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A new species of *Liobagrus* Hilgendorf, 1878 (Teleostei, Siluriformes, Amblycipitidae) from the lower Changjiang River basin in southeast China

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Abstract

A new catfish species, *Liobagrus chenhaojuni* Chen, Guo & Wu, **sp. nov.**, is described from the Tiaoxi River, a tributary of Taihu Lake, located in Zhejiang Province, China. This description is based on morphological characteristics and phylogenetic analysis. This species belongs to a group defined by the presence of a smooth posterior edge of the pectoral-fin spine and can be distinguished from other species in the group by a unique combination of characteristics, including: an upper jaw longer than the lower jaw; maxillary barbels reaching the middle of the pectoral fin; irregular blotches present on the lateral body; a rounded caudal-fin with a length ranging from 16.5% to 19.9% of the standard length; 39 to 41 post-Weberian vertebrae; and 15 to 17 anal-fin rays. The validity of this new species is further supported by the molecular phylogenetic analysis based on *Cytb* sequences.

Key Words

catfish, phylogeny, taxonomy, Zhejiang Province

Introduction

The genus *Liobagrus* comprises a group of small freshwater catfish endemic to East Asia. To date, 20 species have been described, with 12 of them found in mainland China: *Liobagrus marginatus* (Günther, 1892), *Liobagrus nigricauda* Regan, 1904, *Liobagrus styani* Regan, 1908, *Liobagrus anguillicauda* Nichols, 1926, *Liobagrus marginatoides* (Wu, 1930), *Liobagrus kingi* Tchang, 1935, *Liobagrus aequilabris* Wright & Ng, 2008, *Liobagrus chenghaiensis* Sun, Ren & Zhang, 2013; *Liobagrus huaiheensis* Chen, Wu & Wen, 2021; *Liobagrus pseudostyani* Chen & Guo, 2021; *Liobagrus brevispina* Xie, Cao & Zhang, 2022; and *Liobagrus chengduensis* Chen, Guo, Wu & Wen, 2022 (He 1999; Wright and Ng 2008; Sun et al. 2013; Chen and Guo 2021; Chen et al. 2021; Chen et al. 2022; Xie et al. 2022). Research on the taxonomy of Chinese *Liobagrus* has mainly focused on the upper and middle reaches of the Changjiang River (Yangtze) and the Huaihe River basin. However, the *Liobagrus* species in the lower Changjiang River have not been thoroughly surveyed or studied, potentially leaving other undescribed species undiscovered.

The Tiaoxi River is a small river situated in the western part of the Hangjiahu Plain in Zhejiang Province and is one of the main tributaries of Taihu Lake. Despite its small

watershed area, the Tiaoxi River boasts a diverse fish population, with a total of 84 recorded fish species (Li and Shimatani 2016; Zhang et al. 2022). Among these species, the presence of *L. styani* is highly questionable, as it is believed to be narrowly distributed in the Juanshui River basin in the middle Changjiang River (Wu et al. 2013). Moreover, the *L. styani* recorded in the basin is not only far from its type locality but also from the distribution regions of all other *Liobagrus* species in China. Based on collections made between 2022 and 2024, it has been discovered that *Liobagrus* specimens with a smooth posterior edge of the pectoral-fin spine, distributed in the Tiaoxi River, represent an undescribed species. Here, we introduce this species as new to science. The discovery of this new taxon contributes to our understanding of the high level of fish endemism in the lower Changjiang River basin.

Materials and methods

Specimens were manually collected from the Tiaoxi River between 2022 and 2024. Thirty-six type specimens were initially preserved in 10% formalin and subsequently transferred to 70% ethanol for long-term storage. Additionally, seven type specimens were preserved in 99% ethanol for molecular phylogenetic analyses. Vertebrae and fin rays were detected and counted using X-ray imaging. Measurements were obtained using digital calipers, with values recorded to the nearest 0.1mm.

