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A timeline of pharyngeal endoskeletal condensation and differentiation in the shark, *Scyliorhinus canicula*, and the paddlefish, *Polyodon spathula*

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Summary

The lesser-spotted dogfish (*Scyliorhinus canicula*) and the North American paddlefish (*Polyodon spathula*) are two emerging model systems for the study of vertebrate craniofacial development. Notably, both of these taxa have retained plesiomorphic aspects of pharyngeal endoskeletal organization, relative to more commonly used models of vertebrate craniofacial development (e.g. zebrafish, chick and mouse), and are therefore well suited to inform the pharyngeal endoskeletal patterning mechanisms that functioned in the last common ancestor of jawed vertebrates. Here, we present a histological overview of the condensation and chondrogenesis of the most prominent endoskeletal elements of the jaw, hyoid and gill arches – the palatoquadrate/Meckel's cartilage, the hyomandibula/ceratohyal, and the epi-/ceratobranchial cartilages, respectively – in embryonic series of *S. canicula* and *P. spathula*. Our observations provide a provisional timeline and anatomical framework for further molecular developmental and functional investigations of pharyngeal endoskeletal differentiation and patterning in these phylogenetically informative taxa.

Introduction

The vertebrate craniofacial skeleton may be broadly subdivided into dermal and endoskeletal components, based on mode of embryonic skeletogenesis (reviewed in Hall, 2005). The dermal skeleton includes most of the plate-like bones of the skull and face, and forms by direct or "intramembranous" ossification. The endoskeleton, on the other hand, includes the braincase and the pharyngeal arch skeleton – the mandibular, hyoid and gill arches – and forms by endochondral ossification. The mandibular, hyoid and gill arch endoskeleton of gnathostomes was ancestrally segmented along the dorsal-ventral axis into two prominent dorsal and ventral elements – the palatoquadrate and Meckel's cartilage, the hyomandibula and ceratohyal, and the epibranchial and ceratobranchial elements, respectively – with a variable number of pharyngobranchial and hypobranchial elements on the gill arches (for an overview of gnathostome pharyngeal endoskeletal organization, see Gillis et al., 2009a). However, few extant bony fishes exhibit this plesiomorphic organization. For example, in zebrafish, the dorsal (epibranchial) segments of the gill arches

are greatly reduced, while in mammals, the palatoquadrate and Meckel's cartilage no longer function as the support skeleton for the adult jaw (the palatoquadrate gives rise to the incus and the ala temporalis component of the alisphenoid, while Meckel's cartilage gives rise to the malleus) (Fig. 1). As a consequence of the derived nature of the pharyngeal endoskeleton in the three vertebrate model systems most commonly used for studies of craniofacial development – namely mouse, chick and zebrafish – comparative studies in these taxa can yield only limited insight into the developmental mechanisms that ancestrally patterned the pharyngeal endoskeleton in jawed vertebrates.

Recently, the lesser-spotted dogfish, *Scyliorhinus canicula*, and the North American paddlefish, *Polyodon spathula*, have emerged as valuable model systems for craniofacial development (O'Neill et al., 2007; Gillis et al., 2011; Modrell et al., 2011a,b). As cartilaginous (chondrichthyan) and non-teleost ray-finned (actinopterygian) fishes, respectively, these taxa occupy important phylogenetic positions that may permit the inference of plesiomorphic gnathostome character states. Additionally – and importantly for studies of pharyngeal patterning – both chondrichthyans and paddlefishes have retained certain plesiomorphic aspects of jaw, hyoid and gill arch endoskeletal organization, such as exclusive (*S. canicula*) or substantial (*P. spathula*) contributions of the palatoquadrate and Meckel's cartilage to the upper and lower jaw skeleton, and the presence of prominent epibranchial and ceratobranchial elements in their gill arches (Daniel, 1934; Grande and Bemis, 1991) (Fig. 1). Unlike teleost fishes, neither *S. canicula* nor *P. spathula* have undergone an additional round of whole genome duplication (Venkatesh et al., 2007; Hurley et al., 2007), which simplifies the cloning and characterization of candidate developmental genes, and protocols for molecular developmental analysis by mRNA *in situ* hybridization are well-established for both species (Freitas et al., 2006a,b; Freitas et al. 2007; Davis et al., 2007; O'Neill et al., 2007; Coolen et al., 2007, 2008; Gillis et al., 2011; Modrell et al., 2011a,b; Oulion et al., 2011). Finally, both taxa have proven amenable to experimental manipulation (Davis et al., 2007; Sakamoto et al., 2009; Modrell et al., 2011b – see also Dahn et al., 2007; Gillis et al., 2009b; Rotenstein et al., 2010 for examples of experimental embryological manipulation in other oviparous chondrichthyan species), and may therefore offer a unique opportunity to carry out functional studies in the context of plesiomorphic anatomical conditions.

