Developmental stages of endemic bilih fish larvae (*Mystacoleucus padangensis*) from Singkarak Lake, West Sumatra, Indonesia

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Abstract. Larvae developmental stages was studied in the endemic bilih fish (*Mystacoleucus padangensis* Bleeker, 1852) from Singkarak Lake, West Sumatera, Indonesia. We obtained the study material by artificial insemination. The fertilized eggs were incubated in dechlorinated tap water under temperature 26-28^{o}C. Ten to twenty larvae were collected everyday on the first week and then weekly till juvenile. Larvae development stages was assigned using dissecting microscope, determined and named by body total length and morphological features. The result showed that the early juvenile reached at 11.1 mm (5 week post hatching, wph) and scale juvenile at 35.36 mm total length (16 wph) through stages are total length 2.44 mm (pectoral fin bud), 2.85 mm (gill vesicle), 3.24 mm (caudal fin development biginning), 3.60 mm (pectoral fin ray), 3.75 mm (jaw has formed completely), 3.9 mm (caudal fin primordia rounded), 4.12 mm (beginning of notochord flexi), 4.69 mm (dorsal and anal fin development), 6.18 mm (primordia hypural bone), 11.1 mm (skin finfold has disappeared), 14.92 mm (caudal fin completely developed), 16.85 mm (primordia scale developed), 18.32 mm (scale pattern pigmented area has reached lateral line).
Key Words: total body length, weight, morphological characters, metamorphic, ontogeny.

Introduction. Singkarak is the largerst lake in West Sumatera, Indonesia (Figure 1). The size of the lake is about 13,665 Ha, with 160 m in deep, 21 km long, and 16 km wide. There are three major rivers drain into the lake; Sumpur, Paningahan and Sumani River. Many fishes live in this lake, one of them is the endemic fish *Mystacoleucus padangensis* (Bleeker, 1852) (Webert & Beaufort 1916; Kottelat et al 1993), a member of Cyprinidae family, named by local people as bilih fish. It has the highest socio-economic value among fishes living in the Singkarak lake. Fishermen do fishing throughtout the year for consumption and sold in fresh form or in processed form to other region (Syandri 1996a). So fisheries secure family's food supply and provides cash income. Such dual purpose is facing fish exploited and caught in big number.

Bilih fish exploitation level has reached 77.88%, while maximum limit of exploitation is 60% only. As result of over maximum limit exploitation, fish production declined, which is indicated by decreased catches and size become smaller (Purnomo et al 2006). Syandri et al (2011) stated that the decrease of fish production is mainly caused by over exploitation and the use of non selective fishing gears, such small mesh size (1/2 inch), lead small and immature fish also caught. This condition certainly causes the fish do not have chance to breed (Arsil 1999; Yuerlita & Perret 2010). Consequently the fish population significantly decreased and is endangered (Yuerlita & Perret 2010).

In order to maintain the bilih fish sustainable, the fishery resources management needs to be based on local wisdom. One of management priorities in fish population is through hatchery and restocking (Syandri et al 2011). The ecology and reproduction of bilih fish primarily on mature fish has been reported in the past (Kartamihardja 1996; Syandri 1996b; Arsil 1999; Purnomo et al 2006; Yuerlita & Perret 2010; Syandri et al 2013) but there is no imformation about the stages of larval development. As part of

study on postembryonic and gonad development, we present postembryonic (larvae) developmental stages based on body total length and morphological features as a basic information needed in fish culture and conservation.

Figure 1. Map of Singkarak Lake(left) and spawner capturing area (right).

Material and Method

Spawner collection and fertilization. To obtain eggs and sperm wild living spawner were cautgh directly at the meeting point of inlet Bahiang River and the Singkarak Lake (located 00°38'25.2" S, 100°31'20.1" E), Nagari (name of traditional village) Muara Pingai (Figure 1) West Sumatra, Indonesia. The eggs and sperm were hand-striped and collected from 2-3 large spawner and taken into 15 cm watch glass diameter, and then fertilized by dry methods. After 5 minutes, fertilization medium (4% NaCl and 3% urea) was added (Syandri 1999) and kept until 5 minutes, then washed two times to realease unused sperm. Then the fertilized eggs were brought into the Laboratory of Structure and Animal Development, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University in aerated container.

