*Zootaxa* 5141 (4): 341–357
https://www.mapress.com/zt/


**Article**

ISSN 1175-5326 (print edition)
**ZOOTAXA**
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5141.4.3
http://zoobank.org/urn:lsid:zoobank.org:pub:9BFE8D4B-67EA-4440-96DF-74DF5ECB5C10

# Revalidation and redescription of *Sarcocheilichthys sciistius* (Abbott, 1901) (Cypriniformes: Cyprinidae) from the northern China

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## Abstract

*Sarcocheilichthys sciistius* was originally described by Abbott in 1901. It was considered as a synonym of *S. nigripinnis* by later researchers. However, some recent studies suggested that *S. nigripinnis* from the Haihe River Basin and Yellow River Basin were different to *S. nigripinnis* distributed in southern China, and that these populations show a closer relationship with the Heilongjiang (Amur) River endemic species—*S. czerskii* and *S. soldatovi*. In this study, we examined one hundred and twenty-five specimens from nine sites of six river basins. Based on morphological and molecular systematic studies, we confirmed *S. sciistius* is a valid species, and *S. czerskii*, *S. soldatovi* were synonyms of *S. sciistius*. *Sarcocheilichthys sciistius* can be distinguished from *S. nigripinnis* by having more lateral-line scales (40–42 vs. 38–40), shorter lower lip and different pectoral and pelvic fins color. The phylogenetic trees reconstructed by Bayesian Inference and Maximum Likelihood based on the Cyt *b* gene also support *S. sciistius* as a distinct species.

**Key words:** East Asia, freshwater fish, morphology, taxonomy

## Introduction

The genus *Sarcocheilichthys* was established by Bleeker (1859) based on *Leuciscus variegatus* (Temminck & Schlegel 1846). They inhabit the middle and lower reaches of freshwater systems (Zhang *et al*., 2008). Similar to fishes of the Acheilognathidae, they also have an aptitude that involves laying their eggs inside the freshwater mussels (Bănărescu *et* Nalbant, 1973). Currently, there are 13 valid species in the genus *Sarcocheilichthys*, among which 11 species are distributed in China (Zhang *et al*., 2020; An *et al*., 2020): *S. sinensis* Bleeker, 1871, *S. lacustris* (Dybowski, 1872), *S. nigripinnis* (Günther, 1873), *S. davidi* (Sauvage, 1878), *S. czerskii* (Berg, 1914), *S. soldatovi* (Berg, 1914), *S. hainanensis* Nichols & Pope, 1927, *S. kiangsiensis* Nichols, 1930, *S. parvus* Nichols, 1930, *S. caobangensis* Nguyen & Vo, 2001, *S. vittatus* An, Zhang & Shen, 2020.

*Sarcocheilichthys sciistius* was originally described as *Leuciscus sciistius* by Abbott in 1901 based on specimens collected from Pei-Ho River in the northern China. Later, two species from the Heilongjiang (Amur) River Basin were described by Berg (1914) as *Chilogobio czerskii* and *C. soldatovi*. After the revision by Bănărescu and Nalbant (1967), *L. sciistius* and *C. soldatovi* were then synonymized as *S. nigripinnis* and *C. czerskii* was regarded as a subspecies of *S. nigripinnis*. Since then, *L. sciistius* was considered invalid. *Sarcocheilichthys czerskii* was considered valid (Kottelat, 2006, Bogutskaya *et al*., 2008, Zhang et al., 2016; An *et al*., 2020) and *S. soldatovi* was also considered valid in some studies (Kottelat, 2006, Bogutskaya *et al*., 2008, An *et al*., 2020).

Zhang *et al*. (2008) investigated the molecular phylogenetic relationships of the genus *Sarcocheilichthys*, the phylogenetic tree showed that samples of *S. nigripinnis* and *S. czerskii* from different river basins were clearly divided into two clades: the northern clade contains *S. nigripinnis* from the Yellow River Basin and *S. czerskii*;

*Accepted by J. Armbruster: 26 Apr. 2022; published: 27 May 2022*

samples from the Yangtze River Basin and coastal rivers in Southeast China form the southern clade. The results showed that *S. nigripinnis* from the Yellow River has a closer relationship with *S. czerskii* than with southern-clade *S. nigripinnis*. Phylogeographic analysis of *S. nigripinnis* by Liu *et al*. (2013) also shares similar results with Zhang *et al*. (2008), which indicates that cryptic species close to *S. czerskii* may exist. Li (2015) reported specimens of *S. nigripinnis* from the Yellow River Basin can be easily distinguished from the specimens of the type locality due to its red paired fins and anal fin. An *et al*. (2020) doubts the validity of *S. soldatovi* through molecular analysis, suggesting that *S. soldatovi* with *S. czerskii* might be the same species.

Studies above doubted the validity of *S. soldatovi* and *S. czerskii* in varying degrees and implied *S. sciistius* could be a valid species. Therefore, to confirm the validity of *S. sciistius*, *S. czerskii* and *S. soldatovi*, and clarify their relationship with *S. nigripinnis*, a large number of specimens of the genus *Sarcocheilichthys* were examined. Morphological comparison and molecular phylogenetic analysis have also been carried out.

## Materials and methods

**Measurements and counts.** This study was approved by the Ethical Review Committee of laboratory animal welfare, Institute of zoology, Chinese Academy of Sciences (Grant No. IOZ-IACUC-2021-171). Field collections followed the Guide to Collection, Preservation, Identification and Information Share of Animal Specimens (Xue, 2010) and Implementation rules of Fisheries Law of the People's Republic of China. All activities followed Laboratory animal—Guideline for ethical review of animal welfare (GB/T 35892–2018).

A total of 151 specimens of the genus *Sarcocheilichthys* were examined in this study, among them, specimens of *S. sciistius* were collected from five rivers within four river basins (Amur River basin, Haihe River basin, Yellow River basin and Huaihe River basin), specimens of *S. czerskii* were collected from the Songhuajiang River (Amur River basin)**,** specimens of *S. nigripinnis* were collected from three rivers or lakes within two regions (Yangtze River Basin and coastal rivers in Southeast China), detailed information of all specimens involved in this study is provided in the Comparative Materials. Specimens in this study were collected by using traps and hand nets. Freshly caught fish were euthanized with eugenol. The right pelvic-fin was cut off and preserved in pure ethanol for DNA extraction, then the specimens were fixed in 10% formaldehyde solution for three days, followed by 70% ethanol for morphological studies and long-term storage. All specimens examined in this study were finally deposited at the National Zoological Museum, Institute of Zoology, Chinese Academy of Sciences (ASIZB), Beijing, China.

All morphological work except the lip structure was performed on the left side of specimens. Measurement was taken point to point with digital calipers to 0.01mm. The measurements and counts followed Sun *et al*. (2021). The measurements of the lip structure are shown in Figure 1.

**Molecular phylogenetic analysis.** The molecular studies used mitochondrial DNA Cytochrome *b* (Cyt *b*) sequences. DNA was extracted from the pectoral fin on the right side of the fish. Cyt *b* was amplified using the primers L14724 (5'–GACTTGAAAAACCACCGTTG–3') and H15915 (5'–CTCCGATCTCCGGATTACAAGAC–3') followed Xiao *et al*. (2001); sequencing reactions were performed according to the operating instruction of BigDye Terminator v3.1 (BDT), with 1 μL of primer (3.2 pmol/μL), 1 μL template DNA, 2 μL BigDye® Terminator v3.1 and 6 μL double distilled water (dd $H_2O$) for a total reaction volume of 10 μL. The thermocycling conditions were as follows: initial denaturation for 2 min at 96 °C, denaturation for 10 s at 96 °C, annealing for 10 sec at 50 °C and extension for 1 min at 60 °C. After 30 cycles, the final extension was done at 60 °C for 3 min and the PCR product was preserved at 4 °C. Sequencing was carried out by the Beijing TsingKe Biotech Co., Ltd.

