

RESEARCH ARTICLE

First record of *Pampus minor* (Actinopterygii: Perciformes: Stromateidae) from the coastal waters of Wenzhou, China

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Abstract – *Pampus* fishes (Perciformes: Stromateidae) are important commercial species and include six valid species in China. The southern lesser pomfret (*Pampus minor* Liu and Li, 1998) is a species of *Pampus* for which knowledge is incomplete. This report confirms the occurrence of *P. minor* in the coastal waters of Wenzhou, China, by analyzing five specimens of *P. minor* obtained in Wenzhou in March 2019. Morphometric measurements and meristic counts were performed. The diagnostic morphological features of these species were consistent with those of the neotype specimen of *P. minor* and sufficient for separating the examined specimens from other *Pampus* species. This study was based on the Cytochrome Oxidase Subunit 1 (CO1) gene, which was sequenced for the purpose of identification. The genetic distances between *P. minor* and other *Pampus* species ranged from 13.4% to 15.5%, and the mean genetic distance within the *P. minor* group was 0.2%. Given that *P. minor* has not been reported in this region, our findings represent the first record from the coastal waters of Wenzhou and extend the distribution range of this species to the central and northern East China Sea. The reason for the observed northward migration of some *P. minor* individuals from their northernmost recorded habitat (Taiwan Strait) might be global warming. The collection of additional specimens is needed to further study the geographic limits of *P. minor* and its presumed northern expansion.

Keywords: *Pampus minor* / coastal waters of Wenzhou / first record / global warming / distribution expansion

1 Introduction

The genus *Pampus* (Perciformes: Stromateidae) includes important commercial fishes, including six valid species in China (Li et al., 2019b): *Pampus minor* Liu and Li, 1998, *Pampus argenteus* (Euphrasen, 1788), *Pampus chinensis* (Euphrasen, 1788), *Pampus cinereus* (Bloch, 1795), *Pampus punctatissimus* (Temminck et Schlegel, 1845) and *Pampus echinogaster* (Basilewsky, 1855). According to previous reports (Cheng, 1962; Nakabo, 2002; Liu and Li, 2002; Yang et al., 2006; Dolganov et al., 2007; Yamada et al., 2009), *P. echinogaster*, *P. punctatissimus* and *P. chinensis* are common species in the coastal waters of Wenzhou. In recent

years, a variety of molecular markers have been successfully applied to study the taxonomy and genetic structure of the genus *Pampus*. In a taxonomic study of *Pampus* fishes, Divya et al. (2017) utilized the CO1 gene to establish the molecular identity of *P. argenteus* in Indian waters. Li et al. (2017) used the CO1 gene to correct the morphological redescription and DNA barcoding of *P. echinogaster*. In the most recent study, Li et al. (2019b) combined the CO1 gene with morphological characteristics to reidentify *Pampus* species, and preliminarily confirmed the existence of seven valid species. In a study on the genetic structure of *Pampus* species based on the mitochondrial DNA control region (CR) and microsatellite markers, Li et al. (2018) found that the complex migratory behavior and high dispersal ability of ichthyoplankton may have played important roles in shaping the current genetic structure of *P. echinogaster*. Meanwhile, in research on

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P. chinensis, based on the same methods, (Li et al., 2019a) pointed out that the *P. chinensis* populations in different geographical regions constituted a single panmictic stock with high gene flow.

P. minor was initially reported to be widely distributed in the southern part of the Taiwan Strait and to probably occur in Southeast Asian seas (Liu and Li, 1998). *P. minor* was once considered the juvenile of *P. argenteus* and *P. cinereus* (Liu and Li, 1998). Guo et al. (2010) used DNA barcoding to confirm the differences between *P. minor* and other species, reducing confusion over its identification. However, in our recent fishery resource survey in Zhejiang Province (Wentai fishing ground) conducted in March 2019, a total of 65 fish species, including 110437 individuals, were caught by bottom trawling, all of which belonged to class Actinopterygii, none of Chondrichthyes fishes, and 26 species of which were in the order Perciformes. In this survey, we collected five samples of *P. minor*. Although this species is rarely observed, for further studies, it is important to determine whether the species does indeed occur in these offshore waters. To this end, our purpose here was to use traditional taxonomy to accurately identify *P. minor* and then use DNA barcoding to confirm its occurrence.

