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A new record of blue-spotted seabream *Pagrus caeruleostictus* from Chinese coastal waters documented from morphology and DNA barcoding*

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Abstract A new record of *Pagrus caeruleostictus* (Valenciennes, 1830), collected from the Beibu Gulf, South China Sea in April 2013, was documented based on morphology and cytochrome oxidase I subunit (COI) gene barcoding analyses. It can be distinguished by a combination of the following characteristics: head scaled to above eyes; cheeks with 5 or 6 rows of scales; lateral line scales 51–52; 5 rows of scales above the lateral line; 4 and 6 cuspidate teeth in front of upper and lower jaws, respectively, followed by 2 rows of blunter teeth posteriorly; gill rakers on first arch 12 to 15; D XI–XII +9–11; A III +8–9; the first two dorsal spines very short, the third to fifth extended, filamentous in the young; the first pelvic ray filamentous; silvery pink with dark blue spots on back and sides; caudal fin pinkish; other fins bluish or pinkish; the blue spots generally disappear in large specimens. The present report of *P. caeruleostictus* suggests that its distribution in Chinese coastal waters may be attributed to human effort, since this species is unlikely to have expanded naturally from the eastern Atlantic to the South China Sea, with no other records from the Indian or Pacific Oceans. We recommend that a precautionary approach should be adopted for the management of *P. caeruleostictus*.

Keyword: Sparidae; *Pagrus caeruleostictus*; cytochrome oxidase I subunit (COI); Chinese Seas

1 INTRODUCTION

Fishes of the genus *Pagrus* (Sparidae) are commonly known as seabreams or porgies and are distributed in the tropical and temperate waters of the Atlantic and West-Pacific oceans. A number of species within the genus are commercially important, both in fisheries and in aquaculture. Six species have been recognized within the genus *Pagrus* (Wu et al., 2012; Froese and Pauly, 2014): *P. pagrus* (Linnaeus, 1758), *P. auratus* (Forster, 1801), *P. caeruleostictus* (Valenciennes, 1830), *P. auriga* Valenciennes, 1843, *P. major* (Temminck et Schlegel, 1843), and *P. africanus* Akazaki, 1962. Of these species, only two, the red seabream *P. major* and the Australian snapper *P. auratus*, are native to the western Pacific, whereas the other species are native to the Mediterranean Sea and the Atlantic. The blue-spotted seabream *P. caeruleostictus* is distributed in the eastern Atlantic, from Portugal to Angola, including the area around the Canary Islands, and also in warmer parts of the Mediterranean, but not the Black Sea (Whitehead et al., 1986).

During a study of sparid fishes in Chinese Seas, a collection trip was organized in April 2013, during which two unusual specimens were collected by otter trawl at depths of 30–80 m in the Beibu Gulf (18°30′N, 107°45′E), South China Sea. Based on morphological and mitochondrial DNA (mtDNA) cytochrome oxidase I subunit (COI) barcoding analyses, these two

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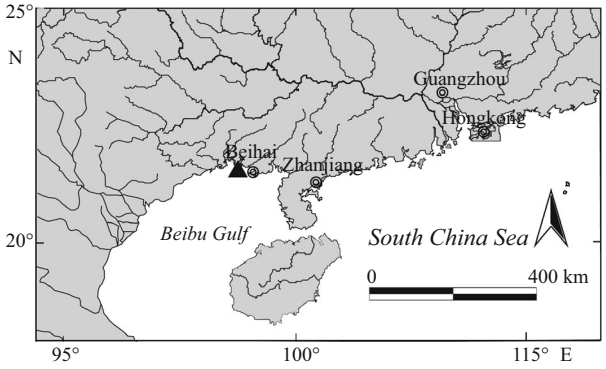


Fig.1 Map showing the collection sites in Chinese Seas

▲: locality of *Pagrus caeruleostictus*.

specimens were identified as *P. caeruleostictus* and may represent the first documented occurrence of this species in Chinese Seas.

2 MATERIAL AND METHOD

2.1 Sampling

The sample materials were collected by otter trawl from the Beibu Gulf (18°30′N, 107°45′E), South China Sea in April 2013 (Fig.1). These two sparid specimens were preserved in 95% ethanol, and deposited in the College of Life Science, Hebei University (HU) under the specimen identification numbers HU 2013092 and HU 2013093.

