



# Morphological Re-Description and DNA Barcoding of *Platycephalus indicus* Collected from Beihai, China

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## ABSTRACT

We collected *Platycephalus indicus* individuals from Beihai and accurately described the species. The diacritically meristic counts were listed as follows: first dorsal fin with a single small isolated spine anteriorly; gill rakers 2-3+4-6=6-9; pored lateral line scales 73-80 and caudal fin with a yellow marking on the middle when fresh. These conclusive characters were consistent with typical *Platycephalus indicus* individuals and could thoroughly separate them from other *Platycephalus* species. The fragment of cytochrome oxidase subunit I (COI) gene of mitochondrial DNA was also sequenced for the classification of specimens. The mean genetic distance within *Platycephalus indicus* was 0.8%, net genetic distance between *Platycephalus indicus* and other 13 species of the genus *Platycephalus* ranged from 10.3% to 26.1%. The phylogenetic analysis similarly supported the validity of *Platycephalus indicus* existed in the coastal waters of Beihai. Comments were made on some of the characters to more fully characterize the species and for phylogenetic studies.

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## Authors' Contribution

ZC and TG presented concept, analyzed the data and wrote the manuscript. TG supervised the study and arranged financial support.

## Key words

Chinese taxonomy of flathead fishes, Isolated spine anteriorly, Yellow marking, Phylogenetic analysis.

## INTRODUCTION

Flathead fishes of the genus *Platycephalus*, family Platycephalidae, were widely distributed in the tropical and temperate areas of the Indo-West Pacific and eastern Mediterranean (Hureau, 1986; Shao and Chen, 1987; Imamura, 1996; Knapp, 1999; Qin *et al.*, 2013). Before 2013, only one species of this genus, *Platycephalus indicus* (Linnaeus 1758), had been recorded and studied in China (Zhu *et al.*, 1963; Chang *et al.*, 1980; Chen, 1982; Chen and Zhao, 1986; Kong *et al.*, 1994; Zhang *et al.*, 1994; Jin, 2006; Tang, 2006; Qin *et al.*, 2013). Qin *et al.* (2013) revealed that the common *Platycephalus* species widely distributed in the coastal waters of China was *Platycephalus* sp.1 in reality (Note: *Platycephalus* sp. 1 as well as *Platycephalus* sp. 2 was two valid but undescribed species. These two species were long-termly recognized and classified by Japanese ichthyologists with the Japanese names Yoshino-gochi and Ma-gochi (see Kamei and Ishiyama, 1968; Masuda *et al.*, 1991, 1997; Osatomi *et al.*, 2001; Nakabo, 2002; Yamada *et al.*, 2007; Qin *et al.*, 2013)). Further studies also indicate that all Chinese description of *Platycephalus indicus* was *Platycephalus* sp. 1 actually (Qin *et al.*, 2013). Due to the undiscovered or rare existence

of *Platycephalus indicus* in Chinese coastal waters, Qin *et al.* (2013) didn't describe native *Platycephalus indicus* but just referred to foreign study of this species found locally. Thus his corrections created the description vacuum of *Platycephalus indicus* recorded in Chinese ichthyology.

Meeting this need, our purpose here was confirming the existence of true *Platycephalus indicus* and providing plentifully morphological characters of this species. Due to the complexities of morphological characters used in traditional taxonomy and taxonomic confusion as well as cryptic species has arisen in *Platycephalus* concerning the nomenclature (Imamura, 2006, 2008, 2012, 2013a, 2013b, 2015; Imamura *et al.*, 2006; Imamura and Knapp, 2009). Employing solely morphological means to solve chaotically taxonomic problems was improper sometimes in traditional taxonomy. The mitochondrial cytochrome oxidase I gene (COI) varies noticeably between species and very little between the individuals of a given species (Gross, 2012). Therefore, a fragment of COI gene, as DNA barcoding (Hebert *et al.*, 2003), has proven to be extremely effective at discriminating species (Domingues *et al.*, 2013; Puckridge *et al.*, 2013; Ming *et al.*, 2015), discovering new-recorded and new species (Gao *et al.*, 2011; Qin *et al.*, 2013), uncovering cryptic species (Hajibabaei *et al.*, 2007; Zemlak *et al.*, 2009), identification of ichthyoplankton (Bian *et al.*, 2008; He *et al.*, 2011). In the present study, a mitochondrial DNA barcoding approach was also employed in order to better solve the taxonomic problems

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of *Platycephalus indicus* at genetic level. The results will contribute to Chinese *Platycephalus* species identification and be helpful to native fishery management, biodiversity conservation, and sustainable exploitation of this species.

## MATERIALS AND METHODS

### Sampling

Specimens were collected from the coastal waters of Beihai (Fig. 1) from August 2012 to October 2016 (3 on August 17, 2012; 2 on November 08, 2014; 4 each on January 12 and October 23, 2016). All 13 individuals were identified based on morphological characteristics commonly-used by Imamura (2012, 2015). The body color and pigmentation were pictured in fresh fish and all measurements were made on preserved specimens. For genetic study, a piece of muscle tissue was obtained from each individual and preserved in 95% ethanol or directly extracted from frozen samples. All specimens examined were frozen and preserved at the Fishery Ecology Laboratory, Fisheries College, Ocean University of China in Qingdao.

