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Morphological Description and Molecular Identification of a Newly Recorded Curved-back Ricefish, *Oryzias curvinotus* (Nichols & Pope, 1927), from Western Taiwan and Lieyu Island

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ABSTRACT

The curved-back ricefish, *Oryzias curvinotus* (Nichols & Pope, 1927), is newly recorded in Taiwan, with specimens collected from the Puzi River in western Taiwan and from Lieyu Island, one of the 12 islands of Kinmen. Identification was based on morphological characters and molecular analysis of mitochondrial DNA COI sequences. Phylogenetic analysis in the present study revealed that samples from the Puzi River formed a unique haplotype, differing from other published haplotypes from China. In contrast, samples from Lieyu Island shared a haplotype identical to seven published COI sequences generated from voucher specimens from China. On the island of Taiwan, *O. curvinotus* is rather rare and currently known only from the Puzi River. However, a ricefish specimen collected in 1916 from the Dajia River in central Taiwan, originally identified as *Oryzias latipes* (CAS-SU 23115), was re-identified as *Oryzias curvinotus* based on diagnostic morphological features examined in this study. *O. curvinotus* is believed to have been widely distributed in western Taiwan in the early 20th century, but its populations have since declined, likely due to the invasion of mosquito fish first introduced to Taiwan in 1911. This study also updates the geographic distribution of *O. curvinotus*, with Lieyu Island representing its northernmost distribution and western Taiwan its easternmost limit worldwide.

Keywords: Hainan medaka, taxonomy, new record, freshwater, COI.

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1 INTRODUCTION

The curved-back ricefish (Hainan medaka), *Oryzias curvinotus* (Nichols & Pope, 1927), is a small fish known to occur in China and Vietnam. In China, the fish is known to be distributed in Guangdong, Guangxi, and Hong Kong, with its type locality on Hainan Island (Uwa & Parenti, 1988; Parenti, 2008; Zhang et al., 2017). This species has been reported to inhabit freshwater environments, including rice fields, pools, reservoirs, and slow-flowing streams (Zhang et al., 2017; website of the Agriculture, Fisheries and Conservation Department, The Government of the Hong Kong Special Administrative Region, 2024). Tran and Ta (2016) observed that *O. curvinotus* inhabits both freshwater and brackish estuarine habitats in northern Vietnam.

In addition to the original description of *O. curvinotus* by Nichols and Pope in 1927, the morphological characters of the species have been redescribed only a few times. Parenti (2008) reported several diagnostic meristic characters of *O. curvinotus*, such as: pectoral fin rays 10–11, anal fin rays 17–20, and lateral scales 27–28. Zhang et al. (2017) further refined these morphological features based on 51 specimens from Guangdong, China, reporting: (1) pre-dorsal length measured as 81.6% ± 1.3% of standard length, (2) dorsal fin rays counted as 6.04 ± 0.20 (range: 6–7), (3) anal fin rays counted as 19.60 ± 0.80 (range: 19–22), (4) pectoral fin rays counted as 8.69 ± 0.47 (range: 8–9), and (5) lateral scales counted as 28.51 ± 0.67 (range: 27–30). These important morphological characters serve to distinguish this species from other congeners.

Zhang et al. (2017) reported that the decline in *O. curvinotus* populations in China was attributable to the invasion of mosquitofish (*Gambusia affinis*). They also noted that this species is considered to be endangered in China. In Guangdong, only sporadic populations persist (Zhang et al., 2017).

In Taiwan, two nominal species of *Oryzias* have been reported so far, including the Chinese ricefish (*Oryzias sinensis*; Chen et al., 1989) and the Chenglong ricefish (*Oryzias chenglongensis*; Chen & Lai, 2024). The Chinese ricefish was listed as vulnerable (VU) in 2017 (Yang et al., 2017). Similar to the decline of *O. curvinotus* populations in China, Huang et al. (2022) proposed that the invasion of poeciliid fish such as mosquitofish and green swordtail was one of the major reasons contributing to the decline of the wild populations of *O. sinensis* in Taiwan.

Between 2021 and 2024, several expeditions were conducted to survey freshwater fish in Taiwan and its adjacent islands. During these surveys, ricefish samples were collected from western Taiwan and Lieyu Island, and were later identified as *O. curvinotus* based on morphological comparisons and molecular identification. The present study describes the morphological characters of these newly identified samples, as well as the results of molecular identification analysis performed on these newly discovered specimens and other comparative samples.

While *O. sinensis* now only inhabits northern Taiwan (Tzeng et al., 2006; Huang et al., 2022), this species was previously reported to be widely distributed in northern and central Taiwan (Chen & Fang, 1999). In order to further investigate the species diversity of the genus *Oryzias* in Taiwan, the current study examined a historical ricefish specimen originally identified as *O. latipes* (CAS-SU 23115), which was collected from the Dajia River in central Taiwan in 1916 and is currently deposited at the Department of Ichthyology, California Academy of Sciences (CAS). The species identification of this specimen is further discussed herein.

2 MATERIALS AND METHODS

2.1 Sample Collection

Sampling localities are shown in Figure 1. The ASIZP (Biodiversity Research Museum, Academia Sinica, Taipei) specimens examined were collected by hand nets. For morphological studies, specimens were fixed in 5% formalin solution and then transferred to 70% ethanol for long-term preservation. For molecular analyses, muscle tissues were preserved in 99.5% ethanol. No live specimens were used in experiments in this study; therefore, no IACUC protocol was required.

