

# Leis' Conundrum: Homology of the Clavus of the Ocean Sunfishes. 1. Ontogeny of the Median Fins and Axial Skeleton of *Monotretete leiurus* (Teleostei, Tetraodontiformes, Tetraodontidae)

Ralf Britz<sup>1,2\*</sup> and G. David Johnson<sup>1</sup>

<sup>1</sup>Division of Fishes, National Museum of Natural History, Smithsonian Institution, Washington DC, USA

<sup>2</sup>Department of Zoology, The Natural History Museum, London, UK

**ABSTRACT** We describe the ontogeny of the axial skeleton and median fins of the Southeast Asian freshwater puffer *Monotretete leiurus*, based on a reared developmental series. Most elements of the axial skeleton in *M. leiurus* arise in membrane bone. Only the base of the anterior three neural arches, the base of the hemal arches of the third preural centrum, the neural and hemal arches and spines of the second preural centrum, the parhypural, the two hypural plates, and the single epural are preformed in cartilage. In contrast to most teleosts, the proximal-middle radials of the dorsal and anal fins are upright and symmetrical and their distal tips coalesce during development to form a deep band of cartilage, from which the spherical distal radials are spatially separated. *J. Morphol.* 266:1–10, 2005. © 2004 Wiley-Liss, Inc.

**KEY WORDS:** vertebral column; median fins; pterygiophores; ontogeny; homology; Tetraodontidae

The Tetraodontiformes are a monophyletic group of teleosts, comprising 10 families with approximately 100 genera and 340 species (Nelson, 1994), the relationships of which to other bony fishes remain unclear. They are well known at the alpha-level, and their anatomy has been described in great detail in numerous articles, obviously because of their unusual and diverse structure. The most recent and comprehensive anatomical monographs on tetraodontiforms are those of Winterbottom (1974) on the musculature and Tyler (1980) on the skeleton.

Although adult Tetraodontiformes have received considerable interest and study from taxonomists and comparative morphologists, their larvae have not been studied so extensively. Leis (1984) and Aboussouan and Leis (1984) summarized the state of our knowledge of tetraodontiform larvae and provided a list of larval descriptions published up to that time. More recent information about the external morphology of the larvae of representatives of six Indo-Pacific families can be found in Leis and Carson-Ewart (2002).

When we began our project on the homology of the clavus of the ocean sunfishes, family Molidae, we

sought information on the ontogeny of the axial skeleton and median fins of less modified tetraodontiforms. Remarkably, we found that, throughout the entire history of the tetraodontiform literature only three articles (Leis, 1977; Matsuura and Katsuragawa, 1985; Fujita, 1992) dealt specifically with developmental osteology of tetraodontiforms, and even those focused only on selected complexes.

Leis (1977) illustrated and described four stages of the developing axial skeleton and unpaired fins of the molid *Ranzania laevis*; Matsuura and Katsuragawa (1985) studied the developing axial skeleton and fin supports in cleared and double-stained specimens of the balistid *Balistes capricus*; and Fujita (1992) described the ontogeny of the caudal skeleton of the tetraodontid *Takifugu niphobles*.

A large comprehensive ontogenetic series from a captive breeding of the Asian freshwater puffer *Monotretete leiurus* forms the basis for a detailed comparison with the development of the axial skeleton and median fins in the ocean sunfish, *Ranzania laevis*, in the second part of our study (Johnson and Britz, 2004).

## MATERIALS AND METHODS

We follow Kottelat (2001) in accepting *Monotretete* as the generic name and spelling for the species *leiurus*. The developmental series of *Monotretete leiurus* was preserved from different captive rearings of a pair of adult, wild-caught specimens purchased from a local aquarium dealer. They were maintained and spawned at 24–26°C in a 100-L tank. Larvae were preserved at different intervals after hatching. No anomalies were encountered among

Contract grant sponsor: Office of Fellowships and Grants in the Division of Fishes, National Museum of Natural History, Smithsonian Institution, Washington DC (Visiting Scientist Fellowship to R.B.).

\*Correspondence to: Ralf Britz, Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK. E-mail: R.Britz@nhm.ac.uk

Published online 17 November 2004 in  
Wiley InterScience (www.interscience.wiley.com)  
DOI: 10.1002/jmor.10243

the specimens of the developmental series used for this study and the meristic values of fully developed larvae and juveniles matched those of the adults.

We use the following abbreviations in the text: NL, notochord length; PU, preural centrum; SL, standard length. The term "membrane bone" was adopted from Patterson (1977). We studied cleared and double-stained (Taylor and Van Dyke, 1985) specimens of the following percomorph taxa:

#### Gasterosteiformes

Solenostomidae: *Solenostomus paradoxus*; AMS I.18314002, 55 mm SL.

#### Perciformes

Moronidae: *Morone americana*; USNM 322796, 14.5 mm SL; USNM 315315, 28.5 mm SL.

#### Tetraodontiformes

Triacanthidae: *Triacanthus biaculeatus*; USNM 071055, 72 mm SL.

Triacanthodidae: *Parahollardia schmidti*; ANSP 100511, 2 specimens, 53.2-55.3 mm SL.

Monacanthidae: *Monacanthus* sp.; USNM uncataloged, 18.8 mm SL.

#### Triodontidae: *Triodon*; AMS 25802006, 108 mm SL.

Tetraodontidae: *Monotretete leiurus*; 38 specimens from 3.8 mm NL-91 mm SL, of which the following stages are illustrated and described: 3.8 mm NL; 4.0 mm NL; 4.3 mm NL; 4.4 mm NL; 4.4 mm SL; 4.6 mm SL; 6.1 mm SL; 10.3 mm SL.

Bone and cartilage were distinguished reliably not only through the differential staining obtained by alizarin red and Alcian blue, but also by the structural differences of the two tissues at high magnifications (80 $\times$  and above): a cellular substructure in cartilage, absent in bone.

## RESULTS

### 3.8 mm NL (Fig. 1A)

The first four neural arches are present in membrane bone. The anterior three have a small cartilaginous base from which the thin and narrow neural arches curve laterally and dorsally, but their tips remain widely separated from each other in the dorsal midline. Seven soft dorsal-fin pterygiophores (hereafter "dorsal pterygiophores") are developed as short thin rods of cartilage that represent proximal-middle radials, the distal radials of which are not yet developed. Only the anteriormost proximal-middle radial of the anal-fin pterygiophores (hereafter "anal pterygiophores") is present as an elongate rod of cartilage just posterior to the anus. The caudal fin is not developed and the chorda shows no flexion.

### 4 mm NL (Fig. 1B)

Only the first four neural arches are present. They are relatively longer compared to the previous stage, but remain widely separated in the dorsal midline. Ten dorsal proximal-middle radials are developed, all still lacking distal radials. The first and the last two proximal-middle radials are stained only faintly, and the fourth to seventh are the largest. At the level of the posterior dorsal pterygiophores, four cylindrical cartilaginous proximal-middle radials of the anal pterygiophores are now present. The first of these is larger than the others and bifurcates dis-

tally at about mid-length, and the remaining three decrease progressively in size. The caudal-fin skeleton is not developed.

