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An unexpected distribution record of the cold water fish *Pholis fangi* (Pholidae) from southern Taiwan

KEITA KOEDA[1,2,]* & NOZOMU MUTO[3]
[1] *National Museum of Marine Biology & Aquarium, 2 Houwan Road, Checheng, Pingtung, 94450, Taiwan*
[2]*Present address: Kuroshio Biological Research Foundation, 560 Nishidomari, Otsuki, Kochi 788-0333, Japan*
[3] *Department of Marine Biology and Sciences, School of Biological Sciences, Tokai University, 5-1-1-1 Minamisawa, Minami-ku, Sapporo, Hokkaido 005-8601, Japan*
**Corresponding author. E-mail: hatampo@gmail.com*

Abstract

Gunnel species (family Pholidae), characterized by an elongated bar-like body and usually found in shallow coastal waters, are mostly restricted to temperate to subarctic waters in the Northern Pacific Ocean, although some in the Western Pacific are distributed southward to the northern East China Sea. Despite the absence of previous records from subtropical and tropical waters, a single specimen of the fish family Pholidae was recently trawled off southwestern Taiwan. Examination on the morphological characteristics and DNA barcoding revealed this is a *Pholis fangi* (Wang & Wang 1935), a species previously recorded only from cold waters in the Bohai and Yellow seas. As a consequence, the present specimen represents the first record of the species from the subtropical South China Sea coast of Taiwan, as well as an isolated southernmost record of this species, more than 1,000 km southward from the otherwise known distribution. Detailed morphology and fresh coloration are described.

Keywords: *Pholis fangi*, gunnels, South China Sea, deep sea, taxonomy, distribution

Introduction

The perciform fish family Pholidae, known as gunnels, are distributed in the northern Pacific, northern Atlantic, and Arctic oceans, and generally inhabiting bottom littoral and coastal sea shore habitats. The phylogeny of the family has long been confused, although recently challenged by several osteological and genetic studies (Yatsu 1981; Yatsu 1985; Radchenko *et al.* 2012; Sweetser & Hilton 2016). The genus *Pholis* Scopoli 1777, characterized by 6 infraorbital pores, the first and second anal-fin spines equal in size, and lack of interorbital pores (Yatsu 1985), currently comprises 11 valid species, of which seven are distributed in the northwestern and northern Pacific Ocean (Fricke *et al.* 2018).

A single specimen of *Pholis fangi* (Wang & Wang 1935) was recently collected from several tens of meters depth off Dong-gang, southwestern Taiwan (subtropical South China Sea coastal waters) by bottom or mid-water trawl. Previously known only from cold waters of the Bohai and Yellow seas, the species has at no time been collected from the East China Sea (Yamada *et al.* 2007). Therefore, detailed morphology of the present specimen, the first record of *P. fangi* from subtropical waters and an isolated southernmost distributional record of the species, supported by a mitochondrial DNA analysis, is described herein.

Methods and materials

Counts, measurements, and terminology of cephalic sensory canal openings follow Yatsu (1981). Median fin rays and vertebrae were counted from a radiograph. Measurements were made to the nearest 0.1 mm with needle-point calipers under a dissecting microscope. Standard and head lengths are abbreviated as SL and HL, respectively. The

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morphological description is based on the specimen collected from southwestern Taiwan, which is deposited in the fish collection of the National Museum of Marine Biology & Aquarium, Pingtung, Taiwan (NMMB-P).

