Phylogenetic aspects of tooth and jaw structure of the Medaka, *Oryzias latipes,* and other beloniform fishes

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(With 3 plates and 2 figures in the text)

Tooth structure is described for adult male, female, and juvenile *Oryzias latipes* (Temminck & Schlegel), the Medaka. Adult males have enlarged, unicuspid teeth posteriorly on the premaxilla and dentary. Oral teeth are smaller and more numerous in females, in which no tooth is notably larger than the others. Juveniles have numerous small teeth from about 3 mm SL (standard length) onwards. By about 16 mm SL, males begin to develop the large posterior teeth, as well as other secondary sexual characters. Lower and upper pharyngeal teeth of both males and females are fine, and in numerous even rows.

The large, posterior oral teeth of males are fully-ankylosed to the attachment bone, and, hence, are not depressible. In female Medaka, as in the Halfbeak *Dermogenys pusillus* van Hasselt, the oral teeth have a ring of unmineralized collagen at the base, and are not depressible. Pharyngeal teeth of Medaka have a ring of unmineralized collagen at the base, and a distinct wedge of collagen absent posteriorly, such that the pharyngeal teeth may be depressed.

Bone in adult Medaka is acellular. Incompletely mineralized teeth, acellular bone, a protrusible upper oral jaw, and a mobile branchial apparatus with an interhyal bone, form a complex characteristic of advanced teleosts. The Medaka differs in several ways from the model advanced teleost: absence of an interhyal bone, ascending and articular processes of the premaxilla, and the rostral cartilage, as well as presence of cartilaginous symphyses between the dentaries and premaxillae, all contribute to the fixed or nonprotrusible jaws.

Reduction in the premaxilla is a derived character within beloniform fishes for which an enlarged, beaked outer jaw is considered plesiomorphic.

Contents

Introduction

Theories of comparative vertebrate anatomy have been based traditionally upon development and morphology of tetrapods, in particular mammals. Studies of the comparative anatomy of bony fishes often use so-called lower (primitive) teleosts, such as the Goldfish (*Carassius auratus*), or higher (advanced) teleosts, such as the Mummichog (*Fundulus heteroclitus*), or the Medaka (*Oryzias latipes*), and present the results as applicable to all teleosts, and sometimes also as primitive for tetrapods. However, advanced teleosts are distinguished by a set of anatomical, functional morphological and physiological characters (see Moss, 1961*a*, *b*, 1965; Fink, 1981; Lauder, 1983; Rosen, 1985; Parenti, 1986) which means that generalizations about all teleosts cannot always be made from one representative species alone.

The Adrianichthyidae (*sensu* Rosen & Parenti, 1981), the Ricefishes and allies, had long been considered Killifishes, order Cyprinodontiformes, until a recent series of papers enunciated their close relationship to the exocoetoids, including Flying fishes, Halfbeaks, Sauries and Needle-fishes, in the order Beloniformes (Parenti, 1981; Rosen & Parenti, 1981; Collette *et al.*, 1984). Adrianichthyids comprise approximately 20 nominal species in four genera, *Oryzias*, *Adrianich-thys*, *Xenopoecilus* and *Horaichthys* (see Rosen, 1964).

The Medaka, *Oryzias latipes* (Temminck & Schlegel) is an advanced teleost that is well known to both research biologists and aquarists. This species is a popular aquarium fish that has been used actively in experimental embryology and genetics throughout this century (see Briggs & Egami, 1959; Yamamoto, 1967, 1975; Uwa & Ojima, 1981; Sakaizumi *et al.*, 1983; Sakaizumi, 1985).

However, the recognizable species of Ricefishes and their relationships to each other have never been defined clearly. There are no published analyses of Ricefish species. Furthermore, in spite of the extensive data available on genetics and experimental embryology of the Medaka, few or no comparative anatomical data exist. Basic comparative developmental anatomy is also only poorly known (Rugh, 1948; Iwamatsu & Hirata, 1980; Rosen & Parenti, 1981; Yabumoto & Uyeno, 1984).

