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A comparative ontogenetic study of the tetraodontiform caudal complex

Peter Konstantinidis[1] and G. David Johnson[2]

[1]Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK; [2]Department of Zoology, Division of Fishes, Smithsonian Institution, P.O. Box 37012, National Museum of Natural History, MRC 0159, Washington, District of Columbia 20013-7012, USA

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Abstract

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Interpretation of the caudal complex of adult Tetraodontiformes has proven problematic because of the consolidation of the component elements. Here, we show that an ontogenetic approach offers considerable elucidation of the homology of the caudal complex, resulting in a new understanding of the grundplan of these fishes. The reductions of structures of the caudal complex are interpreted in a phylogenetic context. The caudal skeleton of larval triacanthodids resembles that of many adult percomorphs; however, during subsequent development epural 3 disappears, while epural 2 is reduced so that it can hardly be distinguished from the uroneural remnants. Juvenile triacanthids have an epural 2 that is lost in ontogeny, and the cartilaginous parhypural becomes integrated into the large hypural plate. In ostraciids and diodontids, the parhypural is absent throughout development. The hypural plates of adult balistids, monacanthids and tetraodontids have a conspicuous diastema between the dorsal and ventral portions. However, in early stages of the former two, the dorsal and ventral portions are continuous in cartilage proximally and remain fused in the adults. In tetraodontids, the two hypurals are separate from their initial appearance in cartilage and never fuse, raising the question of homology of the individual hypurals among the different families.

Peter Konstantinidis, Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK. E-mail: Peter.Konstantinidis@uni-jena.de

'*This type of research [i.e. morphological work] is laborious and requires specialized training, especially in the dissection and identification of minute nubbins of developing cartilage and bone that are usually overlooked by reasonable people.*'

(Leis *et al.* 1997; in Proceedings of the symposium Fish Larvae and Systematics: Ontogeny and Relationships).

Introduction

The Tetraodontiformes are a small order of highly derived teleosts, which comprise nine families with around 350 species (Nelson 2006). Members of the Tetraodontiformes can be found in all major marine habitats. Representatives of the families Tetraodontidae, Diodontidae, Balistidae, Monacanthidae and Ostraciidae are mostly coral reef associated and occur in the Atlantic, Indian and the Pacific Ocean. The Triacanthidae inhabit the shallow waters over sandy and muddy bottoms of the Indo-Pacific Ocean. A few members of the Ostraciidae, Diodontidae inhabit the epipelagic zone and *Triodon macropterus*, the sole member of the family Triodontidae the benthos between 10–300 m deep. One species of the Triacanthodidae (*Atrophacanthus japonicus*) can also be found in the bathypelagic zone down to 2000 meters. Members of the Molidae undertake vertical migrations in the pelagic zone worldwide. Only representatives of the Tetraodontidae have invaded freshwaters of South East Asia, Africa and South America. The diversity of the Tetraodontiformes is also reflected in the wide size range of members of this order. It includes one of the largest and most massive of recent teleosts, the ocean sunfish, *Mola mola*, with a length of up to 3 m and a weight of up to 2300 kg, and at the same time one of the smallest, the dwarf puffer, *Carinotetraodon travancoricus*, with a standard length (SL) of around 25 mm.

Our understanding of the inter- and intrarelationship of Tetraodontiformes is in flux, and many hypotheses have been published in recent years (Winterbottom 1974; Tyler 1980; Leis 1984; Rosen 1984; Tyler and Sorbini 1996; Holcroft 2005; Tyler and Holcroft 2007; Alfaro *et al.* 2007; Yamanoue

et al. 2008). The most comprehensive cladistic analysis was conducted by Santini and Tyler (2003; Fig. 1) based on 210 morphological characters of 20 extant and 36 fossil taxa. In their phylogenetic hypothesis, the Triacanthodidae are the sistergroup of two suborders, the Balistoidei and the Tetraodontoidei. The Balistoidei contain the Triacanthidae, Balistidae, Monacanthidae and Ostraciidae, and the Triodontidae, Molidae, Tetraodontidae and Diodontidae are combined in the Tetraodontoidei. The primary incongruence is in the phylogenetic position of the Ostraciidae and of *Triodon*. The ostraciids have been variously assigned to the Balistoidei (Fig. 1; Winterbottom 1974; Tyler 1980; Tyler and Sorbini 1996, Santini and Tyler 2003) or to certain families of the Tetraodontoidei (Leis 1984; Britz and Johnson 2005b; Holcroft 2005; Alfaro *et al.* 2007; Yamanoue *et al.* 2008). *Triodon* has been, because of its unique combination of primitive and derived characters, a long-standing subject of debate. Although placed at the base of the Tetraodontoidei, *Triodon* was also variously assigned to different other groups (Dareste 1850; Regan 1902; Holcroft 2005; Alfaro *et al.* 2007; Yamanoue *et al.* 2008).

Because of its complexity, the caudal skeleton of teleost fishes has often been used as a source of phylogenetic information. The caudal skeleton of primitive taxa comprises many individual elements (e.g., *Hiodon* and *Elops*; Schultze and Arratia 1988), and there is a general reductive trend in that number as we ascend the teleost tree, caused by the fusion and/or loss of elements. A similar trend of loss and consolidation of caudal skeleton elements often occurs independently within smaller taxonomic groups (e.g., families), as they become more specialized in their locomotory modes (e.g., *Thunnus atlanticus*; Potthoff 1975), and/or for no obvious functional reasons. Gosline (1961: 268) stated for the Percoidei that '*this fusion progresses over different routes in various groups. However, the endpoint, i.e. a fused hypural plate, is approximately the same in all*.' The 'endpoint' in the sense of Gosline is the consolidation of the caudal complex through fusion of elements so that it consists of a few large elements as seen in scombroids, e.g., *Thunnus* sp. (Potthoff 1975), some Gasterosteiformes and Syngnathiformes, *Acanthocephola limbata*, *Poecilia reticulata* or *Diodon* sp. (for a full spectrum see Monod 1968; Fujita 1990).

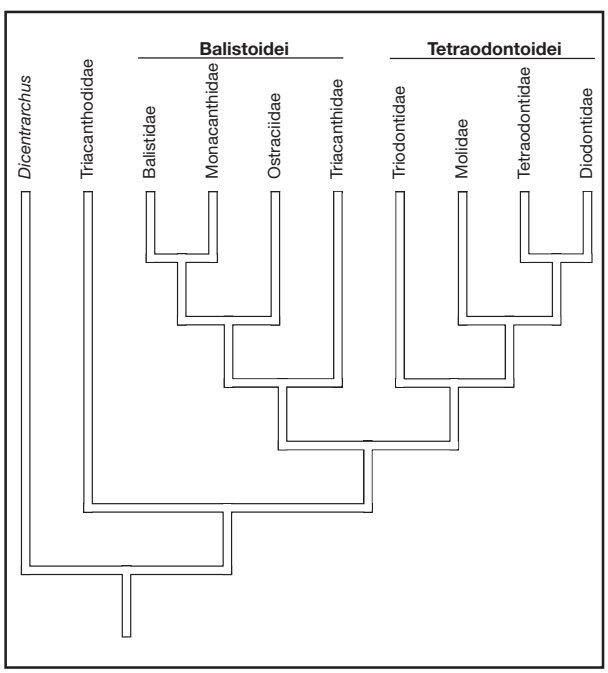

Fig. 1—Phylogenetic hypothesis of the order Tetraodontiformes. The generally accepted phylogenetic hypothesis based on Santini and Tyler (2003).

The caudal skeleton of adult representatives of Tetraodontiformes has been studied by various authors (Monod 1968; Tyler 1968, 1970, 1980; Rosen 1984; Fujita 1990) and thoroughly by Tyler (1970), who reviewed the caudal skeletons of 136 representatives of the Tetraodontiformes and discussed their remarkable diversity and the progressive reduction of elements in an evolutionary context within the order. Adults of the tetraodontiform family Triacanthodidae exhibit a caudal skeleton that is similar to a typical percomorph caudal complex (Tyler 1970, 1980), while members of the more derived tetraodontiform families have a caudal skeleton characterized by a high degree of fusion and reduction of elements, seen at its most extreme in the Molidae, which lack the caudal skeleton entirely (Johnson and Britz, 2005), because of developmental truncation.

Despite extensive published descriptions of the caudal complex in adult tetraodontiforms, its development has only been described for two members of the Tetraodontidae (Fujita 1992; Britz and Johnson 2005a), one balistid (Matsuura and Katsuragawa 1985) and the molid *Ranzania laevis* (Johnson and Britz 2005).

In morphological complexes such as the caudal skeleton, the Weberian apparatus of the Otophysi and the skull and head musculature of teleosts in general, ontogenetic information has often provided the most insightful data, concerning the composition and homology of complex structures (Schultze and Arratia 1988, 1989; Arratia and Schultze 1991; Britz and Johnson 2005b; Johnson and Britz 2005; Britz and Hoffmann 2006; Hoffmann and Britz 2006; Geerinckx and Adriaens 2007; Hilton *et al.* 2007; Hilton and Johnson 2007; Huysentruyt *et al.* 2007; Geerinckx *et al.* 2009; Hilton and Britz 2010; Johnson and Britz 2010; Konstantinidis and Harris 2010). The diversity of the tetraodontiform caudal skeleton makes it makes it an ideal complex for ontogenetic studies.

