

Development of the Osteocranium in *Corydoras aeneus* (Gill, 1858) Callichthyidae, Siluriformes

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ABSTRACT Development in the osteocranium of *Corydoras aeneus* was studied based on 48 cleared and stained specimens and 10 series of serial sections. Development overall follows the general trends observed in siluriform development, with ossifications appearing as a response to functional demands. Early development of the skull occurs in two distinct phases. In a first phase, several new bony elements, all of dermal origin and related to feeding, appear shortly after yolk depletion (4.4 mm SL). Between 5 and 8 mm SL, developmental priorities seem to shift to size increase of the cartilaginous skull and no new bony elements appear. Finally, a second phase of osteogenesis occurs from 8 to 18 mm SL, in which all remaining dermal and perichondral bones appear. *J. Morphol.* 272:573–582, 2011. © 2011 Wiley-Liss, Inc.

KEY WORDS: ontogeny; osteology; functional morphology; catfishes

INTRODUCTION

The Loricarioidea comprise a large clade of South American catfishes containing six families with roughly 1,200 species (Nelson, 2006). Two of these families, Astroblepidae and Loricariidae, have evolved toward a ventrally oriented sucker-mouth, which is used to attach to a substrate while respiration continues (Geerinckx et al., 2007). In addition, loricariids have evolved a highly specialized feeding mechanism for algae scraping, during which they are able to respire while being attached to a substrate with their sucker-mouth (Adriaens, 2003). Despite extreme specialization in behavior and morphology, loricariid catfishes are able to utilize diverse habitats. These specializations are also thought to have led to the subsequent radiation in the family Loricariidae, which comprises over 700 known species (Ferraris, 2007). The evolutionary morphological changes characterizing the ancestral condition of the loricarioid clade are of great importance for understanding the morphological processes driving phenotypic evolution in such highly successful lineages. A recent study on the ontogeny of the osteocranium of the loricariid *Ancistrus* cf. *triradiatus* was performed by Geerinckx et al. (2007). To understand the evolution of such morphological specializations, however, the study of a more basal representative within the loricarioid lineage is nec-

essary. In particular, the comparison of the ontogenetic transformations in such a plesiomorphic morphology to that of a derived one, as in *A. cf. triradiatus* could yield valuable information.

For this study, we chose *Corydoras aeneus*. It belongs to the Callichthyidae, the earliest lineage of the more derived loricarioid clade (Callichthyidae, Scoloplacidae, Astroblepidae, Loricariidae). Members of this family exhibit a plesiomorphic morphology where a sucker-mouth or algae scraping apparatus is lacking but in which the mouth already has a more ventral position. In addition, breeding protocols for this species were readily available as it is well known in ornamental fish trade, so obtaining ontogenetic stages was possible (Tamura et al., 1997).

The aim of this study is to describe the development of the osteocranium in a *C. aeneus* and compare the results to those of *A. cf. triradiatus* and other siluriform species. An additional aim is to link the anatomical transformations of the developing skull to important functional changes in the early life history of the species (Huysentruyt et al. 2009). This approach provides knowledge on morphological trends guided by possible functional constraints and environmental preferences at different developmental stages (Fukuhara, 1992). It is also expected that species-specific differences in functionality of the skull during early ontogeny would be reflected in the timing and sequence of the developing structures associated with these specific functions.

MATERIALS AND METHODS

To induce breeding, 35 adult specimens of *C. aeneus* (Gill, 1858), of which 25 were male, were kept in a separate tank

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with a water temperature of 24–26°C, pH level of 8–8.5, and global hardness of 9–12°. Breeding was induced following the protocol by Fuller (2001). Offspring specimens were removed at different ages and SL, euthanized with an overdose of MS-222 (3-aminobenzoic acid ethyl ester, Sigma), measured and preserved in paraformaldehyde fixative (4%). A total of 48 specimens, ranging in age from 1 to 70 days posthatching and in SL from 4.0 to 30.0 mm, were selected for clearing and staining using the method described by Taylor and Van Dyke (1985). These specimens were studied using an Olympus SZX7 stereomicroscope. Ten additional specimens (SL 3.3, 4.0, 4.4, 4.9, 5.3, 6.3, 9.3, 15.5, 19.5, and 26.0 mm) were embedded in Technovit 7100 and 2–5 µm slices (depending on specimen size) were made using a Reichert-Jung Polycut microtome, which were stained with toluidin blue. These serial sections were further studied using a Reichert-Jung Polyvar microscope and pictures were taken using a ColorView8 digital with AnalySIS-software (Soft Imaging Systems, Germany). Bone terminology used throughout this paper follows Harrington (1955), Adriaens and Verreaes (1998) and Arratia (2003).

RESULTS

Stage 1: 4.4 mm SL (SL 3.3–4.4 mm; $n = 4$)

Neurocranium. No ossifications were observed (Fig. 1a,b).

Splanchnocranium. Teeth primordia are present on both lower and upper jaw, but no supportive ossifications are present. The autopalatine still articulates with the maxillary barbel through the submaxillary cartilage and the cartilaginous hyosymplectic-pterygoquadrate plate has developed an articulation with the opercular bone, the first ossified element to appear.

Gill arches. Teeth are present in both the ventral and dorsal part of the branchial basket, at the level of the fourth and fifth arch, respectively but again without supportive ossifications.

Stage 2: 4.9 mm SL (SL 4.5–4.9 mm; $n = 3$)

Neurocranium. No ossifications were observed (Fig. 1c,d).

Splanchnocranium. Both a thin layer of dentary bone and the maxillary and premaxillary bones are present, all of which are dermal ossifications supporting teeth. The maxillary also supports the maxillary barbel at this point and articulates through a double dorsal process and two submaxillary cartilages with the autopalatine. The maxillary further has a medial articulation with the premaxillary, which in turn bears a dorsal process for articulation with the ethmoid cartilage. In addition, the parurohyal bone has started to ossify in the tendon connecting the sternohyoideus muscle to the hyoid.