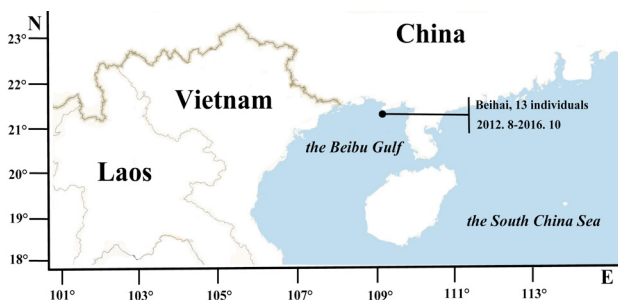


Fig. 1. Sampling location, date and number of *Platycephalus indicus* individuals collected in this study.

### Morphological study

Counts and measurements followed the standard methods given by Imamura (2012, 2015). The detailed guidance was listed as follows: gill rakers, defined as depressible bony elements and not including tooth plates, were counted on the right side. Other counts were routinely taken from the left side. Measurements of body lengths were done on a measuring board graduated in 1.0 mm intervals. All other measurements were taken using dial calipers and recorded to the nearest 0.1 mm. Fin rays were counted using a magnifier when specimens were too small. Small isolated anterior and posterior first dorsal-fin spines are given before and after the first dorsal-fin ray count (in Roman numerals), respectively. Orbital diameter was measured from the anteroventral to posteromedial portions, which was the greatest distance in many species of *Platycephalus*. Interorbital width was measured level

with the eye center. When two small isolated anterior dorsal-fin spines were present, predorsal length was measured from the tip of the snout to the base of the second spine, since the latter was homologous with the single small isolated anterior spine present in many species of *Platycephalus* (see Imamura, 1996 for homology of such spines). Terminology of head spines follows Knapp *et al.* (2000) and Imamura (2015). The following abbreviations for counts and measurements were used: first dorsal-fin rays (D1), second dorsal-fin rays (D2), anal-fin rays (A), pectoral-fin rays (P1), branched caudal-fin rays (C), pored lateral line scales (LLS), oblique body scale rows slanting downward and backward above lateral line (OBS), gill rakers (GR), total length (TL), standard length (SL), head length (HL), predorsal length (PDL), length of first dorsal-fin base (LD1B), length of second dorsal-fin base (LD2B), length of anal-fin base (LAB), snout length (SNL), orbital diameter (OD), upper-jaw length (UJL), lower-jaw length (LJL), interorbital width (IW), postorbital length (POL), suborbital width (SW), pectoral-fin length (P1L), pelvic-fin length (P2L) and caudal-fin length (CL) (all words above were from Imamura, 2012, 2015). Some counts could not be determined because of the poor condition of this individual. In such instances the reported values were taken from the original description (for holotypes) or the specimen was discarded from analysis.

### DNA extraction and sequencing

After morphometric measurements, all 13 specimens were selected for genetic studies. The classical phenol-chloroform technique was used for DNA extraction. Polymerase chain reaction (PCR) was subsequently conducted. The F and R sequences of the primers used for COI amplification were 5'-TCGACTAATCATAAAGATATCGGCAC-3' and 5'-ACTTCAGGGTGACCGAAGAATCAGAA-3' (Ivanova *et al.*, 2007), respectively. PCR was carried out in a 25  $\mu$ L reaction mix containing DNA template (1  $\mu$ L, 50 ng/ $\mu$ L), forward primer (F, 1  $\mu$ L, 10 uM/L), reverse primer (R, 1  $\mu$ L, 10 uM/L), dNTPs (2  $\mu$ L, 2.5 mM/L each), EasyTaq DNA Polymerase (0.15  $\mu$ L, 5 U/ $\mu$ L) and 10 $\times$  PCR buffer (2.5  $\mu$ L, 25 uM/L). A Biometra thermal cycler (Göttingen, Germany) with the following given procedure: one initial denaturation (95°C, 5 min), thirty-five cycles consisting of denaturation (94°C, 50 s), annealing (54°C, 50 s) and extension (72°C, 48 s), and one final extension (72°C, 10 min), was employed to put PCR amplification into effect. PCR products were sent to Shanghai Majorbio Bio-Pharm Technology Co., Ltd. to get original COI sequences.

### COI analysis

All 13 individuals' original sequences were successfully obtained and revised by DNASTAR software (DNASTAR Inc., Madison, WI, USA). One COI sequence

**Table I.- GenBank accession numbers of related COI sequences downloaded from NCBI for phylogenetic tree study.**

Species	GenBank accession number
<i>Platycephalus aurimaculatus</i> Knapp, 1987	JX488155, JX488255
<i>Platycephalus bassensis</i> Cuvier in Cuvier and Valenciennes, 1829	DQ107991
<i>Platycephalus caeruleopunctatus</i> McCulloch, 1922	DQ107994
<i>Platycephalus conatus</i> Waite and McCulloch, 1915	JX488183
<i>Platycephalus endrachtensis</i> Quoy and Gaimard, 1825	DQ108000
<i>Platycephalus fuscus</i> Cuvier in Cuvier and Valenciennes, 1829	DQ107974, DQ107989
<i>Platycephalus grandispinis</i> Cuvier in Cuvier and Valenciennes, 1829	DQ107959, DQ107960
<i>Platycephalus indicus</i> (Linnaeus, 1758)	JX972212
<i>Platycephalus laevigatus</i> Cuvier in Cuvier and Valenciennes, 1829	DQ107966, DQ107980
<i>Platycephalus marmoratus</i> Stead, 1908	DQ107950, DQ107964
<i>Platycephalus orbitalis</i> Imamura and Knapp, 2009	JX488284, JX488286, JX488150
<i>Platycephalus richardsoni</i> Castelnau, 1872	JX488214
<i>Platycephalus westraliae</i> (Whitley, 1938)	DQ107985, DQ107997
<i>Platycephalus</i> sp. 1	JX972210, JX972199
<i>Cocceilla crocodila</i> Cuvier, 1829	JQ349911

