

# Molecular and Morphological Analyses Suggest Cryptic Diversity of Eel Gobies, Genus *Taenioides* (Gobiidae), in Coastal Waters of China

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**Abstract**—The current classification of eel goby of genus *Taenioides* (Gobiidae: Amblyopinae) recognizes two species in coastal waters of China, which is based on morphological differences only. However, such classification could be dubious since the morphological differences among species are usually faint, and more cryptic species have been frequently identified in this genus around the region. To clarify the species diversity of *Taenioides* in China, we collected specimens that conformed to the definition of *Taenioides* from the full range of coastal waters in China, and estimated the species diversity and taxonomic validity of the genus proposed based on both molecular and morphological data. As a result, four distinct species were identified which were distinguished from each other on the basis of genetic divergences (0.047–0.128) estimated from all 13 mitochondrial PCGs sequences. The external morphology were generally conservative, but some distinct diagnostic morphological characters (i.e., total number of the dorsal-fin elements, numbers of vertebrae and barbels on the ventral surface of head) that delimit each species were also observed. The combined molecular and morphological data assigned three of them to be *T. anguillaris*, *T. gracilis* and *Taenioides* sp. (clade Thailand sensu Kurita, Yoshino 2012). The remaining one is considered a new confirmed candidate species, instead of the previously recognized *T. cirratus*. Our analyses provide the first evidence for an underestimation of the species richness in genus *Taenioides* in China coastal waters, and a taxonomic revision is in need for this genus around the region.

**Keywords:** cryptic diversity, eel goby, genus *Taenioides*

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## INTRODUCTION

The eel goby, genus *Taenioides* (Gobiidae: Amblyopinae) are endemic to coastal waters from South Africa eastward to Fiji (Murdy and Randall, 2002). These small to medium-sized muddy-bottoms-dwelling marine species are characterized by several conservative external morphology such as their elongated body, small eyes covered with skin, and numerous distinct dermal folds, lobes, or barbels on the head (Kurita and Yoshino, 2012). Some common meristic characteristics, such as the counts of the dorsal- and anal-fin elements and body proportions, that widely used in teleost taxonomy are so conservative among species that it may sometimes hamper the proper morphological analyses and species validation for this genus (Kurita and Yoshino, 2012). Furthermore the difficulties in collecting eel gobies due to their subterranean mud-dwelling life style, has led to their rarity in museum collections and hence their taxonomic

uncertainty on species level (Murdy and Randall, 2002).

The taxonomy of *Taenioides* has long been considered to be problematic (Murdy and Randall, 2002), and over time some flux in numbers of recognized species is evident (Murdy and Randall, 2002; Kurita and Yoshino, 2012). To date, fourteen nominal species in total were recognized in the genus *Taenioides* globally (Murdy, 2018). An extra cryptic species of *Taenioides* sp. (clade Thailand sensu Kurita, Yoshino 2012) was also recognized in Songkhla, Thailand by Kurita and Yoshino (2012) based on both molecular and morphological analyses. Most of them (fourteen) have been identified in coastal waters of Indo-Pacific Ocean (Murdy and Randall, 2002), which may represent a hot-spot zone with the richest diversity of *Taenioides* species in the world. In Asia, 7 *Taenioides* species have been identified. Among them, four species, *T. anguillaris* (Linnaeus, 1758), *T. snyderi* (Jordan and Hubbs, 1925), *T. gracilis* (Cuvier and Valenciennes

et al., 1837) and *T. cf. kentalleni* (Murdy and Randall, 2002) have been described in Japan and five species, *T. esquivel* (Smith, 1947), *T. anguillaris*, *T. coecula* (Bloch and Schneider, 1801), *T. gracilis*, *T. cirratus* (Blyth, 1860), *T. brachygaster* (Günther, 1861) has been recognized in India, though the exact taxonomy of these species has not been intensively re-examined. However, in China with a wider coastal waters of Indo-Pacific Ocean, only two *Taeniooides* species have been recognized, which involves *T. cirratus* and *T. anguillaris* (Zhu et al., 1963; Wu and Zhong, 2008). Again both of these species are mainly described on external morphology only, and confusions has been evident for *Taeniooides* taxonomy in this region (Wang et al., 2017). For instance, Wu and Zhong (2008) have described a form of *T. cirratus* in China, possessing 45–49 dorsal-fin, 38–45 anal-fin elements, 16–18 pectoral-fin rays and 6 barbels on the ventral surface of the head, which is differed from holotype, originally identified in India by Blyth (1860), which possessed 13 pectoral-fin elements only and 7 barbels. Zhu et al (1963) also recognized a holotype, more conforming to the definition of *T. cirratus* around the same region, which possessed 14 pectoral-fin rays only, but still distinct in number of barbels (6) on the ventral surface of the head. Wang et al. (2017) have suspected the diagnosis of some *T. cirratus* in China, and inferred it to be actually some other forms of *Taeniooides* species that were falsely identified. Such situation indicates that the appropriate name and the actual number of *Taeniooides* species distributed in China coastal waters should be re-examined.