In all gnathostome embryos, skeletal elements (dermal and endoskeletal) differentiate from preskeletal mesenchymal condensations (reviewed by Hall and Miyake, 2000). Dermal condensations differentiate directly into bone, while endoskeletal condensations differentiate first into a cartilaginous model, which is subsequently replaced by bone (though in chondrichthyans, endoskeletal elements remain cartilaginous; Eames et al., 2007). Here, we present an overview of the condensation and chondrogenesis of the major cartilaginous elements of the pharyngeal endoskeleton in the shark, *S. canicula*, and the paddlefish, *P. spathula*. It is our hope that the timeline we present here will be a useful starting point for future molecular developmental and experimental studies of pharyngeal endoskeletal patterning in these phylogenetically informative taxa.

Material and Methods

Embryos of *Scyliorhinus canicula* were obtained from the Station Biologique de Roscoff, France. *S. canicula* egg cases were reared in a flow-through seawater system to the desired embryonic stage. Staging was done according to Ballard et al. (1993). Egg cases were opened with a razor blade and embryos were removed and euthanized with an overdose of MS-222 (Sigma). Embryos were then fixed in 4% paraformaldehyde overnight at 4°C, rinsed in 1× phosphate-buffered saline (PBS), dehydrated into 100% methanol and stored at −20°C. Embryos of *Polyodon spathula* were purchased from Osage Catfisheries (Osage Beach, MO). Embryos were raised at approximately 22°C in tanks with filtered and recirculating water (pH 7.2 ± 0.7, salinity of 1.0 ± 0.2 ppt) to desired stages. Staging was done according to Bemis and Grande (1992). Embryos were fixed in 4% paraformaldehyde for 4 hours at room temperature or overnight at 4°C. Specimens were then rinsed in PBS, dehydrated stepwise into 100% methanol or ethanol and stored at −20°C.

From 100% methanol, embryos of both species were cleared, infiltrated with paraffin, embedded and sectioned as previously described (Gillis et al., 2009a). Sections were stained with a modified Mayer's Haematoxylin and Eosin Y staining protocol, which includes an additional staining step in Alcian blue (as described in Davis et al., 2004). All embryos were sectioned horizontally. For *S. canicula*, one embryo at each stage from 26-31 was examined, while for *P. spathula*, one embryo at each stage from 36-46 was examined.

Results

Pharyngeal endoskeletal condensation and differentiation in *Scyliorhinus canicula*

