### **PENSOFT**.



## *Gobiobotia lii*, a new species of gudgeon (Teleostei, Gobionidae) from the middle Chang-Jiang Basin, central China, with notes on the validity of *G. nicholsi* Bănărescu & Nalbant, 1966

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### Abstract

*Gobiobotia lii* is described from the Qi-Shui, a stream tributary on the northern bank of the middle Chang-Jiang mainstem in Hubei Province and Lake Dongting in Hunan Province, central China. The new species is distinguished from all other congeneric species by possessing a combination of the following characters: a naked region of the abdomen adjacent to the ventral mid-line extending to the vent and the vertebral count (4+31-32). The validity of *G. lii* is confirmed by its monophyletic nature recovered in a phylogenetic analysis, based on the *cyt b* gene and its significant sequence divergence with sampled congeneric species. Critical notes were given on the species recognition of historically documented eight-barbel gudgeons co-existing in Lake Dongting. *Gobiobotia nicholsi* Bănărescu & Nalbant, 1966 should be a valid species distinct from *G. filifer* (Garman, 1912) and both *G. pappenheimi* Kreyenberg, 1911 and *G. boulengeri* (=*Xenophysogobio boulengeri* (Tchang, 1929)) have an erroneous record from the Lake.

## Key Words

cyt b gene, new taxon, morphology, species identification, taxonomy

## Introduction

The gudgeon genus *Gobiobotia* Kreyenberg, 1911 (type species *G. pappenheimi*) comprises about 19 species (Fricke et al. 2021), characterised by the combination of four pairs of barbels; a gas bladder with a transversely widened or oval encapsulated anterior chamber and a minute free posterior chamber, but without pneumatic duct; and large scales, with five to six scale rows above the lateral line (He and Chen 1998). They are typically small-sized bottom dwellers hitherto recorded from the major river basins of the Korean Peninsula, Taiwan and Hainan Island, continental China and northern Vietnam (He and Chen 1998; Kottelat 2001a; Kim and Park 2002). The taxonomic history of *Gobiobotia* has been uneven, with the enigmatic genus often placed in a separate subfamily or family of its own (Kottelat 2001a). Species of

this genus and *Xenophysogobio* Chen & Cao, 1977, an endemic Chinese genus currently known from the upper Yangtze River (= Chang-Jiang in Chinese) Basin, were referred to the subfamily Gobiobotinae of the Cyprinidae (He and Chen 1998). All eight-barbel gudgeons were recently referred to the family Gobionidae (Tan and Armbruster 2018). Nonetheless, the monophyletic nature of the either Gobionidae or *Gobiobotia* has been confirmed in many molecular phylogenetic studies of the order Cypriniformes (Yang et al. 2006; Tang et al. 2012; Zhao et al. 2016; Li et al. 2018).

Gobiobotia, especially from China, has a taxonomic inertia despite He and Chen's (1998) taxonomic revision of the genus. For example, the eight-barbel gudgeons from the mid-lower Chang-Jiang Basin are traditionally classified as four species: *G. brevirostris* Chen & Cao, 1977, *G. filifer* (Garman, 1912), *G. meridionalis* Chen & Cao, 1977 and *G. tungi* Fang, 1933 (Zhang and Zhao 2016). However, *G. jiangxiensis*, originally described by Zhang and Liu (1995) from the Poyang Lake Basin, was not included in *Gobiobotia* by He and Chen (1998). *Gobiobotia nicholsi*, initially described by Bănărescu and Nalbant (1966) from Lake Dongting, was regarded as a synonym of *G. ichangensis* Fang, 1930 (Chen and Cao 1977), a species subsequently synonymised with *G. filifer* (Ding 1994). Although this synonym is widely accepted by succeeding Chinese workers, the validity of *G. nicholsi* requires re-evaluation, based on examination on its type specimens.

Fish surveys were conducted during 2011–2015 and 2017–2018 in Lake Dongting in Hunan Province and during 2021 in some tributaries on the northern bank of the middle Chang-Jiang in Hubei Province, central China. These surveys yielded eighty-five specimens referred to as *Gobiobotia*, conforming to *G. filifer* and *G. meridionalis*, as well as a morphologically and genetically distinct subsample which represents an undescribed species. The present study aims to provide a description of this unnamed species. Some notes on the validity of *G. nicholsi* and the identification of other historically documented co-existing eight-barbel gudgeons in Lake Dongting are also provided.

### Material and methods

### Specimen sampling and preservation.

Specimens utilised for this study were sampled in accordance with the Chinese Laboratory Animal Welfare and Ethics animal welfare laws (GB/T 35892–2018). After being anaesthetised, all captured individuals were fixed by immersion in ethanol or formalin. Specimens were collected using gill nets, trap nets and electrofishing. Caught specimens of *Gobiobotia* were stored in 10% formalin for morphological examination or 95% ethyl alcohol for DNA extraction. All sequences amplified in this study were submitted to GenBank. Their voucher specimens are deposited in the collection of the Museum of Aquatic Organisms at the Institute of Hydrobiology (**IHB**), Chinese Academy of Sciences (**CAS**).

### Morphological analysis

Measurements were taken point to point with a digital caliper connected directly to a data-recording computer and data recorded to the nearest 0.1 mm. Measurements were taken on the left side of specimens whenever possible, following methods used by Kottelat (2001b) and Song et al. (2018). The head length and measurements of other parts of the body are given as percentages of standard length (**SL**). Measurements of parts of the head are given as proportions of the head length (**HL**) (Tables 1, 2). The counts of vertebrae were taken from radiographs of Micro-CT or X-rays. The specimens examined in this study are deposited in the collections of:

AMNH	American Museum of Natural History, New
	York;
BMNH	Natural History Museum, London;
IHB	Institute of Hydrobiology, Wuhan;
MNHN	Muséum National d'Histoire Naturelle, Paris;
ZMB	Museum für Naturkunde-Leibniz Institute for
	Evolution and Biodiversity Science, Berlin.

#### DNA extraction, amplification and sequencing

Genomic DNA was extracted from fin clips stored in ethanol using the TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing) with the recommended protocol.. The cyt b gene was amplified by using primers L14724 (GACTTGAAAAACCACCGTTG) and H15915 (CTC-CGATCTCCGGATTACAAGAC) adopted from Xiao et al. (2001). The mitochondrial gene was performed in 25 µl volumes with 12.5 µl Master mix Taq (Beijing TsingKe Biotech Co., Ltd.), 1 µl of each primer, 1 µl template DNA, adding double distilled water (dd H<sub>2</sub>O) to supply the volume. The thermocycling conditions were as follows: initial denaturation for 4 min at 94 °C, denaturation for 50 s at 94 °C, annealing for 50 s at 55 °C and extension for 1 min, in terms of the product length at 72 °C. After 34-35 cycles, the final extension was done at 72 °C for 10 min; the product was then stored at 4 °C. The sequencing was finished by Tianyihuiyuan Biotechnology Company.