Sequencing results were assembled using SeqMan II, other sequences were acquired from NCBI. The code of each individual and GenBank accession numbers used in this studies are given in Table 1. Forty-three Cyt *b* sequences of *Sarcocheilichthys* species were added, *Hemibarbus maculatus* and *Rhinogobio typus* were used as outgroup. Nucleotide sequence alignment was verified using MEGA v6.0 (Tamura *et al*., 2013) with the software ClustalW. ModelFinder (Kalyaanamoorthy *et al*., 2017) was used to select the best-fit model using BIC criterion. Bayesian Inference phylogenies were inferred using MrBayes 3.2.6 (Ronquist *et al*., 2012). Model for maximum likelihood method was selected by MEGA v6.0 and the ML tree was reconstructed by MEGA v6.0. Bayesian Inference (BI) phylogenies were inferred using MrBayes 3.2.6 (Ronquist *et al*., 2012) under HKY+F+G4 model (2 parallel runs, 1000000 generations), in which the initial 25% of sampled data were discarded as burn-in. The phylogenetic analyses using maximum likelihood (ML) was also provided using MEGA 6.0 under HKY+G model (1000 bootstrap

replications). In addition, two independent methods, the assemble species by automatic partitioning (ASAP) and the Poisson tree process (PTP) model, relying on different operational criteria were applied to infer molecular species delineation for the *Sarcocheilichthys* species (Kapli *et al*., 2017; Puillandre *et al*., 2020). Aligned sequences were uploaded to the online server of ASAP using Jukes-Cantor (JC69) model (https://bioinfo.mnhn.fr/abi/public/asap/) and rooted phylogenetic trees (BI [PTP] and ML [mPTP]), without outgroup, were uploaded to the online server of PTP (http://species.h-its.org/ptp/). Estimates of evolutionary divergence over sequence pairs between groups and within groups were conducted using the Kimura 2-parameter model (Kimura, 1980).

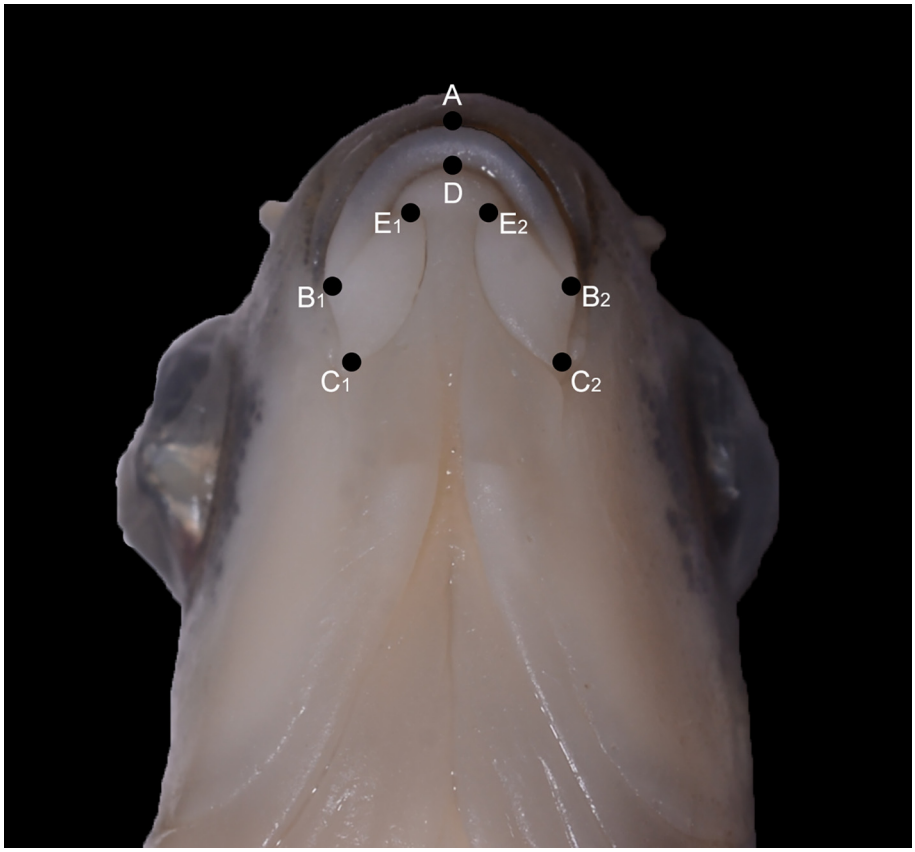


**FIGURE 1** Morphometric measurements of lip structure. $AC_1$: Upper lip length, $DB_1$: Mouth depth, $B_1B_2$: Mouth width, $C_1E_1$: Lower lip length, $C_1C_2$: Posterior inter-lateral lobes distance, $DE_1$: Explored lower jaw length, $E_1E_2$: Anterior inter-lateral lobes distance.

**TABLE 1.** Code, sampling localities and accession numbers of *Sarcocheilichthys* species and outgroup for molecular analysis.

| Code | Species | Species based on this study | Locality | Basin | Accession no. | Source |
|---|---|---|---|---|---|---|
| ASIZB 221752 | *Sarcocheilichthys sciistius* | *Sarcocheilichthys sciistius* | Shatuozi Village, Miyun District, Beijing | Baihe River, Haihe River Basin | - | This study |
| ASIZB 221753 | *Sarcocheilichthys sciistius* | *Sarcocheilichthys sciistius* | Shatuozi Village, Miyun District, Beijing | Baihe River, Haihe River Basin | - | This study |
| ASIZB 221754 | *Sarcocheilichthys sciistius* | *Sarcocheilichthys sciistius* | Shatuozi Village, Miyun District, Beijing | Baihe River, Haihe River Basin | - | This study |
| ASIZB 221759 | *Sarcocheilichthys sciistius* | *Sarcocheilichthys sciistius* | Yichuan County, Henan Province | Yihe River, Yellow River Basin | - | This study |
| ASIZB 221760 | *Sarcocheilichthys sciistius* | *Sarcocheilichthys sciistius* | Yichuan County, Henan Province | Yihe River, Yellow River Basin | - | This study |
| ASIZB 221779 | *Sarcocheilichthys sciistius* | *Sarcocheilichthys sciistius* | Shangcheng County, Henan Province | Guanhe River, Huaihe River Basin | - | This study |

*.....Continued on the next page*

**TABLE 1.**

| Code | Species | Species based on this study | Locality | Basin | Accession no. | Source |
|---|---|---|---|---|---|---|
| ASIZB 221780 | *Sarcocheilichthys sciistius* | *Sarcocheilichthys sciistius* | Shangcheng County, Henan Province | Guanhe River, Huaihe River Basin | - | This study |
| ASIZB 221781 | *Sarcocheilichthys sciistius* | *Sarcocheilichthys sciistius* | Shangcheng County, Henan Province | Guanhe River, Huaihe River Basin | - | This study |
| Unknown | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys sciistius* | Luonan County, Shaanxi Province | Luohe River, Yellow River Basin | EF193430 | Zhang *et al*., 2008 |
| Unknown | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys sciistius* | Luonan County, Shaanxi Province | Luohe River, Yellow River Basin | EF193431 | Zhang *et al*., 2008 |
| Unknown | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys sciistius* | Luonan County, Shaanxi Province | Luohe River, Yellow River Basin | EF193432 | Zhang *et al*., 2008 |
| ASIZB 221901 | *Sarcocheilichthys czerskii* | *Sarcocheilichthys sciistius* | Harbin City, Heilongjiang Province | Hukou Wetland, Heilongjiang (Amur) River Basin | - | This study |
| ASIZB 221902 | *Sarcocheilichthys czerskii* | *Sarcocheilichthys sciistius* | Harbin City, Heilongjiang Province | Hukou Wetland, Heilongjiang (Amur) River Basin | - | This study |
| ASIZB 221903 | *Sarcocheilichthys czerskii* | *Sarcocheilichthys sciistius* | Harbin City, Heilongjiang Province | Hukou Wetland, Heilongjiang (Amur) River Basin | - | This study |
| Unknown | *Sarcocheilichthys czerskii* | *Sarcocheilichthys sciistius* | Zhuaji Village, Heilongjiang Province | Heilongjiang (Amur) River Basin | EF193463 | Zhang *et al*., 2008 |
| Unknown | *Sarcocheilichthys czerskii* | *Sarcocheilichthys sciistius* | Nenjiang City, Heilongjiang Province | Nenjiang River, Heilongjiang (Amur) River Basin | EF193464 | Zhang *et al*., 2008 |
| Unknown | *Sarcocheilichthys czerskii* | *Sarcocheilichthys sciistius* | Nenjiang City, Heilongjiang Province | Nenjiang River, Heilongjiang (Amur) River Basin | EF193467 | Zhang *et al*., 2008 |
| Unknown | *Sarcocheilichthys soldatovi* | *Sarcocheilichthys sciistius* | Unknown | Unknown | JN003337 | Tang *et al*., 2011 |
| ASIZB 221793 | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Wuyuan County, Jiangxi Province | Raohe River, middle Yangtze River Basin | - | This study |
| ASIZB 221794 | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Wuyuan County, Jiangxi Province | Raohe River, middle Yangtze River Basin | - | This study |
| ASIZB 221795 | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Wuyuan County, Jiangxi Province | Raohe River, middle Yangtze River Basin | - | This study |
| ASIZB 221796 | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Wuyuan County, Jiangxi Province | Raohe River, middle Yangtze River Basin | - | This study |
| ASIZB 221713 | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Huzhou City, Zhejiang Province | Taihu Lake, lower Yangtze River Basin | - | This study |
| ASIZB 221714 | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Huzhou City, Zhejiang Province | Taihu Lake, lower Yangtze River Basin | - | This study |
| ASIZB 221716 | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Huzhou City, Zhejiang Province | Taihu Lake, lower Yangtze River Basin | - | This study |
| ASIZB 221717 | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Huzhou City, Zhejiang Province | Taihu Lake, lower Yangtze River Basin | - | This study |
| ASIZB 221720 | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Shengzhou City, Zhejiang Province | Cao'ejiang River Basin | - | This study |
| ASIZB 221724 | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Shengzhou City, Zhejiang Province | Cao'ejiang River Basin | - | This study |
| Unknown | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Yueyang City, Hunan Province | middle Yangtze River Basin | EF193436 | Zhang *et al*., 2008 |

