SHORT REPORT

ScienceAsia 34 (2008): **416–423**

doi: 10.2306/scienceasia1513-1874.2008.34.416

Development and identification of the ricefish *Oryzias* in Thailand

Apichart Termvidchakorn[a,*], **Wichian Magtoon**[b]

[a] Prachinburi Inland Fisheries Station. Inland Fisheries Research and Development Bureau, Department of Fisheries. Krabinburi, Prachinburi Province 20500, Thailand

[b] Department of Biology, Faculty of Science, Srinakharinwirot University, Sukhumvit 23, Bangkok 10110, Thailand

[*] Corresponding author, e-mail: inland_larvae@yahoo.com

Received 23 May 2008
Accepted 13 Nov 2008

ABSTRACT: Ricefish larval specimens were collected from breeding tanks and rearing ponds. The larvae were divided into 3 developmental stages i.e. larval, post-larval, and juvenile stages, and their morphometric and meristic characters, as well as chromatophore pigment patterns were studied. The chromatophore pigment pattern, number of myomeres, dorsal fin rays and anal fin rays were characteristics used for species identification. For *Oryzias* larvae, the chromatophore pigmentation on the gut, midline, and dorsal and ventral parts of the body were important characteristics. The caudal, dorsal, and anal fin rays were also the characteristics used in the case of identifying the juvenile fish. Using the above criteria, four species of ricefish, i.e. *O. javanicus*, *O. dancena*, *O. mekongensis*, and *O. minutillus*, were identified.

KEYWORDS: medaka, fish larvae, chromatophore pigment

INTRODUCTION

Ricefish of the genus *Oryzias* Jordan and Snyder[1] are small freshwater fish, consisting of 21 species found in standing waters in South, Southeast and East Asia[2–5]. There are four species of ricefish in Thailand[6]. The Thai medaka or dwarf medaka (*O. minutillus* Smith, 1945)[4], a small fish commonly found in ponds, ditches, and paddy fields in the central, north, northeast, and southern parts of Thailand[6–11], is also found in other places such as Xishuangbanna, Yunan, China and Rangoon, Myanmar[12]. The Mekong ricefish (*O. mekongensis* Uwa & Magtoon, 1986)[13], another small species, occurs in the northeastern part of Thailand and the Mekong river basin area. The Indian ricefish (*O. dancena* Hamilton, 1822)[14] is a brackish water and coastal area species occurring in India, Sri Lanka, Myanmar and Southern Thailand[10]. Another species occurring in similar habitat, the Javanese ricefish (*O. javanicus* Bleeker, 1848)[15] is found in Southern Thailand, Malaysia, Singapore, and Indonesia[8,10].

The typical characteristics of the ricefish include a laterally compressed body, depressed head, moderately large eyes, and 28–31 vertebrae. The dorsal fin base is from the 17th to 20–21st vertebrae spines, and the anal fin base is from the 10–11th to 19–20th vertebrae spines. The respective numbers of dorsal fin rays in *O. javanicus*, *O. dancena*, *O. minutillus*, and *O. mekongensis* are 6 (6–7), 7, 6 (5–7), and 6 (5–7), and the respective numbers of anal fin rays are 23 (20–23), 24 (22–24), 19 (18–21), and 14 (13–16)[6,10,12,16].

Iwamatsu[17] reported the developmental stages of the (Japanese) medaka (*O. latipes*) from unfertilized eggs, fertilized eggs to hatching stages of embryo, and hatching larvae to young stage. However, morphological information on *Oryzias* larvae and juveniles in Thailand has not yet been reported. In this study, we attempt to use the number of fin rays and the chromatophore pigment pattern to identify species of the larva.

MATERIALS AND METHODS

Ricefish specimens at different ages from hatching to juvenile stages were collected from breeding tanks. The larvae were divided into various stages, i.e. just-hatched larva (larva stage), post larva stage at which the pelvic fin just began to develop, and juvenile stages of which all the meristic characters were completely developed and scales began to develop. Five specimens of each stage were collected and preserved with 10% formalin solution, which was changed to 4% formalin solution after 2 weeks. All specimens in this study were deposited at the National Inland Fisheries Institute, Department of Fisheries, Ministry of Agriculture and Co-operatives, Thailand.

