Mysterious multifarious mullets – morphology and relationships of mugiliforms

Dissertation

To Fulfill the Requirements for the Degree of „doctor rerum naturalium“ (Dr. rer. nat.)

Submitted to the Council of the Faculty of Biological Sciences of the Friedrich Schiller University Jena

by Philipp Thieme, M. Sc.

born on 16th May 1992 in Hohenmölsen

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Gutachter:
Prof. Dr. Lennart Olsson, Friedrich-Schiller-Universität Jena
Prof. Dr. Rainer Schoch, Friedrich-Schiller-Universität Jena
Prof. Dr. Gerhard von der Emde, Universität Bonn
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Declaration of contribution

The cumulative dissertation presented herein includes four peer-reviewed publications and two submitted manuscripts and I, Philipp Thieme, hereby declare that:

All publications and manuscripts presented were designed by Philipp Thieme and the Ph.D. tutor Timo Moritz. Most of the lab-work (e.g., clearing and staining), data collection and processing, data analyses, manuscript preparation and publishing related work was carried out by Philipp Thieme under the guidance of Timo Moritz and the corresponding co-authors.

A summary of the individual contributions of each author to the respective publication or manuscript is listed below.

Chapter 1:

- Clearing and staining of examined material **PT**
- Dissection of examined material **PT, TM**
- Data acquisition **PT, TM**
- 3D-reconstruction **PT**
- manuscript preparation **PT, TM**
- publishing related work **PT**

Chapter 2:

- Rearing of larvae and providing larval material **DV**
Chapter 3:

- Clearing and staining of examined material KK, PT
- Data acquisition KK
- Data analysis KK, PT, TM
- Manuscript preparation KK, PT, TM
- Publishing related work PT

Chapter 4:

- Field work (e.g., specimen collection) RKH, FH
- Clearing and staining of examined material PT
- CT-scanning JK
- Data acquisition JK, PT
- Data analysis JK, PT, FH
- Manuscript preparation JK, PT, RKH, FH
- Publishing related work JK
Chapter 5:

- Larval rearing PT
- Field work (i.e., specimen collection) PW
- Clearing and staining of examined material PT
- Data acquisition PT
- Data analysis PT, PW, TM
- Manuscript preparation PT, PW, TM
- Publishing related work PT

Chapter 6:

- Clearing and staining of examined material PT, NS
- Data acquisition PT, NS, KP
- Data analysis PT
- Data interpretation PT, NS, TM
- Manuscript preparation PT, NS, KP, TM
- Publishing related work PT

__________________________  ____________________________
Philipp Thieme                      Place, Date

__________________________  ____________________________
Prof. Dr. Lennart Olsson           Place, Date
Summary

The Mugiliformes are a taxon of marine, estuarine and freshwater fishes comprising 27 genera and 79 species. They are a dominant group in most regions where they occur and are commercially important for fisheries and aquaculture. While the ecology of mugiliforms is well investigated, multiple questions on their morphology, phylogeny and taxonomy remain. The last morphological studies featuring the Mugiliformes date back almost 20 years and the evolution of characters within this taxon are yet to be examined. Meanwhile, recent molecular analyses provided new insights into the phylogenetic position of mugiliforms as well as their taxonomy. However, these studies were not able to sufficiently resolve the phylogenetic intra- and interrelationships of mugiliforms. Many new phylogenetic hypotheses have been postulated of which the most conclusive assigned the Mugiliformes to the Ovalentaria in unresolved relationships to multiple other taxa.

This dissertation generally focuses on the phylogenetic intra- and interrelationships of the Mugiliformes using different morphological approaches and analysing a wide spectrum of mugiliform and possible closely related taxa. The evolution of the mugiliform skeleton is examined and discussed in the light of recent phylogenetic hypotheses and the larval development. Furthermore, previously suggested relationships of mugiliforms and atherinomorphs, which were based on morphological data, are revised based on newly gathered data. Lastly, the latest and most extensive molecular-genetic hypothesis positioning the Mugiliformes within the Ovalentaria is evaluated on the basis of morphological data.

The first section of this thesis concentrates on the skeleton of mugiliforms. The osteology of the species *Liza aurata* is described and the evolution of traits within the Mugiliformes are discussed based on comparisons to previously described morphological structures in other mugiliform species. Several characters were revealed that show varying character states in different species, e.g., connection of the basisphenoid to the prootic, shape of the metapterygoid, and articulation of the pelvic girdle halves. Furthermore, the development of the postcranial skeleton of *Mugil cephalus* is examined in detail and unveiled the fusion of single elements during ontogeny, which provides new insights for the interpretation of the adult anatomy.
The second section deals with the morphology of atherinomorph taxa, which previously were hypothesized to be closely related to mugiliforms. Various skeletal characters are analysed to find similarities and differences to the mugiliform skeleton and development. The development of scales in atheriniform taxa, the composition of the anal fin in beloniform taxa, and the development of the caudal fin skeleton in atheriniform, beloniform and cyprinodontiform taxa are examined. Among other things, similarities to mugiliforms are observable in the caudal fin development, e.g., two ural centra fuse to form the compound centrum.

Finally, in the third section the phylogenetic position of mugiliforms within the Ovalentaria is analysed using multiple characters from the caudal fin skeleton as well as a broad taxon sampling including all ovalentarian families. The retrieved phylogenies overall correspond well to results from phylogenetic analyses based only on molecular-genetic data. Furthermore, the grundplan of the caudal fin skeleton for a last common ancestor of all ovalentarian taxa is reconstructed and character evolution within the Ovalentaria is discussed.

This dissertation contributes to the understanding of the intra- and interrelationships of mugiliforms and our knowledge on the evolution of the skeleton within this taxon. Moreover, it is shown that morphological studies significantly contribute essential data to discuss and evaluate phylogenetic hypotheses and evolutionary processes.
Zusammenfassung


Diese Dissertation trägt zum Verständnis der phylogenetischen Beziehungen innerhalb der Meeräschen und zum Wissen um die Verwandtschaftsverhältnisse der Meeräschen zu anderen Familien innerhalb der Ovalentaria bei. Außerdem wird gezeigt, dass morphologische Studien bedeutende Erkenntnisse zur Diskussion und Beurteilung phylogenetischer Hypothesen und evolutionärer Prozesse beitragen.
Introduction

Fishes in Evolution and Systematics

Within the last decade, 4,329 new fish species have been described (Fricke et al. 2020). While in 2010 roughly 29,716 valid fish species were known, there were about 34,044 accepted species listed at the end of 2020, which corresponds to an increase of almost 15%. Thus, the Actinopterygii, the ray-finned fishes, are the vertebrate clade with the highest addition of newly described species. Also, it is the vertebrate taxon with the most species in total. This almost comes as no surprise as the diversity of habitats for fishes seems endless. But not only the exploration of previously inaccessible areas in the oceans of the world, especially various deep-sea habitats, revealed new species. Many of the recently described species inhabit regions which seemed to be well known to researchers. But they remained hidden until detailed morphological and mostly molecular-genetic examinations revealed differences to their kin.

Our interest in recognizing all extant and also all fossil species on earth comes along with the urge of sorting, ordering and classifying these species. Already in ancient times scholars like Aristotle (384-322 BC) started to categorize species based on their appearance (eidos) and typical characters (e.g. giving birth/laying eggs, blooded/bloodless) (Wiesemüller et al. 2013; Voultsiadou et al. 2017). One of the most famous naturalists, Carl von Linné (1707-1778), developed the binominal nomenclature in which each species is assigned to a genus and a species name – the system that is still used today (Wiesemüller et al. 2013). Furthermore, he presented hierarchical categories, i.e., species, genus, ordo, and classis, to order the living nature by common phenotypes. In the following century, biologists like Jean-Baptiste de Lamarck (1744-1829), Charles Darwin (1809-1882), and Ernst Haeckel (1843-1919) [and many others] not only wanted to categorize animals and plants anymore but rather searched for the relationships linking species to each other (Wiesemüller et al. 2013). Darwin’s theory of evolution (descent with modification and natural selection) probably is the most famous biological concept to this day (Darwin 1859). It also caused a shift in the way species were categorized: while Linné’s system was based on a God-given order, everything needs to be ordered in the light of evolution based on Darwin’s theory. In the 20th century, the evolutionary theory was revised and refined
by the likes of Theodosius Dobzhansky (1900-1975), Ernst Mayr (1904-2005), and Iwan Iwanowitsch Schmalhausen (1884-1963), to name only a few, who, influenced by the emergence of new disciplines (e.g., developmental biology, experimental genetics, molecular biology, mutation biology), advanced this theory into, what we now call, the Modern Synthesis (Wiesemüller et al. 2013). One result was the definition of the biological species concept in which “[s]pecies are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups” (Mayr 1942). Although species were no longer solely defined by a common phenotype, on higher taxonomic levels, this systematic concept was not applicable. Based on the Homology concept defined by Richard Owen (1804-1892), Willi Hennig (1913-1976) developed his theory of the Phylogenetic Systematic (Hennig 1950). Its goal is to find monophyletic taxa which are crown groups with a common descent defined by homologous characters. Further, Hennig declared that it is necessary to ascertain if a homologous character is apomorphic or plesiomorphic as only apomorphic characters can define a monophyletic taxon (Hennig 1950). Adolf Remane (1898-1976) addressed the concept of homology at around the same time and defined three homology criteria, i.e., position, development, and composition, which were practical guidelines to determine if characters are homologues or analogues (Remane 1952). By the end of the 20th century multiple homology concepts were heavily debated (Brigandt 2003; Hoßfeld and Olsson 2005) and discussions continue until today (e.g., Brower and de Pinna 2012; Nixon and Carpenter 2012; Richter 2017; Suzuki and Tanaka 2017; Vogt 2017).

Many biologists started to apply Hennigs phylogenetic systematic concept to reconstruct evolutionary relationships of species and higher taxonomic levels of all life-forms (e.g., Bremer and Wannstorp 1978; Wiley and Mayden 1985; Mabee 2000). In the 20th century these analyses were based on morphological data, but with the advances made in molecular-genetic techniques, these were quickly utilized for the reconstruction of phylogenetic trees. Soon after, studies based on genetic data largely replaced morphology-based approaches. This had a huge influence on how the evolution of fish was investigated. While morphology-based studies were heavily restricted by time-consuming and effortful comparative analyses, which only allowed for few taxa to be examined at a time, gene-based studies enable the evaluation of much more taxa and data, which results in larger phylogenies. In 2013 Betancur-R et
al. published “The Tree of Life and a New Classification of Bony Fishes”, an extensive analysis of genetic data from 1,410 bony fish taxa that represented 1,093 genera and 369 families. It was the most comprehensive study of vertebrates until then and provided a completely new insight in the evolutionary relationships of the Actinopterygii. In terms of fish, one question may arise: Is this the end of a seemingly endless journey of sorting, ordering and classifying?

Well, it is not. Although many backbone nodes in the phylogenetic tree are well supported in this and subsequent studies, new taxa combinations were hypothesized, many previously hypothesized taxa were discarded and many nodes remain unresolved (Betancur-R et al. 2013; Betancur-R et al. 2017). Furthermore, by turning away from morphology-based approaches, the evolution of morphological characters was treated rather carelessly. However, these new hypotheses yield great advantages as a base for new comparative morphological studies. Also, the hypotheses provided by molecular-genetic analyses need to be reviewed and unresolved relationships within these hypotheses need to be attended with alternative, e.g., morphological approaches.

In this thesis, one of these newly hypothesized taxa combinations, the Ovalentaria, will be examined carefully. Although subsequent studies supported the validity of this taxon, the relationships within the Ovalentaria remain largely unresolved (Betancur-R et al. 2017; Hughes et al. 2018). The starting point of my studies will be the Mugiliformes, a taxon which previously was hypothesized to occupy a rather significant position in the phylogeny of fishes as the sister-taxon to the Percomorpha (Stiassny 1990, 1993). Within the Ovalentaria, the position and phylogenetic relationships of the Mugiliformes are unknown and, therefore, became the focus of the morphological studies of the herein presented thesis.

The Mugiliformes

What are grey mullets?

The Mugiliformes or grey mullets are a fish taxon that currently comprises one family, 27 genera and 79 species (Fig. 1; Xia et al. 2016; Fricke et al. 2020). They are native in all seas around the world. Most mugiliform species inhabit coastal waters in
tropical and subtropical regions, but there are also species that are common in temperate zones. In general, the grey mullets are considered to be an opportunistic fish family as they show a high adaptability to varying environmental conditions. In their distribution area, they often dominate the fish fauna in terms of population size which is attributed to their primarily detritivorous feeding habit (Crosetti and Blaber 2015). Mugiliforms are easily recognized by their uniform eidonomy: the body is slender, cylindrical and grey-silvery; the head of many species is flattened and broadened; two dorsal fins, one spiny and the other soft, are widely separated (Fig. 1, 2; Crosetti and Blaber 2015; Nelson et al. 2016).

**Taxonomy of grey mullets**

*Mugil cephalus* Linnaeus, 1758 was the first mugiliform species scientifically described. By the end of the first half of the 19th century more than half of today’s valid species (43 of 79) were described. In 1836, Cuvier and Valenciennes attempted to systematically classify the Mugiliformes for the first time. Many reviews of the taxonomy of grey mullets were subsequentially published mainly focusing on the generic level (e.g., Jordan and Swain 1884; Jordan and Evermann 1917; Mohr 1927; Schultz 1946; see also Ghasemzadeh and Ivantsoff 2004 for a brief outline of mugilid taxonomy). Probably the most extensive taxonomic study was conducted by Thomson (1997). Based on over 25 years of detailed morphological analyses, his revision

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*Figure 1: Drawing of *Muge tetragonure* by Georges Cuvier (adopted from *Le règne animal distribué d’après son organisation* [Planche N°76], second edition from 1828).*
caused a major shake-up of the generic affiliation of mugiliform species (Table 1). Further, many species names were synonymised which caused a lot of confusion regarding collection material and subsequent analyses of these specimens. Until the end of 2010s all taxonomic studies published on mugiliforms were based on morphological or morphometric data. It was in 2012 when Durand et al. (2012a) and Durand et al. (2012b) presented the first molecular-genetic data of mugiliforms on a taxonomic level. Both studies analysed mitochondrial loci which resulted in general disagreement with previously accepted mugiliform taxonomy. In the following years additional analyses revealed a high proportion of cryptic species within some genera (e.g., Cheilon) and species (e.g., Mugil cephalus), which priorly went unnoticed due to a lack of morphological differences (Whitfield et al. 2012; Durand et al. 2013; Durand and Borsa 2015). Furthermore, a revision of the mugiliform genera was provided based on a combination of morphological and molecular-genetic data (Table 1; Durand 2015; Xia et al. 2016). Currently, one family, the Mugilidae, is recognized within the taxon Mugiliformes and four subfamilies are distinguished, of which the Myxininae comprise two genera with two species (Fig. 2A), the Mugilinae comprise six genera with 24 species (Fig. 2B), the Rhinomugilinae comprise 13 genera with 21 species (Fig. 2C) and the Cheloninae comprise six genera with 32 species (Fig. 2D).

Many attempts on inferring the systematic relationships of mugiliform taxa were included in the taxonomic reviews mentioned in the previous paragraph. A first extensive hypothesis was provided by Schultz (1946) based on four morphological characters and one life history traits (Fig. 3A). Many of the following phylogenetic hypotheses included more taxa and different morphological characters (Fig. 3B, C; Senou 1988; Harrison and Howes 1991; Thomson 1997; Ghasemzadeh 1998). One common assumption made by all these studies was the basal position of the genera Agonostomus and Joturus as sister taxa (Agonostomus more basal in Senou, 1988; Joturus not included in Ghasemzadeh, 1998), followed by Cestraeus (derived position in Schultz (1946), not included in Senou (1988)) and Aldrichetta (not included in Senou, 1988). Thomson (1997) called this paraphyletic taxon Agonostominae (Fig. 3C). For the remaining taxa, all the phylogenetic hypotheses differed greatly (Fig. 3). Already in the 1990s, first molecular-genetic analyses were conducted studying the phylogeny of mugiliforms (Caldara et al. 1996; Rossi et al. 1998). But until the end of the 2010s these studies comprised either only few mugiliform species or were limited to species
Figure 2: Preserved specimen representing the four mugiliform subfamilies. A) Myxinae: *Myxus elongatus* (AM I.39547-008, SL = 124.5mm); B) Mugilinae: *Mugil curema* (DMM IE/16061, SL = 91.7mm); C) Rhinomugilinae: *Osteomugil perusii* (DMM IE/16087, SL = 127.5mm); D) Cheloniae: *Oedalechilus labeo* (DMM IE/14977, SL = 79.3mm).
Table 1: Overview on all mugiliform genera and there taxonomic status (*according to Xia et al., 2015).

<table>
<thead>
<tr>
<th>Described Genera</th>
<th>Author &amp; Date</th>
<th>Type Species</th>
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<th>Currently assigned Genus*</th>
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<td>Trachystoma</td>
<td>Ogilby [J. D.] 1888</td>
<td>Trachystoma multipten Ogilby</td>
<td>Myxus</td>
<td>Trachystoma</td>
</tr>
<tr>
<td>Valamugil</td>
<td>Smith [J. L. B.] 1948</td>
<td>Mugil crenilabis sehei Forskál</td>
<td>Valamugil</td>
<td>Crenimugil</td>
</tr>
<tr>
<td>Xenomugil</td>
<td>Schultz [L. P.] 1946</td>
<td>Mugil thoburni Jordan &amp; Starks</td>
<td>Mugil</td>
<td>Mugil</td>
</tr>
<tr>
<td>Xenorhynchichthys</td>
<td>Regan [C. T.] 1908</td>
<td>Joturus stipes Jordan &amp; Gilbert</td>
<td>Joturus</td>
<td>Joturus</td>
</tr>
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</table>
from one geographical area (Papasotiropoulos et al. 2001; Papasotiropoulos et al. 2002; Turan et al. 2005; Papasotiropoulos et al. 2007; Aurelle et al. 2008; Liu et al. 2010). The latest molecular-genetic study on mugiliforms provided an update on the phylogenetic relationships based on twelve nuclear loci and three mitochondrial genes and included 54 species representing 25 genera (Fig. 4; Xia et al. 2016). In this study, the Myxinae, i.e., *Myxus elongatus* and *Neomyxus leuciscus*, were retrieved as the most basal taxa within the Mugiliformes. These two taxa were previously thought to be rather derived and not only distantly related species (Schultz 1946; Ghasemzadeh 1998). Within the Mugilinae the genera *Agonostomus*, *Dajaus*, *Cestraeus* and *Joturus* are closely related, which resembles the results from previous morphological studies (Schultz 1946; Senou 1988; Harrison and Howes 1991; Thomson 1997; Ghasemzadeh 1998). Further, the Mugilinae include *Mugil* and *Chaenomugil* which supposedly are sister-taxa (Xia et al. 2016). The results from Thomson (1997) also suggest a close relationship of these two genera. The Rhinomugilinae and Cheloninae seemingly are the two most derived taxa within the Mugiliformes. A similar constellation was proposed by Thomson (1997). He hypothesized that *Sicamugil* and *Rhinomugil* are closely related while *Valamugil* and *Crenimugil* are sister-taxa. The molecular-phylogeny presented by Xia et al. (2016) grouped these taxa within the Rhinomugilinae and retrieved similar relationships between them. Furthermore, Thomson (1997) suggested that *Chelon*, *Liza* and *Oedalechilus* are closely related, which again was supported by molecular-genetic results. The phylogenetic hypothesis provided by Xia et al. (2016) will be basis for the phylogenetic discussion in this thesis.
Biogeography and distribution of grey mullets

Mugiliform species are common in coastal waters in most temperate, sub-tropical and tropical seas (Fig. 5, 6; Crosetti and Blaber 2015). While the species *Mugil cephalus* occurs worldwide in the latitudinal range of 51°N to 42°S (Thomson 1963; Ghasemzadeh 2015a), other species have much smaller distribution ranges. And some genera even have clear distribution boundaries separating them from other genera.

