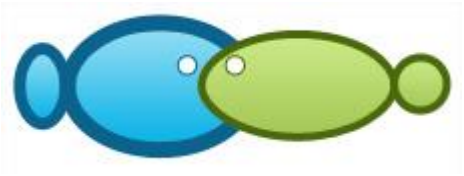

Biometric characterization and morphogenesis of Anasa ornamental fish (*Nomorhamphus* sp.) endemic to Lake Lindu, Central Sulawesi, Indonesia

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Abstract. Anasa (*Nomorhamphus* sp.) is an ornamental fish, endemic to the Lake Lindu inlet river, with high potential economic value, thus it needs to be maintained through cultivation activities. This research aims to explore the biometric information on the growth and morphogenesis of *Nomorhamphus* sp. as a basis for cultivation. The study was conducted in the laboratory using *Nomorhamphus* sp. stadia larval fish that were reared for 30 days under controlled conditions using a container of 35x25x15 cm^3 with aeration. The biometrics characters, namely the length of the dorsal fin, abdominal fin, and tail fin, were measurably differentiated in stadia, from larval to seeds, while the meristic phenotypes remained unchanged. The body length of *Nomorhamphus* sp. of 30 days age ranged from 20.4 to 23.32 mm and indicated sexual dimorphism and dichromatism as secondary sexual characteristics. *Nomorhamphus* sp. has a unique morphology of the jaw, with a lower jaw size longer than the upper jaw. Sexual dichromatism of the female fish is characterized by an orange color on the abdomen and caudal fins. The male fish has a whitish silver color, and the pectoral fin base is pigmented with light yellowish and black color on the edge of the anal fin. The male *Nomorhamphus* sp. also shows secondary sexual characteristics in the form of andropodium, derived from the modification of the anal fin part.
Key Words: growth, morphomeristic, dichromatism, sexual dimorphism.

Introduction. The diversity of endemic freshwater fish species on the island of Sulawesi is very high, covering 68 species from seven families in the lake and river habitats, one of which is the Anasa fish (*Nomorhamphus* sp.), which lives in Lake Lindu, Central Sulawesi (Hadiaty 2018). The genus *Nomorhamphus* belongs to the family Zenarchopteridae and it is known as Julung-Julung or halfbeak and it consists of 13 to 20 endemic species on the island of Sulawesi (Meisner et al 2000; Meisner 2001; Huylebrouck et al 2012, Huylebrouck et al 2014; Kraemer et al 2019). The Anasa species, endemic in Sulawesi, has potential as an ornamental fish commodity because it has a diversity of body color and morphological uniqueness in the lower jaw of the mouth, shaped like a beak or halfbeak (Kusumah et al 2014). In addition, *Nomorhamphus* sp. also has historical value because it is only found in Lake Lindu Central Sulawesi. Overfishing of some species (*N. celebensis* and *N. liemi*) threatens the preservation of endemic fish (Mokodongan 2019a; Daniels 2020). In addition, endemic fish habitats have also reported a decrease in quality due to pollution from waste community activities, such as agricultural waste, plantations, and invasive fish (Mokodongan 2019b). Some of the invasive fish in Lake Lindu reported include *Osphronemus gouramy*, *Oreochromis niloticus* and *Anabas testudineus* (Herjayanto et al 2019). Efforts to manage the genetic resources of endemic species as ornamental fish commodities must be carried out through cultivation activities, simultaneously seeking for sustainability and productivity maximization.