The identification and determination of each de-

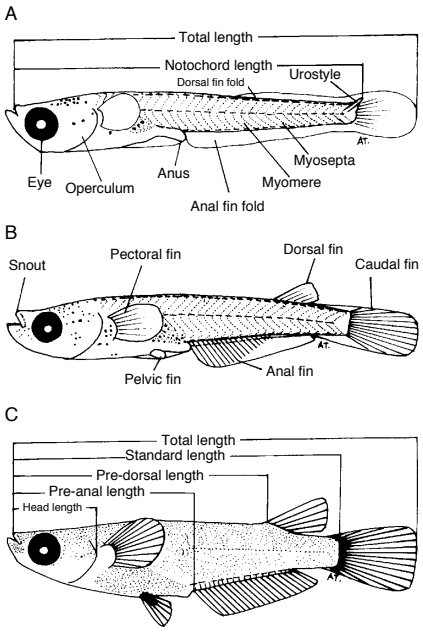

Fig. 1 Morphological characteristics of the ricefish larvae: (A) larval stage (B) post-larval stage (C) juvenile.

velopmental stage were carried out in the laboratory under a stereomicroscope and drawing was performed with a camera lucida. The differences among meristic characters and chromatophore pigment patterns were employed for the identification of the fish species. Fig. 1 shows the three stages of the ricefish larva.

RESULTS

The development of the ricefish larva

Ricefish of the genus *Oryzias* showed a long embryonic development during their incubation period. When the larva hatched from the egg shell, part of the body had already developed further than the yolk sac stage and showed larval period characteristics.

Javanese ricefish (*O. javanicus*)

A 4.10 mm larva that has just hatched (Fig. 2A) shows a large and laterally compressed body. The head is slightly depressed, the mouth open, maxilla and mandible developed, and a large eye occupies about one third of the head. The gut opening occurs at about one third of the way along the body from the head. The urostyle is flexed and the support bones (pleural and hyplural bones) developed. The pectoral, dorsal, and anal fanfolds are present. The caudal fin rays have started to develop. Chromatophore pigment is present on the head, operculum, midline, and dorsal

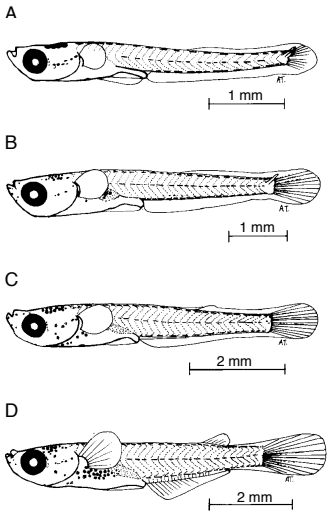

Fig. 2 Developmental stages of *O. javanicus*: (A) 4.06 mm just hatched larva (B) 5.37 mm 1-day-old larva (C) 6.47 mm 1-day-old larva (D) 7.75 mm 5-day-old larva.

and ventral parts of the body. A characteristic feature of a 5.37 mm 1-day-old larva (Fig. 2B) is the mouth part which is more practical. The support caudal bone and rays are developed. The chromatophore pigment has increased on the head, mouth, midline,

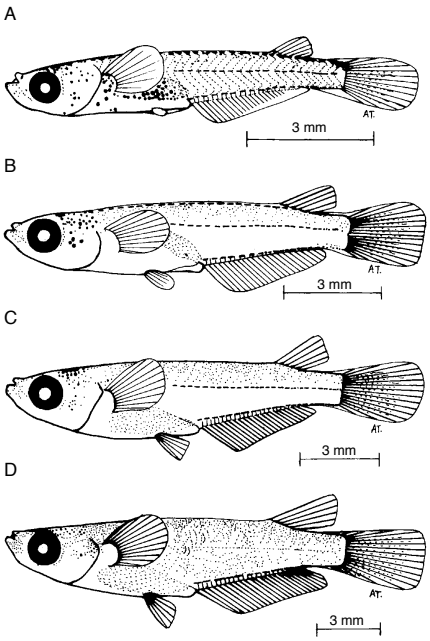

Fig. 3 Developmental stages of *O. javanicus*: (A) 9.67 mm 7-day-old larva (B) 12.94 mm 9-day-old larva (C) 15.88 mm 12-day-old larva (D) 19.67 mm 15-day-old juvenile.

