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Revision of *Schizopygopsis chengi* Fang 1936 (Cypriniformes: Cyprinidae), with a description of a new subspecies

FEI TIAN[1], SIJIA LIU[1], SHENGXUE CHEN[1,2] & KAI ZHAO[1,3*]

[1]*Key Laboratory of Adaptation and Evolution of Plateau Biota, Qinghai Key Laboratory of Animal Ecological Genomics, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China*
tianfei@nwipb.cas.cn; https://orcid.org/0000-0002-6020-1459
liusj@nwipb.cas.cn; https://orcid.org/0000-0003-3302-9128
[2]*University of Chinese Academy of Sciences, Beijing, China*
chenshengxue@nwipb.cas.cn; https://orcid.org/0009-0008-0698-5337
[3]*Northwest Institute of Plateau Biology, Chinese Academy of Sciences No. 23 Xinning Road, Xining, Qinghai 810001, China*
**Corresponding author: zhaokai@nwipb.cas.cn; https://orcid.org/0000-0001-9262-3157*

Abstract

The species status of *Schizopygopsis chengi*, which is defined as a subspecies of *Schizopygopsis malacanthus*, is under debate. In the present study, comprehensive morphological and molecular analyses were performed on *S. chengi*, its closest relatives *S. malacanthus* and other *Schizopygopsis* fishes. The results showed that *S. chengi* did not form a sister lineage to *S. malacanthus*, with morphological differences in unbranched rays of the dorsal fin. The morphological and molecular evidence indicated that *S. chengi* was a valid species and was separated from *S. malacanthus*. By examining specimens of *S. chengi* from the Marke River, Keke River, Duoke River and Baoxing River, populations from the Duoke River showed morphological characteristics of mouth inferior, transverse oral fissure, relative long predorsal length than other geographic populations. The monophyly of population from Duoke River was strongly supported by mitochondrial sequence datasets. Based on morphological and molecular evidence, specimen from Duoke River is considered a newly identified subspecies and named as *Schizopygopsis chengi duokeheensis*.

Key words: *Schizopygopsis chengi duokeheensis,* Qinghai-Tibetan Plateau, Taxonomy, species delimitation, subspecies

Introduction

The cyprinid genus *Schizopygopsis* was established by Steindachner in 1866. It is a group of benthopelagic fish species that is characterized by an almost entirely naked body, no barbels, two rows of pharyngeal teeth, inferior mouths, lower jaws with sharp horny layers and sparse gill rakers (Nichols and Chu 1935; Wu and Wu 1992; Chen and Cao 2000). *Schizopygopsis* species are extensively found in the rivers and lakes across the Qinghai-Tibetan Plateau (QTP), encompassing the headwaters of the Yellow River, Yangtze (Chang-jiang) River, Lancang River, Indus River, and the Yarlung Zangbo River (Wu and Wu 1992). Chen and Cao (2000) recognized 12 species and subspecies in this genus in the latest review of Schizopygopsinae by depicting variations in gill raker numbers, the commencement of the ventral fin, the horny lay of the lower jaw and the spine of the dorsal fin (Chen and Cao 2000).

A Chinese ichthyologist, Fang, named a new species in 1936 as *Chuanchia chengi* based on only one specimen collected from Songpan (=Sung-pan, 松潘), Sichuan Province, China (Fang 1936) . However, its taxonomic status is controversial. Fang recognized it as a species of the genus *Chuanchia* based on the characteristics of mouth inferior, nearly straightly transverse oral fissure, the origin of the ventral fin below the 4–5th branched ray of the dorsal fin, and a lower interorbital space wider than the snout length. Nevertheless, it exhibited morphological distinctions from *Chuanchia labiosa*. In contrast to *C. labiosa*, which possesses seven branched rays of the dorsal fin and a continuous posterior groove on the lower lip, *S. chengi* is characterized by eight branched rays of the dorsal fin, a discontinued posterior groove on the lower lip, as well as a greater body depth and a shorter caudal peduncle. The morphology of

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the posterior groove of the lower lip is used to distinguish the genera *Chuanchia* and *Schizopygopsis*. Cao and Deng (1962) therefore recognized *Chuanchia chengi* as a subspecies of *Schizopygopsis malacanthus* Herzenstein, 1891. *Schizopygopsis malacanthus chengi* has soft unbranched rays of the dorsal fin without serratures in both large and small individuals, whereas *S. m. malacanthus* has strong unbranched rays of the dorsal fin with obvious serratures in small individuals. In addition, Fu, Ding, and Ye (1994) published *Schizopygopsis malacanthus baoxingensis,* a subspecies of *S. malacanthus,* from the Baoxing (BX) River, one of tributaries of the Dadu River (Ding 1994; Yu, *et al.* 2006; Hou, *et al.* 2013). Most ichthyologists agree that *S. malacanthus* includes three subspecies, *S. m. malacanthus* from the Tongtian River, *S. m. chengi* from the Dadu River, and *S. m. baoxingensis* from the Baoxing River (Cao and Deng 1962; Liu 1964; Cao, *et al.* 1981; Ding 1994) (Zhang and Zhao, 2016).

On the other hand, Wu and Wu (Wu and Wu 1992) considered *Schizopygopsis malacanthus chengi* to be an independent species, *S. chengi*, based on its morphological characteristics in the unbranched ray of the dorsal fin and the terminal position of the anal scale. Guo *et al.* (Guo, *et al.* 2021) also recognized it as *S. chengi* and meantime designated *S. m. baoxingensis* as a subspecies of *S. chengi*, without the declaration on their basis. Moreover, phylogenetic analyses revealed that *S. chengi* and *S. malacanthus* formed monophyletic groups without a sistership relationship, suggesting that they were two different species (Yu, *et al.* 2006; Qi, *et al.* 2015). However, in the absence of morphological examination and comparisons of specimens, these studies have not convincingly revised or redescribed the taxonomic status of *S. chengi*. Consequently, the species status of *S. chengi*, whether it is a subspecies of *S. malacanthus* or a distinct species, remains ambiguous, as does its relationship with other *Schizopygopsis* species.

To address this taxonomic issue, we conducted extensive surveys and collections in the known distribution areas of these two species from 2020 to 2023. Morphological, genetic, and phylogenetic analyses have enabled us to confirm that *Schizopygopsis chengi* is an independent species. At the same time, we have identified a new subspecies from specimens collected from the Duoke River, which we report here together.

Materials and methods

Sampling and ethics statement

Schizopygopsis chengi and *S. m. malacanthus* were collected in upstream of the Dadu River and the Yangtze River in Qinghai and Sichuan Provinces from 2020–2023 (Table 1 and Fig. 1). Samples of *S. chengi* were collected from the headstream of Dadu River, including the Marke River (MK), the Keke River (KK) and the Duoke River (DK). Samples of *S. m. baoxingensis* were collected in the Baoxing River (BX). Samples of *S. m. malacanthus* (YS) were collected from the headstream of the Yangtze River, including the Tongtian River (AnC), the Batang River (BaT) and the Chumaer River (ChuM) River. The field investigation and sampling procedures were issued and supervised by the Qinghai Provincial Bureau of Fishery. The present study was performed based on guidelines described in the "Guidelines for Animal Care and Use" manual approved by the Animal Care and Use Committee, Northwest Institute of Plateau Biology, Chinese Academy of Sciences.

TABLE 1. Sample information of *S. chengi* and *S. malacanthus.*

Species	Sampling locality	Basin	Code	NMA	NGA
S. chengi	Marke River,	Dadu River	MK	19	35
	Keke River,	Dadu River	KK	10	28
	Duoke River,	Dadu River	DK	25	38
	Baoxing River	Qingyijiang River	BX	15	7
S. malacanthus	Tongtian River,	Yangtze River	AnC	19	30
	Batang River,	Yangtze River	BaT	23	44
	Chumaer River	Yangtze River	ChuM	24	33

NMA, the number of specimens for morphological analysis; NGA, the number of specimens for genetic analysis.