DNA barcoding is a taxonomic approach that uses a short genetic marker in an organism's DNA to identify that organism as belonging to a certain species (Hebert et al., 2003a; Hua et al., 2004). This method differs from molecular phylogenetics in that the main goal is not to determine relationships but to identify an unknown sample according to a preexisting classification system (Ali et al., 2014). In this study, to ensure the accuracy of species identification, the distribution range of *P. minor* was supplemented by combining morphological identification with DNA barcoding.

2 Materials and methods

2.1 Sample collection

In March 2019, five samples of *P. minor* were collected by bottom trawling in the coastal waters of Wenzhou

(121°05.494'E, 27°01.648'N) (Fig. 1). Other *P. minor* individuals were collected from different geographical locations (Tab. 1), bought at the dock or obtained through fishery resource surveys. In addition, *P. argenteus*, *P. cinereus*, and *P. echinogaster* were collected to construct a phylogenetic tree (Tab. 1). All individuals were identified based on morphological characteristics (Li et al., 2019b), and a piece of muscle tissue was obtained from each individual and preserved in 95% ethanol. All examined specimens were preserved at -20 °C and stored in the Laboratory of Marine Biology and Ecology, Third Institute of Oceanography, Ministry of Natural Resources.

2.2 Morphological analysis

We measured the morphological characteristics of five individuals of *P. minor*. Counting and measurement were performed as described by Elliott et al. (1995) and Li et al. (2017). The countable traits included the

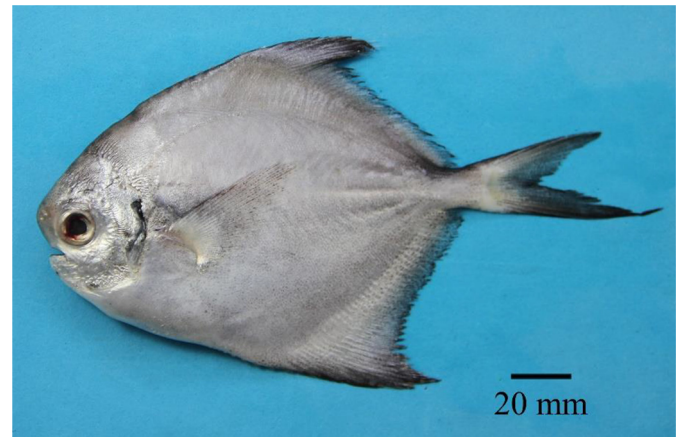


Fig. 1. *P. minor* collected from the coastal waters of Wenzhou, China (standard length: 101 mm).

Table 1. Information on the *Pampus* samples and sequences in this study.

Species	This study		Cited accession no.
	Sampling site	Number	
<i>P. minor</i>	Wenzhou	WZ1~WZ5	MT316581, MT316582, MT316583, MT316584, MT316585, MT316586
	Xiamen	XM1~XM2	
	Zhangpu	ZP1~ZP2	
	Taiwan	TW1~TW2	
	Zhuhai	ZH1~ZH2	
	Zhanjiang	ZJ1~ZJ2	
	Beihai	BH1~BH2	
	Haikou	HK1~HK2	
	Sanya	SY1~SY2	
	Weizhoudao	WZD1~WZD2	
	Fangchenggang	FC1~FC2	
South China Sea	–	FJ434346, FJ434347, MH037007	
<i>P. argenteus</i>		PA1~PA2	KF192337, KF192338
<i>P. cinereus</i>		PC1~PC2	FJ652434, FJ652435
<i>P. echinogaster</i>		PE1~PE2	KJ539190, KJ539191

Table 2. Comparative counts and measurements of *P. minor* (including the data from Liu and Li (1998) and this study) and other *Pampus* species from Wenzhou, China.

