Identity of a Unique Cartilage in the Buccal Cavity of Gars (Neopterygii: Lepisosteiformes: Lepisosteidae)

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The lower jaw of *Lepisosteus osseus* has been described as containing a U-shaped cartilaginous structure identified as a detached portion of Meckel's cartilage. We investigated this structure through study of a growth series of *L. osseus*, including cleared-and-stained specimens and histological preparations. Meckel's cartilage is well developed by 17.2 mm SL, and is formed by typical hyaline cartilage. The left and right Meckel's cartilage are continuous across the anterior midline in most small specimens (<35 mm SL), although the robustness of this continuity is variable and in some specimens the two sides are separate. In larger individuals (>85 mm SL) the left and right sides are separate from one another and end far posterior to the tip of the dentaries. In specimens between 17.6–22.1 mm SL, there is a diffuse, V-shaped patch of connective tissue between the rami of the lower jaws. By 35 mm SL this patch becomes more consolidated and in histological sections of a 44.6 mm SL specimen, it is revealed to contain chondrocytes. In even larger individuals, this becomes a well-defined U-shaped structure. Because both this structure and a Meckel's cartilage that crosses the anterior midline are present in the same individuals, we conclude that this cartilage is not homologous to the anterior portion of Meckel's cartilage (i.e., it fails the test of conjunction) but is rather a neomorphic structure, herein termed the rostrohyal.

THE lower jaw of bony fishes displays a broad array of morphological forms of varying degrees of complexity, and this structural and functional unit contains significant phylogenetic signal at several levels among actinopterygians (Nelson, 1973). There is a general trend toward the loss, reduction, or consolidation of bony elements, particularly of the dermal bones, across the phylogeny of fishes, although some of the most basal extant forms (e.g., Acipenseridae) may be the simplest in terms of number of elements included in the lower jaw. Meckel's cartilage, the cartilaginous precursor of all chondral elements present in the lower jaw of fishes, is the only element of the oral jaws that is consistently present throughout the diversity of Actinopterygii, and indeed, throughout the diversity of gnathostomes generally. This cartilage and its associated ossifications provide the scaffold around which the toothed dermal elements of the lower jaw develop, and they serve as the attachment site for much of the jaw musculature. As such, Meckel's cartilage is the structural foundation for feeding in gnathostomes, and the variation in its form and connections with other musculoskeletal elements can reveal much about the feeding ecology of an organism.

The family Lepisosteidae, the gars, has attracted a great deal of attention among systematic ichthyologists (Wiley, 1976; Grande, 2010) and comparative anatomists generally (Parker, 1882; De Beer, 1937; Jollie, 1984), owing to their relatively basal position among actinopterygians and their striking ''prehistoric'' appearance. Lepisosteids have a heavy scale jacket of ganoid scales, abbreviated heterocercal caudal fins, and elongate snouts with a series of toothed infraorbital bones forming the margin of the upper jaw, and a greatly elongated lower jaw (Suttkus, 1963). In his recent monograph on the osteology and systematics of Lepisosteiformes, Grande (2010:fig. 51) identified a U-shaped cartilage between the left and right lower jaws of *Lepisosteus osseus* as a portion of Meckel's cartilage, suggesting that the ''sudden allometric hyperelongation of the anterior dentaries is not matched with a proportionate elongation of Meckel's cartilage and thus, the Meckel's symphysis may have become detached from the dermal jaw and left well posterior and slightly ventral to the dentary symphysis'' (Grande, 2010:81). This element struck us as highly unusual and its identification as a portion of Meckel's cartilage to be questionable.

In this paper we present new observations on the jaw of *L. osseus*, including data from a newly collected growth series representing early life history stages through large adult skeletal specimens, and serially sectioned specimens of larvae and juveniles. For comparisons we made new observations on the structure and development of the lower jaw of other gars and representatives of several lineages of basal actinopterygians. Based on these observations, we discuss the homology and identification of this peculiar element found in lepisosteids.