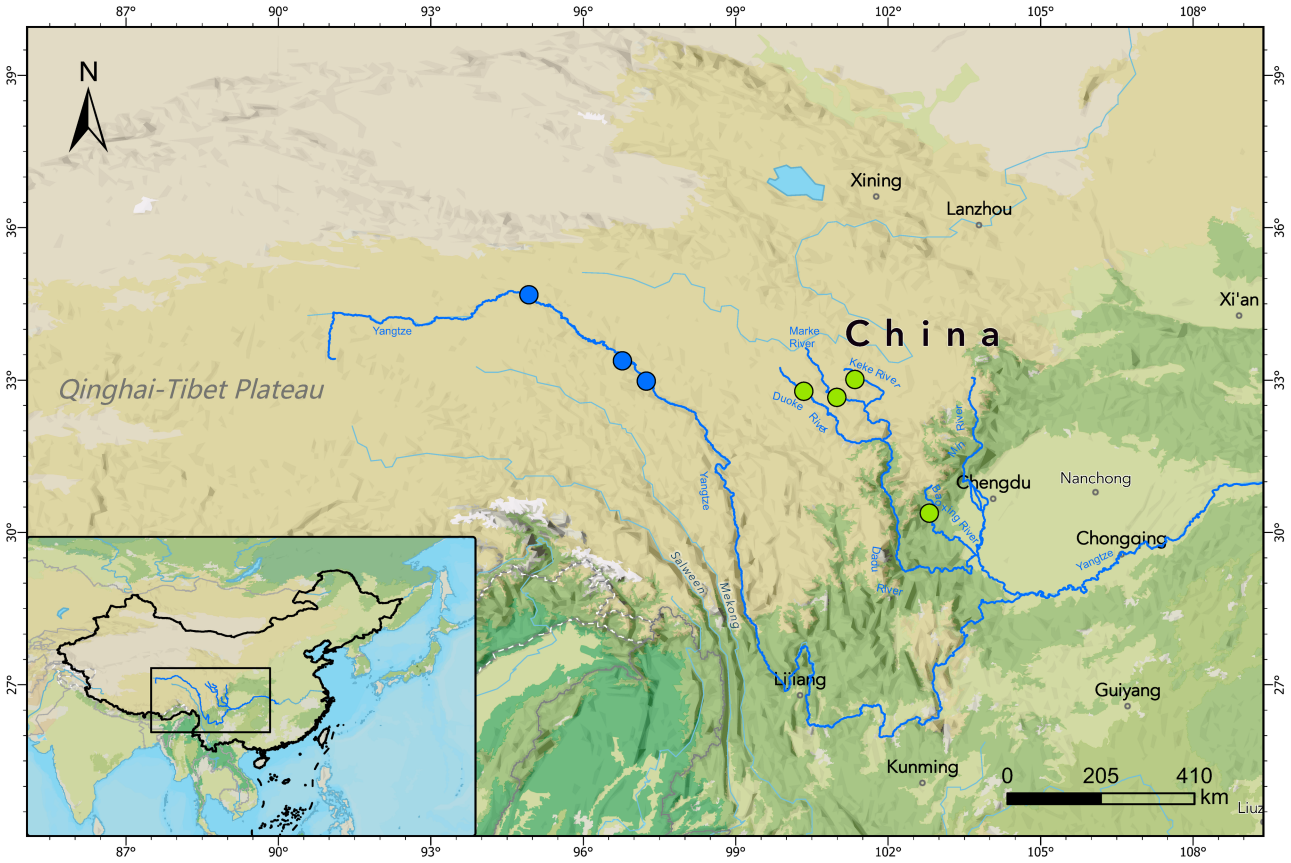

FIGURE 1. Sampling localities. The green and blue circles indicate the sampling sites for *S. chengi* and *S. malacanthus*.

In total, 69 specimens of *S. chengi* and 66 specimens of *S. malacanthus* (details in the comparative materials section) were collected and euthanized using 200 mg/L MS222 (Sigma, USA), after which the caudal fins were excised and preserved in 95% ethanol for DNA extraction. For morphological examination, the specimen was initially preserved in 10% formaldehyde solution. Voucher specimens were labeled and deposited at the Northwest Institute of Plateau Biology, Chinese Academy of Sciences.

Morphometrics

Based on the descriptions by Chen *et al.* (Chen and Cao 2000) and Wu *et al.*(Wu and Wu 1992), characters, including the presence or absence of serratures on the posterior edge of the dorsal spine, the ending edge of the anal scaly sneath in the base of the ventral fin or near the midpoint between the ventral and anal fins, were examined in both small and large specimens. The measurements of nineteen morphological characteristics were taken on the right side of well-preserved specimens with digital calipers and were recorded to the nearest 0.1 mm. The length of the head and other measurements of the body part were calculated as the proportion of the standard length (SL). The measurements of the head part are shown as the proportion of the head length (HL). Additionally, the ratios of caudal-peduncle length (CPL) to caudal-peduncle depth (CPD), of snout length (SL) to mouth width (MW), and of interorbital width (IEW) to eye diameter (ED) were calculated. The number of gill rakers of the first gill arch was counted, including the number of inside and outside of the first arch on the right side of the specimen. Principal component analysis (PCA) was applied to summarize the morphometric data that were expressed as percentages in R (v 1.4.17).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from the caudal fins of the fish using the standard phenol–chloroform method (Sambrook, *et al.* 1982). The mitochondrial Cyt *b* sequence was amplified using PCR with the universal primers L14724 (5'-GACTTGAAAAACCACCGTTG-3') and H15915 (5'-CTCCGATCTCCGGATTACAAGAC-3') according to the description by Xiao *et al.* (Xiao, *et al.* 2001). PCR was performed in a 35 μl reaction volume with the following cycling conditions: initial denaturation at 95°C for 5 min; 35 cycles of a denaturation step at 95°C for 30 sec, an annealing step at 55°C for 35 sec and an extension step at 72°C for 1 min; and a final extension step at 72°C for 10 min. PCR products were visualized by electrophoresis on a 1.0% low-melting agarose gel. The target band of 1140 bp was fractionated and purified using a gel extraction kit (Sangon Biotech, China) and then sequenced in an ABI 3730 sequencer (Agilent Technologies, USA).

Population genetics

Cyt *b* sequences of *Schizopygopsis* fishes were assembled using the SeqMan program (DNAstar) (Burland 2000) and aligned using MAFFT in PhyloSuite (Katoh, *et al.* 2005; Zhang, *et al.* 2020). The aligned sequences were trimmed to generate a length of 1140 bp for the Cyt *b* gene, which was deposited in the GenBank library under Accession Nos listed in Table 2. To infer phylogenetic relationships, sequences of Cyt *b* genes were retrieved from GenBank (Table 2), which included all *Schizopygopsis* fishes and outgroups of *Gymnodiptychus pachycheilus*, *Oxygymnocypris stewartii*, *Diptychus maculatus*, *Gymnodiptychus dybowskii* and *Gymnodiptychus integrigymnatus* (Du, *et al.* 2016; Li, Peng, *et al.* 2016; Li, Tang, *et al.* 2016; Li, Huang, *et al.* 2016; Zeng, *et al.* 2016). Genetic differentiation (F_{st}) and genetic distance were calculated using DnaSP (v 6.12) and MEGA X (Rozas, *et al.* 2017; Kumar, *et al.* 2018).

TABLE 2. Information of Specimens analyzed in the study.

Specimens	Voucher number	Sampling location	Basin	Cyt *b* (Genbank No)	Source
S. chengi	NWIPB2107002–NWIPB2107018	Marke River,	Dadu River	OR972943–OR972949	This study
	NWIPB2107019–NWIPB2107034	Keke River,	Dadu River	OR972936–OR972942	This study
	NWIPB2107036–NWIPB2107054	Duoke River,	Dadu River	OR972926–OR972935	This study
	NWIPB231201–NWIPB231217	Qingyijiang River	Dadu River	DQ533797, DQ533798	Genbank
S. malacanthus	NWIPB2206001–NWIPB2206019	Tongtian River,	Yangtze River	OR972950–OR972953	This study
	NWIPB2206020–NWIPB2206042	Batang River,	Yangtze River	OR972954–OR972957	This study
	NWIPB2206043–NWIPB2206068	Chumaer River	Yangtze River	OR972958–OR972961	This study
S. kessleri	NWIBP2007004, NWIBP2007006, NWIBP2007051, NWIBP2007054	Golmud River	Qaidam Basin	MN267668, MN267669	Genbank
S. pylzovi	NWIPB1205412–5	Yellow River	Yellow River	KY461363, KY461364	Genbank

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TABLE 2. (Continued)

Specimens	Voucher number	Sampling location	Basin	Cyt *b* (Genbank No)	Source
S. stoliczkai	NWIPB1007024, NWIPB100705	Qaraqash River	Hotan River		Genbank
	NWIPB1160487	Pangong Co	Pangong Co	KY461356, KY461357	Genbank
	NWIPB1160383	Lake Manasarovar	Lake Manasarovar		Genbank
S. thermalis	NWIPB1170189–NWIPB1170192	Yuqu River	Nu Jiang	KY461318, KY461319	Genbank
S. younghusbandi	NWIBP1160962–3	Pengqu River	Ganges River		Genbank
	NWIPB0906028–9	Nyang River	Yarlung Zangbo River	KY461322, KY461323	Genbank
S. kialingensis	NWIPB1108004–007	Bailong River	Jialing River	KY461338–KY461340	Genbank
S. anteroventris	NWIPB1108432–5	Lancang River	Lancang River	KY461341–KY461343	Genbank
S. malacanthus	T290–2	Jinsha River	Yangtze River	KY461344–KY461346	Genbank
H. microcephalus	T240, T242	Tuotuo River	Yangtze River	KY461331, KY461333	Genbank
O. stewartii	T78	Yarlung Zangbo River	Yarlung Zangbo River	KY461387	Genbank
G. pachycheilus	T621	Yellow River	Yellow River	KY461377	Genbank
G. dybowskii	1007174	Kaidu River	Tarim River	KJ081379	Genbank
D.maculatus	1305134	Ili River	Ili River	KX022699	Genbank
G. integrigymnatus	T1530	Longchuan Jiang	Jinsha Jiang	KY461321	Genbank

Phylogenetic analysis

The phylogenetic relationships of *Schizopygopsis* fishes were analyzed in PhyloSuite (1.3.2) (Zhang, *et al.* 2020). Multiple sequences were imported into PhyloSuite for alignment and the construction of maximum likelihood (ML) and Bayesian inference (BI) trees using the Bayesian information criterion (BIC) (Kalyaanamoorthy, *et al.* 2017). ML analysis was run in PhyloSuite with a model of TN+F+G4 and 1000 bootstrap replications to evaluate the support for each node (Guindon and Gascuel 2003). Haplotype network was plotted based on 40 haplotypes from using Popart (Leigh and Bryant 2015). BI analysis was carried out using Mrbayes (v.3.2.6) (Ronquist, *et al.* 2012), which included two independent runs of 3×10^6 generations with four Markov chain Monte Carlo (MCMC) chain sampling every 100 generations with 25% burn-in. Convergence was assessed by Trace (v 1.7.1) through the examination of the average standard deviation of split frequencies (< 0.01) and the effective sample size (ESS) values (i.e., ESS > 200) (Andrew, *et al.* 2018; Luo, *et al.* 2023).