One purpose of this report is to describe the structure and development of the oral and pharyngeal jaws, including teeth, of the Medaka. These descriptions, along with comparative anatomical data from other beloniform fishes, are used to infer the systematic significance of this aspect of the anatomy of the Medaka to address the question: how is the Medaka representative of advanced teleosts, and how is it different? Thus, the object of this report, and others in progress, is to increase the comparative anatomical data, along with systematic inferences on the evolution of advanced teleost fishes. A further goal is to provide a phylogenetic framework within which the immense amount of embryological and genetic information may be interpreted.

Materials and methods

Tooth and bone structure was observed in juvenile and adult male and female Medaka, *Oryzias latipes*. Alcohol-preserved specimens and those cleared and stained solely with alizarin, American Museum of Natural History (AMNH) 26760SW, or counterstained with alcian blue (AMNH 10344SW, 38404SW), California Academy of Sciences (CAS) 57464, according to the method of Dingerkus & Uhler (1977), were examined.

Freshly preserved specimens from commercially imported laboratory stocks of the orange-red variety of *Oryzias latipes*, maintained in the Aquatic Research facility, Steinhart Aquarium, and ontogenetic series

of wild-type Medaka raised in the Lake Biwa Aquarium, Ohtsu City, Japan, now at CAS (57464, CAS 57465), were used for dissection and histological examination.

For histology, live fish were preserved in 10% formalin, decalcified in 3% HCl in 70% EtOH, and embedded in paraplast. Serial sections, approximately 15 μm thick, were counterstained with haematoxylin and eosin according to the schedule of Humason (1979).

One premaxillary and one upper pharyngeal bone of the trypsin and KOH cleared and stained male and female *Oryzias latipes* (AMNH 26760SW and 10344SW), and one premaxilla of the Halfbeak *Dermogenys pusillus*, Stanford University (now at CAS) SU 34945, were removed, cleaned manually of flesh, dehydrated in 95% EtOH and also air dried. Bones and teeth were observed using a Zeiss SV8 stereomicroscope and an Hitachi S520 Scanning Electron Microscope (SEM).

Museum register numbers of additional species of comparative lots of alcohol-preserved and cleared and stained atherinomorph (beloniform, cyprinodontiform and atherinoid, following the classification of Rosen & Parenti, 1981) fishes are mentioned in the text or are available on request from the author.

Standard length (SL), rather than age, is given to indicate ontogenetic stage of Ricefishes so that data are comparable with those of previous studies on Medaka development (e.g. Egami & Ishii, 1956; Takeuchi, 1967, 1968). Also, age at preservation was not recorded for specimens in the ontogenetic series raised in the Lake Biwa Aquarium.

Body length (BL), the distance from the posterior margin of the opercular membrane to caudal flexure, is used instead of SL for beloniforms with elongate oral jaws (Collette, 1982).

Results and comparisons

Oral jaw

Bone structure

The upper oral jaws of both male and female Medaka include an edentulous maxilla, and a dentigerous premaxilla that lacks the elongate ascending and prominent articular processes characteristic of other neoteleost, including atherinomorph, fishes (Figs 1, 2; Yabumoto & Uyeno, 1984: figs 5 and 6). In males, the posterior ramus of the maxilla is reduced relative to the size of the ramus in females (compare Figs 1 & 2). There is no rostral cartilage in any developmental stage examined.

The lower oral jaw is composed of a dentigerous dentary, an anguloarticular, and a distinct, small retroarticular (Figs 1, 2; Yabumoto & Uyeno, 1984: figs 5 and 6). In adults, Meckel's cartilage is just one-half the length of the dentary, as opposed to approximately the length of the dentary in, for example, the atherinoid *Menidia*, the cyprinodontiform *Aphyosemion* (Parenti, 1981: fig. 31), and the Halfbeak, *Dermogenys pusillus* (CAS-SU 34945). In juvenile Medaka, 4–7 mm SL, Meckel's cartilage extends nearly the entire length of the dentary. Thus, in Ricefishes, resorption and differential growth of Meckel's cartilage is judged to be an ontogenetic reduction, whereas, in contrast, loss of the rostral cartilage is judged to be a phylogenetic reduction because it is not present in any ontogenetic stage.

