

When Tradition Meets Technology: Systematic Morphology of Fishes in the Early 21st Century

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Many of the primary groups of fishes currently recognized have been established through an iterative process of anatomical study and comparison of fishes that has spanned a time period approaching 500 years. In this paper we give a brief history of the systematic morphology of fishes, focusing on some of the individuals and their works from which we derive our own inspiration. We further discuss what is possible at this point in history in the anatomical study of fishes and speculate on the future of morphology used in the systematics of fishes. Beyond the collection of facts about the anatomy of fishes, morphology remains extremely relevant in the age of molecular data for at least three broad reasons: 1) new techniques for the preparation of specimens allow new data sources to be broadly compared; 2) past morphological analyses, as well as new ideas about interrelationships of fishes (based on both morphological and molecular data) provide rich sources of hypotheses to test with new morphological investigations; and 3) the use of morphological data is not limited to understanding phylogeny and evolution of fishes, but rather is of broad utility to understanding the general biology (including phenotypic adaptation, evolution, ecology, and conservation biology) of fishes. Although in some ways morphology struggles to compete with the lure of molecular data for systematic research, we see the anatomical study of fishes entering into a new and exciting phase of its history because of recent technological and methodological innovations. With each new advance of technology and with each new generation of researcher, systematic morphology becomes a new and vibrant science.

[Morphology] is one of the most interesting departments of Natural History, and may almost be said to be its very soul. What can be more curious than that the hand of a man, formed for grasping, that of a mole for digging, the leg of the horse, the paddle of the porpoise, and the wing of the bat, should all be constructed on the same pattern, and should include similar bones, in the same relative positions?

Charles Darwin (1859 [1958:403])

HERE is something intrinsically appealing in the anatomical form of fishes that has captured the human imagination, both from a scientific perspective as well as that of a general audience. For instance, Hemingway's (1952:70) protagonist in *The Old Man and the Sea*, Santiago, reflects that fishes "are not as intelligent as we who kill them, although they are more noble and more able," suggesting a reverence and appreciation for fishes that is found in many works of art, literature, and other aspects of culture and human thought. From both popular and scientific perspectives, part of this appeal, perhaps, is the complexity that is inherent in the anatomy of fishes. For the scientist, there is a large source of descriptive and comparative data in the skeleton of fishes alone. Grande and Bemis (1998) note that there are more than 680 individual ossified bones (excluding scales, fin rays, teeth, etc.) in the skeleton of the holostean *Amia calva*. Variation in each of these elements carries with it the potential for phylogenetic information. While the skeleton of teleostean fishes is generally marked with reduction in number of elements, a conservative estimate for even taxa such as Tetraodontiformes, notoriously reductive in nature, puts this figure at over 230 bones or cartilages. Therefore, even the skeleton of the most paedomorphic or otherwise anatomically reduced taxa "provides effectively an infinite number of potential characters for analysis" (Grande and Bemis, 1998:ix). With this as a starting frame of reference, coupled with their taxonomic diversity—

over 30,000 extant species and an unparalleled (among vertebrates) fossil record—fishes generally, and actinopterygians in particular, offer an endless source of intrigue for the morphologist and evolutionary biologist.

The purpose of this paper is two-fold. First, we give a brief history of the morphology of fishes and its impact in comparative morphology, focusing on some of the individuals and their works from which we derive our inspiration. This is not an exhaustive discussion, as there are numerous historical figures that are not included whose works we greatly admire and have contributed to our own development as morphologists (e.g., Edward Phelps Allis, 1851–1947). It is our contention that no such historical review can be comprehensive, so we have rather picked and chosen among what we consider to be the highlights. Further, there are useful and important reviews that focus on various aspects of this history of morphological research on fishes from historical (e.g., Cuvier and Valenciennes, 1828; see Pietsch, 1995) and contemporary times (e.g., many papers in Pietsch and Anderson, 1997), as well as aspects of the science of morphology generally (e.g., Nyhart, 1995). However, we feel that this history provides an important backdrop for where comparative morphological study of fishes currently lies and where it can go in the future. We use our own experiences with actinopterygian fishes as the basis for this discussion, but also note that modern morphology is relevant for the systematic study of other groups of fishes as well, including chondrichthyans (e.g., Carvalho et al., 2012; Maisey, 2012; Soares and Carvalho, 2013a, 2013b; Silva and Carvalho, 2015) and sarcopterygians (e.g., Northcutt and Bemis, 1993; Forey, 1998; Arratia et al., 2001). Our second goal for this paper is to discuss what is possible at this point in history in the anatomical study of fishes, and to speculate on the future of systematic morphology of actinopterygian fishes, which, to extend from the quotation from Darwin presented as the epigraph, forms the very soul of ichthyology.

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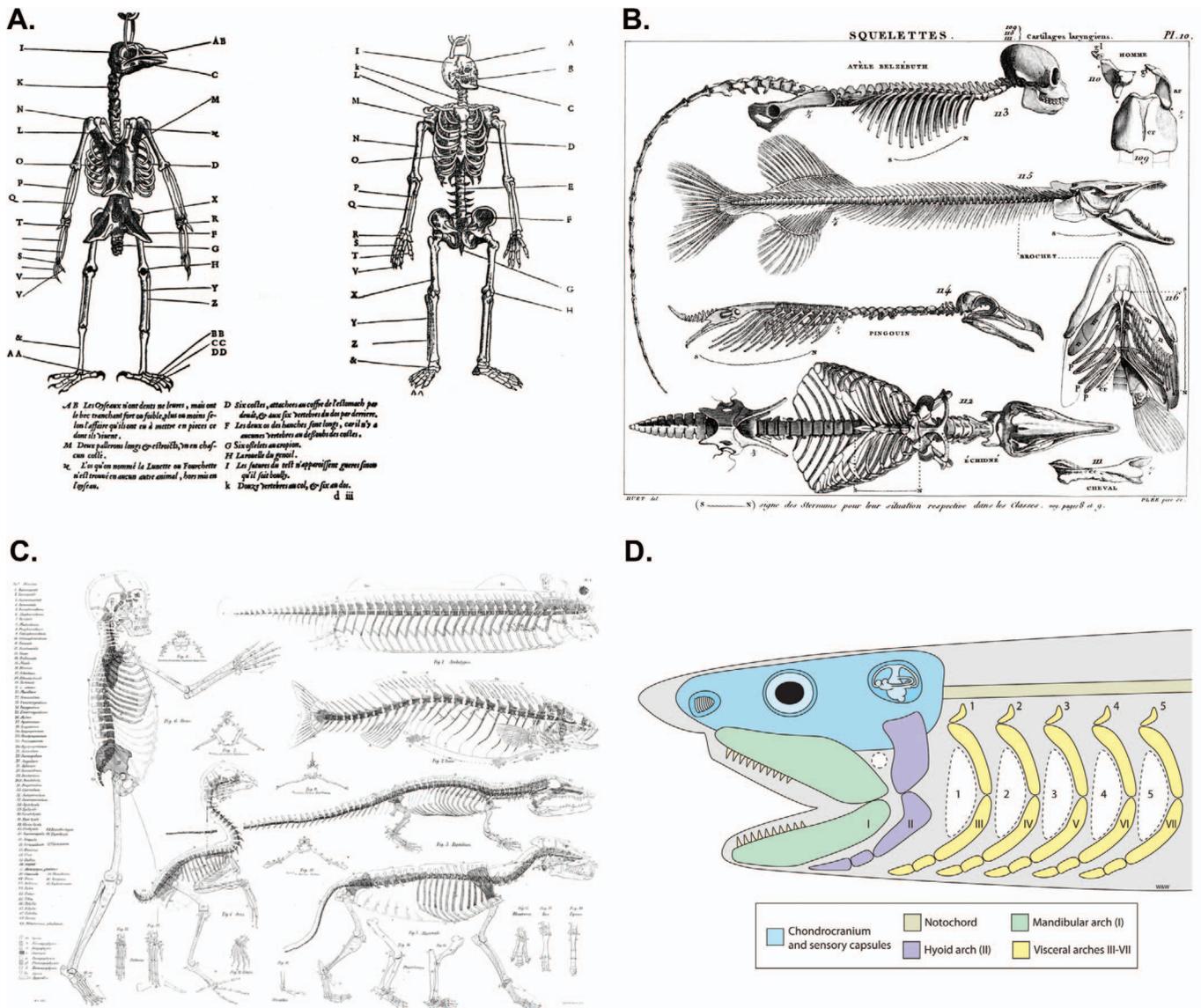