Species delimitation

The Bayesian implementation of the Poisson tree processes (bPTP) model (https://species.h-its.org/ptp/) (Zhang, *et al.* 2013) and an Assemble Species by Automatic Partitioning (ASAP) (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) (Puillandre, *et al.* 2021) were adopted for species delimitation. For the bPTP model, an unrooted ML tree was used as a guide tree, and the MCMC was set as 100,000 generations. In ASAP, the aligned fasta file was selected as the input and run with the default parameter using three substitution models, including the p-distances, the Jukes-Cantor (JC69) distances and Kimura (K80) TS/TV distances.

TABLE 3. Morphological characters in the genus *Schizopygopsis*.

species	The commencement of ventral fin	Unbranched dorsal fin ray	Mouth	Horny layer of lower jaw	Fin ray	ING/ ONG	Anal scales	Predorsal length (% of L_s)	Body depth (% of L_s)	HL (% of L_s)	CPD/CPL
Schizopygopsis stoliczkai (n=4)	under the 4–5th branched ray of dorsal fin	Strong with obvious serratures	Inferior, transverse or slightly hooked	Wide, straight and sharp	D vi, 8; P i, 17–19 V i, 8–9; A iii, 5	29–33/ 15–18	reaching the base of ventral fin	48.15–50.48	19.02–20.56	21.07–22.49	44.89–45.72
S. s. chengi (n=29)	under the 4–5th branched ray of dorsal fin	Soft with few and tiny serratures	Inferior, straightly transverse	Wide, straight and sharp	D vi, 8; P i, 19–21 V i, 9; A iii, 5	25–37/ 15–20	reaching the base of ventral fin	41.30–48.41	18.32–26.78	23.51–28.32	38.51–59.80
S. c. duokehenesis (n=25)	under the 4–5th branched ray of dorsal fin	Soft with few and tiny serratures	Inferior, transverse	Wide, straight and sharp	D vi, 8; P i, 18–21 V i, 9–10; A iii, 5	27–36 /17–21	reaching the midpoint between ventral and anal fins	45.25–53.05	17.89–26.60	24.09–26.18	41.73–55.38
S. c. baoxingensis (n=17)	under the 4th branched ray of dorsal fin	Soft with few and tiny serratures	Inferior, slightly hooked	Wide, straight and sharp	D vi, 8; P i, 17–20 V i, 9–10; A iii, 5	16– 23/10–15	reaching the midpoint between ventral and anal fins	35.79–47.38	20.41–25.00	20.40–25.64	44.31–50.00
S. malacanthus (n=61)	under the 4–5th branched ray of dorsal fin	Strong with serratures	Inferior, transverse or slightly hooked	Wide, straight and sharp	D vi, 8; P i, 16–18 V i, 8–10; A iii, 5	26–38/ 16–25	reaching the midpoint between ventral and anal fins	43.70–50.12	17.24–25.98	19.83–26.84	34.26–56.94
Schizopygopsis kessleri (n=4)	under the 2–3th branched ray of dorsal fin	weak with tiny serratures	Inferior, transverse or slightly hooked	Sharp and narrow	D vi, 8; P i, 17–19 V i, 8–9; A iii, 5	27–31 /16–18	reaching the base of ventral fin	47.81–48.85	18.00–18.05	22.28–23.76	39.10–40.91

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TABLE 3. (Continued)

species	The commencement of ventral fin	Unbranched dorsal fin ray	Mouth	Horny layer of lower jaw	Fin ray	ING/ ONG	Anal scales	Predorsal length (% of L_s)	Body depth (% of L_s)	HL (% of L_s)	CPD/CPL
Schizopygopsis pylzovi (n=4)	under the 2–3th branched ray of dorsal fin	Strong with obvious serratures	Inferior, transverse and big	Sharp and narrow	D vi, 8; P i, 15–17 V i, 8–9; A iii, 5	29–31/16–18	reaching the base of ventral fin	47.27–47.34	18.69–20.38	22.05–23.12	43.19–45.51
Schizopygopsis thermalis (n=4)	under the 4–5th branched ray of dorsal fin	Strong with obvious serratures	Inferior or sub-inferior	Thin and sharp	D vi, 8; P i, 19 V i, 9; A iii, 5	19–20/11–12	reaching the midpoint between ventral and anal fins	47.10–47.58	17.89–20.05	23.59–25.67	42.44–43.16
Schizopygopsis younghusbandi (n=4)	under the 4–5th branched ray of dorsal fin	weak and smooth	Inferior, transverse or hooked	Sharp and narrow	D vi, 8; P i, 18–19 V i, 8–9; A iii, 5	19–20/11–12	reaching the base of ventral fin	45.05–47.62	18.76–23.66	21.22–23.51	39.65–42.00
Schizopygopsis kialingensis (n=4)	under the 2–3th branched ray of dorsal fin	weak and smooth	Inferior or sub-inferior, deeply hooked	Thin and sharp	D vi, 8; P i, 17–19 V i, 8–9; A iii, 5	14–16/10–12	reaching the base of ventral fin or midpoint between ventral and anal fins	49.26–49.50	46.89–51.03	24.28–25.60	40.13–43.48
Schizopygopsis anteroventris (n=4)	under the 1st branched ray of dorsal fin	Strong with obvious serratures	Inferior, transverse or hooked	Sharp	D vi, 7; P i, 17–18 V i, 8–9; A iii, 5	13–15/20–23	reaching the base of ventral fin	46.95–48.12	19.28–23.15	22.98–23.72	40.76–48.14

Results

Validation of *Schizopygopsis chengi*

Morphological, genetic and phylogenetic evidence supports *Schizopygopsis chengi* as a valid species.

Schizopygopsis chengi is distinguished from other *Schizopygopsis* fishes by having an outside gill raker number of the first gill arch greater than 10, an inside gill raker number of the first gill arch greater than 16 and the commencement of a ventral fin under the 4–5th branched ray of the dorsal fin (Table 3). *Schizopygopsis chengi* differs from *S. malacanthus* in the following characteristics: soft unbranched rays of dorsal fins with small and fewer serratures at the posteriors edge (*vs.* strong unbranched rays of dorsal fin with obvisous serratures in *S. malacanthus*); no black dots on the dorsal fin (*vs.* black dots on the dorsal fin of *S. malacanthus*); large black spots on the body (*vs.* small dots on the back of the body or above the lateral line in *S. malacanthus*); and an independent distribution area in the headstream of the Dadu River in western China (*vs.* the distribution of *S. malacanthus* in the upstream of the main stream of the Yangtz River in western China) (Fig. 2 and Tables 3–4).

FIGURE 2. Pictures of *S. chengi* and *S. malacanthus.* a. The lateral view of *S. chengi*. **b.** Dorsal fin of *S. chengi.* **c.** The lateral view of *S. malacanthus*. **d.** Dorsal fin of *S. malacanthus.*

The measurements of morphological characteristics are shown in Table 4. PCA revealed that 49% of the total variance was explained by the first three components, including 24%, 14% and 11% for PC1, PC2 and PC3, respectively (Table 5). Along PC1, the specimens were clearly separated into two groups, corresponding to *S. chengi* and *S. malacanthus* (Fig. 3a). PC1 loaded heavily on head length, preventral length, eye diameter, and IEW/ED, which distinguished *S. chengi* from *S. malacanthus*. Morphometric analysis indicated that *S. chengi* had longer predorsal, prepectoral, and preventral lengths than *S. malacanthus*. Furthermore, PCA revealed that DK populations of *Schizopygopsis chengi* were separated from *S. c. baoxingensis,* MK and KK populations, and the latter two populations could not be partitioned by traditional morphometric data (Fig. 3b and Table 6).

TABLE 4. The results of traditional measurement of *S. chengi* and *S. malacanthus.*

Measurements	*S. chengi* (n=71)				*S. malacanthus* (n=66)			
	Min	Max	Mean	SD	Min	Max	Mean	SD
L_t (mm)	40.0	308.0	147.15	80.63	85.0	294.6	174.3	25.70
L_s (mm)	33.0	287.0	120.99	67.25	67.2	253.0	141.9	34.90
L_s /L_t	0.73	0.90	0.82	0.01	0.81	0.90	0.84	0.02
% of L_s								

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TABLE 4. (Continued)

Measurements	*S. chengi* (n=71)				*S. malacanthus* (n=66)			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Body depth	17.89	26.78	22.18	1.35	17.24	25.98	20.00	2.61
Head length	23.51	28.76	26.29	1.07	19.83	26.84	24.19	1.47
CPL	14.12	19.27	16.57	1.31	14.55	20.06	16.99	1.35
CPD	6.91	9.06	7.84	0.58	5.87	8.44	7.52	0.53
Predorsal length	35.79	49.05	45.90	1.50	43.70	50.12	45.69	2.43
Prepectoral length	22.83	29.96	27.19	1.38	19.79	27.56	24.05	1.56
Preventral length	46.04	58.74	53.28	1.98	45.30	55.45	51.72	2.16
Preanal length	68.22	77.70	74.22	1.17	65.66	78.60	73.37	2.45
% of HL								
Head depth	55.43	74.52	63.51	3.38	56.33	68.70	62.60	2.71
Head width	43.39	68.13	56.18	3.69	50.91	73.71	61.71	5.19
Eye diameter	17.05	28.02	21.46	1.17	13.06	21.88	16.72	2.13
Interorbital width	32.37	47.39	39.49	2.09	29.55	46.19	39.68	3.42
Snout length	21.61	33.88	28.33	2.47	21.49	35.70	29.80	3.28
Mouth width	22.01	38.65	31.51	2.69	30.09	37.47	32.07	2.21
Postorbital length	44.50	55.70	49.53	1.92	46.29	58.84	53.15	2.89
Ratio (%)								
CPD/CPL	38.51	59.80	47.90	3.57	34.26	56.94	44.49	4.27
Mouth width/Snout length	103.18	262.14	170.82	49.05	108.70	254.53	186.98	22.19
Interorbital width/ Eye diameter	134.43	231.33	174.27	11.76	145.59	314.29	240.96	35.62
Count								
ONG	10	27	16.44	3.46	16	25	18.08	2.47
ING	16	37	26.92	7.17	26	38	30.25	4.52

TABLE 5. Results of PC1–PC5 based on 19 measurements between *S. chengi* and *S. malacanthus*.