To clarify this, we therefore re-examined all possible cryptic *Taeniooides* species collected from the full range of coastal waters in China. Considering the difficulties in recognizing *Taeniooides* species by morphological data alone (Kurita and Yoshino, 2012), We therefore first detected distinct *Taeniooides* species on the basis of mitochondrial DNA sequences, and then delimited the range of meristic characteristics for each species recognized by molecular markers. The purpose of the present study was to estimate the species diversity and the taxonomic validity of the *Taeniooides* species proposed in China. The outcome of the present research would provide valuable information for the scientific management and conservation for these species in the future.

## MATERIALS AND METHODS

### *Sampling, DNA Extraction, and Sequence Amplification*

Specimens conforming to the definition of *Taeniooides* were collected from various locations in coastal waters of China (Table 1, Fig. 1). Muscle tissues were removed from each sample and stored in 100% ethanol. Genomic DNA was isolated from 59 of them using standard phenol-chloroform method (Sambrook et al., 1989) and partial fragments of the *COI*

gene were firstly amplified using the universal *COI* barcoding primers F1: 5'-TCAACCAACCACAAAG-ACATTGGAC-3' and R1: 5'-TAGACTT CTGG-GTGG CCAAAGAATCA-3' (Ward et al., 2005) to identify the possible cryptic species. To further confirm these candidate cryptic species, the whole mitogenomes of the candidate cryptic species were thereafter amplified using the primers designed specifically for eel gobies (Supplement 1). The PCR analysis was conducted in 50- $\mu$ L volume containing 50 ng template DNA, 1 $\times$  reaction buffer, 2.0 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.2 mM each primer, and 4.0 units Taq DNA polymerase (Promega, USA) using a PTC-200 (BIORAD, USA) PCR machine. Each reaction was performed under the following conditions: 5 min initial denaturation at 94°C, 40 cycles of 1 min at 94°C for denaturation, 1 min at 50–53°C for annealing, and 1 min at 72°C for extension, and a final extension at 72°C for 5 min. All sets of PCR included a negative control reaction in which all reagents were included, except for the template DNA. The amplifications were examined on a 1.5% agarose gel and stained with ethidium bromide. The Gel Extraction Mini Kit (Watson Bio Technologies, China) was used to purify the PCR products. Thereafter, the PCR products were sequenced with the Sanger sequencing Technology procedure at Invitrogen Ltd., China.

### *Phylogenetic Analysis*

The obtained partial fragments of the *COI* sequences (GeneBank accession nos.: ON524578–ON524592, ON524748–ON524762, ON524734–ON524747, ON524801–ON524815) were firstly edited and aligned using MEGA 6.0 software (Tamura et al., 2013). A phylogenetic tree was inferred from these sequences and from that of seven Gobiidae species downloaded from GeneBank using the neighbor-joining (NJ) methods with a bootstrap of 1000 replicates to identify the cryptic species among the specimens. To further confirm these candidate cryptic species, the complete mitogenome was assembled for them (GeneBank accession nos.: MK541900, OL625024, MK541898, MW682859) using the gene fragments sequenced were used to assemble the complete mitogenome using CodonCode Aligner 5.1.5 (CodonCode Corporation, Dedham, MA). The complete mitogenome was annotated using MitoZ, a mitogenome toolkit (Meng et al. 2019). The protein-coding and ribosomal RNA genes were delimited using NCBI-BLAST. Transfer RNA genes and their clover leaf structures were identified using tRNAscan-SE 1.21 (Lowe and Chan 2016), with cut-off value set to 1 when necessary. The putative OL and control region were identified by sequence homology and supposed secondary structures. Sixteen mitogenomes of other Gobiidae species were downloaded from the GenBank and combined for phylogenetic analyses. Phylogenetic trees were inferred from all 13 annotated protein-cod-

**Table 1.** Sampling localities and size of *Taenioides* specimens used in this study

Clade	Locality	Collection numbers
Changjiang	Nansi Lake (Jining), Luoma Lake (Suqian), Gaoyou Lake (Huaian), Tai Lake (Suzhou), Chao Lake (Hefei), Changjiang Estuary (Chongming), Aojiang Estuary (Wenzhou)	65
Ruosonghe	Ruosonghe Estuary (Wenling)	31
Zhujiang	Zhujiang Estuary (Guangzhou), Nanduijiang Estuary (Haikou), Beibu Bay (Beihai),	27
Nanduijiang)	Nanduijiang Estuary (Haikou)	32

ing gene sequences using the NJ methods with a bootstrap of 1000 replicates using MEGA 6.0 (Tamura et al., 2013). The pairwise genetic distances were also determined to qualify the differentiation between taxa using MEGA 6.0 (Tamura et al., 2013).