Genomic DNA was extracted from the ventral fin of specimens preserved in 99% ethanol using the Baypure Magnetic Bead Method Animal Genomic DNA Extraction Kit (BayBio, Guangzhou, China). The quality and concentration of the DNA were checked using 1% agarose gel electrophoresis and NanoDrop 2000 (Thermo Scientific, USA). *Cytb* sequences were amplified using primers L14724 (GACTTGAAAAACCACCGTTG) and H15915 (CTCCGATCTCCGGATTACAAGAC). Polymerase chain reaction (PCR) amplifications of *Cytb* were performed in a final 25-μL volume mixture containing 1 μL of template DNA, 1 μL of each pair of primers, 12.5 μL of Green Taq Mix (Vazyme, China), and 9.5 μL ddH2O. Thermal cycling began with one cycle at 95 °C for 10 s, followed by 35 cycles of denaturation at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, with a final extension step at 72 °C for 10 min. PCR products were purified and sequenced using an ABI 3730XL analyzer by Sangon Biotech (China). Accession numbers of all newly obtained sequences are provided in Table 1. Sequences were aligned using MEGA v. 6.0 (Tamura et al. 2013) and manually checked. Genetic distance, based on the uncorrected p-distance model, was calculated using MEGA v. 6.0. Phylogenetic relationships were reconstructed using Bayesian inference (BI) and maximum likelihood (ML). *Xiurenbagrus xiurenensis* (Yue, 1981), *X. gigas* Zhao, Lan & Zhang, 2004, *Akysis brachybarbatus* Chen, 1981, *Ictalurus furcatus* (Valenciennes, 1840), and *Noturus taylori* Douglas, 1972, were used as the outgroup for rooting the tree. ML analyses were performed in IQ-TREE v. 1.6.12 (Minh et al. 2013) using the Ultrafast Fast Bootstrap approach (Minh et al. 2013) with 10,000 reiterations. The most appropriate model of sequence evolution (GTR+I+G) was selected using PartitonFinder2 v. 1.1 (Robert et al. 2017). Bayesian inference (BI) was conducted in MrBayes v. 3.2.6 (Ronquist et al. 2012). The most appropriate model of sequence evolution (GTR+I+G) was selected under ModelFinder (Subha et al. 2017). Four simultaneous runs with four independent Markov Chain Monte Carlo (MCMC) algorithms were executed for 10 million generations, and trees were sampled every 1000 generations with a burn-in of 25%. The convergence was verified with the average standard deviation of split frequencies of <0.01 and the potential scale reduction factor (PSRF) of ~1. Trees were visualized using FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). Institutional abbreviations used: NCU_XPWU Laboratory of Xiao-Ping Wu, Nanchang University (Nanchang, Jiangxi, China); IHB Museum of Aquatic Organisms, Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, Hubei, China).

Results

***Liobagrus chenhaojuni* Chen, Guo & Wu, sp. nov.**

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Figs 1, 2A–C, Table 2

Liobagrus styani Li & Shimatani, 2016: 165–167 (Tiaoxi River, Zhejiang, China).

Type material. ***Holotype.*** 24_NCU_XPWU_Y01, Siling Reservoir [四岭水库], Tiaoxi River [苕溪], Yuhang district [余杭区], Hangzhou City [杭州市], Zhejiang Province [浙江省], China, 30°25'42"N, 119°45'18"E, leg. Hao-Jun Chen, February 2024.

September 2022. ***Paratypes.*** 24_NCU_XPWU_Y02–16, n=15, other information same as holotype; 22_NCU_XPWU_Y01–17, n=17, leg. Zhong-Guang Chen & Hao-Jun Chen, September 2022, other information same as holotype; 22_NCU_XPWU_Y18–25, n=8, IHB-T-A0000007–8, n=2, Tiaoxi River [苕溪], Deqing County [德清县], Huzhou City [湖州市], Zhejiang Province [浙江省], China, leg. local people, September 2022.

Diagnosis. *Liobagrus chenhaojuni* sp. nov. is a member of the group defined by the presence of a smooth posterior edge of the pectoral-fin spine (i.e., *L. reinii*, *L. formosanus*, *L. styani*, *L. nantoensis*, *L. anguillicauda*, *L. marginatoides*, and *L. aequilabris*). It can be distinguished from all other species in this group by the following characteristics: the upper jaw is longer than the lower jaw (vs. equal in *L. aequilabris* and *L. formosanus*; shorter in *L. marginatoides*); the maxillary barbels reach the middle of the pectoral fin (vs. reach the pectoral-fin

Table 1. GenBank accession numbers of the sequences for this study.

