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Osteological Development of the Lophiid Anglerfish, *Lophius gastrophysus*

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Abstract The osteological development of the head skeleton and dorsal, pectoral, and anal fin supports, are described from cleared and stained specimens of *Lophius gastrophysus* larvae, ranging from 4.6 to 21.8 mm NL; the results are compared with those of juvenile (79.8 mm SL) and adult (398 mm SL) specimens. Tiny conical teeth are present on the premaxillary, dentary, palatine and vomer since early stage. The first three dorsal fin spines are initially positioned on the midline of body posterior to the supraoccipital, but they migrate forward with growth and become cephalic in juveniles. The forward movement of the dorsal spines is produced by the forward extension of the cartilaginous basal inside the subepidermal space. During the planktonic larval stage the pectoral fins are on the sides of body as in ordinary fishes, but they move ventrad and become leg-like in bottom living juveniles and adults. Ossification of the caudal complex of *L. gastrophysus* larvae proceeds very slowly and only the 21.8 mm NL larva has an almost completely ossified caudal complex. Eight principal caudal rays are loosely attached on the posterior edge of the hypurals and no procurrent rays are present. Larvae have well developed parhypurapophysis at the mid-portion of the urostyle which transforms into keel-like structure in juveniles and adults.

Due to their peculiar appearance and conspicuous feeding strategy, the lophiiform fishes have been extensively studied; however, most osteological studies have concentrated on adults (Morrow, 1882; Regan, 1912; Gregory, 1933; Field, 1966; Monod, 1968; Rosen and Patterson, 1969; Le Danois, 1974; Pietsch, 1974, 1981; Caruso, 1977, 1985). Osteological development of ceratioid larvae has been described by Regan and Trewavas (1932) and Bertelsen (1951, 1984), but no one has studied the osteological development of lophiid anglerfishes. The purpose of this paper is to document the osteological development and anatomy of *Lophius gastrophysus* based on specimens collected from southern Brazilian waters.

Materials and methods

The larval specimens in this study were the same ones used by Matsuura and Yoneda (1986) with some additional large specimens from the collection of the Ichthyoplankton Laboratory of the Instituto Oceanográfico da Universidade de São Paulo. The larvae were collected from the southern Brazilian coast using a 61 cm Bongo net during the survey cruises carried out from November 1969 to March 1982.

Notochord length (NL) and standard length (SL) were measured using a micrometer on a dissecting microscope. NL was taken from the anterior tip of the upper jaw to the posterior end of notochord and SL from the same place to the posterior end of the hypurals (Moser and Ahlstrom, 1970).

Seventeen larvae from 4.6 to 21.8 mm NL were cleared and stained with alcian blue and alizarin red S for cartilage and bone (Dingerkus and Uhler, 1977). The uptake of alizarin is not the first indication of ossification, but for practical purposes the uptake of this stain is usually considered to represent the onset of ossification (Dunn, 1983).

Two juvenile specimens were collected in December 1971 from the bottom, by otter trawl at Lat. 24° 29′S, Long. 46°26′W in 47 m depth. One juvenile of 79.8 mm SL was cleared and stained for bone by the enzyme method of Taylor (1967).

Material examined is deposited in the Ichthyoplankton Laboratory of the Instituto Oceanográfico da Universidade de São Paulo (IOUSP) under following catalogue numbers: L-0789-DY01, 3.2 mm NL; L-1313-DY02, 4.4 mm NL; L-0789-DY03, 4.6 mm NL; L-0336-DY04, 5.7 mm NL; L-0313-DY05, 6.2 mm NL; L-0793-DY06, 8.7 mm NL; L-0313-DY07, 8.3 mm NL; L-1351-DY08, 10.6 mm NL; L-1422-DY09, 11.5 mm NL; L-0495-DY10, 15.6 mm NL; L-0787-DY11, 7.0 mm NL;

L-0259-DY12, 6.0 mm NL; L-0334-DY13, 5.0 mm NL; L-0261-DY14, 5.2 mm NL; L-1604-DY15, 12.5 mm NL; L-2713-DY16, 14.0 mm NL; L-1596-DY17, 21.8 mm NL; J-151-DY18, 79.8 mm SL.