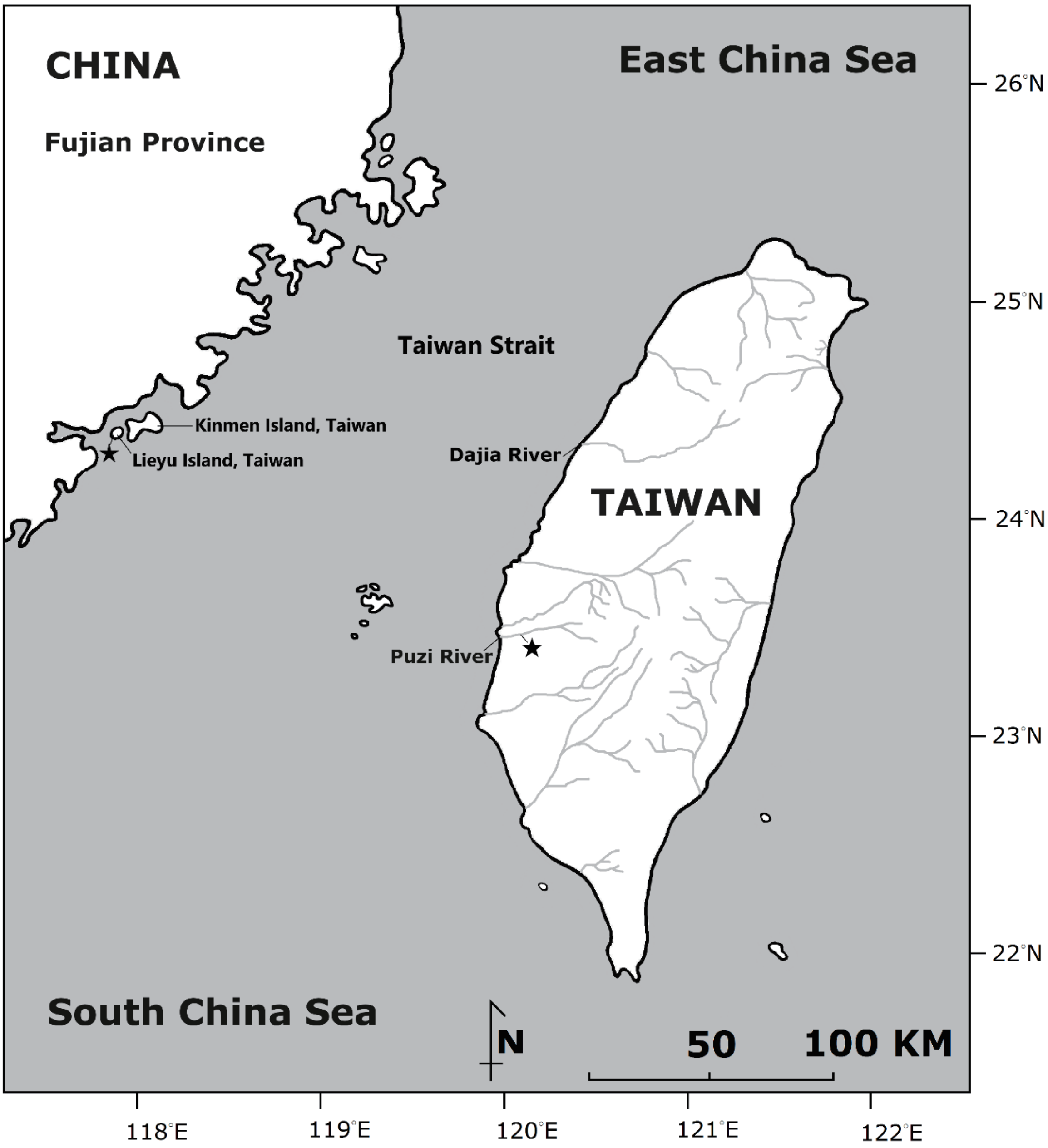

Figure 1. Sampling sites of *Oryzias curvinotus* in the Puzi River, Dajia River, and Lieyu Island, Taiwan. Asterisks indicate the sampling sites.

2.2 Morphological Studies

All morphometric measurements and meristic counts followed Asai et al. (2011) and Zhang et al. (2017). Standard length (SL) was used for all morphometric measurements. All specimens examined were deposited at ASIZP and in the Department of Ichthyology, California Academy of Sciences, California, USA (CAS). Abbreviated names of all institutional codes follow Fricke and Eschmeyer (2024).

2.3 Molecular Analyses

Partial mitochondrial DNA (mtDNA) cytochrome oxidase subunit I (COI) sequences were used as molecular evidence. DNA from the samples was extracted using QuickGene DNA Tissue Kit S (DT-S) (KURABO, Japan). A pair of primers (FishF2 and FishR2), following Ward et al. (2005), was used for PCR and sequencing.

