

Two New Records of *Upeneus Spottocaudalis* and *U. Sundaicus* (Perciformes: Mullidae) from the Northern South China Sea Based on the Morphology and DNA Barcoding

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Abstract

In this study, two new records of goatfishes *Upeneus spottocaudalis* and *U. sundaicus* from the South China Sea, combining evidence from morphology and DNA barcodes for species identification. ML tree and NJ tree result showed that the sequences of *U. spottocaudalis* and *U. sundaicus* were clustered with the homologous sequences from GenBank, respectively, and the intraspecific genetic distances of *U. spottocaudalis* (0.2%) and *U. sundaicus* (0.3%) were less than 2%. Automatic Barcode Gap Discovery (ABGD) analysis also supported this result of classification.

Main Text

The genus of *Upeneus* (Perciformes: Mullidae) up to 46 goatfish species worldwide and widely distributed in tropical and subtropical ocean area (Uiblein and Gouws 2014; Nelson and Mark 2016; Uiblein and Motomura 2021). In China, the goatfishes species diversity were decreased with latitude, such as only one species *U. japonicus* was recorded in the Bohai Sea and the Huanghai Sea (Jiao and Chen 2000), including *U. tragula*, *U. vittatus*, *U. subvittatus*, *U. sulphureus*, *U. quadrilineatus*, *U. moluccensis*, *U. japonicus*, *U. luzonius* and *U. heterospinus* (Cheng et al. 1962; Shen 1993; Chen and Zhang 2015; Uiblein et al. 2019). Goatfishes sometimes are difficult to accurately identify based on the morphology method because the color patterns of different stages of development was unclear, and fading and discoloring of body color after death (Pavlov and Emel Yanova 2018). Using DNA barcoding technology can provide some level to reduce the difficulty of identification and enhance the accuracy of identification (Yi et al. 2018; Sharifuzzaman et al. 2021).

The South China Sea, as the most significant water area in China, possesses complex habitats to provide various marine organisms with a well living environment, leading to abundant biological resources in the South China Sea (Shan et al. 2021). However, an underestimated species diversity is not conducive to the protection of biodiversity and biological resources. Therefore, our study intends to close the knowledge gap by contributing to the taxonomy of the genus *Upeneus* in the South China Sea.

Specimens were collected from local fish markets near the northwestern South China Sea. Specimens and tissue samples were deposited at the College of Fisheries, Guangdong Ocean University (sampling sites see table S1). The counts and measurements were based on Uiblein and Heemstra (Uiblein and Heemstra 2010). COI gene was amplified by PCR using the two pairs universal primers from Ward et al. (Ward et al. 2005), and the PCR products were sequenced by Sangon Biotech (Shanghai). Thirty-three sequences were aligned and manually edited using Sequencher 5.4.5 (Gene Codes Corp., America). These sequences were deposited in GenBank (MZ513942-MZ513973 and MZ514010). Kimura 2-parameter (K2P) model was employed to construct the neighbour-joining (NJ) tree and calculated the genetic distances by MEGA 6.0. The maximum likelihood (ML) tree was constructed using IQ-TREE (Nguyen et al. 2015) under the best-fit model, HKY+G4+F selecting BIC criterion by ModelFinder (Kalyaanamoorthy et al. 2017) for 5000 ultrafast bootstraps (Minh et al. 2013) and 1000 replicates of Shimodaira-Hasegawa test (SH-aLRT) (Guindon et al. 2010). The IQ-TREE and ModelFinder were plugins in PhyloSuite v1.2.2 (Zhang

et al. 2020). All homologous sequences of *Upeneus* were from GenBank. Automatic Barcode Gap Discovery (ABGD) was carried out on the website with default parameter (<https://bioinfo.mnhn.fr/abi/public/abgd/>). Information of specimens and COI sequences are provided in Table S1.

