

Morphological and skeletal comparison and ecological adaptability of Mandarin fish *Siniperca chuatsi* and big-eye Mandarin fish *Siniperca kneri*

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ABSTRACT

Most of the closely related fish species are similar in appearance, with slight differences. This study evaluated 35 morphological characteristic parameters and the number, shape, and spatial location of bones in the skull and vertebrae of the mandarin fish *Siniperca chuatsi* and big-eye mandarin fish *Siniperca kneri*. We can understand their ecological adaptability, compare their morphological and skeletal differences, and elucidate the relationship between their phenotypic trait differences and skeletal characteristics. The results were as follows: 1. The Student's *t*-test results revealed significant differences between *S. chuatsi* and *S. kneri* in terms of head depth, head back length, snout length, interorbital distance, distance of the eyes from the oral fissure, and width between the posterior trunk and base of the caudal fin. Principal component and discriminant analysis showed that the main morphological differences between *S. chuatsi* and *S. kneri* were in the eye position, head hump and body shape. 2. There was no significant difference in the number and shape of the skull and vertebrae between *S. chuatsi* and *S. kneri*, but they differed in spatial position. The terminus of the maxilla in *S. chuatsi* is located at the lower border of the postorbital bone, but at the anterior in *S. kneri*. The size of head hump is closely related to the spatial location of the splanchnocranium in *S. chuatsi* and *S. kneri*. The angles between the caudal vertebrae centrum and each of the following: the neural arch and spine; haemal arch and spine of *S. chuatsi* are larger than those of *S. kneri*. In conclusion, the morphological differences between *S. chuatsi* and *S. kneri* are closely related to the spatial position of skeleton. These phenotypic differences reflect the ecological adaptation of mandarin fish to different natural environments.

1. Introduction

The Sinipercinae, comprising three genera and 12 species, are freshwater fishes endemic to East Asia (Zhou, Yang, & Cai, 1988). Their interspecific and intergeneric morphological differences are an important research subject in studies about adaptation and evolutionary biology of freshwater fishes in East Asia. Geographical isolation and a high number of ecological niches are one of the factors contributing to formation and divergence (Chen, Zhang, & Huang, 1982; Song, 2017).

Mandarin fish *Siniperca chuatsi* Basilewsky (1855) and big-eye mandarin fish *Siniperca kneri* Garman (1912) are closely related species from the subfamily Sinipercinae. They are both elongated and lateral flattened, with a brown stripe from the snout to the front of the dorsal fin base. They have supplementary maxilla and retractable

maxilla. The teeth of the two jaws were small, the anterior of the maxilla and bilateral teeth of mandible were slightly enlarged, and the vomer and palatine have villous teeth. The posterior margin of the preopercle is finely serrated. The posterior margin of the opercle has one to two flat spines, the body is covered with small round scales, and the dorsal fin has 11–12 spines (Zhu & Jin, 1984). Using the shape of entire bones of sinipercine fishes, Liu and Chen (1994) revealed a close relationship between *S. chuatsi* and *S. kneri*. This is supported by the cytogenetic and molecular genetic studies, which have reported the same chromosome number ($2n = 48$), nearly identical karyotypes and chromosome arm number (NF = 54) in the two species (Yu, Zhou, Li, & Li, 1989). *S. chuatsi* and *S. kneri* were resolved in sister clades in phylogenetic analyses based on mitochondrial DNA gene sequences (Chen et al., 2012; Chen, Guo, & Nie, 2007; Zhao, Wang, Li, & Cai, 2006), and clustered together in a tree

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topology inferred from nuclear gene sequences (Li, Orti, & Zhao, 2010; Song, Zhao, & Li, 2017).

Nevertheless, there are some evident morphological differences between the two species, and they are considered two independent species by traditional taxonomists. Their main distinction is based on the relative position of the eye and oral fissure, the size of the eye, the head hump, etc (Cheng & Zheng, 1987). The shape and structure of the skeleton could determine species-specific phenotypes (fish shape). Cheng and Zheng (1987) considered that the anterior canine teeth in the jaw bones of *S. kneri* are more notable than those of *S. chuatsi*. Kong and Zhou (1993), who studied the bone morphology of Siniperinae, reported that the dentate zone of the palatine in *S. chuatsi* is slightly wider than that of *S. kneri*, and found no significant differences in other entire bones.

The two species differ in their geographical distribution (Zhou et al., 1988). In China, the natural distribution area of *S. chuatsi* extends south from Heilongjiang to the north of the Pearl River system, and *S. kneri* is found in the Huaihe-Honghe River system (Zhou et al., 1988). Moreover, the growth performance of the two species is significantly different—under the same aquaculture pond conditions, the growth rate of *S. chuatsi* was 3–5 fold higher than that of *S. kneri* (Chen, Zheng, Wu, Fang, & Xiao, 2003; Li, Yang, & Tao, 1987).

S. chuatsi inhabits clean water with abundant aquatic plants and stealthily preys on live prey, such as fish and shrimp, at night. It possesses tracking and aggressive behavior, but its ability for continuous swimming is weak (Jiang, 1959; Liang, 1995b). *S. kneri* preferably inhabits flowing water rich in dissolved oxygen and feeds mainly on aquatic animals, and few aquatic insects, plant debris, etc (Li, 2008).

The ecological habitat of fish and their predation and movement, are the result of long-term evolution and adaption to a local environment and are displayed as a series of body shape, mouth shape, eyes position, etc. (Piet, 1998; Wainwright & Richard, 1995; Winemiller, Kelso-Winemiller, & Brenkert, 1995). For example, over the past few million years, more than 500 species of cichlids in Lake Malawi (Africa) have evolved from a common ancestor and exhibit a range of adaptive features in jaw morphology and body color (Albertson, Markert, Danley, & Kocher, 1999). It has been presumed that *S. chuatsi* and *S. kneri* have evolved from a common ancestor 4.3 million years ago (Song et al., 2017), and evolved certain adaptive characters to different environments.

The difference in the number of vertebral column between *Cyprinus carpio* var. *Singonensis* and *Cyprinus carpio* var. *wuyuanensis* caused the difference in their body shape. We used multivariate analysis to conduct a comprehensive analysis of the morphological characteristics in *S. chuatsi* and *S. kneri*. Also, we compared their skeletal characteristics to elucidate the correlation between external phenotypic differences and internal phenotypic differences of the skeleton. Concomitantly, we discussed ecological adaptation based on the phenotypic differences between *S. chuatsi* and *S. kneri*, which will further our understanding of the adaptation and microevolutionary biology in fish.