At stage (St.) 26 of *Scyliorhinus canicula* development, the mandibular (Fig. 2a), hyoid (Fig. 2b) and gill arches (Fig. 2c-g) have formed. The arches are bound laterally by ectoderm, medially by endoderm, and contain undifferentiated mesenchyme and a core of paraxial mesoderm. At this stage, there is not yet any indication of mesenchymal condensation at the future sites of endoskeletal differentiation. By St. 28, sparse mesenchymal condensations have formed at the future sites of Meckel's cartilage (Fig. 2h) and the palatoquadrate (not shown) in the mandibular arch, the hyomandibula (Fig. 2i) and the ceratohyal (not shown) in the hyoid arch, and epibranchials (not shown) and ceratobranchials 1-3 (Fig 2j-l) in the first three gill arches. Epibranchial and ceratobranchial condensations are not yet visible in gill arch 4 (Fig. 2m), nor is a ceratobranchial condensation visible in gill arch 5 (Fig. 2n). Note that in elasmobranchs, there is no discrete epibranchial element present in gill arch 5 (the epibranchial primordium fuses with a pharyngobranchial element – Gillis et al., 2009a).

By St. 29/30, dense preskeletal condensations are present in the mandibular arch (Fig. 2o), hyoid arch (Fig. 2p) and gill arches 1-5 (Fig. 2q-u). Condensed mesenchyme has not yet differentiated into cartilage, though faint Alcian blue staining of the condensations in the rostral-most arches (mandibular, hyoid and gill arches 1-3 – Fig. 2o-s) indicates some secretion of proteoglycans that are characteristic of cartilage extracellular matrix. By late St. 31, mesenchymal condensations have differentiated into cartilage in the mandibular (Fig. 2v), hyoid (Fig. 2w) and five gill arches (Fig. 2x-b¹), as indicated by the presence of differentiated chondrocytes within lacunae, a well-defined perichondrium and strong

staining of extracellular matrix with Alcian blue. Note that at St. 31, branchial ray cartilages have also differentiated (see Fig. 2y-a¹).

Pharyngeal endoskeletal condensation and differentiation in *Polyodon spathula*

At St. 36 of *Polyodon spathula* development, the palatoquadrate (Fig. 3a) and Meckel's cartilage (not shown) have condensed within the mandibular arch, but no condensations are detected in the hyoid (Fig. 3b) or gill arches (Fig. 3c-e). By St. 40, Meckel's cartilage and the palatoquadrate remain condensed, and mesenchymal condensations are also observed at the sites of the future hyomandibula (Fig. 3g) and ceratohyal (not shown) within the hyoid arch, and at the sites of the future epibranchials (not shown) and ceratobranchials in gill arches 1 and 2 (Fig. 3h-i, and not shown). At this stage, gill arches 3-5 still contain undifferentiated mesenchyme (Fig. 3j).

By St. 44, Meckel's cartilage (not shown) and the palatoquadrate (Fig. 3k) have differentiated into cartilage. These cartilages exhibit a well-defined perichondrium and chondrocytes nested in lacunae. The endoskeletal elements in the hyoid (Fig. 3l) and gill arches 1-2 (Fig. 3m-n) have also begun to differentiate, though the extent of differentiation lags behind that seen in the mandibular arch. In gill arch 3, the epibranchial (not shown) and ceratobranchial (Fig. 3o) have condensed, and faint staining with Alcian blue indicates some secretion of a proteoglycan-enriched extracellular matrix. In gill arch 4, the epibranchial (not shown) and ceratobranchial (Fig. 3o) have condensed. There is no indication of condensed mesenchyme in gill arch 5.

By St. 46, endoskeletal elements have differentiated into cartilage in the mandibular arch (Fig. 3p), hyoid arch (Fig. 3q), and gill arches 1-3 (Fig. 3r-t). Differentiation of the epibranchial and ceratobranchial elements in gill arch 4 is underway (Fig. 3t), and the ceratobranchial in gill arch 5 has condensed (Fig. 3t).