Sequences were aligned using BIOEDIT 5.09 (Hall, 1999) prior to phylogenetic analysis. Sequence analyses were conducted using Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0 (Kumar et al., 2016). The maximum likelihood (ML) method was employed for phylogenetic analyses. ML analyses were carried out using MEGA 7.0 (Kumar et al., 2016). Branch support for ML trees was established via bootstrap analyses (with 1,000 replications). The best-fit model for ML analyses was selected using MEGA 7.0. For all phylogenetic analyses, 159 accession numbers of COI sequences of *O. curvinotus* from China were downloaded from GenBank as comparative molecular material. Additionally, *O. chenglongensis* collected from its type locality, the Chenglong Wetland in western Taiwan, was sequenced and used as an outgroup in this study. Nine species of ricefish, including *O. celebensis*, *O. dancena*, *O. javanicus*, *O. latipes*, *O. luzonensis*, *O. melastigma*, *O. minutillus*, *O. sarasinorum*, and *O. sinensis*, were also included as outgroups using sequences downloaded from GenBank (Miya et al., 2003; Setiamarga et al., 2008; Setiamarga et al., 2009; Yoon et al., 2011; Hwang et al., 2012; Ma et al., 2020; Huang et al., 2022).

2.4 Examined Specimens of *Oryzias curvinotus*

ASIZP0082339, male, 18.5 mm SL (22.1 mm TL); lower reach of the Puzi River, Puzi, Chiayi County, Taiwan; collected by Shih-Pin Huang, December 17, 2022.

ASIZP0082340, male, 18.1 mm SL (21.6 mm TL); lower reach of the Puzi River, Puzi, Chiayi County, Taiwan; collected by Shih-Pin Huang, August 6, 2022.

ASIZP0082341, 6 specimens, male, 18.1–29.4 mm SL (33.7–35.7 mm TL); Lieyu Island, Kinmen County, Taiwan; collected by Kwang-Yiao Chen, October 31, 2021.

ASIZP0082342, 2 specimens, female, 26.0–31.1 mm SL (31.6–37.6 mm TL); Lieyu Island, Kinmen County, Taiwan; collected by Kwang-Yiao Chen, October 31, 2021.

CAS-SU 23115, male, 22.0 mm SL (26.5 mm TL); Taiko, Formosa (= Dajia River, Taichung, Taiwan); collected by Masamitsu Oshima, 1916. Examination of diagnostic external morphological features of this specimen was based on detailed specimen images photographed by Jon D. Fong.

3 RESULTS AND DISCUSSION

3.1 Morphological Description of *O. curvinotus* from the Puzi River and Lieyu Island

The meristic counts of *O. curvinotus* collected from the Puzi River and Lieyu Island are as follows: dorsal fin rays 6 (in 10); anal fin rays 18–21: 18 (in 2), 19 (in 4), 20 (in 3), 21 (in 1); pectoral fin rays 9–10: 9 (in 12), 10 (in 8); pelvic fin rays 6 (in 20). Lateral scales 27–30: 27 (in 2), 28 (in 2), 29 (in 9), 30 (in 7). Morphometric measurements are shown in Table 1.

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Table 1. Morphometric measurements of *Oryzias curvinotus* from the Puzi River and Lieyu Island. Mean values are shown in parentheses.

Localities	**Puzi River**					**Lieyu Island**							
Characters	male (n=2)					male (n=2)				female (n=2)			
Total length (mm)	21.6–22.1					35.0–35.4				31.6–37.6			
Standard length (mm)	18.1–18.5					29.1–29.4				26.0–31.1			
Percentage in standard length (%)													
Body depth	20.4	–	21.1	(20.7)		22.3	–	24.8	(23.6)	22.2	–	24.6	(23.4)
Body width	11.4	–	12.2	(11.8)		11.0	–	11.6	(11.3)	12.5	–	13.5	(13.0)
Head length	25.4	–	25.9	(25.7)		23.7	–	24.5	(24.1)	25.4	–	26.5	(26.0)
Depth of caudal peduncle	8.3	–	9.7	(9.0)		9.3	–	9.5	(9.4)	9.0	–	10.4	(9.7)
Length of caudal peduncle	13.8	–	15.7	(14.7)		14.3	–	15.1	(14.7)	13.2	–	14.6	(13.9)
Height of dorsal fin	18.9	–	19.3	(19.1)		17.3	–	19.6	(18.5)	12.5	–	14.6	(13.6)
Length of dorsal fin base	7.6	–	7.7	(7.7)		6.5	–	7.1	(6.8)	6.1	–	6.9	(6.5)
Height of anal fin	14.6	–	17.1	(15.9)		15.5	–	18.7	(17.1)	10.0	–	10.8	(10.4)
Length of anal fin base	27.0	–	28.2	(27.6)		29.6	–	29.9	(29.7)	25.4	–	26.0	(25.7)
Pectoral fin length	21.1	–	21.5	(21.3)		22.1	–	23.0	(22.6)	20.6	–	22.7	(21.6)
Length of pectoral fin base	7.2	–	7.6	(7.4)		6.5	–	6.8	(6.7)	6.4	–	6.9	(6.7)
Pelvic fin length	12.4	–	13.3	(12.9)		12.0	–	12.9	(12.5)	12.5	–	13.5	(13.0)
Pre-dorsal length	81.1	–	83.4	(82.3)		81.3	–	82.5	(81.9)	81.4	–	81.9	(81.6)
Pre-anal length	57.3	–	58.0	(57.7)		56.7	–	57.5	(57.1)	57.2	–	61.2	(59.2)
Pre-pelvic length	44.3	–	45.9	(45.1)		42.3	–	44.2	(43.2)	50.5	–	51.2	(50.8)
Length from pectoral base to ventral base	19.9	–	20.0	(19.9)		20.4	–	21.6	(21.0)	22.8	–	23.5	(23.1)
Length from dorsal fin base to caudal fin base	21.1	–	21.5	(21.3)		21.1	–	22.7	(21.9)	21.9	–	22.3	(22.1)
Percentage in head length (%)													
Head width	63.0	–	64.6	(63.8)		62.5	–	65.2	(63.9)	63.8	–	64.6	(64.2)
Occiput depth	65.2	–	66.7	(65.9)		65.2	–	65.3	(65.2)	65.2	–	65.8	(65.5)
Snout length	28.3	–	29.2	(28.7)		27.5	–	29.2	(28.4)	29.0	–	29.1	(29.1)
Interorbital width	50.0	–	52.1	(51.0)		50.0	–	50.7	(50.4)	49.4	–	50.7	(50.0)
Eye diameter	41.3	–	41.7	(41.5)		36.2	–	37.5	(36.9)	38.0	–	39.1	(38.6)

