

Original Research Articles

Threatened fishes of the world: *Schizothorax* (Racoma) *biddulphi* (Günther, 1876) (Cyprinidae)

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In order to study the individual biology of endemic fish in China, lay a foundation for the conservation of its germplasm resources, and consolidate the basic biological data of endemic fish in the world. According to the ethical code and fishing permit, 21 samples of *Schizothorax* (Racoma) *biddulphi* (Günther, 1876) were collected from the Muzati River in Tarim River system from 2021 to 2022, and their morphological characteristics, age identification and anatomical observation were analyzed by classical biological methods. *S. biddulphi* has a long body, a conical head, a pointed snout and a lower mouth. Requires 2 pairs. The scales are small and neatly arranged; Bare or scaly chest; The lateral line scale is slightly larger. The lateral line is complete. The correlation equation between body length and body weight is: $W = 4.148 \times 10^{-5} L^{2.791}$. There were 5 kinds of age identification materials: 15⁺ for lapillus and asteriscus, 14⁺ for vertebra, 11⁺ for opercular bone, and 9⁺ for anal scale. The peritoneal wall of *S. biddulphi* is black; The tooth type is 2-3-5-5-3-2; The gill harrow was 11~13 for laymen and 15~18 for experts. Diphsocyte; Gallbladder oval; The number of vertebrae is 4+42-43+1, and the number of ribs is 23 or 24. *S. biddulphi* is a special species in the Tarim River system, and its evolutionary adaptation is closely related to the uplift of the Qinghai-Tibet Plateau.

INTRODUCTION

Schizothorax (Racoma) *biddulphi* (Günther, 1876), belonging to the Cypriniformes, Cyprinidae, Schizothoracinae, *Schizothorax*, *Schizopyge* Heckel, used to be called *Schizothorax* (Schizopyge) *biddulphi*,¹ *Schizothorax biddulphi* Günther²; Place name: Pipefish, Xinjiang fish, the iconic flagship species of Tarim River system, the cold-water unique fish distributed in alpine waters, and one of the rare and unique fish in China.³ Based on specimens collected from Kashgar River and Shache, Day.F first recorded and described *Schizothorax biddulphi* and named it *Schizothorax biclulphi* (Günther, 1876).⁴ After that, Kessler (1879) and Herzenstein (1889) also found *Schizothorax biddulphi* (Günther, 1876) in the Kashgar River, Yeqand River, Tarim River, Kaidu River and Lop Nur River²; According to relevant studies, *Schizothorax biddulphi* belongs to *Schizothoracinae*, *Racoma*, *Racoma* McCl. Therefore, Wu *et al.* named it *Racoma* (Racoma) *biddulphi* (Günther).⁵

S. biddulphi, as the flagship species of Tarim River system, is a unique cold-water fish distributed in alpine waters.³ In the 1950s, the population was large. Accounts for about 80% of the Bosten Lake.⁶ Due to late sexual maturity, low fertility, slow growth and higher requirements for

the living environment, as well as the intensification of human activities and ecological environment in recent years, the habitat range of *S. biddulphi* has been shrinking and the population has declined sharply. At present, it is only distributed in Aksu River, Weigan River, Yeqiang River and Hetian River in Tarim River system.^{3,7,8} As an indigenous species and ecologically sensitive species only existing in the Tarim River system, studies have only been made on its distribution investigation, external morphology, physiological ecology, artificial reproduction and disease control.^{7,9-13} Due to its endangered status, *S. biddulphi* has attracted wide attention, has been listed as a key protected wild animal in many protection lists at home and abroad, and has been listed as a critically endangered species by the International Union for Conservation of Nature in 2022.¹⁴⁻¹⁸ With the increase of its endangered level, it is of great importance to protect the natural habitat ecosystem of *S. biddulphi*, and it is urgent to carry out breeding protection.

The Tarim River system is the longest inland river in China, located on the northern edge of the Qinghai-Tibet Plateau. The crustal movement of the earth plate raised up the Qinghai-Tibet Plateau, and then the Tarim Basin was formed. The Tarim River system formed a closed and unique freshwater ecosystem, with an aquatic ecosystem