The goal of this study was to analyse the ontogeny of the caudal skeleton of tetraodontiforms and interpret it within a phylogenetic context. The result is a new understanding of the grundplan of the caudal skeleton for the entire order. Possible evolutionary scenarios of the reduction of the caudal

fin elements are discussed in the light of the topology of Santini and Tyler (2003; Fig. 1).

Material and Methods

Specimens were cleared and double stained (c&s) for bone and cartilage following Taylor and Van Dyke (1985). For histological transverse sections (10 μm), a specimen of *Atrophacanthus japonicus* (see Material examined) was embedded in Paraffin and stained by the Azan-Domagk procedure (Romeis 1986).

Photographs of most of the cleared and double-stained specimens were taken either with a ProgRes C 12 plus digital camera attached to a Zeiss Tessovar microscope or with a Zeiss digital camera attached to a Zeiss Discovery V20 dissecting scope. Photographs of the histological sections and the smaller cleared and double-stained specimens were taken with a Nikon Coolpix E4500 attached to a Nikon Microscope Eclipse E600.

For the analysis of the character evolution of the epurals, uroneurals, parhypural, and the hypural series, a simple taxa/character matrix was created and parsimoniously mapped onto the topology of Santini and Tyler (2003) in MacClade (Maddison and Maddison 2005).

Institutional abbreviations

AMS, Australian Museum, Sydney; ANSP, Academy of Natural Science, Philadelphia; BMNH, The Natural History Museum, London; NSMT, The National Museum of Science and Nature, Tokyo; SEAMAP, Southeast Area Monitoring and Assessment Program Ichthyoplankton Archiving Center, Fish and Wildlife Research Institute; USNM, National Museum of Natural History, Smithsonian Institution.

Material examined

Perciformes

Moronidae. *Dicentrarchus labrax* (Linnaeus), BMNH 2009.3.16.16–24, 28 mm SL, c&s.

Tetraodontiformes

Triacanthodidae. *A. japonicus* (Kamohara), BMNH 1987.1.23, one specimen, 58 mm SL, c&s; two specimens, uncatalogued (Chiba Institute of Technology), 14.5–18 mm SL, c&s; one specimen, property of the University of Tuebingen, 15 mm SL, serial sectioned. *Hollardia* sp. (Poey), uncatalogued, 4.9 mm SL, c&s. *Paraholardia* sp. (Fraser–Brunner), one specimen, uncatalogued, 3.9 mm notochord length (NL), c&s. *Triacanthodes anomalus* (Temminck & Schlegel), three specimens, ANSP 101257, 54–60 mm SL, c&s. *Hollaria hollardi* (Poey), one specimen, USNM 187811, photograph only; *Triacanthodes ethiops* (Alcock), one specimen, USNM 93491, photograph only.

Triacanthidae. *Tripodichthys oxycephalus* (Bleeker), two specimens, BMNH 2006.3.280, 16–33 mm SL, c&s. *Tripodichthys* sp. (Tyler), AMS I. 24205–36, 3.9 mm NL, c&s.

Balistidae. *Balistapus undulatus* (Park), four specimens, uncatalogued (NSMT), 3.4 mm NL – 35 mm SL, c&s.

Monacanthidae. *Monacanthus ciliatus* (Mitchill), one specimen, BMNH 1976.6.3, 37 mm SL, c&s. *Stephanolepis* sp. (Gill), one specimen, SEAMAP 10741, 5.4 mm SL, c&s; two specimens, uncatalogued (NSMT), 3.9 mm NL & 14.4 mm SL, c&s.

Ostraciidae. *Lactophrys* sp. (Swainson), one specimen, SEAMAP 25817, 3.5 mm NL, c&s; one specimen, SEAMAP 25776, 4.0 mm SL, c&s; one specimen, SEAMAP 22682, 11.3 mm SL, c&s; one specimen, uncatalogued (SEAMAP), 8.0 mm SL, c&s.

Tetraodontidae. *Carinotetraodon irrubesco* (Tan), uncatalogued, one specimen, 25 mm SL, c&s; *Monotrete suvatii* (Sontirat), uncatalogued, seven specimens, 4.2 mm NL – 16.4 mm SL, c&s. Adult specimens were kept and spawned in captivity. Larvae were preserved on a daily basis in 4% formalin and 2 days later transferred into 70% ethanol.

Diodontidae. *Diodon hystrix* (Linnaeus), SEAMAP 14506, 5.5 mm SL; SEAMAP 22672, 15 mm SL, c&s.

Figure abbreviations

For the additional cartilages in the caudal skeleton that support some of the fin rays, the general term distal caudal radial (adopted from Nybelin 1971) is used.

For the cartilaginous precursor and subsequent ossified element, the same abbreviation is used. The abbreviations, nspu2 and hspu2 apply to both the neural spine and arch and hemal spine and arch, respectively.

Distal caudal radial	dcr
Epural (cartilage)	ep
Hemal spine and arch of preural centrum 2	hspu2
Hemal spine and arch of preural centrum 3	hspu3
Hypural (cartilage)	hu
Neural spine and arch of preural centrum 2	nspu2
Neural spine and arch of preural centrum 3	nspu3
Parhypural (cartilage)	phu
Parhypurapophysis	pphu
Preural centrum 2	pu2
Preural centrum 3	pu3
Ural centrum	uc
Uroneural	un

Terminology of the hypurals

In Teleostei in which the number of hypurals is reduced to fewer than five, the homology assignment and with that the terminology of the remaining hypurals can be problematic. In most previous studies of such taxa (see citations in the

Discussion), a large hypural plate has been interpreted as a result of fusion of several hypurals, but it often remains unclear whether a phylogenetic or ontogenetic fusion has led to the reduction of hypurals.

Herein, where there is no evidence of ontogenetic fusion, the terminology (number 1–5) of each hypural plate follows the hypothesis that hypural elements have been lost rather than fused to form a compound element. We do this because it is not possible to test the hypothesis of phylogenetic fusion, while allowing that such fusion is a possibility (see Discussion about the homology of the hypurals).

In the text, the term diastema refers to the space that divides the supports for the upper lobe of the caudal fin from the lower and is usually located between hypurals 2 and 3 (Fig. 2).

Results – Comparative Ontogeny and Review of the Literature of the Caudal Complex

Because the quantity and the developmental degree of the semaphoronts used in this study differ greatly between the taxa, the results for the Tetraodontiformes are arranged according to anatomical structures, rather than taxonomically. The figures, however, are arranged in taxonomic context following the phylogenetic hypothesis of Santini and Tyler (2003; Fig. 1).

The caudal skeleton of a basal percomorph

In basal percomorphs, such as the moronid sea bass, *Dicentrarchus labrax*, three vertebrae are associated with the caudal complex: preural centrum 3, preural centrum 2 and the ural centrum. The neural spine of preural centrum 3 is long and supports some of the procurrent fin rays. The neural spine of preural centrum 2 is short and does not reach the procurrent fin rays but is formed by a lamina of membrane bone. The hemal spines of preural centrum 2 and 3 remain autogenous from the associated centra. The proximal tips of both hemal spines support some of the procurrent and two principal caudal fin rays. The ural centrum tapers caudally and is associated with the parhypural and five hypurals. The three epurals are median elements that are preformed in cartilage. The three epurals decrease in length posteriorly. The two uroneurals are paired elements that consist entirely of membrane bone (Fig. 2).

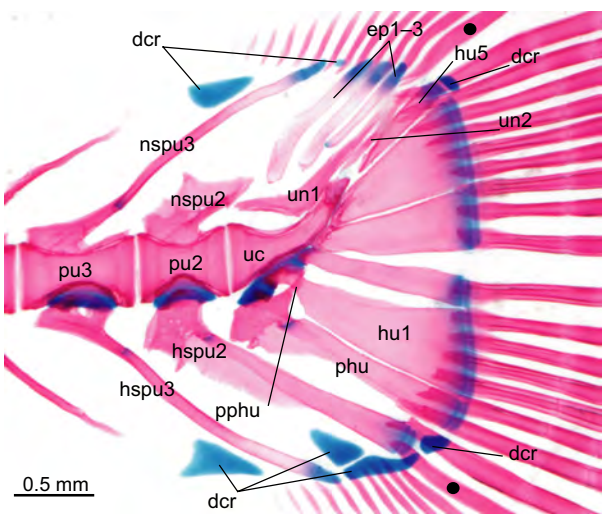

Fig. 2—Moronidae. *Dicentrarchus labrax* (28 mm SL). The black dots indicate the uppermost and lowermost principal caudal fin rays.

On the ventral side of the vertebral column, a parhypural and five hypurals are present, and all six elements are of endoskeletal origin. The parhypural is the last element in the series of hemal spines and arches that provide a canal for the caudal artery. The parhypural bears on each side a hypurapophysis for insertion of the flexor ventralis hypochordal and longitudinalis muscles. The cartilaginous precursors of the parhypural and the first two hypurals are connected via a cartilaginous band proximally. Several distal caudal radials support the fin rays of the caudal skeleton: a large triangular one just anterior to neural spine 3 and hemal spine 3, a smaller one between the tip of neural spine 3 and epural 1, and at the distal tip of hypural 5; two between hemal spines 2 and 3; and one at the tip of hemal spine 2. Seventeen principal caudal fin rays are present, flanked dorsally and ventrally by numerous procurrent fin rays.