The cultivation of fish of the *Nomorhamphus* genus has not developed (Kusumah et al 2014). Some species of Nomorhamphus that have been cultivated as ornamental fish include *N. celebensis*, *N. ebrardtii*, *N. liemi* (Magyar et al 2007; Kusumah et al 2014). The cultivation stage begins with domestication to adapt *Nomorhamphus* sp. from its natural habitat to be cultivated for mass-production (Telechea et al 2012). The success of the domestication process is evaluated based on the growth and development of fish and their reproductive performance on maintenance outside their habitat (Milla et al 2020). Endemic fish ex-situ conservation activities are more appropriate than in-situ conservation, so fish are not disturbed by environmental changes, they do not require large spaces, and their life cycle can easily be monitored. The study of fish morphology aims to determine the development of fish body shape and size, sexual dimorphism and reproductive function for adjustment to aquaculture technology (Telechea et al 2012). The morphometric and meristic characterization of *Nomorhamphus versicolor* indicates sexual dimorphism (Kramer et al 2019). Sexual dimorphism and dichromatism are secondary sexual characteristics that indicate differences in growth patterns and colors between the sexes (Hussy et al 2012; Cervino 2014). Each species of fish has different secondary sexual characteristics (Pulungan 2015). Morphological characteristics develop from larva to juvenile and become definitive in adult fish (Baras et al 2012). Sexual dimorphism is linear with gonad maturity levels and is influenced by niches of each sex, natural selection, and intrasexual competition (Habibie et al 2018). Morphometric changes of protrusions on the forehead and lower lip in male gourami are thicker than in females (Sularto et al 2016). Typical sexual characteristics in ornamental fish are necessary for selecting genetic sources and managing reproduction to produce seeds.

Fish endemic of *Nomorhamphus* sp. that live in the inlet river of Lake Lindu need to be protected from the threat of exploitation and environmental damage through cultivation activities so that their existence is sustainable. Cultivating *Nomorhamphus* sp. by applying the right technology is hoped to maximize production and preserve genetic plasma. The current research aimed to explore biological information of *Nomorhamphus* sp., including biometric characteristics and morphogenesis, dichromatism, and dimorphism, as the foundation of genetic resource management strategies and cultivation development to obtain a high-quality production.

Material and Method

Fish collection. *Nomorhamphus* sp. were collected at the inlet river of Lake Lindu, Central Sulawesi (Figure 1). The fish collected were 8-9 cm in size and are pregnant female parents. Fish were caught using a 5.5 mm mesh shovel net and then leaved for 24 hours in a temporary storage container and given aeration without feeding. The fish are then packaged and transported to the Aquaculture Laboratory, Sultan Ageng Tirtayasa University (Serang, Banten Province) for rearing in controlled containers.

Figure 1. Sample location of *Nomorhamphus* sp. endemic to Lindu Lake, Central Sulawesi, Indonesia (1°12'14.7"S 120°09'47.4"E).

Fish rearing. The *Nomorhamphus* sp. samples used in the current research were larvae born from natural catch breeders and reared for 30 days in a 35x25x15 cm^3 plastic container with a density of 1 fish L^{-1} and were given aeration and feed based on *Artemia* sp. nauplii and *Tubifex* sp. 2 times a day in the morning and evening, according to the size of the fish's mouth. Water quality management is carried out by cleaning fish feces and the rest of the feed two times a day and measuring daily water quality parameters, including temperature (22.30-29.20°C), pH (7.08–9.74), dissolved oxygen (DO, ranging from 3.1-8.9 mg L^{-1}) and total dissolved particles (TDS, ranging from 209 to 701 mg L^{-1}), including minerals, salts and metals. Fish rearing is carried out at the Aquaculture Laboratory, Sultan Ageng Tirtayasa University (Serang, Banten Province).

Biometric characterization. Biometric characteristics include morphometric and meristic fish measurements, referring to Huylebrouck et al (2012) and Baras et al (2012). for each fish stadia (larvae, juveniles, adults), four individuals were selected. The morphometric characterization of Anasa fish (Figure 2) consisted of 22 characters measured using the calipers. In comparison, meristic characters are observed by microscope and counted by the number of rays of each fin and the spinal segments of the fish, which include four fin characters (anal, dorsal, pelvic, pectoral). Each morphometric character has a rational percentage value against the standard length (Huylebrouck et al 2012). In addition, the growth rate of each stadium is measured based on the increase in the length and weight of the fish, each of the four individuals of each stadium performed at the beginning and end of rearing. Observations of the measure of mouth width are carried out at the beginning and end of each stadium, on four tails, by measuring the width of the mouth opening, which is assumed to be open as wide as 90°, multiplied by the length of the upper jaw, by referring to Hulyebrouck et al (2019).