and dorsal and ventral parts of the body and on the caudal ray. By day 3, the 6.47 mm 3-day-old larva (Fig. 2C) shows anal fins which have started to develop from the anterior part of the dorsal and anal fin folds. The chromatophore pigment has intensified on head, cheek, and anterior part of the gut. Dorsal, anal, and pectoral fin rays develop by day 5, when the larva has reached 7.75 mm (Fig. 2D), and after the fin folds have developed. The fin fold has also decreased in size. The chromatophore pigment has continued to spread on the head, gut, and caudal fin rays. By day 7, the 9.67 mm larva (Fig. 3A) shows the development of the dorsal, anal, and pectoral fin rays while the pelvic fin has started to form at the ventral part of the body in front of the anus. The chromatophore pigment has increased on the head, operculum, mandible, gut, dorsal part of the body, and caudal fin rays. A 12.94 mm 9-day-old larva (Fig. 3B) has developed a carpet of dorsal, anal, pectoral, and caudal fin rays while the pelvic fin is developing in size and number of rays. The chromatophore pigment is still increasing on the head, operculum, mandible, gut, and dorsal part of the body. A 15.88 mm 12-day-old larva (Fig. 3C) has its fin rays, and 6 dorsal rays, 23 anal rays, 10 pectoral rays, and 6 pelvic rays have developed completely. The chromatophore pigment has increased on the dorsal part of the body above the mid-body line. The juvenile ricefish have their meristic characters fully developed by day 15, and have reached 19.67 mm (Fig. 3D). The chromatophore pigment has increased on their head and body, and has started to appear on the caudal rays, while scales cover the body.

Indian ricefish (*O. dancena*)

The 1-day-old 5.00 mm *O. dancena* larva (Fig. 4A) shows a body that is laterally compressed, a slightly depressed head, a large eye, and an open and functional mouth. The gut opening occurs at about one third of the way along the body. The urostyle has flexed, and the supported caudal bone has developed. The pectoral fin has formed with its base anchored above the midline of the body. The caudal fin rays are developed. The dorsal and anal fin fold are present. Chromatophore pigment cover the dorsal part of head, operculum, anterior part of the gut, midline, and dorsal and ventral parts of the body. A 3-day-old larva has reached 6.64 mm (Fig. 4B). The dorsal and anal fins have developed from the anterior part of dorsal and anal fin folds. At this stage, the caudal fin rays have formed. Chromatophore pigment has increased on the head, operculum, maxilla, mandible,

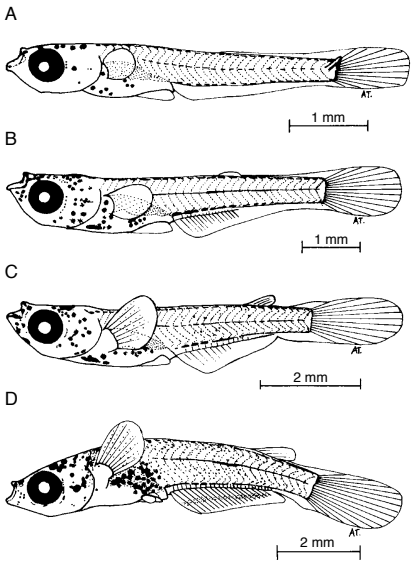

Fig. 4 Developmental stages of *O. dancena*: (A) 5.00 mm 1-day-old larva (B) 6.64 mm 3-day-old larva (C) 7.64 mm 5-day-old larva (D) 9.53 mm 7-day-old larva.

anterior part of the gut, midline, and dorsal and ventral parts of the body. When the larva has reached 7.64 mm at 5 days of age (Fig. 4C), the dorsal and anal fin folds have turned into dorsal and anal fins,

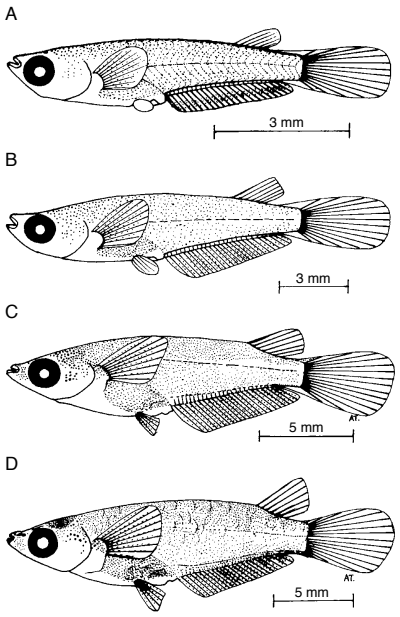

Fig. 5 Developmental stages of *O. dancena* (A) 13.07 mm 9-day-old larva (B) 16.58 mm 12-day-old larva (C) 20.48 mm 15-day-old larva (D) 24.35 mm 19-day-old juvenile.

