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Skeletal Structure of the Branchial Arches in *Paralichthys olivaceus* (Temminck et Schlegel)

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Skeletal structure of the pharyngeal apparatus in *Paralichthys olivaceus* is described with additional terminology for detailed anatomy. SEM observation revealed that the upper pharyngeal is comprised of four pairs of toothplates: the toothplate on the third epibranchial, the toothplate on the second pharyngobranchial, the toothplate on the third pharyngobranchial and the toothplate on the fourth pharyngobranchial. All the pharyngeal toothplates have conical teeth. Special attention was paid to the interarcual connection between the first epibranchial and the second pharyngobranchial for comparison with percoids.

Key words: branchial arch, interarcual cartilage, *Paralichthys olivaceus*, pharyngeal toothplate, principal process, uncinate process

Introduction

The Japanese flounder, *Paralichthys olivaceus* (Temminck et Schlegel) is a typical predacious species among flatfishes (Tsuruta and Omori, 1976; Kikuchi, 1981). The flounder drastically switch their food preference during growth and development, starting with zooplankton as larvae and finally coming to be piscivorus as adults. As this serial feeding system was being established, the feeding habit was empirically confirmed by culturists. However, information is still limited about the feeding mechanism, particularly the anatomical structure (*i.e.* Hikita, 1934; Amaoka, 1969) which may contribute to the mass production of larvae and juveniles of the present species.

We provide here an exhaustive osteological description for the branchial arches of *P. olivaceus*, referring to the terminology and homology for each skeletal component in comparison with the cases of ctenosquamates.

Materials and Methods

Fish were obtained by beam-trawl at 10 m depth from natural population indigenous to the Sea of Japan off the coast of Kasumi, Hyogo Prefecture, in 1972. The fish were preserved in a 10% formalin solution and deposited at the Fisheries Research Station, Kyoto University, Maizuru. Skeletal specimens were prepared according to an improved version of the double staining method (Kawamura and

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Hosoya, 1991). Dried toothplates were coated by ion-sputter and observed with a JSMT220A scanning electron microscope. Osteological terminology on the upper pharyngeal units basically follows that of Travers (1981), while terminology on chondral units basically follows that of Nelson (1969). Anatomical abbreviations are as follows:

BB	basibranchial
BH	basihyal
CB	ceratobranchial
CP	cartilaginous copula
EB	epibranchial
EB1	first epibranchial
EB2	second epibranchial
EB3	third epibranchial
EB4	fourth epibranchial
HB	hypobranchial
IAC1*	first interarcual cartilage
IAC2*	second interarcual cartilage
IBAP*	interbasibranchial articulatory process
LP	lower pharyngeal
PB	pharyngobranchial
PB1	first pharyngobranchial
PB2	second pharyngobranchial
PB3	third pharyngobranchial
PB4	fourth pharyngobranchial
PPEB*	principal process of epibranchial
SCJ*	synchondral joint
TEB3	toothplate on third epibranchial
TPB2	toothplate on second pharyngobranchial
TPB3	toothplate on third pharyngobranchial
TPB4	toothplate on fourth pharyngobranchial
UNPEB	uncinate process of epibranchial
UNPEB3	uncinate process of third epibranchial
UP	upper pharyngeal

Abbreviations with asterisk show the new terminology proposed in the present paper.

Results

Skeletal composition of branchial arch. In *P. olivaceus,* three basibranchial bones (BB) are mesially aligned to compose a pivot of the basibranchial series with associated elements (Fig. 1). The