Stage 3: 5.3 mm SL (SL 5.0–5.3 mm; $n = 4$)

Neurocranium. No ossifications were observed (Fig. 1e,f).

Gill arches. In the middle of the fifth ceratobranchial, along its medial side, a thin bony plate supporting the ventral pharyngeal teeth has started to develop. Along the ventromedial side of

infrapharyngobranchials III and IV, a similar ossification was observed, supporting the dorsal pharyngeal teeth.

Stage 4: 5.4–8.2 mm SL ($n = 17$)

No additional ossifications were observed.

Stage 5: 8.2 mm SL ($n = 1$)

Neurocranium. In the skull roof, three ossification centres are present. Rostrally, the dorsal perichondral supraethmoid bone expands over the ethmoid cartilage. Next to this, on the dorsomedial side of the taeniae marginales, just behind the epiphyseal bridge, the frontal bones appear. On the caudal margin of the neurocranium, the parieto-supraoccipital bone has started to ossify at the level of the tectum synoticum. The skull floor is made up of the ventral hypoethmoid bone (not illustrated), caudally contacting the prevomer bone, which is flanked by two palatal splints at its posterior margin. Caudal to this ossification, the borders of the hypophyseal fenestra have started to ossify, forming the early parasphenoid bone. Caudolaterally, the early posttemporo-pterotic-supracleithra are present, in which the separate elements composing this complex bone cannot be distinguished (Figs. 2, 5a, 6a).

Splanchnocranium. Ossification of the palatine has started at its posterior margin and, in the lower jaw, the Meckel's cartilage has also started to ossify. This way, at its anterior margin, the mentomeckelian appears, while posteriorly, the articular and retroarticular bone become apparent, still separated at this point. In the suspensorium, the hyomandibular and quadrate parts show ossification centres, enforcing articulations with the opercular and articular bone, respectively. In the hyoid arch, the ventral hypohyal and anterior and posterior ceratohyals ossify, as do the branchiostegal rays. Finally, in the opercular series, the interopercle and suprapreopercle have formed. The opercle has now developed its typical oval shape and oblique dorsoventral orientation.

Stage 6: 9.7 mm SL (SL 8.3–9.2 mm; $n = 4$)

Neurocranium. The supraethmoid bone has expanded caudo-laterally and ventrally, it has fused to the hypoethmoid bone, forming the mesethmoid bone. Laterally, this complex shows two expansions of dermal origin, which represent the laterodermethmoid parts. The frontals have expanded in both the anterior and posterior direction, next to a small expansion which covers the epiphyseal bridge and connects both at the midline. At the caudal margin of the neurocranium, anterior expansions of the parieto-supraoccipital bone almost reach the frontal bones. In the skull