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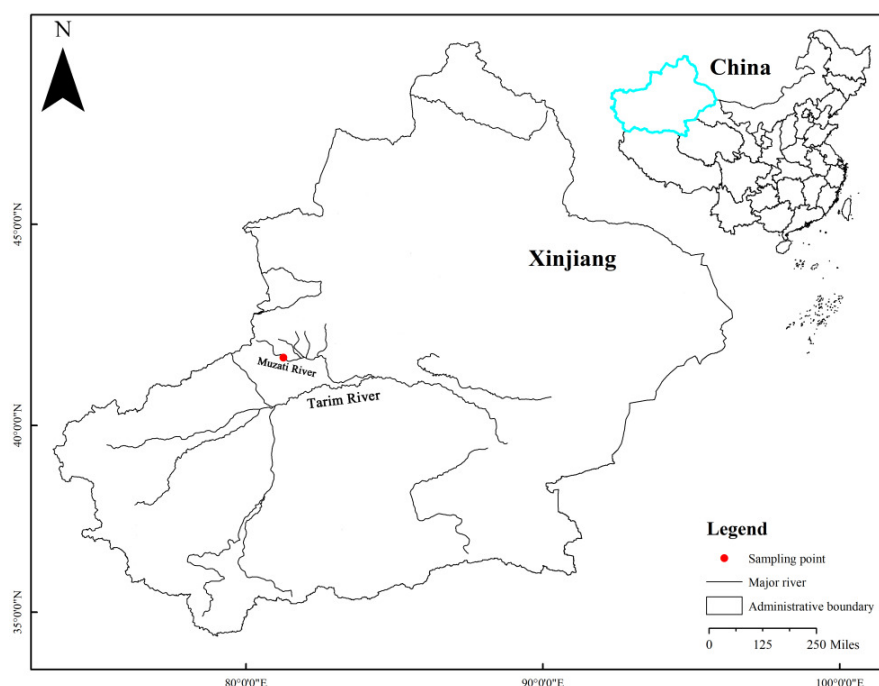


Figure 1. Schematic diagram of the sampling sites of *Schizothorax biddulphi*.

similar to but different from the Qinghai-Tibet Plateau. Due to the special geographical environment and climate, the indigenous fishes distributed here are more differentiated than the fishes of the same genus in the Qinghai-Tibet Plateau. Therefore, it has been paid close attention by fish scholars at home and abroad.¹⁹ Based on the particularity of the Tarim River system, biosecurity and germplasm protection, the stability of the fragile ecosystem in the Tarim Basin can be further maintained. The biological system of *S. biddulphi* was collected from the Muzati River in Tarim River system from 2021 to 2022. The morphological characteristics, age and growth characteristics of *S. biddulphi* were identified and analyzed, and the adaptive behavior of *S. biddulphi* in the Muzati River in Tarim River system was understood, so as to protect the theoretical data of germplasm resources accumulation and lay a foundation for the protection of the germplasm of plateau fish.

MATERIALS AND METHODS

SAMPLE COLLECTION

In this study, which was approved by the Science and Technology Ethics Committee of Tarim University (approval code: TDDKYXF 20200426). From 2021 to 2022, 21 samples of *S. biddulphi* were collected in the Muzati River (81°15'E, 41°43'N) of Tarim River system in China (Figure 1) by means of gillnets and ground cages (mesh 2a = 2 cm). Biological determination and gender identification were performed on site, and three pairs of otoliths were removed and stored in 0.2ml centrifuge tubes; 4-9 vertebrae and anal scale were fixed with 95% ethanol in 2 ml centrifuge tubes for use; and other tissues were fixed with 10% formalin so-

lution and brought back to the laboratory for further treatment.

METHODS

According to Yin,²⁰ 15 measurable traits (length accurate to 0.01 mm, mass accurate to 0.01 g) of *S. biddulphi* were determined (Figure 2).

The relationship between body length and body weight $W = aL^b$ (a and b are constants) was used to fit the growth type. Pauly- t test was used to check the correlation difference between b value and 3, and determine whether the growth was uniform.

Using the formula $K = W/L^{2.1} \times 100000$, where W is body weight (g) and L is body length (mm), the fullness index was calculated and the growth characteristics were observed.

Treatment with 5 kinds of age identification materials, embedding and fixing of lapillus, sandpaper grinding, polishing, acetone dissolution, turning and fixing, continue polishing until the center core is clear; The growth rings were observed with xylene drops in asteriscus and photographed with SMZ 1270i. The vertebra were boiled in water for 5-10 min, the connective tissue was removed, and the xylene was transparent. The opercular bone was boiled for 1 min, and the connective tissue was removed and photographed by microscope. The anal scale was washed with warm water and the mucous membrane was removed.