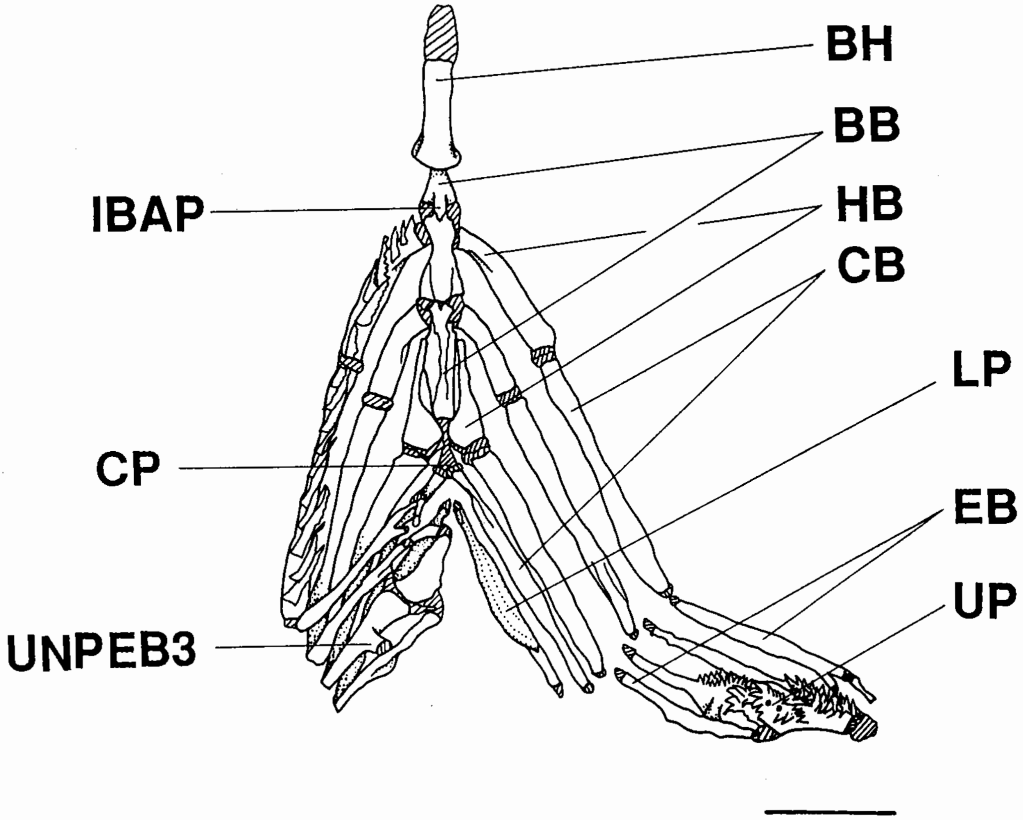

Fig. 1. Branchial arches of the Japanese flounder *Paralichthys olivaceus*, general dorsal view. Cartilaginous portion is cross-hatched. Scale indicates 3 mm. Abbreviations are shown in the text.

anteriormost in the series is occupied by the basihyal (BH), while the posteriormost by a cartilaginous copula (CP). With the posterior vertical plain, viz. interbasibranchial articulatory process (IBAP), the first and second basibranchials are mesially fastened to the anterior crevice of the subsequent basibranchial respectively. This condition is called merely "suture" in some sinistral flounders by Amaoka (1969), or "wedge-like articulation" in the pleuronectines such as *Psettichthys melanosticus, Pleuronectes punctatissimus* and *Paralichthodes algoensis* by Sakamoto (1984). Amaoka (1969) also denoted in *P. olivaceus* that the suture was formed only between the first and the second basibranchial bones, but not between the second and the third. This is not the case in our present observation as has described above. The first basibranchial lacks the lateral foramen (See Chapleau and Keast, 1988). All the basibranchial bones and the basihyal are completely edentate.

The basibranchial series support four pairs of branchial arches. No asymmetrical distinction could be observed in the gross morphology of branchial arches between both sides. The branchial arch of each side is basically composed of the hypobranchial (HB), ceratobranchial (CB), epibranchial (EB), and pharyngobranchial (PB) as generally observed in euteleosteans (Figs. 1–3). The fourth branchial arch lacks the well-ossified hypobranchial as in other adult teleosts as Nelson (1970) stressed (See Roberts,