2.2 Morphology

Twenty-two morphometric variables were measured. Measurements were made in the laboratory using digital calipers (KENTA, KT5-230-63) to the nearest 0.1 mm. Total, standard, predorsal, prevental, preanal, head, and preorbital lengths were measured from the tip of snout to the end of the longest caudal end lob, to the extremity of the hypural complex at the mid-height of the caudal fin base, to the anterior origin of the dorsal fin, to the anterior origin of the pelvic fin, to the anterior of the anal fin, to the mid-lateral end of the operculum, and to the nearest point of the eye rim, respectively. The length of the caudal peduncle was measured from the base of the last anal ray to the extremity of the hypural complex, at the lower edge of the caudal base. Dorsal spine III, IV, and V, anal spine I, II, and III, and pelvic spine I lengths were measured from the base of spine to the end of the tip. Ventral and pectoral ray lengths were measured as the greatest distance from the base of first ray to the end of the tip. The depth of the body and least depth of the caudal peduncle were measured as the vertical distance from the mid-line of insertion of the anterior dorsal fin to the midline position of its opposite ventral surface, and at the narrowest part of the caudal peduncle. The eye diameter was taken at the longitudinal length of the eye. The interorbital width was determined as the narrowest distance between the orbital rims. Body width was determined as the widest distance between the body sides. Morphometric data are presented as percentages of standard length (SL). Measurements were made on the left side of the specimens.

2.3 DNA extraction, amplification and sequencing

Total DNA was extracted from muscle tissue using the standard phenol-chloroform method (Sambrook and Russell, 2001). The mtDNA COI gene was using for identifying the species of these two specimens with the following primers: F1: (5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3') and R1: (5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3') (Ward et al., 2005). The polymerase chain reaction (PCR) was carried out in 25 μL volumes containing 20–50 ng/μL template DNA, 2.5 μL of 10× reaction buffer, 1 μL of $MgCl_2$ (2.5 mmol/L), 1 μL of dNTPs (5 mmol/L), 1 μL of each primer (10 pmol/μL), 1 unit of *Taq* DNA polymerase (TaKaRa Co., Japan), and dH_2O. Amplification of the products was carried out in a Biometra thermal cycler (Biorad, California, USA) under the following conditions: 3 min initial denaturation at 95°C, followed by 35 cycles of 30 s at 94°C for denaturation, 30 s at 52–60°C for annealing, and 1 min at 72°C for extension; and a final extension at 72°C for 10 min. PCR products were purified using a Gel Extraction Mini Kit (Tiangen BioTech Co., Beijing, China) and sequenced on an ABI Prism 3 730 (Applied Biosystems, USA) automatic sequencer.

2.4 Sequence analysis

The sequence was aligned and compared to a homologous sequence of *Pagrus* species from GenBank (http://www.ncbi.nlm.nih.gov). Another species from the genus *Dentex* in the family Sparidae, *Dentex macrophthalmus*, was used as an outgroup. The species and their GenBank accession numbers are listed in Table 1. Base composition and genetic distance based on Kimura-2 parameter (K2P) was calculated using MEGA 5.05 (Tamura et al., 2011). The phylogenetic relationship among *Pagrus* species was reconstructed using the neighbor-joining (NJ) method implemented in MEGA 5.05 and evaluated with 1 000 bootstrap replicates.

Table 1 Information of the *Pagrus* species analyzed in this study, including sample sites and their GenBank accession numbers

Species	Locality	Accession Nos.	Reference
HU 2013092	Beibu Gulf, South China Sea	KF857267	This study
HU 2013093	Beibu Gulf, South China Sea	KF857268	This study
P. caeruleostictus	Italy	JN868714	GenBank
*P. major*1	Nagasaki, Japan	JF952801	Zhang and Hanner, 2011
*P. major*2	South China Sea	FJ237870	Zhang et al., 2007
*P. major*3	Japan	GU207341	GenBank
P. pagrus	Turkey	KC501091	Keskın and Atar, 2013
P. augiga	Turkey	KC501071	Keskın and Atar, 2013
Chrysophrys auratus (=*P. auratus*)		EU752073	Yancy et al., 2008
Dentex macrophthalmus	Turkey	KC500492	Keskın and Atar, 2013

