Spatiotemporal Profile of Skeletal Development in Bonylip Barb, *Osteochilus vittatus* (Valenciennes, 1842)

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Abstract

Skeletal development is essential in supporting aquaculture success, as failure and deformities reduce fish viability and growth. Herein, the cartilage and bone formation in the larva and juvenile of the bonylip barb, *Osteochilus vittatus* (Valenciennes, 1842), is described in detail for the first time. Mature fish were injected with ovaprim at 0.5 mL.kg^{-1} body weight, followed by artificial spawning. After fertilisation, the embryos were reared in a glass container at 28 ± 1 °C. The first hatchling was observed at 26 h and 15 min post-fertilisation (pf), and the last was observed at 48 hpf. The cartilage and bone development were observed from 1 to 30 days post-hatching (dph) by alcian blue and alizarin red S staining. Freshly hatched fish larvae had not undergone either chondrification or ossification. The cartilage was observed at 1 dph, marked with Meckel's cartilage, ethmoid plate, and pectoral fin, as the pre-larva had a standard length (SL) of 5 mm. At 5 dph (SL of 5.6 ± 0.55 mm), 3 to 4 pairs of basidorsal and basiventral were observed. The cartilaginous development ended at 26 dph as the SL reached 14.3 ± 0.57 mm. The ossification started at 20 dph (SL of 9.24 ± 0.68 mm) and was marked with opercle and cleithrum. At 26 dph, as the post-larvae length 14.3 ± 0.57 mm, dentary, pterotic, and parasphenoid were ossified. Juvenile fish at 30 dph (SL of 21.2 ± 3.9 mm) showed completed ossification processes in the cranium and vertebrae.

Keywords: skeleton, chondrogenesis, Cyprinidae, larvae, osteogenesis

Introduction

Bonylip barb, *Osteochilus vittatus* (Valenciennes, 1842; Cyprinidae), is an endemic fish in Southeast Asia, as reported found in Indochina (Rainboth, 1996), Indonesia (Muchlisin et al., 2014; Roesma et al., 2016; Hasan et al., 2019; Jusmaldi et al., 2020), Thailand (Saenjundaeng et al., 2020), Laos (Kottelat, 2016), and Malaysia (Székely et al., 2009). It inhabits a wide range of freshwater habitats, including lowland marshlands, peat swamps, rivers and tributaries, and hill streams (Lumbantobing and Vidthayanon, 2020). These fish are herbivores and are often introduced into several water bodies (lakes and reservoirs) as biological agents to reduce algal blooms and for restocking (Putri et al., 2015; Syandri et al., 2015).

Cyprinidae is an economically important taxonomic group, and bonylip barb has the potential as an alternative to diversifying consumed fish commodities (Mulyasari et al., 2010). Several efforts have been made to optimise the cultivation protocol to promote its culture (Andriani et al., 2019; Herawati et al., 2019; Ahmad et al., 2020), reproductive performance (Sunarno et al., 2019), and control the parasitic infection (Fira et al., 2021). Although seed production has been successful, the result was inconsistent due to issues in the larval rearing protocol. Studies of the early stages in the life cycle of fish were performed to obtain information such as species-specific characteristics and developmental traits that can be observed during early organ development (Park et al., 2016a). Moreover, detailed knowledge of skeletal

development is important so that the early-stage deformities in a hatchery can be identified and controlled. Knowing the specific sequence of skeletal development provides taxonomical, developmental, and ecological knowledge, that can be used to ensure proper protocols for monitoring hatcheries and nurseries, including quality assessment to optimise rearing conditions, feeding strategies, and high-quality diets (Park et al., 2016b; Fernández et al., 2021).

The study on the skeletal development of the larval and juvenile bonylip barb is still limited. Setiadi et al., (2020) reported vertebral ossification from day 5 until 30 with different hardness level treatments. Therefore, this study aims to observe the development of larval and juvenile skeletal of bonylip barb using double-staining alcian blue-alizarin red S. In addition, it attempts to show the correlation between sequence/profile of skeletal development, age period, including its duration to complete the bone formation process and compare with other fish species. These data are important as a basis for the developmental study and to provide information that might be useful for taxonomic research and optimisation of the cultivation protocols of this fish.

Materials and Methods

Ethical approval

Ethical approval for this study was obtained from the Medical Faculty, Universitas Gadjah Mada, Yogyakarta, Indonesia (Approval no. KE/FK/0166/EC/2020).