Rearing embryo and larvae. Fertilized egg were transfered to semicirculation system aquarium (50 x 30 x 30 cm), 20 cm depth aerated tap water and debit 200-300 mL min^{-1}, and kept under temperature 26-28°C as previously described by Syandri (1999). From 3 day post hatching (dph) (yolk suplay is exhausted and mouth has opened) till 20 dph the larvae were fed with commercial diets (Euguchi BP) according to Rahmat (2011), starting from 5 week post hatching (wph) feed gradually replaced with commercial diets. Feed was given 4 times a day according to Syandri & Usman (1997). Water is replaced every 48 hours. The first water replacement was done 2 dph with maximum volume 25% and then gradually increased to 50% up to one month.

Observation. Ten to 20 larvae were killed periodically every day in the first week, then weekly till juvenil. Larvae total length (the distance from the tip of snout to posterior end of the caudal fin or fin fold) less than 1 cm were measured and photographed using Optic Lab camera, for larvae body total length 1 cm or more used digital caliper to an accuracy of 0.01 mm, weighed using analytical balance (Metler). Development stages were determined and named based on fish larvae body total length, and morphological features readily identified by observation of larvae under stereomicroscope (Parichy et al 2009) were considered to be achieved when at least 50% of specimens attained relevant characters. After obsevation larvae were fixed in 10% paraformaldehide.

Results

Hatching stage (0 dph). Larva hatched 19 hours after fertilization at 2.02 mm in body length. Bilih fish larvae body surface were devoid of melanophore and transparant. The head face down, in contact with yolk. The eyes and lens have not developed completely and are not pigmented yet. The mouth and jaw apparatus are not formed. The otolith of the inner ear can be seen clearly on both sides of the head. The fins and gill have not yet developed. The median fin fold relatively same depth. Yolksac oval in shape (Figure 2A). Yolksac oval in shape. Larvae settled at the bottom of aquarium.

The new hatched larvae appereance differ from mature fish in both outer and inner structure. Larvae will undergo gradual changes in dimensions of body parts and appearance, includingthe loss of some larval features, remodeling, the acquisition of new adult features (Urho 2002). Morphological changes were observed daily on the first week, then weekly up to 16 weeks (112 days).

2.44 mm total length stage (1 dph). The eye pigmented black but not distributed completely yet. The embryo shows a distinct reduction in the size of yolk sac which has now become almost tubular due to its greater absorbtion anteriorly. Mouth and anus not open yet. Pectoral bud has formed posterio-lateral of eye on both sides of the head (Figure 2B). Other morphological characters still the same with hatching stage (2.02 mm total length). This development stage is called pectoral bud stage.

2.85 mm total length stage (2 dph). Larvae mouth not open yet, the eye pigment has spread completely and lens clearly seen. Gill vesicle was observed anterior to pectoral fin (Figure 2C). Pectoral fin larger than previously. Larvae weight reached 0.243+0.11 mg. This stage is called gill vesicle stage.

3.22 mm length stage (3 dph). Larvae weight 0.252 mg. The mouth has opened, but jaws are not yet shaped. The gill vesicle has opened also with the rudiments of the gill arch can be seen clearly. The highly pigmented eyes bulging like frog eyes. Pectoral fins reached maximal body total length but have no rays yet. The caudal finfold form change due the edge broader distinctly as result of caudal fin growth. The air bladder consists of one oval tube (Figure 2D). This stage is called caudal fin development beginning.

3.60 mm total length stage (4 dph). Larvae form not much differ from previously larvae form (average length 3.22 mm, 3 dph). Pectoral fin has two rays. Pigment can be observed in gall bladder and at ventral area between two pectoral fins. This stage is called pectoral fin rays (Figure 2E).

3.75 mm total length stage (5 dph). Upper and lower jaw have formed completely. Pectoral fin has 8-9 rays, has no nodes yet. Gut still straight tube shaped. Larvae still transparant, and pigmented air bladder clearly seen. At dorsal body surface pigment dots appear. Larvae weight reach 0.28 mg. This stages is called jaw complete (Figure 2F).

3.90 mm total length stage (6 dph). Rays on pectoral fin are completely shaped, but nodes not appeared yet. Primordia caudal fin rounded in form, has no rays and nodes yet, liver and gut bladder clearly seen. Larvae weight reach 0.30 mg. This stage is called caudal fin primordia rounded (Figure 3G).

4.12 mm total length stage (1 wph). Morphologycally not differerent from fish larvae average total length 3.90 mm (6 dph). The notochord posterior end begins flexion upward. Caudal fin has 2-3 rays. Larvae weight 0.343 mg. This stage is called the flexion of the notochord (Figure 3H).

4.69 mm total length stage (2 wph). Average larvae weight 0.6 mg. Primordia dorsal fin have developed. Flexi in the caudal end of the notochord (urostyle) reached $\pm 70^{o}$. Primordia hypural plate at the bottom of caudal fin seen clearly. Caudal fin rays have developed completely and the fin has one node on every ray and its form change began.

