

**Morphological and Systematic Studies of the Musculoskeletal
System of the Highly Derived Teleost Order Tetraodontiformes**

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P. Konstantinidis and M. P. Harris

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A Comparative Ontogenetic Study of the Tetraodontiform Caudal Skeleton

P. Konstantinidis and G. David Johnson

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The Ontogeny of the Jaw Apparatus and the Suspensorium of the Tetraodontiformes

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Dr. Matthew P. Harris contributed to the publication with his participation in the experimental design and helped to write the manuscript.

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Dr. G. David Johnson provided rare larval material and helped in the data analysis.

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11 ZUSAMMENFASSUNG

Obwohl die Tetraodontiformen (Kugelfische und ihre Verwandten) zu den am besten untersuchten Knochenfischen gehören, sind das Schwestergruppenverhältnis der Ordnung und die Phylogenie innerhalb der Ordnung weitgehend ungeklärt. Zum Teil kann dieser Umstand dadurch erklärt werden, dass die Adulti der Tetraodontiformen, sowohl eidonomisch als auch im anatomischen Detail sehr unterschiedlich sind. Komplexe morphologische Strukturen, wie der *Musculus adductor mandibulae*, die Kiefer einschließlich des Hyopalatinalbogens und das Schwanzflossenskelett, sind (beispielsweise) hochgradig abgeleitet und erschweren die Identifikation von homologen Merkmalen im zwischenartlichen Vergleich.

Meine Dissertation beschäftigt sich daher mit der vergleichenden Ontogenese des *M. adductor mandibulae*, der Kiefer einschließlich des Hyopalatinalbogens und des Schwanzflossenskelettes der Tetraodontiformen. Ein Vorteil des ontogenetischen Ansatzes ist es, dass die Ausbildung komplexer morphologischer Strukturen vom Entstehen bis zur endgültigen Ausdifferenzierung verfolgt werden kann. Damit können primäre und sekundäre Homologiekriterien (Nelson, 1978) gewonnen werden, die im Adultzustand nicht fassbar sind.

Publikation I - The Same but Different: Ontogeny and Evolution of the *Musculus adductor mandibulae* in the Tetraodontiformes

Der *M. adductor mandibulae* der Teleostei dient primär als Kieferschließer und ist häufig in phylogenetischen Arbeiten besprochen worden. Innerhalb der Tetraodontiformes ist der Muskel hoch spezialisiert und sehr unterschiedlich ausgebildet. Der *M. adductor mandibulae* der Triacanthodidae ist am einfachsten und gleicht mit seinen drei Teilen dem eines basalen Percomorphen; das stimmt mit der mutmaßlich basalen Stellung des Taxons überein. Mit der Ausnahme der Triacanthidae, bei denen der *M. adductor mandibulae* sekundär vereinfacht ist (er besteht dort noch aus drei Portionen), ist dieser bei allen anderen Vertretern mehrfach untergliedert - in bis zu sechs Portionen bei Tetraodontiden und bis zu neun bei Monacanthiden.

Diese Komplexität des Muskels erschwert das Erkennen von homologen Muskelpartien bei Vertretern verschiedener Familien, wie frühere Arbeiten belegen, welche nur die anatomischen Verhältnisse der adulten Stadien zum Vergleich heranzogen (Friel and Wainwright, 1997; Winterbottom, 1974). Sowohl Winterbottom als auch Friel und Wainwright stützten ihre Aussagen über die Homologie einzelner Muskelpartien zum einen auf den Ursprung und Ansatz, zum anderen auf die Funktion bestimmter Portionen. Eine Muskelportion, die beispielsweise am Maxillare ansetzt, wird als A1 und eine die am Unterkiefer ansetzt, als A2 bezeichnet. Setzen mehrere Portionen am Maxillare an, gehen die Autoren von einem gleichen Ursprung dieser beiden Portionen aus (A1 α und A1 β bzw. A2 α und A2 β). Beide Arbeiten kommen jedoch zu unterschiedlichen Ergebnissen.

Um diese Homologie-Fragen zu klären, haben wir, mit Hilfe der Immunfluoreszenz und der Eigenfluoreszenz von Gewebe, Ontogenesestadien von jeweils einem Vertreter der Balistoidei (*Balistapus undulatus*) und Tetraodontoidei (*Monotrete suvattii*) analysiert. Mittels dieser vergleichend-ontogenetischen Untersuchung haben wir nachweisen können, dass A1 α und A2 α -Portionen und A1 β und A2 β -Portionen jeweils auf einen Ursprung zurückzuführen sind. Der ontogenetische Ansatz zeigt somit, dass das Kriterium der Lage oder auch der Funktion zur Beurteilung der Homologie einzelner Muskelpartien irreführend sein kann. Somit sind Ursprung und Ansatz und die Funktion von Muskelpartien wie im Falle des *M. adductor mandibulae* keine guten Kriterien für die Begründung von Homologien. Bei phylogenetisch-systematischen Fragestellungen muss daher die Ontogenese des *M. adductor mandibulae* im Vergleich analysiert werden.

Publikation II – A Comparative Ontogenetic Study of the Tetraodontiform Caudal Complex

Das Schwanzflossenskelett der adulten Tetraodontiformen ist durch Reduktionen geprägt und daher in einem so abgeleiteten Zustand, dass eine Interpretation und Identifikation der beteiligten Strukturen nur bedingt möglich ist (Tyler, 1970). Die Ontogenese des Schwanzflossenskelettes liefert Informationen über den Grundplan des Schwanzflossenskelettes der Ordnung und zeigt durch die Reduktion von skeletalen Elementen eine evolutive Tendenz zur Vereinfachung. Bezogen auf die Anordnung einzelner Strukturen gleicht das Schwanzflossenskelett früher ontogenetischer Stadien eines Triacanthoiden dem eines basalen Percomorphen; dies ist ein neuer Beleg für das Prinzip der Rekapitulation. Während der Ontogenese wird jedoch das Epurale 3 reduziert und das Epurale 2 ist in der weiteren Entwicklung gehemmt; es kann daher in späteren Stadien nicht mehr von den Relikten des einzigen vorhandenen Uroneurales unterschieden werden. Frühe Stadien der Triacanthidae besitzen zwei Epuralia, von denen sich das Epurale 2 nicht über die initiale Anlage hinaus entwickelt und später reduziert wird. Des Weiteren wird das Parhypurale in die Hypural-Platte integriert, sodass im adulten Zustand nur noch das Foramen für die Schwanzarterie einen Hinweis auf ein Parhypurale liefert. Zu keinem Zeitpunkt der Ontogenese besitzen Ostraciiden und Diodontiden ein Parhypurale. Adulte Vertreter der Balistiden und Monacanthiden auf der einen Seite und der Tetraodontiden auf der anderen haben ein Diastema, welches die Hypural-Platte in einen dorsalen und einen ventralen Abschnitt teilt. Die beiden Loben sind bereits in frühen Ontogenesestadien der Balistiden und Monacanthiden durch eine Knorpelbrücke verbunden, was die Hypothese einer Reduktion von den weiter caudal angeordneten Hypuralia 3 – 5 stützt, da im Grundplan der Teleostei nur die Hypuralia 1 und 2 eine knorpelige Verbindung eingehen. Bei den Tetraodontiden gehen die beiden Hypuralia zu keinem Zeitpunkt der Ontogenese eine knorpelige Verbindung ein.

Mit diesem erhobenen Sachverhalt stellt sich die Frage nach der Homologie der Hypuralia zwischen Vertretern der Balistiden und Monacanthiden einerseits und der Tetraodontidae andererseits. Entweder haben die Tetraodontiden die Knorpelbrücke reduziert oder aber Hypurale 1 und 2 sind

während der Evolution miteinander verschmolzen und in der Ontogenese nicht mehr nachweisbar. Zur Analyse der Evolution der Epuralia, Uroneuralia, Hypuralia und des Parhypurales haben wir mit Hilfe der Software MacClade eine Merkmals- und Taxa-Analyse entworfen und die Ergebnisse auf die phylogenetisch-systematische Hypothese von Santini und Tyler kodiert. Die Ergebnisse zeigen, dass die Reduktion der Elemente innerhalb der Balistoidei und der Tetraodontoidei auf einer Konvergenz beruhen.

Publikation III – The Ontogeny of the Jaw Apparatus and the Suspensorium of the Tetraodontiformes

Durch ihren besonderen Kieferapparat und Hyopalatinalbogen sind adulte Vertreter der Tetraodontiformen an durophage (hartschalige) Nahrung angepasst. Morphologische Indizien dafür sind die kurzen und starken Kiefer sowie die weit nach rostral verlagerte Pars quadrata/Quadratum; diese sind etwa in derselben vertikalen Ebene angeordnet wie die Pars autopalatina). Im Gegensatz dazu gleicht das Palatoquadratum von larvalen Vertretern der Tetraodontiformen dem von larvalen Percomorphen, die nicht an eine durophage Ernährungsweise angepasst sind: Die Pars quadrata liegt weit caudal der Pars autopalatina und ein schlanker Meckelscher Knorpel repräsentiert den Unterkiefer. Die ontogenetische Veränderung des Kieferapparates und des Hyopalatinalbogens während der Ontogenese weist auf eine Veränderung der Ernährungsweise während der Lebensgeschichte hin.

Innerhalb der Tetraodontiformen besitzen die Triacanthodidae noch einen vorstülpbaren Oberkiefer und einen beweglichen Hyopalatinalbogen. Durch die Reduktion des proximalen Teils des Autopalatinum, das dadurch den knöchernen Kontakt zum Hyopalatinalbogen verliert, besitzen Vertreter der Balistoidei sehr bewegliche Kiefer. Vertreter der Tetraodontoidei dagegen besitzen keine Einzelzähne wie die Vertreter der Balistoidei, dafür aber starke schnabelähnliche Beißkiefer. Bei Vertretern dieser Unterordnung ist das Autopalatinum stark vergrößert, was zu einer Immobilisierung der ethmopalatinalen Gelenkung führt, die eine Fixierung des Hyopalatinalbogens zur Folge hat. Die Ostraciiden nehmen eine Sonderstellung ein und zeigen sowohl Ähnlichkeiten mit den Tetraodontoidei als auch autapomorphe Merkmale. Vertreter dieser Familie haben den distalen Teil des Autopalatinum reduziert, während der proximale Abschnitt fest mit dem Hyopalatinalbogen verbunden ist. Bezogen auf die phylogenetisch-systematische Hypothese von Santini und Tyler (2003) wird deutlich, dass innerhalb der Ordnung Tetraodontiformes zwei unabhängige und konvergente Wege der evolutiven Spezialisierung der Kiefer und des Hyopalatinalbogens in Anpassung an die Durophagie verfolgt worden sind.

3.1 Publication I

RESEARCH ARTICLE

Same but Different: Ontogeny and Evolution of the *Musculus adductor mandibulae* in the Tetraodontiformes



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ABSTRACT

The morphological diversity of fishes provides a rich source to address questions regarding the evolution of complex and novel forms. The Tetraodontiformes represent an order of highly derived teleosts including fishes, such as the pelagic ocean sunfishes, triggerfishes, and pufferfishes. This makes the order attractive for comparative analyses to understand the role of development in generating new forms during evolution. The adductor mandibulae complex, the main muscle associated with jaw closure, represents an ideal model system within the Tetraodontiformes. The adductor mandibulae differs in terms of partitions and their attachment sites between members of the different tetraodontiform families. In order to understand the evolution of the jaws among the Tetraodontiformes, we investigate the development of the adductor mandibulae in pufferfishes and triggerfishes as representatives of two different suborders (Balistoidei and Tetraodontoidei) that follows two different adaptations to a durophagous feeding mode. We show that the varied patterns of the adductor mandibulae derive from similar developmental sequence of subdivision of the partitions. We propose a conserved developmental program for partitioning of the adductor mandibulae as a foundation for the evolution of different patterns of subdivisions in Tetraodontiformes. Furthermore, we argue that derived conditions in the higher taxa are realized by supplementary subdivisions and altered attachment sites. These findings support a reinterpretation of homology of different muscle partitions among the Tetraodontiformes, as muscle partitions previously thought to be disparate, are now clearly related. *J. Exp. Zool. (Mol. Dev. Evol.)* 316:10–20, 2011. © 2010 Wiley-Liss, Inc.

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Although these new homologies may not change the phylogeny itself, they may radically affect any hypotheses based on the refuted homologies.

(Friel and Wainwright, '97; p 460)

It is generally accepted that the evolution of the feeding apparatus is strongly linked with the great success of the ray-finned fishes (Actinopterygii), which represents, with more than 28,000 (Nelson, 2006) species, more than half of all living vertebrates. To understand the evolution of the actinopterygians, many studies have dealt with the morphological adaptations of the jaws in either a phylogenetic context (Schaeffer and Rosen,

'61; Lauder, '79, '80, '82; Westneat, 2004) or with function in highly derived taxa, such as the flatfishes (Gibb, '97; Gibb and Ferry-Graham, 2005), frogfishes (Pietsch and Grobecker, '87), tube-eyes (Pietsch, '78), seahorses (Roos et al., 2008, 2009), and members of the Tetraodontiformes (Turingan and Wainwright,

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: Turingan et al., '95; Friel and Wainwright, '98, '99; inwright and Friel, 2000).

The evolution of the free protrusible jaw was one of the key evolutions of ray-finned fishes. In the bowfin *Amia calva*, as the group of all teleosts, the maxilla has become free from the skull, allowing the ventral end of the maxilla to swing forward (the maxillomandibular ligament). The detachment of the maxilla from the skull was the key step that has led to the advent of a complex mechanism of upper jaw protrusion, evolved at least three times independently during teleost evolution (for details, see Schaeffer and Rosen, '61; Lauder, '82, '85; Johnson and Patterson, '93; Westneat, 2004).

Together with the alterations in the skeleton, muscles, and motor neurons associated with these skeletal elements show great diversity to enable a new function of the jaw. In non-teleost and basal teleost fishes, as well as in basal terrestrial vertebrates, the adductor mandibulae facilitates the closing of the lower jaw. With the advent of the protrusible upper jaw in higher teleosts, the arrangement of the *Musculus adductor mandibulae* has been modified, and acquired the retraction of the upper jaw as an additional novel function. For example, a general teleost fish has

the adductor mandibulae subdivided into four portions, of which one is attached to the maxilla (portion A1). The other subdivisions remain attached to the lower jaw (A2 and A3) and one portion runs along the medial side of the dentary (A ω).

The Tetraodontiformes are well known for their highly specialized jaws to facilitate durophagy, or grasping and processing of hard-shelled prey (Tyler, '80; Turingan and Wainwright, '93; Turingan et al., '95). Although members of all families of the Tetraodontiformes are durophagous, the basal Triacanthodidae exhibit the least feeding specializations of the musculoskeletal jaw apparatus and resemble a general perciform however lacking the A ω (Winterbottom, '74a). We consider this pattern as ancestral for the tetraodontiform order (Fig. 1). The most derived adaptations of the jaws are found in the suborder Tetraodontoidei, in which all members have parrot beak-like jaws without individual teeth. This is in contrast to the sister suborder, Balistoidei, which retain individual teeth as an oral dentition (Fig. 1).

Overall, the Tetraodontiformes show a trend toward morphological simplification, shown prominently in the loss or fusion of skeletal elements. However, in tandem, the more derived families

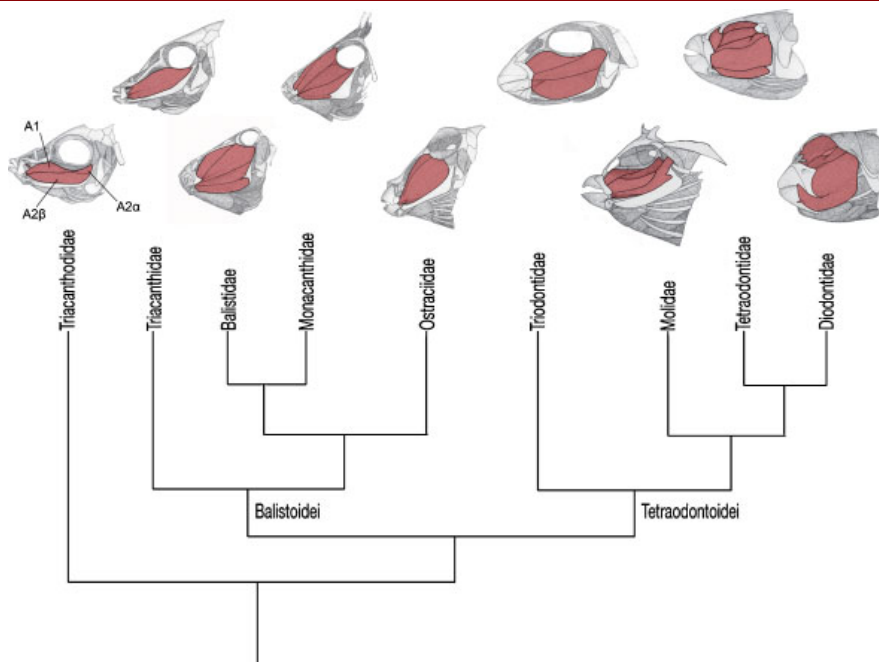


Figure 1. Tetraodontiformes phylogeny. Widely accepted phylogenetic hypothesis of Santini and Tyler (2003) based on morphological characters. Mapped onto this phylogeny is the form of the adductor mandibulae complex in the different taxa (traced in red). The triacanthodidae are the sistergroup within the order, which is subdivided into two suborders, Balistoidei and Tetraodontiidei. According to Santini and Tyler, the triacanthodids represent the most basal forms. Except for *Triodon*, all other members show a highly derived arrangement of the adductor mandibulae complex. Drawings are modified after Winterbottom ('74b). A1 and A2 α +A2 β are different adductor mandibulae portions.

harbor many novelties and specialized structures. For example, representatives of all families of the Tetraodontiformes, except the triacanthodids and triacanthids, have secondarily lost the ability to protrude the upper jaw as seen in most percomorph fishes (Turingan and Wainwright, '93; Turingan, '94; Turingan et al., '95; Wainwright and Friel, 2000). In contrast, the jaw musculature has become extensively elaborated when compared with the common percomorph situation. The basal Triacanthodidae show a similar configuration of the adductor mandibulae than a general percomorph (see above). The adductor mandibulae has evolved up to six portions in puffers and up to eight in some monacanthids (Winterbottom, '74a; Fig. 1; Friel and Wainwright, '98). The complexity and variety (up to 350 species) within this group provides a good case study in which to address the changes in specification and patterning of the musculoskeletal system underlying the evolution of these novel structures.

Attempts to assign homology to the different subdivisions of the adductor mandibulae have led to contrasting hypotheses of the evolution of the musculoskeletal system associated with the morphological diversity of these fishes (Winterbottom, '74a; Friel and Wainwright, '97; Nakae and Sasaki, 2004). Winterbottom ('74a) tried to homologize muscle portions using the origin and insertion of the individual subdivisions. In addition, Friel and Wainwright ('97) analyzed the pattern of subdivision of the muscle in which the authors differentiated between orthologous subdivisions (homologous partitions among species, e.g. A1 in *S. hepatus* and *Perca fluviatilis*) and paralogous subdivisions (subdivisions of a common partition within a species, e.g. A1 α and A1 β in *Microgadus tomcod*). However, the results are incongruent with that of Winterbottom ('74a). Nakae and Sasaki (2004) used an alternative approach and tried to solve the homology of the adductor mandibulae subdivisions via the innervation pattern of the *Ramus mandibularis trigemini* of the trigeminal nerve, but failed to clarify the earlier difficulties of the homology of the partitions. All three cases show the obvious limits in the analysis of adult structures in classifying homology, especially in highly derived forms. However, determining homology of elements is essential to understand the evolution of anatomical structures.

We approached the question of the evolution of the adductor mandibulae in tetraodontiform fishes by analyzing the ontogeny of the adductor mandibulae complex of the freshwater pufferfish, *Monotretes suvattii*, a marine species of the genus *Lagocephalus*, and the triggerfish *Balistapus undulatus*. These genera represent different feeding specializations within the tetraodontiform clade. We looked at ontogeny of the partitions to identify (1) the homology of the different adductor mandibulae subdivisions between members of the different families (orthologs sensu, Friel and Wainwright, '97), (2) the developmental lineage of the individual muscle subdivisions within the balistids and the tetraodontids (paralogs sensu, Friel and Wainwright, '97), and (3) the developmental history that underlies the evolution of

the subdivisions. Through tracing the development of the different partitions in the adductor mandibulae in larvae, we identified a conserved developmental sequence of muscle specification that is specifically altered within the different lineages leading to unique muscle arrangements in the "beaked" and "toothed" Tetraodontiformes.

MATERIAL AND METHODS

Specimens

Reared larvae of *M. suvattii* (size range between 3 mm notochord length (NL) and 8 mm standard length (SL)) were preserved in 4% paraformaldehyde (PFA) at 4°C between 24 and 72 hr and subsequently dehydrated to 100% methanol and stored at -20°C. Larvae larger than 4 mm were preserved in PFA for 4 days and then dehydrated. *Lagocephalus* sp. and *B. undulatus* larvae were obtained from unsorted material from the National Science Museum, Tokyo.

Immunolabeling

We analyzed the early development of the adductor mandibulae in larvae by antibody staining against myosin heavy chain (fast twitch). All larvae were carefully skinned and connective tissue was removed under a dissecting scope (ZEISS Stemi 2000, ZEISS DRC) before antibody labeling. The eyes were removed either before or after the staining. To specifically detect myofibrils, specimens were incubated with a primary antibody specific to fast twitch myosin (MF20, Developmental Studies Hybridoma Bank, 1:500). This was followed by secondary antibody detection conjugated with FITC (1:500, ALEXA 488, Invitrogen) or alkaline phosphatase antibody (1:5000, Dianova). The larvae were rehydrated in phosphate saline buffer (PBS) with several changes over the course of 2 hr. To make the cell membranes permeable, the larvae were treated for 1 hr with Proteinase K (10 μ g/mL) and afterwards with Collagenase (10 μ g/mL) for 30 min. Both steps were carried out at 37°C. Subsequently, the larvae were treated with Acetone for 1 hr at -20°C. The specimens were then washed several times with PBS at room temperature (RT). To block the unspecific antigens, a 10% normal goat serum in tris buffered saline with 0.1% TWEEN-20 (TBST) was applied for 3 hr at RT. Color reaction for alkaline phosphatase activity was done by washing the specimens in TBST+Levamisol (L; 0.5%) (TBST+L) o/n at 4°C, 5 hr with a basic buffer (pH 8) containing Levamisol and then placed in BCIP/NBT color substrate (Sigma FASTTM) at 4°C until visible to eye.

Confocal Imaging

Formalin-fixed tissues exhibit autofluorescence. The high degree of organization of the muscle tissue causes less scattering and allows these structures to be differentially silhouetted against less organized tissue, such as connective tissue or skeletal elements. We used this advantage to trace the ontogeny of the adductor

mandibulae in museum specimens of *Lagocephalus* sp. and *B. undulatus*. The specimens were skinned only.

Specimens were mounted for observation in a small glass ring filled with 0.8% low melting agarose attached to a glass slide. Images of the whole mount specimens were taken with a ZEISS AxioCam HR digital camera attached to a ZEISS Discovery V20 dissecting scope.

Images of immunolabeled and formalin-fixed specimens were taken with a ZEISS LSM 510 confocal microscope controlled by the ZEISS ZEN software. Tile and z-stacks were aligned automatically.

Materials Examined

Balistidae. *B. undulatus* (Park), uncataloged from the collection of National Science Museum, Tokyo, five specimens, 2.6 mm NL–8.8 mm SL; Tetraodontidae: *Lagocephalus* sp. (Swainson), uncataloged from the collection of National Science Museum, Tokyo, two specimens, 3.1 mm NL and 4.0 mm NL; *M. suvattii* (Sontirat): uncataloged, three specimens, 4.5 mm NL–7.1 mm SL, labeled with ALEXA 488; *M. suvattii* (Sontirat): uncataloged, three specimens, 3.2 mm NL–4.1 mm NL, labeled with goat anti-mouse AP.