of *Cocceilla crocodila* and 23 sequences of *Platycephalus* were also downloaded from NCBI for phylogenetic tree study (Table I). These 37 COI sequences were then aligned using the above DNASTAR software. MEGA 5.0 (Tamura *et al.*, 2011) was used to construct neighbor-joining (NJ) tree under the Kimura 2-parameter (K2P) model.

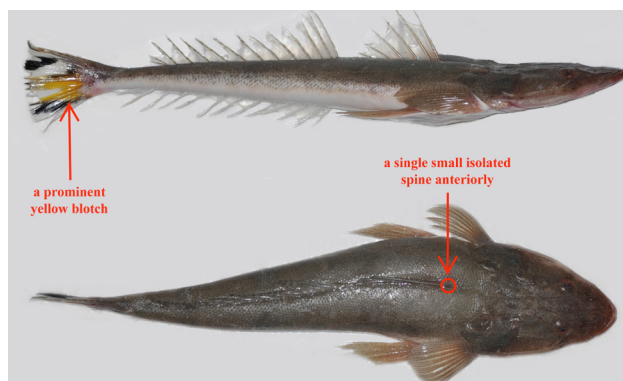


Fig. 2. Lateral (upper) and dorsal (lower) views of *Platycephalus indicus*, 336.8 mm SL.

## RESULTS

### Morphological characters

The generally morphological features were shown in Figure 2. Body was elongate and head strongly depressed. Rear edge of maxilla reached to about below middle of eye. Preopercular spines were 2, and the lower was longer than the upper. A trace of an accessory spine usually present on base of upper spine. Supraorbital ridge was pretty smooth. Spines and ridges on top and side of head weakly developed. Preorbital spine was lacking and a single

preocular spine obscure in large adults. Suborbital ridge was smooth in adults, bearing a spine below rear of eye in juveniles. Teeth on vomer were in a single transverse band. Upper iris lappet was a simple, triangular lobe. Interopercular flap presented, finger-like in shape. Total gill rakers on first gill arch ranged from 6 to 9 (usually 8 or 9). In addition to 13 second dorsal-fin rays and 13 anal-fin rays, first dorsal-fin spines and pectoral-fin rays were I+VII+I and 18 to 19, respectively. Oblique body scale rows slanting downward and backward above lateral line ranged from 86 to 108. Lateral line scales were usually 73 to 80. Anterior-most scale usually had a one or two spine or ridge. Scale pores of lateral line had a single opening to the outside.

Besides above description, these specimens were also a species of *Platycephalus* with the following combination of characters: upper jaw without large caniniform teeth. Teeth absent on dorsal surface of anterolateral edge of upper jaw. Lip margins without papillae. First and second dorsal fin narrowly separated. Head and body covered with small brown flecks, whitish below, several indistinct dark bands crossing back in some; upper surface of eye without papillae; scales covering snout, a small area anteroventral to eye, interorbit, occipital region, nape, postorbital and opercular regions; suborbital region naked; first dorsal fin with a single small isolated spine anteriorly; body with two dark brown bands below second dorsal fin; first and second dorsal, pectoral and pelvic fins with small, dark brownish spots along rays; caudal fin with 2 or 3 horizontal dark bars, a prominent yellow blotch near middle of fin when fresh; head length 29.7–33.7% in SL; pectoral fin length 11.7–16.2% SL. Pelvic fin length 21.7–25.5% SL. Caudal fin usually slightly rounded or mostly straight posteriorly,

length 15.0-19.7% SL. Postorbital length 52.2–59.6% HL; snout length 24.5-29.7% HL; interorbit narrower than orbital diameter in smaller specimens, becoming equal to or wider than orbital diameter with the development of specimens, width 22.7-28.0% HL; orbital diameter 12.6-18.4% HL.

#### Sequence analysis of the COI gene

Thirteen 654-bp-long sequences of COI gene fragments were obtained. After combined the downloaded

COI sequences of *Platycephalus*, a total of 37 sequences were used for analysis. Table II reported the genetic distances between all species. The mean distance among species was 19.5%. Genetic distance between our 13 specimens and *Platycephalus indicus* downloaded from NCBI was only 0.8%. Net genetic distances between *Platycephalus indicus* and other 13 species of the genus *Platycephalus* ranged from 10.3% to 26.1%, which vastly exceeded the threshold of species delimitation.