2. Materials and methods

2.1. Sample collection and measurement of morphological parameters

In mid-March 2018, 1–2 years old *S. chuatsi* and *S. kneri* were collected from Poyang Lake (28°53'N, 116°25'E), China's largest freshwater lake. A total of 43 *S. chuatsi* (body length: 126.27–266.69 mm, mass: 97.00–477.50 g) and 41 *S. kneri* (body length: 74.83–161.41 mm, mass: 23.80–103.00 g) individuals without any classification ambiguity were collected, mainly relying on the relative position of the eye to the posterior end of the oral fissure (The oral fissure in *S. chuatsi* reaches below the posterior edge of the eye or extends farther behind, but ends at the anterior edge of the eye in *S. kneri*.) (Zhou et al., 1988). All individuals were euthanized with 200 mg/L MS-222 (Produced by Hangzhou Animal Pharmaceutical Factory) prior to testing, which took

about 4 min. A total of 35 parameters were measured, 13 conventional quantitative traits and 22 truss parameters (Containing 11 landmarks) used in the analyses of body shape (He, Li, Wang, Blanchet, & Lek, 2013; Vera-Duarte, Bustos, & Landaeta, 2017; Yakubu & Okunsebor, 2011; Yang et al., 2016). All traits and landmarks were manually measured consistently on left side of the specimens.

Conventional quantitative traits included total length (TL), body length (BL), body depth (BD), body width (BW), head length (HL), head depth (HD), oral fissure length (OFL), snout length (SL), eye diameter (ED), distance from the snout to the posterior edge of the eye (SL + ED), interorbital distance (ID), caudal peduncle length (CPL), and caudal peduncle width (CPW) (Fig. 1). For 11 landmarks, we measured the distance of 22 parameters, and they included (1-2), (1-11), (2-3), (2-11), (2-10), (3-4), (3-11), (3-10), (3-9), (4-5), (4-10), (4-9), (4-8), (5-6), (5-9), (5-8), (5-7), (6-7), (7-8), (8-9), (9-10), (10-11) (Fig. 2). The definition and reference of traits and landmarks see Table S1.

The individuals were weighed by an electronic balance (accuracy 0.1 g), and the lengths were measured with a digital vernier caliper (accuracy 0.01 mm).

2.2. Skeletal preparation and measurement

From the above-mentioned morphometric specimens, twenty individuals of each *S. chuatsi* and *S. kneri* were randomly selected. The muscles and viscera were removed and the bodies were simmered in boiling water until the remaining flesh came off easily. Next, the whole skeletons were observed and subsequently disarticulated. The number and shape of the bones in the skull and the vertebrae were compared between the two species by manual observation.

From the above-mentioned morphometric specimens, fifteen individuals of each *S. chuatsi* and *S. kneri* were randomly selected for measurement of skeletal spatial position by X-ray imaging at the Sixth People's Hospital in Shanghai with a molybdenum target film. Adobe Photoshop software was used to measure 12 parameters (see Table 1). The measurement schematics are shown in Fig. 3, Fig. 4, Fig. 8, and Fig. S1, S2.

2.3. Data analysis

2.3.1. Data standardization and parameter selection

To eliminate the difference in size between individuals of the same species, all parameters, excluding body length, were standardized by using the following formula:

$$M_{adj} = \lg \left(\frac{M}{BL} + 1 \right)$$

where M_{adj} represents the standardized data, M is the original measurement, and BL is body length (mm). A standardized parameter is represented by a “parameter abbreviation/BL”. Moreover, after

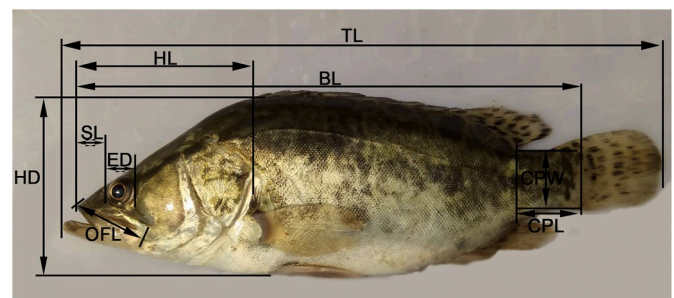


Fig. 1. Morphological measurements in mandarin fish. BL body length; CPL caudal peduncle length; CPW caudal peduncle width; ED eye diameter; HD head depth; HL head length; OFL oral fissure length; SL snout length; TL total length.

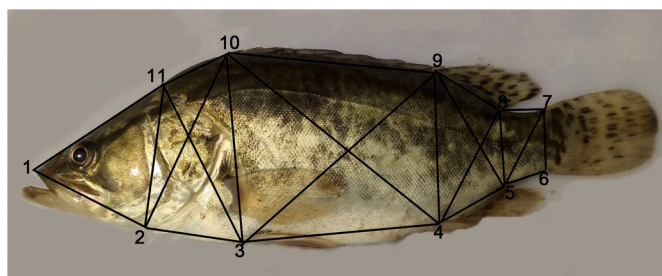


Fig. 2. The measurements of landmarks in mandarin fish.

standardization, the head parameters were compared with head length (Represented by parameter abbreviation/HL), and the SL + ED was compared with the OFL (Represented by SL + ED/OFL).

The standardized data was normally distributed and homoscedastic, and subjected to independent sample analysis (*t*-test) to select parameters with significant differences between the two populations. Based on the results of the *t*-test, the Pearson correlation coefficient was used to screen out independent parameters.

2.3.2. Principal component analysis

The standardized data of the selected parameters that showed a significant difference and independent information were analyzed by principal component analysis (PCA). Kaiser-Meyer-Olkin (KMO) index and Bartlett's sphericity test were used to determine whether the analyzed data were suitable for PCA. Principal components and their contribution rates were calculated by selecting the factors whose eigenvalues were greater than one and by rotating the factor load matrix using the varimax method. Principal component scatter diagram was created based on the factor scores.

2.3.3. Stepwise discriminant function analysis

The stepwise discriminant function was used to select the key difference parameters among the selected independent parameters.

All the statistical analyses were conducted in Microsoft Excel 2010 and SPSS 22.0 (IBM Inc; www.ibm.com), and the differences were considered significant at the 0.05 level.

