

Head Morphology of the Ricefish, *Oryzias latipes* (Teleostei: Beloniformes)

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ABSTRACT The medaka, *Oryzias latipes*, is a model organism in experimental biology. Its ontogeny and osteology have been studied frequently, but no detailed description of its cranial anatomy exists. Based on manual dissections, histological slide sections, and 3D-reconstructions, we describe the anatomy of the jaw apparatus of *O. latipes*, focusing on musculature, connective tissue elements, and the nervous system. The soft tissue anatomy of the head of *O. latipes* is characterized by several derived characters that seem to be related to the modification of the upper jaw and a reduced mobility of its bones, which is an evolutionary novelty of the Beloniformes. These apomorphies could also be influenced by the small size of *O. latipes*. The maxilla is medial to the premaxilla. The presence of extensive connective tissue elements severely limits the mobility of both bones against each other and against the neurocranium. The external section of m. adductor mandibulae is partly fused with the internal section, originates from the quadrate, and inserts to the lower jaw. The intermediate head of the internal section is narrow and forms a bend along the ventral margin of the orbit. The intramandibular section is a separate narrow muscle between retroarticular and dentary. These characters need to be verified in other representatives of Beloniformes to test their contribution to the disputed phylogenetic relationships within this taxon and to improve the understanding of the evolution of beak-like jaws in beloniform fishes. *J. Morphol.* 270:1095–1106, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: Japanese ricefish; Adrianichthyidae; cranial morphology; jaw musculature; musculus adductor mandibulae

INTRODUCTION

The Japanese ricefish or medaka, *Oryzias latipes* (Temminck and Schlegel, 1846), is an established model organism in experimental biology. The osteology, ontogeny, reproductive biology, and external morphology are well known, but there is a surprising lack of basic data concerning most aspects of its anatomy (Kulkarni, 1948; Langille and Hall, 1987; Tanaka et al., 2001; Iwamatsu, 2004). The phylogenetic relationships of the genus *Oryzias* and the closely related genera *Adrianichthys*, *Horaichthys*, and *Xenopoecilus*, which together comprise the taxon Adrianichthyidae, have been discussed controversially since the discovery of this

group of teleostean fishes. Until the description of the genus *Oryzias* (Jordan and Snyder, 1906), the ricefish was considered a species of the genus *Poecilia*, and later attributed to *Aplocheilus*, both genera are classified today as Cyprinodontiformes (Temminck and Schlegel, 1846; Yamamoto, 1975). Later authors highlighted the obvious morphological differences between *Oryzias* and the remaining Cyprinodontiformes (Sethi, 1960; Rosen, 1964). Later, Rosen and Parenti (1981) demonstrated, on the basis of external morphological characters, that the Adrianichthyidae should be placed rather into the taxon Beloniformes (see Fig. 1).

The question of whether the taxon Beloniformes is indeed a natural group seems to be complicated by the fact that this group represents a heterogeneous assemblage of teleostean fishes, which display impressive diversity as regards to cranial morphology. Beside *Oryzias* and its closest relatives, the taxon also includes flying fishes, halfbeaks, sauries, and needlefishes (see Fig. 1), whereas the latter three assemblages are characterized by elongated beak-like jaws and by unique developmental changes in their cranial morphology (Boughton et al., 1991; Lovejoy, 2000; Lovejoy et al., 2004). Most authors consider the taxon Adrianichthyidae to be a sister group of the remaining beloniform fishes and the monophyletic Beloniformes and Cyprinodontiformes to be sister taxa (Rosen and Parenti, 1981; Collette et al., 1984; White et al., 1984; Naruse et al., 1993; Dyer and Chernoff, 1996; Naruse, 1996; Hertwig, 2008; Fig. 1). In contrast, the recent analyses of Si-Zhong (2001) and Steinke et al. (2006) challenge this phylogenetic hypothesis based on osteological and molecular data.

Although most previous studies on the phylogeny of bony fishes have predominantly used external

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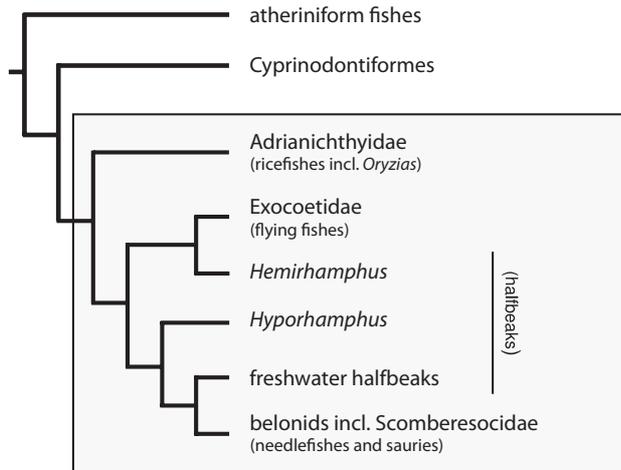


Fig. 1. Phylogenetic hypothesis for the arrangement of beloniform fishes following Lovejoy (2004). Hemiramphids and belonids, that were previously assumed to be monophyletic (Collette et al., 1984), form paraphyletic assemblages in the analysis of Lovejoy et al. (2004).

morphological characters, osteology, and molecular data, information about the contribution of soft tissue characters is limited. Beside the early classical descriptions and comparisons of the anatomy of selected actinopterygian fishes (Vetter, 1878; Allis, 1897, 1903; Dietz, 1914, 1921; Takahasi, 1925; Edgeworth, 1935; Kesteven, 1943; Karrer, 1967), some encouraging works about the utility of soft tissue characters in phylogenetic studies have been published, however (Winterbottom, 1974; Gosline, 1981, 1986, 1989; Stiassny, 1981, 1982; Marino and Dooley, 1982; Howes, 1985a,b, 1989; Gill and Mooi, 1993; Parenti and Song, 1996; Diogo and Chardon, 2000; Diogo, 2004; Parenti and Grier, 2004; Springer and Johnson, 2004; Wu and Shen, 2004; Hertwig, 2008).

This study was undertaken to study the soft tissue anatomy of the head of *O. latipes*. The combined use of manual dissection, staining techniques, histological slide sectioning, and 3D-reconstruction widens the scope of available morphological examinations of the jaw apparatus in small teleostean fishes. We provide a detailed description of the configuration of musculature, connective tissue elements, and nervous system of the cranium of this species. The results provide the basis for comparative studies on the developmental biology of this species and its relatives. Furthermore, it can serve as a starting point for analyses of the phylogenetic relationships of the Beloniformes.