*.....Continued on the next page*

**TABLE 1.**

| Code | Species | Species based on this study | Locality | Basin | Accession no. | Source |
|---|---|---|---|---|---|---|
| Unknown | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Wuhan City, Hubei Province | middle Yangtze River Basin | EF193437 | Zhang *et al*., 2008 |
| Unknown | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Nanchang City, Jiangxi Province | middle Yangtze River Basin | EF193440 | Zhang *et al*., 2008 |
| Unknown | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Huangshan City, Anhui Province | lower Yangtze River Basin | EF193442 | Zhang *et al*., 2008 |
| Unknown | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Shangrao City, Jiangxi Province | middle Yangtze River Basin | EF193443 | Zhang *et al*., 2008 |
| Unknown | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Yichang City, Hubei Province | middle Yangtze River Basin | EF193444 | Zhang *et al*., 2008 |
| Unknown | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Fuchunjiang Township, Zhejiang Province | Qiantangjiang River Basin | EF193449 | Zhang *et al*., 2008 |
| Unknown | *Sarcocheilichthys nigripinnis* | *Sarcocheilichthys nigripinnis* | Shangrao City, Jiangxi Province | middle Yangtze River Basin | KF013952 | Liu *et al*., 2013 |
| Unknown | *Sarcocheilichthys kiangsiensis* | *Sarcocheilichthys kiangsiensis* | Shangrao City, Jiangxi Province | middle Yangtze River Basin | EF193427 | Zhang *et al*., 2008 |
| ASIZB 221817 | *Sarcocheilichthys vittatus* | *Sarcocheilichthys vittatus* | Wuyuan County, Jiangxi Province | Raohe River, middle Yangtze River Basin | - | This study |
| Unknown | *Sarcocheilichthys lacustris* | *Sarcocheilichthys lacustris* | Fuyuan City, Heilongjiang Province | Heilongjiang (Amur) River Basin | EF193412 | Zhang *et al*., 2008 |
| Unknown | *Sarcocheilichthys sinensis* | *Sarcocheilichthys sinensis* | Hukou County, Jiangxi Province | middle & lower Yangtze River Basin | EF193418 | Zhang *et al*., 2008 |
| Unknown | *Sarcocheilichthys parvus* | *Sarcocheilichthys parvus* | Yanshan County, Jiangxi Province | Xinjiang River, middle Yangtze River Basin | - | This study |
| ASIZB 221787 | *Sarcocheilichthys parvus* | *Sarcocheilichthys parvus* | Yanshan County, Jiangxi Province | Xinjiang River, middle Yangtze River Basin | - | This study |
| ASIZB 221788 | *Sarcocheilichthys parvus* | *Sarcocheilichthys parvus* | Shangrao City, Jiangxi Province | middle Yangtze River Basin | EF193420 | Zhang *et al*., 2008 |
| **Outgroup** | | | | | | |
| Unknown | *Rhinogobio typus* | - | Wuhan City, Hubei Province | middle Yangtze River Basin | EF193455 | Zhang *et al*., 2008 |
| Unknown | *Hemibarbus maculatus* | - | Wuhan City, Hubei Province | middle Yangtze River Basin | EF193456 | Zhang *et al*., 2008 |

## Results

### *Sarcocheilichthys sciistius* (Abbott, 1901) (Figures 2, 3, 4 and 5; Table 2).

*Leuciscus sciistius* Abbott (1901): 487 (original description); Böhlke (1953): 33.
*Chilogobio czerskii* Berg (1914): 490; Berg (1949): 661.
*Chilogobio soldatovi* Berg (1914): 492; Berg (1949): 662.
*Sarcocheilichthys nigripinnis sciistius* Nichols (1943): 191.
*Sarcocheilichthys nigripinnis* Bănărescu *et* Nalbant (1967): 294; Luo *et al*. (1977): 474; Yue in Chen *et al*. (1998): 277.
*Sarcocheilichthys nigripinnis czerskii* Bănărescu *et* Nalbant (1967): 294; Luo *et al*. (1977): 476; Zhu (1995): 76; Kim (1997): 207.
*Sarcocheilichthys czerskii* Bogutskaya *et* Naseka (1996): 40; Naseka (1996): 156; Reshetnikov *et al*. (1997): 741; Naseka (1998): 86; Yue in Chen *et al*. (1998): 279; Bogutskaya *et al*. (2001): 44; Kim & Park (2002): 112; Bogutskaya *et* Naseka (2004): 71; Kottelat (2006):88; Bogutskaya *et al*. (2008): 327; Parin *et al*. (2014): 92; Zhang *et al*. (2016): 78; An *et al*. (2020): 217.
*Sarcocheilichthys soldatovi* Bogutskaya & Naseka (1996): 42; Naseka (1996): 155; Reshetnikov *et al*. (1997): 741; Naseka (1998): 86; Bogutskaya *et al*. (2001): 44; Bogutskaya & Naseka (2004): 72; Kottelat (2006): 49; Ocock *et al*. (2006): 46; Bogutskaya *et al*. (2008): 328; An *et al*. (2020): 217.

**Holotype.** USNM 49548 (Figure 2 a, b and c), 53.4 mm SL; From Pei-Ho River (Grand Canal), Tien-Tsin (Tianjin), China.

**Additional material examined.** ASIZB 221731–221744, 221752–221755, 221779–221781, 40.0–71.5 mm SL, eighteen specimens, from Baihe River, Miyun District, Beijing City, 19 October 2020 (River of type locality); ASIZB 221901–221921, 38.5–44.8 mm SL, twenty-one specimens, from Songhuajiang River, Hulan District, Harbin City, 15 June 2021; ASIZB 220835–220855, 51.2–74.8 mm SL, twenty-one specimens, from Liuhe River, Xinglong County, Chengde City, Hebei Province, 26 September 2020; ASIZB 221766–221777, 27.6–71.6 mm SL, twelve specimens, from Qinhe River, Jiyuan City, Henan Province, 3 September 2020. ASIZB 221759–221761, 50.9–53.1 mm SL, three specimens, from Yihe River, Yichuan County, Henan Province, 4 September 2020. ASIZB 221779–221786, 44.1–49.4 mm SL, eight specimens, from Guanhe River, Shangcheng County, Henan Province, 17 September 2020.