### Phylogenetic analyses

The *cyt b* gene of around 1100 bp base was chosen for phylogenetic analysis. The amplified 48 gene sequences were utilised for molecular phylogenetic analysis along with another 11 GenBank-retrieved sequences of the same gene from six congeneric species and two outgroups (*Microphysogobio xianyouensis* and *M. fukiensis*). Detailed information about samples, used here for molecular analysis, is given in Table 3. The sequences were aligned using MAFFT 7.0 (Katoh and Standley 2013) and ends trimmed. The genetic distance was calculated with MEGA 7.0, based on the uncorrected p-distance model (Kumar et al. 2016).

PhyloSuite (Zhang et al. 2020) was used for phylogenetic analyses. The selection of the best-fit model of nucleotide evolution, based on Akaike's Information Criterion was performed in ModelFinder (Kalyaanamoorthy et al. 2017). MrBayes 3.2.6 (Ronquist et al. 2012) was utilised for Bayesian analysis with the selected model: GTR+I+G+F, applying the optimal nucleotide evolution model and the MCMC method with four chains (three hot chains and one cold chain) running simultaneously for 20,000,000 generations to calculate posterior probability. Trees were sampled for every 1000 cycles. The initial 25% of sampled data were discarded as burn-in. Sufficient mixing of the chains was considered to be reached when the average standard deviation of split frequencies was below 0.01.

**Table 1.** Morphometry of *Gobiobotia lii*. Measurements are in percentage of standard length, except for standard length (SL). SD, standard deviation; r, Pearson's correlation coefficient; p, significance; linear regression parameters calculated from measurements. HT = Holotype.

Measurements	Ν	HT	Min	Max	Mean	SD	slope (b)	intercept (a)	r (SL)	р
SL (mm)	14	48.6	42.1	51.2	46.8	2.7				
Body depth	14	22.6	15.6	22.3	19.6	2	0.5461	5.7924	0.701	0.005
Head length	14	25.1	22.5	25.1	23.5	0.8	0.0124	23.043	0.037	0.9
Pre-dorsal length	14	49.5	44.7	51.2	47.4	1.7	-0.029	48.906	-0.04	0.883
Pre-pectoral length	14	21.3	20.4	23.2	21.9	0.9	-0.154	29.126	-0.47	0.091
Pre-pelvic length	14	48.7	44.9	52.9	48.1	1.9	-0.1722	56.232	-0.25	0.398
Pre-anal length	14	74.8	70.4	78.4	74	2.4	-0.0537	76.607	-0.06	0.837
Dorsal length	14	25.2	21.1	26	24	1.6	0.3178	9.2449	0.541	0.046
Pectoral length	14	22.8	18.1	23.4	21.5	1.6	-0.1957	30.756	-0.34	0.24
Pelvic length	14	19.6	15.9	19.6	18	1.2	-0.1486	24.994	-0.33	0.257
Anal length	14	20.1	16.2	21.4	18.4	1.4	-0.0407	20.437	-0.08	0.795
Pectoral-pelvic distance	14	29.2	26	30.3	28.4	1.3	-0.0534	30.95	-0.11	0.705
Pelvic-anal distance	14	27.2	24.9	30	27.5	1.5	-0.2996	41.551	-0.54	0.046
Pelvic-anus distance	14	13.2	9.6	13.1	11.6	1.1	-0.0514	14.082	-0.12	0.682
Anus-anal distance	14	16.4	12.7	18.1	15.4	1.7	0.1019	10.664	0.164	0.575
Head depth	14	61.6	52	69.1	61.5	4.9	0.7576	39.821	0.54	0.046
Head width	14	73.1	65.4	80.8	75.4	3.8	-0.066	64.639	-0.04	0.901
Snout length	14	40.1	38.9	43.2	41.8	1.1	0.007	41.347	0.017	0.955
Interorbital width	14	20.8	21.1	28.6	24.6	2	0.0054	24.079	0.007	0.982
Eye diameter	14	19.1	20	25.8	22.5	1.7	-0.2117	32.172	-0.3	0.29
Maxillary barbel length	14	28.7	23.9	67.9	33	10.5	0.6906	0.2997	0.18	0.539
Post-orbital length	14	33.8	31.5	46.6	39.5	5	-0.355	55.727	-0.19	0.526

Table 2. Morphometric measurements for four species of Gobiobotia: G. jiangxiensis, G. pappenheimi, G. meridionalis and G. filifer.

Character	G. jiangxiensis (n = 6)		G. pappenho	eimi (n = 14)	G. meridion	alis (n = 19)	G. filifer (n = 21)		
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	
SL (mm)	35.2-52.5	40.2±5.8	27.9-56.1	43.7±8.7	54.0-99.5	80.4±10.8	52.2-96.5	75.5±11.7	
Morphometric data									
% of SL									
Body depth	12.2-22.5	17.1±3.3	27.9–56.1	43.7±8.7	14.9–20.8	17.9±1.5	15.4–22.5	18.2±1.9	
Head length	22.0–25.9	24.4±1.3	12.5–14.5	13.6±0.7	24.4-28.1	26.7±1.0	17.9–28.2	25.7±2.1	
Pre-dorsal length	46.8–50.5	48.6±1.6	46.2–51.8	49.2±1.6	43.8–51.9	46.5±2.0	41.2-52.7	46.8±2.4	
Pre-pectoral length	19.8–24.1	22.9±1.6	18.8–28.1	23.6±2.8	24.6-27.3	26.0±0.9	23.2–28.5	25.6±1.3	
Pre-pelvic length	44.6-50.8	48.3±2.3	44.7–50.7	47.7±1.9	45.0-52.4	48.3±1.7	43.5-51.1	47.8±2.0	
Pre-anal length	71.0–78.1	74.7±2.2	69.4–79.0	75.2±2.4	72.7–77.5	75.0±1.4	69.0–77.3	73.3±2.1	
Dorsal length	27.1-31.2	28.8±1.6	22.8-28.1	24.7±1.5	23.4–27.5	25.6±1.1	19.8–25.6	23.1±1.6	
Pectoral length	25.1-28.2	26.5±1.0	17.0-22.5	20.1±1.6	21.9–29.1	25.0±1.5	22.9–31.8	26.1±2.1	
Pelvic length	18.1–23.4	20.6±1.7	16.0-21.4	19.2±1.4	18.0-22.1	20.5±1.2	15.2-20.0	17.8±1.2	
Anal length	18.1–21.3	19.1±1.0	16.0-21.4	19.2±1.4	17.5–20.9	19.0±0.9	15.5–19.2	17.0±0.9	
Pectoral-pelvic distance	26.3–28.7	27.3±0.8	22.9–27.1	25.1±1.4	21.0-28.1	24.2±1.7	21.9–26.0	24.1±1.1	
Pelvic-anal distance	24.4-26.1	25.5±0.6	25.7–31.9	28.1±1.9	26.9-31.1	28.5±1.2	23.9–29.9	26.2±1.6	
Pelvic-anus distance	11.7–14.9	12.9±1.1	8.2-15.4	11.0±1.7	9.0–14.5	11.8±1.2	7.6–13.1	10.8±1.3	
Anus-anal distance	11.3–14.3	12.7±0.9	14.0-18.1	16.0±1.2	14.2–19.6	16.8±1.4	14.4–19.9	16.4±1.6	
% of HL									
Head depth	54.4–60.3	57.1±1.9	47.5–59.0	53.6±3.7	51.3–59.0	55.4±2.2	47.1–76.1	$53.9 \pm 6.1$	
Head width	60.9-82.1	70.4±6.2	51.3-73.2	67.4±5.3	55.4–70.9	63.0±4.0	49.8–79.4	59.4±7.2	
Snout length	40.5-46.4	42.6±2.0	33.7–41.8	38.0±2.0	41.6-62.7	46.0±4.8	34.7–55.1	40.2±4.2	
Interorbital width	21.5-29.3	24.8±2.6	17.0-29.1	21.8±3.6	16.6–29.1	21.0±3.8	24.9-40.7	28.8±3.2	
Eye diameter	19.3–25.2	22.5±2.0	16.1-27.4	21.3±3.7	22.1–35.8	26.0±3.7	12.1–23.2	16.1±2.6	
Maxillary barbel length	15.4–19.0	16.9±1.3	20.1-45.4	31.6±6.9	20.3–39.5	35.2±2.7	25.6-37.4	31.1±3.8	
Post-orbital length	35.7–38.2	37.1±1.0	33.5–44.4	39.1±2.9	32.1-50.4	37.2±4.7	42.0-60.5	46.7±3.7	
Meristic counts									
Lateral-line scales	38–39	38.4±0.5	37–39	38.1±0.7	41–43	41.8±0.6	40-42	40.8±0.7	
Scale rows above	5–6	5.5±0.5	5–6	$5.5 \pm 0.5$	5–6	5.3±0.4	5–6	5.6±0.5	
lateral line									
Pre-dorsal scales	12	12	13	13	12–13	12.6±0.5	12–13	12.8±0.4	
Circumpeduncular scales	12	12	12-13	12.4±0.5	12	12	12	12	