respectively. The dorsal, anal, and pectoral fin rays have developed. Chromatophore pigmentation occurs on the pectoral fin and has increased on the head, operculum, mouth parts, gut, and on the body. A 9.53 mm 7-day larva (Fig. 4D) has an elongated shape. It shows the formation of the pelvic fin, which occurs on the ventral part of the body before the anus. The dorsal, anal, and pectoral fin rays are more developed. The chromatophore pigmentation has increased on the gut, dorsal, ventral, and pectoral parts of the body, and a longitudinal band occurs in the middle of the anal fin. After that, by 9 days, when the larva has reached 13.07 mm (Fig. 5A), the anal fin rays are completely developed, containing the basic number of 23 rays. However, the dorsal and pectoral fin rays are still developing. The pelvic fin has increased in size. At this stage, the chromatophore pigmentation has increased on the head, dorsal, and ventral part of the body. A 16.58 mm 12-day-old larva (Fig. 5B) has a more pointed head than before. The fin rays are completely developed as their basic number for the dorsal and pectoral fins is 7 and 10, respectively. Also, the size of pelvic fin has increased and its rays have started to develop. The chromatophore pigmentation has increased on the head, body, and pectoral and anal fins. By 15 days, the larva has reached 20.48 mm (Fig. 5C), the inferior mouth are clearly visible, and all fin rays are completely developed. The chromatophore pigmentation has increased on the head, body, and pelvic and anal fins. The complete development of the character occurs by day 19 when the larva has reached 24.35 mm (Fig. 5D). At this stage, the scales are still forming and the reproduction organs have not yet been developed.

Mekong ricefish (*O. mekongensis*)

A 4.95 mm 1-day-old Mekong ricefish larva (Fig. 6A) presents an elongated and laterally compressed body. The head is slightly depressed and the mouth has already been formed. The eye diameter spans more than one third of the head length. The urostyle has been flexed and the support caudal bone has been formed. The caudal fin has been developed from the fin fold at the caudal part. The pectoral, dorsal and anal fin fold are clearly present. The caudal rays, however, are still forming. The chromatophore pigmentation occurs on the head, snout, anterior part of the gut, midline, and dorsal and anal regions of the body, and also on the caudal ray. A 6.63 mm 1 day larva (Fig. 6B), on the other hand, shows dorsal and anal fins formed from the fin fold. The chromatophore pigmentation is increasing on head, gut, midline, and dorsal and ventral

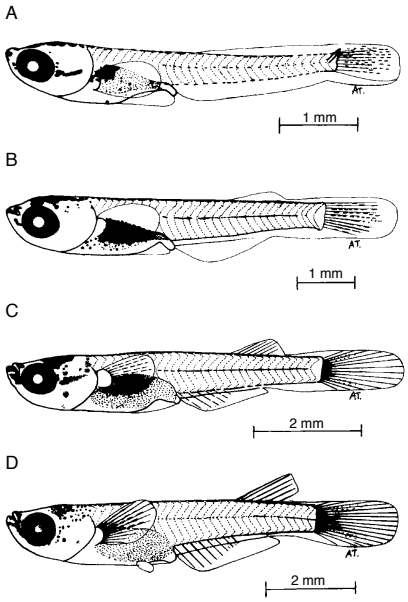

Fig. 6 Developmental stages of *O. mekongensis*: (A) 4.95 mm 1-day-old larva (B) 6.63 mm 3-day-old larva (C) 7.33 mm 5-day-old larva (D) 9.53 mm 7-day-old larva.

parts of the body. A 7.33 mm 5-day-old larva (Fig. 6C) has started to form the pectoral, dorsal, and anal rays. The chromatophore pigmentation has increased on the

