

Ontogeny of the intermandibular and hyoid musculature in the suckermouth armoured catfish *Ancistrus cf. triradiatus* (Loricariidae, Siluriformes)

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Abstract—Loricariidae or suckermouth armoured catfishes are the most speciose catfish family, displaying morphological specializations toward the attachment onto substrates with their sucker mouth, and the scraping of algae and other food items off these substrates. The intermandibular and hyoid musculature differs from the general siluriform situation. This detailed study on several developmental stages of a loricariid representative aims to provide insight in the ontogenetic origin of these muscles, as well as on their morphology and homology. Serial sections and 3D-reconstructions are used to visualize the early muscle configurations. The intermandibularis anterior muscle develops two parts, inserting on the lower jaw but also on the lower lip tissue. A similar differentiation into a dentary and a labial part occurs in the intermandibularis posterior (usually erroneously referred to as protractor hyoidei in loricariids). The protractor hyoidei has a compound nature in teleosts, but in loricariids no interhyoideus portion fuses to the posterior intermandibularis portion. Several arguments, including the absence of a myocomma and a double innervation, indicate the absence of an interhyoideus portion. A double innervation has been found in the hyohyoideus inferior. The posteriormost muscles in the hyoid region are relatively small during early ontogeny: the sternohyoideus halves fuse relatively late; the hyohyoidei adductores develop latest of all ventral head muscles. A remarkable shift in orientation characterizes the hyohyoideus abductor.

Keywords: catfishes; development; homology; myology; Loricarioidea.

INTRODUCTION

The present paper is part of a study on the ontogeny of hard and soft cranial structures of a highly specialized teleost taxon: the neotropical family Loricariidae or suckermouth armoured catfishes. These catfishes are special in having their lower

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lip folded back against the ventral side of the head, and the lower jaws rotated medially and ventrally, so that the teeth are pointed toward the substrate on which they feed (mostly algae and other encrusted matter and detritus) (Alexander, 1965; Schaefer and Lauder, 1986; Geerinckx et al., 2007a). These and other authors (e.g., Howes, 1983a) also elaborated on the high number of cranial muscles serving the highly mobile jaw elements. The specializations of the loricariid head, related to this feeding mode, but also their ability to suck onto substrates and maintain position even in torrential rivers, certainly have to involve modifications in the musculature of the ventral head region. It has already been shown that the so-called protractor hyoidei differs from the general catfish morphology, as it is connected to the modified lower jaw as well as the lower lip tissue (Schaefer and Lauder, 1986; Geerinckx et al., 2007a). Differences in other hyoid muscles have been observed as well (e.g., a forked hyohyoideus inferior, and a transversely oriented hyohyoideus abductor connected to the sternohyoideus) (Howes, 1983a; Schaefer, 1997; Diogo, 2005; Geerinckx et al., 2007a).

The evolutionary origin and transformations of some of the loricariid cranial musculature have been hypothesized (Schaefer and Lauder, 1986, 1996), while the ontogenetic transformations have not yet been studied. Studies on the ontogeny of cranial musculature of other taxa have, however, yielded important results concerning muscle homologies, early larval muscle functions, and changes in muscle morphology and function (Otten, 1982, 1983; Surlemont et al., 1989; Surlemont and Vandewalle, 1991; Adriaens and Verraes, 1996, 1997a, b, c; Hunt von Herbing et al., 1996a, b; Schilling and Kimmel, 1997; Hernández et al., 2002).

Here we present a detailed study of the muscles on the ventral aspect of the head of the bristlemouth catfish *Ancistrus cf. triradiatus* Eigenmann, a representative of the Loricariidae. The muscles found in this region are various parts of the intermandibular muscle, several hyoid muscles and the sternohyoideus. Developmentally, these muscles arise from three different muscle plates (Edgeworth, 1935; Miyake et al., 1992): the intermandibularis anterior and posterior arise from the ventral portion of the mandibular muscle plate, the hyohyoideus inferior, abductor and adductores belong to the ventral part of the hyoid muscle plate, and the sternohyoideus originates from the hypobranchial muscle plate. The protractor hyoidei, present in most teleosts, is composed of the intermandibularis posterior and an anterior division of the hyoid muscle plate, the interhyoideus anterior. The muscles of the branchial arches are not dealt with in this paper.

Our major objectives are a detailed analysis of the ontogeny of the ventral head muscles, as well as providing hypotheses on the identity and homology of the intermandibularis and 'protractor hyoidei' muscle divisions, by comparing them with the same muscles or muscle divisions in non-loricariid catfishes. To do this, we examined not only the muscles and their insertions, but also the paths of and innervations by the relevant nerve branches. Finally, we add a few brief considerations on the functional-morphological aspects of some muscles. Our discussion on the functionality of muscles is based on the anatomy only, as no

biomechanical studies including EMG could be done on such small specimens. An excessive functional interpretation of the results is not appropriate here, not only because of this reason, but also because a comprehensive analysis should include the jaw, suspensorial and opercular musculature as well.

MATERIAL AND METHODS

The bristlenose catfish *Ancistrus* cf. *triradiatus* was chosen for this study because of its fairly typical loricariid habitus and medium size. Moreover, it could be bred fairly easily in aquarium conditions in our laboratory. Specimens throughout early ontogeny (fathered by the same male) were used to study the morphology of the musculature. Five specimens were serially sectioned (standard length and age post-fertilization: 6.1 mm 4 days, 7.0 mm 6 days, 8.0 mm 7 days, 10.2 mm 14 days and 12.4 mm 43 days). Technovit 7100 was used as plastic embedding medium; 2 μm thick slices were obtained using a Reichert-Jung Polycut microtome, and were stained with toluidine blue. Examination was done using a Reichert-Jung Polyvar light microscope, equipped with a camera lucida and a digital camera (Colorview 8; with Olympus AnalySIS 5.0 software). Three of the stages are described in detail, with reference to the other stages when relevant. Earlier stages were not used, as the toluidine didn't stain the undifferentiated muscle tissue. Two adult specimens (70 mm SL, 94 mm SL) were used to compare the early ontogenetic with the adult musculature. One subadult of 35 mm SL was serially sectioned as well (slice thickness 5 μm), in order to obtain detailed information on the innervation of the muscles. 2 μm serial sections of 5.6 and 7.2 mm specimens of the clariid *Clarias gariepinus* from Adriaens and Verraes (1997a) were examined for a short comparison of the intermandibularis posterior innervation (see discussion). 3D-reconstructions were made from digital images of serial sections of the 6.1 and 8.0 mm stages, using the software packages Amira 3.1.1 (T.G.S.) and Rhinoceros 3.0 (McNeel).