Table 3. Detailed information on specimens used in this study. The species with \* means the samples available in GenBank.

Species	Specimen voucher	Sampling location	GenBank no.	
G. lii	IHB201711015435	Yuanjiang, Hunan Prov. (Lake Dongting, Chang-Jiang)	OM275368	
G. lii	IHB2017101929	Yuanjiang, Hunan Prov. (Lake Dongting, Chang-Jiang)	OM275369	
G. lii	IHB202103050300	Yuanjiang, Hunan Prov. (Lake Dongting, Chang-Jiang)	OM275370	
G. lii	IHB202103050303	Yuanjiang, Hunan Prov. (Lake Dongting, Chang-Jiang)	OM275371	
G. lii	IHB201801016013	Yuanjiang, Hunan Prov. (Lake Dongting, Chang-Jiang)	OM275372	
G. lii	IHB2017097256	Qichun, Hubei Prov. (Qi-Shui, Chang-Jiang)	OM275373	
G. lii	IHB2017097257	Qichun, Hubei Prov. (Qi-Shui, Chang-Jiang)	OM275374	
G. lii	IHB2017097258	Qichun, Hubei Prov. (Qi-Shui, Chang-Jiang)	OM275375	
G. lii	IHB202103050858	Qichun, Hubei Prov. (Qi-Shui, Chang-Jiang)	OM275376	
G. lii	IHB202103050859	Qichun, Hubei Prov. (Qi-Shui, Chang-Jiang)	OM275377	
G. filifer	IHB201711015758	Maocaojie, Hunan Prov. (Lake Dongting, Chang-Jiang)	OM275378	
G. guilingensis	IHB20161062A	Rongshui, Guangxi Prov. (Zhu-Jiang)	OM275379	
G. tungi	IHB202108056037	Shangrao, Jiangxi Prov. (Chang-Jiang)	OM275380	
G. tungi	IHB202108056038	Shangrao, Jiangxi Prov. (Chang-Jiang)	OM275381	
G. meridionalis	IHB2017040446	Hengdong, Hunan Prov. (Xiang-Jiang, Chang-Jiang)	OM275382	
G. meridionalis	IHB2017040447	Hengdong, Hunan Prov. (Xiang-Jiang, Chang-Jiang)	OM275383	
G. meridionalis	IHB2016106403	Chenxi, Hunan Prov. (Yuan-Jiang, Chang-Jiang)	OM275384	
G. meridionalis	IHB2016106404	Chenxi, Hunan Prov. (Yuan-Jiang, Chang-Jiang)	OM275385	
G. meridionalis	IHB2017104358	Kaili, Guizhou Prov. (Yuan-Jiang, Chang-Jiang)	OM275386	
G. meridionalis	IHB2017104362	Kaili, Guizhou Prov. (Yuan-Jiang, Chang-Jiang)	OM275387	
G. meridionalis	IHB2017040817	Nanxiong, Guangdong Prov. (Zhu-Jiang)	OM275388	
G. meridionalis	IHB2017040832	Shixing, Guangdong Prov. (Zhu-Jiang)	OM275389	
G. meridionalis	IHB2017013891	Suichuan, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275390	
G. meridionalis	IHB2017013893	Suichuan, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275391	
G. meridionalis	IHB201804014151	Fuzhou, Jiangxi Prov. (Fu-He, Chang-Jiang)	OM275392	
G. meridionalis	IHB201804014152	Fuzhou, Jiangxi Prov. (Fu-He, Chang-Jiang)	OM275393	
G. meridionalis	IHB201707014638	Ningdu, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275394	
G. meridionalis	IHB201707012675	Ningdu, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275395	
G. meridionalis	IHB201707018995	Taihe, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275396	
G. meridionalis	IHB2017075488	Longnan, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275397	
G. meridionalis	IHB201707017921	Shicheng, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275398	
G. meridionalis	IHB201707017918	Shicheng, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275399	
G. meridionalis	IHB201707013207	Huichang, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275400	
G. meridionalis	IHB201707012757	Pingshan, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275401	
G. meridionalis	IHB2016104697	Ji'an, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275402	
G. meridionalis	IHB201707018980	Taihe, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275403	
G. meridionalis	IHB201707018994	Taihe, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275404	
G. pappenheimi*	Uncatalogued	Tieling, Liaoning Prov. (Liao-He)	NC032293.1	
G. naktongensis*	Uncatalogued	Korean Peninsula	JX104484.1	
G. naktongensis*	Uncatalogued	Korean Peninsula	JX104485.1	
G. macrocephala*	Uncatalogued	Korean Peninsula	JX046845.1	
G. macrocephala*	Uncatalogued	Korean Peninsula	JX046846.1	
G. brevibarba*	Uncatalogued	Korean Peninsula	JX040840.1 JX094047.1	
G. brevibarba*	Uncatalogued	Korean Peninsula	JX094047.1	
Xenophysogobio boulengeri*	Uncatalogued	Upper Chang-Jiang Basin	AF375868.1	
Xenophysogobio boulengen Xenophysogobio nudicorpa*	•		NC_025300.1	
	Uncatalogued NTOUP 201011-534	Upper Chang-Jiang Basin Mulan Yi, Fujian Prov		
Microphysogobio xianyouensis*	NTOUP 201011-534 NTOUP 2015-10-001	Mulan-Xi, Fujian Prov. Shaowu City, Fujian Prov.	KM999931.1 KT877353.1	
Microphysogobio fukiensis*	1000F 2013-10-001	Shauwu Gity, i ujiali Fiuv.	1.077505.1	