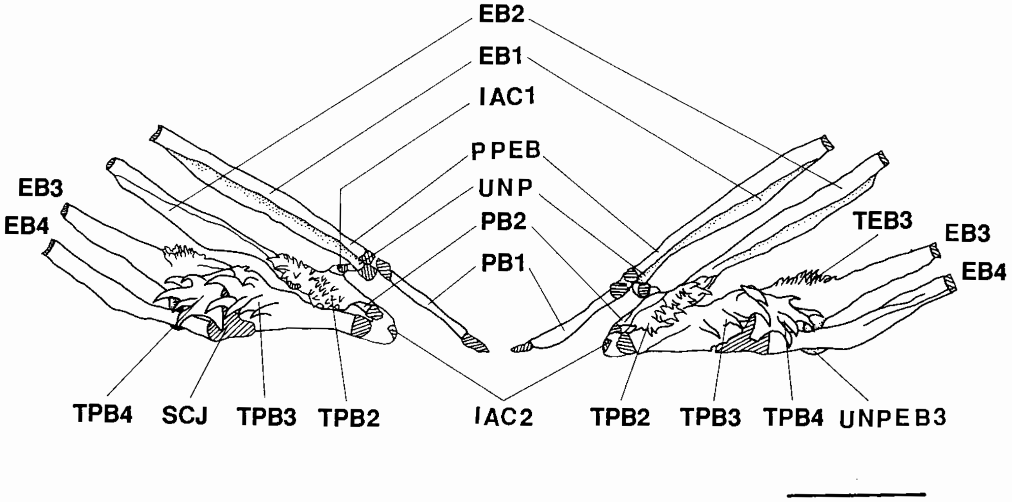

Fig. 2. Dorsal parts of branchial arches in *Paralichthys olivaceus*, ventral view. Left, blind side; right, ocular side. Cartilaginous portion is cross-hatched. Scale indicates 2 mm. Abbreviations are shown in the text.

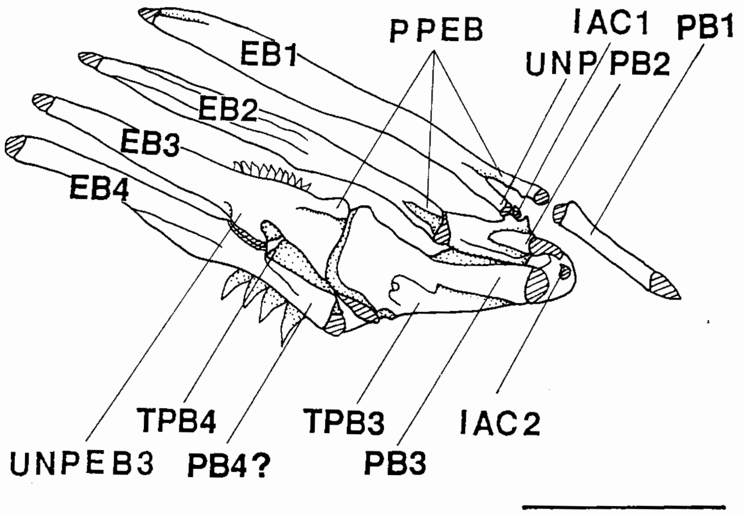

Fig. 3. Dorsal parts of left branchial arches in *Paralichthys olivaceus*, dorsal view. Cartilage is cross-hatched. Scale indicates 2 mm. Abbreviations are shown in the text.

1984, p. 203 as well). Each branchial bone in an arch is connected through the chondral articulation. The first pharyngobranchials, often called the suprapharyngobranchials (*i.e.* Nelson, 1969), stand straight dorsad to suspend the whole pharyngeal apparatus. The second pharyngobranchial is articulated with the third pharyngobranchial at its distal end via a cartilaginous ligament (Figs. 2 and 3). Gill-rakers are located on the membranous coverage over the hypobranchial, the ceratobranchial, and the epibranchial. The fifth ceratobranchials are transformed to a pair of the lower pharyngeals. This is the basic skeletal disposition.

Structural modifications were noted in the 2nd–4th branchial arches of *P. olivaceus* (Figs. 2 and 3). The upper tip of first epibranchial bifurcates to two remarkable processes, viz. anterodorsal and postero-ventral ones. The postero-ventral process has already been given a term "uncinate process (UNP)" by Rosen (1973), while the antero-dorsal process remains to be unnamed. So, we propose a new terminology "principal process (PPEB)" for the antero-dorsal process in the present paper. .The principal process of epibranchial is articulated with the freestanding first pharyngobranchial, while the uncinate process contacts the second pharyngobranchial-toothplate complex at its distal end via an interarcual ligament (IAL). This ligament usually contains a small interarcual cartilage (Fig. 2, IAC1), however it often lacks the cartilage in cultured flounder. Another interarcual ligament associated with the second interarcual cartilage (Fig. 2, IAC2), connects the second and the third pharyngobranchials at each distal end. The bifurcation in the second epibranchial became indistinct by a skeletal outgrowth or membranous ossification which turned out to interconnect both processes (Fig. 3). In the third epibranchial, its distal end is uniquely transformed by the additional skeletal outgrowth: a prominent process projects caudad from the lateral half way and meets its short counter process from the fourth epibranchial. This prominent process seems to be identical with the uncinate process as described by Rosen (1974) on every epibranchial of salmoniform fishes.