**Table II.- Comparative counts of *Platycephalus indicus* and *P. sp. 1* from different records.**

		Records in this study		Records in references			
		Specimens		Qin, (2013)			
		<i>P. indicus</i> (n = 13)		<i>P. sp. 1</i> (n = 174)			
				Imamura, (2015)			
				<i>P. indicus</i> (n = 41)			
Counts	SL (mm)	148.0-546.7		122.0-482.8		40.2-379	
	D1	I+VII+I		II+VI-VII+0-I (usually II + VII+I)		I+VI-VIII+0-I	
	D2	13		13-14 (usually 13)		13-14 (usually 13)	
	A	13		13-14 (usually 13)		13	
	P1	18-19		17- 19		18-20 (usually 19)	
	C	11-12(usually 12)		11-14		9-12 (usually 12)	
	LLS (spines)	73-80 (1-2)		83- 99 (1-2)		67-84 (1-3)	
	OBS	86-108		103-121		83-112	
	GR	6-9		11-17		7-10	
	As % SL:	HL	29.7-33.7		26.3-31.3		29.2-34.7
PDL		28.7-34.2		27.8-41.0		29.9-38.1	
LD1B		16.2-22.3		14.0-22.1		14.8-21.8	
LD2B		30.7-34.2		30.2-38.0		31.8-36.2	
LAB		35.0-39.6		33.5-43.6		34.1-41.1	
SNL		7.4-8.8		7.6-9.8		7.6-9.5	
OD		3.2-6.7		3.1-9.9		3.9-8.5	
UJL		10.9-12.8		9.8-14.3		10.6-13.2	
LJL		14.9-17.6		13.5-19.9		14.8-18.4	
IW		2.7-5.2		2.6-6.1		2.5-5.5	
POL		16.8-19.4		15.9-22.3		16.5-19.3	
SW		2.6-3.3		2.3-3.7		2.5-3.1	
P1L		11.7-16.2		12.2-17.9		12.4-17.7	
P2L		21.7-25.5		19.6-26.7		21.2-25.6	
As % HL:		CL	15.0-19.7		14.1-20.8		14.7-19.9
	SNL	24.5-29.7		23.8-31.3		25.3-28.4	
	OD	12.6- 18 .4		10.9-17.2		13.0-23.6	
	UJL	30.5-34.9		30.3-58.9		35.0-40.4	
	LJL	48.9-52.9		46.8-57.1		48.6-54.3	
	IW	22.7-28 .0		9.8-19.6		7.2-18.4	
	POL	52.2-59.6		52.6-66.7		51.4-61.6	
SW	6.9-9.6		6.7-11.2		7.1-10.1		

D1, first dorsal-fin rays; D2, second dorsal-fin rays; A, anal-fin rays; P1, pectoral-fin rays; C, branched caudal-fin rays; LLS, pored lateral line scales; OBS, oblique body scale rows slanting downward and backward above lateral line; GR, gill rakers; TL, total length; SL, standard length; HL, head length; PDL, pre-dorsal length; LD1B, length of first dorsal-fin base; LD2B, length of second dorsal-fin base; LAB, length of anal-fin base; SNL, snout length; OD, orbital diameter; UJL, upper-jaw length; LJL, lower-jaw length; IW, interorbital width; POL, postorbital length; SW, suborbital width; P1L, pectoral-fin length; P2L, pelvic-fin length; CL, caudal-fin length



**Table III.- Net genetic distances (K2P) within (on the diagonal, bold font) and between (below the diagonal, normal font) species.**

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
<b>A</b>	<b>0.002</b>													
<b>B</b>	0.2065	-												
<b>C</b>	0.267	0.2701	-											
<b>D</b>	0.2023	0.1932	0.3269	<b>0.011</b>										
<b>E</b>	0.2348	0.2233	0.3132	0.2201	<b>0.003</b>									
<b>F</b>	0.2287	0.2259	0.3245	0.216	0.0856	-								
<b>G</b>	0.2266	0.2027	0.3257	0.2159	0.0854	0.0866	-							
<b>H</b>	0.1201	0.2254	0.2895	0.2285	0.2542	0.2618	0.2585	<b>0.005</b>						
<b>I</b>	0.1028	0.2097	0.2744	0.237	0.261	0.248	0.2608	0.1896	<b>0.008</b>					
<b>J</b>	0.1909	0.181	0.2966	0.0964	0.2199	0.215	0.2111	0.2221	0.2172	<b>0.002</b>				
<b>K</b>	0.2148	0.2171	0.2902	0.1994	0.2131	0.2354	0.224	0.2255	0.2476	0.1575	<b>0.003</b>			
<b>L</b>	0.1816	0.1895	0.2815	0.1643	0.193	0.215	0.1947	0.2272	0.1955	0.1365	0.152	<b>0.002</b>		
<b>M</b>	0.1167	0.1986	0.2881	0.1857	0.2441	0.2439	0.2335	0.1124	0.1629	0.1814	0.2132	0.2013	<b>0.002</b>	
<b>N</b>	0.2067	0.1804	0.3003	0.1963	0.2207	0.2145	0.2084	0.2161	0.2155	0.1768	0.1124	0.1633	0.2054	-
<b>O</b>	0.1823	0.2155	0.3277	0.1876	0.2379	0.2292	0.228	0.1961	0.243	0.1456	0.1594	0.1691	0.1831	0.1804

A, *P. westraliae*; B, *P. endrachtensis*; C, *Coceilla crocodila*; D, *P. orbitalis*; E, *P. aurimaculatus*; F, *P. conatus*; G, *P. richardsoni*; H, *P. sp. 1*; I, *P. indicus*; J, *P. marmoratus*; K, *P. grandispinis*; L, *P. laevigatus*; M, *P. fuscus*; N, *P. bassensis*; O, *P. caeruleopunctatus*.

