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Oryzias songkhramensis, A New Species of Ricefish (Beloniformes; Adrianichthyidae) from Northeast Thailand and Central Laos

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ABSTRACT.– A new ricefish, *Oryzias songkhramensis* n. sp., is described on the basis of fifty-one specimens (12.9 - 19.0 mm SL) from three localities in the Songkhram and Mekong basins in northeast Thailand and from two localities in Laos. The new species is distinguished from all other congeners by possessing the following combination of characters: anal-fin rays 15 - 16 (modally 16), bony contact organs on the anal-fin rays in males; a relatively anterior dorsal fin origin, opposite anal fin 13 - 14 (modally 14); vertebrae 27 - 29 (modally 28); cycloid scales 26 - 29 (modally 27) in lateral series, and predorsal scales 19-21 (modally 21). *Oryzias songkhramensis* n. sp. is similar to *O. mekongensis* in the number of dorsal-fin rays and branchiostegal rays, but differs from the latter in having fewer vertebrae, a lower number of scales in the predorsal and lateral scale series, and black and pale yellow marginal bands on the dorsal and ventral portions of the caudal fin (vs. bright orange and black marginal bands on the dorsal and ventral portions of the caudal fin in *O. mekongensis*). In addition, both the principal component analysis (PCA) and canominal discriminant analysis (CDA) for morphometric characters showed that *O. songkhramensis* and *O. mekongensis* differed from each other, although the morphological differences in *O. songkhramensis* were larger than those in both *O. minutillus* and *O. mekongensis*. Analysis of the phylogenetic relationships of six *Oryzias* species, based upon partial sequences of the mitochondrial control region, revealed three important groups; the javanicus, celebensis and mekongensis or latipes groups, and a close relationship between *O. songkhramensis* from Thailand and Laos (bootstrap support of 100%) with *O. mekongensis* as a sister group. The results of the phylogenetic study showed a tendency to classify *O. songkhramensis* as a member of the latipes group.

KEY WORDS: *Oryzias*, Adrianichthyidae, bony contact organs, Thailand, Laos

INTRODUCTION

Ricefish of the genus *Oryzias* belong to the family Adrianichthyidae and are widely distributed in South, East and Southeast Asia and southwards to Sulawesi and the Timor islands (Yamamoto, 1975; Labhart, 1978; Nelson, 2006). The genus *Oryzias* includes about 24 species in Asia (Kotellat, 1990, 2001a, b; Naruse, 1996; Roberts, 1998; Parenti and Soeroto, 2004; Nelson, 2006; Parenti, 2008). In Southeast Asia, there are four species known from Thailand, Laos, Myanmar and Vietnam, but eleven species are found from Indonesia, and one species each in Singapore and the Philippines (Herre and Ablan, 1934; Alfred, 1966; Magtoon, 1986; Roberts, 1998; Kotellat 2001a, b; Parenti and Soeroto, 2004; Parenti, 2008; Magtoon and Termvidchakorn, 2009).

Within Southeast Asia, the occurrence of *Oryzias* in Thailand was first described as *O. minutillus* (Smith), based on specimens collected from Central Thailand. Since then, *O. mekongensis* has been described as a

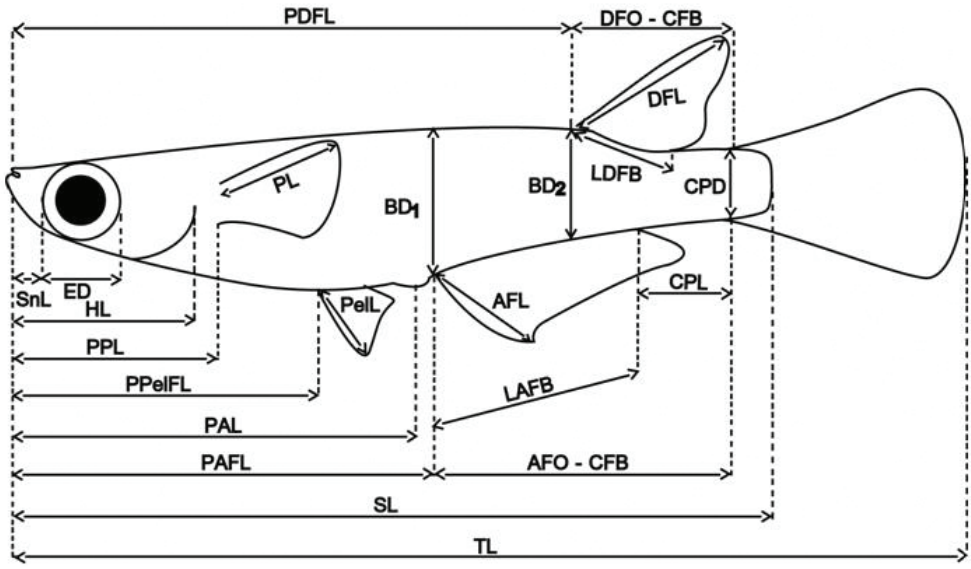

FIGURE 1. Outline drawing of *Oryzias songkhramensis* n. sp. indicating points of measurements. (See the text for explanations of abbreviations).

new species from the Mekong basin in the Northeastern Thailand by Uwa and Magtoon, 1986, whilst *O. javanicus* (Bleeker) and *O. dancena* (Hamilton) were reported from Peninsular Thailand (Smith, 1945; Magtoon, 1986; Uwa, 1986), and *O. minutillus* and *O. mekongensis* were described from the middle Mekong basin in Laos (Roberts, 1998; Kotellat, 2001b). Two species, *O. javanicus* and *O. mekongensis*, were reported in the lower Mekong in Cambodia (Rainboth, 1996). It is interesting to note that some Southeast Asian *Oryzias* species have anal-fin rays which are all small and in males these anal-fin rays also have bony contact organs.

During an intensive field survey of the rice fish of northeast Thailand, an undescribed species of *Oryzias* was collected from the Songkhram and the Mekong River basins. Although similar to *O. mekongensis* in having a comparable number of dorsal-fin rays and principal caudal-fin rays, these fish (here named *O. songkhramensis*) differed in body depth at the anterior anal fin and had a lower number of lateral scales series. Under close examination of these specimens, although the fish shared some characters of both *O. mekongensis* from Thailand and *O. pectoralis* from northern Vietnam, they were revealed to be new to science.

MATERIALS AND METHODS

Morphometrical analyses.– Twenty-two body dimensions were measured by electronic calipers (to 0.01 mm) after fixation in 70% (v/v) ethanol. Methods of measurement generally follow those of Hubbs and Lagler (1958) and Nakabo (2002), except for the following: body depths 1 and 2 (BW1 and BW2) were measured at the anterior origin of the anal and dorsal fins, respectively (Fig. 1). Proportions are expressed in terms of the

TABLE 1. Meristic and morphometric characters for holotype and paratype of *Oryzias songkhramensis* n. sp., from northeast Thailand.

Character	Holotype	**Paratype** (n = 50)	
		range	mean ± SD
Meristics counts			
Dorsal-fin rays	6	5 - 7	6.0 ± 2.0
Anal-fin rays	16	15 - 16	15.5 ± 0.8
Pectoral-fin rays	7	7 - 8	7.2 ± 0.4
Pelvic-fin rays	5	5 - 6	5.9 ± 0.2
Branchiostegal rays	4	4	4
Principal caudal- fin rays	I,4/5,I	I, 3 - 4/3 -5,I	I,3.9 ± 0.3/4.9 ± 0.4,I
Morphometrics			
Standard length (mm)	16.7	12.9 - 19.0	15.8 ± 1.6
Percent of standard length			
Total length	122.6	93.9-141.1	124.2 ± 5.7
Snout length/HL	19.3	12.9 - 31.2	21.7 ± 3.3
Eye diameter/HL	35.6	35.2-53.4	43.2 ± 3.9
Head length	26.2	17.5-29.4	25.0 ± 2.0
Preanal length	54.3	39.9 - 62.2	54.4 ± 3.6
Preanal fin length	58.3	42.1 - 69.9	57.9 ± 4.1
Predorsal fin length	78.8	57.8 - 82.7	77.1 ± 3.4
Prepelvic fin length	45.9	33.7 - 53.9	45.1 ± 3.7
Length of dorsal fin base	6.4	4.4 - 10.3	7.2 ± 1.4
Length of anal fin base	23.7	19.4 - 30.0	25.6 ± 2.5
Pectoral fin length	19.2	13.1 - 24.9	19.7 ± 2.2
Pelvic fin length	11.2	7.5 - 16.6	11.7 ± 2.1
Caudal peduncle depth	10.4	6.2 - 13.4	9.8 ± 1.1
Caudal peduncle length	16.7	11.3 - 20.3	16.7 ± 1.7
Body depth at anterior anal fin	20.3	9.6 - 22.3	17.2 ± 3.4
Body depth at anterior dorsal fin	13.9	10.4 - 24.9	15.1 ± 3.2
Anal fin length	15.5	15.4 - 26.5	19.5 ± 2.8
Dorsal fin length	16.3	14.2 - 23.9	18.7 ± 2.4
Prepectoral fin length	29.2	22.1 - 32.5	28.5 ± 1.8
Dorsal fin origin to caudal fin base	3.7	17.5 - 25.1	22.2 ± 1.6
Anal fin origin to caudal fin base	6.7	30.3 - 47.3	41.8 ± 2.6

standard length (SL) and head measurements as a percentage of the head length (HL) (Tables 1 and 2). Methods for clearing and staining specimens were those described by Dingerkus and Uhler (1977). Fin rays were counted using a binocular microscope. The meristic characters recorded were the dorsal-, anal-, pelvic-, pectoral- and caudal-fin rays; including all branched caudal fin rays plus one upper and one lower branched ray; the number of vertebrae (total = precaudal + caudal) and branchiostegal rays. Morphometric and meristic variation amongst the four species were tested by analysis of covariance (ANCOVA), whilst meristic variation were also tested by analysis of variance (ANOVA). Principal component analysis (PCA) and Canonical discriminant analysis (CDA) were adopted to separate *Oryzias* individuals, based on 22 morphometric characters after log transformation. Significance levels for all tests were set at 0.01.