RESULTS

6.1 mm SL – 4 days post-fertilization (fig. 1)

In this early embryonic stage, with a still incomplete chondrocranium, most muscles of the ventral head region are already present. The intermandibularis anterior is a broad transverse muscle sheet that broadens at both lateral ends. The caudoventral fibres, representing the pars labialis, run almost straight (in a transverse plane), ventral to the anterior margin of the hyoid bar. No insertion is observed on the lower lip tissue, which lies ventral to the muscle. The rostradorsal fibres diverge somewhat rostrally on both sides, running in the direction of Meckel's cartilage, without, however, reaching it. These fibres form the pars dentalis of the intermandibularis anterior (fig. 1a).

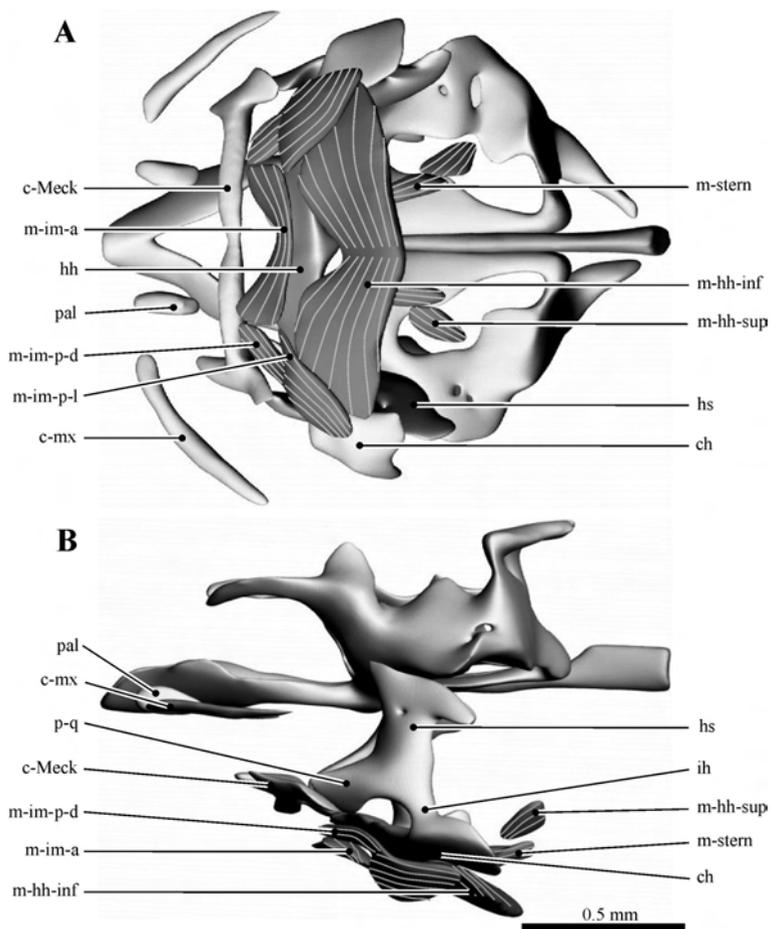


Figure 1. 3D-reconstruction of the head skeleton and ventral musculature of a 6.1 mm SL *Ancistrus* cf. *triradiatus* larva. The branchial basket and the few bony elements (premaxilla, maxilla, opercle, lateralmost branchiostegal ray) are not shown. A. Ventral view; B. lateral view. *c-Meck* cartilago Meckeli; *c-mx* cartilago maxillaris; *ch* ceratohyale; *hh* hypohyale; *hs* hyosymplecticum; *ih* interhyale; *m-hh-inf* musculus hyohyoideus inferior; *m-hh-sup* musculus hyohyoideus superior; *m-im-a* musculus intermandibularis anterior; *m-im-p-d* musculus intermandibularis posterior pars dentalis; *m-im-p-l* musculus intermandibularis posterior pars labialis; *m-stern* musculus sternohyoideus; *p-q* pars quadrata of palatoquadratum; *pal* palatinum.

The intermandibularis posterior is a paired muscle, originating at the ventral face of the lateral part of the hyoid bar. It probably already attaches to the cartilage. Anteriorly, two parts can be distinguished that can't be separated near the posterior origin. The intermandibularis posterior pars dentalis is a dorsal group of fibres that runs mediorostrally, and ends halfway between the hyoid bar and Meckel's cartilage, lateral to the pars dentalis of the intermandibularis anterior. Another part of the muscle, corresponding to the intermandibularis posterior pars labialis, runs more ventrally, in the direction of the lower lip. Its anteriormost end almost meets

the lateral end of the intermandibularis anterior pars labialis. There is no doubt, however, that there is no contact between fibres of both muscles.

The hyohyoideus inferior is a single, broad muscle plate with a slightly V-shaped appearance. The fibres originate at the ventral side of the hyoid bar just medial to the intermandibularis posterior, and run posteromedially until both halves unite ventral to the branchial region. At this moment only the anterior copula of the branchial basket has developed, which is continuous with the hyoid bar.

The sternohyoideus has arisen as a small, paired muscle, that lacks any insertion at this moment. The muscle stretches from the level of the anterior copula (lying ventrolateral to it) almost to the level where the cleithrum of the pectoral girdle will develop. The paired muscle is still very small, and round in transsection.

The hyohyoideus superior is an equally rudimentary muscle. The fibres start just lateral to the sternohyoideus, halfway along the length of the latter muscle. They then run obliquely in the direction of the branchiostegal membrane. This membrane is still only partly formed, and only the lateralmost branchiostegal ray is developing at this moment (not shown on fig. 1). The muscle has not yet reached this branchiostegal ray. It has not yet divided into abductor and adductores parts.