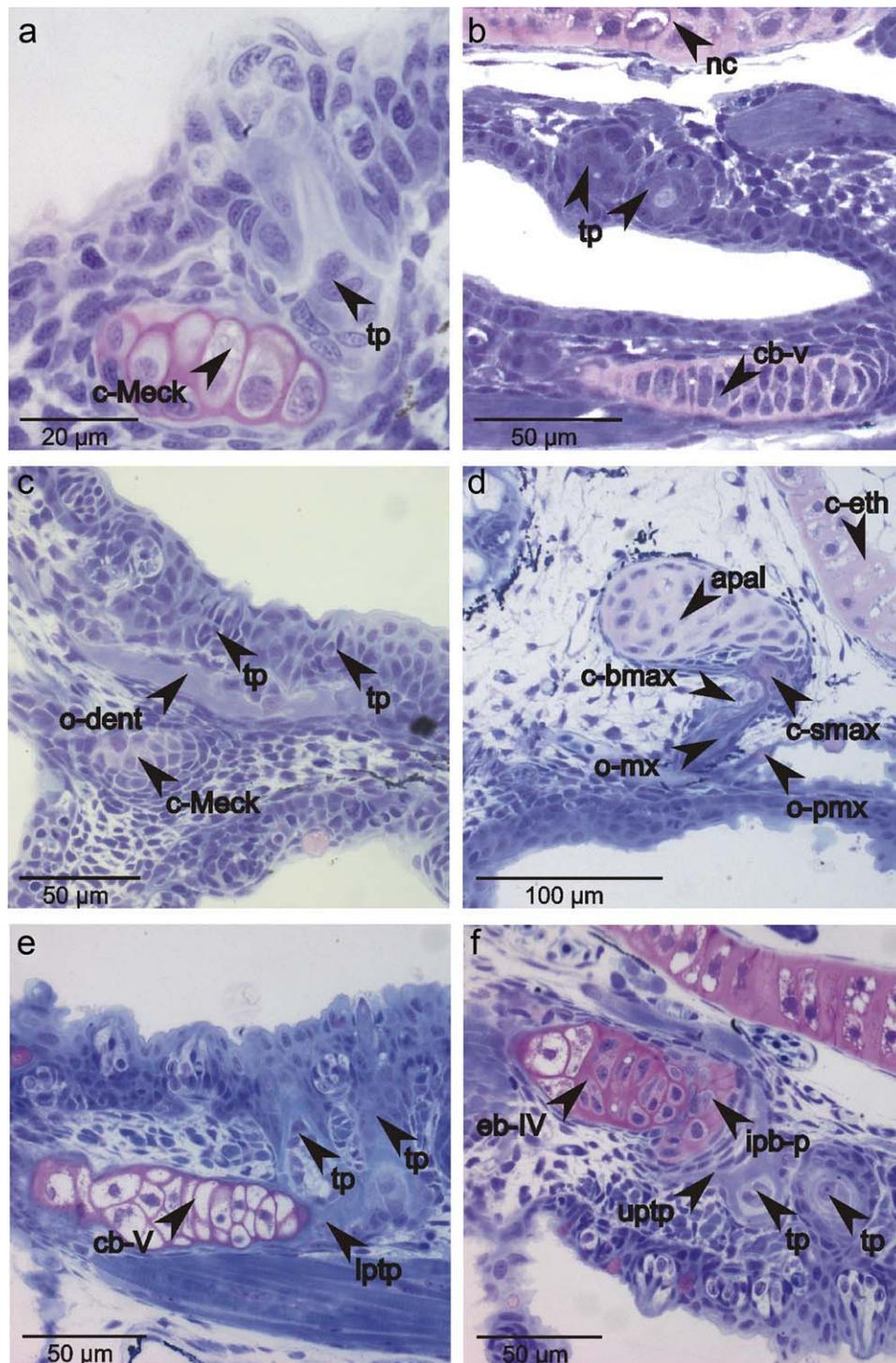


Fig. 1. *Corydoras aeneus*, histological sections showing details of osteocranium development: (a) (4.4 mm SL) lower jaw showing tooth primordia, bone tissue absent; (b) (4.4 mm SL) primordia of dorsal pharyngeal teeth, bone tissue absent; (c) (4.9 mm SL) lower jaw showing tooth primordia, a thin layer of dentary bone present; (d) (4.9 mm SL) palatine–maxillary articulation, showing submaxillary cartilage and an ossified maxillary and premaxillary bone; (e) (5.3 mm SL) primordia of ventral pharyngeal teeth with an ossified lower pharyngeal tooth plate present; (f) (5.3 mm SL) primordial of dorsal pharyngeal teeth with an ossified upper pharyngeal tooth plate present. apal, autopalatine; c-bmax, maxillary barbel cartilage; cb-V, ceratobranchiale V; c-eth, cartilage ethmoideum; c-Meck, Meckel's cartilage; c-smax, cartilago submaxillaris; eb-IV, epibranchiale IV; ipb-p, infrapharyngobranchiale posterior; lptp, lower pharyngeal toothplate; nc, neurocranium; odent, os dentale; o-max, os maxillare; o-pmx, os premaxillare; tp, tooth primordium; uptp, upper pharyngeal toothplate.

floor, the hypophyseal fenestra has started to close, due to further ossification of the parasphenoid. Next to this, the orbitosphenoids have started ossifying at the anterior border of the sphenotic fenestra. In the otic region, both the prootics in the skull floor as well as the sphenotics in the skull roof are present. In the latter bones, the dermosphenotic and autosphenotic parts are already fused. At the caudal margin of the otic region, the posttemporo-pterotic-supracleithrum has expanded dorsally, housing the posttemporal branch of the cranial lateral line system. Ventrally, the basioccipitals and exoccipitals are present (Figs. 2b, 4a, 5b, 6b).

Splanchnocranium. The pterygoid process has started to ossify at its tip, forming the metapterygoid and, next to this, the preopercular bone is present.

Gill arches. Ceratobranchials III–IV, epibranchials I–IV, infrapharyngobranchial III (anterior infrapharyngobranchial), and infrapharyngobranchial IV (posterior infrapharyngobranchial) have all started to ossify in the centre of their respective cartilaginous elements.

Stage 7: 11.2 mm SL (SL 9.8–11.2 mm; $n = 4$)

Neurocranium. All skull roof bones have expanded, strengthening the entire skull. The lateral ethmoid bone is present, with the prefrontal and parietal parts directly appearing as fused elements. Further, the pterosphenoide ossifies at the posterodorsal margin of the fenestra sphenoida and, in the skull floor, the hypophyseal fenestra has closed entirely (Figs. 2b, 4a, 5b, 6b).

Splanchnocranium. Ossification of the interhyal bone appears.

Gill arches. Ceratobranchials and hypobranchials I–II have started to ossify. In contrast to the gill arch bones formed earlier, ossification starts at the rostro-lateral side of the cartilaginous hypobranchials.

Stage 8: 13.9 mm SL (SL 11.3–13.9 mm; $n = 6$)

Neurocranium. Bone expansions have further reinforced skull structure (Figs. 3a, 4c, 5d, 6d).

Splanchnocranium. All ossified parts have expanded. In the hyoid arch, with the appearance of the dorsal hypohyal, all bones are present.

Gill arches. Basibranchials II and III are the last elements to ossify at this stage.

Stage 9: 16.0 mm SL (SL 14.0–16.0 mm; $n = 5$)

Neurocranium. At this stage, only the nasal and first infraorbital appear as new ossifications in the neurocranium (Fig. 3b).