Description of morphological characters: Body small, elongated, and laterally compressed. Snout prominent. Eyes relatively large; eye diameter measured as 36.2–41.7% of head length. Belly slightly flattened in males and rounded in adult females. Pre-dorsal fin region noticeably bulged in adults. Pre-dorsal length comprising 81.1–83.3% of standard length. Length of dorsal fin in males greater than in females; height of dorsal fin measured as 17.3–19.6% of standard length in males and only 12.5–14.6% in females. Rear margin of dorsal fin reaching caudal fin base when compressed in males but not in females. Rear margin of dorsal fin rays filamentous in adult males. Anal fin parallelogram-shaped in males while right-angled triangular-shaped in females. Caudal fin truncated in both sexes. Body covered with relatively large, cycloid scales; lateral lines absent.

Head and body translucent and generally pale yellowish-brown. Belly pale yellowish-white. Anterior region of the snout yellow to saffron yellow. Pre-dorsal fin region with a distinct longitudinal stripe extending to the rear margin of the inter-orbital region. Two pairs of bright yellowish-white marks on dorsal side of neck. Dorsal fin membrane generally grayish-white to pale yellowish-white; anterior region usually deep yellow in adult individuals. Caudal fin membrane generally grayish-white to pale yellowish-white with a distinct yellowish-brown stripe along its upper and lower margins in adult males; stripes usually yellow in females. Anal fin membrane usually gray in adult males and grayish-white in females. Live and preserved specimens of *O. curvinotus* collected from the Puzi River and Lieyu Island are shown in Figure 2.