MATERIALS AND METHODS

Nine adult specimens of *O. latipes* (one for histological examination and 3D-reconstruction, the remaining for manual dissections) of the laboratory strain of the University of Würzburg, as well as two *O. cf. melastigma* (McClelland, 1839) and three *O. nigrimas* (Kottelat, 1990) for comparative purposes were used

in this study. For manual dissection, specimens were fixed in 10% neutrally buffered formalin and then preserved in 70% EtOH. For serial slide sectioning, the selected specimen was fixed in Bouin's fixative modified according to Duboscq-Basil (Romeis, 1989). Manual dissection following the protocol of Hertwig (2008) and drawings were done using a ZEISS Stemi SV stereo microscope with a camera lucida apparatus and a LEICA MZ 16 stereo microscope. Before manual dissection, specimens were stained with alizarin-red in a diluted Borax solution (Borax 1:water 5) on an automated shaker for about 12–36 h, resulting in an irreversible staining of the bones in red, while muscles, nerves, and other soft tissue elements kept their whitish coloration. For histological serial slide sectioning, specimens were decalcified in a 5% nitric acid solution for about 24 h, then stored in a sodium sulfate solution for further 24 h, dehydrated, and embedded in paraffin following standard protocols (Romeis, 1989). Using a Microm HM360 rotary microtome, 10 μ m sections were prepared, stained with Heidenhain's Azan (Romeis, 1989), and embedded with Euparal.

Every second section was digitized using a ZEISS Axioskop 40 using a ColorView III camera and the software analySIS Pro 3.2[®]. The color images were saved in tif format at 2576 \times 1932 pixel resolution including a micrometer scale. The software Autoaligner 2.0 (BITPLANE, 2000) was used to align the images. The technical limitations of the software and available computing power necessitated the conversion of images to grayscale mode and limited the total number of processed images, with the result that the 3D-reconstruction was restricted to the posterior end of the palatine arch. Based on manually defined contour lines derived interactively from the digital images, surface renderings were provided by the software Imaris 4.2 (BITPLANE, 2004). Artifacts in the histological sections caused unevenness in the rendered surfaces of the anatomical structures. However, smoothing was avoided to reduce changes of the raw data. Screenshots of the 3D-reconstructions (see Fig. 2) correspond to five 80 s lasting movies and one interactive animation generated with Imaris software (Werneburg, 2007).

Nomenclature of the bones follows Weitzman (1962, 1974), of muscles Winterbottom (1974) and Karrer (1967), and of the nervous system Allis (1903) and Holje et al. (1986). The osteology of this species has been described previously by Kulkarni (1948) and Langille and Hall (1987) described the development of the cranial bones.

RESULTS

The Jaws

In the upper jaw of *O. latipes*, the maxilla is situated medial and slightly posterior of the premaxilla and carries a lateral projecting ridge that limits the mobility of the premaxilla in a caudal direction. The bones lie close to each other and are connected near their ventral tips by connective tissue (lig. praemaxillo-maxillare) between the lateral face of the maxilla and the medial face of the premaxilla (Figs. 2A,D, 3A, and 4B). The maxilla is attached to the mandible by a broad resilient ligament (lig. maxillo-mandibulare), which stretches between the medial face of the maxilla and posterior edge and lateral face of the dentary. This ligament is connected with the short tendon of the external section of m. adductor mandibulae by connective tissue.

The broad medially projecting tips of the transversally oriented dorsal preopercular arms have short ascending processes that reach posteriorly above the mesethmoid (Fig. 2A). The ventral surfa-

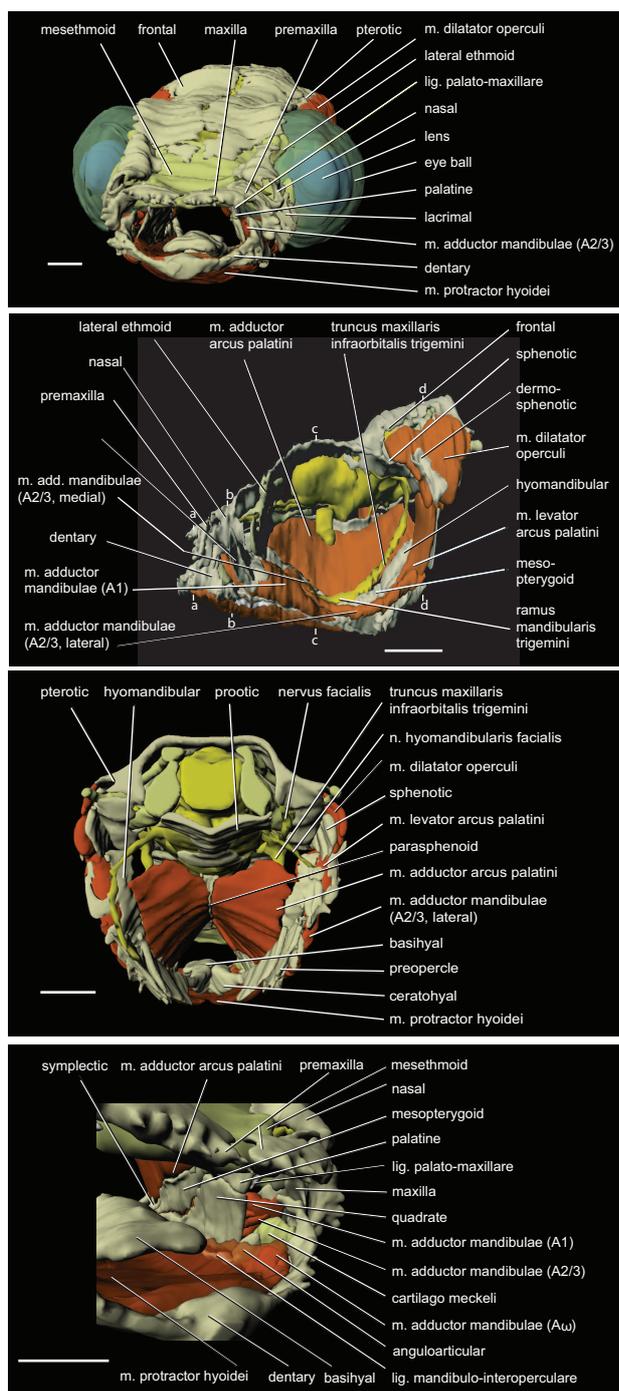


Fig. 2. *Oryzias latipes*—3D views of the head from **A**: left frontolateral; **B**: lateral, eye removed; **C**: caudal; **D**: left frontolateral into the open mouth; m. protractor hyoidei is depicted as transparent throughout. a–d: position of sections (see Fig. 4). Bar scale: 1 mm. 3D-reconstructions correspond to five movies and one interactive animation provided by Werneburg (2007).