Species	Access number	Locality	Reference
Liobagrus chenhaojuni sp. nov. 1	PP446311	Huzhou, Zhejiang, China	This study
Liobagrus chenhaojuni sp. nov. 2	PP446312	Huzhou, Zhejiang, China	This study
Liobagrus chenhaojuni sp. nov. 3	PP446313	Huzhou, Zhejiang, China	This study
Liobagrus chenhaojuni sp. nov. 4	PP446314	Hangzhou, Zhejiang, China	This study
Liobagrus chenhaojuni sp. nov. 5	PP446315	Hangzhou, Zhejiang, China	This study
Liobagrus chenhaojuni sp. nov. 6	PP446316	Hangzhou, Zhejiang, China	This study
L. styani 1	KY653576	Xianning, Hubei, China	NCBI
L. styani 2	KY653577	Xianning, Hubei, China	NCBI
L. aequilabris 1	KY653673	Unknown	NCBI
L. aequilabris 2	KY653674	Unknown	NCBI
L. anguillicauda 1	KY653651	Wuyishan, Fujian, China	NCBI
L. anguillicauda 2	KY653652	Wuyishan, Fujian, China	NCBI
L. marginatus 1	KY653578	Chongqing, China	NCBI
L. marginatus 2	KY653579	Chongqing, China	NCBI
L. kingi	KC193779	Unknown	NCBI
L. huaiheensis 1	ON638213	Xinyang, Henan, China	NCBI
L. huaiheensis 2	ON638214	Xinyang, Henan, China	NCBI
L. obesus	DQ321752	Korea	NCBI
L. andersoni	DQ321753	Korea	NCBI
L. pseudostyani 1	ON638209	Chengdu, Sichuan, China	NCBI
L. pseudostyani 2	ON638210	Chengdu, Sichuan, China	NCBI
L. brevispina 1	ON638211	Chengdu, Sichuan, China	NCBI
L. brevispina 2	ON638212	Chengdu, Sichuan, China	NCBI
L. chengduensis 1	ON638203	Chengdu, Sichuan, China	NCBI
L. chengduensis 2	ON638204	Chengdu, Sichuan, China	NCBI
L. mediadiposalis 1	KX265422	Korea	NCBI
L. mediadiposalis 2	KX265423	Korea	NCBI
L. hyeongsanensis	MZ066608	Korea	NCBI
L. geumgangensis 1	KX265431	Korea	NCBI
L. geumgangensis 2	KX265433	Korea	NCBI
L. somjinensis	MN756661	Korea	NCBI
L. reinii 1	LC333217	Japan	NCBI
L. reinii 2	LC333224	Japan	NCBI
Xiurenbagrus xiurenensis	DQ192464	Guangxi, China	NCBI
Xiurenbagrus gigas	EU490936	Guangxi, China	NCBI
Akysis brachybarbatus	AF499603	Yunnan, China	NCBI
Ictalurus furcatus	KM576102	Unknown	NCBI
Noturus taylori	KP013089	Unknown	NCBI

insertion in *L. styani*, *L. reinii*, and *L. nantoensis*); presence of irregular blotches on the lateral body (vs. absence in *L. formosanus*, *L. nantoensis*, *L. anguillicauda*, *L. marginatoides*, and *L. aequilabris*); the caudal fin is rounded (vs. sub-truncate in *L. marginatoides*); the caudal fin length ranges from 16.5% to 19.9% standard length (vs. 13.1–16.2 in *L. styani*, 20.3–27.0 in *L. anguillicauda* and 20.1–26.9 in *L. aequilabris*); it possesses 39–41 post-Weberian vertebrae (vs. 35–37 in *L. aequilabris*), the anal-fin rays range from 15 to 17 (vs. 12 in *L. nantoensis*) (Table 3).

Description. Morphometric data for type specimens are shown in Table 2. Body elongated, anteriorly depressed (wider than deep), and posteriorly evenly compressed to the tail. Lateral line short, with 6–9 pores. Head depressed and broad when viewed dorsally, with a broadly rounded snout in dorsal view. Anterior nostril tubular, with a rim bearing a fleshy flap forming a short tube; posterior nostril pore-like, with the rim posteriorly confluent with the base of the nasal barbel. Eyes small, dorsolateral, and subcutaneous. Mouth terminal, with the upper jaw noticeably longer than the lower jaw, lips thickened. Premaxillary and mandibular tooth pads curved, bearing small and setiform teeth; palatine teeth absent. Four pairs of barbels: the maxillary barbel long, extending to the pectoral-fin insertion; nasal barbel short, not reaching the gill-membrane margin; inner mandibular barbel approximately half the length of the outer mandibular barbel and does not extend to the pectoral-fin insertion; outer mental barbel longest, reaching the middle of the pectoral fin.