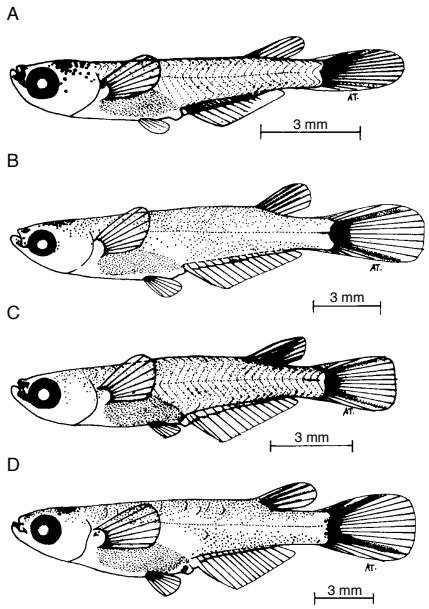

Fig. 7 Developmental stages of *O. mekongensis*: (A) 12.10 mm 9-day-old larva (B) 14.04 mm 12-day-old larva (C) 18.25 mm 15-day-old larva (D) 20.40 mm 19-day-old juvenile.

head, operculum, maxilla, mandible, and pectoral fin. The pigmentation pattern on the upper and larva rim of the caudal fin has changed from scattered points to stripes. By day 7, when the larva has reached 9.53 mm (Fig. 6D), the pelvic fin has started to form at the ventral part of body before the anus. The dorsal rays are completely developed whereas the pectoral and anal rays are still developing. The chromatophore pigmentation has increased on the head and mandible in front of the eye. The larva has reached 12.10 mm by day 9 (Fig. 7A). The pectoral fin rays have developed completely while the size of pelvic fin has increased and its rays are still forming. The chromatophore pigmentation has increased on the head and dorsal and ventral parts of the body. Complete development of all fin rays occurs by day 12, when the larva has reached 14.04 mm (Fig. 7B). At this stage, the fin ray numbers for the dorsal fin, anal fin, pectoral fin, and pelvic fin are 6, 14, 8, and 6, respectively. The chromatophore pigmentation has occurred on the dorsal fin and increased on head, caudal fin, and dorsal and ventral parts of the body. A 18.25 mm 15-day-old larva (Fig. 7C) shows an increase in chromatophore pigmentation on the body covering all myomeres. By this stage, the chromatophore pigmentation stripes on the caudal fin are clearly visible. When the larva reaches day 19, it is 20.40 mm (Fig. 7D). The meristic characters have developed completely. The scales are still developing at this stage and the larva has changed from post-larva to juvenile.

Thai medaka (*O. minutillus*)

A 5.28 mm 1-day-old Thai ricefish larva (Fig. 8A) shows an elongated and laterally compressed body and slightly depressed head. The superior mouth is small and the eye diameter covers about one third of the head length. The urostyle had already flexed and the caudal supported bone has been formed. The pectoral, dorsal, and anal fin folds are present. The caudal fin has developed from the fin fold, but the caudal part and the caudal rays are still forming. Chromatophore pigmentation is present on head, operculum, pectoral fin base, midline, and dorsal and ventral parts of the body as a small dash. By 3 days, the larva has reached 5.88 mm (Fig. 8B). The dorsal and anal fin folds have turned into dorsal and anal fins. The chromatophore pigmentation has increased on the head, gut, midline, and dorsal and ventral parts of the body. A 8.09 mm 5-day-old larva (Fig. 8C) has a small and pointed head. The pectoral and anal fin rays have started to develop, but not the dorsal ray. The chromatophore pigmentation has increased on the dorsal, anal, and

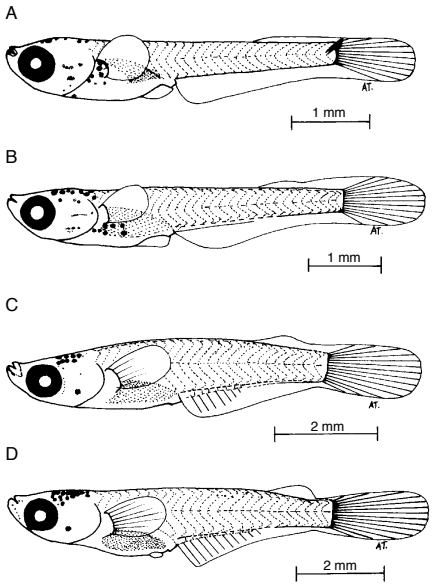

Fig. 8 Developmental stages of *O. minutillus*: (A) 5.28 mm 1-day-old larva (B) 5.88 mm 3-day-old larva (C) 8.09 mm 5-day-old larva (D) 9.57 mm 7-day-old larva.