ces of both ascending processes are attached to the mesethmoid (anterior dorsal face of the supraethmoid and anterior edge of the hypoethmoid following Langille and Hall (1987) by an extended pad of

connective tissue, which also connects the medial edges of the premaxillaries to each other. This connective tissue also includes the medial tips of the maxillaries, which are located directly below the premaxillaries. The dorsal part of the palatine articulates with the maxilla and is connected by the lig. palato-maxillare to a pit on the ventral face of the maxilla. Medially and posteriorly, the palatine head is connected by elastic connective tissue to the ethmoid cartilage. The posterior dorso-lateral tip of the palatine and the dorsal tip of the mesopterygoid are attached to the lateral ethmoid by elastic connective tissue. The lacrimal and anterior face of the lateral ethmoid are connected by connective tissue. The nasal ethmoid is connected posteriorly to the lateral one by a thin layer of superficial connective tissue and is also connected ventrolaterally to the lacrimal.

Adductor mandibulae complex. A separate small muscle, which we consider homologous to the intramandibular section of the m. adductor mandibulae (A ω) in other teleostei, originates with a short tendon from the anterior edge of the quadrate, the connective tissue capsule of the jaw joint, and from the anterior face of the retroarticular (Figs. 2D, 3B, and 4B). The isolated retroarticular that is embedded in connective tissue elements like a meniscus is itself connected posteriorly to the interopercular by the lig. mandibulo-interopercular (2A). The parallel fibers of A ω course along the medial face of Meckel's cartilage and insert directly to the rostral part of Meckel's cartilage and to the medial face of the dentary (Figs. 2D and 3B).

The lateral or external section of m. adductor mandibulae (A1) consists of parallel anterodorsally running fibers, which originate mainly from the dorsolateral edge of the elongated caudal process of the quadrate and of posterior fibers from the cartilaginous interspace between the symplectic, quadrate, and preopercle (Figs. 2B, 3A,B, and 4C). In both manual dissection and histological sections, A1 cannot be separated entirely along its course from the intermediate head of the internal section of m. adductor mandibulae (A2/3), as the two muscles run close to each other and share some crossing fibers in their anterior parts. Near their insertion to the lower jaw, they are fully separated by the ramus (r.) mandibularis trigemini, which descends to the lower jaw between these sections (Fig. 4C). A1 inserts via a short tendon terminally or slightly subterminally to the posterior edge of the dentary at two-thirds of its height below the coronoid process of this bone (Fig. 2D), and has contact via connective tissue to the lig. maxillo-mandibular (Fig. 4B).

The internal section of m. adductor mandibulae (A2/3) consists of three muscle heads. In using the term A2/3, we refer to Karrer (1967), who already described a fusion of A2 and A3 in *Belonesox* and

