

The erectile cheek-spine apparatus in the bristlenose catfish *Ancistrus* (Loricariidae, Siluriformes), and its relation to the formation of a secondary skull roof

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Abstract

In the South American catfish family Loricariidae, the opercle has been decoupled from the lower jaw, and has also lost its function in expiration. While many loricariid species have a small and slightly mobile opercle with reduced opercular musculature, within the hypostomine subfamily a novel opercular mechanism has developed that erects a tuft of enlarged odontodes anterior to the opercle. This defensive mechanism is examined in *Ancistrus* cf. *triradiatus*. The opercle has a prominent anterior process and the orientation of the reinforced articulation hinge to the hyomandibular bone has shifted. The opercular musculature is well developed, with a hypertrophied dilatator operculi that extends deep inside the skull roof bones and toward the midline, over the brain, but below the superficial skull roof. Hence the frontal, sphenotic, parieto-supraoccipital and compound pterotic bones consist of a dorsal, superficial part and a deeper part separating the brain from the muscle: two functional skull roofs are thus formed. The impact on the path of the cranial sensory canals is substantial, moving canals away from the skull surface. Hypertrophy of cranial muscles is known from many teleosts, but the invasion of such large muscles into the skull, which is drastically modified and literally hollowed out, has never been described before. These cranial modifications are greater in males than in females, related to the territorial behavior of the former, in which the erectile spines are usually used.

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Introduction

Among the Neotropical fish taxa, the suckermouth armored catfishes or Loricariidae are remarkable for their atypical catfish morphology, specialized feeding apparatus and high species and shape diversity. Their armor, covering almost the whole body, is formed by numerous dermal bone plates carrying odontodes, tooth-like structures composed of dentine, covered by a hypermineralized

(enameloid) substance. These are firmly ankylosed to the bone or anchored by connective tissue fibers (Bhatti, 1938; Sire and Huysseune, 1996). This spiky external skeleton might well be an effective protection against predation. Odontodes are thought to have evolved independently in the loricarioid lineage (Bhatti, 1938; Reif, 1982), and are also found in e.g. callichthyids, scoloplacids, and the almost naked astrolepids, related loricarioid families (Schaefer et al., 1989; Sire and Huysseune, 1996; Schaefer and Buitrago-Suárez, 2002).

In some loricariid genera, the tuft of large, spiny odontodes anterior to the opercle can be erected.

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Alexander (1965) and Howes (1983) mentioned the role of the dilatator operculi muscle in the erection of the odontodes. In *Ancistrus*, where this mechanism is most developed, both authors noted the impressive size of this muscle, reaching toward the dorsal midline of the skull, between the braincase and the dermal roof of the skull.

All loricariid odontodes, including the erectile ones, can be shed; marks on the dermal ossicles of *Ancistrus* cf. *triradiatus* and *Chaetostoma* sp. provide evidence of odontode loss or shedding (Howes, 1983; Geerinckx, pers. obs.). The fixed cheek odontodes of *Loricaria uracantha* develop in ca. 48 days and are shed after ca. 148 days (Moodie and Power, 1982). The number of erectile spines of the cheek-spine apparatus in *Ancistrus* and related genera is taxonomically informative (Eigenmann and Eigenmann, 1890; Muller, 1989; Miqularena et al., 1994; Ceas and Page, 1996; Fisch-Muller et al., 2001). Their movements, however, have not yet been studied.

The present paper aims to describe the morphology, function, and use of the opercular odontode-erecting apparatus in *A. cf. triradiatus* Eigenmann, as well as discuss the seemingly indeterminately growing opercular musculature and its drastic impact on the spatial design of the skull roof bones.

Material and methods

Specimens of *A. cf. triradiatus* and other loricariid species (see below) were commercially obtained. Live observations of the fast movements of the mechanism were aided by the use of high-speed filming from different perspectives (using a Redlake Motionscope digital high-speed camera set at 200 frames s⁻¹). Muscle dissections were performed on several subadult to adult specimens (maturity is reached at 5–6 cm standard length). The clearing and staining method of Taylor and Van Dyke (1985) was applied for the osteology. One subadult specimen was selected for serial sectioning, using a Technovit 7100 plastic embedding, a Reichert-Jung Polycut microtome, and toluidine blue stain for visualization. Manually sliced sections (ca. 2 mm thickness) were produced from one large specimen. Specimens and sections were examined using an Olympus SZX9 stereoscopic microscope and a Reichert-Jung Polyvar light microscope, equipped with a digital camera.

Examined specimens of *A. cf. triradiatus* (with standard length): clearing and staining: 4 (male: 101.9, 95 mm; female: 76.6, 69.6 mm); dissection: 4 (male: 86, 94 mm; female: 71, 70 mm); manual sectioning: 1 (male: 108 mm); 5 µm serial sections: 1 (gender unknown: 33.5 mm). Some specimens of other loricariids were studied for comparison: *Ancistrus ramunculus* (58 mm),

Ancistrus dolichopterus (93 mm), *Pterygoplichthys lituratus* (63, 150 mm); *Farlowella acus* (124, 109 mm), *Sturisoma aureum* (83, 85 mm), *Rineloricaria parva* (75 mm) and *Otocinclus vestitus* (23, 24 mm).

Results

The following description is based on mature males, where the cheek-spine apparatus is best developed, and the associated structural implications for the neurocranium are most pronounced (see figure captions for length of specimens).

Bones of the cheek-spine apparatus

The cheek-spine apparatus consists of several bones in the lateral cheek region of the head (Fig. 1). The key element in the functioning of the cheek-spine apparatus is the opercle (Figs. 2(a)–(c)). Its articulation with the hyomandibular bone is reinforced by an anterior serration (two small ‘processes’), and a more weakly serrated, ligamentous connection between both bones posteriorly (Figs. 2(b) and 3(c)–(d)). The result is one long, strong and more or less horizontally oriented articulatory hinge around which the opercle pivots outwardly and inwardly (Figs. 3(a)–(d)). On the dorsal side insertion facets are present for the dilatator and levator operculi muscles, while the lateralmost hyohyoidei adductores bundle and the adductor operculi insert at the medioventral side (Figs. 2(b) and (c)). A very prominent anteroventral process articulates with the caudalmost ossicles carrying odontodes (Figs. 2(c) and 3(b)).

There are about 14 of these thick bony ossicles, most of which carry only one large odontode (Fig. 2(d)). To ease interpretation, and to avoid confusion with the larger cheek plates more anteriorly, we will refer to these structures as cheek spines throughout the text. The odontodes are immobile relative to the ossicles to which they are anchored.

There are four small and two larger cheek plates anterior to the cheek spines that, like other dermal bones in loricariids, carry only minute odontodes (not shown in the figures). The largest of these plates bears the distal portion of the preopercular canal (Fig. 2(e)). Its anterior edge articulates with the quadrate. All cheek plates and cheek spines are embedded in thick connective tissue, facilitating a chain of articulations (see below).