Species of the most basal subfamily, the Myxinae, are present in the coastal waters of Australia (*Myxus elongatus*; not along the north coast) and around the Polynesian Islands (*Neomyxus leuciscus*) (Fig. 5A; Ghasemzadeh 2015a; Shen and...
Durand 2015). In these regions, many species belonging to the Rhinomugilinae are present too (Fig. 5B). The monotypic genera *Aldrichetta*, *Gracilimugil*, and *Trachystoma* are also distributed around the Australian coast down to New Zealand and east to the Polynesian Islands (Ghasemzadeh 2015a). Most other Rhinomugilinae species can be found in the South-East Asian waters from New Guinea to India and Japan. The genera *Minimugil* (India to Vietnam), *Paramugil* (New Guinea to North Australia), *Rhinomugil* (India to Vietnam), and *Squalomugil* (New Guinea) have rather limited dis-

Figure 5: Distribution maps of A) Myxinae species: *Myxus elongatus*, orange; *Neomyxus elongatus*, brown; and B) Rhinomugilinae genera and species: *Aldrichetta + Gracilimugil*, white blocks; *Crenimugil + Ellochelon + Osteomugil*, light violet; *Osteomugil robustus*, dark-violet; *Paramugil*, black dots; *Plicomugil labiosus*, violet lines.
distribution ranges, while *Plicomugil* is present all around the South-East and the East Asian seas (Fig. 5B). The two genera *Crenimugil* and *Osteomugil* occur in much larger areas, from the West-Pacific to the east coast of Africa (e.g., *C. crenilabis* and *O. perusi*). The species *Ellochelon* also inhabits this large area (Durand and Whitfield 2015; Ghasemzadeh 2015a; Shen and Durand 2015). A huge overlap between species of the subfamily Rhinomugilinae and the subfamily Cheloninae occurs in the Indic and the West-Pacific (Fig. 5B, 6A). The genus *Planiliza* is distributed from the Polynesian Is-
lands (P. alata) up to Japan (P. macrolepis) as far as to the Red Sea (e.g., P. subviridis) and down to South Africa (e.g., P. melinoptera) (Fig. 6A; Durand and Whitfield 2015; Ghasemzadeh 2015a; Shen and Durand 2015). The genus Chelon also inhabits the coastal waters of South Africa, where they overlap with Planiliza species (Durand and Whitfield 2015). From there Chelon is common in the coastal zones along West-Africa (e.g., C. dumerili) up to the North Atlantic around the North-West European coast (e.g., Chelon aurata) and in the Mediterranean Sea (e.g., Chelon ramada). The four monotypic genera Neochelon (West-Africa), Oedalechilus labeo (Mediterranean Sea), Parachelon (West-Africa), and Pseudomyxus (South Africa) share their distribution areas with Chelon (Fig. 6A; Durand and Whitfield 2015; Turan 2015). Few Mugilinae species, i.e. Mugil bananensis, Mugil capuri, and Mugil curema are also present along the West-African coast (Fig. 6B; Durand and Whitfield 2015) and the genus Agonostomus even reaches east as far as Madagascar (Fig. 6B; Thomson 1997). Most Mugil species, however, occur around the coasts of North (e.g., M. curema), Central (e.g., M. hospes) and South America (e.g., M. liza) as well as in the Caribbean Sea (Fig. 6B; Barletta and Dantas 2015). The two genera Dajaus and Joturus are only present in freshwater streams in the Caribbean Islands and Central America. However, larvae of these genera were also found in offshore waters around the respective coasts (Barletta and Dantas 2015). The genera Chaenomugil and Xenomugil are restricted to the Pacific coast of Central America (Fig. 6B) and the Galapagos Islands and only the genus Cestraeus occurs far west around Australia and Indonesia.

Most mugiliform species can be found in coastal regions. They prefer shallow littoral zones and often stay near the surface in the upper 10m of the water column. The grey mullets show a high degree of adaptability and versatility: they are present in a variety of habitats with different temperatures, sedimentary environments or dissolved oxygen regimes (Nordlie 2015; Whitfield 2015). They can also tolerate a huge span of salinity levels and turbidity. That is why grey mullet populations are common in the clear waters of coral reefs, but also in highly turbid estuaries and polluted harbours (Crosetti and Blaber 2015). Their adaptability to different salinities allows them to inhabit brackish waters (e.g., estuaries, lagoons) and marine waters (i.e., seas and oceans) and, additionally, freshwaters (e.g., rivers, lakes; e.g., Dajaus monticola, Rhinomugil corsula, Planiliza abu) and even hypersaline waters like salt marsh creeks (e.g., Chelon ramada in Mont Saint Michel, France) (Whitfield 2015). All mugiliforms are considered to display a high degree of residency.
Reproduction and larval development of grey mullets

Major migration events in grey mullets seem to happen only during the spawning periods in the adult life stage (Fig. 7). In general, spawning happens offshore in marine environments. Only few exceptions are known: *Rhinomugil corsula*, a species common in freshwater habitats in South Asia, can reproduce in rivers and lakes (Kurian 1975); and, the mountain mullet *Dajaus monticola* may reproduce in brackish waters, which was indicated by early larval stages present in estuaries (Kenny 1995). It is believed that other species common in freshwater habitats may also reproduce in freshwater or brackish water environments (Whitfield 2015). However, it seems like spawning success is enhanced in marine environments. Experimental studies showed that sea water salinities are necessary for successful spawning and embryonic development, and that the survival of embryos is negatively affected if the eggs get in contact with bottom sediment, which is much more likely in estuaries, rivers and lakes (Kuo et al. 1973; Van der Horst 1981; Walsh et al. 1989; Walsh et al. 1991). Adult specimens that reach sexual maturity migrate offshore during their spawning period (Fig. 7). The lengths of the spawning seasons range from three to seven months (Whitfield 2015). There are indications that the timing and length of the spawning periods depend on the water temperature. Observations allow for a rough generalisation: species between the equator and 30° latitude are found to be spawning in winter, when water temperatures are down; species between latitudes of 30° and 40° spawn in late summer and fall, when water temperatures are falling; species inhabiting areas above 40° latitude spawn in summer, when water temperatures are high (Van der Horst 1981; Whitfield 2015). The consensus from multiple studies on *Mugil cephalus*

![Figure 7: Reproduction cycle of Mugil cephalus depicting different life stages and their distribution area (adopted from Whitfield and Durand, 2012).](image-url)
indicates that the optimal temperature range for spawning is between 20-26°C (Whitfield 2015). After spawning adults tend to return to their previous estuarrian habitats (Fig. 7; Whitfield et al. 2012). The fertilized eggs remain offshore and float near the water-surface (Liao 1975). The early development of the embryos within the eggs was described in detail by many authors (Sanzo 1936; Vodyanitskii and Kazanova 1954; Anderson 1957a; Tang 1964; Burdak 1969; Yashouv and Berner-Samsonov 1970; Kuo et al. 1973; Tung 1973; Liao 1975; Boglione et al. 1992; El-Gharabawy and Assem 2006; Miller and Kendall 2009) and summarized by González-Castro and Minos (2015). The eggs of mugiliforms in general can be described as pelagic, buoyant, spherical and transparent. Early developmental steps are reached in short periods of time: Cleavage occurs about 15 minutes after fertilization, the blastula stage occurs after about 4.5 hours and the gastrula stage occurs around 7.5 hours after fertilization. Then the development of organs begins and afterwards, about 13.5 hours after the egg was fertilized, the neurulation starts. The early embryonic developmental stages happen within the egg and hatching of pre-larvae occurs at around 36 – 50 hours after fertilization depending on the water temperature (Kuo et al. 1972; Kuo et al. 1973).

After hatching, the pre-larvae feed on the yolk-sac as their feeding apparatus is not yet fully developed and the mouth is still closed (González-Castro and Minos 2015). With the opening of the mouth and the commence of external feeding, the larval stage begins. Koutrakis (2015) summarized the external development of mugiliform larvae (Sanzo 1936, 1937; Anderson 1957a, b, 1958; Tang 1964; Yashouv and Berner-Samsonov 1970; Cassifour and Chambolle 1975; Cataudella et al. 1988; Boglione et al. 1992; El-Gharabawy and Assem 2006; Khemis et al. 2006; Miller and Kendall 2009; Khemis et al. 2013): The larval stage is characterized by the development of the median and paired fins as well as the flexion of the notochord. The larvae enter the post-larval stage once all median fin elements are developed. Another character associated with the transition from larva to post-larva is the development of scales, which in mugiliforms occurs at around 7-11 mm (total length) and around 24-25 days post hatching (Anderson 1957a; Khemis et al. 2013). It was observed that post-larvae already make their way into estuaries by actively swimming against outgoing water currents and by utilizing flood tidal transport, to reach their nursery areas (Fig. 7; Torricelli et al. 1982; Harrison and Cooper 1991; Trancart et al. 2011). According to Blaber (1987) and Trape et al. (2009) mugiliforms recruit in estuaries between 10-20 mm (standard length) in the post-larva and early juvenile stage.
The juvenile stage, as proposed by different authors (Demir 1971; Balon 1975; Bensam 1989), begins after definitive organs have replaced temporary organs, general morphometrics resemble adults, and, in case of mugiliforms, the third anal fin spine is developed. In mugiliforms this stage is reached at around 28–45 mm (Anderson 1958; Khemis et al. 2013). Growth and maturation take place in the nursery areas, e.g., estuaries, rivers and lakes (González-Castro and Minos 2015; Koutrakis 2015).

*Morphology of grey mullets*

Mugiliforms are a taxon characterized by their extremely uniform eidonomy and their equally similar anatomy. While distinguishing grey mullets from other species is relatively easy, being able to set apart mugilid species from each other can be a virtually unsolvable issue.

Characters useful to recognize a mugiliform fish include: an elongated and subcylindrical body; two dorsal fins which are widely separated and of which the first is spiny with four fin rays and the second is soft with nine to eleven fin rays; anal fin with two to three fin spines and eight to twelve fin rays; pectoral fins that insert high on body; forked caudal fin; broad and often dorsally flattened head; adipose tissue covering the eyes partially; absence of lateral line on body (Fig. 8; Thomson 1997; Harrison and Senou 1999; González-Castro and Ghasemzadeh 2016; Nelson et al. 2016). The skeleton of mugilids, too, has many traits which set them apart from other species: specialized pharyngobranchial organ, which is varying in its form between mugilid species; pelvic girdle connected to the ventral postcleithrum by a ligament; three extrascapular bones associated to the pectoral girdle; 24 – 26 vertebrae; neural spines of the anterior seven vertebrae enlarged and plate-like; lateral processus on the second vertebrae (Harrison and Howes 1991; Thomson 1997; Ghasemzadeh 1998; Harrison and Senou 1999; González-Castro and Ghasemzadeh 2016; Nelson et al. 2016).

Multiple morphological characters were examined in comparative analyses concerned with the phylogenetic position of the Mugiliformes and their relationships to other taxa (Boulenger 1904; Myers 1935; Berg 1940; Gosline 1962; Rosen 1964; Gosline 1968; Stiassny 1990; Stiassny and Moore 1992; Stiassny 1993). Also, extensive analyses were conducted concerned with their external and internal morphology for
classification purposes (e.g., Senou 1988; Thomson 1997; Xia et al. 2016). Schultz (1946), for example, found differences in the scale type (ctenoid-cycloid), the adipose eye-lid (absent-present), the form of the lower lip (folded downward-projecting forward-thickened), and the shape of the preorbital (front edge straight-front edge concave/notched) of different mugiliform genera. These characters are still of use in identifying mugiliform species. In more detailed studies with larger taxon samplings, numerous other varying features were presented, e.g., position of the nostrils (in relation to each other and the eyes), position of the eyes (lateral-dorsal), presence of sessile teeth, length-ratio of pectoral and pelvic axillary scale, and number of pyloric caeca (Thomson 1997). In addition to morphological studies, many morphometric analyses were conducted on different mugiliform species (Corti and Crosetti 1996; Ibáñez Aguirre and Lleonart 1996; Cousseau et al. 2005; Ibáñez-Aguirre et al. 2006; González-Castro 2007; González-Castro et al. 2008; González-Castro et al. 2012). The morphometric concept was also adapted for mugiliform scales (Ibanez et al. 2007; Pacheco-Almanzar et al. 2020). The evaluation of mugiliform scales in general has a long history. Already Jacot (1920) examined scale characters and scale

Figure 8: Depiction of characteristic features employed for identification of mugiliform species (adopted from Harrison and Senou, 1999).
shape of *Mugil cephalus* and *Mugil curema*. Subsequent studies used scales and scale characters for species delimitation and species identification (e.g., Ibáñez and Gallardo-Cabello 2005; Mussarat-Ul-Ainb et al. 2015), for growth analyses (e.g., Cech Jr and Wohlschlag 1975; Elaine and Guadalupe 2005; Ellender et al. 2012), and examined the scale development and squamation (e.g., Pillay 1951; Burdak 1969).

The skeletal anatomy of mugiliforms was often studied in broader phylogenetic approaches and studies focusing on mugiliforms itself are rather scarce. Hollister (1937a) studied the caudal fin skeleton of three *Mugil* species also including some developmental stages. Rosen (1964) included *Mugil cephalus* in his analysis of the Atherinomorpha and compared multiple skeletal structures, e.g., the pharyngobranchial apparatus, the operculum, and the shoulder-girdle. Capanna et al. (1974) and later Harrison and Howes (1991) focused on mugiliforms and examined the pharyngobranchial organ (PBO) in detail. While Capanna et al. (1974) studied this structure in *Chelon ramada* presenting detailed characterizations of the skeletal anatomy and the soft tissue, Harrison and Howes (1991) compared the PBO of different mugiliforms and hypothesized about the potential function and evolution of the organ. In summary, the PBO is a filter apparatus that comprises the upper gill arches and the tooth-plates of the pharyngobranchials, which are covered by a mucosa cushion of thick, epithelial tissue (Harrison and Howes 1991). Stiassny (1990, 1993), again, included the Mugiliformes in her comparative analyses in which the branchial musculature, the pelvic girdle and the neural arch morphology, among other characters, were studied.

Ghasemzadeh (2015b) gave a precise description of the osteology of *Mugil cephalus* and also included notes on the musculature. This publication was based on his unpublished doctoral thesis (Ghasemzadeh 1998), which comprised a much broader morphological analysis of mugiliforms. Unfortunately, he did not include the comparison to other mugiliform taxa and the discussion of the evolution of characters or phylogenetic relationships of mugiliforms in the above-mentioned publication (Ghasemzadeh 2015b). Because the doctoral thesis is unpublished it is not incorporated in the discussion of the herein presented publications.
Phylogenetic Relationships of the Mugiliformes

In the 19th and the first half of the 20th century, the Mugiliformes were positioned in the suborder Percesoces (e.g., Starks 1899; Boulenger 1904; Jordan and Hubbs 1919; Myers 1935; Hollister 1937a; Berg 1940). Jordan (1905) stated that the taxon was originally proposed by Cope. Starks (1899) provided several characters which characterized the Percesoces (e.g., two dorsal fins, pelvic fin abdominal with one spine and five rays). The taxa assembled within the Percesoces significantly varied between different authors. While some only included the Sphyraenidae, Mugilidae and Atherinidae (Starks 1899; Jordan 1905; Jordan and Hubbs 1919; Hollister 1937a; Berg 1940), others added the Polynemidae (Gosline 1962) and the Phallostethidae.

![Phylogenetic hypotheses including the Mugiliformes (red) provided by different authors (A-F)](image)

**Figure 9:** Phylogenetic hypotheses including the Mugiliformes (red) provided by different authors (A-F) within the last thirty years based on morphological data (A, B), morphological and molecular-genetic data (C), and molecular-genetic data (D-F). All taxa assigned to the clade Ovalentaria are marked in blue. The Percomorpha are indicated by a green dot.
(Myers 1935), or even expand the group up to twelve families (Boulenger 1904). Starks (1899, pp. 1), however, already noted that the “study of the skeletons of several representatives of the families Atherinidae, Mugilidae, and Sphyraenidae […] reveals the fact that they are not so closely allied to each other as their external similarity would lead one to suppose”.

In the middle of the 20th century, studies including a broader taxon sampling brought forth new relationship hypotheses. Gosline (1963) provided evidence of a closer relationship of atherinids with cyprinodontids. Rosen (1964, pp. 260) added to this hypothesis and concluded that the “exocoetoids, scomberesocoids, adrianichthyoids, cyprinodontoids, atherinoids, and phallostethoids form a phylogenetically natural group” which he named Atheriniformes. Although different morphological evidence was provided by Rosen (1964), Gosline (1971) remained convinced that mugilids, sphyraenids, polynemids and atherinids were closely related and included them in the suborder Mugiloidei which again was positioned within the Perciformes. The phylogenetic position of the Mugiliformes remained questionable and Nelson (1984) positioned them within the Perciformes together with multiple other fish taxa (including sphyraenids and polynemids) and uncertain relationships. Stiassny (1990) then provided morphological evidence for a closer relationship of atheriniforms and mugiliforms. In a subsequent analysis, she hypothesized that these two taxa should be considered as sister-taxa based on seven common morphological characters and she placed them as sister-taxa to all percomorphs (Fig. 9A; Stiassny 1993). In the same year, Johnson and Patterson (1993) proposed a different taxon assemblage including the Mugiliformes: the Smegmamorpha. The Smegmamorpha, which they considered to be the basal taxon within the Percomorpha and sister-taxa to all Perciformes, comprise five taxa, the Synbranchiformes, Ellassoma, the Gasterosteiformes, the Mugiloidae and the Atherinomorpha (Fig. 9B).

In the following years, many new phylogenetic hypotheses based on molecular genetic data emerged and provided updates on the systematic position of the Mugiliformes (e.g., Wiley et al. 2000 (Fig. 9C); Miya et al. 2003; Miya et al. 2005; Mabuchi et al. 2007; Lavoué et al. 2008). Although some of these studies strengthened a closer relationship of mugiliforms and atheriniforms/atherinomorphs (e.g., Wiley et al. 2000 (Fig. 9C); Chen et al. 2003; Dettai and Lecointre 2005), in most other studies the phylogenetic position of the Mugiliformes remained uncertain (e.g., Miya et al. 2003;
Miya et al. 2005; Mabuchi et al. 2007; Lavoué et al. 2008). Other taxa possibly related to mugiliforms included the Blennoidei, Gobiesocoidei and Pseudochromidae. Setiamarga et al. (2008) investigated a large data set in order to resolve the phylogenetic relationships of the Atherinomorpha and also included mugiliforms in their analysis. Their results suggested even more possible taxa that are more closely related to the Mugiliformes than the Atherinomorpha, including the aforementioned taxa and additionally the Embiotocidae, Pomacentridae and Cichlidae (Fig. 9D). In 2012 Wainwright et al. provided evidence for a new clade, which they named Ovalentaria (Fig. 9E). Besides the Mugiliformes and their proposed sister-taxa mentioned in the molecular analyses above, this taxon comprises the Chaenopsidae, Dactyloscopidae, Labrisomidae, Grammatidae, Opistognathidae, Pholidichthyidae, Plesiopidae, and Polycentriridae (Wainwright et al. 2012). In subsequent molecular analyses, Betancur-R et al. (2013) and Betancur-R et al. (2017) were also able to retrieve the Ovalentaria as a monophyletic clade and provided convincing support values for this taxon (Fig. 9F). However, in the results provided by these three studies, the phylogenetic relationships within the Ovalentaria were not sufficiently resolved and therefore remain questionable (Wainwright et al. 2012; Betancur-R et al. 2013; Betancur-R et al. 2017).

The Caudal Fin Skeleton

The caudal fin of fish has been keeping scientists busy for almost 200 years (Agassiz 1833-1843). Many biologists have addressed the composition of the caudal fin skeleton and the homology of the fins and its skeletal elements in the late 19th and early 20th century (e.g., Kölliker 1860; Cope 1890; Regan 1910; Whitehouse 1910). Especially the caudal fin skeleton appealed to a lot of morphologists and detailed descriptions of many species are available today (Hollister 1936, 1937a, b, 1940, 1941; Gosline 1961; Nybelin 1963; Greenwood 1966; Monod 1968; Schultze and Arratia 1986, 1988, 1989; Fujita 1990). The reason for the great interest in the caudal fin and its skeleton may be due to its function, its high variability, its evolution, and the fact that it is easy to examine.