Dorsal fin II, 5–6 rays, with a convex distal margin; tip of adpressed fins does not reach the pelvic-fin insertion. Dorsal-fin spine covered by thick, straight skin with smooth anterior and posterior margins, slightly shorter than the pectoral-fin spine. Adipose fin high,

Figure 1. *Liobagrus chenhaojuni* sp. nov. **A–C.** Dorsal, lateral, and ventral view of holotype (24_NCU_XPWU_Y01); **D.** Dorsal view of pectoral-fin spine of paratype (22_NCU_XPWU_Y31). Arrows show the anus.

with its base longer than the anal-fin base, confluent with the caudal fin without a marked incision at the confluence. Pectoral fin I, 7–8 rays, with its origin at the vertical through the edge of the operculum, partially covered by the opercular membrane. Pectoral-fin spine long and sharp, with smooth anterior and posterior margins (Fig. 1D), reaching the dorsal-fin insertion. Pelvic fin i, 5–6 rays, short, with the adpressed tip not reaching the anal-fin origin. Anal fin 15–17 rays with a rounded distal margin, and its tip approaches the origin of the ventral procurrent caudal-fin rays, longer than the dorsal-fin base but shorter than the adipose-fin base, with a convex distal edge. Anus closer to the pelvic-fin insertion than to the anal-fin origin. Caudal fin rounded, with 43–50 rays. Vertebral column consists of 39–41 post-Weberian elements.

Body generally dark brown to brownish red, adorned with irregular yellowish blotches that fade to light yellow ventrally. All barbels grayish white to light yellow, while dorsal fins dark brown, and adipose and caudal fins grayish white to light brown. All fins exhibit narrow, grayish white to light yellowish distal margins (Fig. 2A, B).