MATERIALS AND METHODS

All specimens of *L. osseus* were collected from the York River system of Virginia (inclusive of the Pamunkey, Mattaponi, and York rivers) by dip net (eggs, larvae, and small juveniles) or anchored gill nets (adults). Larval and juvenile specimens were cleared and stained following protocols modified from Dingerkus and Uhler (1977). Adult specimens were dissected fresh, with some individuals prepared as dry skeletons following the methods of Bemis et al. (2004). Comparative cleared-and-stained specimens of other taxa were examined. Institutional abbreviations follow Sabaj Pérez (2013).

Specimens were examined using a Zeiss Discovery V20 stereomicroscope, and photographed with an attached AxioCam high-resolution digital camera. Images were adjusted for contrast and color balance, and backgrounds were cleaned using Adobe Photoshop CS3.

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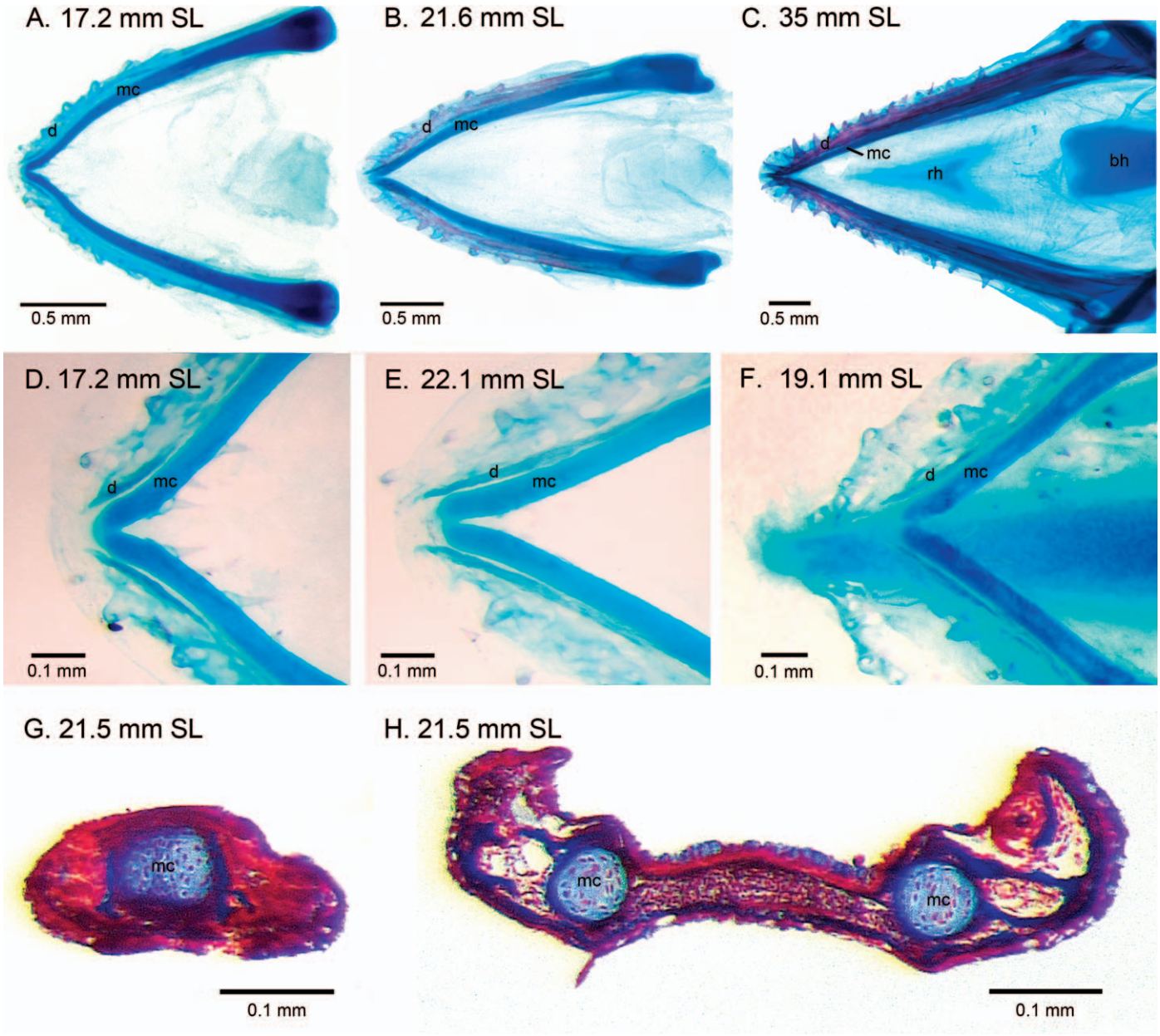