Rearing condition and artificial spawning procedure

Fish broodstock (120–160 g body weight; 20–21 cm total length; age >1 year old) were collected from their cultivation site at the Southern Regional Office of the Ministry of Marine Affairs and Fisheries Singaparna, West Java Province, Indonesia. The rearing condition, artificial spawning, embryo collection and incubation were performed based on published protocols (Madihah et al., 2021). Briefly, females and males were reared separately in a concreted pond (2 m × 1 m × 0.5 m) containing 6000 L water under natural photoperiod and ambient temperature (22–27 °C) with acceptable water quality parameters. They were fed ad satiation twice daily with 5 % of the total biomass. After showing external signs of gonad maturity, the broodstocks' were injected intramuscularly with ovaprim (0.5 $mL.kg^{-1}$ body weight; Syndel Laboratories Ltd., Canada) for induced spawning. At 12 h post-injection, females and males were stripped, and the gametes fertilised. Fertilised eggs were incubated in a glass container (100 cm × 80 cm × 60 cm) with 30 cm water depth (± 500 embryos per container) with strong aeration to minimise clumping the egg. Water temperatures during embryonic development were maintained in the range of 28 ± 1 °C. The embryo did not hatch simultaneously. The first hatchling was observed at 26 h and 15 min pf and the last at 48 hpf.

Larval rearing condition

The newly hatched pre-larvae were fed at 4 dph, after the egg yolk was completely absorbed. The post-larvae aged 4 dph were fed with the suspension of boiled egg yolk, at 6 dph with newly-hatched *Artemia*, and at 8 dph with pellet powder (Fengli-1, PT. Matahari Sakti, Indonesia) at 5 % of the total biomass. The fish samples were nurtured for 30 days to observe cartilage and bone formation. The rearing conditions were at a natural photoperiod with acceptable water quality parameters, namely pH of 7.0–7.2, ammonia levels <0.03 $mg.L^{-1}$, nitrite <0.06 $mg.L^{-1}$ and dissolved oxygen >4 $mg.L^{-1}$.

Alcian blue-alizarin red S double staining

The protocol for alcian blue-alizarin red S double staining of the fish and post-larvae was based on Dingerkus and Uhler (1977). Fixation: A total of 90 bonylip barb larvae (aged 0, 1, 3 dph (days post-hatch)) and 150 bonylip barb fry (aged 5, 10, 20, 26, and 30 dph) were preserved in 10 % formalin solution for 48–72 h. Washing: All larvae were washed in distilled water for 48–72 h. Cartilage staining: All larvae were transferred into an alcian blue solution (100 $mg.L^{-1}$ alcian blue 8GX (Merck, Germany), 800 $mL.L^{-1}$ 95 % ethanol, and 200 $mL.L^{-1}$ acetic acid) for 24–48 h. Rehydration: Larvae were rehydrated in decreasing ethanol series (95 %, 95 %, 75 %, 40 %, and 15 %) and distilled water for 2–3 h each. Bleaching: The residual acid of larvae tissue was cleared in trypsin solution (1 $g.L^{-1}$ trypsin, Merck, Germany), 300 $mL.L^{-1}$ saturated borax, 700 $mL.L^{-1}$ distilled water) for 48–72 h. Bone staining: Bone larvae were stained in alizarin red S solution (5 $g.L^{-1}$ alizarin red S, Merck, Germany and 1000 $mL.L^{-1}$ 1 % KOH) for 24 – 48 h. Pigmented larvae were incubated in bleaching bath series (3:1, 1:1, 1:3) of a mixture of KOH 1 % and glycerol (added with 3–4 drops of 3 % H_2O_2) for several days or until dark colours were removed. Final preservation: Stained larvae were preserved in 100 % glycerol, with the addition of a few thymol crystals. Observation: The preserved larvae were transferred into a small jar and observed under a stereomicroscope (Nikon SMZ445, Japan) with 35× magnification, then photographed using Samsung A20 (Samsung, South Korea).

Results

The post-hatching development

The growth of bonylip barb larvae was measured by the increase in standard length. The correlation between the standard length (mean ± standard deviation) and day post-hatch is presented in Figure 1.