### Results

#### Gobiobotia lii sp. nov.

http://zoobank.org/0061399E-1D8E-4764-A475-9FFC29A6490F Figs 1, 2

Gobiobotia pappenheimi Chen & Cao, 1977: 556 (Lake Dongting), Synonym

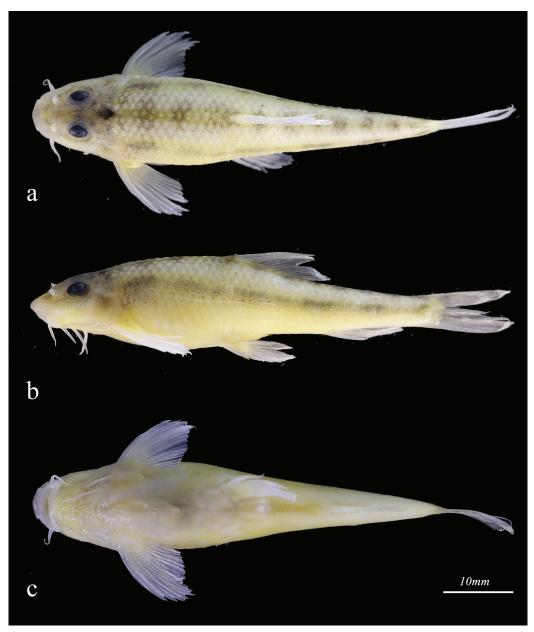
**Holotype.** IHB 202103051401, 48.6 mm SL. P. R. China: Hubei Province: Qichun County: Xiangqiao Town: Chang-Jiang Basin, Qi-Shui stream, 30°20'31"N,

115°43'43"E; D. M. Guo, X. Gong and Y. Liu; 5 March 2021.

**Paratypes.** IHB 202103051399-1400, 202103050858-0859, 4 specimens, 45.1–48.0 mm SL, other data same as holotype. IHB 2014070560439-0447, 9 specimens, 42.1–51.2 mm SL. P. R. China: Hunan Province: Hanshou City: Potou Town: Chang-Jiang Basin, Lake Dongting, 29°00'05"N, 111°58'31"E; L. Cao, Z. G. Jiang, S. J. Ren, R. X. Xie and X. Wang; 5 July 2014. IHB 2017101929, 201711015435, 201801015997; 6012–6015; 6143–6146, 11 specimens, 35.0–40.1 mm SL. P. R. China: Hunan Province: Yuanjiang City: Liaodaokou: Chang-Jiang



**Figure 1.** *Gobiobotia lii*, holotype, IHB 202103051401, 48.6 mm SL, photographed alive immediately upon capture. P. R. China: Hubei Province: Qichun County: Xiangqiao Town: Chang-Jiang Basin, Qi-Shui.



**Figure 2.** Dorsal (**a**), lateral (**b**) and ventral (**c**) views of *Gobiobotia lii*, holotype, IHB 202103051401, 48.6 mm SL. P. R. China: Hubei Province: Qichun County: Xiangqiao Town: Chang-Jiang Basin, Qi-Shui.

Basin, Lake Dongting, 28°51'39.02"N, 112°33'37.87"E; X. Chen, C. T. An, W. H. Shao and Z. T. Wang; 19 October 2017 and 1 January 2018.

**Diagnosis.** Gobiobotia lii is distinct from all other congeneric species, except *G. brevirostris* Chen & Cao, 1977, *G. homalopteroidea* Rendahl, 1933, *G. jiangxiensis* Zhang & Liu, 1995 and *G. pappenheimi* Kreyenberg, 1911, in having a naked region of the abdomen adjacent to the ventral mid-line extending to or beyond the vent (vs. to or away from the pelvic-fin base) (Figs 2c, 3). It differs from these four species in having 4+31–32 (vs. 4+33–37) vertebrae. The new species shares with *G. homalopteroidea* and *G. pappenheimi* the presence of smaller

eyes (diameter less than the interorbital width), maxillary barbels longer than the eye diameter and the third pair of longer mental barbels extending to the pectoral-fin insertion, these three characters separating them from *G. brevirostris* and *G. jiangxiensis*. The new species further differs from *G. homalopteroidea* in possessing a smaller (vs. larger) naked region of the abdomen adjacent to the ventral mid-line extending to the anus (vs. to the anal-fin origin) and the eye diameter 20.0–25.8% of HL (vs. 10.8–13.9%); and from *G. pappenheimi* in having pectoral fins extending away from (vs. beyond) the pelvic-fin insertion, the second branched pectoral-fin ray not prolonged (vs. prolonged) and a longer (vs. shorter) snout than the post-orbital length.



**Figure 3.** Ventral view of body in: (**a**) *G. guilingensis* IHB 202104053183, 86.7 mm SL. P. R. China: Guangxi Province: Rongshui County: Zhu-Jiang Basin, Rong-Jiang; and (**b**) *G. meridionalis*, IHB 201609021174, 84.8 mm SL. P. R. China: Hunan Province: Chenxi County: Chang-Jiang Basin: Yuan-Jiang.

**Description.** Morphometric data for type specimens given in Table 1. General body appearance of holotype shown in Figs 1 and 2. Body small and slender, with maximum depth at dorsal-fin origin. Pre-dorsal body profile depressed. Ventral profile slightly compressed. Abdomen flat or slightly convex from pectoral-fin insertion to anal-fin origin and slightly concave from anal-fin origin to caudal-fin base.

Head depressed and broad. Snout pointed in lateral view and slightly bluntly rounded in dorsal view, longer than post-orbital head. Eyes small and dorsolateral; diameter less than interorbital width. Interorbital space flattened. Some irregular papillae present on the mental region.

Mouth inferior, with opening laterally extending to the vertical line of front edge of nostril. Four pairs of barbels: one pair of maxillary barbels and three pairs of mental barbels. Maxillary barbels extending closely to a vertical line through middle of eye; first pair of mental barbels short, inserted at the same level passing through maxillary-barbel roots and anterior to roots of second mental barbels and reaching anterior margin of eye; second pair of mental barbels extending beyond bases of third pair of mental barbels to hind margin of pre-opercula; third pair of mental barbels long, reaching pectoral-fin insertion.