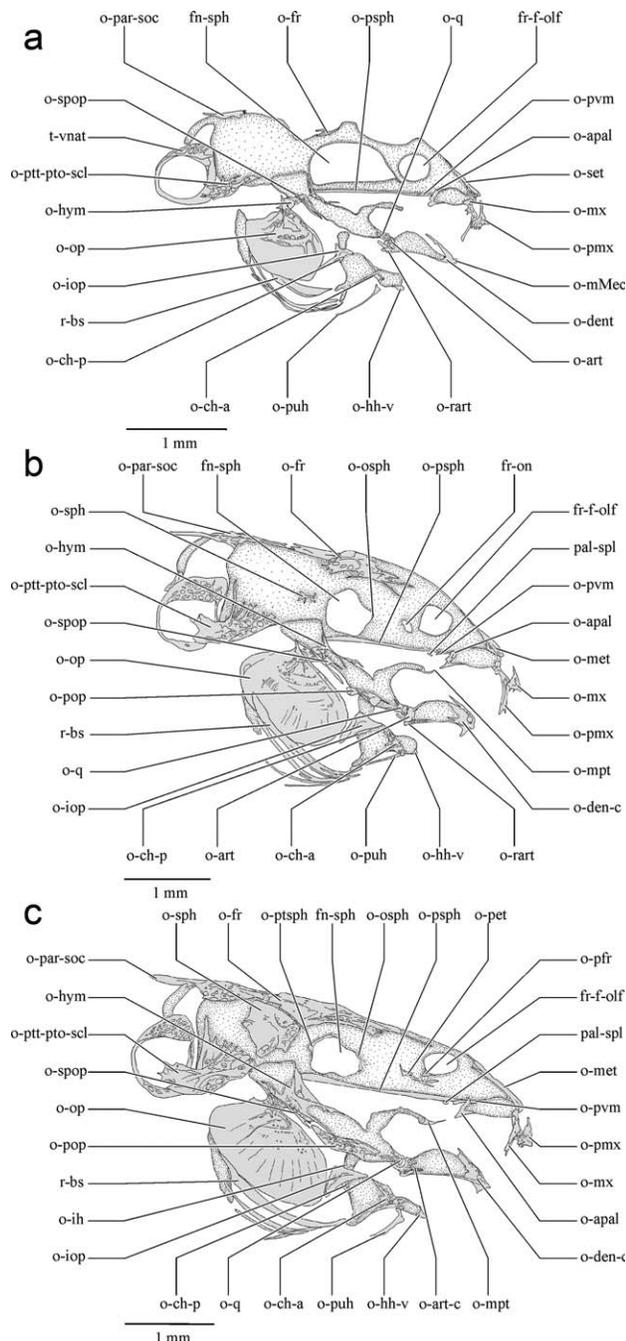


Fig. 2. *Corydoras aeneus*, lateral view of the skull at different developmental stages: (a) 8.2 mm SL; (b) 9.7 mm SL; (c) 11.2 mm SL. fn-sph, fenestra sphenoida; fr-f-olf, foramen fila olfactoria; fr-on, foramen orbitonasalis; o-apal, os autopalatium; o-art, os articulare; o-art-c, os articulare complex; o-ch-a, os ceratohyale anterior; o-ch-p, os ceratohyale posterior; o-den-c, os dentale complex; o-dent, os dentale; o-dsph, os dermosphenoticum; o-fr, os frontale; o-ih, os interhyale; o-hh-v, os hypohyale ventrale; o-hym, os hyomandibulare; o-iop, os interoperculare; omMeck, os mento-meckelium; o-met, os mesethmoideum; o-mpt, os metapterygoidium; o-mx, os maxillare; o-op, os operculare; o-osph, os orbitosphenoidum; o-par-soc, os parietosupraoccipitale; o-pet, os parietosphenoidum; o-pfr, os prefrontale; o-pmx, os premaxillare; o-pop, os postoperculare; o-psph, os parasphenoidum; o-ptsph, os pterosphenoideum; o-ptt-pt-scl, os posttemporo-pterotic-supracleithrum; o-puh, os parurohyale; o-pvm, os prevomerale; o-q, os quadratum; o-rart, os retroarticulare; o-set, os supraethmoideum; o-sph, os sphenoticum; o-spop, os supraoperculare; pal-spl, palatal splint; r-bs, radius branchiostegalis; t-vnat, tunica vesica.