Opercular musculature

The dilatator operculi is the largest opercular muscle, and, in adult males, even the largest of all cranial

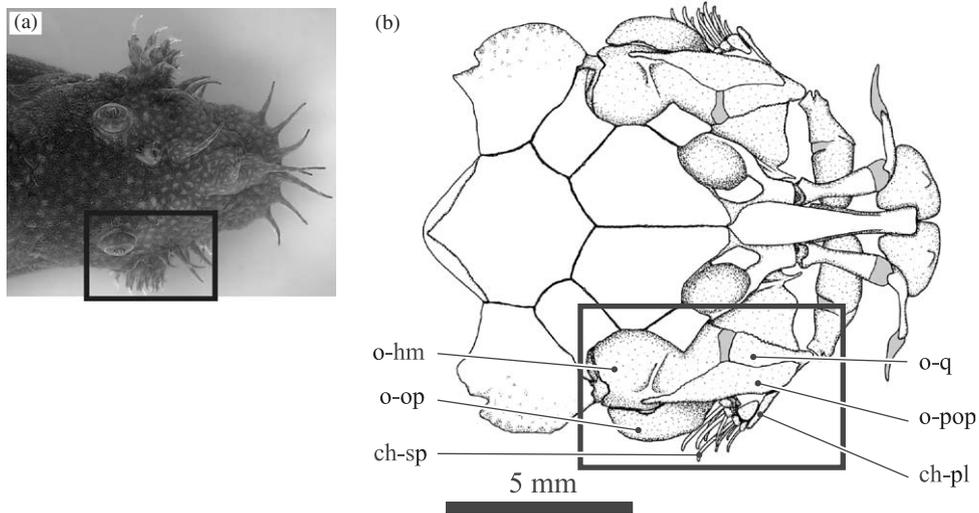


Fig. 1. (a) Head of a 85.4-mm male *Ancistrus* cf. *triradiatus* with cheek spines partially (right side) and completely (left side) erected; (b) dorsal view of neurocranium of a 44-mm subadult. Scale bar applies to figure b only. ch-pl, cheek plate; ch-sp, cheek spine; o-hm, os hyomandibulare; o-op, os operculare; o-pop, os praeoperculare; o-q, os quadratum.

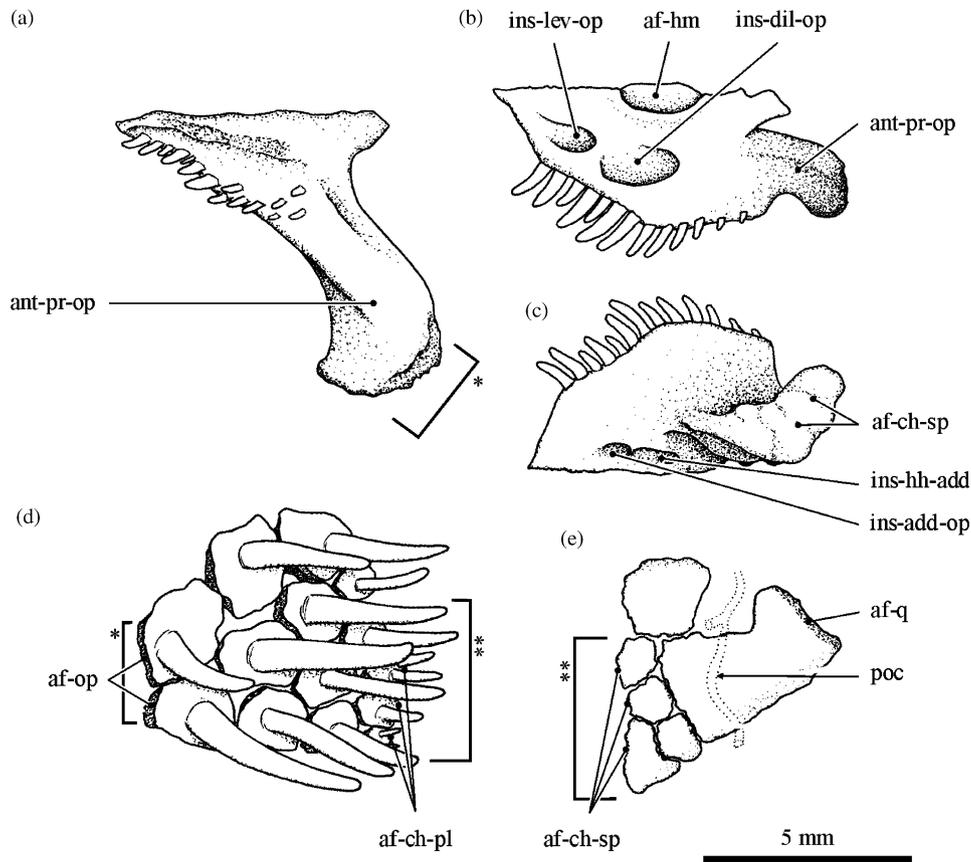


Fig. 2. Right opercle in (a) lateral; (b) dorsal; (c) ventral view; (d) right cheek spines; (e) right cheek plates, of a 95-mm SL male. Asterisks mark corresponding articular facets. Please refer to ‘Functioning and use of the cheek-spine apparatus’ for details. af-ch-pl, articular facet with cheek plates; af-ch-sp, articular facet with ossicles carrying cheek spines; af-hm, articular facet with hyomandibular; af-op, articular facet with opercle; af-q, articular facet with quadrate; ant-pr-op, anterior process of opercle; ins-add-op, insertion site of the adductor operculi; ins-dil-op, insertion site of the dilatator operculi; ins-hh-add, insertion site of the lateralmost hyohyoidei adductores bundle; ins-lev-op, insertion site of the levator operculi; poc, postotic canal.

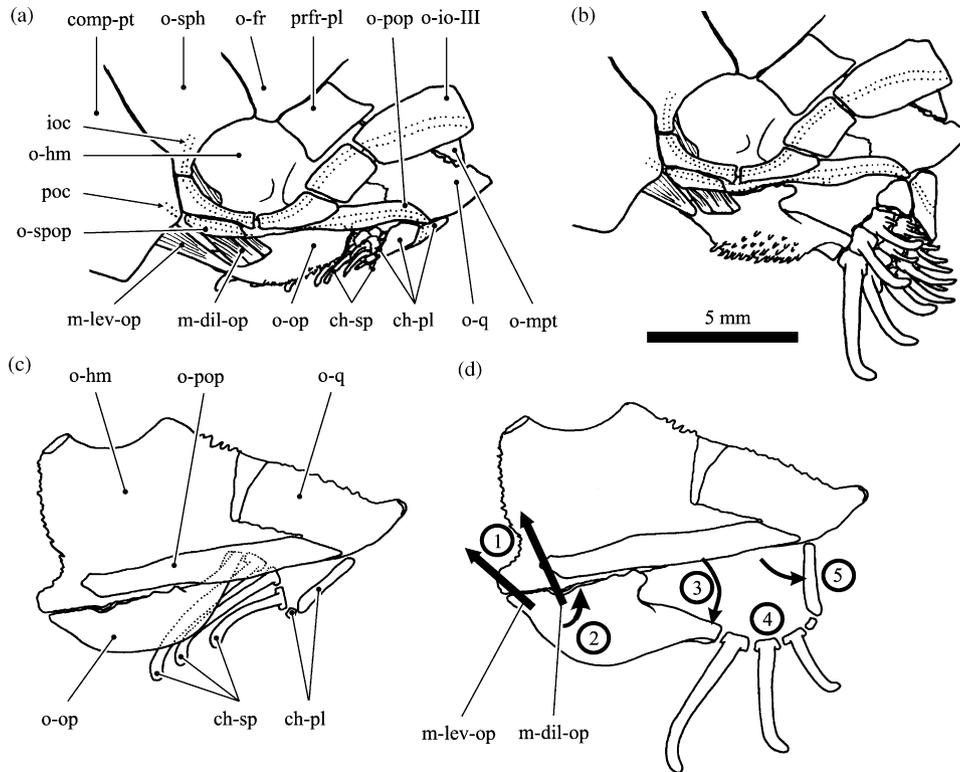


Fig. 3. Dorsal view of the right cheek-spine apparatus of a 76.6-mm female *Ancistrus* cf. *triradiatus*, (a) retracted, (b) erected. Schematic dorsal view, (c) retracted, (d) erected. Only three cheek spines and the largest and one small cheek plate are shown. Please refer to ‘Functioning and use of the cheek-spine apparatus’ for details. ch-pl, cheek plate; ch-sp, cheek spine; comp-pt, compound pterotic bone; ioc, infraorbital canal; m-dil-op, musculus dilatator operculi; m-lev-op, musculus levator operculi; o-fr, os frontale; o-hm, os hyomandibulare; o-io-III, os infraorbitale III; o-mpt, os metapterygoideum; o-op, os operculare; o-pop, os praeoperculare; o-q, os quadratum; o-sph, os sphenoticum; o-spop, os suprapraeoperculare; poc, postotic canal; prfr-pl, prefrontal plate.