All larval and juvenile specimens were examined in 100% glycerin. Illustrations were made with a camera lucida attached to a binocular dissecting microscope.

In order to show the histological structure of the cartilaginous basal of the dorsal spines, formalin preserved larvae were refixed in Bouin's solution and serial sections of the head and trunk were prepared using standard histological techniques. The 7 micron thick sections were stained in Hematoxylin-Eosin.

Two adult specimens were collected in March 1982 by an otter trawl at Lat. 26°06′S, Long. 46°46′W in 125 m depth. A specimen of 398 mm SL was prepared by boiling in water, removing the flesh and reconstructing skeletal structure using all the bones.

Osteological terms and abbreviations used in this study follow those of Gregory (1933), Regan and Trewavas (1932), Bertelsen (1951), Monod (1968) and Rosen and Patterson (1969).

Description

Head skeleton and pectoral girdle. Our smallest specimens (3.2 and 4.4 mm NL) were not stained well with alcian blue, but 4.6 mm NL specimen already has the cleithrum, preopercle, hyomandibular, premaxillary, maxillary, dentary, articular and six branchiostegal rays (Fig. 1A). There are several conical teeth on the premaxillary and dentary. At 5.6 mm NL, next to appear in the mouth region is the vomer with tiny conical teeth (Fig. 1B). The fibrous ossification of the premaxillary and maxillary starts at 8.0 mm NL. Soon after, other bones of the mouth region start ossifying, but the interhyal and epihyal are still cartilaginous (Fig. 1D).

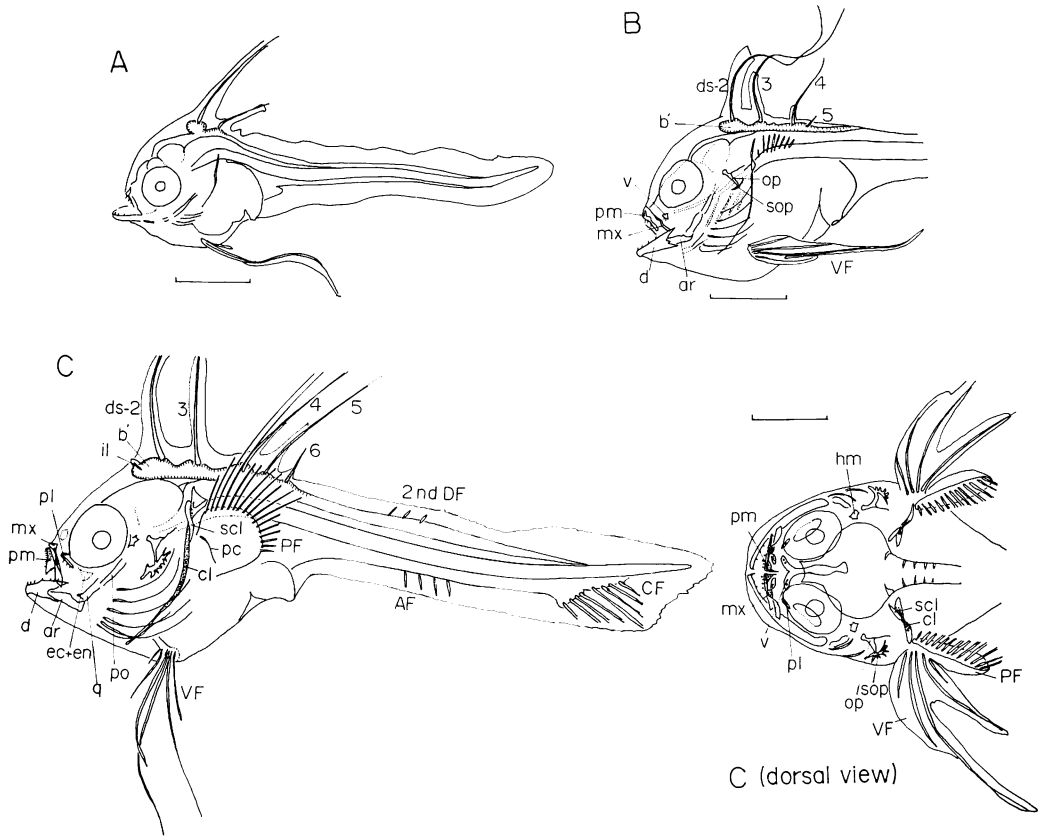
Only the posterior half of the ceratohyal is ossified at 8.0 mm NL and the anterior half remains cartilaginous until 11.8 mm NL. The size and number of conical teeth on both jaws increase with larval size. The entopterygoid and ectopterygoid appear as a single thin plate at 8.0 mm NL and their division appears clear at about 20 mm NL.