Character	Species			
	<i>P. minor</i> (n = 32) (Liu and Li, 1998)	<i>P. minor</i> (n = 5)	<i>P. argenteus</i> (n = 16)	<i>P. cinereus</i> (n = 30)
Total weight [g]		19.9–30.1	25.3–106.2	29.1–38.9
Fork length [mm]		84.6–102.0	94.3–156.2	82.9–110.4
Standard length (SL) [mm]	63.0–134.4	82.1–122.6	88.1–142.6	72.1–94.3
Head length (HL) [mm]		20.1–26.0	22.0–32.8	21.2–25.9
Eye diameter [mm]		5.9–10.1	7.1–10.2	6.2–9.1
Postorbital length [mm]		11.2–12.2	10.1–15.2	11.0–12.3
Body width [mm]		11.2–14.9	9.1–20.2	11.2–18.9
Caudle peduncle length [mm]		7.9–16.9	10.0–20.1	8.1–18.2
Caudle peduncle width [mm]		3.1–4.2	2.5–5.8	3.2–4.1
Dorsal fin rays	VII-IX-34~39	VII-IX-34~39	VII-VIII-39~43	VI-VIII-38~43
Pectoral fin rays	22~24	22~24	21~29	21~23
Anal fin rays	V-VII-35~39	V-VII-35~39	V-VI-35~41	V-VII-38~42
Caudal fins	18~20	18~20	26~28	22~25
Dorsal fin length [mm]		40.2–48.1	42.1–73.2	41.2–50.2
Anal fin length [mm]		43.0–49.2	42.2–72.8	40.9–50.1
Pectoral fin length [mm]		23.2–32.1	31.2–49.9	23.0–32.1
Tail length [mm]		32.5–52.1	38.1–66.1	34.9–62.1
Gill rakers	12–16	11–14	10–12	8–12
Vertebrae	29–30	29–31	37–38	37–38

following: dorsal fin rays, pectoral fin rays, anal fin rays, caudal fin rays, gill rakers on the first gill arch, and vertebrae. The measurable traits included the following: total weight, fork length, standard length, head length, eye diameter, postorbital length, body width, caudle peduncle length, caudal peduncle width, dorsal fin length, anal fin length, pectoral fin length, and tail length. All measurements were performed using calipers to the nearest 0.1 mm. All remaining measurements were obtained using preserved specimens.

2.3 Molecular analyses

Molecular methods were implemented as described by Li et al. (2017). Genomic DNA was isolated from muscle tissue with an Easy Pure[®] Marine Animal Genomic DNA Kit (Beijing TransGen Biotech). A fragment of CO1 mitochondrial DNA was amplified using the primers F1: 5'-TCAACCAACCA-CAAAGACATTGGCAC-3' and R1: 5'-TAGACTTCTGGG TGGCCAAAGAATCA-3' (Ward et al., 2005). Each polymerase chain reaction (PCR) was performed in a 25 µL reaction mixture containing 17.5 µL of ultrapure water, 2.5 µL of 10× PCR buffer, 2 µL of dNTPs, 1 µL of each primer (5 µmol/L), 0.15 µL of Taq polymerase, and 1 µL of DNA template. PCR amplification was performed in a Biometra thermal cycler under the following conditions: 5 min of initial denaturation at 94 °C; 32 cycles of 30 s at 94 °C for denaturation, 30 s at 52 °C for annealing, and 40 s at 72 °C for extension; and a final extension at 72 °C for 10 min. The PCR products were preserved at 4 °C. After agarose gel electrophoresis, the PCR products were sequenced by Personal Biotechnology Co., Ltd.