A neighbor-joining phylogenetic tree was constructed using MEGA 5.0 (Fig. 3). *Coceilla crocodila* was chosen as the out-group to root the tree. All COI sequences of specimens in present study clustered in the same group, and 7 haplotypes were defined. All haplotype sequences were submitted to GenBank with the following accession numbers: KY463436-KY463442. The haplotype 2 (Hap-2), haplotype 4 (Hap-4) and haplotype 5 (Hap-5) were severally shared by 2 specimen and haplotype 7 (Hap-7) by 4 specimen. Remaining haplotypes were unique and each of them was shared by one specimen. At the same time, a large genetic distance (19.0%) between specimens and *Platycephalus sp.1* indicated that they couldn't be the same species.

## DISCUSSION

The morphological characters of specimens used in this study were photographed, counted and compared with previously representative records on Table I. These individuals were characterized by: first dorsal fin with a single small isolated spine anteriorly; gill rakers 2-3+4-6=6-9; pored lateral line scales 73-80 and caudal fin with a yellow marking on the middle when fresh. These phenotypic traits were consistent with the descriptions of typical *Platycephalus indicus* described by Imamura (2015).

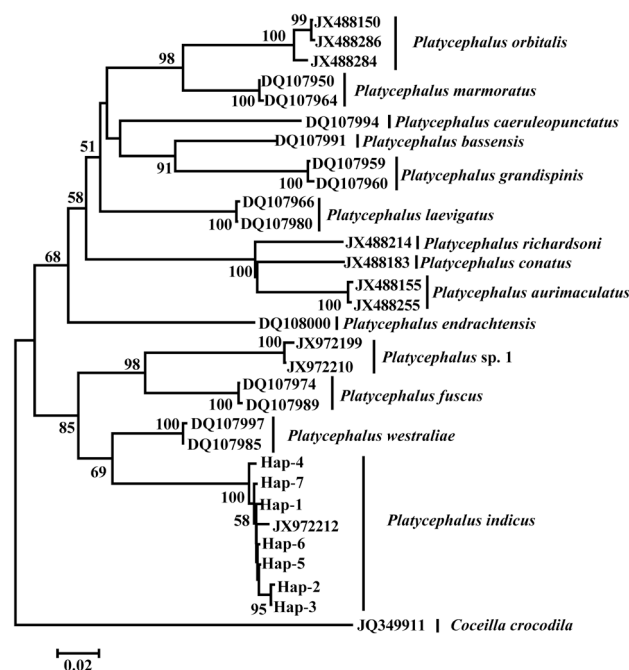


Fig. 3. Phylogenetic tree based on Neighbor-joining analysis of COI sequence. *Coceilla crocodila* (JQ349911) was chosen as the out-group to root the tree. Numbers above branches indicate Neighbor-joining bootstrap percentages. Only Bootstrap values of >50% are shown in the above NJ tree.

*Platycephalus* sp. 1 distributed in China had previously been misidentified as *Platycephalus indicus* (Zhu *et al.*, 1963; Chang *et al.*, 1980; Chen, 1982; Chen and Zhao, 1986; Kong *et al.*, 1994; Zhang *et al.*, 1994; Tang, 2006; Jin, 2006), both species being characterized by usually 13 second dorsal- and anal-fin rays, interorbit and occipital region scaled, large caniniform teeth absent on the upper jaw, a finger-like interopercular flap and so on (Imamura, 2015; Qin *et al.*, 2013). Besides, by reviewing all known references including our present results, it could be obviously found that *Platycephalus indicus* and *Platycephalus* sp. 1 were very similar in meristic values. Almost all morphological characteristics were overlapped. Such overlaps appeared to be very common among *Platycephalus* that were either closely related or lived in similar habitats (Imamura, 2015). These might be the reason why all *Platycephalus* species from the coastal waters of China permanently and routinely misidentified as *Platycephalus indicus* by native ichthyologists.

Although *Platycephalus indicus* was similar with *Platycephalus* sp. 1 and long-termly lacked correct descriptions, there was still some obvious difference that could separate *Platycephalus indicus* from other *Platycephalus* species. Contrast to the concentrated distribution in Australia, only four species (*Platycephalus indicus*; *Platycephalus cultellatus*; *Platycephalus* sp.1 and *Platycephalus* sp. 2) of whole *Platycephalus* were recorded in Northwest Pacific (Imamura 2006, 2015; Nakabo, 2002; Qin *et al.*, 2013). Among above four species, *Platycephalus indicus* was the only one that had the first dorsal fin with a single small isolated spine anteriorly and the caudal fin with a yellow marking on the middle when fresh (Fig. 2). The other three species uniformly usually had a caudal fin without yellow color and two small isolated spines in front of first dorsal fin. As for commonly-seen and previously misidentified *Platycephalus* sp.1, the range of gill rakers and pored lateral line scales could also distinguish *Platycephalus indicus* from it (Table I).