Discussion

Here, we have presented an overview of the condensation and chondrogenesis of major components of the pharyngeal endoskeleton in the shark, *Scyliorhinus canicula*, and the paddlefish, *Polyodon spathula*. We have shown that St. 28-30 are key stages for *S. canicula* pharyngeal endoskeletal condensation, and that by St. 31, many of these condensations have differentiated into cartilage. For *P. spathula*, we have shown that St. 36-40 represent key stages for pharyngeal skeletal condensation, with chondrogenic differentiation of these condensations initiating rostrally (i.e. in the mandibular arch) prior to St. 44, and proceeding beyond St. 46 in the caudal gill arches. Due to the length of the relevant developmental stages in *S. canicula*, we have not figured the precise sequence of endoskeletal condensation and chondrogenesis along the anterior-posterior axis of the head. However, it should be noted that a similar sequence of mandibular, hyoid and gill arch endoskeletal chondrogenesis has been noted in another elasmobranch species (the little skate, *Leucoraja erinacea* – Gillis et al., 2009a). This sequence may, therefore, represent this ancestral sequence of pharyngeal endoskeletal chondrogenesis in jawed vertebrates. While a number of pharyngeal endoskeletal elements have not been discussed (e.g. basibranchials, hypobranchials and pharyngobranchials), it is our hope that the data presented here will

provide a useful starting point for further histological/molecular studies of skeletal elements of interest, and for experimental investigation of pharyngeal endoskeletal patterning in these taxa. This may be particularly useful for future work with *S. canicula*, which has an *in ovo* period of several months (Ballard et al., 1993).

One particular aspect of craniofacial development that will benefit from studies in *S. canicula* and *P. spathula* is the molecular basis of gill arch dorsal-ventral endoskeletal identity. In mammals, dorsal and ventral identity is specified in the mandibular and hyoid arch by regional, nested expression of the *Dlx* family of homeodomain-containing transcription factors – the ‘*Dlx* code’ – within arch mesenchyme (Qiu et al., 1995, 1997; Beverdam et al., 2002; Depew et al., 2002, 2005). Recent work has demonstrated a conserved role for *Dlx* genes in patterning the dorsal-ventral axis of the mandibular and hyoid arch endoskeleton in zebrafish (Talbot et al., 2010), though there is currently no indication that an equivalent *Dlx* code patterns the post-hyoid pharyngeal (i.e. branchial) arches of teleosts (Verreijdt et al., 2006; Renz et al., 2011 – see also Kuraku *et al.* 2010 and Cerny *et al.* 2011 for alternative interpretations of branchial arch *Dlx* gene expression in jawless vertebrates). It is possible that the *Dlx* code arose as an evolutionary novelty in association with the osteichthyan (or perhaps the gnathostome) jaw and hyoid arch. Alternatively, a *Dlx* code may have functioned ancestrally to pattern the dorsal-ventral axis of all gnathostome pharyngeal arches, having been lost or modified beyond recognition in the derived post-hyoid pharyngeal arches of mammals and teleosts. We are currently performing studies using *S. canicula* and *P. spathula* embryos to address this question, as well as other important questions relating to the evolution and development of the gnathostome craniofacial skeleton.

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References

- Ballard WW, Mellinger J, Lechenault H. A series of normal stages for the development of *Scyliorhinus canicula*, the lesser spotted dogfish (Chondrichthyes: Scyliorhinidae). *J. Exp. Zool.* 1993; 267:318–336.
- Bemis WE, Grande L. Early development of the actinopterygian head. 1. External development and staging of the paddlefish *Polyodon spathula*. *J. Morphol.* 1992; 213:47–83.
- Beverdam A, Merlo GR, Paleari L, Mantero S, Genova F, Barbieri O, Janvier P, Levi G. Jaw transformation with gain of symmetry after *Dlx5/Dlx6* inactivation: mirror of the past? *Genesis.* 2002; 34:221–227. [PubMed: 12434331]
- Cerny R, Cattell M, Sauka-Spengler T, Bronner-Fraser M, Yu F, Medeiros DM. Evidence for the prepatterning/cooption model of vertebrate jaw evolution. *Proc. Natl. Acad. Sci. USA.* 2010; 107:17262–17267. [PubMed: 20855630]
- Coolen M, Sauka-Spengler T, Nicolle D, Le Mentec C, Lallemand Y, Da Silva C, Plouhinec JL, Robert B, Wincker P, Shi DL, Mazan S. Evolution of axis specification mechanisms in jawed vertebrates: insights from a chondrichthyan. *PLoS One.* 2007; 2:e374. [PubMed: 17440610]