The caudal skeleton of the Tetraodontiformes

Uroneurals. Larval *Parahollardia* sp. (Fig. 3A) and *Hollardia* sp. (Fig. 3B) have a thin uroneural just posterior to the epurals. In juvenile and adult *Atrophacanthus japonicus*, small and irregularly shaped slivers of bone represent the uroneural(s) (Fig. 3C,D,F–I). These bony fragments are of small size and not necessarily arranged pairwise (Fig. 3C,D,F). Because of a lack of larvae within the size range of 5–15 mm, the documentation of the fragmentation of the uroneural(s) was not possible in this study. Tyler (1970) interpreted the single uroneural of some specimens as uroneural 2, and in cases in which additional fragments are present, these have been interpreted as remnants of uroneural 1 and probably 3.

Based on the ontogenetic material examined herein and the re-examination of the triacanthodids that Tyler (1970) used, as well as the photographs of the specimens used by Rosen (1984), the uroneural of triacanthodids is best interpreted as a single uroneural that represents uroneural 1.

In the Triacanthidae, the single uroneural is a small, stout and somewhat triangular element (Tyler 1968). In *Tripodichthys oxycephalus*, the left and right halves are fused in the midline anteriorly and diverge caudally to make space for the neural canal (Fig. 4B–D). The members of the remaining tetraodontiform families lack uroneurals.

Epurals. In the triacanthodid *Parahollardia* sp. at 3.9 mm, epurals 1 and 2 are present in cartilage of which epural 2 is the smaller (Fig. 3A). Epural 2 is arrested in its development and

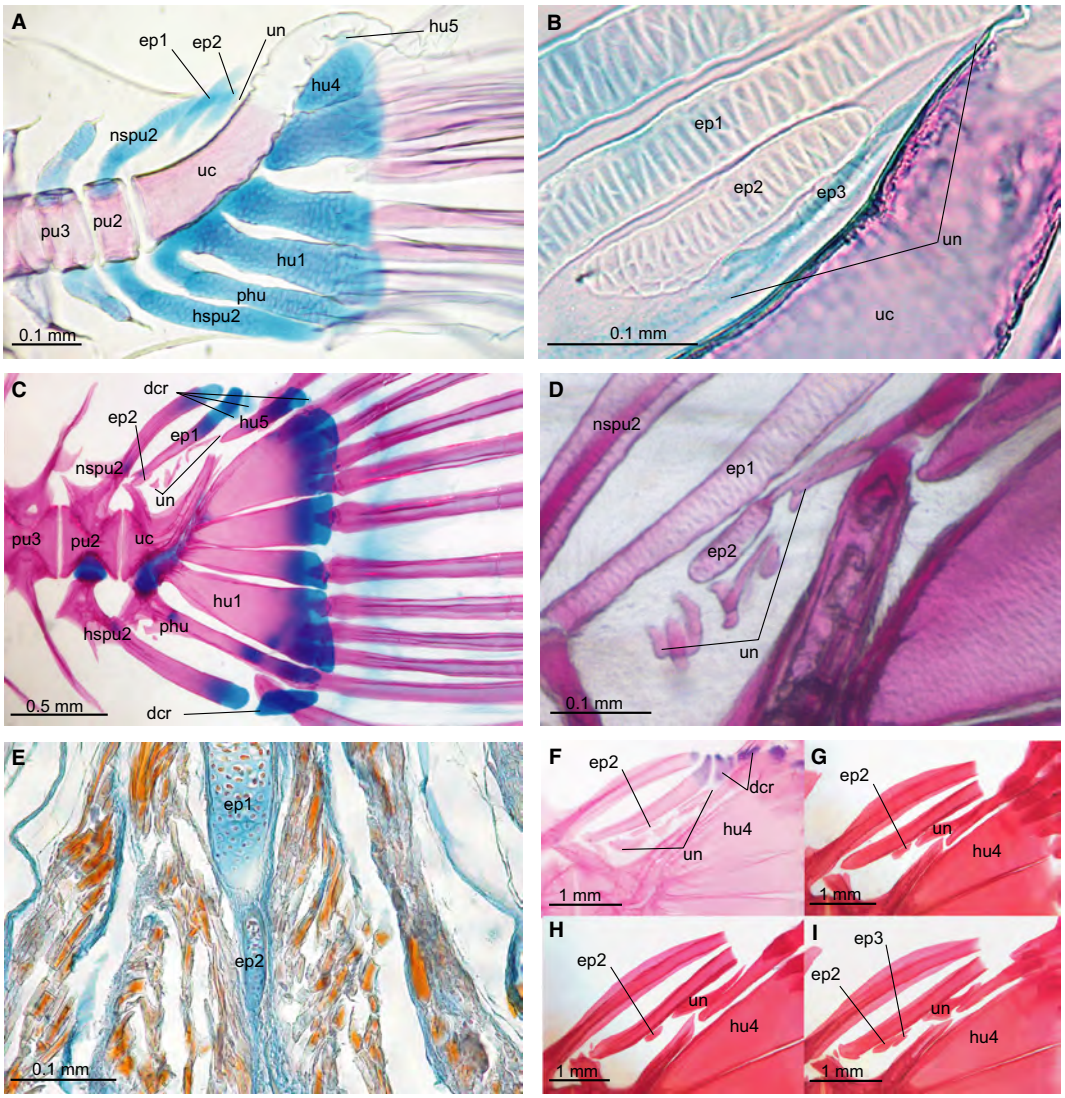

Fig. 3—Triacanthodidae. **A**. *Parahollardia* sp. (3.9 mm SL). **B**. Close-up of the dorsal region of *Hollardia* sp. (4.9 mm SL). **C**. *Atrophacanthus japonicus* (18 mm SL). **D**. Close-up of the dorsal region of *A. japonicus* (14.5 mm SL). **E**. Serial section of an *A. japonicus* (15 mm SL). **F**. Close-up of the dorsal region of *A. japonicus* (58 mm SL). **G–I**. Close-up of the dorsal region of the caudal complex of three *Triacanthodes anomalus* (54–60 mm SL).

remains as a small knob of perichondral bone in adult specimens (Fig. 3C–I).

For adult Triacanthodidae, various authors have reported a single epural that serves at the same time as an autapomorphic character for the order Tetraodontiformes (Tyler 1968, 1970, 1980; Rosen 1984; Fujita 1990; Santini and Tyler 2003). A re-examination of the two photographs of the two Rosen specimens (not shown) and the Tyler specimens of *T. anomalus* (Fig. 3G–I) reveals that Rosen (1984) failed to identify each of the small unlabelled elements as epural 2, while Tyler (1970) apparently misinterpreted epural 2 as a fragment of a uroneural. Because what appears to be the same element we described earlier in *Parahollardia* develops in cartilage and is thus unequivocally an epural, we conclude that these small unpaired elements also represent epural 2.

In an unidentified triacanthodid larva (*Hollardia* sp.) of a slightly larger size, a third epural is present (Fig. 3B). This epural 3 consists of only a few cartilage cells and is located between the two halves of the uroneural. Either this epural 3 fails to ossify or it becomes indistinguishable from the uroneural slivers in older specimens (see section on uroneurals). We interpret a small element just ventral to epural 2 in one of the *T. anomalus* (Fig. 3I) as a third epural. The occurrence of an epural 3 is apparently intraspecifically variable in this taxon, while epural 2 is constantly present in the specimens investigated herein.

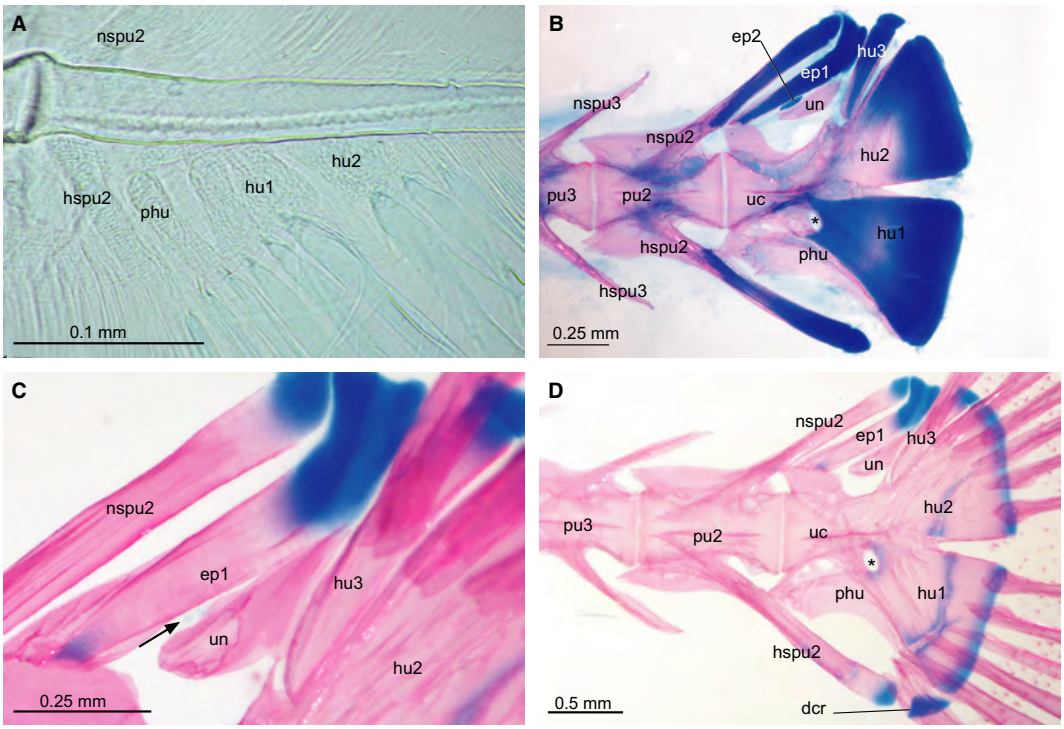

Fig. 4—Triacanthidae. **A**. *Tripodichthys* sp. (3.9 mm NL). **B**–**D**. Two developmental stages of *Tripodichthys oxycephalus* **B**. 16 mm SL; **C**. Close up of **D**. 33 mm SL. Arrow indicates the blue-stained remnant of the resorbed epural 2. Asterisk marks the foramen for the caudal artery.