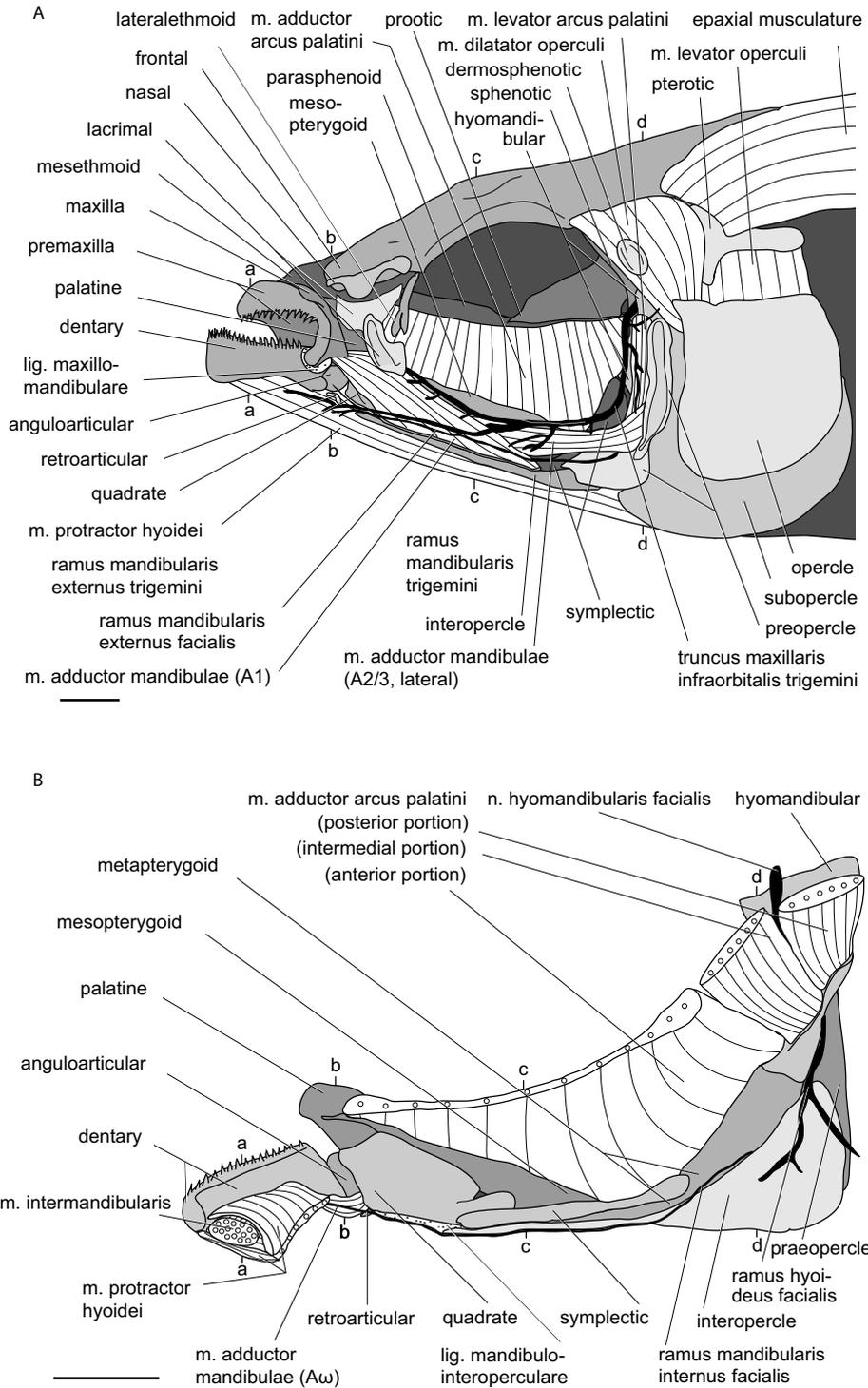


Fig. 3. *Oryzias latipes*—**A**: Lateral view of the head. The integument and the eye have been removed. Solution: 3.2×10 ; **B**: Medial view of the jaw and the suspensorium. Solution: 5.0×10 . Markings a–d indicate the position of the transverse sections in Figure 4. Bar scale: 1 mm.

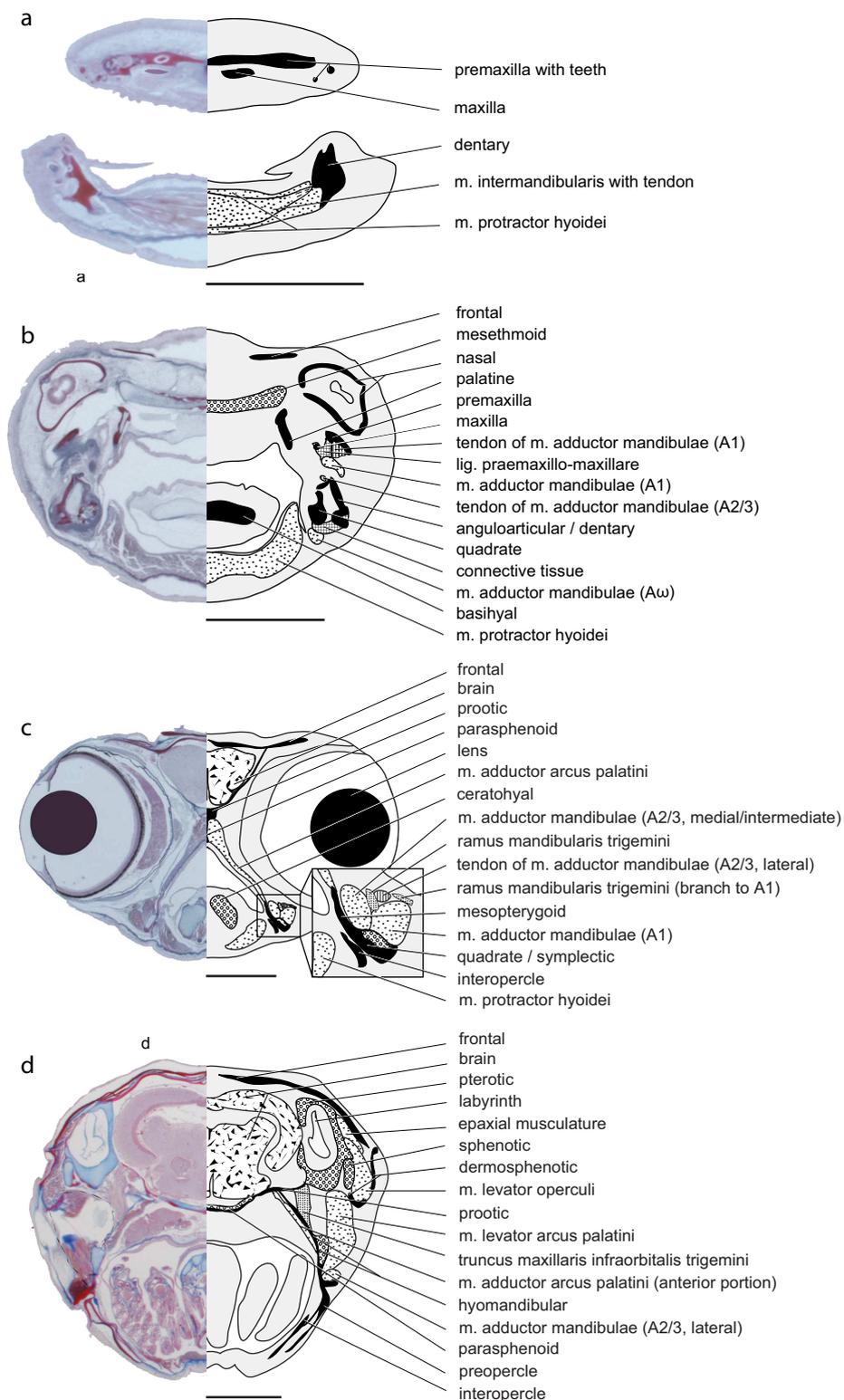


Fig. 4. *Oryzias latipes*—**A**: Transverse section through the anterior part of upper and lower jaw, compare with Figures 2B and 3A,B. **B**: Transverse section through the posterior part of upper and lower jaw, compare with Figures 2B and 3A,B. **C**: Transverse section through eye region, compare with Figures 2B and 3A,B. **D**: Section through ear region, compare with Figures 2B and 3A,B; thickness of slides: 12 μ m. Bar scale: 1 mm.

Xiphophorus (Cyprinodontiformes). The fibers of the narrow intermediate head of A2/3 originate from the caudal process of the quadrate medial to the A1-fibers and from the cartilage that covers the lateral face of the symplectic, run anterodorsally, and fuse with the medial head. The medial head arises from the lateral faces of the quadrate, the symplectic and the cartilaginous interspaces of the hyopalatine arch, and from the tendon of the m. adductor arcus palatini and runs anteriorly. The third, lateral head is narrow, round in cross section, and arises from the lower third of the dorsal arm of the preopercle (Figs. 2B, 3A, and 4D), curves below the ventral margin of the eye, then runs straight in an anterior direction, and develops a very long, thin tendon. This tendon runs parallel to A1 and lateral to the truncus maxillaris trigemini dorsomedially, continues dorsal of the r. mandibularis trigemini, and fuses with the medially situated common tendon of the other two heads of A2/3 above the jaw joint. The resulting tendon inserts to the coronomeckelian bone medial to the anguloarticular. Except for the posterior part of the lateral head, A2/3 is covered laterally in its entirety by A1 (Figs. 2B, 3A, and 4C).