#### *Morphological Observation of Cryptic Species*

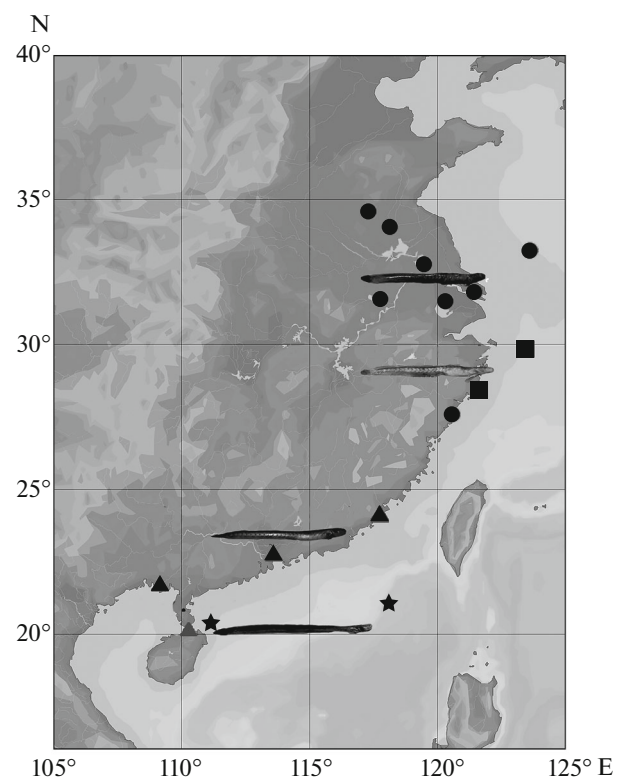
The external morphology of the DNA-recognized cryptic species was further examined with the aid of the stereomicroscopy (Leica s9i). Five metric characteristics including total number of the dorsal-fin, anal-fin elements, pectoral fin elements, and number of vertebrae and barbels on the ventral surface of head, were counted following Murdy and Shibukawa (2001). Extra distinct characteristics that distinguished each species, such as the external morphology of the whole fish, number of teeth on the jaws and other metric characteristics were also examined. These distinct traits of species were photographed under a stereomicroscope using a image capturing software (Leica LAS v4.12).

## RESULTS

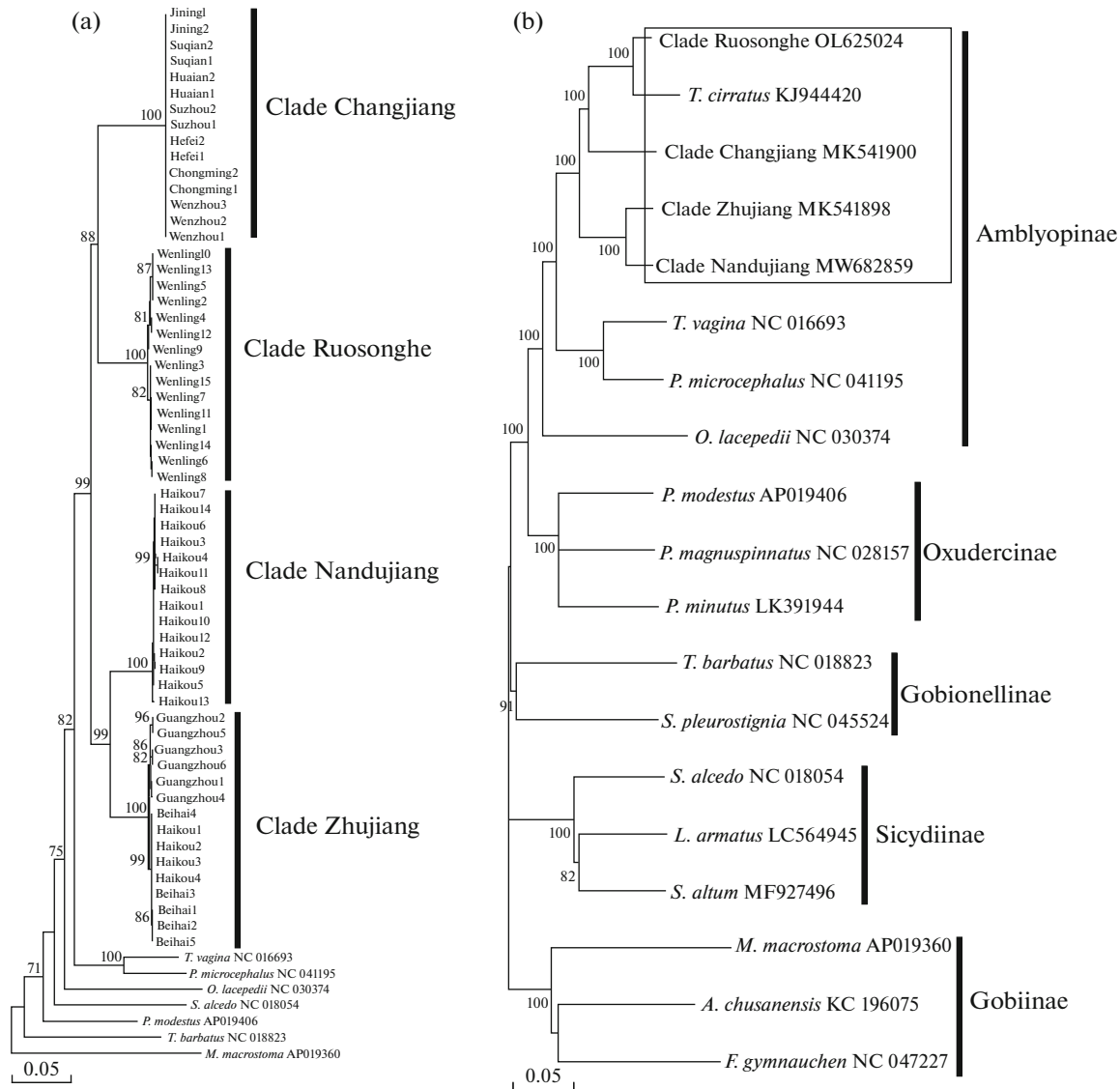
#### *Phylogenetic Analysis and Cryptic Diversity*