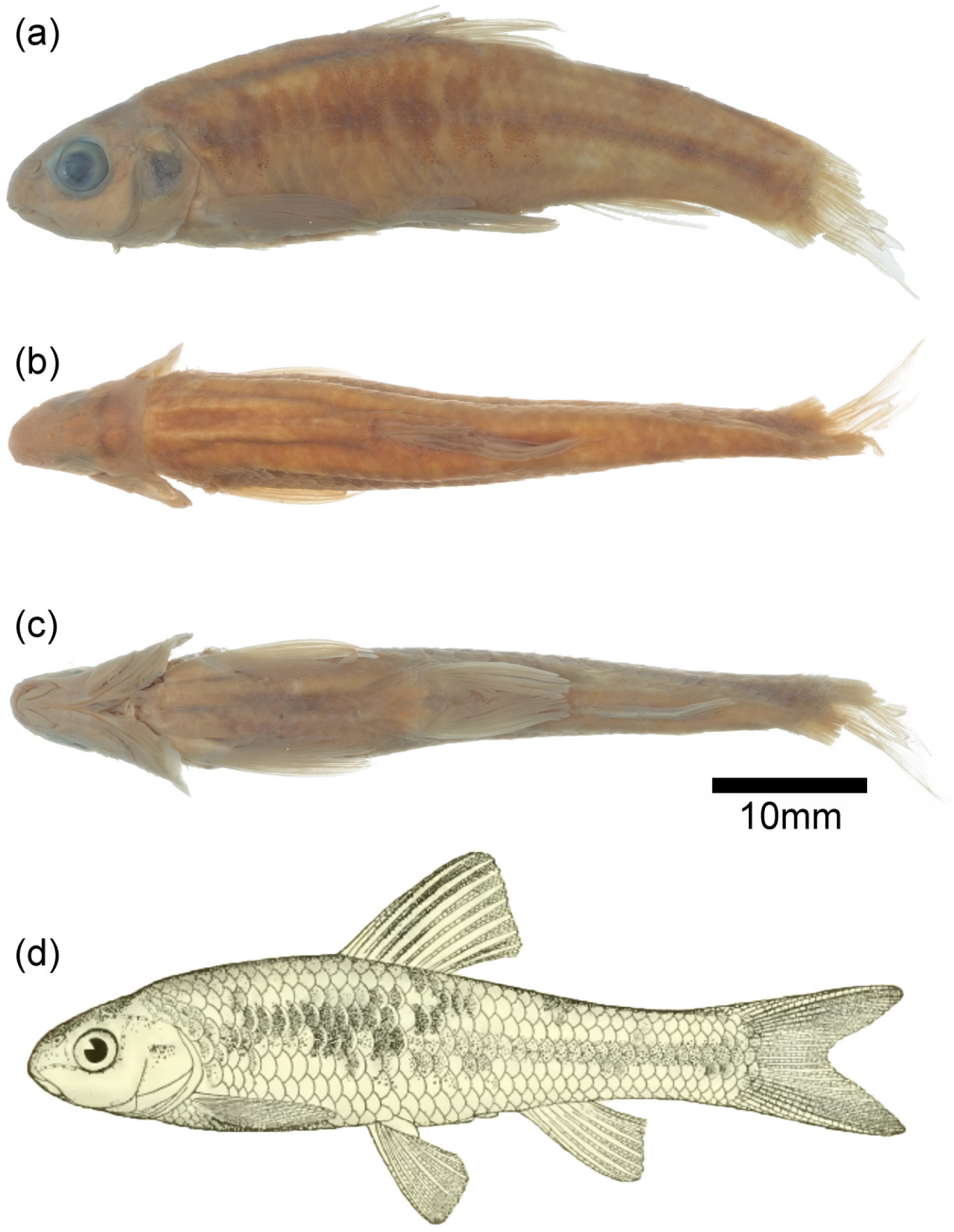


**FIGURE 2** *Sarcocheilichthys sciistius*, Abbott (1901). Holotype, USNM 49548, 53.4 mm SL: (a) lateral view, (b) dorsal view, (c) ventral view, (d) original drawing. Fig a, b and c were taken by Rob Robins and Sandra Raredon, Fig d drawn by Abbott (Download from Biodiversity Heritage Library, www.biodiversitylibrary.org).

**Diagnosis.** *Sarcocheilichthys sciistius* can be separated from *S. parvus*, *S. caobangensis*, *S. vittatus, S. lacustris* and *S. sinensis* by several irregular black vertical blotches on lateral side of body (vs. a longitudinal black band or four wide black vertical bars on lateral side of body), it can be further separated from *S. parvus*, *S. caobangensis* and *S. vittatus* by 40–42 lateral-line scales (vs. 34–36 in *S. parvus*, 38 in *S. caobangensis*, 37–38 in *S. vittatus*), it can be further separated from *S. lacustris* and *S. sinensis* by barbels absent (vs. a pair of short barbels) and last dorsal-fin ray soft (vs. stiff). *Sarcocheilichthys sciistius* can be separated from *S. davidi* by dorsal-fin origin closer to snout tip than to caudal-fin base (vs. equal) and 40–42 lateral-line scales (vs. 38–39), from *S. kiangsiensis* by 12–14 pectoral-fin rays (vs. 15–17) and barbels absent (vs. a pair of short barbels), from *S. hainanensis* by two rows of pharyngeal teeth (vs. one row), from *S. nigripinnis* by 40–42 lateral-line scales (vs. 38–40), lower lip length 61.1%–72.8% of upper lip length (vs. 73.2%–90.2% of upper lip length), paired fins orange-red in color (vs. dark brown or black) (Figure 4).

**Description.** Body elongated and slightly compressed, greatest body depth at dorsal-fin origin and lowest anterior to caudal-fin base; dorsal body profile rising smoothly from nostrils to dorsal-fin base, then sloping gradually to caudal-fin base; ventral body concave from lower jaws to the end of anal-fin base and slightly concave from the end of anal-fin base to caudal-fin base. Anus positioned slightly closer to anal-fin origin than to pelvic-fin insertion. Head short and compressed, length about 22.7−26.1% of body depth and depth greater than width. Snout bluntly pointed, shorter than postorbital head length, basically equal to interorbital width. Eye normal sized, diameter about 24.0−30.5% of head length, smaller than interorbital width, placed in dorsal half of head. Nostrils positioned closely, in front of eyes. Mouth sub-terminal, arc shaped; lips well developed, upper lip smooth and thick, lower lip modified to form two relatively lateral lobes confined only to sides of lower jaw, length 61.1−72.8% of upper lip length; postlabial groove with a narrow interruption, width 23.8−29.8% of upper lip length; lower jaw without developed horny sheath on the cutting edge. No barbels.

Dorsal fin iii, 7 (18 specimens); posterior edge slightly concave, its origin anterior to vertical line of pelvic-fin origin and closer to snout tip than to caudal-fin base. Pectoral fin i, 12 (1), i, 13 (12), or i, 14 (5), tips extending beyond half of distance between pectoral-fin and pelvic-fin origins. Pelvic fin i, 7 (18), tips extending beyond anus but not reaching anal-fin origin, length shorter than others fins. Anal fin iii, 5 (18) branched rays; posterior edge slightly concave, origin closer to the pelvic-fin origin than to caudal-fin base, its length close to caudal peduncle length. Caudal fin forked with 9 branched rays on upper lobes and 8 branched rays on lower lobes, tips slightly blunt, upper and lower lobes are equal in length.

Body covered with medium-sized scales. Thorax before pectoral fins naked. Lateral line complete, slightly bent ventrally at beginning, then almost straight to the end of caudal peduncle. Lateral-line scales 40 (4), 41 (12), 42 (2); scales above lateral line 4.5 (18); scales below lateral line 4 (18); predorsal scales 12 (15), 13 (3); circumpeduncular scales 16 (18).

**Coloration in life.** Dorsal and mid-lateral side of head yellow or light gray, ventral side grayish white. Operculum margin red or yellow. A black vertical marking behind the head. Dorsal and lateral side of the body golden yellow with irregular deep gray blotches, black blotches scattered gradually becomes dense from head margin to caudal-fin base, ventral side grayish white. Dorsal fin translucent or orange-red with two rows of black spots, caudal fin translucent or orange-red and sometimes with a large black spot on lower lobe. Pectoral fin, pelvic fin and anal fin having orange-red median part and hyaline distal edge. (Figure 4a, 4b).

**Coloration in preservative.** Dorsal and mid-lateral side of head light brown, ventral side grayish white. A black or dark gray vertical marking behind the head. Dorsal and lateral side of the body yellow with irregular brown blotches, black blotches scattered gradually become dense from head margin to caudal-fin base, ventral side greyish white. Dorsal fin and caudal fin with brown basal part and white distal margin. Pectoral fin, pelvic fin and anal fin having a yellow median part and a hyaline distal edge. (Figure 3, 5).

**Sexual dimorphism.** In breeding season, adult male with bead pearls on snout and cheek. Margin of operculum, dorsal, lateral side of body and all fins bright red (lateral side of body fades quickly when caught) (Figure 4a); adult female with elongated ovipositor extending beyond origin of pelvic-fin (Figure 5).

**Distribution.** *Sarcocheilichthys sciistius* is widely distributed in the northern China and the border of China and Russia Far East, including Heilongjiang (Amur) River Basin, Yalu River Basin, Liaohe River Basin, Haihe River Basin (including the type locality), middle and lower reaches of Yellow River Basin and Huaihe River Basin (Figure 6).