Total genomic DNA of the specimen was extracted from a fin clip preserved in 99.5% ethanol, using the Wizard Genomic DNA Purification Kit (Promega) and following the manufacturer's protocol. Approximately 650 bp of the 5' end of the mitochondrial Cytochrome Oxidase c subunit I (*COI*) gene was amplified with a primer cocktail ["COI-3" in Ivanova *et al.* (2007)], comprising two forward and two reverse primers. PCR was conducted in a 6.0 µl reaction volume containing 3.0 µl of 2X KAPATaq HS ReadyMix with dye (KAPA BIOSYSTEMS), 0.125 µM of each primer, and 1.0 µl (approximately 20 ng) of template DNA. The PCR profile included initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 40 s and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. The PCR product was purified with ExoSAP-IT (Thermo Fisher Scientific). DNA sequencing was performed using the Big-Dye Terminator Cycle Sequencing Kit v.3.1 (Thermo Fisher Scientific) on an Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific). The resulting sequence was edited with BioEdit 7.0.5.3 (Hall 1999), and deposited in the International Nucleotide Sequence Database Collaboration (INSDC) under Accession No. LC440107. The sequence was aligned with reference sequences of western Pacific congeners, using CLUSTAL W (Larkin *et al.* 2007) implemented in BioEdit. Reference sequences were derived from the INSDC or the Barcode of Life Database (BOLD), and listed in Appendix 1. A neighbor joining (NJ) tree was constructed using MEGA 7 (Kumar *et al.* 2016), based on the uncorrected p-distance between sequence pairs. Node support was assessed using bootstrap resampling (1,000 replicates).

Taxonomy

Family Pholidae

Pholis Scopoli 1777

Pholis fangi (Wang & Wang 1935)
Figs. 1–2

Enedrias fangi Wang & Wang 1935: 215, fig. 39 (type locality: Chefoo, China); Yatsu 1981: 177.
Pholis fangi: Yatsu 1985: 281; Radchenko *et al.* 2010: 736; Sweetser & Hilton 2016: 1687.

Specimen examined. NMMB-P28512, 131.5 mm SL, off Dong-gang (ca. 22°39′N, 120°24′E), Pingtung, South China Sea, Taiwan, 8 February 2018, obtained at fish-landing ground at Dong-gang.

Description of NMMB-P28512. Dorsal-fin rays 81; anal-fin rays II, 44; pectoral-fin rays 15; pelvic-fin rays I, 1; caudal-fin rays 8 (upper) +7 (lower) with 6 (upper) +5 (lower) procurrent rays; vertebrae 86 (36+50). Measurements: pre-dorsal-fin length 13.5% SL; pre-anus length 51.2%; body depth 13.0%; head length 14.0%. Snout length 18.0% HL; eye diameter 18.9%; upper jaw length 28.3%; interorbital width 14.9%; pectoral-fin length 61.3%; caudal-fin length 57.1%. Cephalic sensory canal openings: nasal series 2; interorbital pore 0; infraorbital series 6; anterior postorbital series + posterior postorbital series 3+3; occipital series 1-1-1; mandibular + preopercle series 4+5.

Body elongated, strongly compressed laterally, bar-like. Dorsal and ventral outline of body almost straight. Body and head covered with tiny scales. Lateral line absent. Head small, snout rounded. Mouth strongly oblique, forming an angle of ca. 60° to horizontal axis, lower jaw projecting slightly; maxilla extending posterior to vertical through anterior edge of orbit. Lips thick. Jaws with minute conical teeth, those on premaxilla and mandible in a single row with a patch anteriorly; teeth on maxilla few, widely spaced from each other; vomer and palatines toothless. Branchiostegal membranes on both sides joined.

Dorsal and anal fins low, bases very long; posterior end of dorsal and anal fins connected with caudal fin by membrane. Origin of dorsal fin above posterior tip of operculum; all dorsal fin rays spinous, similar length. Origin of anal fin at middle of body; first and second spines of anal fin shorter than soft rays. Pectoral fin rounded; base just below posterior tip of operculum. Pelvic fin tiny, its base anterior to pectoral-fin base. Caudal fin smaller than head, truncated with rounded uppermost and lowermost corners.