It has two pigment dot lines that is one along primordia lateral line area and other on ventro lateral body. This stage is called dorsal development stage (Figure 3I).

6.18 mm total length stage (3 wph). Average weight 1,03 mg. The eye has developed into sinking eye as in mature fish. Pigment dot on body surface more than on 2 wph larvae, it has one pigment dot line along lateral line and one other at ventro-lateral body, on head and caudal fin. Hypural bone has shaped. Pectoral ray nodes has formed. It has 6 rayed dorsal fin, caudal fin has 1-2 ray nodes, caudal fin groove deeper. The median fin fold dorsally between the dorsal and caudal fins and beween the caudal and anal fin ventrally still exist. Anal fin and pelvic fins have no rays yet. This stage is called hypural bone shaped (Figure 3J).

9.74 mm total length stage (4 wph). Morphologycally like larvae total length 6.18 mm (3 wph), the difference is on rays and nodes number. Dorsal fin has 7-9 rays. Caudal fin incised deeply and full ray, has 4-5 ray nodes. Anal fin has 2-3 ray nodes while pelvic fin nodes not appeared yet. Average weight 3.13 mg. This stage is called caudal fin full ray (Figure 3K).

11.11 mm total length stage (5 wph). External feature does not differ from previous average total length of larvae 9.74 mm (4 wph). Skin finfold has disappeared completely. Larvae still seen transparant. The first ray nodes appeared on dorsal and anal fins, 3-4 ray nodes on caudal fin. Average weight 6.54 mg, larvae has become early juvenile stage (Figure 3L).

13.35 mm total length stage (6 wph). Larvae had the same morphology as average body total length of larvae 11.1 mm (5 wph). It differs with earlier larvae on the nodes numbers. Dorsal fin has 2-3 ray nodes, caudal fin has 4-5 ray nodes, anal fin has 2 nodes, while pelvic fin rays have no nodes yet. Air bladder seen bigger than before and larvae average weight 10.4 mg (Figure 3M).

14.92 mm total length stage (7 wph). Larvae weight 29,12 mg. Caudal fin attaining full complement, has 7-8 rays nodes, dorsal fin has 5 ray nodes, while anal fin has 4 ray nodes. Pelvic fin and fectoral fin have 4 ray nodes each. The eyes developed completely. One-two pigmented lines along latero-dorsal and ventral body. This stage called caudal fin full complement (Figure 3N).

16.85 mm total length stage (8 wph). Pigment primarily found at dorsal body, at the basal dorsal fin, buccal area and a little at ventro-lateral body. Pigment at dorsal body has pattern scale like, it indicates the begining of development of scale primordium. This is called the beginning scale development stage. Pectoral fin has 5 ray nodes, dorsal fin has 5 ray nodes, caudal fin has 8 ray nodes, anal fin has 3 ray nodes and pelvic fin has 5 ray nodes (Figure 4O). Larvae average weight 44,19 mg.

18.32 mm total length stages (9 wph), 19.42 mm (10 wph), 21.15 mm (11 wph), 26.21 mm (12 wph), 27.48 mm (13 wph), 29.55 mm (14 wph), 36.08 mm (15 wph). Morphology of larvae during these stages is almost the same. The differences were found on the spread of pigment on body surface and ray node numbers. Overall they are called scale development phases.

At larvae 18.32 cm total length (9 wph), scale pigmented pattern area has reached lateral line. All of the fins have ray nodes, pectoral fin has 9 ray nodes, dorsal fin has 5 ray nodes, caudal fin has 10 rays nodes, 5 rays nodes of anal and pelvic fin. Larvae weight reached 76.9 mg (Figure 4P).

At the larvae 19.42 mm total length (10 wph), average larvae weight 97.7 mg, pigment spread in the head area and on dorsal body surface reached 2-3 lines under lateral line, darker than larvae 18.32 mm (9 wph). Pectoral fin has 8 ray nodes, dorsal fin has 6 ray nodes, caudal fin has 10 ray nodes, pelvic fin has 6 rays nodes and anal fin ray has 5 nodes (Figure 4Q).

At the larvae 21.15 mm total length (11 wph) pigment has reached 3-5 lines over lateral line. Caudal fin has 13 ray nodes, dorsal fin has 7 ray nodes and anal fin has 6 ray nodes, pelvic fin has 6 ray nodes and pectoral fin has 8 ray nodes, average larvae weight 105.4 mg (Figure 4R).