Fins rays flexible, dorsal fin with 3 simple and 7 (14 specimens examined) branched rays; pectoral fin with 1 simple and 12 (6) or 13 (8) branched rays, extending about two-thirds of the distance to pelvic-fin insertion. Pelvic fin with 1 simple and 7 (14) branched rays, reaching beyond the midway to anal-fin origin and surpassing anus; inserted closer to anal-fin origin than to anterior end of pectoral-fin base; located opposite to that of dorsal-fin base. Anal fin with 3 simple and 6 (14) branched rays; origin equidistant from pelvic-fin insertion and caudal-fin base. Anus positioned closer to the pelvic-fin

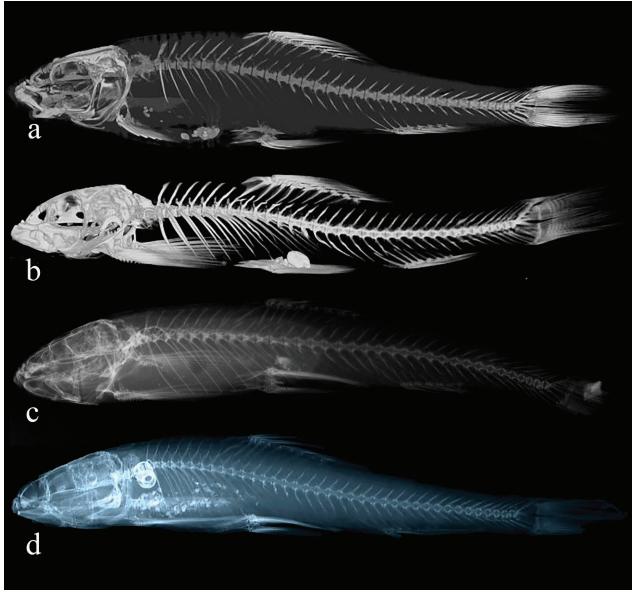


Figure 4. Micro-CT images of lateral body in: (a) *G. lii*, IHB 202103051401, holotype and (b) *G. jiangxiensis*, IHB 90IV1256, holo-type. X-rayed photographs of lateral body in: (c) *G. pappenheimi*, ZMB 18466, holotype and (d) *G. nicholsi*, AMNH 20523, holotype.

insertion than to anal-fin origin. Caudal fin moderately forked; lower lobes are longer than upper lobes.

Lateral line complete and almost straight, extending along mid-lateral of body, with 37 (7) or 38 (7) pored scales; scale rows above and below lateral lines 5 and 3; circumpeduncular scales 11 (7) or 12 (7) and pre-dorsal mid-line scales 13 (14). Body covered with moderately-sized scales; no scales on breast and belly in front of vent. Vertebral counts 4+31–32 (modally 4+32, see Fig. 4).

**Colouration.** In freshly-collected specimens, head and dorsum of body golden yellow, underside and abdomen golden grey; back and lateral head peppered with dark flecks. Back darker and belly lighter, with a dark spot back of the head, 12–13 dorsal dark brown spots extending along mid-line of dorsum from nape to caudal-fin base and 9–10 large dark brown spots along middle of side from gill opening to caudal-fin base. Fin golden yellow, distal margin hyaline.

In formalin-stored specimens, ground colour slightly faded; body dorsally greyish and ventrally greyish-white; dorsal and lateral dark brown spots not clear and the back of the head becoming yellowish-brown. Dorsal, pectoral, pelvic and anal fins light greyish; caudal fin with numerous narrow dark crossbars (Fig. 2).

Sexual dimorphism. No sexual dimorphism was observed in the specimens examined.

**Distribution and habitat.** *Gobiobotia lii* is known from the Qi-Shui, a stream tributary to the middle Chang-Jiang mainstem at Qichun County, Hubei (Fig. 5). The new species was found in turbid, slow-running water with mixed substrates including sand and gravel (Fig. 6); and Lake Dongting in Hunan, where it was collected in lotic habitats or estuaries of effluents. Co-existing species

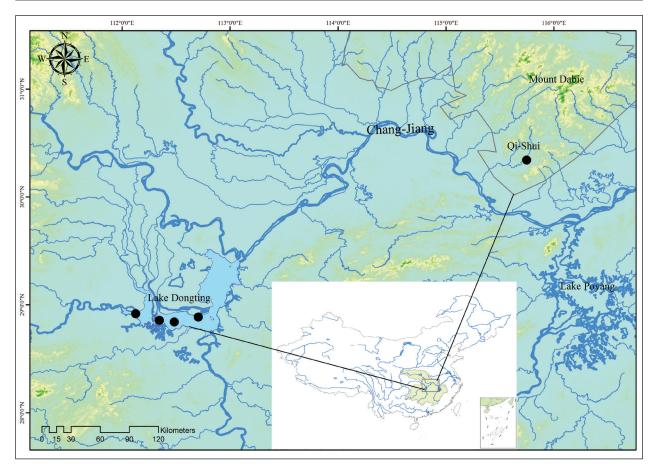


Figure 5. Map showing the distribution of type series of Gobiobotia lii in the middle Chang-Jiang Basin.



**Figure 6.** Sampling locality of the holotype (IHB 202103051401) of *Gobiobotia lii* in the Qi-Shui stream, tributary to the middle Chang-Jiang mainstem in Xiangqiao Town, Qichun County, 5 March 2021.

Table 4. Main diagnostic characters among G. lii and closely-related eight-barbel gudgeons. Number of specimens examined for vertebrae are given in parentheses.

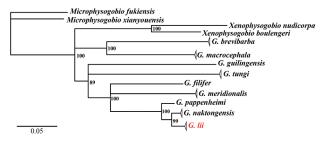
	G. lii^	G. brevirostris	G. homalopteroidea°	G. jiangxiensis <sup>A</sup>	G. pappenheimi
1. Scaleless region of the abdomen adjacent to the ventral mid-line	To anus	To anus	To anal-fin origin	To anus	To anus
2. Snout length/post-orbital head length	> 1	< 1	< 1	> 1	> 1
3. Eye diameter/interorbital width	< 1	> 1	< 1	> 1	< 1
4. Pupil shape	Round	Elliptical	Round	Round	Round
5. Eye diameter % of HL	20.0-25.8	20.0-26.3	10.8-13.9	19.3-25.2	16.1-27.4
6. Maxillary-barbel length/eye diameter	> 1	< 1	> 1	< 1	> 1
7. Extension of third pair of mental barbel	To base of pectoral fin	To middle of opercula	To base of pectoral fin	To front margin of pre-opercula	To base of pectoral fin
8. Pectoral-fin length/distance between pectoral- and pelvic-fin insertions	< 1	< 1	< 1	< 1	> 1
9. Second branched pectoral-fin ray	Not elongated	Elongated	Not elongated	Not elongated	Elongated
10. Vertebral counts	4+31-32 (3)	4+34 (2)	4+36-37	4+33-34 (6)	4+34-35 (5)
11. Lateral-line pored scales	37–38 (14)	38–39 (2)	41–43	38–39 (6)	37-39 (14)

° From He and Chen (1998); <sup>A</sup> from this study.

included *Misgurnus anguillicaudatus*, *Opsariichthys bidens* and *Parabotia fasciata*.

Etymology. The specific epithet is named after Shi-Zhen Li, a native of Qichun County where the holotype and partial paratypes were caught. Li was a well-known medical scientist in the Ming Dynasty, who compiled "Compendium of Materia Medica" ('本草纲目' in Chinese) - one of the most valuable pieces of literature of traditional Chinese medicine. He had a typical image as an old man with a long white dense beard, just like the eight-barbel gudgeon. The common Chinese name '李氏鳅鮀' in here proposed for *Gobiobotia lii*.