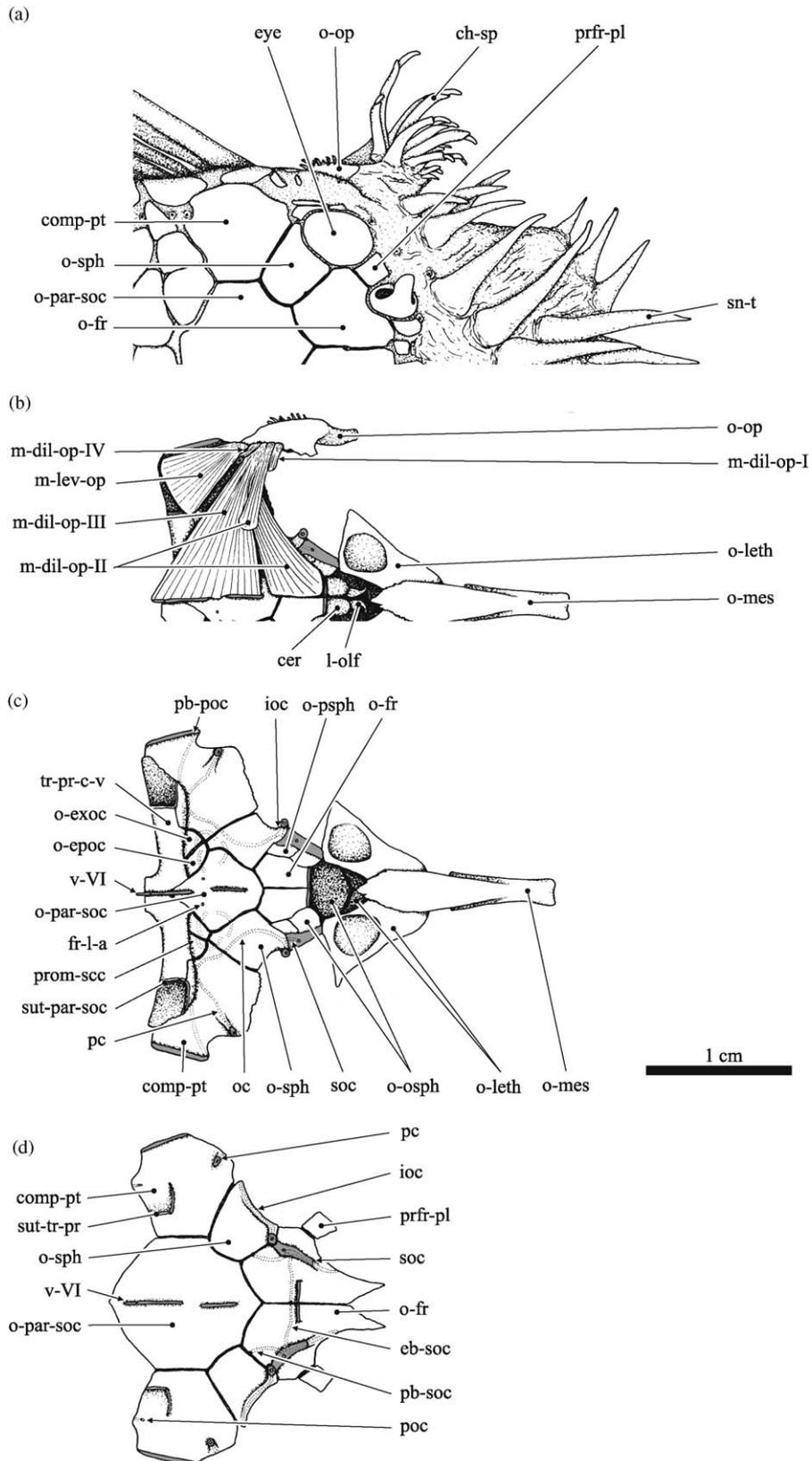
muscles. In all male and female specimens four bundles are discernable (*m-dil-op-I-IV* in Figs. 4–6). The larger the specimen, the larger the relative size and area of origin of these bundles. All bundles insert next to each other on the dorsal aspect of the opercle, lateral to the main articulation with the hyomandibular bone. Their origins, however, differ substantially. The first, anteriormost bundle is short, inserts on the opercle without an obvious tendon, and originates on the

ventral edge of the hyomandibular (this bone is not shown in Fig. 4). The second bundle is larger, and contains a distinct tendon that continues as an aponeurosis through most of the muscle length. It originates on the sphenotic and frontal bones. Both of these bones are modified to accommodate the large bundle (see below). Medially, the second bundles of both sides of the head contact each other. A thick median myocomma is present, which ossifies in the

Fig. 4. Partial dissection of a 86-mm SL male *Ancistrus* cf. *triradiatus* specimen, in dorsal view. (a) Dorsal view of head. (b) Dorsal view of neurocranium and opercle after removal of ‘primary’ skull roof. Except for the brain and the opercular muscles, no soft tissues are featured. (c) Dorsal view of ‘secondary’ skull roof. (d) Ventral view of ‘primary’ skull roof. Gray coloring indicates cut-through bone parts. cer, cerebrum; ch-sp, cheek spine; comp-pt, compound pterotic bone; eb-soc, epiphysial branch of the supraorbital canal; fr-l-a, foramen ramus lateralis accessorius nervus facialis; l-olf, lobus olfactorius; ioc, infraorbital canal; m-dil-op-I-IV, musculus dilatator operculi bundles I–IV; m-lev-op, musculus levator operculi; o-epoc, os epioccipitale; o-exoc, os exoccipitale; o-fr, os frontale; o-leth, os latero-ethmoideum; o-mes, os mesethmoideum; o-op, os operculare; o-osph, os orbitosphenoideum; o-par-soc, os parieto-supraoccipitale; o-psph, os pterosphenoideum; o-sph, os sphenoticum; oc, otic canal; pb-poc, parietal branch of the postotic canal; pb-soc, pterotic branch of the supraorbital canal; pc, preopercular canal; poc, postotic canal; prfr-pl, prefrontal plate; prom-scc, prominences of semicircular canals; sn-t, snout tentacle; soc, supraorbital canal; sut-par-soc, suture with parieto-supraoccipital; sut-tr-pr, suture with transverse process of complex vertebra; tr-pr-c-v, transverse process of complex vertebra; v-VI, (part of) dorsal process of sixth vertebra.

largest specimens. The third bundle is even more hypertrophied, and also contains a broad aponeurosis throughout most of its length. The tendon contacts the

tendon of the second bundle when reaching the opercle. The bundle originates mostly from the compound pterotic and the parieto-supraoccipital bones, but also