3. Results

3.1. Morphological parameters selection

Based on the *t*-test results, we found that there were significant differences in 11 of the 35 parameters (Table 2). The results showed significant differences between the two species in BW/BL, CPL/BL, (4-9)/BL, HD/HL, SL/HL, ID/HL, and SL + ED/OFL ($P < 0.01$), and in CPW/BL, (1-11)/BL, (4-8)/BL, and (6-7)/BL ($P < 0.05$). Compared with *S. kneri*, the snout of *S. chuatsi* is shorter, the distance between the left and right eyes is smaller, the eyes are positioned relatively forward, the head back length (point 1-11; Fig. 2) is shorter, the head hump is higher, the body width is larger, base of the caudal fin and posterior trunk is larger, and the caudal peduncle is longer and higher. There was no significant difference in ED/HL between the two populations, which was different from previous studies (Cheng & Zheng, 1987).

According to the Pearson correlation analysis, no parameter with a correlation coefficient greater than or equal to 0.80 was found. Therefore, the above parameters were used as alternative parameters for PCA and discriminant function analysis.

3.2. Principal component analysis

The KMO sampling suitability was 0.764 and the Bartlett sphericity test was 0.000, indicating that the selected data parameters were suitable for PCA. The accumulative contribution rate of the first six principal

Table 1

Skeletal measurement parameters and their description.

Parameters	Description
The ratio of the orbital to skull contour area	The orbital area was calculated as 1/2 of the orbital diameter, the skull contour area included the sum of $\triangle ABC$ and $\triangle BCD$ (Fig. 4)
The angle of the head hump	Fig. 3 $\angle BAC$
The intersection angle of the neurocranium	Fig. 3 $\angle BAE$
The intersection angle of the splanchnocranium	Fig. 3 $\angle EAC$
The length-to-width ratios of bones	The quadrate, metapterygoid, symplectic, hyomandibular, preopercle, opercle, interopercle, and subopercle of the splanchnocranium, which have a supporting structure, were measured (Fig. 4)
The angle of the preopercle	The angle between the long and short diameter of the preopercle were measured and calculated (Fig. 4).
The spatial position of the area encircled with data points JHGF	JHGF, consisting of quadrate, metapterygoid, symplectic, hyomandibular, and preopercle of the splanchnocranium, the bones that have mainly supporting structure, was measured (Fig. 8).
The gill arch curvature	Supplementary Fig. 1 $\angle A_1B_1C_1$
The ratio of curvature width to body depth	The distance between the upper and lower tangents of the spine compared to body depth
The caudal vertebrae depth-to-body depth ratio	The maximum distance between the neural spine and haemal spine of the caudal vertebrae compared to body depth (Fig. 9)
The angles between the caudal vertebrae centrum and each of haemal arch and spine	Supplementary Fig. 2, the angle shown by the red line
The angles between the caudal vertebrae centrum and each of the neural arch and spine	Supplementary Fig. 2, the angle shown by the red line.

components was 85%. Following the principle of common factor extraction, three items with an eigenvalue greater than one were extracted (Table 3), resulting in the cumulative contribution rate of 65.845%. Among them, the contribution rate of the first principal component was 39.224%. The SL/HL, SL + ED/OFL, HD/HL, BW/BL, (4-9)/BL, and (6-7)/BL contributed the most to the differences in population morphology and were mainly reflected in the snout length, position

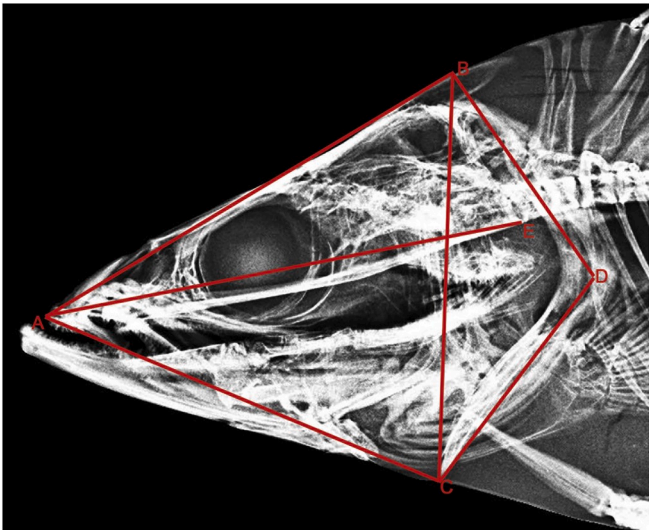


Fig. 3. Diagram of the head measurement in mandarin fish.

of the eyes in relation to the oral fissure, head hump, body thickness, and the width of posterior trunk and base of the caudal fin. The contribution rate of the second principal component was 17.387%. The (1-11)/BL and CPW/BL played the most important roles in the population morphology differentiation, which mainly reflected the difference in the head back length and caudal peduncle width. The contribution rate of the third principal component was 9.233% and it was strongly correlated with ID/HL, the character that mainly reflected the difference in the magnitude of the distance between left and right eye.

Two-dimensional scatter plots were drawn for *S. chuatsi* and *S. kneri* populations based on the three principal components (Fig. 5). The results showed a clear distinction between the two species, as *S. chuatsi* mainly clustered above factor1 = 0 and *S. kneri* below factor1 = 0. The main morphological traits differentiating *S. chuatsi* and *S. kneri* were: snout length, distance between left and right eye, position of the eyes in relation to the oral fissure, size of head hump, head back length, body width, and the width of posterior trunk and base of the caudal fin.

3.3. Stepwise discriminant analysis

The results of the stepwise discriminant analysis (Table 4) showed that HD/HL, SL/HL, ID/HL, and SL + ED/OFL were significant trait

parameters for discriminating the two populations. When these four variables were included in the discriminant function, the multivariate significance test of discriminant effect between the two populations produced the following results: eigenvalue = 5.127, canonical correlation coefficient = 0.91, Wilks' λ = 0.163, χ^2 = 126.895 (P < 0.0001), indicating that the discriminant function established by using these four variables had a significant effect.