CT scanning was performed on the whole fish (Micro CT µ80 microCT instrument).²² Scanning conditions were as follows: voltage (70 kVp), current (114 µA), 360° rotation scanning, scanning 180 min, resolution 14 µm, average frame 4, Angle gain 0.4°. The skeletal system was reconstructed by pCT Ray v4.0-1.

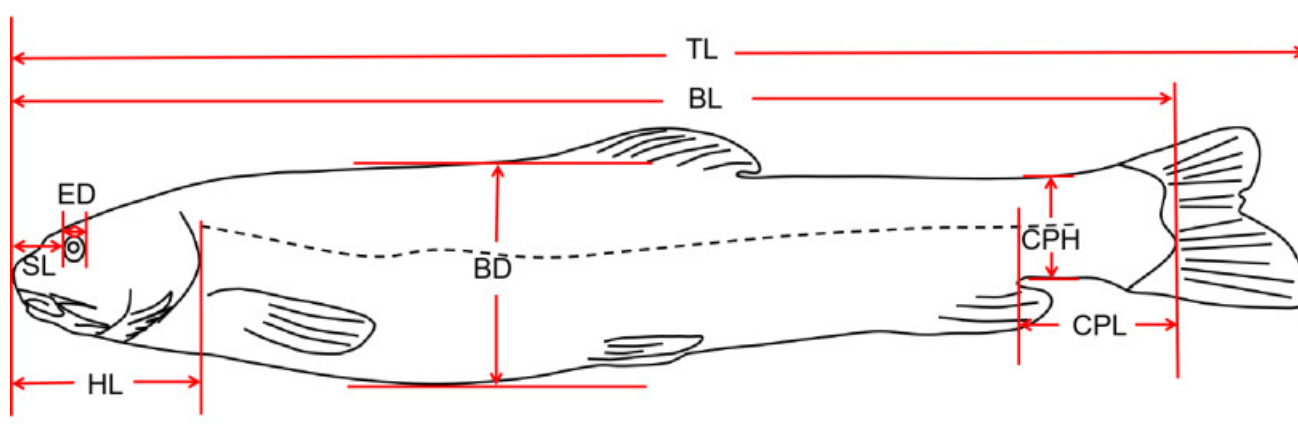


Figure 2. Traditional morphological measurements of *Schizothorax biddulphi*

TL: Total length; BL: Body length; BD: Body depth; BW: Body width; HL: Head length; SL: Snout length; ED: Eye diameter; EI: Eye interval; CPL: Caudal peduncle length; CPH: caudal peduncle height



Figure 3. *S. biddulphi* (This picture shows the largest captured individual)

DATA PROCESSING AND ANALYSIS

SPSS 18.0 and ORIGIN 9.0 were used and expressed as Mean and standard deviation (Mean $\pm$ S.D.).

RESULTS

MORPHOLOGICAL CHARACTERISTICS

S. biddulphi is elongated and slightly flattened. The head is tapered and the snout is pointed. The nostrils are close to the eyes, the eyes are slightly smaller, lateral upper, close to the rostral end. Inferior mouth, horseshoe shape or approximately arc; Maxillary longer than mandibular, mandibular without horn margin; The lower lip is narrow and the posterior labial groove is interrupted. There must be 2 pairs, the front must reach about the base of the back, and the back must reach about the lower edge of the eye. The scales are small and neatly arranged; Bare or scaly chest; Gluteal scale does not reach forward to ventral fin base; Shoulder scale is not obvious; The lateral line scale is slightly larger. Lateral line complete, lateral median (Figure 3, Figure 4).

Dorsal fin pattern III, 7-8, anal fin pattern II, 5, pectoral fin pattern I, 17-20, ventral fin pattern I, 8-9; The starting point of the dorsal fin is less than or equal to the distance between the base of the tail fin, and the final hard

spine has a serrate 20-30, which is larger than, equal to or slightly less than the head length.

QUANTIFIABLE CHARACTER ANALYSIS

Among the 15 measurable traits of *S. biddulphi* (Table 1), the body height was higher than the body width, and the tail stalk length was higher than the tail stalk height. Among the standard deviation of measurable traits, only weight, total length and body length were larger, while others were smaller. The standard deviations of body height, body width, head length, snout length, eye diameter, eye distance, mouth split, caudal stalk length and caudal stalk height were 14.88, 13.60, 16.60, 5.76, 1.60, 6.42, 6.04, 11.76 and 6.62, respectively. The standard deviation of body mass is 218.82, indicating that the size of body mass is different. The standard deviations of total length and body length were 89.10 and 80.53, respectively, indicating that the population size of *S. biddulphi* covered a wide range.