8.0 mm SL – 7 days post-fertilization (fig. 2)

At this moment the chondrocranium is more or less complete, and several bony elements are already present, e.g., the premaxillary and dentary parts supporting the teeth (Geerinckx et al., 2005; Geerinckx et al., 2007b). Both parts of the intermandibularis anterior are now well discernable, as the muscle is now clearly forked, and both parts contact each other only at the midline. The pars dentalis is becoming more C-shaped, as both lateral ends grow anteriorly. The absence of most of the dentary bone suggests this muscle is not fully functional at this moment. The pars labialis still is a transverse sheet, somewhat narrower medially, and lying caudoventral to the pars dentalis. It contacts the lower lip tissue not only at both lateral ends, but along the whole of its length.

The intermandibularis posterior now originates on the ventral face of the cartilaginous ceratohyal. Both muscle parts have grown anteriorly. The pars dentalis has arrived at the level of the dentary, without any clear sign of insertion though. The ventral and more flattened pars labialis, still confluent with the pars dentalis caudally, now reaches to the lateral end of the intermandibularis anterior pars labialis. Neither in this stage, nor in any older stage, fibres have been found that are continuous between both muscles. The intermandibularis posterior pars labialis thus ends exactly at the place where the lower lip is folded backwards (the lower lip is folded back posteroventrally and forms the posterior half of the sucker that surrounds the loricariid mouth). From this stage on it is clear that the fibre diameter is significantly larger in the pars dentalis than in the pars labialis of both the intermandibulares anterior and posterior muscles.

Since the 6.1 mm stage the ceratohyal has become notably broader laterally. Coupled to this, the hyohyoideus inferior has extended its insertion posteriorly, and

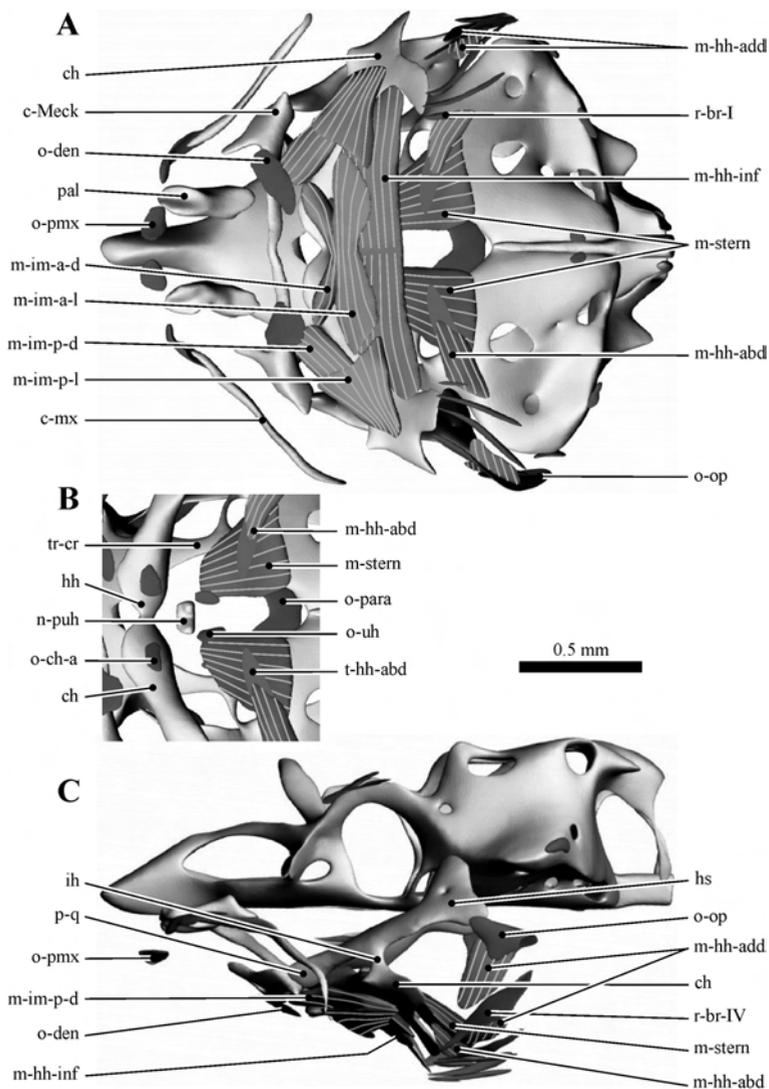


Figure 2. 3D-reconstruction of the head skeleton and ventral musculature of a 8.0 mm SL *Ancistrus* cf. *triradiatus* larva. The branchial basket is not shown. A. Ventral view; B. detail of ventral view, with several muscles removed to show the parurohyal elements; C. lateral view. *c-Meck* cartilago Meckeli; *c-mx* cartilago maxillaris; *ch* ceratohyale; *hh* hypohyale; *hs* hyosymplecticum; *ih* interhyale; *m-hh-inf* musculus hyohyoideus inferior; *m-hh-abd* musculus hyohyoideus abductor; *m-hh-add* musculus intermandibularis anterior pars dentalis; *m-im-a-l* musculus intermandibularis anterior pars labialis; *m-im-p-d* musculus intermandibularis posterior pars dentalis; *m-im-p-l* musculus intermandibularis posterior pars labialis; *m-stern* musculus sternohyoideus; *n-puh* cartilago nucleus of parurohyale, associated to anterior copula (branchial basket not shown); *o-ch-a* os ceratohyale anterior; *o-den* os dentale; *o-op* os operculare; *o-para* os parasphenoideum; *o-pmx* os praemaxillare; *o-uh* sesamoid (urohyal) part of os parurohyale; *p-q* pars quadrata of palatoquadratum; *pal* palatinum; *r-br-I-IV* radius branchiostegus I-IV; *t-hh-abd* tendon of hyohyoideus abductor; *tr-cr* trabecula cranii.

has thus also broadened laterally. It is now the largest cranial muscle, being more voluminous even than the adductor mandibulae.

Considerable growth has occurred in the sternohyoideus. Both halves still remain separate. Each half, however, has expanded substantially: anteriorly, insertion is on the dorsal surface of each urohyal half of the developing parurohyal; posteriorly, the broad muscle inserts somewhat dorsally on the anterior edge of the cleithrum. As yet, there is no articulation between the urohyal halves and the hyoid bar, but a connection of the urohyal to the anterior copula ensures an indirect connection between the sternohyoideus and the hyoid bar (Geerinckx et al., 2007b). Both the hyohyoideus inferior and the sternohyoideus could well be functional by now.