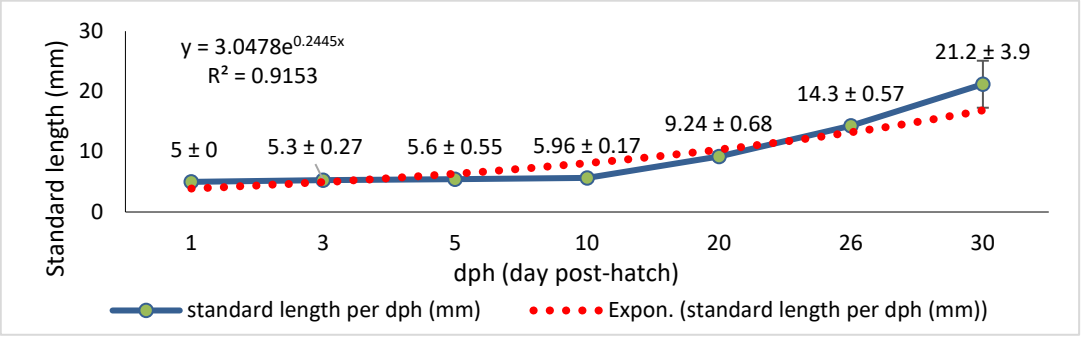

Fig. 1. Post-hatching standard length of bonylip barb, *Osteochilus vittatus* larvae and fry (aged 1–30 dph) (n = 5).

Cartilaginous development in bonylip barb

The cartilage formation in the cranial

The cranial skeleton of the bonylip barb consists of the neurocranium and the viscerocranium. The results showed that cartilage formation in the cranial began at 1 dph, and a complete structural form was seen at 20 dph (Fig. 2). At 1 dph, the neurocranium structure, such as ethmoid plate and auditory capsule were observed (Fig. 2a). The other neurocranium structures, such as lamina orbitonasales and taenia marginalis posterior were observed at 3 dph (Fig. 2g). Laminae orbitonasales develop as small structures from the posterolateral side of the ethmoid plate. Taenia marginalis posterior (orbital cartilage) extends the anterior structure to the auditory capsule. This structure develops anterior to the midorbit and later connected by the epiphysial bar at 10 dph. Figure 2c shows the occipital arch, trigemino-facialis chamber, and lateral commissure, with a neurocranial origin developed at 5 dph. The development of neurocranium cartilage also continues at 10 dph by forming the epiphyseal bar (Figs. 2d and 2i). It continues until 20 dph with the formation of taenia marginalis anterior, as seen in Figure 2j.

The cartilage development of the viscerocranium also occurs at the age of 1 dph, marked with the formation of palatoquadrate, Meckel's cartilage, ceratohyal, and ceratobranchial (Fig. 2k). At 3 dph, viscerocranium developed the last cartilage structure called hyosymplectic (Fig. 2l) and the pterygoid process of pterygoquadrate cartilage (Fig. 2b). After that, the viscerocranium no longer developed new structures. Finally, at 20 dph the cartilage structures in the cranial are completely formed and distinct from one structure to the other (Figs. 2e, 2j, and 2o).