Pharyngeal toothplate. *P. olivaceus* has upper and lower pairs of pharyngeal toothplates as

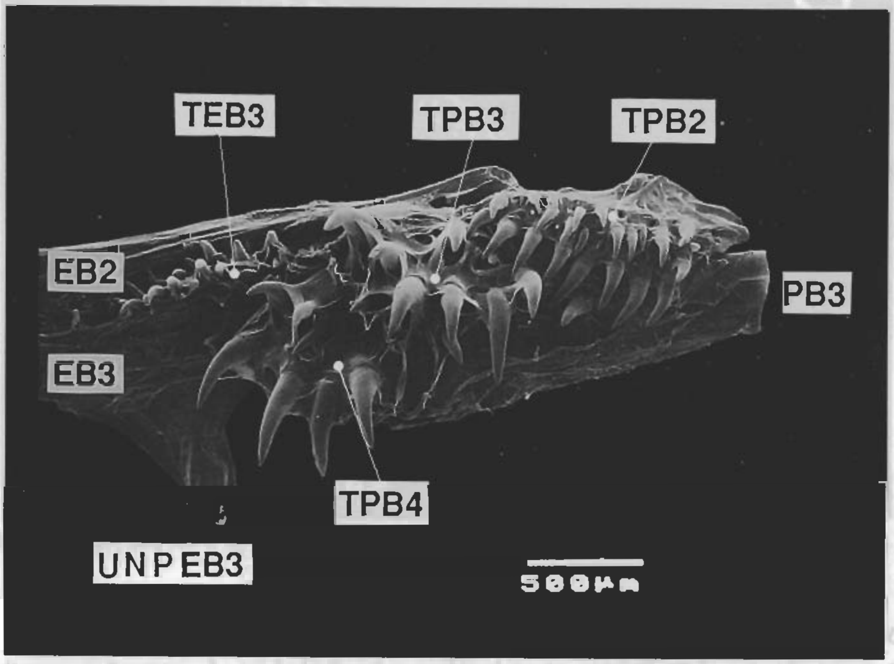

Fig. 4. Upper pharyngeals on right branchial arches in *Paralichthys olivaceus*, ventral view by SEM. Abbreviations are shown in the text.