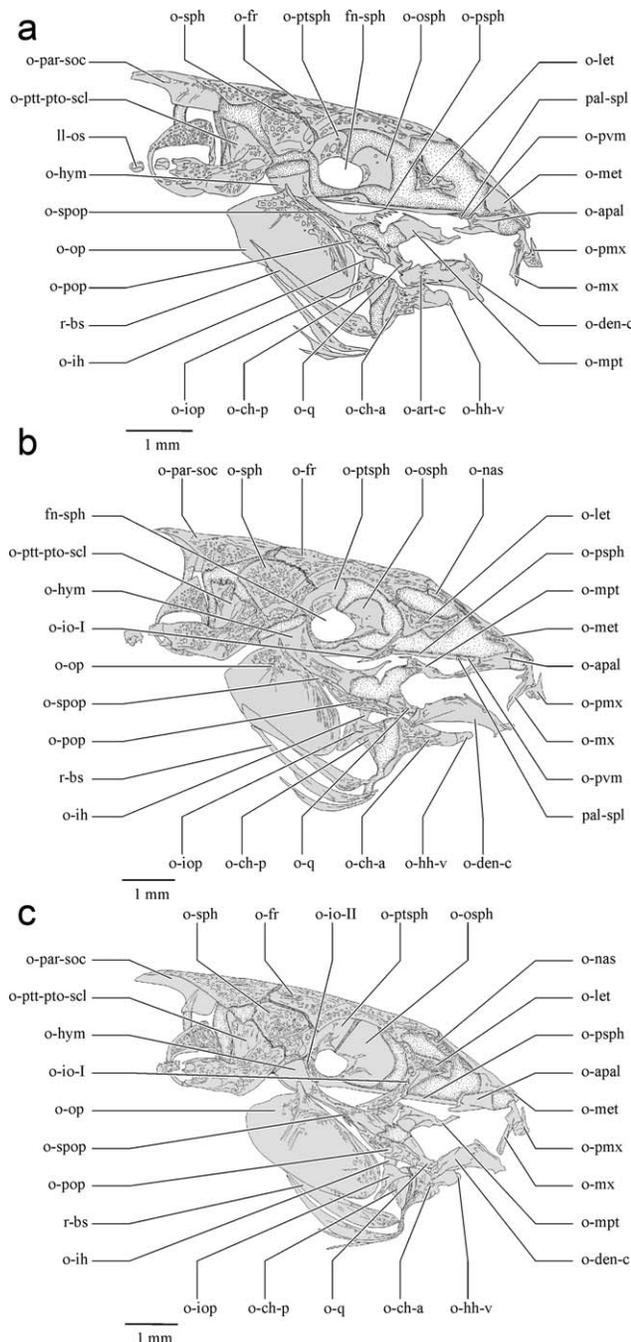


Fig. 3. *Corydoras aeneus*, lateral view of the skull at different developmental stages: (a) 13.9 mm SL; (b) 16 mm SL; (c) 18 mm SL. fn-sph, fenestra sphenoida; ll-os, lateral line ossicle; o-apal, os autopalatinum; o-art-c, os articulare complex; o-ch-a, os ceratohyale anterior; o-ch-p, os ceratohyale posterior; o-den-c, os dentale complex; o-fr, os frontale; o-hh-v, os hypohyale ventrale; o-hym, os hyomandibulare; o-ih, os interhyale; o-io-I, os infraorbitale I; o-io-II, os infraorbitale II; o-iop, os interoperculare; o-let, os lateroethmoideum; o-met, os mesethmoideum; o-mpt, os metapterygoideum; o-mx, os maxillare; o-nas, os nasale; o-op, os operculare; o-osph, os orbitosphenoidum; o-par-soc, os parieto-supraoccipitale; o-pmx, os premaxillare; o-psph, os parasphenoidum; o-pop, os preoperculare; o-ptsph, os pterosphenoidum; o-ptt-pto-scl, os posttemporo-pterotico-supracleithrum; o-pvm, os prevomerale; o-q, os quadratum; o-sph, os sphenoticum; o-spop, os supraoperculare; pal-spl, palatal splint; r-bs, radius branchiostegalis.

Stage 10: 18.0 mm SL (SL 16–18 mm; $n = 2$) (SL 18.1–30.0 mm; $n = 8$)

With the second infraorbital present, the skull resembles the adult configuration. Next to this, the palatal splints have merged to the prevomer, although serial sections show them to still be present in some adult specimens, albeit highly diminished in size. This suggests that their reported absence in other callichthyid genera such as *Callichthys*, *Dianema*, *Lepthoplosternum*, *Hoplosternum*, and *Megalichthys* is probably the result of a fusion or even secondary loss during ontogeny (Reis, 1998) (Fig. 3c).

DISCUSSION

Ossification sequence in teleosts has been related to functional demands arising in developing organisms (Cubbage and Mabee, 1996; Adriaens and Verreaes, 1998). The moment of yolk sac depletion is critical, as, from that point on, exogenous feeding becomes obligatory and starvation becomes a major threat for larval survival (Bailey and Houde, 1989; Pedersen et al., 1990; Jardine and Litvak, 2003). From the onset of exogenous feeding, the larval skull thus faces new and increased functional demands on the structures directly associated with feeding (van Snik et al., 1997; Jardine and Litvak, 2003). In most teleost larvae the common feeding method is suction feeding, which results in the necessity to be able to create and maintain a negative buccal cavity pressure (Wagemans and Vandewalle, 2001). Therefore, it is expected that development in both the chondro- and osteocranium reflects the timing of these demands and that priorities during early cranial ontogeny would be focused on the completion of a functional feeding apparatus, prior to neurocranial fortification.

As expected, in *C. aeneus* the full cartilaginous splanchnocranium is present at the time of yolk sac depletion (4.4 mm SL). At this point also, the opercle is the first ossified element to be present. Teeth are already present on both lower and upper oral and pharyngeal jaws, although not yet erupted and not yet supported by ossified elements. Buccal teeth appearing prior to ossification of their supporting elements is a situation uncommon in teleost fishes, where teeth usually appear at the same time or after premaxillary and dentary bones (Vandewalle et al., 2005). In contrast to this, pharyngeal teeth do generally appear separated from their respective supportive skeletal elements (Vandewalle et al., 2005), as is also the case here.

It has been noted that the first bones that appear during teleostean development always seem to be of dermal origin (Wagemans et al., 1998). Indeed, throughout studies dealing with osteological development in teleosts, onset of ossification generally involves the maxilla, premaxilla, dentary, upper and lower pharyngeal jaws, branchiostegal rays and opercular elements, most of