Members of the family Triacanthidae have two epurals. In the smallest triacanthid available for clearing and staining, an epural cartilage is not yet developed (Fig. 4A). In a 16-mm *T. oxycephalus*, two epurals are present in cartilage, of which epural 1 is nearly as long as the second preural neural spine, whereas epural 2 is a very small, elongate nubbin (Fig. 4B) wedged between epural 1 and the single uroneural. Obviously, epural 2 fails to ossify and is absent in the 33-mm *T. oxycephalus* (Fig. 4C,D). Accordingly, Tyler (1970, 1980) did not identify a second epural in adult triacanthids.

Balistidae have a single epural, already present in cartilage in a 3.5 mm *Balistapus undulatus* (Fig. 5B). The epural appears as a cartilaginous rod just posterior to the second preural neural spine (Fig. 5B–D). In adults, it is expanded by laminae of membrane bone anteriorly and posteriorly (Fig. 5D). According to Matsuura and Katsuragawa (1985), the epural apparently develops at a later stage (4.9 mm) in *Balistes capriscus* than in *B. undulatus*, although the subsequent development is identical. The development of the epural in Monacanthidae resembles that of the balistids, but the distal end of the epural is broader (Fig. 5E–H).

In the 3.5-mm *Lactophrys* sp., an epural is not yet developed (Fig. 6A), and the first and only vestige of a free epural in ostraciids was seen in the 4 mm specimen of *Lactophrys* (Fig. 6B), wherein a comma-shaped cartilaginous rod is positioned dorsal to the flexed notochord. In larger specimens, a free epural is apparently absent (Fig. 6C,D). In larger specimens (Fig. 6C,D), the ural centrum bears a horizontally oriented bony ridge, but there is no clear evidence to suggest that this represents an ossified epural. Based on adult specimens, Tyler (1970) concluded that in ostraciids the epural is fused to the ural centrum. Although not impossible, it seems unlikely because the fusion of the epural to the ural centrum is not reported for any other teleost so far.

In the tetraodontid *Monotrete suvattii*, the single epural appears as the last dorsal element of the caudal complex as a rhomboidal-shaped cartilaginous block between the neural spine of preural centrum 2 and the notochord (Fig. 7D). In older specimens, the distal tip broadens and is closely associated with the second preural neural spine anteriorly and the flexed notochord posteriorly (Fig. 7E–G). In adults, the base of the epural articulates with the dorsal ridge on the ural centrum. The development of the epural is identical to that of *Monotrete leiurus* (Britz and Johnson 2005a) and *Takifugu niphobles* (Fujita 1992). However, according to Fujita (1992), later stages of *T. niphobles* differ from the two *Monotrete* species, in that the distal part of the cartilaginous epural is fused to the distal part of the enlarged neural spine of preural centrum 2.

The smallest diodontid available has a small cartilaginous element just above the notochord (Fig. 8A) that we interpret as an epural. The next available stage has a fully ossified

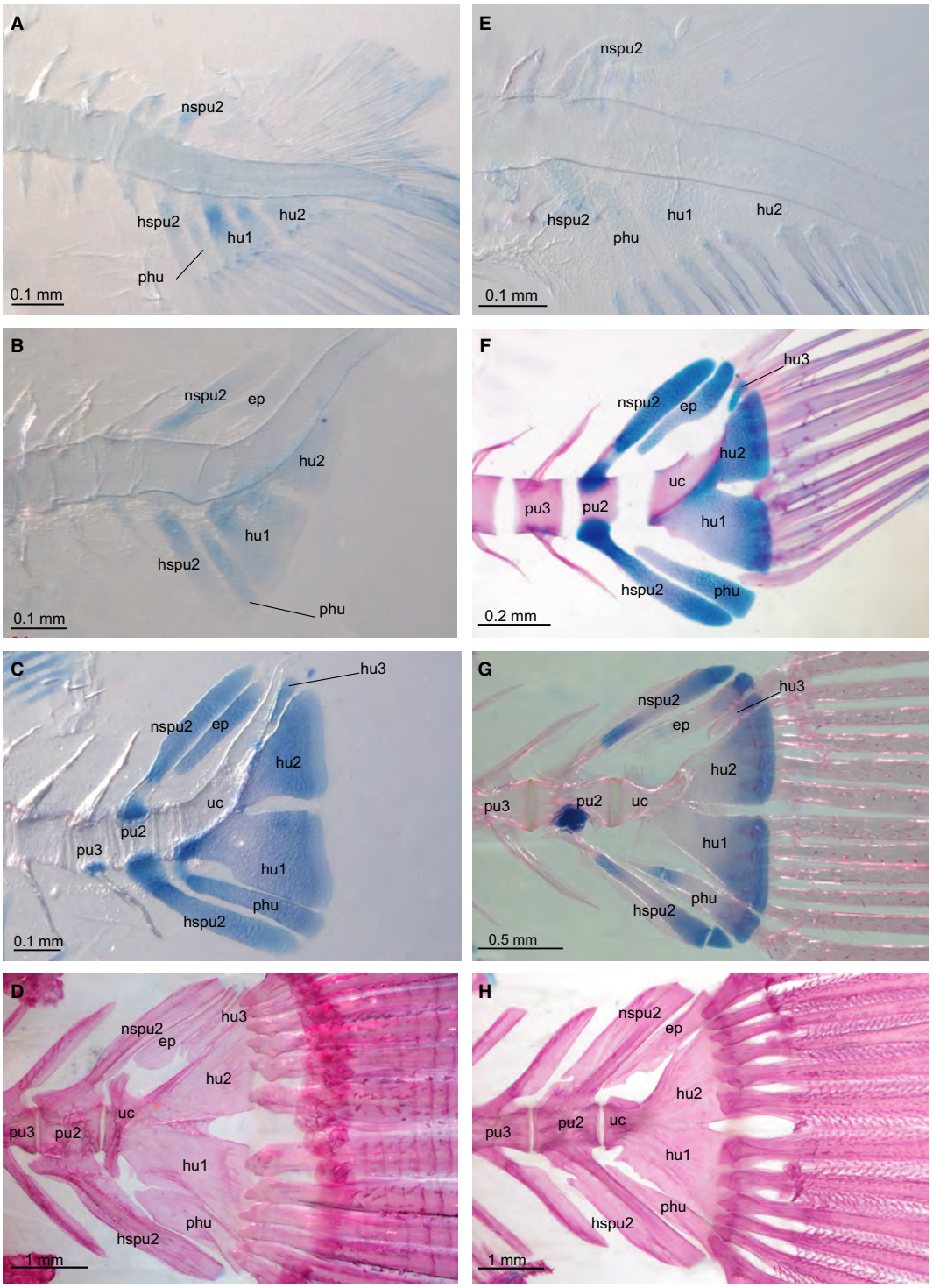

Fig. 5—Balistidae and Monacanthidae. **A**–**D**. Developmental series of *Balistapus undulatus*. **A**. 3.5 mm NL; **B**. 3.2 mm SL; **C**. 4.2 mm SL; **D**. 35 mm SL. **E**–**G**. Developmental series of *Stephanolepis* sp. **E**. 3.9 mm NL; **F**. 5.4 mm SL; **G**. 14.4 mm SL. **H**. *Monacanthus* sp. (37 mm SL).