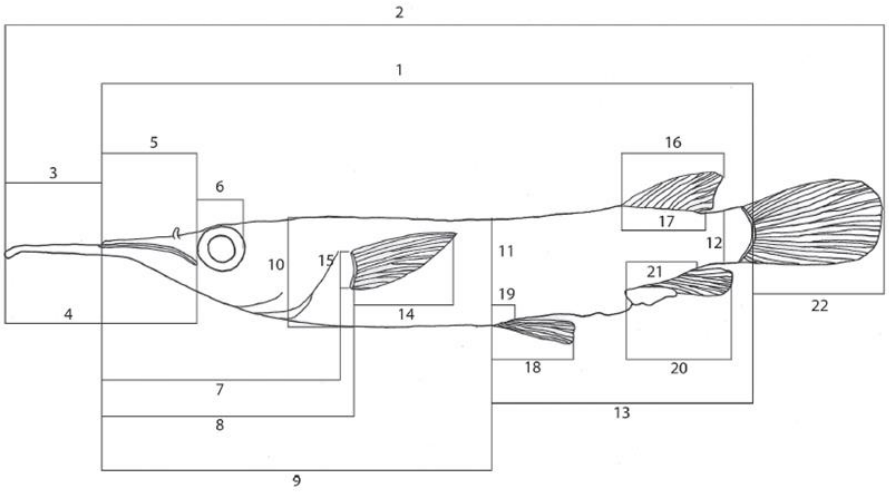

Figure 2. Morphometric measures of *Nomorhamphus* sp.:1) standard length; 2) the total length including the jaw; 3) the length of the lower jaw brembach; 4) the length of the lower jaw; 5) the length of the upper jaw; 6) eye diameter; 7) the length of the head; 8) the distance of the mouth to the pectoral fin; 9) the distance of the mouth to the abdominal fins; 10) the height of the body on the pectoral fins; 11) the height of the body on the abdominal fins; 12) the height of the tail stem; 13) the distance of the abdominal fin to the tip of the tail; 14) the length of the end of the pectoral fin; 15) the height of the pectoral fin; 16) the length of the dorsal fin; 17) the length of the base of the dorsal fin; 18) the length of the abdominal fins; 19) the length of the base of the abdominal fin; 20) the length of the fins; 21) the length of the base of the fin; 22) the length of the tail fin.

Morphological phenotype. Morphological characteristics were observed on the larval stadia (H0), juvenile (H15), and seed stadia (H30) against the shape and color of the body as well as the phenotype of secondary sex characteristics referring to the halfbeak species *Dermogenys pusilla* (Meisner 2001; Kraemer et al 2019).

Data analysis. Biometric characteristics that include morphometric and meristic character data and *Nomorhamphus* sp. morphogenesis were analyzed descriptively.

Results

Morphometric characters of Nomorhamphus sp. The newborn *Nomorhamphus* sp. larvae have a standard length ranging from 13.57 to 14.42 mm, which in the juvenile phase is of 16.53 to 17.55 mm, while the seeds size is in the range of 20.4 to 23.32 mm. The morphometric characters development of *Nomorhamphus* indicate an increase in the fin distance to the abdomen, in the length of the dorsal fin, in the length of the abdominal fin, in the length of the base of the anal fin, and in the length of the tail fin, as the standard-length increases (Table 1). The truss of the head grows in the diameter of the eye, in the size of the lower jaw, and in the width of the mouth opening. In the newly born larvae, the ratio of the eye diameter to the length of the head is larger than in the juvenile and seed; by contrast, the size of the mouth opening and the length of the lower jaw increase in juveniles and seeds.