The symphyses of the left and right premaxilla, and of the left and right dentary, are formed by a large block of cartilage on the anterior extent of each of these bones (Figs 1, 2; Yabumoto & Uyeno, 1984: figs 5 and 6). All bone in the oral jaws of adult Medaka is acellular (Parenti, pers. obs.; see Parenti, 1986). Bone of at least the premaxilla is acellular in the Needlefish *Belone belone* (Linnaeus) (Astakhov, 1979: fig. 2), and in the operculum and lower oral jaw elements of the Needlefishes *Potamorrhaphis guianensis* (Schomburgk) and *Strongylura notata* (Poey) (Moss, 1961b). Development of bone in Medaka, using ontogenetic series of wild-type specimens from
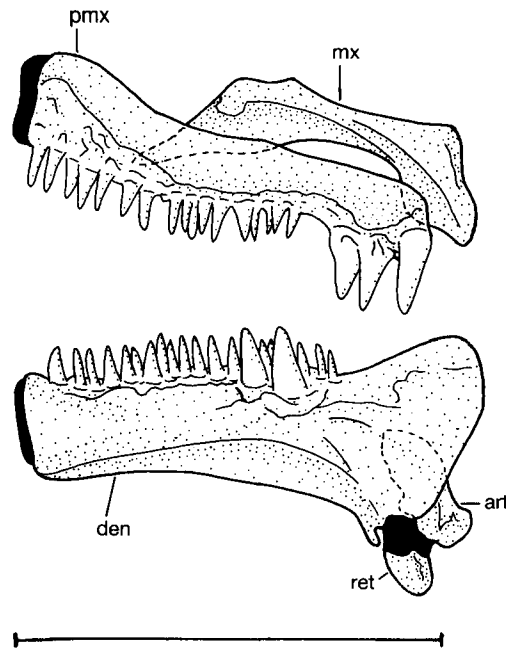
FIG. 1. Left lateral view of upper and lower oral jaw, adult male, *Oryzias latipes*, AMNH 10344SW, 24 mm SL. Anterior is to the left. Cartilage blackened, bone stippled. Scale bar represents 1 mm. art, anguloarticular; den, dentary; mx, maxilla; pmx, premaxilla; ret, retroarticular.

northern and southern Japan currently being raised at the California Academy of Sciences, will be described as the results of a separate study.

Tooth structure

The unicuspid, uniserial, oral jaw teeth appear initially in males and females of about 2–3 mm SL as cones of dentine that eventually grow to meet the pedicels of attachment bone. Females (Fig. 2) have smaller and more numerous teeth than do males (Fig. 1). Large teeth on the posterior extent of the premaxilla and dentary of males (Fig. 1; Yabumoto & Uyeno, 1984: fig. 6) appear at about 16 mm SL.

Premaxillary teeth of females have a ring of unmineralized collagen between the dentine cone and bone of attachment (Plate Ia). The anterior teeth of males also have a fibrous connection to the attachment bone, but the enlarged, posterior teeth are fully-ankylosed to the attachment bone (Plate Ib).

Teeth on the premaxilla and dentary of the Halfbeak, *Dermogenys pusillus*, are arranged in two rows (Plate IIa). All oral jaw teeth have a fibrous connection to the pedicels of attachment bone; there is a distinct wedge of collagen between the cap of dentine and the bone (Plate IIb). Moy-Thomas (1934) reported that teeth of the Needlefish *Belone* sp. attach to the dentary either by direct fusion to upgrowths from the dentary or by what he viewed as separately developed pedicels of bone. Whether the teeth themselves are responsible for inducing (or producing) attachment bone, or if attachment bone is an outgrowth of the dentary has not been resolved;
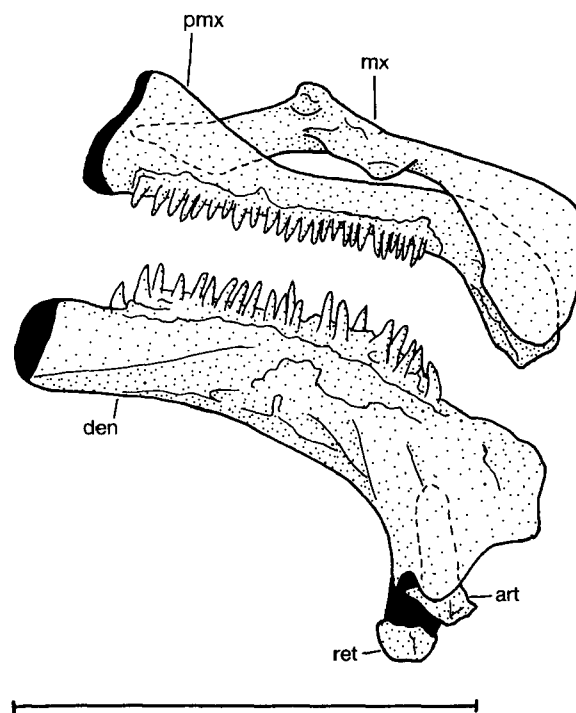
Fig. 2. Left lateral view of upper and lower oral jaw, adult female, *Oryzias latipes*, AMNH 10344SW, 25 mm SL. Anterior is to the left. Cartilage blackened, bone stippled. Scale bar represents 1 mm. Abbreviations as in Fig. 1.