At the average larvae 26.21 mm body lenght (12 wph), pectoral fin has 9 ray nodes, dorsal fin has 8 ray nodes, caudal fin has 13 ray nodes, anal fin has 7 ray nodes, pelvic fin has 9 ray nodes. Larvae weight 197.5 mg. Pigment more darker and the first hard rays of dorsal fin have pigmented. Primordia scale become more clearer, average larvae weight 181.9 mg (Figure 4S).

At larvae 27.48 mm body lenght (13 wph) found caudal fin has 15 ray nodes, anal fin has 6-7 ray nodes, dorsal fin has 8 ray nodes, pelvic fin has 10 ray nodes and pectoral fin has 10 ray nodes. Larvae reached 208.53 mg average weight (Figure 4T).

While at larvae 29.55 mm total length (14 wph) pectoral pin has 10 ray nodes, caudal fin has 18 ray nodes, anal fin has 8 ray nodes, dorsal fin has 10 ray nodes, pelvic fin has 10 ray nodes. At the ventro-lateral body pigment has change white iridofor (silvery colour). Larvae reached 321.53 mg average weight (Figure 4U).

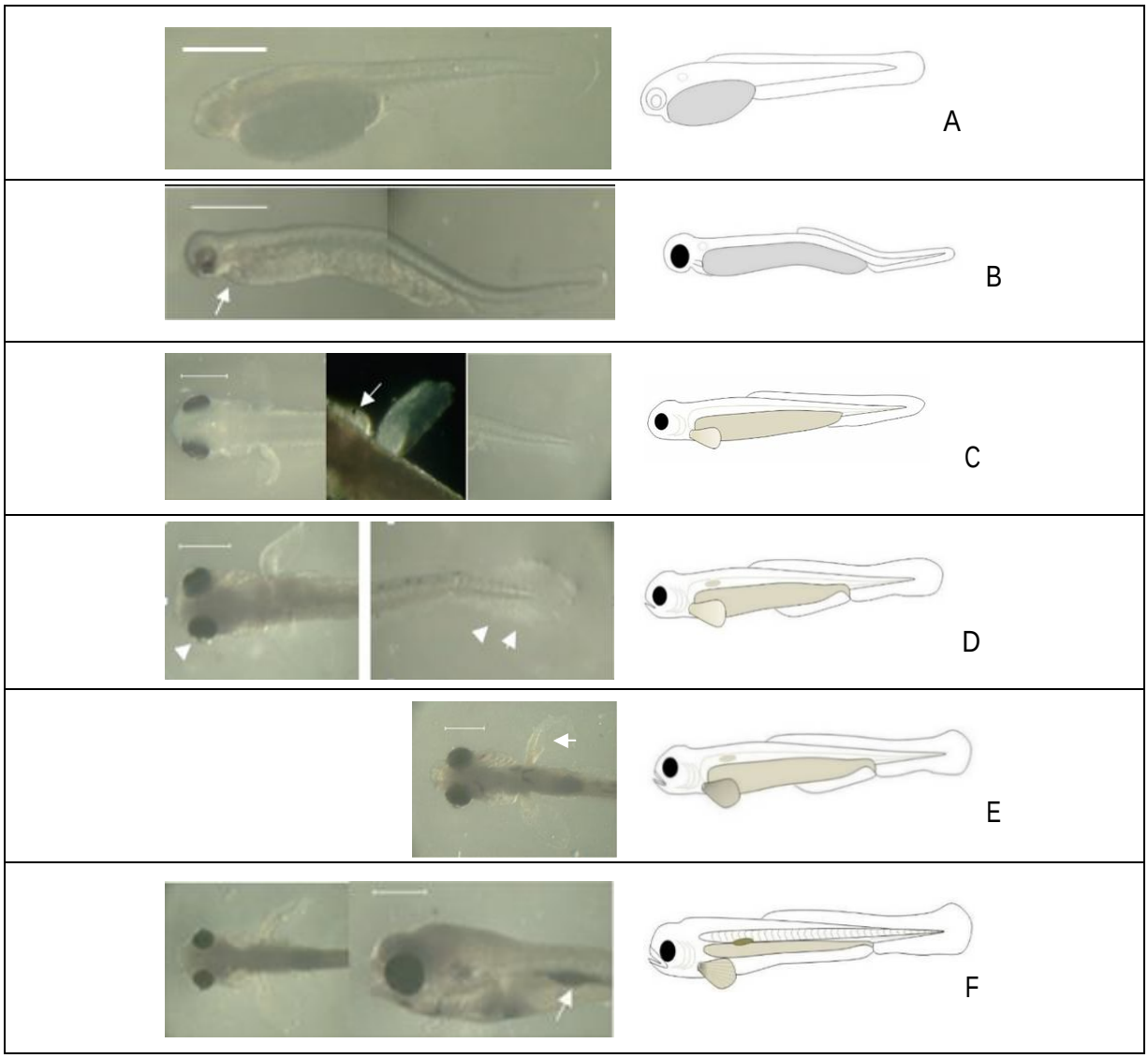

Figure 2. Larvae development of *Mystacolecous padangensis* at different lengths. Scale bar (white): 400 µm. A. 2.02 mm total body length, the hatching larvae (0 dph); B. 2.44 mm total length (1 dph), arrow: pectoral fin vesicle; C – 2.85 mm total length (2 dph), arrow: gill vesicle; D – 3.22 mm total length (3 dph), digestive tract opened; E – 3.60 mm total length (4 dph); F – 3.75 mm total engths (5 dph), arrow: swimblader.