Morphometric measurements of *U. spottocaudalis* and *U. sundaicus* was shown in Table 1. *Upeneus spottocaudalis* was firstly recorded in the southern Indonesia and the northeastern Australia (Uiblein et al. 2017), and Fricke (Fricke et al. 2019) found the distribution extended to the New Ireland. This species with VII spines in the first dorsal-fin are divided into *japonicus*-group; head and body dorsally reddish; mid-lateral body with a faint reddish band extending the upper base of caudal-fin; series red blotches on the body; barbels yellow; lower caudal-fin with rounded or triangular dark spots or blotches is the key taxonomic features to distinguish with other species of *japonicus*-group, which only are with oblique stripes in lower caudal-fin (Fig. 1a). *Upeneus sundaicus* with VIII spines in the first dorsal-fin belongs to *tragula*-group; head and body dorsally pale brown; a blonder brown stripe on the mid-lateral body from behind the upper operculum to the base of the caudal-fin; barbels yellow; lower caudal-fin without any spot or stripe; upper caudal-fin with series weak stripes (Fig. 1c).

The phylogenetic tree (Fig. 2) shows that our sequences of *U. spottocaudalis* were clustered with the sequence (GU673189) of paratype of *U. spottocaudalis*. Furthermore, the *U. sundaicus* sequences in this study of were clustered with the sequences identified as *U. sundaicus* from GenBank. These two results were also presented in the NJ tree. All of these clades were supported with high value of SH-aLRT, and ultrafast bootstrap. The intraspecific genetic distances of *U. spottocaudalis* (0.2%) and *U. sundaicus* (0.3%) were much less than 2% (Table S1). Result of classification based on ABGD was concordant with the ML tree and the NJ tree (Fig. 2).

Taxonomy is the most basic subject in biological studies, and accurate species identification determines the feasibility of subsequent studies. Two new records species of *U. spottocaudalis* and *U. sundaicus* were identified integrating morphology and DNA barcodes, which enrich the diversity of ichthyofauna of the South China Sea. It is of certain reference value to the taxonomy of the genus *Upeneus* in the South China Sea.

Declarations

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Authors' contributions Zhisen Luo investigated, collected the specimens, conducted the experiments, finished the drawing, analyzed data, edited the original draft and reviewed the manuscript. Murong Yi,

investigated, collected the specimens, provided the methodology, supervised the experiments, designed, reviewed and edited the manuscript, decided on the final manuscript. Kangwen Qiu assisted to the experiments, reviewed and edited the manuscript. Sibiao Liu analyzed data, reviewed and edited the manuscript. Sui Gu, supervised the experiments, reviewed and edited the manuscript. Yunrong Yan provided experimental conditions, supervised, reviewed and edited the manuscript.

Availability of data and material Information of COI sequences are available in the NCBI website (<https://www.ncbi.nlm.nih.gov/>). Information of collection of specimens of this study are provided in the Supplementary Table Captions. All measurement and analyzed data are presented in this manuscript. Automatic Barcode Gap Discovery (ABGD) analysis is conducted on the ABGD website (<https://bioinfo.mnhn.fr/abi/public/abgd/>).

Ethical Approval All animal experiments were carried out in accordance with the guidelines and approval of the Animal Research and Ethics Committee of College of Fisheries, Guangdong Ocean University (permissions, B20181030-01).

Consent to participate On behalf of all authors, consent to participate this manuscript writing and contribute to Conservation Genetics Resources.

Consent to publication On behalf of all authors, consent to publish this article.

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Tables

Table 1 Morphometric measurements and meristic counts of *Upeneus spottocaudalis* and *Upeneus sundaicus* (% in SL, average value in parentheses)