Character	Component				
	PC1	PC2	PC3	PC4	PC5
L_s/L_t	0.18	0.06	-0.03	-0.09	-0.38
Body depth	0.12	0.36	-0.05	-0.17	0.05
Head length	-0.33	0.00	0.32	-0.09	0.11
CPL	0.01	0.08	-0.19	0.29	0.63
CPD	-0.29	-0.33	-0.10	0.04	0.03
Predorsal lenght	-0.28	0.03	0.34	-0.08	0.14
Prepectoral length	-0.28	0.06	-0.01	-0.13	0.13
Preventral length	-0.33	-0.07	0.26	-0.04	0.09
Preanal length	-0.18	-0.08	0.11	-0.12	0.22
Head depth	0.02	-0.17	-0.53	-0.03	0.06
Head width	0.23	-0.42	0.10	0.04	0.08
Eye diameter	-0.36	0.00	-0.35	0.17	-0.09
Interorbital width	0.03	-0.42	-0.01	0.04	0.18
Snout length	0.13	-0.15	0.22	0.57	-0.12
Snout length	0.18	-0.39	-0.05	-0.04	0.06

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TABLE 5. (Continued)

Character	Component				
	PC1	PC2	PC3	PC4	PC5
Postorbital length	0.26	0.11	0.16	-0.24	0.09
CPD/CPL	-0.23	-0.32	0.05	-0.19	-0.46
MW/SL	0.03	-0.16	-0.23	-0.59	0.17
IEW/ED	0.33	-0.17	0.32	-0.16	0.18
Variance (%)	0.24	0.14	0.11	0.10	0.08
Cumulative variance (%)	0.24	0.38	0.49	0.59	0.66

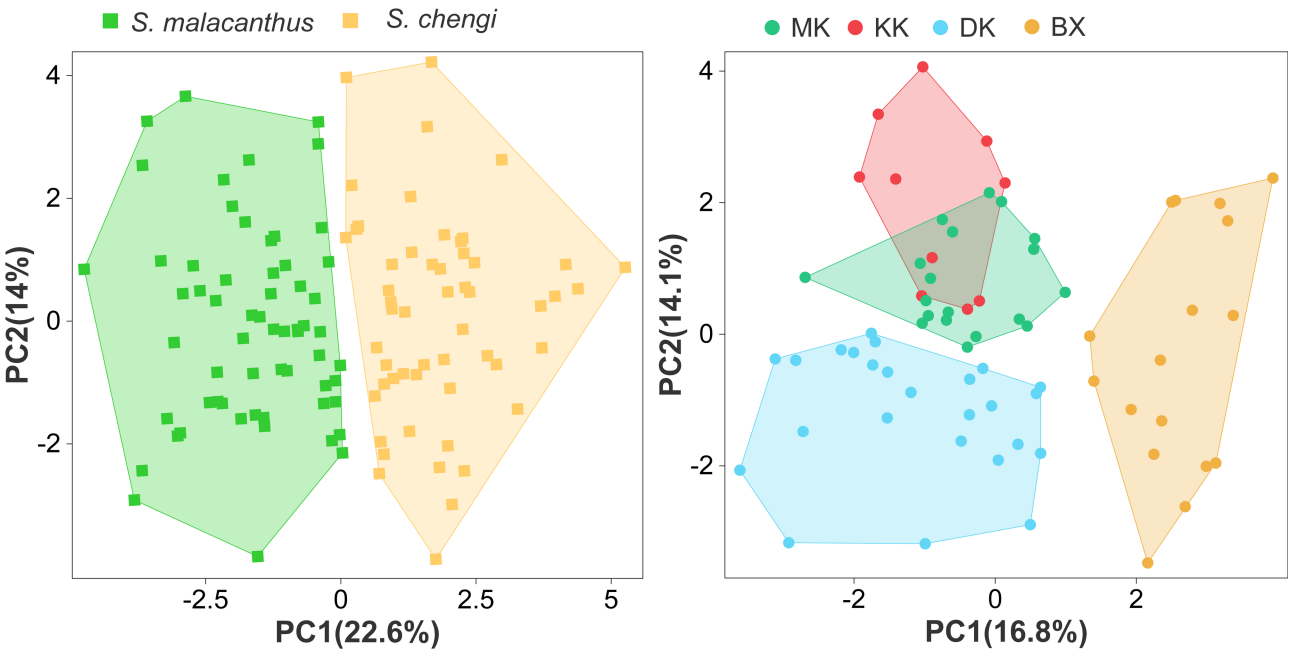

FIGURE 3. PCA analysis. a. The PCA analysis for *S. chengi* (orange circles) and *S. malacanthus* (green circles). **b.** The PCA analysis for *S. chengi* populations of Marke River (light blue circles), Keke River (red circles) and Duoke River (orange circles).

TABLE 6. Results of PC1–PC5 based on 19 measurements among three geographic populations of *S. chengi*.

Character	Component				
	PC1	PC2	PC3	PC4	PC5
BL/TL	0.12	0.12	-0.02	-0.26	0.11
BL/BH	-0.05	0.26	-0.20	-0.05	-0.24
HL/BL	0.00	-0.36	-0.38	-0.02	-0.15
CPL/BL	0.05	0.05	-0.23	0.43	0.51
CPD/BL	0.16	-0.36	0.19	0.01	0.17
PDL/BL	-0.24	-0.35	-0.19	-0.05	0.10
PPL/BL	0.07	-0.18	-0.29	0.17	-0.16
PEL/BL	-0.28	-0.32	-0.14	0.15	-0.01
PAL/BL	-0.20	-0.31	0.01	0.16	-0.08
HD/HL	0.13	0.24	0.31	0.22	-0.02
HW/HL	-0.15	-0.15	0.41	0.34	0.09
ED/HL	0.47	-0.01	-0.19	0.09	-0.02

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TABLE 6. (Continued)

Character	Component				
	PC1	PC2	PC3	PC4	PC5
IEW/HL	0.13	-0.04	0.07	0.28	-0.54
SL/HL	-0.15	0.19	-0.02	0.41	-0.27
MW/HL	0.35	-0.15	0.17	0.31	-0.01
PL/HL	-0.20	0.07	0.10	-0.10	0.27
CPD/CPL	0.09	-0.29	0.34	-0.37	-0.31
MW/SL	0.39	-0.27	0.15	-0.05	0.20
IEW/ED	-0.38	-0.04	0.32	0.06	0.00
Variance(%)	0.17	0.14	0.13	0.09	0.09
Cumulative variance (%)	0.17	0.31	0.43	0.53	0.61

Sequencing of the Cyt *b* gene generated 36 haplotypes, including 24 in *S. chengi* and 12 in *S. malacanthus*. Additionally, we included 2 haplotypes of *S. c. baoxingensis* and *S. m. malacanthus* obtained from public databases (Liu, *et al.* 2015). Therefore, 26 and 14 haplotypes of *S. chengi* and *S. malacanthus*, respectively, were subjected to phylogenetic analysis. The haplotype network demonstrated the clear separation between *S. chengi* and *S. malacanthus* without any common haplotypes (Fig. 4a). The BI and ML trees yielded identical topologies, revealing that *S. chengi* did not form a sister lineage with *S. malacanthus* (Fig. 4b). *Schizopygopsis malacanthus* was grouped with other *Schizopygopsis* fish and *Heizensteinia microcephalus,* while *S. chengi* formed an independent lineage with strong support. Within *S. chengi*, samples from the DK River formed a monophyletic group, and the MK and KK populations were grouped together without phylogenetic differentiation. Two haplotypes of *S. c. baoxingensis* formed a monophyletic group in *S. c. chengi* (Fig. 4b).

Based on Cyt *b* haplotypes, the interspecific genetic distance among these species ranged from 0.019 (between *S. pylzovi* and *S. microcephalus*) to 0.091 (between *S. anteroventris* and *G. potanini*). The genetic distance between *S. chengi* and *S. malacanthus* was 0.076, which was greater than that of the other 13 comparisons (Table 7). The genetic differentiations (F_{st}) ranged from 0.654 (between *S. malacanthus* and *S. microcephalus*) to 0.966 (between *S. kialingensis* and *S. chengi*). The genetic differentiation between *S. chengi* and *S. malacanthus* was 0.834, which was greater than that between *S. malacanthus*-*S. kialingensis*, *S. malacanthus*-*H. microcephalus*, *S. malacanthus*-*S. anteroventris*, and *S. malacanthus*-*S. pylzovi*. (Table 7). The genetic distance was 0.022 between the DK and MK populations and reached 0.024 between the DK and KK populations (Table S1). The genetic distances within *S. chengi* were even greater than that between *H. microcephalus* and *S. pylzovi* (0.019) as well as those between *H. microcephalus* and *S. kessleri* (0.018). The F_{st} values were 0.473 and 0.487 between DK populations and and *S. c. chengi* as well as between DK populations and *S. s. baoxingensis*, suggesting genetic differentiation among the geographic populations (Table S1). Additionally, we found that the nucleotide and haplotype diversities were greater in the MK/KK populations than in the DK populations, which might be associated with the more restricted range of the DK population (Table S2).