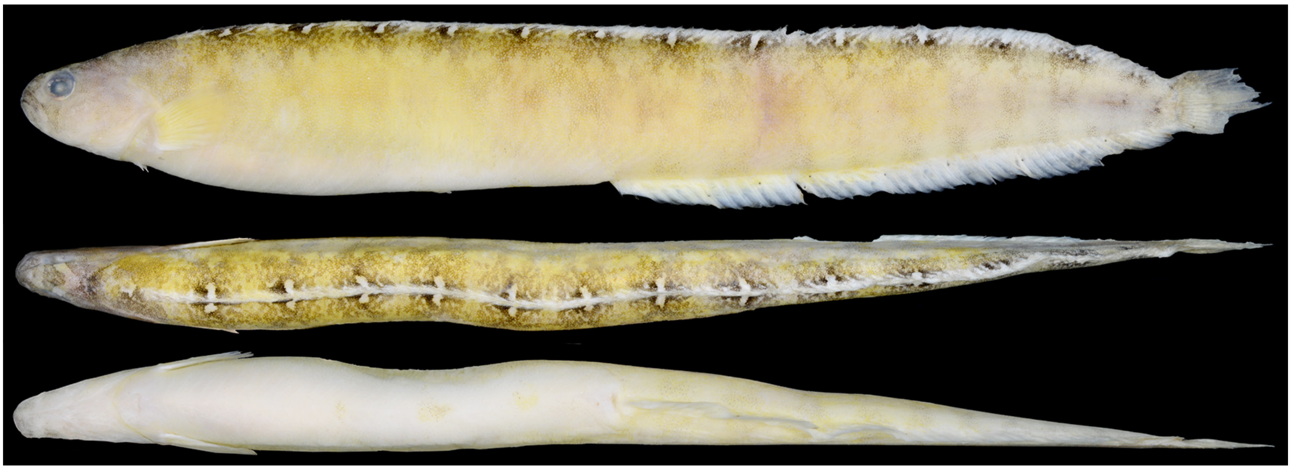

FIGURE 1. Lateral, dorsal, and ventral views (top to bottom) of a fresh specimen of *Pholis fangi* collected from southwestern Taiwan. NMMB-P28512, 131.5 mm SL.

FIGURE 2. Lateral and ventral views (top and bottom) of head of *Pholis fangi* collected from southwestern Taiwan. NMMB-P28512, 131.5 mm SL.

Fresh coloration. Body and head uniformly yellow, abdomen whitish; faint H-shaped dusky marking on dorsal and mid-lateral aspects; occipital, snout, lips, and ventral surface of lower jaw faintly dusky; fins generally pale yellow, with 13 equally-spaced short vertical white bands, margined with dark brown, on dorsal fin.