Table 1

Morphometric characters of *Nomorhamphus* sp. endemic to Lake Lindu, Central Sulawesi reared under laboratory condition

Morphometric characters	*Day 0 (n=2)*	*Day 15 (n=3)*	*Day 30 (n=3)*
Standard length (mm)	13.57-14.42	16.53–17.55	20.4–23.32
	Standard length		
Total length (%)	120.76±0.05 (120.73–120.79)	122.89±1.0 (121.7–123.89)	120.73±0.24 (120.45–120.9)
Brembach's lower jaw length (%)	1.59±0.3 (1.37–1.8)	3.1±0.28 (2.78–3.3)	2.62±0.49 (2.43-3.17)
Head length (%)	23.18±1.99 (21.77–24.58)	26.05±1.09 (24.8–26.83)	23.50±2.60 (20.88–26.07)
Pectoral fin length (%)	11.67±0.04 (11.36–11.98)	13.43±0.47 (13.16–13.97)	14.3±0.43 (14.03-14.79)
The length of the pectoral fin tip (%)	7.06±1.03 (6.33–7.78)	5.69±0.82 (5.0–6.59)	5.31±0.32 (5.04–5.66)
Dorsal fin length (%)	17.16±1.35 (16.20–18.11)	17.76±0.1 (17.64-17.84)	18.35±0.86 (17.59–19.29)
Length of base of the dorsal fin (%)	14.19±0.78 (13.63–14.73)	14.12±0.8 (13.49–15.02)	14.33±0.36 (14.11–14.75)
Length of abdominal fins (%)	5.3±0.78 (4.47–5.58)	7.71±0.46 (7.33-8.22)	7.82±0.11 (7.69–7.9)
Length of base of the abdominal fin (%)	2.82±0.38 (2.54–3.08)	2.33±0.51 (1.93-2.9)	1.97±0.42 (1.51–2.35)
Anal fin length (%)	17.08±1.66 (15.9–18.25)	18.2±2.68 (15.84–21.11)	17.5±1.5 (16.38–19.4)
Length of anal fin base (%)	14.6±0.29 (14.39-14.8)	15.62±2.52 (13.04–18.08)	16.17±1.86 (14.95–18.31)
Tail fin length (%)	14.09±0.53 (13.71–14.46)	16.67±0.23 (16.42–16.87)	18.42±1.35 (17.12–19.81)
	Head length		
Head length (mm)	21.77–24.58	24.8–26.83	20.88–26.07
Eye diameter (%)	36.29±2.99 (34.17–38.4)	22.5±4.06 (19.53–25.27)	26.78±2.37 (24.35–28.28)
Length of the lower jaw (%)	6.95±1.91 (5.6–8.3)	11.87±0.16 (11.21–12.31)	11.10±0.33 (10.33–12.71)
Length of the upper jaw (%)	18.14±6.64 (13.44–22.83)	16.07±2.9 (10.97-20.65)	19.80±0.28 (19.57–20.11)
Width of mouth opening (mm)	0.81±0.18 (0.67–0.93)	0.65±0.44 (0.63–1.32)	1.47±0.25 (1.18–1.63)

The value is the mean and standard deviation; the range is the min-max value.

Meristic characters Nomorhamphus sp. The meristic characters of the larval, juvenile, and seed phase Anasa fish do not show differences in the number of fins, for each observed meristic character (Table 2). Ray fins of Anasa fish included pectoral fins (paired), pelvic fins (paired), dorsal fin, anal fin, and caudal (tail) fin. The number of fins fingers are: 14-15 in anal fins (A.14-15), 11 in dorsal fins (D.11), 13 in pectoral fins (P.13), and 6 in pelvic fins (Pe.6).

Table 2

Meristic characters of *Nomorhamphus* sp. endemic to Lake Lindu, Central Sulawesi

Meristic characteristic	*Day 0 (n=2)*	*Day 15 (n=3)*	*Day 30 (n=3)*
Anal fin rays (A)	14-15	14-15	14-15
Dorsal fin rays (D)	11	11	11
Pectoral fin rays (P)	13	13	13
Pelvic fin rays (Pe)	6	6	6

Morphogenesis of Nomorhamphus sp. The body color of *Nomorhamphus* sp. larval changes shortly after birth to the juvenile of 15 days age, then to the adult of 30 days age (Figure 3).