A 1554bp segment of the mtDNA *COI* gene was sequenced in all the 59 specimens. The NJ tree constructed from these sequences generally revealed four highly-diverged clades, indicating four possible cryptic *Taenioide* species in specimens collected from the full range of coastal waters in China (Fig. 2a). Samples of these four candidate species were further used to sequence and assemble the complete mitogenomes. As a result, a total length of 16710–17069 bp mitogenomes were assembled from the four candidate species, which were comprised of 13 PCGs, 22 tRNAs, 2 rRNAs, one origin of replication on the light-strand (OL), and 2 control regions (CRs) (Supplements 2–9). A NJ tree was constructed using all 13 mitochondrial

PCGs sequences from these candidate species and 15 other Gobiidae species downloaded from NCBI database. The data resulted in a stable topology of NJ trees with high bootstrap support (100%) for most internodes (Fig. 2b). In the phylogenetic trees, all the four candidate species clustered together to form a *Taenioides* clade, suggesting their constituent of genus *Taenioides*. However, sequence divergences among the four candidate species were also evident, because the pair-



**Fig. 1.** Sampling localities of *Taenioides* gobies observed in this study: (■), clade Ruosonghe; (●), clade Changjiang; (▲), clade Zhujiang; (★), clade Nanduijiang.

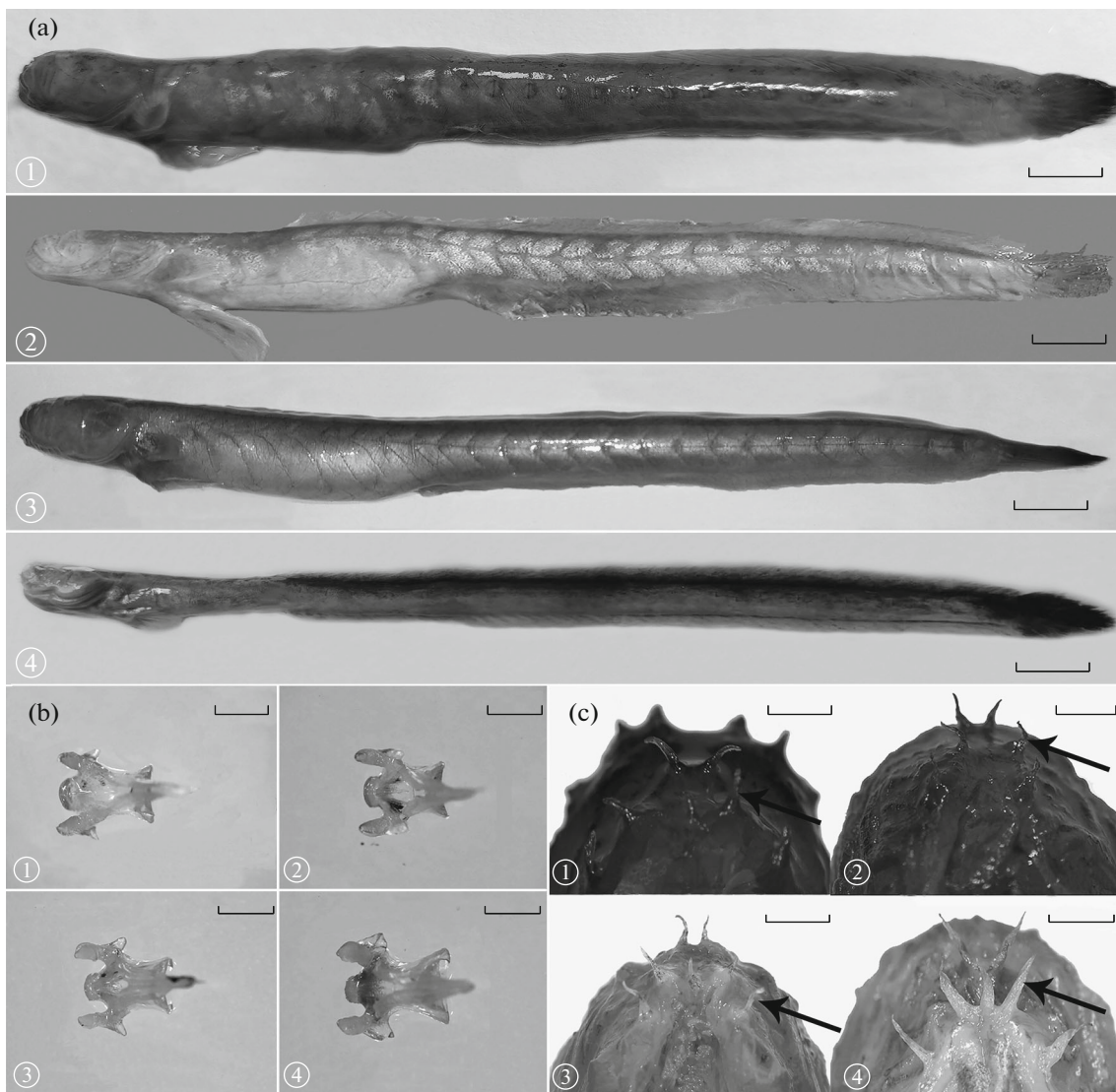


**Fig. 2.** NJ phylogenetic tree constructed from these *Taenioides* gobies and their closely related species in Gobiidae using *COI* sequences (a), and 13 PCGs sequences data (b). Only bootstrap supports >70% in each node were shown. The species in genus *Taenioides* are marked by a black box. The numbers following the species name are the accession numbers of mitochondrial sequences deposited in NCBI database for each species.