All four branchiostegal rays have now developed, and the hyohyoideus superior now consists of two distinct parts, the medial hyohyoideus abductor and the more lateral hyohyoidei adductores. The hyohyoideus abductor reaches more or less up to the dorsal aspect of the first, medialmost ray, which is just appearing in this stage (fig. 2a). The muscle still runs somewhat anteromedially, but the insertion on the ventral fascia of the sternohyoideus has shifted to the posterior part of the latter muscle (probably due to allometric growth of this muscle instead of to a real migration of the hyohyoideus abductor tendon). The hyohyoideus abductor is the only hyoid muscle that has a substantial tendon (fig. 2b). Other muscle insertions, including the posterior hyohyoideus abductor insertion, are all primarily muscularous.

The hyohyoidei adductores have differentiated between the 6.1 and 7.0 mm stages, as shown by serial sections of these specimens. The plural name reflects the fact that several short muscle bands, running from one branchiostegal ray to another, together constitute the adductor of the branchiostegal membrane. Two of these parts are already observed in this stage: a first muscle, stretching between the opercle and the lateralmost, fourth branchiostegal ray, and a second, still smaller muscle, connecting the fourth to the third ray. The third, medialmost part, connecting the third to the second ray, has not yet appeared.

12.4 mm SL – 43 days post-fertilization (fig. 3)

By the moment most of the osteocranium elements are present, muscle differentiation has more or less reached its completion. Only minor changes in relative size or orientation of muscles are observed between this and older stages.

The teeth-bearing part of the dentary has the form of an oval basket, suspended to a lateral handle including the angulo-articular, part of the dentary, and the mentomeckelium that has fused dorsally to the dentary. The intermandibularis anterior pars dentalis inserts in a shallow fossa on the lateral aspect of the dentary, anteroventral to the fusion of the dentary to the angulo-articular. As such, the curved pars dentalis surrounds the teeth-bearing baskets of both lower jaws caudally. The pars labialis of the intermandibularis anterior still contacts the pars dentalis medially, but can be considered as a separate functional unit.

Both parts of the intermandibularis posterior have completely separated: posteriorly some of the fibres touch, but they are not continuous. The pars dentalis is

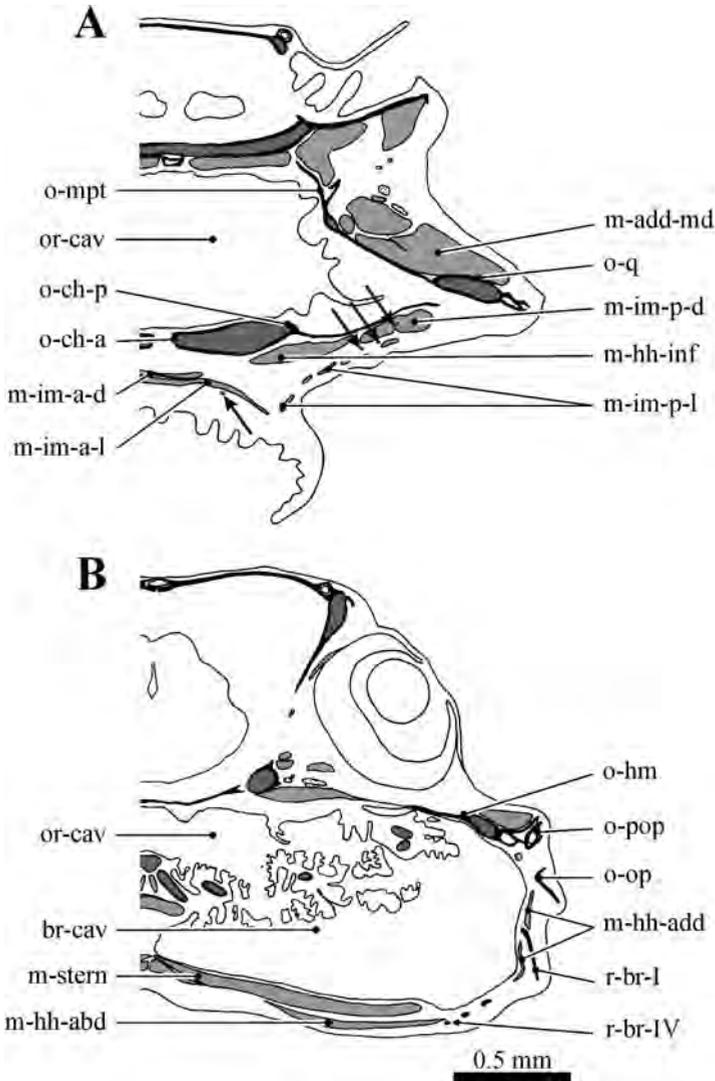


Figure 3. Cross sections of a 12.4 mm SL *Ancistrus* cf. *triradiatus* larva. A. At posterior portion of hyoid bar; B. at branchiostegal rays (black indicates bone, dark grey indicates cartilage, light grey indicates muscle, stippled areas indicate nerves). Arrows indicate the portions of the interior mandibular nerve branch of the trigeminal nerve. *br-cav* branchial cavity; *m-add-md* musculus adductor mandibulae; *m-hh-abd* musculus hyohyoideus abductor; *m-hh-add* musculi hyohyoidei adductores; *m-hh-inf* musculus hyohyoideus inferior; *m-im-a-d* musculus intermandibularis anterior pars dentalis; *m-im-a-l* musculus intermandibularis anterior pars labialis; *m-im-p-d* musculus intermandibularis posterior pars dentalis; *m-im-p-l* musculus intermandibularis posterior pars labialis; *m-stern* musculus sternohyoideus; *o-ch-a* os ceratohyale anterior; *o-ch-p* os ceratohyale posterior; *o-hm* os hyomandibulare; *o-mpt* os metapterygoideum; *o-op* os operculare; *o-pop* os praepopulare; *o-q* os quadratum; *or-cav* oral cavity; *r-br-I-IV* radius branchiostegus I-IV.

a compact muscle, originating posterolateral to the pars labialis on the posterior ceratohyal, and inserting on the lateral aspect of the dentary. The corresponding fossa in this bone is minute, lying just anterior to that of the pars dentalis of the intermandibularis anterior. The pars labialis of the intermandibularis posterior is a thin muscle, and has formed several separate small bundles that look like diverging threads, all inserting on the lower lip (fig. 3a).

