surface of body from after neck to before tail is rust red, irregular vaporous black blobs appear near the gap of ventral scales; ventral tail gray and covered with dark brown spots.

Morphological variation. Morphometric data are summarized in Table 1. The dorsal background coloration of paraneotype SYNU900703 is close to earthy yellow. Postoculars 3/3 in paraneotype SYNU13090001.

Distribution and ecology. *Gloydius shedaoensis qianshanensis* is distributed in the Liaodong Peninsula mountainous area north to Liaoyang County, east to Xiuyan County, and south to Wafangdian City of Liaoning Province (Fig. 9).

Gloydius shedaoensis qianshanensis dwells in the deciduous broad-leaved forest in mountainous and hilly areas and the microhabitats contain forest edges, shrubs, grass, and gravel hillsides (Li 1999). Adults are easier to find in autumn because at that time they climb up branches to prey on avians, mainly Passerines (Fig. 10). The predation sites are usually selected on twigs above puddles or near the branches bearing berries, and other structures that attract avians. Sometimes two *G. s. qianshanensis* ambush on the same branch or adjacent branches in highly similar positions. In this study, two adult male *G. s. qianshanensis* were collected in Wafangdian City in early October 2019. One of them was collected from the ground bushes, and the other was collected from a branch not far away. It was established

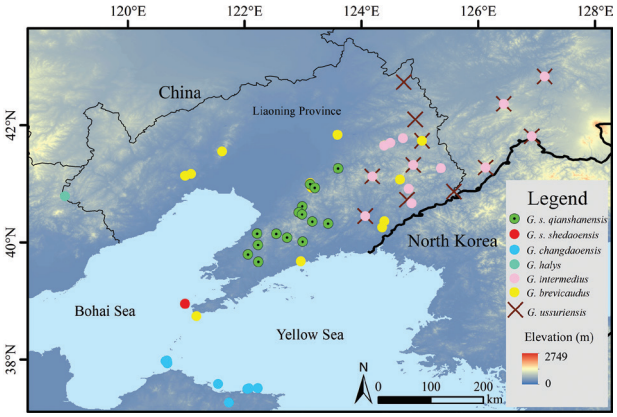

Figure 9. Collection localities of *Gloydius shedaoensis qianshanensis* (black center green circle) and some other congeneric species (subspecies) in Liaoning Province and surrounding areas.

that both of their excrements had bird feathers that have not been completely digested. The excrements of adult individuals collected in Liaoyang City in October 2022 are mostly bird feathers, and only one sample excrement contains the hair of small mammals. *G. s. qianshanensis* do not estivate like *G. s. shedaoensis* on Snake Island. They prey on small mammals such as mice in midsummer. The main activity period of *G. s. qianshanensis* is from May to October of the year. They begin to hibernate in early November. The distribution of *Gloydius shedaoensis qianshanensis* is limited to the mountains of the Liaodong Peninsula, which may be related to their avian feeding habits. The East Asian-Australasian Flyway supports the greatest diversity and populations of migratory birds globally (Yong et al. 2015). The Liaodong Peninsula is located in the East Asian-Australasian Flyway and it is the necessary passage for some

Figure 10. A. The habitat of *Gloydius shedaoensis qianshanensis* in Wafangdian, Liaoning; **B.** Ambush state of *G. s. qianshanensis* on the branch, Xiang-Shu Meng photographed in Wafangdian, Liaoning.

south-north migratory passerine birds to cross the Bohai Strait to reach the southern part of the Chinese Mainland (Yong et al. 2021).

Mating occurs in spring and autumn, and hatch from late August to mid-September, with 3–16 offspring per adult. The total length of the baby snakes is 192–247 mm and they weigh 4.5–6.8 g (Li 1999). Two juveniles were collected in Wafangdian City in June 2013. One of the samples vomited a centipede, and found centipedes in the stomach of the other sample, indicating that centipedes are an important diet composition for juvenile *G. s. qianshanensis*. In addition, juveniles do not prey on avians, but on juvenile mice of *Mus musculus* Linnaeus, 1758 in our captivity observations.

Discussion

In previous decades, the population density of *Gloydius shedaoensis qianshanensis* was larger. People in some places call it "Tie shu pi (Stick on the bark)", describing the snake's posture of lurking on the branches to ambush avians. *G. s. qianshanensis* is described as "very common" in literature from the last century. Ji and Wen (1996) collected it every year during their internship with students from 1964 to 1994. In addition, Jian-Li Li also found a large number of *G. s. qianshanensis* during field surveys at the Liaodong Peninsula in the 1980s and 1990s (interview to Jian-Li Li). In recent years, the population density of *G. s. qianshanensis* has decreased rapidly in Qianshan Mountain, Wafangdian City, and other places. The main reasons include the destruction of the habitat caused by the development of local tourism and farmland. *G. s. qianshanensis* depend on primary forest to survive. Human activities have led to the continuous reduction of such environments, and the population of *G. s. qianshanensis* has thus declined. Additionally, people of the area hunt *G. s. qianshanensis* for reasons including the need for exotic dishes, and for traditional medicine. Therefore, conservation strength needs to be further enforced. Now, *G. shedaoensis* has been listed in category □ of wild animals under the special state protection of China. The population on Snake Island has been well protected by establishing a national nature reserve. Therefore, we suggest establishing nature reserves where *G. s. qianshanensis* is concentrated.

The formation of Snake Island is closely related to the emergence of the Bohai Sea and geological changes. The geographical status of Snake Island was unstable during neotectonic movements, ice ages, and interglacials. The formation of insular in this case went through stages between being connected directly to the mainland, or disconnected, alternatively. Snake Island was last separated from the mainland 15,000 years ago, and such a short period of geographic isolation is insignificant for species formation (Zhou et al. 2000). Based on molecular phylogenetic analyses of ND4 and cytb genes, *Gloydius shedaoensis qianshanensis* constitutes a sister clade with *G. s. shedaoensis*. They form a monophyletic group with *G. intermedius*, and the three are closely related (Shi et al. 2016). However, *G. s. shedaoensis* adapted the insular climate, distinct from the mainland population. In both taxonomic traits (external morphology and skeletal morphology), and behaviors, they can be considered as eco-species (Shi et al. 2016). According to O'Brien and Mayr (1991), a subspecies is characterized by having a unique geographic range or habitat, a group of phylogenetically concordant phenotypic characters, and a unique natural history relative to other subdivisions of the species (Auliya 2002). Therefore, this study supports Li's (1999) subspecies delimitation of *G. shedaoensis*. Further research based on genomic information should be approached, in order to resolve the evolutionary relationships of *Gloydius* species around the Bohai Rim.

Acknowledgments

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