wise genetic distance among them (0.047–0.128) was high and generally equal to the differentiation among known species in family of Gobiidae (Supplement 10). Avise and Walker (1999) have assigned an inter-specific differentiation of mitogenome >2% for vertebrate species. If such threshold values for inter-specific differentiation were also accepted for teleost Gobiidae, then the observed candidate species may represent four different cryptic species in genus *Taenioides* (Gobiidae: Amblyopinae). For the convenience of narrating, we therefore assigned them as *Taenioides* sp. (clade Changjiang), *Taenioides* sp. (clade Ruosonghe), *Taenioides* sp. (clade Zhujiang) and *Taenioides* sp. (clade Nanduijiang), according to sampling localities of the specimens.

#### Morphological Observation of the Cryptic Species

Morphological examinations were also conducted to validate these DNA-recognized cryptic species. The results also supported that these candidate species may represent four cryptic species in genus *Taenioides* (Gobiidae: Amblyopinae), since a combination of three characteristics, i.e., the total number of dorsal-fin elements, the number of barbels on the ventral surface of head, and the total number of vertebrae could easily distinguish these four species from each other (Fig. 3). For instance, the numbers of vertebrae in each of the four candidate species were 29, 30, 27 and 29–30 for *Taenioides* sp. (clade Changjiang), *Taenioides* sp. (clade Ruosonghe), *Taenioides* sp. (clade Zhujiang),



**Fig. 3.** The morphological observation of four candidate *Taenioides* species: ①, clade Changjiang; ②, clade Ruosonghe; ③, clade Zhujiang; ④, clade Nanduijiang (a); the morphology and number of vertebra in each species: ①, 29; ②, 30; ③, 27; ④, 29–30 (b), only the first vertebra were shown for each species in the present study; the barbels (→) on the ventral surface of head in each species: ①, ③, ④, 7; ②, 6 (c). Scale: (a) 1 cm; (b, c) 1 mm.

and *Taenioides* sp. (clade Nanduijiang), respectively (Fig. 3b, Table 2). *Taenioides* sp. (clade Changjiang), *Taenioides* sp. (clade Ruosonghe) and *Taenioides* sp. (clade Zhujiang) had generally the similar range of dorsal-fin elements number, which was 46–48, 43–48 and 47–51, respectively. However, *Taenioides* sp. (clade Nanduijiang) had much higher number of dorsal-fin elements with 56–59, which was distinct from all other three species (Table 2). The genus *Taenioides* may also has different number of short barbels on the ventral surface of the head. *Taenioides* sp. (clade Ruosonghe) had three pairs of barbels arranged anteroposteriorly, totaling six barbels on the tip of the lower jaw

(Fig. 3c). This arrangement was represented as 2–2–2, in the direction from the tip of lower jaw to anus. In contrast, *Taenioides* sp. (clade Changjiang), *Taenioides* sp. (clade Zhujiang) and *Taenioides* sp. (clade Nanduijiang) all had seven barbels on the tip of the lower jaw, with three pairs of barbels and one additional barbels located between the second pair of barbels (Fig. 3c). The arrangement was thus represented as 2–3–2. The morphological distinction among them provided further supports for the inference that the observed candidate species may represent four cryptic species in genus *Taenioides*.

**Table 2.** Counts of five meristic characteristics for the 14 nominal species, *Taenioides* sp. (clade Thailand sensu Kurita, Yoshino 2012) and four candidate *Taenioides* species distributed in China

Nominal species	Year	Type locality	Elements			Vertebrae	Barbels
			dorsal-fin	anal-fin	pectoral-fin		
<i>T. anguillaris</i> Linnaeus	1758	China	51	43	15 <sup>I</sup>	30	6 <sup>II</sup>
<i>T. hermannii</i> Lacepède	1800	Chinese, painting	Unknown	Unknown	Unknown	Unknown	Unknown
<i>T. coecula</i> Bloch et Schneider	1801	Tranquebar, India	50	43			
<i>T. gracilis</i> Cuvier et Valenciennes	1837	Pondicherry, India	54	48	15–18 <sup>II</sup>	29	7 <sup>III</sup>
<i>T. rugosus</i> Richardson	1846	China	45	40			
<i>T. eruptionis</i> Bleeker	1849	Java, Indonesia	56	48	16 <sup>I</sup>	29	
<i>T. cirratus</i> Blyth	1860	Calcutta, India	47	44	13 <sup>IV</sup>		7 <sup>IV</sup>
<i>T. brachygaster</i> Gunther	1861	East Indies	51	46		27	
<i>T. mordax</i> DeVis	1884	Queensland, Australia	48	40		28	6
<i>T. purperascens</i> DeVis	1884	Brisbane, Australia	43	36	16–17 <sup>II</sup>		6 <sup>II</sup>
<i>T. snyderi</i> Jordan et Hubbs	1925	Fukuoka, Japan	54	47	17 <sup>V</sup>	32	6 <sup>V</sup>
<i>T. caniscapulus</i> Roxas et Ablan	1938	Negros, Philippines	48	41			7
<i>T. esquivel</i> Smith	1947	Mozambique	54	48		29	
<i>Taenioides</i> cf. <i>kentalleni</i> Murdy et Randall	2002	Jubail, Saudi Arabia	72	65	20–21 <sup>II</sup>	45	10
<i>Taenioides</i> sp. (clade Thailand sensu Kurita, Yoshino)	2012	Songkhla, Thailand				28	7
<i>Taenioides</i> sp. (clade Changjiang)		Chongming, China	46–48	40–41	17	29	7
<i>Taenioides</i> sp. (clade Ruosonghe)		Wenling, China	43–48	41–45	17	30	6
<i>Taenioides</i> sp. (clade Zhujiang)		Guangzhou, China	47–51	41–43	17	27	7
<i>Taenioides</i> sp. (clade Nanduijiang)		Haikou, China	56–59	47–48	17	29–30	7