Anatomical Abbreviations

Winterbottom ('74a,b) followed the nomenclature introduced by Vetter (1878) and labeled the different partitions of the *M. adductor mandibulae* based on relative position, origin, and insertion site in the adult; this classification also implies function. To demonstrate homology, portions of the adductor mandibulae were named as followed: A1, a portion that has its origin lateral on the suspensorium and its insertion point on the maxilla (either directly or dorsally on the primordial ligament that is associated with the maxilla) of the upper jaw; portions named A2 and A3 attach always on the lower jaw and A ω runs along the meckelian fossa. In puffers and other tetraodontiformes, more than one

portion inserts on the upper jaw. Therefore, Winterbottom ('74a,b) extended the nomenclature and added a Greek letter after the abbreviation, e.g. A1 α and A1 β for the portions that insert on the maxilla of the upper jaw. This system was adopted by Friel and Wainwright ('97) and Nakae and Sasaki (2004); however, several additions were made to clarify different homology assessments including species/sub-order specific identifiers to “establish” functional identity in the adult.

Although the previous nomenclature (A1–A ω) is sufficient for analysis of the functional changes of the adductor mandibulae complex, it is not suitable for discussions of homology—especially in cases of complex specialization. By looking at the observed homology of different partitions of the adductor mandibulae (see Table 1), the result is a lack of clarity in the identification of muscle homology. Thus, we suggest a revised or complementary system of nomenclature that takes into consideration the developmental history as the primary measure of homology when available. It is likely that these systems of nomenclature will be similar in many cases, *except* in cases of diversification and novelty.

The base abbreviation for the adductor mandibulae subdivisions that we use here is AM. We introduce the term AM_{PRE} for the initial stage of the adductor mandibulae. Separation of the AM_{PRE} is denoted with a Greek symbol (e.g. AM α). Further separation of the partitions is labeled with hash marks (e.g. AM α' or AM α'' for the two partitions resulting from separation of the AM α). In discussing variation in placement or function of a partition, we follow Winterbottom ('74b) who defined the origin of a muscle as the attachment closer to the centre (e.g. suspensorium) and, consequently, the insertion as the point farther away from the center (e.g. maxilla).

Although A ω was described for trigger- and pufferfishes and the A1 β of the triggerfishes, we were not able to detect them in the particular age specimens we examined.

Table 1. Comparative classification of adult adductor mandibulae partitions.

This study (lineage)	Winterbottom (function and lineage inferred)	Friel and Wainwright (function and lineage inferred)	Nakae and Sasaki (function and lineage inferred)
AM α'	A2 α /A2 β	A2 β' t&tA2 β'' t/A2 $\beta'b$	A2 α /A2 β
AM α''	A1 α /A2 γ	A1 β t/A2 $\beta''b$	A1 α /A2 γ
AM α'''	A3/A3	A3/A3	A3/A3 α
AM β'	A2 β /A2 α	A2 α /A2 α	A2 β t&tA1 β' /A2 α
AM β''	A1 β /A1 α	A1 α t/A1 α b	A1 β /A1
N/A	absent/A1 β *	Absent/A1 β *	A3 β *

Partitions of the adductor mandibulae are classified using nomenclature according to the pattern of divisions (lineage) of the partitions or with previous classification scheme based on attachment site. For classification based on function, tetraodontids and balistids are shown (t/b); in classification based on ontogeny, the partitions are identical. *N and S argue that the A1 β of the balistids is a subdivision of A3; F and W did not discuss the unusual position of this muscle.

RESULTS

Development of the Adductor Mandibulae in Pufferfishes

Comparative studies of the development of Tetraodontiformes are hindered by the availability of larvae of different taxa of the group. When available, the method of fixation of the specimens often does not support immunological or in situ methods necessary for visualization of specific tissues in younger specimens. However, we were able to investigate the early formation of the adductor mandibulae using immunolabeling of muscles in

a developmental series of larvae of the freshwater puffer *M. suvattii* bred in the lab.

Unlike the adults with several separate and distinct partitions of the adductor mandibulae, we find that the adductor mandibulae in early larvae of *M. suvattii* is represented by a single portion here referred to as AM_{PRE} (3.2 mm NL; Fig. 2A, B). This portion has its origin on the hyomandibula, projecting anteriorly and bending slightly upward. The dentary is not yet ossified and AM_{PRE} inserts at this stage on the posterior end of the meckelian cartilage (not shown). By 3.5 mm NL (Fig. 2C),

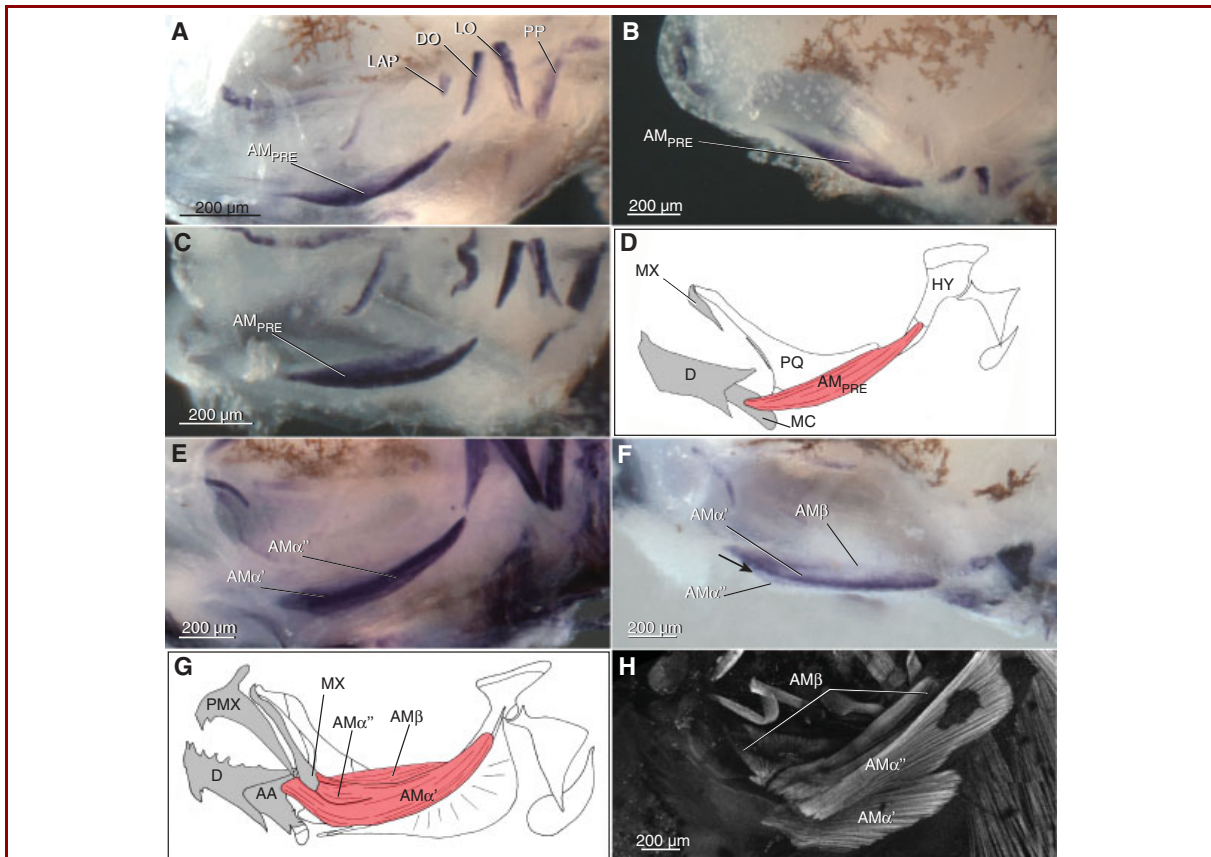


Figure 2. The formation and early partitioning of the adductor mandibulae of *Monotretes suvattii*. **A.** Lateral view of a 3.2 mm NL specimen. AM_{PRE} is formed as a single primordia attaching to the lower jaw anteriorly and to the suspensorium posteriorly. **B.** Dorsal view of the same specimen as in **A.** Only the adductor mandibulae is marked. **C.** Lateral view of a 3.5 mm NL specimen. AM_{PRE} has broadened mediadorsally. **D.** Schematic drawing of the suspensorium of a specimen of the size represented in **C** to visualize the attachment of AM_{PRE} . **E.** Lateral view of a 4.1 mm NL specimen. AM_{PRE} is now subdivided and represented by three portions. In lateral view only $AM_{\alpha'}$, $AM_{\alpha''}$ can be seen. $AM_{\alpha''}$ is attached to the maxilla. **F.** Dorsal view of the same specimen as in **E** in which the third subdivision, AM_{β} , can be seen. The attachment of $AM_{\alpha''}$ to the maxilla is indicated by the red arrow. **G.** Schematic drawing of the suspensorium of a specimen of the size represented in **E** and **F** to visualize the three different subdivisions. **H.** Lateral view of a 4.5 mm SL specimen. $AM_{\alpha''}$ has shifted its origin and AM_{β} now occupies most of the dorsal part of the suspensorium. AA, anguloarticular; AM_{PRE} , precursory portion of the adductor mandibulae; $AM_{\alpha'}$, $AM_{\alpha''}$, AM_{β} , subdivisions of the adductor mandibulae; D, dentary; DO, dilatator operculi; HY, hyomandibula; LAP, levator arcus palatini; LO, levator operculi; MC, meckelian cartilage; MX, maxilla; NL, notochord length; PMX, premaxilla; PP, protractor pectoralis; PQ, palatoquadrate; SL, standard length.

AM_{PRE} has broadened posteriomedially, but still remains associated with the meckelian cartilage (Fig. 2C and D). In a 4.1 mm NL specimen, AM_{PRE} shows an initial subdivision (Fig. 2E and F). This separation of the AM_{PRE} is denoted as AM α (AM α' and AM α'' ; see below) and AM β . The portion that is defined as AM β occupies the dorsal part of the palatoquadrate (Fig. 2F and G). Where the posterior end of the maxilla covers AM α laterally, a small bundle of muscle fibers encroached on the posteroventral end of the maxilla; this is the initial stage of AM α'' (Fig. 2E–G). The remaining portion of AM α is now termed AM α' . By 4.5 mm SL, AM β is now fan-shaped expanded and covers most of the suspensorium (Fig. 2H). In a slightly larger larva (5.2 mm SL), AM α' and AM β are fully separated (Fig. 3A).

At this juvenile stage, the anterior part of AM β has extended anterior and covers the entire dorsal and most anterior part of the suspensorium (Fig. 3A), and is attached to the parasphenoid dorsally. The anterior most part of AM β is larger and can be clearly distinguished from the more posterior portion (Fig. 3A). The subdivision AM α'' partially covers AM α' laterally (Fig. 3A). At this stage, a further division of AM α occurs medially and is termed AM α''' (not shown); this is the partition previously denoted as A3

by Winterbottom ('74a), Friel and Wainwright ('97), and Nakae and Sasaki (2004). This arrangement of the portions of the adductor mandibulae resembles the situation of adult puffers as described by the aforementioned authors, with the exception that the muscle portion that is attached to the maxilla, the ethmoid and the lateral ethmoid, has not developed. As development proceeds (7.1 mm SL; Fig. 3B), the more massive anterior part of AM β separates and forms a discrete entity, AM β'' (7.1 mm SL; Fig. 3B and C). Because of the separation, the posterior part of the former AM β is now renamed to AM β' . The new partition AM β'' is attached to the maxilla ventrally and to the ethmoid and lateral ethmoid dorsally. Because this portion is attached to the maxilla Winterbottom ('74a), Friel and Wainwright ('97), and Nakae and Sasaki (2004) believed that this portion is homologous to a subdivision that serves already the upper jaw (herein AM α'' ; see Table 1 for the nomenclature of the three aforementioned studies).

The development of the adductor mandibulae in *M. suvattii* larvae suggests that (1) the major muscle partitions of the adductor derive from a common precursor and (2) that the diversity in the number of partitions is owing to successive divisions of this precursor. Furthermore, our data show that muscles with different

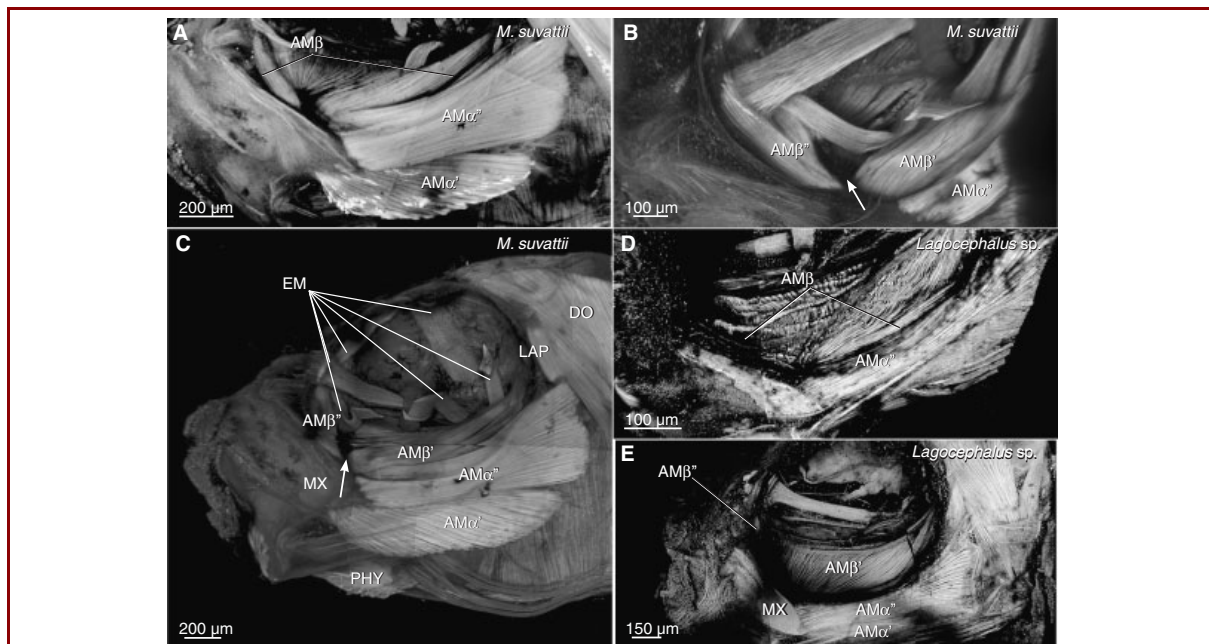


Figure 3. Comparative analysis of the development of the adductor mandibulae in puffers. A. Lateral close up of a 5.2 mm SL *Monotrete suvattii*. AM β occupies the entire dorsal part of the suspensorium. B. Close up of C, the head of a 7.2 mm SL *M. suvattii*. The most anterior part of AM β (now AM β') is separated and forms an individual portion. The arrow marks the gap between AM β' and AM β'' . D. Lateral close up of a 3.1 mm SL *Lagocephalus* sp. The adductor mandibulae arrangement at this stage is comparable to the *M. suvattii* in Figure 2H. E. Lateral close up of a 4.0 mm SL *Lagocephalus* sp. is comparable to the *M. suvattii* in Figure 3A. AM α' , AM α'' , AM β , AM β' , AM β'' , subdivisions of the adductor mandibulae; DO, dilatator operculi; EM, eye muscles; LAP, levator arcus palatini; MX, maxilla; NL, notochord length; PHY, protractor hyoidei; SL, standard length.

attachment sites in the adult arise from similar antecedent partitions. This last fact has important implications for the analysis of homology of the adductor mandibulae complex (see below).

To further verify these results, we looked at the development of a marine puffer, *Lagocephalus* sp., as another example of the tetraodontid family. We were able to detect the structure of early developing muscles in batch-preserved museum specimens using simple autofluorescence of the muscle fibers (see Material and Methods). Although we were not able to obtain specimens of *Lagocephalus* sp. at small enough stages to detect the initial subdivisions of the adductor mandibulae, the smallest specimens we recovered showed partitioning of the $AM\beta$ and $AM\alpha$ as seen in *M. suvattii*. The earliest larval stages of *Lagocephalus* sp. (Fig. 3D) were comparable to the *M. suvattii* larva of Fig. 2E. $AM\beta$ covers the suspensorium and reaches far rostrally. In the older specimen, the most anterior part of $AM\beta$ is attached to the maxilla and to the ethmoid region of the skull, but not yet separated as an individual $AM\beta''$ (Fig. 3E). $AM\beta''$ is a fully separated portion in adult *Lagocephalus* sp. (Winterbottom, '74a); therefore, this partition likely separates in older juveniles.

The similarity of the development of the adductor mandibulae of the two puffer species, *M. suvattii* and *Lagocephalus* sp.,

suggests that the developmental trajectory of the adductor mandibulae, and in particular that the $AM\beta''$ binds to the maxilla, is conserved among members of this "beaked" family, the Tetraodontidae (Fig. 1).

Development of the Adductor Mandibulae Complex in the Triggerfish, *Balistapus undulatus*

The jaws of members of the balistid suborder do not form a beak, such as the puffers, but rather retain a dentition. However, this suborder also exhibits a complicated, elaborate adductor mandibulae complex that functions to support derived feeding behaviors of the group (Turingan and Wainwright, '93; Turingan, '94; Ralston and Wainwright, '97). We, therefore, broadened our ontogenetic analysis to a member of this family to compare with the puffers. Such as *Lagocephalus* sp., we detected developing muscles using differential autofluorescence of formalin-fixed museum specimens using confocal microscopy.

Larval balistids are exceptionally rare in collections. Although we were able to get a series of developmental stages, the smallest balistid obtained (2.6 mm NL) showed the development of three adductor mandibulae portions (Fig. 4A). All teleosts analyzed to date show that the initial subdivisions derive from a single

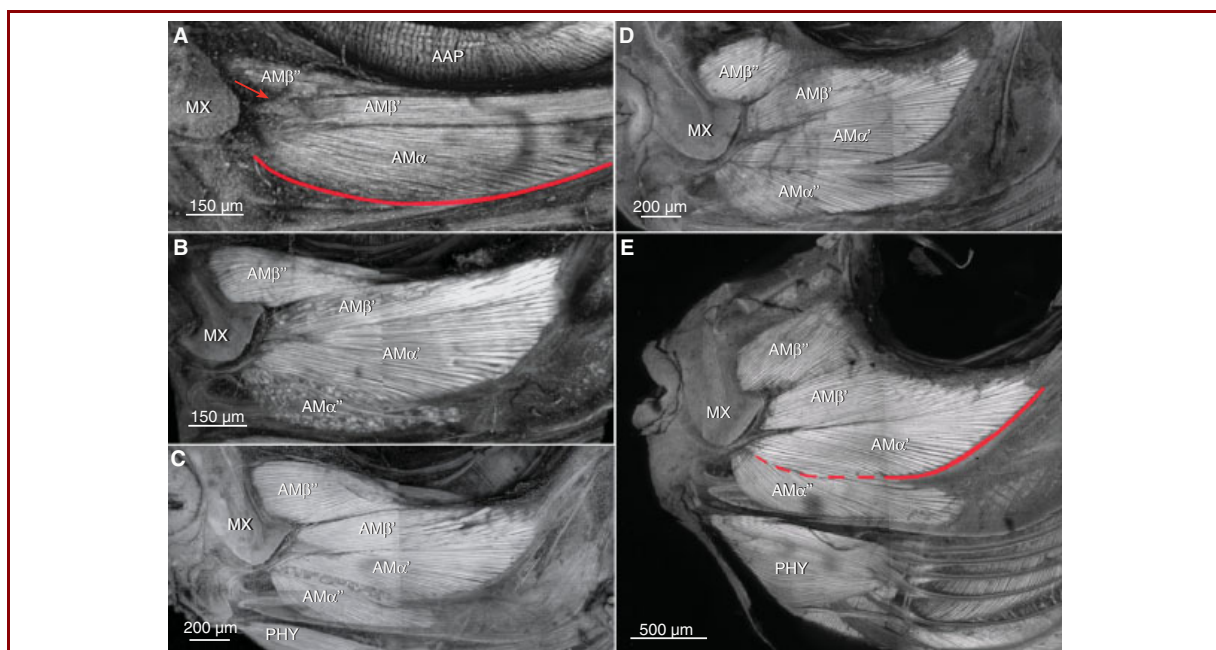


Figure 4. The development of the adductor mandibulae in the balistid, *Balistapus undulatus*. A. Close up of a 2.6 mm NL specimen, *B. undulatus*. $AM\beta''$ is still connected to $AM\beta'$ anteriorly (red arrow). The attachment of $AM\alpha'$ to the lateral crest of the preopercle is outlined to show the similarity to the puffers. B. Close up of a 3.4 mm NL specimen. C. Close up of a 4.9 mm SL specimen. D. Close up of a 5.9 mm SL specimen. E. Lateral view of the head of a 8.8 mm SL specimen. This stage closely resembles the adult. The attachment of $AM\alpha'$ to the lateral crest of the preopercle is outlined. AAP, adductor arcus palatini; $AM\alpha'$, $AM\alpha''$, $AM\beta'$, $AM\beta''$, subdivisions of the adductor mandibulae; MX, maxilla; NL, notochord length; PHY, protractor hyoidei; SL, standard length.

progenitor; thus, we identified the subdivisions in this early larval stage of *B. undulatus* by the position and similar fiber direction as seen in the first division of AM_{PRE} in the puffers (Fig. 2A and C). We identified the two ventral portions as AM α , AM β ', and the dorsal portion as AM β ' (Fig. 4A). AM β ' is a narrow band that is connected to the preopercle and the dorsal part of the suspensorium. AM α is the largest muscle and, as in the puffers, it has its origin on the lateral crest of the preopercle (Fig. 4A). Importantly, these small larvae AM β ' is seen to derive from the most anterior part of AM β (then becoming AM β ' ; Fig. 4A)—identical to the case in the puffers. In a 3.4 mm SL larva, AM β ' extends caudally (Fig. 4B) and occupies the dorsal part of the metapterygoid. In larger specimens, AM β ' becomes smaller in a rostrocaudal direction, shifts its position, and changes its attachment to the lateral ethmoid and ethmoid exclusively (Fig. 4C and D). AM α ' appears last as an offshoot of AM α originating on the preopercle ventral to the lateral crest (Fig. 4B–D).

DISCUSSION

The adductor mandibulae complex of the Tetraodontiformes presents an ideal model for the study of the evolution of morphological novelty. In particular, the homology and evolution of the highly derived adductor mandibulae has been the focus of previous studies (Winterbottom, '74a; Friel and Wainwright, '97; Nakae and Sasaki, 2004). Given the complexity of the adductor complex of the Tetraodontiformes, the question of homology of the subdivisions within and among species becomes important in order to interpret the transformation of characters in a phylogenetic context. Does the ancestral muscle portion predetermine the pattern and functional attributes of the muscle or can descendant subdivisions adopt a new function? To allow an in-depth evaluation of the role of topological characters, such as origin, attachment, and innervation, as well as the function of specific muscle groups in the evolution of new feeding strategies, we compared the development of the adductor mandibulae of a balistid, as a representative of the suborder Balistoidei (toothed forms) with two tetraodontids, as representatives of the Tetraodontoidei (beaked forms; Fig. 1).

Developmental Program and the Evolution of Complexity of the Adductor Mandibulae in Tetraodontiformes

The multiple-partitioned adductor mandibulae seen in Tetraodontiformes derives from a single myogenic anlage and not from multiple muscle primordia that integrate into a complex. This property of the development of the adductor mandibulae complex is conserved throughout the teleosts and reported for a wide variety of taxa, e.g. *Danio rerio* (Hernandez et al., 2005; Diogo et al., 2008; Staab and Hernandez, 2010), Loricarioidei (Geerinckx et al., 2007; Huysentruyt et al., 2007), *Kneria* sp. (personal observation), the Japanese flounder *Paralichthys olivaceus* (Uji et al., 2010), as well as for *Amia* (Jarvik, '80), as the sistergroup of the teleosts. Although pufferfishes and their

relatives are highly derived, the initial development of the adductor mandibulae resembles the common ontogenetic trajectory among teleosts.