Fig. 1. Illustrations from the early history of the morphology of fishes, showing concepts of homology and archetypes. (A) Illustration from Belon (1555; modified from Grande, 2010:fig. 557) showing homology of the vertebrate skeleton. (B) Illustration from Geoffroy Saint-Hilaire (1818) showing homology among the axial skeletons of vertebrates. Image courtesy of T. W. Pietsch. (C) The archetype of the vertebrate (Owen, 1849; also used by Owen, 1848). (D) Conceptualized/simplified schematic of the vertebrate head; modified from Liem et al. (2001:fig. 7.3) by William E. Bemis.

THE MORPHOLOGICAL TRADITION

Historically, the basic framework of the classification and evolution of fishes has come from analysis of their morphology. Today, molecular data provides additional information, resolution, or in the very least additional testable hypotheses for various problematic taxa (e.g., *Stylephorus*; Miya et al., 2007), or helps to understand patterns within large groups of fishes (e.g., Alepocephali-formes; Lavoué et al., 2008). Recent broad scale phylogenetic analyses of actinopterygian fishes (e.g., Smith and Craig, 2007; Near et al., 2012; Betancur-R. et al., 2013) have added to the understanding of the relationships of fishes, improving the resolution for unwieldy and seemingly intractable groups such as Percomorpha. However, it remains that many of the primary groups of actinopterygian fishes have been established through an iterative process of anatomical study and comparison of fishes that has spanned a time period approaching 500 years.

Through the effort of early workers, such as Pierre Belon (1517–1564), some of the basic groups of fishes (and animals generally) that are still accepted and considered valid today, were first described (e.g., in Belon’s 1553 *De aquatilibus, libre duo* he identified Pleuronectiformes—his “Spiny oviparous fishes of a flat form”; Pietsch, 1995). This was accomplished by personal observation of specimens. Also from the studies of Belon, we have the development of a formalized and systematic method for the homology of elements within the skeleton (Belon, 1555; Fig. 1A). Hypotheses of homology, as the basic conceptual unit of morphology, provide the core point of discussion for comparative statements linking taxa through hypotheses of common ancestry. These hypotheses of homology are fundamental to all comparative works. Belon’s image comparing the skeleton of a bird and that of a human can be seen as the basis for all that follows in comparative anatomy. Similar comparative figures to this adorn most published comparative studies since, and show, through visual connections, the hypotheses of homology

that are being advanced. Simply, the images of specimens are at once both the raw data being presented for documentation and consideration, as well as the interpretation of “meaning” of the data. In a similar way, Geoffroy Saint-Hilaire (1772–1844) presented his views of the homology of the axial skeleton of vertebrates in a figure published in his *Anatomical Philosophy* (1818; Fig. 1B). By removing the appendicular skeletons from the specimens and arranging the skeletons in similar positions—unnatural in some cases (e.g., the tetrapods)—the similarities between the different taxa are immediately conveyed. Sir Richard Owen (1804–1892) formalized the “similarity” that struck Belon and Geoffroy Saint-Hilaire, and introduced to biology the word “homology”: “The same organ in different animals under every variety of form and function” (Owen, 1843:379). These similarities are the data that support homology statements. Without a doubt, the concept of homology is not that simple as evidenced by the volume of literature debating its meaning and nuance (see papers in Hall, 1994; also Remane, 1952, 1955; Rieppel and Kearney, 2002). However, homology is the basis for all comparative works, historical and modern.

Owen also advanced the concept of the archetype (Owen, 1848; see Panchen, 1994), which provides a conceptual organization of the vertebrate bauplan (Fig. 1C). Although this form of idealistic morphology can mask and overly atomize particular structures, we do see some use in it as an organizing construct for approaching the morphology of fishes, particularly in studies of development. Further, the archetype provides a simplification of the body plan that allows for effective conceptualization of vertebrate anatomy, and a modern equivalent to Owen’s depiction of the archetype is still used for its didactic value (e.g., Liem et al., 2001:fig. 7-3; Fig. 1D). The concept of an archetype was, in part, built upon generalities seen in nature and derived from embryology. Before Owen’s introduction of the term homology and the concept of the archetype, Karl Ernst von Baer (1792–1876) emphasized the importance of embryology (i.e., ontogeny). Through his broad zoological knowledge, he became aware of the importance of character transformation during ontogeny and described his “Four Laws of Embryology” (Baer, 1828) stating that 1) general characters develop before the more specific, 2) general relationships between elements develop before more specific relationships, 3) the anatomy of an embryo of a given taxon is not the same as that of other forms but rather separates itself from them, and 4) the embryo of a derived taxon never resembles the adult of an ancestral taxon, but rather its embryo. Although neither Owen nor Baer were approaching the anatomy of fishes (and vertebrates generally) from an evolutionary perspective, they provided these great insights in comparative morphology. From these laws, Ernst H. P. A. Haeckel (1834–1919), who was strongly influenced by Darwinian evolution, derived his Biogenetic Law (Haeckel, 1866). This has famously and succinctly been restated as “ontogeny recapitulates phylogeny.” Haeckel artistically illustrated his works to show the repeating patterns he saw in ontogeny, combining the beauty and scientific content of comparative anatomy (Fig. 2). These guiding principles inspired the works of those that followed, including such as Gegenbaur (1872, 1888), Fürbringer (1897), Goodrich (1930), de Beer (1937), and others. Notably, for instance, Charles Tate Regan (1878–1943), whose inroads into systematic ichthyology and the classification of fishes were

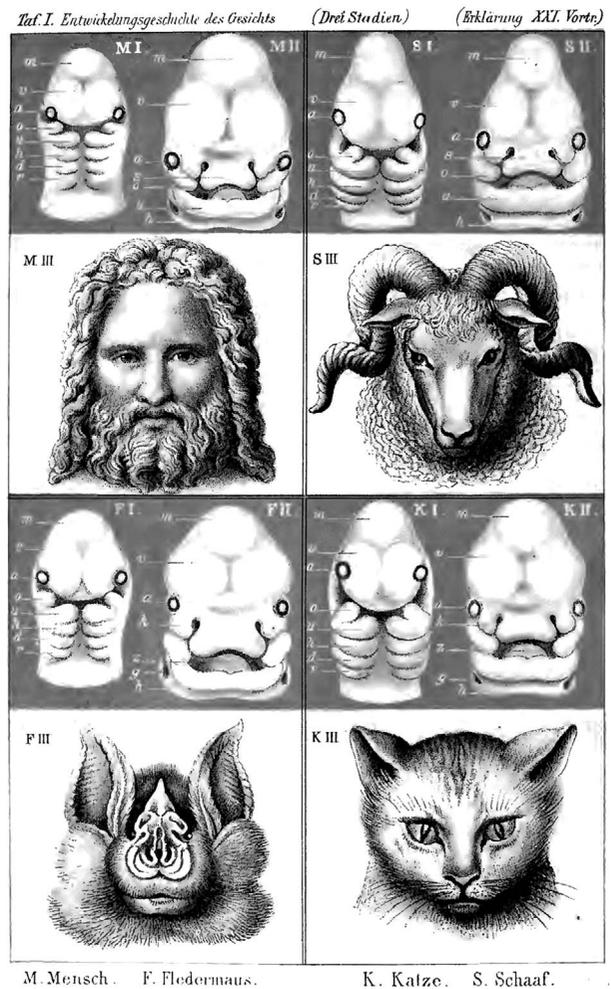


Fig. 2. Illustration from Haeckel’s *Antropogenie oder Entwicklungsgeschichte des Menschen* (1877). The image shows early ontogenetic stages of four mammals (human, bat, cat, and sheep). Although not directly associated with the biogenetic law, the illustration clearly yet artistically shows Haeckel’s hypothesis of repeating ontogenetic stages across phylogeny.