The discrimination formulas based on the above four parameters were for the *S. chuatsi* population:

$$Y = -242.665 + 298.323X_1 + 545.209X_2 + 48.5511X_3 + 137.333X_4$$

Table 2
Parameters (mean ± SD) with significant differences of morphological proportions between *S. chuatsi* and *S. kneri* populations.

parameters	<i>S. chuatsi</i>	<i>S. kneri</i>
SL/HL	0.266 ± 0.0308	0.302 ± 0.025
ID/HL	0.316 ± 0.036	0.353 ± 0.030
SL + ED/OFL	0.887 ± 0.076	1.081 ± 0.103
HD/HL	0.697 ± 0.096	0.628 ± 0.050
BW/BL	0.057 ± 0.007	0.051 ± 0.010
CPL/BL	0.055 ± 0.008	0.050 ± 0.009
CPW/BL	0.046 ± 0.004	0.043 ± 0.008
(1-11)/BL	0.096 ± 0.007	0.102 ± 0.015
(4-9)/BL	0.117 ± 0.018	0.105 ± 0.016
(4-8)/BL	0.097 ± 0.009	0.092 ± 0.113
(6-7)/BL	0.049 ± 0.005	0.045 ± 0.009

Table 3
The factor loadings for 11 parameters of *S. chuatsi* and *S. kneri* on the first three principal components.

parameters	the first principal component (PC1)	the second principal component (PC2)	the third principal component (PC3)
SL/HL	−0.684*	0.361	0.035
ID/HL	−0.428	0.349	0.707*
SL + ED/OFL	−0.789*	0.060	−0.058
HD/HL	0.686*	−0.045	0.301
BW/BL	0.760*	0.023	0.172
CPL/BL	0.570	−0.279	−0.346
CPW/BL	0.439	0.794*	0.103
(1-11)/BL	−0.260	0.814*	−0.377
(4-9)/BL	0.727*	0.059	0.158
(4-8)/BL	0.548	0.476	−0.300
(6-7)/BL	0.760*	0.230	0.063

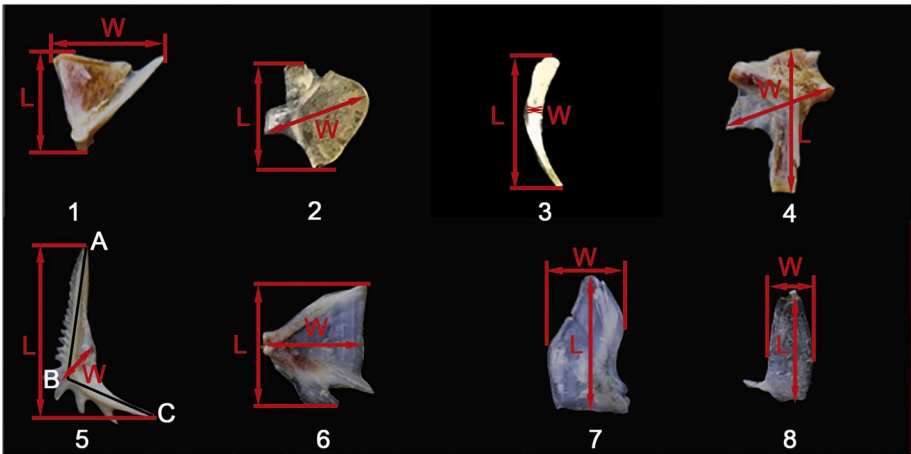


Fig. 4. Diagram of bones measurement in splanchnocranium. 1 quadrate; 2 metapterygoid; 3 symplectic; 4 hyomandibular; 5 preopercle; 6: opercle; 7 interopercle; 8 subopercle; L the length of bone; W the width of bone; A terminus of the long diameter in the preopercle; B middle of the 1st and 2nd spine in preopercle; C terminus of the short diameter in the preopercle.

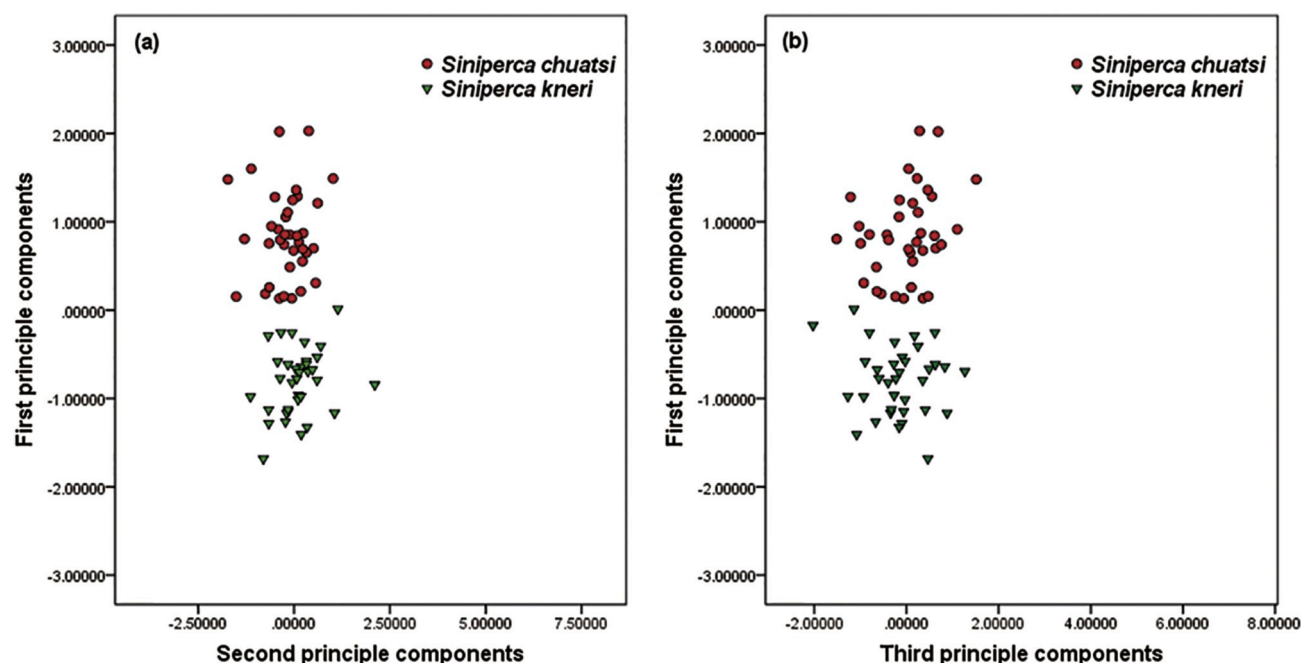


Fig. 5. Morphologic principal component scatter plot of *S. chuatsi* and *S. kneri*. (a) Scatter diagram of principal component 1 and principal component 2. (b) Scatter diagram of principal component 1 and principal component 3.