Fig. 1. Early ontogeny of the lower jaw of *Lepisosteus osseus*. (A–C) Ventral view of entire lower jaw (A, from VIMS 12968; B, from VIMS 12762; C, VIMS 13554). (D–F) Close-up views of the anterior tip of lower jaw (D, from VIMS 12958; E, from VIMS 12968; F, from VIMS 12762). (G, H) Histological cross sections through the lower jaw near the anterior tip (G) and further posteriorly (H); both from VIMS 13559. Anterior facing left in A–F. Abbreviations: bh, basihyal; d, dentary; mc, Meckel's cartilage; rh, rostrohyal.

RESULTS

***Jaws of* Lepisosteus osseus.**—In the earliest stages included in this study (17.2–22.1 mm SL), Meckel's cartilage is already well formed (Fig. 1A, D). There are three primary rami of Meckel's cartilage: a short but distinct retroarticular process that undercuts the articular surface to extend ventral to the *pars quadrata* of the palatoquadrate; an elongate coronoid process that is directed anterodorsally from the articulatory surface; and a greatly elongate mandibular ramus that extends the length of the lower jaw. No chondral bones are present at these stages. The Meckel's cartilages of the left and right jaws are continuous across the midline in most specimens examined between 17.2–35 mm SL (Fig. 1D–G). The robustness of the commissure, however, is variable, with several specimens (17.5, 19.2, 21.4 mm SL) having only a weak connection between the two sides. In some specimens the left and right sides are separate (e.g., a 17.8 mm SL specimen from VIMS 12762). The anterior portion of the left and right Meckel's cartilage are strongly recurved, as seen in ventral view, in the smallest specimens (Fig. 1A, D). This recurve begins to straighten by 21.6 mm SL as the jaws grow anteriorly (Fig. 1B, E, F), and Meckel's cartilages are more or less straight by 35 mm SL (Fig. 1C).

In larger specimens (≥85 mm SL; Fig. 2), the left and right Meckel's cartilages are broadly separated and the mandibular rami taper strongly anteriorly; there is a gap in the sizes of our sample between 35 and 85 mm SL, so it is unclear when this separation occurs. In our 85 mm SL specimen, Meckel's cartilage ends at 27.1–28.6% the length of the lower jaw (right and left sides, respectively), and much of the length of the dentaries anterior to the tips of Meckel's