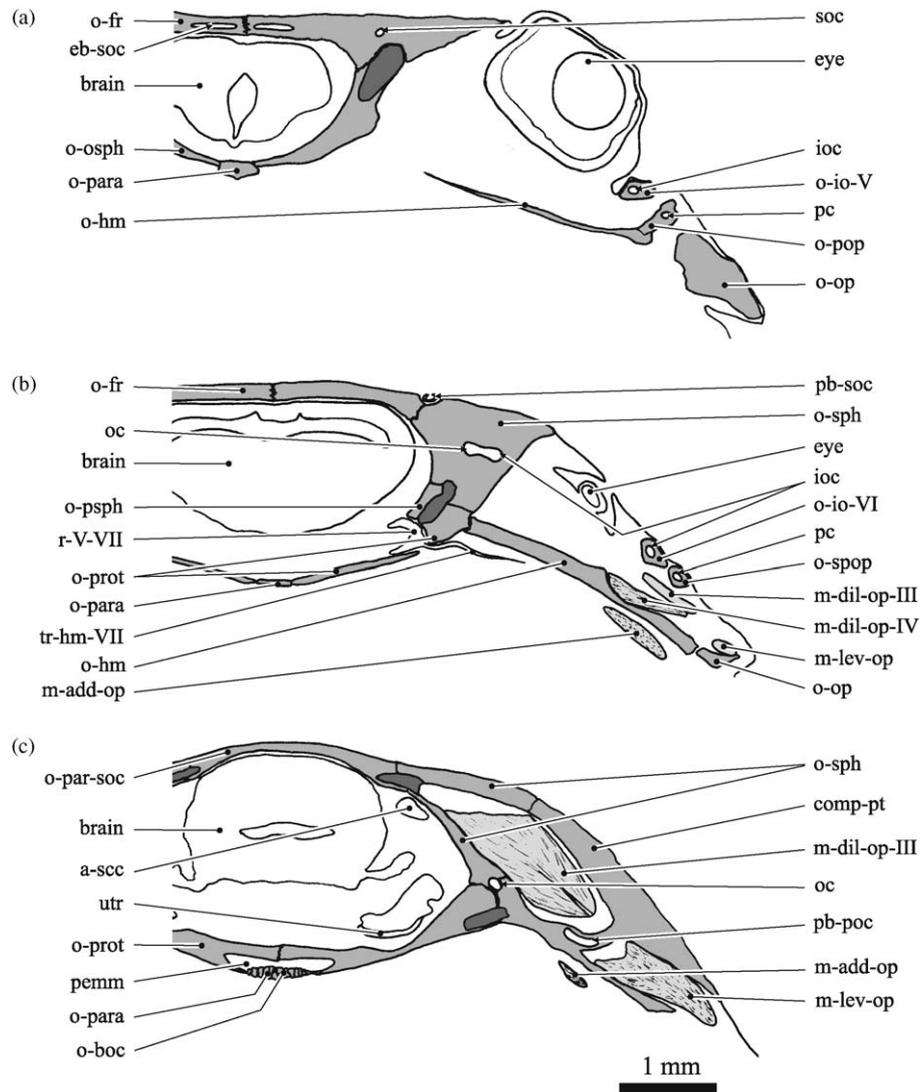


Fig. 5. Sections of a 33.5-mm SL unsexed subadult of *Ancistrus* cf. *triradiatus*, at the level of (a) the epiphysial canal branch, (b) the parietal canal branch exit, and (c) the posterior eye muscle myodome. Musculature other than the opercular, and most other soft tissues are not figured. a-scc, anterior semicircular canal; comp-pt, compound pterotic bone; eb-soc, epiphysial branch of the supraorbital canal; ioc, infraorbital canal; m-add-op, musculus adductor operculi; m-dil-op-III/IV, musculus dilatator operculi bundles III/IV; m-lev-op, musculus levator operculi; o-boc, os basioccipitale; o-fr, os frontale; o-hm, os hyomandibulare; o-io-V/VI, os infraorbitale V/VI; o-op, os operculare; o-osph, os orbitosphenoidum; o-par-soc, os parieto-supraoccipitale; o-para, os parasphenoidum; o-pop, os praeoperculare; o-prot, os prooticum; o-psph, os pterosphenoidum; o-sph, os sphenoticum; o-spop, os suprapraeoperculare; oc, otic canal; pb-poc, parietal branch of the postotic canal; pb-soc, pterotic branch of the supraorbital canal; pc, preopercular canal; pemm, posterior eye muscle myodome; r-V-VII, trigeminofacial nerve root; soc, supraorbital canal; tr-hm-VII, truncus hyomandibularis nervus facialis; utr, utricle of inner ear.

from the posterior part of the sphenotic bone, as well as from the dorsal process of the sixth vertebra. The compound pterotic and parieto-supraoccipital bones are significantly modified in large adults. Both bundles touch each other medially. The small fourth bundle lacks a real tendon, and originates on the caudal margin of the hyomandibular below the passage of the third bundle. Manipulating any of the dilatator operculi bundles results in an upward pivoting of the opercle along the hyomandibular articulation (Fig. 3(d)).

The levator operculi muscle inserts on the dorsal aspect of the opercle, posterior to the dilatator operculi, and similarly diverges broadly into a cranial cavity. It consists of a single bundle with a plate-like aponeurosis throughout the muscle and originates broadly on the compound pterotic bone, without reaching the midline. Manipulation results in the same effect as with the dilatator operculi: the opercle pivots upward (Fig. 3(d)).

The adductor operculi muscle originates with a long, narrow tendon on the ventral surface of the compound