based primarily in comparative osteology, understood and incorporated larval characters into his studies (e.g., Regan, 1916). However, until the proceedings of the ASIH Ahlstrom symposium were published (Moser et al., 1984), the inclusion of larval fishes in morphological systematic studies to test homology and common ancestry remained scarce.

This interplay between ontogeny and phylogeny offers compelling support for evolution. It is therefore ironic that this support was greatly extended by a steadfast special creationist, Jean Louis Rodolphe Agassiz (1801–1878). Agassiz (1857; Fig. 3) conceptualized his so-called “three-fold parallelism,” in which he saw a reciprocal relationship between morphology, paleontology, and ontogeny. As pointed out by Grande and Bemis (1998), Agassiz’s three-fold parallelism (=“three-fold congruence” in modern terminology) actually included a fourth distinct line of evidence, biogeography: “Even now, some curious coincidences may be pointed out which go far to show that the geographical distribution of animals [=biogeography] stands in direct relation to their standing in their respective classes [=phylogeny], and to the order of their succession in past geological ages [=paleontology, or stratigraphy], and more indirectly, also to their embryonic growth

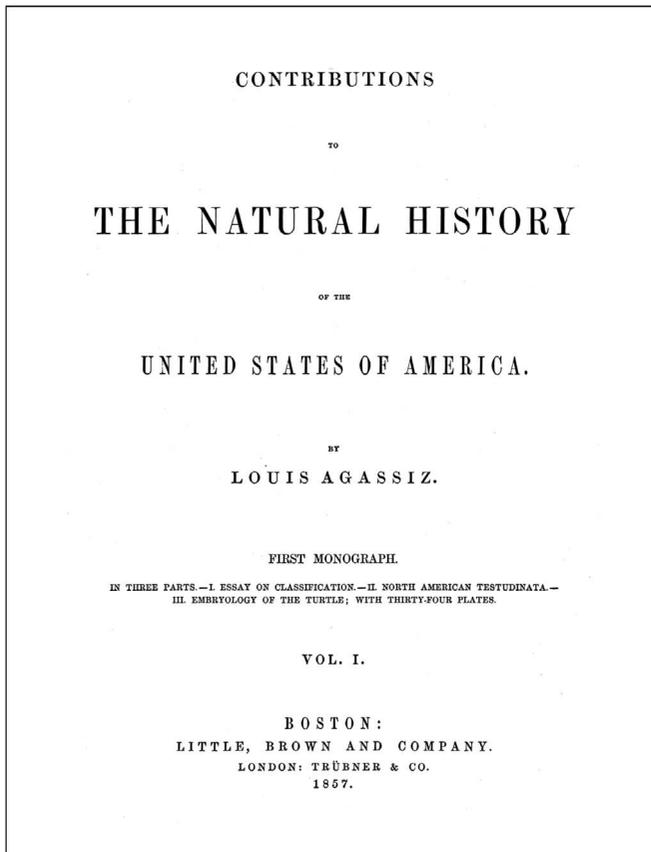


Fig. 3. Title page of Agassiz (1857) in which he outlined his views on the congruence between comparative anatomy, embryology, stratigraphy, and biogeography.

[=ontogeny]” (Agassiz, 1857:120–121). Collectively, these four avenues of study offer explanation for the order observed in nature, and although Agassiz would deny it, evolution is the uniting principle among them (Grande and Bemis, 1998). Moreover, there is now a fifth source of information available—molecular data—that must be considered (Grande and Bemis, 1998), yet comparative anatomy remains a linking discipline for all these areas of evolutionary biology.

We would be remiss if we did not include discussion of the role that morphology plays in understanding the geological history of fishes, namely through the fossil record. More so than any other group of vertebrates, the preservation bias that fishes have because of their habitat (i.e., aquatic habitats are more likely to be fossiliferous than terrestrial habitats) has produced a long and important fossil record of fishes. Their study, however, and especially for actinopterygians, is disproportionately low relative to their abundance (Grande and Bemis, 1998). The so-called Stockholm School of paleoichthyology, centered around Erik Stensiö (1891–1984), epitomizes the importance of the morphology of fishes not only for understanding the morphology of fossil forms in and of themselves but also for putting the fossil taxa into a broader context (e.g., see Stensiö, 1963; Fig. 4). Stensiö, and his students and fellow researchers such as Erik Jarvik (1907–1998) and others approached the study of fossil fishes from the perspective of an anatomist, using the works of Allis (e.g., 1897, 1920, 1921) and others on extant fishes such as *Polypterus*, *Polyodon*, and *Amia* as guidance (Schultze, 2009). It is from this anatomical perspective, including a strong

embryological and developmental perspective (e.g., Stensiö, 1947), combined with painstaking preparation methods that Stensiö could extract such a high level of information from his fossils (Fig. 4). This Stockholm School had broad international influence during its heyday, and its influence persists through to the present day (Schultze, 2009).

Ultimately, morphology of fishes has served as the basis of much of what is known about the evolutionary relationships among fishes, and these hypotheses of relationships are then translated into classifications. From the earliest stages of fish classifications, beginning with C. Linnaeus and P. Artedi moving through the reigns of G. Cuvier, L. Agassiz, J. Müller, E. D. Cope, C. T. Regan, and others in late 19th Century and early 20th Century, when, of course, there was no alternative. Greenwood et al. (1966) in their synthesis of teleostean classification summarized some of this history. Some of the groups identified by Greenwood et al. (1966) have withstood the test of time, such as the Osteoglossomorpha and the Ostariophysi, specifically from a cladistic approach to analysis, while others have changed (e.g., the Myctophoidae). More importantly these authors offered discrete morphological characters as evidence for the groups they were proposing that served as a morphological fundament for future generations to come. This process is inherent in the progress of science. Wiley and Johnson (2010) continue this process, now with an expanded literature including molecular analyses. Even with modern hypotheses from “modern” data sets, these authors never lose sight of the morphology of fishes and offer morphological characters supporting their classification.

CONTINUING THE TRADITION

Broadly based integrated studies have as their basis descriptive and comparative morphological data. Few, if any, descriptive studies, however, are pure descriptions (i.e., “morphology for morphology’s sake”) and are often related to a comparative (i.e., systematic or evolutionary) study. A basic tenet of systematic morphology is that there are more data to be gathered from every specimen. With each new specimen collected, prepared, examined, and analyzed, new data become available for inclusion in the total sum of knowledge of fishes. Further, different methods and different perspectives may lead to different interpretations of morphology. These different interpretations are, in turn, at the heart of the morphology of fishes.