All specimens examined were deposited in the collections of the Museum of Fisheries, Kasetsart University, Bangkok (KUMF), National Science Museum,

TABLE 2. Comparison of the meristic and morphometric data between *O. mekongensis*, *O. pectoralis* and *O. minutillus*.

Character	*O. minutillus* (n = 26)		*O. mekongensis* (n = 57)		*O. pectoralis* (n = 16)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Meristic counts						
Dorsal-fin rays	6	6	5 - 6	5.9 ± 0.3	5 - 6	5.9± 0.3
Anal-fin rays	17 - 21	18.9 ± 0.9	13 - 16	14.5 ± 1.0	17 - 20	19.2 ± 0.9
Pectoral-fin rays	7 - 8	7.1 ± 0.2	6 - 8	7.5 ± 0.6	8 - 10	9.2 ± 0.6
Pelvic-fin rays	5 - 6	5.1 ± 0.3	6 - 7	6.0 ± 0.2	6	6
Branchiostegal rays	4 - 4	4	4	4	5	5
Principle caudal-fin rays	I,3/4,I	I,3/4.,I	I, 4 - 5/5 - 6, I	I, 4.0 ± 0.2/5.1 ± 0.3, I	I, 4/5,I	I, 4/5, I
Morphometric measurment						
Standard length (mm)	8.7 - 12.9	10.2 ± 0.9	10.0 - 12.6	10.9 ± 0.6	19.9-28.7	24.7 ± 2.5
Percent of standard length						
Total length	122.7 - 131.9	127.6 ± 2.3	100.0 - 129.6	120.8 ± 4.2	120.1 - 131.1	125.3 ± 3.3
Snout length/HL	18.9 - 31.3	25.3 ± 3.2	11.7 - 15.9	13.1 ± 0.9	17.8 - 31.8	23.8 ± 4.0
Eye diameter/HL	33.6 - 46.8	39.7 ± 2.9	45.6 - 51.5	48.9 ± 1.3	37.9 - 55.3	43.7 ± 4.3
Head length	20.1 - 25.2	23.2 ± 1.4	20.7 - 25.7	24.1 ± 0.8	18.6 - 24.7	21.7 ± 1.4
Preanal length	38.7 - 50.8	47.4 ± 2.4	44.9 - 57.7	53.6 ± 1.5	51.3 - 57.3	53.5 ± 1.6
Preanal fin length	46.6 - 54.1	50.8 ± 1.9	48.8 - 69.0	58.1 ± 2.3	53.9 - 60.6	57.2 ± 1.9
Predorsal fin length	76.6 - 84.6	79.9 ± 2.1	64.4 - 79.5	76.5 ± 2.2	76.2 - 81.1	78.6 ± 1.6
Prepelvic fin length	36.2 - 45.2	40.6 ± 2.0	36.7 - 46.1	44.3 ± 1.3	40.5 - 45.9	42.8 ± 1.4
Length of dorsal fin base	6.1 - 8.0	6.9 ± 0.6	5.2 - 7.2	6.4 ± 0.4	4.9 - 10.2	7.2 ± 1.4
Length of anal fin base	28.4 - 34.9	31.6 ± 1.6	18.2 - 25.5	23.9 ± 1.2	27.8 - 32.2	30.3 ± 1.6
Pectoral fin length	17.6 - 24.1	20.4 ± 1.5	11.9 - 19.3	15.2 ± 1.2	17.4 - 25.7	21.8 ± 2.3
Pelvic fin length	6.4 - 19.4	9.7 ± 2.5	7.1 - 10.8	8.8 ± 0.9	9.1 - 14.1	11.5 ± 1.3
Caudal peduncle depth	8.3 - 10.6	9.1 ± 0.6	8.1 - 10.8	9.8 ± 0.5	9.0 - 10.6	9.1 ± 0.6
Caudal peduncle length	12.3 - 19.5	14.9 ± 1.8	16.9 - 21.7	20.5 ± 0.7	10.5 - 17.2	13.9 ± 1.8
Body depth at anterior anal fin ray	16.2 - 20.9	18.6 ± 0.9	15.2 - 20.5	18.7 ± 0.9	17.8 - 22.6	20.2 ± 1.2
Body depth at anterior dorsal fin ray	9.4 - 12.9	11.7 ± 0.8	10.6 - 14.8	13.4 ± 0.8	12.4 - 14.6	13.6 ± 0.7
Anal fin length	14.5 - 21.3	17.4 ± 2.0	10.4 - 20.0	13.3 ± 2.4	13.1 - 17.7	15.8 ± 1.0
Dorsal fin length	13.2 - 22.2	16.8 ± 2.8	9.3 - 18.6	12.1 ± 2.1	15.7 - 18.9	16.8 ± 0.9
Prepectoral fin length	17.8 - 30.9	27.8 ± 2.7	23.3 - 29.5	27.7 ± 0.9	15.5 - 27.1	24.9 ± 2.7
Dorsal fin origin to caudal fin base	17.2 - 22.4	19.6 ± 1.5	18.4 - 23.6	22.0 ± 0.9	18.8 - 23.7	21.2. ± 1.4
Anal fin origin to caudal fin base	42.4 - 50.6	45.5 ± 1.9	33.9 - 43.1	40.7 ± 1.3	39.9 - 45.2	42.9 ± 1.7

Tokyo (NSMT), National Inland Fisheries Institute (NIFI), Ministry of Agriculture, Thailand, and the laboratory, Department of Biology, Srinakharinwirot University, Bangkok (LBSWU).

Other abbreviations used: SL, standard length; TL, total length; SnL, snout length; ED, eye diameter; HL, head length; PAL, preanal length; PAFL, preanal fin length; PDFL, predorsal fin length; PpelFL, prepelvic fin length; LDFB, length of dorsal fin base; LAFB, length of anal fin base; PL, pectoral fin length; PelL, pelvic fin length; CPD, caudal peduncle depth; CPL, caudal peduncle length; BD1, body depth at anterior anal fin; BD2, body depth at anterior dorsal fin; AFL, anal fin length; DFL, dorsal fin length; PPL, prepectoral fin length; DFO- CFB, dorsal fin origin to caudal fin base; AFO-CFB, anal fin origin to caudal fin base.

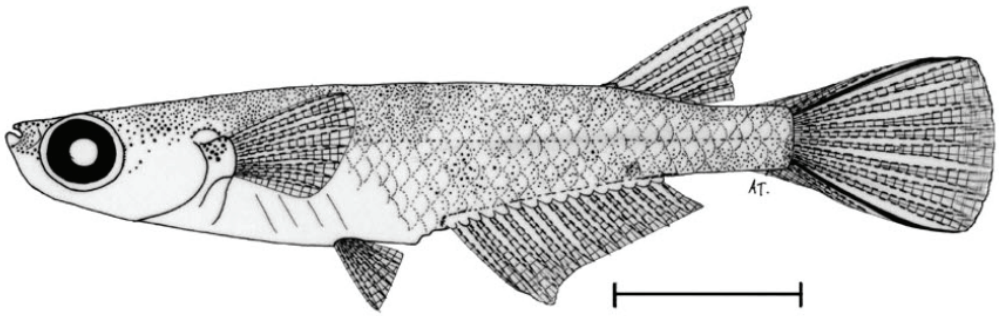

FIGURE 2. *Oryzias songkhramensis* sp. nov., holotype, KUMF 7021, 16.7 mm SL, male; Thailand: Rattanawapi, Nakhon Phanum province, Mekong basin, lateral view. Bar indicates 4 mm.

Mitochondrial DNA sequence analyses.– Six species of *Oryzias* were obtained from various localities shown as follows; *O. minutillus* from the Chao Phraya basin (Ayuthaya), *O. javanicus* from the Peninsula basin (Phuket), *O. dancena* from the Peninsula basin (Ranong) and Phetburi basin (Phetburi), *O. celebensis* from Indonesia, *O. mekongensis* from the Mekong basin (Nongkhai and Sakonnakon), and *O. songkhramensis* from the Mekong basin (Nongkhai, Thailand and Vientian, Laos). Methods used to study were summarized as follows: Total genomic DNA was extracted using a DNeasy® Tissue Kit (OIAGEN) following the menufacture' protocol. Partial sequences of control region mitochondrial DNA for six species of *Oryzias* were amplified by PCR using the following primer pairs: L15923 (5'- TTA AAG CAT CGG TCT TGT AA -3') and H16498 (5'- CCT GAA GTA GGA ACC AGA TG -3') (Iguchi and Nishida, 2000) PCR consisted of 1 minute denaturing at 94°C, 1 minute annealing at 50 °C and 1 minute extension at 72 °C repeated 30 times on a Minicycler™ MJ Research. The PCR product were purified with QIAquick™ Gel Extraction (QIAGEN). Sequencing at Macrogen.Sequences were aligned using ClustalX (Jeanmougin et al., 1998) and checked by eye. The phylogenetic relationships among individual of the partial control region mtDNA were inferred by maximum Parsimony method in PAUP* v. 4.0 (Swofford, 1998). The outgroups used *Cololabis saira* (AP002392) were chosen according to the following phylogenetic hypothesis study base on complete mtDNA of the three beloniform families (Miya et al., 2005)

KEY TO THE SPECIES OF *ORYZIAS* IN THAILAND AND VIETNAM

1a Anal-fin rays 17 - 21……....…….....……..2
1b Anal-fin rays 13 - 16 …….....3
2a Anal-fin rays 18 - 20, last ray branched; branchiostegal rays 5; pectoral-fin rays 9 - 10; a black spot on upper part of pectoral fin base; principal caudal-fin rays i,4/5,i; bright orange submarginal bands on the dorsal and ventral portions of caudal fin (when alive); maximum adult size nearly 29 mm SL... *Oryzias pectoralis*
2b Anal-fin rays 17 - 21, last ray unbranched, anal-fin without horny process in male; branchiostegal rays 4; pectoral-fin rays 7 - 8, no black spot on upper part of pectoral fin base; principal caudal-fin rays i,3/4,i; without color submarginal bands on the dorsal and ventral portions of caudal fin (when

FIGURE 3. *Oryzias songkhramensis* n. sp., upper: holotype, KUMF 7021, male; lower: paratype, NIFI 3390, female. Bar indicates 5 mm.

alive); maximum adult size about 16 mm SL.…….……. *Oryzias mintiullus*

3a Precaudal vertebrae 10 - 12 (modally 11); anterior dorsal-fin origin, opposite vertebrae 18 - 20 (modally 19); anterior anal-fin origin, opposite vertebrae 10 - 12 (modally 11); anal-fin rays without bony process in male; scales in a lateral series 29 - 32 (modally 31); bright orange submarginal bands on the dorsal and ventral portions of caudal fin (when alive)..... *Oryzias mekongensis*

3b Precaudal vertebrae 11 - 12 (modally 12); anterior dorsal fin origin, opposite vertebrae 19 - 20 (modally 20); anterior anal fin origin, opposite vertebrae 11 - 12 (modally 12), anal-fin rays with bony process in males; scales in a lateral series 26 - 29 (27); black and pale yellow submarginal bands on the dorsal and ventral portions of the caudal fin (when alive)…….....…… ……..… *Oryzias songkhramensis* n. sp.