**Habitat and biology.** *Sarcocheilichthys sciistius* usually inhabits slowly flowing rivers with clear, shallow wa-

ters with a sand-gravel substrate (Figure 7a). In the Baihe River, coexisting species include *Opsariichthys bidens*, *Zacco platypus*, *Rhynchocypris lagowskii*, *Rhodeus sinensis, Hemibarbus labeo*, *Squalidus wolterstorffi*, and *Microphysogobio chinssuensis*. In addition, species of freshwater mussel like *Unio douglasiae* can also be found in this habitat. *Sarcocheilichthys sciistius* usually feeds on the algae growing on the surface of pebbles (Figure 7b).


**FIGURE 3** *Sarcocheilichthys sciistius*, ASIZB 221744, 71.5 mm standard length: (a) lateral view, (b) dorsal view, (c) ventral view

**Genetic comparisons.** The molecular phylogenetic results (both BI and ML) based on Cyt *b* sequence shows that *Sarcocheilichthys sciistius*, *S. czerskii* and *S. soldatovi* form a monophyletic group which is sister to *S. nigripinnis*, and *S. czerskii* together with *S. soldatovi* nested within *S. sciistius* (Figure 8). The interspecific genetic distances between *S. sciistius* and its closest two congeners *S. czerskii* and *S. nigripinnis* are 0.6% and 6.6% for Cyt *b* based on K2P model respectively. The intraspecific genetic distance in *S. sciistius* is 0.6% and in *S. nigripinnis* is 1.1%, which are much lower than the inter-species genetic distances (6.6%) between these two species; however, the intraspecific genetic distance in *S. sciistius* is much higher than that of *S. czerskii* (0.6% vs. 0.03%, Table 3). The only available sequence from *S. soldatovi* is also located inside the linage of *S. sciistius* - *S. czerskii*. The ASAP analysis recognized six molecular operational taxonomic units (MOTUs) among the given nine species, and PTP method for BI tree supported eight MOTUs with the posterior probabilities of 1.00, 1.00, 1.00, 0.90, 1.00, 0.88, 0.57, and 0.95* (* refers to the node of *S.sciistius, S. czerskii and S. soldatovi*). The mPTP analysis for ML tree supported seven MOTUs with the posterior probabilities of 1.00, 0.98, 0.98, 0.70, 1.00, 0.76 and 0.96* respectively (* refers to the node of *S. sciistius, S. czerskii and S. soldatovi*). Both methods showed *S. sciistius* a distinct taxa and the genetic evidence supported *S. sciistius* to be a possible distinct species and *S. czerskii* with *S. soldatovi* may just be geographic populations of *S. sciistius* based on the current available sequences. The phylogenetic trees reconstructed by the BI and ML methods showed almost the same tree topology and the support values of each method are shown in the tree (Figure 8). The genetic distances of the Cyt *b* gene amongst nine species of *Sarcocheilichthys* that distributed in China are given in Table 3.


**FIGURE 4** (a) Live individual of *Sarcocheilichthys sciistius* in breeding season, male, from Yanjiahe River, Huaihe River Basin, Guangshan County, Henan Province, 26 April 2021; (b) Live individual of *S. sciistius*, male, from Jumahe River, Haihe River Basin, Fangshan District, Beijing, 22 October 2020; (c) Live individual of *S. nigripinnis*, male, from the Taihu Lake, lower Yangtze River Basin, Huzhou City, Zhejiang Province, 31 October 2021.


**FIGURE 5** *Sarcocheilichthys sciistius*, female, ASIZB 221728, 71.2 mm standard length; from Liuhe River, Luanhe River Basin, Xinglong County, Hebei Province; 27 September 2020.

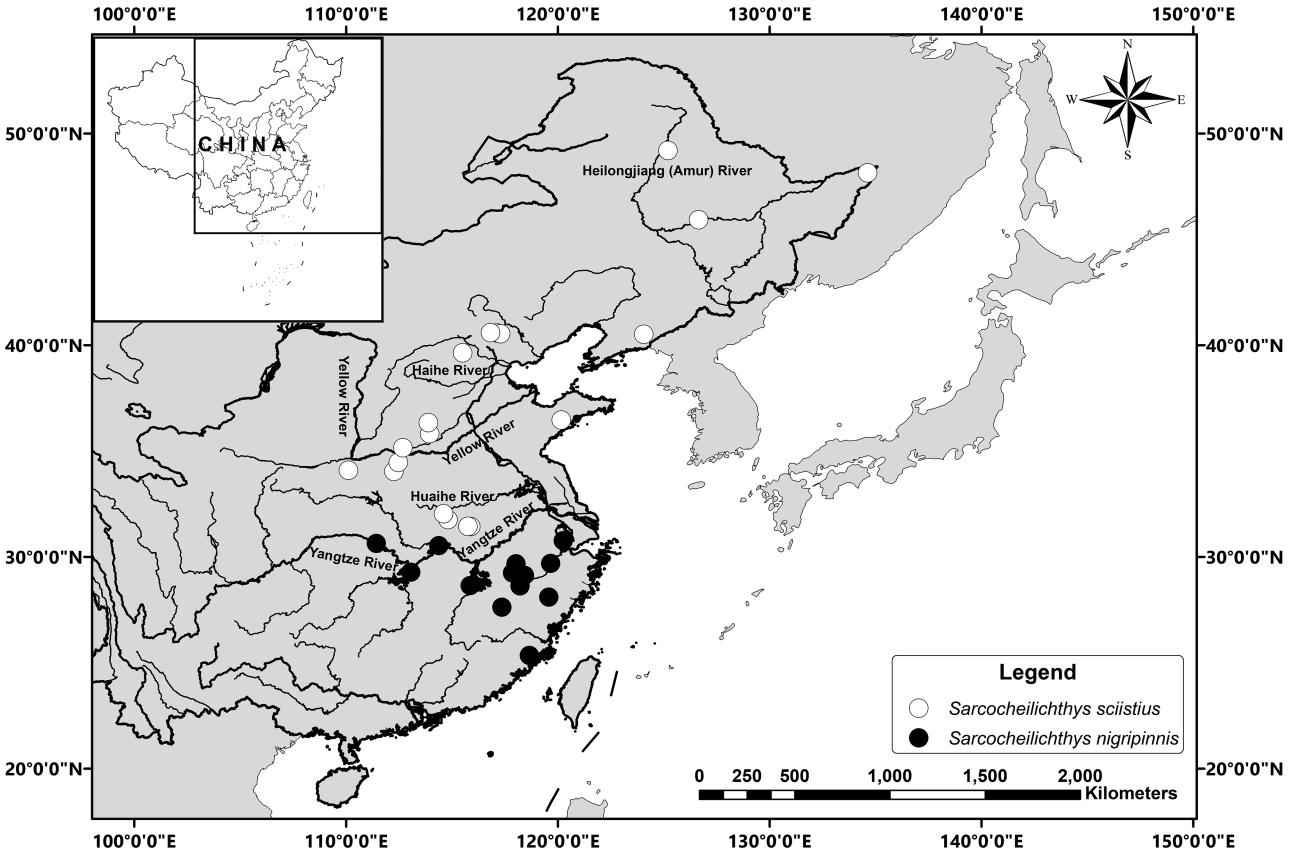


**FIGURE 6** Collection sites and distribution of *Sarcocheilichthys sciistius* and *S. nigripinnis*.

**TABLE 2.** Morphometric measurements of *Sarcocheilichthys sciistius*, and *S. nigripinnis*.

| Characters | *S. sciistius* (Holotype) | *S. sciistius* (n=18) | | | *S. nigripinnis* (n=18) | | |
|---|---|---|---|---|---|---|---|
| | | Range | Mode | | Range | Mode | |
| Dorsal-fin rays | 7 | 7 | 7 | | 7 | 7 | |
| Anal-fin rays | 5 | 5 | 5 | | 6 | 6 | |
| Pectoral-fin rays | / | 12−14 | 13 | | 12–14 | 13 | |
| Pelvic-fin rays | 7 | 7 | 7 | | 7 | 7 | |
| Lateral line scales | 40 | 40−42 | 41 | | 38−40 | 38 | |
| Scales above lateral line | 4.5 | 4.5 | 4.5 | | 4.5−5.5 | 5 | |
| Scales below lateral line | 3 | 3 | 3 | | 3 | 3 | |
| Pre-dorsal scales | 13 | 12−13 | 12 | | 11−13 | 12 | |
| Circumpeduncular scales | / | 17 | 17 | | 17 | 17 | |
| | | Range | Mean | SD | Range | Mean | SD |
| Standard Length (mm) | 53.4 | 40.0−71.5 | 57.5 | | 54.4−96.2 | 67.9 | |
| **In percentage of SL** | | | | | | | |
| Body depth | 25.2 | 22.8−27.2 | 24.1 | 1.4 | 22.0-25.5 | 23.6 | 1.1 |
| Head length | 24.2 | 22.7−26.1 | 24.5 | 1.0 | 22.6-26.4 | 25.1 | 1.1 |
| Dorsal-fin length | / | 22.8−27.0 | 24.9 | 1.2 | 22.1−27.4 | 25.2 | 1.4 |
| Dorsal-fin base length | 15.1 | 13.6−16.1 | 14.9 | 0.7 | 13.4−15.9 | 14.4 | 0.8 |
| Pectoral-fin length | 20.1 | 20.0−22.3 | 21.3 | 0.6 | 17.3−23.6 | 20.5 | 1.7 |
| Pectoral-fin base length | 4.7 | 3.7−5.3 | 4.5 | 0.5 | 3.7−5.0 | 4.4 | 0.4 |