The caudal skeleton of actinopterygian fishes, and in particular Teleostei, is the source of many phylogenetically informative characters (e.g., Patterson and Rosen, 1977; Arratia, 1991; Schultze and Arratia, 2013; Arratia, 2015, in this volume) because it offers such broad diversity across fishes (Fujita, 1990) and is open to so many different interpretations (Fig. 5). As such, it provides a good example of how the systematic morphology of fishes can reach different conclusions regarding the “same” elements. By labeling in a figure or referencing an element in text as a hypural, for instance, there is a fundamental, implied hypothesis that a specific element is, in an evolutionary sense, the “same” hypural of another taxon. In basal teleosts there are typically two hypurals ventral to the diastema (h1 and h2) and a variable number of hypurals dorsal to the diastema (Fig. 5B), if one exists (e.g., see Fig. 5C). Frequently, and particularly in more derived taxa, there is only a single hypural ventral to the diastema (e.g., Fig. 5D–F), and this element loses its relationship to supporting centra or

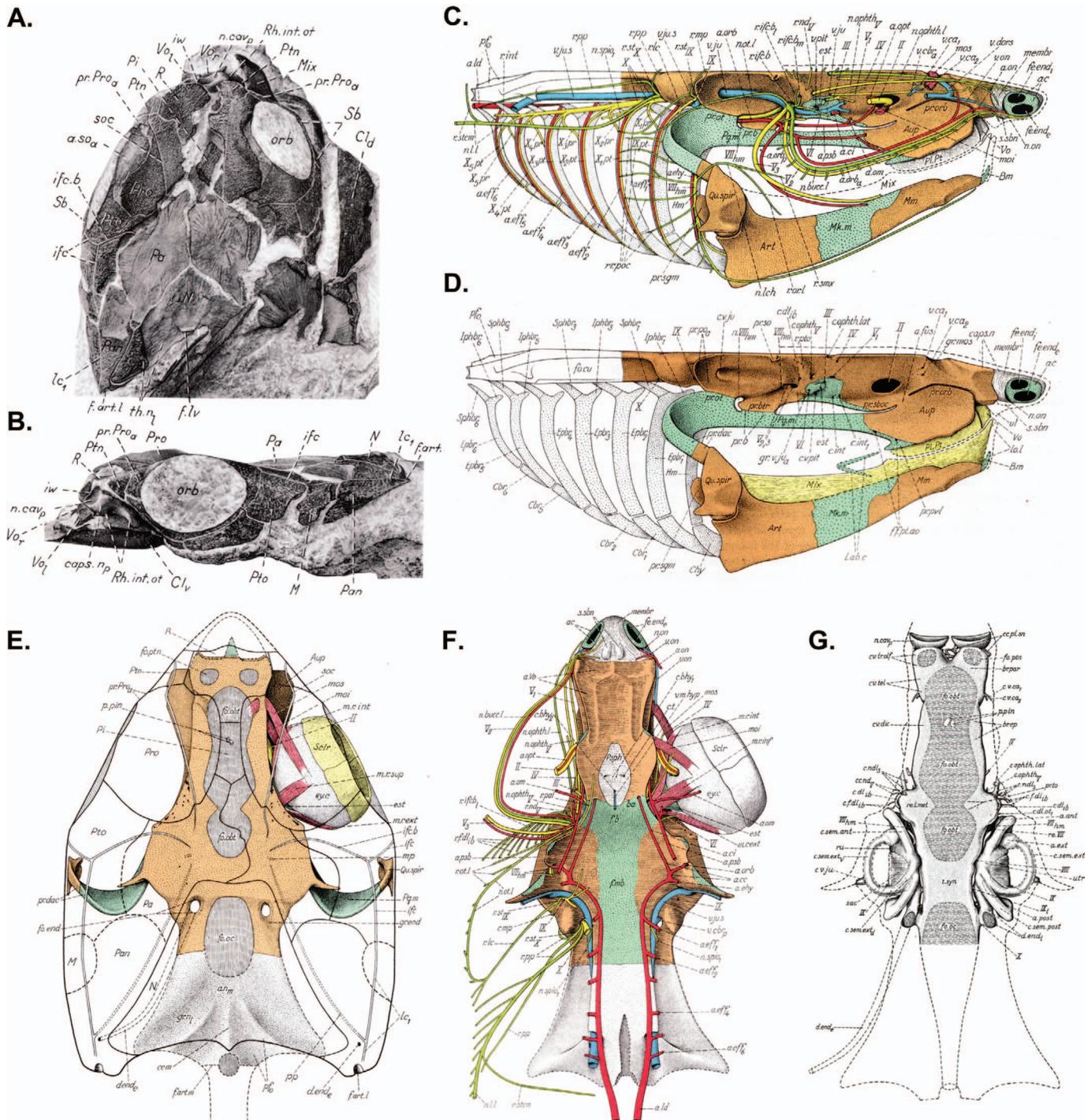


Fig. 4. Illustrations showing a specimen (A, B) and reconstructions (C–G) of the head of the coccosteomorph arthrodire †*Tapinosteus heintzi* Stensiö, 1959. From Stensiö (1963:pl. 7 figs. 2, 3, and figs. 70, 76, 47, 71A, 62A, respectively).

centrum, as these are often reduced or consolidated. This raises the question of what that single element represents when compared to the two ventral hypural condition found in more basal taxa. Is it an enlarged h1? Is it a fusion between h1 and h2? Does the fact that there is one element ventral to the diastema in one taxon and two in the other imply homology between the two conditions (i.e., the separate elements h1 and h2 in one taxon are equivalent to a combined h1+2)? Similarly, the parhypural may be also incorporated in this compound element; inclusion of the parhypural can be identified by the presence of a canal for the caudal vessels (Fig. 5F). Comparative ontogeny offers

one potential determining factor, although often there is only a single cartilage found in this ventral position at all points in ontogeny (e.g., Hilton and Britz, 2010; Konstantinidis and Johnson, 2012; Wiley et al., 2015, in this volume), resulting in a so-called “phylogenetic fusion” (see discussion of this phenomenon by Hilton, 2002:129–132). Topological relationships of the elements relative to other structures is another potential source of homology statements.

Highlighting the different possibilities in identification of hypurals serves as an example of the continued need for careful morphological analyses to explore such issues and the origin of different interpretations of anatomical