In basal Craniata, i.e., hagfish (Myxinoidea) and lampreys (Petromyzontida), only a median fin fold (without skeletal elements) is present around the caudal portion of the body. In the more derived Chondrichthyes a heterocercal caudal fin (exter-
nally asymmetric caudal fin) is supported by multiple cartilaginous skeletal elements (Agassiz 1833-1843; Lauder 2000). In basal Actinopterygii, e.g., Acipenseriformes, *Mimipiscis*, and *Cheirolepis*, a heterocercal fin is present too (Gardiner 1984a, b; Arratia and Cloutier 1996; Choo 2012). In actinopterygians, however, bony skeletal structures support the caudal fin. While the basal Actinopterygii account only for a minimal percentage of the recent actinopterygian taxa, the vast majority, the Teleostei, are characterized by a homocercal caudal fin (externally symmetric caudal fin) (Agassiz 1833-1843; Schultze and Arratia 1989; Arratia 1999; Lauder 2000). The transition from a heterocercal to a homocercal tail is believed to be a key innovation promoting the diversification of teleosts. The shapes of the caudal fins as well as their functions in
teleosts are just as diverse as the clade itself. Its main function is aquatic propulsion, other functions in correspondence to the multiple shapes have been proposed: steering, manoeuvring, and holding position within the water column (Lauder 1989; Gosline 1997).

While the shape of the tail changed from basal actinopterygians to teleosts, heterocercal to homocercal, the caudal skeleton remained asymmetric. In simplified terms, the caudal skeleton of basal actinopterygians, e.g., *Amia* (Fig. 10A), is a slightly dorsally bend prolongation of the vertebral column with multiple centra and their appendages supporting the fin rays of the caudal fin (Grande and Bemis 1998). These centra, either called preural (haemal arch surrounds ventral artery) or ural (ventral artery passes hypurals bilaterally) have ventrally attached either haemal spines or, their serial homologues, the hypurals in a one-to-one ratio (Hilton 2011). In basal teleosts such as *Elops* (Fig. 10C), the number of preural and ural centra supporting the caudal fin are greatly reduced in adults, where only two ural centra can be seen. Furthermore, the one-to-one ratio of ural centra and the appending hypurals seems dissolved. While the caudal fin skeleton of basal actinopterygians can be described as polyural, in teleosts the caudal fin skeleton is termed diural. Based on the position of preural centrum 1 (the most-caudal centrum with a haemal arch surrounding the ventral artery), the number of hypurals connecting to each ural centrum, and ontogenetic data of *Elops* and *Hiodon*, it was hypothesized that the first ural centrum in teleosts corresponds to ural centra 1 and 2 in more basal actinopterygians and the second ural centrum to ural centra 3 to 5 (Fig. 10; Schultze and Arratia 1986, 1988). Another apomorphy of teleosts is the presence of uroneurals (Hilton 2011). These are paired and elongated bones positioned lateral to the ural centra. They are thought to be remnants of neural arches formerly connected to the supernumerary ural centra in basal actinopterygians (Schultze and Arratia 2013). Furthermore, the caudal fin skeleton of basal teleosts includes up to three epurals and neural spines of preural centra.

In many teleost taxa, multiple elements fuse together (e.g., hypurals, ural centra), get reduced or enlarged (e.g., epurals, uroneural 1), or are completely absent (e.g., hypural 5, uroneural 2). While there seem to be no limits to the possible simplification of the caudal fin skeleton, there remain some highly derived species which still represent a rather basal condition of the caudal fin skeleton (e.g., *Polymixia*), which are helpful corner stones in analysing evolutionary scenarios of caudal fin skeleton modifications (Fig. 10).
Objectives

This thesis aims to study the phylogenetic position of mugiliforms. To accomplish this task, the skeletal morphology of mugiliform species and a variety of outgroup taxa (members of the Ovalentaria) was analysed. Furthermore, one complex, the caudal fin skeleton was analysed in detail in all ovalentarian taxa. The thesis is structured in three sections which approach these different aspects:

Section 1: The osteology of mugiliforms was previously only poorly studied and this thesis aims to gain a better knowledge on the skeletal composition of mugiliforms. This includes the adult anatomy but also the skeletal development which is needed to determine the origin of compound structures and to understand developmental patterns. All this data is necessary to analyse phylogenetic relationships within the taxon Mugiliformes and to reconstruct the evolution of morphological structures within this taxon.

Chapter 1 provides insights into the osteology of the species *Liza aurata*. All skeletal elements are analysed and comparisons to other mugiliform species are drawn. In Chapter 2 the skeletal development of *Mugil cephalus* is examined and its implications on the perception of the adult mugiliform morphology are discussed. Furthermore, developmental stages of other mugiliform species are compared to find similarities and differences in the development within the Mugiliformes.

Section 2: Previously, morphological characters suggested a close relationship between mugiliforms and atherinomorphs. Molecular-genetic data, however, refuted these hypotheses. This thesis aims to study different morphological structures within atherinomorph taxa, which then can be adduced to reanalyse the relationships of mugiliforms and atherinomorphs based on morphological data.

Chapter 3 analyses the scale morphology and development in the atheriniform genus *Atherina*. Scales have been shown to present phylogenetically important characters and so can provide new insights into the relationship of taxa. In Chapter 4 the composition of the anal fin in the beloniform genus *Nomorhamphus* was studied in detail. This genus belongs to a taxon of live-bearing fish, which has implications on the anal fin skeleton of male specimen. Chapter 5 deals with the development of the...
caudal fin skeleton in the three major taxa of the Atherinomorpha, the Atheriniformes, Beloniformes and Cyprinodontiformes. The caudal fin skeleton is a character complex frequently used in morphological phylogenetic analyses. It presents multiple features which can be compared between higher level taxa. Additional developmental data can improve the comparison of atherinomorphs and mugiliforms based on the results of Chapter 2.

Section 3: Molecular-genetic analyses resulted in hypotheses placing the Mugiliformes within the taxon Ovalentaria. No morphological analyses dealt with this taxon as a whole so far. In this section a morphological study shall provide new insights into the relationship of these previously phylogenetically far-flung taxa. The morphological structure ideally suited for such an approach is the caudal fin skeleton. It was already successfully used in multiple phylogenetic analyses and provides several characters that can be compared on higher taxonomic levels. Further, results of an analysis of the caudal fin skeleton will give a first impression on the phylogenetic relationships of ovalentarian taxa.

Chapter 6 presents new data on the relationships of ovalentarian taxa based on an extensive analysis of caudal fin skeleton characters. Multiple characters are compared using different methods, i.e., ancestral character state reconstruction, phylogenetic analyses and morphological comparisons.
Results

Section 1: Analyses of the Mugiliform Skeleton and the Implications for Phylogenetic Relationships
The osteology of the golden grey mullet *Liza aurata* (Teleostei: Mugiliformes: Mugilidae) including interactive three-dimensional reconstructions

Philipp Thieme¹² | Timo Moritz¹²

¹Department of Science, Deutsches Meeresmuseum, Stralsund, Germany
²Institute for Zoology and Evolutionary Research, Friedrich-Schiller-University Jena, Jena, Germany

Correspondence
Philipp Thieme, Deutsches Meeresmuseum, Katharinenberg 14-20, 18439 Stralsund, Germany.
Email: philipp.thieme@meeresmuseum.de

Abstract
Grey mullets are remarkably characterized by their overall uniform external morphology. Identifying species as well as positioning the Mugiliformes in a phylogenetic context is rather difficult. Most recently they were placed in the newly erected Ovalentaria, but more detailed relationships to potential sister taxa were not resolved. Studying the internal morphology, especially the osteology, might provide new insights into the evolution of the Mugiliformes as well as help clarify their phylogenetic position within the Ovalentaria. A detailed osteology of the golden grey mullet *Liza aurata* is presented. The use of cleared and stained specimens allowed for a complete examination of bony and cartilaginous structures, and a 3D reconstruction from a µCT data set provided additional information on the positional relationships of the bones. Following this, the data obtained were compared with different mugilid species, particularly with the flathead grey mullet *Mugil cephalus*. Several differences between these species could be identified, such as the position of the basisphenoid, the shape of the hyomandibular and the composition of the branchial arches. These characters might help in understanding the evolutionary changes happening within the mugiliforms and will provide the basis to study this taxon in detail, finally allowing the reconstruction of the body plan of grey mullets.

**KEYWORDS**
anatomy, morphology, Mugil cephalus, Ovalentaria, phylogeny

1 | INTRODUCTION

Members of the Mugiliformes comprise some of the most common teleost fishes in coastal marine waters. They also inhabit brackish waters and lagoons, and some members even live in fresh waters in tropical, subtropical and temperate regions (Crosetti & Blaber, 2015; Durand et al., 2012; Durand & Borsa, 2015; Thomson, 1997). Because of their size, worldwide distribution and adaptability to different aquatic conditions, they are of interest to fisheries and aquaculture.

Grey mullets are commercially fished in different parts of the world, for example, Australia and the United States, and quite intensively cultured in Egypt and other Mediterranean countries (Crosetti, 2015; Crosetti & Blaber, 2015; Oren, 1981).

Currently 79 species in 27 genera are recognized in the Mugiliformes (Fricke et al., 2019). Nonetheless, the diversity of these...
species in terms of their external morphology is rather low, often complicating the correct identification of the species (Crossetti & Blaber, 2015). Accordingly, only few obvious characters are present for analysing the phylogenetic relationships within this taxon (Durand, 2015; Gonzalez-Castro & Ghasemzadeh, 2015). This had led to a number of contradicting phylogenetic hypotheses being published in the past century (Berg, 1940; Ghasemzadeh & Ivantsoff, 2004; Gosline, 1968; Parenti, 1993; Rosen, 1964; Rosen & Parenti, 1981; Stiassny, 1990, 1993; Stiassny & Moore, 1992). During the past decade, molecular studies tried to overcome this shortcoming and tried to revise the systematics of grey mullets (Durand et al., 2012; Durand & Borsa, 2015; Heras et al., 2009; Xia et al., 2016). The most recent study conducted by Xia et al. (2016) used a combined approach of analysing molecular data as well as external morphological characters of 54 mugilid species. This approach resulted in a detailed phylogeny, including a list of possible synapomorphies on different taxonomic levels.

Considering the overall position of the Mugiliformes in the tree of fishes, recent studies provided phylogenies in which the grey mullets are positioned within the newly erected taxon Ovalentaria(e) (Betancur-R et al., 2013, 2017; Hughes et al., 2018). Nonetheless, based on the low support values, no definite statement on closely related taxa to the Mugiliformes within the Ovalentaria can be inferred (Betancur-R et al., 2017). In previous analyses (Stiassny, 1993; Wiley et al., 2000), the Atherinomorpha (respectively the Atheriniformes) were considered to be the sister taxon to the Mugiliformes, which cannot be ignored because of the results of studies by Betancur-R et al. (2017).

To get a better understanding of the position of the Mugiliformes within the taxon Ovalentaria, a morphological analysis over a broad spectrum of taxa seems necessary. For such an approach, external and internal morphological characters should be analysed together. Especially skeletal structures can provide more useful traits and result in a better understanding of the phylogenetic relationships within the Ovalentaria. To perform such a study, more data on the skeletal morphology of representatives from this taxon, and thus also from Mugiliformes, are necessary.

A comprehensive osteology of the flathead grey mullet Mugil cephalus L. is already available (Ghasemzadeh, 2015), and selected characters, such as the pelvic girdle or pharyngobranchial organ, have been investigated in other studies (Harrison & Howes, 1991; Parenti, 1993; Stiassny, 1993; Stiassny and Moore, 1992). Nonetheless, more data are needed to reconstruct the mugiliform body plan as a basis for comparison with other ovalentarian taxa. The first step involves the detailed osteological descriptions of other mugiliform species, incorporating individual variability and cartilaginous structures. Based on the most recent phylogenetic analysis by Xia et al. (2016), M. cephalus is distinctly related to the golden grey mullet Liza aurata (Risso 1810), the focus species of the present study, within the Mugiliformes.

Some structures in fish morphology, especially the neurocranium, show a complex 3D structure which often poses problems when studying the osteology of a species. When studying smaller specimens, a reconstruction based on real or virtual sections is helpful to gain a better understanding of positional relationships. Using CT and μCT data sets for virtual 3D-reconstructions has become a regular method in morphological studies (e.g., Antunes-Carvalho et al., 2016; Brocklehurst et al., 2019; Werneburg & Hertwig, 2009a). Even if the investigator gains a good impression of the anatomical conditions, the person still faces the problem of communicating these findings to the readers using a 2D medium. Integrating 3D models in publications, accessible through freely available software, can offer a possibility to overcome this drawback.

In the herein-presented osteology of L. aurata, a 3D reconstruction of the neurocranium, allowing for zooming, moving, rotating and fading out single elements, is included. More 3D reconstructions of the gill arches, the axial skeleton and the suspensorium are available as supplementary material. Further focus was on the 3D relations of skull bones as well as on cartilaginous structures. This data pointed out the skeletal characters which showed remarkable variability between the investigated species and thus may serve as phylogenetic informative characters in future studies.

2 | MATERIALS AND METHODS

Specimens used in this study (Table 1) were obtained from Deutsches Meeresmuseum (DMM). No living specimens were collected, killed or examined during this study. For examination, specimens were either cleared and double stained or scanned using a μCT and digitally reconstructed. Clearing and double staining for cartilage (blue) and bone (red) principally followed the protocols of Dingerkus and Uhler (1977) and Taylor and Van Dyke (1985). Then, the specimens were dissected under binoculars and photographed using a Canon EOS 80D with a Canon MP-E 65 mm objective and a Sigma EX 105 mm objective as well as with a Leica M165 C stereomicroscope equipped with a dedicated Leica DFC425 using the software Leica Application Suite (Leica Microsystems, version: 4.9.0). The pictures were cropped, colour values were adjusted using Adobe Photoshop CC (version: 20.0.5) and plates were arranged in Adobe Illustrator CC (version: 23.0.3). The μCT scans of L. aurata were obtained at the Zoologisches Forschungsmuseum Alexander Küng in Bonn using a 100 kV SkyScan 1272 desk-top X-ray microtomography (Bruker microCT, Kontich, Belgium) with the following scanning parameters: 56 kV source voltage, 166 μA source current, 110 ms exposure time and 9 μm voxel size. Three-dimensional reconstruction was performed using Amira (version: 6.0.0), exported to Autodesk Maya (Thermo Fisher Scientific, version: 2019) for smoothing and assembling and then saved as 3D PDF using the 3D-PDF Exporter for Autodesk Maya (SimLab Soft, version: 8.0). The herein-implemented interactive 3D reconstructions (Figure 4 and Supporting Information Figures S1–S3) can be rotated, zoomed in and out and moved. Furthermore, using the model tree option, the reader can select single bones and can opt to hide or show the respective bones.
RESULTS

3.1 Neurocranium

The neurocranium of *L. aurata* is dorso-ventrally flattened, with the lateral axis widening in the posterior direction (Figure 1). The anterior border of the neurocranium is formed by the maxillary condyles of the vomer and the posterior border by the occipital condyles of the basioccipital and the exoccipitals that connect the neurocranium to the vertebral column (Figures 1 – 3). The roof of the neurocranium is formed by the nasals, frontals, parietals and the supraoccipital. The nasal is a flat trapezoid-shaped bone that is slightly curved ventro-anteriorly forming the dorsal border to the nasal sacs (Figures 1, 2 and 4). The nasal bears the anterior end of the supraorbital canal. The canal is open dorsally and only partly enclosed in its middle. A ligament connects the antero-lateral corner of the nasal to the preorbital. The frontal is a paired, elongated trapezoid bone (Figures 1, 2 and 4). Its anterior tip is positioned beneath the posterior part of the nasal (Figure 4). The portion of the supraorbital canal that runs along the dorsal surface of the frontal delimits the lateral part from the rest of the frontal. The lateral part forms the dorsal margin of the orbit (Figures 1a and 2a). Posterior to the orbit, the frontal is prominently notched. Ventrally on the frontal, a large crest runs beneath the supraorbital canal forming the dorso-medial border of the orbit (Figures 1c and 4). The crest starts shortly behind the anterior tip of the frontal and connects to the cartilage.

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*Only head length available.*
which links the frontal to the lateral ethmoid. From this ventral crest, two small, medially directed crests project (Figure 4). The frontals overlie each other medially; however, this overlap shows a high intra-specific variability. Two extremes that were observed (intermediate stages were present as well) are as follows: (a) The middle part of the medial margin of the right frontal has a flat, roundish protrusion that overlays the left frontal, which has a recessus of the same shape at the according position. Anterior and posterior to that, the left frontal has a flat, triangular (anterior) and a flat, roundish (posterior) protrusion that overlays the right frontal. (b) The left frontal has no protrusion, whereas the right one has a large triangular protrusion which runs along the whole medial margin and covers the left frontal which has a recessus of the same shape. Along the dorsal surface of the frontal, the supraorbital canal runs from the anterior tip of the frontal to the posterior end of the orbit. Anteriorly the canal connects to the supraorbital canal on the nasal, whereas posteriorly it connects to the infraorbital canal on the dermosphenotic as well as the temporal canal that runs further posterior along the postero-lateral margin of the frontal. The parietal is an almost rectangular bone with curvy margins (Figures 1a,b and 2a,b). Its midlateral part is bent downward and ventrally contacts the pterotic, forming a part of the temporal fossa (Figures 1b, 2b and 4), which is an insertion area for the epaxial musculature. Posteriorly the parietal covers the antero-medial part of the epioccipital. Medially the parietal covers a small part of the supraoccipital, whereas the antero-lateral margin of the parietal connects to the pterotic through a thin layer of cartilage and the antero-

**FIGURE 1** Cleared and stained neurocranium of *Liza aurata* (IE/15832, SL = 39 mm). (a) Dorsal view, (b) lateral view and (c) ventral view. Abbreviations: bo: basiscapital; bs: basisphenoid; et: ethmoid; es: epistoma; et: ethmoid; ex: exoccipital; fro: frontal; ic: intercalar; let: lateral ethmoid; na: nasal; par: parietal; pas: parasphenoid; pro: prootic; ps: pterosphenoid; ptm: posttemporal; pto: pterotic; sos: supraoccipital; sp: sphenotic; vo: vomer. Scale bar = 1 mm

**FIGURE 2** Drawings of the neurocranium of *Liza aurata* (IE/15832, SL = 39 mm). (a) Dorsal view, (b) lateral view and (c) ventral view. Bone, white; cartilage, grey. Abbreviations: bo: basiscapital; bs: basisphenoid; et: ethmoid; ex: exoccipital; fro: frontal; ic: intercalar; let: lateral ethmoid; na: nasal; par: parietal; pas: parasphenoid; pro: prootic; ps: pterosphenoid; ptm: posttemporal; pto: pterotic; sos: supraoccipital; sp: sphenotic; vo: vomer. Scale bar = 1 mm
lateral tip can slightly cover the sphenotic in some specimens (Figures 1a, 2a and 4). A small portion of the anterior margin is covered by the frontal. The left and right parietals are widely separated from each other (Figure 2a).