The dermal opercle and subopercle are present behind the eye at about 5 mm NL and the posterior side of the former bears a thin process which extends posteriorly to the upper portion of the cleithrum at 11.8 mm NL. The crescent-shaped subopercle is attached at the pointed ventral edge of the opercle and remains in this position through the larval and juvenile stages. At 8.0 mm NL, the ventral part of the subopercle becomes ramified (Fig. 1C). At about 10 mm NL, the opercle and subopercle become stained with alizarin and ramified processes at the ventral edge of the subopercle attain the same number as in adults (17–20).

One of the most outstanding features of Lophiiformes is the peculiar nature of the exit from the gill chamber. The usual slit behind the gill cover of either side is replaced by a slit which opens behind the base of the pectoral fin. In early stages (Fig. 1A, B), the distal tips of the elongated branchiostegals overlap the cleithrum and they become longer and more curved during later larval stage. The result is that the opercular-branchiostegal flap forms a channel and the respiratory cleft opens behind the pectoral fin base.

Origins of the preopercle and quadrate can be recognized in the smallest specimen, but the hyomandibular-preopercle-quadrate complex is formed only at 11.8 mm NL when ossification begins (Fig. 1D). The 13.0 mm NL larva has an ossified symplectic at the interspace between the eye and preopercle and a partly ossified sphenotic and pterotic on the side of the midbrain. The interopercle is also ossified at this size. The metapterygoid is the last cranial bone to ossify at about 21.8 mm NL (Fig. 1F).

The anlage of cleithrum is present in the smallest specimen (Fig. 1A). Fibrous ossification starts centrally at about 7 mm NL and proceeds dorsally and ventrally. The 8.0 mm NL larva has a membranous supracleithrum which articulates on the dorsal part of the cleithrum (Fig. 1C). At this size the postcleithrum is also visible behind the cleithrum. Pectoral fin rays first appear at about 5 mm NL and the adult complement of rays is attained at about 10 mm NL. Uptake of alizarin stain of the pectoral fin rays starts at about 13 mm NL and only the 21.8 mm NL larva has fully stained pectoral fin rays. Origins of the cartilaginous pectoral radials are visible at 11.8 mm NL and they become faintly stained with alcian blue at about 13 mm NL. In the largest larva (21.8 mm NL) these two elements are articulated with cartilaginous coraco-scapular blade which is shown



with dotted line in Fig. 1F.

The pectoral fins of *L. gastrophysus* larvae are positioned on the sides of body as in ordinary fishes, but they move ventrad and become leg-like in adults (Fig. 2). The downward movement seems to occur during metamorphosis when the planktonic larvae change to a bottom living juveniles. The dorsal part of the pectoral fin (= accessory pectoral radial) twists in a forward direction and there is a remarkable reorientation of the cleithrum on which the pectoral fin support is articulated.

Dorsal spines and their supports. *Lophius gastrophysus* larvae have well developed dorsal and pelvic fins at an early stage (Matsuura and Yoneda, 1986). Our smallest 4.6 mm NL specimen has two well developed dorsal spines which are supported by cartilaginous basal (Fig. 1A). Elongate pelvic fins are also present, but there are no pelvic bones.