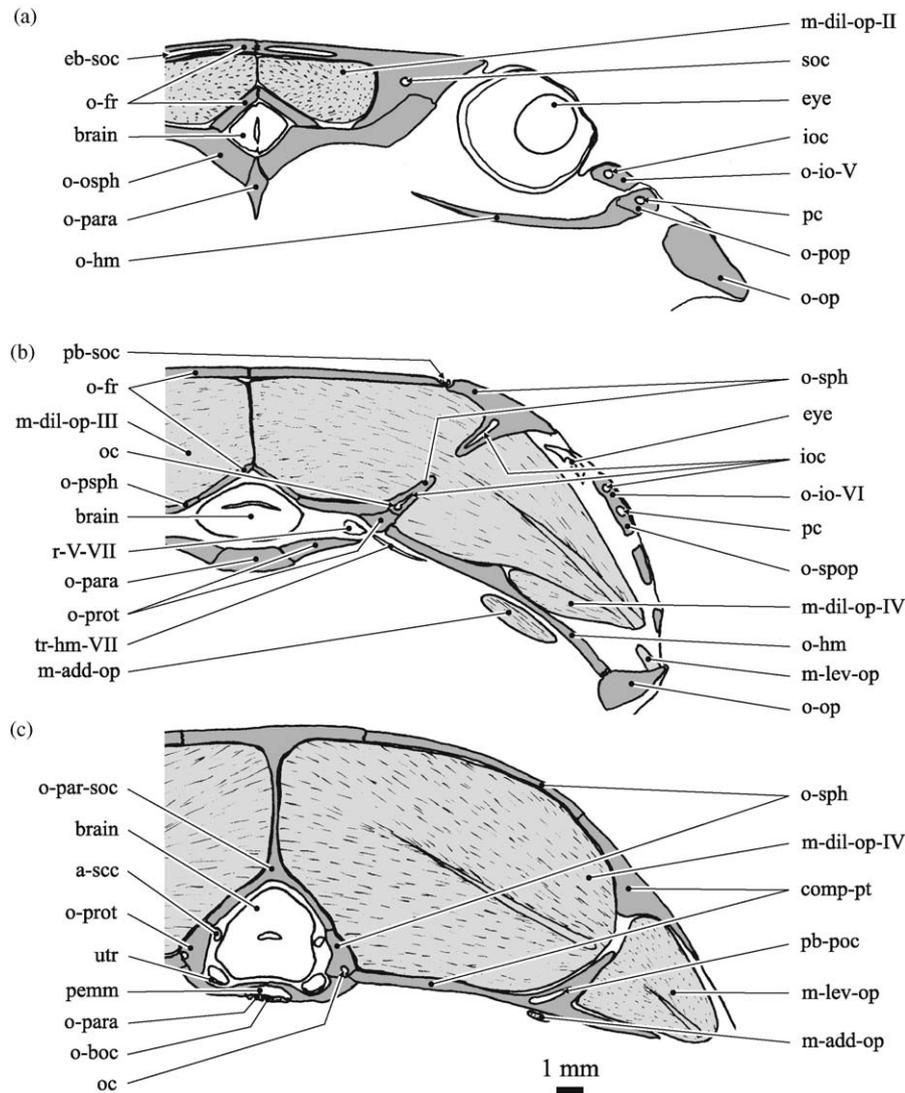


Fig. 6. Sections of a 108-mm SL (fully grown) male specimen of *Ancistrus* cf. *triradiatus*, at the level of (a) the epiphysial canal branch, (b) the parietal canal branch exit, and (c) the posterior eye muscle myodome. Muscles other than the opercular ones, and most other soft tissues are not figured. a-scc, anterior semicircular canal; comp-pt, compound pterotic bone; eb-soc, epiphysial branch of the supraorbital canal; ioc, infraorbital canal; m-add-op, musculus adductor operculi; m-dil-op-II/III/IV, musculus dilatator operculi bundles II/III/IV; m-lev-op, musculus levator operculi; o-boc, os basioccipitale; o-fr, os frontale; o-hm, os hyomandibulare; o-io-V/VI, os infraorbitale V/VI; o-op, os operculare; o-osph, os orbitosphenoideum; o-par-soc, os parieto-supraoccipitale; o-para, os parasphenoideum; o-pop, os praeoperculare; o-prot, os prooticum; o-psph, os pterosphenoideum; o-sph, os sphenoticum; o-spop, os suprapraeoperculare; oc, otic canal; pb-poc, parietal branch of the postotic canal; pb-soc, pterotic branch of the supraorbital canal; pc, preopercular canal; pemm, posterior eye muscle myodome; r-V–VII, trigeminofacial nerve root; soc, supraorbital canal; tr-hm-VII, truncus hyomandibularis nervus facialis; utr, utricle of inner ear.

pterotic. The muscle contacts the fascia of the protractor pectoralis muscle, the fibers of which run perpendicular to the direction of the adductor operculi. The muscle becomes somewhat broader before inserting on the ventromedial aspect of the opercle, posterior to the insertion of the lateralmost of the hyohyoidei adductores bundles (that originate on the fourth, lateralmost branchiostegal ray). Manipulation results in a downward rotation of the opercle, so that the muscle can be considered as the antagonist of the dilatator and levator operculi.

Growth of the dilatator operculi and the formation of a secondary skull roof

When comparing small and larger adults, a drastic change in the skull roof structure and the paths of the sensory canals is noted. This change does not seem to take place abruptly at maturation or at a certain standard length, but occurs progressively during subadult and adult growth. Until maturation, the major skull roof bones have a normal, more or less uni-layered

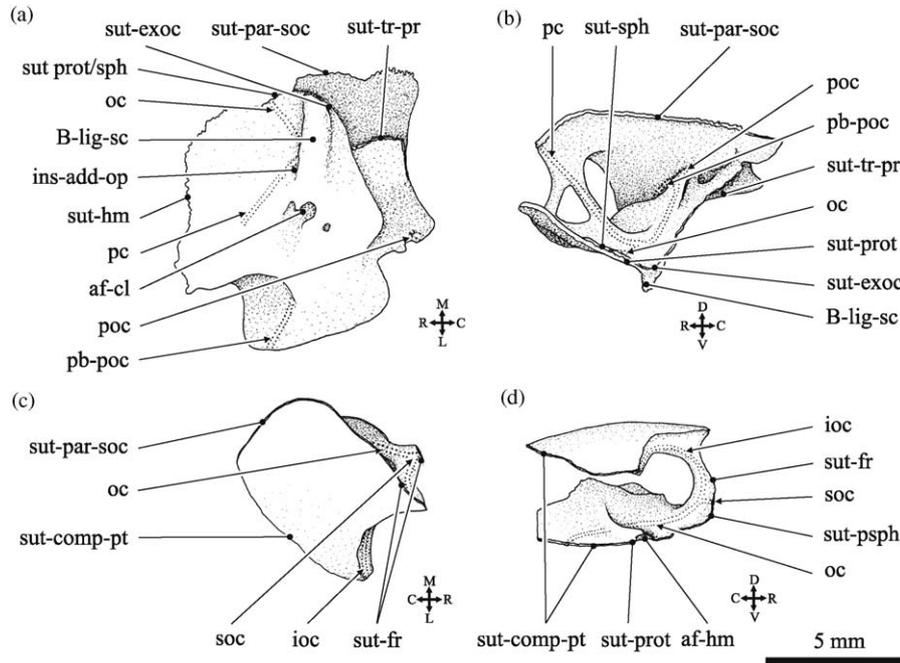


Fig. 7. Right compound pterotic in (a) ventral, (b) medial view; right sphenotic in (c) dorsal, (d) (slightly ventro)lateral view, of a 95-mm SL male *Ancistrus* cf. *triradiatus*. af-cl, articular facet with cleithrum; af-hm, articular facet with hyomandibular; B-lig-sc, supracleithral part of Baudelot's ligament; ins-add-op, insertion site of the adductor operculi; ioc, infraorbital canal; oc, otic canal; pb-poc, parietal branch of the postotic canal; pc, preopercular canal; poc, postotic canal; soc, supraorbital canal; sut-comp-pt, suture with compound pterotic; sut-exoc, suture with exoccipital; sut-fr, suture with frontal; sut-hm, suture with hyomandibular; sut-par-soc, suture with parieto-supraoccipital; sut-prot, suture with prootic; sut-psph, suture with pterosphenoid; sut-sph, suture with sphenotic; sut-tr-pr, suture with transverse process of complex vertebra.

architecture, as has been the case during the whole of the earlier development. Only the compound pterotic and, partly, the sphenotic bone arise as a ventral and a dorsal layer.

Figs. 5 and 6 compare transections of a subadult unsexed specimen and an adult male. As the relative size of the dilatator operculi increases, it invades the skull more deeply. In subadult specimens, sufficient insertion surface is present on the compound pterotic bone. The architecture of this bone is very complex (see Figs. 5(c), 6(c), 7(a) and (b)). The ventral layer carries the otic and postotic canals of the cranial lateral line system, and demarcates the brain laterally and posteriorly. The ventral layer contacts the dorsal surface layer laterally and caudally. During further growth, when the muscle becomes thicker, both layers are separated at their lateral contact zone with only two bony trabecles connecting both layers anteriorly. One of these carries the preopercular canal branch, bringing it to the surface in the dorsal bone layer (Fig. 7(b)). The pterotic branch of the postotic canal exits via the ventral layer, and the remainder of the postotic canal itself is seen uncovered on top of the ventral floor of the transverse process of the complex vertebra, lateroventral of the swim bladder. Further posteriorly, the postotic canal rises toward the dorsal layer of the compound pterotic bone, and then continues as the lateral line canal, passing through the body armor.