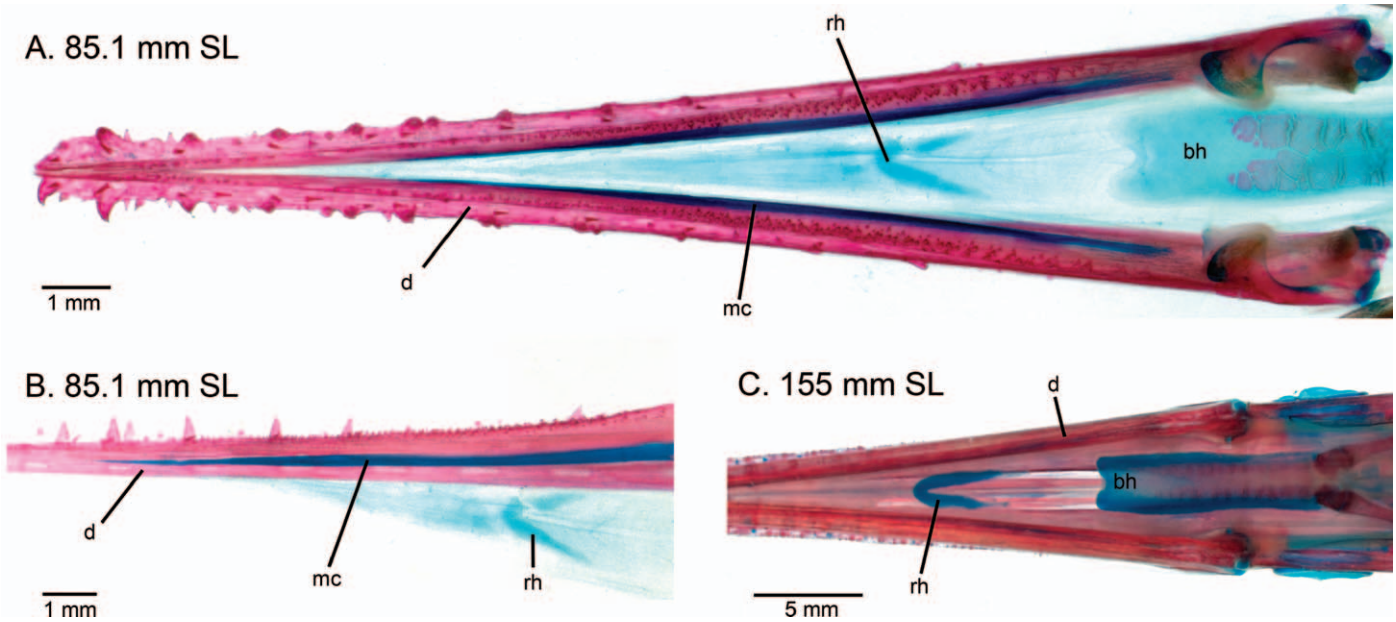

Fig. 2. Lower jaw of juvenile *Lepisosteus osseus*. (A) Dorsal view of entire lower jaw (VIMS 9078). (B) Dorsomedial view of the anterior portion of the right lower jaw (VIMS 9078). (C) Ventral region of the lower jaw showing position of the rostrohyal relative to the basihyal in a larger juvenile individual (VIMS 13551). Anterior facing left in all. Abbreviations: bh, basihyal; d, dentary; mc, Meckel's cartilage; rh, rostrohyal.

cartilages have flattened medial surfaces where the two sides meet (Fig. 2A, B). In larger specimens, Meckel's cartilages end further posteriorly along the jaw (up to 37.6% from the anterior tip in a 155 mm SL specimen), and the left and right dentaries are in contact anteriorly for about 15% of the length of the jaws.

In a group of specimens that stained particularly well for cartilage (17.6–22.1 mm SL; not illustrated), there is a diffuse, roughly V-shaped patch of darkly stained connective tissue positioned in between the left and right lower jaws anterior to the level of the hyoid arch. This is not apparent in all specimens of this size range (Fig. 1A, B) nor is it obvious in histological sections of a 21.5 mm SL specimen (Fig. 1G, H). In a 44.6 mm SL specimen, the left and right Meckel's cartilages are clearly continuous across the midline (Fig. 3A). Further posteriorly, there is a medial, poorly circumscribed structure that contains a few chondrocytes (Fig. 3B). More posteriorly, this structure divides such that there is a left and right component of this loosely consolidated element (Fig. 3C). In histological sections of a specimen approximately 200 mm SL, there is a structure that is median anteriorly (Fig. 3D) and paired posteriorly