- Coolen M, Menuet A, Chassoux D, Compagnucci C, Henry S, Lévêque L, Da Silva C, Gavory F, Samain S, Wincker P, Thermes C, D'Aubenton-Carafa Y, Rodriguez-Moldes I, Naylor G, Depew M, Sourdain P, Mazan S. The dogfish *Scyliorhinus canicula*: A reference in jawed vertebrates. CSH Protoc. 2008; 2008
- Dahn RD, Davis MC, Pappano WN, Shubin NH. Sonic hedgehog function in chondrichthyan fins and the evolution of appendage patterning. Nature. 2007; 445:311–314. [PubMed: 17187056]
- Daniel, JF. The Elasmobranch Fishes. University of California Press; Berkeley, CA: 1934.
- Davis MC, Shubin NH, Force A. Pectoral fin and girdle development in the basal actinopterygians *Polyodon spathula* and *Acipenser transmontanus*. J. Morphol. 2004; 262:608–628. [PubMed: 15376275]
- Davis MC, Dahn RD, Shubin NH. An autopodial-like pattern of *Hox* expression in the fins of basal actinopterygian fish. Nature. 2007; 447:473–476. [PubMed: 17522683]
- Depew MJ, Lufkin T, Rubenstein JLR. Specification of jaw subdivisions by *Dlx* genes. Science. 2002; 298:381–385. [PubMed: 12193642]
- Depew MJ, Simpson CA, Morasso M, Rubenstein JL. Reassessing the *Dlx* code: the genetic regulation of branchial arch skeletal patterning and development. J. Anat. 2005; 207:501–561. [PubMed: 16313391]
- Eames BF, Allen N, Young J, Kaplan A, Helms JA, Schneider RA. Skeletogenesis in the swell shark *Cephaloscyllium ventriosum*. J. Anat. 2007; 210:542–554. [PubMed: 17451531]
- Freitas R, Zhang G, Cohn MJ. Biphasic *Hoxd* gene expression in shark paired fins reveals an ancient origin of the distal limb domain. PLoS One. 2007; 2:e754. [PubMed: 17710153]
- Freitas R, Zhang G, Cohn MJ. Evidence that mechanisms of fin development evolved in the midline of early vertebrates. Nature. 2006a; 442:1033–1037. [PubMed: 16878142]
- Freitas R, Zhang G, Albert JS, Evans DH, Cohn MJ. Developmental origin of shark electrosensory organs. Evol. Dev. 2006b; 8:74–80. [PubMed: 16409384]
- Gillis JA, Dahn RD, Shubin NH. Chondrogenesis and homology of the visceral skeleton in the little skate, *Leucoraja erinacea* (Chondrichthyes: Batoidea). J. Morphol. 2009a; 270:628–643. [PubMed: 19117064]
- Gillis JA, Dahn RD, Shubin NH. Shared developmental mechanisms pattern the vertebrate gill arch and paired fin skeletons. Proc. Natl. Acad. Sci. USA. 2009b; 106:5720–5724. [PubMed: 19321424]
- Gillis JA, Rawlinson KA, Bell J, Lyon WS, Baker CVH, Shubin NH. Holocephalans embryos provide evidence for gill arch appendage reduction and opercular evolution in cartilaginous fishes. Proc. Natl. Acad. Sci. USA. 2011; 108:1507–1512. [PubMed: 21220324]
- Grande L, Bemis W. Osteology and phylogenetic relationships of fossil and Recent paddlefishes (Polyodontidae) with comments on the interrelationships of Acipenseriformes. Soc. Vert. Paleont. Mem. (suppl. to vol. 11, no. 1, J. Vert. Paleont.). 1991:1–121.
- Hall, BK. Bones and cartilage: developmental and evolutionary skeletal biology. Elsevier; San Diego, CA: 2005. p. 760
- Hall BK, Miyake T. All for one and one for all: condensations and the initiation of skeletal development. Bioessays. 2000; 22:138–147. [PubMed: 10655033]
- Hurley IA, Mueller RL, Dunn KA, Schmidt EJ, Friedman M, Ho RK, Prince VE, Yang Z, Thomas MG, Coates MI. A new time-scale for ray-finned fish evolution. Proc. Roy. Soc. B. 2007; 274:489–498.
- Kuraku S, Takio Y, Sugahara F, Takechi M, Kuratani S. Evolution of oropharyngeal patterning mechanisms involving *Dlx* and *endothelins* in vertebrates. Dev. Biol. 2010; 341:315–323. [PubMed: 20171204]
- Modrell MS, Buckley D, Baker CVH. Molecular analysis of neurogenic placode development in a basal ray-finned fish. Genesis. 2011a; 49:278–294. [PubMed: 21381180]
- Modrell MS, Bemis WE, Northcutt RG, Davis MC, Baker CVH. Electrosensory ampullary organs are derived from the lateral line placodes in bony fishes. Nat. Commun. 2011b; 2:496. [PubMed: 21988912]