Table 4

Fisher linear discriminated functions of *S. chuatsi* and *S. kneri* populations.

characters	parameters	population	
		<i>S. chuatsi</i>	<i>S. kneri</i>
X ₁	HD/HL	298.323	239.910
X ₂	SL/HL	545.209	660.653
X ₃	ID/HL	48.5511	74.909
X ₄	SL + ED/OFL	137.333	179.592
	constant	-242.665	-288.0091

and for the *S. kneri* population:

$$Y = -288.009 + 239.910X_1 + 660.653X_2 + 74.909X_3 + 179.592X_4$$

The above formulas were used to identify each fish in each population. The discriminant accuracy and comprehensive discriminant rate of *S. chuatsi* and *S. kneri* were 100% in non-interactive and interactive validation, indicating that the discriminant formula was stable and reliable. Discriminant analysis showed that the main morphological differences between *S. chuatsi* and *S. kneri* were in the eye position, interorbital distance and head hump.

3.4. Bone morphology and number

The skull of *S. chuatsi* and *S. kneri* consists of neurocranium and splanchnocranium. The 38 neurocranial bones were divided into four areas: ethmoid, frontal, ear, and occipital regions. The ethmoid region includes paired nasal, paired lateral ethmoid, one vomer, and one ethmoid. The frontal region includes paired frontal, paired parietal, and five pairs of orbital bones. The ear region consists of paired alisphenoid, paired sphenotic, paired pterotic, paired prootic, paired epiotic, paired opisthotic, one basisphenoid, and one parasphenoid. The occipital region includes one supraoccipital, one basioccipital, and a pair of exoccipital. The splanchnocranium comprises 96 bones and three cartilages in the skull, and they are divided into three zones: mandibular arch, hyoid arch, and branchial arch. The mandibular arch includes a pair of each premaxilla, maxilla, supplementary maxilla, palatine, ectopterygoid, endopterygoid, metapterygoid, quadrate, articular, angular, and

dentary. The hyoid arch consists of a pair of each hyomandibular, symplectic, preopercle, interopercle, opercle, subopercle, epihyal, hypohyal, interhyal, ceratohyal and urohyal, one basihyal, and seven pairs of branchiostegal ray. The branchial arch includes three basibranchial, three pairs of hypobranchials, four pairs of each a ceratobranchial, an epibranchial, and a pharyngobranchial, and a pair of epipharyngeals and hypopharyngeals. Some bones are shown in Fig. 6. The terminology of skull see the reference of Cobcroft, Pankhurst, Sadler, and Hart (2001); Carvalho and Vari (2015), Boyle and Herrel (2018).

The number of vertebrae (including urostyle) in *S. chuatsi* and *S. kneri* are 27–28, with 11–12 precaudal vertebrae, and 16–17 caudal vertebrae. The first and second vertebrae have two dorsal ribs, and starting from the third vertebra, a total of 10 ventral ribs are formed. The terminology of the vertebral column references of Bird and Mabee (2003), Britz and Bartsch (2003), Bensimon-Brito, Cardeira, Cancela, Huysseune, and Witten (2012).

There was no significant difference in the number and shape of the skull bones and vertebrae between *S. chuatsi* and *S. kneri*.

3.5. Spatial differences in the skull

The terminus of the maxilla of *S. chuatsi* is located at the lower edge or farther behind the postorbital bone, while the terminus of the maxilla of *S. kneri* is in front of the postorbital bone (Fig. 7).

The ratio of the orbital to skull area was 0.079 ± 0.017 in *S. chuatsi* and 0.128 ± 0.023 in *S. kneri*. These results suggest that the skull area was larger in *S. chuatsi* than in *S. kneri* ($P < 0.01$).

The head hump angle in *S. chuatsi* is $59.43 \pm 3.20^\circ$, and that in *S. kneri* is $52.80 \pm 1.82^\circ$; the intersection angle of the neurocranium is $20.87 \pm 1.16^\circ$ in *S. chuatsi* and $18.70 \pm 1.10^\circ$ in *S. kneri*; the intersection angle of the splanchnocranium is $38.77 \pm 1.50^\circ$ in *S. chuatsi*, while that in *S. kneri* is $34.07 \pm 0.80^\circ$. There was a significant difference between the head hump angle and the intersection angle of the splanchnocranium ($P < 0.05$). The result showed that the head hump of *S. chuatsi* was significantly greater than that of *S. kneri*, and this difference was contributed to the supporting bones in the splanchnocranium.

There was no significant difference in the length-to-width ratios of



Fig. 6. Comparison of skull bones between *S. chuatsi* and *S. kneri*.

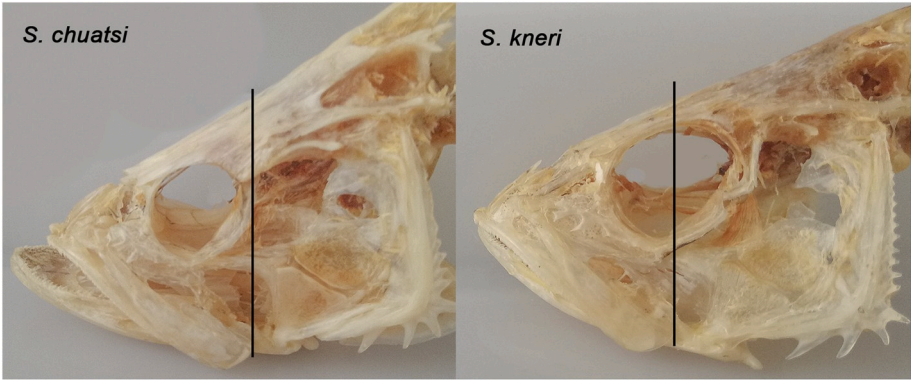


Fig. 7. The relative position of maxilla and postorbital bone in *S. chuatsi* and *S. kneri*. The line indicates the position of terminus of the maxilla.

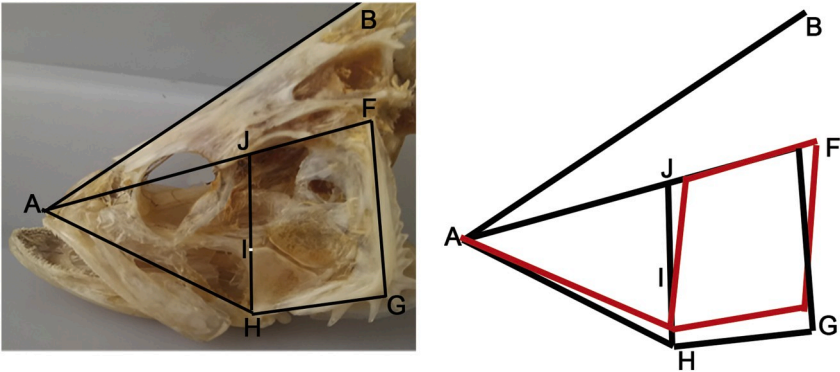


Fig. 8. Positional differences of splanchnocranial bones between *S. chuatsi* and *S. kneri* (red represents *S. kneri*, black represents *S. chuatsi*). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the supporting bones in the splanchnocranium between *S. chuatsi* and *S. kneri* (Table 5). The angle between the length and the short diameter of the preopercle was $103.02 \pm 0.89^\circ$ in *S. chuatsi*, and $102.42 \pm 0.23^\circ$ in *S. kneri*, and they were not significantly different between the two species.