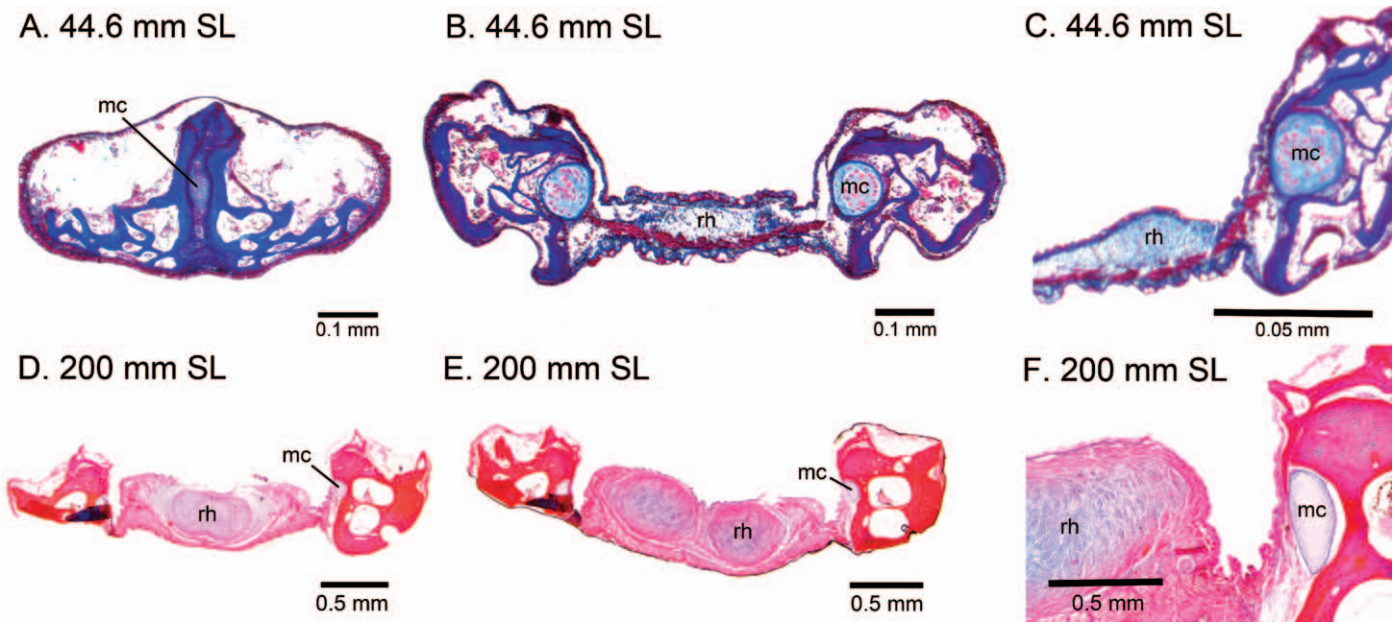

Fig. 3. Histological cross sections of the lower jaw of *Lepisosteus osseus* from an individual 44.6 mm SL (A–C; from VIMS 13559) and an individual approximately 200 mm SL (D–F; from an uncataloged specimen, University of Tuebingen). (A, D) Sections from near the anterior tip of the lower jaw. (B, E) Sections more posterior. (C, F) Close-up view of sections from posterior on the lower jaw showing different structure of the cartilage in Meckel's cartilage and the rostrohyal. Abbreviations: d, dentary; mc, Meckel's cartilage; rh, rostrohyal.

(Fig. 3E) that appears to have a distinct histology from hyaline cartilage (e.g., the cartilage of Meckel's cartilage; Fig. 3F). Rather, this structure, which we term the rostrohyal, is formed primarily by connective tissue fibers with relatively few cartilage cells, and resembles fibrohyaline-cell cartilage (Benjamin, 1990; Fig. 3F).

In our available whole-mount cleared-and-stained specimens, the rostrohyal is present and somewhat more defined in the 35 mm SL specimen examined (Fig. 1C), and is in the same relative position as the clearly defined U-shaped structure identified by Grande (2010) as a portion of Meckel's cartilage. In specimens larger than 85 mm SL (Fig. 2A–C) the rostrohyal becomes progressively more defined, and in adult specimens (≥500 mm SL; not illustrated) it is present as a thick cord-like band of cartilage about equidistant between the intermandibular joint and the anterior extent of the basihyal toothplate. At no stage is there a direct connection of this structure to Meckel's cartilage, the basihyal, or any other element of the hyoid arch. It was not possible to trace which muscles attach to the rostrohyal, but given its topographic position it likely serves as an attachment site for portions of the *musculus intermandibulus posterior* and/or the *m. interhyoideus*.