Species delimitation was carried out using bPTP and ASAP, both of which decisively supported that *S. chengi* and *S. malacanthus* represented two distinct species (Fig. 5). Based on the bPTP model, 14 species were defined, with Bayesian support values ranging from 0.93 to 1.00. *Schizopygopsis chengi* and *S. malacanthus* were delimited as two species, which was supported by the posterior probabilities of 0.98 and 0.97 for the two lineages. ASAP identified the 14 best partitions based on pairwise genetic distance using Cyt *b* sequences (Table S3). According to the lowest score of 2.0, the ASAP method delimited all the samples into 13 species, and *S. chengi* and *S. malacanthus* were separated into two taxonomic classifications (Fig. 5).

It has three subspecies, i.e. *Schizopygopsis chengi chengi*, *Schizopygopsis chengi baoxingensis* and *Schizopygopsis chengi duokeheensis,* subsp. nov.

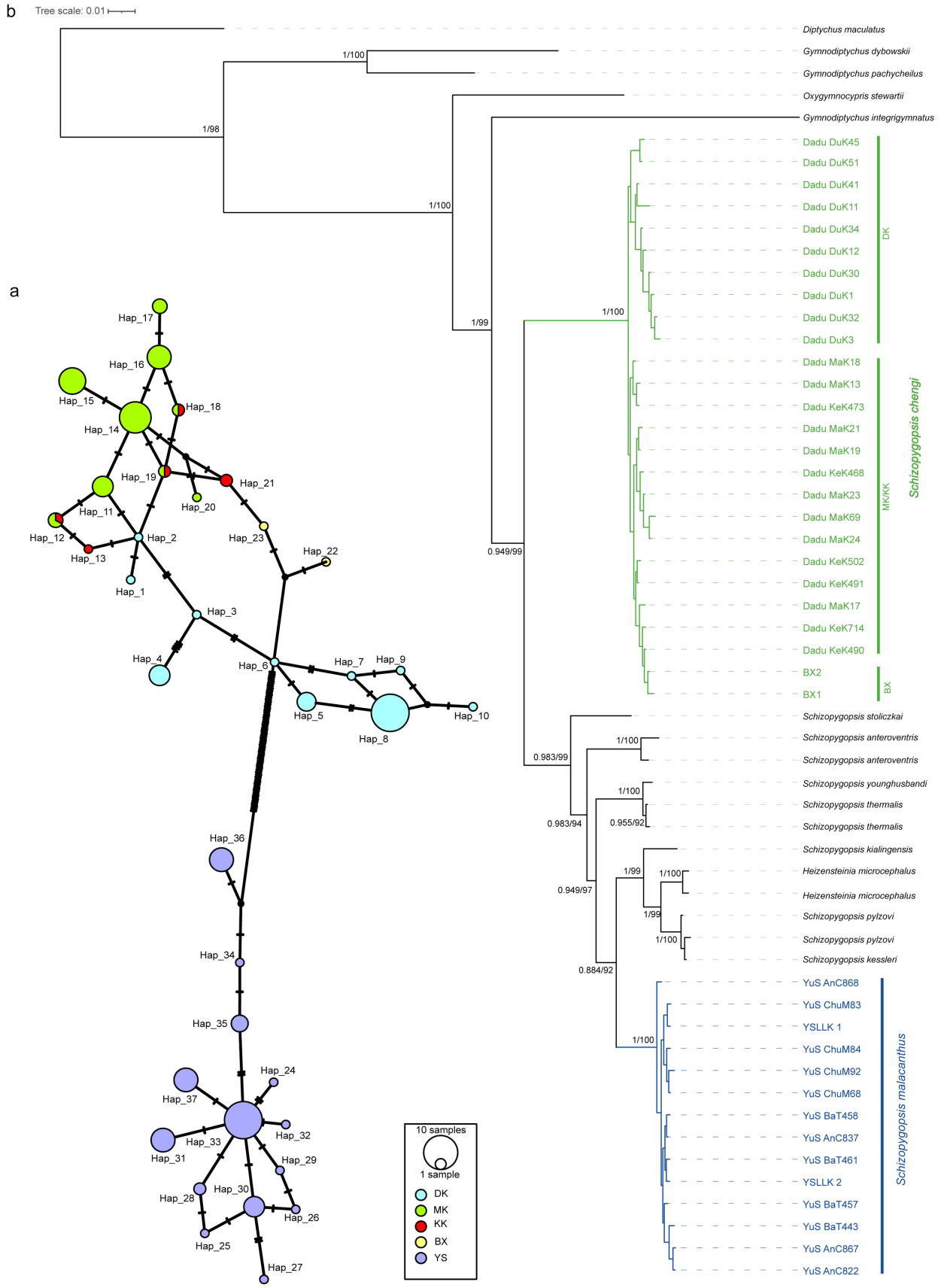

FIGURE 4. Population genetic analyses. a Haplotype network. Green, red, yellow and blue circles indicated haplotypes from MK, KK, BX, DK of *S. chengi*, and purple circles indicated haplotypes identified in *S. malacanthus*. **b.The maximum likelihood tree.** ML and BI trees demonstrated the same topology. The posterior probabilities and Bayesian supported values were labeled on the branches. The green and blue squares indicated samples from *S. chengi* and *S. malacanthus.*

TABLE 7. K2P genetic distance (lower left diagonal) and genetic differentiation (F_{st}, upper right diagonal).

	S. chengi	*S. malacanthus*	*S. kialingensis*	*H. microcephalus*	*S. anteroventris*	*S. pylzovi*	*S. kessleri*	*S. stoliczkai*	*S. thermalis*	*S. younghusbandi*	*G. potanini*
S. chengi		0.834	0.966	0.934	0.927	0.950	0.966	0.967	0.961	0.967	0.928
S. malacanthus	0.078		0.724	0.654	0.735	0.705	0.964	0.972	0.949	0.964	0.851
S. kialingensis	0.080	0.039		0.931	0.942	0.964	0.981	0.989	0.990	0.989	0.975
H. microcephalus	0.072	0.041	0.027		0.899	0.852	0.900	0.967	0.950	0.962	0.948
S. anteroventris	0.085	0.048	0.057	0.052		0.915	0.935	0.935	0.923	0.936	0.937
S. pylzovi	0.075	0.042	0.026	0.019	0.050		0.008	0.982	0.966	0.979	0.961
S. kessleri	0.076	0.042	0.025	0.018	0.050	0.001		0.991	0.990	0.989	0.974
S. stoliczkai	0.079	0.055	0.063	0.060	0.050	0.054	0.054		0.990	0.995	0.975
S. thermalis	0.082	0.039	0.048	0.049	0.049	0.043	0.044	0.047		0.889	0.968
S. younghusbandi	0.079	0.042	0.051	0.051	0.050	0.046	0.046	0.049	0.047		0.974
G. potanini	0.064	0.086	0.085	0.082	0.091	0.082	0.083	0.085	0.082	0.081	

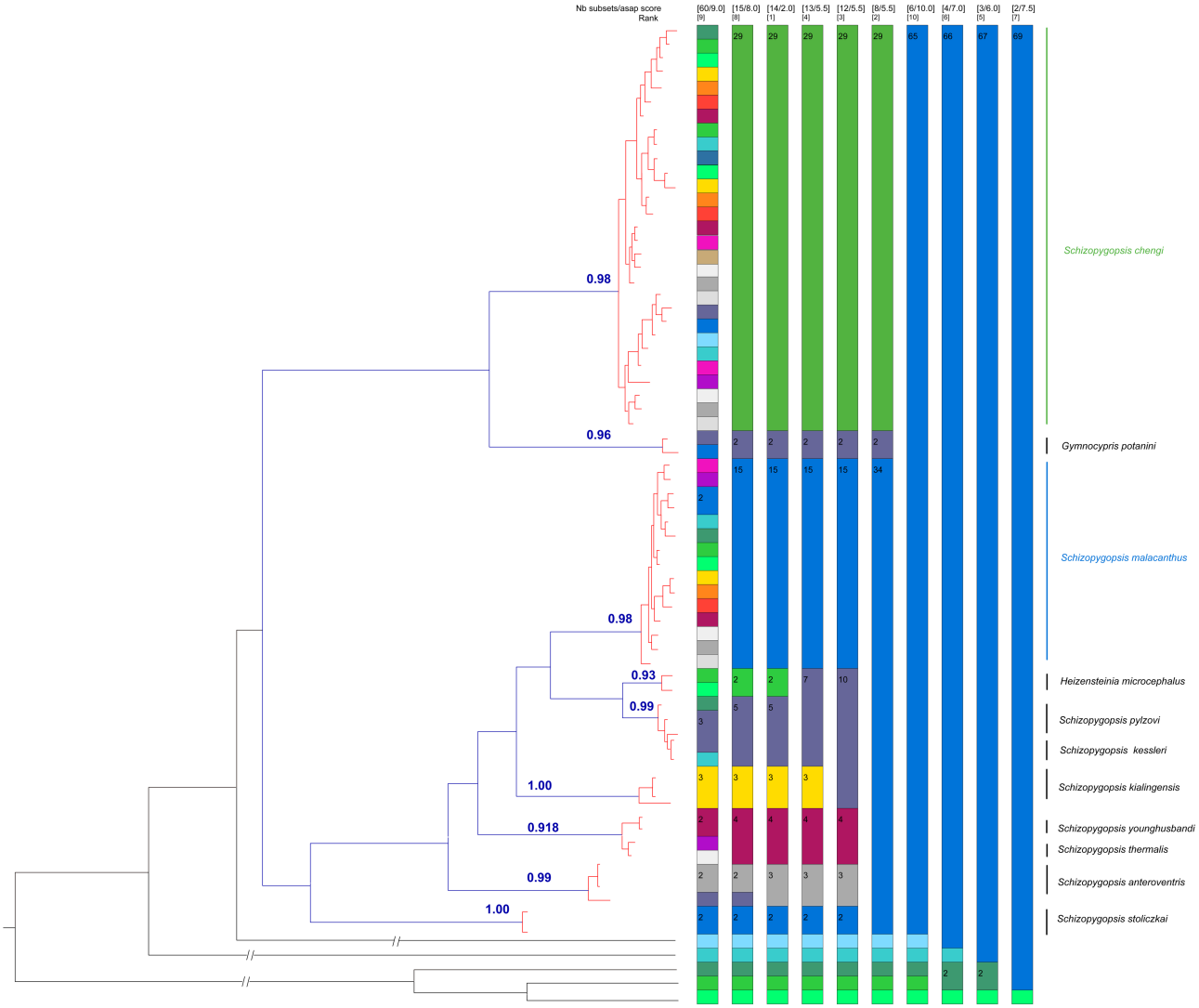

FIGURE 5. The species delineation. a. Branch in blue was defined as a single species by bPTP analysis, and branch in red was rejected as a species by bPTP analysis. The numbers on branches indicated Bayesian support values. **b.** Squares in colors indicated all samples. The ASAP scores, the number of subsets, and the corresponding rank for were recorded on the top. The square with the same color is defined as a species. The top 10 partition results were listed.