The ethmoid, the vomer and the paired lateral ethmoids form the anterior portion of the neurocranium, the ethmoidal region. The ethmoid (=mesethmoid) is an unpaired bone with a flattened antero-laterally directed part, which shows a sponge-like structure, as well as a more massive posterior part that triangularly extends dorsally with two latero-dorsal directed tips (Figures 1a, 2c and 4). Laterally on each side, a cavity is formed in which the ethmoidal cartilage persists. A posteriorly opened cone is part of the ethmoid, which originates from the tip on the posteromedial surface (Figure 4). In these small specimens, the ethmoid is still largely embedded in the ethmoid cartilage; ethmoid, vomer and lateral ethmoid are still separated by this cartilage (Figure 1). Also, between the frontals and the ethmoid, a piece of the ethmoidal cartilage is present in all specimens of this study. The vomer has a laterally extended anterior part, which is characterized by the maxillary conyles formed by its antero-lateral tips (Figures 1 and 2). Between these tips the vomer is notched on its anterior margin. Because of the dorso-ventral expansion of the conyles, a cavity posterior to them is created. The ethmoidal cartilage lies in this cavity. Otherwise, the dorsal surface behind the conyles is flattened. Posteriorly, the vomer runs out into a pointed process that fits ventrally into a depression of the parasphenoid (Figures 1c and 2c). The ventral side of the vomer is porous and sparsely occupied by small teeth along its anterior part (Figure 4). The lateral ethmoid forms the antero-dorsal border of the orbit and the posterior border of the nasal sacs (Figure 1b). Antero-ventrally, a remnant of the lamina orbitonasalis cartilage persists inside an ossified duct and laterally articulates with the preorbital. Along the dorsal margin of the lateral ethmoid, a deep cavity between the anterior and posterior surface is present in which the dorsal portion of the lamina orbitonasalis cartilage persists in the contact area towards the frontal (Figures 1b and 4). Medially, a vertical crest with the foramen for the olfactory nerve forms the medial border of the nasal sacs (Figures 1b and 4). Anteriorly, this crest forms a small, anterior-opened hollow. Beneath this vertical crest, two horizontal plates with a hollow in between surround the remaining ethmoidal cartilage anteriorly and the remaining trabecula communis cartilage posteriorly (Figures 1c and 4). The posteromedial edge of the more ventral plate contacts the parasphenoid. The ventral surface of the lateral ethmoid is concave and forms a hollow for the palatine.

The parasphenoid runs ventrally over most of the neurocranium’s length and is toothless (Figures 1c and 2c). It is an elongated bone that can be divided into an anterior, a middle and a posterior part. The anterior part, largely in the orbital region, is long and splits anteriorly into two tips, between which the fossa for the vomer is formed ventrally. Dorsally it contacts the remains of the trabecula communis cartilage, which has two halves each connecting to the medio-ventral corner of the lateral ethmoids (Figure 1b). In the middle part, the parasphenoid forms the wing-like processus ascenden s laterally on both sides that contact the prootics. Dorso-medially in front of these processus, a small remnant of the trabecular cartilage persists. A ventral crest, starting behind the fossa for the vomer, stretches until the end of the middle part of the parasphenoid (Figure 2b). The posterior part of the parasphenoid is shorter than the anterior part and ventrally concave and splits into two tips posteriorly. It is positioned below the prootics and the basisphenoid. Along its antero-lateral margins, the posterior part of the parasphenoid is separated from the prootics by some cartilage. This cartilage does not reach the processus ascendens, resulting in a foramen between this cartilage, the processus ascendens, the posterior part of the parasphenoid and the prootic (Figures 1b and 2b). Above the middle of the parasphenoid sits the small basisphenoid. This is a T-shaped bone with a cartilaginous ventral tip connecting to the remaining trabecular cartilage dorsally of the
parasphenoid (Figures 1b and 2b). The basisphenoid is not directly connected to any other bone but surrounded by connective tissue, which separates the brain cavity from the orbital cavities as well as the left and right orbital cavities (Figure 4). This connective tissue extends between the ventral crests of the frontals, the pterosphenoids, the prootics, the parasphenoid and the remaining cartilages anteriorly.

The prootic forms a large ventral portion of the neurocranium and the posterior border of the orbit together with the sphenotic and pterosphenoid (Figure 4). The prootic connects to the parasphenoid medio-ventrally, and the antero-ventral margins each have a mould for the wing-like processus ascender of the parasphenoid (Figures 1c, 2c and 4). Above the notched ventral lamina, a medial protrusion is present, and a hollow is formed in between the lamina and this protrusion (Figure 4). The prootics connect to each other with the medial margins of these protrusions, forming the roof for the anterior myodom, with a remnant of the cartilaginous neurocranium persisting between them. Laterally to the protrusion, a crest separates an anterior hollow with several foramina from the posteriorly positioned anterior part of the otic bulla (Figure 4). The prootic connects through its postero-ventral margin to the basioccipital, which forms the posterior part of the otic bulla. Another small hollow, for the lapillus, is present ventrally to the otic bulla (Figure 4). Posterior to the prootic forms an arch, through which the horizontal semicircular canal runs towards the exoccipital. Antero-laterally a large articular surface towards the sphenotic borders the jugular canal (Figures 1c and 4). Antero-dorsally another facet connects the prootic to the pterosphenoid. Posteriorly the prootic connects to the pterotic and the exoccipital (Figures 1b,c and 2b,c). The pterosphenoid is a trapézoid bone whose medial margin is concave posteriorly. Its anterior part connects to the ventral crest of the frontal (Figures 1c, 2c and 4). The lateral margin of the pterosphenoid connects to the sphenotic. The sphenotic (=autosphenotic) forms the antero-lateral border of the brain cavity (Figure 4). The internal surface of the sphenotic is characterized by two medium-sized hollows which are separated by a vertical crest. Laterally, the sphenotic has a large process with several foramina in its anterior surface (Figures 1, 2 and 4). Postero-ventrally to this protrusion, the sphenotic forms the anterior articulatory groove for the hyomandibular (Figures 1b,c, 2b,c and 4). The ventral surface of the concave protrusion is the origin of the levator arcus palatini. Medial to the protrusion, the sphenotic is connected to the prootic. Along the split antero-medial margin, a part of the neurocranial cartilage persists and links the sphenotic to the pterosphenoid and the frontal (Figures 1 and 2). The dorsal margin of the sphenotic directly contacts the frontal and the pterotic. The pterotic has a rather flat dorsal surface that is elongated posteriorly (Figure 1a,b). Anteriorly a process extends and contacts the postero-dorsal corner of the frontal as well as the sphenotic. Latero-ventrally the pterotic forms the posterior articulatory groove for the hyomandibular (Figures 1b, 2b and 4). Medially the dorsal surface contacts the parietal as well as the epi-occipital (Figures 1a, 2a and 4). The internal structure of the pterotic is dominated by two cone-like hollow structures in its anterior half that are connected through a small duct at their ends (Figure 4). Beneath these cone-like hollows, a flat ventral surface connects the pterotic to the prootic and the exoccipital. The enclosed temporal canal runs along the first third of the dorso-lateral margin of the pterotic, anteriorly connecting to the temporal canal on the frontal and posteriorly to the antero-lateral extrascapular canal. On each side of the skull, three separated parts of the extrascapular canal are present (Figures 1a and 2a). The antero-lateral part is L-shaped and anteriorly connects to the sensory canal of the pterotic. Its medial tip connects to the medial extrascapular, which slightly covers the parietal. These two extrascapulars together with the pterotic and the epi-occipital delimit an opening which is filled with epaxial musculature. The posterior extrascapular carries the sensory canal towards the lateral line of the trunk. The intercalar is a flat triangular bone that ventrally overlays the pterotic and exoccipital and touches the prootic with its anterior tip and the epipptic with its dorsal tip (Figures 1c, 2c and 4). At its posterior margin, a vertical extension covers the space between the pterotic and exoccipital. From there a posteriorly-directed processus protrudes interlocking with the ventral arm of the posttemporal.

The basioccipital is positioned posterior to the parasphenoid and the prootic and ventral to the exoccipitals (Figures 1–4). It is wide anteriorly, gets narrower posteriorly and is laterally notched at the transition between the anterior and posterior parts. The posterior part
of the basioccipital forms the occipital condyle which connects to the first vertebral centrum through a roundish, concave articular facet (Figure 4). Its dorsal surface forms the ventral border to the foramen magnum. A transition zone marked by two transverse crests separates the posterior and anterior parts (Figure 4). The anterior part forms the postero-ventral portion of the otic bullae; they are separated by a medial ridge. This medial crest does not reach the transverse crest of the transition zone, leaving a hollow in the middle of the bone dorsally. Ventrally, between the left and right otic bulla, a canal for the myodome is present, which is antero-ventrally covered by the parasphenoid. Laterally on each side and posterior to the respective otic bulla, a short postero-lateral-directed process serves as an attachment site for Baudelot’s ligament (Figure 1c). The exoccipital forms the posterior border of the neurocranium, the postero-dorsal roof of the otic bullae, the lateral borders of the posterior spinal cord canal and the lateral and dorsal borders of the foramen magnum (Figures 1b, 2b, 3 and 4). Furthermore, the postero-ventral tip of the exoccipital forms an additional small occipital condyle which articulates with the respective articular facet of the first vertebra. Antero-medially another condyle towards the transition zone of the basioccipital is present. The exoccipital is a highly complex bone, which will be described using its antero-postero midline axis as a reference (Figure 4). From this axis, four crests can be delimited: an antero-ventral crest, a medial horizontal crest, a lateral semispherical crest and a dorsal vertical crest (Figure 4). The antero-ventral crest forms the lateral border of the otic bulla and fills the gap between the basioccipital and prootic. In addition, a small arch embeds the horizontal semicircular canal at the antero-dorsal tip of the midline of the exoccipital. The medial crest...
forms the postero-dorsal roof of the otic bulla also connecting to the basioccipital. The lateral semispherical crest forms the ventral and posterior borders to the hollow between the epioccipital, exoccipital and pterotic. The dorsal crest is large and forms the wall of the spinal cord canal. This vertical crest has a triangular opening anteriorly, through which three foramina are visible. Two more foramina are present in the middle part of the dorsal crest. The lateral surface of the dorsal crest is marked by a ridge running from the postero-dorsal tip towards the lateral extension. The epioccipital is positioned in between the pterotic, exoccipital and supraoccipital and is anteriorly overlapped by the parietal (Figures 1–3). The internal part is characterized by a vertical crest in the middle separating an anterior hollow from a posterior hollow (Figure 4). The crest has a large foramen dorsally. The anterior hollow is directed towards the cranial cavity. The posterior hollow is formed like a ventrally opened cone because of another medial crest. It functions as a dorsal roof to the cavity between the exoccipital and pterotic. From the lateral corner of the posterior margin, a small protrusion extends towards the dorsal arm of the posttemporal. From the postero-medial corner of the epioccipital, a rod-like extension that expands into the large horizontal arm with a notched posterior margin emerges (Figures 1a,b, 2a,b and 3). The supraoccipital is a large bone covering the dorsal back of the brain-case (Figures 1 and 3). It is largely covered by the frontals and parietals. The posterior part of the supraoccipital is bent ventrally providing a surface for the insertion of the epaxial musculature. Postero-medially an enlarged vertical crest with a serrated posterior margin emerges. The postero-lateral margins contact the antero-lateral margins of the epioccipitals (Figure 3). The posterior margin of the supraoccipital is elongated and connects to the exoccipitals but does not reach down to the foramen magnum.

Two of the three pairs of otoliths are positioned in the otic bullae: the large sagitta anterior and the smaller astericus posterior (Figure 4). The sagitta stands in the anterior otic bulla, with the longer margins ventrally and dorsally. The astericus stands in the posterior otic bulla, with the shorter margin ventrally. Whereas the astericus is dorsally covered by the exoccipital, the sagitta is covered only by a layer of connective tissue. The smallest of the three otoliths is the lapillus. It lies in the dorsal hollow of the prootic (Figure 4).

3.2 | Infracraniial and orbital bones

The infraorbital series consists of five bones numbered antero-posteriorly, the last also named dermosthinopterotic (Figure 5a). The infraorbitals are dermal, laminar bones arranged around the ventral and posterior portions of the eye/orbit. The anterior-most infraorbital (infraorbital 2) is the smallest in the infraorbital series, and the following ones gradually get larger (Figure 5c). The opened infraorbital canal runs through the infraorbitals. Only the dorsal part of this canal on the dermosthinopterotic is enclosed with an opening for the branch towards the supraoccipital sensory canal on the frontal. The preorbital is a triangular bone that is positioned anterior to infraorbital 2 and in front of the eye sockets (Figure 5b). Its antero-ventral margin is heavily serrated, whereas its other margins are smooth. The anterior margin of the preorbital is positioned dorsal to the maxilla, and its anterior tip contacts the antero-dorsal process of the palatine. Medially the preorbital has a groove that articulates with the cartilage from the lateral ethmoid. On the preorbital, the infraorbital canal runs from postero-ventrally to antero-dorsally. This canal is closed only on its anterior end. The sclerotic ring is largely made of cartilage with two rather small, roundish laminar bones, equal in size, positioned at the anterior and posterior parts of the eyeball (Figure 5d).

3.3 | Jaw bones, hyoplatine arch and opercular series

The premaxilla is the anterior-most bone of the upper half of the skull (Figure 6a). It is a dermal, alveolar and curved bone with one row of setiform teeth originating from its antero-ventral margin (Figure 6c,f, g). The posterior part of the premaxilla is extended in and functional connection with the maxillary. At the posterior tip, a pointed, triangular projection is present. Antero-medially an ascending process emerges, which glides over the rostral cartilage. The ascending process of the premaxilla bears a foramen in its middle. Medially a symphyses connects both premaxillae to each other through a ligament. The maxilla is an elongated dermal and toothless bone with a complex 3D structure (Figure 6c,e). Its posterior end is twisted medially forming an attachment site for a ligament, which connects the maxilla to the posterior margin of the premaxilla. Posteriorly to the anterior tip is a dorsally curved arch-like process which encompasses the ascending process of the premaxilla. Furthermore, the posterior tip of this arch-like process is bent medially and is positioned ventral to the rostral cartilage to which it is connected through ligaments. The posterior surface of the arch-like process (Figure 6e: arrow) functions as an attachment site to the intermaxillary ligament which connects the maxilla to the lateral condyle of the vomer as well as to the maxilla from the other body side.

The lower jaw comprises the dentary, anguloarticular and the retroarticular (Figure 6a,b). The dentary is an elongated bone whose anterior part is slightly curved medially forming a roundish anterior margin. At the symphysis, a small piece of cartilage connects the left and right halves of the lower jaw. Along the anterior margin, two rows of tightly set setiform teeth-like structures with long bases are arranged. These teeth-like structures apparently have no dentin and no enamel. In the middle of the dentary, a dorsally directed arm that transitions into a posteriorly directed coronoid process is positioned (Figure 6b). The premaxilla and maxilla glide over this process (Figure 6a). Postero-ventrally this arm forms the angular fossa. Laterally, along the anterior–posterior axis of the dentary runs the mandibular sensory canal, which has three openings (Figure 6b). Meckel’s cartilage is covered by the dermal dentary antero-medially, where it inserts into the angular fossa, and proceeds posteriorly to the anguloarticular, where it ossifies as part of the articular. Antero-medially to this ossification, the small coronomeckalian is present. The anguloarticular is a triangular bone with a dermal and chondral
component that has a pronounced concave anterior margin resulting in an anteriorly pointing angular process (Figure 6b). It is positioned medial to the dentary, with its ventral margin proceeding above the sensory canal of the dentary. Its posterior-most part forms the articular facet for the articular condyle of the quadrate (Figure 6a,b). A dorsally bent postero-lateral process limits the articulation of the quadrate and the angularoarticular. On the postero-ventral end of the lateral surface, a portion (open in some specimens) of the mandibular sensory canal connects the sensory canal on the dentary to the opercular canal on the preopercle. Between the angularoarticular and the postero-ventral-positioned retroarticular, a small cartilage persists, which is also part of the articulation of the quadrate with the lower jaw. The retroarticular is an almost triangular bone, which connects to the interopercle through a ligament (Figure 6a,b).

The suspensorium comprises the chondral hyomandibular, symplectic, quadrate, metapterygoid and palatine, as well as the dermal endo- and ectopterygoid (Figure 6a,d). The hyomandibular is a deltoid-shaped bone, which has two condyles dorsally for articulation with the sphenotic and pterotic of the neurocranium (Figures 4 and 6a,d). A third condyle, the processus opercularis, on the postero-dorsal margin articulates with the opercle. A large crest runs along the lateral surface of the hyomandibular. It starts beneath the sphenotic condyle and continues downward towards the posterior margin, fading out ventrally. The posterior surface of the crest is perforated, whereas the anterior surface is concave, forming a depression anteriorly. A smaller crest arising from the pterotic condyle unites with the larger one. A distinct foramen for the ramus hyomandibularis of the nervus fascialis is located dorsally at the juncture of both crests. Antero-dorsally of the main body is a large outgrowth of membrane bone with a serrated ventral margin. These serrations interdigitate with fitting serrations along the dorsal margin of the metapterygoid. The postero-ventral corner of the suspensorium remains cartilaginous. Through this cartilage, the ventral tip of the hyomandibular, the symplectic and the interhyal are connected. The symplectic is a rod-like bone positioned horizontally beneath the metapterygoid and quadrate (Figure 6a,d). Its anterior tip is cartilaginous and fits into a notch formed by the quadrate. The symplectic becomes broader posteriorly and has a ventral extension, which can have a small anterior-pointing thorn, at the posterior end of the ventral margin. The quadrate is triangular in shape with a convex postero-dorsal margin (Figure 6a,d). The articular head is medio-laterally broadened and shaped like an hourglass, forming the jaw articulation with the angularoarticualr. Along the posterior margin of the quadrate, the palatoquadrate cartilage persists as a band between the quadrate and metapterygoid and continues up to the palatine connecting also to the endo- and ectopterygoid. The postero-ventral process of the quadrate has a dorsal groove that surrounds the symplectic ventrally. The metapterygoid is pentagon shaped with a slightly concave antero-dorsal margin (Figure 6a,d). A flat postero-dorsal process stretches medial to the hyomandibular. A small piece of cartilage remains at the postero-ventral corner that connects to the cartilaginous part between the hyomandibular and symplectic. The endopterygoid is an ovoid-shaped bone positioned horizontally and stretching medially towards the parasphenoid (Figure 6a,d). It is slightly convex in the middle and inclined antero-ventrally. A patch of teeth directed to the buccal cavity is positioned medio-ventrally. Along the lateral edge, a ventrally directed lamina is attached. This lies medially to the quadrate as well as, partly, to the meta- and ectopterygoid. The ectopterygoid is a rod-like bone, inclined antero-dorsally, with a broad posterior laminar outgrowth, which ends medial to the quadrate and contacts the ventral lamina of the endopterygoid anteriorly (Figure 6a,d). The palatine is a rectangular bone that is curved antero-laterally and has a ventral lamina as well as a pointing postero-ventral process (Figure 6a,d). Its anterior tip is cartilaginous and connects to the preopercle. A small notch is present between the main portion of the palatine and anterior to the ventral lamina. In some specimens, the ventral margin of the lamina is covered with teeth. The postero-ventral process of the palatine is positioned lateral to the anterior part of the ectopterygoid. Along the posterior margin, the palatoquadrate cartilage persists connecting the palatine to the endopterygoid as well as the ectopterygoid.

The opercular series comprises the opercle, subopercle, interopercle and preopercle (Figure 6a). The opercle is a large, thin, slightly triangular bone that has a horizontally oriented dorsal and vertically orientated anterior margin (Figure 6a). The posterior margin and the lateral surface are convex. An articulation socket is present medially to the antero-dorsal corner connecting to the processus opercularis of the hyomandibular. This portion is the most massive of the otherwise rather thin opercle. The anterior margin bends slightly postero-
ventrally forming a rim for the dorsal process of the subopercle, which functions as an extension to the anterior margin of the opercle (Figure 6a). The subopercle is curved postero-dorsally, with its ending transitioning into a band of connective tissue which surrounds a small accessory bone (Figure 6a; asterisk). The subopercle is a thin, laminar bone that is located beneath the opercle, which slightly overlaps the subopercle's dorsal margin. The subopercle covers the cleithrum laterally. The interopercle is a triangular-shaped bone slightly elongated in the anterior direction (Figure 6a). Its posterior margin lies above the anterior margin of the subopercle. The dorsal margin is thicker than the rest of the bone. The interopercle is anteriorly curved with a dorso-medial articulation facet towards the posterior ceratohyal in its middle. The anterior tip of the interopercle is connected to the retroarticular through a strong ligament. The dorso-lateral surface of the interopercle is covered by the preopercle. The preopercle is a large L-shaped bone that is placed anterior to the opercle and dorsal to the interopercle (Figure 6a). In contrast to the other opercular bones, it is much thicker, because it carries the preopercular-mandibular canal, which runs from the dorsal tip vertically to the level of the anterior tip and from there horizontally to the anterior tip. The vertical part of the canal is opened posteriorly, whereas the horizontal part is opened ventrally. The postero-medial outgrowth of the preopercle partly covers the opercle as well as the dorsal process of the subopercle. A small, perforated anterior outgrowth at the curve from the ventral to the dorsal part covers the connection of the hyomandibular, interhyal and symplectic.