The meristic characteristics for the possible nominal species were recompiled from Murdy and Randall (2002) with additional information from other authors: <sup>I</sup> Koumans, 1953; <sup>II</sup> Murdy, 2018; <sup>III</sup> Chabanaud, 1927; <sup>IV</sup> Blyth, 1860; <sup>V</sup> Kurita, 2012.

#### Species Assignment of the Cryptic Species

In order to reveal the exact species of these observed specimen may represent, all mitochondrial gene sequences of *Taenioides* species available in NCBI database were downloaded and compared to the mitogenomes assembled for the four cryptic species. Based on the NJ phylogenetic trees constructed and the pairwise genetic distance, *Taenioides* clades Ruosonghe, Zhujiang and Nanduijiang were assigned to be *T. anguillaris*, *Taenioides* sp. (Thailand clade sensu Kurita, Yoshino 2012), and *T. gracilis*, respectively (Supplements 11–16). The remaining one, *Taenioides* sp. (clade Changjiang), corresponding to none

of the known *Taenioides* species (Supplements 10–16), may represent an undescribed species in this genus. To support the species assignment indicated above, a more detailed morphological diagnosis was further conducted and compared to the distinct meristic characteristics of 14 nominal species in genus *Taenioides* such as the total number of the dorsal-fin, anal-fin element, and pectoral-rays, the number of vertebrae and barbels on the ventral surface of the head. Additional traits such as the external morphology of the whole fish; teeth on the upper and lower jaws that was frequently used to delimit the species in genus *Taenioides* were also examined. The results indi-

cated that the values of these meristic characters measured above in *Taenioides* clades Ruosonghe, Zhujiang and Nanduijiang generally corresponding to that of *T. anguillaris*, *Taenioides* sp. (Thailand clade sensu Kurita, Yoshino 2012), and *T. gracilis*, respectively (Table 2). However, the values of meristic characters of *Taenioides* sp. (clade Changjiang) corresponded to none nominal species known to date (Table 2). This results provide further support for the inference that *Taenioides* sp. (clade Changjiang) may represent a new constituent of genus *Taenioides*.

## DISCUSSION

Our study demonstrates the strengths of combining molecular and morphological analyses to shed lights on the cryptic diversity and hence taxonomy of uncertain teleost taxa, such as eel gobies that are morphologically conservative and hard to delimit with morphological data alone (Kurita and Yoshino, 2012). Based on both molecular and morphological data, we revealed four cryptic species of eel gobies, genus *Taenioides* in China in stead of the previously recognized two of *T. cirratus* and *T. anguillaris* (Zhu et al., 1963; Wu and Zhong, 2008) for the first time.

However, none of the observed four cryptic species seem to be appropriate to *T. cirratus* (Blyth, 1860) due to their distinctness in pectoral-fin rays number (17 vs 13) (Table 2). The mitogenome of *T. cirratus* has already been sequenced (Chen and Wen, 2016), and again none of the mitogenome of the four species seem to accord with that of *T. cirratus*. The four cryptic species were assigned as *T. anguillaris*, *T. gracilis*, and *Taenioides* sp. (clade Thailand sensu Kurita, Yoshino 2012) and *Taenioides* sp. (clade Changjiang), respectively according to our molecular and morphological analyses. This is contrast with the fact that *T. cirratus* is the most frequently recorded *Taenioides* species in the coastal waters of China (Zhu et al., 1963; Wu and Zhong, 2008; Qin et al., 2019; Liang et al., 2020). But a detailed comparison indicated that none of the these previously recorded holotypes seem to be appropriate to *T. cirratus* described by Blyth (1860) due to their distinctness both in number of pectoral-fin rays (14 by Zhu et al., 1963; 17–18 by Wu and Zhong, 2008; and 13 by Blyth, 1860) or barbels on the ventral surface of the head (6 by Zhu et al., 1963; 6 by Wu and Zhong, 2008; and 7 by Blyth, 1860). Therefore, whether the failure in collecting *T. cirratus* in the present study arises from the insufficient sample collections that may miss some important cryptic species of *Taenioides*, or it may simply reflect that the previously recognized *T. cirratus* in coastal waters of China was just a false species identification, still await further investigations in the future.

