Tropical Natural History 11(1): 9-20, April 2011


# First Record of the Ricefish, *Oryzias haugiangensis* (Adrianichthyidae: Oryziinae), from Southeastern Thailand

**WICHIAN MAGTOON***

*Department of Biology, Faculty of Science, Srinakharinwirot University, Sukhumvit 23, Bangkok 10110, THAILAND*
* Corresponding author. E-mail: wichian@swu.ac.th
Received: 5 November 2010; Accepted: 12 January 2011

**ABSTRACT.– Specimens of the ricefish, *Oryzias haugiangensis*, reported here for the first time from the brackish waters in the southeastern Thailand, were caught in a mangrove forest habitat. This study compares the morphometric and meristic characters and the karyotype of *O. haugiangensis* with those of the Andaman sea specimens of *O. javanicus* which are clearly distinguishable by the length of the snout, head, preanal, preanal fin, prepelvic fin and anal fin base, plus the body depth at the anterior of the anal fin origin. *Oryzias haugiangensis* is similar to *O. javanicus* in the number of pelvic fin and branchiostegal rays present, and in the diploid chromosome number (2n = 48), but differs from the latter in that it has a lower number of anal fin and pectoral fin rays and has a different karyotype, with 2n = 48 (2 SM + 23 A; NF = 54). *Oryzias haugiangensis* belongs to monoarmed chromosome group within the genus *Oryzias*.**

**KEY WORDS: *Oryzias haugiangensis*, mangrove habitat, morphology, karyotype**

## INTRODUCTION

Ricefish of the genus *Oryzias* are widely distributed in the South, East and Southeast Asia and southwards to Sulawesi and the Timor islands (Yamamoto, 1975; Labhart, 1978; Nelson, 2006; Berra, 2001). *Oryzias* includes 27 known species in Asia (Nelson, 2006; Parenti, 2008; Magtoon, 2010, Parenti and Hadiaty, 2010; Herder and Chapuis, 2010). In Southeast Asia, there are five species known from Thailand and four from Laos, Myanmar and Vietnam, but thirteen species are found from Indonesia, two species in Malaysia and one species each in Singapore and the Philippines (Herre and Ablan, 1934; Alfred, 1966; Magtoon, 1986; Roberts, 1998; Kotellat, 2001a, 2001b; Parenti and Soeroto, 2003; Parenti, 2008; Magtoon and Termvidchakorn, 2009).

Five nominal species have been described in Thailand for the ricefish genus *Oryzias*, i.e., *O. minutillus* Smith, 1945, *O. mekongensis* Uwa and Magtoon, 1986, *O. songkhramensis* Magtoon, 2010, *O. javanicus* (Bleeker, 1854), and *O. dancena* (Hamilton-Buchanan, 1822), the former three species are distributed from Thailand to Laos and Cambodia. The latter two species, on the other hand, have been distributed from the Indian Ocean throughout Peninsular Thailand in the western Andaman Sea and in the eastern part of the Gulf of Thailand (Smith, 1945; Uwa, 1986; Magtoon, 1986; Roberts, 1998).

In recent years, the first report of *Oryzias haugiangensis* was described in the Mekong delta in southern Vietnam by Roberts (1998), yet, interestingly, *O. haugiangensis* has not been reported in Thai waters. Since the locality of the south-eastern Thailand represents a new record for *O. haugiangensis* in Thailand, a detailed description of the specimens are given.

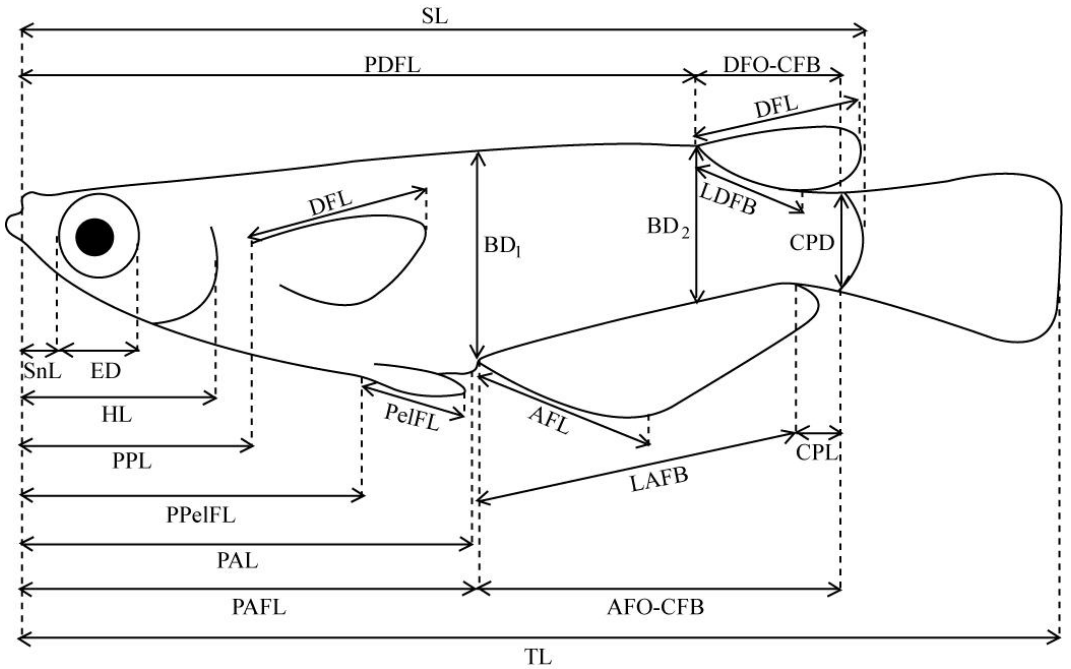


**FIGURE 1.** Outline drawing of a male *Oryzias* indicating the points of measurements. Abbreviations used are: SL. (standard length), TL (total length), SnL (snout length), ED (eye diameter), HL (head length), PAL (preanal length), PAFL (preanal fin length), PDFL (predorsal fin length), PPelFL (prepelvic fin length), LDFB (length of dorsal fin base), LAFB (length of anal fin base), PL (pectoral fin length), PelFL (pelvic fin length), CPD (caudal peduncle depth), CPL (caudal peduncle length), BD1 (body depth at anterior origin of anal fin), BD2 (body depth at anterior origin of dorsal fin), AFL (anal fin length or height of anal fin), DFL (height of dorsal fin or dorsal fin length), PPL (prepectoral fin length), DFO – CFB (dorsal fin origin to caudal fin base) and AFO - CFB (anal fin origin to caudal fin base).