One of the most remarkable changes during the larval stage is the anterior migration of the three anterior dorsal spines. Initially the second and third dorsal spines are on the dorsal midline posterior to the supraoccipital and advance anteriorly with growth (Fig. 1A–F). The forward movement of the dorsal spines is produced by the forward extension of the cartilaginous basal inside the subepidermal space. The ventral surface of the basal of the 5.6 mm NL larva starts to depart from the dorsal surface of the body, and the "worm-like" basal migrates to a forward position (Fig. 1D). The cartilaginous basal of the 11.8 mm NL larva carries all five dorsal spines on it, but in the 13.0 mm NL larva the cartilage is discontinuous. It seems that the intermediate portions of the basal between the second and third dorsal spines and between the third and fourth dorsal spines have been absorbed at about this stage. The 15.2 mm NL larva has three cartilaginous basals: the first carries the illicium and the second dorsal spine; the second one, only the third dorsal spine; and the

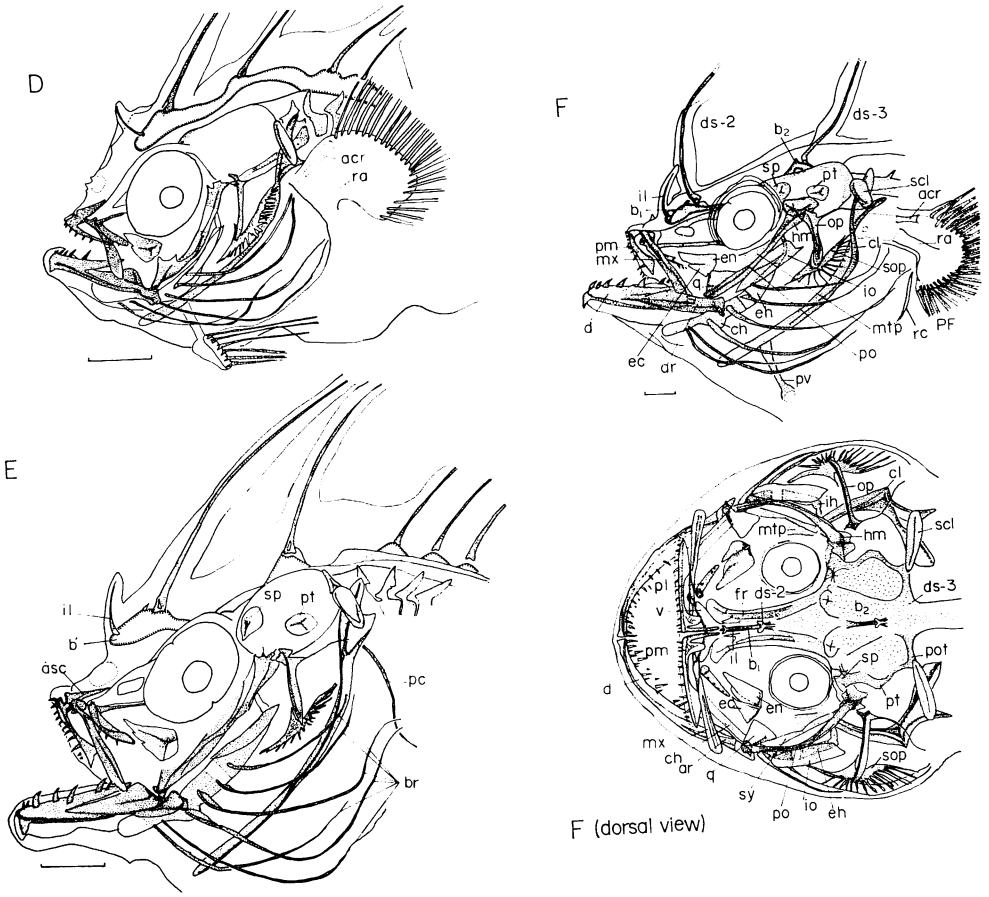


Fig. 1. Cleared and stained specimens of *Lophius gastrophysus* larvae. Alcian blue stained structures are shown in white and ossifying ones stippled. The cartilaginous basal is ticked with short lines. A, 4.6 mm NL; B, 5.6 mm NL; C, 8.0 mm NL; D, 11.8 mm NL; E, 15.2 mm NL; F, 21.8 mm NL. Scale bar=1.0 mm. Symbols: acr, accessory upper pectoral radial; ar, articular; asc, ascending process of premaxillary; b′, cartilaginous basal; br, branchiostegals; bl, basal bone of illicium and 2nd dorsal spine; b2, basal bone of 3rd