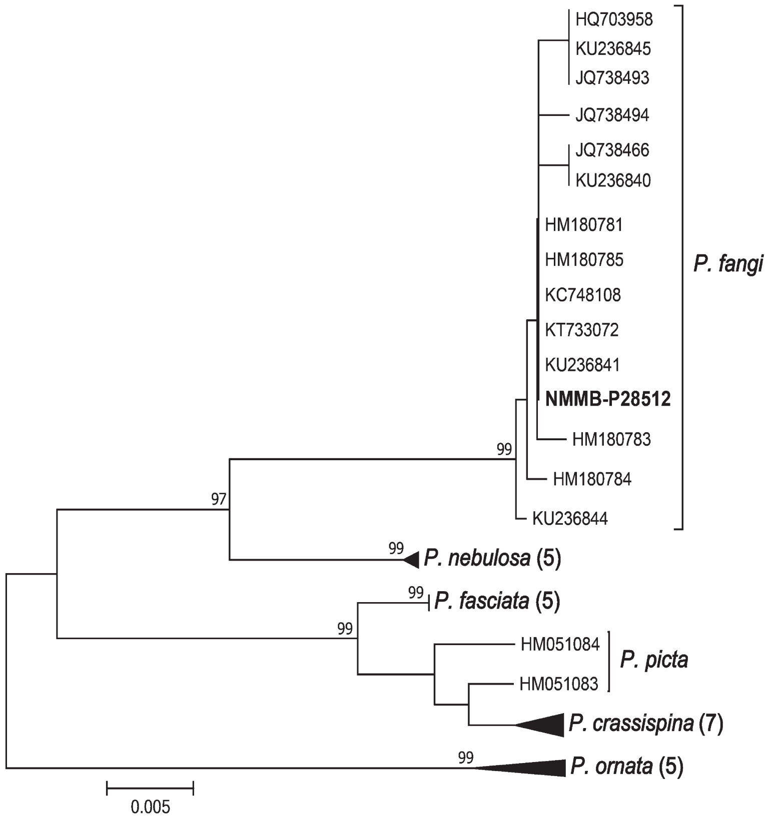

FIGURE 3. Neighbor joining tree based on partial (569 bp) sequences of the *COI* gene of *Pholis fangi* (NMMB-P28152) and other reference specimens. Node support indicated by bootstrap values (> 90 %) based on 1,000 replications. Sequence numbers in parentheses beside each species' name. Scale bar represents the number of base differences per site based on the uncorrected *p*-distance.

Distribution. *Pholis fangi* is currently known from the Bohai and Yellow seas (Yatsu 1981; Yamada *et al.* 2007), newly collected from off southern Taiwan, northern South China Sea, but presently not known from the East China Sea.

Remarks. The morphological characters of the present specimen agree well with the key characters of *P. fangi* given by Yatsu (1981; as *Enedrias fangi*), viz. interorbital pore absent, pectoral-fin length 61.3% of HL (vs. 58–64 % in the latter), and 13 equally-spaced short vertical white bands, margined with dark brown, on dorsal fin. All other count and measurement characters were also consistent with those given in previous studies (Yatsu 1981; Yamada *et al.* 2007).

Within the amplified region of the COI gene sequence obtained from the present specimen, 569 bp were successfully aligned with those of 38 reference specimens (from INSDC or BOLD), including 14 specimens of *P. fangi* (Appendix 1). Five sequences from the latter 14 reference specimens were identical with that of the present specimen, suggesting their conspecificity. Furthermore, all of those 14 reference specimens and the present specimen together formed a monophyletic clade with a high bootstrap value (99) on a NJ tree (Fig. 3).

The entire topology of the NJ tree was also largely consistent with the identification of the specimens. Each species formed a monophyletic clade with a high bootstrap value (99), except for *P. picta* (Kner 1868) and *P. crassispina* (Temminck & Schlegel 1845), the former being paraphyletic with respect to the latter, with only a low bootstrap value (68) supporting the monophyly of the latter. *Pholis fangi* and *P. nebulosa* (Temminck & Schlegel 1845) together constituted a monophyletic clade (supported by a bootstrap value of 99), implying their status as closest relatives. This was matched by the proximity of their distributional ranges (the latter from the northwest Pacific coast southward from Hokkaido, Japan to the Bohai and Yellow Seas) as well as their morphological similarity (see below). Radcheko *et al.* (2012) also suggested that the two species may be the most closely related *Pholis* congeners, based on a phylogenetic analysis of mitochondrial Cytochrome b sequences (approximately 1,100 bp).

Pholis fangi is most similar to *P. nebulosa* but can be clearly distinguished in having a pectoral-fin length greater than 58% of HL (vs. less than 56% in *P. nebulosa*), and a series of short vertical white bands, margined with dark brown, on the dorsal fin (vs. a series of triangular black markings) (Yatsu 1981).