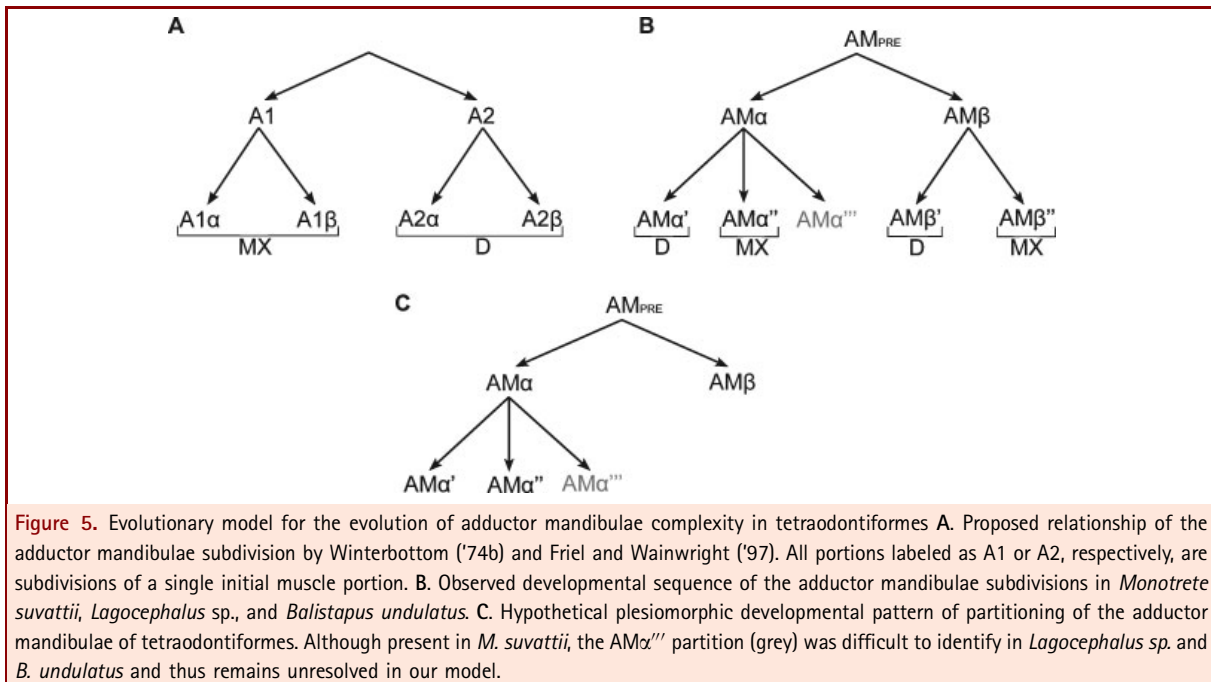
The initial anlage of the adductor mandibulae divides into two partitions, AM α and AM β , of which AM α subsequently gives rise to three portions (Fig. 5B). This early state of complexity of the adductor resembles the adult pattern of the triacanthodids and the most plesiomorphic arrangement of the tetraodontiformes (Fig. 5C). This pattern of subdivision of the adductor can be applied to the triacanthids as well, although AM α ' and AM α ' are not completely separated in this group. This is either the result of an uncompleted separation of the two muscle subdivisions or a secondary fusion.

Although the balistids and the tetraodontids have very different adaptations of the skeletal elements of the jaws and arrangement of the associated musculature (e.g. AM α ''), the developmental history of the partitions are the same. Both groups share an additional subdivision of AM β that differentiates them from the pattern in the basal triacanthodids (Fig. 5B). This model would also explain the pattern in representatives of the families, Diodontidae and Molidae. In contrast to the balistids, the Monacanthidae show a tripartite AM β , suggesting another duplication event of one of the AM β partitions. Members of the family Ostraciidae are exceptional in having AM β subdivided as the more derived Tetraodontiformes but have only an anteriorly bifurcated AM α .

Homology of the Adductor Mandibulae Subdivisions in the Tetraodontiformes

Winterbottom ('74a) and Friel and Wainwright ('97) used the pattern of duplicated partitions to understand the evolutionary trajectory of the adductor mandibulae of the tetraodontiformes. Winterbottom's ('74a) analysis does not present a hypothesis about character transformation between species. Friel and Wainwright ('97) were able to, through an analytical approach to provide a more parsimonious hypothesis than Winterbottom, explain the evolution of the adductor mandibulae complex.

When only adult specimens are used, as it is the case in Winterbottom ('74a) and Friel and Wainwright ('97), the only option to assess homology between an antecedent muscle and its decedent subdivisions is via anatomical similarity, e.g. relative position and anatomical "connectivity" (Remane, '52; Patterson, '82), which implies a correlation to a similar function of the related portion, e.g. A1 α and A1 β serve the maxilla and are the result of a duplication event of A1, which was already attached to the maxilla. The hypotheses of Winterbottom ('74a) and Friel and Wainwright ('97) are based on this correlation and state that all muscle portions that bind to the upper jaw (and, therefore, have also a certain function assigned) are offshoots from a single subdivision termed A1 by these authors. Additionally, all muscle portions that bind to the lower jaw derive from another subdivision (their A2). This, then, implies homology (Fig. 5A).



As the ontogeny of the adductor mandibulae in the pufferfishes demonstrates, the contrary is the case: both subdivisions that bind to the upper jaw (AM α'' and AM β'') have their origin from different precursory muscle portions (Figs. 3A–C and 5B). The earlier view that the descended partitions of AM α (A1) and AM β (A2) were distinct separable units with a defined function is, therefore, no longer supported. In addition, a particular function of a partition does not indicate homology, e.g. AM α'' attached to the maxilla in the pufferfishes and to the lower jaw in the triggerfish has a similar developmental history and, therefore, represents in our view a homologous partition between the groups. The overlap between function and homology is common, but in cases of diversification the difference in these two properties become apparent.

We are aware that for many taxa it is almost impossible to obtain larval material for ontogenetic studies and that in such cases topological characters, such as similarity and connectivity of structures (Remane, '52; Patterson, '82), are often the only way to assess homology. Analysis of ontogeny allows tracing complicated structures back to a more simple state and is independent of function and to a certain degree of connectivity and position. This, therefore, provides the most conclusive data for the identification of homologous structures.

Our findings show that topological characters, such as origin, attachment, and function of adductor mandibulae subdivisions, are not predetermined by the topology and the functional role of the antecedent precursor. This has a wider implication for

evolutionary studies because the adductor mandibulae has often been used to elucidate the evolution of the highly kinetic jaws across teleosts. Gosline ('86) hypothesized that the complicated and highly derived subdivision pattern of the adductor mandibulae of higher teleosts (Acanthomorpha) are modifications of a basal pattern, already present in the ancestral sistergroup (Myctophidae). Our findings support this hypothesis.

Development and the Evolution of Feeding Specializations

Members of the tetraodontiform order show unique evolutionary specializations of the jaw that are defining traits for the different suborders. The increase in the complexity of the adductor mandibulae is directly linked with the evolution of feeding specializations in the order. This functional articulation is in part owing to the increase in complexity of the number of partitions of the adductor mandibulae. By addressing the comparative development of the adductor mandibulae among groups with specialized feeding structures (e.g. beak or dentition), we show that the various patterns are due from differential partitioning of a single muscle primordia. Complexity arises in the derived tetraodontiform suborders owing to further subdivision of the AM β portion. It is noteworthy that the timing of specification of AM α'' is quite different between the puffer and triggerfish, as it develops early in *M. suvattii* and it is the last subdivision that appears in *B. undulatus*.

It is not partitioning that differentiates the adductor mandibulae complex between the different suborders, rather the

varied attachment of the AM α' . This defines a specific character change underlying these specializations. Although pufferfishes and triggerfishes have lost the ability to protrude their upper jaw, the AM α' partition in pufferfishes retains the ancestral attachment site on the upper jaw as seen in higher teleosts (A1), whereas in triggerfishes AM α' is attached to the lower jaw. Turingan ('94) observed that the mass of the adductor mandibulae portions that serves the upper jaw (mass is linked with bit force) do not vary between different taxa within the tetraodontids. He inferred that these portions are not important for the different modes of processing prey (crushing vs. biting). Different articulation of the muscle to the skeletal system, however, might represent alternative evolutionary trends to increase the mobility (not protrusion) of the upper jaw. This is supported by the fact that tetraodontiformes process prey with their oral jaws exclusively (Turingan, '94; Wainwright and Friel, 2000), instead of with the pharyngeal jaws as is common for most of the teleosts.

CONCLUSION

A conserved pattern of subdivision of the adductor mandibulae is seen between the development of diverse tetraodontiform groups, as the balistids and tetraodontids—groups with varied feeding behaviors and jaw specializations. Thus, it is most likely that the common ancestor to both groups shared this rudimentary process of muscle patterning. Complexity in the adductor mandibulae in some of the tetraodontiformes apparently has arisen from added partitioning of a single portion (AM β) and subsequent alteration of positioning of attachments of AM α' in the triggerfish.

These observations are in contrast to the previous hypotheses of Winterbottom ('74a) and Friel and Wainwright ('97) that the adductor mandibulae is comprised of two muscle partitions, A1 and A2, which are distinct in function and their evolutionary history. This last concept was used as the foundation to homologize different muscle partitions between species. However, by looking at the development of the complex, we clearly show that the different portions of the adductor mandibulae are not functionally related to their particular antecedent muscle Anlagen. Although our work focuses on the diversity of the adductor mandibulae of a highly derived order within the teleost fishes, our results suggest that the condition in even generalized teleosts may not be as straightforward as implied by the nomenclature (e.g. A1–3) applied in previous studies to infer to homologous subdivisions.

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LITERATURE CITED

- Diogo R, Hinitz Y, Hughes SM. 2008. Development of mandibular, hyoid and hypobranchial muscles in the zebrafish: homologies and evolution of these muscles within bony fishes and tetrapods. *BMC Dev Biol* 8:24.
- Friel PJ, Wainwright PC. 1997. A model system of structural duplication: homologies of adductor mandibulae muscles in tetraodontiform fishes. *Syst Biol* 46:441–463.
- Friel JP, Wainwright PC. 1998. Evolution of motor patterns in tetraodontiform fishes: does muscle duplication lead to functional diversification? *Brain Behav Evol* 52:159–170.
- Geerinckx T, Brunain M, Adriaens D. 2007. Development of the osteocranium in the suckermouth armored catfish *Ancistrus* cf. *triradiatus* (Loricariidae, Siluriformes). *J Morphol* 268:254–274.
- Gibb AC. 1997. Do flatfish feed like other fishes? A comparative study of percomorph prey-capture kinematics. *J Exp Biol* 200:2841–2859.
- Gibb AC, Ferry-Graham LA. 2005. Cranial movements during suction feeding in teleost fishes: are they modified to enhance suction production? *Zoology* 108:141–153.
- Gosline WA. 1986. Jaw muscle configuration in some higher teleostean fishes. *Copeia* 3:705–713.
- Hernandez LP, Patterson SE, Devoto SH. 2005. The development of muscle fiber type identity in zebrafish cranial muscles. *Anat Embryol* 209:323–334.
- Huysentruyt F, Geerinckx T, Adriaens D. 2007. A descriptive myology of *Corydoras aeneus* (Gill, 1858) (Siluriformes: Callichthyidae), with a brief discussion on adductor mandibulae homologies. *Anim Biol* 57:433–452.
- Jarvik E. 1980. Basic structure and evolution of vertebrates. London, New York: Academic Press. p i–xvi, 1–575.
- Johnson GD, Patterson C. 1993. Percomorph phylogeny—a survey of acanthomorphs and a new proposal. *Bull Mar Sci* 52:554–626.
- Lauder GV. 1979. Feeding mechanics in primitive teleosts and in the halecomorph fish *Amia calva*. *J Zool* 187:543–578.
- Lauder GV. 1980. Evolution of the feeding mechanism in primitive actinopterygian fishes—a functional anatomical analysis of *Polypterus*, *Lepisosteus*, and *Amia*. *J Morphol* 163:283–317.
- Lauder GV. 1982. Patterns of evolution in the feeding mechanism of actinopterygian fishes. *Am Zool* 22:275–285.

- Lauder GV. 1985. Aquatic feeding in lower vertebrates. In: Hildebrand M, Bramble DM, Liem KF, Wake DB, editors. Functional vertebrate morphology. Cambridge, MA, and London: Belknap Press. p 210–229.
- Nakae M, Sasaki K. 2004. Homologies of the adductor mandibulae muscles in tetraodontiformes as indicated by nerve branching patterns. *Ichthyol Res* 51:327–336.
- Nelson JS, editor. 2006. *Fishes of the world*, 4th edition. New York: Wiley. p 1–601.
- Patterson C. 1982. Morphological characters and homology. In: Joysey KA, Friday AE, editors. *Problems of the phylogenetic reconstruction*. London: Academic Press. p 21–74.
- Pietsch TW. 1978. The feeding mechanisms of *Stylophorus chordatus* (Teleostei: Lampridiformes): functional and ecological implications. *Copeia* 1978:255–262.
- Pietsch TW, Grobecker DB. 1987. *Frogfishes of the world. Systematics, zoogeography, and behavioural ecology*. Stanford: Stanford University Press. p i–xxiv, 1–420.
- Ralston KR, Wainwright PC. 1997. Functional consequences of trophic specialization in pufferfishes. *Funct Ecol* 11:43–52.
- Remane A. 1952. *Die Grundlagen des natürlichen Systems, der vergleichenden Anatomie und Phylogenetik*. Leipzig: Geest & Port.
- Roos G, Leysen H, Van Wassenbergh S, Herrel A, Jacobs P, Dierick M, Aerts P, Adriaens D. 2008. Linking morphology and motion: a test of a four-bar mechanism in seahorses. *Physiol Biochem Zool* 82:7–19.
- Roos G, Van Wassenbergh S, Herrel A, Aerts P. 2009. Kinematics of suction feeding in the seahorse *Hippocampus reidi*. *J Exp Biol* 212:3490–3498.
- Santini F, Tyler JC. 2003. A phylogeny of the families of fossil and extant tetraodontiform fishes (Acanthomorpha, Tetraodontiformes), Upper Cretaceous to Recent.
- Schaeffer B, Rosen D. 1961. Major adaptive levels in the evolution of the actinopterygian feeding mechanism. *Am Zool* 1:187–204.
- Staab KL, Hernandez LP. 2010. Development of the cypriniform protrusible jaw complex in *Danio rerio*: constructional insights for evolution. *J Morphol* 271:814–825.
- Turingan RG. 1994. Ecomorphological relationships among Caribbean tetraodontiform fishes. *J Zool* 233:493–521.
- Turingan RG, Wainwright PC. 1993. Morphological and functional bases of durophagy in the queen triggerfish, *Balistes vetula* (Pisces, tetraodontiformes). *J Morphol* 215:101–118.
- Turingan RG, Wainwright PCC, Hensley DA. 1995. Interpopulation variation in prey use and feeding biomechanics in Caribbean triggerfishes. *Oecol* 102:296–304.
- Tyler JC. 1980. Osteology, phylogeny, and higher classification of the fishes of the order Plectognathi (tetraodontiformes). NOAA Tech Rep NMFS Circ 434:1–422.
- Uji S, Kurokawa T, Suzuki T. 2010. Muscle development in the Japanese flounder, *Paralichthys olivaceus*, with special reference to some of the larval-specific muscles. *J Morphol* 271:777–792.
- Vetter B. 1878. *Untersuchungen zur vergleichenden Anatomie der Kiemen und Kiefermuskulatur der Fische*. 2. Theil. *Jen Z Nat* xii:431–450.
- Wainwright PC, Friel JP. 2000. Effects of prey type on motor pattern variance in tetraodontiform fishes. *J Exp Zool (Mol Dev Evol)* 286:563–571.
- Westneat MW. 2004. Evolution of levers and linkages in the feeding mechanisms of fishes. *Integr Comp Biol* 44:378–389.
- Winterbottom R. 1974a. The familial phylogeny of the tetraodontiformes (Acanthopterygii: Pisces) as evidenced by their comparative myology. *Smithson Contr Zool* 155:1–201.
- Winterbottom R. 1974b. A descriptive synonymy of the striated muscles of the teleostei. *Proc Acad Nat Sci Phila* 125:225–317.

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A comparative ontogenetic study of the tetraodontiform caudal complex

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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.

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'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 1950; 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 Percoidae 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, *Acanthocephala limbata*, *Poecilia reticulata* or *Diodon* sp. (for a full spectrum see Monod 1968; Fujita 1990).

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 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

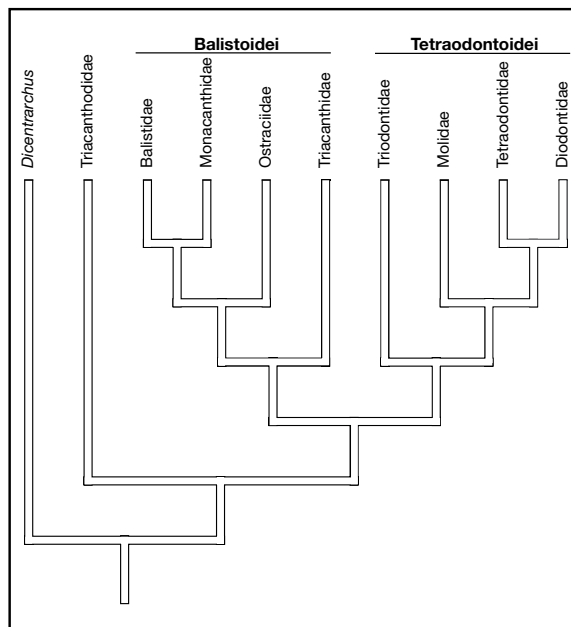


Fig. 1—Phylogenetic hypothesis of the order Tetraodontiformes. The generally accepted phylogenetic hypothesis based on Santini and Tyler (2003).

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. *Parahollardia* 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 irubescens* (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

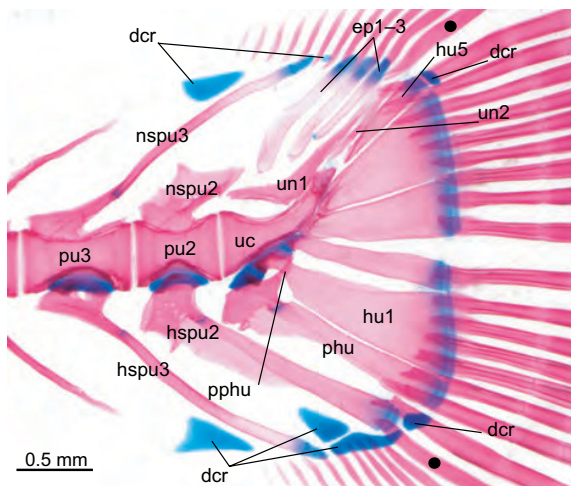


Fig. 2—Moronidae. *Dicentrarchus labrax* (28 mm SL). The black dots indicate the uppermost and lowermost principal caudal fin rays.

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).

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 hypochochordal 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

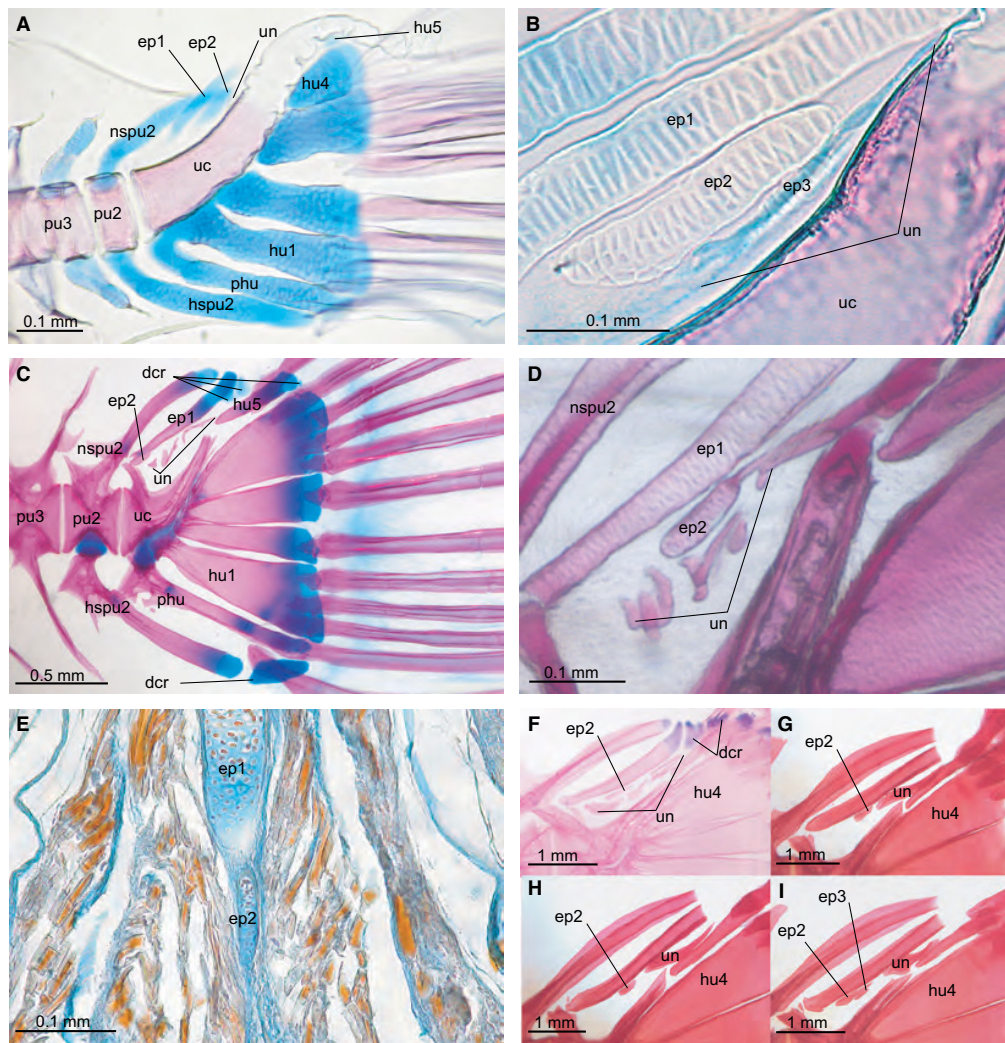


Fig. 3—Triacanthodidae. **A.** *Parahollandia* sp. (3.9 mm SL). **B.** Close-up of the dorsal region of *Hollandia* sp. (4.9 mm SL). **C.** *Avrophacanthus japonicus* (18 mm SL). **D.** Close-up of the dorsal region of *A. japonicus* (14.5 mm SL). **E.** Serial section of an *A. japonicus* (15 mm SL). **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 *Parahollandia* 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 (*Hollandia* 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.