Comparative observations.—Among Lepisosteidae, we discovered this element in *L. osseus* (above), *L. platostomus*, and *Atractosteus spatula*. Specimens of other lepisosteids were not available for dissection, although it is likely that this element is present in all members of this family.

While a complete survey of the development of the lower jaw, and Meckel's cartilage specifically, across the diversity of Actinopterygii is beyond the scope of the present study, we examined available specimens representing large taxonomic groupings, and did not find any evidence of a similar structure to the rostrohyal of Lepisosteidae in any other taxa. In particular, we focused our cursory survey on the smallest specimens readily available to determine the distribution of the condition of having the left and right Meckel's cartilages continuous across the anterior midline (admittedly, many of these specimens are in advanced stages of development). In a small specimen of *Polypterus delhezi* (VIMS 13573, 32.0 mm SL), the left and right Meckel's cartilages approach each other but are not fused, and a median cartilage is not present; a similar condition is found in other smaller specimens of *Polypterus* spp. (T. Moritz, pers. comm., 2012). In Acipenseridae, closely staged developmental series for several species were available for study (e.g., *Acipenser fulvescens* and *Scaphirhynchus albus*), including pre- and post-hatching stages when Meckel's cartilage was first developed; the left and right Meckel's cartilages were never found to be continuous across the midline (Fig. 4A). Similarly, the smallest specimens of *Amia calva* prepared for this study also showed clearly separate left and right Meckel's cartilages (Fig. 4B), although this may be a function of the size of these specimens (G. Arratia, pers. comm., 2013). Within basal teleostean groups there appears to be variation in the condition of Meckel's cartilage. For example, in some taxa such as the osteoglossomorph *Hiodon* and the clupeomorph *Alosa* (Fig. 4C), the left and right Meckel's cartilages are separate in the midline (although the smallest specimen of *Hiodon* available, 32.6 mm SL, is advanced in development). In 15–17 mm specimens of *Elops* and *Hucho*, 7–9 mm NL (notochord length) specimens of *Chanos*, *Dorosoma*, *Clupea*, *Sardinops*, and *Catostomus*, and 3–5 mm NL specimens of *Danio* and *Luxilus*, there is a single continuous Meckel's cartilage that begins to separate into left and right portions just before the dentary begins to ossify (G. Arratia, pers. comm., 2013). In other taxa examined here, such as the osteoglossomorph *Arapaima* (Fig. 4D) and the esociform *Esox* (Fig. 4E), both of which have moderately elongate jaws, there is a median cartilage at the symphysis of the lower jaws (after ossification of the dentary); this is considered to be a portion of Meckel's cartilage. A more detailed comparative study of the early ontogeny of the lower jaw in fishes is beyond the scope of this study, but is warranted.

Although clearly an independent occurrence of jaw elongation from that found in lepisosteids, the Belonidae (Beloniformes) offer an interesting comparison to the condition found in lepisosteids. We examined small, post-larval specimens of *Strongylura marina*, and found a very different condition of how the left and right portions of the lower jaws come together in the anterior midline. In *Strongylura* (Fig. 4F) the left and right dentaries meet in the midline for more than 50% of the length of the lower jaw, through a robust, tight suture. The left and right Meckel's cartilages curve slightly medially posterior to this suture, but are widely separated in the midline.