TAXONOMY

Oryzias songkhramensis n. sp. (Figures 2 and 3; Table 1)

Material examined.– Holotype: KUMF 7021, 1 ex., 16.7 mm SL, Thailand: Ban Nong Phai, Ratanawapi, Nong Khai Province; 18º 13′ 12″ N, 103º 10′ 48″ E; W. Magtoon and T. Deesin, 19 Apr. 2004.

Paratypes: KUMF 7022, 1 ex., 14.9 mm SL, same data at holotype; NSMT-P 92823, 1 ex., 16.3 mm SL; NSMT-P 92824, 1 ex., 17.0 mm SL; NIFI 3391, 28 ex., 12.9 - 19.0

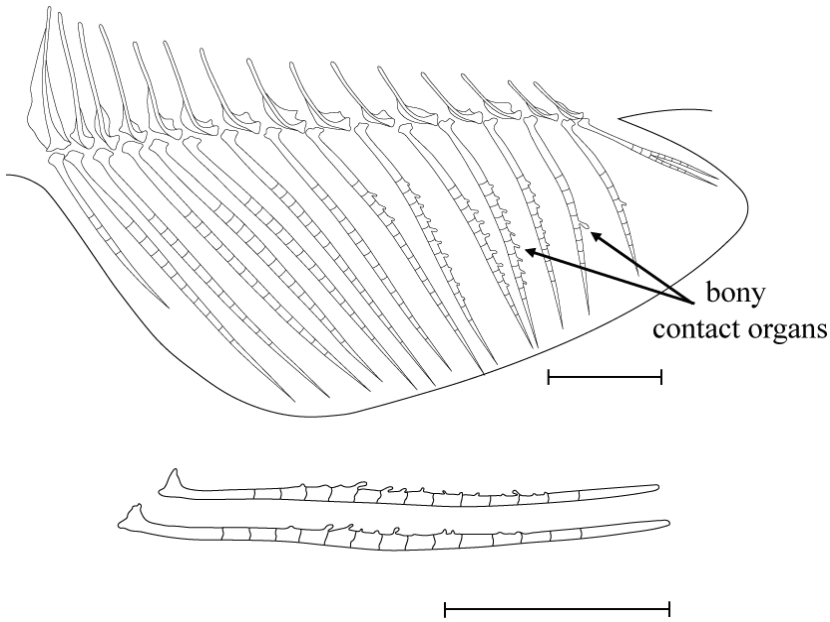

FIGURE 4. Diagrammatic representation of the modified and the modified anal-fin rays with bony contact organ of *Oryzias songkhramensis* n. sp., male, paratype NIFI 3208. Bar indicates 1 mm.

mm SL, NIFI 3392, 20 ex., 13.2 - 18.3 mm SL; Ban Nong Phai, Ratanawapi, Nong Khai Province; 18º 13′ 12″ N, 103º 10′ 48″ E; W. Magtoon and T. Deesin, 19 Apr. 2004.

Diagnosis.– *Oryzias songkhramensis* is distinguished from all other congeners by possessing the following combination of characters: anal-fin rays 15 - 16 (modally 16), bony contact organs on the anal-fin rays in males; a relatively anterior dorsal fin origin, opposite anal fin 13 - 14 (modally 14); vertebrae 27 - 29 (modally 28); fourth vertebra without neural prezygapophysis; scale relatively large, cycloid; 26 - 29 (modally 27) in a lateral series, predorsal scale 19 - 21 (modally 21); snout length shorter than eye diameter, snout length 12.9 - 31.2% (mean 21.7%) of HL; eye diameter 35.2 - 53.4% (mean 43.2%) of HL; a black spot at the base of pectoral fin, pectoral fin length 13.1 - 24.9% (mean 19.7%) of SL; length of anal fin base 19.4 - 30.0% (mean 25.6%) of SL; caudal peduncle length 11.3 - 20.3% (mean 16.7%) of SL; caudal fin slightly, rounded, length 3.6 - 3.8 (mean 3.7) in SL, black and pale yellow marginal bands on the dorsal and ventral portions of the caudal fin.

Description.– Meristic and morphometric data of 51 specimens, 12.9 - 19.0 mm SL appear in Table 1 and general appearance in Figs. 2 and 3.

Body compressed laterally; body depth at anterior dorsal fin: 6.6 (4.8 - 9.0) in standard length (SL), body depth at anterior anal fin: 5.8 (5.0 - 8.7) in SL. Caudal peduncle compressed, long, its length 2.6 (2.2 - 2.9) in head length (HL). Head not very compressed, with depressed anterior part, length 3.9 (3.8 - 4.1) in SL. Dorsal body profile straight from head to dorsal-fin origin; ventral body profile convex from head to anal-fin origin. Interorbital space

TABLE 3. Frequency distribution of the number of anal-fin rays counts in four *Oryzias* species from Southeastern Asia.

Species	Anal-fin rays								Mean ± SD, n
	13	14	15	16	17	18	19	20	
O. songkhramensis	-	-	12	16	-	-	-	-	15.6 ± 0.5, 28
O. minutillus	-	-	-	-	5	14	5	1	18.0 ± 0.7, 25
O. mekongensis	-	13	9	3	-	-	-	-	14.5.± 0.7, 25
O. pectoralis	-	-	-	-	-	-	3	2	19.6 ± 0.6, 16

TABLE 4. Frequency distribution of the number of precaudal and caudal vertebrae in four *Oryzias* species from Southeastern Asia.

Species	Precaudal vertebrae				Mean ± SD, n
	9	10	11	12	
O. songkhramensis	-	-	7	31	11.7 ± 0.4, 38
O. minutillus	2	22	2	-	10.0 ± 0.4, 26
*O. mekongens*is	-	1	19	11	11.3 ± 0.5, 31
O. pectoralis	-	-	1	4	10.8 ± 0.4, 16

TABLE 5. Frequency distribution of the dorsal position, recorded as the anal-fin ray opposite the dorsal fin origin, in four *Oryzias* species from Southeastern Asia.

Species	Anal fin ray									Mean ± SD, n
	12	13	14	15	16	17	18	19	20	
O. songkhramensis	-	7	23	-	-	-	-	-	-	13.7 ± 0.4, 30
O. minutillus	-	-	-	-	-	7	11	4	1	17.7 ± 1.0, 22
*O. mekongens*is	15	8	-	-	-	-	-	-	-	12.4 ± 0.5, 23
O. pectoralis	-	-	-	-	3	2	-	-	-	16.6 ± 0.5, 16

broad. Snout length 5.1 (4.1 - 6.1) in HL, snout short, 12.9 - 31.2% (mean 21.7%) of HL, snout shorter than eye diameter. Eye diameter 2.3 (2.2 - 2.4) in HL; eye relatively large 35.2 - 53.4% (mean 43.2%) of HL, the pupil situated in the anterior part of head. Mouth terminal, almost horizontal; upper jaws short, not protractile; lower jaw projects slightly beyond upper jaw. Each jaw with two rows of caniniform teeth; large teeth on premaxillary and dentary in males, only on dentary in females. First pleural rib on parapophysis of second vertebra; lateral process of pelvic bone attached to fourth pleural rib; basihyal bone relatively short triangular, basihyal cartilage extremely elongate and rectangular (Fig. 6). Caudal skeleton with two epural bones: one ventral accessory bone and a second accessory cartilage or bone. Body covered with large cycloid scales, 26 - 27 (modally 27) in lateral series, predorsal scales 19 - 21 (modally 21) (Table 8). Principal caudal-fin rays i, 4/5, i; procurrent fin-rays, dorsal 4 - 5 (modally 4) and ventral 4 - 5 (modally 5), circumpeduncular scales 14. Lateral line absent. Vertebrae 27 - 29 (modally 28), fourth vertebra without neural prezygapophysis (Fig. 7); branchiostegal rays 4. Peritoneum dense black within, the outer surface next body wall silvery. Absence of melanic pigments on vent or genital area.