Sequence variation and molecular phylogeny. A total of 37 cyt b gene sequences from five Chinese species of Gobiobotia: G. filifer (one sequence), G. guilingensis (one), G. lii (10), G. meridionalis (23) and G. tungi (two) were amplified in this study. These sequences were used for phylogenetic analysis along with another 11 GenBank-retrieved sequences from six congeneric species and two out-groups (Microphysogobio fukiensis and *M. xianyouensis*) (Table 3). A total length of 1038 bp gene sequence was obtained after sequence alignment and trimming, including 643 conserved sites, 396 variable sites, 349 parsimony informative sites and 47 singleton sites. The mean frequency of four nucleotides of the new species was A = 30.7%, T = 27.5%, C = 15.5% and G = 26.4%; the base composition was A-T rich (58.2%). The Bayesian Inference tree, based on the cyt b gene for G. lii and other 10 congeners, is shown in Fig. 7, with node support values displayed. Intraspecific genetic distance values for here-recognised species of the Gobionidae ranged from 0.0% to 1.8%. The interspecific genetic distance of G. lii with all other sampled congeneric species varied from 2.6% to 22.1% (mean 16.8%) and the intraspecific genetic distance value of this species was 0.8% (Table 5). In the Bayesian 50% majority-rule consensus tree, samples of G. lii constituted a robustly-supported (100% Bayesian posterior probability, bpp) lineage, strongly supported by 100% bpp to be sister of G. naktongensis Mori, 1935.



**Figure 7.** Bayesian Inference tree inferred from the *cyt b* gene for 11 putative species of eight-barbel gudgeons. Nodal numbers are posterior probability values greater than 50%.

## Photographic examination on relevant specimens

Some of specimens, utilised in Banărescu & Nalbant's (1966) taxonomic revision of *Gobiobotia*, are from Huping (now Yueyang City) on Lake Tungting (= Dongting). These specimens were not examined by Chinese workers when they revised species of this genus. In this study, photographic examinations are made on them and also on the holotype of *Xenophysogobio boulengeri* and *Gobiobotia pappenheimi* and their topotypical specimens are examined as well.

Both the holotype (AMNH 20523; Fig. 8) and the paratype (AMNH 20524) of *G. nicholsi*, as stated in its original description, have 44–45 lateral-line pored scales, four scale rows below the lateral line, the eye diameter about half of the interorbital width and the second branched pectoral-fin ray not prolonged. Nevertheless, the naked region of the abdomen adjacent to the ventral mid-line extends away from or to the pelvic-fin insertion rather than to or behind the vent in the original description.

Nineteen specimens (AMNH 10311) of 32.2–41.0 mm SL, under the name of *G. pappenheimi* from Lake Dongting, have a naked region of the abdomen adjacent to the ventral mid-line extending to the pelvic-fin insertion

Species	Within Group	1	2	3	4	5	6	7	8	9	10
1. G. lii	0.0076										
2. G. naktongensis	0.0078	0.0260									
3. G. pappenheimi	n/c	0.0387	0.0350								
4. G. filifer	n/c	0.1296	0.1212	0.1395							
5. G. meridionalis	0.0029	0.1358	0.1356	0.1352	0.1424						
6. G. guilingensis	n/c	0.1721	0.1649	0.1824	0.1575	0.1805					
7. G. tungi	0.0029	0.1736	0.1660	0.1757	0.1856	0.1890	0.1925				
8. G. brevibarba	0.0029	0.1826	0.1835	0.1853	0.1926	0.1864	0.2009	0.1927			
9. G. macrocephala	0.0019	0.1856	0.1776	0.1814	0.1684	0.1740	0.1881	0.1993	0.1631		
10. X. boulengeri	n/c	0.1833	0.1849	0.1800	0.1787	0.1804	0.1979	0.1935	0.1914	0.1915	
11. X. nudicorpa	n/c	0.1964	0.1822	0.1847	0.1996	0.1883	0.1912	0.2209	0.1199	0.2070	0.1199

Table 5. Genetic distances (uncorrected p-distance) of cyt b gene computed by MEGA 11 amongst 11 species.

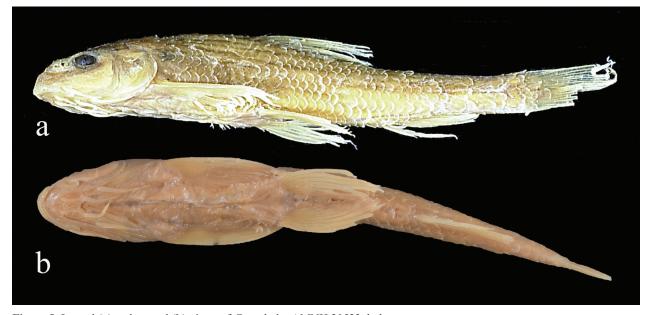


Figure 8. Lateral (a) and ventral (b) views of G. nicholsi, AMNH 20523, holotype.

and more than 40 lateral-line pored scales (Fig. 9a, b). By contrast, the holotype of *G. pappenheimi* (ZMB 18466) has a naked region of the abdomen adjacent to the ventral mid-line extending to the vent and 38–39 lateral-line pored scales (Fig. 9c, d), as found in specimens of this species from northern China.

Two small specimens (AMNH 20522) of 30.0 mm and 31.2 mm SL, under the name of G. boulengeri (= Xenophysogobio boulengeri) from Lake Dongting, have larger eyes (diameter about one-fifth head length), the adpressed tip of the pectoral fin reaching or slightly beyond the pelvic-fin insertion, the adpressed tip of the pelvic fin reaching the anal-fin origin and the upper extremity of the gill opening aligned with the upper margin of the eye (Fig. 10a, b). Nevertheless, three available topotypical specimens and the holotype (MNHN-IC-1934-0180; photograph examined) have smaller eyes (diameter about one-tenth head length), the adpressed tip of the pectoral fin not reaching the pelvic-fin insertion, the adpressed tip of the pelvic fin not reaching the anal-fin origin and the upper extremity of the gill opening aligned with the lower margin of the eye (Fig. 10c, d).

### Discussion

The generic concept of Gobiobotia still remains contentious. All eight-barbel gudgeons were traditionally classified into three subgenera, namely Gobiobotia, Progobiobotia and Xenophysogobio (Chen and Cao 1977). The generic rank was subsequently assigned to Xenophysogobio and Progobiobotia was rendered subgeneric to Gobiobotia (He and Chen 1998), a classification widely accepted by succeeding authors, for example, Zhang and Zhao (2016) and Wu et al. (2021). The generic status of Gobiobotia and Xenophysogobio was verified in molecular phylogenetic analyses of the gobiobotine fishes (Wang et al. 2002) or gudgeons (Tang et al. 2012; Li et al. 2018). However, the taxonomic status of the subgenus Progobiobotia (including two species G. (P.) abbreviata Fang & Wang, 1931, type species and G. (P.) guilingensis Chen, 1989) remained yet to be evaluated in these analyses as a result of failure to sample its included species. In the BI tree yielded from the *cyt b* gene (Fig. 7), G. (P.) guilingensis was distantly related to G. (G.) brevibarba Mori, 1935 and G. (G.) macrocephala Mori, 1935, but constituted a polytomy with G. (G.) tungi and a lineage



Figure 9. Lateral (a) and ventral (b) views of *G. pappenheimi*, AMNH 10311, from Lake Dongting; lateral (c) and ventral (d) views of *G. pappenheimi*, ZMB 18466, holotype.

consisting of G. (G.) filifer, G. (G.) lii, G. (G.) meridionalis, G. (G.) naktongensis Mori, 1935 and G. (G.) pappenheimi. Evidently, G. (P.) guilingensis was intertwined with sampled species of the subgenus Gobiobotia. For this reason, the subgeneric status of Progobiobotia is not warranted. The generic definition of Gobiobotia (sensu He and Chen 1998) is followed in this study. The presence of four pairs of barbels can easily distinguish it and Xenophysogobio from all other genera of the Gobionidae. The genus Gobiobotia includes those eight-barbel gudgeons with a tiny free posterior chamber of the gas bladder without pneumatic duct and large scales, with five or six scale rows above the lateral line.