3.4 | Hyoid, urohyal and branchial arches

The basihyal is distinctly divided into a large cartilaginous anterior part and a small ossified posterior part. The posterior part is a rather short bone that is broadened anterior and posterior giving the bone an hourglass-shaped appearance (Figure 7a). Postero-ventrally two articulation sites towards the dorsal hypohyals are present. On the dorsal surface, the basihyal toothplate with several teeth is present. The anterior part is elongated and broadens anteriorly similar to a shovel blade (Figure 7a). On the antero-dorsal surface of the cartilage as well as around its antero-dorsal margin, multiple small toothplates are scattered. Many small toothplates run along the connective tissue towards the anterior ceratohyal. The dorsal hypohyal, a small triangular-shaped bone, is connected to the basihyal at its antero-dorsal edge (Figure 7c). The foramen for the afferent hyoidean artery forms an opening in the lateral–medial axis anterior to the condyle connecting the dorsal hypohyal to the anterior ceratohyal. Beneath the dorsal hypohyal lies the ventral hypohyal, which has an articular
surface antero-medially to the ventral hypohyal of the other body side (Figure 7). Cartilage remains between the hypohyals and the anterior ceratohyal. The **anterior ceratohyal** is hatchet shaped with a medium-sized anterior part, a narrow middle and a dorso-ventrally expanded posterior part (Figure 7). The antero-dorsal surface is notched, and the lateral dorsal margin forms a process to which the connective tissue from the anterior basihyal attaches. Two distinct articulation sites on the anterior surface (one towards the dorsal and one towards the ventral hypohyal) are present. Cartilage remains around the posterior and postero-ventral margin of the anterior ceratohyal. This connects the anterior to the posterior ceratohyal. The **posterior ceratohyal** is shaped like a trapezoid, and the cartilage separating it from the anterior ceratohyal surrounds its antero-ventral margin (Figure 7). A posterior-directed projection from the anterior ceratohyal and an anterior-directed projection from the posterior ceratohyal interrupt their cartilaginous connection and interdigitate the two bones. Ventral to the postero-dorsal corner, an articulation facet towards the interopercle is present. In front of the postero-dorsal corner of the posterior ceratohyal, an evagination forms a connection site for the **interhyal**, a short, rod-like bone with two cartilaginous tips (Figures 6a,d and 7). Dorsally the interhyal connects to the cartilage between the symplectic and hyomandibular. Four branchiostegal rays articulate at the anterior ceratohyal: two smaller ones ventro-medially to its middle part and two larger ones ventro-laterally to the posterior part (Figure 7). In addition, two more branchiostegal rays articulate ventro-laterally at the posterior ceratohyal. The branchiostegals become longer posteriorly, and the two most anterior ones are rather slender, whereas the following branchiostegals possess broadened articulation heads. The **urohyal** is an elongated, laminar bone (Figure 7a). Anteriorly it is split into two portions that are implemented in strong connective tissue, which connects the urohyal to both ventral hypohyals. Along the ventral margin, two wing-like laminae extend latero-ventrally. The upper part is expanded in ventral–dorsal direction and has two attachment sites: one antero-
dorsally, which contacts the second basibranchial, and the other on the posterior tip which links to the cleithral symphysis through ligaments.

Four basibranchials are present (Figure 8a–d). The first one is a small ossified cup around the anterior tip of the cartilage connecting it to the second basibranchial. This one is an hourglass-shaped bone with symmetrical laminar outgrowths, which becomes broader posteriorly. Another cartilage connects the second to the third basibranchial. This bone is elongated and has a broad middle part, and its posterior end transitions into a cartilaginous cusp. Behind that is the cartilaginous basibranchial four which is hexagon shaped. Three hypobranchials are connected to the basibranchials on each side (Figure 8a,b,d). The first one has a broad medial margin with a cartilaginous edge and tapers off laterally before a stout anterior process and a rectangular posterior plate, which has a cartilaginous edge, emerge. It is connected to the second basibranchial and partly to the cartilage in front of it. Laterally, the first hypobranchial is ventrally inclined. The second hypobranchial is similar to the first one. It is connected to the third basibranchial and, again, partly to the cartilage in front of it. Anteriorly, the second hypobranchial has a cartilaginous cusp that protrudes towards the first hypobranchial. The third hypobranchial is an irregular-formed bone with a rod-like and ventrally orientated anterior part, which transitions into a broad triangular plate with a curved posterior margin. The cartilaginous tips of the anterior parts almost connect beneath the third basibranchial. Along the posterior margin, an elongated cartilage which connects to the cartilage behind basibranchial three is present. Five ceratobranchials are present, of which the first three are connected to the first three hypobranchials, respectively, with their cartilaginous antero-medial margins (Figure 8a–d). The fourth ceratobranchial contacts the postero-lateral corner of the fourth basibranchial with its cartilaginous antero-medial edge, whereas the last ceratobranchial overlays this edge with its cartilaginous tip. The first four ceratobranchials are dorsally bent, elongated bones which latero-dorsally connect to the epibranchials through cartilage. The fourth ceratobranchial is slightly broader than the third one. Ceratobranchial five is again even broader dorsally but has a narrow antero-ventral part. The dorso-medial margin of the fifth ceratobranchial is covered by small teeth. The cartilage between

**FIGURE 10** Cleared and stained pectoral and pelvic girdles of *Liza aurata*. (a) Pectoral girdle in lateral view (IE/15455, SL = 39 mm; right side), (b) drawing of the pectoral girdle in medial view (IE/15455, SL = 39 mm; right side), (c) pelvic girdle in situ (IE/15832, SL = 39 mm; lateral view) and (e) pelvic girdle in situ with oblique light and dark background (IE/15832, SL = 39 mm; lateral view). Abbreviations: app: anterior process of basipterygial plate; ba: basipterygial arm; bp: basipterygial plate; cl: cleithrum; co: coracoid; dr: distal radial; ew: external wing of basipterygial arm; fr: fin ray; fs: fin spine; iw: internal wing of basipterygial arm; pcd: dorsal postcleithrum; pcv: ventral postcleithrum; ppp: posterior process of basipterygial plate; pr: proximal radial; ptm: posttemporal; sc: scapula; su: supracleithrum. Scale bar = 2 mm
ceratobranchial two and epibranchial two is dorso-laterally elongated, and an additional small cartilage (Figure 8c; asterisk), which has a broad roundish base and is almost T-shaped, is located above it. The cartilage between ceratobranchial three and epibranchial three is dorso-laterally elongated and bent medially. The cartilage between ceratobranchial four and epibranchial four is broader than the postero-dorsal edge of the ceratobranchial and becomes bigger posteriorly. In addition, from its postero-medial corner a straight hook is oriented medially towards ceratobranchial five. Four epibranchials are present, all with complex and irregular shapes (Figure 8a,d–f). The first epibranchial has a broad lateral part and a narrower medial part, with both ends being cartilaginous. From its postero-dorsal margin, a dorso-lateral process emerges from which a hook-like posterior-directed cartilage originates. The second epibranchial transitions from a rod-like lateral part to a broad medial part. In addition, a thorn-like, laterally orientated process is present. The medial margin of the second epibranchial is surrounded by a large cartilage. The third epibranchial is again a rod-like bone that becomes broader medially. It has a postero-dorsal process that is broadened distally and has a cartilaginous posterior corner. This process is in close contact with the fourth epibranchial. The fourth epibranchial has a narrow lateral part and a broader medial part. Its lateral process is short and has a cartilaginous tip and an additional posteriorly directed arm. A thick cartilage surrounds the medial margin of the fourth epibranchial that has a groove for the connecting pharyngobranchial organ in the middle. The first pharyngobranchial is a short, rod-like bone directed dorsally and connected to the first epibranchial as well as the prootic (Figure 8c,d).

The second pharyngobranchial is a bean-shaped, trabecular bone antero-ventral to the second epibranchial. Its anterior tip is cartilaginous, and lateral to that, connected through a ligament, is a large, tooth-like cartilage extending into the space between epibranchial one and two. The pharyngobranchial organ is a complex structure (Figure 8d–g). An anterior toothplate (pharyngobranchial toothplate associated with pharyngobranchial three) and a posterior toothplate (pharyngobranchial toothplate associated with pharyngobranchial four) form the buccal surface of the pharyngobranchial organ (Figure 8d). Both are equipped with a large number of thin long teeth along the medio-ventral surface. The teeth become larger towards the margins of the toothplate. The anterior pharyngobranchial toothplate is triangular and points antero-medially. A thin groove along its posterior margin separates the anterior from the posterior toothplate. This latter one is almost rectangular with a convex posterior margin. Dorsally a compounded supporting structure is part of the pharyngobranchial organ and connects both toothplates to epibranchials two to four (Figure 8e–g). This supporting structure presumably represents pharyngobranchial three and can be described as follows: A medial portion of the supporting structure is connected to the medial margin of the third toothplate and ascends posteriorly. Its anterior and postero-dorsal tips are cartilaginous. From the middle where the margins of toothplates three and four join each other, a stout portion with a cartilaginous tip arises. Two braces connect this stout portion to the medial portion, one medio-ventrally directed and the other postero-dorsally directed. The cartilage posterior to the postero-dorsal tip of the median portion may represent the remnants of the fourth pharyngobranchial (Figure 8f,g; arrows). The large medial cartilage of the second epibranchial encompasses the postero-dorsal portion of this part of the medial portion anteriorly. The third epibranchial is connected to the cartilaginous tip of the stout portion. And the fourth epibranchial connects to the cartilage that presumably is pharyngobranchial four. Gill rakers are present along the anterior and posterior edges of all ceratobranchials as well as partly along the anterior margin of all hypobranchials (Figure 8a–c). The anterior margins of the first two epibranchials are also covered along their whole length by gill rakers, whereas the anterior margins of epibranchials three and four are only partly covered. The gill rakers have a U-shaped base that grabs around the margin of the respective bone and an extended shaft with an elongated tip.

3.5 | Median fins

Two dorsal fins are present. The first dorsal fin is composed of four pterygiophores with one fin spine each (Figure 9a,b). The first pterygiophore is extremely enlarged: twice as high and 10 times as broad as the second one. It is positioned between the seventh and the eighth neural spine and supports the first and largest fin spine. This pterygiophore has two processes, one antero-dorsally and one antero-ventrally (in close contact with the posterior border of the seventh neural arch), which are connected by membrane bone. The anterior margin of this membrane bone fits around the edge of the seventh neural spine. In addition, the antero-ventral process has bilateral extensions. The first fin spine articulates on the dorsal process of the first pterygiophore with its ventral foramen. Strong ligaments connect the lateral wing-like processes of the fin spine to the bilateral
extension of the antero-ventral process of the first pterygiophore. Pterygiophore two and three are much smaller, are positioned around the eighth neural spine and have their distal tips bent in posterior direction. They also have bilateral extensions, which, however, are much smaller than the ones of the first pterygiophore. The fin spines of pterygiophores two and three have a broad base with wing-like processes on each side. Whereas the second fin spine articulates with the pterygiophore in a similar way as the first one, the third fin spine remains on its pterygiophore and is only connected to it through ligaments. The first three pterygiophores are positioned close to each other, whereas the fourth one is positioned at some distance behind the ninth neural spine. It has nearly the same shape as pterygiophores two and three without bilateral extensions. Nonetheless, it supports a much smaller fin spine than the first three pterygiophores. This fourth spine has a broad ventral base. None of the pterygiophores is divided into separate radials. Between the first and the second dorsal fins are three interdorsal pterygiophores. These pterygiophores are characterized by the absence of any associated ray or spine (Figure 9a,b). Their shape is similar to the last pterygiophore of the first dorsal fin, except for their distal tips which are not as prominently bent posteriorly as in the fourth pterygiophore of the first dorsal fin. The interdorsal pterygiophores are positioned between the 10th and the 13th neural spines. The second dorsal fin is composed of nine pterygiophores which support 10 fin rays (Figure 9a). Only the last fin support bears two fin rays, which are clearly separate on their bases (Figure 9e). The pterygiophores of the second dorsal fin are divided into two parts: a small distal radial and an elongated proximal radial. The distal portions of the proximal radials are bent posteriorly. In addition, beneath this flexion, each radial has a triangular-shaped outgrowth of membrane bone posteriorly, whereas pterygiophore one additionally has one in the anterior direction. The cartilaginous distal tips of the proximal radials and the cartilaginous proximal margin of the distal radials are orientated towards each other (Figure 9a). A fin stay is connected to the last fin support through a cartilaginous bridge (Figure 9a,e). The distal radials ossify bilaterally first, forming attachment processes for the fin rays. The first and the second pterygiophores are positioned between the 13th and the 14th neural spines, whereas the last pterygiophore is positioned behind the 16th neural spine. The first fin ray is shorter than the following ones. And all fin rays are supported by the distal radial of the respective pterygiophore and partly by the dorsal part of the proximal radial of the following pterygiophore (except for the last two fin rays). The anal fin is composed of 10 pterygiophores which support 2 fin spines and 11 fin rays (Figure 9a,c). The two fin spines and one spine-like fin ray are connected to the enlarged first pterygiophore. This pterygiophore has an elongated antero-dorsal process and a plate of membrane bone anteriorly. Distally, a large anterior foramen is present to which the first small spine articulates through U-shaped proximal processes that encompass the antero-ventral margin of the pterygiophore (Figure 9c). Posterior to that, another foramen that is posteriorly opened and could also be described as posterior hook is present. Just like the first spine, the second spine articulates with this foramen. Behind the opening of the foramen, an ossified distal radial supports the spine-like first fin ray, which has two separated halves distally and is segmented (Figure 9c). This fin ray has two wing-like lateral extensions proximally that surround the distal radial. The other pterygiophores are divided into proximal radials and distal radials, and each pterygiophore supports one fin ray each, except for the last which supports two fin rays. A fin stay is positioned posterior to the last fin support and connected to it through a cartilaginous bridge (Figure 9c). The proximal radials become shorter in posterior direction. Their distal portions are bent in the postero-distal direction, and each of them has a triangular-shaped, posteriorly orientated outgrowth of membrane bone (Figure 9c). The second proximal radial is positioned behind the first haemal spine. The distal tip of this spine is in contact with the pterygiophore. The last two pterygiophores are positioned between the fifth and sixth haemal spines.

### 3.6 | Supraneurals, vertebral column and intermuscular bones

Above the vertebral column and in front of the first dorsal fin, three supraneurals are present (Figure 9a). The first one is T-shaped, with the horizontal portion being slightly curvy and following the dorsal body outline. The vertical portion is positioned between the neural plates of vertebrae two and three. The second supraneural has a shorter horizontal portion than the first one. The vertical portion is positioned between the neural plates of vertebrae four and five. The third supraneural is rod-like and sigmoidal. It is positioned in front of the first pterygiophore of the first dorsal fin and between the neural plates of vertebrae six and seven.

The vertebral column comprises 24 vertebrae, of which the compound centre of the caudal fin complex is counted as a single vertebra (Figure 9a,d). The total number of vertebrae does not vary between the investigated specimens. In general, the vertebral centra are hour-glass shaped and approximately the same size, although the most posterior ones are slightly shorter (Figure 9a,f). Dorsally, the neural arches are fused to the vertebra centra. They are large anteriorly and become smaller in the posterior direction. The first seven anterior neural spines expand, forming plates which become smaller and longer in posterior direction. Of the 24 vertebrae, 11 can be classified as abdominal vertebrae and 13 as caudal vertebrae (Figure 9a). The first vertebra has two additional antero-dorsally directed articular facets which connect to special processes of the exoccipitals (Figure 4). The neural spine of the first vertebra is split into a right and a left part anteriorly, which fuse to one plate posteriorly. The anterior surface of the first vertebra connects to the roundish articular facet of the basioccipital. The parapophyses of the first three vertebrae are located laterally to the neural arches and are bent latero-ventrally, with the first being the shortest and the second being the longest (Figure 9a). The following parapophyses are again shorter, and their origin shifts in posterior direction from lateral of the neural arches to ventral of the vertebra centra. Epicentrals are positioned behind each
of the first five parapophyses, with the last one being very small (Figure 9a). Ventrally connected to the medial tip of the second episternal is the first short rib. In total, nine pairs of ribs are present. They contact the parapophyses laterally and have a broad proximal and a thin distal part. The last rib contacts the parapophysis of the 10th centrum medially; no rib is connected to the parapophysis of the last abdominal centrum. Postzygapophyses are distinctly present at the second vertebra, where they are pronounced, and again from the sixth vertebra on backwards (Figure 9f). Prezygapophyses are present from the fourth vertebra on and become more pronounced posteriorly.

Starting with the eighth vertebra, small postexapophyses emerge, which become larger and more distinct posteriorly (Figure 9f). The first caudal vertebra has a broad and elongated haemal arch that continues into a relatively short haemal spine (Figure 9a). This spine is closely associated with the first anal pterygiophore. The following vertebrae have smaller haemal arches although longer haemal spines. In addition, starting with the second caudal vertebra, distinct preexapophyses are present. The neural and haemal spines of the 12th caudal vertebra (preural centrum 3) and the 13th caudal vertebra (preural centrum 2) contribute to the caudal fin (see the “Caudal Fin” section).

3.7 | Caudal fin

The caudal fin skeleton supports 15 (8 dorsal and 7 ventral) principal fin rays as well as 8–9 dorsal and 10 ventral procurrent rays in the herein-examined specimen (Figure 9a,d). The ventral procurrent rays are supported by the haemal spines of preural centra 2 and 3, both of which have cartilaginous tips, and two ventral inter-haemal spine cartilages (anterior and posterior to the haemal spine of preural centrum 3). The dorsal procurrent rays are supported by the neural spine of preural centra 3, which also has a cartilaginous tip, two epurals as well as the inter-neural spine cartilage. Preural centrum 2 is characterized by an enlarged haemal spine with an anterior outgrowth and an extremely reduced neural spine. Between the anterior dorsal zygapophysis and the neural spine, an anterior orientated protrusion is present with membrane bone connecting it to the neural spine remnant. The nomenclature of the compound centre and its attachments is based on the description of the caudal fin development of Mugil curema by Hollister (1937) with slight changes as he named the parhypural “hypural 1.” Therefore, the compound centre is presumably formed by ural centrum 1 and ural centrum 2; hypurals 1 and 2 as well as hypurals 3 and 4 are fused to uniform plates and together with the parhypural are fused to the compound centre (Figure 9d). Only the fifth hypural, which is positioned above the postero-dorsal tip of the compound centre, remains as a separate entity. A uroneural is positioned above the remaining parts of the neural arch of the compound centre and hypural 5. It is a paired bone that has a hook-like shape and partly encompasses hypural 5. Two large epurals are between the neural arch of preural centrum 3 and hypural 5. Epural 1 is longer and narrower than epural 2. They both have cartilaginous tips.