Schizopygopsis chengi chengi (Fang, 1936) (Figure 6, Tables 3 and 8)

Chuanchia chengi (Fang Bingwen), 1936, Sinensin, 7(4): 454 (Western Sichuan Province)
Schizopygopsis malacanthus chengi: Cao Wenxuan, Deng Zhonglin, 1962, Acta Hyfrobiogica Sinica 2: 45 (The Dadu River); Cao Wenxuan, 1964, edited by Wu Xianwen *et al.*, The Cyprinid Fishes of China I: 189 (The Dadu River).

Materials examined

NWIPB2107001–19 (19 specimens), 67–253 mm SL, collected in Western China: Qinghai Province: Kepei Village: Marke River, one of the headstreams of the Dadu River; 100.92 E, 32.66 N; collected by S. Liu and K. Zhao, July 2021.

NWIPB2107019–28 (10), collected in Western China: Qinghai Province: Jiangritang Village: Keke River, one of the headstreams of the Dadu River; 101.12E, 32.24 N; collected by S. Liu and K. Zhao, July 2021.

FIGURE 6. Holotype of *S. c. chengi*. a. The lateral view of holotype *S. c. chengi*. **b.** The dorsal view of holotype *S. c. chengi*. **c.** The ventral view of holotype *S. c. chengi*. **d.** The morphology of anal fin in holotype *S. c. chengi*. **e.** The inner side of gill arch from left side of *S. c. chengi*. **f.** The outer side of gill arch from left side of *S. c. chengi*.

Diagnosis

Schizopygopsis chengi chengi is distinguished from *Schizopygopsis chengi baoxingensis* by having a greater number of gill rakers in the first gill arch. The numbers of outside and inside gill rakers ranged from 15–20 and 25–37, respectively, in *S. c. chengi* and from 10–15 and 16–23, respectively, in *S. c. baoxingensis*. It differed from *S. c. duokeheensis* by having anal scaly sneath terminating at the base of the ventral fin.

Description

D. iv, 8; P. i, 19–21; V. i, 9; A. iii, 5; vertebrae 4 + 42–46.

The morphometric measurements are shown in Table 7.

Body elongate, slightly flat. Snout obtuse and round. No barbels. Mouth inferior, oral fissure nearly straightly transverse, inner side of the lower jaw sharp with a strong horny layer. Head large and convex. Dorsal profile convex and sloping. Ventral profile flat. Nostrils 2 on each side, close together, near eye edge relative to tip of snout. Gill rakers of the first gill arch long and dense. Pharyngeal teeth in 2 rows, 4.3/3.4; slightly hooked and pointed at the tip and with a concave grinding surface. Last unbranched dorsal fin ray weak with small and few serratures at the posterior edge. Body naked with a group of minute scales bordering the scapular region. The lateral line is complete and straight along the middle of the body and tail. The origin of the dorsal fin closer to the tip of the snout than to the base of the caudal fin. Commencement of the ventral fin under the 4–5th dorsal branched ray. The anterior and posterior angles of dorsal fin squarish. Anal opening proximity to the origin of the anal fin. The tip of the anal fin reaches the base of the caudal fin. Caudal forked. Anal sneath consisting of enlarged scales on each side, extending anteriorly and terminating at the base of the ventral region.

Color pattern

Alcohol-preserved specimens black-gray, abdomen yellowish, whole body with black spots. The dorsal and caudal fins yellow to pale brown, and the ventral and anal fins yellow.

Distribution and habitats

Schizopygopsis chengi chengi is distributed in the headstreams of the Dadu River, the Marke River and the Keke River in Bama County, Qinghai Province, China (Fig. 7). The coexisting fishes included *Gymnocypris potanini, Schizothorax davida*, *Schizothorax prenanti*, *Triplophysa markehenensis*, *Triplophysa stenure*, *Triplophysa leptosome, Triplophysa orientalis* and *Triplophysa microps.*

Schizopygopsis chengi chengi dwells in cold highland streams with a substrate of sand, pebbles and gravels at altitudes of 3000–4000 m above sea level. It feeds on plant fragments and algae on pebbles and gravels as well as insects such as *Gammaridea* sp. (gammarid) and *Chironomus* sp. (chironomid).

FIGURE 7. The habit of *S. c. chengi* in the Marker River.

Etymology

The subspecies *Schizopygopsis chengi chengi* was named after W. C. Cheng (郑万钧), an assistant botanist at the Herbarium of the Biological Laboratory of Science Society of China, who identified and presented the specimen. The Chinese name for this species is suggested as 大渡裸裂尻鱼指名亚种.

Schizopygopsis chengi baoxingensis Fang, Ding *et* Ye, 1994 (Fig. 8, Tables 3 and 8)

Schizopygopsis malacanthus baoxingensis (Fu, Ding and Ye), 1994, The Fishes of Sichuan, 399–401 (The Baoxing River).

FIGURE 8. Holotype of *S. c. baoxingensis*. a. The lateral view of holotype *S. c. baoxingensis*. **b.** The dorsal view of holotype *S. c. baoxingensis*. **c.** The ventral view of holotype *S. c. baoxingensis*. **d.** The morphology of anal fin in holotype *S. c. baoxingensis*. The inner **(e)** and outer **(f)** side gill arch from left side of *S. c. baoxingensis*.

TABLE 8. The results of traditional measurement of *S. c. chengi*, *S. c. baoxingensis* and *S. c. duokehenesis*.

Measurements	*S. c. chengi* (n=29)				*S. c. duokehenesis* (n=25)				*S. c. baoxingensis* (n=17)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
L_t (mm)	127.0	308.0	210.7	48.07	101.0	287.0	174.3	25.70	40.0	98.0	56.45	11.78
L_s (mm)	103.0	264.0	175.3	41.23	82.0	256.0	141.9	34.90	33.0	82.0	45.77	10.17
L_s /L_t	0.80	0.89	0.83	0.03	0.80	0.90	0.83	0.02	0.73	0.86	0.81	0.03
% of L_s												
Body depth	18.36	26.68	23.04	3.26	17.89	26.78	20.63	1.76	20.41	25.00	22.87	4.21
Head length	23.51	27.16	25.07	0.93	25.26	28.32	27.02	0.88	24.07	28.76	26.79	1.79
CPL	14.12	19.27	17.72	1.32	15.29	18.30	16.83	0.92	14.25	16.39	15.15	2.02
CPD	6.91	9.06	8.23	0.59	7.45	8.58	8.11	0.37	6.93	8.43	7.17	1.42
Predorsal length	41.30	48.41	44.33	1.89	46.45	49.05	47.31	1.74	35.79	47.38	46.06	2.82
Prepectoral length	22.83	28.65	25.60	1.39	24.43	29.18	28.09	5.03	24.60	29.96	27.89	1.58
Preventral length	49.83	57.21	52.82	1.71	51.32	58.74	55.44	1.60	46.04	56.54	51.57	2.34
Preanal length	70.89	77.09	74.11	1.48	71.51	77.70	75.44	1.56	68.22	78.10	73.10	2.74
% of HL												
Head depth	59.21	74.52	65.78	3.10	55.43	66.06	59.63	2.96	58.82	71.06	65.13	3.55
Head width	43.39	68.13	56.97	1.55	49.79	62.40	55.70	3.38	50.23	61.13	55.88	2.10
Eye diameter	17.29	28.02	22.37	2.20	17.05	26.41	21.86	3.03	17.86	24.61	20.14	2.63
Interorbital width	32.37	47.39	39.59	2.44	35.50	43.84	39.41	2.26	33.84	44.29	39.48	3.55
Snout length	21.61	31.79	27.79	2.50	24.28	33.88	28.64	2.76	26.45	31.78	28.56	3.48
Mouth width	22.01	38.65	30.45	1.03	23.24	33.56	29.51	2.42	32.83	37.54	34.56	2.68
Postorbital length	44.88	55.70	50.33	1.43	46.71	53.44	49.72	2.08	44.50	53.43	48.53	2.62
Ratio (%)												
CPD/CPL	38.51	59.80	48.54	1.59	43.59	53.88	47.72	2.51	44.31	50.00	47.44	2.08
Mouth width/ Snout length	147.96	242.84	189.65	13.42	172.97	262.42	207.67	24.43	103.18	130.00	115.15	16.28
Interorbital width/ Eye diameter	145.07	215.25	178.08	20.30	134.43	231.39	183.65	27.76	143.30	206.67	161.08	19.60
Count												
ONG	15	27	18.78	2.67	15	24	18.08	2.00	10	15	12.47	1.76
ING	27	37	33.33	3.63	25	36	28.25	4.52	16	23	19.18	2.18

Materials examined

NWIPB231201–17 (17), collected. 45–54 mm SL. collected in Western China: Sichuan Province: Baoxing County; 102.81E, 30.38N.