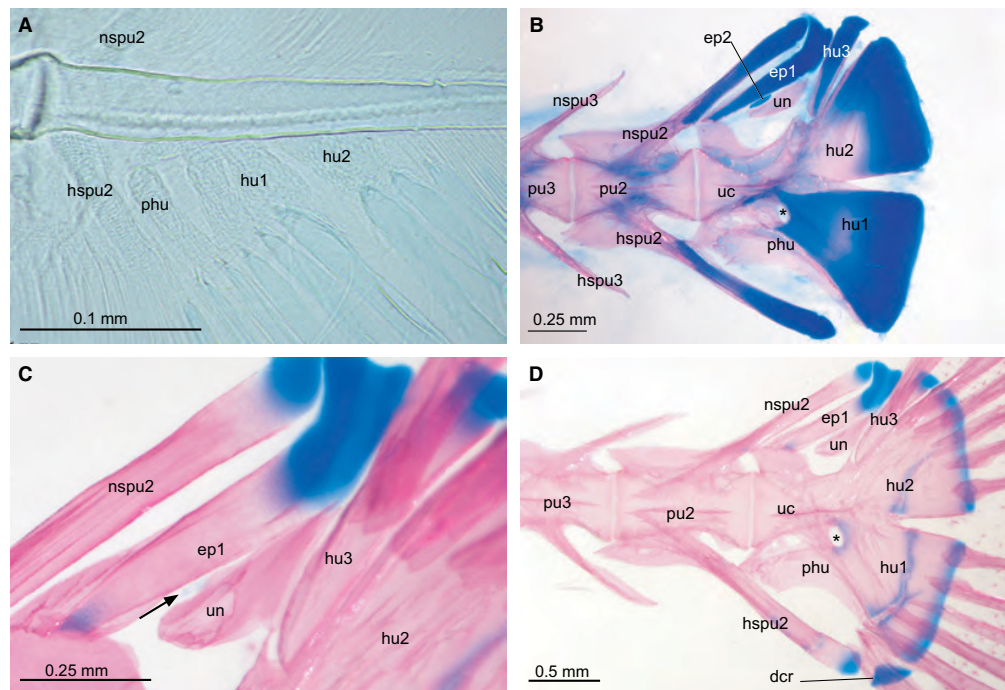


Fig. 4—Triacanthidae. **A.** *Tripodichthys* sp. (3.9 mm NL). **B–D.** Two developmental stages of *Tripodichthys oxycephalus* **B.** 16 mm SL; **C.** Close up of **D.** 33 mm SL. Arrow indicates the blue-stained remnant of the resorbed epural 2. Asterisk marks the foramen for the caudal artery.

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 capricus* 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 *Monotretes 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 *Monotretes leiurus* (Britz and Johnson 2005a) and *Takifugu niphobles* (Fujita 1992). However, according to Fujita (1992), later stages of *T. niphobles* differ from the two *Monotretes* 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

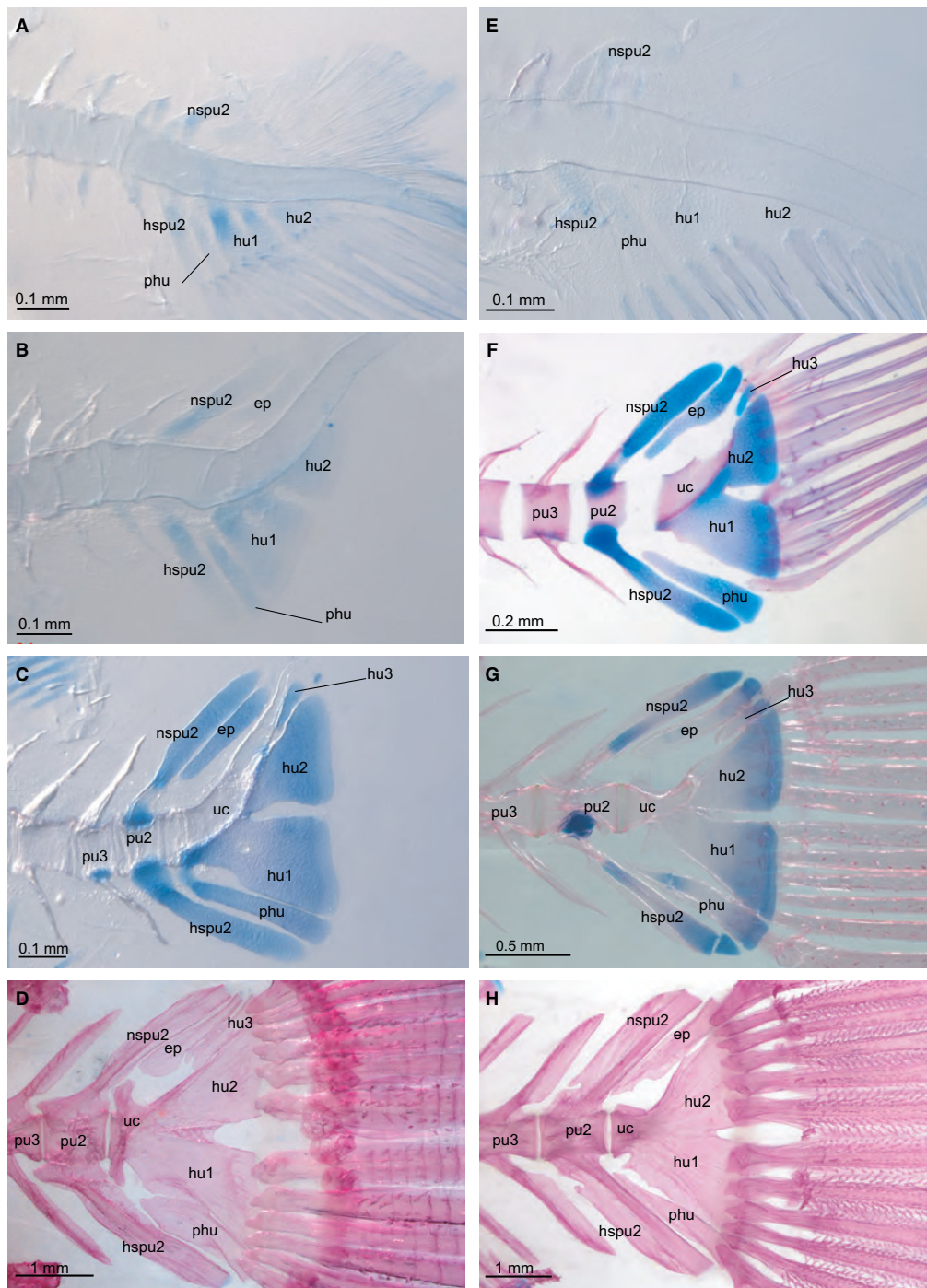


Fig. 5—Balistidae and Monacanthidae. **A–D**. Developmental series of *Balistapus undulatus*. **A**. 3.5 mm NL; **B**. 3.2 mm SL; **C**. 4.2 mm SL; **D**. 35 mm SL. **E–G**. Developmental series of *Stephanolepis* sp. **E**. 3.9 mm NL; **F**. 5.4 mm SL; **G**. 14.4 mm SL. **H**. *Monacanthus* sp. (37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

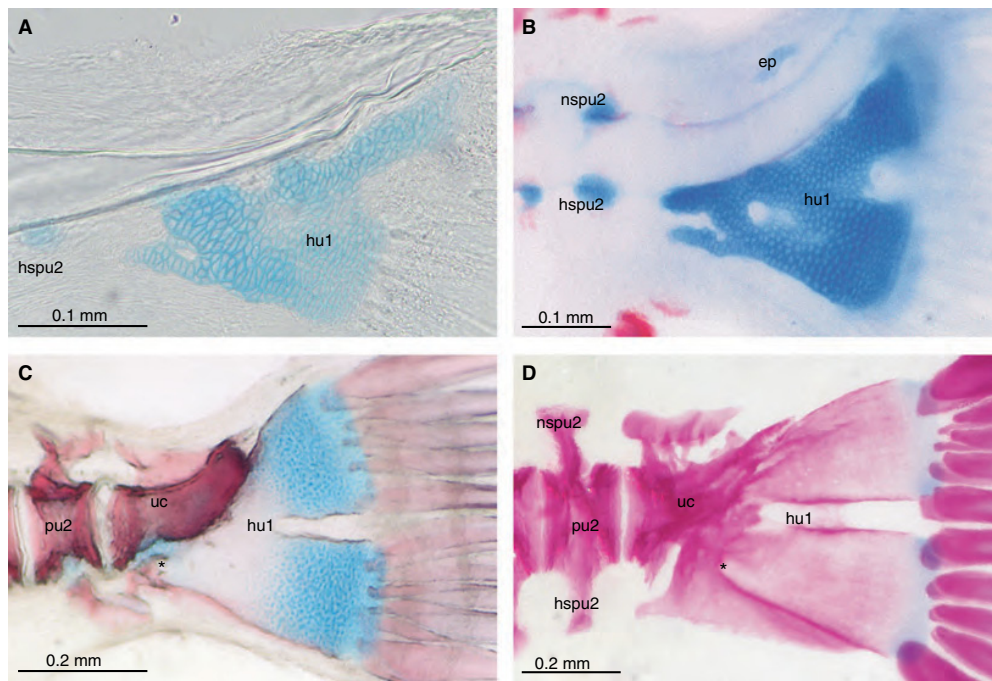


Fig. 6—Ostraciidae. Developmental series of *Lactophrys* sp. **A**. 3.5 mm NL; **B**. 4.0 mm NL; **C**. 8.0 mm SL; **D**. 11.3 mm SL. Asterisk marks the foramen for the caudal artery.

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

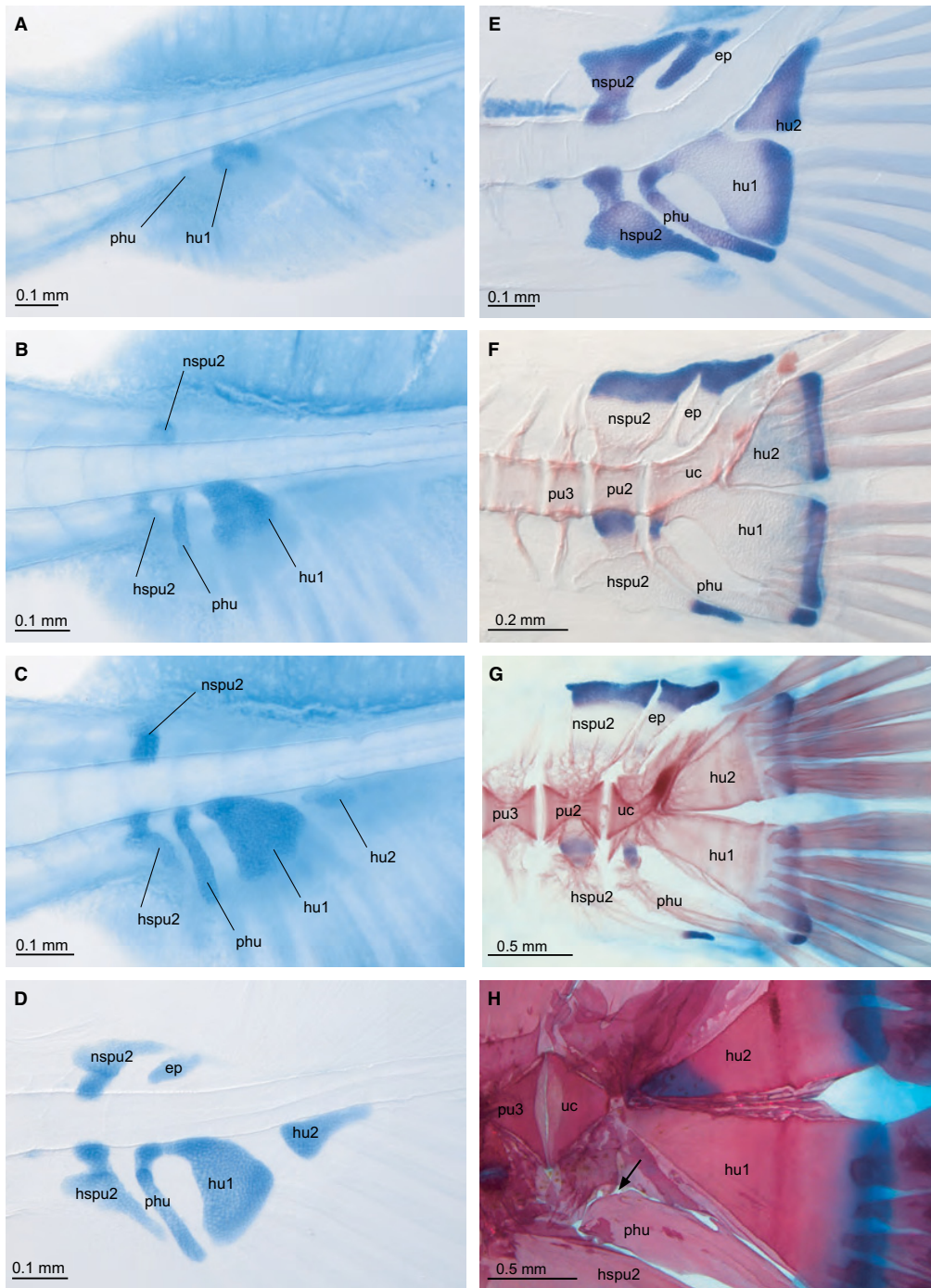


Fig. 7—Tetraodontidae. **A–G**. Developmental series of *Monotretes suvattii*. **A**. 4.6 mm NL; **B**. 4.7 mm NL; **C**. 4.7 mm NL; **D**. 4.9 mm NL; **E**. 4.4 mm SL; **F**. 5.7 mm SL; **G**. 16.4 mm SL. **H**. *Carinotetraodon irubescens* (25 mm SL). Arrow marks the gap between the foreshortened parhypural 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*

capricus 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

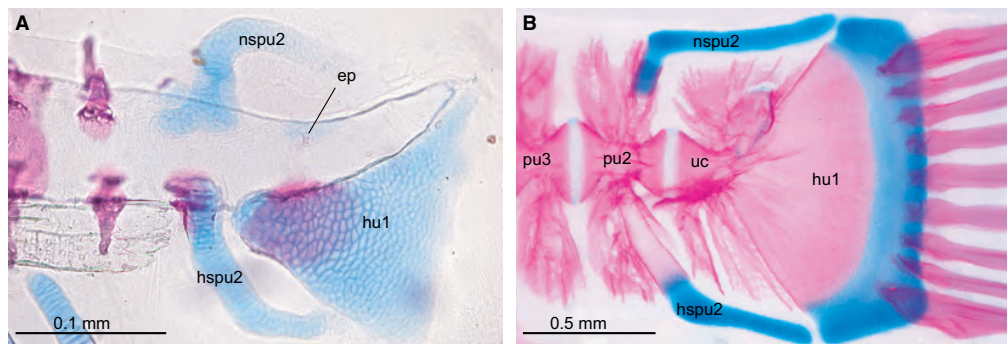


Fig. 8—Diodontidae. **A.** and **B.** Two developmental stages of *Diodon hystrix* **A.** 5.5 mm SL; **B.** 15.0 mm SL.

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

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. suwattii*, 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 *Monotretete*, 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 *Monotretete*.

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 *Chilomyxterus 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. capricus*. 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 Aracaniinae (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), gobioides (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.

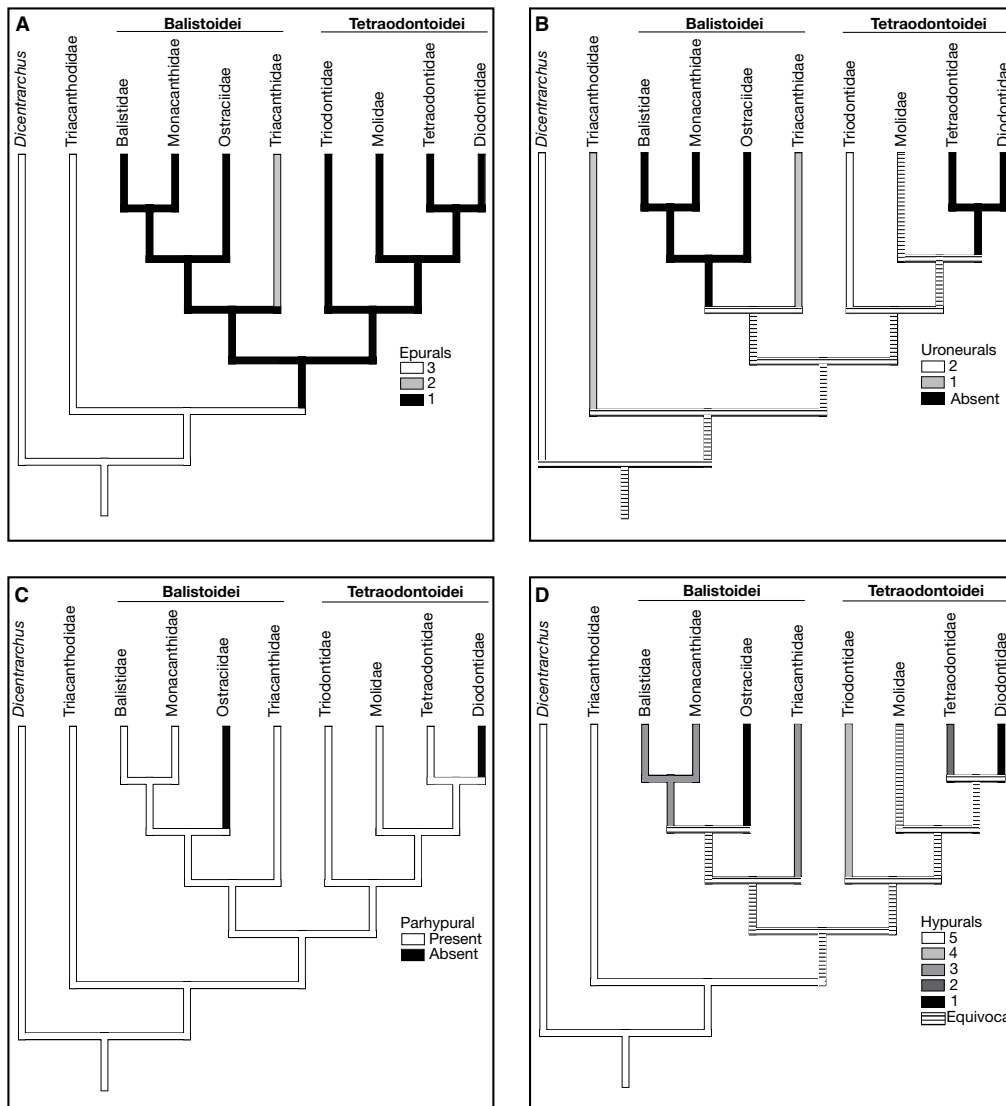


Fig. 9—Character evolution. The four major elements in the caudal skeleton of the Tetraodontiformes mapped onto Santini and Tyler’s (2003) phylogenetic hypothesis. **A.** Epurals, **B.** Uroneurals, **C.** Parhypural and **D.** Hypurals. Colour of the branches indicates the different character states.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

Alfaro, M. E., Santini, F. and Brock, C. D. 2007. Do reefs drive diversification in marine teleosts? Evidence from the pufferfish and their allies (Order Tetraodontiformes). – *Evolution* **61**: 2104–2126.

Arratia, G. and Schultze, H. P. 1991. Palatoquadrate and its ossifications: development and homology within osteichthyans. – *Journal of Morphology* **208**: 1–81.

Bird, N. C. and Mabee, P. M. 2003. Developmental morphology of the axial skeleton of the zebrafish, *Danio rerio* (Ostariophysi: Cyprinidae). – *Developmental Dynamics* **228**: 337–357.

Britz, R. and Hoffmann, M. 2006. Ontogeny and homology of the claustra in otophysan Ostariophysi (Teleostei). – *Journal of Morphology* **267**: 909–923.

Britz, R. and Johnson, G. D. 2005a. Leis' conundrum: homology of the clavus of the ocean sunfishes. 1. Ontogeny of the median fins and axial skeleton of *Monotretus leiurus* (Teleostei, Tetraodontiformes, Tetraodontidae). – *Journal of Morphology* **266**: 1–10.

Britz, R. and Johnson, G. D. 2005b. Occipito-vertebral fusion in ocean sunfishes (Teleostei: Tetraodontiformes: Molidae) and its phylogenetic implications. – *Journal of Morphology* **266**: 74–79.

Dareste, C. 1850. Recherches sur la classification des poissons de l'ordre des Plectognathes. – *Annales des Sciences Naturelles. B. Zoologie* **3**: 105–133.

Fritzsche, R. A. and Johnson, G. D. 1980. Early osteological development of white perch and striped bass with emphasis on identification of their larvae. – *Transaction of the American Fisheries Society* **109**: 387–406.

Fujita, K. 1989. Nomenclature of cartilaginous elements in the caudal skeleton of teleostean fishes. – *Japanese Journal of Ichthyology* **36**: 22–29.

Fujita, K. 1990. *The Caudal Skeleton of Teleostean Fishes*. Tokai University Press, Tokyo.

Fujita, K. 1992. Development of the caudal skeleton in the tetraodontid fish, *Takifugu niphobles*. – *Japanese Journal of Ichthyology* **38**: 438–440.

Geerinckx, T. and Adriaens, D. 2007. Ontogeny of the intermandibular and hyoid musculature in the suckermouth armoured catfish *Ancistrus cf. viridiatus* (Loricariidae, Siluriformes). – *Animal Biology* **57**: 339–357.

Geerinckx, T., Huysentruyt, F. and Adriaens, D. 2009. Ontogeny of the jaw and maxillary barbel musculature in the armoured catfish families Loricariidae and Callichthyidae (Loricarioidea, Siluriformes), with a discussion on muscle homologies. – *Zoological Journal of the Linnean Society* **155**: 76–96.

Geoffroy, S.-H. E. 1830. *Principes de Philosophie Zoologique*. Pitcher and Didier, Rosseau Paris.

Gosline, W. A. 1961. The perciform caudal skeleton. – *Copeia* **1961**: 265–270.

Hilton, E. J. and Britz, R. 2010. The caudal skeleton of osteoglossomorph fishes, revisited: comparisons, homologies, and character. In: Nelson, J. S., Schultze, H. P. and Wilson, M. V. H. (Eds): *Mesozoic Fishes 4 – Homology and Phylogeny*, pp. 219–237. Dr. Friedrich Pfeil, München.

Hilton, E. J. and Johnson, G. D. 2007. When two equals three: developmental osteology and homology of the caudal skeleton in carangid fishes (Perciformes: Carangidae). – *Evolution & Development* **9**: 178–189.

Hilton, E. J., Britz, R., Johnson, G. D. and Forey, P. L. 2007. Clarification of the occipito-vertebral region of *Arapaima gigas* (Osteoglossomorpha: Osteoglossidae) through developmental osteology. – *Copeia* **2007**: 218–224.

Hilton, E. J., Johnson, G. D. and Smith-Vaniz, W. F. 2010. Osteology and systematics of *Parastromateus niger* (Perciformes: Carangidae), with comments on the carangid dorsal Gill-Arch skeleton. – *Copeia* **2**: 312–333.

Hoffmann, M. and Britz, R. 2006. Ontogeny and homology of the neural complex of otophysan Ostariophysi. – *Zoological Journal of the Linnean Society* **147**: 301–330.

Holcroft, N. I. 2004. A molecular test of alternative hypotheses of tetraodontiform (Acanthomorpha: Tetraodontiformes) sister group relationships using data from the RAG1 gene. – *Molecular Phylogenetics and Evolution* **32**: 749–760.

Holcroft, N. I. 2005. A molecular analysis of the interrelationships of tetraodontiform fishes (Acanthomorpha: Tetraodontiformes). – *Molecular Phylogenetics and Evolution* **34**: 525–544.

Huysentruyt, F., Geerinckx, T. and Adriaens, D. 2007. A descriptive myology of *Corydoras aeneus* (Gill, 1858) (Siluriformes: Callichthyidae), with a brief discussion on adductor mandibulae homologies. – *Animal Biology* **57**: 433–452.

Johnson, G. D. and Britz, R. 2005. 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). – *Journal of Morphology* **266**: 11–21.