COI sequence was recognized as an effective and reliable method for species identification (Domingues *et al.*, 2013; Hebert *et al.*, 2003; Masuda and Ozawa, 2000; Qin *et al.*, 2013). The validity of *Platycephalus indicus* has also been demonstrated from genetically reconstructed phylogenetic relationships of *Platycephalus*, showing that specimens from Beihai, with a yellow marking on the mid-caudal fin and *Platycephalus indicus* sequence from NCBI were included in a same monophyletic clades. As an indicator of speciation, Hebert *et al.* (2004) proposed the '10×rule', whereby barcoded individuals are flagged as possible another species if they diverge by 10 times or more the average intraspecific variability of the group. A different approach was taken by Ward *et al.* (2009),

who analyzed barcode data from about 1000 fish species and showed that at a level of 2% distance or greater, individuals were much more likely to be congeneric than conspecific. We identified that *Platycephalus indicus* and other *Platycephalus* species were distinguished by distance = 0.103–0.261. All other pairwise divergence among who species exceeded distance = 0.195, and the highest values were observed between *Platycephalus indicus* and *Platycephalus aurimaculatus* (distance = 0.261). The mean evolutionary distance within the species *Platycephalus indicus* was 0.8%, use of either the 10× or 2% rule suggests that the genetic distance between groups was significantly higher than the average genetic distance within the group, which indicated that the COI gene used as a barcode of *Platycephalus indicus* was effective at identifying *Platycephalus* species. Thus, these reality provided our studies a strongly morphological and genetic level support that the species *Platycephalus indicus* in the coastal waters of China was correctly identified and described.

It was also reported that *Platycephalus indicus* was widely distributed in the coastal waters of China (Zhu *et al.*, 1963; Chang *et al.*, 1980; Chen, 1982; Chen and Zhao, 1986; Kong *et al.*, 1994; Zhang *et al.*, 1994; Jin, 2006; Tang, 2006). We have also tried our best to collect more specimens from all Chinese seas. But it seemed that fishermen and researchers could hardly see the occurrence of *Platycephalus indicus*. Only 13 *Platycephalus indicus* was successfully collected in the past four years and all of them came from Beihai. The inundant distribution of *Platycephalus* sp. 1 and infrequent presence of *Platycephalus indicus* made a sharp contrast. A single South China Sea distribution of *Platycephalus indicus* suggested this species might prefer warm water and live in lower latitude areas. Further domestic and overseas specimen collection is also indispensable in order to define its clearly geographic limits.

Accurate identification of fish is essential and would assist in managing fisheries for long-term sustainability, and improve ecosystem research and conservation. Resolution of cases of this nature will require careful morphological analysis from expert taxonomists before any final recommendations can be made (Ward *et al.*, 2005; Xiao *et al.*, 2016). Mitochondrial sequence divergences are strongly linked to the process of speciation, DNA barcoding and morphological analysis should go hand-in-hand. The data presented here would aid more information and explicit species taxonomy and avoid numerous misidentification and erroneous distributional records within *Platycephalus* genus. We hope this study will not only promote the sustainable exploitation, biodiversity conservation and fisheries management of *Platycephalus*

distributed in China but also contribute to species identification within this genus in the future.

## CONCLUSION

The morphological characters and COI sequence analysis revealed that specimens collected from the coastal waters of Beihai were consistent with typical *Platycephalus indicus* individuals and could thoroughly separate them from other *Platycephalus* species. Our present study confirmed the existence of true *Platycephalus indicus* and providing plentifully morphological characters of this species. We hope this study will not only promote the sustainable exploitation, biodiversity conservation and fisheries management of *Platycephalus* but also contribute to species identification within this genus in the future.

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### Statement of conflict of interest

Authors have declared no conflict of interest.