## MATERIALS AND METHODS

**Morphometrical analyses.**– Specimens of *O. haugiangensis* from the south-eastern region of Thailand were caught with a hand net during a biological survey of the mangrove forest area around Trat province between March 2009 and March 2010. Methods of measurement generally follow those of Hubbs and Lagler (1958) and Nakabo (2002) (Fig. 1). The clearing and staining of specimens was performed as described by Dingerkus and Uhler (1977). Fin rays were counted with a binocular microscope. The meristic characters recorded were the dorsal-, anal-, pelvic-, pectoral- and principal caudal-fin rays plus one upper and one lower branched; the number of vertebrae (total = precaudal + caudal) and branchiostegal rays. Morphometric and meristric data of *O. javanicus* from the Andaman site at Phuket and Ranong, and *O. haugiangensis* from the Gulf of Thailand site, 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.

**Chromosomal analyses.**– Chromosome preparations were made using the method of


**FIGURE 2.** *Oryzias huagiangensis* Roberts, 1998. Material specimens (**A**) NIFI 4260 from Thailand (fresh condition) and (**B**) CTU-P 2167 from Vietnam (fixed condition). Bars indicate 5 mm.

Ojima and Kurishita (1980). Classification of chromosomes was determined following the method as described by Levan et al., (1964). Metacentrics and submetacentrics are described as two-arm chromosomes, and submetacentrics as one-arm chromosomes.

## RESULTS

### *Oryzias haugiangensis* Roberts, 1998 (Figure 2; Table 1)

*Oryzias javanicus*–Rainboth, 1996: 177 (field records from Vietnam in 1974).

*Oryzias haugiangensis* Roberts, 1998; 222. Type locality: south Vietnam, Bassac River (Hua Giang) at Can Tho. Parenti, 2008: 563, fig. 41 (redescription of paratype, CAS 93898, female, 16.0 mm SL).

**Material examined.**– NIFI 4260, 22, 13.4-19.7 mm SL, Thailand; Bangkaja Sub-district, Mueang, Trat Province 12º 17’ N, 102º 65’ E; Magtoon, W, 3 March 2010.

**Description.**– (based on 22 specimens, 13.4-19.7 mm SL). Counts of materials are in Table 1: Dorsal-fin rays 7; anal-fin rays [19-22 (mode 21)]; pectoral-fin rays [10-11 (10)]; pelvic-fin rays 6; body covered with cycloid scales, (27-31) in a lateral series; transverse scales 7-9 (8); predorsal scales 22-24 (23); transverse scales 8-9 (8); principal caudal-fin rays i,4/5,i; procurent fin dorsal 4, ventral 5; vertebrae 27-28.

Measurements in percent of SL and HL are shown in Table 1. Results of ANCOVAs are shown in Table 2. TL (124.2-153.1); body depth at anterior origin of anal fin (20.1-29.0); body depth at anterior origin of dorsal fin (14.7-20.9);

**TABLE 1.** Meristic and morphometric characters of *O. haugiangensis* and *O. javanicus* from Phuket and Ranong. * Indicates n for number of specimens, ND is no data.