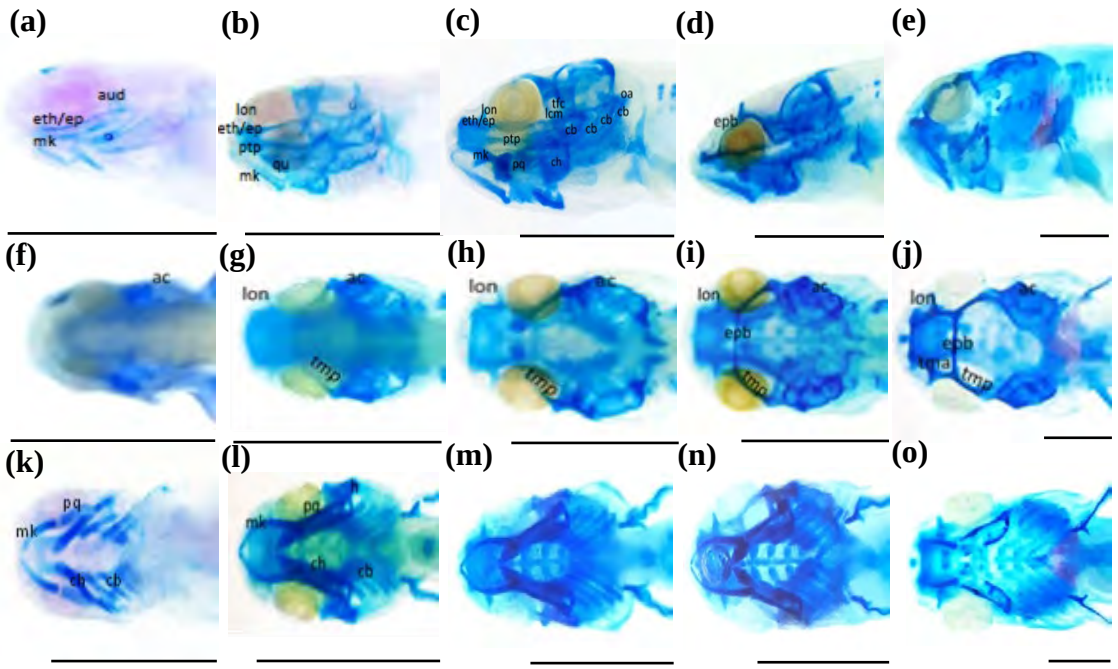

Fig. 2. The cartilage formation in the cranial of bonylip barb, *Osteochilus vittatus* (age of 1–20 dph) viewed from multiple angles under stereo microscope Nikon SMZ445 (magnification 35x). (a, b, c, d, e) age 1, 3, 5, 10, and 20 dph respectively, in lateral view. (f, g, h, i, j) age 1, 3, 5, 10, and 20 dph respectively, in dorsal view. (k, l, m, n, o) age 1, 3, 5, 10, and 20 dph, respectively, in ventral view. Abbreviations: dph, days post-hatch; aud/ac, auditory capsule; eth/ep, ethmoid plate; mk, Meckel's cartilage; pq/qu, palatoquadrate; ch, ceratohyal; cb, ceratobrancial; lon, laminae orbitonasales; ptp, pterygoid process of pterygoid cartilage; epb, epiphyseal bar; lcm, lateral commisure; tfc, trigemino-facialis chamber; oa, occipital arch; tmp, taenia marginalis posterior; tma, taenia marginalis anterior; h, hyosymplectic. Scale bar in the bottom of each photo = 1 mm.

The cartilage formation in the vertebral, fins, and tail

The cartilage formation in the vertebral and tail started at 5 dph and ended at 26 dph, while the cartilaginous structure of fins started developing at 1 dph and ended at 26 dph (Fig. 3). In the vertebral column, the first cartilaginous structures observed at 5 dph are called basidorsal and basiventral, this was seen from several vertebrae in the abdomen, coloured blue (Fig. 3c). The initial total number observed of basidorsal and basiventral at 5 dph are 3 to 4 pairs (Fig. 3f). The number increased into 8 pairs at 20 dph (Figs. 3h and 3k). At 26 dph, the total number reached 13 pairs and completed the cartilaginous development (Figs. 3i and 3l).

The first development of cartilaginous structure in the fins was marked with pectoral fins formation at 1 dph, followed by the structures called dorsal radials (dorsal fins) and anal radials (anal fins), which started to develop at 5 dph and 20 dph, respectively (Figs. 3c and 3h). From 1 dph forward, the pectoral fins develop into their complete formation at larvae of 26 dph (Fig. 3i). A total of 4 dorsal radials were observed at 5 dph (Fig. 3c), which increased into 8, 13, and 15 fin rays at 10, 20, and 26 dph subsequently (Figs. 3g, 3h, and 3i, respectively). Figures 3h and 3i showed that 5 anal radials (anal fins) developed at 20 dph and grew into 6 fin rays at 26 dph. Furthermore, the cartilaginous structure of anal fins started to develop at 26 dph. The fins include pectoral fins, dorsal fins, pelvic fins, and anal fins finished the cartilaginous development at 26 dph (Fig. 3i).

The first cartilaginous structure to develop in (caudal fins) larvae tail at 5 dph is hypural. A total of 6 hypurals at 5 dph develop to the final 8 fin rays at 10 dph (Figs. 3c and 3g). Other caudal fin structures, such as the epural and uroneural, begin to develop at 20 dph, with as many as 2 and 1 fin rays, respectively. No increase in the number of caudal fins was observed in larvae older than 20 dph (Fig. 3h). Cartilage development identified in bonylip barbs aged 1, 3, 5, 10, 20 and 26 dph are summarised in Table 1.

The development of bone formation in bonylip barb

The bone formation in the cranial

The bone formation of bonylip barb in the cranial occurred at 20 dph (Figs. 4a, 4d, and 4g). The neurocranium and viscerocranium developed from 20 dph and completed the ossification processes at 30 dph. The bone structure in the neurocranium that undergoes an early ossification process at 20 dph is basioccipital articulatory. The ossification process is marked by the appearance of purplish red colour due to the alizarin red S dye (Fig. 4a).

At 26 dph, the ossification in the neurocranium was observed in broader areas such as pterotic, epioccipital, exoccipital, basioccipital, and parasphenoid (Fig. 4b). The neural origin structures continued to ossify until 30 dph. These last structures were frontal, sphenotic, and supraoccipital bones (Fig. 4c).

At 20 dph, the ossification in viscerocranium structures also began to occur, and the structures which partially ossified were opercle (Fig. 4a). The other viscerocranium structures, such as premaxilla, dentary, and subopercle ossified at the age of 26 dph (Fig. 4b). The last structures ossified at 30 dph are articular, retroarticular, quadrate, symplectic, and interopercle (Fig. 4c). Finally, the pectoral girdle structures include cleithrum, supracleithrum, and posttemporal bones ossified at 20, 26, and 30 dph (Figs. 4a, 4h, and 4f, respectively).