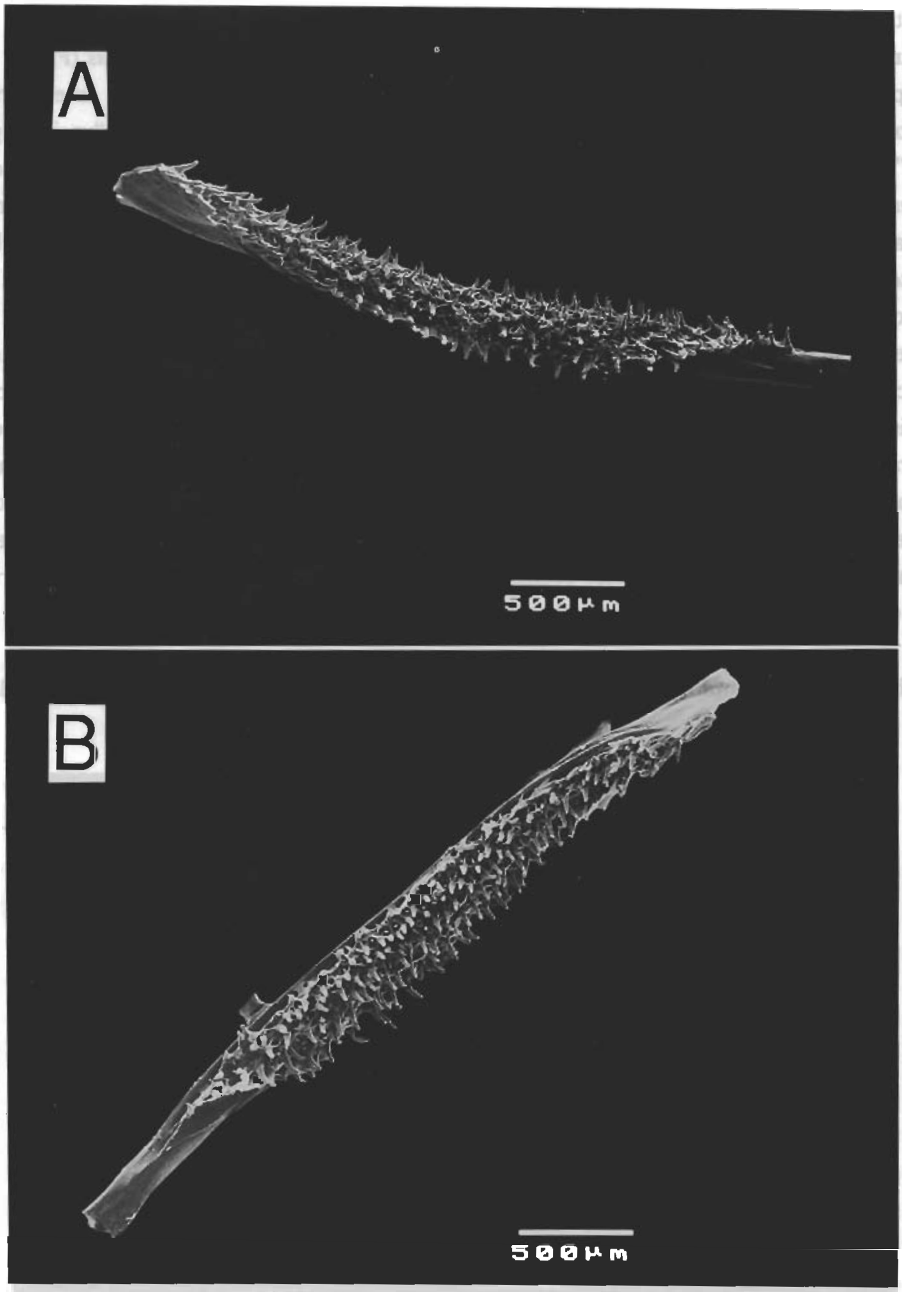

Fig. 5. Lower pharyngeals of *Paralichthys olivaceus*, dorsal view by SEM. A, left lower pharyngeal; B, right lower pharyngeal.

generally seen in teleosts (Fig. 1).

The upper pharyngeal toothplates on each side can be divided into four morphotypes: the third epibranchial toothplate (TEB3), the second pharyngobranchial toothplate (TPB2), the third pharyngobranchial toothplate (TPB3), and the fourth pharyngobranchial toothplate (TPB4) which all occupy the dorsal surface of the pharyngeal cavity (Figs. 2–3).

The third epibranchial toothplate is the smallest and is fused with the anterior margin of the third epibranchial bone. The second pharyngobranchial toothplate is the second smallest, enclosing the second pharyngobranchial bone at its dorsal base (Fig. 3). The third pharyngobranchial toothplate is rather developed, enclosing the third pharyngobranchial bone as well. The fourth pharyngobranchial toothplate is the largest, attached to both the third and fourth epibranchials by the synchondral joint (Fig. 3, SCJ). All the upper pharyngeal teeth project ventro-laterally. As for the number of the upper pharyngeal teeth, SEM observation reveals 15–16 in the third epibranchial, 24 in the second pharyngobranchial, 7–10 in the third pharyngobranchial, and 5–7 in the fourth pharyngobranchial (Fig. 4). Neither difference in dental count there seems to be, nor shape between each side. All the upper pharyngeal teeth are conical: teeth on the fourth pharyngobranchial toothplate are the largest, while those on the third epibranchial toothplate are the smallest.

Numerous denticles closely cover the three-fourth of the length of the lower pharyngeal and project over irregular directions (Fig. 5).

Discussion

Although there have been many reports in the various fields of flatfish biology, osteological information for the comparison is still limited. This makes it difficult to polarize the characters clarified in *P. olivaceus.*

As far as the branchial arches are concerned, *P. olivaceus* seems to be rather more primitive than any other flatfishes for retaining the toothplate on the third epibranchial. Its single derived feature is the loss of the toothplate lying on the second and third hypobranchials (See Amaoka, 1969), which is apomorphous only to *Psettodes erumei.* We provide comments on the characteristics notable in an anatomical aspect as listed below.