midline areas of the body. By day 7, the larva has reached 9.57 mm (Fig. 8D). The shape of the dorsal fin is clearer than in the previous stage and the pelvic

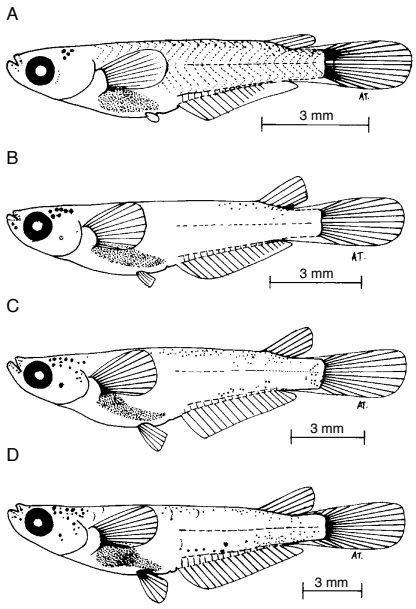

Fig. 9 Developmental stages of *O. minutillus*: (A) 11.13 mm 9-day-old larva (B) 12.82 mm 12-day-old larva (C) 16.15 mm 15-day-old larva (D) 19.86 mm 19-day-old juvenile.

Table 1 Key to identify the larval, post-larval, and juvenile stages of the *Oryzias* species that occur in Thailand.

Key to identify the larval stages of *Oryzias* (Fig. 10)

1a Chromatophore pigment present on caudal fin 2
1b Chromatophore pigment absent from caudal fin 3
2a Chromatophore pigment present on the base of the pectoral fin and preanal myomere ..... *O. javanicus*
2b Chromatophore pigment absent from base of pectoral fin and preanal myomere ..... *O. mekongensis*
3a Chromatophore pigment present on base of the pectoral fin and preanal myomere ..... *O. minutillus*
3b Chromatophore pigment absent on base of the pectoral fin and preanal myomere ..... *O. dancena*

Key to identify the post-larval stages of *Oryzias* (Fig. 11)

1a Chromatophore pigment present on caudal fin 2
1b Chromatophore pigment absent from caudal fin 3
2a Heavy pigment on gut and body ..... *O. javanicus*
2b Little pigment on gut and body ..... *O. mekongensis*
3a Heavy pigment on gut and body ..... *O. minutillus*
3b Little pigment on gut and body ..... *O. dancena*

Key to identify the juvenile stages of *Oryzias* (Fig. 12)

1a Chromatophore pigment present on caudal fin 2
1b Chromatophore pigment absent from caudal fin 3
2a Chromatophore pigment scattered on caudal fin and absent on dorsal fin; 23 anal fin rays ..... *O. javanicus*
2b Chromatophore pigment shows stripes on dorsal and ventral part of caudal fin and dorsal fin; 14 anal fin rays ..... *O. mekongensis*
3a Chromatophore pigment present on anal fin; 24 anal fin rays ..... *O. minutillus*
3b Chromatophore pigment absent on anal fin; 18 anal fin rays ..... *O. dancena*

fin is forming as a small double fin fold at the ventral part of the body in front of the anus. The pectoral and anal rays are still developing. The chromatophore pigmentation has increased on the head and mandible.

The 11.13 mm 9-day-old larva (Fig. 9A) has developed its fin rays. The size of the pelvic fin is still increasing while the fin fold between gut and anus is absent. The chromatophore pigmentation is increasing on the dorsal and lateral parts of the body. When the larva reaches 12.82 mm by day 12 (Fig. 9B), the pectoral and dorsal fin rays have completely developed. The anal fin rays are still developing and the pelvic fin has started to form. The chromatophore pigmentation occurs on the head, mandible, midline, and dorsal and lateral parts of the body. By day 15, the larva has reached 16.15 mm (Fig. 9C) and all fins have completely developed. The number of pectoral fins, dorsal fins, anal fins, and pectoral fins are 8, 6, 18, and 5, respectively. The chromatophore pigmentation occurs on the head, midline, and dorsal and ventral parts of the body. A 19.86 mm 19-day-old larva (Fig. 9D) has changed from post larva to juvenile as evidenced by the formation of scales. The chromatophore pigmentation has increased on head, dorsal, and ventral part of the body. The scales have formed on the skin where the pigment is dense.