### 4.3 mm NL (Fig. 1C)

Nine bony neural arches are now present, decreasing in size posteriorly. The anterior four have broadened slightly. Twelve proximal-middle radials are developed, the first and the last are only faintly stained, and all still lack distal radials. The 13th is about to chondrify and can be barely seen at high magnifications (80 $\times$ ). The distal tips of the second through sixth have started to coalesce along the dorsal midline. Seven cartilaginous proximal-middle radials of the anal pterygiophores are developed, decreasing abruptly in size posteriorly. The first is comparatively longer than in the previous stage and the bifurcation starts at the proximal third of its length. Two cartilages are now well developed in the caudal area. The first, the parhypural, is situated with its hemal arch ventrally on the chorda. The second, the lower hypural, is larger and almost rectangular, with the distal edge rounded. Slightly more caudally a tiny nodule of cartilage, the future upper hypural, is beginning to chondrify. Two faintly stained caudal-fin rays are developed in the caudal larval finfold, one slightly posteriorly in the space between the lower and the upper hypural cartilage and one at the level of the upper hypural cartilage.

### 4.4 mm NL (Fig. 1D)

Eleven neural arches are present, with the anterior five broadened. Short hemapophyses of membrane bone are developed on vertebrae 6-9. The anterior six vertebrae show some signs of differentiation of centra. The full complement of 13 dorsal proximal-middle radials is now chondrified. Numbers 1-9 are subequal in length, which then decreases posteriorly, so that the last proximal-middle radial is about one-third the length of each of the anterior nine. Numbers 2-9 show some traces of fusion of their distal tips. Distal radials are developed in association with the proximal-middle radials of the first five pterygiophores. Ten fin rays are ossified in the dorsal larval fin fold. The anal fin consists of seven proximal-middle radials, with the first and last bifurcated distally. Their length decreases greatly posteriorly, with the last less than one-third the length of the first. Eight distal radials are associated with the anterior seven proximal-middle radials. Ten anal-fin rays have formed, the first two articulating through distal radials with the two arms of the first pterygiophore, the last two lacking associated distal radials. The distal tips of the two arms of the first, and those of the subsequent three proximal-middle radials have begun to fuse along their ventral midline. In the caudal area the fully cartilaginous neural and hemal arches and

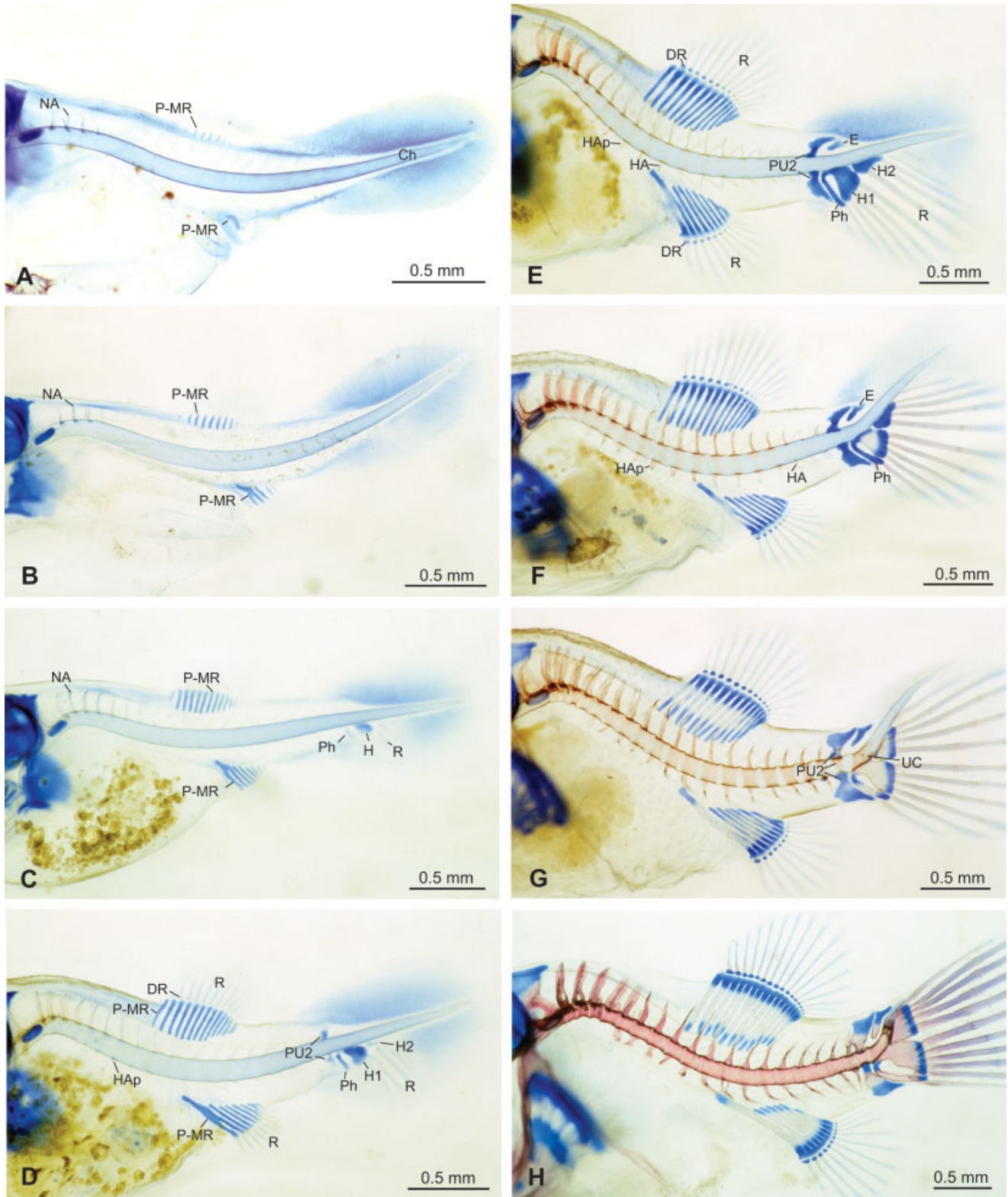


Fig. 1. Axial skeleton and median fin supports of cleared and stained specimens of *Monotretete leiurus*. **A:** 3.8 mm NL. **B:** 4.0 mm NL. **C:** 4.3 mm NL. **D:** 4.4 mm NL. **E:** 4.5 mm NL. **F:** 4.4 mm SL. **G:** 4.6 mm SL. **H:** 6.1 mm SL. DR, distal radial; E, epural; H, hypural; HA, hemal arch; Hap, hemapophysis; NA, neural arch; P-MR, proximal-middle radial; Ph, parhypural; R, soft fin ray.



spines of the second preural centrum have developed anterior to the parhypural cartilage. The latter has elongated slightly. The lower hypural cartilage has enlarged and the upper hypural cartilage is developed as an elongate chondrification parallel to the lower edge of the chorda. Three caudal-fin rays articulate with the lower hypural cartilage and two are ossified in the area of the upper hypural cartilage.