Dorsal-fin rays 6 - 7 (modally 6), 3rd rays longest, 3rd -6th branched rays, a relatively

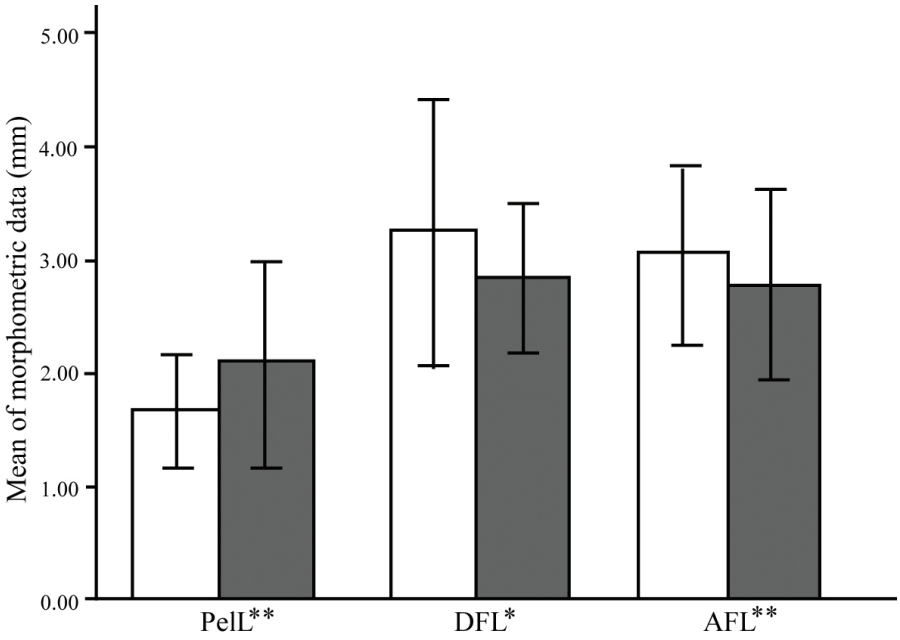

FIGURE 5. Sex dimorphism of *Oryzias songkhramnensis* n. sp. of male and female. PelL, pelvic fin length; DFL, dorsal fin length and AFL, Anal fin length. Male (white filled box) and female (gray filled box). Data are shown as the mean ± SD, and are derived from 51 samples. * and ** represent a significant difference between male and females at $P<0.05$ and $P<0.01$, respectively.

anterior dorsal-fin origin, opposite vertebrae 20 - 21 (modally 20) (Table 7), a relatively anterior dorsal-fin origin, opposite anal-fin rays 13 - 14 (modally 14) (Table 5); dorsal fin in some males is longer than those of females, reaching at least the base of the caudal fin; dorsal fin length 1.3 (1.2 - 1.5) in HL, 5.4 (4.7 - 6.1) in SL and 0.8 (0.7 - 0.9) in depth of body dorsum to belly in front of dorsal fin; dorsal fin length much longer than base, long of dorsal fin base 14.5 (10.9 - 18.1) in SL and 18.4 (13.7 - 23.1) in TL; dorsal fin length much shorter than predorsal length, predorsal length or distance 1.3 (1.2 - 1.3) in SL, 1.6 in TL.

Anal-fin rays 15 - 17 (modally 15) (Table 3), 4th rays longest, anal fin slightly convex, in some males, the rays are slightly filamentous, a relatively anterior anal-fin origin, opposite vertebrae 11 - 12 (modally 12) (Table 6), anal-fin rays with bony contact organs in males (Fig. 4); anal fin length 1.3 (1.1 - 1.4) in HL, 5.0 (4.3 - 5.7) in SL, 6.4 (5.5 - 7.2) in TL and 0.8 (0.7 - 0.9) in depth of body dorsum to belly in front of anal fin; anal fin length much shorter than anal fin base, long of anal fin base 4.3 (3.7 - 4.9) in SL and 5.5 (4.7 - 6.3) in TL; anal fin length much shorter than predorsal length. Predorsal length 1.7 (1.6 - 1.8) in SL, 2.2 (2.2 - 2.3) in TL.

Pectoral-fin rays 7 - 8 (modally 7), 3rd longest, 2nd-6th branched rays, falcate, reaching vertical of pelvic fin origin, a black spot at the base of pectoral fin; pectoral fin length 1.3 (1.2 - 1.5) in HL, 5.5 (4.5 - 6.4) in SL and 6.9 (5.7 - 8.1) in TL; pectoral fin length shorter than head length; pectoral fin length shorter than prepectoral length;

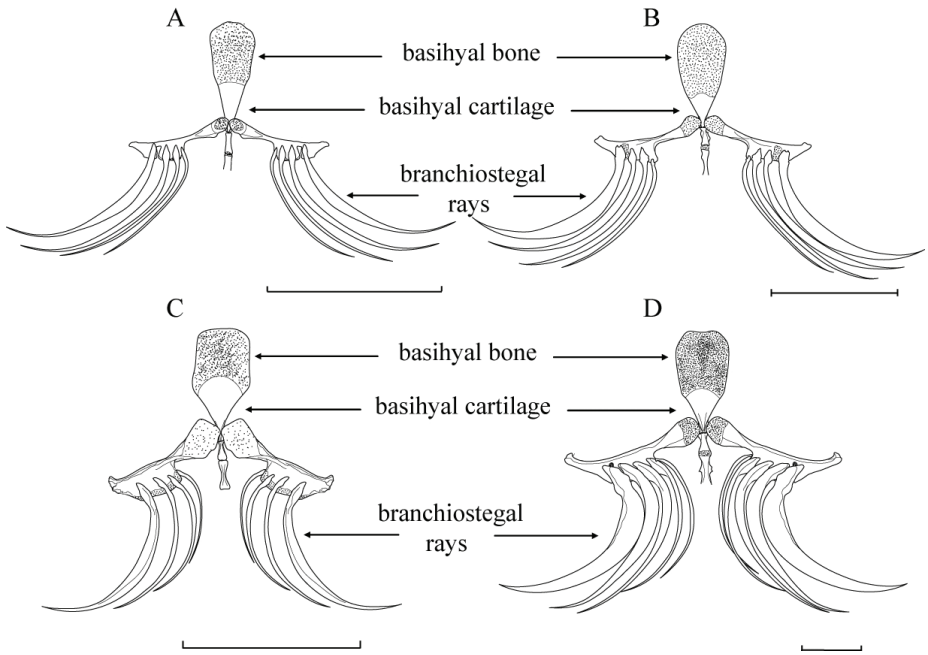

FIGURE 6. Anterior section of the ventral portion of gill arches in (**A**) *Oryzias songkhramensis* n. sp. LBSWU 0404, (**B**) *O. minutillus*, (**C**) *O. mekongensis* LBSWU 0201 and (**D**) *O. pectoralis* NIFI 3406. Bar indicates 1 mm.

prepectoral length 3.5 (3.4 - 3.5) in SL, 4.3 (4.3 - 4.4) in TL.

Pelvic-fin rays 5 - 6 (modally 6), 2nd- 5th branched rays, small, not reaching anal origin, anterior pelvic-fin rays origin, opposite vertebrae 7 - 9 (7), pelvic fin with pale yellow in live; pelvic fin length 2.3 (1.8 - 2.8) in HL, 9.2 (6.8 - 11.6) in SL and 11.7 (8.6 - 14.8) in TL, pelvic fin much shorter than head length; pelvic fin length much shorter than prepelvic length, prepelvic length 2.2 (1.9 - 2.4) in SL, 2.8 (2.5 - 3.0) in TL.

Outer margins of caudal fins rounded, black and yellow submarginal bands on the dorsal and ventral portions of caudal fin; caudal peduncle length 1.6 (1.3 - 1.9) in HL, 6.4 (5.0 - 7.8) in SL, 8.2 (6.2 - 10.2) in TL; caudal peduncle length shorter than head length; caudal peduncle moderately slender; caudal peduncle depth 2.6 (2.2 - 2.9) in HL, 10.4 (8.4 - 12.3) in SL, 13.1 (10.7 - 15.6) in TL.

Origin of first dorsal fin slightly anterior to caudal fin base 1.2 (1.1 - 1.2) in HL, 4.6 (4.4 - 4.8) in SL, 5.8 (5.6 - 6.1) in TL; origin of anal fin anterior to caudal fin base 0.6 in HL, 2.4 (2.4 - 2.5) in SL, 3.1 (3.0 - 3.2) in TL.

Color of fresh specimens.– Based on color observations of a material specimen (NIFI 3208, 16.8 mm SL): body brownish dorsally and belly pale yellow. As a small, numerous pigment spreading over the head, trunk and fins. Pelvic-fin slightly pale yellow. Males manifesting black and yellowish marginal bands on the dorsal and ventral portions of the caudal fin.

Color of preserved specimens.– Body brownish dorsally, pale ventrally. Melanophores forms blackish line above the

TABLE 6. Frequency distribution of the anal position, recorded as the vertebrae opposite the anal fin origin, in four *Oryzias* species from Southeastern Asia.

Species	Vertebrae				Mean ± SD, n
	9	10	11	12	
O. songkhramensis	-	-	5	25	11.8 ± 0.4, 30
O. minutillus	2	23	-	-	9.8 ± 0.4, 24
*O. mekongens*is	-	-	22	2	11.1 ± 0.1, 24
O. pectoralis	-	-	1	4	9.8 ± 0.4, 24

TABLE 7. Frequency distribution of the dorsal position, recorded as the vertebrae opposite the dorsal fin origin, in four *Oryzias* species from Southeastern Asia.

Species	Vertebrae					Mean ± SD, n
	18	19	20	21	22	
O. songkhramensis	-	-	24	8	-	20.3 ± 0.4, 32
O. minutillus	-	3	19	-	-	19.9 ± 0.5, 22
*O. mekongens*is	1	20	5	-	-	19.1 ± 0.5, 26
O. pectoralis	-	-	-	-	5	19.9 ± 0.5, 22

TABLE 8. Frequency distribution of the number of predorsal scales and scales in a lateral series in four *Oryzias* species from Southeastern Asia.

Species	Predorsal Scales								Mean ± SD, n	Scales in a lateral series								Mean ± SD, n
	19	20	21	22	23	24	25	26		25	26	27	28	29	30	31	32	
O. songkhramensis	2	2	20	-	-	-	-	-	20.7 ± 0.6, 24	-	8	12	2	2	-	-	-	26.9 ± 0.9, 24
O. minutillus	-	-	9	12	6	-	-	-	21.9±0.7, 26	3	6	-	-	-	-	-	-	25.7±0.5, 26
*O. mekongens*is	-	-	1	5	14	-	-	-	22.6 ± 0.5, 20	-	-	-	-	1	2	15	2	30.9 ± 0.6, 20
O. pectoralis	-	-	-	-	7	3	3	3	24.1 ± 1.2, 16	-	-	-	-	3	3	10	-	30.4 ± 0.8, 16

anal fin base and middle line, along the anal-fin base. Black submarginal bands are present on the dorsal and ventral portions of the caudal fin. Cluster of black specks at the pectoral fin base. Peritoneal membranes blackish.