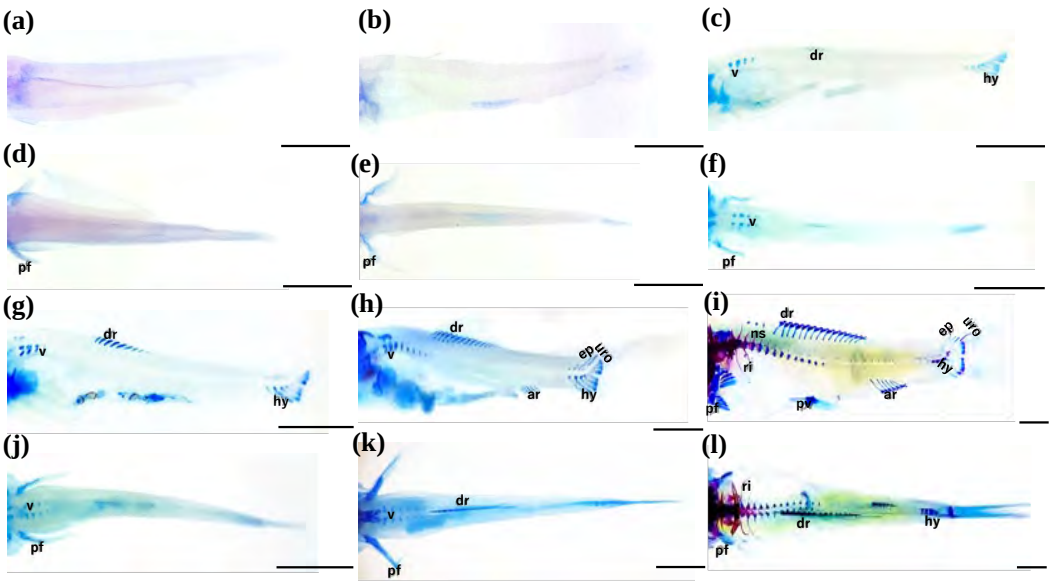

Fig. 3. The cartilage formation in the vertebral, fins and tail of bonylip barb *Osteochilus vittatus* (age of 1–26 dph) viewed from multiple angles under stereo microscope Nikon SMZ445 (magnification 35×). (a, b, c) age 1, 3, and 5 dph, respectively in lateral view. (d, e, f) age 1, 3, and 5 dph, respectively in dorsal view. (g, h, i) age 10, 20, and 26 dph, respectively in lateral view. (j, k, l) age 10, 20, and 26 dph, respectively in dorsal view. Abbreviations: dph, days post-hatch; v, vertebral; pf, pectoral fin; pv, pelvin fin; ri, ribs; dr, dorsal radial; ar, anal radial; ns, neural spine; ep, epural; hy, hypural; uro, uroneural. Scale bar in the bottom of each photo = 1 mm.

Table 1. Summary of the cartilage development identified in bonylip barb, *Osteochilus vittatus* larvae and fry (aged 1–26 dph).

Cartilage elements	Day post-hatch (dph)					
	1	3	5	10	20	26
Cranial						
Ethmoid plate	■	■	■	■	■	■
Palatoquadrate	▒	▒	▒	▒	▒	▒
Meckel's cartilage	■	■	■	■	■	■
Auditory capsule	▒	▒	▒	▒	▒	▒
Pterygoid process of pterygoquadrate cartilage	—	■	■	■	■	■
Ceratohyal	▒	▒	▒	▒	▒	▒
Ceratobranchial	■	■	■	■	■	■
Occipital arch	—	—	▒	▒	▒	▒
Hyosymplectic	—	■	■	■	■	■
Trigemino-facialis chamber	—	—	▒	▒	▒	▒
Lateral commissure	—	—	■	■	■	■
Epiphyseal bar	—	—	—	■	■	■
Laminae orbitonasales	—	▒	▒	▒	▒	▒
Taenia marginalis posterior	—	■	■	■	■	■
Taenia marginalis anterior	—	—	—	—	▒	▒
Vertebral, fins, and tail						
Vertebral column	—	—	3-4	3-4	8 pairs	13 pairs
Pectoral fin	■	■	■	■	■	■
Dorsal radial	—	—	4	8	13	15
Anal radial	—	—	—	—	5	6
Hypural	—	—	6	8	8	8
Epural	—	—	—	—	2	2
Uroneural	—	—	—	—	1	1

Note: the number in the column shows the total number of particular structures mentioned.