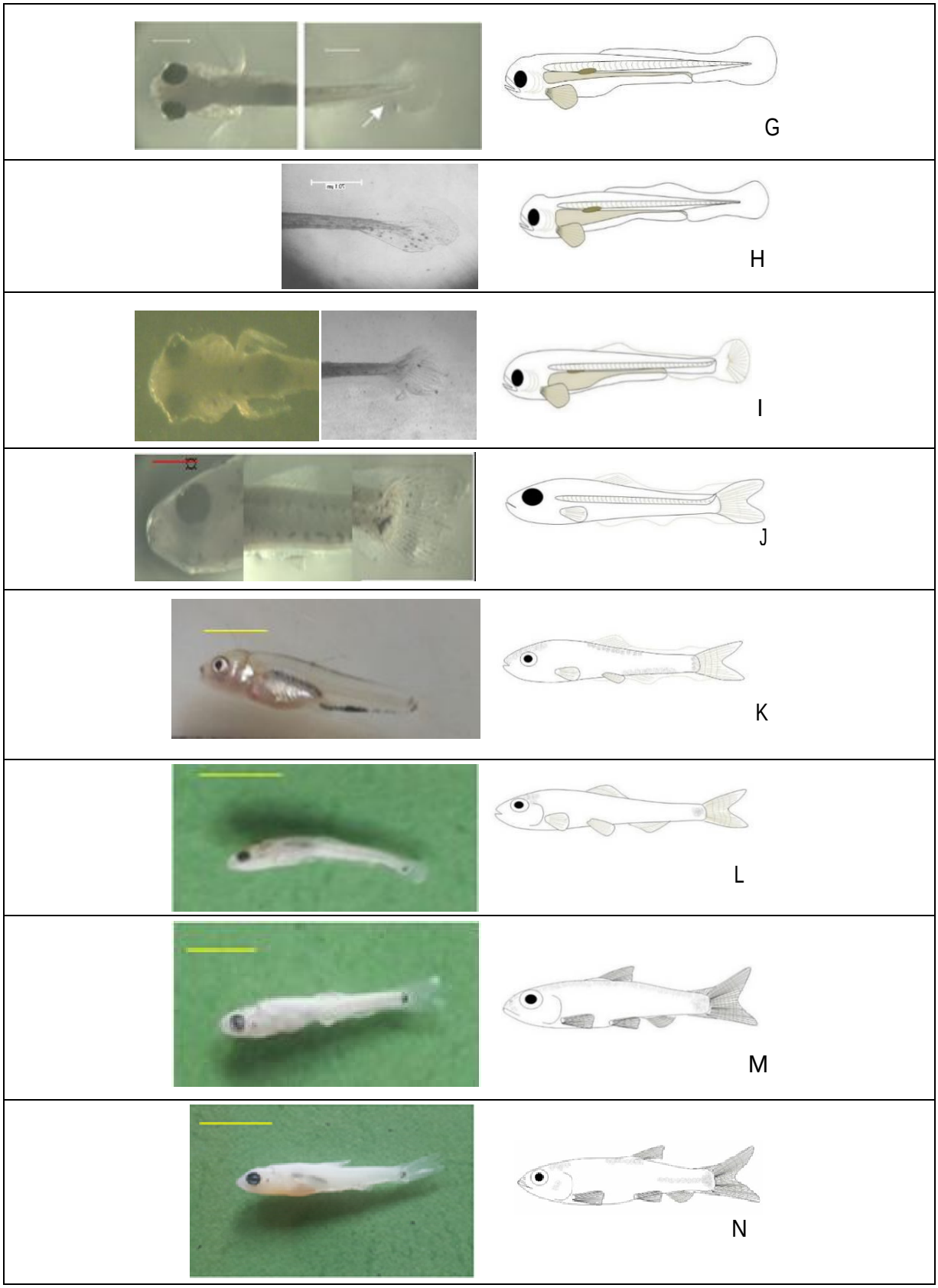

Figure 3. Development of *Mystacoleucus padangensis* at different lengths. Scale bar: white - 400 µm, red - 100 µm, yellow - 3 mm. G – 3.90 mm total length (6 dph), arrow: primordium caudal fin; H – 4.12 mm total length (1 wph); I – 4.69 mm total length (2 wph); J – 6.18 mm total length (3 wph); K – 9.74 mm total length (4 wph), single arrow - cornea, and double arrow - primordium caudal fin; L – 11.11 mm total length (5 wph); M – 13.35 mm total length (6 wph); N – 14.92 mm total length (7 wph).

