


First record of the Javanese ricefish, *Oryzias javanicus* (Bleeker, 1854) (Beloniformes: Adrianichthyidae) in the natural waters of India

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1 | INTRODUCTION

The Javanese ricefish, *Oryzias javanicus* (Bleeker, 1854), (Beloniformes: Adrianichthyidae) has a natural distribution in the brackishwaters of South East Asia from Peninsular Thailand, Malaysia, Singapore, Western Borneo and Indonesia east to Sulawesi and Lombok (Fricke, Eschmeyer, & Laan, 2018), and in Bangladesh (Ahmed, Islam, Sanzida, Akter, & Nahar, 2017, Unpublished; based on sequences available in GenBank) (Figure 1). Together with *O. dancena*, they are two of the most adaptable ricefish species, and highlighted as new experimental models (Imai, Koyama, & Fujii, 2005; Kang, Tsai, Liu, Lee, & Hwang, 2010; Parenti, 2008; Yusof, Ismail, Koito, Kinoshita, & Inoue, 2012). Here, we record for the first time, the occurrence of *O. javanicus* from natural waters in the south-eastern coast of India, ~1,000 km away from its natural distribution range, and separated by the Bay of Bengal.

2 | MATERIALS AND METHODS

Live samples ($n = 15$) of *Oryzias* sp. were collected on 14th April 2018 from the Muttukadu lagoon (12°48'31.38"N, 80°14'42.51"E) (Figure 1) near the experimental station of ICAR-Central Institute of Brackishwater Aquaculture (CIBA), Chennai, India. The collection location was 250 m away from the mouth of the Muttukadu lagoon, which opens to the Bay of Bengal. Fish was encountered mainly in the shallow water zone near mangrove areas with slow water current

and muddy substratum. They were found to be swimming in small schools close to the water surface at a depth of <50 cm. At the time of collection, water salinity was around 33 ppt. The total length of male and female fish ranged from 32.5–36.8 mm and 30.2–34.5 mm respectively. For morphological identification, 10 samples were preserved in 10% formaldehyde mixed with seawater and five samples were preserved in 90% ethanol for molecular studies. Two samples (one male and one female) were deposited in the collections of the Zoological Survey of India (ZSI) with the accession number MBRC/ZSI/F1853. To validate the morphological identification, the partial sequences of cytochrome c oxidase subunit I (COI) gene was generated. The genomic DNA was extracted from ethanol preserved muscle tissue using a standard DNA proteinase K digestion/phenol-chloroform extraction procedure. A degenerated primer was designed by aligning COI sequence of *Oryzias* spp. available in NCBI GenBank. The fragments of COI gene (532 bp) were amplified using primer; COI_OJ_F: 5' AAGCCTGCTAATTCGAGCWG 3' and COI_OJ_R: 5' GGGTCGAAGAATGTGGTRTT 3'. PCR amplification was carried out in a reaction mixture containing 10 ng template DNA, 12.5 µl of Taq DNA polymerase master mix containing 1.5mM MgCl₂ (Ampliqon), 0.4 µM of each primer, and nuclease free water added up to 25 µl. The PCR amplification was carried out with the following conditions: 35 cycles of denaturation (93°C for 30 s), annealing (50°C for 30 s) and extension (72°C for 45 s) with an initial denaturation step of 93°C for 2 min and a final extension step at 72°C for 5 min. The purified PCR products were bi-directionally sequenced using corresponding primer sets. A neighbour-joining (NJ) phylogenetic tree and a maximum-likelihood (ML) tree were constructed