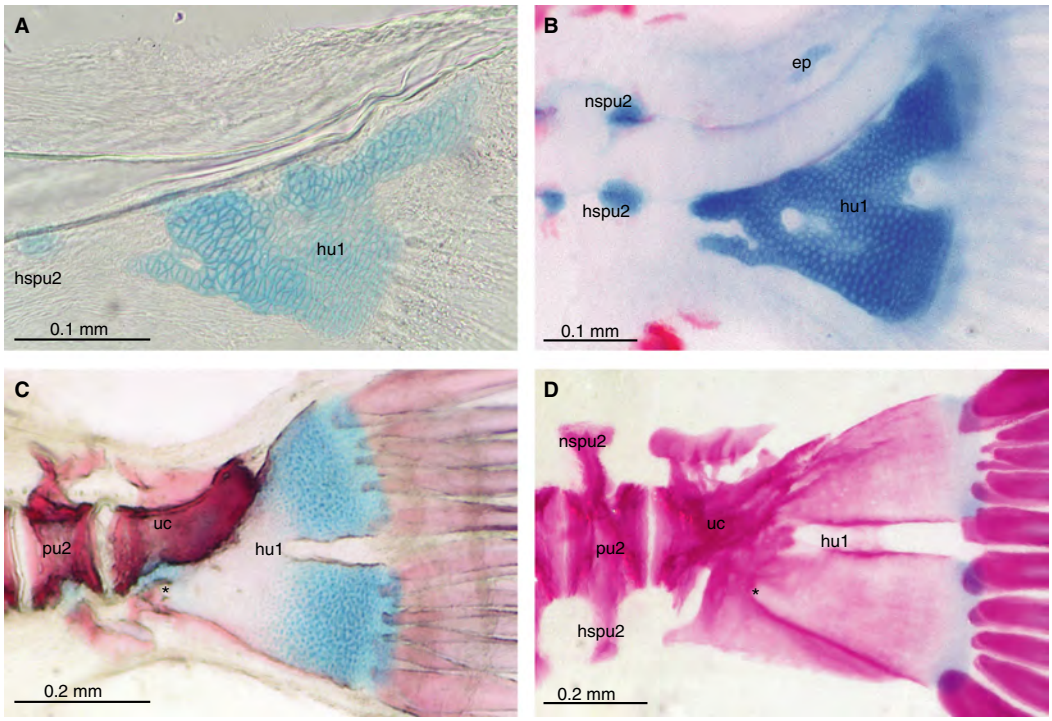

Fig. 6—Ostraciidae. Developmental series of *Lactophrys* sp. **A**. 3.5 mm NL; **B**. 4.0 mm NL; **C**. 8.0 mm SL; **D**. 11.3 mm SL. Asterisk marks the foramen for the caudal artery.

hypural plate with a large lamina of membrane bone at its anterodorsal margin (Fig. 8B), and no real trace of an epural can be observed. It is not clear whether the epural will be reduced or incorporated into the caudal complex. Tyler (1970) assumed that the epural in adult diodontids is fused to the neural spine of preural centrum 2.

Ural centrum, parhypural and hypurals. As observed by Tyler (1968, 1970), adult specimens of triacanthodids have an autogenous parhypural and five individual hypurals, of which hypural 5 is the smallest. In the 3.9-mm *Parahollardia* sp., the parhypural and the five hypurals are already present (Fig. 3A). The bases of the cartilaginous parhypural, hypural 1 and hypural 2 are fused to each other, while the cartilaginous precursors of hypurals 3–5 remain separate, even in larger triacanthodids (Fig. 3A,C). In juvenile and adult *A. japonicus*, the ossified parhypural and hypurals 1 and 2 are separate, and only a remnant of the cartilage remains as evidence of the early fusion of the three elements (Fig. 3C).

The 3.9 mm *Tripodichthys* sp. has two cartilaginous hypurals of which hypural 1 is fused with the parhypural proximally (Fig. 4A). In the triacanthids, the number of hypurals is reduced to three of which hypural 1 and 2 form a large plate (Fig. 4D; Tyler 1968, 1970). It is uncertain whether the cartilaginous hypurals fuse proximally prior to ossification or remain separate until ossification begins. The diastema at the posterior margin marks the position where the two hypurals are fused (Fig. 4D). In the 16-mm *T. oxycephalus*, the parhypural is completely fused to the hypural plate which, in turn, has started to fuse to the ural centrum (Fig. 4B–D). The foramen for the caudal artery (Fig. 4B,D) within the lower part of the hypural plate is the only evidence that a separate parhypural was present in an earlier stage (Fig. 4D), and Tyler (1968, 1970) suspected the fusion of the parhypural to the lower hypural because of the exit of the caudal artery in the anteroventral part of the hypural plate. An anterior extension of laminar membrane bone extends the hypural plate anteroventrally. A small, third hypural is present just dorsal to the large hypural plate (Fig. 4B–D).

Two of the three hypurals are already present in a 3.5-mm *B. undulatus* (Fig. 5A). At this stage, the parhypural is foreshortened and does not enclose the caudal artery. The first hypural bears a large foramen (Fig. 5A). Before ossification begins, hypurals 1 and 2 fuse together proximally (Fig. 5B–D). A small hypural 3 develops after flexion of the notochord but remains much smaller than the first two hypurals (Fig. 5C,D). In the 4.2 mm *B. undulatus*, the cartilaginous parhypural is connected to hypural 1. In the 4.2-mm specimen, the parhypural encloses the caudal artery, but in later stages the parhypural is again foreshortened (Fig. 5D; Tyler 1970; Matsuura 1979); instead, the ural centrum develops a ventrally oriented crest of membrane bone that encloses the caudal artery (Fig. 5D), here referred to as the 'hemal arch element' following the nomenclature introduced by Matsuura

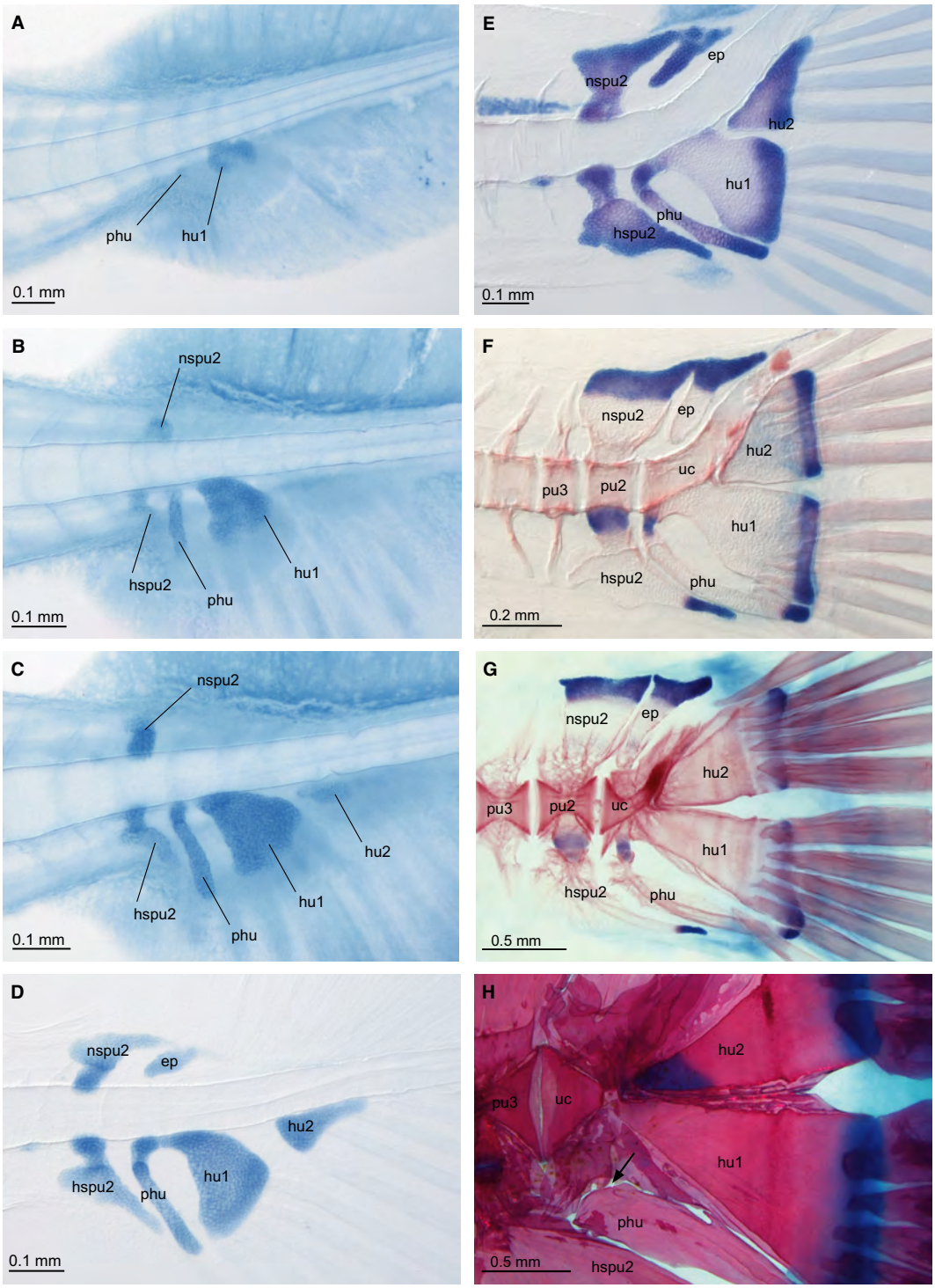

Fig. 7—Tetraodontidae. **A**–**G**. Developmental series of *Monotrete suvattii*. **A**. 4.6 mm NL; **B**. 4.7 mm NL; **C**. 4.7 mm NL; **D**. 4.9 mm NL; **E**. 4.4 mm SL; **F**. 5.7 mm SL; **G**. 16. 4 mm SL. **H**. *Carinotetraodon irrubesco* (25 mm SL). Arrow marks the gap between the foreshortened parhypural and the ural centrum.