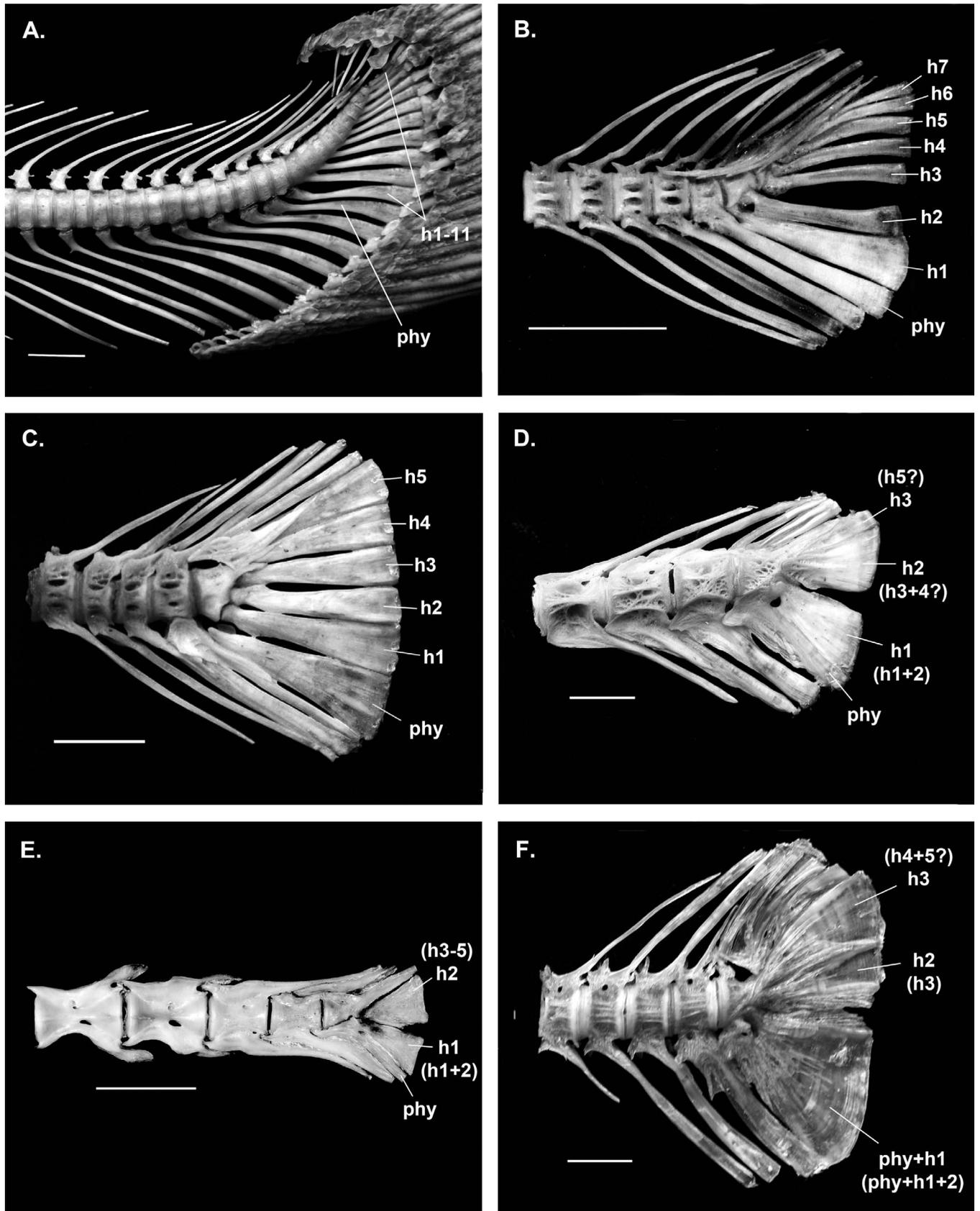


Fig. 5. Caudal skeletons of actinopterygian fishes, showing different numbering schemes and inferences of homology. (A) *Amia calva*, VIMS 19093. (B) *Hiodon alosoides*, UMA F10592. (C) *Channa argus*, VIMS 19091. (D) *Astroscopus guttatus*, VIMS 19094. (E) *Caranx crysos*, UMA F11596. (F) *Zaprora silenus*, VIMS 19093. Scale bars equal 10 mm. Anatomical abbreviations: h, hypural; phy, parhypural. Institutional abbreviations: UMA, University of Massachusetts Amherst; VIMS, Nunnally Ichthyology Collection, Virginia Institute of Marine Science.

structure. Recent studies of euteleostean fishes (e.g., Doosey and Wiley, 2015; Wiley et al., 2015, in this volume) further document the development of various skeletal elements (e.g., epurals) and discuss alternate ways of naming skeletal elements across phylogenetically disparate taxa. The presence of so-called “condensation fields” as precursors for cartilaginous structures in early development known from studies of the model organism *Danio* also offers new avenues for homology statements for the evolutionary anatomy of fishes (Crotwell and Mabee, 2007; Wiley et al., 2015, in this volume). The use of gene expression patterns to trace the homology of structures during development potentially offers a very powerful tool to add to the robustness of these hypotheses, although it is not yet commonly employed. Part of the problem derives from a general lack of communication between researchers working in broadly different fields, although there are a number of labs that are bridging historical divides between comparative and systematic morphology and developmental genetics. Online community resources, such as Phenoscope (Mabee et al., 2012), the Teleost Anatomy Ontology (Dahdul et al., 2010, 2012), and the Zebrafish Information Network (Bradford et al., 2011), offer potential mechanisms to bridge systematic morphology with research on the genetic underpinnings of morphology. Cass et al. (2013) provide a good example of the power of this approach in a study showing that the zebrafish swimbladder and the tetrapod lung share the same unique gene expression during development (although the onset time of some late genes is slightly altered). These authors conclude that the similarity of gene expression pattern is due to common ancestry. This only reiterates our thesis—there is still much to discover about the fundamental morphological structure of actinopterygian fishes generally.

PERSPECTIVES ON THE FUTURE OF MORPHOLOGICAL DATA COLLECTION FOR THE SYSTEMATICS OF FISHES

. . . get the anatomy wrong and the rest is meaningless.

Colin Patterson (attributed to him by Stiassny et al., 1996:xii)

From our perspective, the advancement of the field of morphology of fishes, and morphology generally, lies along three fronts: 1) development of new approaches and technology to understand anatomical structure more fully; 2) new hypotheses that can be critically examined with morphology, as well as revisiting hypotheses proposed in the literature; and 3) there are new reasons for studying the anatomy of fishes in descriptive, systematic, and comparative frameworks. Advancements in the field of morphology have been tied to advancements in the preparation of specimens. Alizarin staining for visualization of bone has been used since the early 1900s, and although by today’s standards these early attempts at clearing and staining were rather primitive, their results added substantially to the understanding, for instance, of the development and structure of the caudal skeleton of teleostean fishes (Hollister, 1936). With the addition of trypsin clearing (Taylor, 1967) and alcian staining for cartilage (e.g., Dingerkus and Uhler, 1977; Potthoff, 1984; Taylor and Van Dyke, 1985), new avenues, including observations on even earlier developmental stages (e.g., Walker and Kimmel, 2007), could be included in descriptive and comparative studies (Fig. 6D). Although precise data

documenting the effect that clearing and staining as a method had on the field of morphology of fishes (e.g., number of papers employing these techniques following the publication of the protocols) would be difficult if not impossible to generate (because of such a scattered literature), it is generally held that these methodological improvements in the morphology of fishes have been the basis of an increase in the number of specimen-based studies and the ways researchers have used specimens (G. D. Johnson, pers. comm.). Indeed, there are many research programs that produce exquisite morphology through the use of clearing and staining, combined with modern microscopes with different optical capabilities, innovative cameras, new imaging software capabilities (e.g., Z-stacking, which produce microscopic images with increased depth of field) and new illustration techniques (e.g., recent examples include Arratia and Bagarinao, 2010; Hilton et al., 2011; Britz and Johnson, 2012; Mattox et al., 2014; Carvalho and Vari, 2015; Kubicek and Conway, 2015; Johnson and Schnell, 2015, in this volume; Wiley et al., 2015, in this volume). Numerous modifications of the clearing-and-double-staining protocols exist adapted to the specific needs, e.g., the use of ethanol solutions of alizarin red instead of a KOH-alizarin solution dye for staining bone in fishes in order to preserve skeletal muscles (Springer and Johnson, 2000; Datovo and Bockmann, 2010; Datovo et al., 2014). Complementary methodological developments including the whole-mount staining of nerves (Freihofer, 1966; Filipinski and Wilson, 1984; Song and Parenti, 1995) offer extensions to clearing and staining, and insight into homology and the systematic morphology of fishes (Parenti and Song, 1996; Nakae and Sasaki, 2004; Kenaley, 2010; Schnell et al., 2010). Similar “revolutions” are seen also in paleoichthyology, with the development of acid preparations of Toombs and Rixon (1959), as perfected by Grande and Bemis (1998) and Grande (2010); unfortunately, this method of preparation is applicable for relatively few fossil taxa, as clearing and staining is practical for fishes up to a certain size. Although large specimens can be cleared and stained and important morphological data can be derived uniquely from such specimens, this process can be time intensive (e.g., two or more years for 25 cm specimens of *Elops* sp. and 50 cm specimens of *Chanos* sp.; G. Arratia, pers. comm., 2015). Therefore, for the study of numerous large specimens, radiography is much more convenient.