#### 4.5 mm NL (Fig. 1E)

Nineteen neural arches, the full complement, are developed, decreasing slightly in size from 1–13, but more so from 14–18. All neural arches, except the last on PU2, are formed in membrane bone. The anterior four neural arches are expanded. The neural arch on vertebra 18 is only a narrow, needle-like splint of bone above the chorda. The anterior nine neural arches are still separated in the midline, followed by three arches that have fused in the midline and bear a short neural spine, again followed by six arches separated in the midline. The last neural arch and its spine have grown considerably and are fully cartilaginous. Vertebrae 6–10 bear short hemapophyses in membrane bone ventrolaterally, continued by hemal arches in membrane bone on vertebrae 11–17. Vertebra 18 bears small cartilaginous anlagen of hemal arches, followed by a large, fully cartilaginous hemal arch bearing an anteriorly expanded large spine on PU2 (vertebra 19). The long parhypural, fully cartilaginous, is fused to the lower hypural cartilage at the very base, with which both attach to the chorda. The first signs of chorda flexion can be observed. The lower hypural is a roughly rectangular cartilage with an expanded distal portion. The upper hypural cartilage is elongate and triangular. A single elongate epural cartilage is developed between the neural spine of PU2 and the chorda. The full complement of 11 caudal-fin rays is present, the ventral two articulating with the tip of the parhypural, followed dorsally by four rays at the lower hypural, three rays at the upper hypural, and the two uppermost rays in the larval caudal fin with no immediate articulation. There are 13 dorsal proximal-middle radials, the last two being fused at their proximal tips. Thirteen fin rays are ossified, of which all but the last two are associated with distal radials. Seven anal proximal-middle radials are present, of which the first and the last are bifurcated distally. There are 10 anal-fin rays, the anterior eight associated with a distal radial. The distal tips of all anal and all but the first dorsal proximal-middle radials have begun to fuse.

#### 4.4 mm SL (Fig. 1F)

The first neural arch has a fine, needle-like, dorsally directed process at its posterior base, which probably forms the posterior border of the foramen

for the first spinal nerve (dorsal root?). The first five neural arches are widely separated in the dorsal midline, those of the sixth and seventh vertebrae are approaching each other, and those of vertebrae 8–14 are fused in the dorsal midline and bear short neural spines. Posterior to neural arch 14 all subsequent arches are still separated dorsally and the 17th consists only of the right half arch in this specimen. The neural arches that are fused dorsally are those between which the proximal tips of the dorsal proximal-middle radials interdigitate. Centra have ossified on vertebrae 1–15. The neural arch and spine of the 19th (second preural) vertebra is much larger than those of the preceding 18 and forms completely in cartilage. Its neural spine is inclined posteriorly. Short hemapophyses are developed on abdominal vertebrae 5–10 and are joined in the ventral midline forming a complete arch on vertebrae 6–10. All caudal vertebrae bear short hemal arches that are closed in the ventral midline on vertebrae 11–14, and 16–17, but still open on 15–18. The hemal arch of vertebra 18 has tiny nodules of cartilage at its base. The hemal arches and spine of the second preural vertebra (19) are large and fully cartilaginous, like its corresponding neural arches and spine. The parhypural is well developed, still cartilaginous, and fused proximally with the now expanded lower hypural cartilage, the middle portion of which is ossified. The upper hypural cartilage has also expanded and is roughly the shape of an acute triangle. Of the 11 caudal-fin rays, two articulate with the parhypural, four with the lower hypural, and four with the upper hypural; the uppermost ray in the larval fin fold has no immediate articulation. A single cartilaginous epural is developed. It is slightly bifurcated distally and shows some signs of fusion with the anteriorly located distal end of the neural spine of PU2. The 13 dorsal proximal-middle radials are larger and their distal tips are clearly fused along the dorsal midline. Fifteen dorsal-fin rays are present, the anterior 12 of which embrace the small spherical cartilaginous distal radials. The proximal tip of the first anal proximal-middle radial is approaching the hemapophyses of the 11th vertebra closely, slightly posterior to it. There are seven fully cartilaginous anal proximal-middle radials, with the last bifurcated distally. Their distal tips are fused. Twelve anal-fin rays are present and the anterior eight articulate with spherical distal radial cartilages, decreasing in size posteriorly.

#### 4.6 mm SL (Fig. 1G)

All neural arches posterior to the seventh, including those of the caudal area, are fused in the dorsal midline and possess short neural spines. The large cartilaginous neural arch of PU2 is perichondrally ossified at its base. All hemapophyses and hemal arches except that of the 11th are closed in the ventral midline. Neural arches of vertebrae 17–18

bear small nodules of cartilage at their bases. All centra are formed now, including the ural centrum. In the caudal skeleton, the middle part of the parhypural and most areas of the lower and upper hypural cartilage are perichondrally ossified, and the base of the lower hypural is fused to the ural centrum. Only the distal tips of both hypurals that articulate with the caudal-fin rays remain cartilaginous. Articulation of the caudal-fin rays is identical to that of the previous stage. Dorsal proximal-middle radials 1–9 are perichondrally ossified along their mid-length. The small, posteriormost proximal-middle radial has started to fuse proximally with the penultimate. Fourteen dorsal-fin rays are present and all except the last articulate with cartilaginous distal radials. The first and largest proximal-middle radial of the anal fin is also perichondrally ossified along its mid-length. Its proximal tip approaches the distal end of the hemal arch of the 11th vertebra and is located slightly anterior to it. The last and shortest proximal-middle radial is fused proximally to the penultimate. Eleven anal-fin rays are developed and all except the last articulate with cartilaginous distal radials.

### 6.1 mm SL (Fig. 1H)

At the posterior face of the base of the first neural arch, a pointed posterior process has developed, the postzygapophysis of the first vertebra, which extends laterally beyond the articulation of the first and second centra. The neural arches on vertebrae 1–6 have broadened further, forming blade-like dorsal processes on their respective centra. Through this lateral growth, the bases of neural arches 1–4 now completely enclose the foramina for the spinal nerve roots. The base of the fifth neural arch bears a short thin dorsal process that marks the posterior border of the developing foramen. It is still separate from the neural arch on the left side but has fused with it on the right side, thus completely enclosing that foramen. The sixth neural arch bears a short posterior process on the base of the right neural arch only. The left and right neural arch of the seventh vertebra are fused in the dorsal midline, forming a short spine. The neural spines of all subsequent vertebrae are comparatively longer, and that of the third preural centrum (vertebra 18) is expanded laterally. Ossification of the first preural neural arch and spine now reaches up to half of its length. All hemapophyses are slightly larger and broader, as are the hemal arches of the caudal vertebrae. The latter have well-developed hemal spines. The small cartilages at the base of hemal arches 17–18 are ossified. The cartilage of the expanded hemal arch and spine of the second preural centrum is ossified in its middle part with the base and distal tip still cartilaginous. The parhypural is ossified over most of its length, with the base and the distal tip still cartilaginous. Its dorsal margin bears a narrow lam-

ina of membrane bone. The cartilage of the base is still connected to the lower hypural. The ural centrum, formerly restricted to the level of the lower hypural now extends up around the chorda to the dorsal base of the upper hypural. The distal tip of the chorda dorsalis shows signs of resorption and does not project as far beyond the upper hypural as in the previous stage. The basal two-thirds of the upper hypural are ossified, but its base remains separate from the ural centrum. The uppermost caudal-fin ray now articulates with the dorsal edge of the upper hypural. The proximal half of the single epural is ossified. Sixteen dorsal-fin rays articulating with 16 distal radial cartilages are present. All seven anal proximal-middle radials are ossified along their mid-length, leaving only the proximal and distal tips in cartilage. The proximal tip of the first anal proximal-middle radial has grown further anteriorly and is now located between the hemapophyses of the tenth vertebra. All 11 anal-fin rays embrace distal radial cartilages.