Ventral muscles of the head. On the ventral surface of the jaws, the parallel fibers of m. protractor hyoidei form the muscular floor of the mouth and connect the hyoid arch and lower jaw (Figs. 2A–D, 3A,B, and 4A–C). Posteriorly, the fibers of m. protractor hyoidei attach directly to the ventrolateral face of the ceratohyal and the anterior tips of the branchiostegal rays (Fig. 2C). The origin of this muscle is clearly separated from mm. hyohyoidei inferioris lateralis and medialis and covers the area between the second and the fourth branchiostegal rays. Before the muscle passes the jaw joint, the fibers of the two contralateral counterparts fuse and course anteriorly as a single flat, wide muscle (Figs. 2C,D, 3A, and 4B,C). A weak myocomma is present that runs posterolaterally from the fusion of the contralateral muscles. The anterior attachment of the fused m. protractor hyoidei comprises a broad dorsal and a ventral head that are separated by the transversal m. intermandibularis and inserts to the posterior face of the dentaries beneath the symphysis of the lower jaw (Figs. 2A,D, 3B, and 4A). The dorsolateral fibers run anterolaterally and insert directly to the lateral faces of the dentaries posterior of the m. intermandibularis tendons. Unlike the fibrous larger dorsal head, the ventral head consists of just a few fibers, especially along the midline, and inserts with a very short, laminar tendon.

The spindle-shaped m. intermandibularis stretches transversely between the halves of the lower jaw and transverses the symphysis of the dentaries (Figs. 3B and 4A). It originates tendinously from ridges on the medial faces of the dentaries. The shape of this muscle in cross section behind

the symphysis of the mandible is round to oval (Fig. 3B).

The Palatine Arch

The m. adductor arcus palatini is a large muscle consisting of parallel fibers, which connects the neurocranium with the palatine arch (Figs. 2B,C, 3A,B, and 4C,D). It is composed of three portions (anterior, intermediate, and posterior; Fig. 3B). The anterior portion of the m. adductor arcus palatini is by far the largest portion and represents a wide stretching, thin muscle that forms the floor of the orbit (Figs. 2B,C, 3A,B, and 4C). It originates from the lateral face of the ventral ridge of the parasphenoid and the anterior edge of the ventral face of the prootic. Anteriorly, this muscle inserts via connective tissue to the posterior tip of the palatine, and directly to the mesopterygoid, which forms dorsomedially and dorsolaterally facing attachment sites in its dorsal part. More posteriorly, this muscle inserts partially via a short tendon to the dorsomedial edges of the symplectic and the cartilage of the palatine arch, and directly to a ridge-shaped attachment site on the medial face of the hyomandibular (Fig. 3B). The muscle belly of the m. adductor arcus palatini widens in cross section in its anterior and posterior parts, but in the middle below the orbit it consists of just a few layers of fibers, especially near its insertion to the palatine arch (Figs. 2B and 3B).

The intermediate portion of the m. adductor arcus palatini consists of shorter parallel fibers that originate medial to the origins of the anterior mm. levator arcus branchialis from the prootic. It inserts directly to the dorsal face of the ridge on the medial face of the hyomandibular, medially of the dorsal opening of the canalis facialis of the hyomandibular (Fig. 3B). The intermediate portion of the m. adductor arcus palatini is separated from the posterior portion by the n. hyomandibularis facialis, which emerges ventrolaterally from the neurocranium (Fig. 3B).

The posterior m. adductor arcus palatini is a small muscle consisting of short fibers, which converge weakly ventrally. It arises laterally to the origins of the anterior mm. levator arcus branchialis via a short tendon from the ventral face of the prootic. It runs ventrally and inserts via a short tendon to the dorsomedial face of the medial ridge of the hyomandibular laterally of the dorsal foramen of the canalis facialis (Fig. 3B). Its fibers reach from the foramen of the canalis facialis in a caudal direction to the hyomandibular articulation.

The pinnate m. levator arcus palatini arises via a short tendon from the ventral edge of the transversal process of the sphenotic and runs ventrally along the posterior margin of the orbit (Figs. 2B,C, 3A, and 4D). Its insertion site consists of a medial

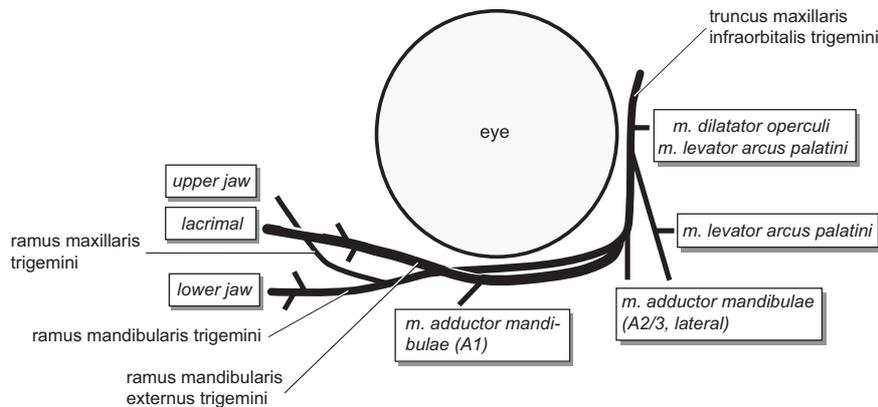


Fig. 5. Course and branching scheme of truncus maxillaris infraorbitalis trigemini (part of V cranial nerve) and its main target areas. For a more detailed description of the nerves and particularly the innervated areas see text. Compare also with Figs. 2B, 3A, and 4C,D.

head comprising the shortest fibers attaching to the anterior and lateral side of the hyomandibular and a lateral head whose more ventral insertion covers the anterior face of the dorsal arm and the lateral face of the preopercle and the posterodorsal part of the lateral face of the symplectic. The belly of the *m. levator arcus palatini* runs along the posterior margin of the orbit as a narrow sheet of muscle fibers (Figs. 2B, 3A, and 4D). The insertion of the lateral head of the *m. levator arcus palatini* is covered by A2/3 in lateral view (Figs. 2B and 3A).