Key to identify genus *Oryzias*

The larva of the genus *Oryzias* was identified to species by using the difference of morphomatics and chromatophore pigment pattern in each stage (Table 1, Figs. 10–12).

DISCUSSION

The larval stage of *Oryzias* had a well developed head, eye, mouth, digestive tract, and caudal part. When it hatches, the larva passed a prelarval stage, and the yolk was completely absorbed. At the caudal part, the flexion of the urostyle could be observed while the

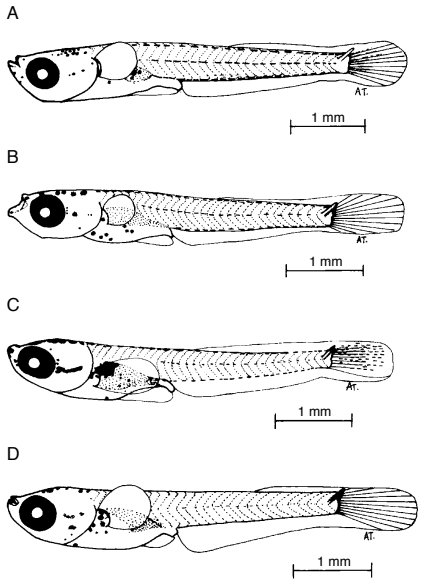

Fig. 10 Meristic characteristics and chromatophore pigment patterns among different species of larval stage ricefish: (A) 5.37 mm *O. javanicus* (B) 5.00 mm *O. dancena* (C) 4.95 mm *O. mekongensi* (D) 5.28 mm *O. minutillus*.

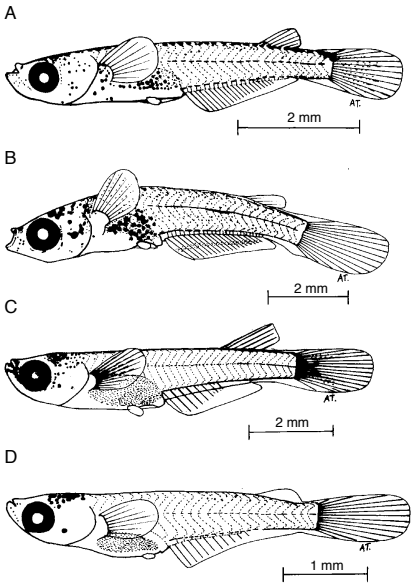

Fig. 11 Meristic characteristics and chromatophore pigment patterns among different species of post-larval stage ricefish: (A) 9.67 mm *O. javanicus* (B) 9.53 mm *O. dancena* (C) 8.61 mm *O. mekongensis* (D) 9.57 mm *O. minutillus*.

supported bone formed. The chromatophore pigmentation appeared on the head, midline and dorsal and ventral of the body. The chromatophore pigmentation was found to increase on the head, body and ray in each stage, which was in agreement with the study reported by Iwamatsu[17] on the normal developmental stage of the medaka (*O. latipes*). The identification was simply based on the meristic characteristics, i.e., number of dorsal, anal, pectoral, and pelvic rays together with the chromatophore pigment patterns on the head, body, and rays.

CONCLUSIONS

Studies of the developmental stages and larva identification of the genus *Oryzias* in Thailand were carried out using specimens collected from breeding tanks or rearing ponds. The larvae were classed as a larva stage because the *Oryzias* larvae did not have a yolk sac stage. The post-larval stage started when the pelvic fin was formed and finished when scales started to develop. The juvenile stage began when the scales started to develop. The just-hatched larva were at the larva stage which showed the development of head, eye, mouth, digestive tract, caudal fin and chromatophore pigmentation on their head and body. The genus *Oryzias* had a short period (about 19 days) for complete development from larva to juvenile.