The spatial position of the area encircled with data points JHGF, consisting of quadrate, metapterygoid, symplectic, hyomandibular, and preopercle of the splanchnocranium, the bones that have mainly supporting structure, was measured (Fig. 8). The results showed that the angle $\angle AFG$ was $78.43 \pm 2.07^\circ$ and $\angle AJH$ was $69.63 \pm 3.12^\circ$ in *S. chuatsi*, and the angle $\angle AFG$ was $72.47 \pm 1.70^\circ$ and $\angle AJH$ was $63.02 \pm 2.35^\circ$ in *S. kneri*. The inclination angle of area JHGF and line AF in *S. chuatsi* is larger than that in *S. kneri* ($P < 0.05$). The degree of head hump in *S. chuatsi* and *S. kneri* depends on different whole inclination angles of the quadrate, metapterygoid, symplectic, hyomandibular, and preopercle in the splanchnocranium.

The gill arch curvature of *S. chuatsi* is $47.43 \pm 6.02^\circ$, while that in *S. kneri* is $33.45 \pm 3.87^\circ$; the gill arch curvature of *S. chuatsi* is significantly larger than that of *S. kneri* ($P < 0.05$).

3.6. Differences in the vertebral column

The ratio of curvature width to body depth of *S. chuatsi* was 0.24 ± 0.04 and the caudal vertebrae were oriented upward. In *S. kneri*, the ratio of spine curvature width to body depth was 0.35 ± 0.04 , and the caudal vertebrae was relatively flat (Fig. 9A). The caudal vertebrae depth-to-body depth ratio of *S. chuatsi* was 0.51 ± 0.01 and that of *S. kneri* was 0.49 ± 0.02 . Although the ratio of curvature width to body depth of *S. chuatsi* was smaller compared with that of *S. kneri*, the caudal vertebrae depth-to-body depth ratio in *S. chuatsi* was slightly larger than that in *S. kneri*. The intersection angles between the caudal vertebrae centrum and each of the following: the neural arch and spine; haemal arch and spine were measured (see Fig. S2). The results showed that in *S. chuatsi* the intersection angles between the caudal vertebrae centrum and the neural arch and spine were larger compared with those in *S. kneri*, with the exception of the first caudal vertebra (Table 6). The intersection angles between the caudal vertebrae centrum and the haemal arch and spine were larger in *S. chuatsi* than in *S. kneri*, with the exception of the second and third caudal vertebrae (Table 6).

The connection between the caudal fin and hypurals differed between *S. chuatsi* and *S. kneri*. The third and fifth hypural from bottom to top were each connected with three fin rays in *S. chuatsi* and with two and four fin rays, respectively, in *S. kneri* (Fig. 10).

The last haemal arches and spines in *S. chuatsi* and *S. kneri* were specialized into modified haemal spines. The ridges of the modified haemal spines were closer to each other in *S. chuatsi*, while those of the *S. kneri* were farther apart (Fig. 10).

4. Discussion

4.1. Morphological differences between *S. chuatsi* and *S. kneri*

Previous studies showed that multivariate analysis can be used to quantify the morphological indicators of species. For example, Gong and

Li (2010) used multivariate analysis to study the bones of *Anguilla marmorata* Quoy & Gaimard (1824), *Anguilla bicolor pacifica* Schmidt (1928), *Anguilla rostrata* Lesueur (1817), *Anguilla anguilla* Linnaeus (1758), *Anguilla japonica* Temminck & Schlegel (1846), and *Anguilla australis* Richardson (1841), thereby distinguishing the six eel species.

Cheng and Zheng (1987) qualitatively described the main morphological differences between *S. chuatsi* and *S. kneri*. Compared with *S. kneri*, the head hump of *S. chuatsi* is higher, the eyes are smaller, the posterior of maxilla reaches below the posterior edge of the eye or extends farther behind. In the present study, we quantified the morphological characteristics of *S. chuatsi* and *S. kneri* by using multivariate analysis. The results revealed additional differences in morphological characteristics between the two populations besides the traditional traits mentioned above: (1) Head: there were significant differences in SL/HL and ID/HL and (1-11)/BL ($P < 0.05$) between *S. chuatsi* and *S. kneri* (see Table 2). Three parameters were significantly smaller in *S. chuatsi* than in *S. kneri*, and they included snout length, interorbital distance, and the head back length. (2) Body shape and tail: Significant differences between *S. chuatsi* and *S. kneri* were detected in BW/BL, (4-9)/BL, CPL/BL, (4-8)/BL, (6-7)/BL, and CPW/BL ($P < 0.05$) (see Table 2). These parameters were significantly greater in *S. chuatsi* than in *S. kneri*. Compared with *S. kneri*, the body of *S. chuatsi* is thicker, the posterior trunk and base of the caudal fin are wider, and the caudal peduncle is longer and higher.

Cheng and Zheng (1987) considered that the eyes of *S. chuatsi* were smaller (head length is 5.3–8.1 the eye diameter) and the eyes of *S. kneri* were bigger (head length is 4.7–5.1 the eye diameter). In this study, the average ED/HL of *S. chuatsi* was 0.223 ± 0.122 and that of *S. kneri* was 0.257 ± 0.029 and thus not significantly different ($P > 0.05$). In contrast, the skull area in *S. chuatsi* was significantly larger compared with that of *S. kneri* ($P < 0.01$), which was one of the reasons for visually bigger eyes when observed by naked eyes. In addition, in the traditional descriptions, the relative position of the oral fissure and the eyes is one of the differentiating characters between *S. chuatsi* and *S. kneri*. The parameter analysis showed a highly significant difference between *S. chuatsi* and *S. kneri* of the SL + ED/OFL ($P < 0.01$), but no significant difference of the OFL/HL ($P > 0.05$). This suggests that the difference in the distance of the eyes from the oral fissure in the traditional description was mainly related to the eye position—in *S. chuatsi*, the eyes are positioned relatively forward, whereas the eyes of *S. kneri* are located more to the back.