### 10.3 mm SL (Fig. 2)

The anterior six and the bases of all subsequent neural arches are greatly expanded (Fig. 2A). Neural arch 7 bears a slightly expanded neural spine. All but the last two subsequent neural spines are rather narrow and needle-like. The neural spine of PU3 is slightly expanded. The basal two-thirds of the large neural arch and spine on PU2 are ossified, and its cartilaginous tip has expanded further. Hemapophyses on vertebrae 5–10 and the subsequent eight hemal arches have expanded and the latter bear narrow spines. The hemal arch and spine on PU2 are more elongate and less expanded (Fig. 2B). The base of the arch, which articulates with the centrum, and its distal area remain cartilaginous. The urostyle bears short dorsal laminae of membrane bone that approach the ventral tip of the single epural, which has a short membrane bone process projecting dorsally from its ventral tip. The parhypural has elongated, its distal and subproximal tips remaining cartilaginous. Ossification from the ural centrum and the lower hypural covers the immediate base of the parhypural. Most of the bodies of the two hypurals are ossified, leaving only their distal margins cartilaginous. The upper hypural remains separate from the ural centrum, but the lower is fused with it.

There are 13 ossified dorsal proximal-middle radials, with their proximal and fused distal tips in cartilage (Fig. 2C). All except the shorter first and last are subequal in length. Fourteen dorsal-fin rays are present, each associated with a cartilaginous spherical distal radial. There are seven ossified anal proximal-middle radials with cartilaginous proximal and fused distal tips (Fig. 2D). The first and last proximal-middle radials retain bifurcated tips. The first has elongated further and its proximal tip is

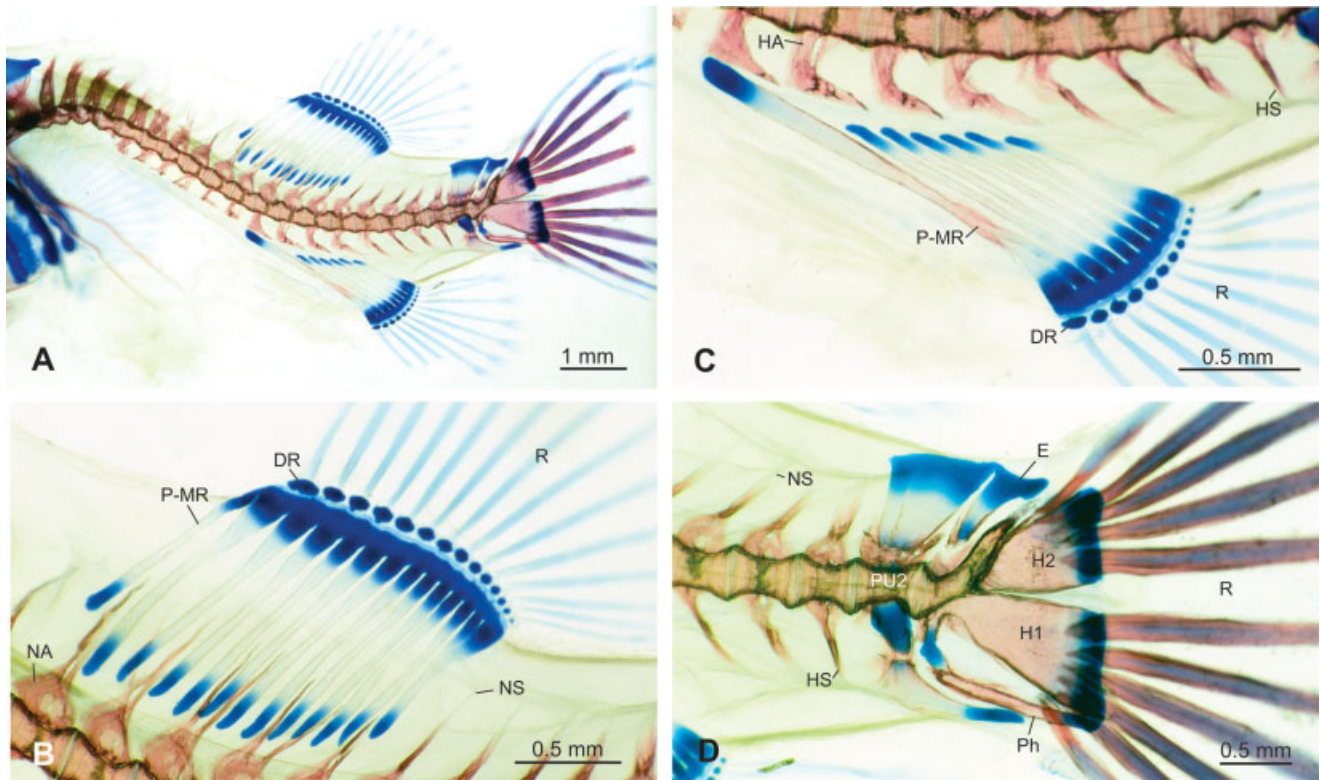


Fig. 2. Axial skeleton and median fin supports of 10.3 mm SL cleared and stained specimen of *Monotretle leiurus*. **A:** Overview. **B:** Close-up of dorsal-fin skeleton. **C:** Close-up of anal-fin skeleton. **D:** Close-up of caudal-fin skeleton. DR, distal radial; E, epural; H, hypural; HA, hemal arch; Hap, hemapophysis; HS, hemal spine; NA, neural arch; NS, neural spine; P-MR, proximal-middle radial; Ph, parhypural; R, soft fin ray.

supported by the hemal arch and spine of the 11th vertebra. There are 11 anal distal radials articulating with the same number of anal-fin rays.

## DISCUSSION

### Ontogeny of Axial Skeleton

Most elements of the axial skeleton in *Monotretle leiurus* arise in membrane bone. Only the base of the anterior three neural arches, the base of the hemal arches of the third preural centrum (also the fourth in some specimens), the neural and hemal arches and spines of the second preural centrum, the parhypural, the two hypural plates, and the single epural are preformed in cartilage.