presence of presumptive teeth may be necessary for the formation of dermal bone (see Huysseune, 1983; Parenti, 1986).

In all beloniforms examined so far, oral jaw teeth are attached either directly or indirectly to pedicels of bone. Pedicellate teeth may be an additional derived character to be added to the list of those defining beloniforms as proposed by Rosen & Parenti (1981) (see *Pharyngeal jaw*, below), although pedicellate teeth among advanced teleosts are not limited to beloniforms, being present in, for example, cichlids (Huysseune, 1983).

Pharyngeal jaw

Bone structure

Six derived characters of the branchial apparatus were given by Rosen & Parenti (1981:17) to define beloniform fishes: three involve the dorsal portion of the gill arches (Rosen & Parenti, 1981: figs 11–17) (a small or absent interarcual cartilage, relatively small second and third epibranchials, and vertical reorientation of the second pharyngobranchial); one the ventral portion of the gill arches (large, ventral flanges on the fifth ceratobranchials); and two the hyoid bar (only a single, ventral, hypohyal bone, and no interhyal bone) (see also Yabumoto & Uyeno, 1984: figs 8–9.)

The lower pharyngeal (fifth ceratobranchial) bones are closely apposed or fused, although these conditions are not unique to beloniforms (Rosen, 1964). The upper pharyngeal (third infrapharyngobranchial) bones are also closely apposed, as they are in the Needlefishes and
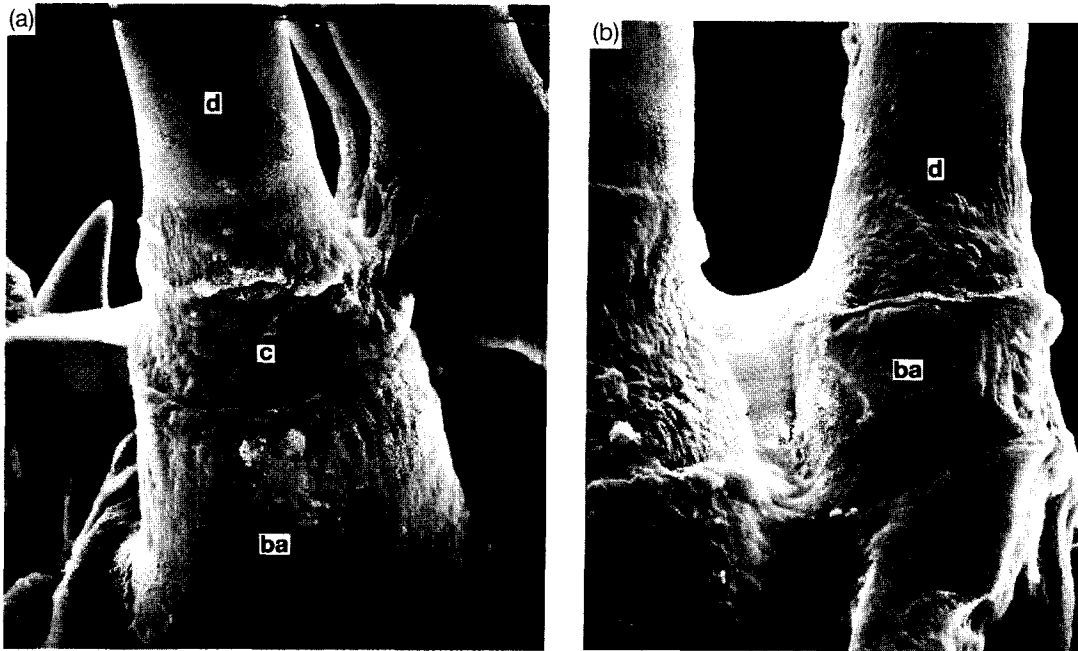