DISCUSSION

Grande's (2010) identification of the U-shaped cartilage in the floor of the mouth of *L. osseus* as the anterior portion of Meckel's cartilage stemmed in part from his reading of Jollie (1984), which commented on and drew from the study of head development in *L. platostomus* by Hammarberg (1937). Hammarberg (1937:20; description of a 16 mm specimen) noted that ''*Vorn hängen die beiden Meckelschen Knorpel miteinander ununterbrochen zusammen*'' [Anteriorly, the two Meckel's cartilages are continuous]. Jollie disagreed, reporting that in his specimens he never found the left and right Meckel's cartilage to be continuous across the midline. Veit (1911) in his study on the development of the *L. osseus* reported the same phenomenon in his 11–12 mm and 14 mm specimens. We have confirmed that the left and right Meckel's cartilage is continuous across the midline in lepisosteids and in at least some teleostean fishes, although the diversity of ontogeny of the lower jaw needs to be further examined. While we agree with Hammarberg (1937) and Veit (1911) on this phenomenon, we disagree on their report of the fusion of the palatoquadrate with the Meckel's cartilage in some stages. Hammarberg, in his 12.0 and 16.0 mm specimens, and Veit, in his 11–12 mm and 14 mm specimens, describe a fusion between the palatoquadrate and Meckel's cartilage, which we regard as highly unlikely, and not seen in our specimens or reported elsewhere within lepisosteids (Jollie, 1984; Grande, 2010) or even gnathostomes, as far as we are aware.

The rostrohyal of lepisosteids appears to form relatively late in ontogeny (ca. 17.6 mm SL), well after Meckel's cartilage has developed and ossification of the dermal bones of the lower jaw has already begun. Because both the rostrohyal (as a diffuse, primarily connective tissue, fibrohyaline structure) and a Meckel's cartilage that crosses the anterior midline are present in the same individuals, we conclude that this cartilage is not homologous to the anterior portion of Meckel's cartilage as proposed by Grande (2010; i.e., it fails the test of conjunction; Patterson,