As can be seen from the ratio of measurable traits (Table 2), body length/eye diameter had the largest variation range, and head length/caudal stalk length and caudal stalk length/caudal stalk height had the smallest variation range. Body length was 4.80-6.36 times of body height, body length was 3.92-4.87 times of head length, and body length was 5.72-8.73 times of caudal stalk length. The head length is 4.18-8.26 times the eye diameter, and the caudal stalk length is 1.29-1.97 times the caudal stalk height. It can be

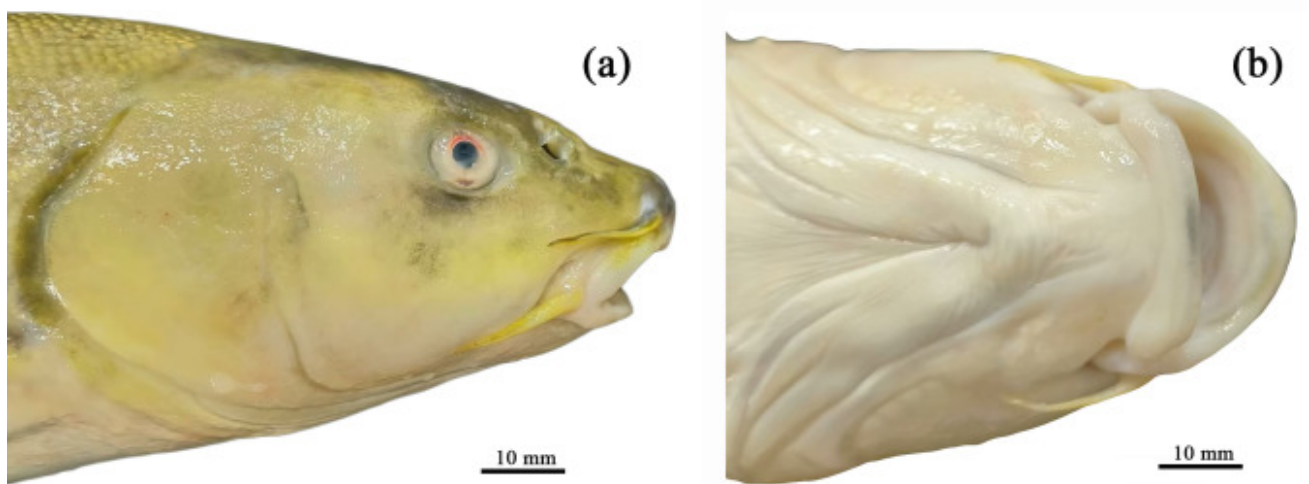


Figure 4. Lateral view and ventral view of head of *S. biddulphi*

Table 1. Measurable Characters of *S. biddulphi*

Measurement project	Mean $\pm$ S.D.	Range
TW	94.38 $\pm$ 218.12	2.54 - 1022.83
TL	179.08 $\pm$ 89.10	67.11 - 497.69
BL	152.77 $\pm$ 80.53	56.18 - 444.91
BD	27.78 $\pm$ 14.88	9.63 - 80.91
BW	20.96 $\pm$ 13.60	7.50 - 70.52
HL	34.81 $\pm$ 16.60	13.92 - 91.32
SL	11.47 $\pm$ 5.76	4.83 - 31.62
ED	6.09 $\pm$ 1.60	3.32 - 11.06
EI	10.40 $\pm$ 6.42	3.44 - 33.71
SLL	8.32 $\pm$ 6.04	3.15 - 31.79
CPL	22.16 $\pm$ 11.76	8.58 - 59.99
CPH	13.53 $\pm$ 6.62	5.79 - 35.91
Pectoral fin length	7.76 $\pm$ 11.95	2.56 - 59.35
Ventral fin length	7.28 $\pm$ 9.50	2.34 - 48.16
Anal fin length	13.45 $\pm$ 14.58	6.08 - 75.95

seen that *S. biddulphi* has a long body, a thin body, a relatively small head, small eyes in the front of the head, and a slightly rectangular tail stalk.

LENGTH-WEIGHT RELATIONSHIP

The relationship between body weight and body length of *S. biddulphi* was fitted with $W = aL^b$ as follows (Figure 5): $W = 4.148 \times 10^{-5}L^{2.791}$ ($R^{15} = 0.998$), there was no significant difference between b value and 3 ($P > 0.05$), belonging to uniform growth type.

CONDITION FACTOR

Statistical analysis using the formula $K = W/L^{2.1} \times 100000$ shows that the fullness of *S. biddulphi* fluctuates from 0.63 to 0.90, with an average value of 1.29.