Sexual dimorphism.– Anterior rays in dorsal and anal fin rays are greatly elongated in males, whereas the pelvic fin is elongate in female (Fig. 5). Distal margin of anal fin is convex in males, straight to slightly concave in females. Pelvic-fin rays in females are greatly elongated. Males yellowish at the pelvic fin; pelvic fin in females, pale yellowish. Genital papilla bilobed in females, but a short and conical tube in males. In males, the posterior and anal fin rays have a bony contact organ. During the breeding season, the melanophores of males remain scattered but increase in density on the body side, dorsal and anal caudal fins. Interneural spines and wings of basipterygium are more developed in males.

Ecology.– The fish were collected in clear water from ditches and paddy fields by dip nets in the Songkhram and Mekong River basins, Northeastern Thailand. The type locality is a small ditch, 3 - 6 m wide and 0.5 to 2 m deep; the substrate ranges from

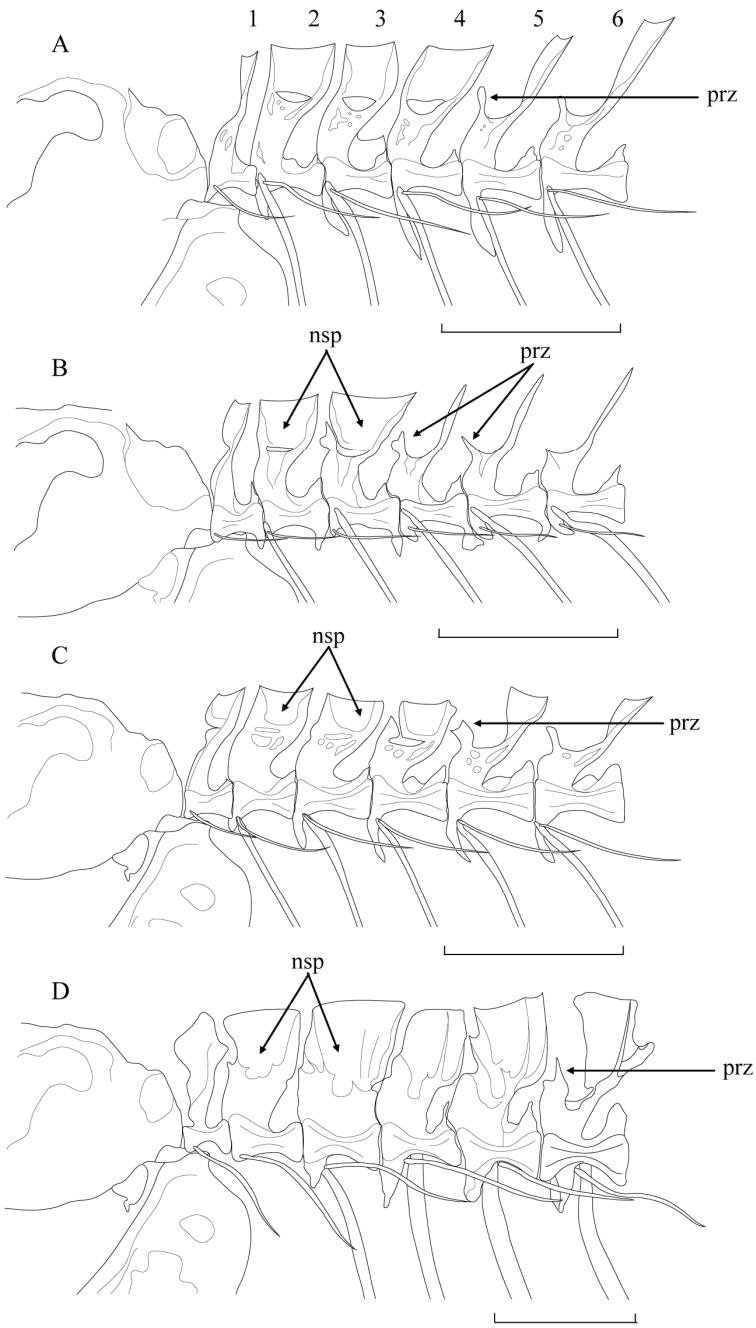

FIGURE 7. Diagrammatic representation of the left anterior view of (**A**) *Oryzias songkhramensis* n. sp., (**B**) *O. minutillus*, (**C**) *O. mekongensis* and (**D**) *O. pectoralis*. Abbreviations: nsp, neural spine; prz, neural prezygapophysis. Bar indicates 1.0 mm.

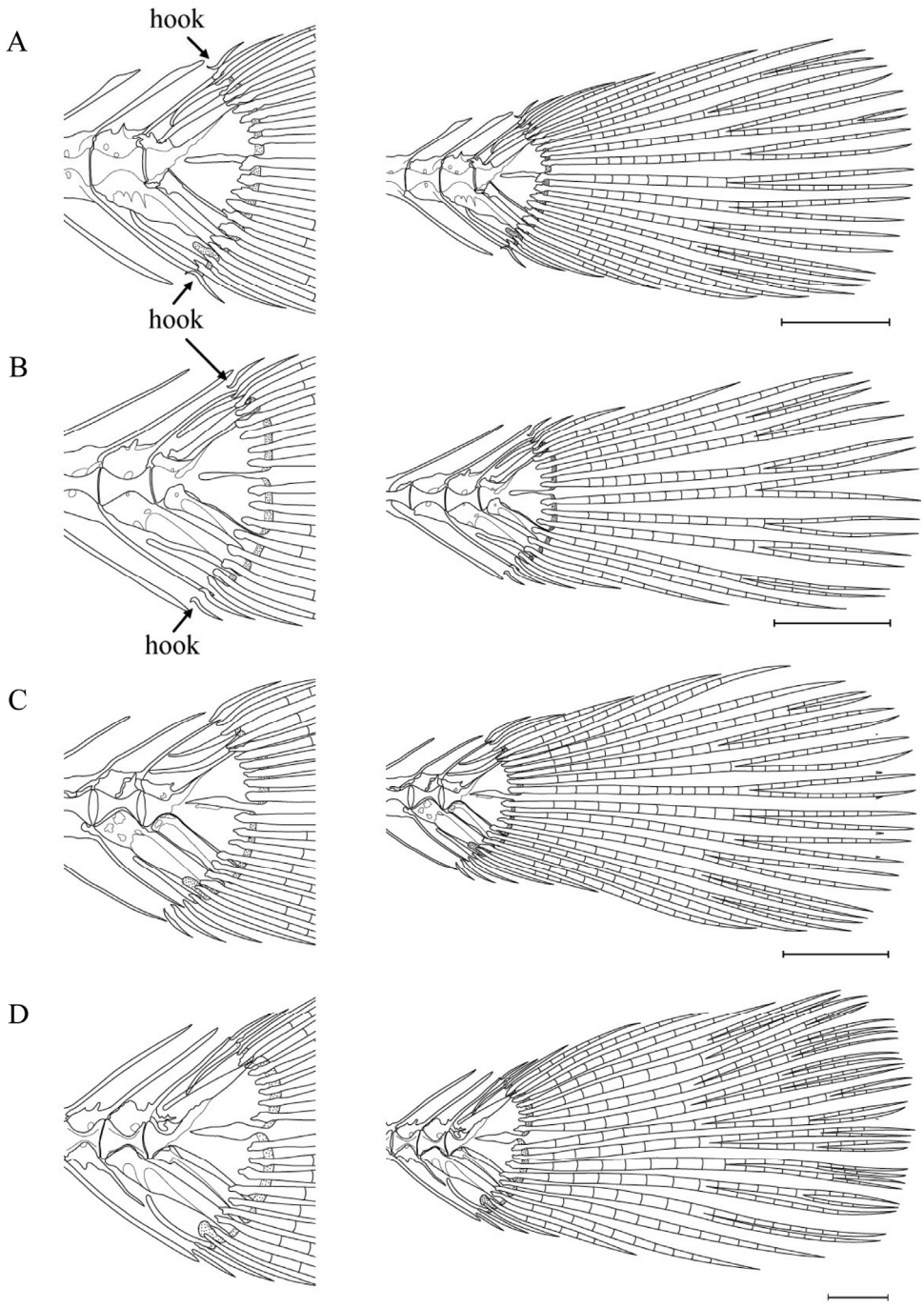

FIGURE 8. Caudal fin osteology for (**A**) *Oryzias songkhramensis* n. sp., (**B**) *O. minutillus* (**C**) *O. mekongensis* and (**D**) *O. pectoralis.* Bar indicates 1.0 mm.