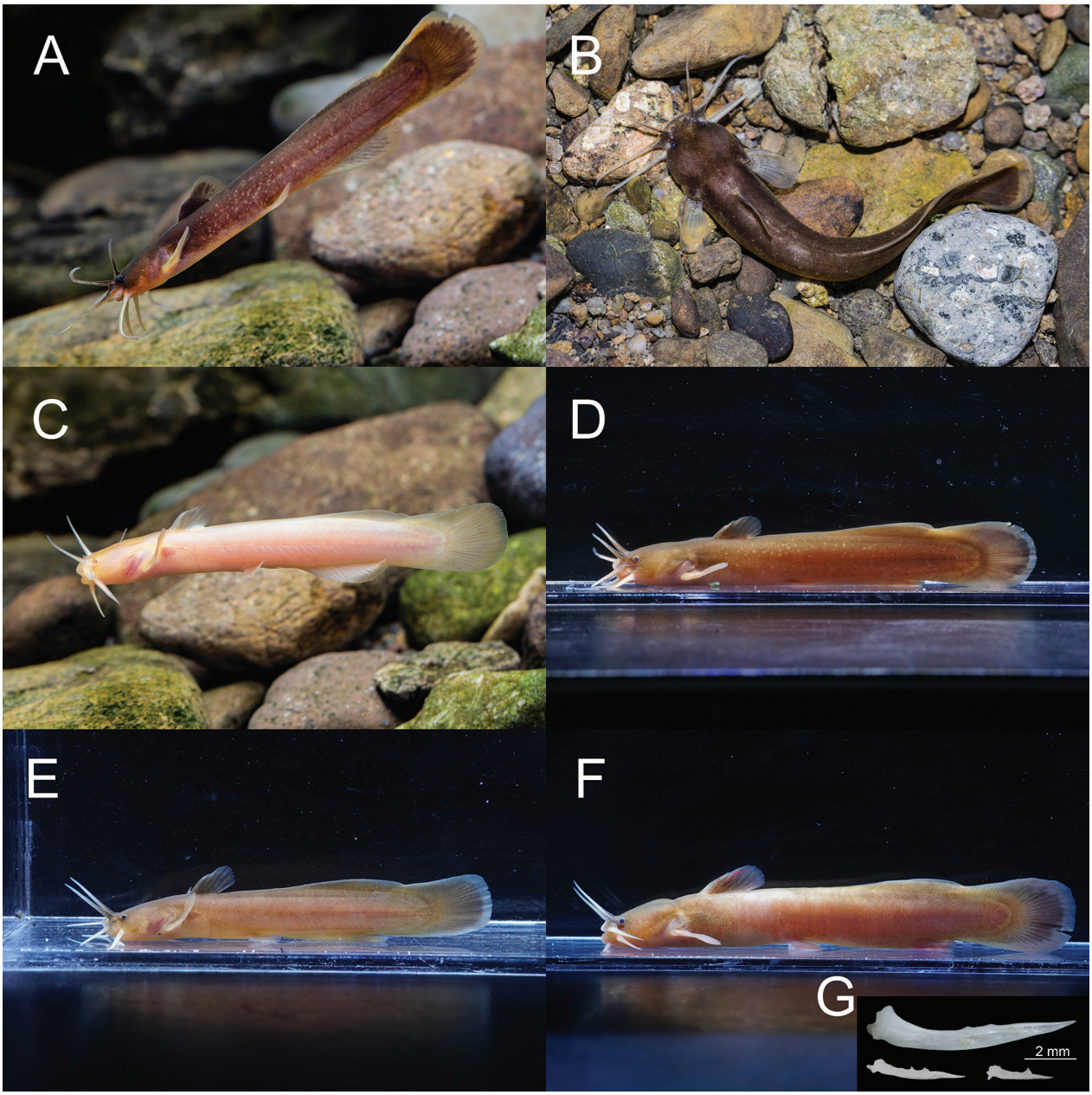

Figure 2. Living specimens of *Liobagrus chenhaojuni* sp. nov. and its similar congeneric species. **A, B.** *Liobagrus chenhaojuni* sp. nov.; **C.** *Liobagrus chenhaojuni* sp. nov. albino individual; **D.** *L. styani*; **E.** *L. anguillicauda*; **F.** *L. brevispina*; **G.** dorsal view of pectoral-fin spine of *L. brevispina*.

An albino individual was found, exhibiting a generally pink body without irregular yellowish blotches (Fig. 2C).

Etymology. This species is named after Mr. Hao-Jun Chen, who assisted in the field survey.

Vernacular name. 浙江鮡 (Pinyin: zhe jiang yang).

Distribution and ecology. *Liobagrus chenhaojuni* sp. nov. is exclusively found within the Tiaoxi River basin (Fig. 3). Within this habitat, it typically resides at the bottom of the stream with medium pebbly substrates, together with *Rhinogobius leavelli* (Herre, 1935), *Microphysogobio bicolor* (Nichols, 1930), *Vanmanenia stenosoma* (Boulenger, 1901), *Acrossocheilus fasciatus* (Steindachner, 1892), *Opsariichthys bidens* Günther, 1873, and *Zacco tiaoxiensis* Zhang, Zhou & Yang, 2022 (Fig. 4).

Molecular analyses. A dataset consisting of 33 *Cytb* sequences and five outgroup taxa was employed for phylogenetic analyses (Table 1). The alignment of *Cytb* exhibited a length of 1116 characters, with 343 variable sites and 313 sufficiently informative sites. Phylogenetic analyses generated ML and BI trees with largely congruent topologies (Fig. 5). Notably, species from China with a smooth posterior edge of the pectoral-fin spine formed a monophyletic group, while those with a serrated posterior edge of the pectoral-fin spine constituted a paraphyletic group. The validity of *Liobagrus chenhaojuni* sp. nov. is supported by the molecular-phylogenetic result. It belongs to the group with a smooth posterior edge of the pectoral-fin spine, and the phylogenetic relationship with-

Table 2. Morphometric data for type specimens of *Liobagrus chenhaojuni* sp. nov.