AGE DETERMINATION

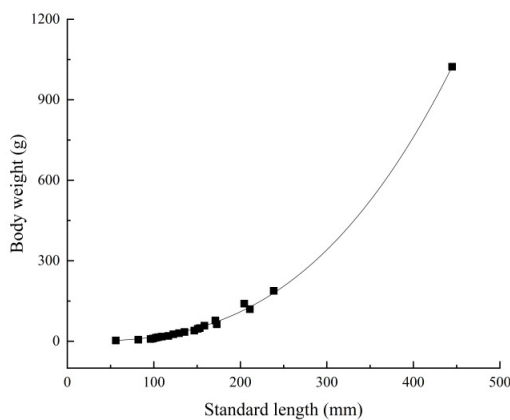
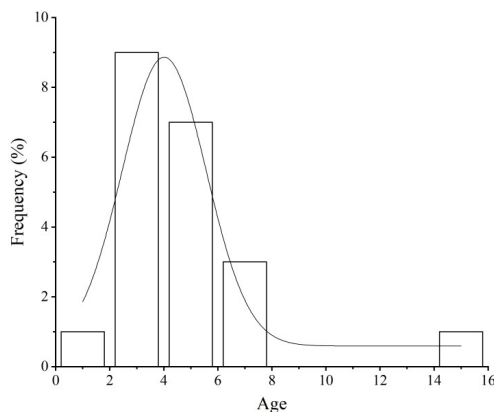
The age frequency distribution of 21 *S. biddulphi* is shown in Figure 6. The largest individual of *S. biddulphi* was identified by age (TL: 444.91 mm; TW: 1022.83 g) for age identification (Figure 7), 15⁺ (a) for lapillus, 15⁺ (b) for asteriscus, 14⁺ (c) for vertebra, 11⁺ (d) for opercular bone, and 9⁺ (e) for anal scale. The five age identification materials all showed irregular secondary wheel structure at 4-5 ages.

ANATOMICAL OBSERVATION

The anus of *S. biddulphi* is not advanced and the abdominal cavity is large, the peritoneal wall is black, and the mesangium keeps the organs in a relatively fixed position in the body cavity (Figure 8-a). There are no gnathic teeth, oral teeth and free tongue in the mouth, but there are pharyngeal teeth in the oropharyngeal cavity, only on the fifth

Table 2. Ratio of measurable characters of *S. biddulphi* (n=21)

Trait Ratio	Mean±S.D.	Range
BL / BD	5.56 ± 0.45	4.80 - 6.36
BL / HL	432 ± 0.26	3.92 - 4.87
BL / CPL	7.01 ± 0.75	5.72 - 8.73
BL / ED	24.06 ± 5.66	16.92 - 40.23
HL / ED	5.54 ± 1.07	4.18 - 8.26
HL / CPL	1.63 ± 0.19	1.27 - 1.97
HL / CPH	2.62 ± 0.28	2.26 - 3.45
CPL / CPH	1.62 ± 0.19	1.29 - 1.97
BD / TW	1.39 ± 0.21	1.12 - 1.79

**Figure 5. Length-weight relationships of *S. biddulphi*****Figure 6. Age frequency distribution of *S. biddulphi***

branchial arch (pharyngeal bone), columnar, apical, and hooked, powerful teeth with few grains and distinct grains, teeth of 2.3-5 - 5.3-2 (**Figure 8-b**); It has three rows of pharyngeal teeth on the inside: the first row has the largest five, the second row has three and the third row has two smaller ones. Gill harrows are 11 to 13 for outsiders and

15 to 18 for insiders (**Figure 8-c**). Anterior chamber (bladder body) 124.20 mm, posterior chamber 62.50 mm (**Figure 8-d**).

S. biddulphi has no stomach; the intestine is closely connected with the esophagus and coiled in the abdominal cavity. The intestinal recursion area is smaller than other parts, so the intestine is divided into four parts: anterior intestine, midintestine, posterior intestine and rectum. The intestinal length is 959.16 mm (**Figure 8-e**). The gallbladder is elliptical and large in volume (**Figure 8-f**). The number of vertebrae is 4+42-43+1, and there are 23 or 24 ribs between the 5th and 17th or 18th cones (**Figure 8-g**).

OBSERVATION OF GONADAL TISSUE

The gonads of *S. biddulphi* are attached in pairs to the ventral sides of the swim bladder and extend after confluent at the ends (**Figure 9**).