The hyohyoideus inferior expands as the hyoid (now mostly ossified) becomes broader. Due to its posterior expansion on the caudoventral process of the hyoid, the muscle almost reaches the bases of the branchiostegal rays.

The left and right halves of the sternohyoideus have fused medially, now also ensuring a direct mechanical couple between the pectoral girdle and the hyoid: the parurohyal bone is well developed, and two anterior processes are firmly connected to, and articulate with, the hypohyals.

The hyohyoideus abductor has come to lie in an almost transverse plane, although not yet as in adult specimens (fig. 4). It attaches tendinously to the sternohyoideus

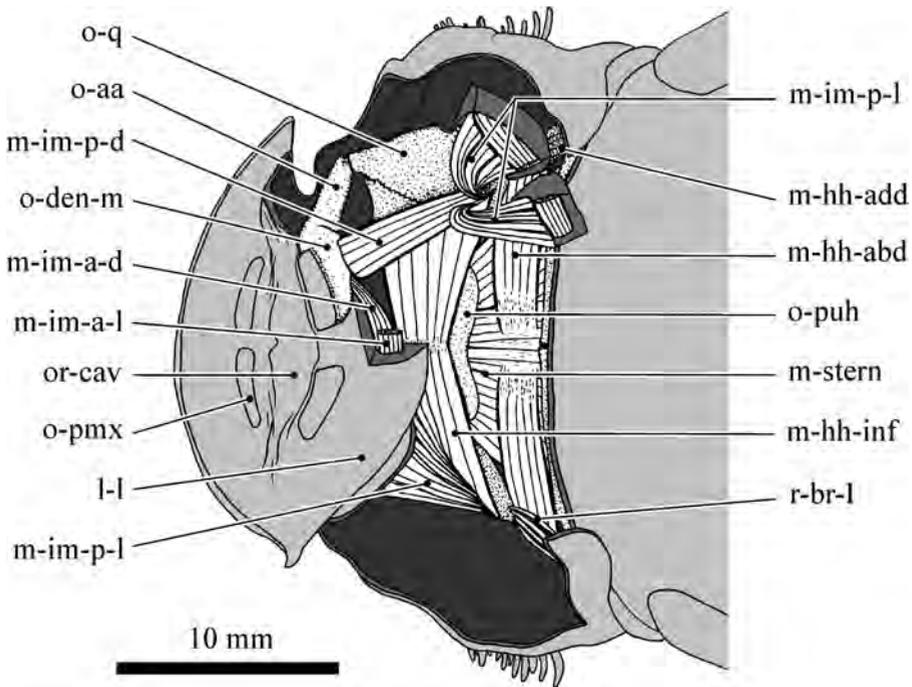


Figure 4. Ventral view of the cranial musculature of adult *Ancistrus* cf. *triradiatus* (94 mm SL). Part of the skin, left half of the lower lip and associated muscles removed. *l-l* lower lip; *m-hh-inf* musculus hyohyoideus inferior; *m-hh-abd* musculus hyohyoideus abductor; *m-hh-add* musculi hyohyoidei adductores; *m-im-a-d* musculus intermandibularis anterior pars dentalis; *m-im-a-l* musculus intermandibularis anterior pars labialis; *m-im-p-d* musculus intermandibularis posterior pars dentalis; *m-im-p-l* musculus intermandibularis posterior pars labialis; *m-stern* musculus sternohyoideus; *o-aa* os angulo-articulare; *o-den-m* os dento-mentomeckelium; *o-pmx* os praemaxillare; *o-puh* os parurohyale; *o-q* os quadratum; *or-cav* oral cavity; *r-br-I*, radius branchiostegus I.

medially. The fibres primarily insert on the medial, first branchiostegal ray, and a few tendinous fibres stretch somewhat further, toward the second ray. Except for these few fibres, no muscle, but a short ligamentous band connects the first and second branchiostegal rays, both in this and adult specimens (fig. 3b).

In the 10.2 mm stage, the third part of the hyohyoidei adductores has appeared. In the 12.4 mm and later stages the three parts, connecting the opercle to the fourth ray, the fourth to the third ray, and the third to the second ray, continue to broaden as the branchiostegal rays elongate.

Nerve patterns and muscle innervations

Both parts of the intermandibularis anterior are innervated by the inferior mandibular branch of the trigeminal nerve, that originates from the infraorbital nerve trunk, passes over the lower jaw and runs back somewhat caudally, finally reaching the muscle. This nerve also enters the intermandibularis posterior, innervating both of its parts (fig. 5a-b-c). It is the only innervation of these muscles; no twig of the hyoid branch of the facial nerve enters the caudal portion of the intermandibularis posterior. The hyohyoideus inferior receives a branch from the hyoid branch of the facial nerve (fig. 5d), but also, remarkably, receives a thin branch of the inferior mandibular branch of the trigeminal nerve (fig. 5c). This latter innervation was unambiguously observed in all examined specimens except the 6.1 mm specimen (where most nerves are still poorly visible). Hyoidei abductor and adductores are innervated by posterior branches of the hyoid branch of the facial nerve (hyomandibular trunk portion). A branch of the occipito-spinal nerve supplies each half of the sternohyoideus.

DISCUSSION

Ancistrus cf. *triradiatus* is the second siluriform species of which the ontogeny of the intermandibular and hyoid musculature is described. A few comparisons can be made with the African catfish *Clarias gariepinus*, that has been the focus of Surlemont et al. (1989), Surlemont and Vandewalle (1991) and Adriaens and Verraes (1997a). In *A.* cf. *triradiatus* all ventral head muscles except the hyohyoidei adductores are observed in the 6.1 mm SL specimen. Of these, the sternohyoideus is least developed, as its size relative to the muscle in juvenile and adult specimens is almost negligible. The other muscles are already more substantial, compared to their final sizes. Insertions are observed in the hyohyoideus inferior and intermandibularis posterior (posteriorly). In *C. gariepinus*, the intermandibularis anterior, protractor hyoidei and hyohyoideus inferior are present in the 4.7 mm TL specimen (Surlemont and Vandewalle, 1991), while in the 5.2 mm TL specimen the sternohyoideus is seen (Surlemont et al., 1989). Only in the 7.2 mm SL specimen the hyohyoidei abductor and adductores are observed (Adriaens and Verraes, 1997a). Notice that Surlemont and collaborators used total length and not standard length to denominate their stages.