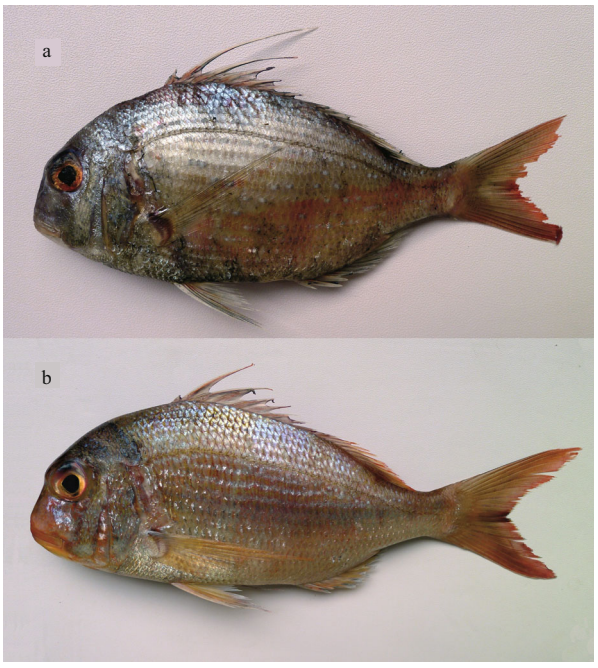


Fig.2 *Pagrus caeruleostictus* (Valenciennes, 1830)

a. lateral view (HU 2013092, 120.5 mm SL); b. lateral view (HU 2013093, 234.3 mm SL).

3 RESULT

3.1 Diagnosis

Pagrus caeruleostictus (Fig.2) is distinguished from other congeners by the following combination of characters (Whitehead et al., 1986): body oblong, deep, compressed. Head scaled to above eyes. Cheeks with 5 or 6 rows of scales. Lateral line scales 51 to 52, five rows of scales above the lateral line. Four and six cuspidate teeth in front of upper and lower jaws, respectively, followed by blunter teeth that become progressively molar-like and are arranged in 2 rows. Gill rakers 12 to 15 on first arch. Dorsal fin with XI to XII spines and 9 to 11 soft rays; anal fin with III spines and 8 to 9 soft rays; the first two dorsal spines very short, the third to fifth longest and filamentous in the young; the first pelvic ray filamentous. Silvery pink with dark blue spots on back and sides; caudal fin pinkish; other fins bluish or pinkish; old individuals with numerous irregular dark blotches on head.

3.2 Description

Counts and measurements are given in Table 3. Body oblong, deep, compressed. Profile of head and occiput rising less steeply than its related species of the genus *Evynnis*, interorbital space not as highly arched. Width of preopercle considerably less than that of the scaled cheek. Posterior nostril slit-like, its distance from the eye about 3.3–5.1 in orbital length. Mouth low, terminal, slightly oblique. Jaws very strong; lips thick. Vomer toothless. Teeth in upper jaw in two rows posteriorly, the inner molar, the outer less so, the latter becoming more conical towards the anterior, the former being replaced by a band of minute granular teeth in three series. Two canines present on either side anteriorly. In lower jaw, dentition similar, but with three anterior canines on side. Dorsal fin with 11 or 12 spines and 9 or 10 soft rays; the first 2 spines always very short, the third and fifth longest, filamentous in the young; third, longer than head by half length of eye in the young, and less than in the adult, about 0.6 of head; fourth, length less than head both in young and adult, about 0.8 of head in young and 0.6 in adult. Anal fin with 3 spines and 8 or 9 soft rays; second and third anal spines usually equal, second sometimes longer, 0.3 of head. Pelvic fin with 1 spine and 5 soft rays; the first soft ray