Nevertheless, *T. gracilis* and *Taenioides* sp. (clade Thailand sensu Kurita, Yoshino, 2012) were, for the first time, recognized in China coastal waters in the present study. *T. gracilis* was previously identified in wide coastal waters of the Indo-Pacific ocean from east coast of Africa and Madagascar to India, north-eastward to Japan, and southward to Australia (Murdy, 2018). *Taenioides* sp. (clade Thailand sensu Kurita, Yoshino, 2012) was only identified in a much narrower coastal waters of Indo-Pacific ocean, in songkhla of Thailand (Kurita and Yoshino, 2012), a sea area that is close to where the South China Sea locates. Therefore, it is quite reasonable that we collected both species in South China Sea in the present study. *Taenioides* sp. (clade Thailand sensu Kurita, Yoshino, 2012) is a cryptic species of genus *Taenioides* that was recognized only recently by Kurita and Yoshino (2012), for their distinctness in the number of vertebrae ( $V = 28$ ) and unique composition (*ND2*) in mitogenome based on the examination of two specimen only. But they were collected in large numbers in the present study, with identical morphological traits and molecular composition (Table 2; Supplements 13, 16). This may indicate that *Taenioides* sp. (clade Thailand sensu Kurita, Yoshino, 2012) should represent a valid constituent of genus *Taenioides*. Surprisingly, *Taenioides* sp. (clade Thailand sensu Kurita, Yoshino, 2012) observed in the present study possessed only 27 vertebrae in stead of 28, as described by Kurita and Yoshino (2012). Such differentiation in vertebrae number may represent a morphological variance between geographic populations within species, that was frequently observed in species of genus *Taenioides* (Kurita and Yoshino, 2012; Murdy, 2018).

Intriguingly, *Taenioides* sp. (clade Changjiang) recognized in the present study seems to be appropriate to none of the previously known nominal *Taenioides* species (Table 2; Supplements 10–16). It distributes widely in coastal estuary waters of China, and was collected in multiple locations from East China Sea southward to South China Sea (Table 1). It even invaded many inland freshwater lakes, such as Tai lake, Gaoyou Lake, Luoma Lake and Nansi Lake, and had become a very common species in these regions (the present study; Qin et al., 2019; Liang et al., 2020). And there, it was frequently falsely recognized as a form of *T. cirratus* (Wang et al., 2017; Qin et al., 2019; Liang et al., 2020). However this recognition was not supported by both of our molecular and morphological data, as indicated above. Due to the lack of the molecular data for most of the other nominal species in genus *Taenioides*, we compared the detailed meristic characters of *Taenioides* sp. (clade Changjiang) with that of 14 known nominal species worldwide (Table 2). The values of five meristic characters (i.e., total number of dorsal-fin and anal-fin elements, the

number of the pectoral-fin rays, and the number of vertebrae and barbels on the ventral surface of the head) seems to fall into the scope of none of these species except for *T. rugosus* (Richard, 1846), and *T. caniscapulus* (Roxas and Ablan, 1938). However, *T. rugosus* was now recognized as synonym of *T. anguillaris* (Wu and Zhong, 2008). *T. caniscapulus* is distinct for the presence of an extra three teeth at the tip of the snout, which distinguish it from any known *Taenioides* species (Roxas and Ablan, 1938), including *Taenioides* sp. (clade Changjiang). Therefore, the cryptic species, *Taenioides* sp. (clade Changjiang) recognized in the present study may be considered a new confirmed candidate species in genus *Taenioides*. Further investigation are in need to validate its taxonomic status in the future.

In conclusion, the present study provided the first evidence for an underestimation of the species richness in genus *Taenioides* in coastal waters of China. Four cryptic species were recognized in stead of previously recognized two in this genus, and one of the them, *Taenioides* sp. (clade Changjiang), may represent a new confirmed candidate species. A careful taxonomic revision is thus called for in genus *Taenioides* worldwide in the future.

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#### COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of interests.* The author declares that he has no conflicts of interest.

*Statement on the welfare of humans or animals.* All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

#### SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at <https://doi.org/10.1134/S0032945222060315>

#### REFERENCES

Avise, J.C. and Walker, D., Species realities and numbers in sexual vertebrates: Perspectives from an asexually transmitted genome, *Proc. Natl. Acad. Sci. USA.*, 1999, vol. 96, pp. 992–995.  
<https://doi.org/10.1073/pnas.96.3.992>

Bloch, M.E. and Schneider, J.G., *Systema Ichthyologiae Iconibus Cx Illustratum*, Post obitum auctoris opus inchoatum absolutum, correxit, interpolavit jo, Gottlob Schneider, Berolini: Bibliopolio Sanderiano Commisum, 1801.

Blyth, E., Report on some fishes received chiefly from the Sitang River and its tributary streams, Tenasserim Provinces, *J. Asiatic Soc. Bengal.*, 1860, vol. 29, pp. 138–174.

Chabanaud, P., Sur diverses espèces du genre *Taenioides* Lacep. (Poissons Gobiiformes), *Bull. De La Soc. Zool. De France*, 1927, vol. 52, pp. 404–415.