PLATE I. *Oryzias latipes*, AMNH 26760SW, premaxillary teeth. (a) Female, 24 mm SL, SEM × 1500; (b) Male, 24 mm SL, SEM × 1500. Abbreviations: ba, pedicel of bone of attachment; c, unmineralized collagen; d, dentine. Anterior is to the left.
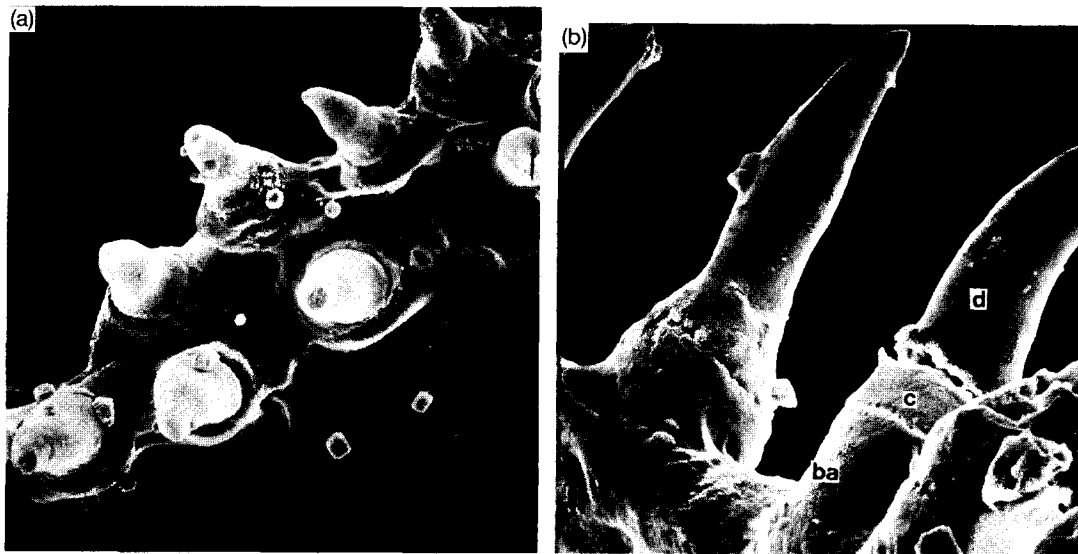

PLATE II. *Dermogenys pusillus*, CAS-SU 34945, male, 22 mm SL, premaxillary teeth. (a) Double row of pedicelate teeth, SEM × 500. Anterior is to the lower left. (b) Several premaxillary teeth, SEM × 1000. Anterior is to the right. Abbreviations as in Plate I.

Sauries. The upper pharyngeals are fused in Halfbeaks and Flying fishes (see Collette *et al.*, 1984; Nelson, 1969: plate 84, figs 6–8).

All bone in the pharyngeal jaws of adult Medaka is acellular (Parenti, pers. obs.; see *Oral jaw*; *Bone structure*, above).

Tooth structure

Pharyngeal teeth in Ricefishes and primitive beloniforms are arranged in even rows (Plate IIIa; Yabumoto & Uyeno, 1984: fig. 8a). In the Medaka, the teeth are angled slightly anteriorly, and have small, posteriorly hooked cusps (Plate IIIa). Fully-erupted teeth in a single row are all approximately the same height. Erupting replacement teeth occur just posterior to the mature-tooth row. In histological section, these erupting teeth can be identified as cones of dentine not attached firmly to the toothplate, as in developing pharyngeal teeth of the cichlid, *Astatotilapia elegans* (Trewavas) (Huysseune, 1983: fig. 3). Huysseune also reported that dentine cones of unerupted upper pharyngeal teeth develop ventral to the cartilaginous precursors of the endo-chondral pharyngeal bones; therefore, tooth development (and eruption) must coincide with resorption of such initial cartilage.