	<i>Upeneus sundaicus</i>	<i>Upeneus spottocaudalis</i>
n	13	24
SL	100.3-146.5 (120.5)	87.9-122.7 (105.4)
in % SL		
Body depth at first dorsal-fin origin	24.9-31.3 (27.9)	24.3-28.2 (25.7)
Body depth at anal-fin origin	21.3-25.9 (23.6)	20.1-23.5 (21.8)
Half body depth at first dorsal-fin origin	19.6-25.9 (22.5)	18.2-25.7 (20.5)
Half body depth at anal-fin origin	15.7-20.5 (17.1)	13.9-23.2 (15.9)
Caudal-peduncle depth	10.8-13.7 (12.1)	9.4-11.3 (10.4)
Caudal-peduncle width	4.3-4.9 (4.7)	3.2-4.5 (3.8)
Maximum head depth	22.8-28.9 (25.1)	22.5-27.9 (24.2)
Head depth through eye	15.0-20.0 (17.4)	14.9-18 (16.2)
Suborbital depth	8.2-12.8 (10.8)	6.3-11.3 (8.1)
Interorbital length	6.7-8.3 (7.7)	7.1-8.5 (7.6)
Head length	22.3-29.2 (25.7)	22.6-27.7 (25.7)
Snout length	6.7-9.7 (8.4)	5.2-9.4 (7.6)
Postorbital length	9.0-12.6 (10.7)	9.5-11.5 (10.4)
Orbit length	5.1-7.9 (6.8)	6.9-8.5 (7.7)
Orbit depth	5.0-6.5 (5.7)	5.4-8.0 (7)
Upper-jaw length	7.2-11.7 (9.2)	6.5-10.0 (8.2)
Lower-jaw length	5.1-9.1 (7.3)	5.7-9.3 (7.1)
Snout width	6.4-8.8 (7.9)	5.6-9.1 (8.1)
Barbel length	15.3-20.0 (17.5)	15.0-19.0 (16.6)
Maximum barbel width	0.8-1.3 (1.0)	0.9-1.2 (1.0)
First pre-dorsal length	30.8-35.5 (32.8)	31.1-36.8 (33.5)
Second pre-dorsal length	59.4-70.1 (63.6)	61.9-66.5 (63.9)
Interdorsal distance	10.6-15.4 (13.0)	11.5-18.4 (15.5)
Caudal-peduncle length	20.6-27.1 (23.3)	19.2-29.1 (22.9)
Pre-anal length	62.1-70 (65.5)	63.5-72.8 (66.2)

Pre-pelvic length	16.2-32.8 (28.5)	26.9-31.4 (29.4)
Pre-pectoral length	24.4-30.4 (27.3)	25.5-32.9 (27.4)
Second dorsal-fin depth	22.4-25.9 (24.1)	20.6-23.9 (22.6)
Pelvic-fin depth	23.8-29.6 (26.6)	24.0-29.5 (25.7)
Pectoral-fin depth	13.8-19 (16.5)	13.0-18.1 (16.4)
Length of first dorsal-fin base	13.6-20.5 (17.3)	12.5-19.7 (14.4)
Length of second dorsal-fin base	12.4-17.1 (14.8)	12.0-15.6 (13.7)
Caudal-fin length	24.2-30 (27.4)	24.8-32.4 (29.1)
Length of anal-fin base	10.0-10.7 (10.3)	7.7-11.9 (10.2)
Anal-fin height	6.9-10.3 (9.0)	6.4-16.4 (9.9)
Pelvic-fin length	14.2-22.1 (18.9)	14.3-21.3 (18.6)
Pectoral-fin length	18.6-23.3 (20.3)	16.6-22.1 (19.9)
Pectoral-fin width	4.5-6.7 (5.6)	3.8-6.1 (4.5)
First dorsal-fin height	19.8-27.1 (23.7)	17.6-21.7 (19.6)
Second dorsal-fin height	14.2-16.5 (15.2)	15.9-17.1 (16.5)
Meristic characters		
Dorsal-fin spines	VIII+9	VII+9
Anal-fin spines	I-6	I-6
Pelvic-fin spines	I-5	I-5
Pectoral-fin spines	14	14
Scales along lateral line	28-31	26-30
Scales above lateral line	2.5	2
Scales below lateral line	4.5	3-4
Total gill rakers	4-6+13-15	3-5+10-15

Figures



Figure 1

Upeneus spottocaudalis, (a) fresh specimens, MuSY001, 107.1mm SL, Sanya, China; (b) preserved specimens, MuHK003, 104.2mm SL, Haikou, China. *Upeneus sundaicus*, (c) fresh specimens, GOU104525, 106.9mm SL, Zhanjiang, China; (d) preserved specimens, GOU104525, 106.9mm SL, Zhanjiang, China