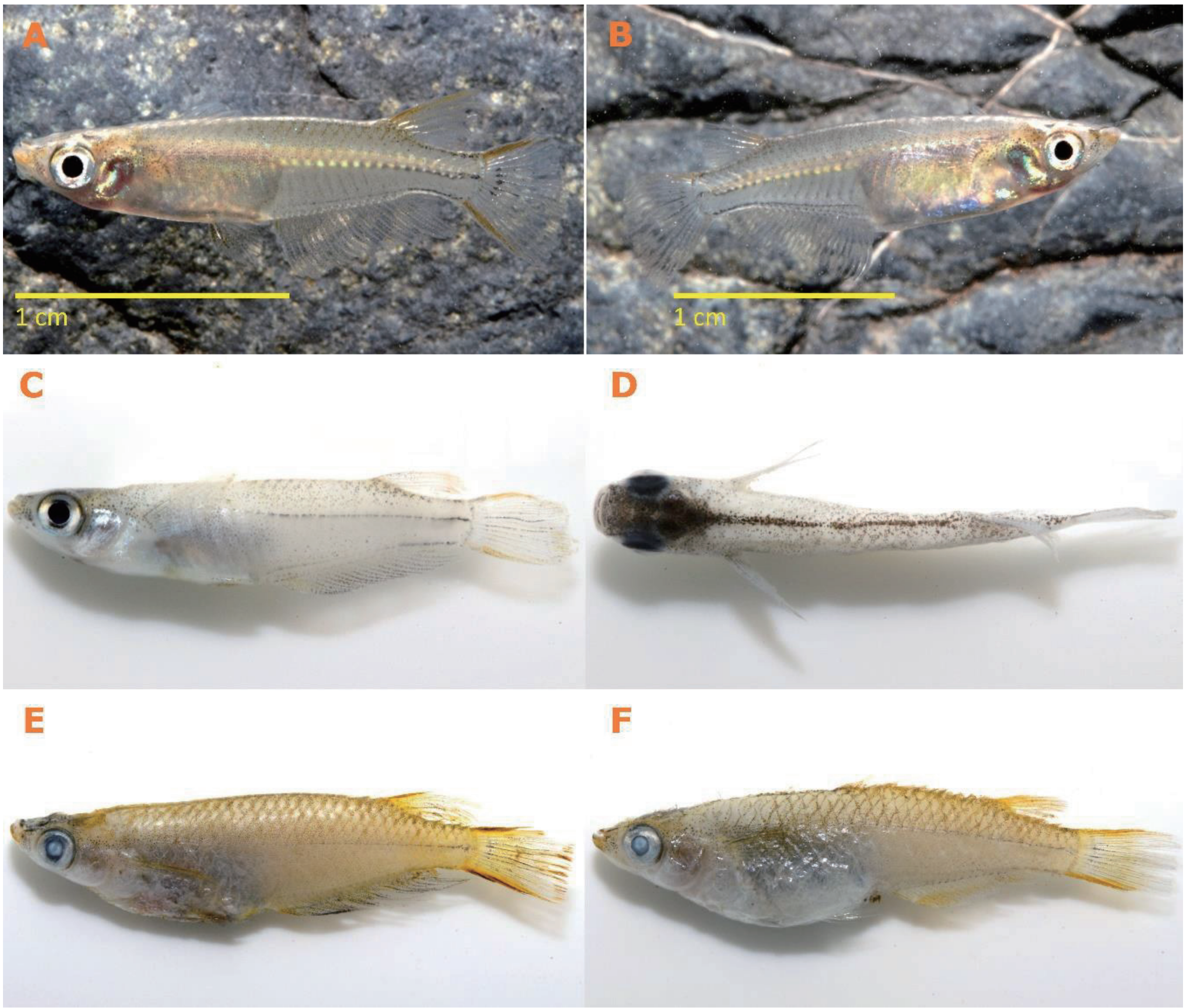

Figure 2. Live individuals of *Oryzias curvinotus* collected from the Puzi River (A. male, B. female; not listed in examined materials); specimen photographs of *O. curvinotus* collected from the Puzi River (C. lateral view, D. dorsal view of ASIZP0082340, male, 18.1 mm SL); specimen photographs of *O. curvinotus* collected from Lieyu Island (E. ASIZP0082341, male, 29.4 mm SL; F. ASIZP0082342, female, 31.1 mm SL).

Based on morphological characters of specimens collected from the Puzi River and Lieyu Island, the fish is identified as *O. curvinotus*. A higher proportion of pre-dorsal length, a diagnostic feature of *O. curvinotus* described in Zhang et al. (2017), was also observed in the examined specimens collected from the Puzi River and Lieyu Island (81.6% ± 1.3% in Zhang et al. vs. 81.1–83.3% in the present study).

3.2 Molecular Analyses

Partial COI sequences from the examined *O. curvinotus* specimens, along with comparative COI sequences downloaded from GenBank, were analyzed. A total of 65 COI gene haplotypes, derived from 165 individuals, were included in this analysis. The accession numbers of *O. curvinotus* and outgroups used for molecular analysis are shown in Table 2. The length of COI sequences was 537 bp in total. The alignment contained 86 total mutations and 84 polymorphic (segregating) sites. The phylogenetic tree (Figure 3) was reconstructed by the maximum likelihood (ML) method under the HKY+G+I model.

Table 2. Accession numbers of *Oryzias curvinotus* and outgroups used for molecular analysis in this study.

Species	Accession number / Isolate	Sample size	Locality	Same haplotype	Source
O. curvinotu	PQ443705	6	Puzi River, Taiwan	—	This study
	PQ443706	3	Lieyu Island, Taiwan	MK049268.1-GZ6; MF928998.1-ST7; MF928995.1-ST4; MF928993.1-RP6; MF928991.1-RP4; MF928989.1-ST3; MF928986.1-RP2	
O. curvinotus	MF928906.1-LG1	8	China	MF928907.1-LG2; MF928908.1-LG3; MF928909.1-LG4; MF928910.1-LG5; MF928911.1-LG6; MF928913.1-LG8; MF928976.1-FC14	Long and Wang, 2019, unpublished data
	MF928897.1-LS5	3	China	MF928898.1-LS6; MF928905.1-LS13	
	MF928930.1-LS3	4	China	MF928901.1-LS9; MF928903.1-LS11; MF928964.1-DZG7	
	MF928958.1-DZG1	5	China	MF928959.1-DZG2; MF928960.1-DZG3; MF928962.1-DZG5; MF928967.1-DZG10	
	MF928886.1-CM1	5	China	MF928887.1-CM2; MF928893.1-CM8; MF928894.1-CM9; MF928895.1-CM10	
	MF928951.1-DHD7	3	China	MF928936.1-GQ4; MF929021.1-HG9	
	MF928943.1-HG4	5	China	MF928947.1-HG8; MF928946.1-HG7; MF928944.1-HG5; MF928945.1-HG6	
	MF928935.1-GQ3	4	China	MF928937.1-GQ5; MF928938.1-GQ6; MF929017.1-GQ10	
	MK049258.1-SY1	3	China	MK049259.1-SY2; MK049260.1-SY3	
	MF928929.1-LS2	7	China	MF929020.1-GQ13; MF929019.1-GQ12; MF928940.1-GQ8; MF928904.1-LS12; MF928900.1-LS8; MF928899.1-LS7	
	MF928917.1-WC2	8	China	MF928918.1-WC3; MF928920.1-WC5; MF928921.1-WC6; MF928922.1-WC7; MF928923.1-WC8; MF928924.1-WC9; MF928925.1-WC10	
	MF928969.1-FC5	8	China	MF928956.1-DHD12; MF928942.1-HG3; MF928972.1-FC8; MF928977.1-FC15; MF928978.1-FC16; MF928980.1-FC18; MF928984.1-FC23	
	MF928928.1-LS1	3	China	MF928896.1-LS4; MF928902.1-LS10	
	MF928970.1-FC6	2	China	MF928975.1-FC12	
	MF928931.1-FC1	6	China	MF928968.1-FC4; MF928971.1-FC7; MF928979.1-FC17; MF928981.1-FC20; MF928982.1-FC21	
	MF928927.1-DHD2	5	China	MF928932.1-FC2; MF928933.1-FC3; MF928948.1-DHD4; MF928949.1-DHD5	