Morphometrics	Holotype	Paratypes
Standard length (mm)	70.2	38.8–79.3
% of standard length		
Head length	22.4	20.6–23.0
Body depth	18.8	13.6–18.4
Dorsa-fin base length	10.0	9.1–10.0
Anal-fin base length	16.5	12.3–19.7
Adipose-fin base length	28.8	27.7–37.2
Caudal peduncle length	20.4	19.3–23.1
Caudal peduncle depth	17.2	14.0–17.7
Dorsal-fin spine length	7.5	6.3–7.4
Pectoral-fin spine length	9.3	7.2–9.6
Caudal-fin length	18.4	16.5–19.9
Anus to pelvic-fin insertion	3.6	3.4–6.4
Anus to anal-fin origin	4.3	4.9–7.5
Predorsal length	28.8	27.0–30.5
Prepectoral length	18.4	17.7–21.4
Prepelvic length	46.0	42.9–45.5
Preanal length	58.4	57.3–60.1
Dorsal to adipose-fin origin	29.3	25.3–31.8
% of head length		
Head width	89.2	83.0–93.3
Head depth	61.8	50.0–59.4
Snout length	31.8	26.3–32.0
Mouth width	69.4	68.0–76.1
Interorbital width	35.7	33.1–40.0
Nasal barbel length	73.2	67.6–73.9
Maxillary barbel length	94.9	81.4–95.4
Inner mandibular barbel length	61.8	50.0–62.1
Outer mandibular barbel length	99.4	93.0–98.7
Width between anterior nares	12.7	12.5–16.1
Width between postoral nares	28.7	28.0–36.6

in the group is represented as *Liobagrus chenhaojuni* sp. nov. + (*L. anguillicauda* + (*L. aequilabris* + *L. styani*)). The genetic distances between *Liobagrus chenhaojuni* sp. nov. and other congeneric species ranged from 5.8% to 14.2% (Suppl. material 1).

Discussion

The placement of the new species within *Liobagrus* is supported by both its morphology and phylogeny. Species of *Liobagrus* can be divided into two groups based on the possession of a smooth or serrated posterior edge of the pectoral-fin spine. Xie et al. (2022) described *L. brevispina* as having a smooth posterior edge of the pectoral-fin spine. However, based on the examination of specimens, *L. brevispina* was found to have a serrated posterior edge of the pectoral-fin spine (Fig. 2G). The serrations on its pectoral-fin spine weaken but do not disappear as it grows. Currently, the group defined by the presence of a smooth posterior edge of the pectoral-fin spine comprises only seven species: *L. reinii*, *L. formosanus*, *L. styani*, *L. nantoensis*, *L. anguillicauda*, *L. marginatoides*, and *L. aequilabris*. In this group, *Liobagrus chenhaojuni* sp. nov. can be easily distinguished from *L. aequilabris*, *L. formosanus*, and *L. marginatoides* by having the upper jaw longer than the lower (vs. shorter or equal). *Liobagrus chenhaojuni* sp. nov. is similar to *L. reinii*, *L. styani*, *L. nantoensis*, *and L. anguillicauda* by the similar upper and lower jaw positions, but differs based on the maxillary barbels reaching the middle of the pectoral fin (vs. reaching the