Pharyngeal teeth also have a fibrous connection to the attachment bone. There is a small section of collagen absent from the large, *posterior* border between the dentine and the collagenous ring, such that the teeth are depressible (Plate IIIb). This condition is similar to, but not exactly like, that described by Fink (1981) as Type 4 tooth attachment in neoteleosts, in which the tooth has a relatively larger posterior collagen area with a section of collagen absent from the *anterior* tooth border. Therefore, development of the Ricefish pharyngeal teeth may be said to differ from
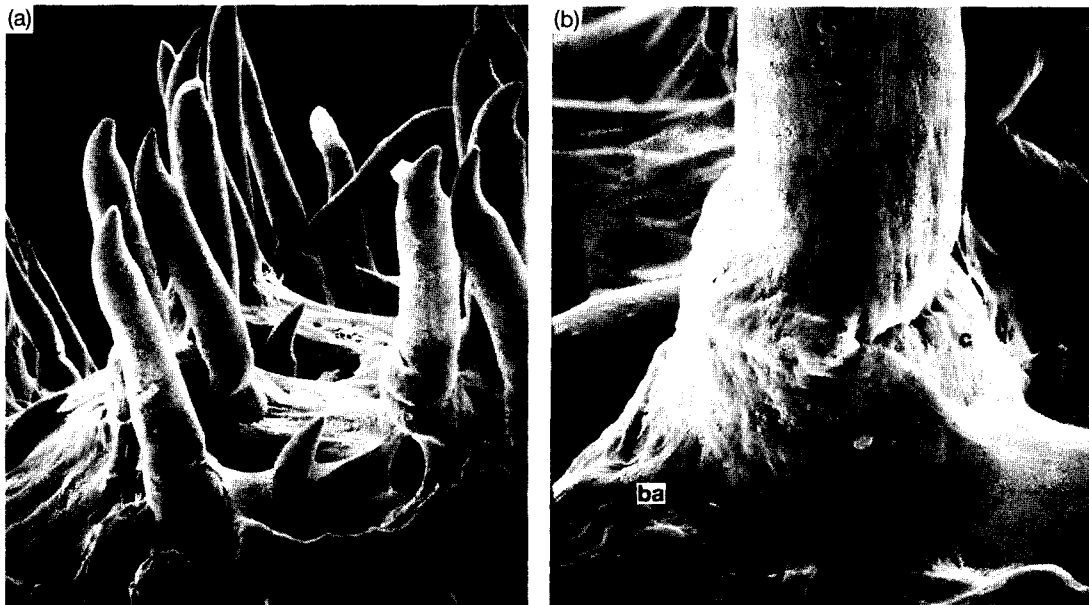

PLATE III. *Oryzias latipes*, AMNH 10344SW, male, 23 mm SL, upper pharyngeal teeth. (a) Lateral view between two rows of teeth, SEM × 350. Note erupting replacement teeth. (b) Base of one pharyngeal tooth, SEM × 1500. Abbreviations as in Plate I. Anterior is to the left.

that of typical Type 4 teeth. But, rather than define Ricefish pharyngeal teeth as a different, perhaps a fifth, type, I conclude for the time being that there is more variation on tooth attachment modes among teleosts than previously described (see Moy-Thomas, 1934; Fink, 1981).

Discussion

Sexual dimorphism of teeth

Differentiation of the large posterior oral teeth of male Medaka occurs along with the expression of other secondary sexual characteristics, such as elongation of the dorsal and anal fin rays, formation of bony processes or contact organs (Oka, 1931; Wiley & Collette, 1970) on the posterior anal fin rays, and thickening and enlarging of anal and dorsal fin pterygiophores (Egami, 1956; Egami & Ishii, 1956; Yamamoto, 1975). Development of these secondary sexual characteristics is labile and under hormonal control (Yamamoto, 1975). For example, Takeuchi (1968) demonstrated that administration of oestradiol to young genetic males (XY) prior to complete sexual differentation inhibited the large posterior oral tooth formation. Conversely, administration of methyl testosterone to young females (XX) promoted large posterior oral tooth formation (Takeuchi, 1967).