| Parameter | ***O. haugiangensis*: Vietnam** | ***O. haugiangensis*: Trat, Thailand** | ***O. javanicus*: Phuket, Thailand** | ***O. javanicus*: Ranong, Thailand** |
|---|---|---|---|---|
| | **(n* = 5)** | **(n* = 22)** | **(n* = 31)** | **(n* = 30)** |
| Standard length (mm) (SL) | 20.1 - 21.9 (21.3 ± 0.7) | 13.4 - 19.7 (16.6 ± 1.3) | 20.5 - 30.9 (24.9 ± 2.8) | 23.7 - 30.1 (27.4 ± 1.7) |
| Dorsal-fin rays | 7 | 7 | 6 - 8 (7) | 7 |
| Anal-fin rays | 20 - 22 (20) | 19 - 22 (21) | 17 - 25 (23) | 21 - 26 (23) |
| Pectoral-fin rays | 10 - 12 | 10 - 11 (10) | 11 | 11 - 12 (11) |
| Pelvic-fin rays | 6 | 6 | 6 | 6 |
| Branchiostegal rays | ND | 5 | 5 | 5 |
| Upper caudal fin ray number | 4 - 5 (4) | 4 | 4 - 5 (4) | 4 |
| Lower caudal fin ray number | 4 - 6 (5) | 5 | 5 | 5 |
| Abdominal vertebrae | ND | 10 - 11 (11) | 10 - 11 (11) | 10 |
| Caudal vertebrae | ND | 16 - 17(16) | 17 - 19 (19) | 19-20(19) |
| **Measurements (in % of SL)** | range (mean ± SD) | range (mean ± SD) | range (mean ± SD) | range (mean ± SD) |
| Total length (TL) | 126.3 - 130.7 (127.6 ± 1.8) | 124.2 - 153.1 (128.5 ± 5.7) | 112.9 - 127.7 (122.1 ± 3.9) | 101.2 - 126.5 (121.9 ± 4.7) |
| Snout length/HL | 27.6 - 33.3 (31.0 ± 2.5) | 23.4 - 31.2 (27.9 ± 2.2) | 13.0 - 25.0 (18.6 ± 3.1) | 14.0 - 25.3 (19.6 ± 2.8) |
| Eye diameter/HL | 37.8 - 42.2 (40.6 ± 1.9) | 35.9 - 45.9 (40.2 ± 2.7) | 35.2 - 61.9 (46.1 ± 5.6) | 37.8 - 50.4 (42.7 ± 3.0) |
| Head length (HL) | 25.2 - 26.4 (25.9 ± 0.5) | 23.6 - 29.6 (25.5 ± 1.2) | 22.9 - 31.0 (27.3 ± 2.1) | 21.2 - 34.2 (27.1 ± 2.1) |
| Preanal length | 47.7 - 54.1 (50.0 ± 2.4) | 47.5 - 62.4 (51.5 ± 3.0) | 49.0 - 58.8 (54.4 ± 2.3) | 46.3 - 61.1 (55.1 ± 3.0) |
| Preanal fin length | 51.1 - 56.8 (53.7 ± 2.4) | 49.0 - 64.5 (54.3 ± 3.3) | 54.1 - 63.0 (58.8 ± 2.2) | 48.2 - 62.8 (57.7 ± 2.7) |
| Predorsal fin length | 81.8 - 82.4 (82.0 ± 0.3) | 80.5 - 101.4 (82.9 ± 4.2) | 74.4 - 84.0 (80.4 ± 2.1) | 65.7 - 84.0 (80.6 ± 3.2) |
| Prepelvic fin length | 41.1 - 45.3 (43.0 ± 1.7) | 40.9 - 55.8 (44.7 ± 3.1) | 37.8 - 48.0 (43.9 ± 2.5) | 22.8 - 49.1 (43.6 ± 4.5) |
| Length of dorsal fin base | 6.6 - 9.4 (8.0 ± 1.2) | 6.0 - 8.9 (7.2 ± 0.7) | 5.89 - 9.02 (7.4 ± 0.7) | 6.4 - 8.2 (7.3 ± 0.5) |
| Length of anal fin base | 31.0 - 34.8 (33.2 ± 1.6) | 29.9 - 39.7 (32.6 ± 2.2) | 22.0 - 36.7 (30.7 ± 3.3) | 23.6 - 36.3 (31.3 ± 2.6) |
| Pectoral fin length | 23.7 - 30.9 (26.4 ± 2.7) | 21.3 - 32.3 (25.0 ± 2.1) | 19.5 - 32.9 (26.2 ± 3.8) | 20.8 - 32.1 (25.3 ± 2.7) |
| Pelvic fin length | 11.3 - 13.3 (12.2 ± 0.9) | 11.0 - 15.9 (12.7 ± 1.2) | 9.1 - 15.5 (12.7 ± 1.4) | 9.3 - 13.8 (12.1 ± 1.0) |
| Caudal peduncle depth | 10.3 - 12.8 (11.7 ± 1.1) | 10.2 - 12.7 (11.0 ± 0.6) | 10.1 - 18.5 (12.2 ± 1.6) | 10.8 - 13.7 (12.1 ± 0.7) |
| Caudal peduncle length | 10.9 - 14.2 (12.1 ± 1.4) | 8.8 - 13.1 (10.8 ± 0.9) | 9.7 - 13.8 (12.0 ± 1.1) | 8.9 - 14.1 (11.9 ± 1.0) |
| Body depth at anterior origin of anal fin | 25.4 - 28.4 (26.9 ± 1.1) | 20.1 - 29.0 (23.4 ± 2.2) | 20.4 - 29.5 (24.6 ± 2.5) | 18.0 - 29.4 (25.8 ± 2.6) |
| Body depth at anterior origin of dorsal fin | 16.2 - 21.3 (18.8 ± 1.9) | 14.7 - 20.9 (16.5 ± 1.4) | 15.4 - 23.1 (18.4 ± 1.7) | 11.7 - 23.3 (19.0 ± 2.3) |
| Height of anal fin | 22.4 - 28.6 (25.1 ± 2.7) | 16.0 - 38.1 (25.0 ± 7.9) | 15.2 - 27.1 (19.9 ± 3.4) | 13.2 - 27.0 (20.9 ± 4.2) |
| Height of dorsal fin | 18.1 - 24.6 (21.7 ± 2.4) | 14.1 - 33.2 (21.2 ± 5.1) | 14.0 - 24.9 (18.5 ± 3.2) | 12.6 - 20.1 (17.3 ± 2.0) |
| Prepectoral fin length | 28.8 - 30.8 (30.1 ± 0.9) | 28.7 - 37.8 (30.5 ± 1.7) | 22.1 - 31.8 (27.8 ± 2.3) | 23.1 - 35.1 (29.7 ± 2.6) |
| Dorsal fin origin to caudal fin base | 17.1 - 21.6 (18.8 ± 1.7) | 17.3 - 22.2 (19.0 ± 1.2) | 16.7 - 41.2 (19.8 ± 24.2) | 14.5 - 28.5 (19.7 ± 2.4) |
| Anal fin origin to caudal fin base | 42.7 - 46.5 (44.8 ± 1.5) | 38.9 - 52.4 (43.1 ± 2.8) | 37.0 - 48.1 (41.5 ± 2.7) | 34.3 - 46.1 (42.2 ± 3.0) |

**TABLE 2.** Analysis of covariance (ANCOVA) of morphometric measurements of *O. haugiangensis*; *O. javanicus*: Phuket and *O. javanicus*: Ranong.