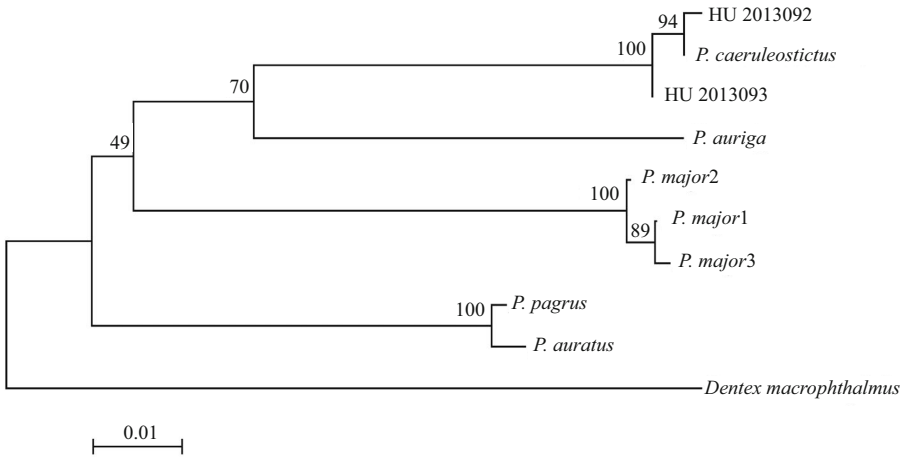


Fig.3 Neighbor-joining tree for *Pagrus* species based on a Kimura-2 parameter model calculated in MEGA 5.05 based on mtDNA COI sequences. Bootstrap percentages are shown on branches

Table 2 Pairwise net genetic distances among species of *Pagrus* estimated using the Kimura-2 parameter model based on mtDNA COI gene sequences

Species	1	2	3	4	5	6	7	8
1 HU 2013092								
2 HU 2013093	0.005							
3 *P. caeruleostictus*	0.002	0.004						
4 *P. major*1	0.133	0.126	0.131					
5 *P. major*2	0.128	0.122	0.126	0.004				
6 *P. major*3	0.135	0.128	0.133	0.002	0.005			
7 *P. pagrus*	0.107	0.100	0.105	0.108	0.104	0.110		
8 *P. auriga*	0.097	0.095	0.095	0.109	0.111	0.111	0.126	
9 *P. auratus*	0.109	0.102	0.107	0.110	0.106	0.112	0.005	0.128

filamentous, extending near anal fin insertion, 0.8 in head. Pectoral extending slightly beyond anal fin insertion, longer than head by half length of eye. Caudal deeply forked.

Body scales ctenoid, weak and moderate in size, extending onto bases of dorsal and anal rays. Head scaled to above eyes; cheeks with 5 or 6 rows of scales. Preopercular unscaled. Lateral line scales 51 to 52; five rows of scales above the lateral line.

Color of fresh specimen: fresh specimens pink with silvery reflections and large numerous small blue spots on the upper sides; belly silvery. In large specimens, the blue spots are not generally found. A dark blotch at base of last dorsal soft rays, Caudal fin pinkish; other fins bluish or pinkish. Old individuals with numerous irregular dark blotches on head. Our formalin specimens are nearly uniform in color, with a few traces of dark spots.

3.3 Sequence analyses of the COI gene

The mtDNA COI gene (657 bp) was used for phylogenetic reconstruction. Topology of the tree was provided by Neighbor-joining (NJ) analyses based on K2P model with 1 000 replications of a bootstrapping test. *Dentex macrophthalmus* was used as outgroup (Fig.3). The phylogenetic tree consists of four main branches. The two specimens (HU 2013092, HU 2013093) and *P. caeruleostictus* cluster together with high bootstrap support (100%) (Fig.3), with a genetic distance of 0.02%–0.05% (Table 2 and a genetic distance of 9.5%–9.7% from the sister lineage of *P. auriga* (bootstrap value=70%) (Table 2). This clade was placed in a sister position to *P. major* (boot strap value =49%), with a genetic distance between them of 11.0% (*P. auriga* versus *P. major*1) to 13.5% (HU 2013092 versus *P. major*3). *P. pagrus* and *P. auratus* cluster together with a very high bootstrap value (100%) in the lower branches, with a genetic distance among other species of 0.05% (*P. pagrus* versus *P. auratus*) to 12.8% (*P. auriga* versus *P. auratus*).

Both the phylogenetic position of the two specimens and the genetic distances between interspecies of *Pagrus* support the conclusion that the two specimens examined in this study are *P. caeruleostictus*.