Large posterior oral teeth are present in adult males, and sometimes females, of other adrian-ichthyid species, such as *Oryzias melastigma* (McClelland) (AMNH 20650SW), *Horaichthys setnai* Kulkarni (AMNH 36576SW, CAS 56255CS), and *Xenopoecilus sarasinorum* (AMNH 20481SW). Presence of large posterior oral teeth may define a group of adrianichthyid species as monophyletic, with their enlargement lost secondarily in some species. However, resolution of the composition of this group awaits results of a comparative anatomical study of all known Ricefish species currently in preparation.

The fully-ankylosed teeth of male Medaka apparently have no special role in feeding (see *Phylogenetic significance*), but may be another character believed to be important in sexual recognition. However, secondary sexual characters in beloniforms are not necessarily associated with tooth attachment. For example, *Dermogenys pusillus* is a viviparous species that exhibits marked sexual dimorphism associated with reproduction, yet, I have observed no structural difference between oral teeth of adult males and females.

Phylogenetic significance

Tooth and jaw structure

The interhyal, a small, chondral bone that joins the hyoid bar to the hyomandibula and the symplectic, is a synapomorphy of the Teleostomi (Gardiner, 1973:29): acanthodians and osteichthyans (*sensu* Lauder & Liem, 1983). Presence of an interhyal facilitates movement of the hyoid bar (composed of an anterior and a posterior ceratohyal) and, hence, of the entire branchial apparatus (Lauder, 1980). Movement of the branchial apparatus is correlated in turn with depression of the lower oral jaw, the 'mandible', and is, therefore, an integral component of the feeding mechanism in fishes (Lauder, 1983).

Absence of an interhyal is a synapomorphy of beloniform fishes (Rosen & Parenti, 1981), in which the posterior ceratohyal is joined firmly to the hyomandibular-symplectic junction by

ligaments. One non-experimental functional inference of this observation is limited movement of the hyoid bar, hence, limited movement of the entire branchial apparatus. It is not my purpose here to speculate at length on the feeding mechanism of the Medaka, as such an inference could be contradicted by experimental evidence. But, this inference has a notable correlation with movement of the oral jaws; the upper oral jaw of Ricefishes, as well as other beloniforms, as far as is known, is not protrusible (see also Alexander, 1967b). The premaxillae do not have the long ascending processes characteristic of neoteleosts; these processes are inferred to have been lost secondarily. Also, in Ricefishes, the rostral cartilage is absent, and the left and right premaxilla and dentary are joined firmly together at their symphyses by cartilage.

Foster (1973) observed feeding behaviour in laboratory stocks of the Needlefish, *Xenentodon cancila* (Hamilton-Buchanan). This piscivore reportedly swallows whole prey head first after catching it sideways and manipulating it in the outer jaws or beak. Feeding is said to be inertial, with prey items entering the mouth as the Needlefish swims forward (Foster, 1973).

The firmly attached, non-protrusible upper oral jaw was used by earlier fish systematists (e.g. Myers, 1931) to separate *Oryzias* from other egg-laying Microcyprini, once including the Killifishes and the North American amblyopsid cavefishes. Descriptive and functional anatomical investigations of the protrusible upper oral jaw and of the pharyngeal jaw of Killifishes, such as, for example, the North American *Fundulus*, are numerous (e.g. Rosen, 1964; Alexander, 1967a, b; Parenti, 1981; Lauder, 1983). To follow the cladistic classification of Ricefishes in the Beloniformes, rather than the Cyprinodontiformes, means avoiding misleading comparisons of *Fundulus* and *Oryzias* as more closely related to each other than either is to, say, the Halfbeak *Dermogenys*. *Oryzias* and *Dermogenys*, with their highly derived nonprotrusible or fixed jaws, are the close relatives in such a comparison.