dorsal spine; cl, cleithrum; ch, ceratohyal; co, coracoid; d, dentary; ds, dorsal spine; ec, ectopterygoid; eh, epihyal; en, entopterygoid; fr, frontal; hm, hyomandibular; il, illicium; ih, interhyal; io, interopercle; mtp, metapterygoid; mx, maxillary; op, opercle; pl, palatine; pc, postcleithrum; pm, premaxillary; po, preopercle; pt, pterotic; pot, posttemporal; pv, pelvic bone; q, quadrate; ra, pectoral radial; rc, respiratory cleft; scl, supracleithrum; sop, subopercle; sp, sphenotic; sy, symplectic; v, vomer; AF, anal fin; CF, caudal fin; DF, dorsal fin; PF, pectoral fin; VF, ventral fin.

third one, the posterior three dorsal spines. The posterior end of the first basal is fused on the dorsal surface of the frontal between the eyes; its anterior part is free from the frontal. In the 21.8 mm NL larva the anterior part of the first basal is approximate to the frontal, but only in the juvenile of 79.6 mm SL there is complete articulation of it in the region between the ascending processes of the premaxillaries.

In order to determine the histological structure of the basal, we prepared a series of histological sections of 7.5 mm NL larva. The cartilaginous basal appears in cross section as an elliptical structure above the skull inside the subepidermal space, surrounded by connective tissue and muscle fibers (Fig. 3). The ventral edge of the cartilaginous basal is separate from the surface of neurocranium. Figure 3B shows a cross section of basal parts of

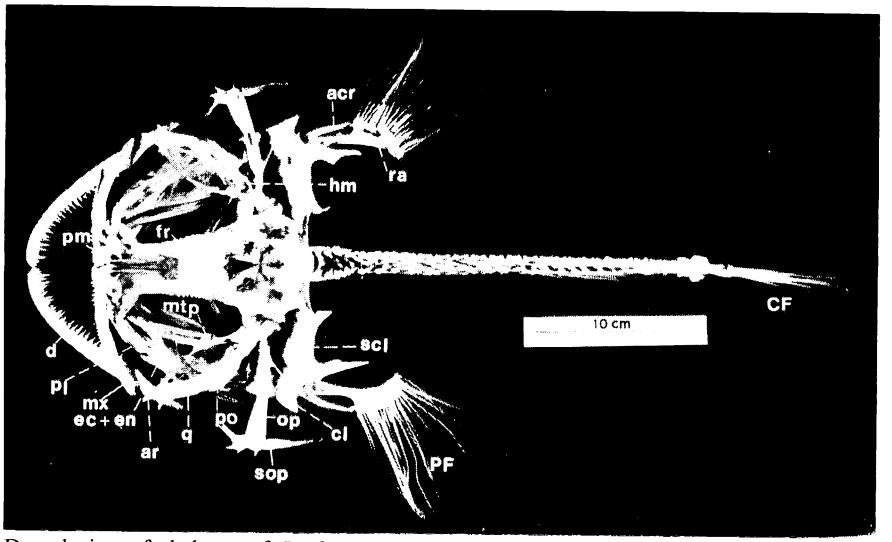


Fig. 2. Dorsal view of skeleton of *Lophius gastrophysus* adult, 398 mm SL (Photo: R. Carneiro). For abbreviations see Fig. 1.