In contrast, neural and hemal arches of *Engraulis* (Balart, 1995), *Ictalurus* (Grande and Shardo, 2002), *Pagrus* (Matsuoka, 1982), *Sparus* (Faustino and Power, 1998), various scombroids (Potthoff, 1975; Potthoff and Kelley, 1982; Potthoff et al., 1986), *Scombrobrax* (Potthoff et al., 1980), *Anisotremus* (Potthoff et al., 1984), *Microspathodon* (Potthoff et al., 1987), *Morone* (Fritzsche and Johnson, 1980), and *Centropomus* (Potthoff and Tellock, 1993) are always preformed in cartilage. In the percomorph taxa listed, the neural and hemal spines of at least

posterior abdominal and all caudal vertebrae are also preformed in cartilage.

Other percomorph taxa, in which all abdominal and most caudal neural and hemal arches and spines form in membrane bone, include gobioids (Johnson and Brothers, 1993) and the gasterosteoid *Indostomus* (Britz and Johnson, 2002). Grassi (1883) noted that some neural arches in cyprinids are not preformed in cartilage, and Emelianov (1928, 1935) pointed out that cartilaginous precursors of endoskeletal elements of the axial skeleton in several groups of fishes may be lost during evolution and replaced by membrane bone. He cited *Gobius* as one example in which cartilage is completely absent from the ontogeny of all but the tail region of the axial skeleton, and *Atherina* and *Syngnathus* as taxa in which he could not detect any cartilage during the development of the hemal arches. Cartilaginous precursors also seem to be missing in the development of the axial skeleton of *Gadus* (Faruqi, 1935).

Substitution of cartilaginous precursors by membrane bone has also been reported in the skull region of teleosts, in which sometimes the basisphenoid and invariably the intercalary ossify in membrane bone (Patterson, 1975, 1977). Comprehensive onto-



genetic studies dealing with this issue are still lacking, and we anticipate that when ontogenetic series from a broad taxonomic sample of teleosts are investigated, substitution of cartilaginous precursors by membrane bone will emerge as a recurring theme in the evolution of the teleost skeleton. Interestingly, however, in gobioids and *Indostomus*, taxa with neural and hemal arches and spines developing in membrane bone, those on PU2 and the elements of the caudal-fin skeleton such as the parhypural, hypurals, and epurals are always preformed in cartilage.

Another unusual character of the axial skeleton in *Monotretes leiurus* is that the anterior neural arches fail to meet in the dorsal midline to form a neural spine. In later stages than those figured here in detail, a horizontal lamina of membrane bone develops from each half arch just above the neural canal. During subsequent development these laminae meet in the midline and form a complete bony roof above the neural canal from which the two distal tips of the neural arches still project. Commonly among teleosts the neural arches meet in the dorsal midline to form a neural spine, which can be paired in the anterior region of the axial skeleton of basal teleosts or unpaired as in most derived teleosts.

### Direction of Development of Median Fins

The proximal-middle radials of the dorsal fin develop in a bidirectional fashion in *Monotretes leiurus*, but those of the anal fin chondrify in an anteroposterior direction. This is in contrast to Mabee et al.'s (2002:82) recent statement that "The skeletal elements of dorsal and anal fins differentiate in the same directions in all actinopterygians." They cited only two engraulids as exceptions to this rule, in which the dorsal elements develop posteroanteriorly and those of the anal fin bidirectionally. However, the majority of taxa cited in support of Mabee et al.'s (2002) assertion include representatives of only two of the percomorph suborders, the Percoidae and Scombroidei. Obviously, it is premature to generalize about highly conserved patterns in actinopterygian fin patterning before the huge taxonomic diversity of this extremely speciose group is adequately covered.

We also take issue with another of Mabee et al.'s (2002:82) statements that the "hypurals and fin rays of the caudal fin develop bidirectionally in all actinopterygians." Although the caudal-fin rays in our series of *M. leiurus* form in a bidirectional fashion, as in the taxa cited by Mabee et al. (2002), the hypurals develop in an anteroposterior direction. This is also evidently the case in articles cited by Mabee et al. (2002) to support the opposite fact, as in *Polypterus* (Bartsch and Gemballa, 1992), *Engraulis* (Balart, 1995), *Chanos* (Taki et al., 1986), *Pagrus* (Matsuoka, 1982), *Scombrolabrax* (Potthoff et al., 1980), *Coryphaena* (Potthoff, 1980), *Centropomus*

*undecimalis* (Potthoff and Tellock, 1993), *Lutjanus* (Potthoff et al., 1988), and several scombroids (Potthoff et al., 1986). We did not check every article cited by Mabee et al. (2002), but our sample clearly shows that Mabee et al. (2002) incorrectly attributed a bidirectional development of hypurals to these taxa.

### Ontogeny of Caudal Skeleton

Although two comprehensive, monographic studies of the adult teleost caudal skeleton have been published (Monod, 1968; Fujita, 1990), comparable ontogenetic studies are lacking. Exceptions are the recent articles by Schultze and Arratia (1988) and Arratia and Schultze (1992) on basal teleosts.

The only study of the development of the caudal fin of a tetraodontid is that of Fujita (1992) on *Takifugu niphobles*. His account differs in some important aspects from what we have seen in *Monotretes leiurus*. Fujita (1992:439) reported anterior and posterior neural and hemal arches on PU2 in some of his specimens and therefore concluded that PU2 forms "from two incipient centra." We have found no indication that PU2 forms from more than one centrum in *M. leiurus*. The neural and hemal arches and spines of that centrum appear very early in ontogeny (at 3.7 mm SL) before any of the other preural caudal axial elements have formed. These arches are also much larger and more expanded than all the others. However, the centrum of PU2, as well as its arches, ossifies from single, not double, centers. Because we have not studied Fujita's material, we can only point out these discrepancies. In *T. niphobles*, as in *M. leiurus*, the neural and hemal arches of PU3 are preformed in cartilage, the parhypural is fused at the base with the lower hypural, and the single epural coalesces distally with the tip of the hemal spine on PU2. This coalescence was present in a few, but not all specimens of *M. leiurus*. Fujita described a fusion of the anlagen of what he called hypural 1 and 2 into a single hypural, corresponding in position to what he called the lower hypural. In *M. leiurus* this element arises from a single anlage. Fujita (1992:438) also stated that the upper hypural element comprises hypural 3 "fused with the posterior hypurals," although he did not provide any evidence for such a fusion in ontogeny. To the contrary, he found only a single cartilage anlage for this element, as did we in our ontogenetic series of *M. leiurus*.

Adult tetraodontids consistently have two hypural elements, the lower fused to the ural centrum and the upper separate from it. Because up to five individual hypurals occur in basal tetraodontiforms, such as triacanthodids (Tyler, 1970, 1980; Fujita, 1990) and *Triodon* (Tyler, 1970, 1980), homology of the two elements of tetraodontids remains unclear. Nonetheless, Tyler (1980) and Fujita (1990) considered the lower hypural elements in tetraodontids to

be fused hypurals 1 and 2, and the upper to be fused hypurals 3 and 4. We can only state that the two hypurals in *Monotrete leiurus* arise from only two anlagen, and we did not observe any fusion.