During ontogeny, the cavity (or 'myodome') in the compound pterotic bone soon becomes insufficiently small to house the dilatator operculi. In adults, modifications are seen on the sphenotic bone as well. When the sphenotic bone develops, it consists of a superficial layer and a deeper oblique part. These two parts are still in close contact anteriorly and medially, and suture to the superficial and deeper layer of the compound pterotic, respectively. In subadults, the space between the two sphenotic layers increases, and the medial contact is soon lost (Fig. 5(c)). As the dilatator operculi grows further, it reaches between the surface layer and the deeper canal-bearing part, elongating and thinning out the rostral contact zone, so that only a thick bony trabecle remains between both diverging layers (Fig. 7(d)). The otic canal is found deep inside the skull, below the muscle. The infraorbital canal, which surfaces and exits the bone laterally, ascends through the bony trabecle (Fig. 6(b)). Both bone layers serve as points of insertion for the dilatator operculi in large specimens (Figs. 6(b) and (c)). Fig. 8(a) shows the cavity in the sphenotic bone, here situated between the diverging otic and infraorbital canals.

As the muscle invades the skull more deeply, it grows between the brain and the dorsal skull roof. At the same time, the parieto-supraoccipital bone expands dorsoventrally. The result is a deepened,

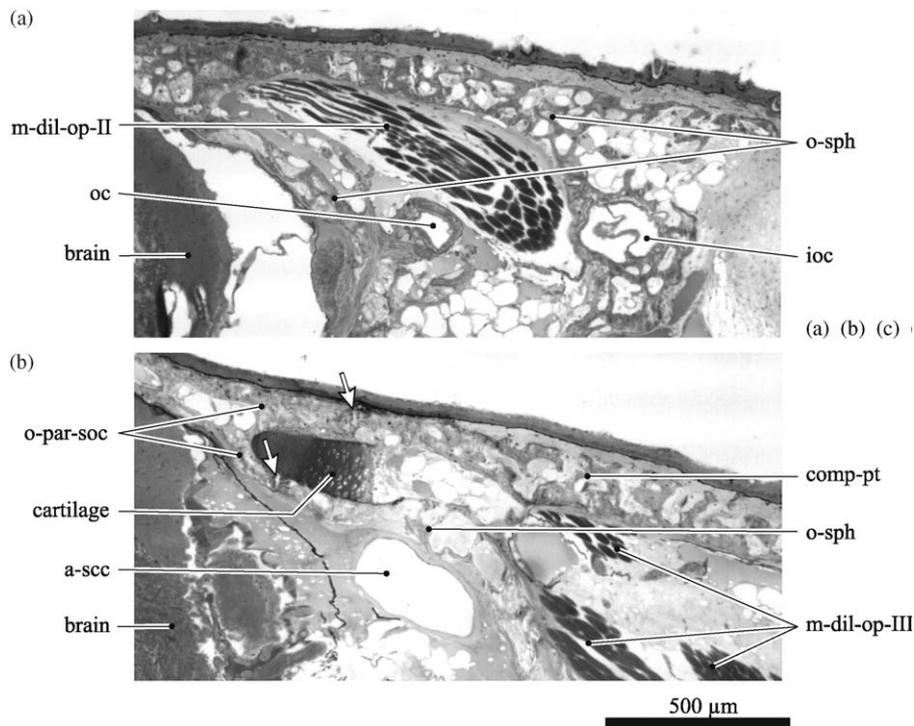


Fig. 8. (a) Section of a 33.5-mm SL subadult posterior to the orbit, showing the dilatator operculi myodome within the sphenotic bone. (b) Section of same specimen at the level of the posterior eye muscle myodome indicating the onset of the separation of both layers of the parieto-supraoccipital bone (slightly posterior to the sphenotic bone). Arrows mark the sutures between the parieto-supraoccipital bone and the compound pterotic (above) and the sphenotic (below), respectively. a-scc, anterior semicircular canal; comp-pt, compound pterotic bone; ioc, infraorbital canal; m-dil-op-II/III, musculus dilatator operculi bundles II/III; o-par-soc, os parieto-supraoccipitale; o-sph, os sphenoticum; oc, otic canal.

two-layered bone, hiding the brain deep below the dorsal skull surface. The bone is first split laterally, at the level of the dorsalmost part of the cartilaginous otic capsule remnant (Fig. 8(b)). The dorsal and ventral layers of the parieto-supraoccipital bone contact each other only at the midline, where a sagittal fragmented bony sheet is present (Fig. 9(c)). The dilatator operculi inserts on both layers, as well as on the sagittal sheet (Fig. 6(c)). Where this sheet is incomplete, and foramina are present (Fig. 9(c)), the left and right muscles touch each other.

The last bone that is drastically modified is the frontal, to which most muscle fibers of the second dilatator operculi bundle run (Figs. 6(a) and (b)). Only the posterior half of the bone is modified. The muscle enters the bone caudally, wedging in between a ventral layer of the bone covering the braincase and a dorsal layer at the dorsal skull surface. The lateral portion of the frontal, connecting the ventral and dorsal layers and carrying the supraorbital canal of the cranial lateral line system, is deepened, and a vertical ridge is formed (Figs. 9(a) and (b)). This ridge is deepest posteriorly, where the supraorbital canal continues into the sphenotic bone. Still, the epiphysial and parietal branches of the supraorbital canal both exit dorsally in all specimens, i.e., passing through the surface layer. The epiphysial

branch splits off the supraorbital canal where it still lies near the surface; the parietal branch, however, starts somewhat further posteriorly, where the main canal is found deeper in the skull (Fig. 9(b)). Hence, in large specimens, the parietal branch is seen rising back up to the surface layer of the frontal, where it exits at the frontal-sphenotic suture.

An additional effect of the extreme hypertrophy of the dilatator operculi is the flattening of the anterior braincase itself. While the pterosphenoid is a vertical bone in young specimens, it is squeezed and moved into an almost horizontal plane in large adults. Similarly, the plane of the epioccipital is shifted more horizontally (Fig. 4(c)). The tight contact between many of the skull bones makes some of the sutures hard to discern in most specimens, e.g. between the prootic and the sphenotic bones. This tight contact might be promoted by the fact that a substantial part of several skull bones serves as point of muscle insertion, although similar tight contacts and even fusions in this skull region are seen in other loricarioids as well (Reis, 1998; Huysentruyt and Adriaens, 2005).

Thus, below the 'primary' skull roof, which forms the dorsal roof of the skull, a 'secondary' skull roof is formed between the brain and the dilatator operculi muscles. The bones contributing to this extra roof are