4 DISCUSSION

The blue-spotted seabream, *Pagrus caeruleostictus*, was originally described by Valenciennes (1830) under the name of *Chrysophrys caeruleostictus* based on one syntype from the Canary Islands, eastern Atlantic. Steindachner (1868) synonymized *C. caeruleostictus* under *Pagrus caeruleostictus*, but he

Table 3 Counts and measurements of *Pagrus caeruleostictus* collected from the Beibu Gulf, South China Sea

Morphometric (mm)/meristic characters	HU 2013092	HU 2013093
Total length	155.5	303.6
Standard length (SL)	120.5	234.3
Counts		
Dorsal fin rays	VI, 9	VII, 10
Anal fin rays	III, 8	III, 9
Pectoral fin rays	15	16
Lateral line scales	51	52
Scales above the lateral line	6	5
Transverse scales	6+0.5	6+0.5
Gill rakers	15	12
Measurements, as %SL		
Head length	32.4	32.6
Preorbital length	10.5	10.5
Body depth	15.7	15.9
Body width	43.6	42.2
Eye diameter	9.5	8.4
Interorbital width	10.3	11.0
Predorsal length	44.6	47.0
Preanal length	62.6	64.7
Prevental length	36.9	36.6
Dorsal spine III	36.2	20.7
Dorsal spine IV	27.3	19.2
Dorsal spine V	19.2	13.6
Anal spine I	6.1	4.0
Anal spine II	10.5	9.3
Anal spine III	9.6	8.5
Ventral spine I	17.7	16.6
Ventral rays length	24.7	26.4
Pectoral length	37.6	36.3
Caudal peduncle depth	11.2	16.2
Caudal peduncle length	16.4	10.8

did not provide an adequate description and explanation. Thereafter, subsequent authors have followed his view (Fowler, 1936; Whitehead et al., 1986; Wu et al., 2012; Froese and Pauly, 2014).

According to Whitehead et al. (1986), the blue-spotted seabream is restricted to the tropical and warm temperate waters of the Mediterranean Sea and eastern Atlantic, occurring from Portugal and the Strait of Gibraltar to Angola. There is no record of its presence in the Indian and Pacific oceans. It is neither listed in a recent checklist of marine species of Chinese Seas (Liu, 2008) nor in the checklist of the fishes of the South China Sea (Randall and Lim, 2000). No further findings were reported in Chinese Seas until the present study. Our record of two specimens from April 2013 in the Beibu Gulf, South China Sea, demonstrates the presence of the species in this area, although the capture during this study should be regarded as accidental. *P. caeruleostictus* is unlikely to have extended its range naturally from the eastern Atlantic to the South China Sea, especially given the lack of other records from the Indian or Pacific Oceans. The most probable explanation for this record is the escape or dispersal of *P. caeruleostictus* during introduction or transportation in Chinese Seas or adjacent waters. It is unknown which Pacific Northwest country introduced the fertilized eggs or live-product of this species, though frozen products of this species have been imported into Japan, Korea and Hong Kong from some eastern Atlantic countries (Y. Iwatsuki, personal communication) in recent years. Despite this, it is clear this species has not been imported into the Chinese mainland. Based on our observation of young and adult specimens of this species, we believe that this species is reproducing in the wild in the Beibu Gulf, South China Sea.

This study documents the first occurrence of *P. caeruleostictus* from Chinese Seas, although it is well documented in the Mediterranean Sea and eastern Atlantic regions (Whitehead et al., 1986). As our report is based on two specimens, additional catches of specimens are needed to confirm whether there is an established population of this species in this area. If so, the potential impacts of these species on the native fish community should be assessed.

We conclude that two species of the genus *Pagrus*, *P. caeruleostictus* and *P. major*, are found in Chinese Seas. *P. caeruleostictus* can be easily distinguished from *P. major* by the following characters: 1) the third to fifth dorsal spines extended and filamentous versus not extended; 2) the first pelvic ray filamentous versus not filamentous; 3) lateral line scales 51–52 versus 56–63; 4) cheeks with 5 or 6 rows of scales versus 7 or 8 rows of scales; 5) back silvery pink with dark blue spots on sides versus back reddish with blue spots on sides.

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