Matsuura and Katsuragawa (1985) also assumed fusion of posterior hypurals in their skeletal developmental study of *Balistes* without having observed it in ontogeny. Their smallest specimen was 4.5 mm and exhibited a neural arch with a full neural spine and an autogenous hemal arch and spine on preural centrum 2, an autogenous parhypural, and 3 hypural cartilage plates, of which the lower two are subequal in size, and the third comparatively smaller. At 4.9 mm, the lower two hypural plates fuse, and an additional small hypural cartilage develops below the distal tip of the chorda. The authors interpreted the anterior element as fused hypural 1+2, the second as fused hypural 3+4, and the smallest as hypural 5, but they never observed fusion of hypural 3 and 4. *Balistes* thus differs from *Monotrete* in having four hypural cartilages, of which the lower two fuse during ontogeny, and in having the parhypural develop autogenously, remaining separate from the ural centrum and the lower hypural. As in *Monotrete*, a single epural cartilage is present above the chorda and posterior to the neural arch and spine of PU2 in *Balistes*.

Ontogenetic fusion of hypurals into larger plates does occur among teleosts, and has been demonstrated for different scombroids (Potthoff, 1975; Potthoff and Kelley, 1982; Potthoff et al., 1986), and *Coryphaena* (Potthoff, 1980). In other cases, as, e.g., in *Indostomus*, in which fusion of hypurals was hypothesized from the adult condition (Johnson and Patterson, 1993), an ontogenetic study has contradicted this claim (Britz and Johnson, 2002). As we have detailed recently (Britz and Johnson, 2002), we see no reason for assuming fusion, unless it has been demonstrated through developmental studies. We note again that the concept of phylogenetic fusion, often invoked when fusion is assumed, but has not or could not be demonstrated, is not a testable hypothesis.

### Ontogeny of Dorsal- and Anal-Fin Skeleton

All pterygiophores of the dorsal and anal fins in *Monotrete leiurus* are preformed in cartilage, as is the case in all teleosts studied to date (e.g., *Hiodon*: Hilton, 2002; *Engraulis*: Balart, 1995; *Chanos*: Taki et al., 1986; *Ictalurus*: Grande and Shardo, 2002; *Salmo*: Harrison, 1895; Schmalhausen, 1912; *Indostomus*: Britz and Johnson, 2002; *Morone*: Fritzsche and Johnson, 1980; *Pagrus*: Matsuoka, 1985; *Sparus*: Faustino and Power, 1999; *Balistes*: Matsuura and Katsuragawa, 1986; *Coryphaena*: Potthoff, 1980; *Scombrolabrax*: Potthoff et al., 1980; various scombroids: Potthoff et al., 1986; *Anisotremus*: Potthoff et al., 1984; *Microspathodon*: Potthoff et al., 1987; *Centropomus*: Potthoff and Tellock, 1993; *Paralichthys*: Balart, 1985).

In most teleosts, as shown for the basal percoid *Morone* in our Figure 3A,B, each pterygiophore comprises a cylindrical rod of cartilage representing the proximal-middle radial, and a small spherical cartilage at its distal tip representing the serially associated distal radial. The latter becomes bilaterally embraced by the bases of the two hemitrichs of its serially associated fin ray. Each proximal-middle radial is bent posteriorly near its distal end, and as it develops and ossifies (Fig. 3B), its serially associated distal radial comes to rest on the anterodistal corner of the succeeding proximal-middle radial.

Most teleosts exhibit the distally asymmetrical configuration described above, but distinctive modifications in shape, sequential arrangement, and other structural aspects characterize the pterygiophores of numerous groups. In all tetraodontiforms, for example, the proximal-middle radials do not bend at their distal tips, being instead upright and distally symmetrical (Figs. 2A–C, 3C,D), and within this group, two discretely modified pterygiophore configurations are found. The more primitive of these two conditions was described and illustrated by Bridge (1896) for the balistid *Balistes* and the monacanthid *Monacanthus*, and by Rosen (1984: figs. 9, 10) for the triacanthodids *Hollardia* and *Parahollardia* in our Figure 3C,D. In these taxa, each somewhat triangular distal radial rests between and articulates equally with the cartilaginous tips of sequential pairs of the upright proximal-middle radials (the latter may contact one another distally but always remain separate). In addition to the taxa mentioned above, this condition characterizes the triacanthid *Triacanthus* (USNM 071055), and the triodontid *Triodon* (AMS 25802006).

The more derived configuration found in tetraodontiforms is the one we describe above for *Monotrete leiurus* (Figs. 1, 2). Here, the proximal-middle radials are also upright and distally symmetrical, but their cartilaginous distal tips coalesce ontogenetically, so that they are eventually connected by a deep continuous band of cartilage. The distal radials, rather than being roughly triangular, are spherical and are spatially separated from the proximal-middle radial band of cartilage (Fig. 2B,C). This very striking condition is highly unusual among teleosts, and we know of only two non-tetraodontiform taxa in which a similar state occurs, solenostomid (pers. obs. AMS 18314002) and syngnathid (Bridge, 1896; Rauther, 1925) gasterosteiforms. Fusion of several, but not all proximal-middle radials was reported in the posteriormost dorsal pterygiophores of osmerids and salangids (Johnson and Patterson, 1996), in a few posterior dorsal or anal pterygiophores of early stages of three aulopiforms (Baldwin and Johnson, 1996), and in *Metavelifer* (Baldwin and Johnson, 1996).

The highly unusual distal coalescence of the proximal-middle radials we found in *Monotrete lei-*