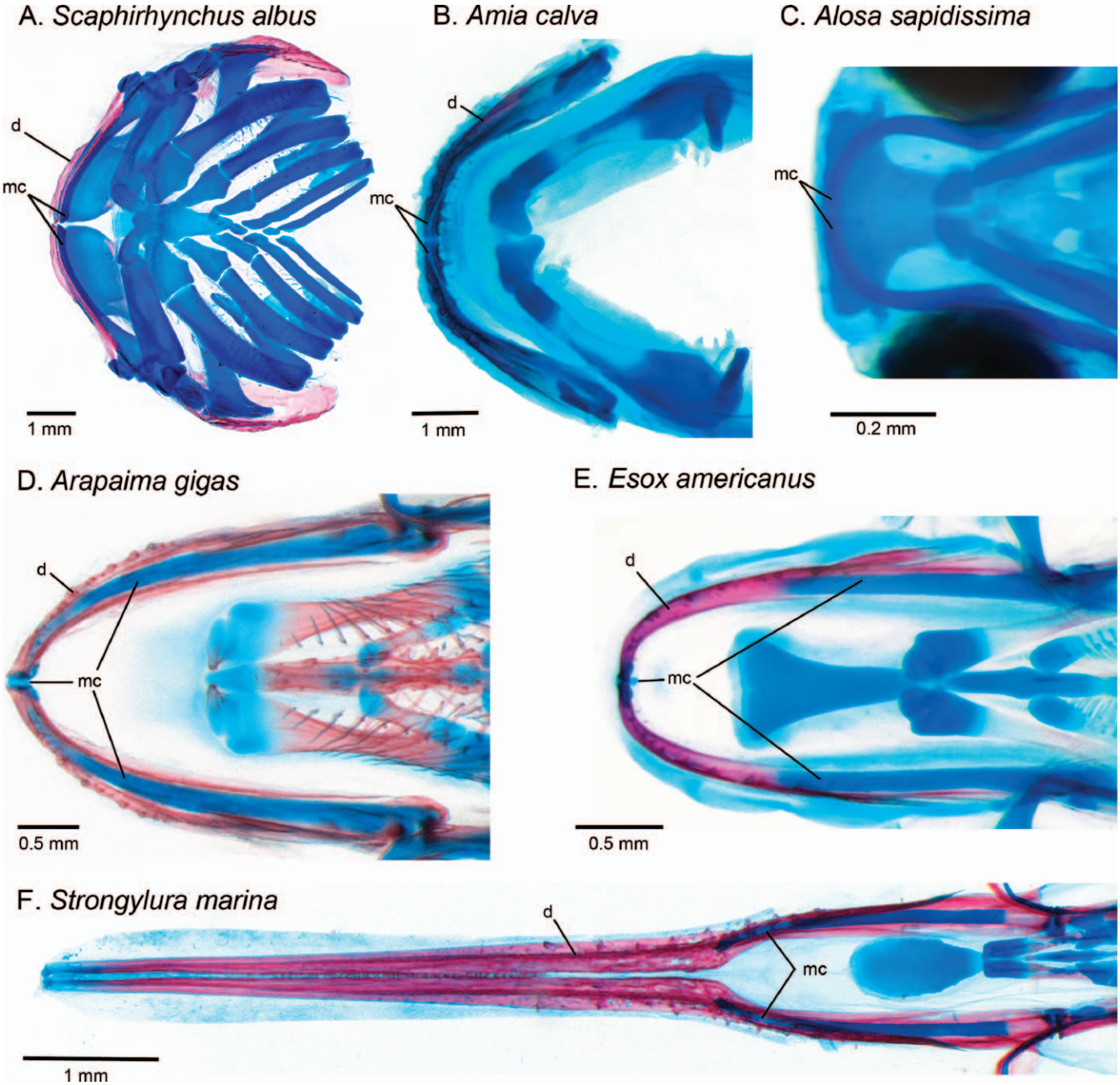

Fig. 4. Lower jaws from a diversity of actinopterygian fishes showing relationship between the left and right sections of Meckel's cartilage. (A) *Scaphirhynchus albus* (from VIMS 12193; 43.9 mm SL). (B) *Amia calva* (VIMS 13561; 26.1 mm NL). (C) *Alosa sapidissima* (VIMS 13556; 9.1 mm NL). (D) *Arapaima gigas* (INPA 22785; 27.3 mm SL). (E) *Esox americanus* (VIMS 13568; 20.6 mm SL). (F) *Strongylura marina* (VIMS 13558; 30.5 mm SL). Anterior facing left in all. Abbreviations: d, dentary; mc, Meckel's cartilage.

1982). We rather identify the U-shaped cartilage in the floor of the mouth between the two rami of the lower jaw in members of Lepisosteidae as a neomorphic structure that is likely associated with the general elongation of the lower jaw in gars (as suggested by Grande, 2010), and forming an attachment site for musculature involved with protraction of the hyoid arch. As we have found no name to describe this structure in the literature, we term it the rostrohyal as an antithesis of the urohyal, which serves as an attachment site for musculature involved with retracting the hyoid arch, and to reflect its proposed function of stabilizing the anterior musculature that protracts the hyoid arch.

MATERIAL EXAMINED

Acipenser fulvescens: VIMS 13577 (developmental series of 260 individuals, including 87 CS).

Alosa sapidissima: VIMS 13555 (2 CS); VIMS 13556 (1 CS); VIMS 13557 (1 CS).

Amia calva: VIMS 13560 (9 CS); VIMS 13561 (1 CS); VIMS 13562 (1 CS).

Arapaima gigas: INPA 22779 (5, including 2 CS); INPA 22785 (15, including 3 CS).

Atractosteus spatula: UAIC 12439.01 (1 of 2 a).

Esox americanus: VIMS 13568 (1 CS)

Hiodon alosoides: UAMZ 4041 (5 CS); VIMS 13576 (3 CS).

Lepisosteus osseus: VIMS 9078 (1 CS); VIMS 12762 (5 CS); VIMS 12968 (6 CS); VIMS 13551 (1 CS); VIMS 13554 (1 CS); VIMS 13559 (developmental series of 285 individuals, including 4 CS and 2 serially sectioned specimens); VIMS 13571 (1 ds); VIMS 13572 (1 ds); 1 uncataloged serially section specimen at University of Tuebingen.

Lepisosteus platostomus: VIMS 13414 (1 a); VIMS 13570 (1 ds).

Polypterus delhezi: VIMS 13573 (1 CS).

Scaphirhynchus albus: VIMS 12193 (113, including 21 CS).

Strongylura marinus: VIMS 13558 (1 CS).

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