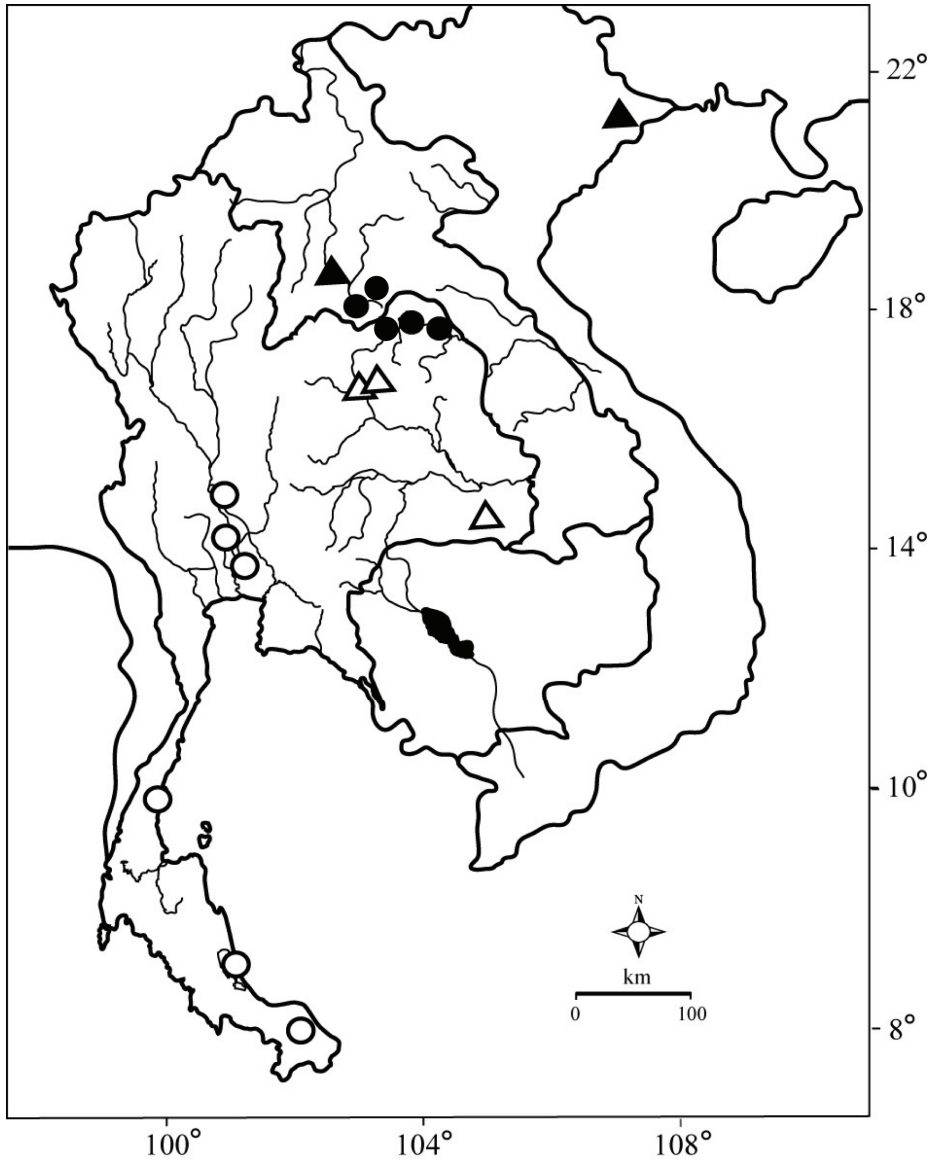

FIGURE 9. Geographic distribution of *Oryzias* sp. in S.E.Asia. *O. songkhramensis* n. sp. (solid circles), *O. minutillus* (open circles), *O. mekongensis* (open triangles) and *O. pectoralis* (solid triangles), based on the specimens examined in these studies.

sand to detritus or muds. Other species encountered were *Trichogaster trichopterus* and *Trichopsis vittatus* (Belontidae); *Rasbora rubrodorsalis* (Cyprinidae); *Channa striata* (Channidae) and *Machrobrachium lanchesteri* (De Man). Aquatic plant species encountered were *Nymphaea lotus* L., *Jussiaea repens* L. and *Hydrilla verticillata* (L.f.) Royle. The range of water quality value parameter were: water temperature 27.5 - 28.7 ºC, conductivity

TABLE 9. Eigenvectors for the first three principal component (PC), based on 22 morphometric characters, in *Oryzias songkhramensis* n. sp., *O. mekongensis*, *O. minutillus* and *O. pectoralis.*

Measurements	PC I	PC II	PC III
Standard length	0.128	0.003	-0.043
Total length	0.114	0.025	-0.051
Snout length	0.049	-0.154	0.185
Eye diameter	-0.189	0.090	0.283
Head length	-0.017	0.123	-0.003
Preanal length	0.180	-0.068	-0.041
Preanal fin length	0.189	-0.051	-0.072
Predorsal fin length	0.185	0.000	-0.132
Prepelvic fin length	0.091	0.037	-0.046
Length of dorsal fin base	-0.186	0.211	0 .146
Length of anal fin base	-0.008	0.099	0.028
Pectoral fin length	0.217	-0.048	-0.156
Pelvic fin length	-0.247	0.436	-0.092
Caudal peduncle depth	-0.136	0.248	0.025
Caudal peduncle length	0.264	-0.307	0.098
Body depth at anal fin origin	0.037	0.057	0.019
Body depth at dorsal fin origin	-0.089	0.246	-0.046
Anal fin length	-0.127	0.045	0.227
Dorsal fin length	-0.197	0.000	0.366
Prepectoral fin length	0.209	-0.221	0.065
Dorsal fin origin to caudal fin base	0.011	0.004	0.108
Anal fin origin to caudal fin base	0.163	-0.115	0 .044
Eigenvalues	13.918	2.066	1.009
% of variance	63.26	9.39	4.59

41.5 - 124.4 µm/l, dissolved oxygen 2.05 - 3.97 mg/l, pH 7.1 - 7.9 and salinity 0.1 ppt.

Molecular analyses.– Using the nucleotide sequences of the partial mitochondrial CR (821 characters), maximum parsimony based analysis of the phylogenetic relationships of six *Oryzias,* with *Cololabis saira* as the out group, revealed two deep clades and three groups (Fig. 13). The first clade comprised of the Javanicus group, made up of *O. javanicus* from the Peninsula basin (Phuket), and *O. dancena* from Peninsula basin (Ranong) and Petchaburi basin (Petchaburi), with slightly more distantly related *O. minutillus* from the Chao Phraya basin (Ayuthaya). The second clade was comprised of two groups. The first, the celebensis group consists of *O. celebensis* (Indonesia). The secondand sister group is the mekongensis, or latipes, group which comprises of *O. mekongensis* from the Mekong basin (Nongkhai and Sakonnakhon, Thailand) and *O. songkhramensis* from the Mekong basin (Nonhkhai, Thailand and Vientiane Laos). The results show the tendency to classify *O. songkhramensis* within the latipes (mekongensis) group. This result was in agreement with the results from previous analyses of the sequences of cytochrome *b*, 12S rDNA (Naruse, 1996) and tyrosinase (Takehana et al., 2005).

Distribution.–The geographic distribution of this species is limited to the Songkhram and Mekong River basin in northeast Thailand and central Laos (Fig. 8).

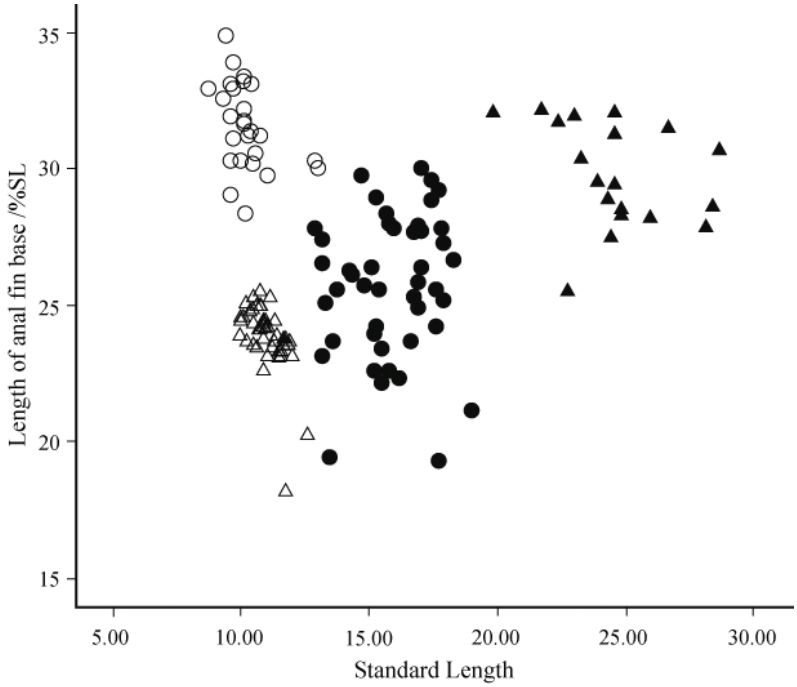

FIGURE 10. Relationships between standard length (SL) and the percentage of the length of the anal fin base to the SL of *O. songkhramensis* n. sp. (solid circles), *O. minutillus* (open circles), *O. mekongensis* (open triangles) and *O. pectoralis* (solid triangles).

Etymology.– The specific name, *songkhramensis*, refers to the Songkhram River basin region of Thailand.

Comparisons.– *Oryzias songkhramensis* is similar to *O. mekongensis* in general body appearance, especially in the same number of dorsal-fins, branchiosteagal and principal caudal-fin rays. However, the former is distinguished from the latter in: (i) having more precaudal vertebrae [11 - 12 (modally 12) vs. 10 - 12 (modally 11)] (Table. 4), (ii) fourth vertebra without neural prezygapophysis (vs. fourth vertebra with neural prezygapophysis) (Fig. 7), (iii) an anterior dorsal fin, original, opposite anal-fin rays [13 - 14 (modally 14) rays vs. 13 - 15 (modally 12) rays], (iv) a larger eye diameter [35.2 - 53.4% (mean 43.2%) of SL vs. 33.6 - 46.8% (mean 39.7%) of SL], (v) a longer snout length [12.9 - 31.2% (mean 21.7%) of HL vs. 11.7 - 15.9% (mean 13.1%) of HL], (vi) a shorter length of anal fin base [19.4 - 30.0% (mean 25.6%) of SL vs. 28.4 - 34.9% (mean 31.6%) of SL], (vii) a shorter pectoral fin 13.1 - 24.9% (mean 19.70%) of SL vs. 17.6 - 24.1% (mean 20.4%) of SL], (viii) the appearance of bony contact organs on the anal-fin rays in males (vs. the absence of bony contact organs on anal-fin rays in males of *O. mekongensis*), (ix) the appearance of hooked proximal portion of the procurrent rays (vs. the absence of hooked proximal portion of the procurrent rays in *O. mekongensis*) (Fig. 8) and (x) the appearance of black and pale yellow marginal bands on the dorsal and ventral portion of the caudal fin (in contrast to the bright orange and black marginal