Johnson, G. D. and Britz, R. 2010. Occipito-vertebral fusion in actinopterygians: conjecture, myth and reality. Part 2: Teleosts. In: Nelson, J. S., Schultze, H. P. and Wilson, M. V. H. (Eds): *Mesozoic*

- Fishes 4 – Homology and Phylogeny*, pp. 95–110. Dr. Friedrich Pfeil, München.
- Johnson, G. D. and Patterson, C. 1993. Percormorph phylogeny – a survey of acanthomorphs and a new proposal. – *Bulletin of Marine Science* **52**: 554–626.
- Klassen, G. J. 1995. Phylogeny and biogeography of the Ostraciinae (Tetraodontiformes, Ostraciidae). – *Bulletin of Marine Science* **57**: 393–441.
- Konstantinidis, P. and Conway, K. W. 2010. The median-fin skeleton of the Eastern Atlantic and Mediterranean Clingfishes *Lepadogaster lepadogaster* (Bonnaterre) and *Gouania wildenowi* (Risso) (Teleostei: Gobiessocidae). – *Journal of Morphology* **271**: 215–224.
- Konstantinidis, P. and Harris, M. P. 2010. Same but different: ontogeny and evolution of the musculus adductor mandibulae in the Tetraodontiformes. – *Journal of Experimental Zoology Part B*. in press.
- Leis, J. M. 1984. Tetraodontiformes: Relationships. In: Moser, H. G., Richards, W. J., Cohen, D. M., Fahay, M. P., Kendall, A. W. J. and Richardson, S. L. (Eds): *Ontogeny and Systematics of Fishes*, pp. 459–463. Allen Press, Lawrence, KS.
- Leis, J. M., Olney, J. E. and Okiyama, M. 1997. Proceedings of the symposium fish larvae and systematics: ontogeny and relationships. The International Larval Fish Conference, June 16–30, 1995 held in Sydney, Australia, at the 19th Annual Meeting of the Early Life History Section of the American Fisheries Society. – *Bulletin of Marine Science* **60**: 1–212.
- Maddison, D. R. and Maddison, W. P. 2005. *MacClade 4: Analysis of Phylogeny and Character Evolution, Version 4.08*. Sinauer, Sunderland, MA.
- Matsuura, K. 1979. Phylogeny of the superfamily balistoidea pisces Tetraodontiformes. – *Memoirs of the Faculty of Fisheries Hokkaido University* **26**: 49–170.
- Matsuura, Y. and Katsuragawa, M. 1985. Osteological development of fins and their supports of larval grey triggerfish, *Balistes capriscus*. – *Japanese Journal of Ichthyology* **31**: 411–421.
- Monod, T. 1968. Le complex urophore des poissons ‘téléostéens’. – *Mémoires Institut France Afrique Noire* **81**: 1–705.
- Nelson, J. S. 2006. *Fishes of the World*, 4th edn, pp. 1–601. Wiley & Sons, New York.
- Nybelin, O. 1971. On the caudal skeleton in *Elops* with remarks on other teleostean fishes. – *Acta Regiae Societatis Scientiarum et Litterarum Gothoburgensis. Zoologica* **7**: 1–78.
- Patterson, C. 1968. The caudal skeleton in Lower Liassic pholidophorid fishes. – *Bulletin of the British Museum (Natural History) Geology* **16**: 201–239.
- Patterson, C. 1982. Morphological Characters and Homology. In: Joysey, K. A. and Friday, A. E. (Eds): *Problems of the Phylogenetic Reconstruction*, pp. 21–74. Academic Press, London.
- Potthoff, T. 1975. Development and structure of caudal complex, vertebral column, and Pterygiophores in Blackfin Tuna (*Thunnus atlanticus*, Pisces, Scombridae). – *Bulletin of Marine Science* **25**: 205–231.
- Potthoff, T. 1980. Development and structure of fins and fin supports in dolphin fishes *Coryphaena hippurus* and *Coryphaena equiselis* (Coryphaenidae). – *Fishery Bulletin* **78**: 277–312.
- Potthoff, T. and Kelley, S. 1982. Development of the vertebral column, fins and fin supports, branchiostegal rays, and squamation in the Swordfish, *Xiphias gladius*. – *Fishery Bulletin* **80**: 161–186.
- Potthoff, T. and Tellock, J. A. 1993. Osteological development of the snook, *Centropomus undecimalis* (Teleostei, Centropomidae). – *Bulletin of Marine Science* **52**: 669–716.
- Potthoff, T., Richards, J. W. and Ueyanagi, S. 1980. Development of *Scombrobrax heterolepis* (Pisces, Scombrobracidae) and comments on familial relationships. – *Bulletin of Marine Science* **30**: 329–357.
- Potthoff, T., Kelley, S., Saksena, V., Moe, M. and Young, F. 1987. Description of larval and juvenile yellowtail damselfish, *Microspathodon chrysurus*, Pomacentridae, and their osteological development. – *Bulletin of Marine Science* **40**: 330–375.
- Potthoff, T., Kelley, S. and Collins, L. A. 1988. Osteological development of the Red Snapper, *Lutjanus campechanus* (Lutjanidae). – *Bulletin of Marine Science* **43**: 1–40.
- Regan, C. T. 1902. On the classification of the fishes of the suborder Plectognathi; with notes and description of new species from specimens in the British Museum collection. – *Proceedings of the Zoological Society of London* **2**: 284–305.
- Remane, A. 1952. *Die Grundlagen des natürlichen Systems, der vergleichenden Anatomie und Phylogenetik*. Geest & Port, Leipzig.
- Richards, W. J. 2006. Early stages of Atlantic fishes: an identification guide for the western central North Atlantic. In: Richards, W. J. (Ed.): *Early Stages of Atlantic Fishes: an Identification Guide for the Western Central North Atlantic*, **2**, pp. i–vi, 1337–2640. CRC Tyler and Francis, Boca Raton.
- Romeis, B. 1986. *Mikroskopische Technik*. Urban & Schwarzenberg, Muenchen.
- Rosen, D. 1984. Zeiformes as primitive plectognath fishes. – *American Museum Novitates* **2782**: 1–45.
- Rosen, D. E. and Patterson, C. 1969. The structure and relationship of the paracanthopterygian fishes. – *Bulletin of the American Museum of Natural History* **141**: 361–469.
- Santini, F. and Tyler, J. C. 2003. A phylogeny of the families of fossil and extant tetraodontiform fishes (Acanthomorpha, Tetraodontiformes), Upper Cretaceous to recent. – *Zoological Journal of the Linnean Society* **139**: 565–617.
- Schultze, H. P. and Arratia, G. 1988. Reevaluation of the caudal skeleton of some actinopterygian fishes .2. Hiodon, Elops, and Albula. – *Journal of Morphology* **195**: 257–303.
- Schultze, H. P. and Arratia, G. 1989. The composition of the caudal skeleton of Teleosts (Actinopterygii, Osteichthyes). – *Zoological Journal of the Linnean Society* **97**: 189–231.
- Taylor, W. R. and Van Dyke, C. C. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. – *Cybiurn* **9**: 107–120.
- Tyler, J. C. 1968. A monograph on plectognath fishes of the superfamily Triacanthoidea. – *Monographs of the Academy of Natural Sciences of Philadelphia* **16**: 1–364.
- Tyler, J. C. 1970. The progressive reduction in number of elements supporting the caudal fin of fishes of the order Plectognathi. – *Proceedings of the Academy of Natural Sciences of Philadelphia* **122**: 1–85.
- Tyler, J. C. 1980. Osteology, phylogeny, and higher classification of the fishes of the order Plectognathi (Tetraodontiformes). – *NOAA Technical Report NMFS Circular* **434**: 1–422.
- Tyler, J. C. and Sorbini, L. 1996. New superfamily and three new families of tetraodontiform fishes from the Upper Cretaceous: the earliest and most morphologically primitive plectognaths. – *Smithsonian Contributions to Paleobiology* **82**: i–iii, 1–59.
- Tyler, J. C. and Holcroft, N. 2007. Tetraodontiformes - triggerfishes, boxfishes, puffers (fugu), molas and allies. DOI: <http://tolweb.org/Tetraodontiformes/52153/2007.02.19>.
- Tyler, J. C., O’Toole, B. and Winterbottom, R. 2003. Phylogeny of the genera and families of zeiform fishes, with comments on their relationships with tetraodontiform and caproids. – *Smithsonian Contributions to Zoology* **618**: 1–110.
- Wiley, E. O. and Johnson, G. D. 2010. A teleost classification based on monophyletic groups. In: Nelson, J. S., Schultze, H. P. and

- Wilson, M. V. H. (Eds): *Mesozoic Fishes 4 – Homology and Phylogeny*, pp. 123–182. Dr. Friedrich Pfeil, München.
- Winterbottom, R. 1974. The familial phylogeny of the Tetraodontiformes (Acanthopterygii: Pisces) as evidenced by their comparative myology. – *Smithsonian Contributions to Zoology* **155**: 1–201.
- Yamanoue, Y., Miya, M., Matsuura, K., Katoh, M., Sakai, H. and Nishida, M. 2008. A new perspective on phylogeny and evolution of tetraodontiform fishes (Pisces: Acanthopterygii) based on whole mitochondrial genome sequences: basal ecological diversification? – *BMC Evolutionary Biology* **8**: 1–14.

3.3 Publication III



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Ontogeny of the jaw apparatus and suspensorium of the Tetraodontiformes

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Abstract

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The jaw apparatus and suspensorium of adult Tetraodontiformes are well adapted to a durophagous feeding habit. Anatomical indicators are the short, stout jaws and a suspensorium in which the quadrate lies in the same vertical plane as the autopalatine. In contrast, the palatoquadrate of larval Tetraodontiformes generally resembles that of larval percomorphs – a more posteriorly positioned quadrate and a slender and long Meckelian cartilage. Among Tetraodontiformes, the Triacanthodidae retain a protrusible upper jaw and a versatile suspensorium. The jaws of the Balistoidei have greater mobility achieved by a reduced autopalatine that has lost its bony contact with the suspensorium. In contrast to the Balistoidei, the beak-like jaws of the Tetraodontoidae lack individual teeth in the biting part of the jaws. The autopalatine is enlarged, which results in immobilization of the ethmopalatine articulation. The Ostraciidae are exceptional in having the distal part of the autopalatine reduced, while the proximal part remains attached to the suspensorium.

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Introduction

The Tetraodontiformes have attracted considerable attention in diverse fields of biological research such as genetics (Brainerd and Murray 2000; Amemiya *et al.* 2001; Aparicio *et al.* 2002; Amores *et al.* 2004), functional morphology (Brainerd 1994; Turingan and Wainwright 1993; Turingan *et al.* 1995; Wainwright *et al.* 1995; Wainwright and Turingan 1996, 1997; Brainerd and Patek 1998; Bartol *et al.* 2003), comparative anatomy (Tyler 1968; Winterbottom 1974; Fujita 1992; Santini and Tyler 2003; Nakae and Sasaki 2004; Britz and Johnson 2005a,b; Johnson and Britz 2005), and systematics (Winterbottom 1974; Tyler 1980; Leis 1984; Rosen 1984; Holcroft 2004, 2005; Yamanoue *et al.* 2007, 2008). Accordingly, they are one of the most extensively studied teleost groups.

Tetraodontiformes are characterized by the absence of parietals, nasals, extrascapulars, infraorbitals, anal-fin spines, ribs, fewer than 21 vertebrae, and 12 or fewer caudal fin rays, among other features (Tyler 1980; Tyler and Holcroft 2007; Wiley and Johnson 2010). These reductions are accompanied

by a high degree of specialization of all sorts of structures in particular in the more derived taxa, e.g. the inflatable stomach of the Tetraodontidae and Diodontidae (Breder and Clark 1947; Brainerd 1988, 1994), the locking mechanism of both the pelvic and dorsal fin spines of the Triacanthodidae, Triacanthidae, or only the latter in the Balistidae and Monacanthidae (Tyler 1968, 1980), the expandable skin flap of the Balistidae, Monacanthidae, and *Triodon macropterus* (Breder and Clark 1947), and the subdivision of the adductor mandibulae muscle into six to eight portions (Winterbottom 1974).

The jaw apparatus of teleost fishes has attracted much interest, because of the surmise that it is intrinsically tied to the evolution and tremendous diversification of this vertebrate taxon (Schaeffer and Rosen 1961; Lauder 1980, 1982, 1985; Westneat 2003, 2004; Wilga 2008). Suction feeding, through generating negative pressure, represents the plesiomorphic mode for teleost fishes (Schaeffer and Rosen 1961; Lauder 1980, 1982, 1985; Westneat 2003, 2004; Wilga 2008) and is facilitated by a rapid expansion of the buccal cavity (Lauder 1980, 1985; Lauder and Liem 1980, 1981). During their evolution, the jaw apparatus has become elaborated and

optimized (Schaeffer and Rosen 1961; Wainwright and Bellwood 2002; Westneat 2003). The premaxillae and maxillae, which form the upper jaw, have become free from the braincase to allow a rostral protrusion. The freeing of the upper jaws from the braincase also allows a greater lateral expansion of the buccal cavity facilitated by the suspensorium, a functional unit formed by endoskeletal and exoskeletal elements of the viscerocranium. Two joints enable the lateral movement of the suspensorium, one anteriorly between the palatine and the ethmoid region and one posteriorly between the hyomandibular and the otic capsule.

The jaws of the Tetraodontiformes have drastically departed from the aforementioned grundplan because of a durophagous feeding habit (Tyler 1968; Targett 1978; Lauzanne 1988; Turingan and Wainwright 1993; Turingan 1994; Duncan and Szelistowski 1998). Their jaws are specialized and well adapted for this mode of feeding, specifically through the preceding reduction in upper jaw protrusibility and mobility of the suspensorium. Many studies have investigated the evolution, diversity, and/or functional relevance of the motor pattern of the musculature for durophagy in these fishes (Turingan and Wainwright 1993; Wainwright and Turingan 1993, 1997; Turingan 1994; Turingan *et al.* 1995; Ralston and Wainwright 1997; Friel and Wainwright 1998, 1999; Wainwright and Friel 2000). However, in these studies, the anatomical differences of the jaws and suspensorium have often been simplified and treated as similar among the different families of the suborder.

The goal of this study is to evaluate the published information about anatomy of the jaws and suspensorium of the Tetraodontiformes and to add new information by investigating the ontogeny of members of most of the families. We also show that two completely different anatomical specializations have evolved within the Tetraodontiformes, each derived from the grundplan represented by the Triacanthodidae. In light of the new data, we discuss problems with some previous morphological and phylogenetic analyses.

Material and Methods

Specimens were cleared and double stained (c&s) for bone and cartilage following Taylor and Van Dyke (1985). Photographs 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. The suspensoria and jaws were dissected under a Zeiss DRC dissecting scope where possible. In *Diodon hystrix* and *Ranzania laevis*, the jaws were excluded because of the fusion of the dentaries and premaxillae with their contralateral members. In our *Parahollardia* material, the jaws were very fragile, and accordingly, we left them attached to the neurocranium.

For analysis of the character evolution of the rostral cartilage, the ethmoid region, the autopalatine and its connection

to the ectopterygoid, and the ethmopalatine articulation, a simple taxa/character matrix was created and mapped onto the topology of Tyler and Holcroft (2007).

Institutional abbreviations

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 standard length (SL), c&s.

Tetraodontiformes. Triacanthodidae. *Atrophacanthus japonicus* (Kamohara), BMNH 1987.1.23, one specimen, 58 mm, c&s; one specimen, uncatalogued (Chiba Institute of Technology), 18 mm SL, c&s; *Parahollardia* sp. (Fraser-Brunner), one specimen, CA 89144605, 3.9 mm notochord length (NL), c&s. Triacanthidae. *Tripodichthys oxycephalus* (Bleeker), two specimens, BMNH 2006.3.280, 16 and 33 mm SL, c&s. Balistidae. *Balistapus undulatus* (Park), two specimens, uncatalogued (NSMT), 2.7 & 4.9 mm NL, c&s; one specimen, BMNH 1974.2.25, 29.0 mm, c&s. Monacanthidae. *Stephanolepis* sp. (Gill), one specimen, SEAMAP 10741, 5.1 mm SL, c&s; two specimens, uncatalogued (NSMT), 3.4 and 14.4 mm SL, c&s. Ostraciidae. *Lactophrys* sp. (Swainson), one specimen, SEAMAP 25817, 2.3 and 3.5 mm NL, c&s; one specimen, SEAMAP 22682, 11.3 mm SL, c&s. Molidae. *Ranzania laevis* (Pennant), two specimens, uncatalogued (USNM), 2.5 and 22.0 mm SL, c&s. Tetraodontidae. *Monotretu suvatii* (Sontirat), four specimens, uncatalogued, 3.7 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 (not buffered) and 2 days later transferred into 70% ethanol. Diodontidae. *Diodon hystrix* (Linnaeus), one specimen, uncatalogued (NSMT), 2.7 mm NL, c&s; one specimen, SEAMAP 19379, 5.9 mm SL, c&s; one specimen, SEAMAP 22672, 15 mm SL, c&s.

Figure abbreviations

Autopalatine	ap
Angular	ang
Dentary	d
Ectopterygoid	ecpt
Endopterygoid	enpt
Ethmoid	eth
Ethmoid plate	epl

Frontal	f
Hyomandibular	hy
Internasal septum	sint
Interopercle	iop
Lamina orbitonasalis	lorb
Lateral ethmoid	leth
Meckelian cartilage	mc
Maxilla	mx
Metapterygoid	mpt
Opercle	op
Parasphenoid	psph
Pars autopalatina	pap
Preopercle	pop
Preopercular spinules	spop
Premaxilla	pmx
Quadrate	q
Retroarticular	ra
Rostral cartilage	rc
Subopercle	sop
Symplectic	sy
Vomer	v

Results

Triacanthodidae – *Parahollandia* sp. (3.9 mm; Fig. 1A,B): In this larval stage of *Parahollandia* sp., the suspensorium consists of the palatoquadrate and the pars hyomandibularis. The quadrate and the autopalatine have started to ossify within the pars quadrata and the pars autopalatina, respectively. The posteroventral process of the quadrate is fully developed and attached to the symplectic and preopercle. The pars metapterygoidea defines the posterior end of the palatoquadrate and tapers caudally, and its posterior tip is positioned at about the same level as the posterior margin of the symplectic. The premaxilla and maxilla are slender, well-ossified elements. The dentary has two teeth on each side of the symphysis. The hyomandibular consists of a thin perichondral layer of bone. A

foramen, serving as a passage for the mandibular branch of the facial nerve, pierces the hyomandibular. A crest of membrane bone extends the dorsal part of the hyomandibular anteriorly. The opercular head is oriented ventrally. The opercular series is present. The horizontal arm of the preopercle is longer than the vertical arm.

The autopalatine articulates with a laterally expanded ethmoid plate. The lamina orbitonasales and the internasal septum are very close together. The lateral ethmoid and the ethmoid have started to ossify in the lamina orbitonasalis and the internasal septum, respectively.

***Atrophacanthus japonicus* (18.0 and 58.0 mm; Fig. 1C,D):** All ossifications of the suspensorium are present. The z-shaped autopalatine bears a prominent processus maxillaris. The dorsoposterior margin of the autopalatine houses an indentation for the lateral ethmoid. The quadrate has shifted its position and is now located roughly below the autopalatine. The pars autopalatina persists posteroventrally to the autopalatine. The posteroventral process of the quadrate covers the anterior part of the symplectic laterally. The pars metapterygoidea has become larger, and the metapterygoid is present.

The triangular ectopterygoid is in loose contact with the autopalatine and the processus pterygoideus. The anterior end of the endopterygoid is covered by the autopalatine laterally, while the posterior two-thirds of the endopterygoid covers the metapterygoid dorsally. The premaxilla has a long ascending process that is extended at its proximal base and housed in a groove formed by the dorsomedial and dorsolateral processes of the maxilla. The dentary is a stout and triangular element with conical teeth. A large coronomeckelian is present at the posterior end of the meckelian cartilage, and medial to the dentary. A small retroarticular at the caudoventral end of the articular points ventrally.

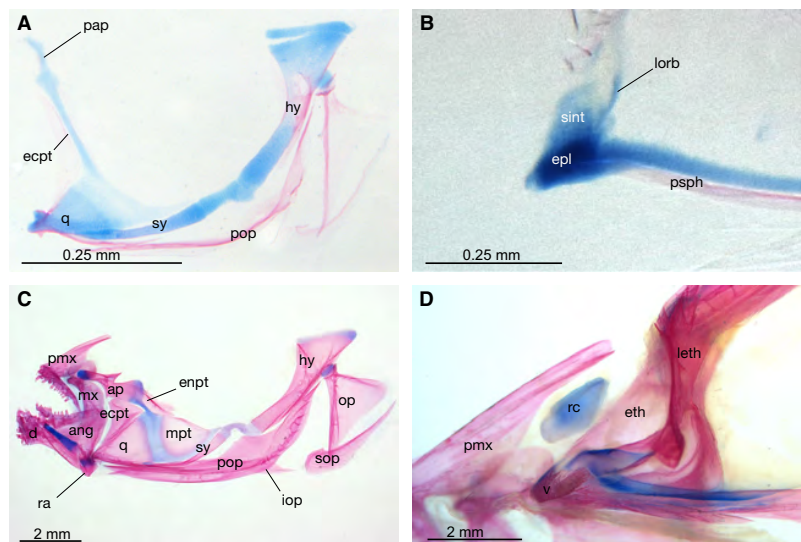


Fig. 1—Triacanthodidae. —**A** and **B**. *Parahollandia* sp. —**A**. Suspensorium, jaws removed, and —**B**. Ethmoid region of a 3.9 mm SL specimen. —**C** and **D**. *Atrophacanthus japonicus*. —**C**. Suspensorium of a 18 mm SL specimen. —**D**. Ethmoid region of a 58 mm SL specimen. All figures in lateral view.

The dorsal margin of the hyomandibular articulates along its entire edge with a facet on the otic capsule formed by the sphenotic, prootic, and autopterotic. The cartilaginous connection between the symplectic and the hyomandibular is arched dorsally, where the dorsal tip of the interhyal articulates.

The triangular opercle has a dorsoventrally oriented ridge at its anterior margin that extends dorsally beyond the opercular head of the hyomandibular. The subopercle is broad anteriorly and tapers posterodorsally. The interopercle reaches from the anterior end of the subopercle to the anterior end of the preopercle.

The autopalatine articulates with the lateral expansion of the vomer and the anteroventral part of the lateral ethmoid. Anterior to the ethmoid a large oval rostral cartilage articulates with the ascending processes of the premaxillae.

Triacanthidae – *Tripodichthys oxycephalus* (16.0 and 33.0 mm; Fig. 2): The two individuals do not differ remarkably, and both show juvenile to adult characters. The autopalatine is a squarish element with a well-developed processus maxillaris. The posterior margin of the autopalatine has an indentation for articulation with the vomer. The processus pterygoideus is a short curved bar, forming the only connection between the autopalatine and the suspensorium. The autopalatine articulates with the maxilla anteriorly and the vomer posteriorly. Posteriorly, the triangular ectopterygoid does not reach the ventral margin of the autopalatine. The metapterygoid has a blunt posterior cartilaginous end and reaches to the level of the ventral end of the pars hyosymplectica. Posterodorsally, the metapterygoid bears a crest of membrane bone.

The shaft of the hyomandibular is roughly as wide as the head, of which the dorsal margin forms a single articular condyle articulating with a groove formed by the prootic, sphenotic, and pterotic of the neurocranium. The opercular head is oriented dorsoventrally. In both specimens, the pars hyomandibular is separated from the pars symplectica.

A dorsally oriented process from the opercle covers the opercular head of the hyomandibular laterally. Anteriorly, the subopercle bears a process and tapers posterodorsally. The preopercle is slender and consists mainly of the ridge where the adductor mandibulae is attached. The thin interopercle is pointed anteriorly and broadened posteriorly.

The ovoid rostral cartilage is situated between the ethmoid and the ascending processes of the premaxilla. The ethmoid is

a massive block-like bone flanked by the lateral ethmoids. The lateral ethmoid itself is roughly vertically oriented and in these specimens does not reach the anterior end of the ethmoid plate and does not contact the vomer.

Balistidae – *Balistapus undulatus* (2.7 mm; Fig. 3A): The quadrate is the only ossification present in the palatoquadrate at this stage. The posteroventral process of the quadrate is fully developed and embraces the anterior tip of the symplectic. The pars autopalatina is slightly curved and pointed anterodorsally. As in the triacanthodids, the pars metapterygoidea tapers caudally to a point and ends in the region of the posterior margin of the symplectic. The ectopterygoid has started to ossify as a small strip of bone anterior to the processus pterygoideus. The dentary is a massive element that embraces the articular caudally. The retroarticular at the posterior tip of Meckel's cartilage points ventrally. Both the dentary and the premaxilla bear conical teeth.

The posterior end of the symplectic is only slightly higher than its anterior tip. The perichondrally ossified hyomandibular is discernible with the opercular head projecting posterodorsally. All elements of the opercular series are present. The preopercle bears about thirty ossified, segmented spinules at the angle of the vertical and horizontal arm.