The Opercle

The conical *m. dilatator operculi* originates directly from the lateral edge of the frontal, the lateral face of the pterotic, the lateral face of the sphenotic, and the posterior face of the transversal process of the sphenotic, and via some ventral fibers from the lateral face of the hyomandibular (Figs. 2A,B and 3A). The large origin of this muscle completely covers the lateral face of the sphenotic. The *m. dilatator operculi* inserts with its apex on the medial face of the weakly developed dorsal process of the opercle (Figs. 2A and 3A). The dermosphenotic is embedded in the lateral fascia of this muscle (Figs. 2B and 3A).

The parallel-fibered *m. levator operculi* originates directly from a narrow posterior face of the hyomandibular and from the ventral face of the transverse process of the pterotic, runs ventrally, and inserts directly on the medial face of the opercle (Figs. 3A and 4D). Its posterior part covers the *m. adductor operculi*.

The *m. adductor operculi* lies medial to the *m. levator operculi* and consists of parallel fibers that arise directly from the ventral face of the pterotic, and run parallel to the *m. levator operculi* ventrally. This muscle inserts via a short tendon more

ventrally than the *m. levator operculi* on the weakly developed crista on the medial face of the opercle. The origin of *m. adductor operculi* on the transverse process of the pterotic is situated more dorsally than the origin of the *m. levator operculi*.

Nervous System

After leaving the neurocranium, the truncus maxillaris infraorbitalis trigemini courses ventrally along the posterior margin of the orbit (Figs. 2B, 3A, 4D, and 5). Two smaller nerves branch off that run posterolaterally and ventrolaterally, respectively, and innervate *mm. dilatator operculi et levator arcus palatini* and posterior parts of A2/3 of the adductor mandibulae complex (see Fig. 5). Further anterior, a smaller branch runs ventrolaterally and innervates also A2/3. Then, the truncus maxillaris infraorbitalis trigemini separates into two large branches that run parallel, turn anteriorly below the eye where they meet the dorsomedial surface of the *m. adductor mandibulae* complex and then separate in their courses more anteriorly (Figs. 3A and 5). The medial branch represents the remaining truncus maxillaris infraorbitalis trigemini, whereas the lateral branch could be homologous to the *r. mandibularis externus trigemini* of Holje et al. (1986). Before it reaches the dorsomedial surface of the *m. adductor mandibulae*, it issues a smaller branch that runs laterally, ramifies and innervates the A1. The remaining branch runs anterodorsally along the dorsal surface of the *m. adductor mandibulae* complex between A1 and A2/3. On the lateral and dorsal surface of A1 and medial to the lacrimal, this nerve ramifies into several small branches, but a large branch continues dorsally along the medial face of the lacrimal, innervating the sensory canal of this bone and surrounding tissue (Figs. 2A, 3A, and 5).

The remaining truncus maxillaris infraorbitalis trigemini splits into two separate nerves when it reaches the lateral face of the quadrate (see Fig. 5). The dorsal branch, the r. maxillaris trigemini, is relatively thin. It runs anterodorsally along the dorsal surface of the m. adductor mandibulae complex, passes the medial face of the lacrimal, turns dorsally above the insertion site of A1 on the dentary, turns medially along the medial faces of maxilla and premaxilla, ramifies and innervates the integument of the upper jaw (see Fig. 5).

The ventral branch, the ramus mandibularis trigemini, turns ventrally and crosses the m. adductor mandibulae complex between the lateral surface of A2/3 and the medial surface of A1 (Fig. 4C). It splits off smaller nerves innervating A1 and A2/3 and runs below the long tendon of the lateral head of A2/3. Then it passes the jaw joint dorsally between the insertion of A1 to the dental and the common tendon of A2/3 to the coronomeckelian bone, and enters the medial face of the lower jaw (see Fig. 5). It then runs anteriorly above the dorsal margin of the anguloarticular and coronomeckelian bone, continues anteriorly along the dorsal surface of the Meckel's cartilage, and issues the ramus alveolaris trigemini, which enters a canal in the dentary. The remaining r. mandibularis trigemini turns transversely, ramifies, and innervates the muscles and integument of the lower jaw.

The n. hyomandibularis facialis runs below the neurocranium in a ventrolateral direction and between the origins of the intermediate and posterior portions of the m. adductor arcus palatini (Fig. 3B), enters the short canalis facialis that transverses the ridge on the medial face of the hyomandibular and is nearly parallel in orientation to the main axis of this bone. It emerges from the foramen of this canal on the medial face of the hyomandibular and splits into r. mandibularis facialis and r. hyoideus facialis (Fig. 3B). The r. hyoideus facialis courses ventrally along the medial face of the hyomandibular and issues a small branch that runs posteriorly to muscle fibers on the medial face of the subopercle, whereas the remaining nerve continues ventrally and enters the hyoid arch (Fig. 3B).

The r. mandibularis facialis turns to the lateral face of the hyopalantine arch between the posterior edge of the hyomandibular and the dorsal arm of the preopercle where it is covered by the insertion of the m. levator arcus palatini, runs a short distance ventrally on the lateral face of the preopercle, turns anteriorly and splits into two branches (Fig. 3A,B). The r. mandibularis externus facialis courses along the lateral face of the preopercle and the ventral of the lateral head of A2/3 to the lateral surface of the adductor mandibulae complex, turns anteriorly, runs along the lateral surface of A1, turns ventrally along the lateral surface and

passes the jaw joint dorsally. The r. mandibularis internus facialis enters the medial face of the hyopalantine arch between the anterodorsal edge of the lateral face of the preopercle and the posteroventral margin of the symplectic, runs anteriorly along the medial face of the symplectic, and then along the ventral face of the interopercle, passes the jaw joint ventrally, runs along the medial surface of A ω , and innervates the m. protractor hyoidei (not A ω) at the ventral integument of the lower jaw (Fig. 3B).