| Characters | F-values | *P* |
|---|---|---|
| Morphometric measurements as % of SL | | |
| Total length (TL) | 6.233 | 0.001 |
| Snout length/HL | 1.918 | 0.133 |
| Eye diameter/HL | 0.646 | 0.588 |
| Head length (HL) | 2.542 | 0.062 |
| Preanal length | 5.402 | 0.002 |
| Preanal fin length | 5.751 | 0.001 |
| Predorsal fin length | 6.291 | 0.001 |
| Prepelvic fin length | 2.844 | 0.043 |
| Length of dorsal fin base | 4.288 | 0.007 |
| Length of anal fin base | 0.505 | 0.680 |
| Pectoral fin length | 1.507 | 0.219 |
| Pelvic fin length | 1.253 | 0.296 |
| Caudal peduncle depth | 0.998 | 0.398 |
| Caudal peduncle length | 2.039 | 0.115 |
| Body depth at anterior origin of anal fin | 0.962 | 0.415 |
| Body depth at anterior origin of dorsal fin | 3.200 | 0.028 |
| Height of anal fin | 0.076 | 0.973 |
| Height of dorsal fin | 0.704 | 0.552 |
| Prepectoral fin length | 1.258 | 0.294 |
| Dorsal fin origin to caudal fin base | 0.157 | 0.925 |
| Anal fin origin to caudal fin base | 2.189 | 0.096 |

snout length/HL (23.4-31.2); eye diameter/HL (27.5-45.9); HL (23.6-29.6); preanal length (47.5-62.4); preanal fin length (49.0-64.5); predorsal fin length (80.5-101.4); prepelvic fin length (40.9-55.8); length of dorsal fin base (6.0-8.9); length of anal fin base (29.9-39.7); pectoral fin length (21.3-32.3); pelvic fin length (11.0-15.9); caudal peduncle depth (10.2-12.7); caudal peduncle length (8.8-13.1); anal fin length (16.0-38.1); dorsal fin length (14.1-33.2); prepectoral fin length (28.7-37.8); dorsal fin origin to caudal fin base (17.3-22.2); anal fin origin to caudal fin base (38.9-52.4).

Body compressed laterally, depth of body greatest at anterior origin of anal fin. HL [3.7 (3.5-3.9)] in SL; head less compressed than body, anterior part of head depressed, head short, [23.6 to 29.6 (25.5±1.2)] % of SL. Snout length [3.7 (3.6-3.9)] in HL; snout short, [23.4-31.2 (19.6±2.2)] % of HL, snout shorter than eye diameter. Eye diameter [2.5 (2.4-2.6)] in HL, the pupil situated in the anterior part of head; eye large, [35.9-45.9 (40.2±2.7)] % of HL. Mouth terminal, almost horizontal; lower jaw slightly projecting beyond upper jaw. Each jaw with two rows of caniniform teeth, with large teeth. Dorsal body profile relatively straight from head to dorsal-fin origin; ventral profile convex from head to anal-fin origin. First pleural rib on parapophysis of third vertebrae; lateral process of pelvic bone attaches to third pleural rib. Body covered with large cycloid scale, 27-31 (28) in lateral series, predorsal scale 22-24 (23), transverse scale 7-9 (8).

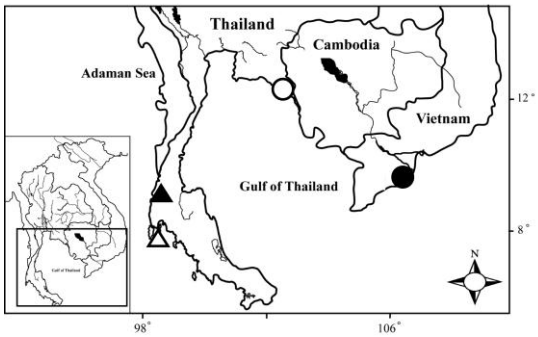


**FIGURE 3.** Geographical distribution of *Oryzias* in south and southeast Thailand. Solid triangle, *O. javanicus* from Ranong; open triangle, *O. javanicus* from Phuket; solid circle, *O. haugiangensis* from Vietnam; open circle, *O. haugiangensis* from Thailand.

Caudal skeleton with two pleural plates; ventral accessory bone and accessory cartilage or bone. Lateral line absent. Caudal peduncle compressed, depth [2.3 (2.2-2.4)] in HL; caudal peduncle length, [2.6 (2.5-2.8)] in HL. Caudal fin truncated. Vertebrae 27-28. Branchiostegal rays 5-6 (5). Presence of numerous small papilla processes on the posterior region of anal fin in male fishes. Peritoneum dense black within, the outer surface next to body wall bright silvery.

**Color of fresh specimens.**– Body translucent, belly more or less silver. Head and body dark greyish-brown in males, light brown in females. Dorsal fin base yellow and filaments yellow or hyaline. Pelvic fin bright yellow and pectoral fin hyaline. Anal fin with filamentous rays bright yellow. Caudal fin with yellowish submarginal bands on the dorsal and ventral lobes and along the distal edge, and the color being more intense in males. Caudal fin with 1 - 3 longitudinal dark black stripes on the proximal half of the membrane between median rays. Dorsal fin base with yellow and filaments yellow or hyaline. Pelvic fin bright yellow and pectoral fin hyaline. Both sexes with silvery operculum.

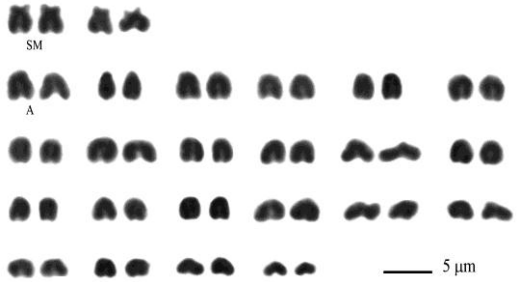


**FIGURE 4.** A karyotype of *O. haugiangensis* from Thailand. SM = submetacentric and A = acrocentric.

**Color of preserved specimens.**– Body pale grey to blackish, brownish dorsally and pale ventrally. Melanophores forming a blackish line along anal fin base and on mid-lateral line. Blackish spots on dorsal part of head, body and caudal peduncle. Dorsal and anal fin interradial membranes with scattered melanophores. The color is more intense in males.