(1979). The large hypural plate, consisting of two hypurals, fuses to the ural centrum (Fig. 5D). Matsuura and Katsuragawa (1985) observed four hypural anlagen in larval *Balistes capriscus* of which the lower two fuse together, forming the first hypural of the adults. This is in contrast to our observation in *B. undulatus*. However, the foramen we observed in

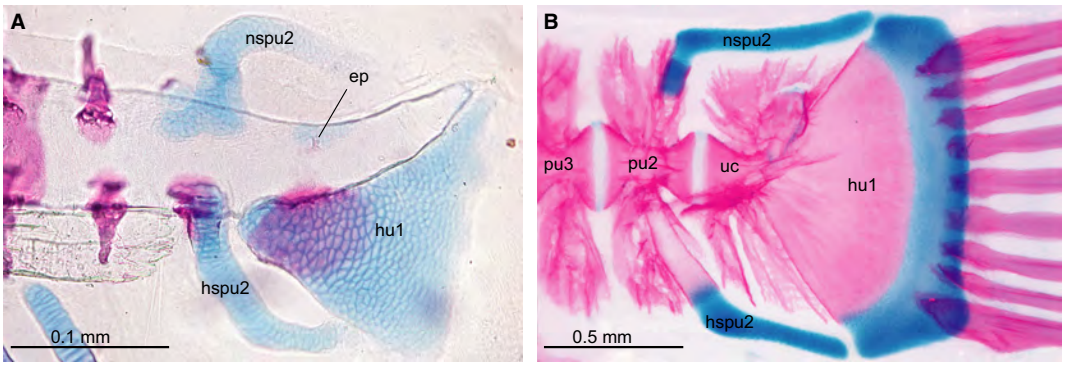

Fig. 8—Diodontidae. **A**. and **B**. Two developmental stages of *Diodon hystrix* **A**. 5.5 mm SL; **B**. 15.0 mm SL.

the smallest *B. undulatus* (Fig. 5A) might indicate a fusion of two individual hypurals as described by Matsuura and Katsuragawa (1985) for *B. capriscus*.

The development of the caudal complex in *Stephanolepis* sp. is very similar to that in *B. undulatus*, but some differences are notable. The parhypural never encloses the caudal artery and contact with the cartilaginous hypural 1 is never established. Hypural 1 does not bear a foramen at any stage of ontogeny (Fig. 5E–H). In adult monacanthids, the hemal arch element is missing or less developed than in the balistids (Matsuura 1979; Tyler 1980). The caudal skeleton of some monacanthid genera lacks hypural 3 as, for example, in *Monacanthus ciliatus* (Fig. 5H; Tyler 1970; Matsuura 1979). It is possible that this difference among the genera might yield phylogenetic information. As far as is known, the balistids, as the proposed sistergroup, have a hypural 3, and therefore, the monacanthid genera that possess hypural 3 probably represent the plesiomorphic state.

Larvae of *Lactophrys* sp. smaller than 3.5 mm do not show any elements of the caudal complex. In the 3.5 mm *Lactophrys* sp., the cartilaginous precursor for the hemal arch is present, and there is an irregular-shaped structure that appears to be the only element that develops in the region of the parhypural and hypurals (Fig. 6A). Anteriorly, the hypural plate has an anterodorsally oriented process (Fig. 6A,B). There is a single origin of ossification of the hypural plate (Fig. 6C). The element becomes larger and foreshadows the shape of a hypural plate seen in adult specimens (Fig. 6B–D). A foramen present in the 4 mm specimen (Fig. 6B) is absent in the smaller as well as in larger specimens. The anterior margin of the cartilaginous process is connected with the base of the hypural plate via a lamina of membrane bone, bearing a foramen that encloses the caudal artery (marked by an asterisk in Fig. 6C,D). Tyler (1970), based on adult specimens, hypothesized a fusion of the parhypural to the hypural plate, but as shown here a separate parhypural never develops. In the largest stage, the hypural plate is fused to the ural centrum (Fig. 6D).

In *M. suvattii*, the first elements to appear are the parhypural and hypural 1 (Fig. 7A). They are followed by the hemal- and neural arch of preural centrum 2 in the next larger specimen (Fig. 7B). At 4.7 mm, the second hypural develops as a roughly triangular-shaped cartilage (Fig. 7C). In the same stage, the proximal base of the parhypural, which forms the hemal canal, curves towards the proximal base of the lower hypural (Fig. 7C) and fuses with it (Fig. 7D). The ossification of the hemal canal of the parhypural occurs from both sides (Fig. 7E,F). A perichondral ossification with its origin at hypural 1 points ventrad but does not approach the parhypural ossification (Fig. 7F), leaving a remnant band of cartilage. The lower hypural fuses to the ural centrum, while the upper hypural remains separate (Fig. 7F,G).

The development of the parhypural and the hypurals are in accordance with the developmental sequence of these elements reported for *M. leiurus* (Fig. 7; Britz and Johnson 2005a). The first elements that appear are the parhypural and hypural 1 (Fig. 7A). They are followed by the neural and hemal spines of preural centrum 2 (Fig. 7B). Hypural 2 is the last element in the ventral series that develops (Fig. 7C,D). In contrast to the puffers of the genus *Monotrete*, Fujita (1992) noted that in *Takifugu niphobles* a second hypural appears before the parhypural. Furthermore, he reported for *T. niphobles* three separate hypurals, of which the first two fuse to form a compound element (his 'hypural 1+2').

The parhypural of adults of the tetraodontid genus *Carinotetraodon* does not bear a hemal canal; instead, a lamina of membrane bone projects ventrad and encloses the caudal artery, similar to the situation in the balistids and monacanthids (Figs 5D,F–H and 7H). The lack of the hemal canal of the parhypural in *Carinotetraodon* and the balistid/monacanthid clade is clearly convergent but helps to distinguish puffers of the genus *Carinotetraodon* from *Monotrete*.

In the caudal region of our 5.5 mm *Diodon hystrix*, a single element (referred to as the hypural plate) is present and has already started to ossify from a single ossification centre anterodorsally (Fig. 8A). The hypural plate does not show any separation of elements nor a foramen for the caudal artery. The 15 mm *D. hystrix* resembles the adult situation closely, and the ossification of the hypural plate is nearly complete (Fig. 8B). The anteroventral margin of the hypural plate is extended by a lamina of membrane bone. In front of the

hypural plate, an unpaired process projects ventrally and fills the gap between the hemal spine of preural centrum 2 and the hypural plate. This ventral outgrowth might become confluent with the hypural plate because it is not present in larger specimens. There are no traces of a cartilaginous preformed parhypural in these two stages. Tyler (1970) noted that in diodontids the parhypural is either fused to the hypural plate in *Diodon holacanthus* or to the hemal spine of preural centrum 2 in *Diodon jaculiferus*, and *Chilomycterus tigrinus*. The ontogeny of the caudal skeleton of *D. hystrix*, however, shows no trace of a parhypural.

Distal caudal radials. Within tetraodontiforms, distal caudal radials are only present in triacanthodids and triacanthids. However, distal caudal radials have not been described for members of these two families so far. Either they were overlooked because of lack of cartilage staining or they were perceived as the distal tips of the corresponding underlying elements of the caudal skeleton.

Atrophacanthus japonicus has four distal caudal radials (Fig. 3C). Ventrally, a large distal caudal radial at the tip of the hemal spine of preural centrum 2 articulates with the lowermost caudal fin ray. Dorsally, there are three distal caudal radials, of which two are situated between epural 1 and hypural 5 in the 18 mm specimen and at the distal tip of epural 1 in the 58 mm specimen. In the larger specimen, the distal caudal radials articulate with the uppermost fin ray (Fig. 3C,F). The third distal caudal radial is the smallest and is situated on the tip of hypural 5. The distal caudal radial at the tip of hypural 5 can be homologized with one in *D. labrax*. The other two in the dorsal part of the caudal skeleton in *A. japonicus* are not present in *D. labrax*. The large ventral distal caudal radial in *A. japonicus* is problematic to homologize with one of the two ventral distal caudal radial between hemal spine of preural centrum 3 and hemal spine of preural centrum 2 in *D. labrax*.

In the triacanthid *Tripodichthys oxycephalus*, the dorsal distal caudal radials are reduced, and only a single distal caudal radial is present in the ventral part that articulates with the lowermost fin ray (Fig. 4D). This distal caudal radial in *T. oxycephalus* is homologous with the single ventral one of *A. japonicus*.

Neural and hemal spines and arches of preural centrum 2. In the larval specimen of *Parahollardia* sp., the cartilaginous neural and hemal arches of preural centrum 2 are already fully developed (Fig. 3A). The hemal spine is more massive and slightly longer than the neural spine. In *A. japonicus*, the hemal and neural spines are perichondrally ossified except at their distal tips. In adult triacanthodids, the neural arch fuses to preural centrum 2, whereas the hemal arch remains free (Tyler 1970). The tips of the neural and hemal spines do not reach the fin rays (Fig. 3C,F).

In the smallest *Tripodichthys* sp., the cartilaginous precursor of the hemal spine of preural centrum 2 is already fully grown, while its associated neural spine has not yet reached its full length (Fig. 4A). The distal tip of the hemal spine approaches the most ventral fin ray in triacanthodids (Fig. 4D; Tyler, 1970). In triacanthids, the hemal and neural arches of the first and second preural centrum are coalesced with their associated centra (Fig. 4D).