## REFERENCES

- Bian, X.D., Zhang, X.M., Gao, T.X. and Xiao, Y.S., 2008. Morphological and genetic identification of Japanese halfbeak (*Hyporhamphus sajori*) eggs. *J. Fish. China*, **32**: 342-352.
- Chang, H., Sha, X., He, G. and Song, L., 1980. A description of the morphological characters of the eggs and larvae of the flathead fish *Platycephalus indicus*. *Oceanol. Limnol. Sin.*, **11**: 161-171.
- Chen, W., 1982. A report on the peculiarities of sexual maturation of flathead fish *Platycephalus indicus* Linnaeus. *Trans. Oceanol. Limnol.*, **1**: 36-40.
- Chen, W. and Zhao, W., 1986. Age and growth of the flathead fish (*Platycephalus indicus* Linnaeus) in Yellow Sea. *J. Fish. China*, **10**: 289-304.
- Domingues, R.R., Amorim, A.F. and Hilsdorf, A.W.S., 2013. Genetic identification of *Carcharhinus* sharks from the southwest Atlantic Ocean (Chondrichthyes: Carcharhiniformes). *J. appl. Ichthyol.*, **29**: 738-742. <https://doi.org/10.1111/jai.12154>
- Gao, T.X., Ji, D.P., Xiao, Y.S., Xue, T.Q., Yanagimoto, T. and Setoguma, T., 2011. Description and DNA barcoding of a new *Sillago* species, *Sillago sinica* (Perciformes: Sillaginidae), from coastal waters of China. *Zool. Stud.*, **50**: 254-263.
- Gross, M., 2012. Barcoding biodiversity. *Curr. Biol.*, **22**: 73-76. <https://doi.org/10.1016/j.cub.2012.01.036>
- Hajibabaei, M., Singer, G.A.C., Hebert, P.D.N. and Hickey, D.A., 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends Genet.*, **23**: 167-172. <https://doi.org/10.1016/j.tig.2007.02.001>
- He, W.P., Cheng, F., Li, Y.X., Liu, M., Li, Z.J. and Xie, S.G., 2011. Molecular identification of *Coilia ectenes* and *Coilia mystus* and its application on larval species. *Acta Hydrobiol. Sin.*, **35**: 565-571.
- Hebert, P.D.N., Cywinska, A., Ball, S.L. and Waard, J.R., 2003. Biological identification through DNA barcodes. *Proc. R. Soc. B Biol. Sci.*, **270**: 313-321. <https://doi.org/10.1098/rspb.2002.2218>
- Hebert, P.D.N., Stoeckle, M.Y., Zemplak, T.S. and Francis, C.M., 2004. Identification of birds through DNA barcodes. *PLoS Biol.*, **2**: 1657-1663. <https://doi.org/10.1371/journal.pbio.0020312>
- Hureau, J.C., 1986. Platycephalidae. In: *Fishes of the North-Eastern Atlantic and the Mediterranean* (eds. P.J.P. Whitehead, M.L. Bauchot, J.C. Hureau, J. Nielsen, E. Tortonese), Volume III. UNESCO, Paris, France, pp. 1241-1242.
- Imamura, H., 1996. Phylogeny of the family Platycephalidae and related taxa (Pisces: Scorpaeniformes). *Species Divers.*, **1**: 123-233.
- Imamura, H., 2006. Re-diagnosis of the marbled flathead, *Platycephalus marmoratus* (Actinopterygii: Teleostei: Platycephalidae), with comments on the composition of the type series. *Species Divers.*, **11**: 295-306.
- Imamura, H., 2008. Synonymy of two species of the genus *Platycephalus* and validity of *Platycephalus westraliae* (Teleostei: Platycephalidae). *Ichthyol. Res.*, **55**: 399-406. <https://doi.org/10.1007/s10228-008-0046-1>
- Imamura, H., 2012. Re-description of *Platycephalus angustus* Steindachner 1886 (Teleostei: Platycephalidae), a valid flathead in northern Australia and New Guinea. *Ichthyol. Res.*, **60**: 112-121. <https://doi.org/10.1007/s10228-012-0319-6>
- Imamura, H., 2013a. *Platycephalus mortoni* Macleary 1883, a junior synonym of *Platycephalus fuscus* Cuvier 1829 (Teleostei: Platycephalidae). *Ichthyol. Res.*, **60**: 282-286. <https://doi.org/10.1007/s10228-013-0348-9>
- Imamura, H., 2013b. Validity of *Platycephalus*