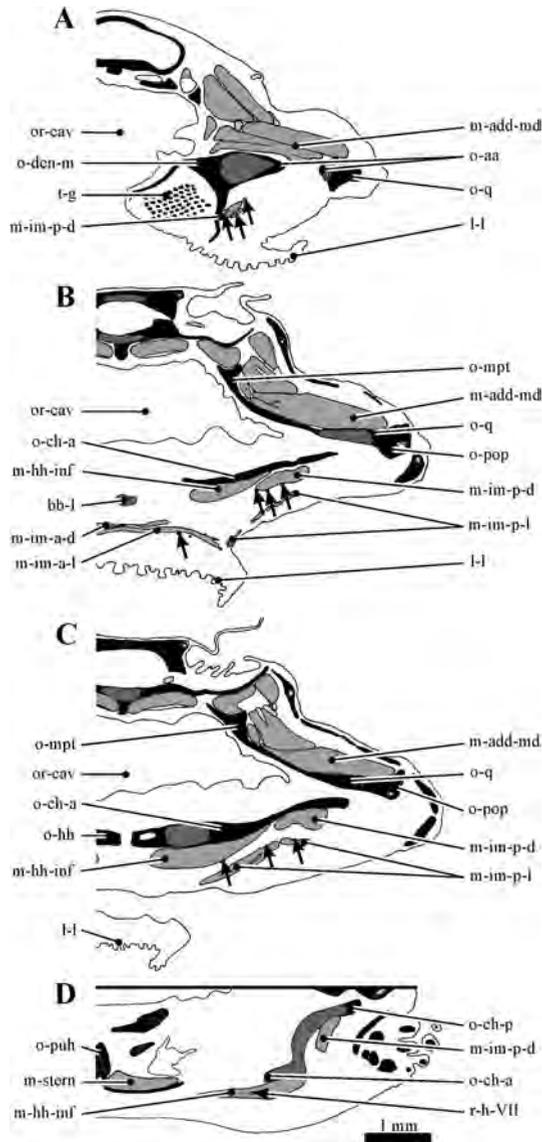


Figure 5. Cross sections of a 33.5 mm SL *Ancistrus* cf. *triradiatus* specimen. Only the right half of each section is shown (lower right portion in D). A. At lower jaw; B. at anterior edge of hyoid; C. at hyoid; D. at posterior edge of hyoid (black indicates bone, dark grey indicates cartilage, light grey indicates muscle, stippled areas indicate nerves). Arrows indicate the portions of the interior mandibular nerve branch of the trigeminal nerve. *bb-l* basibranchiale I; *l-l* lower lip; *m-add-md* musculus adductor mandibulae; *m-hh-inf* musculus hyohyoideus inferior; *m-im-a-d* musculus intermandibularis anterior pars dentalis; *m-im-a-l* musculus intermandibularis anterior pars labialis; *m-im-p-d* musculus intermandibularis posterior pars dentalis; *m-im-p-l* musculus intermandibularis posterior pars labialis; *m-stern* musculus sternohyoideus; *o-aa* os angulo-articulare; *o-ch-a* os ceratohyale anterior; *o-ch-p* os ceratohyale posterior; *o-den-m* os dento-mentomeckelium; *o-hh* os hypohyale; *o-mpt* os metapterygoideum; *o-pop* os praeperculare; *o-puh* os parurohyale; *o-q* os quadratum; *or-cav* oral cavity; *r-h-VII* ramus hyoideus nervus facialis; *t-g*, tooth germs.

Intermandibularis anterior

Both intermandibularis anterior parts, i.e., the pars dentalis and the pars labialis, run transversely in all examined *Ancistrus* cf. *triradiatus* stages. They originate as one, forked muscle, and separate during further ontogeny. In adults, they are completely separated, even in the midline (Geerinckx et al., in press a). The identification of this muscle as intermandibularis anterior (the anteriormost, transversely oriented part of the ventral intermandibularis muscle complex in teleosts) corresponds to the definition introduced, or applied by Vetter (1878), Edgeworth (1935), Greenwood (1971), Anker (1974), Winterbottom (1974), Miyake et al. (1992), Schilling and Kimmel (1997) and Diogo and Vandewalle (2003). The differentiation of the intermandibularis anterior into dentary and labial bundles has not been described in related loricarioid families; detailed accounts on this muscle in loricariids are lacking (Howes, 1983a; Schaefer and Lauder, 1986; Schaefer, 1990). In the related callichthyids only a dentary part is present (Huysentruyt, pers. comm.).

Intermandibularis posterior

The muscle here named intermandibularis posterior is usually termed protractor hyoidei in loricariids and other siluriforms (in loricariids, e.g., Alexander, 1956; Howes, 1983a; Schaefer and Lauder, 1986, 1996; Schaefer, 1997). The term protractor hyoidei (often called geniohyoideus) has been generally used to identify the muscle stretching between the lower jaw (dentary) and the lateral part of the hyoid bar [Greenwood (1971) and Winterbottom (1974) argued why the use of the name geniohyoideus should be avoided in teleosts]. The name protractor hyoidei has been applied first by Holmqvist (1910), and concerns a muscle with an anterior part derived from the intermandibularis posterior and a posterior part derived from an anterior portion of the ventral hyoid muscle plate (interhyoideus; Edgeworth, 1935; Winterbottom, 1974). In zebrafish, the anterior interhyoideus portion of this hyoid muscle plate originates separately from the posterior hyohyoideus portion (Schilling and Kimmel, 1997). Here, and in several other teleosts, the fusion of the intermandibularis posterior and the interhyoideus, connected at the midline, results in an X-shaped protractor hyoidei (Hernández et al., 2002).