Species of the gudgeon genus *Gobiobotia* are subdivided into two groups, based on body squamation. One group includes those species with a naked region of the abdomen adjacent to the ventral mid-line, extending to the vent or even the anal-fin origin. Four species, along

with G. lii, are placed in the group: G. brevirostris, G. homalopteroidea, G. jiangxiensis and G. pappenheimi. The rest of congeneric species are assigned to the other group defined by having an unscaled region of the abdomen adjacent to the ventral mid-line extending to or away from the pelvic-fin base. There are marked variations of the new species with the most similar species, G. homalopteroidea and G. pappenheimi, which are provided in the diagnosis. Gobiobotia brevirostris is presently known only from the upper Han-Jiang of the middle Chang-Jiang Basin, while G. jiangxiensis occurs in the Xin-Jiang, an effluent of Lake Poyang. The new species is further distinct from the two co-existing species in having 4+31-32(vs. 4+33-35) vertebrae, the smaller eyes (diameter less than the interorbital width), maxillary barbels longer than the eye diameter and the third pair of longer mental barbels extending to the pectoral-fin insertion; from G. brevirostris in having a longer (vs. shorter) snout than



Figure 10. Lateral (a) and ventral (b) views of *X. boulengeri*, AMNH 20522, from Lake Dongting; lateral (c) and ventral (d) views of *X. boulengeri*, MNHN-IC-1934-0180, holotype.

post-orbital head and relatively smaller (vs. larger) eye diameter than interorbital width; and from *G. jiangxiensis* in having longer (vs. shorter) maxillary barbels than the eye diameter and the third pair of mental barbels extending to the pectoral-fin insertion (vs. to the front margin of the pre-opercula) (See Table 4 and Fig. 4).

Some of type specimens of *G. lii* were collected from Lake Dongting where five nominal species of eight-barbel gudgeons have been documented: *G. boulengeri* (= *Xenophysogobio boulengeri*), *G. filifer*, *G. meridionalis*, *G. nicholsi* and *G. pappenheimi* (Bănărescu and Nalbant 1966; Chen and Cao 1977; He and Chen 1998; Wu et al. 2021). The identification of all these species, except the third one, indeed requires re-evaluation. From Chen and Cao's (1977) point of view, type specimens of *G. nicholsi* were small and similar to *G. ichangensis* (= *G. filifer*) for the presence of small eyes, the first pair of mental barbels rooted anterior to maxillary-barbel bases and the well-developed keels on pre-dorsal scales of the dorsum; they also had no remarkable differences with small (25.5–32.0 mm SL) topotypes. These

type specimens were juveniles of G. filifer, a species currently known from the Chang-Jiang Basin. Gobiobotia filifer is characterised by having an elongated tip of the second branched pectoral-fin ray (He and Chen 1998). This prolonged tip is definitely not exhibited by type specimens of G. nicholsi (AMNH 20523; Fig. 8). The observation by Gao et al. (1988) revealed that the second branched pectoral-fin ray of G. filifer has an elongated tip at the juvenile stage (16 mm TL). This finding negates Chen and Cao's (1977) hypothesis that the types of G. nicholsi were juveniles of G. filifer. Other characters diagnostic for G. filifer comprise 40-42 lateral-line pored scales, three scale rows below the lateral line and the eye diameter slightly less than the interorbital width (Chen and Cao 1977). They are not shared with the type specimens (photograph examined; Fig. 8) of G. nicholsi, with 44-45 lateral-line pored scales, four scale rows below the lateral line and the eye diameter about half of the interorbital width. It is apparent that G. nicholsi and G. filifer are two distinct species.

characteristics led them to reach a conclusion that these

Bănărescu and Nalbant (1966) followed Nichols (1928; 1943) to identify specimens from Lake Dongting as G. pappenheimi, initially described by Kreyenberg (1911) from Tientsin (now Tianjin) of China, while giving its precise type locality as the Pai-ho (= Bai-He of the Hai-He Basin) in Hopei (now Hebei Province). The original description is vague and, thus, of limited taxonomic use for current species identification. This species, as exhibited in the holotype (ZMB 18466, photograph examined; Fig. 9c, d), has a naked region of the abdomen adjacent to the ventral mid-line up to the vent and a slightly prolonged tip of the second branched pectoral-fin ray and 38 lateral-line pored scales. These characters, however, are not shared with two large specimens (photograph examined; Fig. 9a, b) recognised by Bănărescu and Nalbant (1966) as G. pappenheimi from Lake Dongting. Instead, both have a naked region of the abodmen adjacent to the ventral mid-line up to the pelvic-fin base and more than 40 lateral-line scales and lacks a slightly elongated tip of the second branched pectoral-fin ray (Fig. 9a, b), thus not conspecific with either G. filifer or G. lii, as well as G. pappenheimi. Due to the two specimens and other 17 small specimens (photograph examined) not preserved in good condition, the precise count of lateral-line pored scales for them is impossible. All these specimens from Lake Dongting are probably the misidenfication of G. nicholsi. Nonetheless, the hypothesis needs to be confirmed in a future study. In Chinese literature (Liang and Liu 1966; Chen and Cao 1977; He and Chen 1998; Zhang et al. 2016), the occurrence of G. pappenheimi in Lake Dongting is an erroneous record. This species is not found in the Chang-Jiang and river basin south of the river.

One small specimen of 37.5 mm SL caught from Lake Dongting was referred to as a distinct species by Chen and Cao (1977), who stopped short of describing a new species owing to lack of large specimens. It has a naked region of the abdomen adjacent to the ventral mid-line up to the vent, no prolonged tip of the second branched pectoral-fin ray, the first pair of mental barbels inserted anterior to the maxillary-barbel bases, 38 lateral-line pored scales and a gas bladder with an anterior chamber enclosed in a membranous capsule (Chen and Cao 1977, page 556: table 10-1). All these characters, except the last one, are shared with G. lii. It is possible that the small specimen from Lake Dongting is conspecific with this species. Size-related change is the plausible explanation for the variation in the anterior chamber of the gas bladder between it and type specimens of G. lii.