The two larger Ricefish species from Sulawesi, *Adrianichthys kruyti* Weber and *Xenopoecilus poptae* Weber & de Beaufort, reaching 170 mm SL, have enlarged upper and lower oral jaws (Rosen, 1964: figs 2 & 3) that one might call beaked. Reduction of the ascending processes occurs in *Oryzias* as well as in *Horaichthys*, in which the maxilla is absent (Kulkarni, 1940; Rosen, 1964). Collette et al. (1984) considered an elongate lower jaw or 'half-beak' primitive for exocoetoids, in part because Needlefishes pass through a half-beak stage during ontogeny. The term 'beak' has been applied casually in beloniform systematics, and has never been defined clearly. Some Halfbeaks, such as *Dermogenys pusillus*, have relatively large upper oral jaws, and could just as well be called beaked, were it not for the emphasis on relative sizes of the upper and lower oral jaws. Treating Ricefishes as primitive beloniforms (Rosen & Parenti, 1981; Collette et al., 1984) makes it logical to consider the beak, that is, an elongate upper and lower jaw, a derived character for all beloniforms. Hence, a beak is here considered plesiomorphic for exocoetids. This interpretation is consistent with the observation that beloniform fishes exhibit a transition series in characters associated with fusion of the pharyngeal jaws. In Ricefishes the lower pharyngeal bones are closely apposed, in Sauries and Needlefishes the lower pharyngeal bones are fused, and in Halfbeaks and Flying fishes, both lower and upper pharyngeal jaws are fused.

As far as is known, adult beloniforms have acellular bone (see *Oral jaw; Pharyngeal jaw*). Teeth in adult beloniforms are incompletely mineralized, which is consistent with the observation that teleosts with acellular bone generally have a fibrous connection of dentine to the attachment bone (Parenti, 1986). Adult male Ricefishes of several species with large, posterior oral teeth fully-ankylosed to the attachment bone have a condition like that in *Esox* and *Sphyraena*, large predatory fishes: bone is acellular, and oral teeth are fully-ankylosed to the bone whereas pharyngeal teeth are incompletely mineralized (Fink, 1981; Parenti, 1986).

Assessment of the Medaka as a model higher teleost

Factors that make Killifishes and Ricefishes desirable for study of development, such as small body size, large egg size, ease of maintenance of large stocks in aquaria, and relatively long development time, make them undesirable for, or are irrelevant to, experimental functional morphology. The majority of literature on such experiments focuses on medium to large fishes, such as the Pike *Esox*, the Perch *Perca*, and the Sunfish *Lepomis* (see Lauder, 1980, 1983). Also, comparative anatomical studies using modern techniques such as SEM, often present illustrations of relatively large fish such as *Esox* and the Hake *Merluccius* (see Fink, 1981), or the Spanish mackerel *Scomberomorus* (see Collette & Russo, 1985).

Thus, by necessity or convention, embryological data accumulate on fishes such as the adrian-ichthyids (Yamamoto, 1975), whereas comparative anatomical and especially functional morpho-logical data are lacking or only poorly known. Incompletely mineralized teeth, acellular bone, a protrusible upper jaw, and a mobile branchial apparatus with an interhyal bone, form a complex characteristic found in advanced teleosts. Anatomy of the Medaka may be said to differ in several ways from that of the so-called model advanced teleost, primarily in characters associated with nonprotrusible or fixed jaws: absence of an interhyal bone, the rostral cartilage, and the ascending processes of the premaxillae, as well as presence of cartilaginous symphyses in the upper and lower oral jaws. Comparative functional anatomy, using the larger Ricefish genera endemic to Sulawesi, should live material become available, will help to answer intriguing questions such as, how does the branchial apparatus function without an interhyal bone? Answers to these and similar questions will complement the existing embryological and genetic data to create a comprehensive body of knowledge on a single group of fishes, which may then be used to create and test theories of evolution within advanced teleosts.

Summary

The Medaka, *Oryzias latipes*, shares with other advanced teleosts: (1) acellular bone, and (2) incompletely mineralized teeth.

The Medaka differs from many other advanced teleosts by having: (1) no rostral cartilage; (2) cartilaginous symphyses between the premaxillae and dentaries; (3) no ascending or articular processes on the premaxilla; and (4) no interhyal bone. The first three characters give Ricefishes and other beloniforms a nonprotrusible oral jaw. The fourth contributes towards the fixed pharyngeal jaws. Inferences about the feeding mechanism of fishes that lack an interhyal bone have been made only through dissection of museum specimens and preliminary observation of aquarium fishes, and need to be confirmed with more sophisticated functional anatomical techniques.

Extreme reduction of the premaxilla, as in *Oryzias*, and *Horaichthys* in which the maxilla is also absent, is viewed herein as a derived character for beloniform fishes for which an enlarged, beaked outer jaw is considered primitive.

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