Fourth basibranchial. In the basibranchial series, a remarkable copula follows the third basibranchial in *P. olivaceus.* The cartilaginous copula is comparable with the fourth basibranchial (see Nelson, 1969, p. 480) which is stained blue by alcian blue. This finding is opposite to that in the Cynoglossidae. All the cynoglossids lack the fourth basibranchial except for *Symphurus* where it degenerates into a small roundish cartilage (Chapleau, 1988). As for the other remaining flatfish members, the cartilage is quite distinct (Cole and Johnstone, 1901; Amaoka, 1969; Sakamoto, 1984). In these flatfishes, the fourth basibranchial generally functions to support the paired fourth ceratobranchials. *P. olivaceus* has a medium adult size of 80 cm TL in maximum as a flatfish with a large gape from the wide oral cavity. Contrarily, cynoglossids, on their way to progenesis grow up to 30 cm TL with a small gape from the narrow oral cavity. These characteristics seem to be incorporated with the contradistinction observed in the way of the fourth basibranchial between *P. olivaceus* and the Cynoglossidae.

Principal process and uncinate process. In *P. olivaceus,* the upper tip of the first epibranchial bifurcates to the principal process and the uncinate process. The bifurcation in the first epibranchial was recognized not only in sinistral flounders such genera as *Psettodes, Citharoides,* and *Lepidoblepharon,* but also in many dextral flounders (Amaoka, 1969; Sakamoto, 1984). It is notable that the uncinate process of the third epibranchial is directly articulated with an unnamed counterpart from the third epibranchial in *P. olivaceus* (Fig. 3).

Interarcual cartilage. Since Rosen and Greenwood (1976) described the presence of a cartilaginous element, the so-called interarcual cartilage between the first epibranchial and the second pharyngobranchial, it has been incorporated in the definition of euteleostean lineages (Travers, 1981).

In the way of connection between the first epibranchial and the second pharyngobranchial in ctenosquamates, Rosen and Patterson (1990) recognized six morpho-, or submorphotypes, attaching importance to the interarcual cartilage: (1) the nodule in some myctophids; (2) the short plug in anoplogasterid beryciforms and kurtids; (3) the rodlike form in more normal percoid fishes; (4) the ossified rod in synbranchids and most carapids; (5a) a short, stout ligament in *Percopsis* ; (5b) a more elongate ligament as in *Raniceps* and *Trichodon,* which may contain a small interarcual cartilage as in certain gadiformes (Markle, 1989).

In *P. olivaceus,* the interarcual ligament interconnects the first epibranchial with the second pharyngobranchial, containing a small interarcual cartilage which is often absent in cultured individuals. Also, Chapleau and Keast (1988, Fig. 18) showed the presence of an interarcual cartilage in an achirid flatfish as in the same way. These observations lead us to assume the case for flatfishes is comparable to type (5b).

Rosen and Patterson (1990) speculated on the transformation series in percoids and related taxa that type (1) is primitive, (2) is somewhat more derived, (3) is the derived percoid condition, (4) is surely derived from (3), and type (5) is derived relative to types (1) to (3). If applying our conclusion to this speculation, Pleuronectiformes can be presumed to have originated ranging from myctophids to the primitive percoid stock at as early a time, *i.e.* as the Eocene (Norman, 1934). What this presumption suggests, is only that they can be backed to the ancestor much earlier than extending to percoid as noted also by Amaoka (1969).

Fourth pharyngobranchial. The primitive percoid branchial skeleton is characterized by having a well-developed toothplate on the fourth pharyngobranchial and lacking its corresponding endoskeletal element (Nelson, 1969; Johnson, 1980). Contrarily, we recognized an elongate endoskeletal element attached onto the toothplate in *P. olivaceus,* which seems to be identical with the fourth pharyngobranchial judging from the outer shape (Fig. 3). It continues ventrad to the the fourth epibranchial, making a single rod. The endoskeletal element may give a key to presume the interrelationships in higher groups. However, prior to systematic application, it is also necessary to be tested on its osteological homology, viz. the fourth pharyngobranchial proper or the uncinate process of the fourth epibranchial from the ontogenetic point of view.

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ヒラメの鰓弓における骨格構成

細谷　和海・河村　功一

従来，情報が限られていたヒラメの鰓弓の骨格構成を，咽頭歯板を中心にSEM観察と透明骨格標本の微解剖により明らかにした。鰓弓は左右相称で，両対の骨要素に大きな形態的分化は認められなかった。上咽頭歯板は，対在する第3上鰓骨咽頭歯板，第2咽鰓骨咽頭歯板，第3咽鰓骨咽頭歯板，第4咽鰓骨咽頭歯板からなり，それぞれ大きさの異なる円錐歯を備えていた。また，第1上鰓骨鈎状突起と第2咽鰓骨間に介在する，鰓弓間軟骨の存在様式をスズキ型魚類と比較し，若干の系統的考察を行った。