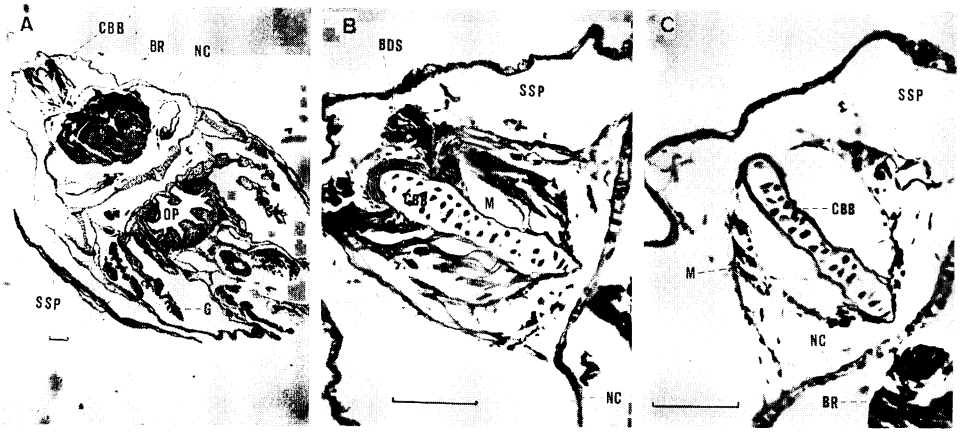


Fig. 3. Cartilaginous basal of *Lophius gastrophysus* larva (7.5 mm NL). A, cross section of basal bone at head region; B, enlarged cross section of the basal bone region of A; C, enlarged cross section of the basal bone at head region where no dorsal spine is found. Symbols: BR, brain; BDS, base of the dorsal spine; CBB, cartilaginous basal; G, gill fillament; M, muscle fibers; NC, neurocranium; OP, oropharyngeal cavity; SSP, subepidermal space. Scale bar = 100 micron.

the second dorsal spine which are articulated dorsolaterally with the cartilaginous basal. Figure 3C shows a cross section of the basal at slightly more posterior position where no dorsal fin spine is found. Here the cartilaginous basal is supported by connective tissue and a small quantity of muscle fibers.

Caudal complex. The caudal fin rays are supported by the bones of the hypural complex, which are the urostyle (= preural centrum 1 plus ural centra 1 and 2), uroneural, parhypural and hypurals. Eight principal caudal fin rays are loosely attached on the posterior edge of the hypurals and no procurrent rays are present (Fig. 4C). The caudal fin rays first appear at about 7.8 mm NL and notochord flexion starts at about 8–9 mm NL. The 8.0 mm NL larva has eight caudal rays, but no caudal bone is visible. The caudal complex of the 11.8 mm NL larva does not show any sign of ossification. In the 15.2 mm NL larva we can recognize divisions between the parhypural, hypural 1 and 2, and hypural 3+X,