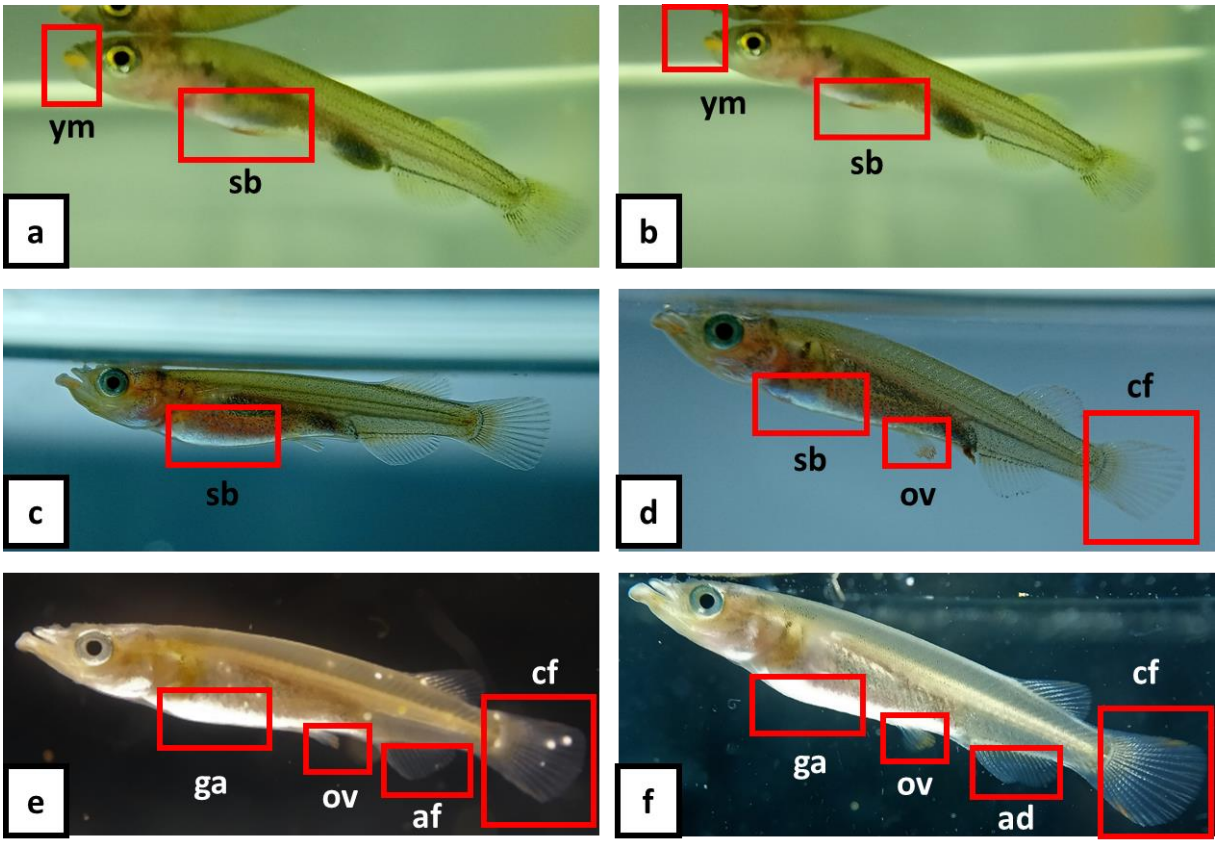

Figure 3. Morphogenesis characteristic color of body parts in the larval phase (a, b), juvenile age 15 days (c, d), and seeds aged 30 days in female fish (e) and male fish (f). Yellow mouth (ym); silver belly (sb); orange ventral (ov); anal fin (a); caudal fin (c); andropodium (ad) modified from anal fin.