Bănărescu and Nalbant (1966) was the first to recognise two specimens from Lake Dongting of the middle Chang-Jiang Basin as *G. boulengeri*, a species that was initially described by Tchang (1929) from Szechwan (today's Sichuan Province in the upper Chang-Jiang Basin). Both (AMNH 20522, photograph examined; Fig. 10a, b) have larger eyes (diameter about one-fifth head length), the adpressed tip of the pectoral fin reaching or slightly beyond the pelvic-fin insertion, the adpressed tip of

the pelvic fin reaching the anal-fin origin and the upper extremity of the gill opening aligned with the upper margin of the eye. All these characters are incongruent with the original description of G. boulengeri (= Xenophysogobio boulengeri). Three available topotypes and the holotype (MNHN-IC-1934-0180, photograph examined; Fig. 10c, d) have small eyes (diameter about one of tenth head length), the adpressed tip of the pectoral fin not reaching the pelvic-fin insertion, the adpressed tip of the pelvic fin not reaching the anal-fin origin and the upper extremity of the gill opening aligned with the lower margin of the eye. Evidently, the aforementioned two specimens from Lake Dongting are not identical to X. boulengeri. To which species they belong still remains unclear, given the poor condition of specimens and no examination on the structure of their gas bladders. In Chinese literature (Chen and Cao 1977; He and Chen 1998; Zhang et al. 2016; Guo et al. 2021), X. boulengeri has an erroneous record from Lake Dongting. This species is endemic to the upper Chang-Jiang Basin.

Based on the above analysis, it is here concluded that Lake Dongting harbours four eight-barbel gudgeons: *G. filifer*, *G. lii*, *G. meridionalis* and *G. nicholsi*. The new species is separated from all other three co-existing congeneric species by the presence of a naked region of the abdomen adjacent to the ventral mid-line extending to the vent (vs. extending away from or to the pelvic-fin base). It lacks an elongated tip of the second branched pectoral-fin ray typical for *G. filifer* and has 37–38 lateral-line pored scales fewer than 44–45 and 40–43 for *G. nicholsi* and *G. meridionalis*, respectively.

The validity of G. lii is further affirmed by its monophyletic nature recovered in *cyt* b gene-based phylogenetic analysis and its significant sequence variation on all sampled congeners. In the BI trees (Fig. 7), Gobiobotia lii formed a strongly-supported exclusive lineage, being sister to the endemic South Korean species G. naktongensis. The new species differs from it in having, amongst others, a naked region of the abdomen adjacent to the ventral mid-line reaching the pelvic-fin base, the pectoral fin extending beyond the pelvic-fin insertion and the third pair of mental barbels reaching over the hind margin of the opercula. The sequence divergence of G. lii with sampled congeneric species varied from 2.6-22.1% (average 12.4%) (Table 5), greater than 2% being utilised as a threshold for vertebrates' species delimitation (Avise and Walker 1999; Hebert et al. 2003).

### Comparative material

- G. abbreviata: IHB2113-2117, 5 specimens, 53.4– 74.8 mm SL, Min-Jiang at Leshan City, Sichuan Province, China.
- G. brevibarba: IHB, uncatalogued, 2 specimens, 61.5– 88.6 mm SL; Misan-Ri, Sangnam-Myon Inje-Gun, Kangwon-Do, South Korea.

- G. brevirostris: IHB 81VII1310-1311, 2 specimens, 39.0–41.7 mm SL; Han-Jiang at Tanghe County, Henan Province, China.
- *G. filifer*: IHB 83IV1438-1439, 83IV1441-1450, 64V2287, 64IV0803-0804; IHB 0305, 53120-53121, 53123, 20 specimens, 52.2–96.5 mm SL; Yichang City, Hubei Province, China. IHB 2017100792, 1 specimen, 78.6 mm SL; Yueyang City, Hunan Province, China. AMNH 79426, 1 specimen, all other data same as Yueyang City, Hunan Province, China (photograph examined).
- *G. guilingensis*: IHB 20161062A, 1 specimen, 86.7 mm SL; Zhu-Jiang at Rongshui County, Guangxi Province, China.
- G. jiangxiensis: IHB 90-IV-1256, holotype, 41.0 mm SL;
  Xin-Jiang at Shangrao City, Jiangxi Province. IHB 90-IV-1257, IHB 90-IV-1377, IHB 90-IV-1731-1732,
  4 paratypes, 37.0–54.0 mm SL; Shangrao City and Guangfeng County. IHB 90-IV-1796, 6 specimens, 35.2–52.5 mm SL; all other data same as holotype.
- G. macrocephala: IHB, uncatalogued, 1 specimen,
   53.8 mm SL; Somaegok-Ri pukpsng-Myon, Hongchon-Gun, Kangwon-Do, South Korea.
- G. meridionalis: IHB 201609021340-1344, 201609056055, 6 specimens, 70.5–85.9 mm SL; Yuan-Jiang at Luxi County, Hunan Province, China. IHB 201609025884-5885, 2 specimens, 95.3–99.5 mm SL; Yuan-Jiang at Mayang County, Hunan Province, China. IHB 201609056048, 201609021171-201609021176, 7 specimens, 75.2–93.7 mm SL; Yuan-Jiang at Chenxi County, Hunan Province, China. IHB 201711056049, 201809036686, 201809036690, 3 specimens, 54.0–74.3 mm SL; Xiang-Jiang at Chalin City, Hunan Province, China.
- *G. naktongensis*: IHB, uncatalogued, 2 specimens, 44.3–47.5 mm SL; Kyongsangbuk-Do, South Korea.
- *G. nicholsi*: AMNH 20523, holotype, 35.7 mm SL; Yueyang City, Hunan Province, China (X-radiograph examined); AMHN 20524, 2 paratypes, 21.9–32.2 mm SL; same locality as holotype (photograph examined).
- G. pappenheimi: ZMB 18466, holotype, 45.0 mm SL, Tianjin City, China (X-radiograph examined); BMNH 1925.8.6.36, Russia (X-radiograph examined); AMNH 10311, 10308, 10553, 19 specimens, 32.2–41.0 mm SL; Yueyang City, Hunan Province, China (photograph examined). IHB 80-VII-607-609, 611–613, 6 specimens, 45.8–56.1 mm SL; Nen-Jiang at Heilongjiang Province, China. IHB 80-II-1282-1283, 92-V-1609–1611, 5 specimens, 27.9–39.5 mm SL; Liao-He at Liaoning Province, China.
- *G. tungi*: IHB 202108056037-6038, 2 specimens, Xin-Jiang, an effluent of Poyang Lake at Shangrao City, Jiangxi Province, China.
- Xenophysogobio boulengeri: MNHN-IC-1934-0180, holotype, Szechwan (now Sichuan Province), China (X-radiograph and photograph examined); AMNH 20522, 2 specimens, 30.0–31.2 mm SL; Yueyang City, Hunan Province, China (photograph examined); IHB78IV0215-16, 0218, 3 specimens, Min-Jiang, an

effluent of the upper Chang-Jiang at Leshan, Sichuan Province, China.

# Ethics approval and consent to participate

All procedures described in this paper were in accordance with Chinese laws and were licensed by the Ministry of Ecology and Environment of the People's Republic of China.

## Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

Xiao Chen and Man Wang conceived the study and analysed the data. Xiao Chen led the writing. Liang Cao and E Zhang revised the manuscript. All authors contributed to the writing of the paper.

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