- grandispinis* Cuvier, 1829, with priority over *Platycephalus longispinis* Macleay, 1884 (Actinopterygii: Scorpaeniformes: Platycephalidae). *Species Divers.*, **18**: 183–192. <https://doi.org/10.12782/sd.18.2.183>
- Imamura, H., 2015. Taxonomic revision of the flathead fish genus *Platycephalus* Bloch, 1785 (Teleostei: Platycephalidae) from Australia, with description of a new species. *Zootaxa*, **3904**: 151–207. <https://doi.org/10.11646/zootaxa.3904.2.1>
- Imamura, H. and Knapp, L.W., 2009. *Platycephalus orbitalis*, a new species of flathead (Teleostei: Platycephalidae) collected from Western Australia. *Zootaxa*, **2271**: 57–63.
- Imamura, H., Komada, M. and Yoshino, T., 2006. Record of the flathead fishes (Perciformes: Platycephalidae) collected from Nha Trang, Vietnam. *Coast. mar. Sci.*, **30**: 293–300.
- Ivanova, N.V., Zemlak, T.S., Hanner, R.H. and Hebert, P.D., 2007. Universal primer cocktails for fish DNA barcoding. *Mol. Ecol. Notes*, **7**: 544–548. <https://doi.org/10.1111/j.1471-8286.2007.01748.x>
- Jin, X.B., 2006. *Fauna Sinica of China, Osteichthyes, Scorpaeniformes*. Science Press, Beijing, China, pp. 529–533.
- Kamei, M. and Ishiyama, R., 1968. *Morphology and ecology of two types of flathead fishes belonging to the genus Platycephalus*. Proceedings of the Meeting of the Japanese Society of Scientific Fisheries, Tokyo, pp. 43.
- Knapp, L.W., 1999. Platycephalidae. In: *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific* (eds. K.E. Carpenter and V.H. Niem), Volume 4. Bony Fishes Part 2 (Mugilidae to Carangidae), FAO, Rome, pp. 2385–2421.
- Knapp, L.W., Imamura, H. and Sakashita, M., 2000. *Onigocia bimaculata*, a new species of flathead fish (Scorpaeniformes: Platycephalidae) from the Indo-Pacific. *Special Publ.*, **64**: 1–8.
- Kong, X., Yu, Z., Xie, Z. and Xu, W., 1994. Study on the karyotype and Ag-NOR banding of *Platycephalus indicus* (Linnaeus). *J. Ocean Univ. Qingdao*, **24**: 344–348.
- Masuda, Y., Haraguchi, M., Ozawa, T., Matsui, S. and Hayashi, S., 1997. Morphological and biochemical comparisons of two flathead species of the genus *Platycephalus* collected from the Western Suonada and Southern Yatsushiro Seas, Japan. *Nippon Suisan Gakkaishi*, **63**: 345–352. <https://doi.org/10.2331/suisan.63.345>
- Masuda, Y. and Ozawa, T., 2000. Age and growth of the flathead, *Platycephalus indicus*, from the coastal water of west Kyushu, Japan. *Fish. Res.*, **46**: 113–121. [https://doi.org/10.1016/S0165-7836\(00\)00138-7](https://doi.org/10.1016/S0165-7836(00)00138-7)
- Masuda, Y., Shinohara, N. and Ozawa, T., 1991. Catches and distributions of two flathead species of genus *Platycephalus* in the southern Yatsushiro Sea, Japan. *Nippon Suisan Gakkaishi*, **57**: 1257–1262. <https://doi.org/10.2331/suisan.57.1257>
- Ming, Q.L., Shen, J.F., Cheng, C. and Feng, Z.J., 2015. Genetic relationships between *Tribolium castaneum* and *T. confusum* based on mitochondrial DNA sequences. *Pakistan J. Zool.*, **47**: 1405–1412.
- Nakabo, T., 2002. *Fishes of Japan with pictorial keys to the species*, English Edition. Tokai University Press, Kanagawa, Japan, pp. 615.
- Osatomi, K., Nozoe, T., Tsunemoto, K. and Hara, K., 2001. PCR-RFLP analysis of two Flathead species of genus *Platycephalus* from the Ariaka Sound, Japan. *Nippon Suisan Gakkaishi*, **67**: 503–504. <https://doi.org/10.2331/suisan.67.503>
- Puckridge, M., Andreakis, N., Appleyard, S.A. and Ward, R.D., 2013. Cryptic diversity in flathead fishes (Scorpaeniformes: Platycephalidae) across the Indo-West Pacific uncovered by DNA barcoding. *Mol. Ecol. Resour.*, **13**: 32–42. <https://doi.org/10.1111/1755-0998.12022>
- Qin, Y., Song, N., Zou, J.W., Zhang, Z.H., Cheng, G.P., Gao, T.X. and Zhang, X.M., 2013. A new record of flathead fish (Teleostei: Platycephalidae) from China based on morphological characters and DNA barcoding. *Chinese J. Oceanol. Limnol.*, **31**: 617–624. <https://doi.org/10.1007/s00343-013-2186-z>
- Shao, K.T. and Chen, J.P., 1987. Fishes of the family Platycephalidae (Teleostei: Platycephaloidei) of Taiwan with descriptions of two new species. *Bull. Inst. Zool. Acad. Sin.*, **26**: 77–94.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S., 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.*, **28**: 2731–2739. <https://doi.org/10.1093/molbev/msr121>
- Tang, Q., 2006. *Marine fishery resources and habitat of China's exclusive economic zone*. Science Press, Beijing, pp. 370.
- Taniguchi, N., Ochiai, A. and Miyazaki, T., 1972. Comparative studies of the Japanese Platycephalid fishes by electropherograms of muscle protein, LDH and MDH. *Japanese J. Ichthyol.*, **19**: 89–96.
- Ward, R.D., Hanner, R. and Hebert, P.D.N., 2009. The campaign to DNA barcode all fishes, FISH-BOL.



- J. Fish Biol.*, **74**: 329-356. <https://doi.org/10.1111/j.1095-8649.2008.02080.x>
- Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. and Herbert, P.D.N., 2005. DNA barcoding Australia's fish species. *Phil. Trans. R. Soc. London Ser. B: Biol. Sci.*, **360** (1462): 1847-1857. <https://doi.org/10.1098/rstb.2005.1716>
- Xiao, J.G., Song, N., Gao, T.X. and McKay, R.J., 2016. Redescription and DNA barcoding of *Sillago indica* (Perciformes: Sillaginidae) from the coast of Pakistan. *Pakistan J. Zool.*, **48**: 317-323.
- Yamada, U., Tokimura, M., Horikawa, H. and Nakabo, T., 2007. *Fishes and fisheries of the East China and Yellow Seas*. Tokai University Press, Kanagawa, Japan, pp. 493-496.
- Zemlak, T.S., Ward, R.D., Connell, A.D., Holmes, B.H. and Hebert, P.D.N., 2009. DNA barcoding reveals overlooked marine fishes. *Mol. Ecol. Resour.*, **9** (Suppl-1): 237-242. <https://doi.org/10.1111/j.1755-0998.2009.02649.x>
- Zhang, C., Cheng, Q., Zheng, B., Li, S., Zheng, W. and Wang, W., 1994. *The fishes of the Yellow and Bohai Sea*. The Sueichan Press, Keelung, Taiwan, pp. 255-256.
- Zhu, Y., Zhang, C. and Cheng, Q., 1963. *The fishes of the East China Sea*. Science Press, Beijing, pp. 485-486.