Chen, I.S. and Wen, Z.H., The complete mitochondrial genome of whiskered eel goby *Taenioides cirratus* (Perciformes, Gobioidae), *Mitochondr. DNA*, 2016, vol. 27, pp. 1509–1511.

<https://doi.org/10.3109/19401736.2014.953115>

Cuvier, G., and Valenciennes, A., *Histoire Naturelle des Poissons*, Tome douzième. Suite du livre quatorzième. Gobioides, Livre quinzisième. Acanthoptérygiens à pectorales pédiculées, Paris: Pitois, 1837.

Günther, A.C.L.G., *Catalogue of the Acanthopterygian Fishes in the Collection of the British Museum*, London: British Museum, 1861.

Jordan, D.S. and Hubbs, C.L., Record of fishes obtained by David Starr Jordan in Japan, 1922, *Mem. Carnegie Mus.*, 1925, vol. 10, pp. 93–346.

Koumans, F.P., *Fishes of the Indo-Australian Archipelago*, vol. 10: *Gobioidea*, Leiden: Brill, 1953.

Kurita, T. and Yoshino, T., Cryptic diversity of the eel goby, genus *Taenioides* (Gobiidae: Amblyopinae), in Japan, *Zool. Sci.*, 2012, vol. 29, pp. 538–545.

<https://doi.org/10.2108/zsj.29.538>

Liang, Y.Y., Fang, T., Li, J., et al., Age, growth and reproductive traits of invasive goby *Taenioides cirratus* in the Chaohu Lake, China, *J. Appl. Ichthyol.*, 2020, vol. 36, pp. 219–226.

<https://doi.org/10.1111/jai.14024>

Linnaeus, C., *Systema Naturae: editio decima reformata*, Holmiae: Impensis Laurentii Salvii, 1758.

Lowe, T.M. and Chan, P.P., tRNAscan-SE on-line: integrating search and context for analysis of transfer RNA genes, *Nucleic Acids Res.*, 2016, vol. 44, pp. 54–57.

<https://doi.org/10.1093/nar/gkw413>

Meng, G., Li, Y., Yang, C., and Liu, S.L., MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization, *Nucleic Acids Res.*, 2019, vol. 47, Article e63.

<https://doi.org/10.1101/489955>

Murdy, E.O., A redescription of the gobiid fish *Taenioides purpurascens* (Gobiidae: Amblyopinae) with comments on, and a key to, species in the genus, *Ichthyol. Res.*, 2018, vol. 65, pp. 454–461.

<https://doi.org/10.1007/s10228-018-0635-6>

Murdy, E.O. and Randall, J.E., *Taenioides kentalleni*, a new species of eel goby from Saudi Arabia (Gobiidae: Amblyopinae), *Zootaxa*, 2002, vol. 93, pp. 1–6.

<https://doi.org/10.11646/zootaxa.93.1.1>

Murdy, E.O. and Shibukawa, K., A revision of the gobiid fish genus *Odontamblyopus* (Gobiidae: Amblyopinae),



*Ichthyol. Res.*, 2001, vol. 48, pp. 31–43.  
<https://doi.org/10.1007/s10228-001-8114-9>

Qin, J., Cheng, F., Zhang, L., et al., Invasions of two estuarine gobiid species interactively induced from water diversion and saltwater intrusion, *Manag. Biol. Invasion*, 2019, vol. 10, pp. 139–150.  
<https://doi.org/10.3391/mbi.2019.10.1.09>

Richardson, J., Report on the ichthyology of the seas of China and Japan, *Rep. British Assoc. Advancement Sci*, 1846, pp. 187–320.

Roxas, H.A. and Ablan, G.L., A new taenioid fish from Occidental Negros, *Philippine J. Sci.*, 1938, vol. 66, pp. 261–265.

Sambrook, J., Fritsch, E.F, and Maniatis, T., *Molecular cloning: a laboratory manual*, N. Y.: Cold Spring Harbor Lab. Press, 1989.

Smith, J.L.B., New species and new records of fishes from South Africa, *Ann. Mag. Nat. Hist.*, 1947, vol. 13, pp. 793–821.

Tamura, K.G., Stecher, D., Peterson, A., et al., MEGA6: molecular evolutionary genetics analysis, version 6.0, *Mol. Biol. Evol.*, 2013, vol. 30, pp. 2725–2729.  
<https://doi.org/10.1093/molbev/mst197>

Wang, Y.N., Li, X.Q., Ke, H., et al., Application of DNA barcoding gene COI for identifying *Taenioides cirratus* in Nansi Lake, *Chin. J. Fish.*, 2017, vol. 30, pp. 12–18.

Ward, R.D., Zemplak, T.S., Innes, B.H., et al., DNA barcoding Australia's fish species, *Proc. Natl. Acad. Sci. USA*, 2005, vol. 360, pp. 1847–1857.  
<https://doi.org/10.1098/rstb.2005.1716>

Wu, H.L. and Zhong, J.S., *Fauna Sinica, Ostichthyes, Perciformes (V), Gobioidae*, Beijing: Sci. Press, 2008.

Zhu, Y.D., Zhang, C.L., and Cheng, Q.T., *Fish Fauna of the East China Sea*, Beijing: Sci. Press, 1963.