The larvae of *Nomorhamphus* sp. at birth have a brightly creamy color, the tip of its jaw is yellow (ym), and the abdomen is gray (ga). In the juvenile phase, the yellow color on the jaw fades and resembles an adult fish, while on the abdomen it is orange in male fish (d) and differentiates in the abdominal fin (bv) and caudal fin (cf). In the 30-day-old seed phase, the color and morphological character already resembles that of adult fish, namely creamy in females and silver with dichromatism in males, in the form of striped patterns,

and have andropodium as a secondary sex characteristic. Sexual dichromatism on the female fish is characterized by an orange color on the abdomen with normal abdominal and caudal fins (e), while in male fish, it is black pigmented at the end of its fins (f).

Discussion. Newborn *Nomorhamphus* sp. larvae had a standard length of 13.57-14.42 mm, while in the juveniles it increased to 16.53–17.55 mm, and at the seed phase reached 20.4-23.32 mm. The longer the fish's standard-length size, the longer the dorsal fin, abdominal fin, and tail fin. The development of the biometric phenotype of *Nomorhamphus* sp., starting from the larval phase to the 30-day-old seed, does not show a difference in meristic cerebral pals. Other meristic finding which did not change were: the radius of anal fin (A.14-15), the radius of the dorsal fin (D.11), the rays of the pectoral fin (P.13), and the rays of the pelvic fin (Pe.6). *Nomorhamphus* sp. has a unique morphology in the jaw, which is in the shape of a beak with a lower jaw size longer than the upper jaw. The lower jaw of the *Nomorhamphus* sp. larvae has a smaller proportion than some other types of *Nomorhamphus*. Until the age of 30 days, the proportion of mandibular length to standard length is 2.43–3.17%. *Nomorhamphus rex* has a mandibular proportion of 11.3–17.5% (Huylebrouck et al 2012). According to Gunter et al (2014), the lengthening of the lower jaw takes place at the stage of juvenile until adult fish, being controlled by the calmodulin gene (calm1), adapted to their habitat. In other *Nomorhamphus* species, the sympatric *N. rex* with *N. aenigma* has an elongated lower jaw, but *N. aenigma* undergoes an evolution towards a short jaw, as an ecological adaptation (Kobayashi et al 2020). *N. rex* species swim in the surface layer of the river, while *N. aenigma* swims in the middle and lower layers.

The size of the fish's mouth width develops from day 0 to day 30. The size of the feed given depends on the size of the fish's mouth width. The newborn larvae (H0) have a range of mouth width of about 0.67–0.93 mm, while the larvae on the 15th day have a mouth width ranging from 0.63–1.32 mm. Based on the size of the fish's mouth width, then the use of appropriate feed is *Artemia* sp. and nauplii of *Artemia* sp. with a length of about 0.5 to 0.6 mm. In contrast to days 0 and 15, the fish enter the juvenile phase to the day 30, with a mouth width size of 1.18–1.63 mm, so that fish can be fed with *Tubifex* sp. or silkworms. By the elongation of the lower jaw, Anasa fish larvae tend to eat food in the water column.

Morphologically, the larval body of *Nomorhamphus* sp. at birth has a bright cream color, the end of the jaw is yellow (ym), and the abdomen is gray (ga). In the juvenile phase, there is a change of the yellow color in the jaws that fades to resemble an adult fish. In contrast, the color of the abdomen (d), abdominal fins (bv), and caudal fins (cf) indicate dichromatism. Male had brighter orange abdominal fin than female. (Similarly, the basic color on the body of an adult *Nomorhamphus* sp. also shows dichromatism (cream color in females and silver color in males). Sexual dichromatism signals mate recognition of the fish associated with diversification in several taxonomic groups, including cichlids (Wagner et al 2012). Dichromatism evolved heterogeneously, influenced by the degree of gene dominance and by the role of natural and sexual selection (Portik et al 2019). In the species of *Oreochromis mossambicus*, the color of the female's body does not give rise to sexual selection by golden or black males (Tave 2021).