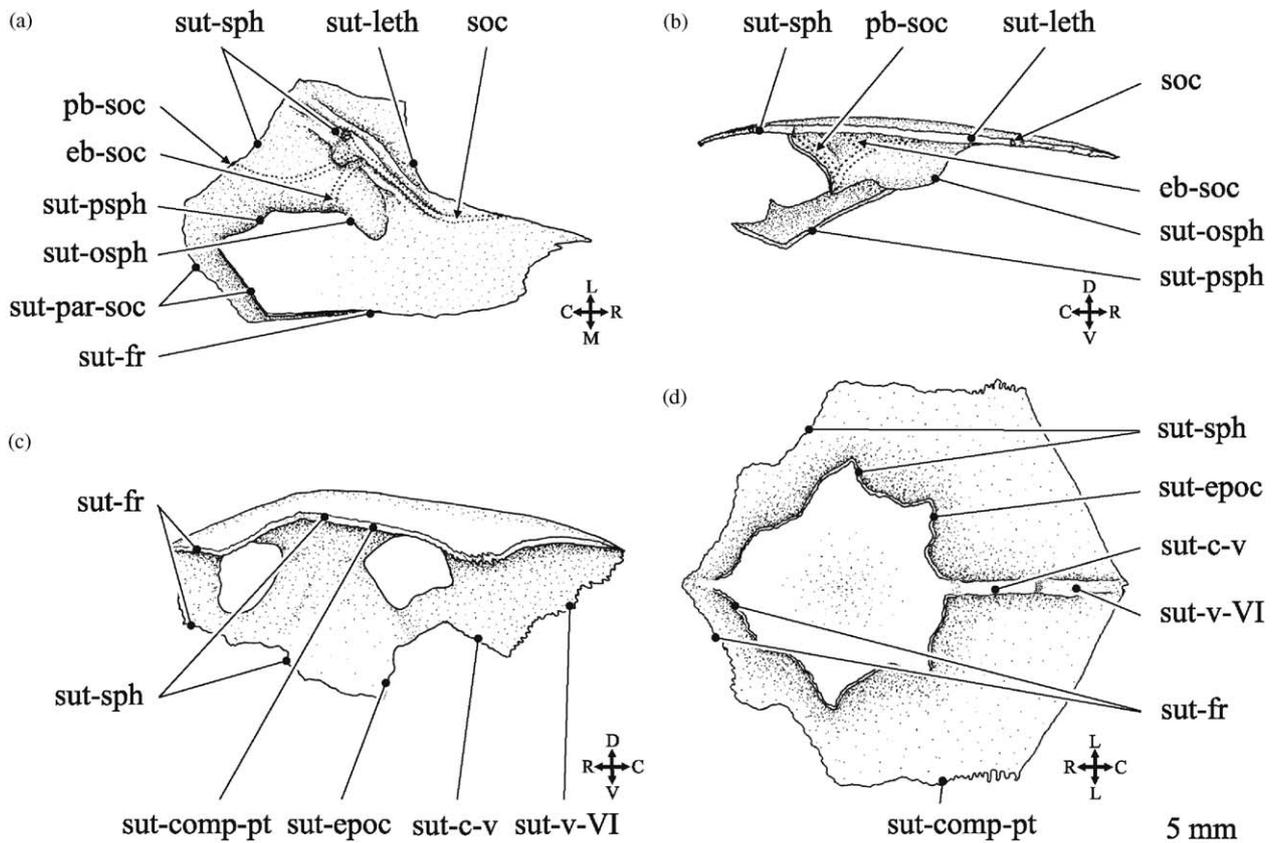


Fig. 9. Right frontal in (a) ventral, (b) lateral view; parieto-supraoccipital in (c) left lateral, (d) ventral view, of a 95-mm SL male *Ancistrus* cf. *triradiatus*. eb-soc, epiphysial branch of the supraorbital canal; pb-soc, pterotic branch of the supraorbital canal; soc, supraorbital canal; sut-c-v, suture with complex vertebra; sut-comp-pt, suture with compound pterotic; sut-epoc, suture with epioccipital; sut-fr, suture with frontal; sut-leth, suture with lateral ethmoid; sut-osph, suture with orbitosphenoid; sut-par-soc, suture with parieto-supraoccipital; sut-psph, suture with pterosphenoid; sut-sph, suture with sphenotic; sut-v-VI, suture with dorsal process of sixth vertebra.

the frontal, sphenotic, parieto-supraoccipital, compound pterotic, pterosphenoid and epioccipital bones (the latter two only due to their altered orientation). The sensory canals are 'forced' deeper inside the skull, and exiting branches rise toward the skull surface via bone trabecles or vertical bone ridges.

Functioning and use of the cheek-spine apparatus

The cheek spines of *A. cf. triradiatus* can be erected and retracted within 100 milliseconds, or can stay erected for a longer period. The functioning of the apparatus can be understood by manipulating the dilatator or levator operculi muscles in freshly killed specimens. Refer to Fig. 3 for an illustration of the functioning of the apparatus. Manipulation of both muscles (1 in Fig. 3(d)) suggests that they have the same function: rotating the opercle upward (2). The direction of this movement is related to the long, hinge-like articulation of the opercle to the hyomandibular bone (see above). The long anterior process of the opercle,

pointing medioventrally at rest, is swung outward (3). The bases (ossicles) of the cheek spines lie in a concave plane when retracted, so that the spines are packed together (Figs. 3(a)–(c)). The opercular motion causes the cheek-spine bases to bulge laterally, directing the spines toward the possible danger or competitor. Also, the plane of the bases is now convex, so that the spines point in various directions (4) (see also Fig. 3(b)). The cheek-spine bases are anchored to the quadrate via the chain of cheek plates, limiting the outward movement, and thus causing them to lie in a convex plane: the divergence of the cheek spines when completely erected is caused by this limitation of outward movement (5). High-speed video images indeed show that the spines are diverged to the utmost only when they are completely erected. Pulling the adductor operculi results in retraction of the whole apparatus. The system appears to be functional from around the 21-mm standard length, when all elements are present, and manipulation results in erection and retraction of the (still small) spines.

The suturing of the hyomandibular to the compound pterotic bone is present in all loriciariids examined by us,

as well as in *Hypostomus plecostomus* (Schaefer, 1987), but is shorter and not as tight as in *Ancistrus*. The strong suture in *Ancistrus* can be functionally interpreted as a fortification preventing excessive outward movement of the hyomandibular bone when the opercular muscles pull the opercle outward. Also, some of the dilatator bundles originate on the hyomandibular instead of on the neurocranium; thus, a rather immovable hyomandibular is advantageous for the functioning of these bundles.

Among the examined species, the cheek-spine apparatus and the hypertrophied opercular musculature are also present in *A. ranunculus* and *A. dolichopterus*. The impact on the skull roof is similar, though slightly less pronounced: the two layers of the frontal are slightly less separated in adults of these species, and the medial contact zone between both layers of the parieto-supraoccipital bone is somewhat broader (the bone is less deep).

Aquarium observations demonstrate the biological role of the cheek-spine apparatus in *A. cf. triradiatus*. In case of a limited food supply, individuals might be willing or unwilling to share resources. In the latter case, a sudden lateral bending of the body is performed so that the anterior body region is rammed toward the competitor. The erected pectoral fins are used as well. This behavior has also been seen in *A. ranunculus*, *P. lituratus*, *S. aureum*, *R. parva* and *F. acus*. The three examined *Ancistrus* species, however, often combine the ramming of the anterior body region with the erection of the cheek spines, and usually only at the side facing the competitor. The fishes also erect the spines when threatened, or when two males compete for the same cavity (which provides protection and nesting opportunity to the winning male). The males face each other snout to snout, then position their head next to each other, and attempt to hit the opponent and push him away. Such territorial fights can continue for several minutes. Most likely, the cheek spines have a display function as well. The large, fleshy snout tentacles (Fig. 1(a)) might well have the same purpose of display. Sabaj et al. (1999) suggested the use of these tentacles as larval mimicry to attract females. Surely, neither hypothesis excludes the other.