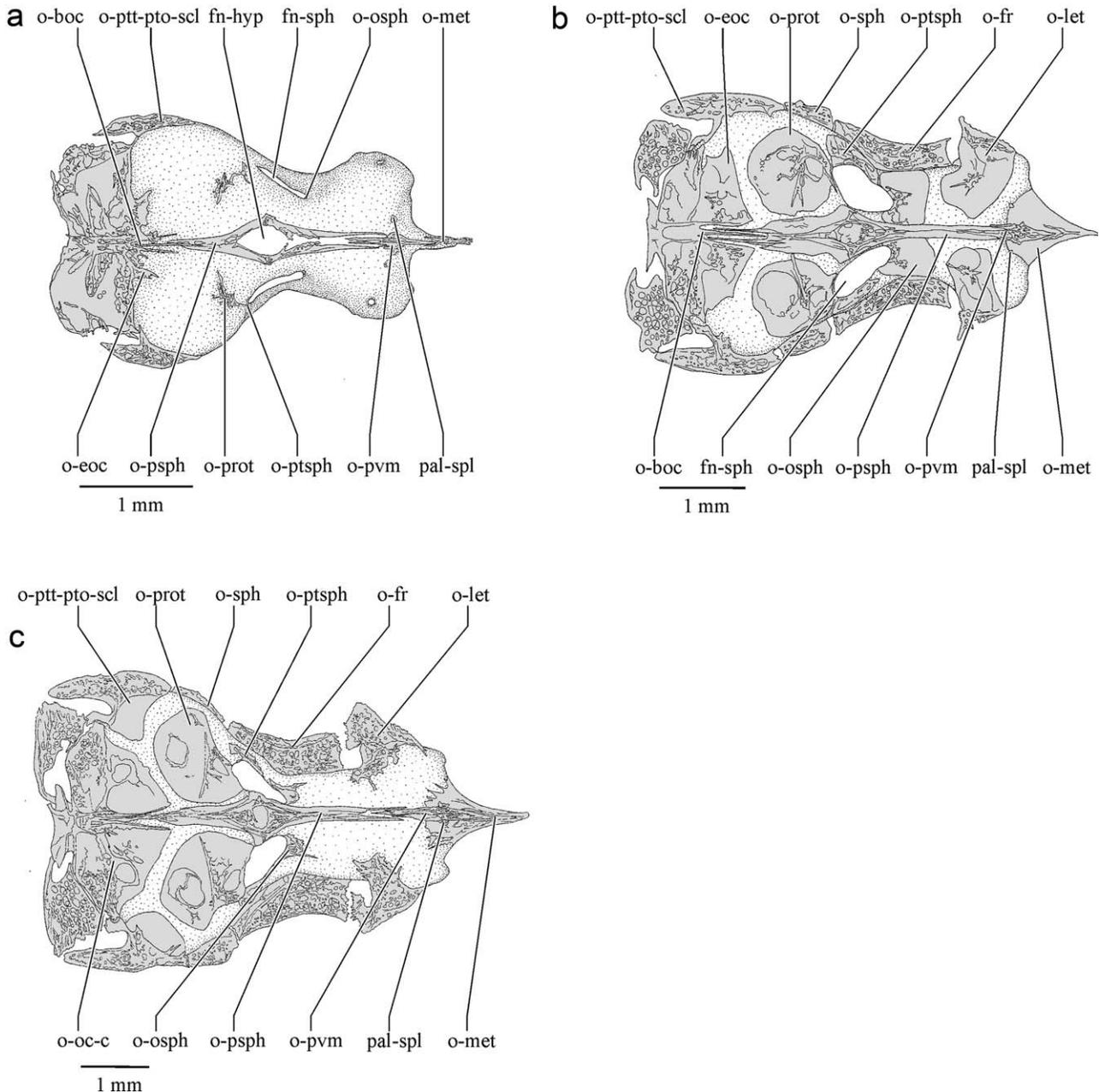


Fig. 4. *Corydoras aeneus*, ventral view of the neurocranium at different developmental stages: (a) 9.7 mm SL; (b) 11.2 mm SL; (c) 13.9 mm SL. fn-hyp, fenestra hypophyseae; fn-sph, fenestra sphenoidae; o-boc, os basioccipitale; o-eoc, os exoccipitale; o-fr, os frontale; o-let, os lateroethmoideum; o-met, os mesethmoideum; o-oc-c, os occipitale complex; o-osph, os orbitosphenoidae; o-psph, os parasphenoidae; o-prot, os prooticum; o-ptsph, os pterosphenoidae; o-ptt-pto-scl, os posttemporo-pterotico-supracleithrum; o-pvm, os prevomerale; o-sph, os sphenoticum; pal-spl, palatal splint.

which are of dermal origin and all of which are associated with jaw movement and the allied act of respiration (Weisel, 1967; Kobayakawa, 1992; Tilney and Hecht, 1993; Mabee and Trendler, 1996; Adriaens and Verraes, 1998; Faustino and Power, 2001; Vandewalle et al., 2005; Geerinckx et al. 2007). Shortly after yolk depletion, at 4.9 mm SL, the appearance of the parurohyal may indicate a response to mechanical stress experienced due to

activity of the sternohyoideus muscle (Adriaens and Verraes, 1998). Such a response would imply an increase in lower jaw mobility shortly after this dietary shift. In siluroids, the parurohyal is described to be the result of a fusion between a tendon bone and the basibranchial cartilage (Arratia and Schultze, 1990). Adriaens and Verraes (1998) therefore argue that the absence of the first basibranchial in *Clarias gariepinus* is the

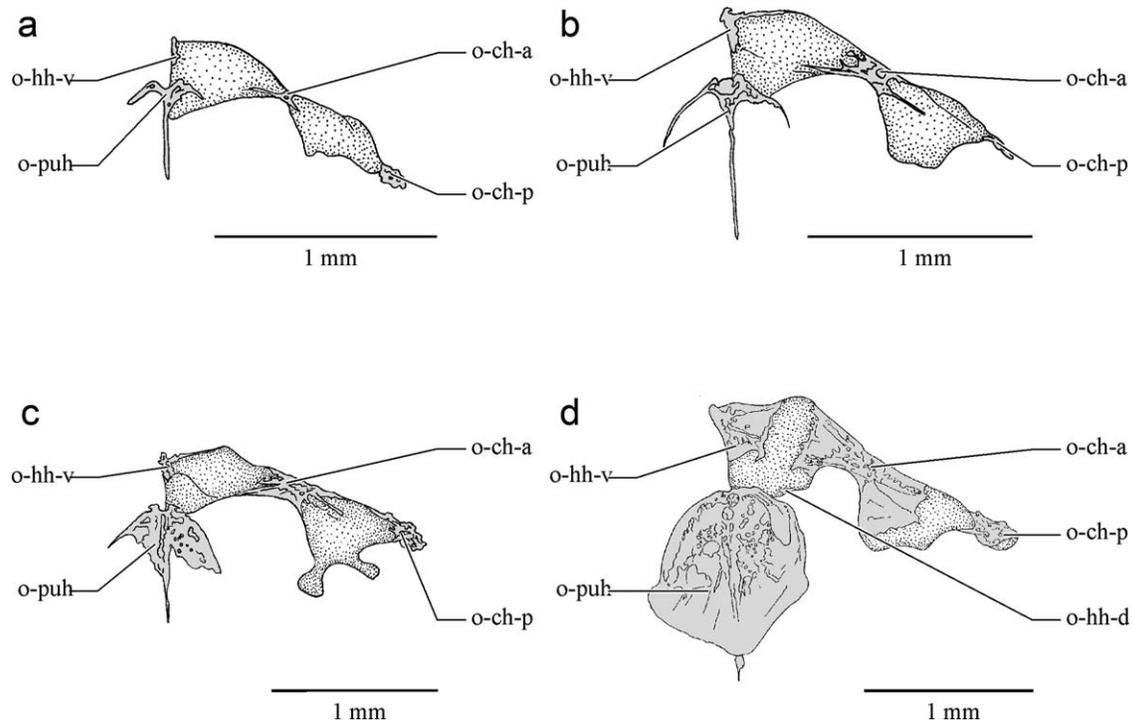


Fig. 5. *Corydoras aeneus*, ventral view of the hyoid at different developmental stages: (a) 8.2 mm SL; (b) 9.7 mm SL; (c) 11.2 mm SL; (d) 13.9 mm SL. o-ch-a, os ceratohyale anterior; o-ch-p, os ceratohyale posterior; o-hh-d, os hypohyale dorsalis; o-hh-v, os hypohyale ventralis; o-puh, os parurohyale.

result of a similar fusion. Geerinckx et al. (2007) corroborate this hypothesis based on observations on serial sections of *A. cf. triradiatus*. Although no such fusion was observed in this study, the absence of the first basibranchial also indicates a parurohyal of mixed origin in *C. aeneus*.