Sex recognition is easy to do after entering the juvenile phase or when the sex cells are perfectly differentiated, so that interstitial cells in non-germinal tissues begin to produce sex hormones that give rise to secondary sex characteristics in the form of morphological differences (dimorphism) or color (dichromatism) between males and females. The 30-day-old *Nomorhamphus* sp. showed a morphological character that already resembles that of adult fish, with dichromatism and dimorphism in the form of andropodium, as a secondary sex characteristic. The formation of andropodium begins with the presence of hollows in the anal fins of male fish. The basin is formed due to a shortening process of the first 5 fin radii (Kraemer et al 2019). *Nomorhamphus* sp. sex dimorphism was also indicated by the lower jaw (Brembach jaw). Male fish have a longer lower jaw, compared to the females. The differences in the length of the lower jaw are also found in other types of *Nomorhamphus*, such as *N. rex* (Hulyebrouck et al 2012) and *N. versicolor* (Kraemer et al 2019). A common feature of sexual dimorphism in other species of

Nomorhamphus is that females have larger bodies than males. However, at the age of 30 days, there was no difference in body length of *Nomorhamphus* sp.

The sexual phenotypic dimorphism is generally associated with the expression of genes of both sexes. In guppies, *Poecilia reticulata*, sexual dimorphism is expressed in size, ornament, and behavior, due to natural and sexual selection in the wild (Sharma et al 2014). A secondary sexual characteristic in male *Poecilia reticulata* is gonopodium, which modifies the anal fin and serves as a sperm distributor in internal fertilization. In addition, sexual dimorphism is also found in *Adrianichthys oophorus* where female fish have a longer abdominal fin size than male fish that serve as a place to incubate their eggs until hatching (Gundo et al 2013). The secondary sexual characteristics are more developed in males, but females of many species also develop secondary sexual characteristics, including color ornamentation and aggressive behavior (Baldauf et al 2010). Secondary sexual characteristics are important features under hormonal control and undergo evolution through sexual selection between individuals in the process of getting a partner (Wheeler et al 2020). Sexual development is a complex and specific process in each fish species, that involves genes and epigenetic interaction mechanisms that cause phenotypic changes (Budd et al 2015).

Conclusions. Characteristics of biometric *Nomorhamphus* sp. endemic to Lake Lindu, Central Sulawesi, measurably differentiates in-stadia larvae until the seeds of 30 days old, namely at the length of the dorsal fin, abdominal fin, and tail fin, while the meristic phenotype remains unchanged (A.14-15, D.11, P.13, Pe.6). The body length of *Nomorhamphus* sp. of 30 days age ranged from 20.4 to 23.32 mm. Their body also presents sexual dimorphism and dichromatism. *Nomorhamphus* sp. has a unique morphology in the, with a lower jaw longer than the upper jaw. The female fish is characterized by an orange color on the abdomen and caudal fins. The male fish has a whitish silver color, and the pectoral fin base is light yellowish pigmented with black on the edge of the anal fin. The male *Nomorhamphus* sp. also shows secondary sexual characteristics in the form of andropodium, derived from the modification of the anal fin part.

Acknowledgements. The authors would like to thank to the Deputy for Research and Development Strengthening, Ministry of Research and Technology/National Research and Innovation Agency, as the financial support who has funded this research through the basic research scheme for superior universities (PDUPT), for the fiscal year 2021, based on the contract number: 1/E1/KP.PTNBH/2021. Thanks are also given to the Ekspedisi Riset Akuatika (ERA) team for the information regarding *Nomorhamphus* sp. in Lindu, Central Sulawesi.

Conflict of interest. The authors declare no conflict of interest.

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Received: 21 April 2022. Accepted: 07 October 2022. Published online: 22 October 2022.
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How to cite this article:
Wicaksono A. W., Carman O., Soelistyowati D. T., Arfah H., Alimuddin, Herjayanto M., Solahudin E. A., Ahmadi K., 2022 Biometric characterization and morphogenesis of Anasa ornamental fish (*Nomorhamphus* sp.) endemic to Lake Lindu, Central Sulawesi, Indonesia. AACL Bioflux 15(5):2479-2487.