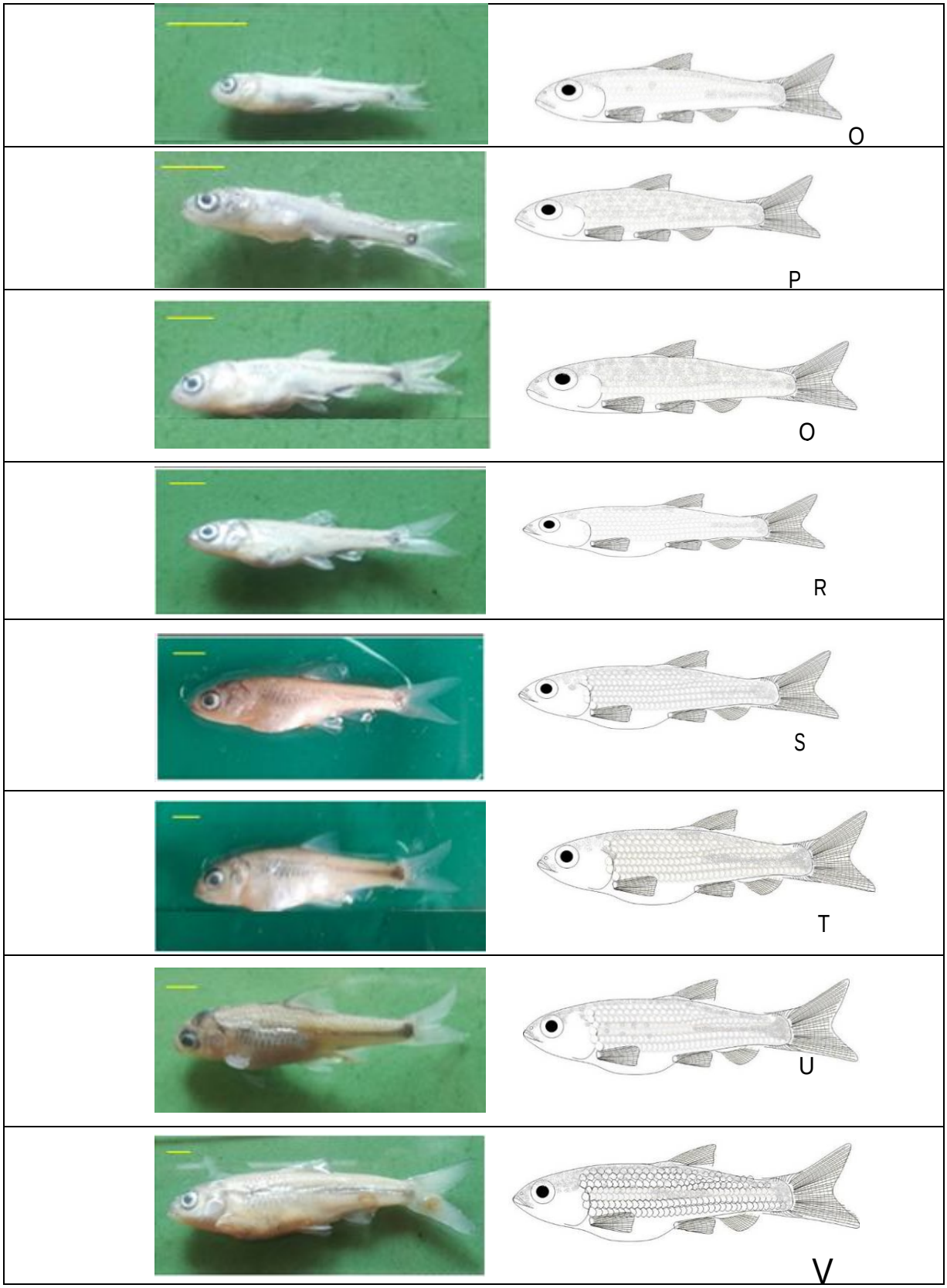

Figure 4. Development of *Mystacolecous padangensis* at different lengths. Scale bar: yellow – 3 mm. O – 16.85 mm total length (8 wph); P – 18.32 mm total length (9 wph); Q – 19.42 mm total length (10 wph); R - body total length 21.15 mm (11 wph); S - body total length 26.21 mm (12 wph); T – 27.48 mm body total length (13 wph); U – 29.55 mm body total length (14 wph); V – 39.16 mm body total length (16 wph).