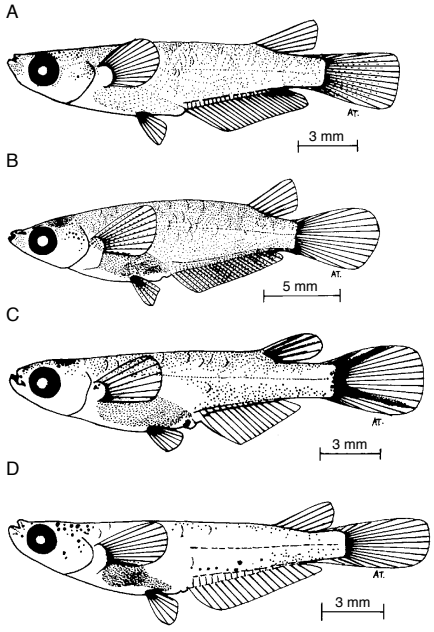

Fig. 12 Meristic characteristics and chromatophore pigment patterns among different species of juvenile stage ricefish: (A) 19.67 mm *O. javanicus* (B) 24.35 mm *O. dancena* (C) 20.40 mm *O. mekongensis* (D) 19.86 mm *O. minutillus*.

Acknowledgements: We wish to express our sincere gratitude to Prof. Yasuhiko Taki, Tokyo University for his helpful suggestions. Thanks are also due to Pingnaphra Krichumphon, Department of Biology, Srinakharinwirot University for helping in collecting specimens of *O. dancena*. We thank Siriwan Suksri, Inland Fisheries Research and Department Bureau, Department of Fisheries, Thailand for helping with the deposition of some *Oryzias* specimens.

REFERENCES

1. Jordan DS, Snyder JO (1906) A review of the Poeciliidae or killifishes of Japan. *Proc US Natl Mus* **31**, 161–246.
2. Labhart P (1978) Die Arten der Gattung *Oryzias* Jordan and Snyder, 1978. *Deutsche Killifish Gemeinschaft J* **10**, 53–8.
3. Nelson JS (2006) *Fishes of the World*, 4th edn, John Wiley & Sons, New York.
4. Smith HM (1945) The fresh-water fishes of Siam, or Thailand. *Bull USA Nat Mus* **188**, 1–622.
5. Yamamoto T (1975) *Medaka (Killifish) Biology and Strains*, Keigaku Publising Co, Tokyo.
6. Magtoon W, Takata K, Uwa H, Taki Y (1993) Distribution and chromosomal diversity of Thai-medaka, *Oryzias minutillus* in Thailand. *Resp Suwa Hydrobiol* **9**, 137–47.
7. Magtoon W, Uwa H (1985) Karyotype evolution and relationship of a small ricefishes, *Oryzias minutillus*, from Thailand. *Proc Jpn Acad* **61B**, 157–60.
8. Magtoon W, Uwa H (1988) First report of *Oryzias melastigma* (Cyprinodontidae: Pisces) in Thailand. 26th Kasetsart University Scientific Symposium, pp 229–33.
9. Magtoon W, Nadee N, Higastitani T, Takata K, Uwa H (1992) Karyotype evolution and geographical distribution of the Thai medaka, *O. minutillus*, in Thailand. *J Fish Biol* **41**, 489–97.
10. Roberts TR (1998) Systematic observations on tropical Asian medakas or ricefishes of the genus *Oryzias*, with descriptions of four new species. *Jpn J Ichthyol* **45**, 213–24.
11. Takata K, Hosshino M, Magtoon W, Nadee N, Uwa H (1993) Genetic differentiation of *Oryzias minutillus* from Thailand. *Jpn J Ichthyol* **4**, 319–27.
12. Uwa H, Wang RF, Chen YR (1988) Karyotypes and geographical distribution of ricefishes from Yunnan, southwestern China. *Jpn J Ichthyol* **35**, 332–40.
13. Uwa H, Magtoon W (1986) Description and karyotype of a new ricefish, *O. mekongensis*, from Thailand. *Copeia* **2**, 473–7.
14. Hamilton F (1822) *An Account of the Fishes Found in the River Ganges and its Branches*, Archibald Constable & Co, London.
15. Bleeker P (1854) Ichthyologische waarnemingen gedaan opverchillende reizen in de residentie Banten. *Nat Tijdschr Ned Ind* **7**, 309–26.
16. Uwa H, Parenti L (1988) Morphometric and meristric variation in ricefishes, genus *Oryzias*: a comparison with cytogenetic data. *Jpn J Ichthyol* **35**, 159–66.
17. Iwamatsu T (1994) Stages of normal development in medaka *Oryzias latipes*. *Zool Sci* **11**, 825–39.