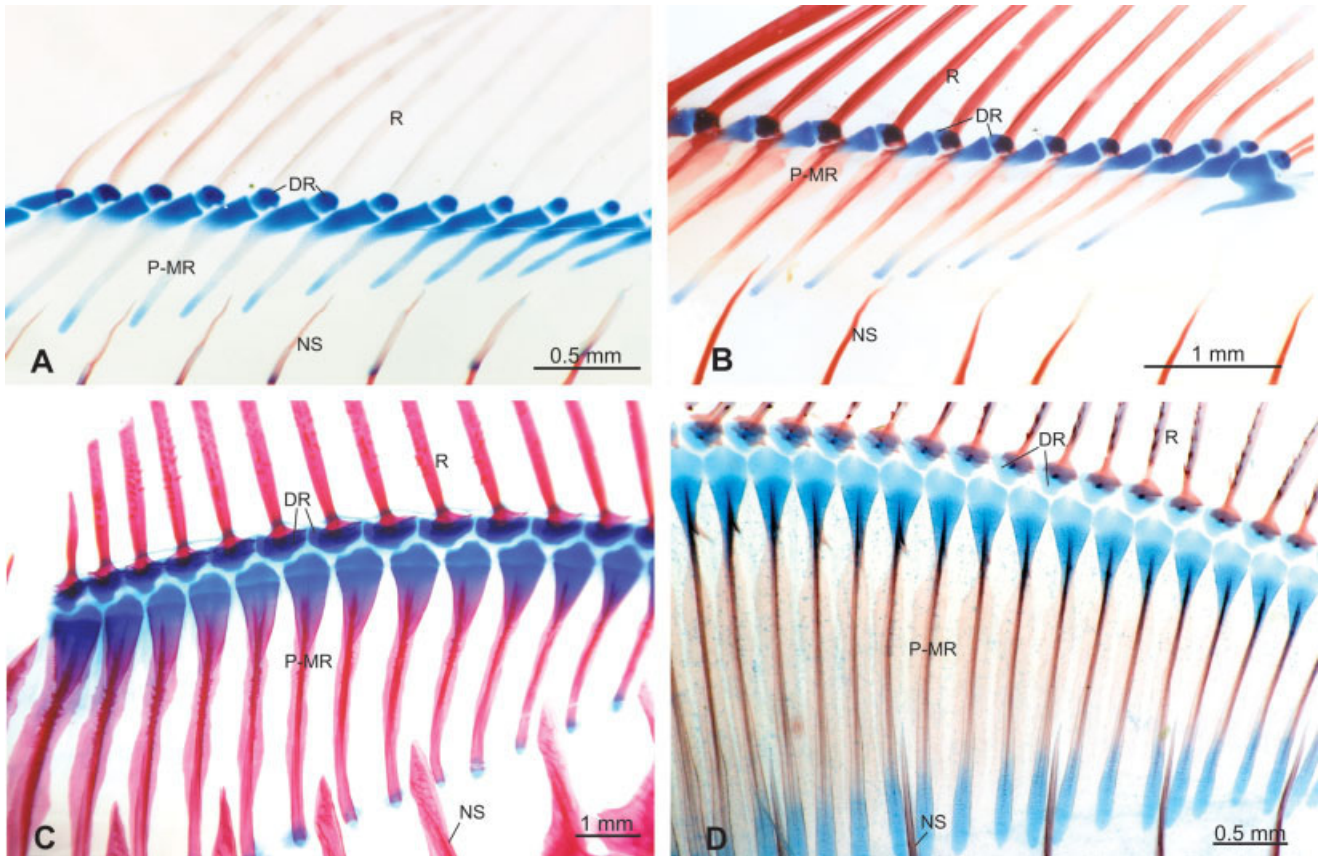


Fig. 3. Soft dorsal-fin skeleton of cleared and stained specimens. **A:** *Morone americana* 14.5 mm SL. **B:** *Morone americana* 28.5 mm SL. **C:** *Parahollardia schmidti* 55.3 mm SL. **D:** *Monacanthus* sp. 18.8 mm SL. DR, distal radial; NS, neural spine; P-MR, proximal-middle radial; R, soft fin ray.

*urus* was also described by Bridge (1896) for *Tetraodon immaculatum*, *Diodon hystrix*, and *Mola mola*. However, in his important tetraodontiform monograph, Tyler (1980), working exclusively with single-stained specimens, X-rays, or dry skeletons, did not observe cartilage and thus failed to recognize the two quite distinctive configurations of soft ray pterygiophores that occur in the Tetraodontiformes. The significance of the unique, more derived configuration will be explored in more detail in Part 2 of our study.

#### ACKNOWLEDGMENTS

We thank Mark Sabaj and John Lundberg (Academy of Natural Sciences, Philadelphia) and Jeff Leis (Australian Museum, Sydney) for access to material under their care. We also thank Jim Tyler (National Museum of Natural History, Smithsonian Institution, Washington DC) and an anonymous reviewer for helpful comments on the manuscript.

#### LITERATURE CITED

- Aboussouan A, Leis JM. 1984. Balistoidei: development. In: Moser HG, Richards WJ, Cohen DM, Fahay MP, Kendall AW,

- Richardson SL, editors. Ontogeny and systematics of fishes. Lawrence, KS: Allen Press. p 450–459.
- Arratia G, Schultze H-P. 1992. Reevaluation of the caudal skeleton of some actinopterygian fishes. III. Salmonidae. *J Morphol* 214:187–249.
- Balart EF. 1985. Development of median and paired fin skeleton of *Paralichthys olivaceus* (Pleuronectiformes: Paralichthyidae). *Jpn J Ichthyol* 31:398–410.
- Balart EF. 1995. Development of the vertebral column, fins, and fin supports in the Japanese anchovy, *Engraulis japonicus* (Clupeiformes: Engraulidae). *Bull Mar Sci* 56:495–522.
- Baldwin CCB, Johnson GD. 1996. Interrelationships of Aulopiiformes. In: Stiassny MLJ, Parenti LR, Johnson GD, editors. Interrelationships of fishes 2. San Diego: Academic Press. p 355–404.
- Bartsch P, Gemballa S. 1992. On the anatomy and development of the vertebral column and pterygiophores in *Polypterus senegalus* Cuvier, 1829 (“Pisces”, Polypteriformes). *Zool Jb Anat* 122:497–529.
- Bridge TW. 1896. The mesial fins of ganoids and teleosts. *J Linn Soc Zool* 25:530–602.
- Britz R, Johnson GD. 2002. “Paradox lost”: skeletal ontogeny of *Indostomus paradoxus* and its significance for the phylogenetic relationships of Indostomidae (Teleostei, Gasterosteiformes). *Am Mus Novitates* 3383:1–43.
- Emelianov SV. 1928. Die Entwicklung der Rippen und ihr Verhältnis zur Wirbelsäule. IV. Die Entwicklung der Rippen und Muskelgräten der Teleostier. *Rev Zool Russe* 8:3–73.
- Emelianov SV. 1935. Die Morphologie der Fischrippen. *Zool Jb Anat* 60:133–262.