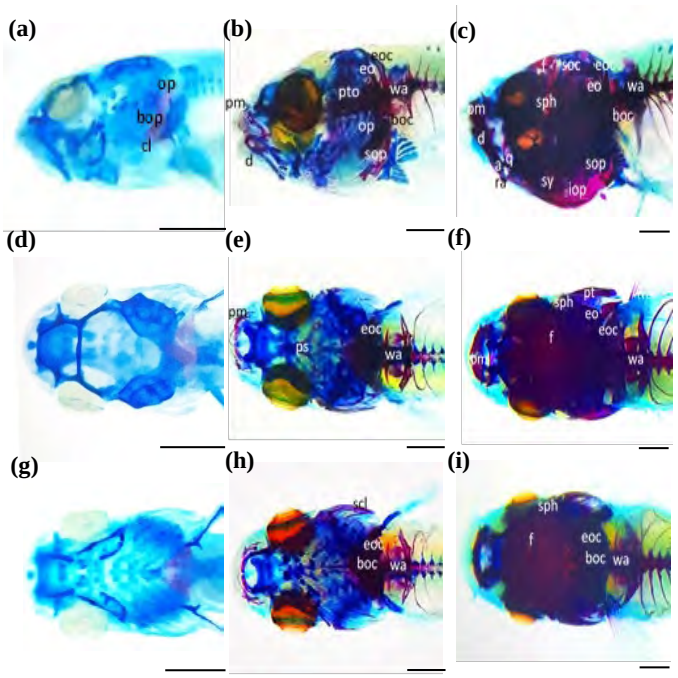

Fig. 4. The bone formation in the cranial of bonylip barb, *Osteochilus vittatus* (age of 20–30 dph) was viewed from multiple angles under stereo microscope Nikon SMZ445 (magnification 35×). (a, b, c) age 20, 26, and 30 dph, respectively, in lateral view. (d, e, f) age 20, 26, and 30 dph, respectively, in dorsal view. (g, h, i) age 20, 26, and 30 dph, respectively, in ventral view. Abbreviations: dph, days post-hatch; op, opercle; bop, basioccipital articulary process; cl, cleithrum; pm, premaxilla; d, dentary; pto, pterotic; eo, epioccipital; eoc, exoccipital; wa, Weberian apparatus; boc, basioccipital; sop, subopercle; a, articular; ra, retroarticular; q, quadrate; f, frontal; sph, sphenotic; sy, symplectic; soc, supraoccipital; iop, interopercle; ps, parasphenoid; pt, posttemporal; scl, supracleithrum; boc, basioccipital. Scale bar in the bottom of each photo = 1 mm.

The bone formation in the vertebral, spine, fins, and tail

The ossification of bonylip barb in the vertebral structure started at 26 dph and was fully formed at 30 dph (Fig. 5). Unlike this result, the fins and tail structure started at 26 dph but were not fully ossified at 30 dph (Fig. 5). Ossification was first observed in the vertebral structures such as the Weberian apparatus, ribs, and neural spine (Fig. 5a). At 30 dph, all vertebral columns, neural spine, haemal spine, and ribs from abdomen to the caudal have completely ossified (Figs. 5b and 5d).

The fins ossification process occurs at 30 dph, and these structures include: 4 dorsal fin rays, 1 urostyle, with hypural and epural observed through the ossification process (Fig. 5b). In the tail structure, ossification starts at 26 dph and develops into a better form at 30 dph. The bone development in bonylip barb aged 20, 26, and 30 dph is summarised in Table 2.

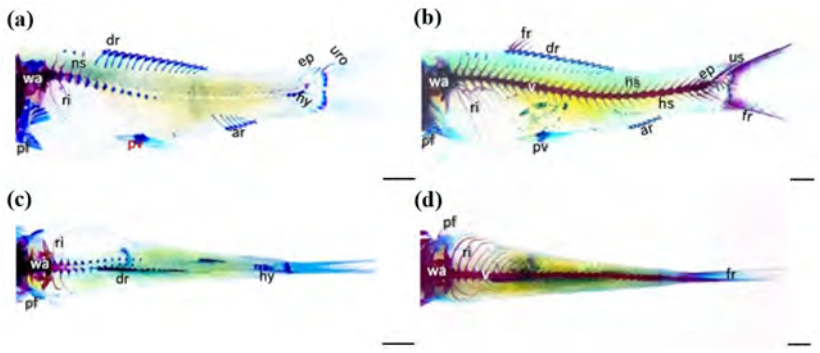

Fig. 5. The bone formation in the vertebral, fins, and tail of bonylip barb, *Osteochilus vittatus* (age of 26–30 dph) viewed from multiple angles under stereo microscope Nikon SMZ445 (magnification 35×). (a, b) age 26 and 30 dph in lateral view. (c, d) age 26 and 30 dph in dorsal view. Abbreviations: dph, days post-hatch; wa, Weberian apparatus; v, vertebral; pf, pectoral fin; pv, pelvin fin; ri, rib; dr, dorsal radial; ar, anal radial; ns, neural spine; hs, haemal spine; ep, epural; hy, hypural; uro, uroneural; us, urostyle; fr, fin ray. Scale bar in the bottom of each photo = 1 mm.