*.....Continued on the next page*

**TABLE 2. (continued)**

| Characters | *S. sciistius* (Holotype) | *S. sciistius* (n=18) | | | *S. nigripinnis* (n=18) | | |
|---|---|---|---|---|---|---|---|
| | | Range | Mode | | Range | Mode | |
| Pelvic-fin length | 16.7 | 17.7−20.0 | 18.9 | 0.6 | 16.6−20.3 | 18.3 | 1.0 |
| Pelvic-fin base length | 5.9 | 4.1−6.1 | 5.0 | 0.5 | 3.5−5.4 | 4.5 | 0.6 |
| Anal-fin length | / | 17.0−21.1 | 19.8 | 1.1 | 16.4−21.3 | 18.2 | 1.3 |
| Anal-fin base length | 9.8 | 8.9−11.5 | 9.9 | 0.7 | 7.7−10.8 | 9.2 | 0.8 |
| Predorsal length | 48.3 | 47.4−50.5 | 48.7 | 0.9 | 46.8−49.8 | 48.2 | 0.8 |
| Postdorsal length | 39.2 | 37.2−43.4 | 39.9 | 1.8 | 37.4−42.5 | 39.7 | 1.3 |
| Snout-pectoral distance | 25.5 | 22.5−25.5 | 24.4 | 0.9 | 23.3−27.5 | 24.8 | 1.0 |
| Snout-pelvic distance | 50.3 | 48.0−52.2 | 50.0 | 1.2 | 47.3−52.4 | 50.4 | 1.2 |
| Preanal length | 70.6 | 70.1−75.5 | 72.7 | 1.5 | 70.7−74.7 | 73.2 | 1.1 |
| Caudal peduncle length | 17.2 | 18.1−21.8 | 19.7 | 1.3 | 17.2−20.0 | 18.9 | 0.9 |
| Caudal peduncle depth | 11.9 | 11.0−13.1 | 12.1 | 0.5 | 11.3−13.4 | 12.1 | 0.5 |
| Head Length (mm) | 12.9 | 10.3−17.4 | 14.2 | | 12.5−25.3 | 17.1 | |
| **In percentage of HL** | | | | | | | |
| Head depth | 77.5 | 72.1−82.8 | 77.4 | 3.1 | 72.6−84.5 | 75.4 | 2.7 |
| Head width | 49.9 | 48.4−62.7 | 54.4 | 3.2 | 48.0−57.8 | 52.4 | 2.7 |
| Snout length | 27.7 | 32.0−44.2 | 34.8 | 3.1 | 29.8−36.4 | 33.6 | 1.8 |
| Postorbital head length | 50.3 | 41.9−51.6 | 46.7 | 2.5 | 45.2−50.3 | 48.3 | 1.4 |
| Eye diameter | 26.4 | 24.0−30.5 | 27.0 | 1.7 | 23.6−31.5 | 25.1 | 1.8 |
| Interorbital width | 38.8 | 29.4−39.9 | 34.0 | 3.2 | 28.5−37.3 | 32.4 | 2.4 |
| Upper lip length (mm) | 3.7 | 2.7−5.4 | 3.8 | | 3.4−7.3 | 4.6 | |
| **In percentage of upper lip length** | | | | | | | |
| Mouth depth | 55.2 | 54.1−64.3 | 59.2 | 3.3 | 55.7−79.4 | 62.1 | 5.1 |
| Explored lower jaw length | 17.2 | 14.9−20.2 | 18.0 | 1.8 | 13.9−24.9 | 18.4 | 2.8 |
| Mouth width | 75.8 | 74.8−94.8 | 85.6 | 5.4 | 74.0−105.7 | 85.4 | 6.7 |
| Posterior inter-lateral lobes distance | 70.1 | 69.6−90.3 | 80.0 | 5.4 | 59.1−91.4 | 73.0 | 7.0 |
| Anterior inter-lateral lobes distance | 24.3 | 23.8−29.8 | 26.8 | 1.8 | 19.5−36.4 | 28.4 | 4.2 |
| Lower lip length | 67.5 | 61.1−72.8 | 66.8 | 3.9 | 73.2−90.2 | 76.4 | 4.5 |

**TABLE 3.** Genetic distances of the Cyt *b* gene computed by MEGA v.6.0 among 9 analyzed species of *Sarcocheilichthys*.

| - | Species | Intraspecific | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Sarcocheilichthys sciistius* | 0.0064 | - | - | - | - | - | - | - | - | - |
| 2 | *Sarcocheilichthys czerskii* | 0.0003 | 0.006 | - | - | - | - | - | - | - | - |
| 3 | *Sarcocheilichthys soldatovi* | n/c | 0.006 | 0.000 | - | - | - | - | - | - | - |
| 4 | *Sarcocheilichthys nigripinnis* | 0.0113 | 0.066 | 0.065 | 0.065 | - | - | - | - | - | - |
| 5 | *Sarcocheilichthys kiangsiensis* | n/c | 0.096 | 0.094 | 0.094 | 0.089 | - | - | - | - | - |
| 6 | *Sarcocheilichthys vittatus* | n/c | 0.163 | 0.161 | 0.162 | 0.172 | 0.151 | - | - | - | - |
| 7 | *Sarcocheilichthys parvus* | 0.0059 | 0.170 | 0.169 | 0.168 | 0.173 | 0.137 | 0.155 | - | - | - |
| 8 | *Sarcocheilichthys lacustris* | n/c | 0.162 | 0.159 | 0.159 | 0.164 | 0.132 | 0.174 | 0.144 | - | - |
| 9 | *Sarcocheilichthys sinensis* | n/c | 0.157 | 0.155 | 0.155 | 0.160 | 0.135 | 0.174 | 0.138 | 0.031 | - |
| | **Outgroup** | 0.1647 | 0.169 | 0.169 | 0.168 | 0.170 | 0.168 | 0.183 | 0.167 | 0.182 | 0.184 |