After the bones associated with the feeding and breathing apparatus have developed (between 5 and 8 mm SL), priorities in development shift to size increase of the cartilaginous skull. After this increase, cartilage resorption and further ossification begins, reinforcing the skull. The skull floor is generally the first neurocranial part to ossify (Vandewalle et al., 1995, 1997; Adriaens and Verraes, 1998). Such an early fortification is necessary as, from the moment of exogenous feeding on, the overlying brain has to be protected from the particles passing below (Adriaens and Verraes, 1998). It has also been hypothesized that respiratory movements and buccal pressure differences would also generate mechanical loadings inducing skull floor ossification (Mabee and Trendler, 1996; Geerinckx et al., 2007). In *C. aeneus*, skull floor ossifications indeed occur early, however, not directly after the shift from endogenous to exogenous feeding, as expected. In this case, skull floor bones like the ventral mesethmoid part, the prevomer, and parasphenoid only start to ossify from 8 mm SL on, well after this shift in feeding style (which occurs at 4.4 mm SL). At this stage, the

prevomer also clearly shows two lateral palatal splints. The presence of such structures in adult callichthyids has been documented in the past for species of *Corydoras* and *Aspidoras* (Reis, 1998), but was not observed in our earlier study on the osteology of adult *C. aeneus* (Huysentruyt and Adriaens, 2005). We now know that these splints fuse to the prevomer bone, although additional observations on serial sections show them to be still present in some adult *C. aeneus* specimens, but, in that case, highly diminished in size. This suggests that the reported absence of such splints in other callichthyid genera like *Callichthys*, *Dianema*, *Leptoplosternum*, *Hoplosternum* and *Megalechis* (Reis, 1998) is probably the result of a secondary loss (through fusion or resorption) during ontogeny. The prevomer bone in *C. aeneus* is toothless, in contrast to the general siluriform state. Also unlike other catfish, the bone arises as an unpaired element, but since it has been argued that the prevomer bone generally consists of an unpaired dermal toothless bone fused to paired autogenous toothplates, it seems that only the latter are absent in *C. aeneus* development (Adriaens and Verraes, 1998). At 8 mm SL, the neurocranium also has started to ossify at its dorsal side, since such reinforcements become necessary due to skull growth (Adriaens and Verraes, 1998). Ossification simultaneously starts at both the level of the frontal bones as well as the level of the pari-

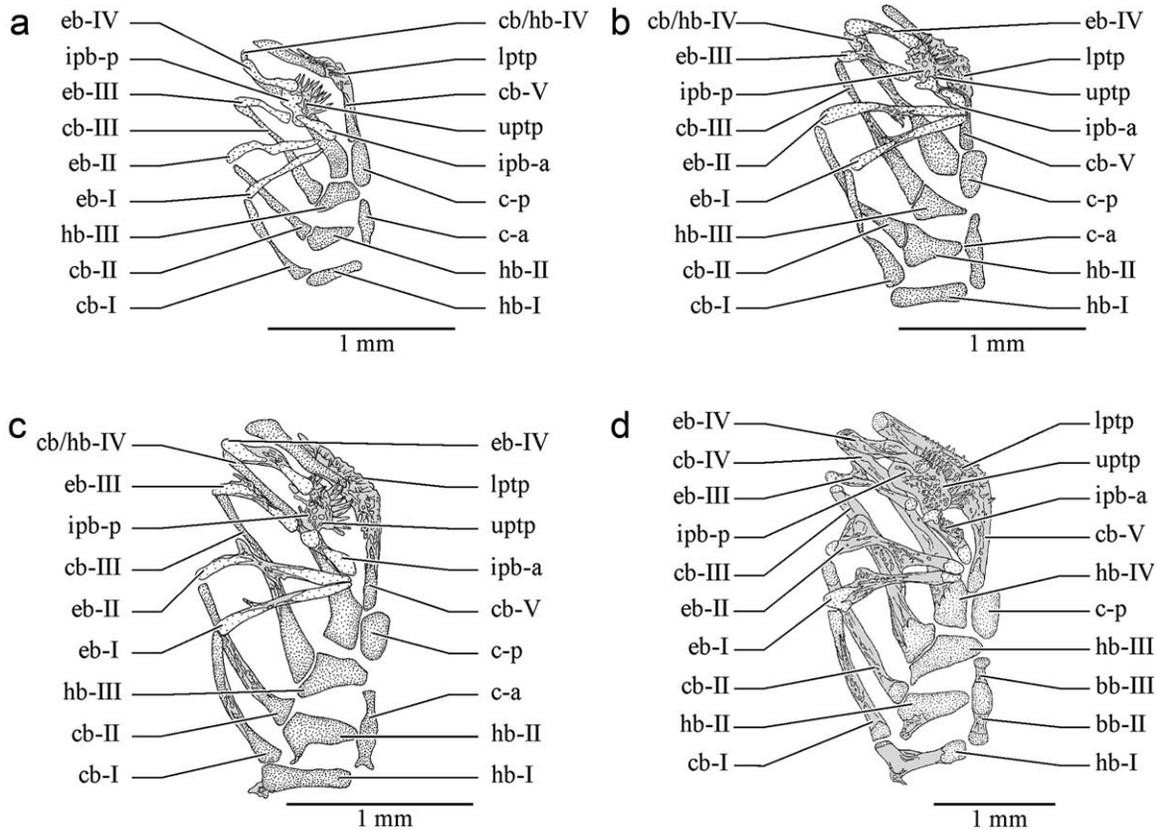


Fig. 6. *Corydoros aeneus*, dorsal view of the right half of the branchial basket at different developmental stages: (a) 8.2 mm SL; (b) 9.7 mm SL; (c) 11.2 mm SL; (d) 13.9 mm SL. bb, basibranchial; c-a, copula anterior; cb, ceratobranchial; c-p, copula posterior; eb, epibranchial; hb, hypobranchial; ipb-a, infrapharyngobranchial anterior; ipb-p, infrapharyngobranchial posterior; lptp, lower pharyngeal toothplate; uptp, upper pharyngeal toothplate. Roman numerals indicate arch number. /-symbol indicates fusion of elements.