Table 2. Summary of the bone development identified in bonylip barb, *Osteochilus vittatus* larvae and fry aged 20–30 dph.

	Day post-hatch (dph)		
Bone elements	20	26	30
Cranial			
Premaxilla			
Articular			
Retroarticular			
Quadrate			
Symplectic			
Dentary			
Sphenotic			
Pterotic			
Frontal			
Epioccipital			
Basioccipital			
Exoccipital			
Supraoccipital			
Parasphenoid			
Subopercle			
Opercle			
Interopercle			
Cleithrum			
Supracleithrum			
Posttemporal			
Vertebral, fins, and tail			
Abdominal vertebral			
Caudal vertebral			
Dorsal fin			
Caudal fin			
Anal fin			
Pelvic fin			
Hypural			
Epural			
Urostyle			

Discussion

The process of cartilage formation begins with the epithelial-mesenchymal interaction, which leads to mesenchymal cells aggregating and condensing. Chondrogen cells formed from condensation then differentiate into chondroblasts and chondrocytes. Meanwhile, the chondrocytes will secrete unique type II collagen and undergo hypertrophy (Akiyama et al., 2004). The cartilage resulting from this process can become permanent or be replaced by bone through ossification (Zinck et al., 2020).

The present study showed *O. vittatus* developed cartilaginous structures since 1 dph, however, the vertebral and tail started at 5 dph. The formation in the cranial takes place from the anterior part in both the anteroventral (olfactory, orbital, auditory), and anterodorsal (gills and jaws) areas to the posterior vital areas. Stiassny (2000) reported that these structures function as nerve and brain protectors, blood vessels and nerve transmission channels. In addition, this study also showed that the first cartilaginous structures in the vertebral column are called basidorsal and basiventral. According to Tonelli et al. (2020), basidorsal and basiventral are the most anterior and posterior cartilaginous structures that later construct neural or haemal arches, respectively. In the vertebral, fins, and tail of fish larvae, the formation of cartilage structures occurs from anterior to posterior. Cartilage formation starts from the pectoral fin, which acts as respiratory support (Zimmer et al., 2020), continues in the spinal area, is marked by basidorsal and basiventral, and ends in the caudal area.

Boglione et al. (2013) described two different mechanisms that can form bone through the development of cartilage precursors or direct mesenchymal condensation. The common ossification in teleost fish such as bonylip barb, is intramembranous and perichondral ossifications. Intramembranous ossification occurs when mesenchymal cells differentiate into osteoblasts and establish bone without cartilage precursors. Meanwhile, perichondral ossification develops when a cartilage precursor is surrounded by the perichondrium, transforming into the periosteum.

In the present study, the cranial structure of the bonylip barb began to ossify at the age of 20 dph, which is later than in other Cyprinidae larvae such as *Danio rerio* (Hamilton, 1822); and *Cyprinus carpio* Linnaeus, 1758, which occur at 8 dph and 17 dph, respectively (Maradonna et al., 2013; Kužir et al., 2020). Further comparison with Gobiidae family such as *Luciogobius guttatus* Gill, 1859 (Kim et al., 1992); *Tridentiger obscurus* (Temminck & Schlegel, 1845) (Hwang et al., 2018); and *Gymnogobius urotaenia* (Hilgendorf, 1879) (Jin et al., 2021); began to ossify quite early, respectively at 11, 8 and 9–10 dph, whilst in Moronidae family, such as *Dicentrarchus labrax* (Linnaeus, 1758) (Darias et al., 2010) ossification starts at 15 dph.