X-ray computed tomography (i.e., CT scanning) was first used in the morphology of fishes on a specimen of *Latimeria chalumnae* (Schultze and Cloutier, 1991). Ten years later, the use of CT scanning for studying the morphology of fishes was further refined and used in the study of a fossil chondrichthyan (Maisey, 2001a) and the inner ear morphology of elasmobranchs (Maisey, 2001b). Since that time, advances in technology and computing power let CT scanning and the resulting three-dimensional digital renderings of hard structures (i.e., bones; Fig. 7) to become a widely used tool in the systematic morphology of fishes (and vertebrates generally). It since has been successfully applied to a number of fossil and living fishes representing a broad taxonomic breadth, including stem gnathostomes (Giles et al., 2015), elasmobranchs (Maisey, 2001a, 2001b, 2004; Pradel et al., 2009; Mollen et al., 2012; Moyer et al., 2015), basal sarcopterygians (Snitting, 2008; Boisvert, 2009), polypterids (Claeson et al., 2007; Claeson and Hagedorn, 2008), catostomids (Fink and Humphries, 2010), gymnotiforms (Carvalho and Albert, 2011; Maxime and Albert, 2014),

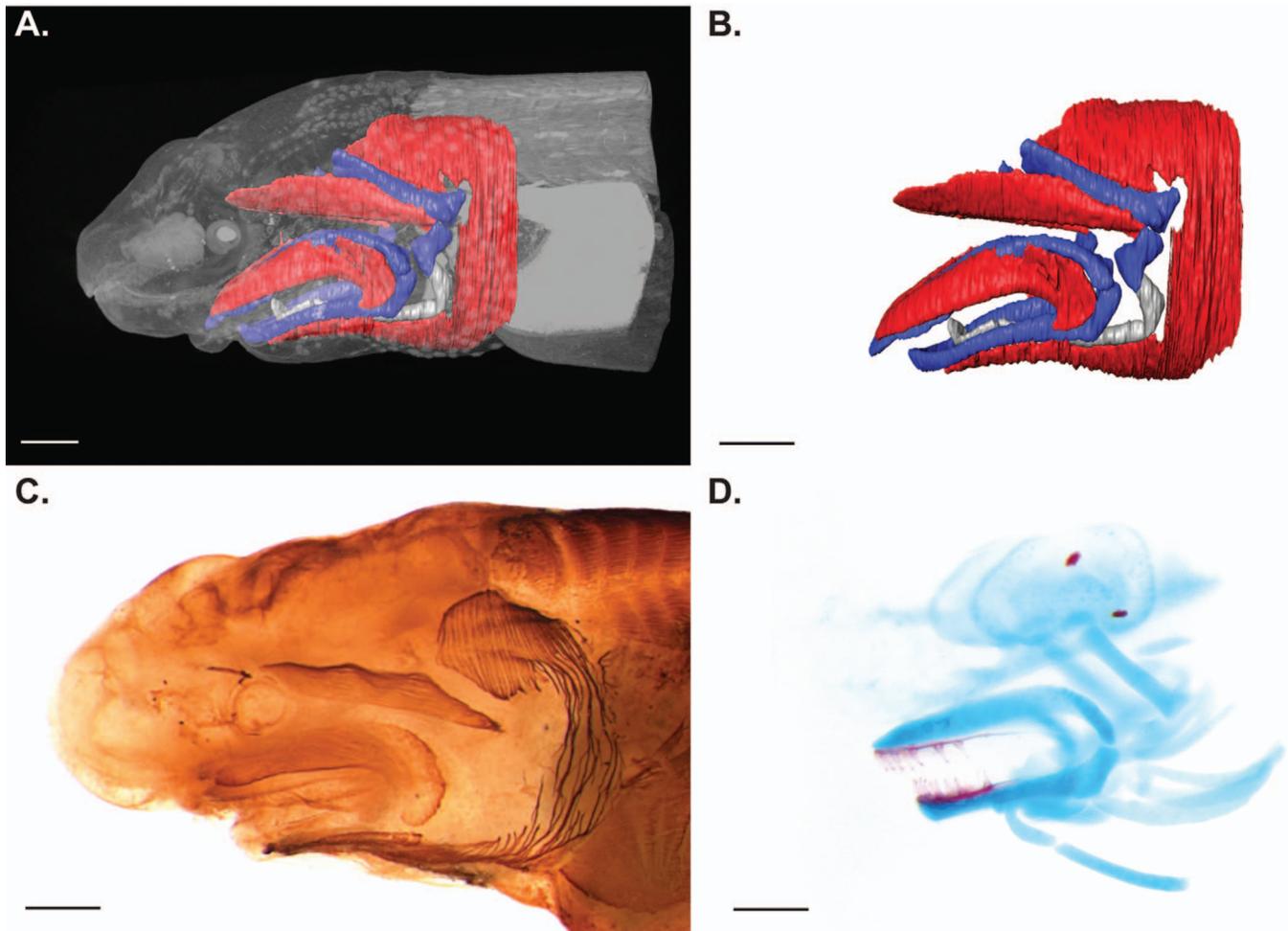


Fig. 6. Different morphological techniques used in early ontogenetic stages of the paddlefish, *Polyodon spatula*. (A, B) Reconstructions of a micro-CT scan. (A) Reconstruction of musculature (red) and skeletal structures (blue and gray) with an overlay of the actual CT scan. (B) Reconstruction as a standalone model. (C) Image of the cranial musculature stained with an antibody specific for muscle tissue. (D) Classical clearing-and-staining method for skeletal structures (bone stained red, cartilage stained blue). Scale bars equal 0.5 mm.

siluriforms (Rodiles-Hernández et al., 2005; Claeson et al., 2008; Lundberg et al., 2012, 2014; Lundberg and Luckenbill, 2015, in this volume), lophiiforms (Pietsch et al., 2009; Chanet et al., 2012), fundulids (Parenti and Hartel, 2011), kurtids (Carpenter et al., 2004), anarhichadids (Bemis and Bemis, 2015, in this volume), and molids (Chanet et al., 2012). CT scanning also has been applied successfully to questions of functional morphology and other aspects of the morphology of fishes. For instance, this technique has been the basis of biomechanical studies of jaws of durophagous elasmobranchs (Summers et al., 2004), the functional properties of cranial sutures in *Polypterus* (Markey et al., 2006), and the feeding mechanics of seahorses (Van Wassenbergh et al., 2013). CT scanning has in some ways replaced the more time consuming histology, although the latter is unparalleled regarding its resolution. As technology has improved, CT scanning has extended to smaller and smaller individuals, such that high-resolution x-ray CT scanning (HRXCT) is becoming the state of the art. For example, Lundberg et al. (2014) used HRXCT to examine the enigmatic miniature catfish *Kryptoglanis shajii*. This study produced remarkable images of a 65 mm SL individual. These fishes are rare, and this methodology allows for non-destructive sampling of morphological data. A study published nearly simultaneously (Britz et al., 2014) that

was based on a single 60 mm SL cleared-and-stained individual of the same species produced anatomical similar results and conclusions—the morphology of this species was highly peculiar and in many ways unlike other catfishes. This example provides a testament to the power of morphology—-independent studies coming to similar conclusions using different methodology and technology.