Diagnosis

Schizopygopsis chengi baoxingensis is distinguished from *S. c. chengi* and *S. c. duokeheensis* by having fewer outside and insider gill rakers on the first gill arch. The numbers of outside and insider gill rakers ranged from 10–15 and 16–23 in *S. c. baoxingensis*, from 15–20 and 25–37 in *S. s. chengi*, and from 17–21 and 27–36 in *S. c. duokeheensis*. The oral fissure is slightly hooked in *S. c. baoxingensis*, and nearly transverse in *S. c. chengi* and *S. c. duokeheensis*.

Description

D. iii, 8; P. i, 17–20; V. i, 9–10; A. iii, 5; vertebrae 4 + 44–46.

The morphometric measurements are shown in Table 7.

Body elongate, slightly flat. Greatest depth at origin of dorsal fin, dorsal profile arched, ventral profile curved. Snout obtuse and round. Lip narrow with two lateral lobes, posterior groove of the lower lip discontinued. Mouth inferior, oral fissure slightly hooked, and the lower jaw sharp with a strong and flat horny layer. No barbels. Eye big, round, forward in the middle of the head. Gill rakers long and sparse, outer gill rakers small. Pharyngeal teeth in 2 rows, 4.3/3.4; hooked, pointed at the tip and with a concave grinding surface. Body entirely naked with 1–4 rows of scales above the pectoral axil. Lateral line complete, along middle of flank and caudal peduncle, anterior portion skinfold-like. The last unbranched dorsal fin ray weak with small serratures at the posterior edge. The origin of the dorsal fin closer to the tip of the snout than to the base of the caudal fin. Commencement of the ventral fin under the 4th branched ray of the dorsal fin. The anterior angle of dorsal fin round and posterior angle squarish. Anal sneath consisting of enlarged scales on each side, and the anal sneath reaching or slightly exceeds the midpoint between the ventral and anal fins. Caudal deeply forked, the lower lobe slightly longer than the upper one.

Color pattern

The back yellowish brown to blackish brown, the abdomen greyish white, without black dots or spots on the body, the dorsal, ventral and anal fins yellow, and the caudal fin light pink in the living specimen.

Distribution and habitats

Schizopygopsis chengi baoxingensis is distributed in the headstreams of Qingyijiang River, the Baoxing River in Baoxing County, Sichuan Province, China (Fig. 9).

Schizopygopsis chengi baoxingensis inhabits cold highland streams with a substrate of pebbles and gravels at altitudes of 1500-2000 m above sea level. It mainly feeds on plant fragments and algae.

Etymology

The name of the subspecies, *baoxingensis*, is derived from the Baoxing River (宝兴河), where the holotype was collected. The Chinese name for this species is suggested as 大渡裸裂尻鱼宝兴亚种.

FIGURE 9. The habit of *S. c. baoxingensis* in the Baoxing River.

Schizopygopsis chengi duokeheensis, subsp. nov., Zhao, *et al.*, (Fig. 10, Tables 3 and 8)

Holotype

NWIPB2107029, total length 218.00 mm; standard length 183.40 mm; Western China: Qinghai Province: Zhiqin Village: Duoke River, one of the headstreams of the Dadu River; 100.46 E, 32.57 N; collected by S. Liu and K. Zhao, July 2021

Paratypes

NWIPB2107030–53 (24), 48–276 mm SL, collected with the holotype.

Diagnosis

Schizopygopsis chengi duokeheensis is distinguished from *S. c. chengi* by having an anal scaly sneath ending nearly or slightly in front of the midpoint between the ventral and anal fins. It differed from *S. c. baoxingensis* by having dense outside and inside gill rakers on the first gill arch.

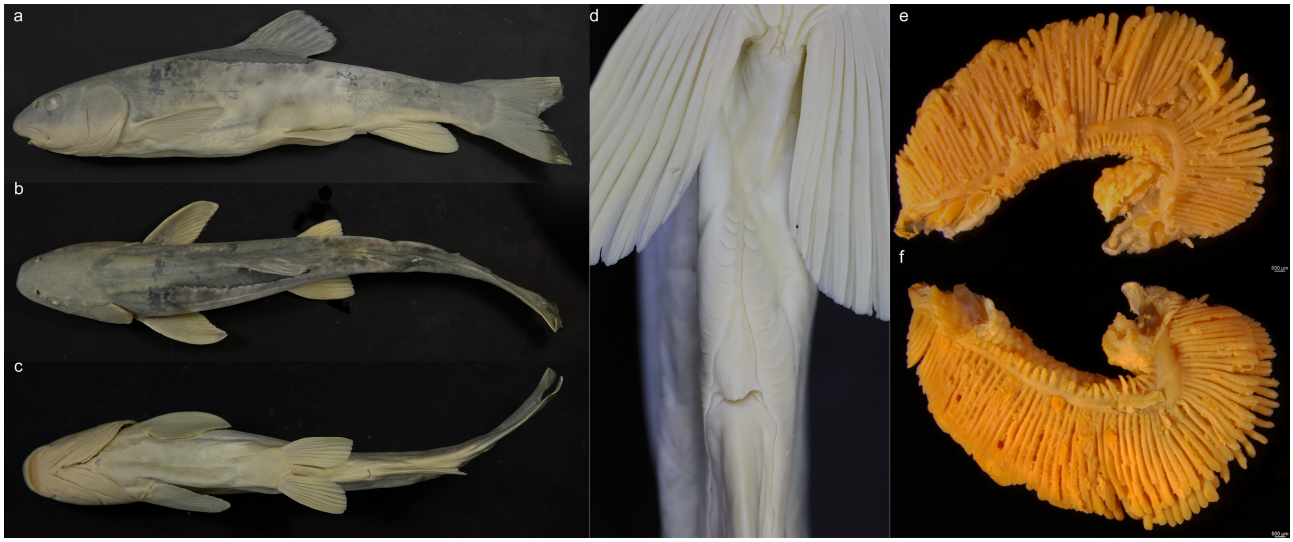

FIGURE 10. Holotype of *S. c. duokeheensis*. a. The lateral view of holotype *S. c. duokeheensis*. **b.** The dorsal view of holotype *S. c. duokeheensis*. **c.** The ventral view of holotype *S. c. duokeheensis*. **d.** The morphology of anal fin in holotype *S. c. duokeheensis*. The inner **(e)** and outer **(f)** side gill arch from left side of *S. c. duokeheensis*.

Description

D. iv, 8; P. i, 18–21; V. i, 9; A. iii, 5; vertebrae 4 + 44–46.

The morphometric measurements are shown in Table 7.

Body elongate, slightly flat. Greatest depth at origin of dorsal fin. Dorsal profile convex and sloping. Ventral profile flat. Head slightly conical. Snout obtuse and round. Mouth inferior, oral fissure transverse, ventral view of margin of lower jaw horseshoe-shaped. Lip narrow, and the inner side of the lower jaw sharp with a strong horny layer. Lower lip with two lateral lobes, posterior groove of the lower lip discontinued. No barbels. Eye round, moderate. Nostrils 2 on each side, above the anterior edge of eye, near eye edge relative to tip of snout. Gill rakers short and dense. Pharyngeal teeth in 2 rows, 4.3/3.4; slightly hooked and pointed at tip and with a concave grinding surface. Body entirely naked with 2–4 rows of scales above the pectoral axil. Lateral line complete, flat and straight, along middle of the body and caudal peduncle. Last unbranched dorsal fin ray weak with small and few serratures at the posterior edge. The anterior and posterior angles of dorsal fin roundish corner. The origin of the dorsal fin almost at the midpoint between the tip of the snout and the base of the caudal fin. Anal scaly sneath ending nearly or slightly in front of the midpoint between the ventral and anal fins. Anal opening proximity to the origin of the anal fin. Caudal forked, the lower lobe slightly longer than the upper one.

Color pattern

For alcohol-preserved specimens, back gray to brown, abdomen yellowish, whole body with blackish brown spots, and the dorsal and caudal fins with black spots.

Ecology

Distribution and habitats

Schizopygopsis chengi duokeheensis is distributed exclusively in the Duoke River in Bama County, Qinghai Province, China (Fig. 11). The coexisting fish was *Triplophysa markehenensis.*

Schizopygopsis chengi duokeheensis inhabits cold highland streams with a substrate of sand, pebbles and gravels at altitudes of 3000–4000 m above sea level. It mainly feeds on plant fragments and algae growing on pebbles and gravels.

FIGURE 11. The habit of *S. c. duokeheensis* in the Duoke River.

Etymology

The name of the new subspecies, *duokeheensis*, is derived from the Duoke River (多柯河), where the species inhabits. The Chinese name for this species is suggested as 大渡裸裂尻鱼多柯亚种.

Discussion

Molecular evidence for species validation

The phylogenetic relationship is crucial for revealing and validating the taxonomic status of cryptic species. In the present study, phylogenetic analysis, genetic differentiation, and species delimitation were combined to reveal the valid taxonomic status of *Schizopygopsis chengi*. The phylogenetic tree showed that *S. chengi* and *S. malacanthus* formed two separate lineages, which is consistent with the taxonomic classification of Wu and Wu and previous studies (Wu and Wu 1992; Yu, *et al.* 2006; Qi, *et al.* 2015). The genetic distance between *S. chengi* and *S. malacanthus* was 0.075, which was greater than the average genetic distance of 0.055 of the examined species. Molecular and phylogenetic results demonstrated high genetic differentiation between *S. chengi* and *S. malacanthus*, which strongly supported that they are two valid species.