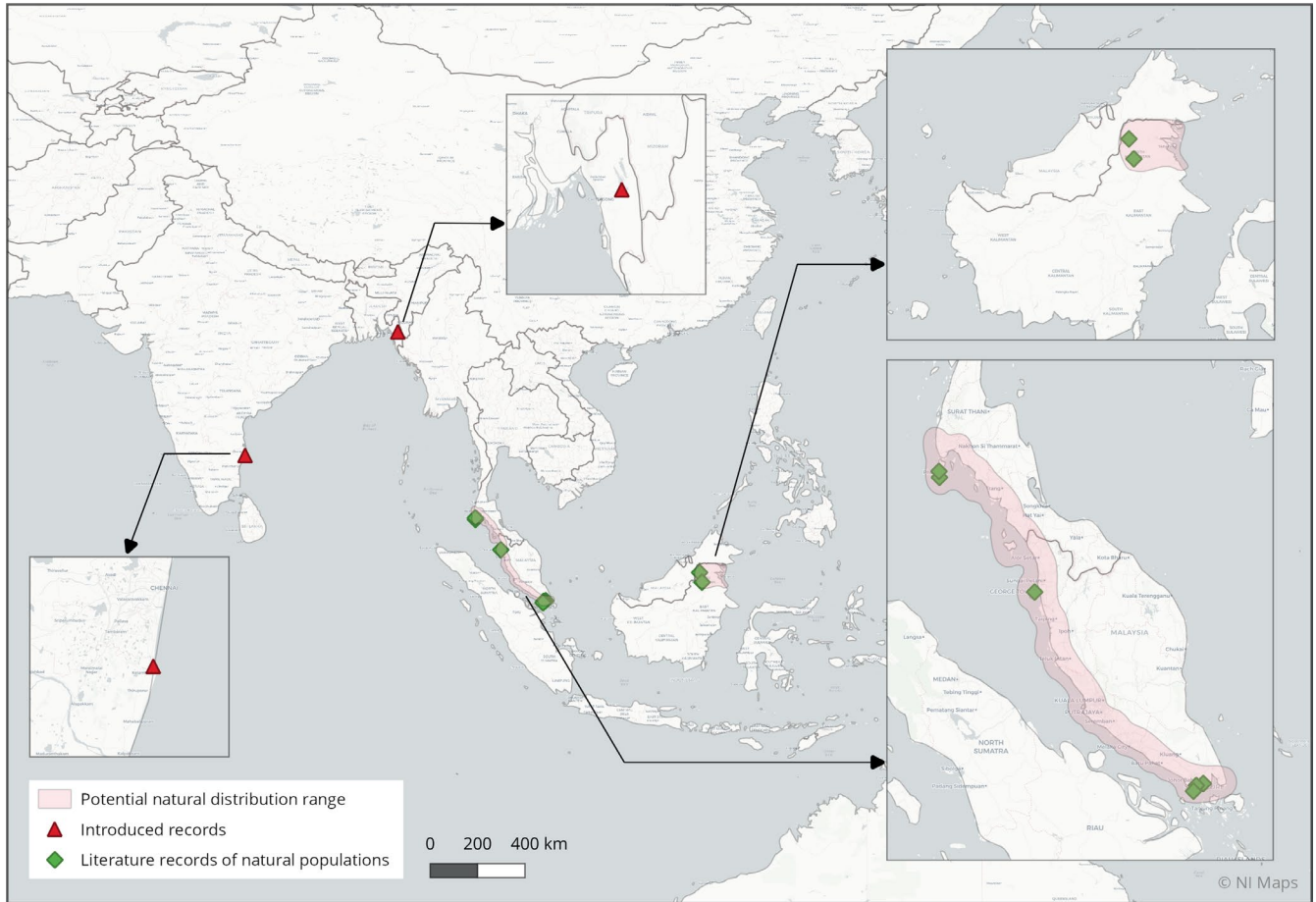


FIGURE 1 Map showing the native distribution range of *Oryzias javanicus* in South East Asia, and the new record from India

TABLE 1 Comparison of meristic counts of *Oryzias javanicus*

Meristic counts	Roberts (1998)	Parenti (2008)	Magtoon and Termvidchakorn (2009)	Yusof et al., (2012)	Present study
Dorsal-fin rays	6–7	6–8	6–8	6	6
Anal-fin rays	21–25	18–25	22–26	21	20–21
Pelvic-fin rays	6	5–6	NA	6	6
Pectoral-fin rays	11	10–13	11–12	10–11	10–12
Principal caudal-fin rays ^a	5/6	i, 4/5, i	i, 4/5, i	i, 5/6, i	i, 4/5, i

Note: Table adapted from Yusof et al., (2012) with observations of the present study: ^aRays of dorsal and ventral lobes are indicated separated by slash. Number of unbranched and branched rays is indicated by Roman and Arabic numerals, respectively.



FIGURE 2 (a) Male and (b) female *Oryzias javanicus* collected from the Muttukadu lagoon, India

from the COI sequences of *Oryzias* species retrieved from NCBI GenBank using MEGA V. 7 (Kumar, Stecher, & Tamura, 2016).