- O'Neill P, McCole RB, Baker CVH. A molecular analysis of neurogenic placode and cranial sensory ganglion development in the shark, *Scyliorhinus canicula*. *Dev. Biol.* 2007; 304:156–181. [PubMed: 17234174]
- Oulion S, Borday-Birraux V, Debais-Thibaud M, Mazan S, Laurenti P, Casane D. Evolution of repeated structures along the body axis of jawed vertebrates, insights from the *Scyliorhinus canicula* *Hox* code. *Evol. Dev.* 2011; 13:247–259. [PubMed: 21535463]
- Qiu MS, Bulfone A, Martinez S, Meneses JJ, Shimamura K, Pedersen RA, Rubenstein JLR. Role of *Dlx-2* in head development and evolution: Null mutation of *Dlx-2* results in abnormal morphogenesis of proximal first and second branchial arch derivatives and abnormal differentiation in the forebrain. *Genes Dev.* 1995; 9:2523–2538. [PubMed: 7590232]
- Qiu MS, Bulfone A, Ghattas I, Meneses JJ, Christensen L, Sharpe PT, Presley R, Pedersen RA, Rubenstein JLR. Role of *Dlx-1* and *Dlx-2* in proximodistal patterning of the branchial arches: mutations alter morphogenesis of proximal skeletal elements derived from the first and second arches. *Dev. Biol.* 1997; 185:165–184. [PubMed: 9187081]
- Renz AJ, Gunter HM, Fischer JM, Qiu H, Meyer A, Kuraku S. Ancestral and derived attributes of the *dlx* gene repertoire, cluster structure and expression patterns in an African cichlid fish. *Evodevo.* 2011; 2:1. [PubMed: 21205289]
- Rotenstein L, Milanes A, Juarez M, Reyes M, de Bellard ME. Embryonic development of glial cells and myelin in the shark, *Chiloscyllium punctatum*. *Gene Expr. Patterns.* 2010; 9:572–585. [PubMed: 19733690]
- Sakamoto K, Onimaru K, Munakata K, Suda N, Tamura M, Ochi H, Tanaka M. Heterochronic shift in *Hox*-mediated activation of sonic hedgehog leads to morphological changes during fin development. *PLoS One.* 2009; 4:e5121. [PubMed: 19365553]
- Schilling TF, Piotrowski T, Grandel H, Brand M, Heisenberg CP, Jiang YJ, Beuchle D, Hammerschmidt M, Kane DA, Mullins MC, van Eeden FJ, Kelsh RN, Furutani-Seiki M, Granato M, Haffter P, Odenthal J, Warga RM, Trowe T, Nüsslein-Volhard C. Jaw and branchial arch mutants in zebrafish. I. Branchial arches. *Development.* 1996; 123:329–344. [PubMed: 9007253]
- Talbot JC, Johnson SL, Kimmel CB. *hand2* and *Dlx* genes specify dorsal, intermediate and ventral domains within zebrafish pharyngeal arches. *Development.* 2010; 137:2507–2517. [PubMed: 20573696]
- Venkatesh B, Kirkness EF, Loh Y-H, Halpern AL, Lee AP, Johnson J, Dandona N, Viswanathan LD, Tay L, Venter JC, Strausberg RL, Brenner S. Survey sequencing and comparative analysis of the elephant shark (*Callorhynchus milii*) genome. *PLoS Biol.* 2007; 5:e101. [PubMed: 17407382]
- Verreijdt L, Debais-Thibaud M, Borday-Birraux V, Van der Heyden C, Sire JY, Huysseune A. Expression of the *Dlx* gene family during formation of the cranial bones in zebrafish (*Danio rerio*): differential involvement in the visceral skeleton and braincase. *Dev. Dyn.* 2006; 235:1371–1389. [PubMed: 16534783]