TABLE 10. Standardized canonical (CAN) coefficients, based on 22 morphometric characters, among *Oryzias songkhramensis* n. sp., *O. mekongensis*, *O. minutillus* and *O. pectoralis.*

Measurements	CAN I	CAN II	CAN III
Standard length	0.468	0.568*	-0.250
Total length	0.505	0.566*	-0.258
Snout length	0.388*	0.312	0 .291
Eye diameter	0.288	0.596*	-0.286
Head length	0.386	0.541*	-0.188
Preanal length	0.460	0.642*	-0.291
Preanal fin length	0.438	0.610*	-0.280
Predorsal fin length	0.493	0.556*	-0.273
Prepelvic fin length	0.408	0.585*	-0.213
Length of dorsal fin base	0.373*	0.327	-0.127
Length of anal fin base	0.575*	0.203	-0.165
Pectoral fin length	0.601*	0.453	0.007
Pelvic fin length	0.252	0.501*	-0.132
Caudal peduncle depth	0.427*	0.391	-0.155
Caudal peduncle length	0.192	0.495*	-0.355
Body depth at anal fin origin	0.212	0.150	-0.311*
Body depth at dorsal fin origin	0.250	0.448*	-0.061
Dorsal fin length	0.458	0.564*	0.261
Prepectoral fin length	0.365	0.536*	-0.069
Dorsal fin origin to caudal fin base	0.394	0.573*	-0.251
Anal fin origin to caudal fin base	0.511*	0.497	-0.259
Eigenvalues	33.075	4.225	3.280
% of variance	81.50	10.40	8.10

* represent a significant difference

bands on the dorsal and ventral portions of the caudal fin in *O. mekongensis*).

Oryzias songkhramensis n. sp. is similar to *O. pectoralis* from northern Vietnam in having a comparable number of dorsal-fin rays and a black spot on the upper part of the pectoral fin. However, the former is distinguished from the latter in: (i) having anal-fin rays which are much lower [15 - 16 (modally 16) vs. 17 - 20 (modally 19)] (Table 3), (ii) a lower number of predorsal scales [19 - 21 (modally 21) vs. 23 - 26 (modally 23) (Table 8), (iii) a lower number of branchiostegal rays (4 vs. 5), (ix) a lower number of the total vertebrae (27 - 29 vs. 30), (v) a longer head length [17.5 - 29.4% (mean 25.1%) of SL vs. 18.6 - 24.7% (mean 21.7%) of SL], (vi) fourth vertebra without neural prezygapophysis (vs. sixth vertebra with neural prezygaphophysis (Fig. 7), (vii) first pleural rib on parapophysis of second vertebrae (vs. first pleural rib on parapophysis of third vertebrae), (viii) a smaller snout diameter [12.9 - 31.2% (mean 21.7%) vs. 17.8 - 31.8% (mean 23.7%) of SL], (ix) a shorter long of anal fin base [19.4 - 30.0% (25.6%) vs 27.8 - 32.2 % (30.3%) of SL (x) a longer caudal peduncle length [11.3 - 20.3% (mean 16.7%) of SL vs. 10.5 - 17.2% (mean 13.8%) of SL], (xi) a shorter pectoral fin length [13.1 - 24.9% (mean 19.7%) of SL vs. 17.3 - 25.7% (mean 21.9%) of SL], and (xii) black and pale yellow marginal bands on the dorsal and ventral portion of the caudal fin (in contrast to the bright orange at marginal bands on the dorsal and ventral portion of the caudal fin in *O. pectoralis*).

Another related species, *Oryzias songkhramensis* n. sp. is similar to *O.*

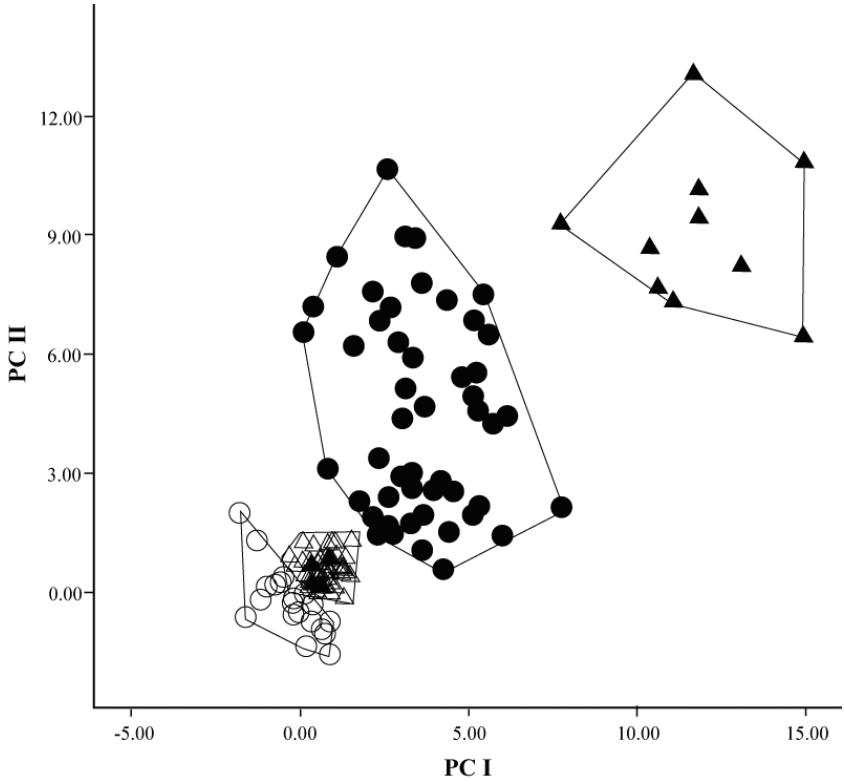

FIGURE 11. Plots of the first two principal component (PC) scores, based on 22 morphometric characters, of *Oryzias songkhramensis* n. sp. (solid circles), *O. minutillus* (open circles), *O. mekongensis* (open triangles) and *O. pectoralis* (solid triangles).

minutillus in general body appearance, especially in having the same number of dorsal-fin, pectoral-fin, and branchiosteagal rays. However, the former is distinguished from the latter in: (i) having a lower number of anal-fin rays [15 - 16 (modally 16) vs. 17 - 20 (modally 18)] (Table 4), (ii) more precaudal vertebrae [11 - 12 (modally 12) vs. 9 - 11 (modally 10)] (Table 5), (iii) more principal caudal-fin rays [i, 4/5,i rays vs. i, 3/4,i rays], (iv) having a neural prezygapophysis origin in the fifth vertebrae (vs. a neural prezygaphophysis origin in the third vertebrae) (Fig. 6), (v) a relatively anterior dorsal fin origin, opposite the anal fin [13 - 14 (modally 14) vs. 17 - 20 (modally 18)] (Table 6), (vi) an anterior anal fin, original, opposite vertebrae [11 - 12 (modally 12) vs. 9 - 10 (modally 10)] (Table 7), (vii) a lower predorsal scale [19 - 21 (modally 21) vs. 21 - 23 (modally 22)] (Table 9), (viii) a longer snout length [12.9 - 31.2% (mean 21.7%) of SL vs. 11.7 - 15.9% (mean 13.1%) of SL], (ix) a smaller eye diameter [25.2 - 53.4% (mean 43.2%) vs. 45.6 - 51.5% (mean 48.9%) of SL], (x) a shorter caudal peduncle length [11.3 - 20.3% (mean 16.7%) of SL vs. 16.9 - 21.7% (mean 20.5%) of SL], (xi) a longer pectoral fin length [13.1 - 24.9% (mean 19.7%) of SL vs. 11.9 - 19.3% (mean 15.2%) of SL], and (xii) the appearance of black and pale yellow marginal bands on the dorsal and ventral portion of the caudal fin (vs. no color marginal bands on the dorsal and ventral portion of the caudal fin).

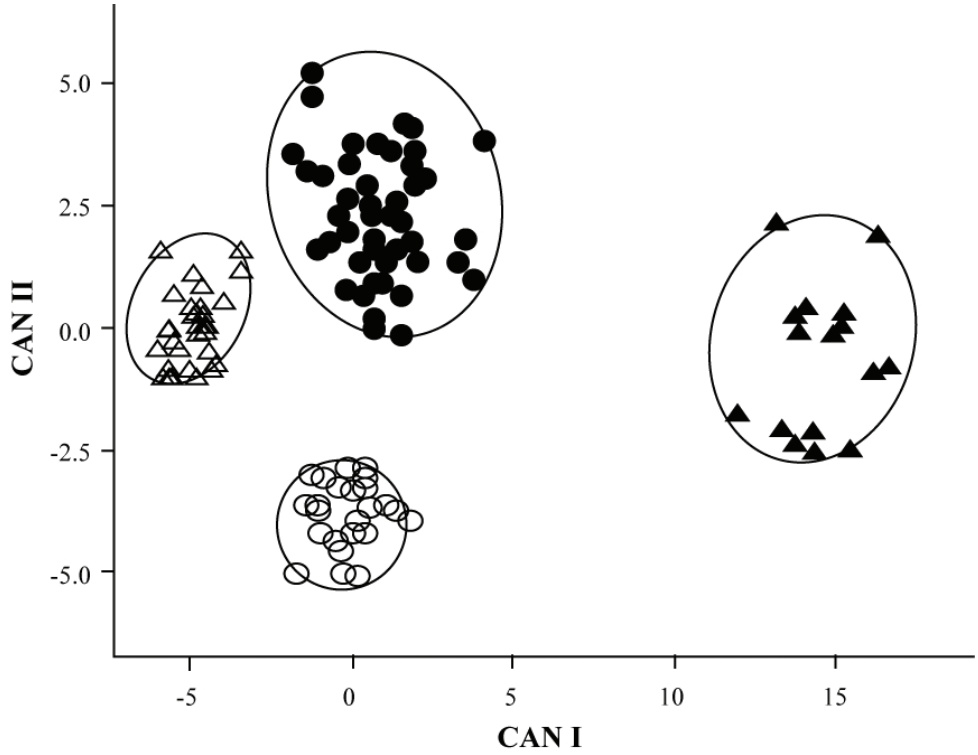

FIGURE 12. Plots of discriminant scores on the first and second canonical (CAN) scores, based on 22 morphometric characters, of *Oryzias songkhramensis* n. sp. (solid circles), *O. minutillus* (open circles), *O. mekongensis* (open triangles) and *O. pectoralis* (solid triangles).