In the present study, the phenotypic characteristics of *S. chuatsi* and *S. kneri* were quantified and the traditional characters were refined and supplemented, thus providing a more effective reference for ecology and microevolution between *S. chuatsi* and *S. kneri*.

4.2. Skeletal differences between *S. chuatsi* and *S. kneri*

A total of 134 skull bones and 28 vertebrae were counted and subsequently measured after disarticulating the skeleton. The careful comparison of the skeletons of the two species revealed no notable differences in the shape and number of the bones, as well as in the length-to-width ratio of some bones in the splanchnocranium.

The spatial position of the bones differed between *S. chuatsi* and *S. kneri*. The difference in the relative position of the maxilla and the posterior orbital bone led to a different position of the oral fissure and the eye. The degree of head hump is related to the whole inclination angle of the quadrate, metapterygoid, symplectic, hyomandibular, and preopercle in the splanchnocranium, and the inclination angle in *S. chuatsi* was much larger. Concomitantly, the gill arch of the two species also underwent corresponding adaptations, with the gill arch of *S. chuatsi* being larger. The intersection angles between the caudal vertebrae centrum and each of the following: haemal arch and spine; neural arch and spine in the posterior trunk of *S. chuatsi* were larger than those of *S. kneri*, resulting in a wider hind trunk and tail in *S. chuatsi*.

According to the morphological and skeletal characteristics, one of

Table 5
The length-to-width ratios (mean $\pm$ SD) of splanchnocranial bones.

Bones	<i>S. chuatsi</i>	<i>S. kneri</i>	Bones	<i>S. chuatsi</i>	<i>S. kneri</i>
quadrate	0.87 ± 0.02	0.90 ± 0.01	preopercle	0.87 ± 0.02	0.90 ± 0.01
metapterygoid	1.07 ± 0.05	1.15 ± 0.02	opercle	1.07 ± 0.05	1.15 ± 0.02
symplectic	6.92 ± 0.02	7.10 ± 0.04	interopercle	6.92 ± 0.02	7.10 ± 0.04
hyomandibular	1.26 ± 0.07	1.29 ± 0.05	subopercle	1.26 ± 0.07	1.29 ± 0.05

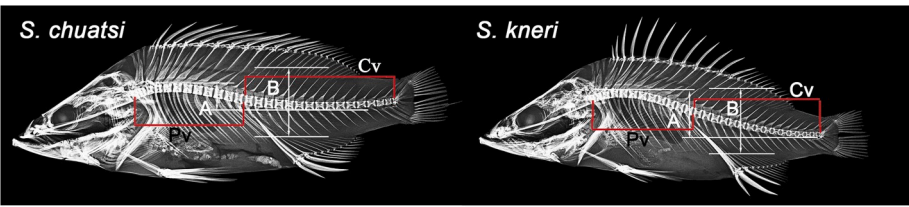


Fig. 9. Difference of spinal curvature between *S. chuatsi* and *S. kneri*.

Table 6
Comparison (mean ± SD) of the angles between the caudal vertebrae centrum and each of the following: the neural arch and spine; hemal arch and spine.

vertebrae	<i>S. chuatsi</i>		<i>S. kneri</i>	
	hemal arch and spine	neural arch and spine	hemal arch and spine	neural arch and spine
1 st caudal vertebra	–	53.9 ± 3.0°	–	57.2 ± 2.5°
2 nd caudal vertebra	38.4 ± 0.1°	58.1 ± 1.2°	39.2 ± 1.5°	56.8 ± 1.0°
3 rd caudal vertebra	41.9 ± 0.2°	57.8 ± 2.4°	42.3 ± 2.9°	52.6 ± 2.6°
4 th caudal vertebra	41.8 ± 0.5°	56.6 ± 2.5°	40.1 ± 1.4°	48.9 ± 2.9°
5 th caudal vertebra	39.0 ± 0.8°	52.8 ± 2.6°	38.2 ± 0.3°	44.0 ± 1.0°
6 th caudal vertebra	40.0 ± 1.1°	48.9 ± 2.2°	37.7 ± 1.7°	43.0 ± 1.9°
7 th caudal vertebra	41.3 ± 2.0°	47.1 ± 3.3°	36.4 ± 0.5°	44.3 ± 0.9°
8 th caudal vertebra	42.9 ± 1.8°	47.0 ± 3.3°	36.4 ± 2.1°	41.6 ± 1.2°
9 th caudal vertebra	39.4 ± 2.1°	44.8 ± 1.7°	35.7 ± 0.5°	40.8 ± 2.3°
10 th caudal vertebra	39.8 ± 1.2°	39.7 ± 0.6°	36.6 ± 1.5°	38.7 ± 0.9°
11 th caudal vertebra	39.5 ± 2.4°	39.4 ± 1.6°	35.8 ± 1.4°	35.9 ± 0.9°
12 th caudal vertebra	37.8 ± 0.3°	35.9 ± 0.7°	32.3 ± 1.8°	34.1 ± 0.6°
13 th caudal vertebra	40.5 ± 3.8°	32.6 ± 0.9°	31.4 ± 3.4°	31.9 ± 2.1°
14 th caudal vertebra	37.4 ± 0.5°	30.8 ± 1.0°	36.7 ± 2.0°	29.6 ± 2.6°
15 th caudal vertebra	42.4 ± 1.4°	–	36.2 ± 2.9°	–

the main factors of the phenotypic difference between the two species is the difference in the spatial position of the internal skeleton.

4.3. Ecological adaptation of morphological differences between *S. chuatsi* and *S. kneri*

Extant sinipercline species inhabit freshwater, but their ancestors were originally marine teleosts, which shifted into fresh water during the long-term transgressions and regressions (relative to sea level) in East Asia in the Tertiary (Li, 1991). From the Miocene to the Pliocene, the sinipercline radiated on a large scale, resulting in the emergence of *Siniperca* fishes in China (Liu, 1993). Song et al. (2017), who estimated the divergence time of *Siniperca* based on fossil information, concluded that the common ancestor of this genus was diverged in the Miocene (12.2 million years ago), and the two species *S. chuatsi* and *S. kneri* evolved 4.3 million years ago.