In the smallest *Balistapus undulatus*, the long hemal spine on preural centrum 2 articulates with the most anterior fin ray (Fig. 5A), whereas in adults it loses contact with the most ventral ray (Fig. 5D; Tyler 1970; Matsuura 1979). Matsuura and Katsuragawa (1985) described a similar development of the second preural hemal and neural arches and spines in *B. capriscus*. Both spines are extended by laminae of membrane bone in fully developed specimens. The neural arch fuses to preural centrum 2, whereas the hemal arch does not (Fig. 5D; Tyler 1970; Matsuura 1979).

In monacanthids, the development of the hemal and neural arches of preural centrum 2 and their associated spines resemble that described for the balistids (Fig. 5E–H).

The Ostraciidae differ from all other tetraodontiform families in having reduced neural and hemal spines on preural centrum 2. The first element to appear in association with preural centrum 2 is the hemal arch (Fig. 6A). In the larger stage, the hemal arch has developed (Fig. 6B). In the next larger stage small, ill-defined neural and hemal spines are present and are probably not preformed in cartilage (Fig. 6C,D). The hemal arch remains free from the second preural centrum (Fig. 6D). According to Klassen (1995), the articulation of the hemal arch with preural centrum 2 and the length of the hemal spine have diagnostic potential and can be used for distinguishing members of the subfamily Aracaninae (long and remains free from preural centrum 2) and the ostraciine genus *Lactophrys* (remains free from preural centrum 2) from all other Ostraciinae (short and fused to preural centrum 2).

In the Tetraodontidae, the hemal arch and spine of preural centrum 2 appear at roughly the same time, although the neural spine lags a bit behind the hemal spine (Fig. 7B). The hemal and neural spines of preural centrum 2 become prominent elements of the caudal skeleton. The cartilaginous precursors of both spines are equal in size until the 4.4 mm specimen (Fig. 7E). During subsequent development, the neural spine becomes more massive than the hemal spine (Fig. 7F,G). The neural arch fuses to the centrum, whereas the hemal arch remains free. The development of the hemal and neural arches and spines of preural centrum 2 of *Monotrete suvattii* resembles that of *M. leiurus* as it was described by Britz and Johnson (2005a).

In *Diodon hystrix*, the distal half of the hemal and neural spine of preural centrum 2 is bent at almost 90° to its base (Fig. 8B). The neural spine is not expanded as in the tetraodontids. In diodontids, the anterior part of the neural arch and spine is extended by a lamina of membrane bone. Both the hemal and neural arches fuse to their associated centrum.

Caudal fin rays. The plesiomorphic situation for the percomorphs is a complement of 17 fin rays (Fig. 2; Johnson and

Patterson 1993). Tetraodontiformes have a reduced number of fin rays. Triacanthodids, triacanthids, balistids, monacanthids and *Triodon* have 12 caudal fin rays, the highest number within the order (Figs 3C, 4D and 5D, H; Tyler 1970; Matsuura 1979), and they are equally distributed over the upper and lower lobe. Among members of the family Ostraciidae, the number of fin rays is variable. Ostraciinae have ten equally distributed rays (e.g., *Lactophrys* sp.; Fig. 6D), whereas members of the Aracaninae have an additional fin ray associated with the lower lobe (Tyler 1970). Tetraodontids have consistently 11 caudal fin rays, of which five are associated with the upper and six with the lower hypural (Fig. 7G) (Tyler 1970). The nine caudal fin rays in *D. hystrix* are equally distributed over the homogenous hypural plate (Fig. 8B). As far as known, only *Chilomycterus reticulatus* differs from the other diodontid species in having ten fin rays (Richards 2006).

Discussion

Before we present our interpretation of the evolution of the individual structures, we feel it is important to address the complex issue of the homology of the hypural elements. As shown in the following paragraphs, there are different interpretations of the evolutionary history of the hypural plates of the taxa in which fewer than five hypurals are present. The significance of the full neural spine on preural centrum 2 is also discussed separately.

Homology of the hypurals

Among the most common reductions in the caudal skeleton of teleosts is the consolidation of hypurals to one or two large plates, e.g., myctophids (Fujita 1990), gobiesocids (Konstantinidis and Conway 2010), scombroids (Potthoff 1975; Fujita 1990), some zeiforms (Tyler *et al.* 2003), some labrids (Fujita 1990), gobiids (Fujita 1990) and some carangids (Fujita 1990; Hilton *et al.* 2010).

The tetraodontiform caudal skeleton shows a wide range of diversity, from the plesiomorphic condition with five hypurals to a single large plate in the ostraciids and diodontids (and the total absence of the caudal skeleton in the molids; Johnson and Britz 2005). In taxa with a consolidated caudal skeleton (e.g., to one or two large hypural plates), the identity of the remaining hypurals is problematic. In this study, the hypurals are sequentially numbered from the most ventral to the most dorsal. This is a simple, practical approach and does not automatically imply homology of hypurals among different taxa.

It has been assumed that the consolidated caudal skeleton evolved either through fusion of individual hypurals into a compound element or through loss of some of the hypurals. However, the caudal skeletons of adults cannot be differentiated from each other.

In cases in which an ontogenetic fusion of hypurals has been documented, e.g., in the blackfin tuna (Potthoff 1975), the swordfish (Potthoff and Kelley 1982), dolphin fishes (Potthoff 1980) and some jacks and pompanos (Hilton and Johnson 2007) the situation is obvious. However, in tetraodontiforms, there is no evidence of ontogenetic fusion of hypurals. Among the taxa of the order with a consolidated caudal skeleton either an evolutionary fusion of hypurals to a compound element or a loss of hypurals has to be proposed. However, a fusion or a loss of hypurals *ab initio* during evolution cannot directly be tested, and only indirect aspects such as topology, shape, relation to other structures, and/or position, *etc.* ('*principe de connexion*': Geoffroy 1830; '*Kriterium der Lage*': Remane 1952; '*special quality*': Patterson 1982) might give an indication of the trajectory (either fusion or loss) that has caused the reduction of hypurals.

Regarding particular cases within the tetraodontiforms, indirect indicators to assign the homology of hypurals are as follows:

1. The fusion of the proximal ends of the parhypural to hypural 1 as well as the cartilaginous connection between hypural 1 and hypural 2. The early fusion of the cartilaginous hypurals 1 and 2 appears to be highly conserved among teleosts and can be found throughout teleostean diversity (Potthoff 1975; Fritzsche and Johnson 1980; Potthoff 1980; Potthoff *et al.* 1980; Potthoff and Kelley 1982; Potthoff *et al.* 1987, 1988; Potthoff and Tellock 1993; Bird and Mabee 2003; Hilton and Johnson 2007) and is demonstrated herein for the triacanthodids (Fig. 3A) as well. Fusion at their first appearance has never been known to occur between hypural 2 and 3 or any other hypurals.
2. The position of the diastema. In teleosts with a more primitive organization of the caudal fin elements (Figs 1 and 2; Monod 1968; Fujita 1989), the diastema is always located between hypurals 2 and 3.
3. The size of the hypurals. In taxa with a reduced number of hypurals, the remaining hypurals are usually larger, and that can be interpreted as the result of a fusion of individual hypurals to a compound element.

The two cartilaginous hypurals in the balistid/monacanthid clade (Fig. 5; the triacanthids are uncertain because of an incomplete ontogenetic series) are connected via a cartilaginous bridge, as is the case for hypural 1 and 2 of many teleosts. This supports the loss of the more dorsally located hypurals (either hypurals 4–5 in balistids and some monacanthids or 3–5 in all other monacanthids) rather than the fusion of individual hypurals. However, this evidence is contradicted by the position of the diastema. One has to postulate a shift of the diastema from the position between hypural 2 and 3 to hypural 1 and 2 and a change in the size of the remaining hypurals. The situation in the tetraodontids contradicts that found in balistids and monacanthids. In *M. suvattii*, the two hypurals are never connected via cartilage at any stage of development (Fig. 7) and together with the size of the remaining hypurals support the fusion theory (the ventral hypural plate of 1 + 2 and the dorsal hypural plate of 3–5).

Ontogeny as a source to identify homologous structures fails to be of great use because a fusion *ab initio* of hypurals cannot be observed. The problem of the homology of the hypurals in the derived clades of the Tetraodontiformes remains ambiguous.

A consolidation of the caudal complex is a general theme within the Teleostei, independent of their phylogenetic relationships (various citations herein). We believe, based on our results, that both the phylogenetic fusion and the reduction of elements are potential programs that have led to an identical appearance of the caudal skeleton in these teleostean groups. Therefore, the use of the number of hypurals to reveal homologous hypurals across taxa is suspicious.

Character evolution of the caudal skeleton

The results of this study are discussed in reference to the phylogenetic hypothesis for tetraodontiforms proposed by Santini and Tyler (2003). Santini and Tyler's study is based on 210 morphological characters exemplified by 36 fossil and 20 extant taxa. This is, by far, the most comprehensive phylogenetic hypothesis published on this group.