Balistapus undulatus (4.9 mm; Fig. 3B): All ossifications in the suspensorium are present. The pars autopalatina now appears as an oblique-oriented 'T'. As a perichondral ossification, the autopalatine surrounds the pars autopalatina except for the most distal tip on either side of the crossbar. The processus pterygoideus is slightly curved and more massive than in the previous stage. It is now embraced by the ectopterygoid (anteroventrally) and endopterygoid (posterodorsally). The pars quadrata and the associated quadrate are elongated caudally. The metapterygoid covers the pars metapterygoidea, and a small crest of membrane bone has started to develop at its dorsal margin. The posterior tip of the pars metapterygoidea is closely associated with the posterior end of the pars hyosymplectica. The maxilla is now firmly attached to the premaxilla. The teeth now have the incisiform appearance of the adults. The pars hyosymplectica is arched, and the posterior part of the symplectic is now oriented more dorsally and is no longer in line with the shaft of the hyomandibular. The spinules on the lateral face of the preopercle are reduced. The distal tip of the triangular opercle projects ventrally and covers the subopercle laterally (displaced in this specimen).

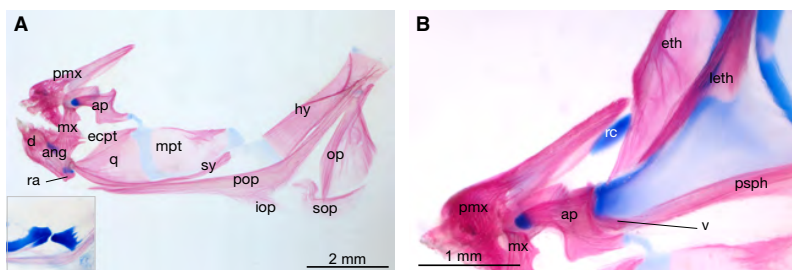


Fig. 2—Triacanthidae. —**A** and **B**. *Tripodichthys oxycephalus*. —**A**. Suspensorium and —**B**. Ethmoid region of a 33 mm SL specimen. Close up in **A**. Close up of the separate pars symplectica and pars hyomandibular of a 16 mm SL specimen. All figures in lateral view.

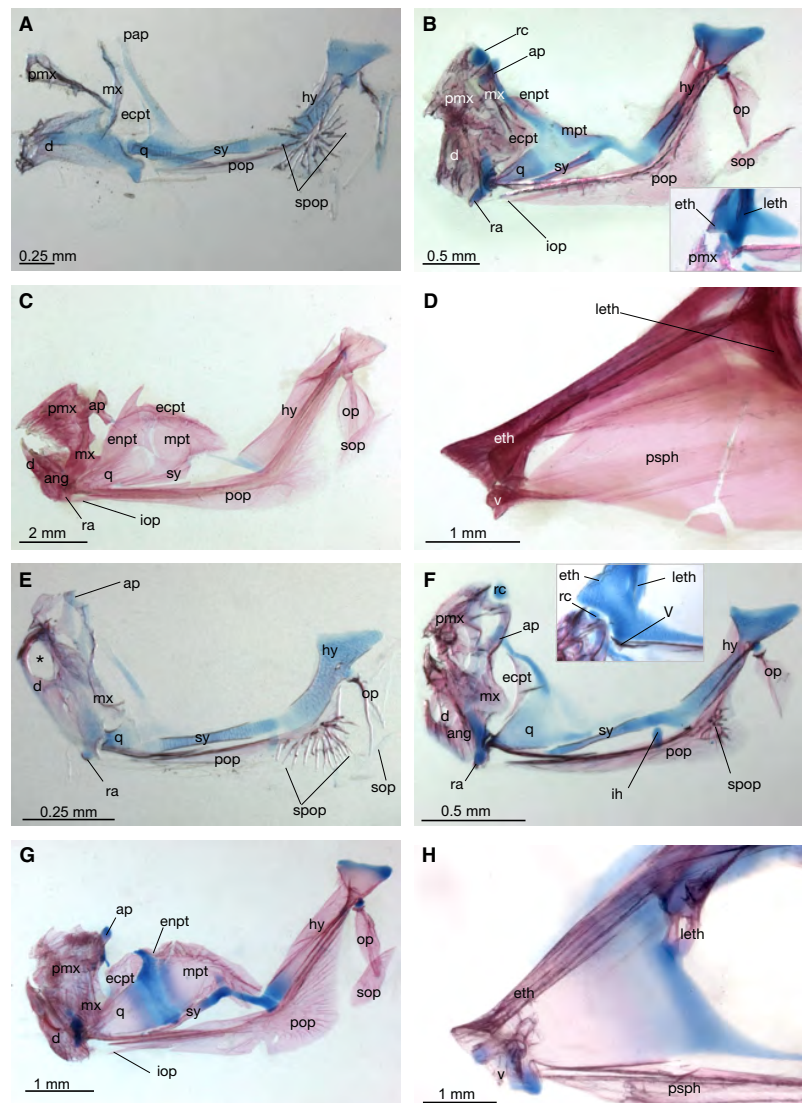


Fig. 3—Balistidae and Monacanthidae. —**A–D.** *Balistapus undulatus*. —**A.** Suspensorium of a 2.7 mm NL specimen. —**B.** Suspensorium and close up of the ethmoid region of a 4.9 mm SL specimen. —**C.** Suspensorium and **D.** Ethmoid region of a 29 mm SL specimen. —**E–H.** *Stephanolepis* sp. —**E.** Suspensorium of a 3.4 mm NL specimen. Asterisk marks the foramen in the dentary. —**F.** Suspensorium and close up of the ethmoid region of a 5.1 mm SL specimen. —**G.** Suspensorium and **H.** Ethmoid region of a 14.4 mm SL specimen. All figures in lateral view.

In the ethmoid region, all elements are present although not yet fully developed. The lamina orbitonasalis and the internasal septum are close together on the dorsal surface of the ethmoid plate. The posterior end of the ethmoid is partially covered by the frontals. The vertical oriented lateral ethmoid is fully formed. A conical rostral cartilage is closely attached and embraced by the premaxillae.

Balistapus undulatus (29.0 mm; Fig. 3C,D): This stage closely resembles the adult condition. All bones are present and well ossified. The T-shaped autopalatine is connected to the rest of the suspensorium only by a persisting part of the processus pterygoideus of the palatoquadrate, which is enclosed proximally by the ectopterygoid. The ectopterygoid bears a long dorsoposteriorly oriented process. A second process of the ectopterygoid extends ventrally between the

quadrate and metapterygoid, medial to the palatoquadrate. The metapterygoid has a blunt posterior end and does not reach the hyomandibular. The ventral margin of the metapterygoid covers the persisting pars hyosymplectica dorsally. Crests of membrane bone broaden the symplectic dorsally and ventrally. The dorsal crest bears a projection directly approaching the ventral projection of the ectopterygoid. In the opercular series only the opercle has changed its shape. It is now an oval element of which its ventral end covers the subopercle laterally.

The ethmoid region has become greatly elongated, and the ethmoid has moved well anterior to the lateral ethmoid. The anterior margin of the ethmoid forms a concave surface and together with the vomer forms a socket for the rostral cartilage. The ethmoid is expanded laterally to form a socket that

articulates with the posterodorsally oriented tip of the crossbar of the autopalatine.

Monacanthidae – *Stephanolepis* sp. (3.5 mm; Fig. 3E): A distinct pars metapterygoidea is absent at this stage, and the palatoquadrate therefore consists only of the pars quadrata, processus pterygoideus, and the pars autopalatina. The quadrate has started to ossify, surrounding the socket that articulates with the lower jaw. The posteroventral process of the quadrate is attached to the symplectic and to the preopercle. The processus pterygoideus projects anterodorsally, ending in an ill-defined pars autopalatina. The dentary is well ossified and pierced by a large foramen. The maxilla is a needle-shaped ossification located posterior to the premaxilla, which bears a single tooth next to the symphysis. The symplectic anteriorly and the hyomandibular posteriorly have started to ossify in the pars hyosymplectica. All elements of the opercular series are present. The preopercle has around 20 bony spinules, which are located roughly at the angle between the horizontal and vertical arms.

Stephanolepis sp. (5.1 mm; Fig. 3F): The pars quadrata has become enlarged relative to the processus pterygoideus. As in the balistids, the pars autopalatina appears as an obliquely oriented 'T'. The autopalatine has started to ossify close to the shaft of the 'T' and at the center of the crossbar. The ectopterygoid fills the indentation between the autopalatine and the quadrate anteriorly. A small pars metapterygoidea is now present, tapers posteriorly, and ends at about the same level as the posterior margin of the symplectic. The elements of the jaws are similar in general appearance to those of a 4.9 mm specimen of *Balistapus undulatus*. The shape of the teeth has now changed to incisiform. Between the head and shaft, the hyomandibular has a crest of membrane bone on the anterior margin.

The opercle articulates with the ventrally oriented opercular head of the hyomandibular. The subopercle is a teardrop-shaped bone just ventral to the opercle (not shown). The preopercular spinules are restricted to the broadened part of the preopercle. The interopercle is a short needle-shaped element medial to the anterior portion of the preopercle. The stout ethmoid region resembles that in *B. undulatus* at around 4.9 mm. A small cone-shaped rostral cartilage is embraced by the premaxillae.

Stephanolepis sp. (14.4 mm; Fig. 3G,H): All elements of the suspensorium are present at this stage. The shaft of the pars autopalatina consists only of a thin strip of cartilage that connects the highly reduced autopalatine to the suspensorium. The processus pterygoideus is embraced by the endopterygoid and ectopterygoid. Neither element approaches the autopalatine. The metapterygoid has a large crest of membrane bone that encloses the pars hyosymplectica caudoventrally. All jawbones are well ossified, and the rostral cartilage is embraced by the premaxillae posteriorly. The persisting pars hyosymplectica is strongly arched, resulting in a less horizontal position of the symplectic. All elements of the

opercular series are well ossified. The preopercle has lost the spinules entirely.

The ethmoid region is elongated, and the ethmoid occupies a large area of the ethmoid cartilage. The lateral ethmoid lags behind relative to the anterior tip of the ethmoid.

Ostraciidae – *Lactophrys* sp. (2.3 mm; Fig. 4A,B): The processus pterygoideus of the palatoquadrate is a massive dorsoanteriorly oriented bar with a blunt end. The pars autopalatina is not yet differentiated from the processus pterygoideus. The pars metapterygoidea has a blunt end, extending posteriorly to the level between the hyomandibular and the symplectic. The quadrate is present and has a large posteroventral process. The premaxilla, maxilla, and all elements of the lower jaw are present. The rectangular Meckel's cartilage bears an anteroventrally oriented retroarticular. Both the symplectic and hyomandibular are beginning to ossify. The opercular head of the hyomandibular faces posteroventrally and articulates with a small opercle. All elements of the opercular series are present. The anteroventral edge of the ethmoid plate articulates with the blunt end of the pars autopalatina.

Lactophrys sp. (3.5 mm; Fig. 4C): The processus pterygoideus has broadened, and the anterior tip of the autopalatine has started to ossify. The metapterygoid is present and has a lamina of membrane bone at the posterodorsal margin. The endopterygoid has formed at the dorsal margin of the pars autopalatina and the pars quadrata. The symplectic is slightly curved anteroventrally and now separated from the hyomandibular. The shaft of the hyomandibular has slightly shifted dorsally, and its distance to the preopercle has increased. Two crests of membrane bone have enlarged the hyomandibular, the larger anteriorly between the head and the shaft and the smaller caudoventrally to the shaft.

The vertical arm of the narrow preopercle is shorter than the horizontal arm. The opercle is the smallest element in the opercular series. The subopercle bears a posterodorsal process, which is covered by the opercle laterally. The interopercle is needle shaped and its anterior tip closely approaches the retroarticular.

Lactophrys sp. (11.3 mm; Fig. 4D,E): The autopalatine lacks the processus maxillaris, and an articulation with the maxilla is not established. Proximally, the autopalatine interdigitates with the ectopterygoid and endopterygoid. The small endopterygoid covers the autopalatine proximally and the metapterygoid anterodorsally. Its extension contacts the ectopterygoid on the medial side of the palatoquadrate. The crest of membrane bone of the metapterygoid approaches the shaft of the hyomandibular closely. Ventrally, the symplectic is extensively enlarged by membrane bone. The anterior crest of membrane bone of the hyomandibular is greatly enlarged. The opercular series does not differ significantly from the previous stage, described before.

The ethmoid region is elongated, and the lateral ethmoid lags behind relative to the anterior tip of the ethmoid. The vomer forms a massive block that covers the ethmoid ventrally. The anterior part of the vomer is aligned with the anterior part

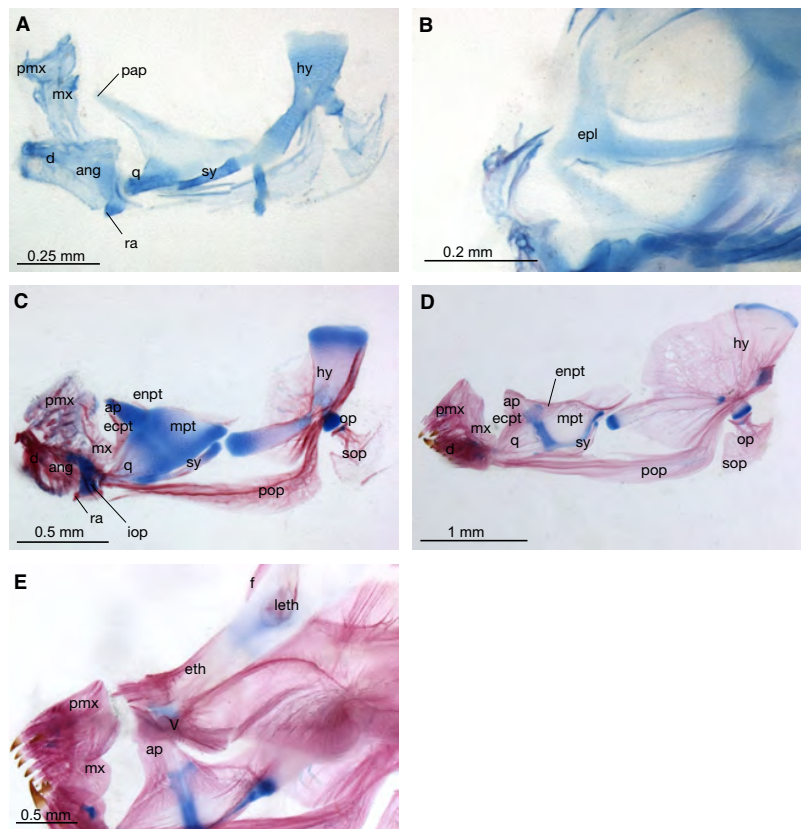


Fig. 4—Ostraciidae. —**A–E.** *Lactophrys*. sp. —**A.** Suspensorium —**B.** Ethmoid region of a 2.3 mm NL specimen. —**C.** Suspensorium of a 3.5 mm NL specimen. —**D.** Suspensorium and **E.** Ethmoid of a 11.3 mm SL specimen. All figures in lateral view.

of the ethmoid, and together, they form an indentation. A shallow, anterolaterally oriented indentation on the vomer provides the articulation surface for the autopalatine.

Molidae – *Ranzania laevis* (2.5 mm; Fig. 5A): The processus maxillaris of the autopalatine bears two wing-shaped crests of membrane bone. The one that projects dorsocaudally is tightly bound to the lateral ethmoid, while the more posteriorly oriented crest is closely attached to the parasphenoid.

The maxilla is closely attached to the premaxilla (not shown). The premaxilla and the dentary bear several small distinct teeth. The posteroventral process of the quadrate covers the symplectic laterally. An endopterygoid is developed on the dorsal surface of the processus pterygoideus. The metapterygoid has started to ossify. The opercular head of the hyomandibular is posteroventrally oriented. The slender symplectic is horizontally oriented. The opercle is a ventrally oriented slender element. The subopercle and interopercle are slender elements. The preopercle bears a large crest ventrally. The lamina orbitonasalis and the internasal septum are positioned at the same plane. A small vomer is present ventral to the ethmoid plate.

Ranzania laevis (22 mm; Fig. 5B,C): The crests of membrane bone of the autopalatine are greatly enlarged. The ectopterygoid now covers the dorsal part of the quadrate

laterally. Ventrally, the autopalatine interdigitates with the ectopterygoid and is also attached to the endopterygoid. The caudal part of the metapterygoid articulates with the shaft of the hyomandibular and at the same time covers the persisting pars hyosymplectica laterally. The hyomandibular bears two laminae of membrane bone of which the anterior is smaller than the posterior.

All elements of the opercular series have become elongated. The subopercle has shifted its position and is now in a more anterior position than in the previous stage. It has lost its connection to the opercle. Most of its length is now covered by the preopercle. The horizontal arm of the preopercle has elongated and is now longer than the vertical arm.

It is not clear to us whether the anterodorsal extension of the vomer represents the ethmoid. However, a development of an ethmoid could not be observed in any of the stages.

Tetraodontidae – *Monotretes suvatii* (3.7 mm; Fig. 6A,B): The processus pterygoideus of the palatoquadrate ends in a slightly angled pars autopalatina. The quadrate has started to ossify. The pars metapterygoida tapers caudally and ends roughly at about the level where the interhyal articulates with the pars hyosymplectica. A faintly stained maxilla is present anterior to the processus pterygoideus. At about the anterior

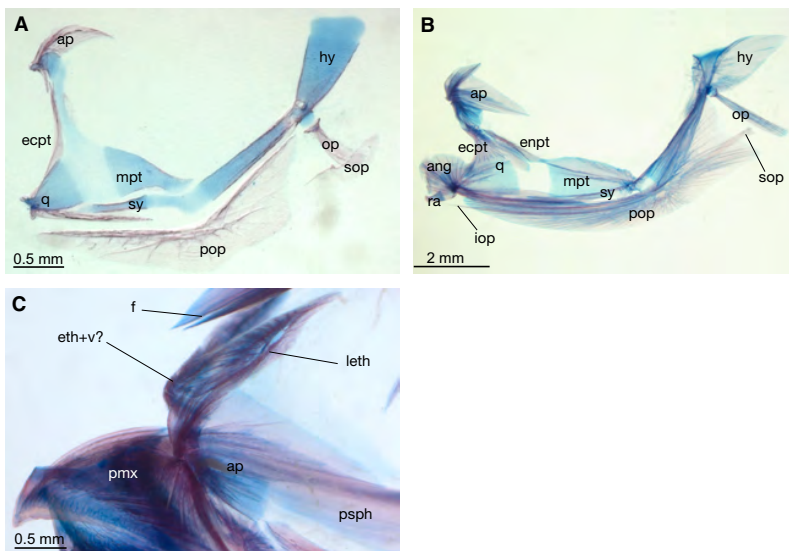


Fig. 5—Molidae. —**A–C.** *Ranzania laevis*. Interopercle entirely covered by the preopercle laterally. —**A.** Suspensorium, jaws removed, of a 2.5 mm NL specimen. —**B.** Suspensorium, jaws removed, and **C.** Ethmoid region of a 22 mm SL specimen. All figures in lateral view.

tip of Meckel's cartilage, two teeth are present. The symplectic and the hyomandibular are ossified within the pars hyosymplectica. Of the opercular series only the opercle and the subopercle are present.

The ethmoid plate is laterally expanded, and the tips are slightly ventrally curved. The internasal septum of the ethmoid plate is not separated from the lamina orbitonasalis.

Monotrete suwattii (4.7 mm; Fig 6C,D): The processus pterygoideus appears shorter and more massive than in the previous stage. The pars autopalatina is covered by the autopalatine. The processus maxillaris of the autopalatine bears a small posterodorsally oriented process of membrane bone that contacts an anterolateral projection of the vomer. The quadrate is now located more anteriorly than in the previous stage. The ectopterygoid has started to ossify. Seven teeth are present on the dentary and a single one on the premaxilla. The maxilla is closely attached to the premaxilla. The metapterygoid has started to ossify in the posterior region of the palatoquadrate. At this stage, the hyomandibular and symplectic are fully formed. The persisting cartilage between the symplectic and hyomandibular is slightly arched.

All elements of the opercular series are fully formed. The opercle is triangular in shape. The pointed caudal end of the subopercle projects dorsally. The interopercle is needle shaped and bears a ventrally oriented projection. A crest enlarges the preopercle at its ventral edge. The horizontal arm of the preopercle is larger than the vertical.

Monotrete suwattii (5.4 mm; Fig. 6E,F): Only slightly notable differences exist between this stage and the 4.7 mm larva. The processus pterygoideus has broadened. The posteroven-tral process of the quadrate is enlarged and attached to the symplectic and preopercle. The endopterygoid is ossified on the posterior margin of the processus pterygoideus.

The lateral ethmoid and the ethmoid are now ossified and located in the same vertical plane. The vomer covers the ethmoid plate ventrally and bears a rostrrolateral projection.

Monotrete suwattii (16.9 mm; Fig. 6G,H): This stage resembles the adult closely, and all elements are fully developed. The dorsal process of membrane bone of the autopalatine has become elongated and interdigitates with the vomer. Ventrally, the autopalatine interdigitates with the ectopterygoid and is closely attached to the endopterygoid. The metapterygoid is enlarged and has a small crest of membrane bone at its dorsal margin. The jaw elements have a beak-like appearance in which individual teeth are still visible. The opercle has a triangular appearance, and its ventral margin is embraced by two dorsally oriented projections of the subopercle. The interopercle is a needle-shaped element with a ventrally oriented extension.

The rostrrolateral projection of the vomer is enlarged and interdigitates with the autopalatine. The ethmoid region has become more elongated, and the ethmoid and the lateral ethmoid are no longer vertically aligned.

Diodontidae – *Diodon hystrix* (2.7 mm; Fig. 7A,B): The short processus maxillaris of the autopalatine has a dorsally oriented splint-like process of membrane bone. The palatoquadrate has a short and wide processus pterygoideus. The ectopterygoid extends from the shaft of the pars autopalatina to the dorsal margin of the quadrate.

The pars hyosymplectica is a broad element in which the symplectic and the hyomandibular have started to ossify. The opercular head of the hyomandibular is pointed posteroven-trally.