Figure 1.

Schematic overview of pharyngeal endoskeletal organization in a shark, paddlefish, zebrafish and mouse. In all images, anterior is to the left. Mandibular arch derivatives are colored in red, hyoid arch derivatives are colored in blue and gill arch derivatives are colored in yellow. The paddlefish schematic was drawn from an original specimen (derivatives of gill arch 4 and 5 are obscured); the shark schematic was redrawn from a specimen figured in Gillis et al. (2011) (note that branchial rays are not shown); the zebrafish schematic was redrawn from a 6 day-post-fertilization specimen figured in Schilling et al. (1996); the mouse schematic was redrawn from Depew et al. (2005). Abbreviations: *at*, ala temporalis; *bh*, body of the hyoid; *cb1-5*, ceratobranchials 1-5; *ch*, ceratohyal; *eb1-4*, epibranchials 1-4; *ghh*, greater horn of the hyoid; *hm*, hyomandibula; *hs*, hyosymplectic; *in*, incus; *lc*, laryngeal cartilages; *lhh*, lesser horn of the hyoid; *ma*, malleus; *mk*, Meckel's cartilage; *mk^{tp}*, rostral projection of Meckel's cartilage; *pq*, palatoquadrate; *sp*, styloid process; *st*, stapes; *thy*, thyroid cartilage.

Figure 2.

Horizontal histological sections through the mandibular (MA), hyoid (HA) and gill arches 1-5 (GA1-5) of the lesser-spotted dogfish, *Scyliorhinus canicula*, at (a-g) stage (St.) 26, (h-n) St. 28, (o-u) St. 29/30 and (v-bi) St. 31. In all images, anterior is to the left. For histological details, see text. Abbreviations: *br*, branchial ray; *cb1-5*, ceratobranchial 1-5; *hm*, hyomandibula; *mk*, Meckel's cartilage. * indicates an as-yet undifferentiated mesenchymal condensation. All scale bars = 20µm.

Figure 3.

Horizontal histological sections through the mandibular (MA), hyoid (HA) and gill arches 1-5 (GA1-5) of the North American paddlefish, *Polyodon spathula*, at (a-e) stage (St.) 36, (f-j) St. 40, (k-o) st. 44 and (p-t) St. 46. In all images, anterior is to the left. For histological details, see text. Abbreviations: *cb1-5*, ceratobranchial 1-5; *hm*, hyomandibula; *pq*, palatoquadrate. * indicates an as-yet undifferentiated mesenchymal condensation. All scale bars = 20µm.