eto-supraoccipital bone. The latter bone arises as a single ossification and a separate parietal and supraoccipital part could not be discerned at any stages. Hoedeman (1960), in his studies on the callichthyids *Callichthys* and *Hoplosternum* did not find ontogenetic evidence for the compound nature of this bone. However, various authors have observed such a fusion between both the dermal parietal and perichondral supraoccipital in various Siluriphysi and have argued this state to be typical for the group (Bamford, 1948; Lundberg, 1975; Fink and Fink, 1996). Therefore, the bone found here was homologized with this fused parieto-supraoccipital. Another example of such a compound bone arising at this stage is the post-temporo-pterotico-supracleithrum. Again, during ontogeny, no signs of fusion of the separate elements constructing this compound bone were observed, but, nonetheless, the compound nature of this bone was accepted based on the arguments provided by Huysentruyt and Adriaens (2005).

From 8 mm SL on, ossification in the splanchnocranium drastically increases, with various centers of ossification in the autopalatine, opercular

series, lower jaw, suspensorial, and hyoid arch. Also at this point all articulatory facets have started ossifying, rigidifying the entire splanchnocranium structure. In the lower jaw, the mentomeckelian, dentary, articular and retroarticular bones, which will later make up the compound dentary bone (*s.l.*), are still present as separate elements. As in all callichthyids and various other loricarioids like loricariids, astroblepids and most trichomycterids, no coronomeckelian bone is present (Mo, 1991; De Pinna, 1993; Geerinckx et al., 2007). At this point in the opercular series, a suprapreopercular bone has also developed. The presence of this bone was already mentioned in adult *C. aeneus* specimens as a condition uncommon for Callichthyidae, and it was suggested that further ontogenetic research on the ontogeny of this bone would have to confirm its hypothesized homology (Huysentruyt and Adriaens, 2005). Given the fact that our data show the bone to develop in close contact to the preopercular canal and given its position, the small bone shown in the figures would indeed appear to be a suprapreopercular bone. However, also as shown in the figures

(Figs. 2, 3), the bone described as the suprapreopercular in the ontogenetic series fuses to the hyomandibula and the preopercular canal does not protrude into it. Next to this, the bone develops early in ontogeny, while in a related species like *A. triradiatus*, it arises much later in ontogeny (Geerinckx et al., 2007) and does not resemble the bone described as suprapreopercle in the adult specimens of *C. aeneus*. In addition, study of additional adult specimens shows the bone to be variably present in these adults. Therefore, it is questionable whether or not the bone described in early ontogeny as the suprapreopercle is homologous to the bone seen in some adult specimens. Nonetheless, since both bones, when present, are in close contact with the preopercular canal and are situated above the preopercular bone, the use of name suprapreopercle seem justifiable in both cases.

In contrast to the 8 mm SL stage, at around 10 mm SL, ossification appears more focused on the neurocranium, with additional ossifications appearing in the skull roof, wall and floor. In the splanchnocranium, however, only the preopercular and metapterygoid bones appear at this stage. The catfish metapterygoid has been described as a fusion of ectopterygoid, entopterygoid, and metapterygoid by Howes and Teugels (1989). Other authors, however, reported the ectopterygoid and entopterygoid to be absent, which we believe to be the case in this species as well (Regan, 1911; Arratia, 1990, 1992; Adriaens and Verraes, 1998; Reis, 1998). Also at 10 mm SL, most elements of the branchial basket start ossifying, all of which start in the center of the respective cartilaginous elements. Shortly after this, at 11 mm SL, ceratobranchials and hypobranchials I–II also start ossifying, although, in this case, ossification starts at the rostralateral side of the cartilaginous hypobranchials. Finally, at 14 mm SL, the basibranchials are the last bony elements to develop. This way, the entire branchial basket, apart from the tooth bearing elements, has completely ossified within the short range of 10–14 mm SL and together with the hyoid arch, it is the last large functional unit to complete ossification. Next to this, the interhyal first ossifies at this point. The late emergence of a bony interhyal indicates that the fact that it is lost in several siluriform catfishes like *C. gariepinus*, Loricariidae and Scoloplacidae (Bailey and Baskin, 1976; Adriaens and Verraes, 1998) could be the result of a truncation in development. This could also explain the variability in the number of bones in the infraorbital series, found throughout siluriform phylogeny.

Only three additional ossifications occur after this point. At 16 mm SL, the nasal and first infraorbital bones are the last bones to appear in the development of the *C. aeneus* cranium.

Conclusively, overall ossification sequence in *C. aeneus* follows the general trends observed in

siluriform development, with ossifications appearing as a response to functional demands (Tilney and Hecht 1993; Vandewalle et al. 1995, 1997; Adriaens and Verraes 1998). This way, early development of the skull is focused on the completion of a functional feeding and respiratory apparatus, prior to rigidification and growth.

In *A. cf. triradiatus*, a similar ossification sequence was observed, but mainly the timing at which the different osteocranial elements arise during ontogeny differed (Geerinckx et al., 2007). Since *A. cf. triradiatus* has a more direct development with an extended embryonic phase and without a true larval phase, most of the ossification sequence has ended by the time of the shift to exogenous feeding (Geerinckx et al., 2007). Therefore, it appears that the emergence of a highly specialized cranial morphology in loricariids has been facilitated by evolutionary shifts in ontogenetic timing rather than sequence.

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