The natural distribution of *S. chuatsi* and *S. kneri* is different in geographical location and water system, but there are overlaps and cross-distributions. In China, *S. chuatsi* are distributed in the northern areas and *S. kneri* in the southern areas. In overlapping distribution areas, *S. kneri* can be present in the upper, middle, and lower reaches and tributaries, while *S. chuatsi* live in the middle and lower reaches or lakes. It is presumed that *S. chuatsi* and *S. kneri* differentiated as the result of adaptation to different freshwater environments. The speciation, in addition to being affected by changes in the sea level, was influenced by geological movements, the uplift of the Himalaya–Tibetan plateau, glacier movements, development of monsoon climate, and so on (Li, 1981; Yin & Harrison, 2000). With the formation of different ecological niches, the fish body displayed corresponding adaptive characteristics.

Fish body shape is closely related to its swimming ability (Lauder, 1986). In the upper reaches and tributaries of the rivers, the water is generally turbulent. Under such conditions, the lower head hump, thinner trunk, and streamlined body shape of the *S. kneri* helps reducing the resistance and increasing swimming speed during predation and enemy evasion. In contrast, *S. chuatsi* inhabit environments with slower water flow, encounter fewer enemies and pests, and therefore do not require long distance movements. Their body is characterized by high head hump, wide trunk, and relatively poor swimming ability (Liang,

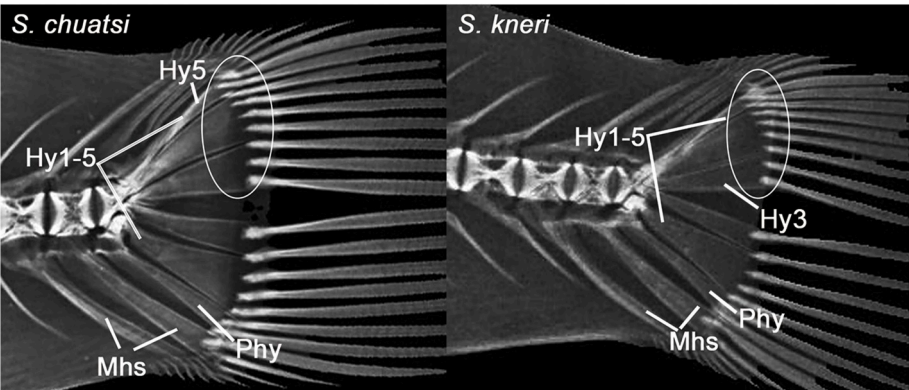


Fig. 10. The difference of caudal skeleton between *S. chuatsi* and *S. kneri*. Hy hypural, Mhs modified hemal spine, Phy parhypural.

1995a), hindering active pursuit of prey. As an ambush predator, the larger caudal area increases the swimming speed during the sudden attack, as the caudal peduncle length was positively correlated with swimming speed (Langerhans, Layman, Shokrollahi, & Dewitt, 2004). Therefore, the posterior trunk of *S. chuatsi* is large and the caudal peduncle is long and high, resulting from their adaptation to middle and lower reaches.

Vision plays a major role in the predation by mandarin fish (Liang, 1995a). Eye size and their position is related to the position of food in water (Hugueny & Pouilly, 1999). The eyes of *S. kneri* occupy a large proportion of the head area, and they are located relatively back on the head, all of which facilitates food identification and search in a flowing water environment.

Both *S. chuatsi* and *S. kneri* are carnivorous fish and they mainly feed on fish and shrimp (Liang, 1996), although the *S. kneri* diet includes a small amount of zooplankton, aquatic insects, and plant debris (Han, Hu, & Hong, 1996; Li, 2008). The difference in their diets may be related to the different ecological niches occupied by these two species. *S. kneri* lives in the upper reaches where food resources are relatively scarce. When food resources are scarce, they will feed on zooplankton, aquatic insects, and plant debris for survival. *S. chuatsi* lives in rivers and lakes with relatively abundant food resources and maintains its lifelong feeding habit of praying on fish and shrimp. In addition, the digestive structure and protease activity of *S. chuatsi* and *S. kneri* reflect their corresponding adaptations (Han et al., 1996; Lv, Chen, Qing, & Fang, 2005).

Ethics statement

All animal handling procedures were approved by the Animal Care and Use Committee of Shanghai Ocean University and followed the guidelines of animal experiments on Shanghai Ocean University.

CRedit authorship contribution statement

XiaoYing Cao: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft. **JinLiang Zhao:** Conceptualization, Resources, Data curation, Writing - review & editing, Supervision, Project administration, Funding acquisition. **ChenHong Li:** Conceptualization, Resources, Writing - review & editing. **ShuQin Zhu:** Investigation, Validation. **YueYue Hao:** Investigation, Data curation. **YaMei Cheng:** Investigation, Data curation. **HongYan Wu:** Resources.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://doi.org/10.1016/j.aaf.2020.04.007>.

Numbers denote landmark number. 1 tip of snout; 2 terminus of angular; 3 origin of pelvic fin; 4 origin of anal fin; 5 posterior end of anal fin base; 6 ventral origin of caudal fin; 7 dorsal origin of caudal fin; 8 posterior end of dorsal fin base; 9 posterior end of dorsal fin spines; 10 origin of dorsal fin; 11 posterior point of the neurocranium.

The skull contour area (the sum of $\triangle ABC$ and $\triangle BCD$), the angle of the head hump ($\angle BAC$), the intersection angle of the neurocranium ($\angle BAE$), and the intersection angle of the splanchnocranium ($\angle EAC$) were measured with Adobe Photoshop software. A front of premaxilla, B

middle of supraoccipital, C terminus of cleithrum, D middle of cleithrum, E terminus of basioccipital.

The bones of *S. chuatsi* are shown left of the number and *S. kneri* are shown right of the number. 1 maxilla, 2 premaxilla, 3 dentary, 4 palatine, 5 articular and angular, 6 quadrate, 7 hyomandibular, 8 endopterygoid, 9 ectopterygoid, 10 frontal, 11 supraoccipital, 12 vomer, 13 basioccipital, 14 parasphenoid, 15 preopercle, 16 opercle, 17 interopercle, 18 subopercle.

A front of premaxilla, B terminus of supraoccipital, F top of preopercle, G middle of the 2nd and 3rd spine in preopercle, H front of quadrate, I terminus of quadrate, J intersection of line HL and AF.

A the distance between the upper and lower tangents of the spine, B the maximum distance between the neural spine and hemal spine of the caudal vertebrae; Pv Precaudal vertebrae: Anterior vertebral region lacking hemal arches and spines, bearing ribs and parapophyses. Cv Caudal vertebrae: the first caudal vertebra has a completely fused hemal arch (see Bird & Mabee, 2003).

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