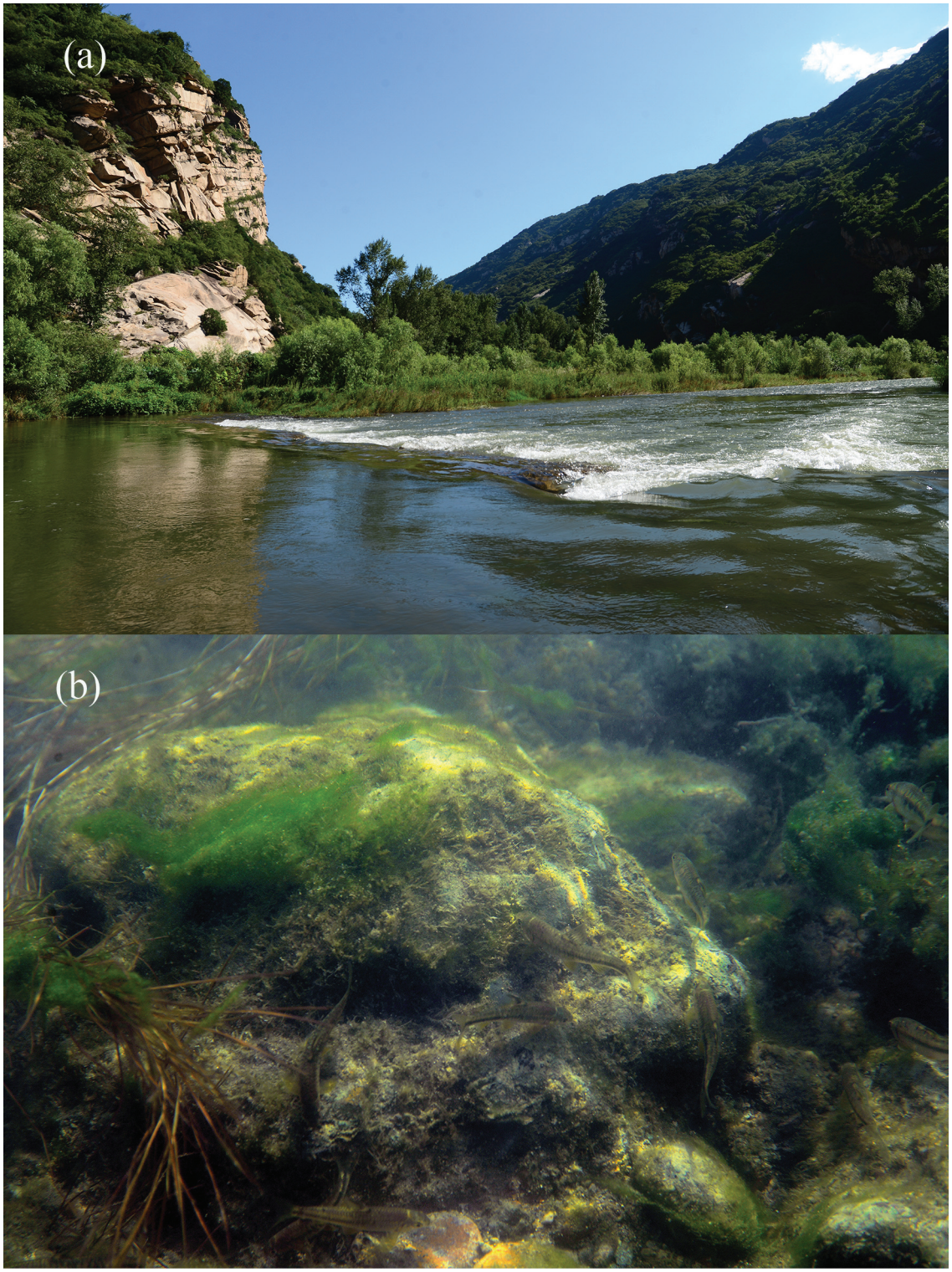


**FIGURE 7** (a) Typical habitat of *Sarcocheilichthys sciistius*, Baihe River, Haihe River Basin, Shatuozi Village, Miyun District, Beijing, China; 10 August 2020; (b) underwater photograph of *S. sciistius*, Chaohe River, Haihe River Basin, Chengzi Village, Miyun District, Beijing, China; 11 August 2020; photographed by Zhi-Xian Sun.

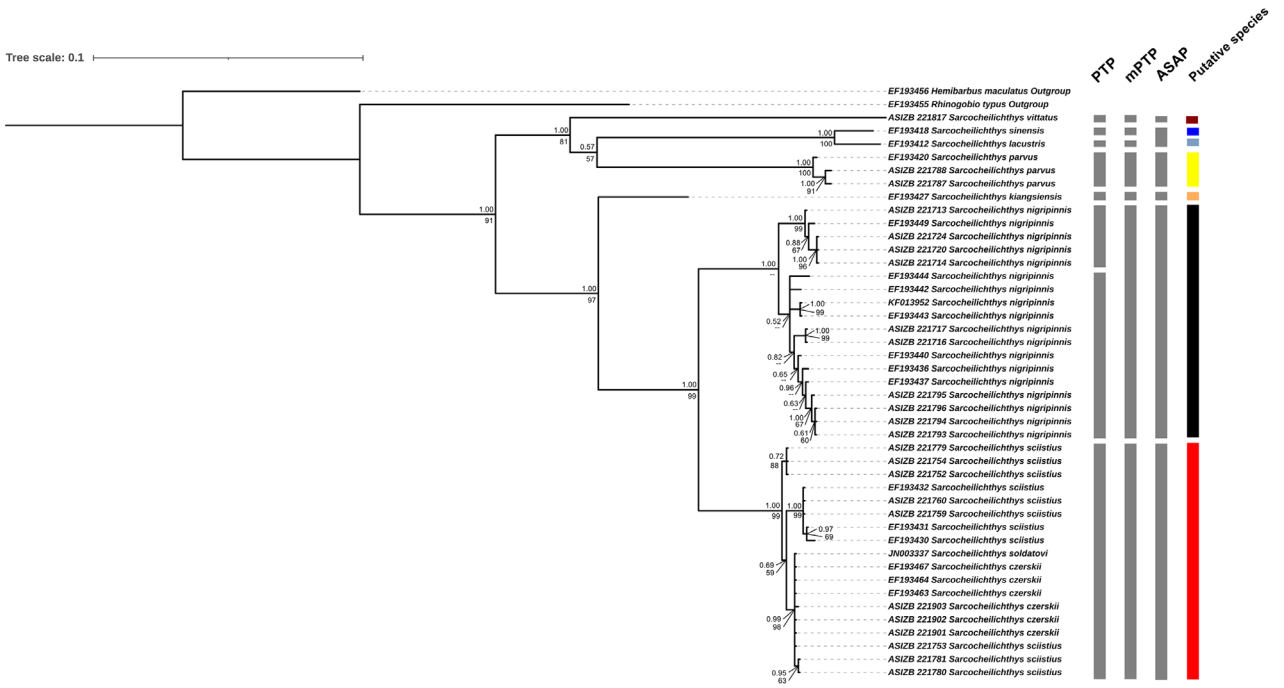


**FIGURE 8** Molecular phylogenetic tree of nine *Sarcocheilichthys* species based on Cyt *b* sequence reconstructed by Bayesian Inference method (Bayesian posterior probabilities values are shown above the branch) and Maximum Likelihood method (bootstrap values are shown below the branch), with the species delimitation results shown on the right.

## Discussion

The type locality of *Sarcocheilichthys sciistius* is the Pei-ho River, Tien-Tsin, China (now as the Tianjin section of the Grand Canal of China). Today, urbanization in both cities, Beijing and Tianjin, leads to habitat loss in the Grand Canal of China, although there are few stream-like habitats in this section. However, during our field collection in the nearby areas, we found that the Baihe River has a large population of *S. sciistius*. The Baihe River drains into the Grand Canal of China (Tianjin section, the type locality). Because of the shortage of the topotypes of *S. sciistius*, the newly captured specimens from the same river system are regarded as representatives of the topotypes. Besides, Shanghai (Lower reaches of the Yangtze River) is the unique record type locality of *S. nigripinnis* without any other clear records (Günther, 1873). Under this circumstance, we select the specimens from Taihu Lake (lake in lower reach of the Yangtze River, close to Shanghai) as typical specimens of *S. nigripinnis* in this study.

Nichols (1943) recognized *Leuciscus sciistius* as a subspecies of *Sarcocheilichthys nigripinnis*, named *S. nigripinnis sciistius*. Bănărescu and Nalbant (1967) synonymized *S. sciistius* with *S. nigripinnis*, which was accepted by Luo *et al.* (1977) and Yue *et al.* (1998). In this study, based on morphological comparison, *S. sciistius* can be distinguished from *S. nigripinnis* by having more lateral line scales (Figure 9), shorter lower lip length and different color of paired fins and anal fin (Figure 4). Molecular phylogenetic analysis also supports *S. sciistius* to be a distinct species from *S. nigripinnis*. The lineage contains *S. sciistius* is sister to *S. nigripinnis* with high Bayesian posterior probabilities values of 1.00 and high bootstrap value of 99% (Figure 8). In addition, the distribution of these two species does not overlap with *S. sciistius* distributed in the north of the Yangtze River Basin while *S. nigripinnis* is distributed in the south. This result is also supported by previous studies (Zhang *et al*., 2008; Liu *et al*., 2013), and we therefore confirm that *S. sciistius* is a valid species in this study.