3.8 | Pectoral and pelvic girdles

The major components of the pectoral girdle are the posttemporal, supracleithrum, cleithrum, coracoid, two postcleithra (dorsal and ventral) and the scapula (Figure 10a,b). One fin spine, four proximal radials and 17–18 distal radials supporting 17–18 fin rays are connected to it. The posttemporal is roughly Y-shaped. It has a narrow and elongated, antero-dorsal orientated arm; a triangular, antero-ventral orientated arm, with a small postero-ventral process; and a broad, postero-ventral orientated part (Figure 10a,b). The antero-dorsal tip is positioned above the epiccipital, whereas the antero-ventral tip is directly connected to the intercalar (Figures 1 and 2). The supracleithrum has a flat anterior part that is overlapped by the posterior part of the posttemporal and ventrally connected to the small postero-ventral process of the posttemporal (Figure 10a,b). Ventrally an antero-median crest emerges that has its largest expansion medially and becomes smaller ventrally. The ventral half overlaps the cleithrum. The cleithrum is curved in the antero-ventral direction in lateral view (Figure 10a,b). The slightly convex dorsal part of the cleithrum, which is posteriorly extended as dorsal cleithral process, forms a shallow hollow medially. It has a pointed dorsal process and is notched behind it. Medio-ventrally to this process, Baudelot’s ligament attaches. The anterior surface of the cleithrum is flat and ventrally transforms into a keel with an anterior crest; it bears posteriorly curved laminae laterally and medially. The lateral lamina has a posteriorly pointing process dorsally and tapers off ventrally. The medial lamina is longer, forming the largest surface of the cleithrum; dorsal to it, a ventrally pointed hook is present (Figure 10a,b). The left and right cleithra are tightly connected through ligaments at the cleithral symphysis. Between the medial and the lateral portions, the scapula is connected to the dorsal as well as partly to the anterior portion of the cleithrum through a cartilaginous band that surrounds the anterior and ventral margins of the scapula (Figure 10a,b). The scapula is a five-sided bone with curved anterior and posterior margins and a large foramen in its middle. Along the posterior margin of the scapula, three proximal radials are present. The fourth as well as a small portion of the third are connected to the coracoid. All four radials have cartilaginous distal margins, become larger ventrally and are dumbbell-shaped. The cartilaginous distal radials are positioned between the proximal tips of the 17–18 fin rays, and all keep the same distance towards the proximal radials. Only the most dorsal fin ray and its distal radial are positioned above the posterior corner of the scapula. The pectoral fin spine is connected to the scapular condyle at the postero-dorsal margin of the scapula. Proximally it forms a complex articulation with a short lateral and an elongated medial part. The spine is closely positioned to the first pectoral fin ray (Figure 10a). Beneath the scapula, the coracoid is positioned (Figure 10a,b). Scapula and coracoid are connected through the cartilaginous band partly surrounding the scapula in the examined specimen; in larger specimens the cartilaginous band disappears. Both the anterior and the posterior margins of the coracoid are S-shaped, with the ventral part elongated. The convex curve of its posterior margin is pronounced. The ventral tip of the coracoid is close to the ventral margin of the medial portion of the
cleithrum. The anterior tips of the pelvic girdle are positioned underneath the coracoids of the pectoral girdle. The basipterygial plates of the pelvic girdle are positioned beneath the sixth vertebra (Figure 10c–e). The pelvic girdle is slightly antero-dorsally inclined. It comprises two elongated and trapezoid-shaped basipterygia. Each of the basipterygia has a rod-like basipterygial arm starting at the cartilaginous anterior tip and running to the posterior basipterygial plate (Figure 10c). Medial to the arm is a broad membranous outgrowth, the internal wing, which is slightly dorsally inclined. The external wing is quite small and positioned lateral to the posterior half of the arm. Its lateral, triangular enlargement functions as process (lateral process) towards the ventral basipterygial plate, to which it is connected through a strong ligament (Figure 10d,e). The basipterygial arm together with the internal and external wings forms a convex profile. Posterothese three portions continue into the thick basipterygial plate. It is connected to the opposite basipterygial plate through a serrated median suture (Figure 10c). In addition, each plate has a notch for the strong interpelvic ligament that connects both halves ventrally. Anterior to the suture are two thin anterior processes positioned in a horizontal plane and beneath the internal wings. Postero-medially, each base has a posterior process, sometimes called isihiac process. At the posterior edge of the basipterygial plates, five fin rays articulate through a cartilaginous bridge, and lateral to them, an additional single fin spine is present. One cartilaginous distal radial is fused to the proximo-dorsal tip of the ventral half of the most medial fin ray. The fin spine articulates with a U-shaped (lateral view) joint lateral to the cartilaginous bridge. Its proximo-dorsal tip has a ventrally orientated hook that reaches into that joint, whereas the proximo-ventral tip reaches around the posterior margin of the ventral side of the plate.

3.9 | Scales

Scales from the anterior flank are ctenoid and show whole cteni (Figure 11a). They are almost quadrangular with a rounded posterior margin. The anterior field is characterized by a striated margin. The margins of the lateral fields are straight with no special features. The posterior field is characterized by distinct cteni. The focus of the scales is slightly shifted posterior. The circuli are clearly visible but are discontinuous anteriorly (interrupted by the striae) and taper off posteriorly. Primary and secondary radii are present on the anterior field. Scales from other lateral body regions are similar in shape and features and will not be described individually. Lateral line scales are present along the dorsal margin of the opercle. They are similar to the scales from the lateral body regions; however, they are characterized by a short canal for the lateral line canal, which is at the position of the focus (Figure 11b). Scales on the head are quite different from the body scales as they are cycloid (Figure 11c). The shape of the head scales is, however, diverse, and only the scales along the midline of the head are circular, whereas the others have more irregular shapes.

4 | DISCUSSION

The herein-presented osteology describes the bony and cartilaginous elements of the skeleton of *L. aurata*. Interactive 3D models are given for the neurocranium (Figure 4), branchial arches (Supporting Information Figure S1), suspensorium (Supporting Information Figure S2) and the anterior part of the axial skeleton, including the pectoral and pelvic girdles (Supporting Information Figure S3). Although pictures of 3D-reconstructions are so far commonly used in morphological descriptions in ichthyology (e.g., He et al., 2016; Lundberg et al., 2017; Werneburg & Hertwig, 2009b), fully interactive models allow better retracing of 3D descriptions and spatial relationships of skeletal structures (e.g., de Boer et al., 2011; Holliday et al., 2013; Ruthensteiner & Hess, 2008; Van De Kamp et al., 2014; Figure 4). The models presented are not raw data but already-processed data, illustrating the view of the respective investigators, which is retrievable. Because of the additional information available to the reader, future morphological studies should aim to include such models to illustrate complex 3D structures.

In the following text, the osteology of *L. aurata* will be mainly compared to that of *M. cephalus* to identify characters and character complexes which show differences between these species. This will help identify possible structures of use for a broader comparison of grey mullets and the reconstruction of their body plan. The skeleton of the two species is quite similar. Therefore, only differences will be discussed in the following paragraphs.

The neurocranium of *L. aurata* and *M. cephalus* is almost identical in shape with similar proportions. In *M. cephalus* the nasal is in contact with the preorbital, but these bones are clearly separated in *L. aurata*. The overlap of the frontals was not explicitly described for *M. cephalus*; however, it can be seen in the drawings by Ghasemzadeh (2015). In the examined specimen of *M. cephalus*, the overlap of the frontals shows a variability that is similar to the conditions found in *L. aurata*. This variability should be examined more intensively using a larger sample size. The vomer of *M. cephalus* has a deep notch anterio-

rily between the maxillary condyles, which is less pronounced in *L. aurata*. When comparing the lateral ethmoid of *M. cephalus* to that of *L. aurata*, the former seems to have the anterior border of the orbit to be more elongate ventrally. Furthermore, the anterior hollow of the medial crest is comparatively larger in *M. cephalus* than in *L. aurata*. The description of the lateral ethmoid of *M. cephalus* given by Ghasemzadeh (2015, p. 133) vaguely describes “an oblique antero-
dorsal lamina (which) forms two funnel-shaped nasal sacs on the
lateral aspect of each lateral ethmoid. In the examined specimen of *M. cephalus* as well as *L. aurata*, such an “antero-dorsal lamina” could not be identified.

Ghasemzadeh (2015; p. 134) described “a foramen between each alar process and prootic” in the paragraph about the para-sphenoid of *M. cephalus*, which is not present in the examined specimen. What was identified for *M. cephalus* was an opening posterior to each processus ascends like the one present in *L. aurata*. A basisphenoid, which is present in *L. aurata*, was not described for *M. cephalus* by Ghasemzadeh (2015). He only remarked that the basisphenoid in other mugiliforms can have a “weak connection with both pterosphenoids, or (is) strongly linked to them” (Ghasemzadeh, 2015; p. 134). Nonetheless, a large, Y-shaped basisphenoid in the specimen of *M. cephalus* could be identified, which is positioned in between the pterosphenoids and prootics and directly connected to them through the dorso-lateral extensions. In addition, two large foramina lateral to the basisphenoid are present in both species through which the eye musculature as well as the ophthalmic nerves passes. The sphenotic is almost quadrangular with a lateral process in *M. cephalus* in the dorsal view. In *L. aurata*, on the contrary, the sphenotic is posteriorly extended and therefore rectangular with a lateral process. The pterotic of *M. cephalus* has a depression on its postero-dorsal elongation for the posttemporal with which it is in contact. In *L. aurata* no direct contact exists between those two bones; however, they are in proximity to each other. The intercalar of *M. cephalus* covers a larger area than that in *L. aurata*, overlying parts of the epipptic and prootic, which are touched only by the margins of the intercalar in *L. aurata*. The basioccipital of *L. aurata* and *M. cephalus* is quite similar in shape and relation to other bones. The origin of Baudelot’s ligament in *M. cephalus* lies in the lateral depressions of the transition zone (Ghasemzadeh, 2015). In the specimen of *M. cephalus*, processes similar to those found in *L. aurata* are present at the lateral depressions of the basioccipital to which Baudelot’s ligaments connect. The shape of the epipptical is similar in *M. cephalus* and *L. aurata*; however, in *M. cephalus* it is connected to the prootic based on its description by Ghasemzadeh (2015), although in the paragraph covering the prootic this connection is not mentioned. The supraoccipital of *M. cephalus* has two earflap-shaped medio-lateral processes which are less pronounced in *L. aurata* and therefore appear inconspicuous. Furthermore, in *M. cephalus* two posterior-directed processes originate from the ventrally bent posterior part of the supraoccipital, which could not be found in *L. aurata*. In the examined specimens of *L. aurata*, the supraoccipital crest does not reach in between the divided anterior margin of the neural plate of the first vertebra. Nonetheless, in larger specimens, the situation could be slightly altered and be more similar between both species.

In *L. aurata* and *M. cephalus* the same number of infraorbitals is present. The respective bones are similar in both species with the exception of the preorbital (Ghasemzadeh, 2015), which is connected to the nasal in *M. cephalus* but not in *L. aurata* where it reaches only the height of the anterior tip of the palatine. The antero-ventral margin of the preorbital is more deeply serrated in *L. aurata* than in *M. cephalus*. In the specimen of *M. cephalus*, the ventral tip is medially displaced, which is not the case in *L. aurata*.

The premaxilla in *M. cephalus* has two rows of teeth, whereas only one row of teeth is present in *L. aurata*. The posterior process of the premaxilla is larger in *L. aurata* than in *M. cephalus*. It extends dorsally more anterior in *L. aurata* and has a greater ventral–dorsal expansion. In addition, the posterior projection is more pronounced in *L. aurata*. All other jaw bones are quite similar in both species.

In *M. cephalus* the hyomandibula anteriorly has a ventrally directed process that is joined by the dorsal process of the metapterygoid (antero-dorsal process according to Ghasemzadeh, 2015). In *L. aurata* the respective process of the hyomandibula is rather a part of the large anterior outgrowth that has a serrated ventral margin. Consequently, the metapterygoid in *L. aurata* has a broader dorsal margin than that in *M. cephalus*, which is why the overall shape of the metapterygoids of *L. aurata* (pentagonal) and *M. cephalus* (quadranular) is different. The dorsal posterior margin of the symplectic in *M. cephalus* is slightly serrated and interdigitates with the ventral margin of the metapterygoid (*Ghasemzadeh, 2015*). In *L. aurata* such serrations are not visible. In addition, it should be noted that in *M. cephalus* the ventral lamina of the endopterygoid, the posterior part of the ectopterygoid and the postero-dorsal process of the metapterygoid are each positioned medial to the quadrant and the hyomandibula.

The opercular series in the two mugilid species is very similar. It can be noted that the dorsal margin of the opercle in *M. cephalus* is bent medially, partly covering the posttemporal apex, which is not the case for the herein-examined specimen of *L. aurata*. Two conspicuous character traits referring to the subopercle and the interopercle are present in both species: first, the postero-dorsal extension of the subopercle (a cartilaginous band that surrounds a small, separated bone) and, second, the dorso-medial articular facet of the interopercle towards the posterior ceratohyal. Both traits should be further investigated in other mugiliform species and could even be useful in higher-level phylogenetic comparisons.

In the hyoid arch, some differences in the basihyal and urohyal of *L. aurata* and *M. cephalus* are observed. The posterior part of the basihyal (basihyal in Ghasemzadeh, 2015) is seemingly shorter in *L. aurata* and bears a toothplate. No toothplate is described for *M. cephalus* (Ghasemzadeh, 2015). In contrast, the anterior part of the basihyal (glossohyal in Ghasemzadeh, 2015) in *L. aurata* is much more elongated and bears several teeth along its antero-dorsal margin. The smaller and semilunar anterior part of the basihyal (basihyal in Ghasemzadeh, 2015) in *L. aurata* has two rows of teeth, whereas only one row of teeth is present in *M. cephalus*. The epiphyal of *Ghasemzadeh (2015)* is synonymous with the posterior ceratohyal in this study. The urohyal of *L. aurata* does not have thin posterior processes extending from the lateral extensions, which, on the contrary, are present in *M. cephalus*. Furthermore, the antero-dorsal process is linked to the postero-ventral portion of the basihyal only in *M. cephalus* (Ghasemzadeh, 2015) but rather contacting the second basibranchial in *L. aurata*. 
The branchial arches represent a highly complex character entity. Between _L. aurata_ and _M. cephalus_, minor and some more elementary differences are present. Of the four basibranchial, the shape of the third, "almost spear shaped" in _M. cephalus_ (Ghasemzadeh, 2015; pp. 149) and more broad in the middle in _L. aurata_, and the shape of the cartilaginous fourth basibranchial, more elongated posteriorly in _M. cephalus_ and with ossified antero-lateral comers in the examined specimen of _M. cephalus_, vary. For the hypobranchials of _M. cephalus_, Ghasemzadeh (2015) described that the anterior process of the third hypobranchial connects to the posterior border of the third basibranchial and that "remnants of the fourth ... hypobranchials were present as alcianophilic cartilaginous nodules" (Ghasemzadeh, 2015; pp. 149). In the examined specimen of _M. cephalus_, both conditions resemble much more the ones observed in _L. aurata_. The process of the third hypobranchial connects to its counterpart beneath the middle of the third basibranchial, and no separated nodules are present but rather enlarged posteriorly directed cartilages connected to the third hypobranchial. The interpretation of these nodules as remnants of the fourth hypobranchials by Ghasemzadeh (2015) seems erroneous as in his drawings (Figure 8.10; Ghasemzadeh, 2015) they are positioned anterior to basibranchial four and with no connection to ceratobranchial four. Differences in the ceratobranchials between _L. aurata_ and _M. cephalus_ are restricted to the row of teeth along the dorso-medial margin of the fifth ceratobranchial, which are present in _L. aurata_ but not in _M. cephalus_. Most differences in the branchial apparatus of _L. aurata_ and _M. cephalus_ can be found in the epibranchials. In _M. cephalus_, epibranchial two has a postero-lateral-directed process originating from the large medial part that is in close contact with epibranchial three. In _L. aurata_ such a process is not present. _L. aurata_, on the contrary, has a lateral thorn-like process emerging from the dorsal side of the rod-like part of epibranchial two, which is absent in _M. cephalus_. For the third epibranchial of _M. cephalus_, Ghasemzadeh (2015) described two ventral wings. Neither in the examined specimen of _M. cephalus_ nor in the analysis by Harrison and Howes (1991) were such wings found on the third epibranchial. It is assumed that in his description, Ghasemzadeh (2015) interconverted the directions and that the larger "wing" corresponds to the medial part whereas the other one probably matches the postero-dorsal process of the third epibranchial of _L. aurata_. In direct comparison the third epibranchial in _M. cephalus_ is more massive than that in _L. aurata_, with both the medial part and the postero-dorsal process being larger (Ghasemzadeh, 2015). One feature of the third epibranchial present in both species is the postero-dorsal process that is in close contact with the fourth epibranchial. The fourth epibranchial of both species is quite similar; even the shape of the cartilage surrounding the medial margin matches up (Harrison & Howes, 1991). The epibranchials of _Chelon ramada_ (Risso 1827) are similar to those of _L. aurata_ (Harrison & Howes, 1991). The pharyngobranchial organ was studied in detail for _C. ramada_ and _M. cephalus_ (Capanna et al., 1974; Harrison & Howes, 1991) and even included a taxonomic survey of its general morphology of mugilid species of 21 currently recognized genera (Harrison & Howes, 1991). This survey impressively showed the huge variability of the pharyngobranchial organ morphology; however, the comparison of the skeletal structures is only limited. The development of the pharyngobranchial organ of _C. ramada_ indicates that pharyngobranchial three and four as well as the corresponding toothplates are incorporated into this complex (Harrison & Howes, 1991). In this case, pharyngobranchial four develops only as a small cartilage and remains as such, sometimes fused to the cartilaginous tip of the flat posterior arm that connects to epibranchial four. The skeletal composition of the pharyngobranchial organ of _M. cephalus_, _L. aurata_ and _C. ramada_ shows only little variation, and the most distinct differences can be seen in the dentition of the toothplates (Ghasemzadeh, 2015; Harrison & Howes, 1991). Some mugilid taxa even lack a pharyngobranchial organ, such as _Agonostomus, Dajaus_ and _Joturus_, whereas others have a rudimentary (Cestraeus) or intermediate (_Aldrichetta_) pharyngobranchial organ (Harrison & Howes, 1991).

The median fins of _L. aurata_ and _M. cephalus_ show only a few differences. In _M. cephalus_ the membrane bone between the antero-dorsal and antero-ventral process of the first pterygiophore of the first dorsal fin is notched, which is not the case in _L. aurata_. In different species descriptions, it is noted that the second dorsal fin of mugiliforms is composed of one fin spine and a different number of fin rays (e.g., Cuvier et al., 1836; Deef, 2018; Günther, 1938; Harrison et al., 2007). As Ghasemzadeh (2015) already showed for _M. cephalus_, no spines are present in the second dorsal fin of mugiliforms: The separation of the two halves of the first fin ray is visible only at its distal tips; however, the fin ray is clearly segmented and even slightly branched distally. A similar problem arises in terms of the number of fin rays supported by the last pterygiophore of the second dorsal fin: Comparing the number of fin rays from literature (e.g., Thomson, 1997; Harrison et al., 2007) to the examined specimen, the number of fin rays is always lower by one in the literature. This is based on the assumption that the last fin ray is split down to its origin, although two separated fin rays are actually present, connected to the distal radial of the last pterygiophore. This results in an overall count of 10 fin rays for the second dorsal fin of _L. aurata_ and matches the results for _M. cephalus_ (Figure 8.13; Ghasemzadeh, 2015). The separation of the two fin rays is not visible in fresh or preserved specimen, and therefore, counting them as one pair seems reasonable. Nonetheless, factually they represent two individual fin rays.