At larvae 36.08 mm body length (15 wph), average larvae weight is 579.7 mg. Pectoral fin has 10 ray nodes, caudal fin has 18 ray nodes. Dorsal fin has 9-10 ray nodes, anal fin has 8 ray nodes and pelvic fin has 10 ray nodes. Scale primordia clearly visible under the skin epidermis and this stage call scale primordia.

39.16 mm total length stage (16 wph). The larvae weight 579.7 mg. The scale has penetrated skin epidermis and covered most of body surface. Ventro-lateral body area has silvery colour. At this stage fish larvae has become scaled juvenile, because morphologycal characters of mature fish completely formed (Figure 4V).

Discussion. After having observation on larvae morphology, we can conclude that larvae undergo gradual changes in external appearance. Significantly changes happened in the first few days of the larvae life. Organs that are formed during metamorphoses were the mouth, fins, gill, pigment, and the last formed were scales. Several studies have been made by various researchers on other carps development: *Carassius auratus* (Battle 1940), *Barbus anoplus* (Cambray 1983), *Barbus trevelyani* (Cambray 1985), *Carassius carassius* (Laurila et al 1987; Laurila & Holopainen 1990), *Cyprinus carpio* (Osse & Van den Boogaart 1995; Nica et al 2012), *Chela dadiburjori* (Sado & Kimura 2005a), *Tanichthys albonubes* (Sado & Kimura 2005b), *Puntius conchonius* (Bhattacharya et al 2005), *Barbodes balleroides* (Damrizal 2006), *Barbus luteus* (Al Hazzaa & Hussein 2007), *Barbus grypus* (Sahinoz et al 2007), *Danio rerio* (Parichy et al 2009), *Barbus sharpeyi* (Mukhaysin & Jawad 2012), *Leuciscus cephalus* (Kupren et al 2015).

At hatching bilih larvae, mouth and digestive tract are not developed yet. It also happens in some other Cyprinidae larvae such as *B. trevelyani* (Cambray 1985), *C. carassius* (Laurila & Holopainen 1990), *C. carpio* (Nica et al 2012), *B. sharpeyi* (Mukhaysin & Jawad 2012), but in *B. anoplus* mouth is incomplete and the gut is well developed (Cambray 1983). In few days early life larvae used yolk that was available (endogenus). Mouth opened at 3.22 mm length stage (3 dph), upper jaw, lower jaw and teeth have not shaped. Mouth and jaws shaped completely at 3.75 cm (5 dph) total length. At this moment, the yolk is absorbed completely and larvae has got outside food completely (exogenous sources). It was slightly different from *T. albonubes* where mouth opened at 3.4 mm body length (Sado & Kimura 2005b), in *C. dadiburjori* 3.5 mm body length (Sado & Kimura 2005a), in *D. rerio* at 4.3 mm (Parichy et al 2009), *B. sharpeyi* at 4.7-5.5 mm (Mukhaysin & Jawad 2012), *Candidia barbata* at 7.6 mm (Sado & Kimura 2002). The timing of the first exogenous feeding appears to depend on the quality and quantity of yolk available to the young during endogenous feeding (Heyer et al 2001).