DISCUSSION

MORPHOLOGICAL STRUCTURE

The shape characteristics of fish can be divided into three categories: morphological traits, countable traits and proportional traits.²³ Comparing the morphological characteristics of other *Schizothorax* species, we found that the *S. pseudaksaiensis* dorsal fin fin form III, 6-8, anal fin fin form III, 5, pectoral fin fin form I, 15-18, ventral fin fin form I, 8²⁴; *S. eurystomus* dorsal fin type III, 8, anal fin type III, 5, pectoral fin type I, 17-20, ventral fin type I, 9.²⁵ This difference may be related to the habitat environment. *S. biddulphi* and *S. eurystomus* distributed in the tributaries and main streams of Tarim River system, while *S. pseudaksaiensis* distributed in the Ili River system.³ Geographical isolation resulted in different habitats and movements, and the difference in swimming speed resulted in the difference in fin patterns. This is also consistent with the correlation between bone growth and environmental factors.²⁶ The number of vertebrae of *S. pseudaksaiensis* is 4+44-45+1,²⁷ which is slightly higher than that of *S. biddulphi*. This may be due to the fact that although the vertical distribution of *S. pseudaksaiensis* lives at an altitude of 650-1500 m, there is little difference from that of *S. biddulphi* (940-1350 m).

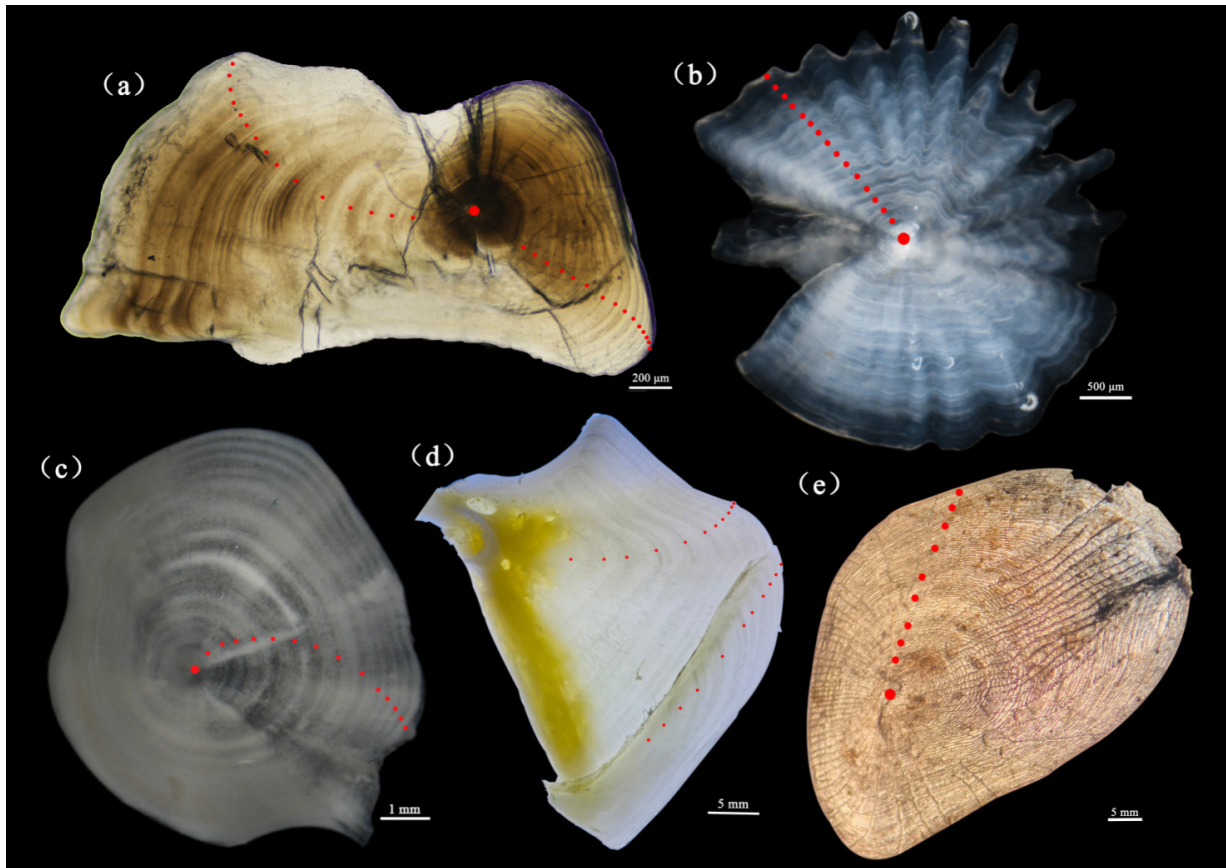


Figure 7. 5 kinds of age identification materials of *S. biddulphi*

Note: (a) Lapillus, (b) Asteriscus, (c) Vertebra, (d) Opercular bone, (e) Anal scale