**Sexual dimorphism.**– In addition to the different body color patters mentioned above, the anal fin has a convex distal margin with bony contact organs in males, whereas the distal margin of the anal fin is straight or slightly concave in females. Anal fin rays are longer and thicker in males. Melanophores on caudal and pelvic fins and on body side are denser in males during the breeding season. Urogenital papilla relatively large, bilobed in females, and a short conical tube in males. Interneural spines and wings of basipterygium more developed in males. Males are distinguished from the females in having (i) a longer height of dorsal fin [4.0-4.6 (mean 4.3) % of SL vs. 2.2-2.3 (3.0) % of SL] (ii) a longer height of anal fin [5.0-6.2 (5.5) % of SL vs. 2.6-3.2 (2.8) % of SL], and (iii) a larger body depth at anal fin origin [3.3-5.4 (4.2) % of SL vs. 3.3-4.0 (3.6) % of SL].

**TABLE 3.** Eigenvectors for the first three principal components (PC) based on 22 morphometric characters of *O. haugiangensis*; *O. javanicus*: Phuket and *O. javanicus*: Ranong.

| Measurements | PC I | PC II | PC III |
|---|---|---|---|
| Standard length (SL) | 0.050 | 0.091 | -0.010 |
| Total length (TL) | 0.015 | 0.144 | -0.088 |
| Snout length | 0.065 | 0.028 | -0.181 |
| Eye diameter | 0.004 | 0.100 | 0.049 |
| Head length (HL) | 0.098 | -0.014 | 0.056 |
| Preanal length | 0.106 | -0.064 | 0.020 |
| Preanal fin length | 0.032 | -0.109 | -0.045 |
| Predorsal fin length | 0.060 | 0.079 | -0.009 |
| Prepelvic fin length | 0.102 | -0.043 | 0.086 |
| Length of dorsal fin base | -0.030 | 0.172 | -0.121 |
| Length of anal fin base | 0.032 | -0.062 | 0.252 |
| Pectoral fin length | 0.072 | -0.027 | -0.131 |
| Pelvic fin length | 0.101 | -0.023 | -0.037 |
| Caudal peduncle depth | 0.088 | 0.029 | -0.098 |
| Caudal peduncle length | -0.106 | 0.087 | -0.106 |
| Body depth at anterior origin of anal fin | 0.074 | 0.056 | 0.001 |
| Body depth at anterior origin of dorsal fin | 0.093 | 0.024 | -0.034 |
| Height of anal fin | 0.002 | 0.063 | 0.117 |
| Height of dorsal fin | -0.046 | 0.110 | 0.042 |
| Prepectoral fin length | 0.082 | 0.024 | 0.049 |
| Dorsal fin origin to caudal fin base | -0.035 | 0.167 | -0.099 |
| Anal fin origin to caudal fin base | 0.027 | -0.074 | 0.263 |
| Eigenvalues | 11.00 | 7.86 | 3.14 |
| % of variance | 50.02 | 35.72 | 14.27 |

**Distribution.**– *Oryzias haugianensis* is known from brackish habitats in the Mekong delta in Vietnam (Robert, 1998; Parenti, 2008). In the present study, *O. haugianensis* is found in brackish habitats of a mangrove forest area at Trat province, south-eastern Thailand (Fig. 3).

**Cytogenetic data.**– *Oryzias haugianensis* from Trat province, south-eastern Thailand had a diploid chromosome number of 48. The karyotype consisted of two metacentric and 22 acrocentric chromosome pairs, NF (chromosome arm number) being 54 (Fig. 4). According to existing karyotype analysis, *Oryzias* can be divided into three major groups, those having monoarmed, biarmed and fused chromosomes (Uwa, 1986). This classification also coincided well with that indicated by electrophoretic studies of allozymes and muscle proteins (Sakaizumi 1985a, b), and with the molecular analysis based upon the DNA sequence of cytochrome B, 12S rDNA (Naruse, 1996) and tyrosinase (Takehana et al., 2005). *O. haugianensis* possessed chromosomes which belonged to the monoarmed chromosome group.

**Comparisons.**– *Oryzias haugianensis* from southeastern Thailand is similar to *O. javanicus* from Phuket and Ranong provinces in general body appearance, especially in having the same number of dorsal-fin, branchiosteagal- and pelvic-fin rays. However, the former is distinguished from the latter in having (i) a more slender body depth at the origin of the dorsal fin [14.7-20.9 (mean 16.5) % of SL vs. 20.4-29.5 (24.6) % of SL and 18.0-29.4 (25.8) % of SL, respectively], (ii) a longer snout

length [23.4-31.2 (27.9) % of HL vs.13.0-25.0 (18.6) and 14.0 - 25.3 (19.6)% of HL, respectively], (iii) a smaller orbit diameter [35.9-45.9 (40.2) % of HL vs. 35.2-61.9 (46.1) and 37.8-50.4 (42.7) % of HL, respectively], (iv) a more slender head length, [23.3-29.6 (25.5) % of SL vs. 22.9-31.0 (27.3) % of SL and 21.2-34.2 (27.1), respectively], and (v) a more slender preanal fin length [49.0-64.5 (54.3) % of SL vs. 54.6-63.3 (58.8) % of SL and 48.2-62.8 (57.7) % of SL, respectively] (Table 1).

Morphometric and meristric characters of the two species of *O. haugiangensis* and *O. javanicus* are shown in Table 1. Results of ANCOVAs are shown in Table 2. Significant differences in elevations among four populations of *Oryzias* were shown in seven morphometric characters. For instance, the head length was shorter [23.6-29.6 (25.5) % of SL] in *O. haugiangensis* from Thailand than in *O. haugiangensis* from Vietnam, [25.2-26.4 (25.9) % of SL], *O. javanicus* from Ranong [21.2-34.2 (27.1) % of SL] and *O. javanicus* from Phuket [22.9-31.0 (27.3) % of SL]. The predorsal fin length was longer in [80.5-101.4 (82.9) % of SL] in *O. haugiangensis* from Thailand than in *O. haugiangensis* from Vietnam, [81.8-82.4 (82.0) % of SL], *O. javanicus* from Ranong [65.7 - 84.0 (80.6) % of SL] and *O. javanicus* from Phuket [74.4-84.0 (80.4) % of SL].