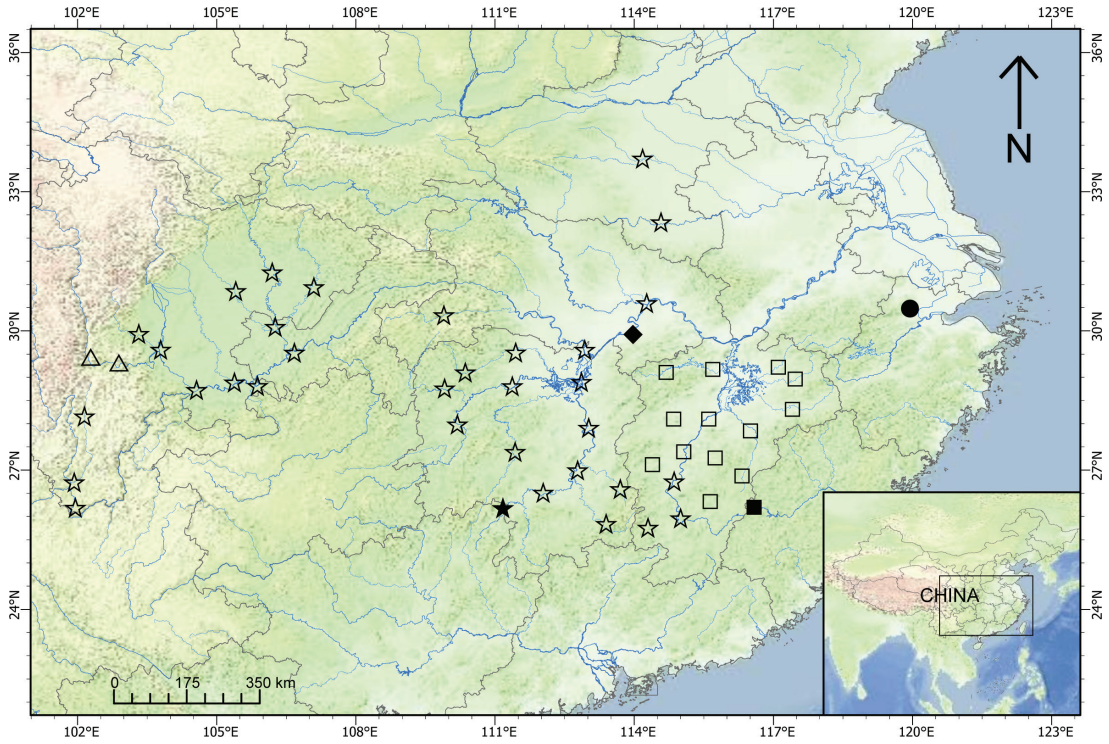

Figure 3. Distribution of *Liobagrus* with a smooth posterior edge of the pectoral-fin spine in Mainland China. **Dot.** *Liobagrus chenhaojuni* sp. nov.; **square.** *L. anguillicauda*; **rhombus.** *L. styani*; **star.** *L. aequilabris*; **triangle.** *L. marginatoides*. Solid show the type localities.

Figure 4. Sampling locality of *Liobagrus chenhaojuni* sp. nov. Siling Reservoir, Tiaoxi River, Yuhang district, Hangzhou City, Zhejiang Province, China.