Based on a large-scale trawl survey of the Yellow Sea (including the Bohai Sea) and East China Sea, Yamada *et al.* (2007) reported that *P. fangi* was restricted to the former, and had at no time been collected in the East China Sea. In the Bohai and Yellow seas, specimens of *P. fangi* were generally collected in water temperatures below 13°C (mostly < 10°C), except for two examples (collected in 16°C and 18°C). The World Ocean Atlas 2013 v.2 (Boyer *et al.* 2013) noted that the water temperature in February (100 m depth) at 23° 5′N, 119°5′E (near the collection site), averaged over 8 years from 2005–2012, was 20.5, well above the known temperature range of the species. This indicates that the species may be able to tolerate higher-temperature waters than previously thought.

The previously-recognized southernmost record of *P. fangi* being from the southern Yellow Sea (33° N)(Yamada *et al.* 2007), the present specimen, collected off southwestern Taiwan, represents the first record of *P. fangi* and the family Pholidae from coastal Taiwanese waters, an extension of the southernmost record of the former by more than 1,000 km. It is also the first record of the typically temperate to subarctic family from subtropical waters (Yatsu 1981).

It is uncertain whether the specimen in question represented a local population or was an example of rare dispersal from a more northern source (Bohai/Yellow Seas), not leading to population establishment. The lack of morphological and genetic differentiation between the former and those from the Bohai/Yellow Seas seems supportive of the latter explanation, although genetic divergence between Taiwanese and the Bohai/Yellow Sea populations may be inhibited by substantial gene flow or migration (seemingly unlikely given the absence of records from the East China Sea, despite intensive fisheries and ichthyofaunal surveys). In any case, the present report is a rare example of range extension of a cold water marine organism towards warmer latitudes, contrary to increasing recent observations of movements in the opposite direction, supposedly in relation to water currents (e.g., Koeda & Hibino 2017; Koeda *et al.* 2017; Koeda & Ho 2018a, b).

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APPENDIX 1. List of reference sequences of the genus *Pholis* occurring in the western Pacific Ocean.

Species	INSDC Accession no.	BOLD ID
Pholis crassispina	-	FOJS064-13
Pholis crassispina	-	FOJS065-13
Pholis crassispina	-	FOJS066-13
Pholis crassispina	-	FOJS089-13
Pholis crassispina	-	FOJS101-13
Pholis crassispina	KC748107	GBGCA4718-13
Pholis crassispina	NC_004410	GBMTG323-16
Pholis fangi	JQ738493	ANGBF2256-12
Pholis fangi	JQ738494	ANGBF4536-12
Pholis fangi	JQ738466	ANGBF4549-12
Pholis fangi	HQ703958	ANGBF5800-12
Pholis fangi	HM180781	ANGBF918-12
Pholis fangi	HM180783	ANGBF920-12
Pholis fangi	HM180784	ANGBF921-124
Pholis fangi	HM180785	ANGBF922-12
Pholis fangi	KC748108	GBGCA4717-13
Pholis fangi	KU236845	GBMIN119979-17
Pholis fangi	KT733072	GBMIN129653-17
Pholis fangi	KU236841	GBMIN130172-17
Pholis fangi	KU236840	GBMIN95312-17
Pholis fangi	KU236844	GBMIN95313-17
Pholis fasciata	HQ712712	DSFAL354-08
Pholis fasciata	HQ712713	DSFAL355-08
Pholis fasciata	HM421789	DSFAL651-09
Pholis fasciata	HM421791	DSFAL653-09
Pholis fasciata	HQ712711	DSFAL673-10
Pholis nebulosa	HM180563	ANGBF857-12
Pholis nebulosa	HM180564	ANGBF858-12
Pholis nebulosa	HM180565	ANGBF859-12
Pholis nebulosa	HM180567	ANGBF861-12
Pholis nebulosa	KC748109	GBGCA4716-13
Pholis ornata	GU440457	MFC148-08
Pholis ornata	GU440458	MFC265-08
Pholis ornata	FJ165014	TZFPB876-08
Pholis ornata	FJ165015	TZFPB886-08
Pholis ornata	FJ165016	TZFPB887-08
Pholis picta	HM051084	ANGBF8450-12
Pholis picta	HM051083	ANGBF8454-12