The enlarged first anal pterygiophore is similar in _L. aurata_ and _M. cephalus_. According to Ghasemzadeh (2015), it resembles the fusion of two pterygiophores. This seems possible; however, before a clear statement on the origin of this structure is made, the ontogeny of the anal fin should be evaluated. In addition, the ontogenetic origin of the third spine in _M. cephalus_ should be studied, as in _L. aurata_, a spine-like fin ray is present at the same position. In total, one additional fin ray is present in the anal fin of _L. aurata_ compared to _M. cephalus_. Just as in the second dorsal fin, the last pterygiophore of the anal fin supports two individual fin rays. A similar discussion as for the second dorsal fin in terms of the overall number of fin rays in species descriptions and identification guides arises; as the separation of the fin rays is not visible in fresh or preserved specimens, counting them as one pair seems reasonable but incorrect.
The supraneurals of *L. aurata* and *M. cephalus* differ only in the shape of their horizontal portions (horizontal ramus in Ghasemzadeh, 2015). In *M. cephalus* the anterior part of the first supraneural is more elongated, reaching anterior to the first neural plate, whereas in *L. aurata*, it reaches only to the level of the posterior tip of the second neural plate. Furthermore, the anterior and posterior tips of the first supraneural in *M. cephalus* are branched. The second supraneural is still T-shaped in *M. cephalus*, whereas an anterior part is absent in *L. aurata*. Both species share the same amount of vertebrae, which is 24, as well as the partition in 11 abdominal and 13 caudal vertebrae (Ghasemzadeh, 2015). For Chelon labrosus (thick-lipped grey mullet, Mugil chelo; Ford, 1937) and the thin-lipped grey mullet (probably *C. ramada*; Mugil spp. according to Ford, 1937), the same number of vertebrae was reported with minor variations (±1 caudal vertebra) in 4 of 116 specimens of *C. labrosus* and 2 of 34 specimens of *C. ramada*, respectively. The number of expanded neural plates varies between *L. aurata* (7) and *M. cephalus* (5). Nonetheless, Ghasemzadeh (2015) remarked that only the eighth neural spine is the first to become truly spinous. From anterior to posterior the parapophyses change their position relative to the vertebrate centre from a more central to a ventral position. For the 10th and 11th vertebrae in *M. cephalus*, Ghasemzadeh (2015) reported an ossified haemal bridge between each pair of parapophyses. In the examined specimen of *L. aurata*, no such haemal bridges were observed; however, Ford (1937; pp. 33–34) also found "a weak transverse bridge across the parapophyses of the 11th vertebra" in six specimens of *C. labrosus*. In *L. aurata*, the first haemal spine is positioned in between the first and the second anal pterygiophores, whereas in *M. cephalus*, it is positioned anterior to the first anal pterygiophore with its distal tip close to this pterygiophore (Ghasemzadeh, 2015). In both species the anterior-most rib has an anterior laminar outgrowth which is more pronounced in *M. cephalus* than in *L. aurata*. The number of epicentrals (=epineurals in Ghasemzadeh, 2015) is equal in *L. aurata* and *M. cephalus*. In the specimen of *L. aurata*, these are positioned within the horizontal septum, which are called epicentrals. Nonetheless, Patterson and Johnson (1995) came to terms to call these intermuscular bones in "percomorphs" epineurals as they were unable to distinguish between epicentrals and epineurals in adult specimen and suggested to study the ontogeny for a concluding classification.

The caudal fin of grey mullets shows few differences between the species. Several characters such as the number of principal fin rays, the fusion of hypurals 1 and 2 as well as hypurals 3 and 4 and the reduction of the neural spine of preural centrum 2 are principally equal in *L. aurata* and *M. cephalus*. Nonetheless, Ghasemzadeh (2015) interpreted the "terminal half centrum" (Ghasemzadeh, 2015; pp. 157), which is termed "compound centre" in this study, as a urostyle. The term "urostyle," however, is not consistently used for the same structure or complex in terms of its composition, that is, the elements fused together, which was recognized in multiple studies. The description of the caudal fin of Mugil curema and its development presents some evidence that maybe only one centrum is incorporated into the compound centre (Hollister, 1937), which would be in contrast to other teleosts (i.e., Doosey & Domke, 2014; Fujita, 1992; Gosline, 1961a, 1961b; Kohn et al., 1983; Kohn & Taki, 1983). Therefore, using the term "urostyle" may falsely indicate the comparability of structures which have different ontogenetic origins. That is why the more neutral term "compound centre" is preferred as it does simply imply that several structures are fused together. In general and as already stated by Gosline (1961a: p. 268), "it would seem best to compare caudal skeletons of half-grown individuals, wherever possible" or, even better, an ontogenetic series to follow the complete development of the caudal fin elements.

The pectoral girdles of *L. aurata* and *M. cephalus* are quite similar. In *M. cephalus* the posttemporal expands anteriorly and overlaps with the posterior corner of the pterotic (Ghasemzadeh, 2015), which is not visible in the studied specimen of *L. aurata*. Nonetheless, in larger specimens such an overlap could be possible. In both species, Baudelot's ligament attaches to the median dorsal surface of the cleithrum. This is in contrast to other studied acanthomorphs, including *Ellochelon vaigiensis* (Quoy & Gaimard 1825), for which it was stated that Baudelot's ligament connects to the supracleithrum (*Liza vaigiensis* in Patterson & Johnson, 1995). The pectoral fin spine, often referred to as marginal spur, because of its uncertain derivation (Ghasemzadeh, 2015; Stiassny, 1993), was described as short in *M. cephalus* and reduced to a small spur in *Agonostomus*. In the examined specimen of *L. aurata*, the fin spine is longer in comparison to that of other mugilid species, covering approximately one-quarter of the length of the first fin ray. The ventral postcleithrum in mugiliforms connects to the lateral process of the pelvic girdle (Stiassny, 1993; Stiassny & Moore, 1992). This can also be seen in *L. aurata* and *M. cephalus* (Ghasemzadeh, 2015). In both species the pelvic girdle is inclined antero-dorsally, although, according to the drawings of Ghasemzadeh (2015; figure 8.5A), in *M. cephalus* the inclination is more pronounced than in *L. aurata*.

The body scales of *L. aurata* are similar to each other, whereas the head scales show some differences. Bräger and Moritz (2016) already noted for *L. aurata* and *C. labrosus* that a high level of morphological similarity exists between the body scales of the two species. Comparing the body scales of species of the genus *Liza* and *Chelon* to that of the species of the genus *Mugil*, no distinct differences can be seen (Bräger & Moritz, 2016; Jacot, 1920; Pillay, 1951). Greater differences can be observed if these scales are compared to that of *Rhinomugil corsula* (=*Mugil corsula* in Pillay, 1951) which have a greater antero-postero extension. A higher degree of difference in the scales of grey mullets was observed by Mussarat-Ul-Ainb et al. (2015), who showed that between the four species *Chelon melinopterus* (=*Liza melinoptera* in Mussarat-Ul-Ainb et al., 2015), *Moolgarda speigleri* (=*Valamugil speigleri* in Mussarat-Ul-Ainb et al., 2015), *M. cephalus* and *Planiliza macrolepis* (=*Liza macrolepis* in Mussarat-Ul-Ainb et al., 2015), the scales (especially head and lateral line scales) can be used for the identification of the species. Nonetheless, as observed in this study, the high diversity of shapes of the head scales makes it necessary to clearly state which particular scales are compared. If this is studied in greater detail using geometric morphometrics, even the body scales of more closely related mugilid species can help in discriminating species (Ibanez et al., 2007).
5 | CONCLUSION

In summary, the skeleton of L. aurata shows only a few characteristic features if compared in detail to other mugilid species. Most of them can be found in the head region, whereas only a few are found in the postcranial skeleton. For example, differences in the neurocranium occur in the positional relation of the nasal to the preorbital and the type of connection of the basi-phenoid to the prootic and sphenotic. Other differences are related to the shape of jaw bones as well as the shape of the elements of the suspensorium. In the postcranial skeleton, the shape of the supraneurals, the amount of broadened anterior neural arches (neural plates) and the position and inclination of the pelvic girdle can vary between mugilid species. Only if studied in great detail these differences in the osteology of mugilids will come to light. Therefore, more detailed studies of these differences using a higher taxon sampling might allow a better understanding of the mugilid evolution and will provide the needed information for the reconstruction of the mugilid body plan. Nonetheless, not only should differences be of interest as some unique mugilid characters, such as the connection between the interopercle and posterior ceratohyal or the appendage connected to the subopercle, represent high-value information that needs to be included when reconstructing their evolutionary history. All together this might provide helpful characters for studying the present poorly resolved phylogeny of the Ovalentaria.

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AUTHOR CONTRIBUTIONS

P.T. cleared and stained part of the listed specimens, dissected the specimens, photographed pictures, performed the 3D reconstruction of Liza aurata and prepared the manuscript. T.M. cleared and stained part of the listed specimens, photographed pictures and prepared the manuscript.

ORCID

Philipp Thieme © https://orcid.org/0000-0002-3065-1272

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Postcranial skeletal development of *Mugil cephalus* (Teleostei: Mugiliformes): morphological and life-history implications for Mugiliformes

PHILIPP THIEME¹,²,*, DARIO VALLAINC³ and TIMO MORITZ¹,²,*

¹Deutsches Meeresmuseum, Katharinenberg 14–20, 18439 Stralsund, Germany  
²Institut für Zoologie und Evolutionsforschung, Friedrich-Schiller-Universität Jena, Erbertstraße 1, 07743 Jena, Germany  
³International Marine Centre IMC, Loc. Sa Mardini 09170, Torregrande, (OR), Italy

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Within the fish taxon Mugiliformes, the larval development of *Mugil cephalus* has been studied most intensively, because it has the widest range of distribution among all mugilids and is of interest to aquaculture all over the world. Although numerous studies have dealt with larval rearing, growth and development, the osteological development of *M. cephalus* and mugiliforms in general has largely been neglected. Herein, we describe the skeletal development of mullets for the first time. Cleared and double-stained specimens of aquaculture-reared *M. cephalus* and wild-caught mugilid larvae were examined to describe the early development of the pectoral and pelvic girdle, the vertebral column and the caudal and median fins. The description of four embryonic and six larval developmental steps within the embryonic and larval period enables us to compare larval sizes of reared and wild-caught larvae. Ontogenetic fusions of ural centra 1 and 2 into a compound centrum, in addition to the fusion of two pterygiophores in the anal fin, have implications for the perception of the adult morphology. Moreover, comparison of mugilid development with that of other ovalentarian taxa shows that recent phylogenetic hypotheses need further morphological investigation.


INTRODUCTION

Mugilids or grey mullets are a globally distributed taxon that is of major interest to fisheries and aquaculture (Crosetti & Blaber, 2015). In the Mediterranean region, mugilids, and particularly *Mugil cephalus* Linnaeus, 1758, are cultured extensively (Whitfield et al., 2012; Crosetti, 2015). They are cultured to a lesser extent in countries such as Taiwan and Japan.

Mugilid species mostly occur in coastal waters, where they inhabit estuaries and lagoons. For *M. cephalus*, it is known that adults migrate to the nearshore marine zone for spawning (Whitfield et al., 2012). A similar pattern was shown for other mugilid species, such as the South American *Mugil liza* Valenciennes, 1836, for which the beginning of the reproductive migration is correlated with environmental changes, i.e. a sudden drop in temperature (Vieira & Scalabrini, 1991; González-Castro, 2007; González-Castro et al., 2011; González-Castro & Minos, 2015). After spawning, eggs and larvae stay close to the surface and drift in ocean currents. On reaching the juvenile stage, they gradually move downwards in the water column and are transported passively to coastal waters, where they migrate into estuaries, grow and reach maturity (Vieira, 1991; Whitfield et al., 2012; González-Castro & Minos, 2015).

The ontogeny of mugilid larvae has been well studied (e.g. Anderson, 1957, 1958; Kuo et al., 1973; Tung, 1973; Boglione et al., 1992). Descriptions of the morphology of the developmental stages and larval growth are available for different species based on artificial rearing, i.e. *M. cephalus* (Sanzo, 1930; Tang, 1964; Yashouv & Berner-Samsonov, 1969; Liao et al., 1972; Tung, 1973; Nash & Shehadeh, 1980; Loi et al., 2020) and *Chelon labrosus* (Risso,
1827) (Cataudella et al., 1988; Boglione et al., 1992; Khemis et al., 2006, 2013), or from wild-caught fry, i.e. Chelon auratus (Risso, 1827) (Vodyanitskii & Kazanova, 1954; Demir, 1971), C. labrosus (Sanzo, 1936; Cassifour & Chambolle, 1975), Chelon saliens (Risso, 1827) (Yashouv & Berner-Samsonov, 1970), Mugil curema Valenciennes, 1836 (Anderson, 1957), Oedalechilus labeo (Cuvier, 1829) (Sanzo, 1937) and Planiliza macrolepis (Smith, 1846) (Sebastian & Nair, 1975), and these were reviewed by González-Castro & Minos (2015). However, with the exception of Loi et al. (2020), who studied the development of the eye and the digestive system, all papers described the embryonic and larval development based only on external morphology, and the skeletal development was neglected. Only one study provides some insight into skeletal development, focusing on the caudal fin structure (Hollister, 1937).

Mugil cephalus is the most-studied mugilid because it is the species with the largest distribution range (Whitfield et al., 2012; Crosetti & Blaber, 2015). However, Durand & Borsa (2015) showed that there is a high proportion of cryptic species in the species complex that is recognized as M. cephalus, but also within grey mullets in general. Xia et al. (2016) studied the phylogenetic relationships within Mugiliformes and reclassified/recombined four subfamilies: Cheloniinae, Mugilinae, Myxininae and Rhinomugilinae. The osteology of M. cephalus, a representative of Mugilinae, was described by Ghasemzadeh (2015). Recently, Thieme & Moritz (2020) described the osteology of Liza aurata (Risso, 1810) (Cheloninae). Despite their distant relationship, there are only few differences between their anatomies. Nevertheless, there are still skeletal elements of unknown developmental origin, such as the enlarged first pterygiophore of the anal fin or the compound centrum of the caudal fin, which can only be resolved using developmental data. Such developmental data are also necessary to analyse the phylogenetic position of Mugiliformes on the tree of fishes. Betancur-R et al. (2013) first placed the mugiliforms within the newly erected taxon Ovalentaria(e), This was supported by Betancur-R et al. (2017) and Hughes et al. (2018), who placed Embiotocidae or Ambassidae as close relatives to mugilids, respectively. Previously, atherinomorphs were thought to be closely related to mugiliforms based on morphological analyses (e.g. Stiassny, 1990, 1993; Johnson & Patterson, 1993). Although atherinomorphs are also part of the Ovalentaria, molecular data suggest that this taxon is more closely related to polycentrids and cichlids (Betancur-R et al., 2013, 2017; Hughes et al., 2018).

For the first time, we describe the development of the postcranial skeleton of M. cephalus based on artificially reared larvae. This is the first study to provide a detailed insight into a previously neglected topic. The skeletal development of M. cephalus will provide information necessary to understand adult morphological characters and is therefore important for systematic analyses. Additionally, these data will help to improve artificial rearing because they provide a baseline for comparison with reared larvae, to identify malformations properly and to assess the developmental stage of the larvae precisely. Lastly, a comparison with wild-caught fry of different mugilid species provides more insight into the general developmental pattern within the family Mugilidae.

MATERIAL AND METHODS

REARING
Brood stocks were collected during the natural spawning season in the lagoon of Tortoli (central-eastern Sardinia, Italy) and induced to spawn as described by Vallainc (2017). Eggs were transported to the facilities of the International Marine Centre (Oriostano, Sardinia, Italy) where they were incubated as described by Loi et al. (2020). For a short time, fertilized eggs were incubated in flow-through (water flow of 4 L min⁻¹), at 560 eggs L⁻¹. At 2 days post-hatching (dph), larvae were transferred into an indoor recirculating aquaculture system and reared at a density of 40 individuals L⁻¹. Phytoplankton [Isochrysis galbana Parke and Tetraselmis suecica (Kylin) Butcher, 1:1 v/v] was added daily to the tanks to maintain a concentration of 400 000 cells mL⁻¹ for a period of 22 days. First feeding started at 2 dph and consisted of rotifers (Brachionus Pallas, 1766 species) fed on I. galbana. The rotifer concentration was adjusted daily to four individuals per millilitre until 22 dph. The administration of enriched (Easy DHA Selco, INVE Aquaculture) Artemia Leach, 1819 nauplii began at 12 dph and continued until 37 dph at an average concentration of 2.5 nauplii mL⁻¹. Artificial feed (Gemma Wean 0.1, Skretting) was added ad libitum to the facilities of the International Marine Centre (Oristano, Sardinia, Italy) where they were incubated at an average concentration of 2.5 nauplii mL⁻¹. Artificial feed (Gemma Wean 0.1, Skretting) was added progressively to the tanks from 22 dph onwards, and larvae were fed ad libitum. Water temperature was set at 22.1 ± 0.7 °C, salinity 36.8 ± 0.4 ppt and dissolved oxygen 83 ± 14%. The water was renewed every 10 days at a rate of 30%. Ammonia and nitrite were kept < 0.25 mg L⁻¹, and nitrate < 25 mg L⁻¹.

MATERIAL EXAMINED
Aquaculture-reared specimens of M. cephalus were collected daily from 0 to 42 dph (exceptions: 15, 16, 21, 22, 23, 29, 30 and 36 dph). The sample size taken varied as follows: 20 samples taken at 0, 2, 3,
10 and 19 dph, 21 samples at 1 dph, 17 samples at 40 dph, 13 samples at 31 dph and five samples on all other days (for a full list of sampled specimen and respective sizes see Table S1). Before the larvae were fixed in 4% formaldehyde, photographs of each larva were taken, which were then used for measuring larvae with Imaged (v.1.52a; US National Institutes of Health, Bethesda, MD, USA). A subsample of the aquaculture-reared specimens was used for clearing and staining to examine the skeletal development of M. cephalus larvae. Clearing and staining principally followed the protocols of Dingerkus & Uhler (1977), Taylor & Van Dyke (1985) and Schnell et al. (2016), with minor adjustments. For staining, the larvae were placed in 100% ethanol for complete dehydration and afterwards stained in an Alcian Blue solution (cartilage staining) for 2 h depending on size. Larger larvae were then put directly into 0.5% KOH, whereas smaller larvae were first transferred into 30% ethanol using a descending alcohol series. Clearing had already happened in the KOH solution. Larger larvae were transferred into a 70% sodium borate solution with trypsin. After clearing, larvae were stained in a 0.25% KOH solution with Alizarin Red (bone staining) for several hours. For examination and storage, the larvae were transferred into 86% glycerin.

The specimens were photographed using a Leica M165C microscope with the Leica application software (LAS v.4.9.0) and with a Canon EOS 80D with a Canon MP-E 65 mm objective. Drawings were carried out with Adobe Photoshop CC (v.21.1.3) and Adobe Illustrator CC (v.24.1.3).

RESULTS

Growth

The development of M. cephalus larvae is categorized principally following the developmental steps previously defined by Peñáz (2001). Main characters for each category are adopted from cyprinid and percid development (Peñáz, 2001) and adjusted to mugilid development. Classification herein includes only larval stages after hatching. Therefore, 12 of the previously recognized 17 developmental steps could be adopted for M. cephalus larvae. Starting in the embryonic period, steps E6–E9 were observed, in addition to steps L1–L6 in the larval period and J1 and J2 in the juvenile period. Main characters for each step can be found in Table 1.

Hatching of the first larvae occurred at 2.646 ± 0.08 mm NL (N = 20). Growth during the embryonic period (E6–E9) was slow, and notochord length between 0 and 5 dph was not significantly different (Fig. 1). The transition from E6 to E9 was fast (~1 day per period), whereas the last embryonic period (~4 days) and the first larval period (~3.4 days) were longer. During L1–L3 the larvae grew much more than in the embryonic period (Fig. 1). The preflexion phase lasted until the end of L2. Flexion of the notochord then started at the beginning of L3 and was finished by the beginning of L4, starting the postflexion phase. There was a large overlap between L2 and L3 larvae in terms of days post-hatching, which was associated with differences in size; L2 larvae were smaller than L3 larvae but could be of the same age. In the remaining larval steps and in J1 and J2, the overlap of dph and the developmental stage of the larvae increased even more. Differences in the size of larvae within each developmental step were small, but size was significantly different between each step (only when comparing L1, L2 and L3 and L4, L5 and L6). After entering the juvenile period, growth increased until the end of the study period.