As for *Sarcocheilichthys czerskii* and *S. soldatovi*, their taxonomic history are also complicated. After its original description, Bănărescu and Nalbant (1967) synonymized *Chilogobio soldatovi* with *S. nigripinnis* and treated *C. czerskii* as *S. nigripinnis czerskii*. Yue *et al*. (1998) raised the subspecies *S. nigripinnis czerskii* to the species level and followed the viewpoint proposed by Luo *et al*. (1977) that *S. czerskii* was only distributed in the Amur River basin. And this result was widely accepted (Kottelat, 2006; Bogutskaya *et al*., 2008; Zhang *et al*., 2016; An *et al*., 2020). Yue *et al*. (1998) considered *S. soldatovi* as a synonym of *S. czerskii*, but many researchers still considered

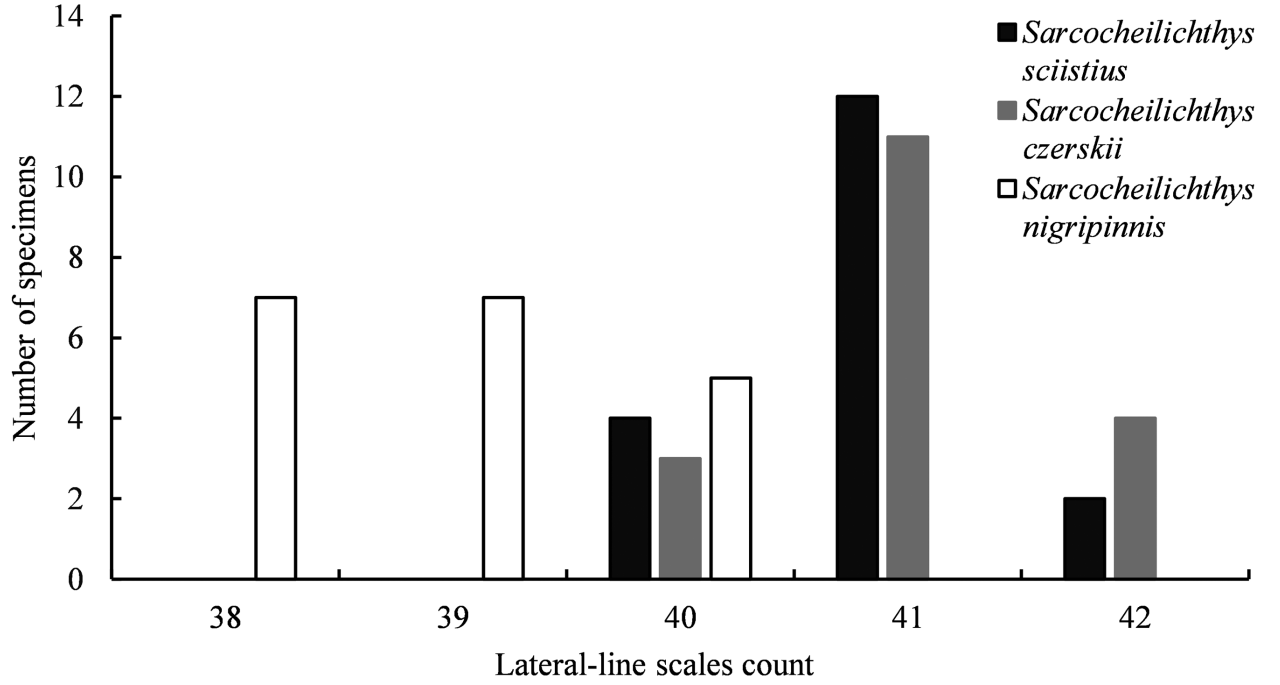


**FIGURE 9** Lateral-line scale counts of *Sarcocheilichthys sciistius*, *S. czerskii* and *S. nigripinnis*.

*S. soldatovi* as a valid species (Kottelat, 2006; Bogutskaya *et al*., 2008; An *et al*., 2020). Although *Sarcocheilichthys czerskii* and *S. soldatovi* have different type localities (Sintuka River and Tschlja Lake respectively), their distributions almost overlap (Bogutskaya *et al*., 2008; Antonov *et al*., 2019). *Sarcocheilichthys czerskii* is reliably known from Khanka Lake and the *S. soldatovi* is distributed from upper reaches (including Buir Nur) of the Amur River down to Ussuri and Khanka Lake (Kottelat, 2006; Bogutskaya *et al*., 2008). Antonov *et al*. (2019) also mentioned that it is quite difficult to accurately identify the species' range for the Amur River Basin because of the morphological similarities between these two species. According to the original description and subsequent studies (Berg, 1914; Bogutskaya *et al*. 2008; An *et al*., 2020), *S. czerskii* can be separated from *S. soldatovi* by the following combination of characters: head length larger than body depth (vs. about equal), mouth subterminal (vs. almost inferior), back and lateral side of breeding males goldish with black vertical stripes, (vs. black with bluish stains, throat and anterior part of belly crimson). In this study, we examined twenty-one specimens from the Heilongjiang (Amur) River and found that those diagnostic characters written above are not able to distinguish those two species. It is difficult to distinguish whether the mouth position was subterminal or almost interior, and specimens with the same mouth position show differences in head length, some with the head length larger than body depth and some with them equal. The genetic distances within groups of sequences from the Heilongjiang River are extremely small (0.0003%), supporting that specimens from Harbin, Nenjiang and Zhuaji are the same population (Figure 8, Table 1). In addition, there were no significant differences in morphology between specimens from the Heilongjiang River and *S. sciistius*. Sequences of *S. czerskii* and *S. soldatovi* are also nested within *S. sciistius* in the phylogenetic tree (Figure 8). The interspecific genetic distances between *S. sciistius*, *S. czerskii* and *S. soldatovi* are quite small (0.6%) and molecular species delineation methods (ASAP and PTP) suggested them to be one species. Therefore, we consider *S. czerskii* and *S. soldatovi* to be the synonyms of *S. sciistius*. *Sarcocheilichthys sciistius* now should be a widely distributed species ranging from the Heilongjiang (Amur) River Basin to Huaihe River Basin.

## Acknowledgments

This work was supported by National Science & Technology Fundamental Resources Investigation Program of China (Grant No. 2019FY101800), Sino BON - Inland Water Fish Diversity Observation Network, and Project of Biological Resources Survey in Wuyishan National Park. We appreciate Rob Robins and Sandra Raredon for providing images of the holotype.

## Compared materials

*Sarcocheilichthys caobangensis*
Data from An *et al*. (2020).

*Sarcocheilichthys davidi*
Data from Ding (1994) and An *et al*. (2020).

*Sarcocheilichthys hainanensis*
ASIZB 11394–11400, 49.6–65.5 mm SL, seven specimens, from Zhongshan County, Guangdong Province, January 1937.

*Sarcocheilichthys kiangsiensis*
ASIZB 64039–64043, 84.9–131.1 mm SL, five specimens, from Oujiang River, Zhejiang Province, 1972; ASIZB 73451–73453 101.0–135.7mm SL, from Zishui River, Hunan Province, 5 April 2002; ASIZB 73444–73446, 94.2–104.6 mm SL, from Hunan Province, 7 April 2002.

*Sarcocheilichthys lacustris*
ASIZB 131051, 61.3 mm SL, one specimen, from Lanxi County, Heilongjiang Province, 20 July 2005; ASIZB 34212, 92.1 mm SL, one specimen, from Jilin Province, 20 June 1950.

*Sarcocheilichthys nigripinnis*
ASIZB 221710–221717, 68.5–96.2 mm SL, eight specimens, from Taihu Lake, Huzhou City, Zhejiang Province, 31 October 2020; ASIZB 221718–221727, 54.4–62.9 mm SL, ten specimens, from Cao'ejiang River, Shengzhou City, Zhejiang Province, 1 January 2020. ASIZB 221793–221816, 49.3–75.1 mm SL, twenty-four specimens, from Xingjianghe River, Wuyuan County, Jiangxi Province, 11 April 2021.

*Sarcocheilichthys parvus*
ASIZB 221787–221788, 39.8–45.1 mm SL, two specimens, from Yanshanhe River, Yanshan County Shangrao City, Jiangxi Province, 11 April 2021.

*Sarcocheilichthys sinensis*
ASIZB 73435–73436, 106.9–140.3 mm SL, two specimens, from Zishui River, Hunan Province, 5 April 2002; ASIZB 39278, 83.2 mm SL, one specimen, from Yangxian County, Shaanxi Province.

*Sarcocheilichthys soldatovi*
Data from Kottelat (2006), Bogutskaya *et al*. 2008 and Antonov *et al*., 2019.

*Sarcocheilichthys vittatus*
ASIZB221817, 50.4 mm SL, one specimen, from Xingjianghe River, Wuyuan County, Shangrao City, Jiangxi Province, 11 April 2021. An *et al*. (2020).

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