Discussion

The hinge direction of the opercle-hyomandibular articulation has undergone an evolutionary shift. In the basal catfish family Diplomystidae and the loricarioid Nematogenyidae, it is almost vertical (Arratia, 1987, 1992). The same configuration is present in most teleosts, i.e., the opercular opening is directed posteriorly. In the loricarioid Trichomycteridae and Callichthyidae, the direction has become more oblique, although not completely horizontal (Arratia, 1992; Huysentruyt and Adriaens, 2005). In most Loricariidae,

the opercular articulation with the hyomandibular is situated dorsally on the opercle, instead of anteriorly, a fact also noticed in other loricariids (Schaefer, 1987). The hinge has become horizontally oriented, so that the opercle swings not only outward and inward, but also upward and downward (Howes, 1983). Hence the hinge has shifted from functioning in a vertical plane, as in most fishes, to a horizontal plane. This situation is at its most extreme in the Ancistrini, e.g. *Ancistrus*, with the strongly reinforced hinge oriented from dorsorostral to ventrocaudal (Fig. 3). This infers an evolutionary rotation of the opercle-hyomandibular articulation of more than 90°, compared to a diplomystid- or trichomycterid-like ancestral configuration. The articulation plane is also reflected in the tendon direction of the dilatator and levator operculi (i.e., running dorsocaudally from the opercle, more or less perpendicular to the hinge; Fig. 3).

We agree with Howes (1983) that the position and mobility of the opercle implies that it takes little or (most probably) no part in opening or closing of the branchiostegal membrane, which is situated at the ventral, and not at the lateral side of the head. The absence of significant movement during normal respiration or feeding is supported by preliminary results from a kinematic study on *A. cf. triradiatus* and *P. lituratus* (Geerinckx, pers. obs.). These and Howes' observations corroborate the hypothesis that the opercle and the three opercular muscles have lost their role in the respiratory mechanism. The branchiostegal membrane slit, well ventral to the opercle, is the only exhalant opening; the membrane is operated by the hyohyoidei musculature.

We define the cheek-spine apparatus as consisting of a series of several loose cheek spines that articulate with the opercle, and can be erected by the movements of this bone. The presence of a large anteroventral opercular process is typical only of those loricariid taxa that possess a cheek-spine apparatus. It is absent in *F. acus*, *S. aureum*, *R. parva*, *O. vestitus*, and *P. lituratus*. The latter species has a minuscule 'opercular apparatus', consisting merely of an evertible opercle (Geerinckx, pers. obs.).

The opercular musculature appears to be highly variable in both presence and size within the loricariid family. In his study of the loricariid cranial muscles, Howes (1983) correlated the atrophy or disappearance of opercular muscles in some species to a small size and immobility of the opercle. Refer to Howes (1983: Fig. 9) for an excellent illustration of the diversity in sizes and shapes of the opercular muscles in loricariids. The adductor operculi, which usually connects the opercle to the neurocranium floor in teleosts (Winterbottom, 1974), is seen inserting on the preopercle instead of the opercle in *Pseudacanthicus*, *Stoniella* and *Panaque*; it is weakly developed in *Loricaria*, *Sturisoma* and *Farlowel-*

la, and is absent in *Pseudohemiodon* and *Hemiodonichthys* (Howes, 1983). It is confluent with fibers of the protractor pectoralis muscle in *Otocinclus* (Schaefer, 1997). The levator operculi muscle, originating from the hyomandibular, sphenotic, pterotic or posttemporal in teleosts (Winterbottom, 1974), is weakly developed in *Loricaria*, *Sturisoma* and *Farlowella*, and absent in *Pseudohemiodon* and *Hemiodonichthys* (Howes, 1983). The dilatator operculi muscle is found in all loricariids examined by Howes (1983) and us, although it is small in *Pseudohemiodon* and *Hemiodonichthys*, where it is virtually reduced to a tendon running in a lateral hyomandibular groove (Howes, 1983). Winterbottom (1974) mentions the sphenotic, frontal, pterotic and hyomandibular bones as possible sites of origin for the dilatator operculi muscle in teleosts. *Ancistrus* is remarkable in having an extremely well developed dilatator operculi originating from all of these bones, as well as from the parieto-supraoccipital bone and the dorsal process of the sixth vertebra.

The invasion of the skull roof bones by the opercular musculature appears to be unique among teleosts; it is more common for large cranial muscles (e.g., the adductor mandibulae) to overgrow the surface of the skull (e.g., Liem, 1980; De Schepper et al., 2005). In *Ancistrus*, invasion starts at cartilaginous zones (taenia marginalis and otic capsule), and from there separates the upper and deeper layer of the bones, using both layers as points of insertion. In later stages, growth and remodulation of bone tissue itself, and not the ossification of growing cartilaginous tissue becomes dominant. This explains, for example, the progressive deepening of the median part of the parieto-supraoccipital bone, without the presence of cartilage parts.

The apparatus was found to be more developed in the male than in the female specimens. Related to this, the dilatator operculi size and the modifications of the skull bones are relatively larger in males. This corresponds well with the fact that the apparatus is used more by the males during territorial behavior, and represents a sexual dimorphism that is mostly unrevealed externally. The female cheek spines never grow larger than those of similar-sized males that just reach maturity: this external difference appears to be better correlated with gross body size (but can still be called sexual dimorphism). The depth of the compound pterotic and the parieto-supraoccipital bones is substantially less in females. Also, only a negligible portion of the dorsal process of the sixth vertebra, and the caudal most end of the frontal bone, serve as insertion for the dilatator operculi; hence, the latter bone is essentially unchanged in females.

Within the Loricariidae, the erectile cheek-spine apparatus has evolved in the hypostomine Ancistrini (Isbrücker, 1980; Montoya-Burgos et al., 1997), and is one of the synapomorphies of this tribe (Schaefer, 1987). The more weakly developed ‘opercular apparatus’, as described

above for *P. lituratus*, is present in the tribe Pterygoplichthini (Armbruster, 2004). Evertible cheek plates as in the cheek-spine apparatus of *Ancistrus*, but lacking hypertrophied odontodes, are present in some *Pterygoplichthys* species (Armbruster, 2004). The ancistrine cheek-spine apparatus could not have evolved as such without the decoupling of the opercular region from the jaw movements. The interoperculo-mandibular ligament has been lost in most loricariids and related astroblepids (Schaefer, 1988; Armbruster, 2004). In most loricariids, the interopercle itself has been lost, too (Schaefer, 1988; Armbruster, 2004). The term interoperculum used by Howes (1983: p. 335), referring to the largest cheek plate, is erroneous. The two largest cheek plates, which have been mentioned in other loricariids as well (Schaefer, 1987, 1988, 1997), might well have contributed to the evolutionary origin of the whole cheek-spine apparatus, as they provided a strong articulatory connection between the cheek spines and the suspensorium (quadrate), which we consider necessary for the apparatus to be used as an aggressive, powerful tool. The homology of this largest plate, carrying the distal portion of the preopercular canal, and the bone carrying the same canal in the other examined loricariids, seems plausible. A phylogenetic discussion on this bone and the possible true interopercle in the loricariid *Delturus* is given by Armbruster (2004).

In conclusion, the evolution toward the ancistrine cheek-spine apparatus has been made possible by the evolutionary decoupling of the opercle from the lower jaw movements, the presence of odontodes on bony platelets in the skin, the possibility to develop a series of articulations between the opercle, the cheek spines, the cheek plates anterior to them, and the quadrate, and, last but not least, the possibility of the opercular musculature to expand substantially inside newly evolved skull cavities.

A remarkable convergence to the loricariid cheek-spine apparatus is seen in a distantly related loricarioid family. Some trichomycterids (*Branchioica*, *Pareiodon*, *Vandellia*) also have an anteroventral opercular process and an enlarged dilatator operculi, growing on, not inside the skull (Howes, 1983). There are no separate erectile odontodes, but both the opercle and interopercle carry large odontodes and are alternately abducted, providing a means of attachment to the substrate of these detritivores (Eigenmann, 1918). Additionally, they function as anchors in the parasitic stegophiline and blood parasitic vandelline trichomycterids (Kelley and Atz, 1964; Baskin et al., 1980; Moodie and Power, 1982; Breault, 1991; Spotte et al., 2001).

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