DISCUSSION

In teleostean fishes, the jaws are closed by the m. adductor mandibulae, which has differentiated in the evolution of bony fishes into several distinct sections (Vetter, 1878). In most species, the external section (A1) originates on the hyopalantine arch and lowers the upper jaw, while the internal section also originates from the hyopalantine arch but raises the lower jaw. In the plesiomorphic state in bony fishes, the internal section includes the separate parts A2 and A3 (Allis, 1897; Winterbottom, 1974), but in numerous species of the Atherinomorpha both are merged and form a complex muscle consisting of several distinct heads which is called A2/3 (Karrer, 1967; Hertwig, 2008). Additionally, a further intramandibular section A ω connects the medial faces of the mandible with the hyopalantine arch (Winterbottom, 1974; Lauder and Liem, 1980; Gosline, 1986). The specific configuration of the m. adductor mandibulae complex in *O. latipes* described in this study differs clearly from that in other species of Atherinomorpha (Alexander, 1967; Karrer, 1967; Aarn and Ivantsoff, 1996, 1997; Aarn and Shepherd, 2001; Hertwig, 2008).

At first glance, section A1 appears to be completely missing in *O. latipes*, as described by Hertwig (2008) in *Nomorhamphus* and *Hemirhamphodon*, because no muscle inserts directly to the upper jaw, and the m. adductor mandibulae and the upper jaw are only connected indirectly via the lig. mandibulo-maxillare. However, here the lateral partition of the m. adductor mandibulae complex is considered as homologous to A1 because of the following reasons. The lig. mandibulo-maxillare was interpreted as a tendon of A1 in Hertwig (2008), but actually has a direct bone to bone attachment to the maxilla and dentary. Despite the lack of a direct insertion to the upper jaw, the m. adductor mandibulae in *O. latipes* consists of two sections with completely separate origin and insertion sites. The lateral or external section originates from the quadrate and inserts to a separate attachment site on the posterior edge of the dentary, whereas the medial section consists of three heads originating more medially from the hyopalantine arch and inserts via a common tendon to the

coronomeckelian bone on the medial face of the anguloarticular. In some atheriniform fishes, an additional intermedial part of the m. adductor mandibulae is found medial to A1 and lateral to the remaining A2/3 with separate insertion to the lower jaw. Its position is comparable to that of the questionable muscle in *O. latipes*, and it has been regarded as the lateral portion of A2/3 (Hertwig, 2008). The lateral section in *O. latipes* could therefore be homologous to one of these two muscles present in atheriniform fishes, which are probably a paraphyletic assemblage and the closest relatives of the clade comprising Beloniformes and Cyprinodontiformes, according to most proposals concerning the phylogenetic relationships within Atherinomorpha (Rosen and Parenti, 1981; White et al., 1984; Parenti, 1993). The situation in *O. latipes* is additionally complicated by the fact that sections A1 and A2/3, although divided by the ramus mandibularis trigemini, are partially fused, as indicated by the presence of a few shared fibers arising from the quadrate, running anteromedially and inserting to the common tendon of A2/3. The usefulness of the course of the ramus mandibularis trigemini as an indicator of the homology of subdivisions of the jaw musculature is controversial (Edgeworth, 1935; Kesteven, 1943; Winterbottom, 1974; Howes, 1985a,b, 1988; Gosline, 1986, 1989; Adriaens and Verreas, 1996; Diogo and Chardon, 2000; Haas 2001; Wu and Shen, 2004). Despite these uncertainties, the lateral section of the m. adductor mandibulae complex described here is considered homologous to A1 on the basis of its separate lateral origin and insertion as well as its anatomical position lateral to A2/3 and the descending ramus mandibularis trigemini. The specific configuration of the m. adductor mandibulae complex clarified here, especially the insertion of A1 to the lower jaw and its partly fusion with the middle part of A2/3, is not present in other groups of the Beloniformes as studied so far and represents therefore an autapomorphic character of *O. latipes* and cannot be used in phylogenetic analyses. The absence of A1 in *Nomorhamphus* and *Hemirhamphodon* is a synapomorphy of these freshwater halfbeaks, particularly as A1 is present in *Hyporhamphus unifasciatus*, which is the sister taxon of the clade consisting of freshwater halfbeaks, needlefishes, and sauries (see Fig. 1). These proposed homology hypotheses have certainly to be tested in a cladistic analysis with a broad sampling strategy including other species of Adianichthyidae as well as representatives of all higher taxa of Beloniformes and Cyprinodontiformes.

The narrow shape and bent course of A2/3 along the ventral margin of the orbit is a peculiar character of the jaw musculature of *O. latipes* as is illustrated by the 3D-reconstructions (Fig. 2B), which has to be regarded as a further apomorphy of this species. However, these anatomical traits of

A2/3 are present in a comparable manner in “dwarf” species of Cyprinodontiformes, e.g., *Cynopocilus melanotaenia*, *Fluviphylax pygmaeus*, and *Leptolebias fractifasciatus* (Weitzman, 1982; Weitzman and Vari, 1988; Hertwig, 2008). *O. latipes* is a small species, which reaches up to 32 mm standard length (SL), but does not fall into the category of miniature fishes defined by Weitzman and Vari (1988) as species exceeding not more than 26 mm SL in the wild. The peculiar configuration of A2/3 described earlier is similar in all these species and could be influenced by secondary miniaturization and represents an independently evolved result of the large size of the orbit in relation to the jaw apparatus. In contrast to the consequences of miniaturization on the head skeleton (Kottelat et al., 2006), the impact of miniaturization on the cranial musculature in fishes has never been investigated in detail. In phylogenetic analyses, therefore, the possible effects of functional constraints coupled with ontogenetic character changes and an evolutionary tendency to decrease in size all have to be examined carefully for homoplastic transformations if characters of the musculature are to be considered as an indicator of phylogeny.

The anatomy of soft tissue elements of the jaw apparatus in *O. latipes* displays several other derived characters. A1 arises completely from the dorsal face of the caudal process of the quadrate, whereas in other atheriniforms, as far as is currently known, A1 originates more caudally and predominantly from the preopercle (Karrer, 1967; Aarn and Ivantsoff, 1996, 1997; Aarn and Shepherd, 2001; Hertwig, 2008). In *O. latipes*, the intramandibular section of the m. adductor mandibulae complex, A_{∞} , consists of parallel fibers and arises mainly from the retroarticular and anguloarticular and via a short tendon from the connective tissue around the jaw joint, and is also connected to the most anterior edge of the quadrate. In most related taxa, however, A_{∞} is a pinnate muscle and originates as a unitary tendon from the medial face of the quadrate or interopercle (Karrer, 1967; Osse, 1969; Hertwig, 2008). In some species of Cyprinodontiformes with a particularly large A_{∞} , a ventral portion of parallel fibers spanning between the retroarticular/anguloarticular and dentary is present, which is comparable to A_{∞} in *O. latipes* (Hertwig, 2008). A separate m. adductor hyomandibulae connecting the neurocranium to the dorsomedial face of the hyomandibular (Winterbottom, 1974) is absent in *O. latipes* but present as a very thin sheet of fibers in other atheriniform fishes such as *Belonesox belizianus* (Karrer, 1967), *Atherina boyeri*, and *Gambusia gagei* (unpublished data). Winterbottom (1974) pointed out that the differentiation of a separate m. adductor hyomandibulae from posterior parts of the m. adductor arcus palatini occurred in many lineages of the teleostean fishes independently, but

could be locally informative in determining the phylogenetic relationships within Atherinomorpha despite its convergent mode of evolution.