Meristic characters are shown in Table 1. *Oryzias javanicus* from Phuket and Ranong were shown to the significantly different from *O. haugiangensis* in the number of anal-fin rays and the scale of lateral line series. *Oryzias haugiangensis* had smaller numbers of pectoral-fin rays than *O. javanicus* from Phuket and Ranong provinces [10-11 (10) vs. 11 and 11-12 (11), respectively]. The mode of anal-fin ray counts of *O. javanicus* from Phuket and *O. javanicus* from Ranong and *O. haugiangensis* were 17-25 (23), 21-26 (23) and 19-22 (21), respectively. The mode of pectoral-fin rays counts of *O. javanicus* from Phuket and Ranong provinces was 11, which differed significantly from that of *O. haugiangensis* (10). A relatively anterior dorsal fin origin, opposite anal fin of *O. haugiangensis* was 16-19 (17), which also differed significantly from that *O. javanicus* from Phuket and Ranong provinces. The basihyal cartilage elongate and rectangular of *O. haugiangensis* was more slender than *O. javanicus* from Phuket (Fig. 5).

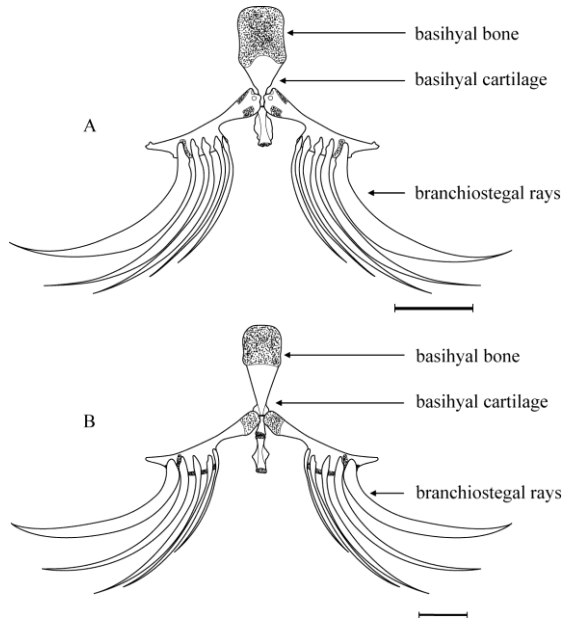


**FIGURE 5.** Anterior section of the ventral portion of gill arches in (**A**) *O. haugiangensis* and (**B**) *O. javanicus*: Phuket. Bars indicate 1 mm.

*Oryzias haugiangensis* from southeastern Thailand is similar to *O. hubbsi* from Indonesia in having the same number of dorsal-fin rays and pelvic-fin rays, but the former is distinguished from the latter in having a relatively high number of anal-fin rays 19-22 (vs. 16-19 in *O. hubbsi*); pectoral-fin rays 10-11 (vs. 8-9) and branchiostegal rays 5 (vs. usually 4-5).

**TABLE 4.** Standardized canonical (CAN) coefficients based on 22 morphometric characters of *O. haugiangensis*; *O. javanicus*: Phuket and *O. javanicus*: Bangban (Ranong).

| Measurements | CAN I | CAN II |
|---|---|---|
| Standard length (SL) | 0.374 | 0.248 |
| Total length (TL) | 0.438 | 0.273 |
| Snout length | 0.087 | 0.303 |
| Eye diameter | 0.539 | -0.107 |
| Head length (HL) | 0.347 | 0.201 |
| Preanal length | 0.433 | 0.293 |
| Preanal fin length | 0.507 | 0.257 |
| Predorsal fin length | 0.469 | 0.281 |
| Prepelvic fin length | 0.272 | 0.243 |
| Length of dorsal fin base | 0.410 | 0.159 |
| Length of anal fin base | 0.274 | 0.433 |
| Pectoral fin length | 0.313 | 0.127 |
| Pelvic fin length | 0.344 | 0.092 |
| Caudal peduncle depth | 0.325 | 0.194 |
| Caudal peduncle length | 0.314 | 0.204 |
| Body depth at anterior origin of anal fin | 0.152 | 0.345 |
| Body depth at anterior origin of dorsal fin | 0.453 | 0.321 |
| Height of anal fin | 0.209 | 0.312 |
| Height of dorsal fin | 0.158 | 0.180 |
| Prepectoral fin length | 0.383 | 0.740 |
| Dorsal fin origin to caudal fin base | 0.137 | 0.404 |
| Anal fin origin to caudal fin base | 0.413 | 0.385 |
| Eigenvalues | 17.561 | 0.781 |
| % of variance | 95.70 | 4.30 |

In the PCA, the first, second and third principal components accounted for 50.0%, 35.7%, and 14.3% of variance, respectively (Table 3), and the first two were taken for component scores. The results showed that the Thai and Vietnamese populations of *O. haugiangensis* were separate but that the Vietnamese population of *O. haugiangensis* and *O. javanicus* population from Phuket overlapped whereas, the Thai population of *O. haugiangensis* and *O. javanicus* from Ranong were separate (Fig. 6). The greatest contribution to the variance along the first and second axis was made by caudal peduncle length (negative) and long of dorsal fin base (positive), respectively.

On the other hand, the CDA results suggest that the respective Thai and Vietnam populations of *O. haugiangensis* overlapped moderately with each other, as did the *O. javanicus* populations from Phuket and Ranong, whereas, *O. haugiangensis* and *O. javanicus* were completely separate (Fig. 7). The first and second axis accounted for 95.7% and 4.3% of variance, respectively. Values of standardized canonical coefficients given in Table 4 indicate that the greatest proportion of variance on the first axis was expressed by differences in eye diameter (positive). The greatest contribution to the variance along the second axis was made by prepectoral fin length (positive).