Species	**Accession number / Isolate**	**Sample size**	**Locality**	**Same haplotype**	**Source**
	MF928926.1-DHD1	9	China	MF928983.1-FC22; MF928974.1-FC11; MF928973.1-FC10; MF928957.1-DHD13; MF928955.1-DHD11; MF928954.1-DHD10; MF928953.1-DHD9; MF928952.1-DHD8	
	MF928939.1-GQ7	2	China	MF929018.1-GQ11	
	MF929000.1-QZ1	3	China	MF929002.1-QZ3; MF929013.1-QZ7	
	MF929001.1-QZ2	5	China	MF929014.1-QZ8; MF929012.1-QZ6; MF929004.1-QZ5; MF929003.1-QZ4	
	MF928992.1-RP5	2	China	MF928994.1-RP7	
	MF928999.1-ST8	2	China	MF929009.1-ST13	
	MF928996.1-ST5	2	China	MF928997.1-ST6	
	MK049268.1-GZ6	7	China	MF928998.1-ST7; MF928995.1-ST4; MF928993.1-RP6; MF928991.1-RP4; MF928989.1-ST3; MF928986.1-RP2	
	MK049263.1-GZ1	1	China	—	
	MK049264.1-GZ2	1	China	—	
	MK049265.1-GZ3	1	China	—	
	MK049266.1-GZ4	1	China	—	
	MK049267.1-GZ5	1	China	—	
	MK049269.1-GZ7	1	China	—	
	MK049270.1-GZ8	1	China	—	
	MK049261.1-SY4	1	China	—	
	MK049262.1-SY5	1	China	—	
	MK049271.1-ZH1	1	China	—	
	MK049272.1-ZH2	1	China	—	
	MK049273.1-ZH3	1	China	—	
O. curvinotus	MF928987.1-ST1	1	China	—	Long and Wang, 2019, unpublished data
	MF928988.1-ST2	1	China	—	
	MF929005.1-ST9	1	China	—	
	MF929006.1-ST10	1	China	—	
	MF929007.1-ST11	1	China	—	
	MF929008.1-ST12	1	China	—	
	MF928985.1-RP1	1	China	—	
	MF928990.1-RP3	1	China	—	
	MF928888.1-CM3	1	China	—	
	MF928889.1-CM4	1	China	—	
	MF928890.1-CM5	1	China	—	
	MF928891.1-CM6	1	China	—	
	MF928892.1-CM7	1	China	—	
	MF928961.1-DZG4	1	China	—	
	MF928963.1-DZG6	1	China	—	
	MF928965.1-DZG8	1	China	—	

Species	Accession number / Isolate	Sample size	Locality	Same haplotype	Source
	MF928966.1-DZG9	1	China	—	
	MF928941.1-DHD3	1	China	—	
	MF928950.1-DHD6	1	China	—	
	MF928916.1-WC1	1	China	—	
	MF928919.1-WC4	1	China	—	
	MF928912.1-LG7	1	China	—	
	MF928914.1-LG9	1	China	—	
	MF928915.1-LG10	1	China	—	
	MF929015.1-QZ9	1	China	—	
	MF929016.1-GQ9	1	China	—	
	MF929010.1-HG1	1	China	—	
O. celebensis	MN064715	1	—	—	Ma et al. 2020
O. chenglongensis	PQ834836	6	Chenglong Wetland, Taiwan	—	This study
O. dancena	NC012976.1	1	—	—	Setiamarga et al. 2009
O. javanicus	NC012981.1	1	—	—	Setiamarga et al. 2009
O. latipes	AP004421.1	1	Japan	—	Miya et al. 2003
O. luzonensis	NC012979.1	1	—	—	Setiamarga et al. 2009
O. melastigma	JQ713914.1	1	—	—	Hwang et al. 2012
O. minutillus	AB498068.1	1	—	—	Setiamarga et al. 2009
O. sarasinorum	AB370891	1	—	—	Setiamarga et al. 2008
O. sinensis	NC013434.1	1	South Korea	—	Yoon et al. 2011
O. sinensis	ON619547	1	Shuanglienpi, Taiwan	—	Huang et al. 2022