Pectoral fin bud is the first primordium fin appearing in bilih fish. Caudal fin is shaped after pectoral fin, followed by dorsal fin, anal fin, and pelvic fins. All the fins are completely formed at 11.1 mm length (5 wph). On *D. rerio* the caudal fin is the first developed and followed by anal fin, dorsal fin, pectoral and the last pelvic fin (Parichy et al 2009) and *C. carasius* (Battle 1940) and *P. conchonius* pectoral fin is well developed before hatching (Bhattacharya et al 2005). The bilih fish pectoral fin primordium appearing at 2.44 mm length (1 dph), fin ray at 3.60 mm length (4 dph), completely growth at 3.90 mm length (6 dph), and ray nodes formed at 3 wph, close resemble to *C. dadiborjuri* primordium pectoral fin appearing at 2.9 mm total length (Sado & Kimura 2005a), and for *D. rerio* at 3.4 mm length (Parichy et al 2009). The bilih fish caudal fin primordium appearing at 3.22 mm length (3 dph), rounded at 3.90 mm and attaining full complement at 14.92 mm total length (7 wph), faster than *C. dadiburjori* caudal fin anlagen appearing at 4.9 mm but slower attaining full complement at 5.9 mm body length and caudal fin rounded immediately after hatching (Sado & Kimura 2005a), *D. rerio* caudal fin anlagen appearing at 4.3 mm standard length, rounded at 5.0 mm (Parichy et al 2009). Dorsal fin primordium appearing at 4.69 mm length (2 wph), attaining full compplement at slightly earlier than in *C. dadiburjori* dorsal fin at 5.8 mm body total length and attaining full complement at 6.6 mm body length (Sado & Kimura 2005a). Anal and pelvic fin primordium have formed at 6.18 mm length (3 wph), while in *D. rerio* are apparent at 7.5 mm standard length, and achieved maximum fin number at 10-12 mm standard length (Parichy et al 2009), in *C. dadiburjori* anal fin anlagen

appearing at 5.6 mm, attaining full complement at 7.1 mm body length (Sado & Kimura 2005a). Finfold disappears completely at 11.1 mm. Nearly similar cases are found in other developing cyprinid such as *C. carpio*, finfold disappeared around 10 mm length (Osse & Van den Boogaart 1995) *T. albonubes* at 6.9-7.9 mm (Sado & Kimura 2005b), *B. sharpeyi* (Mukhaysin & Jawad 2012) and, in *C. barbatus* aggregate number of all pin rays were completed at 12 mm body length (Sado & Kimura 2002). Loss of skin folds and the fins formed completely indicate that the larvae has been early juvenile although some larvae character still remain such as, transparent body and scale have not formed (Balon 1975; Urho 2002).

Primordia eyes seen clearly at hatching, but not completely developed. 2.44 mm length (1 dph) eye pigment has appeared. At 2.85 mm total length (2 dph) pigment has distributed completely and lens can be clearly seen. This also happens in *B. trevelyani* (Cambray 1985) and *P. conchonius* (Bhattacharya et al 2005). Early eye pigmentation has been reported in other cyprinidae such as *C. auratus* (Battle 1940), *B. luteus* (Al Hazzaa & Hussein 2007); at hatching *B. anoplus* eyes are pigmented and the pigment is distributed uniformly (Cambray 1983).

A gill vesicle develops between the eyes and pectoral fin on both sides of the head at 2.85 mm larva body total length (2 dph). It has opened at 3.22 mm total length (3 dph) and branchiale can be seen clearly. The same case was found on *C. dadiburjori,* the gill vesicle has opened at 2.8 mm (Sado & Kimura 2005a), but earlier than in *B. sharpeyi* where has opened at 4.7-5.5 mm total length (Mukhaysin & Jawad 2012).

At hatching, pigmentation is not visible in any region of bilih fish larvae. The first pigmentation occured on the eyes at larvae length 2.44 mm (1 dph), followed by pigmentation on dorsal body at 3.22 mm length (3 dph). Pigment will increase as soon as larvae length increases. The onset of pigment appeared of bilih fish like in *B. luteus* (Al Hazzaa & Hussein 2007), *P. conchonius* (Bhattacharya et al 2005), *B. sharpeyi* (Mukhaysin & Jawad 2012), but is different on *D. rerio* (Kimmel et al 1995), *T. albonubes* (Sado & Kimura 2005b), *C. dadiburjori* (Sado & Kimura 2005a), *B. anoplus* (Cambray 1983) where the eye pigmentation appears at hatching.

Bilih fish scale formation has began at 16.85 mm total length stage (8 wph) and develop completely at 39.16 mm total length stage (16 wph), which is earlier than in *T. albonubes* where squamation was initiated at 8.4 mm and completed at 13 mm body length (Sado & Kimura 2005b); on *C. dadiburjori* was initiated at 8.0 mm and completed at 10 mm body length (Sado & Kimura 2005a), and on *B. sharpeyi* squamation began at 31.3 mm total length, completed in juveniles at 33.9 mm body length (Mukhaysin & Jawad 2012). The squamation timing is close to those of its related Cyprinid genera.

Conclusions. The present paper is the first information of larvae endemic fish *Mystacoleucus padangensis* developmental stages. Based on the study results and informations collected we can conclude that there were variations in timing and sequencing of larvae ectodermal derivate development in Cyprinidae family. Since observations were performed only on larva development stages, further studies are obviously needed on gonadal development and factors affecting embryonic and larval development for management through hatchery and restocking of this species.

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