**Remarks.**– *Oryzias haugiangensis* was first described from southern Vietnam (Roberts, 1998), and can be distinguished by the numbers of dorsal-fin rays (6-7), anal-fin rays (19-23), pectoral-fin rays (1 -11), caudal-fin rays (4-5, 5/6, 5-6) and vertebrae

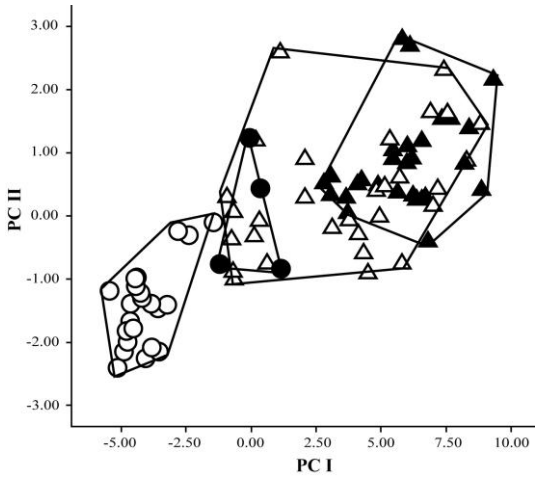


**FIGURE 6.** Scores of principle component 1 plotted against component 2 for pooled measurements of two species of *Oryzias* in Thailand [Solid triangle, *O. javanicus* from Ranong; open triangle, *O. javanicus* from Phuket; solid circle, *O. haugiangensis* from Vietnam; open circle, *O. haugiangensis* from Thailand].

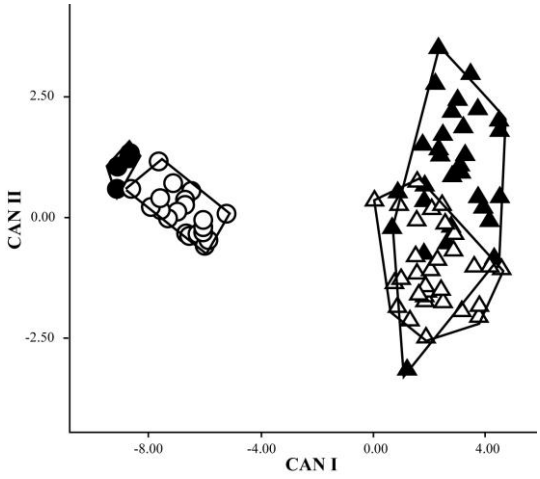


**FIGURE 7.** Plots of discriminant scores on the first and second canonical (*CAN*) axes based on 22 morphometric characters of two species of *Oryzias* in Thailand. [Solid triangle, *O. javanicus* from Ranong; open triangle, *O. javanicus* from Phuket; solid circle, *O. haugiangensis* from Vietnam; open circle, *O. haugiangensis* from Thailand].

(27-28). Later Parenti (2008) described *O. haugiangensis* from southern Vietnam, but with 19-22 anal-fin rays, 27-29 vertebrae and 6 pelvic-fin rays. Subsequent examination of the *O. haugiangensis* specimens from southern Vietnam (CTU-P 2167: 2009) revealed that they had 20-22 (20) anal-fin rays and 10-12 pectoral-fin rays. These *O. haugiangensis* from southeastern Thailand are therefore similar to those described by Roberts, 1998, Parenti, 2008 and material specimens from southern Vietnam, except the SL that, in this study, is smaller than Roberts's and Parenti's specimens.

**Comparative material examined.**– *Oryzias haugiangensis*: CTU-P 2167, 5, 20.1-21.9 mm SL, Vietnam; Vinh province, Duyen Hai district, Xa Dan Thanh, Ap Con Ong, Kenzo Utsugi, 02 April 2009. *Oryzias javanicus*: NIFI 3410, 15, Thailand; Mueang, Phuket, Magtoon and N. Magtoon 1 April 2005; NIFI 3411, 8, Thailand; Mueang, Phuket, W. Magtoon and P. Pongsai, 26 Aug. 1988; CAS 58033, 10: 13.4 - 21.9 mm SL, Thailand; Nai Han, Phuket, N. Tawinsunnuk, H. Uwa and W. Magtoon 2 Feb. 1985; KUMF 2997, 4, Thailand; Saphan-Hin Phuket, W. Magtoon and H. Uwa, 26 Aug. 1988; NIFI 3218, 2, Thailand; Saphan-Hin, Phuket, W. Magtoon and P. Pongsai, 15 May 2005; NIFI 4258, 2, Thailand; Bangban, Ranong, W. Magtoon, 3 March 2010; LBSWU00303, 28, Thailand; Bangban, Ranong, W. Magtoon, 3 March 2010. *Oryzias hubbsi*: NIFI 3415, 23.1 mm SL, Indonesia, S. Hamguchi and W. Magtoon, 6 September 2005.

## ACKNOWLEDGEMENTS

I greatly appreciated receiving specimens loans from Drs. Tran Dac Dinh and Kenzo Utsugi, Can Tho University, College of Agriculture and Fisheries, Vietnam and Nagao Natural Environment Foundation, Japan, respectively. I sincerely thank

Professor Dr. Gordon S. Maxwell, Open University of Hong Kong and Ecosystem Research Center (New Zealand) for his reading of the manuscript. My cordial thanks to Professor Dr. Satoshi Hamagushi and Professor Dr. Mitsuru Sakaizumi for their checking of this species using mtDNA sequence analysis. I also thank Associate Professor Thawat Donsakul, Miss. Wirawan Kamsri and Miss. Sinothai Smithikunanon, Srinakharinwirot University, Thailand for their cooperation in field collections.

## LITERATURE CITED

Alfred, E.R. 1966. The fresh-water fishes of Singapore. Zoologische Verhandelingen, 78: 1-68.

Berra, T.M. 2001. Freshwater fish distribution. Academic Press, San Diego. CA, 615 pp.