We see parallels to the so-called “clearing and staining revolution” (G. D. Johnson, pers. comm.) in recent developments of new approaches to the morphology of fishes, such as whole mount immunohistochemistry for tracing the early development of muscles (Fig. 6C). This technique allows the incorporation of broader taxonomic sampling for comparative and ontogenetic myology than was possible until recently. Immunohistochemistry, such as antibody staining of developmental series, has the potential to resolve long standing questions of homology, as shown in Konstantinidis and Harris (2011) on the homology of the different portions of the adductor mandibulae in Tetraodontiformes. Tetraodontiformes are known for their complex jaw musculature particularly in the case of the adductor mandibulae (Fig. 8). Some monacanthids have up to eight and some pufferfishes up to six partitions. In comparison, generalized percomorphs have only four partitions of the adductor mandibulae. If ontogenetic material and/or a sufficient technique are not

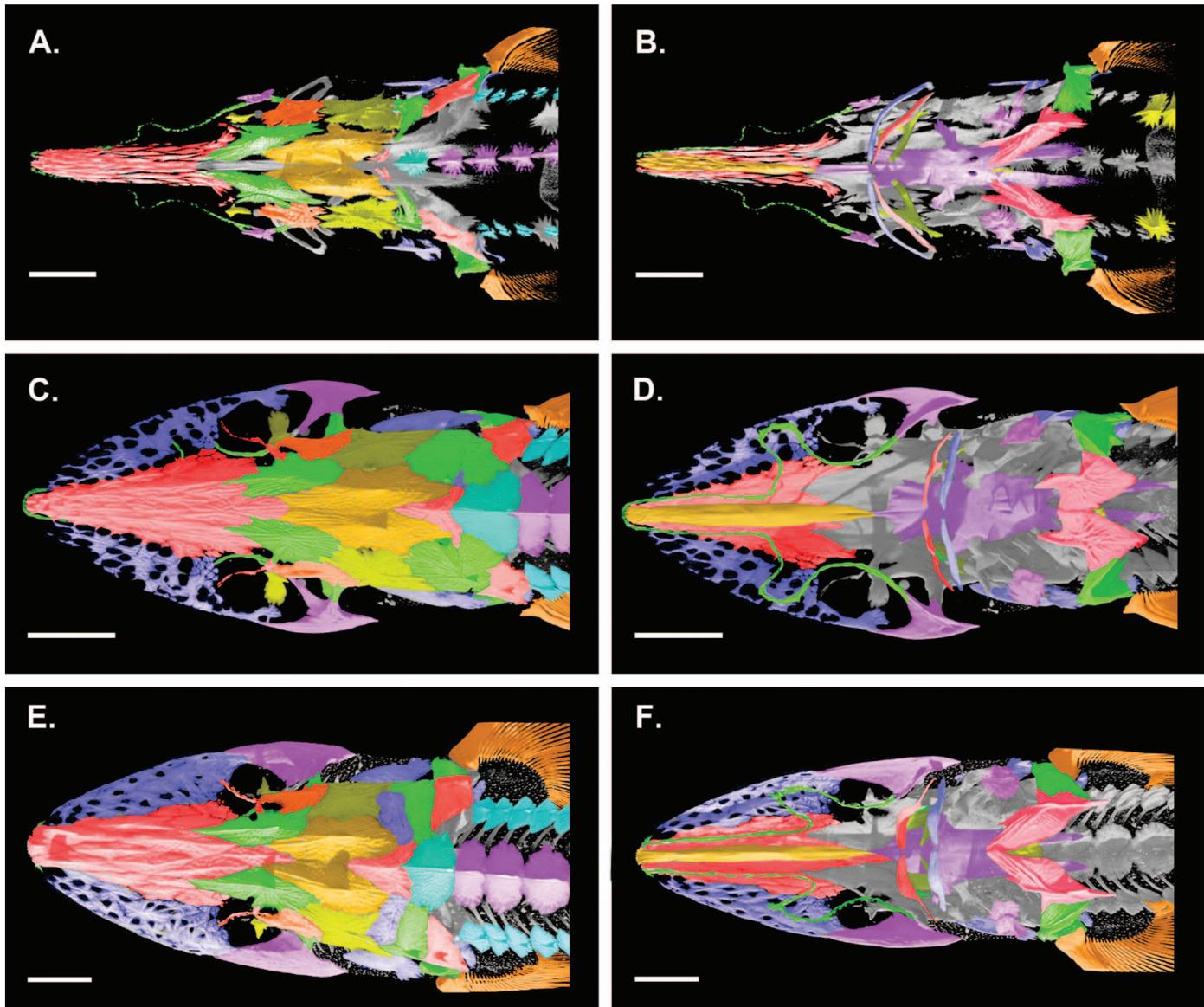


Fig. 7. Computed tomography images of sturgeons. (A) Dorsal and (B) ventral views of the skull of *Huso huso* (MCZ 54269). (C) Dorsal and (D) ventral views of the skull of *Scaphirhynchus platyrhynchus* (FMNH 45024). (E) Dorsal and (F) ventral views of the skull of *Pseudoscaphirhynchus kaufmanni* (UAIC 13265.01). Institutional abbreviations: FMNH, Field Museum of Natural History; MCZ, Museum of Comparative Zoology, Harvard University; UAIC, University of Alabama Ichthyology Collection. Scale bars equal 1 cm.

available, other criteria provide the only evidence of homology. However, Konstantinidis and Harris (2011) were able to demonstrate that the traditional indicators for homology, such as the origin and insertion of muscle partitions as well as function, do not reflect their developmental origin and therefore their homology.

No preparation method should be used exclusively and in isolation, and between this method and other recent advances in visualization of soft tissues, such as confocal microscopy of labeled muscles (Konstantinidis and Harris, 2011; Fig. 8), micro-CT scanning (Fig. 6A, B; Metscher, 2009a, 2009b; Konstantinidis et al., 2015, in this volume), magnetic resonance imaging or MRI (e.g., Perry et al., 2007; Runcie et al., 2009; Chakrabarty et al., 2011; see Berquist et al., 2012 and The Digital Fish Library, 2015), and others, we predict a similar advance in the myology of fishes as that which took place in the late 1970s and early 1980s when clearing and staining became more commonly applied. In 30 to 40 years, it may be as common and “easy” to acquire

myological data as it is now to acquire osteological data. This stands in contrast to the difficult and time consuming, yet elegant, dissection-based collection of myological data (e.g., Springer and Johnson, 2004; Datovo et al., 2014). We predict, therefore, that in the future there will be an increase in new muscular (and other soft-tissue) characters in phylogenetic data sets. Advances in preparation of specimens are being matched by advances in imaging techniques (Lauridsen et al., 2011). Given the large size of the data files generated from all of these modern imaging methods, accessible repositories such as MorphoBank (O’Leary and Kaufmann, 2011; MorphoBank, <http://www.morphobank.org/index.php/Home/Index>, Accessed: 1 June 2015) and Digimorph (<http://digimorph.org/>, Accessed: 1 June 2015) are becoming increasingly important.