Notable differences are found in the relationships between standard length (SL) and percentage of caudal peduncle depth to standard length of *O. songkhramensis* compared to *O. mekongensis* and *O. pectoralis* discriminate *O. songkhramensis* from other species (Fig. 10). Morphological difference between and within *O. songkhramensis*, *O. mekongensis* and *O. minutillus* are clarified. Twenty-two morphometric characters and six meristric characters were examined, and 13 morphometric and 4 meristric characters were found to differ amongst the three species.

In the PCA, the first, second and third principal components accounted for 63.26%, 9.39% and 4.59% of variance, respectively (Table 9), the first two being taken component scores. The results showed that *O. minutillus* and *O. mekongensis* overlapped moderately, whereas *O. songkhramensis*, *O. mekongensis* and *O. pectoralis* were completely separated (Fig. 11). On the other hand, the CDA resulted individuals of the respective ricefishes of *O. songkhramensis*, *O. mekongensis, O. minutillus* and *O. pectoralis* clearly falling into four difference areas (Fig. 12). The first, second and third axis accounted for 81.50%, 10.40% and 8.10% of variance, respectively; standardized canonical coefficients and eigenvalues are given in Table. 10. The greatest proportion of variance on the first axis was expressed by difference in pectoral fin length. The greatest

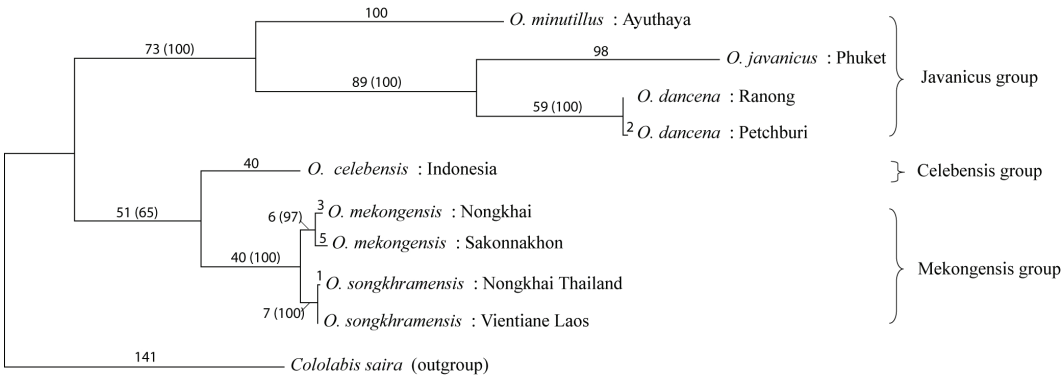

FIGURE 13. Single most parsimonious tree (715 steps) based on 821 charecters from the domain I sequences of the mitochondrial control region of the six indicated *Oryzias* species, including that of the new species of this report, *O. songkhramensis* n. sp. Numbers above branches indicate branch lengths and numbers in parentheses show bootstrap values as a % from 1000 replicates. GenBank accession numbers are given in the text.

contributions to the variance along the second axis was made by preanal length.

There are fourteen characters with main loadings on CNA2: standard length, total length, eye diameter, head length, preanal length, preanal fin length, predorsal fin length, prepelvic fin length, length of dorsal fin base, caudal peduncle depth, body depth at anterior anal fin origin, dorsal fin length, prepectoral fin length and dorsal fin origin to caudal fin base. In order to estimate the importance of the measurements for species identification, a univariate analysis of these fourteen measurements, expressed as percentages of SL or HL, were compared between *O. songkhramensis* with each of *O. mekongensis*, *O. minutillus* and *O. pectoralis*. All of them showed significant differences in the mean values of the indexes. The clear results of CDA in this study suggested that morphometric analyses are in fact more appropriate for discriminating between the different morphologies of *Oryzias*. Meristic characters are shown in Table 4. Anal-fin ray counts of the four species were shown to be significantly different. *Oryzias Mekongensis* and *O. songkhramensis* had smaller numbers of anal-fin rays (14 - 16 and 15 - 16, respectively) than *O. minutillus* and *O. pectoralis* (17 - 20 and 18 - 20, respectively). The mode of anal-fin ray counts of *O. mekongensis*, *O. songkhramensis, O. minutillus* and *O. pectoralis* were 14, 16, 18 and 19, respectively (Table. 4). The mode of a relatively anterior dorsal fin origin, opposite the anal fin of *O. mekongensis*, *O. songkhramensis, O. pectoralis* and *O. minutillus*, were 12, 14, 17 and 18, respectively (Table 6). Thus, the morphological differences detected here suggest that *O. songkhramensis* seems to represent a distinct species.

Remarks.– Roberts (1998) reported that *Oryzias pectoralis* from Nam Theum basin, Central Laos, have 19 - 20 anal-fins, 6 - 7 dorsal-fins and 6 pelvic-fin rays. In Ouang Ninh province, northern Vietnam, the species has been found in paddy fields and was also described with 19 - 20 anal-fin rays (Kotellat, 2001). *O. songkhramensis* is similar to *O. pectoralis* in having somewhat the same number of dorsal-fin rays and a black spot on the upper part of the pectoral fin. However, the former is distinguished

from the latter in having a slender smaller sized body (12.8 - 19.0 mm SL vs. 22.3 - 24.0 mm SL), a lower number of anal-fin rays (14 - 17 vs. 19 - 20), a lower number of total vertebrae (27 - 29 vs. 30), the appearance of black and pale yellow marginal bands on the dorsal and ventral portion of the caudal fin (vs. bright orange marginal bands on the dorsal and ventral portion of the caudal fin in *O. pectoralis*), and the appearance of a bony contact organ on the anal-fin rays in males (vs. the absence of a bony contact organ on anal-fin rays in males of *O. pectoralis*). The phylogenetic relationships based on the mitochondrial control region of six *Oryzias* species revealed three clear groups; the javanicus, celebensis and mekongensis (latipes) groups, in agreement with the results from previous analyses of the partial cytochrome *b*, 12S rDNA (Naruse, 1996) and tryosinase (Takehana et al., 2005) gene sequences. *Oryzias songkhramensis* sp. nov, was placed within the mekongensis (latipes) group.

Comparative material examined.– *Oryzias songkhramensis*: THAILAND: NIFI 3394, 4 ex., 15.0 - 17.0 mm SL; Ban Phang district, 17º 58' N, 104º12' E. Nakhon Phanum; colls: W. Magtoon and T. Deesin, 21 Apr. 2003. NIFI 3395, 4 ex., eka, district, 17º 55' N, 103º 57' E, Nakhon Phanum; colls. W. Magtoon and T. Deesin, 22 Apr.2003. LAOS: NIFI 3414, 14 ex., 14.8 - 16.9 mm SL; Sai Thani, Vientiane; W. Magtoon and T. Deesin, 19 Jun. 2007.

Oryzias minutillus: THAILAND: NIFI 3421, 8 ex., 9.8 - 10.7 mm SL; Muang, 15º 07' N, 100º 13' E, Chi-Nat Province, Thailand; colls. W. Magtoon and T. Deesin, 20 Jun. 2007. NIFI 3396, 3 ex., 12.7 - 13.4 mm SL, Muang, 14 ^{0} 28' N, 100^{0} 08' E, Suphanburi province; colls. W. Magtoon and P. Phaisumphon, 5 Aug. 2006. NIFI 3405, 8 ex., 12.7 - 13.4 mm SL; Klong Luang, 14º 05' N, 100º 33' E, Phathum Thani province; colls. W. Magtoon and T. Deesin, 5 Apr.2006. NIFI 01972, 3 ex, 10.0 - 10.8 mm SL; Sai Buri, 6º 44' N, 101º 82' E, Narathiwat; colls. L. Sankphan, M. Kotellat and T. R. Roberts, 21 Mar.1985. - NIFI 3212, 4 ex., 11.9 - 12.9 mm SL; Sathing Phra, 7º 22' N, 100º 30' E, Songkha province; colls. W, Magtoon and P. Phongsai, 3 Mar. 2006. NIFI 3422, 2 ex, 10.8 - 11.9 mm SL; Muang, 10º 29' N, 99º 12' E, Chumphon; colls. W. Magtoon and P. Kraichumphon, 1 Apr. 2006.

Oryzias mekongensis: THAILAND: NIFI 3209, 2 ex., 12.8 - 16.0 mm SL: Nong Han district, 17º 21' N, 103º 03' E, Udon Thani Province, colls. W. Magtoon and T. Deesin, 1 Oct. 2006. LBSWU 00203, 10 ex., 14.1 - 6.5 mm SL: Nong Han district, 17º 21' N, 103º 03' E, Udon Thani Province; colls. W. Magtoon and T. Deesin, 1 Oct. 2006. CTNRC 42-2612-2620, 9 ex., 11.5 - 14.5 mm SL: Kanthalak, 14º 39' N, 104º 39' E, Srisaket Province, colls. S. Chunchai and W. Magtoon, 15 Mar. 1984.

Oryzias pectoralis: VIETNAM: NIFI 3400, 4 ex., 23.8-25.3 mm SL; Ouang Ninh Province, Vietnam; W. Magtoon and Mai Dinh Yen, 17 March 2003. LAOS: CAS 92320, 21.1 mm SL male, Laksao, Nam Theum basin, central Laos, 19 Nov., T. R. Roberts (from photographic data).

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