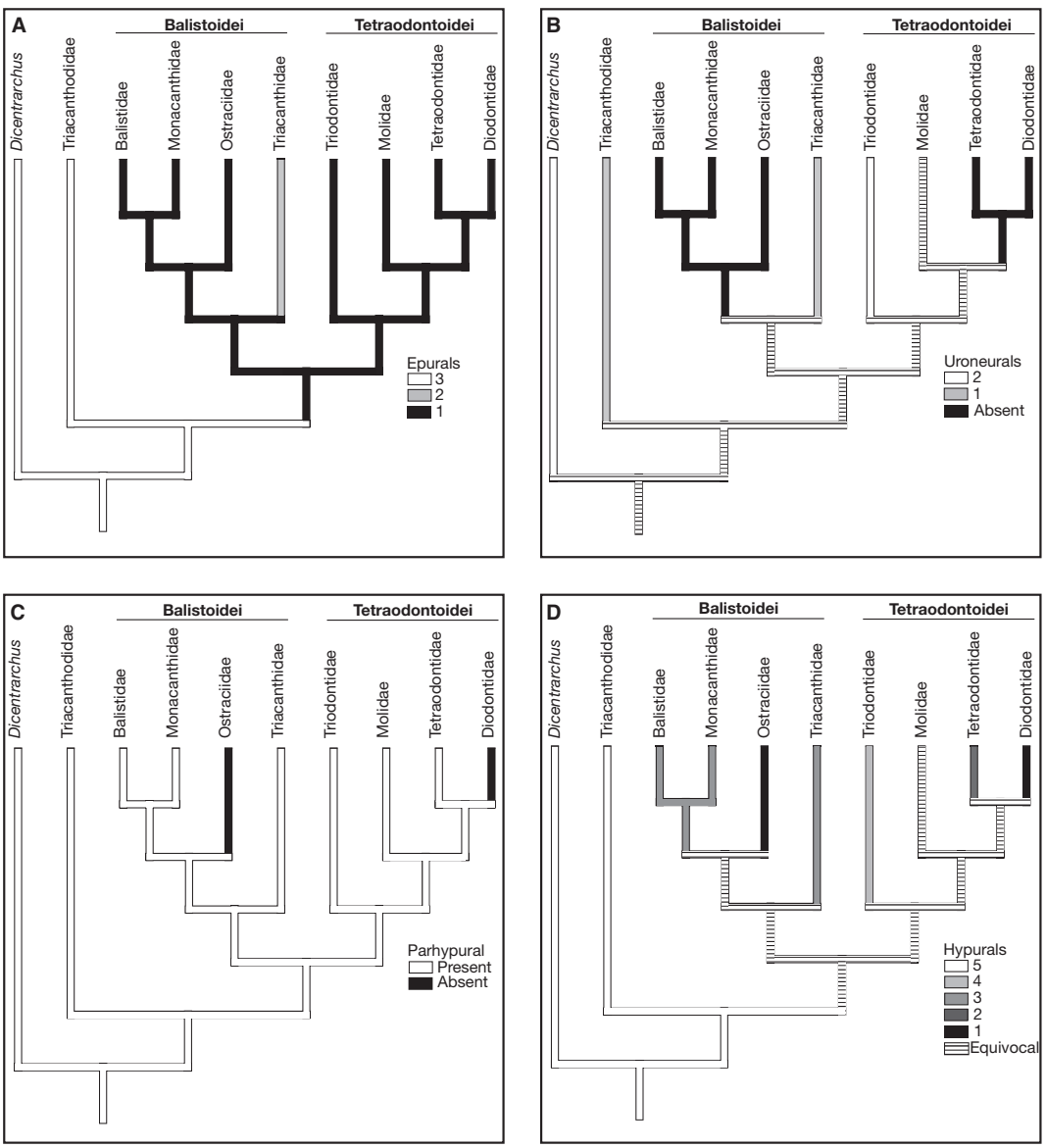

Fig. 9—Character evolution. The four major elements in the caudal skeleton of the Tetraodontiformes mapped onto Santini and Tyler's (2003) phylogenetic hypothesis. **A**. Epurals, **B**. Uroneurals, **C**. Parhypural and **D**. Hypurals. Colour of the branches indicates the different character states. Striated branches in **B**. and **D**. show equivocal alternatives.

The evolution of the caudal skeleton is reconstructed, and the character states for the epurals, uroneurals, the parhypural and the hypurals are mapped parsimoniously at nodes onto Santini and Tyler's phylogenetic hypothesis (the elongated neural spine of preural centrum 2 is discussed but not mapped onto the phylogenetic tree). The characters (number of epurals, number of uroneurals, presence or absence of a parhypural and number of hypurals) are treated as independent evolutionary events and therefore mapped separately. The absence of the caudal complex in the Molidae is most likely not the result of a subsequent loss of individual elements, rather of a single event. Herein, we treated the situation in *Ranzania* as not applicable in the analyses of the character evolution. For a detailed anatomical analysis of the ontogeny of the clavus, see Johnson and Britz (2005).

Extended neural spine of preural centrum 2. A long neural spine on preural centrum 2 characterizes all tetraodontiforms but the ostraciids. A small neural spine on preural centrum 2 is primitive for teleosts and resembles the plesiomorphic situation for derived clades, such as the Acanthomorpha and Percomorpha as well (Patterson 1968; Rosen and Patterson 1969).

Although there is no consensus concerning the relationship among the tetraodontiform families (Winterbottom 1974; Tyler 1980; Leis 1984; Rosen 1984; Santini and Tyler 2003; Holcroft 2004; Alfaro *et al.* 2007; Yamanoue *et al.* 2008), it is unlikely that the ostraciids represent the most basal taxon. The small preural neural spine on preural centrum 2 of the ostraciids is therefore secondary, and the long neural spine of preural centrum 2 represents the plesiomorphic character state.

Among other characters, Rosen (1984) and Tyler *et al.* (2003) designated a fully developed neural spine of preural centrum 2, among other characters, as a synapomorphy for the Tetraodontiformes and Zeiformes. However, even though the sistergroup relationship of the tetraodontiforms is ambiguous (Wiley and Johnson 2010), the fully developed second preural neural spine is treated here as an autapomorphy of the tetraodontiforms.

Epurals. The most parsimonious reconstruction of the evolution of the epurals requires only two steps (characters are unordered) to describe the evolution of the epurals. Three epurals are present in the triacanthodids and the outgroup. The first step implies a reduction of two epurals, i.e. from three epurals directly to one on the branch leading to the common ancestor of the Balistoidei/Tetraodontoidei. The third step involves a reversal of a rudimentary epural 2 in the branch leading to the triacanthids that is reduced in the adult again. Although one step longer, the reconstruction with characters ordered (e.g., from three epurals to two epurals, from two to one and one to absence) provides an alternative explanation: the triacanthids, with two epurals, represent the subsequent step of the reduction of the epurals and an independent loss of epural 2 occurs in the Balistoidei above the triacanthids and in the Tetraodontoidei. Although less parsimonious, it seems more plausible that two epurals are present at the base of the Balistoidei, and the reduction to a single epural appears within the Balistoidei (ostraciids, balistids and monacanthids) and convergently at the base of the Tetraodontoidei (Fig. 9A).

Uroneurals. The most parsimonious reconstruction requires four steps and produces 21 equally parsimonious possibilities to explain the evolution of the uroneurals. According to Tyler (1970, 1980), *Triodon* bears two uroneurals, which represents the most plesiomorphic state within the order and resembles the situation of the outgroup. One uroneural is present in the triacanthodids and triacanthids, whereas all other taxa lack a uroneural. The distribution of the uroneurals makes it difficult to interpret; according to the character optimization, *Triodon* either retains a second uroneural while the triacanthodids have independently lost a second uroneural or, which is unlikely, *Triodon* gains independently a second uroneural (Fig. 9B).

Parhypural. The most parsimonious reconstruction requires two steps and produces a single parsimonious option to express the evolution of the parhypural. The parhypural is reduced two times independently (excluding the Molidae) in the Ostraciidae and Diodontidae (Fig. 9C).

Hypurals. The most parsimonious reconstruction requires five steps and produces 32 equally parsimonious explanations for the evolution of the hypurals. Five hypurals is the primitive condition of the Tetraodontiformes. It is most likely that at the base of the Balistoidei, the hypurals are reduced to three (this is the present condition in triacanthids, balistids and some monacanthids) and then further reduced to a single large element in the Ostraciidae. The Tetraodontoidei show a wide variety of hypural reduction as well. According to Tyler (1970, 1980), *Triodon*, as the basal member of the Tetraodontoidei, has four hypurals. The subsequent step requires the loss of two hypurals and leads to the situation of the Tetraodontidae with two large hypural plates. The reduction to one hypural plate in the Diodontidae appears convergently with the Ostraciidae (Fig. 9D).

Conclusions

Ontogenetic information reveals that the caudal skeleton of the Triacanthodidae is more plesiomorphic than previously reported. It is actually more comparable to a generalized percomorph, such as the common sea bass, *Dicentrarchus labrax*. Nonetheless, the tetraodontiform caudal skeleton bauplan is derived in several features, namely the long neural spine of preural centrum 2 (vs. short), a single uroneural (vs. two) and the lack of procurrent caudal fin rays.

Comparative morphological and molecular studies have failed to fully resolve the interrelationships of the families and

conclusively identify the sistergroup of the Tetraodontiformes. Despite the fact that ontogenetic information cannot discriminate between phylogenetic fusion and loss of bony elements, we have demonstrated here that it is a critical source of information for the elucidation of homology in the composition of complex structures, such as the caudal skeleton. An ontogenetic approach is fundamental for and often the only morphological pathway towards new insight into and solution to longstanding systematic problems, such as those presented by the complex evolutionary history of the Tetraodontiformes, one of the most comprehensively studied groups of acanthomorph fishes.

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