The compound nature of the protractor hyoidei is always coupled to a double innervation. The anterior segment is innervated by the inferior mandibular nerve branch of the trigeminal nerve (V) [the mandibular branch of this nerve serves the whole mandibular muscle plate (Jarvik, 1980)]. Innervation of the posterior segment is by, at least, the hyoidean branch of the hyomandibular nerve trunk of the facial nerve (VII) (Dietz, 1914; Winterbottom, 1974). This nerve trunk innervates all muscles derived from the hyoid muscle plate (Jarvik, 1980). Usually a transverse myocomma is observed on the line separating both muscle parts (Greenwood, 1971; Winterbottom, 1974). The relative contribution of the interhyoideus part in the protractor hyoidei varies widely, from a very large part in, e.g., the osteoglossomorph

Pantodon (Greenwood, 1971), to a small posterior portion in, e.g., the siluriform *Ictalurus* (Winterbottom, 1974).

In Notopteridae and Mormyridae, as well as in some (unspecified) 'siluroid ostariophysans' the interhyoideus portion is absent (Greenwood, 1971: 49), so that there is, in fact, no compound protractor hyoidei, but only an intermandibularis posterior. The same morphology is present in *Ancistrus* cf. *triradiatus*. Two nomenclatural options remain. First, following the terminology of Greenwood (1971), the usage of the name protractor hyoidei could be banned for those ostariophysans with the muscle lacking an interhyoideus portion and the corresponding hyoidean nerve branch innervation. This, of course, would imply some revisions of taxa in which the muscle has been described without coverage of the innervation. Second, the name protractor hyoidei could be expanded to that muscle connecting the lower jaw and the hyoid arch, and consisting of the intermandibularis posterior and the anterior portion of the interhyoideus, or of the intermandibularis posterior alone. As far as is known, the presence of the latter muscle in the protractor hyoidei appears to be almost universal (but see the remark on *Hypophthalmus* below). An expansion of the usage of the name protractor hyoidei has been proposed by Winterbottom (1974), to avoid nomenclatural confusion originating from the names for the intermandibular part (intermandibularis posterior or intermandibularis II) (Holmqvist, 1911; Edgeworth, 1928, 1935).

A revision of descriptions among actinopterygians led us to the conclusion that the first option is preferable. The protractor hyoidei should be used only if evidence of both muscle parts is present. In most taxa that have been thoroughly examined, a compound protractor hyoidei is indeed present, as a transverse myocomma has been observed (e.g., Kirchhoff, 1958; Kampf, 1961; Thiele, 1963; Dutta, 1968), or both innervations have been observed (e.g., Vetter, 1878; Dietz, 1914; Edgeworth, 1935; Datta Munshi and Singh, 1967). In several siluriforms, however, in which the muscle has been described, only the inferior mandibular branch innervation has been found to be present [Juge, 1899 on *Silurus glanis* and Singh, 1967 on *Clarias batrachus*, *Callichrous pabda* (now valid as *Ompok pabda*), *Eutropiichthys vacha*, *Rita rita* and *Wallago attu*]. Thus in several siluriforms there is no compound protractor hyoidei, but an intermandibularis posterior only. An exceptional, opposite configuration appears to be present in the pimelodid catfish *Hypophthalmus edentatus*, in which Howes (1983b) only mentions innervation of the protractor hyoidei by branches of the ramus hyoideus VII. Whether the anterior innervation is present, or the muscle consists of the interhyoideus alone, should be verified, as Howes (1983b) didn't explicitly mention the (aberrant) absence of the inferior mandibular branch innervation.

Winterbottom (1974) found a myocomma in *Diplomystes* and *Ictalurus*. The situation in the latter species differs from the description by Ghiot et al. (1984), who did not mention this myocomma. Neither of these two authors elaborated on the innervation. The nature of the so-called protractor hyoidei in other siluriforms should therefore be verified in future myological studies. It appears that the presence

of an intermandibularis posterior or a protractor hyoidei varies within the siluriform order. The diversity in muscle composition might very well be related to, or even explained by the development of a varying number of mandibular barbels in most catfish taxa (see below).

Verification of the serial sections used by Adriaens and Verraes (1997a) revealed that the ‘protractor hyoidei’ in *Clarias gariepinus* actually lacks the hyoidean branch innervation and the transverse myocomma (pers. ob.). It can thus be concluded that also here, the muscle in fact corresponds to the intermandibularis posterior only. A comparative early embryological study using antibody labelling, as done by Hernández et al. (2002) on zebrafish, carried out on related siluriforms with and without a true protractor hyoidei, would be of most interest.

We were unable to state any unambiguous homology between the bundles of the intermandibularis posterior in *Ancistrus* cf. *triradiatus* and the pars lateralis, pars dorsalis and pars ventralis of the intermandibularis posterior in *Clarias gariepinus* (Adriaens and Verraes, 1997a). Fusion of the pars ventralis and the pars lateralis of the latter species gives rise to four different fields of superficial fibres for the manipulation of the mandibular barbels.

Generally, in non-loricarioid siluriforms there is a secondary subdivision of the intermandibularis posterior/protractor hyoidei, with several bundles serving the bases of the mandibular barbels (Takahasi, 1925; Adriaens and Verraes, 1997a; Diogo and Chardon, 2000). This is not the case in loricarioids (most families of which lack these barbels) (Diogo, 2005). In the loricarioid callichthyids, that possess two pairs of mandibular barbels, these are not served by separate muscles bundles (Huysentruyt, pers. comm.). Loricariids thus are among the most aberrant catfish groups: they have an expanded lower lip that is ‘folded back’ posteroventrally, and the taxon must most probably have evolved muscle fibres connecting it to the hyoid arch (the pars dentalis) independently from non-loricarioid catfishes having mandibular barbels and associated muscle bundles. It is highly probable that the pars labialis of the intermandibularis posterior is essential in moving the lower lip and thus the function of the sucker mouth (Geerinckx et al., 2007a). Saxena and Chandy (1966) described a remarkably diverse protractor hyoidei in the cyprinids *Garra*, *Crossocheilus* and *Psilorhynchus*, fishes that also demonstrate a sucker-like mouth (both the double innervation and the myocomma are present in these species).