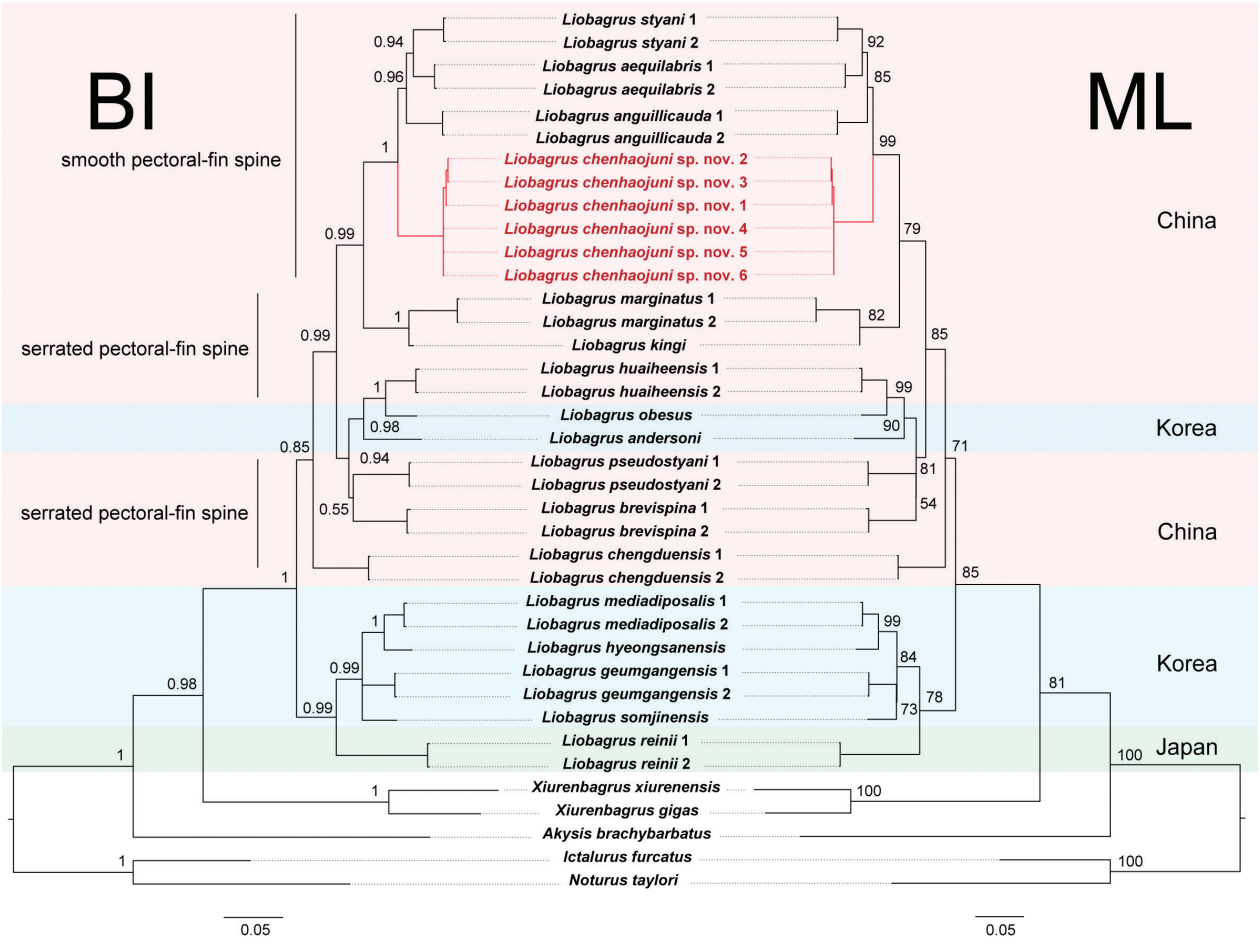

Figure 5. Bayesian inference tree and maximum likelihood tree inferred from *Cytb* gene sequences. Posterior probabilities/bootstrap supports are shown on the left/right of nodes.

pectoral-fin insertion in *L. reinii*, *L. styani*, and *L. nantoensis*), presence of irregular blotches on the body lateral (vs. absence in *L. nantoensis* and *L. anguillicauda*), caudal-fin length is 16.5–19.9% of standard length (vs. 13.1–16.2% in *L. styani* and 20.3–27.0% in *L. anguillicauda*), 15–17 anal-fin rays (vs. 12 in *L. nantoensis*). Furthermore, *Liobagrus chenhaojuni* sp. nov. has a distinctive distribution, being exclusively found in the Tiaoxi River within the lower Changjiang River basin, far from any other congeners.

The discovery of *Liobagrus chenhaojuni* sp. nov., as well as *Zacco tiaoxiensis* in the Tiaoxi River, shows

Table 3. Comparisons of major diagnostic characters of *Liobagrus* with a smooth posterior edge of the pectoral-fin spine.

Characters	*Liobagrus chenhaojuni* sp. nov.	*L. nantoensis*[a]	*L. reinii*[a]	*L. styani*[b]
Upper/lower jaw in length	>1	>1	>1	>1
post-Weberian vertebrae	39–41	Unknown	Unknown	39–41
Anal-fin rays	15–17	12	17	17–19
Caudal-fin length as % of standard length	16.5–19.9	Unknown	Unknown	13.11–16.2
Caudal-fin shape	Rounded	Rounded	Rounded	Rounded
Maxillary barbels	Reaching middle of pectoral fin	Reaching pectoral-fin insertion	Reaching pectoral-fin insertion	Reaching pectoral-fin insertion
Irregular blotches on body lateral	Present	Absent	Present	Present
Characters	*L. anguillicauda*[b]	*L. aequilabris*[a]	*L. formosanus*[b]	*L. marginatoides*[c]
Upper/lower jaw in length	>1	=1	=1	<1
Post-Weberian vertebrae	38–40	35–37	38–39	Unknown
Anal-fin rays	15–17	15–17	15	13–15
Caudal-fin length as % of standard length	20.3–27.0	20.1–26.9	17.7–20.0	18.1–20.1
Caudal-fin shape	Rounded	Rounded	Rounded	Sub-truncate
Maxillary barbels	Reaching middle of pectoral fin	Reaching pectoral-fin insertion	Reaching pectoral-fin insertion	Reaching middle of pectoral fin
Irregular blotches on body lateral	Absent	Absent	Absent	Absent

Notes: data from a, Xie et al. 2022; b, Wu et al. 2016; c, Chen and Guo 2021.

that fish diversity in this area has been underestimated. There is a need for systematic fish surveys in the basin to uncover cryptic species diversity and generate data essential for conservation efforts. Unlike most congeners distributed in streams within sparsely populated regions, the new species was found in the Yangtze River Delta region, which is the economic hub of China. Given its distribution, the new species is exposed to greater risks from human activities compared to other congeners. Therefore, it is imperative to closely monitor and protect it to ensure its survival.

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Supplementary material 1

Genetic distances of Cytb sequences computed by MEGA 6 of *Liobagrus*

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