All four elements of the opercular series are present. The opercle is triangular with a very narrow shaft that leads to the articular facet for the opercular head of the hyomandibular. The subopercle is larger than the opercle, and the interopercle

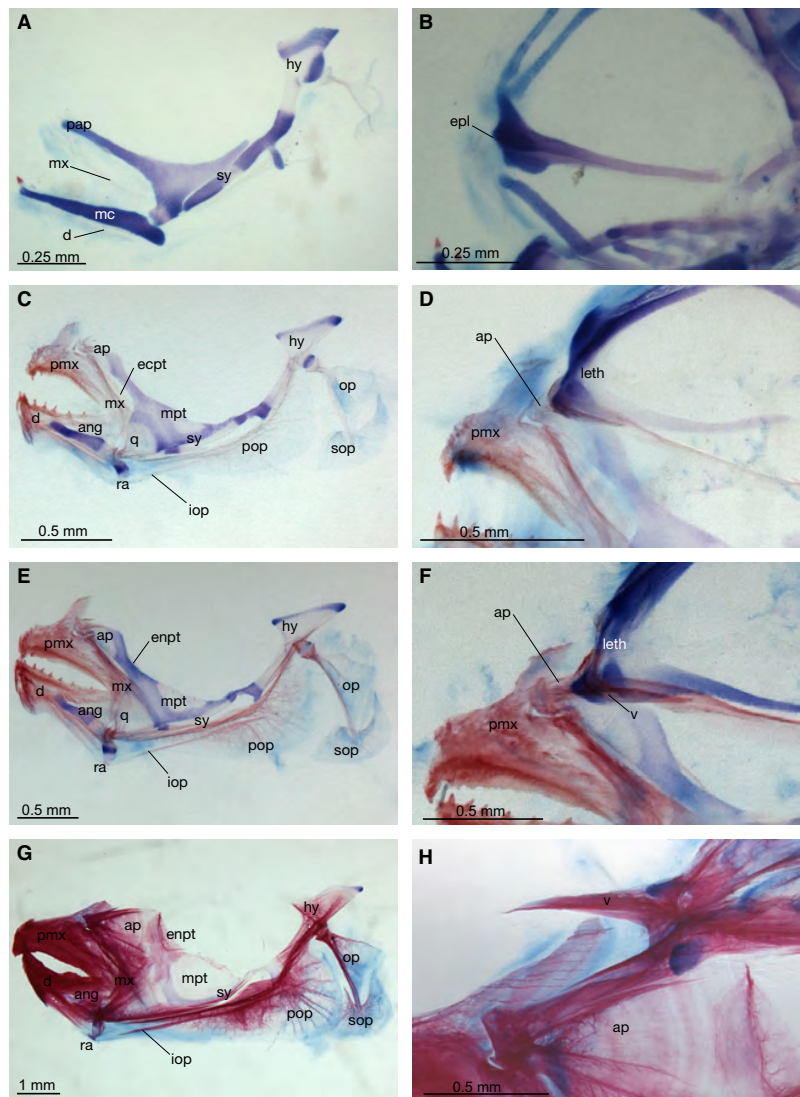


Fig. 6—Tetraodontidae. —**A–H.** *Monotretes swattii*. —**A.** Suspensorium and **B.** Ethmoid region of a 3.7 mm NL specimen. —**C.** Suspensorium and **D.** Ethmoid region of a 4.7 mm NL specimen. —**E.** Suspensorium and **F.** Ethmoid region of a 5.4 mm SL specimen. —**G.** Suspensorium and **H.** Ethmoid region of a 16.4 mm SL specimen. All figures in lateral view.

is triangular in shape. The preopercle is obliquely oriented, and its dorsal margin is roughly a straight line.

The process of membrane bone of the autopalatine surrounds the ethmoid plate. The frontal reaches far anteriorly, and its most anterior tip contacts the dorsally oriented process of the autopalatine. No ossifications within the ethmoid region are present at this stage.

Diodon hystrix (5.9 mm; Fig. 7C): The processus pterygoideus has become broader and shorter compared to the previous stage, and the palatoquadrate is more massive. Three processes of membrane bone are developed on the processus maxillaris of the autopalatine. The quadrate has shifted its position and is now located below the autopalatine. The endopterygoid appears at the posterodorsal edge of the shortened processus pterygoideus. The quadrate is enlarged, and the metapterygoid covers the pars metapterygoidea, which

extends to the anteroventral margin of the hyomandibular. A crest of membrane bone extends the hyomandibular anteriorly. The opercle is triangular in shape. The subopercle bears an anterior process that is covered laterally by the preopercle. The interopercle has a large ventrally oriented crest.

Diodon hystrix (15.0 mm; Fig. 7D,E): The autopalatine is in contact and interdigitates with the ectopterygoid and endopterygoid. The membrane bone processes of the autopalatine are now more distinct, and two of them project dorsally, while the third projects medially. The metapterygoid has a crest of membrane bone at its dorsal margin. The symplectic, hyomandibular, and the opercular series are as described for the previous stage.

The lateral ethmoid is separated from the anterior tip of the ethmoid region. The ethmoid is still absent, and the vomer is only a small, thin element embraced by the anterior part of

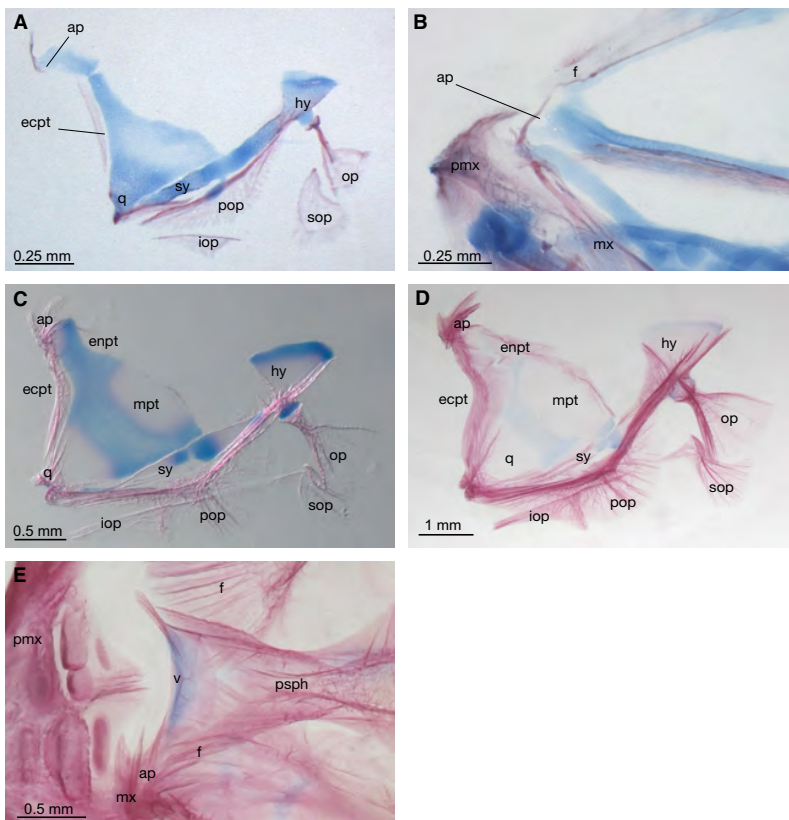


Fig. 7—Diodontidae. —**A–E**. *Diodon hystrix*. —**A**. Suspensorium, jaws removed, and **B**. Ethmoid region of a 2.7 mm NL specimen. —**C**. Suspensorium, jaws removed of a 5.9 mm SL specimen. —**D**. Suspensorium, jaws removed, and **E**. Ethmoid region from a 15 mm SL specimen. All figures but **E** are in lateral view.

the parasphenoid. The frontal and part of the lateral ethmoid are closely attached to the two dorsally projecting processes of the autopalatine.

Discussion

The Tetraodontiformes are well known for their robust jaws adapted to a non-elusive and hard-shelled (durophagous) diet (Tyler 1968; Targett 1978; Lauzanne 1988; Turingan and Wainwright 1993; Duncan and Szelistowski 1998). Although able to engulf prey by producing negative pressure in the buccal cavity, it plays only a minor role in feeding in tetraodontiforms. Suction feeding is described for *Balistes vetula*, when it feeds on soft items (Turingan and Wainwright 1993) and for *Chylomyxterus schoepfi*, where it is related not to feeding but to inflation of the body (Wainwright *et al.* 1995).

Authors of many functional studies have attempted to understand the evolution of muscular control of the highly derived jaws (Turingan and Wainwright 1993; Turingan 1994; Turingan *et al.* 1995; Ralston and Wainwright 1997; Friel and Wainwright 1998, 1999; Wainwright and Friel 2000). In these studies, the skeletal adaptations of the jaws and suspensorium have been simplified and treated as identical among representatives of the order. As we discuss below, adaptations of the jaw and suspensorium for durophagy differ

remarkably among the Tetraodontiformes and follow two different modulations.

Ontogenetic transformation of the suspensoria

Early tetraodontiform larvae have a palatoquadrate and jaws identical to that of many other teleost larvae (de Beer 1937; Fritzsche and Johnson 1980; Cabbage and Mabee 1996; Hunt von Herbing *et al.* 1996) (Fig. 8). Larval *Dicentrarchus labrax* and tetraodontiforms have an elongate processus pterygoideus, and the anterior tip of the pars autopalatina/autopalatine is located far beyond the level of the quadrate. Meckel's cartilage reaches from the quadrate anteriorly to beyond the level of the anterior tip of the pars autopalatina/autopalatine. In *D. labrax*, the shape of the jaws and suspensorium do not change drastically during subsequent development, but in tetraodontiforms, there is a characteristic and significant shape change in which the quadrate becomes much more anteriorly placed, and the processus pterygoideus becomes short and robust.

Although all adult tetraodontoids lack individual teeth (Pflugfelder 1930; Andreucci *et al.* 1982), early larval stages of the Tetraodontidae (Fig. 6), Diodontidae (Fig. 7) and Molidae (personal observation) have them (unknown for *Triodon*).

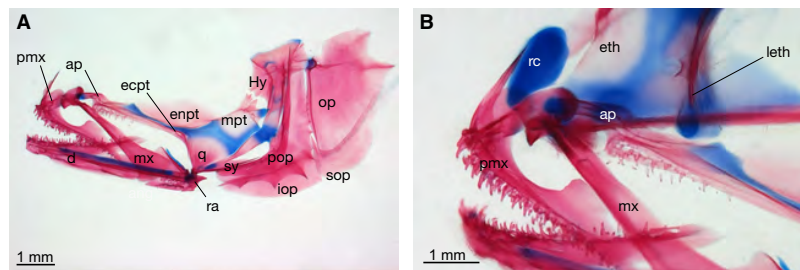


Fig. 8—Moronidae. —**A.** and **B.** *Dicentrarchus labrax*. —**A.** Suspensorium and —**B.** Ethmoid region of a 28 mm SL specimen. All figures in lateral view.

In concert with the ontogenetic shape change of the palatoquadrate is an elongated horizontal arm of the preopercle, which is characteristic for all adult tetraodontiforms. We interpret this unusual shape of the suspensorium and the concomitant elongated horizontal arm of the preopercle as an autapomorphy of the order.

Character evolution

There is no general consensus about the phylogenetic intrarelationship of the Tetraodontiformes (Fig. 9). Herein, we discuss the evolution of the rostral cartilage, autopalatine, and ethmoid region within the context of the phylogenetic hypothesis of Tyler and Holcroft (2007). In that hypothesis, the triacanthodids are the sistergroup of the remaining eight families, which are divided into two suborders: the Balistoidei, in which the Triacanthidae are the sistergroup to the Balistidae, Monacanthidae, and Ostraciidae. In contrast to Tyler and Holcroft, we treat the Aracanidae as a subgroup of the Ostraciidae, Balistidae, and Monacanthidae; and the Tetraodontoidei, comprising Triodontidae (*Triodon*) as the sistergroup to a clade formed by the Molidae, Tetraodontidae, and Diodontidae.

We consider the jaws of the Triacanthodidae as the most plesiomorphic condition within the Tetraodontiformes because: (1) the large ascending process is able to glide over the ovoid rostral cartilage, suggesting upper jaw protrusion similar to that described by Liem (1980) for cichlids with a similar jaw anatomy, (2) the shape and attachment of the autopalatine indicate the possibility of a lateral movement of the suspensorium, (3) characters listed elsewhere and not necessarily involving the jaws (Winterbottom 1974; Tyler 1980; Leis 1984; Rosen 1984; Santini and Tyler 2003).

Rostral cartilage. The Triacanthodidae and Triacanthidae have a primitive rostral cartilage [1(0)], similar in configuration to that of *Dicentrarchus labrax*, reported previously only by Winterbottom (1974) and Rosen (1984) (Fig. 9; [1]). Although several studies have dealt with the osteology of the Tetraodontiformes extensively (Matsuura 1979; Tyler 1980; Santini and Tyler 2003), they have failed to report a rostral cartilage in the Balistidae and Monacanthidae, wherein it is specialized in being conical in shape and fully embraced by the premaxillae and maxillae [1(1)]. The Ostraciidae and the Tetraodontoidei have convergently lost the rostral cartilage [1(2)] (Table 1).

Ethmoid region. In the Triacanthodidae and Triacanthidae, the ethmoid is placed slightly dorsoposterior to the vomer, and its anterior, oblique margin forms a concavity for the rostral cartilage (Fig. 9; [2]). Because a similar configuration of the ethmoid region characterizes *Dicentrarchus labrax* and other generalized percomorphs, we consider this situation to be plesiomorphic for all Tetraodontiformes [2(0)]. In the Balistidae, Monacanthidae, Ostraciidae, and *Triodon* (Tyler 1980: Fig. 184), the anterior end of the ethmoid overlaps the vomer anteriorly [2(1)]. In the Balistidae and Monacanthidae, both elements together form an anteroventrally oriented concavity that abuts the rostral cartilage. The ethmoid region of the Molidae, Tetraodontidae, and Diodontidae is extremely different, making a meaningful comparison between the families impossible. In tetraodontids, the vomer is the prominent element of the ethmoid region and has a forked anterior margin [2(3)]. In contrast, the vomer is reduced to a small plate in molids, where it is fused to the ethmoid [2(2)] and diodontids, which lack the ethmoid entirely [2(4)].

Autopalatine and its contact to the suspensorium. Because of the shape change of the palatoquadrate during ontogeny, the connection between the autopalatine and the ectopterygoid in adult Triacanthodidae is specialized (the autopalatine is broad and in loose contact with the ectopterygoid) compared to the generalized percomorph configuration [3(0)] (Fig. 9; [3]). Nonetheless the triacanthodid configuration [3(1)] is more primitive than the other two configurations within the order. According to Tyler's (1980) description, in *Triodon*, the ectopterygoid is loosely attached to the autopalatine and resembles the Triacanthodidae closely. Two more derived configurations characterize the remaining families of the order: the autopalatine becomes removed from the ectopterygoid and therefore is detached from the suspensorium [3(2)]; or the autopalatine interdigitates with the ectopterygoid and therefore becomes tightly attached to the suspensorium [3(3)]. Loss of the bony connection of the autopalatine to the suspensorium in the Triacanthidae, Balistidae, and Monacanthidae is accompanied by a reduction in size of the autopalatine. In the Monacanthidae, where this reduction is greatest, the processus maxillaris is still complete. The Ostraciidae, in contrast, have the processus maxillaris of the autopalatine reduced and an interdigitation pattern of the autopalatine to

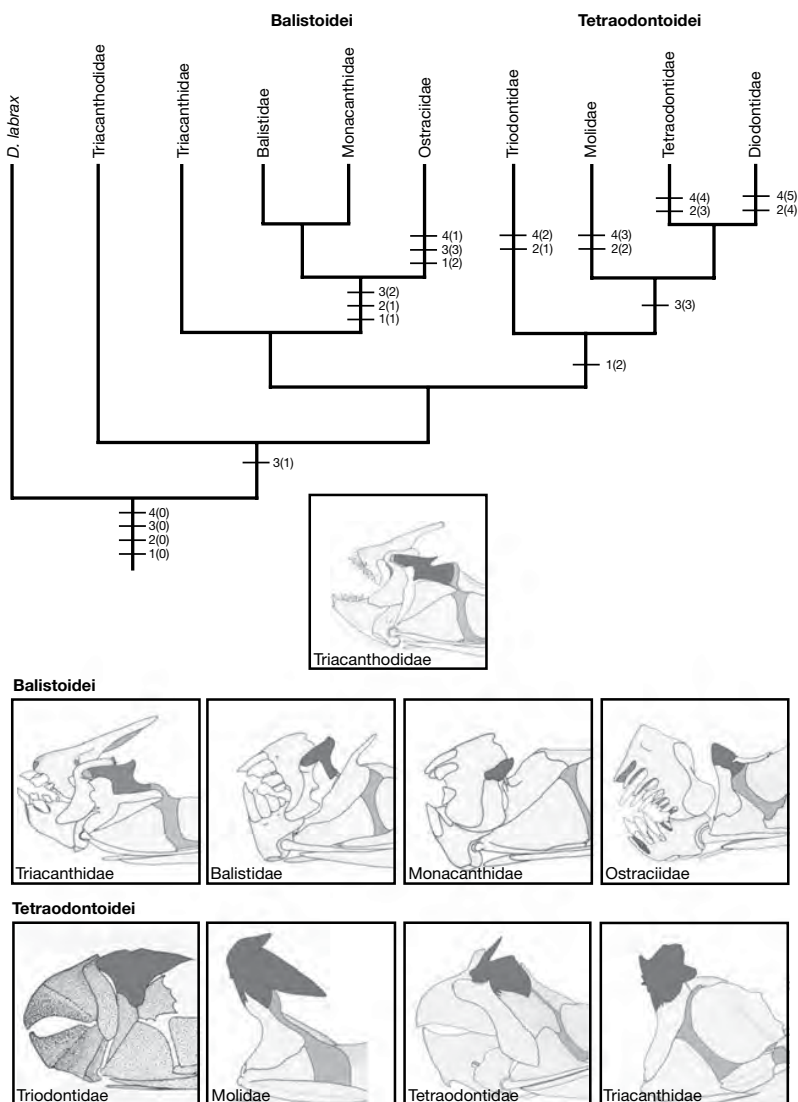


Fig. 9—Character evolution. The four major elements in the caudal skeleton of the Tetraodontiformes mapped onto Tyler and Holcroft's (2007) (only recent taxa are shown) phylogenetic hypothesis. Rostral cartilage 1. Plesiomorphic 1(0); modified 1(1); absent 1(2). Ethmoid region 2. Plesiomorphic 2(0); modified 2(2–4). Autopalatine and its contact to the suspensorium 3. Acanthomorph condition 3(0); plesiomorphic for Tetraodontiformes 3(1); autopalatine detached from suspensorium 3(2); autopalatine interdigitates with ectopterygoid 3(3). Autopalatine and the ethmopalatine articulation 4. Plesiomorphic 4(0); modified 4(1–5). For an explanation about plesiomorphic conditions and modification, see text. Drawings below present the different modifications of the autopalatine. The drawing of *Triodon* is modified after Tyler (1980).

the ectopterygoid identical to the members of the Tetraodontoidei [3(3)] (Table 2).

Autopalatine and ethmopalatine articulation. As in generalized percomorphs, the autopalatine in the Triacanthodidae forms an articulation with the vomer and the lateral ethmoid that enables the suspensorium to move laterally [4(0)] (Fig. 9; [4]). In the Triacanthidae, the ethmoid is excluded from the ethmopalatine articulation, and the autopalatine articulates exclusively with the vomer. In the Balistidae and Monacanthidae, the autopalatine articulates only with the ethmoid. Although the arrangement of articular elements of the ethmoid region differs between the balistids and monacanthids on the one hand and triacanthids on the other, both cases represent the plesiomorphic state for the order. Exclusion of the vomer from the articulation in the former two families may

result from reduction in the autopalatine [4(0)]. The Ostraciidae are unique within tetraodontiforms in having the distal rather than the proximal part of the autopalatine reduced [4(1)], as is the case in the remaining balistoids.

The fixation of the ethmopalatine articulation differs substantially among the four families of the Tetraodontoidei (Table 3). We therefore coded the immobilization of the ethmopalatine articulation with four different states [4(2–5)] and conclude that this fixation cannot be used to support the monophyly of the suborder as has been previously suggested (Fraser-Brunner 1943; Santini and Tyler 2003).

Conflicts and problems with various phylogenetic hypotheses

Since Cuvier (1817) first recognized the fish order Tetraodontiformes ('Plectognathi'), the intrarelationships of this

Table 1 Rostral cartilage

Taxa	Rostral cartilage
<i>Dicentrarchus</i>	Ovoid
<i>Atrophacanthus</i>	Ovoid
<i>Tripodichthys</i>	Ovoid
<i>Balistapus</i>	Conical
<i>Monacanthus</i>	Conical
<i>Lactophrys</i>	Absent
<i>Triodon</i>	Absent
<i>Ranzania</i>	Absent
<i>Monotrete</i>	Absent
<i>Diodon</i>	Absent

Table 2 The autopalatine and its association with the ectopterygoid (suspensorium)

Taxa	Contact autopalatine to the ectopterygoid
<i>Atrophacanthus</i>	Loose contact
<i>Tripodichthys</i>	Detached
<i>Balistapus</i>	Detached
<i>Monacanthus</i>	Detached
<i>Lactophrys</i>	Interdigitates
<i>Triodon</i>	Loose contact
<i>Ranzania</i>	Interdigitates
<i>Monotrete</i>	Interdigitates
<i>Diodon</i>	Interdigitates

Table 3 The autopalatine and its association with different elements of the ethmoid region

	Vomer	Ethmoid	Lateral Ethmoid	Parasphenoid	Frontal
<i>Triodon</i>	Sutured	Sutured	Sutured		
<i>Ranzania</i>			Attached	Attached	
<i>Monotrete</i>	Interdigitated				
<i>Diodon</i>					Interdigitated

morphologically highly specialized group have been a continuous subject of discussion. Winterbottom (1974) was the first to publish a phylogenetic hypothesis based on cladistic principles applied to morphological characters (Hennig 1950). His work on the phylogenetic systematics of the Tetraodontiformes was followed by several others that re-evaluated his conclusions, based on additional morphological characters and taxonomic data sets (Tyler 1980; Leis 1984; Rosen 1984; Tyler and Sorbini 1996; Santini and Tyler 2003). In recent years, with the rapid development of molecular systematics, additional studies aiming to elucidate the interrelationship of the Tetraodontiformes with molecular data sets were added to the existing turmoil (Holcroft 2005; Alfaro *et al.* 2007; Yamanoue *et al.* 2008).

Despite the considerable attention given to the intrarelationships of the Tetraodontiformes over the years, no consensual phylogenetic hypothesis has come to the fore. Among other things, authors continue to disagree on the position of the ostraciids relative to members of the suborder Tetraodontoidae and on the position of *Triodon* (e.g. on the exclusion of *Triodon* from the Tetraodontoidae). To date, neither morphological nor molecular characters have provided unambiguous answers to these problems (Dareste 1849, 1850; Regan 1902; Winterbottom 1974; Tyler 1980; Leis 1984; Rosen 1984; Tyler and Sorbini 1996; Holcroft 2005; Alfaro *et al.* 2007; Yamanoue *et al.* 2008). One reason for the phylogenetic intractability of the tetraodontiforms may be their confusing combination of reductive and highly specialized features. Another is that, in the absence of an ontogenetic perspective, previous studies have sometimes misinterpreted or misidentified skeletal elements (Britz and Johnson 2005a,b; Johnson and Britz 2005; Konstantinidis and Johnson 2011).

As one example, beak-like jaws have been used in various earlier studies to define the suborder Tetraodontoidae (Cuvier 1817, 1829; Fraser-Brunner 1943; Tyler 1980; Santini and Tyler 2003). Although the configuration of this beak has often been interpreted to be the same in all tetraodontoids, a few (mostly ignored) studies shed some doubt on its homology. Several of these comparatively investigated its ultrastructure in the four families (Andreucci 1968; Andreucci and Blumen 1971; Britski and Andreucci 1975; Andreucci *et al.* 1982; Britski *et al.* 1985). Britski *et al.* (1985) summarized the results and concluded that the beak of *Triodon* resembles more closely the jaws of parrotfishes (Scaridae), that the Molidae show no evidence of the incorporated teeth found in the other three families, and that only the beaks of Tetraodontidae and Diodontidae have a cogently similar ultrastructure. In other words, the beaks of *Triodon*, molids, and tetraodontids/diodontids differ enough to invalidate this shared structure as a synapomorphy of the suborder. It is likely that fixation of the ethmo-palatine articulation is related to the beak-like jaws, and, as we concluded above, seems also to have arisen independently, also invalidating earlier hypotheses (e.g. Fraser-Brunner 1943; Santini and Tyler 2003) that it is a unifying character of tetraodontoids.

Other examples of misinterpreted characters can be found in the phylogenetic hypothesis of Santini and Tyler (2003) based on 210 morphological characters. The authors coded fusion of the anterior vertebrae to the basioccipital in the Ostraciidae [127(1)] as originally described by Tyler (1963). Britz and Johnson (2005b) demonstrated that the anteriormost vertebra also fuses to the occiput in the Molidae. Santini and Tyler misinterpreted this character (character 127) for molids, instead describing the molid basioccipital (character 9) as 'prolonged dorsally behind the exoccipital to border the foramen magnum to the exclusion of the exoccipitals'. This prolongation is actually the result of the first vertebra having fused to the basioccipital. Konstantinidis and Johnson (2011) showed that two to three epurals are present in larval stages of

triacanthodids, whereas Santini and Tyler observed only a single epural (character 182) in adult triacanthodids they used in their study.