3 | RESULTS

The Muttukadu lagoon population of *Oryzias* sp. was identified as *Oryzias javanicus* following the taxonomic characters provided by Iwamatsu, Imaki, Kawamoto, and Inden (1982), Uwa and Parenti (1988) and Parenti (2008) and supported by genetic data. The species shows population-level variations in their meristic counts (Table 1).

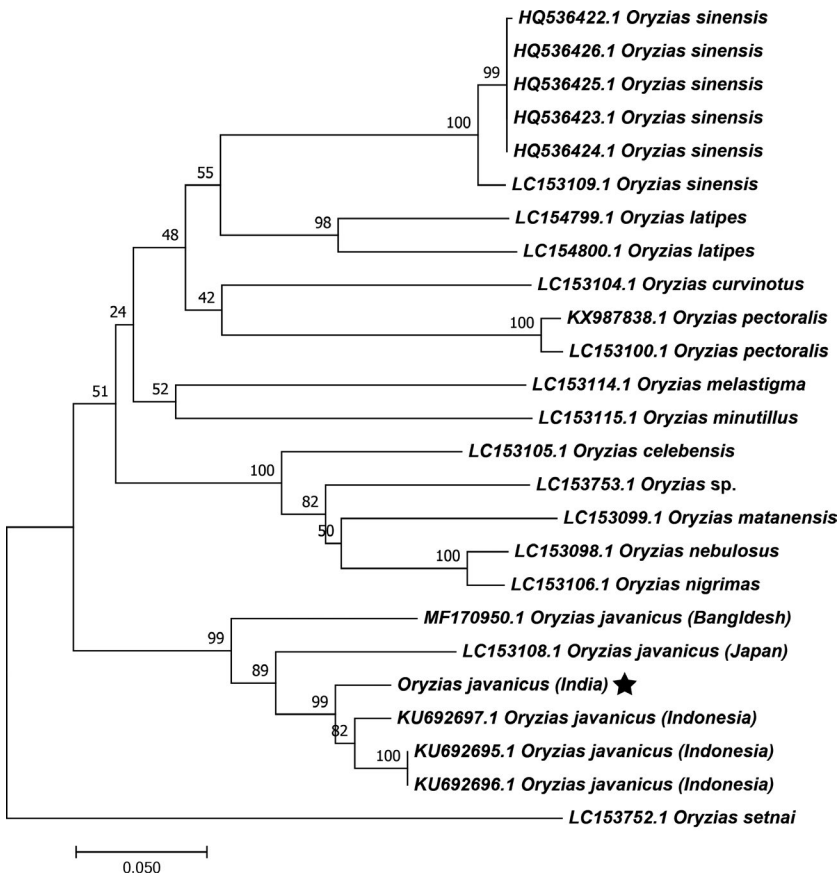


FIGURE 3 Neighbour-joining tree based on mtDNA COI gene of different *Oryzias* species together with the sequence generated in the present study