The present study showed that opercle was the first-ossified cranial element in *O. vittatus*. This specific bone is related to respiration. Identical to bonylip barb, *Sebastes koreanus* Kim & Lee, 1994 (Yu and Kim, 2016); *Clarias gariepinus* (Burchell, 1822) (Adriaens and Verraes, 1998); and *Ancistrus triradiatus* Eigenmann, 1918 (Geerinckx et al., 2007), also reported opercle to be the first element to be ossified. The cranial ossification of bonylip barb sets up from the gill area to the posterior or occipital region. This results from the combined ossification of the parachondral basilar plate, occipital arch, and auditory capsule, which protects nerves, arteries, and blood vessels (Stiassny, 2000). Ossification in the posterior cranial parts coincides with ossification in several anterior parts, such as the infraorbital area (result of intramembranous ossification) (Boglione et al., 2013), maxillary area (result of intramembranous ossification) (Boglione et al., 2013), and mandible (result of Meckel's cartilage ossification) (Stiassny, 2000). This same sequence proceeds for 10 days in bonylip barbs, 12 days in *C. carpio* larvae (Kužir et al., 2020), 14 days in *D. rerio* (Maradonna et al., 2013) and 33 days in *Labeobarbus intermedius* (Rüppell, 1835) (Borisov et al., 2012).

Slightly different from the bonylip barb ossification pattern is reported in primitive species such as bowfin, *Amia calva* Linnaeus, 1766 (Schoch, 2006), golden Nile catfish, *Chrysichthys auratus* (Geoffroy Saint-Hilaire, 1809) (Vandewalle et al., 1995), brook trout, *Salvelinus fontinalis* (Mitchill, 1814) (Steingraeber and Gingerich, 1991), as well as other gadoid fishes such as Alaska pollock (*Theragra chalcogramma* Pallas, 1814) (Brown et al., 2001). In these cases, their cranial ossification process started from bones involved in feeding such as maxilla and dentary followed by opercular bone that support respiration.

Vertebral ontogeny, as seen in *O. vittatus* started with the formation of the neural arches, which are similar to *Dentex dentex* (Linnaeus, 1758) (Koumoundouros et al., 1999); *Solea senegalensis* Kaup, 1858 (Gavaia et al., 2002); and *Lates calcarifer* (Bloch, 1790) (Fraser et al., 2004). This process is proceeded by ossification of the centra. Vertebral centra ossification of bonylip barb continues caudad starting from the anterior vertebral area (Weberian apparatus, ribs, neural spine), whereas preural ossification continues rostrad. That same profile also occurs in common snook *Centropomus undecimalis* (Bloch, 1792) (Potthoff and Tellock, 1993); *Sparus auratus* Linnaeus, 1758 (Faustino and Power, 1998); and *Scophthalmus maximus* (Linnaeus, 1758) (Tong et al., 2012).

The present study showed that the caudal fin was the first locomotory structure to be ossified in bonylip barb, continued with the dorsal fin, the anal fin, and the pelvic fin. The same sequence was also observed in *D. dentex* (Koumoundouros et al., 1999); *Paralichthys olivaceus* (Temminck & Schlegel, 1846) (Hosoya and Kawamura, 1998); and *S. maximus* (Tong et al., 2012).

The results of the present study showed that the ossification of bonylip barb in the vertebral structure started at 26 dph and was fully formed at 30 dph. The ossification of the vertebral structure was relatively slow compared to *C. carpio* for the same structures in the bonylip barb. Carp fish started showing ossification in the vertebral at 17 dph and in the fin at 22 dph (Kužir et al., 2020). In other Cyprinidae members, such as zebrafish, ossification in that similar section occurs at 18 dph (Gavaia et al., 2006). The ossification process of bonylip barb in the spine and fin area occurs about 8–12 days later compared to other Cyprinidae family members. According to Tong et al. (2012), *S. maximus*, which follows the same pattern of bonylip barb, started to ossify in the vertebral region at 19 dph and completed at 24 dph, while identical ossification in *C. undecimalis* occurs at 16–24 dph (Potthoff and Tellock, 1993).

Conclusion

In the bonylip barb, *Osteochilus vittatus*, cartilage formation in the cranial and fins was detected at 1 dph (SL of 5 mm), while the ossification in the vertebrae and the tail was at 5 dph (SL of 5.6 ± 0.55 mm). In all these structures, cartilage formation occurred from anterior to posterior. The development of the skeletal cartilage was completely formed by 26 dph (SL of 14.3 ± 0.57 mm). The cranial structure of the bonylip barb began to ossify at the age of 20 dph (SL of 9.24 ± 0.68 mm), while the ossification in the vertebrae structure and the fin subsequently started at 26 dph and 30 dph. The osteogenesis occurs starting from the cranium, then the vertebrae and the cauda. Both ossification processes in the cranial and vertebral structures were fully formed at 30 dph (SL of 21.2 ± 3.9 mm). These new findings of larval cartilage and bone formation in bonylip barb under normal circumstances could be useful for detailed taxonomic studies of *O. vittatus*, which could lead to optimising its cultivation protocol and increase fish viability and growth.

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