To determine the correct DNA barcoding for *P. minor*, all original sequences were revised by DNASTAR software (DNASTAR, Madison, WI, USA), and some CO1 sequences were downloaded from GenBank for comparative analysis (Tab. 1). All sequences were aligned again using DNASTAR software. A neighbor-joining (NJ) tree was created with all related *Pampus* species, and the distances between and within groups were calculated using MEGA 5.0 (Tamura et al., 2011) with 1000 bootstrap replicates based on evolutionary distances calculated using the best model, i.e., the Kimura 2-parameter (K2P) model.

3 Results

3.1 Morphological analysis

We accurately identified *P. minor* on the basis of biometrical characteristics of the studied specimens. Finally, we summarized its major diagnostic morphological characteristics as follows: body oval; dorsal fins VII-IX 34–39, pectoral fins 22–24, anal fins V-VII 35–39, caudal fins 18–20; transverse occipital canals and dorsal branches of the lateral line canal on top of the head with a truncated rear edge; ventral transverse occipital canals sparse and slightly longer than or equal in length to the dorsal branches; gill rakers thin (delicate), sparse, 3–4 + 8–10 = 11–14; and vertebrae 29–31. The detailed counts and measurements of the morphological characteristics of *P. minor* are provided in Table 2.

According to the data in Table 2, the numbers of caudal fin rays and vertebrae are distinct between *P. minor* and the other two *Pampus* species, while the remaining countable characters

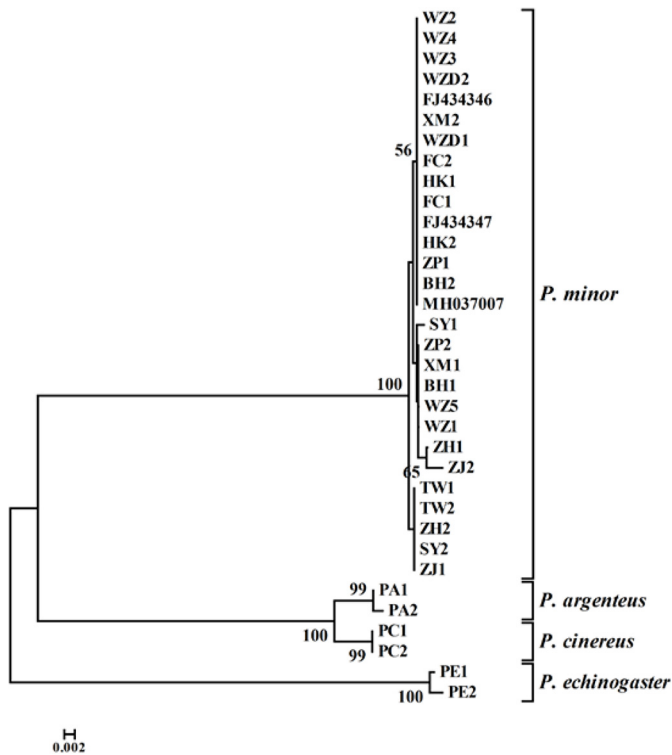


Fig. 2. NJ tree constructed using the K2P model for CO1 gene sequences of *P. minor*. Bootstrap values greater than 50% from 1000 replicates are shown.

overlap. The most distinguishable characters of *P. minor* are the truncated edge of the transverse occipital canal and dorsal branches on the upper back of the head. The transverse occipital canal on the ventral branch is spare and slightly longer than or equal in length to the dorsal branch of the lateral line canal. The composition is similar to that of the intestinal wall, and the main function is to enlarge the absorption surface area, which verifies the identification of this species in the coastal waters of Wenzhou, China.