Within *Schizopygopsis chengi*, a geographic population structure was formed. Samples from the DK River were separated from samples from the MK, KK and BX Rivers, with genetic distances ranging from 0.023–0.024, which is consistent with previous phylogenetic studies (Yu, *et al.* 2006; Liu, *et al.* 2009; Liu, *et al.* 2015). The samples

from the BX River formed a specialized group in the lineage of *S. c. chengi*. This phenomenon may be caused by geographic isolation among river systems, which influences gene flow among geographic populations (Fig. 1). Molecular phylogenetic analyses suggested the presence of three independent subspecies, *S. c. chengi* in the MK/KK River, *S. c. baoxingensis* in the BX River and *S. c. duokeheensis* in the DK River.

Morphological evidence for species validation

A previous study revealed that *Schizopygopsis chengi* has soft unbranched rays of the dorsal fin in both small and large individuals, and *S. malacanthus* had strong unbranched rays of the dorsal fin in only small individuals (Chen and Cao 2000). Therefore, *S. chengi* was defined as a subspecies of *S. malacanthus*. In the present study, we inspected specimens of small and large samples from both *S. chengi* and *S. malacanthus*. Morphological examination confirmed that *S. chengi* has soft rays of dorsal fins with small and few serratures at the posterior edge in both small and large individuals, and *S. malacanthus* has strong unbranched rays of dorsal fin with obvious serratures in small and large individuals. Furthermore, morphometric analysis indicated that *S. chengi* possesses longer predorsal, prepectoral, and preventral lengths compared to *S. malacanthus*, offering supportive evidence for morphological differences between two species (Whelan, *et al.* 2023). These morphological variances are related to the swimming performance of the fish, which may be due to differences in the flow velocities in the Dadu and Yangtze Rivers (Li, *et al.* 2009; Svozil, *et al.* 2020; Garcia-Vega, *et al.* 2023).

Within *S. chengi*, oral fissure, the ending position of the anal scales and the gill rakers on the first gill arch were used to distinguish the three subspecies. The anal scales of *S. c. chengi* reach the base of the ventral fin, and the anal scales terminate at the nearly midpoint between the ventral and anal fins in *S. c. duokeheensis* and *S. c. baoxingensis*. Compared with *S. c. chengi* and *S. c. duokeheensis*, *S. c. baoxingensis* has fewer gill rakers on the first gill arch and slightly hooked oral fissure. *Schizopygopsis chengi duokeheensis* has a relatively longer predorsal length than *S. c. chengi* and *S. c. baoxingensis*. The morphometric analysis also showed that *S. c. chengi*, *S. c. baoxingensis* and *S. c. duokeheensis* exhibited differences in PDL/SL, MW/HL, MW/SL and IEW/ED, which might be related to prey preferences and swimming performance in habitats with different eco-environments.

Conservation implications

The validation of *S. chengi* and identification of *S. c. duokeheensis* in the DK River are also important for local fish conservation. Reports on fish distribution are rare in the DK River, and the discovery of *S. c. duokeheensis* highlights the possibility of unknown fish resources in the QTP. Due to the harsh environment on the QTP, *Schizopygopsis* species grow and reproduce slowly and have restricted distributions. Therefore, most of them are listed as vulnerable and endangered species, including *S. chengi* as an endangered species and *S. malacanthus* as a vulnerable species. *Schizopygopsis chengi duokeheensis* has a more limited distribution and lower genetic diversity, which suggests that it may be at greater risk and more vulnerable to anthropogenic disturbance.

Comparisons with *Schizopygopsis* fishes

Schizopygopsis chengi differs from *Schizopygopsis pylzovi*, *Schizopygopsis kessleri*, *Schizopygopsis kialingensis* and *Schizopygopsis anteroventris* in the commencement of the ventral fin under the 4–5th branched ray of the dorsal fin (Table 3). It is distinguished from *S. malacanthus*, *Schizopygopsis stoliczkai* and *Schizopygopsis thermalis* by differences in strength of unbranched rays and serratures in the dorsal fin. Compared to *Schizopygopsis younghusbandi*, *S. chengi* has a wider mouth and horny layer of the lower jaw and fewer branched rays in the pelvic fin.

Key to the species of the genus of *Schizopygopsis*

1 (14) Inside gill raker of the first gill arch more than 20
2 (11) Commencement of the ventral fin under the 4–5th branched ray of the dorsal fin
3 (6) The strong unbranched rays of the dorsal fin with obvious serratures
4 (5) Anal scale reaching the base of the ventral fin (the distribution in river systems in the western QTP) . . . . . S. *stoliczkai*
5 (4) Anal scales reaching the midpoint between the ventral and anal fins (the distribution in the upstream of Yangtze River) . . . . . *S. malacanthus*
6 (3) The weak unbranched rays of dorsal fins with few serratures or no serratures
7 (8) Anal scales reaching the base of the ventral fin . . . . . *S. s. chengi*
8 (7) Anal scales reaching the midpoint between the ventral and anal fins
9 (10) Oral fissure hooked, outside gill raker on the first gill arch 10–15, inside gill raker on the first gill arch 16–23 (the distribution in the Baoxing River) . . . . . *S. s. baoxingenesis*
10 (9) Oral fissure transverse, outside gill raker on the first gill arch 17–21, inside gill raker on the first gill arch 25–37 (the distribution in the Duoke River) . . . . . *S. s. duokeheensis*
11 (2) Commencement of the ventral fin under the 2nd–3rd branched ray of dorsal fin
12 (13) The last unbranched ray of dorsal fin weak with few tiny serratures, the first outside gill raker outward hooked with small serrations . . . . . *S. kessleri*
13 (12) The last unbranched ray of dorsal fin strong with obvious serratures, the first outside gill raker normal without serrations . . . . . *S. pylzovi*
14 (1) Inside gill raker of the first gill arch less than 20
15 (18) Commencement of the ventral fin under the 4–5th branched ray of the dorsal fin
16 (17) The last unbranched ray of dorsal fin strong with obvious serratures (the distribution in hot springs in Tanggula Mountains) . . . . . *S. thermalis*
17 (16) The last unbranched ray of the dorsal fin weak with few tiny serratures or without serratures . . . . . *S. younghusbandi*
18 (15) Commencement of ventral fin under 2nd–3rd branched ray of dorsal fin (the distribution in the Jialingjiang River). . . . . *S. kialingensis*

Comparative materials

Specimens of *Schizopygopsis* fishes were obtained from the Qinghai-Tibetan Plateau Biota Museum at the Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, Qinghai Province.

Schizopygopsis chengi chengi: NWIPB2107001–NWIPB2107012, 12 specimen, 108.0–264.0 mm SL, the Marke River, Banma County, Qinghai Province; NWIPB2107013–NWIPB2107022, 10 specimen, 103.0–253.0 mm SL, the Keke River, Banma County, Qinghai Province; NWIPB2107023–NWIPB2107029, 7 specimen, 105.0–123.0 mm SL, the Marke River, Daba County, Sichuan Province, kindly provided by Dr. Zuogang Peng from Southwest University.

Schizopygopsis chengi baoxingensis: NWIPB231201–NWIPB231215, 15 specimens, 33.0–82.0 mm SL, the Baoxing River, Baoxing County, Sichuan Province.

Schizopygopsis chengi duokeheensis: NWIPB2107029–NWIPB2107053, 25 specimen, 82.0–256.0 mm SL, the Duoke River, Banma County, Qinghai Province.

Schizopygopsis malacanthus: NWIPB2206001–NWIPB2206019, 19 specimens, 86.0–176.6 mm SL, the Tongtian River, Zhiduo County, Qinghai Province; NWIPB2206020–NWIPB2206042, 23 specimen, 82.1–187.3 mm SL, Batang River, Yushu County, Qinghai Province; NWIPB2206043–NWIPB2206068, 24 specimens, 84.5–206.3 mm SL, Chumaer River, Qumalai County, Qinghai Province.

Schizopygopsis pylzovi: NWIPB1205412–5, four specimens, 146.2–157.3 mm SL, the Yellow River, Gande County, Qinghai Province.

Schizopygopsis kessleri: NWIBP2007004, NWIBP2007006, NWIBP2007051, NWIBP2007054, four specimens, 107.6–128.2 mm SL, the Golmud River, Golmud City, Qinghai Province.

Schizopygopsis kialingensis: NWIPB1108004–007, four specimens, 90.3–95.4 mm SL, the Bailong River, Diebu County, Gansu Province.

Schizopygopsis anteroventris: NWIPB1108432–5, four specimens, 142.3–154.2 mm SL, the Lancang River, Nangqian County, Qinghai Province.

Schizopygopsis stoliczkai: NWIPB1007024 and NWIPB100705, two specimens, 95.0–103.6 mm SL, the Qaraqash River, Pishan County, the Xingjiang Uygur Autonomous Rigon; NWIPB1160487, one specimen, 142.8 mm SL, Pangong Co, Tibet Autonomous Rigon; NWIPB1160383, one specimen, 196.6 mm SL, the Lake Manasarovar, Pulan County, Tibet Autonomous Rigon.

Schizopygopsis thermalis: NWIPB1170189–NWIPB1170192, four specimens, 98.3–1034.4 mm SL, the Yuqu River, Zuogong County, Tibet Autonomous Rigon.

Schizopygopsis younghusbandi: NWIBP1160962–3, two specimens, 87.6–98.2 mm SL, the Pengqu River, Dingri County, Tibet Autonomous Rigon; NWIPB0906028–9, two specimens, 85.3–95.6 mm SL, Nyang River, the Nyingchi City, Tibet Autonomous Rigon.

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Ethnic statement

The field investigation and sampling procedures were issued and supervised by the Qinghai Provincial Bureau of Fishery. The present study was performed based on guidelines described in the “Guidelines for Animal Care and Use” manual (NWIPBACUS-No-2020-07) approved by the Animal Care and Use Committee, Northwest Institute of Plateau Biology, Chinese Academy of Sciences.

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Conflict of interest statement

The authors declare that they have no competing interests.

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