Molecular data sets have also proved incongruent. Holcroft (2005) published the first molecular phylogenetic hypothesis of the Tetraodontiformes based on mitochondrial 12S and 16S genes and the nuclear RAG1 gene. Alfaro *et al.* (2007) re-analyzed Holcroft's data set and added sequences of the enigmatic *Triodon* and a second triacanthid species. The two studies indicate similar relationships, but Alfaro *et al.*'s analysis shows a higher resolution, possibly because of the inclusion of *Triodon*. In their analysis, *Triodon* forms, together with the Molidae and Ostraciidae, the basal sistergroup of the remaining families, suggesting a convergent evolution of the parrot beak-like jaws within the order. Yamanoue *et al.* (2008) analyzed the entire mitochondrial genome and reached a quite different conclusion, which conflicts with previous morphological and molecular hypotheses. Yamanoue *et al.* (2008) recovered a close relationship between the Ostraciidae and *Triodon* that forms together with the Triacanthodidae the basal sistergroup of the remaining families, among which the interrelationships are mostly unresolved. Yamanoue *et al.* discussed most of their findings in light of statistic parameters that makes it difficult for us to interpret and evaluate their findings.

Holcroft (2005) and Alfaro *et al.* (2007) recovered a clade formed by molids and ostraciids and, in the latter analysis, including *Triodon*. A closer relationship of ostraciids to some taxa within the Tetraodontoidei was also proposed by Rosen (1984) and Leis (1984). Rosen placed the ostraciids in a polytomy with the Tetraodontoidei based on one character (character 20; 'dorsal fin and radials remote from occipital region of skull'). Leis placed the ostraciids in a trichotomy with diodontids and molids based on 23 of what he called 'larval' characters (actually, a number of them, e.g. vertebral and fin-ray numbers also characterize adults). *Triodon* was unavailable for both studies.

As noted above, Britz and Johnson (2005b) also presented evidence in support of a close relationship between the Ostraciidae and one tetraodontoid family, the Molidae, i.e. the shared fusion of one or more vertebrae to the basioccipital. This rare fusion is known elsewhere among acanthomorphs only in males of the distantly related Cetomimidae (Johnson *et al.* 2009) and among other teleosts, only in the osteoglossomorph *Heterotis* (Patterson and Johnson 1995; Johnson and Britz 2010), and the elopomorph *Megalops* (Johnson and Britz 2010).

We anticipate that more ontogenetically focused studies will facilitate resolution of some of the phylogenetic conundra of teleost taxa, particular those involving highly specialized taxa, like the Tetraodontiformes, in which the larvae still show more plesiomorphic conditions than their adult congeners (for examples see Johnson 1984 and citations herein).

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References

- Alfaro, M. E., Santini, F. and Brock, C. D. 2007. Do reefs drive diversification in marine teleosts? Evidence from the pufferfish and their allies (Order Tetraodontiformes) – *Evolution* **61**: 2104–2126.
- Amemiya, C. T., Amores, A., Ota, T., Mueller, D., Garraty, J., Postlethwait, J. and Litman, G. 2001. Generation of a P1 artificial chromosome library of the Southern pufferfish. – *Gene* **272**: 283–289.
- Amores, A., Suzuki, T., Yan, Y. L., *et al.* 2004. Developmental roles of pufferfish Hox clusters and genome evolution in ray-fin fish. – *Genome Research* **14**: 1–10.
- Andreucci, R. D. 1968. Macroscopic and microscopic morphology of *Spheroides testudineus* teeth (checkered puffer). – *Acta anatomica* **71**: 448–471.
- Andreucci, R. D. and Blumen, G. 1971. Radioautographic study of *Spheroides testudineus* denticles (checkered puffer). – *Acta anatomica* **79**: 76–83.
- Andreucci, R. D., Britski, H. A. and Carneiro, J. 1982. Structure and evolution of tetraodontoid teeth an auto radiographic study Pisces Tetraodontiformes. – *Journal of Morphology* **171**: 283–292.
- Aparicio, S., Chapman, J., Stupka, E., *et al.* 2002. Whole-genome shotgun assembly and analysis of the genome of *Fugu rubripes*. – *Science* **297**: 1301–1310.
- Bartol, I. K., Gharib, M., Weihs, D., Webb, P. W., Hove, J. R. and Gordon, M. S. 2003. Hydrodynamic stability of swimming in ostraciid fishes: Role of the carapace in the smooth trunkfish *Lactophrys triqueter* (Teleostei : Ostraciidae). – *Journal of Experimental Biology* **206**: 725–744.
- de Beer, G. R. 1937. *The Development of the Vertebrate Skull* [Reprint 1985]. Reprint 1985 edn. University of Chicago Press, Chicago and London.
- Brainerd, E. L. 1994. Pufferfish inflation – functional-morphology of postcranial structures in *Diodon holocanthus* (Tetraodontiformes). – *Journal of Morphology* **220**: 243–261.
- Brainerd, E. L. and Murray, S. S. 2000. Patterns of genome size evolution in tetraodontiform fishes. – *American Zoologist* **40**: 953.
- Brainerd, E. L. and Patek, S. N. 1998. Vertebral column morphology, C-start curvature, and the evolution of mechanical defenses in tetraodontiform fishes. – *Copeia* **4**: 971–984.

- Breder, C. M. and Clark, E. 1947. A contribution to the visceral anatomy, development and relationships of the Pleotognathi. – *Bulletin of the American Museum of Natural History* **88**: 287–319.
- Britski, H. A. and Andreucci, R. D. 1975. Sobre os dentes dos piexes Molidae. – *Ciencia e Cultura (Sao Paulo)* **23**: 287.
- Britski, H. A., Andreucci, R. D., Menezes, N. A. and Carneiro, J. 1985. Coalescence of teeth in fishes. – *Revista Brasileira De Zoologia* **2**: 459–484.
- Britz, R. and Johnson, G. D. 2005a. Leis' conundrum: Homology of the clavus of the ocean sunfishes. 1. Ontogeny of the median fins and axial skeleton of *Monotret leurus* (Teleostei, Tetraodontiformes, Tetraodontidae). – *Journal of Morphology* **266**: 1–10.
- Britz, R. and Johnson, G. D. 2005b. Occipito-vertebral fusion in ocean sunfishes (Teleostei: Tetraodontiformes: Molidae) and its phylogenetic implications. – *Journal of Morphology* **266**: 74–79.
- Cubbage, C. C. and Mabee, P. M. 1996. Development of the cranium and paired fins in the zebrafish *Danio rerio* (Ostariophysi, Cyprinidae). – *Journal of Morphology* **229**: 121–160.
- Cuvier, G. 1817. *Le règne animal*. Deterville, Paris.
- Cuvier, G. 1829. *Le règne animal*, 2 edn. Deterville, Paris.
- Dareste, C. 1849. Observations sur l'ostéologie du poisson appelé *Triodon macroptere*. – *Annales des Sciences Naturelles. B. Zoologie* **3**: 68–83.
- Dareste, C. 1850. Recherches sur la classification des poissons de l'ordre des Plectognathes. – *Annales des Sciences Naturelles. B. Zoologie* **3**: 105–133.
- Duncan, R. S. and Szelistowski, W. A. 1998. Influence of puffer predation on vertical distribution of mangrove littorinids in the Gulf of Nicoya, Costa Rica. – *Oecologia* **117**: 433–442.
- Fraser-Brunner, A. 1943. Notes on the Plectognath fishes. – VIII. The classification of the suborder Tetraodontoidea, with a Synopsis of the Genera. – *Proceedings of the Royal Society London* **10**: 1–18.
- Friel, J. P. and Wainwright, P. C. 1998. Evolution of motor patterns in tetraodontiform fishes: Does muscle duplication lead to functional diversification? – *Brain Behavior and Evolution* **52**: 159–170.
- Friel, J. P. and Wainwright, P. C. 1999. Evolution of complexity in motor patterns and jaw musculature of tetraodontiform fishes. – *Journal of Experimental Biology* **202**: 867–880.
- Fritzsche, R. A. and Johnson, G. D. 1980. Early osteological development of white perch and striped bass with emphasis on identification of their larvae. – *Transaction of the American Fisheries Society* **109**: 387–406.
- Fujita, K. 1992. Development of the caudal skeleton in the tetraodontid fish, *Takifugu niphobles*. – *Japanese Journal of Ichthyology* **38**: 438–440.
- Hennig, E. H. W. 1950. *Grundzuege einer Theorie der Phylogenetischen Systematik*. Deutscher Zentralverlag, Berlin.
- Holcroft, N. I. 2004. A molecular test of alternative hypotheses of tetraodontiform (Acanthomorpha: Tetraodontiformes) sister group relationships using data from the RAG1 gene. – *Molecular Phylogenetics and Evolution* **32**: 749–760.
- Holcroft, N. I. 2005. A molecular analysis of the interrelationships of tetraodontiform fishes (Acanthomorpha: Tetraodontiformes). – *Molecular Phylogenetics and Evolution* **34**: 525–544.
- Hunt von Herbing, I., Miyake, T., Hall, B. K. and Boutilier, R. G. 1996. Ontogeny of feeding and respiration in larval Atlantic cod *Gadus morhua* (Teleostei, gadiformes). 1. Morphology. – *Journal of Morphology* **227**: 15–35.
- Johnson, G. D. 1984. Percoidei: Development and relationship. In: Moser, H. G., Richards, W. J., Cohen, D. M., Fahay, M. P., Kendall, A. W. J. and Richardson, S. L. (Eds): *Ontogeny and Systematics of Fishes*, pp. 464–498. Allen Press, Lawrence, KS.
- Johnson, G. D. and Britz, R. 2005. 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). – *Journal of Morphology* **266**: 11–21.
- Johnson, G. D. and Britz, R. 2010. Occipito-vertebral fusion in actinopterygians: Conjecture, myth and reality. Part 2: Teleosts. In: Nelson, J. S., Schultze, H. P. and Wilson, M. V. H. (Eds): *Mesozoic Fishes 4 – Homology and Phylogeny*, pp. 95–110. Dr. Friedrich Pfeil, München.
- Johnson, G. D., Paxton, J. R., Sutton, T. T., et al. 2009. Deep-sea mystery solved: Astonishing larval transformations and extreme sexual dimorphism unite three fish families. – *Biology Letters* **5**: 235–239.
- Konstantinidis, P. and Johnson, G. D. 2011. A comparative ontogenetic study of the tetraodontiform caudal complex. – *Acta Zoologica* doi: 10.1111/j.1463-6395.2010.00490.x [Epub ahead of print].
- Lauder, G. V. 1980. Evolution of the feeding mechanism in primitive Actinopterygian fishes – a functional anatomical analysis of *Polypterus*, *Lepisosteus*, and *Ama*. – *Journal of Morphology* **163**: 283–317.
- Lauder, G. V. 1982. Patterns of evolution in the feeding mechanism of Actinopterygian fishes. – *American Zoologist* **22**: 275–285.
- Lauder, G. V. 1985. Aquatic feeding in lower vertebrates. In: Hildebrand, M., Bramble, D. M., Liem, K. F. and Wake, D. B., (Eds) *Functional Vertebrate Morphology*, pp. 210–229. Belknap Press, Cambridge, Mass. & London.
- Lauder, G. V. and Liem, K. F. 1980. The feeding mechanism and cephalic myology of *Salvelinus fontinalis*: Form, function, and evolutionary significance. In: Balon, E. K. (Ed.): *Perspectives in Vertebrate Science*, Vol. 1, pp. 365–390. Junk Publishers, Leiden.
- Lauder, G. V. and Liem, K. F. 1981. Prey capture by *Luciocephalus pulcher* – implications for models of jaw protrusion in Teleost fishes. – *Environmental Biology of Fishes* **6**: 257–268.
- Lauzanne, L. 1988. Feeding habits of african freshwater fishes. In: Leveque, C., Ssentongo, G. W. and Bruton, M. N. (Eds): *Biologie et ecologie des poissons d'eau douce Africains*, pp. 1–508. O R S T O M, Paris.
- Leis, J. M. 1984. Tetraodontiformes: Relationships. In: Moser, H. G., Richards, W. J., Cohen, D. M., Fahay, M. P., Kendall, A. W. J. and Richardson, S. L. (Eds): *Ontogeny and Systematics of Fishes*, pp. 459–463. Allen Press, Lawrence, KS.
- Liem, K. F. 1980. Adaptive significance of intraspecific and interspecific differences in the feeding repertoires of cichlid fishes. – *American Zoologist* **20**: 295–314.
- Matsuura, K. 1979. Phylogeny of the superfamily balistoidea pisces Tetraodontiformes. – *Memoirs of the Faculty of Fisheries Hokkaido University* **26**: 49–170.
- Nakae, M. and Sasaki, K. 2004. Homologies of the adductor mandibular muscles in Tetraodontiformes as indicated by nerve branching patterns. – *Ichthyological Research* **51**: 327–336.
- Patterson, C. and Johnson, G. D. 1995. The intermuscular bones and ligaments of teleostean fishes. – *Smithsonian Contributions to Zoology* **559**: i–iv, 1–83.
- Pflugfelder, O. 1930. Das Gebiss der Gymnodonten. Ein Beitrag zur Histogenese des Dentins. – *Zeitschrift fuer die gesamte Anatomie* **93**: 543–565.
- Ralston, K. R. and Wainwright, P. C. 1997. Functional consequences of trophic specialization in pufferfishes. – *Functional Ecology* **11**: 43–52.
- Regan, C. T. 1902. On the classification of the fishes of the suborder Plectognathi; with notes and description of new species from specimens in the British Museum Collection. – *Proceedings of the Zoological Society of London* **2**: 284–305.

- Rosen, D. 1984. Zeiformes as primitive plectognath fishes. – *American Museum Novitates* **2782**: 1–45.
- Santini, F. and Tyler, J. C. 2003. A phylogeny of the families of fossil and extant tetraodontiform fishes (Acanthomorpha, Tetraodontiformes), Upper Cretaceous to recent. – *Zoological Journal of the Linnean Society* **139**: 565–617.
- Schaeffer, B. and Rosen, D. 1961. Major adaptive levels in the evolution of the actinopterygian feeding mechanism. – *American Zoologist* **1**: 187–204.
- Targett, T. E. 1978. Food resource partitioning by the pufferfishes *Sphoeroides spengleri* and *S. testudineus* from Biscayne Bay, Florida. – *Marine Biology* **49**: 83–91.
- Taylor, W. R. and Van Dyke, C. C. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. – *Cybiium* **9**: 107–120.
- Turingan, R. G. 1994. Ecomorphological relationships among Caribbean tetraodontiform fishes. – *Journal of Zoology* **233**: 493–521.
- Turingan, R. G. and Wainwright, P. C. 1993. Morphological and functional bases of durophagy in the queen triggerfish, *Balistes vetula* (Pisces, Tetraodontiformes). – *Journal of Morphology* **215**: 101–118.
- Turingan, R. G., Wainwright, P. C. C. and Hensley, D. A. 1995. Interpopulation variation in prey use and feeding biomechanics in Caribbean Triggerfishes. – *Oecologia* **102**: 296–304.
- Tyler, J. C. 1963. The apparent reduction in number of precaudal vertebrae in trunkfishes (Ostracintoidea, Plectognathi). – *Proceedings of the Academy of Natural Sciences of Philadelphia* **115**: 153–190.
- Tyler, J. C. 1968. A monograph on plectognath fishes of the superfamily Triacanthoidea. – *Monographs of the Academy of Natural Sciences of Philadelphia* **16**: 1–364.
- Tyler, J. C. 1980. Osteology, phylogeny, and higher classification of the fishes of the order Plectognathi (Tetraodontiformes). – *NOAA Technical Report NMFS Circular* **434**: 1–422.
- Tyler, J. C. and Holcroft, N. I. 2007. Tetraodontiformes – triggerfishes, boxfishes, puffers (fugu), molas and allies. Version 19 February 2007. <http://tolweb.org/Tetraodontiformes/52153/2007.02.19>. In: *The Tree of Life Web Project*, [http://tolweb.org/\[www document\]](http://tolweb.org/[www document]).
- Tyler, J. C. and Sorbini, L. 1996. New superfamily and three new families of tetraodontiform fishes from the Upper Cretaceous: The earliest and most morphologically primitive plectognaths. – *Smithsonian Contributions to Paleobiology* **82**: i–iii, 1–59.
- Wainwright, P. C. and Bellwood, D. R. 2002. Ecomorphology of feeding in coral reef fishes. In: Sale, P. F. (Ed.): *Coral Reef Fishes: Dynamics and Diversity in a Complex Ecosystem*, pp. 33–55. Academic Press, San Diego.
- Wainwright, P. C. and Friel, J. P. 2000. Effects of prey type on motor pattern variance in tetraodontiform fishes. – *Journal of Experimental Zoology Part A* **286**: 563–571.
- Wainwright, P. C. and Turingan, R. G. 1993. Coupled versus uncoupled functional systems: Motor plasticity in the queen triggerfish *Balistes vetula*. – *Journal of Experimental Biology* **180**: 209–227.
- Wainwright, P. C. and Turingan, R. G. 1996. Muscular basis of buccal pressure: Inflation behavior in the striped burrfish *Chilomycterus schoepfi*. – *Journal of Experimental Biology* **199**: 1209–1218.
- Wainwright, P. C. and Turingan, R. G. 1997. Evolution of pufferfish inflation behavior. – *Evolution* **51**: 506–518.
- Wainwright, P. C., Turingan, R. G. and Brainerd, E. L. 1995. Functional morphology of pufferfish inflation: Mechanism of the buccal pump. – *Copeia* **3**: 614–625.
- Westneat, M. W. 2003. A biomechanical model for analysis of muscle force, power output and lower jaw motion in fishes. – *Journal of Theoretical Biology* **223**: 269–281.
- Westneat, M. W. 2004. Evolution of levers and linkages in the feeding mechanisms of fishes. – *Integrative and Comparative Biology* **44**: 378–389.
- Wiley, E. O. and Johnson, G. D. 2010. A teleost classification based on monophyletic groups. In: Nelson, J. S., Schultze, H. P. and Wilson, M. V. H. (Eds): *Mesozoic Fishes 4 – Homology and Phylogeny*, pp. 123–182. Dr. Friedrich Pfeil, München.
- Wilga, C. A. D. 2008. Evolutionary divergence in the feeding mechanism of fishes. – *Acta Geologica Polonica* **58**: 113–120.
- Winterbottom, R. 1974. The familial phylogeny of the Tetraodontiformes (Acanthopterygii: Pisces) as evidenced by their comparative myology. – *Smithsonian Contributions to Zoology* **155**: 1–201.
- Yamanoue, Y., Miya, M., Matsuura, K., et al. 2007. Phylogenetic position of tetraodontiform fishes within the higher teleosts: Bayesian inferences based on 44 whole mitochondrial genome sequences. – *Molecular Phylogenetics and Evolution* **45**: 89–101.
- Yamanoue, Y., Miya, M., Matsuura, K., Katoh, M., Sakai, H. and Nishida, M. 2008. A new perspective on phylogeny and evolution of tetraodontiform fishes (Pisces: Acanthopterygii) based on whole mitochondrial genome sequences: Basal ecological diversification? – *BMC Evolutionary Biology* **8**: 1–14.

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Graduate Teaching Assistant: Ichthyological part of a Morphology based graduate course

Curatorial

Curatorial assistance of the ichthyological collection of the Ernst Haeckel Museum, Jena, Germany

Fellowships

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Publications

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A Comparative Ontogenetic Study of the Tetraodontiform Caudal Complex. Konstantinidis P and Johnson GD. *Acta Zoologica*. in press.

Same But Different: Ontogeny and Evolution of the Musculus adductor mandibulae in the Tetraodontiformes. Konstantinidis P and Harris MP. *Journal of Experimental Zoology Part B*. 2011. 316: 10-20.

The Median-Fin Skeleton of the Eastern Atlantic and Mediterranean Clingfishes *Lepadogaster lepadogaster* (Bonnaterre) and *Gouania wildenowi* (Risso) (Teleostei: Gobiesocidae). Konstantinidis P and Conway KW. *Journal of Morphology*. 2010. 271: 215-224.

Zebrafish *eda* and *edar* Mutants Reveal Conserved and Ancestral Roles of Ectodysplasin Signaling in Vertebrates. Harris MP, Rohner N, Schwarz H, Perathoner S, Konstantinidis P and Nüsslein-Volhard C. *Plos Genetics*. 2008. 4 (10): e1000206. doi:10.1371/journal.pgen.1000206

Evolution of high-performance swimming in sharks: transformations of the musculotendinous system from subcarangiform to thunniform swimmers. Gemballa S, Konstantinidis P, Donley JM, Sepulveda C and Shadwick RE. *Journal of Morphology*. 2006. 267: 477-493.

Patterns of red muscle strain/activation and body kinematics during steady swimming in a lamnid shark, the shortfin mako (*Isurus oxyrinchus*). Donley JM, Shadwick RE, Sepulveda CA, Konstantinidis P and Gemballa S. *Journal of Experimental Biology*. 2005. 208: 2377-87.

Convergent evolution in mechanical design of lamnid sharks and tunas. Donley JM, Sepulveda CA, Konstantinidis P, Gemballa S and Shadwick RE. *Nature*. 2004. 429: 61-65.

Presentations

- 2010 Oral** **P. Konstantinidis.** How enigmatic is the Ragfish (*Icosteus aenigmaticus*). Joint Meeting of Ichthyologists and Herpetologists, Providence, Rhode Island, USA.
- 2009 Oral** **P. Konstantinidis.** Ontogenetic studies on certain structures in Tetraodontiformes, puffers and their relatives. Larval fish workshop at the National Museum of Science and Nature, Tokyo, Japan.
- P. Konstantinidis** and R. Britz. Ontogeny and homology of the Musculus adductor mandibulae complex in tetraodontids, an immuno-histochemical study of *Monotrete suvattii* (Tetraodontiformes: Tetraodontidae). Indo Pacific Fish Conference 8, Freemantle, Australia.
- 2008 Oral** **P. Konstantinidis.** Relevance of developmental trajectories to phylogeny and evolution. The case of Tetraodontiformes, pufferfishes and their relatives. Invited seminar at the Museo Nacional de Ciencias Naturales, Madrid, Spain
- 2008 Poster** **P. Konstantinidis.** The Importance of Ontogenetic Studies: New Insights Into the Caudal Fin Skeleton of Tetraodontiformes (Teleostei). Joint Meeting of Ichthyologists and Herpetologists, Montreal, Canada.
- 2007 Oral** **P. Konstantinidis** and K. W. Conway. The dorsal- and anal-fin skeleton of the Mediterranean clingfish *Gouania wildenowi* (Teleostei: Gobiesociformes). Joint Meeting of Ichthyologists and Herpetologists, Montreal, Canada.
- 2006 Poster** **P. Konstantinidis**, R. Britz and G. D. Johnson. Ontogeny of the beak of the gymnodonts and fixation of the ethmopalatine articulation (Teleostei, Tetraodontiformes). 8th International Congress of Vertebrate Morphology, Paris, France.
- P. Konstantinidis** and R. Britz. Development of the hyopalatine arch in tetraodontids: Fixation of the ethmopalatine articulation. Joint Meeting of Ichthyologists and Herpetologists, New Orleans, Louisiana, USA.
- 2005 Poster** **P. Konstantinidis** and Sven Gemballa. Derived trunk morphology in a thunniform swimmer: The musculotendinous system of *Euthynnus alletteratus*. Society for Integrative and Comparative Biology, San Diego, USA.
- 2004 Poster** **P. Konstantinidis**, S. Gemballa, J. M. Donley and R. E. Shadwick. Thunniform swimmers with internalized red muscles: Derived features in the musculotendinous system of Mako sharks. 7th International Congress of Vertebrate Morphology, Boca Raton, USA.

Professional Organizations

American Society of Ichthyologists and Herpetologists
 Society for the Study of Evolution
 Early Life History Section of The American Fisheries Society
 Gesellschaft für Ichthyologie

Professional Service

Invited Reviewer

Acta Zoologica, Ichthyological Research,
 Journal of Fish Biology

Consultancy

The International Union for Conservation of
 Nature. Identification of larval fishes of sea mounts of the
 Southern Ocean

References

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