3.2 Molecular analysis

Thirty-four sequences with a total length of 630 bp were employed in the analysis. The newly obtained CO1 haplotype sequences of *P. minor* have been submitted to GenBank under accession numbers MT316581-MT316586 (Tab. 1). In total, six variable sites, three parsimony-informative sites, and three singleton sites were identified, and no deletions/insertions were observed. The A+T base content (A: 26.2%; T: 33.1%) was higher than the G+C base content (G: 16.8%; C: 23.9%). The NJ phylogenetic tree is shown in Figure 2. All *P. minor* individuals clustered into a group, and *P. echinogaster*, *P. argenteus*, and *P. cinereus* clustered individually. Genetic distances between *P. minor* and the other three species of the genus *Pampus* ranged from 13.4% to 15.5%. The genetic distance within the *P. minor* group was only 0.2%, and the interspecific genetic distance was over 10 times greater than the intraspecific genetic distance, which vastly exceeded the threshold for species delimitation (Hebert et al., 2003b). The

results of the combined morphological and genetic analyses strongly supported the validity of this new record of *P. minor* in Wenzhou.

4 Discussion

In 1998, Liu and Li first described the morphological characteristics of *P. minor* and confirmed its taxonomic status as a new species by comparing its morphological characteristics with those of other *Pampus* species (Liu and Li, 1998). Because of its small size (the adult does not exceed 150 mm in standard length) and the similarity of external characteristics within the genus *Pampus*, *P. minor* was regarded as the larva of *P. argenteus* and *P. cinereus* until Liu and Li (1998) identified it as a valid species (Li et al., 2019b).

Climate may be a significant factor determining the distribution of biomes (Dulvy et al., 2008; Perry et al., 2005). Climate change can influence the adaptability of different species in a region and the competitiveness of different populations within ecosystems (Vanderwal et al., 2013; Hoffmann and Sgro, 2011). Animals and plants in nature may be displaced because they are unable to adapt to global warming at an adequate speed (Wiens, 2016). Similarly, Chen et al. (2018) found that the observed northward migration of some *Platyrrhina sinensis* individuals in Zhejiang from their southern habitat might be because of global warming. Studies have also shown that the distribution of *Larimichthys polyactis* in the central and southern waters of the Yellow Sea has shifted offshore in spring and northward in winter during the past 10 years (Shan et al., 2011). Given the adjacent waters, the changes in the distribution of *P. minor* may be somewhat related to global warming. Temperature is a critical factor for fish physiology (Shan et al., 2011), and an increase in temperature will affect the normal metabolic processes of fish and alter their physiology and life history. *P. minor* always appears in warm coastal waters (Liu and Li, 1998), and the coastal waters of Wenzhou are influenced by the high temperatures of the Taiwan Warm Current and South China Sea warm current, causing warm-water and warm-temperate species to dominate in these waters (Dong et al., 2017). Therefore, climate change may have shifted the distribution of *P. minor* northward to Wenzhou, changing its original distribution area.

As an alternative explanation, *P. minor* may have always been distributed in the coastal waters of Wenzhou, but because of the morphological similarity of *Pampus* fishes, it may have been misidentified as other *Pampus* species (Liu and Li, 1998; Li et al., 2019b). For example, for a long time, *Periophthalmus magnuspinnatus* was misidentified as other species of Gobiidae because of their similar morphological characteristics (Wang and Yang, 2006). Furthermore, it is possible that *P. minor* is rare in the coastal waters of Wenzhou and was ignored in previous surveys. Wang and Fan (2007) once found just five sharks in the southern area of the East China Sea and identified them as a new record, which was named *Megachasma pelagios*. The abundance of this species might be very low, but its occurrence in this area over a long period of time cannot be denied.

5 Conclusions

In this study, we used a combination of biometrical identification and molecular biological methods to identify *P. minor*. Our findings provide the first record of this species from the coastal waters of Wenzhou and revise the distribution range of this species. The species is mainly distributed in the southern part of the Taiwan Strait and the northern part of the South China Sea and Beibu Gulf, with its northernmost distribution reaching the coastal waters of Wenzhou, China. The reason for the observed northward migration of some *P. minor* individuals might be global warming. In summary, this study lays a solid foundation for the in-depth study of *P. minor* and provides evidence that global warming leads to the northward movement of species.

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