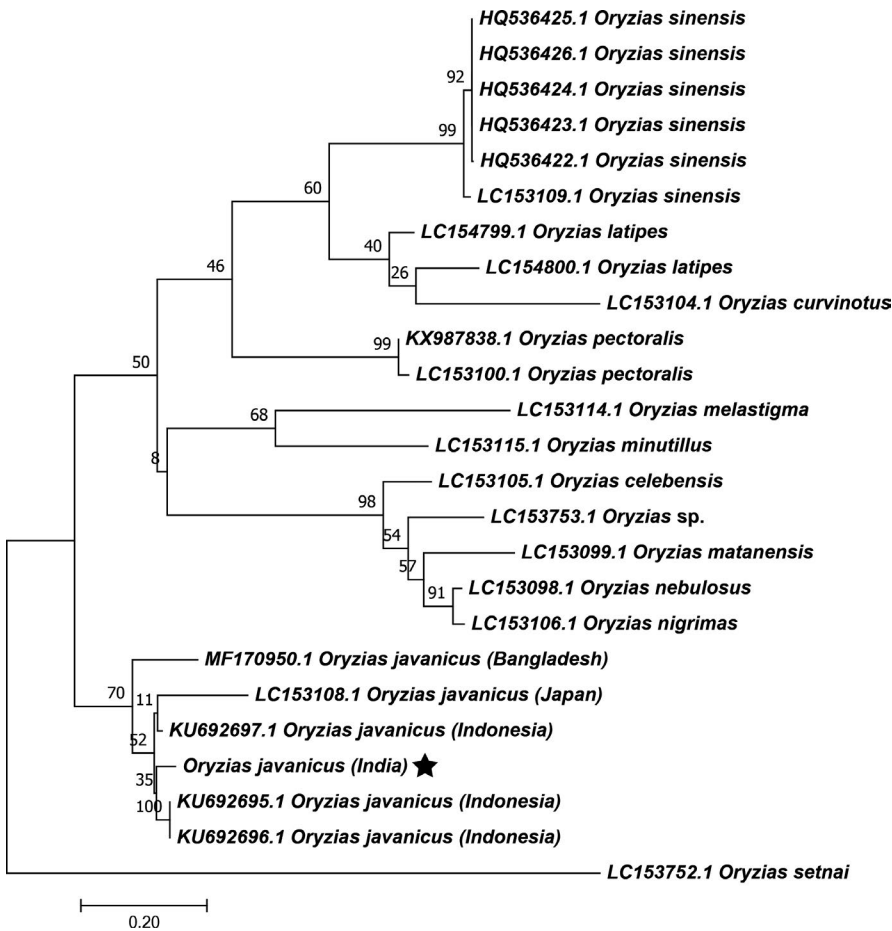


FIGURE 4 Maximum-likelihood tree based on mtDNA COI gene of different *Oryzias* species together with the sequence generated in the present study