- Emelianov SV. 1939. Sequence in the ontogenetic appearance of vertebral arches in teleosts and the omission of chondral stages in their development. *Comp Rend Acad Sci URSS* 23:978–981.
- Faruqi AJ. 1935. The development of the vertebral column in the haddock (*Gadus aeglefinus*). *Proc Zool Soc Lond* 1935:313–332.
- Faustino M, Power DM. 1998. Development of osteological structures in the sea bream: vertebral column and caudal fin complex. *J Fish Biol* 52:11–22.
- Faustino M, Power DM. 1999. Development of the pectoral, pelvic, dorsal, and anal fins in cultured sea bream. *J Fish Biol* 54:1094–1110.
- Fritzsche RA, Johnson GD. 1980. Early osteological development of white perch and striped bass with emphasis on identification of their larvae. *Trans Am Fish Soc* 109:387–406.
- Fujita K. 1990. The caudal skeleton of teleostean fishes. Tokyo: Tokai University Press.
- Fujita K. 1992. Development of the caudal skeleton in the tetraodontid fish, *Takifugu niphobles*. *Jpn J Ichthyol* 38:438–440.
- Grande T, Shardo JD. 2002. Morphology and development of the postcranial skeleton in the channel catfish *Ictalurus punctatus* (Ostariophysi: Siluriformes). *Field Zool* 99:1–30.
- Grassi B. 1883. Beiträge zur näheren Kenntnis der Entwicklung der Wirbelsäule der Teleostier. *Morph Jb* 8:456–473.
- Harrison RG. 1895. Die Entwicklung der unpaaren und paarigen Flossen der Teleostier. *Arch mikr Anat Entwgesch* 46:500–578.
- Hilton EJ. 2002. Osteology of the extant North American fishes of the genus *Hiodon* Lesueur, 1818 (Teleostei: Osteoglossomorpha: Hiodontiformes). *Field Zool* 100:1–142.
- Johnson GD, Britz R. 2004. Leis' conundrum: homology of the clavus of the ocean sunfishes. 2. Ontogeny of the median fins and axial skeleton of *Ranzania laevis* (Teleostei, Tetraodontiformes, Molidae). *J Morphol* (in press). Published online in Wiley InterScience. DOI: 10.1002/jmor.10242.
- Johnson GD, Brothers EB. 1993. *Schindleria*: a paedomorphic goby (Teleostei: Gobioidae). *Bull Mar Sci* 52:441–471.
- Johnson GD, Patterson C. 1993. Percomorph phylogeny: a survey of acanthomorphs and a new proposal. *Bull Mar Sci* 52:554–626.
- Johnson GD, Patterson C. 1996. Relationships of lower euteleostean fishes. In: Stiassny MLJ, Parenti LR, Johnson GD, editors. *Interrelationships of fishes 2*. San Diego: Academic Press. p 251–332.
- Kottelat M. 2001. Nomenclatural status of names of tetraodontiform fishes based on Bibron's unpublished work. *Zoosyst* 23: 605–618.
- Leis JM. 1977. Development of the eggs and larvae of the slender mola, *Ranzania laevis* (Pisces, Molidae). *Bull Mar Sci* 27:448–466.
- Leis JM. 1984. Tetraodontiformes: relationships. In: Moser HG, Richards WJ, Cohen DM, Fahay MP, Kendall AW, Richardson SL, editors. *Ontogeny and systematics of fishes*. Lawrence, KS: Allen Press. p 459–463.
- Leis JM, Carson-Ewart BM. 2002. The larvae of Indo-Pacific coastal fishes. Leiden: Brill.
- Mabee PM, Crotwell PL, Bird NC, Burke AC. 2002. Evolution of median fin modules in the axial skeleton of fishes. *J Exp Zool (Mol Dev Evol)* 294:77–90.
- Matsuoka M. 1982. Development of vertebral column and caudal skeleton of the red sea bream, *Pagrus major*. *Jpn J Ichthyol* 29:285–294.
- Matsuoka M. 1985. Osteological development in the red sea bream, *Pagrus major*. *Jpn J Ichthyol* 32:35–51.
- Matsuura Y, Katsuragawa M. 1985. Osteological development of fins and their supports of larval grey triggerfish, *Balistes capricus*. *Jpn J Ichthyol* 31:411–421.
- Monod T. 1968. Le complexe urophore des poissons téléostéans. *Mém IFAN* 81:1–705.
- Nelson JS. 1994. *Fishes of the world*, 3rd ed. New York: John Wiley & Sons.
- Patterson C. 1975. The braincase of pholidophorid and leptolepid fishes, with a review of the actinopterygian braincase. *Philos Trans R Soc Lond B* 269:275–579.
- Patterson C. 1977. Cartilage bones, dermal bones and membrane bones, or the exoskeleton versus the endoskeleton. In: Andrews MS, Miles RS, Walker AD, editors. *Problems in vertebrate evolution*. Linn Soc Symp Ser 4:77–121.
- Potthoff T. 1975. Development and structure of the caudal complex, the vertebral column, and the pterygiophores in the blackfin tuna (*Thunnus atlanticus*, Pisces, Scombridae). *Bull Mar Sci* 25:205–231.
- Potthoff T. 1980. Development and structure of fins and fin supports in dolphin fishes *Coryphaena hippurus* and *Coryphaena equiselis* (Coryphaenidae). *Fish Bull* 78:277–312.
- Potthoff T, Kelley S. 1982. Development of the vertebral column, fins and fin supports, branchiostegal rays, and squamation in the swordfish, *Xiphias gladius*. *Fish Bull* 80:161–186.
- Potthoff T, Tellock JA. 1993. Osteological development of the snook, *Centropomus undecimalis* (Teleostei, Centropomidae). *Bull Mar Sci* 52:669–716.
- Potthoff T, Richards WJ, Ueyanagi S. 1980. Development of *Scombrobrax heterolepis* (Pisces, Scombrobracidae) and comments on familial relationships. *Bull Mar Sci* 30:329–357.
- Potthoff T, Kelley S, Moe M, Young F. 1984. Description of porkfish larvae (*Anisotremus virginicus*, Haemulidae) and their osteological development. *Bull Mar Sci* 34:21–59.
- Potthoff T, Kelley S, Javech JC. 1986. Cartilage and bone development in scombroid fishes. *Fish Bull* 84:647–678.
- Potthoff T, Kelley S, Saksena V, Moe M, Young F. 1987. Description of larval and juvenile yellowtail damselfish, *Microspathodon chrysurus*, Pomacentridae, and their osteological development. *Bull Mar Sci* 40:330–375.
- Potthoff T, Kelley S, Collins LA. 1988. Osteological development of the red snapper, *Lutjanus campechanus* (Lutjanidae). *Bull Mar Sci* 43:1–40.
- Rauther M. 1925. Die Syngnathiden des Golfes von Neapel. Berlin: R. Friedländer & Sohn.
- Rosen DE. 1984. Zeiforms as primitive plectognath fishes. *Am Mus Novitates* 2782:1–45.
- Schmalhausen JJ. 1912. Zur Morphologie der unpaaren Flossen. I. Die Entwicklung des Skelettes und der Muskulatur der unpaaren Flossen der Fische. *Z wiss Zool* 100:509–587.
- Schultze H-P, Arratia G. 1988. Reevaluation of the caudal skeleton of some actinopterygian fishes. II. *Hiodon*, *Elops*, and *Albula*. *J Morphol* 195:257–303.
- Taki Y, Kohno H, Hara S. 1986. Early development of fin-supports and fin-rays in the milkfish *Chanos chanos*. *Jpn J Ichthyol* 32:413–420.
- Taylor WR, Van Dyke GC. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium* 9:107–119.
- Tyler JC. 1970. The progressive reduction in number of elements supporting the caudal fin of fishes of the order Plectognathi. *Proc Acad Nat Sci Phila* 122:1–85.
- Tyler JC. 1980. Osteology, phylogeny, and higher classification of the fishes of the order Plectognathi (Tetraodontiformes). NOAA Tech Rep NMFS Circ 434:1–422.
- Winterbottom R. 1974. The familial phylogeny of the Tetraodontiformes (Acanthopterygii: Pisces) as evidenced by their comparative myology. *Smithson Contr Zool* 155:1–201.