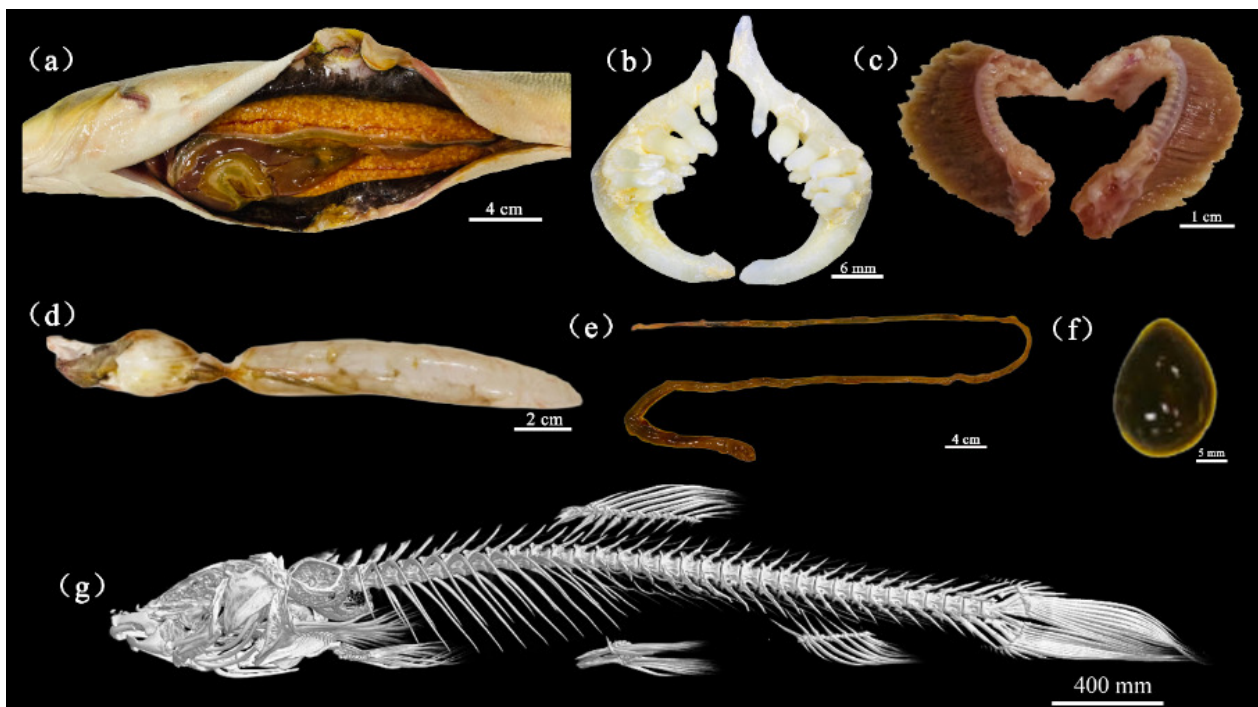


Figure 8. Anatomical observation of *S. biddulphi*

Note: (a) Viscera, (b) Pharyngeal teeth, (c) Gill harrow, (d) Swim bladder, (e) Gut, (f) gallbladder, (g) Whole body skeleton



Figure 9. Anatomical diagram of the gonad of *S. biddulphi*

However, *S. pseudaksaiensis* lives in the rapids or slow currents in the upper reaches of the Ili River system, while *S. biddulphi* lives in the still water of the Tarim River system.

AGE

Age structure or composition is one of the fundamental attributes of fish populations and is associated with biological and ecological characteristics such as feeding, reproduction and population growth.²⁰ In this study, the age structure of *S. biddulphi* ranged from 1 to 15 years old, and the dominant ages were 3 years old. The five age identification materials of the largest individual in the catch showed a difference in age, the youngest age was 9 years old, the oldest age was 15 years old, which was similar to the age identification of *Schizothorax* fish by Ma Baoshan et al. The five age identification materials of *S. biddulphi* showed differences in age, with the youngest being 9 years old and the oldest being 15 years old, which was similar to the age identification of *S. o'connori* by Ma.²⁸ In addition, the five age identification materials of *S. biddulphi* all showed divergent or irregular siderings, which may be consistent with the sexual maturity at 4-5 years of age and had reproductive phenomena, which was consistent with the results of Guo Yan's³ study on *S. biddulphi* that the minimum sexual maturity age of males was 4 years and that of females was 5 years. In this study, *S. biddulphi* is a uniformly growing fish, but the slow growth rate is one of the characteristics of plateau fish. When the body weight of *S. biddulphi* reaches 1000 g or more, it takes about 16 years, which is not only related to its own life history characteristics, but also related to the water environment caused by the habitat altitude (1415 m).