The following characters of the jaw apparatus in *O. latipes* are proposed to be potential synapomorphies or symplesiomorphies of this species or even of Beloniformes and Cyprinodontiformes (see Fig. 1), which should be clarified in cladistic analyses. The lig. maxillo-mandibulare, which is probably a derivative of the primordial membrane and connects the dorsal tip of the maxilla with the anguloarticular (Gosline, 1986), is present in atheriniform fishes (Dyer and Chernoff, 1996) but is completely reduced in the *O. latipes*, Hemiramphidae, and Cyprinodontiformes examined so far (Hertwig, 2008). A potential synapomorphy, respectively, symplesiomorphy of *O. latipes* and Cyprinodontiformes could be the plain shape of the m. protractor hyoidei near its anterior attachment to the dentary. This is also found in *Aplocheilus* and *Pachypanchax* as well as in the remaining rivulid and aplocheiloid fishes, which are characterized by the possession of numerous plesiomorphic traits within the Cyprinodontiformes and are thus regarded as sister taxa to the remaining species of this group even in alternative topologies (Rosen and Parenti, 1981; Costa, 1998; Hertwig, 2008).

The transformation of the mesopterygoid into a small laminar bone in Cyprinodontiformes, in some Hemiramphidae and in *O. latipes*, seems to be coupled with the anterior expansion of the m. adductor arcus palatini, which originally connected the neurocranium and hyomandibular (Dietz, 1914; Edgeworth, 1935; Winterbottom, 1974). In various taxa of teleostean fishes, this muscle expands anteriorly and forms the floor of the orbit between the parasphenoid and the hyopalatal arch (Winterbottom, 1974). In the plesiomorphic condition of the Atherinomorpha, which according to Costa (1998) is found in *Perca fluviatilis*, all atheriniform fishes, and some beloniform fishes, the mesopterygoid is dorsomedially enlarged and contributes to the ventral margin of the infraorbital region. In these taxa, the anterior portion of the m. adductor arcus palatini is thin and does not extend beyond the posterior part of the mesopterygoid, inserting laterally to this bone. In the derived state, the anterior portion of m. adductor arcus palatini is broader and expands rostrally to the anterodorsal part of the palatine arch, even up to the posterior edge of the palatine (Fig. 2B,C), bearing dorsolateral and dorsomedial attachment sites in *O. latipes*, and medial or dorsal attachment sites in Cyprinodontiformes (Hertwig, 2008). This condition could have been evolved also independently in both groups.

In the upper jaw of *O. latipes*, several connective tissue elements are very short, flat, and not shaped as distinctive ligaments. The ventral parts of the maxilla and premaxilla are attached by a

thin sheet of connective tissue that corresponds to the lig. premaxillo-mandibulare. The pad of connective tissue between the ventral faces of the premaxillaries, the tips of the maxillaries, and the dorsal face of the mesethmoid is regarded here as homologous to the ethmomaxillary ligament. This ligament connects the tip of the dorsal process of the maxilla with various elements of the ethmoid region of the skull and is present in atheriniform fishes and in a modified condition in aplocheiloid fishes and, therefore, probably in the ground plan of the Atherinomorpha (Rosen and Parenti, 1981; Dyer and Chernoff, 1996; Costa, 1998; Hertwig, 2008). In species of Hemiramphidae, the bones of the upper jaw are also immobile and connected by connective tissue, and their maxillaries are connected to the lateral ethmoid by short connective tissue elements, whereas a distinct ethmomaxillary ligament is absent.

Certain derived characters of the jaw apparatus of the skeleton (Kulkarni, 1948), the m. adductor mandibulae complex and connective tissue elements in *O. latipes* described in this study were probably coupled or are associated with the principal alteration of the mechanics of the upper jaw present also in other Beloniformes. This evolutionary change included the shift of the maxilla into a position medial to the premaxilla as well as a reduction in the mobility of both upper jaw bones against each other and in relation to the neurocranium. These functional changes permit the indirect movement of the upper jaw as an exclusive result of the internal section of the m. adductor mandibulae inserting to the lower jaw via ligaments between the lower and upper jaw. The external section of the jaw closing muscle complex thus loses its function as a muscle that lowers the upper jaw. Consequently, a general trend toward a reduction of the external section of the m. adductor mandibulae inserting at the upper jaw can be observed in several taxa of Beloniformes: in *H. unifasciatus* the A1 is a thin sheet of a few muscle fibers, it is completely reduced in *Hemirhamphodon* and *Nomorhamphus* (Hertwig, 2008), or the muscle shifts its origin anteriorly and its insertion to the lower jaw as is the case in *O. latipes*. The immobile upper jaw bones are one of the few synapomorphic characters that unite the very heterogeneous Beloniformes (Mickoleit, 2004). The configuration of jaw bones in *O. latipes* could resemble an early stage in the evolution of the elongated beak-like jaws in Hemiramphidae, Belonidae, and Scomberesocidae and could reflect, therefore, a situation similar to the ground pattern of Beloniformes. The observed specific topology of the m. adductor mandibulae complex, however, must currently be regarded as an evolutionary novelty of *O. latipes*, because in *Hyporhamphus*, a species that is a phylogenetically more basal within the hemiramphid fishes (Lovejoy, 2000; Lovejoy et al., 2004; see Fig.

1), A1 is present as mentioned earlier and attaches to the maxilla. That would mean that the tendency toward a reduced mobility of the upper jaw bones is homologous but that the resulting configuration of the jaw musculature has been independently evolved at least two times within the Boloniformes.

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