Bleeker, P. 1854. Ichthyolgische waarnemigen, gedaan op verschillede reizen in de residentie Banten. Natuurkundig tijdschrift voor Nederlandsch-Indie, 7: 309-326.

Dingerkus, G. and Uhler, L.D. 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. Stain Technology, 52: 229-232.

Hamilton-Buchanan, F. 1822. An Account of the Fishes of River Ganges and its Branches. George Ramsay and Co., London, vii+405pp, 39 pls.

Herder, F. and Chapuis, S. 2010. *Oryzias hadiatyae*, a new species of ricefish (Atherinomorpha: Beloniformes: Adrianichthyidae) endemic to Lake Masapi, Central Sulawesi, Indonesia. The Raffles Bulletin of Zoology, 58: 269-280.

Herre, A.W. and Ablan, G.L. 1934. *Aplocheilus luznenensis*, a new Philippine Cyprinodont. Philippine Journal of Science, 54: 275 - 277.

Hubbs, CL and Lagler, K.F. 1958. Fishes of the Great Lakes region. Bulletin - Cranbrook Institute of Science, 26: 213.

Kottelat, M. 2001a. Freshwater fishes of northern Vietnam. Environment and Social Development Unit, East Asia and Pacific Region, World Bank, Washington iii+140 pp., 15 pls.

Kotellat, M. 2001b. Fishes of Laos. Wildlife Heritage Trust Publications, Colombo, 198 pp.

Labhart, P. 1978. Die Arten der Gattung *Oryzias* Jordan and Snyder, 1907. Deutsche Killifisch Gemeinschaft Journal, 10: 53-58.

Levan, A, Fredga, K and Sandberg, A.A. 1964. Nomenclature for centrometric position on chromosomes. Hereditas 52: 201-220.

Magtoon, W. 1986. Distribution and phyletic relationships of Oryzias fishes in Thailand. In: T. Uyeno, R. Arai, T. Taniuchi and K. Matsuura, eds. Indo-Pacific fish biology, proceedings of second international conference on Indo-Pacific fishes. Tokyo. Ichthyological Society of Japan, pp. 859-866.

Magtoon, W. and Termvidchakorn, A. 2009. A revised taxonomic Account of ricefish *Oryzias* (Beloniformes; Adrianichyidae), in Thailand, Indonesia and Japan. The Natural History Journal of Chulalongkhorn University, 9: 35-68.

Magtoon, W. 2010. *Oryzias songkhramensis*, a new species of ricefish (Beloniformes; Adrianich-thyidae) from the northeast Thailand and central Laos. Tropical Natural History, 10: 107-129.

Nakabo, T. 2002. Introduction to ichthyology In: Nakabo T (ed) Fishes of Japan with picturial keys to the species, Enghish edn. Tokai Univerity Press, Tokyo, pp. xxi-xlii.

Naruse, K. 1996. Classification and phylogeny of fishes of the genus *Oryzias* and its relatives. The Fish Biology Journal Medaka, 8: 1-9.

Nelson, J.S. 2006. Fishes of the world. 4th edition., John Wiley and Sons, Inc. New York, 601 pp.

Ojima, Y. and Kurishita, A. 1980. A new method to increase the number of mitotic cells in the kidney tissue for fish chromosome studies. Proceedings of the Japan Academy, 56B: 610-615.

Parenti, L.R. 2008. A Phylogenetic analysis and taxonomic revision of ricefishes, *Oryzias* and (Beloniformes, Adrianichthyidae). Zoological Journal of the Linnean Society, 154: 494-610.

Parenti, L.R. and Hadiaty, R.K. 2010. A New, Remarkably Colorful, Small Ricefish of the Genus *Oryzias* (Beloniformes, Adrianichthyidae) from Sulawesi, Indonesia. Copeia, 2010: 268-273.

Parenti, L.R and Soeroto, B. 2003. *Adrianichthys roseni* and *Oryzias nebulosus*, two new ricefishes (Atherinomorpha: Beloniformes: Adrianich-thyidae) from Lake Poso, Sulawesi, Indonesia. Ichthyological Research, 51: 10-19.

Rainboth, W.J. 1996. Fishes of the Cambodian Mekong. FAO Species identification field guide for fishery purpose, Mekong River Commission, FAO and DANIDA, 265 pp.

Roberts, T.R. 1998. Systematic observations on tropical Asian medakas or ricefishes of the genus *Oryzias*, with descriptions of four new species, Ichthyological Research, 45: 213-324.

Sakaizumi, M. 1985a. Electrophoretic comparison of proteins in five species of *Oryzias* (Pisces: Oryziatidae) Copeia, 521-522.

Sakaizumi, M. 1985b. Species-specific expression of parvalbumins in the genus *Oryzias* and its related species. Comparative Biochemistry and Physiology, 80B: 499-505.

Smith, H.M. 1945. The freshwater fishes of Siam, or Thailand. Bulletin of the United States National Museum, 622 pp.

Takehana, Y., Naruse, K and Sakaizumi, M. 2005. Molecular phylogeny of the medaka fishes genus *Oryzias* (Beloniformes: Adrianichthyidae) based on nuclear mitochondrial DNA sequences. Molecular Phylogenetics and Evolution, 36: 417-428.

Uwa, H. and Magtoon, W. 1986. Description and karyotype of a new ricefish, *Oryzias mekongensis*, from Thailand. Copeia. 1986: 473-478.

Uwa, H. 1986. Karyotype evolution and geographical distribution in the ricefish, genus *Oryzias* (Oryziidae). In: Uyeno T, Arai R, Taniuchi T, Matsuura K, eds. Indo-Pacific fish biology, of the second international conference on Indo-Pacific fishes, Tokyo: Ichtyological Society of Japan, pp 867-876.

Yamamoto, T. 1975. Medaka (killifish) biology and strains. Series of stock culture in biological field. Tokyo: Keiguku Publishing Company, 365 pp.