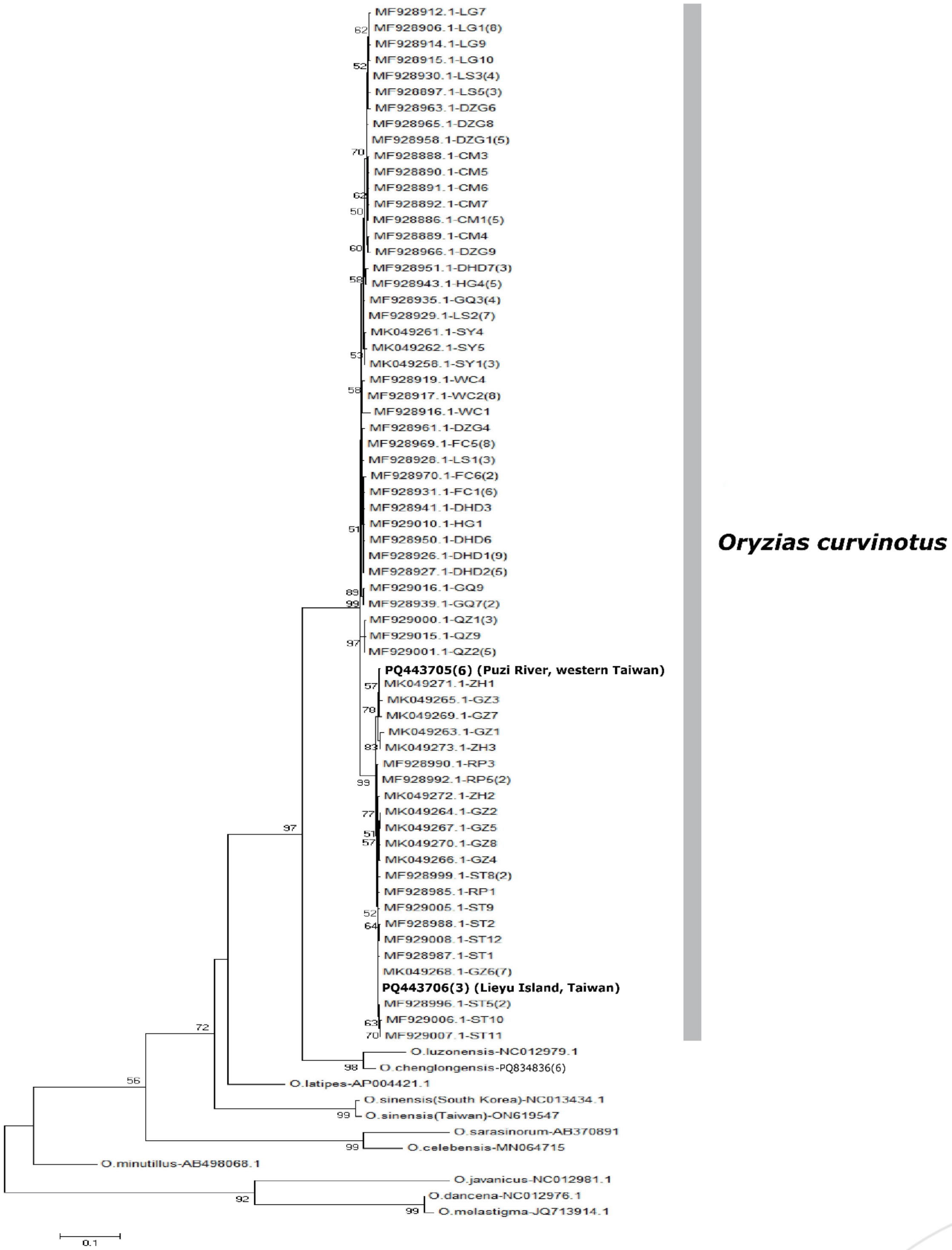

Figure 3. Molecular phylogenetic tree of *Oryzias curvinotus* and other comparative materials based on partial COI sequences, reconstructed using the maximum likelihood method. Bootstrap support values >50% are shown. The sample size for each haplotype is shown after the OTU (shown as accession number).

Results from the molecular analysis revealed that the studied *Oryzias* samples collected from the Puzi River and Lieyu Island were grouped in the *O. curvinotus* clade and confirmed to be the same species.

The phylogenetic tree was divided into two major clades with high bootstrap support (99). The first clade comprised 41 haplotypes (119 accession numbers included) from China. The second clade comprised 23 haplotypes (32 accession numbers included) from China, plus one haplotype each from the Puzi River (6 individuals) and Lieyu Island (3 individuals).

The present phylogenetic analysis revealed that samples from Lieyu Island (PQ443706) and seven published accession numbers from seven Chinese samples formed a single haplotype. In contrast, samples from the Puzi River (PQ443705) formed a unique haplotype that differed from published haplotypes (see Table 2).

Parenti (2008) reported that *O. curvinotus* occurs in freshwater to brackish water, while Zhang et al. (2017) reported that the species is found in freshwater. Even though this species was found in the freshwater habitat of the Puzi River, considering that *O. curvinotus* can also tolerate brackish water, the fish may also be distributed down to the estuary area of the river.

The Taiwan Strait is known as a natural geographical barrier between Taiwan Island and China. The unique haplotype of the Puzi River specimen (PQ443705) may be attributed to the barrier imposed by the Taiwan Strait. A previous study revealed high similarity in freshwater fish fauna between Taiwan Island and southeastern mainland China, which is attributable to the connections between rivers in Taiwan and China when sea levels dropped during the ice ages in the Pleistocene (Chen & He, 2001). Therefore, *O. curvinotus* may have crossed the Taiwan Strait during this epoch and is now distributed in Taiwan's inland waters. In contrast, Lieyu Island is located of the southeastern coast of mainland China and is near the estuary of the Joulong River, Fujian, China. The single haplotype shared between Lieyu Island (PQ443706) and continental China (e.g., MK049268.1, MF928998.1) is likely related to the proximity of these two locations.

3.3 Habitats in Western Taiwan and Lieyu Island

In Taiwan, *O. curvinotus* is known only from the lower reaches of the Puzi River, Puzi City, Chiayi County, located in the Chianan Plain of western Taiwan. The habitat of the Puzi River is shown in Figure 4. Overall, this species is relatively rare in the Puzi River. Water salinity measurements were taken on August 6, 17, and 31, 2024, at the sampling site. The salinity levels were measured as 0.40, 0.13, and 0.14 psu, respectively, indicating a freshwater environment. In addition, a total of 14 sympatric fish species were recorded in the same habitat, including seven native species (*Hemiculter leucisculus*, *Opsariichthys pachycephalus*, *Periophthalmus modestus*, *Pseudogobius javanicus*, *Pseudogobius taijianensis*, *Rhinogobius similis*, and *Rhodeus ocellatus*) and seven introduced species (*Barbonymus gonionotus*, *Channa striata*, *Gambusia affinis*, *Oreochromis niloticus*, *Poecilia reticulata*, *Pterygoplichthys pardalis*, and *Trichopodus trichopterus*). On Lieyu Island, the species was collected from a pond near the coastal area.