Hyohyoideus inferior

The posterior portion of the ventral hyoid muscle plate gives rise to the hyohyoideus inferior, hyohyoideus abductor and hyohyoidei adductores in most teleosts (a few taxa have an undifferentiated interhyoideus posterior) (Takahasi, 1925; Greenwood, 1971; Winterbottom, 1974). The hyohyoideus inferior, connecting both sides of the hyoid bar ventrally, is especially well developed in loricariids, and has been found to be somewhat forked laterally (Schaefer, 1997; Howes, 1983a; Geerinckx et al., 2007a). Ontogenetically, the posterior part develops somewhat earlier than the anterior part, with insertion on the posterior ventral surface of the cartilaginous hyoid

bar, as well as its cartilaginous ventrocaudal process to which the branchiostegal rays attach. The insertion of the anterior part is well rostral to the insertion of the intermandibularis posterior. The unexpected innervation in *Ancistrus* cf. *triradiatus* of the anteriormost fibres of the hyohyoideus inferior by a thin branch of the inferior mandibular branch of the trigeminal nerve, clearly shown in the serial sections, is highly unusual, as it is not known from other teleosts (Winterbottom, 1974). One could speculate on possible different contraction patterns, as well as on the homology of these anteriormost fibres. They do, however, originate together with the remainder of the hyohyoideus inferior, and no visible aponeurosis separates them from it.

Hyohyoideus abductor

In *Ancistrus* cf. *triradiatus* the fibre direction of the paired hyohyoideus abductor changes during ontogeny. The medial insertion shifts backwards, until the muscle lies completely transverse. It then stretches from the medialmost branchiostegal ray straight towards the midline, not reaching it, but inserting on the ventral fascia of the sternohyoideus, whose fibres run perpendicular to it. This transverse direction of the hyohyoideus abductor is intriguing. In most teleosts its medial insertion is more rostral, on the hyoid bar (Winterbottom, 1974). Thus the muscle direction usually is oblique, and the muscle reaches or crosses the midline anteriorly. It has been stated that the expected function of the muscle, i.e., the expansion of the branchiostegal membrane, is not possible when the fibres run transversely (manipulation of the muscle effectively closes the branchiostegal opening) (Geerinckx et al., 2007a). In teleosts without a distinct hyohyoideus abductor, a caudal shift of fibres of the hyohyoideus inferior (undifferentiated interhyoideus posterior?) has been described, inserting on the proximal region of the branchiostegal rays; also the intermandibularis posterior/protractor hyoidei may reach the rays (Winterbottom, 1974). As such, these muscles can generate expansion of the branchiostegal membrane. It might be possible that the insertion of the posterior fibres of the hyohyoideus inferior on the ventrocaudal process of the hyoid bar can generate such an expansion as well; this process is cartilaginous. Schaefer (1990) observed insertion of these posterior fibres on the bases of the branchiostegal rays in loricariids and some other loricarioids. It has to be mentioned that the loricariid branchiostegal membrane is very small compared to that of most other siluriforms, where the number of branchiostegal rays can be much higher (up to 20) (McAllister, 1968; Adriaens and Verraes, 1998). The long cartilaginous ventrocaudal process of the hyoid bar, observed in loricariids, is not present in other siluriforms, where the rays usually articulate with the ceratohyals directly (Arratia, 1987).

Hyohyoidei adductores

The three strands of hyohyoidei adductores on each side arise in the same sequence as the branchiostegal rays: the medialmost strand (between the second and third ray)

in *Ancistrus* cf. *triradiatus* develops latest. The strands are not continuous in any developmental stage nor in adults, as is sometimes the case in teleosts (Winterbottom, 1974). While both the abductor hyohyoideus and hyohyoidei adductores originate from the hyohyoideus superior, the muscle observed in the 6.1 mm specimen could well represent the abductor alone, as its position corresponds to the medial portion of the abductor (while the adductores will develop far more laterally). Also, the adductores strands develop from lateral to medial, suggesting that the separation into the abductor and adductores parts might occur before the muscles can be recognized on toluidine blue stained serial sections.

Sternohyoideus

During development of *Ancistrus* cf. *triradiatus*, the sternohyoideus grows from a pair of narrow muscles to one broad muscle mass stretching from the dorsal aspect of the parurohyal to the edge of the whole horizontal limbs of the cleithra. This muscle is rather conserved among teleosts (Winterbottom, 1974). Its development is more or less identical to that of the muscle in *Clarias gariepinus* (Adriaens and Verraes, 1997a), and its relation to the sesamoid, 'urohyal' part of the parurohyal bone corroborates the thesis that this part develops as an ossification of the paired but fusing anterior tendons of the sternohyoideus muscle (Arratia and Schultze, 1990).

Finally, some brief, functional considerations may be added to this discussion. One could hypothesize that the contribution of the pressure pump, driven primarily by the hyoid movements, is relatively larger in *Ancistrus* cf. *triradiatus* than in other siluriforms (and some other bottom-living teleosts), where the suction pump is more important (Hughes, 1970; Adriaens and Verraes, 1997a). The relative importance of the suction pump system is correlated to the size and mobility of the opercle and branchiostegal membrane. The latter membrane of *A. cf. triradiatus* is exceptionally small for a siluriform (see above). Its relative size also substantially decreases during ontogeny; the size and extent of the branchiostegal rays in the 8.0 mm specimen (fig. 2a) and the 33.5 mm specimen (fig. 4) reflects this. The opercle is also small and is almost not moving during normal respiration (Howes, 1983a; Geerinckx et al., 2007a). In many bottom-living teleosts the branchiostegal membrane is very large (Hughes, 1970). In the siluriform *Clarias gariepinus*, the buccal pressure pump is believed to be most important in early larvae (11.4 mm TL); the importance of the opercular suction pump increases in the 15.5 mm and 21.4 mm TL larvae (Vandewalle et al., 1985). In this species the number of branchiostegal rays (and relative size of the membrane) gradually increases up to 10 during ontogeny (Adriaens and Verraes, 1998).

The shift in orientation of the hyohyoideus abductor in *Ancistrus* cf. *triradiatus* might indicate a functional shift of an abductor of the branchiostegal membrane (as in most teleosts) to an adductor (as hypothesized for adult *A. cf. triradiatus*; Geerinckx et al., 2007a). The respiration and feeding mechanism of *A. cf. triradiatus* (and other loricariids), being well adapted to the need of forceful suction for

attachment, however, most probably involves more modifications than those of the hyoid region alone. The roles of the lower jaws and the oral valve, as well as the associated musculature, hitherto have only been hypothesized for adult specimens (Geerinckx et al., 2007a). A more general study of all head muscle systems, especially in embryonic and juvenile loricariids, will yield a more complete comprehension of the cranial movements and functional shifts during ontogeny.

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