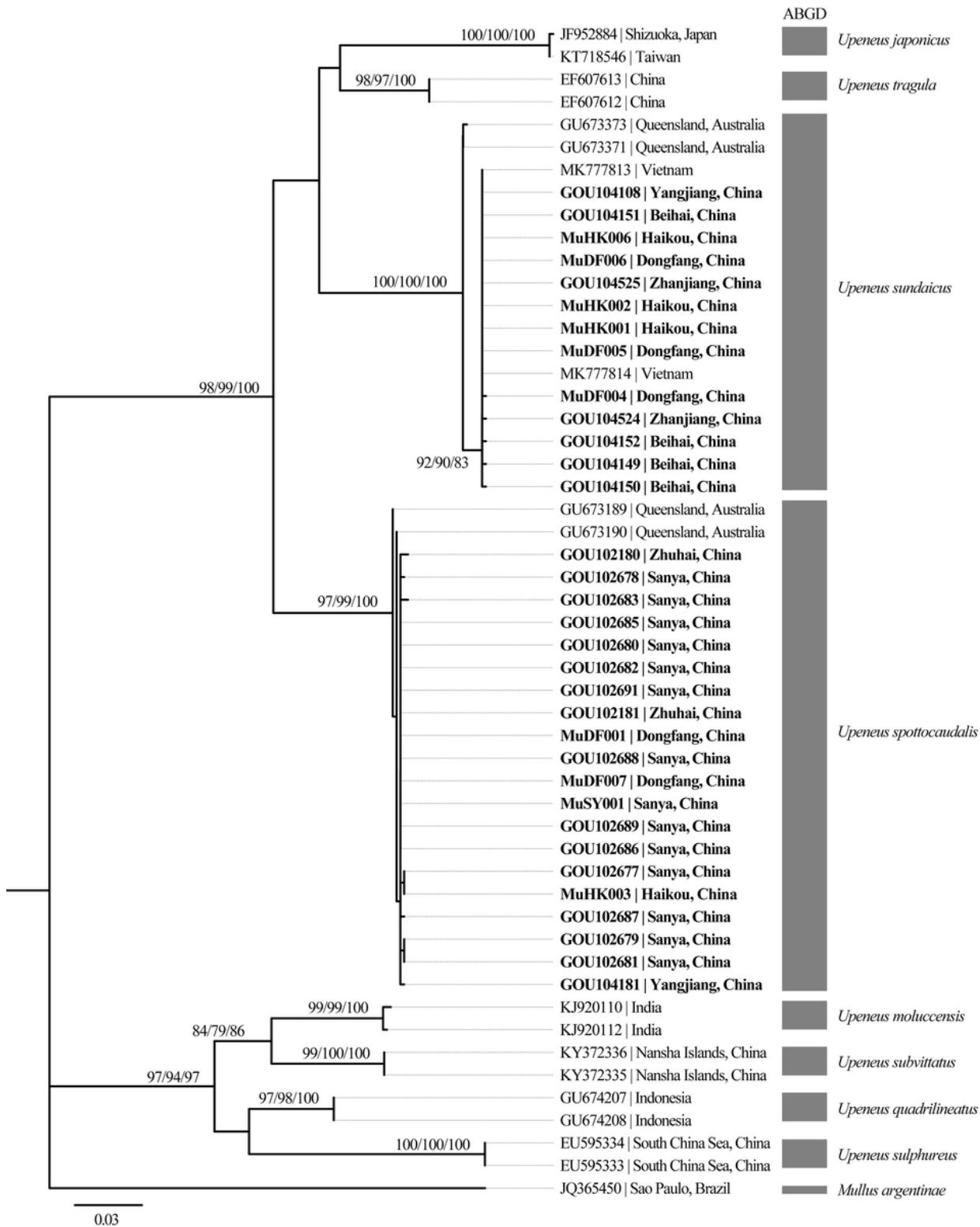


Figure 2

Maximum likelihood (ML) and neighbor-joining (NJ) tree of *Upeneus* based on DNA sequences of the mitochondrial COI gene (bold fonts for this study). Numbers on the branch are SH-aLRT support (%), and the bootstrap values for ML and NJ analyses. Only values above 70% are displayed. Gray rectangles for result of ABGD analysis, and each rectangle represents single species

Supplementary Files

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