Figure 4. Habitat of *Oryzias curvinotus* in the Puzi River, Chiayi, western Taiwan.

3.4 Updated Geographic Distribution

According to previously published information by Zhang et al. (2017), Guangdong represented the northernmost and easternmost distribution limits of *O. curvinotus*. However, this study revealed that, worldwide, its northernmost distribution area is Lieyu Island, and its easternmost distribution area is western Taiwan. These new findings extend the known range of *O. curvinotus*, which now includes Guangdong, Hong Kong, Guangxi, and Hainan in China, extending westward to northern Vietnam, eastward to western Taiwan, and northward to Lieyu Island.

3.5 A Supposition About the Historical Distribution of *O. curvinotus* on the Island of Taiwan

In Taiwan, *Oryzias sinensis* was initially identified as *Aplocheilus latipes* (Chen, 1954), later recorded as *Oryzias latipes* (Shen, 1993; Chen & Fang, 1999), re-identified as *O. latipes sinensis* by Tzeng et al. in 2006, and eventually classified as *O. sinensis* (Huang et al., 2022). This species was considered widely distributed in northern and central Taiwan in the past (Chen & Fang, 1999). The second species of ricefish found in Taiwan is *O. chenglongensis*, described as a new species by Chen and Lai in 2024, known from the brackish waters of Chenglong Wetland in western Taiwan. The present study identified *O. curvinotus* as the third species of ricefish found in Taiwan.

The present study also re-examined a 1916 specimen of *Oryzias* (CAS-SU 23115), collected from the Dajia River in central Taiwan (Figure 5). It was determined to be *O. curvinotus*, clearly distinguishable from *O. sinensis* based on the following diagnostic morphological characters: (1) pre-dorsal length measured as 80.3% of standard length, (2) pre-dorsal fin area with a distinct longitudinal stripe extending to the rear margin of the inter-orbital region, (3) dorsal fin rays with filamentous rear margins in adult males, and (4) a prominently bulged pre-dorsal fin region. In addition, this specimen can also be clearly distinguished from *O. chenglongensis* and identifiable as *O. curvinotus* based on the following diagnostic morphological characters: (1) dorsal fin rays with filamentous rear margins in adult males, and (2) a prominently bulged pre-dorsal fin region. All these specific features were also seen in the *O. curvinotus* specimens examined in this study.

Figure 5. Lateral view (upper) and dorsal view (lower) of *Oryzias curvinotus* collected from the Dajia River, Taichung, central Taiwan in 1916, CAS-SU 23115, male, 22.0 mm SL. Photo by Jon D. Fong.

Field surveys carried out in this study indicate that *O. curvinotus* is rare in western Taiwan at the present time. However, it can be conjectured from the specimen (CAS-SU 23115) of the Dajia River that *O. curvinotus* may have had a broader distribution in western Taiwan in the early 20th century and may have been misidentified as *O. latipes* in earlier classifications.

3.6 Comments on the Conservation of *O. curvinotus* on the Island of Taiwan

The mosquitofish (*Gambusia affinis*), introduced to Taiwan in 1911 (Sato et al., 1972), is considered a threat to *O. sinensis* in Taiwan (Huang et al., 2022) and to *O. curvinotus* in China (Zhang et al., 2017). It is conjectured that the invasion of mosquitofish has resulted in the decline of *O. curvinotus* populations in western Taiwan.

Zhang et al. (2017) mentioned that *O. curvinotus* currently requires conservation in China. This species is also listed as a "key species of conservation concern" in Hong Kong (website of the Agriculture, Fisheries and Conservation Department, The Government of the Hong Kong Special Administrative Region, 2024). Given the limited distribution and low population numbers of *O. curvinotus* in Taiwan, more effective conservation measures and policies are recommended in order to protect this species.

The major threats to *O. curvinotus* population in western Taiwan include the invasion of alien fish species (especially *Gambusia affinis* and *Poecilia reticulata*), habitat destruction, and water pollution caused by domestic wastewater discharge and livestock runoff. Thus, a feasible approach to conserve this population would include removing introduced fish species, avoiding habitat destruction, and preventing water pollution. Furthermore, in order to increase the wild population, habitat creation and artificial restoration should be evaluated.

4 CONCLUSIONS

Based on morphological and molecular evidence, the present study extends the known distribution range of *Oryzias curvinotus* beyond China and Vietnam to include western Taiwan and Lieyu Island. Additionally, this study proposes that *O. curvinotus* was present in central Taiwan's Dajia River in 1916, with the specimen collected there representing the earliest confirmable occurrence of this species in Taiwan. These findings improve our understanding of the distribution range of *O. curvinotus*, identifying Lieyu Island as its northernmost distribution area and western Taiwan as its easternmost distribution area. The updated geographic distribution data, along with molecular analyses of populations in Taiwan and China, provide foundational insights for establishing further conservation strategies for this threatened species.

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