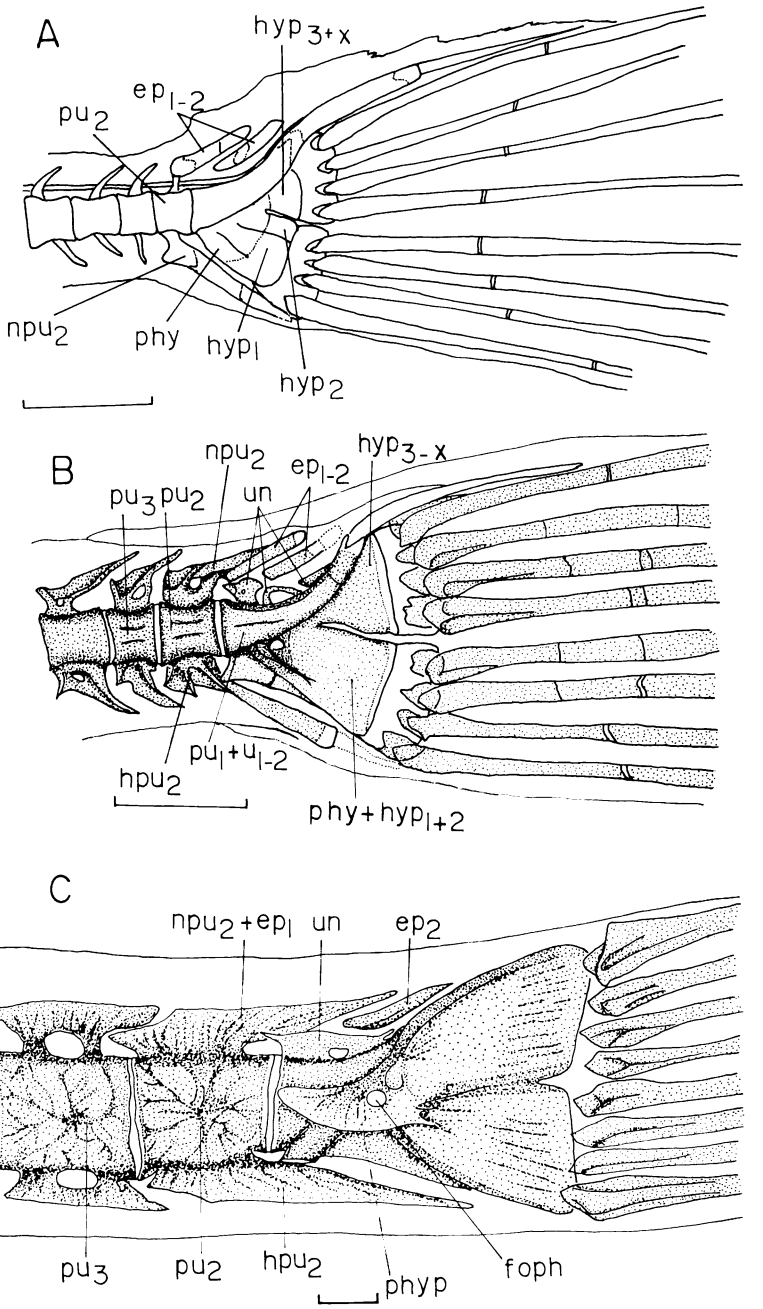


Fig. 4, Caudal complex of *Lophius gastrophysus* larvae and juvenile. A, 15.2 mm NL; B, 21.8 mm NL; C, 79.8 mm SL. Scale bar=1.0 mm. Symbols: ep, epural; foph, foramen of parhypurapophysis; hpu, haemal spine of preural centrum; hyp, hypural; npu, neural spine of preural centrum; phy, parhypural; phyp, parhypurapophysis; pu, preural centrum; u, ural centra; un, uroneural.