FEEDING

S. biddulphi has developed snout and jaw whiskers, so it is strong in detecting and selecting food types. Its mandibular fissure and horseshoe-shaped or arc-shaped morphological structure further enhance its feeding ability, which is consistent with the study on the feeding habits of *Diptychus maculatus*.²⁹ The lower position of the mouth slit enables it to better approach the bottom and scrape the algae attached to the benthic material during feeding. The horseshoe-shaped or curved mouth split increases its feeding area, improves feeding efficiency, and helps it better adapt to the benthic environment when looking for food. This feeding habit is also closely related to the high number

of thin and long gill rakes,²⁰ which increase the contact area with the current and effectively filter and capture phytoplankton and small aquatic insects in the water; The high number of gill rakes also indicated that *S. biddulphi* had higher filter feeding efficiency and higher utilization rate of food resources. There are more developed pharyngeal teeth in the mouth: the tip is pointed and hook-like, and the chewing surface is wide, which is suitable for feeding algae and carnivorous invertebrates. Three lines of hypopharyngeal teeth, the outer line of hypopharyngeal teeth played a major role in feeding, and the most inner line of thin and few hypopharyngeal teeth (only 2) significantly reduced the function of grinding food. The changes of hypopharyngeal teeth further confirmed that in order to adapt to the colder environment, the feeding species increased and the omnivorous degree increased. The morphological structure of fish digestive tract varies with species and is also related to feeding habits and living environment.³⁰ *S. biddulphi* is a gastraceless fish with elongated intestine, anterior enterocoele and complex curvature, which is in line with omnivorous and phytophagous gastraceless fish by increasing intestinal length and thereby increasing absorption area.²¹ And the intestine is full, indicating that the amount of food increases correspondingly to further meet the metabolic consumption of the body under cold conditions. The intestinal length was 2.16 times that of the body length, which further confirmed the omnivorous vegetative feeding habit. The intestinal tract of *S. biddulphi* is longer than the body length, and the degree of intestinal rotation is higher, which is consistent with the fact that some fish prolong the retention time and contact area of food by changing the length of the intestinal tract in order to better digest food.²¹ In addition, the larger gallbladder of *S. biddulphi* is consistent with the study of Ren et al.³¹ that it has a larger feeding capacity, which is consistent with the study of increasing food quantity under cold environment.

CONSERVATION STATUS

In 1977, the largest individual of *S. biddulphi* 3335 g was caught in Bosten Lake, an affiliated water body of Tarim River system.³ In 2005, after 28 years, the largest individual of 2500 g was found in Kizil Reservoir, an affiliated water body of Aksu River in the upper reaches of Tarim River system.¹¹ In 2023, after a gap of 18 years, the largest individual in this study was collected from the Muzati River, a tributary of the upper reaches of the Tarim River system, which was 1022.83 g, which was much smaller than the individual size captured before, but its population decreased sharply. In order to protect the germplasm resources, habitat and breeding ground of *S. biddulphi*, nature reserves should be set up to improve the ecological environment and strengthen the research on their ecological habits and reproductive laws, to provide the basis for their conservation, management and sustainable utilization.

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AUTHORS' CONTRIBUTION

Conceptualization: C.X. Wang (Lead). Writing – original draft: C.X. Wang (Lead). Methodology: Gulden Serekbol (Lead). Formal Analysis: L.H. Hu (Equal), L.T. Yang (Equal). Investigation: L.H. Hu (Equal), L.T. Yang (Equal). Supervision: B. Huo (Equal), Y. Song (Equal). Writing – review & editing: S.A Chen (Lead).

COMPETING OF INTEREST – COPE

No competing interests were disclosed.

ETHICAL CONDUCT APPROVAL – IACUC

All experimental protocols were approved by the Science and Technology Ethics Committee of Tarim University (approval code: TDD-KYXF 20200426, approval date: 26 April 2020) and adhered to animal welfare laws, guidelines and policies.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

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