Given such advancements in data acquisition, it should not be surprising that there has been a similar advancement of novel hypotheses and reexamination of previously proposed hypotheses regarding the systematic relationships of

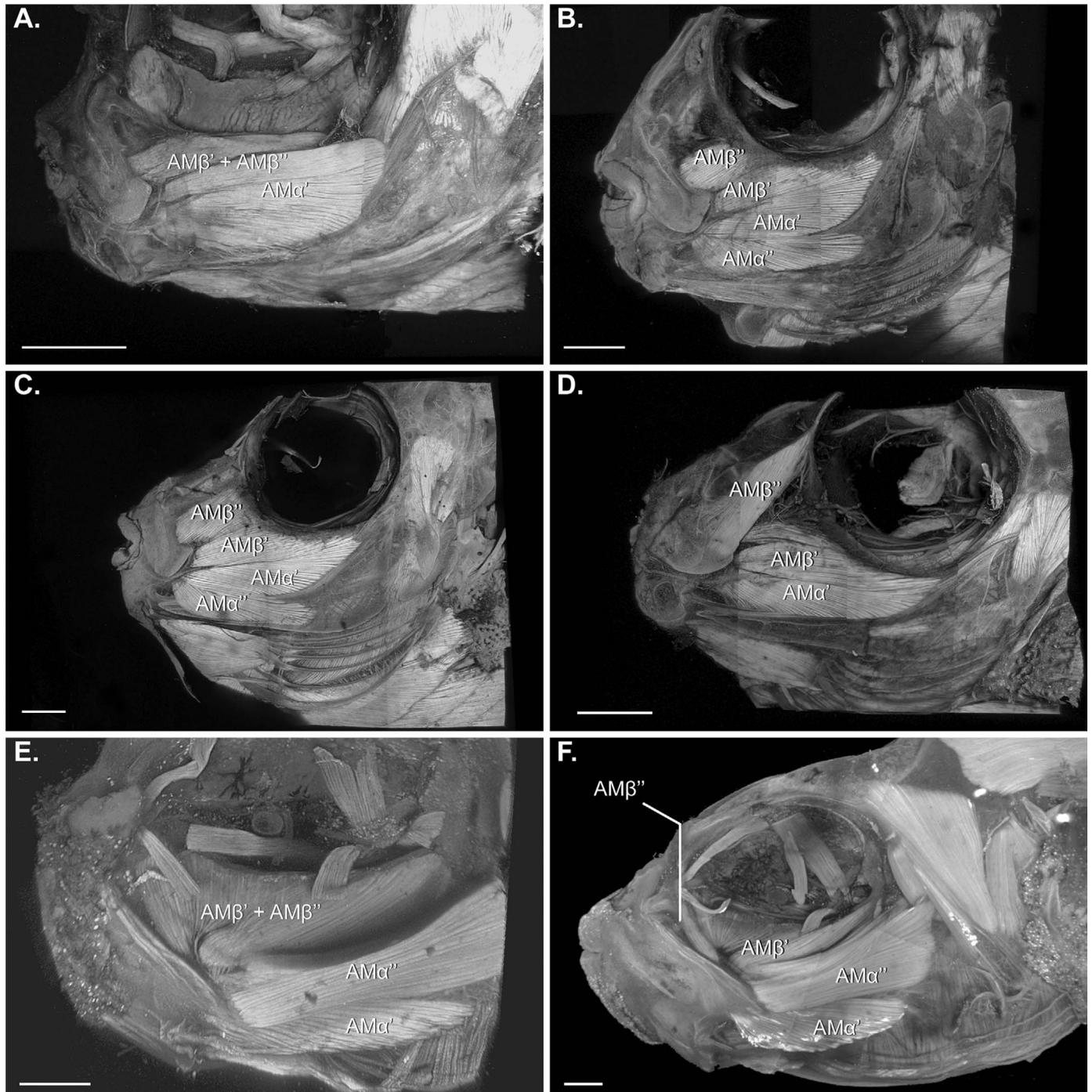


Fig. 8. Confocal microscopy of antibody stained specimens of tetraodontiform fishes showing early stages of development of cranial musculature. (A–C) Ontogenetic series of *Balistapus undulatus*. (D) An unidentified monacanthid larva. (E, F) Two different stages of the freshwater pufferfish *Monotrete suvattii*. Abbreviations represent different muscle portions of the *musculus adductor mandibulae*.

fishes using morphological data. Some of these novel hypotheses regarding the comparative anatomy of fishes (e.g., hypotheses of homology, i.e., synapomorphy) have come from morphological investigation of relationships initially proposed by molecular analysis (e.g., a paracanthopterygian relationship of *Stylephorus*; Miya et al., 2007; Grande et al., 2013). Our intent is not to critically examine any individual example here, but rather to point out that this process (called “pseudomorphology” by some; Mooi and Gill, 2010) is, in principle, no different than examining and comparing the morphology of fishes in light of hypotheses of relationships based on different morphological studies,

historical or current. Data are data, and interpretations of these data can be—and should be—evaluated in the context of *all* data available (Dillman and Hilton, 2011).

Past morphological analyses provide rich sources of hypotheses to test with new morphological data. The phylogenetic placement of the Stomiiformes for example has changed significantly over the past 50 years. Greenwood et al. (1966) placed them as a suborder within the Salmoniformes. Rosen (1973) and Fink and Weitzman (1982) reanalyzed stomiiform interrelationships and considered this group sister to the Eurypterygii and placed them as a separate order of the Neoteleostei. This placement is still the currently accepted

hypothesis, although recent molecular studies recover the Stomiiformes as sister to Osmeriformes (López et al., 2004; Li et al., 2008, 2010; Betancur-R. et al., 2013). Synapomorphies such as the retractor dorsalis muscle shared by the Stomiiformes and all other neoteleosts are striking, but nonetheless there is morphological evidence found in early life history stages of stomiids (e.g., the development of the gill arches and the caudal skeleton) that suggest that the ontogeny of Stomiiformes needs to be further scrutinized (Dosey and Domke, 2014; Schnell and Johnson, unpubl. data). Simply, hypotheses from the literature—whether based on morphological or molecular data—must be reconsidered (supported or refuted) in light of new anatomical data generated by new generations of morphologists.

Finally, the use of morphological data is not limited to understanding phylogeny and evolution of fishes. Personally this is what we find most attractive and compelling for our own research in morphology. However, morphology is the first line of understanding alpha-taxonomic diversity. Further, morphology contributes to understanding the interaction fishes have with their environment, as an organism's anatomy can be the basis for understanding many aspects of its behavior and ecology (i.e., the interrelated approaches of ecomorphology, functional anatomy, and biomechanics). Such subfields of morphology can have meaningful implications for elements of conservation biology (i.e., understanding how an organism uses its environment can be predicted based on morphological analysis). The dimensions of these aspects to which morphology can contribute will only become more diverse as new questions arise, such as how the ontogeny and osteology of teleostean larvae are impacted by environmental changes like ocean acidification (Schnell and Clemmensen, unpubl. data). Recognition of deviation from the normal ontogeny for a taxon can be recognized and greatly informed by an appreciation and understanding of the systematic anatomy of early life history stages.

CONCLUSIONS

But the progress of science needs not only the rare genius who in one leap covers a large step in the ascent to the summit but also the slow, continuous walkers, pushing up in small steps.

Richard Goldschmidt (1956:181)

Morphology, in its strictest sense, is inherently an observational science: the analysis of form is, in its essence, a discussion of facts related to the structure of an organism. Once comparisons are made, however, there are elements of hypothesis-driven science. Ultimately, the strengths and relevance of morphology in the modern comparative biology of fishes lie in the fundamental fact that a thread can be found tying everything in comparative biology of fishes to morphology. Said another way, all facets of comparative biology—no matter what level of organization—can find their roots in morphology. This is because morphology is a broad discipline, each sub-discipline having different goals and different purposes but that all can relate to any level of the phenotype, from cellular to behavioral. In particular, morphology has a unique place in both the history and future of systematic ichthyology, and continues to grow with each new technological advance. With each new generation it returns to become a new and vibrant science.

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