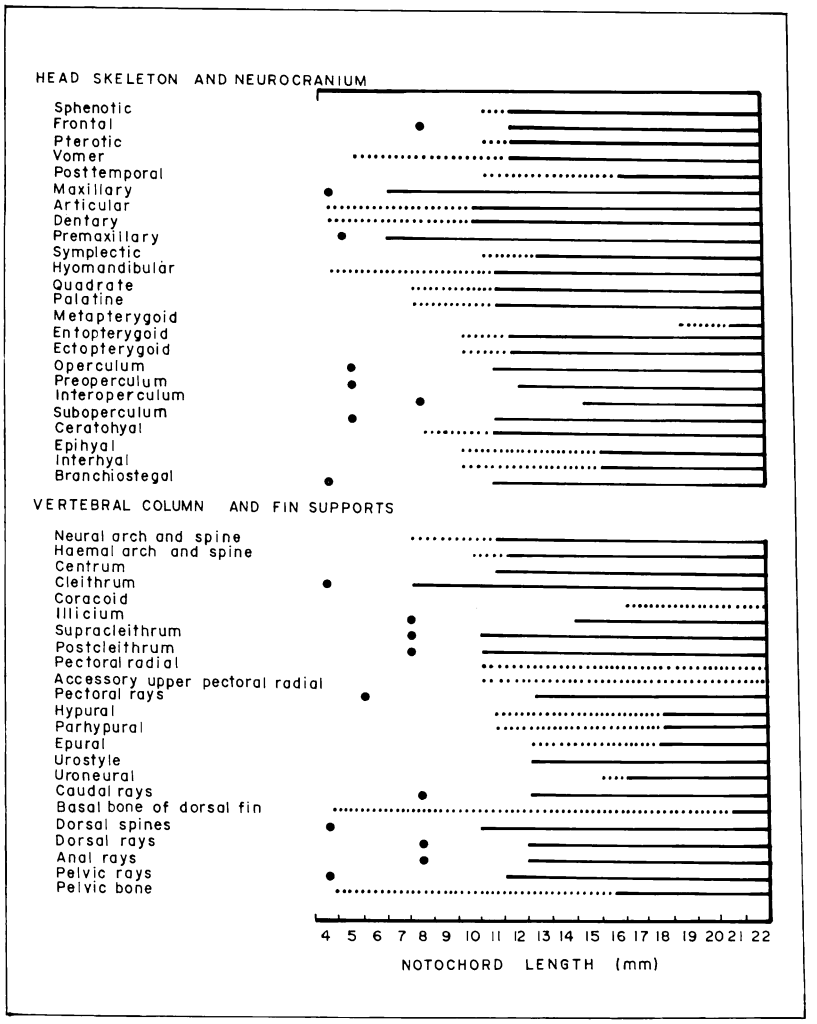


Fig. 5. Developmental sequence of head skeleton, vertebral column and fin supports of *Lophius gastrophysus* larvae. Dotted line, cartilaginous state. Solid line, ossified state. Black circle, first appearance of anlage of membrane bone.

which indicates that these bones developed from separate cartilaginous elements. The cartilaginous epural 1 is attached to the tip of the neural spine of preural centrum 2. In the 16.8 mm NL larva the partially ossified parhypural and hypural 1 and 2 are fusing. The connection between cartilaginous epural 2 and the tip of the ossified neural spine of the preural centrum 2 is more pronounced at 16.8 mm.

Caudal complex of the 21.8 mm NL larva is almost completely ossified except for the posterior tips of epural 1 and 2 and the haemal spine of preural centrum 2. The posterior one-third of the hypural plate is also ossifying (Fig. 4B). A parhypurapophysis is present on the base of the fused parhypural and hypural 1 and it will later extend laterally and form a wing-like flange in juveniles (Fig. 4C). The prezygapophysis of the parhypural extends forward on both sides and forms a haemal channel. As shown by Monod (1968), the haemal chord which runs along the ventral side of the vertebral column through the haemal channel, enters into the tunnel-like haemal channel of the parhypural and bifurcates into two branches. These come out on both sides of hypural plate through the foramina of the wing-like projections of the parhypurapophysis. Three uroneural elements appear on the dorsal side of

urostyle in 21.8 mm NL larva, but they later become fused to the urostyle in juveniles (Fig. 4C). Epural 1 fuses to the neural spine of preural centrum 2 in juveniles, but epural 2 remains autogenous during larval and juvenile stages.

Detailed sequences of cartilaginous-osteological development of *L. gastrophysus* larvae are shown in Fig. 5.

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アンコウ科魚類 *Lophius gastrophysus* の骨格形成

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アンコウ科魚類 *Lophius gastrophysus* の仔魚の頭部骨格と背鰭・胸鰭・尾鰭およびその支持骨の形成過程を記載した．円錐歯は仔魚前期から主上顎骨その他に見られた．背鰭の前方の3棘は前期仔魚では後頭部に位置するが，成長につれて前方に移動し，稚魚期には前頭部に位置する．この前進は皮下間隙中の軟骨性担鰭骨の前方への伸展によってもたらされる．プランクトン生活期の仔魚の胸鰭は，普通の魚類のように体側についているが次第に腹側に移り，底性生活をする稚魚・成魚では完全な腹位となり足状となる．尾鰭支持骨の骨化は非常に遅く始まり，21.8 mm NL の仔魚でようやくほぼ全ての支持骨が骨化する．尾鰭の8軟条は下尾骨後端にゆるやかに接続している．尾骨の側面には発達した parhypurapophysis があり稚魚期になるとキール状になる．