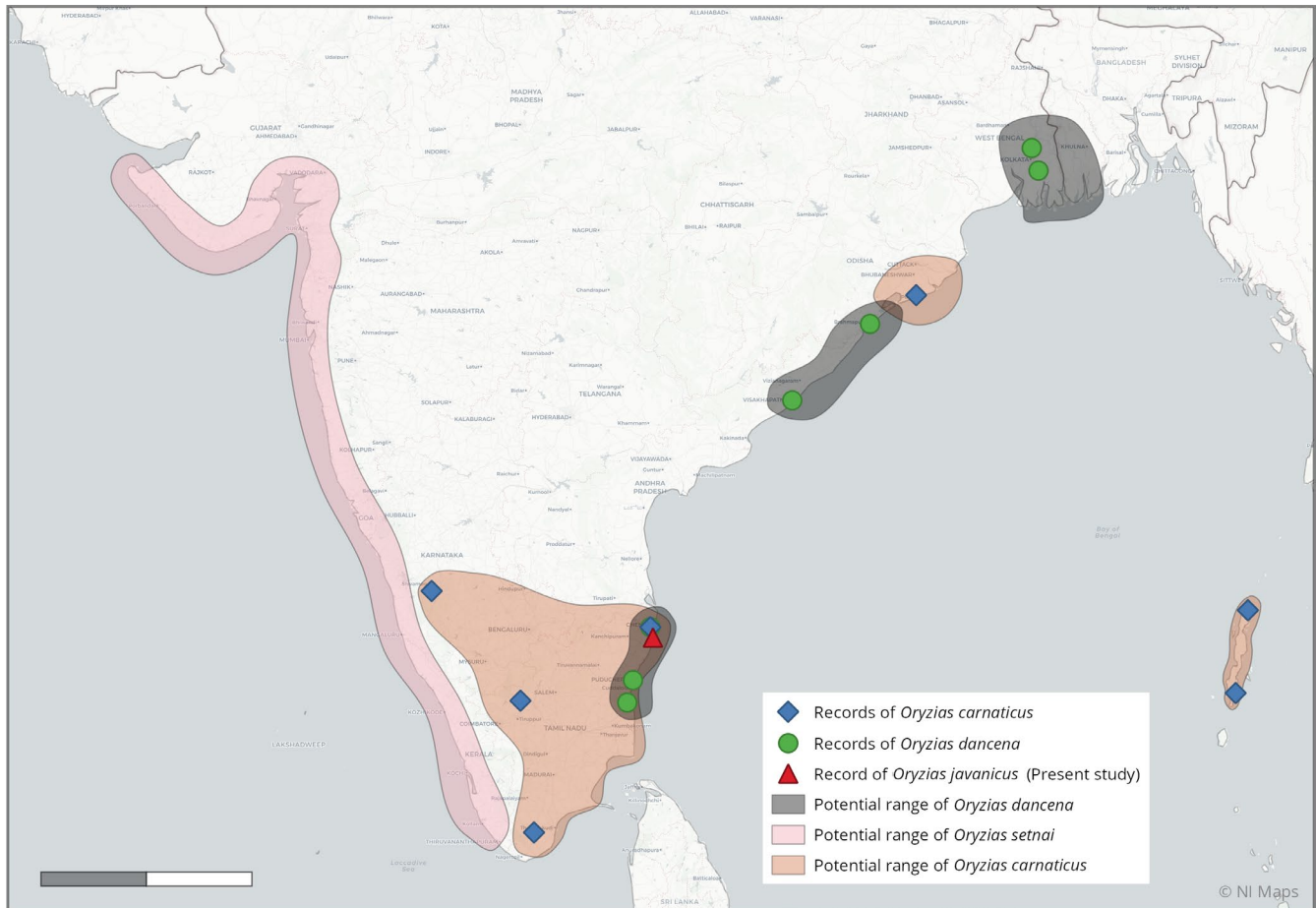


FIGURE 5 Map showing distribution of *O. carnaticus*, *O. dancena*, *O. javanicus*, and *O. setnai* in India

Oryzias javanicus is very similar to *O. dancena* and *O. carnaticus*, species that are native to the Indian subcontinent; but *O. javanicus* can be easily distinguished from the Indian species, by the presence of yellowish sub-marginal bands on the dorsal and ventral sides of the caudal fin (Parenti, 2008; Yusof et al., 2012) (Figure 2).

Phylogenetic analysis using COI sequences generated from Muttukadu samples (GenBank accession number: MK210563), was identical (100%) to the sequences of *O. javanicus* retrieved from GenBank (MF170950; LC153108; KU692695; KU692697; KU692696), and showed no divergence under the Kimura 2-parameter (K2P) distance model. The nucleotide genetic distance between the populations occurring in India and Indonesia was found to be indistinguishable, which showed the same evolutionary relatedness in neighbour-joining and maximum-likelihood analysis (Figures 3 and 4).

4 | DISCUSSION

Outside its natural distribution range (i.e. South East Asia), *O. javanicus* has been recorded from Bangladesh (in Kaptai Chora) (based on sequence MF170950 in NCBI GenBank), and Japan (based on sequence LC153108 in NCBI GenBank). The Bangladesh

population could be introduced similar to those observed in the present study. However, it is likely that the specimens from Japan (Suzuki-Matsubara et al., 2016) could be part of a captive population (sequences in GenBank are from the Higashiyama Zoo) and not recorded from the wild.

The habitat of *O. javanicus* in Muttukadu lagoon is similar to those observed in peninsular Malaysia (Yusof et al., 2012; Yusof, Ismail, & Rahman, 2013). In contrast, *O. dancena* prefers low-saline regions, away from the main river, where water movement is restricted by vegetation (Yusof et al., 2012), and other typical freshwater habitats (Parenti, 2008); but *O. carnaticus* is also found in coastal, brackish as well as freshwater habitats (Parenti, 2008; Roberts, 1998). The distribution ranges of *O. carnaticus*, *O. dancena*, *O. javanicus*, and *O. setnai* in India is shown in Figure 5.

The meristic counts of *O. javanicus* collected from Muttukadu were more or less in the same ranges as those reported by Roberts (1998), Parenti (2008), Magtoon and Termvidchakorn (2009) and Yusof et al., (2012) with minor differences, which could partly be attributed to population-level variations. Both morphological and molecular analysis confirmed the identity of the species recorded from Muttukadu as *O. javanicus*.

The distribution of *O. javanicus* in the natural waters of the Indian peninsula is confirmed for the first time in the present study, bringing

the number of *Oryzias* species recorded from India to five (the others being, *O. dancena* (Hamilton, 1822), *O. melastigma* (McClelland, 1839) (currently in the synonymy of *O. dancena*), *O. carnaticus* (Jerdon, 1849), and the endemic *O. setnai* (Kulkarni, 1940). The water bodies around Muttukadu currently comprise the westernmost distribution range of this species. The presence of considerable number of individuals in the Muttukadu Lagoon and also in the adjacent experimental ponds of ICAR-Central Institute of Brackishwater Aquaculture (CIBA), suggests that *O. javanicus* is well established in the present locality. However, its natural occurrence in this region needs further exploration, and detailed studies are required to understand the larger distribution range of this species on the south-eastern coast of India, and areas beyond, in peninsular India.

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