In L3 larvae, the first cartilaginous structures are visible in the unpigmented caudal region. The first mesenchymal condensations indicate the development of the second dorsal fin and the anal fin. In L4 larvae, pigments start to emerge in the caudal region (for development of pigmentation, see Boglione et al., 1992; Gonzalez-Castro & Minos, 2015), and in some specimens the hypural plates are visible. Owing to the flexion of the notochord, the caudal fin rays point posteriorly and no longer posteroventrally.
Furthermore, the first actinotrichia condensations indicate the development of the first fin rays in the second dorsal fin and the anal fin. Mesenchyme condensations reveal the formation of the first dorsal fin. In L5, lepidotrichia in the median fins develop and, for the first time, the pelvic fin fold appears, which signals the beginning of the development of the pelvic girdle and the pelvic fin. Entering L6, the larvae have fin rays or fin spines developed in all fins, which have yet to reach their final shape. The transition to the juvenile period (J1) is marked by the prolonging of the upper and lower caudal fin rays, changing the shape of the caudal fin. Also, the fin rays of the pectoral and pelvic fins are prolonged. The last and the longest step before reaching maturity is J2, during which the juveniles attain the final fin shape, including the forked caudal fin, and similar body proportions to adult specimens.

### Table 1. Main characters usually appearing during consecutive developmental steps (following Peňáz, 2001) in *Mugil cephalus*, including the mean notochord length (NL*) or standard length (SL) of the aquaculture-reared specimens investigated herein

<table>
<thead>
<tr>
<th>Step</th>
<th>Characters (after Peňáz, 2001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E6</td>
<td>Hatching of larvae begins; unpaired fin fold reaches around trunk from dorsal to ventral to the head, caudal lobe differentiated; eye lenses present, without pigmentation; melanophores on trunk except caudal peduncle</td>
</tr>
<tr>
<td>E7</td>
<td>Eyes pigmented; transition from trunk to head plain; fin fold beneath yolk sac reduced</td>
</tr>
<tr>
<td>E8</td>
<td>Transition from dorsal and ventral fin fold to caudal fin lobe constricted; fin fold anterior to yolk sac reduced; dent behind head; notochord distinctly bent above yolk sac; small mouth pit visible in a few specimens</td>
</tr>
<tr>
<td>E9</td>
<td>Mouth opened, lower jaw moveable; pre-anal and anal fin fold still connected in most specimens</td>
</tr>
<tr>
<td>L1</td>
<td>Mouth fully opened and jaws moveable; start of exogenous feeding; remnants of yolk sac present, with oil globule; fin fold above head reduced</td>
</tr>
<tr>
<td>L2</td>
<td>Yolk sac fully depleted; transition to exogenous feeding; first condensations of mesenchyme in the caudal lobe of fin fold; notochord still straight</td>
</tr>
<tr>
<td>L3</td>
<td>Notochord flexion starts; first caudal fin rays develop (pointing posteroventrally); cartilaginous precursors of hypurals 1–4 emerge; mesenchyme condensations in anal fin and second dorsal fin</td>
</tr>
<tr>
<td>L4</td>
<td>Notochord flexion is completed; more caudal fin rays present and arranged fan-wise pointing posteriorly, caudal fin lobe elongated; caudal peduncle pigmented; mesenchyme condensations in first dorsal fin; first lepidotrichia of anal fin and second dorsal fin</td>
</tr>
<tr>
<td>L5</td>
<td>Lepidotrichia in first dorsal fin start to ossify; pelvic fin bud appears</td>
</tr>
<tr>
<td>L6</td>
<td>All fin rays in anal and dorsal fins developed; median fin folds have disappeared; pectoral and pelvic fin still round, not completely developed; start of squamation</td>
</tr>
<tr>
<td>J1</td>
<td>Transitional step: pectoral and pelvic fins prolonged, with fin shape characterized by length of fin rays; posterior margin of caudal fin no longer straight</td>
</tr>
<tr>
<td>J2</td>
<td>Final fin shape and body proportions are attained (often also body colour); squamation completed; caudal fin forked</td>
</tr>
</tbody>
</table>

### Osteological Development

#### Vertebral Column

The vertebral column of *M. cephalus* comprises 24 vertebrae, including 11 abdominal and 13 caudal vertebrae. The compound centrum of the caudal fin is counted as a single vertebra. The first seven neural spines are expanded anteriorly. In total, nine pairs of ribs and three supraneurals are present. Additionally, *M. cephalus* has five pairs of epicentrals (Ghasemzadeh, 2015; Thieme & Moritz, 2020).

In the smallest larvae examined, no skeletal structures of the vertebral column are present. The first elements to develop are the paired neural arches of vertebrae 1 and 2, which are already ossified above the notochord at ~2.6–2.7 mm NL (Figs 2, 3A). Additional paired neural arches, first of abdominal and then of caudal vertebrae, are continuously developing in the posterior direction (2.9–3.3 mm NL). Segmentation...
of chordablasts within the notochordal sheath starts the formation of the chordacentra (Hall, 2015). In the posterior direction, dorsal and ventral segmentation centres emerge for each centrum (Fig. 3B). They grow towards each other and fuse. The ventral centres develop slightly later than the dorsal ones, with one exception: the ventral segmentation centre of the first vertebral centrum develops only after all abdominal vertebrae are pre-formed (~3.7 mm NL; Fig. 3C). The arcocentral ossification of the vertebral centra starts after the complete segmentation of the chordacentra, which predefine the boundaries of the vertebral centra (Hall, 2015).

Soon after the first caudal neural arches, the first haemal arches begin to form (~3.0 mm NL). Only the nine posteriormost haemal and neural arches have visible cartilaginous precursors, whereas all others are ossified almost immediately (Fig. 3B, C). Together with the first haemal arches, the paraphyses of the tenth and the ninth vertebrae develop ventral to the notochord. In the anterior direction, more paraphyses emerge, but their origin is shifted dorsally from ~3.7 mm NL to 4.6 mm SL (Fig. 3C, D). The haemal and neural arches fuse to their associated vertebral centrum and grow dorsally and ventrally, respectively. Once they have encompassed the neural or the haemal canal, their halves fuse and extend as a single spine. Between the halves of the penultimate haemal and neural arch, an autogenous and cartilaginous spine appears, respectively, that grows out distally (~3.6 mm NL; Figs 3E, 5B). These elements initially ossify medially and afterwards in the proximal and distal directions (Fig. 5C, D). Additionally, paired spines from the haemal and neural arch develop lateral

Figure 1. Age–length relationship during the observed developmental steps of Mugil cephalus larvae. Steps E6–L3 (filled circles) were measured as notochord length; steps L4–J2 (open circles) were measured as standard length. Error bars show the SD from the mean age (horizontal) and the mean length (vertical) for each developmental step. Growth up to L3 is best described by a quadratic function (continuous line), whereas growth afterwards is described by a linear function (dotted line).
to the autogenous spines and later fuse with them. Between the halves of the last haemal and neural arch, cartilaginous autogenous spines also emerge. The ventral one is elongated, whereas the dorsal one remains short (Fig. 5E). The arches fuse with these spines soon after the spines start to ossify. The last haemal arch maintains a cartilaginous proximal base and remains autogenous throughout development.

The paired ribs start to develop at ~3.6 mm NL (Fig. 3D). The first ribs that start to form are associated with the third vertebral centrum and appear ventrolateral to the vertebral column. Seven more pairs of ribs emerge in the posterior direction, and their developmental origin is shifted ventrally (Fig. 3E). All ribs grow bidirectionally: medially towards the respective parapophysis and in the ventrolateral direction.

At ~5.3 mm SL, the anterior epicentals develop within the horizontal septum (Fig. 4A). The epicentral of the second vertebral centrum forms an entity with the only epipleural (Fig. 4). Between 4.8 and 5.1 mm SL, the vertebral centra have their hour-glass shape, and the first prezyg-, postzyg- and postexapophyses develop (Fig. 3F). The development of the anterior
Figure 3. Schematic illustration of the development of the axial skeleton (lateral view) of *Mugil cephalus*. A, L1: 3.1 mm notochord length (NL), 10 days post-hatching (dph). B, L2: 3.2 mm NL, 14 dph. C, L3: 3.7 mm NL, 14 dph. D, L3: 3.6 mm NL, 18 dph. E, L4: 4.4 mm standard length (SL), 19 dph. F, L6: 7.2 mm SL, 25 dph. Blue, cartilage; pink, bone; light grey, notochord. Abbreviations: af, anal fin; ah, autogenous haemal spine; ans, autogenous neural spine; ap, anal fin pterygiophore; cc, compound centrum; d1p, first dorsal pterygiophore; d2p, second dorsal pterygiophore; df2, second dorsal fin; ep, epipleural; eu, epural; ha, haemal arch; hyp, hypural; ip, interdorsal pterygiophore; na, neural arch; pa, parapophysis; ph, parhypural; ri, rib; sn, supraneural; uc, ural centrum; ur, uroneural; vc, vertebral column.
extension of the first seven neural spines begins at ~8.6 mm SL and is not completed in the largest larvae examined. The three supraneurals start to develop at ~3.4 mm SL from anterior to posterior. Cartilaginous precursors develop between the second and third, fourth and fifth, and sixth and seventh neural spine, already representing their final shape (Fig. 3E). Ossification proceeds from the anterior to the posterior supraneurals and starts at ~4.5 mm SL (Fig. 3F).

Caudal fin

In adult specimens, the caudal fin comprises a parhypural, a lower hypural plate (hyp1 + hyp2) and an upper hypural plate (hyp3 + hyp4), in addition to a single hypural (hyp5), a compound centrum (cc), a single pair of uroneurals, two epurals, an interneural spine cartilage and two interhaemal spine cartilages. The haemal spine and neural spines of pre-ural centrum 2 (pu2) and pre-ural centrum 3 (pu3) are also part of the caudal fin structure. The neural spine of pu2 is reduced in size compared to the neural spine of pu3. There are eight dorsal and seven ventral principal caudal fin rays, with up to nine dorsal and up to ten ventral procurent rays (Ghasemzadeh, 2015; Thieme & Moritz, 2020).

Before the flexion of the notochord begins (~2.7 mm NL) the cartilaginous precursors of the first and second hypural emerge (Fig. 3B). The cartilaginous precursor of the parhypural and the third and fourth hypural develop while the first two hypurals fuse distally (~3.2 mm; Fig. 5A). The first fin rays of both the dorsal and the ventral principal rays are already ossifying. At the beginning of notochord flexion (~3.7 mm NL), all previously mentioned elements have grown and, in addition, two cartilaginous epurals have emerged (Fig. 5B). From the proximal part of the first hypural, a cartilaginous bridge towards the parhypural is forming. More fin rays are emerging dorsally and ventrally. Afterwards, the first ural centrum (u1) is marked by the mineralization of the notochordal sheath anterodorsally to the first and second hypural (~4.4 mm SL; Fig. 5C). The cartilaginous bridge from the first hypural now connects to the parhypural and extends posteriorly, leading to the proximal fusion of hyp1 and hyp2 (Fig. 5C). The ossification of hyp1 to hyp4 and the parhypural begins in the middle of each structure. Shortly afterwards, the ossifications of hyp1 and hyp2 merge distally. Next, a small ventral mineralization centre appears anterior to hyp3 and hyp4, representing ural centrum two (u2; Fig. 5D). Dorsal to hyp4, the cartilaginous hyp5 develops. In the following phases, u2 ossifies in the dorsal direction (~4.6 mm SL, Fig. 5E) and then merges with u1 to form the compound centrum (u1 + u2; Fig. 5F). Owing to the continuing ossification of all structures, hyp1 and hyp2 merge in their proximal parts, leading to a large ossified lower hypural plate (Fig. 5G). Likewise, hyp3 and hyp4 fuse distally and proximally and form the upper hypural plate. At ~5.2 mm SL, the uroan develops as a paired bone dorsal to the cc. The parhypural develops its characteristic hypurapophysis. Between the distal tips of the last two haemal spines, an interhaemal spine cartilage develops, and an additional cartilage appears posterior to the distal tip of the last haemal spine (~5.8 mm SL). The fifth hypural ossifies, and the upper hypural plate starts to fuse to the cc (Fig. 5H). A second interhaemal spine cartilage develops anterior to the distal tip of the penultimate haemal spine, while an interneural spine cartilage appears anterior to the distal tip of the autogenous neural spine. An additional cartilage develops posteroventral to the distal tip of the autogenous haemal spine (Fig. 5G, H).

Pectoral girdle and fin

Adult specimens of M. cephalus have a posttemporal, a supracleithrum, a dorsal and a ventral postcleithrum, a scapula, a coracoid, four proximal radials and 16 or 17 distal radials, in addition to one fin splint and 16 or 17 fin rays (Thomson, 1997; Ghasemzadeh, 2015).
The smallest larvae examined have a pectoral fin bud surrounded by a fin fold that is bare of any supporting structures. The first bone of the pectoral girdle to emerge is the cleithrum (~2.7 mm NL; Figs 2A, 6A). It appears as an elongated and thin bone. The coracoscapular cartilage, which is the precursor to the coracoid and scapula, appears shortly after (~2.6 mm NL; Fig. 2). It is positioned posterior to the cleithrum.
and has an anteroventral process and an elongated posteroventral process (Fig. 6B). The posttemporal develops slightly before the supracleithrum appears. The posttemporal is positioned in front of the dorsal tip of the cleithrum, whereas the supracleithrum is positioned lateral to the dorsal tip of the cleithrum (Fig. 6B). The ventral postcleithrum emerges posterior to the coracoscapular cartilage as a thin bone (3.0–3.2 mm NL; Figs 2, 6B). The cleithrum broadens dorsally and ventrally. Posterior to the coracoscapular cartilage, the cartilaginous precursor of the proximal radials is visible as a single entity within the fin bud (~3.7 mm SL; Fig. 2). Meanwhile, the cleithrum grows and develops its three-dimensional shape, and the ventral postcleithrum extends ventrally and dorsally. The dorsal arm of the posttemporal elongates towards the epioccipital (Fig. 6C). A cleavage starts to divide the cartilaginous precursor of the proximal radials (~4.1 mm SL; Figs 2, 6C). Dorsal to the ventral postcleithrum and medial to the proximal radials, the dorsal postcleithrum develops (~4.2 mm SL). The coracoid is the first structure to ossify within the coracoscapular cartilage right at the origin of the anteroventral process. The first fin rays appear dorsally and posteriorly to the dorsalmost proximal radial. At the same time, the first distal radials develop from dorsal to ventral (~5.9 mm SL). The ossification of the coracoid and the scapula proceeds, while the posterior process of the coracoscapular cartilage shortens and will later be reabsorbed almost completely (Fig. 6D). More fin rays develop, and all of them become larger except for the most dorsal one, which develops into the fin splint. The first distal radial is positioned above the posterodorsal corner of the scapula and is distinct from all other distal radials because it is much larger (~4.6 mm SL). In some studies (i.e. Woltering et al., 2018), this first distal radial is called the propterygium. The remaining distal radials develop from dorsal to ventral. The anterior and posterior margins of the proximal radials and the distal radials are still cartilaginous in the largest larvae examined (16.3 mm SL). The first distal radial is fused with the scapula in adult specimens (Ghasemzadeh, 2015; Thieme & Moritz, 2020). After the ossification of the coracoid and the scapula is completed, a cartilaginous band remains between the two bones. The ventral postcleithrum extends towards the pelvic girdle, and a connection to the external wing of the basipterygium is established via connective tissue.

Pelvic girdle and fin

The pelvic girdle of adult M. cephalus is composed of two halves (basipterygia) that approach each other posteriorly. Associated with each basipterygium is a single fin spine and five fin rays. Following the nomenclature used by Thieme & Moritz (2020), each basipterygium can be sectioned into an anterior basipterygial arm, which has an internal and an external wing, in addition to a posterior basipterygial plate, which medi ally has an anterior and a posterior process. The two halves emerge at some distance as cartilaginous precursors (basipterygial cartilages) roughly resembling the shape of the future pelvic girdle (4.1–4.3 mm SL; Fig. 2). The anterior basipterygial arm and the posterior basipterygial plate can already be distinguished. The first ossifications appear lateral and

Figure 7. Drawings of development stages of the pelvic girdle in Mugil cephalus larvae. A, L5: 5.2 mm standard length (SL; DMM IE/16314). B, L6: 7.2 mm SL, 25 dph, C, L6: 7.4 mm SL, 25 dph. D, L6: 8.6 mm SL (DMM IE/16314). Blue, cartilage; pink, bone; light pink, membranous bone. Abbreviations: abp, anterior basipterygial process; ba, basipterygial arm; bp, basipterygial plate; ew, external wing; iw, internal wing; pbp, posterior basipterygial plate; pcv, ventral postcleithrum. Scale bars: 200 μm.
medial at the basipterygial arm, later merging in the middle and spreading anterior and posterior (~5.2 mm SL; Fig. 7A, B). The first fin rays develop posterior to the basipterygial plate from lateral to medial within the fin bud. The anterior and posterior basipterygial processes emerge medial at the cartilaginous basipterygial plate (Fig. 7B, C). Proceeding from the basipterygial arm, the basipterygial plate starts to ossify from anterior to posterior and from lateral to medial. The halves of the most lateral fin ray fuse to form a fin spine. The internal and external wings of the basipterygial arm develop as membranous bone in anteromedial and anterolateral directions, respectively (5.8–6.0 mm SL; Figs 2, 7C, D). The last portions of the pelvic girdle to ossify are the posterior basipterygial processes.

**Median fins**

Two dorsal fins are present in adult *M. cephalus*. The first dorsal fin consists of four unsegmented pterygiophores, of which the first is enlarged, in the anterior to posterior direction, in addition to four fin spines. The second dorsal fin has nine pterygiophores that are segmented into proximal and distal radials and a fin stay that is connected to the last pterygiophore via a cartilaginous bridge. Ten fin rays articulate with the nine pterygiophores. The anal fin comprises nine pterygiophores, which are segmented into proximal and distal radials, a fin stay, three fin spines anteriorly followed by eight fin rays posteriorly. The first pterygiophore is enlarged and has an anterodistal foramen for the base of the first spine and a posterodistal hook, with which the second spine articulates.

A median fin fold is present in the smallest larvae examined. The first structures indicating the development of the posterior dorsal fin and the anal fin are aggregations of actinotrichia in the dorsal and ventral fin folds (~2.8 mm NL). The first cartilaginous, undifferentiated pterygiophores develop above the ventral fin fold. Shortly afterwards, the first cartilaginous pterygiophores develop opposite the ventral ones, representing the second dorsal fin (~3.6 mm NL; Fig. 3D). More pterygiophores are added anteriorly and posteriorly in both the anal fin and the second dorsal fin. The most anterior pterygiophore of the anal fin is smaller than all other pterygiophores and is dorsally in close contact with the second pterygiophore (Fig. 8A). The pterygiophores of the first dorsal fin and the interdorsal pterygiophores appear at around the same time (~4.0 mm NL). Only one of the specimens examined shows three interdorsal pterygiophores, while only the two posterior pterygiophores of the first dorsal fin are present, indicating that they develop as one entity from posterior to anterior. In the second dorsal fin and in the anal fin, the pterygiophores then divide into proximal and distal radials. This proceeds bidirectionally, starting with the pterygiophores in the middle of each fin. As soon as the first distal radials are present, the actinotrichia condense tightly, already resembling the developing fin rays. The lepidotrichia ossify around the condensed actinotrichia (~3.9 mm NL). Shortly after the second anal fin pterygiophore has divided into proximal and distal radials, the proximal radial fuses with the first pterygiophore (Fig. 8B). The distal tip of the first pterygiophore is similar to a distal radial, but there is no separation of this tip and the rest of the pterygiophore. The interdorsal and first dorsal fin pterygiophores do not divide into different radials. The development of fin rays in the second dorsal and the anal fin proceeds bidirectionally, with one fin ray per distal radial. In both fins there is one additional fin ray at the posterior end that cannot be assigned to any pterygiophore (Fig. 3F). Additionally, there is one supernumerary fin ray at the origin of the anal fin (Fig. 8C). The right and left halves of the first two anal fin rays will later fuse and form fin spines (~10.5 mm SL). The fin spines of the first dorsal fin develop simultaneously (~4.7 mm SL). The ossification of the proximal radials of the anal fin and the second dorsal fin...
fin starts in the middle of each radial and proceeds proximally and distally (~4.8 mm SL). The first dorsal fin pterygiophores and the interdorsal pterygiophores begin to ossify afterwards. An extension of membranous bone develops anterior to the first pterygiophore of the first dorsal fin (~5.9 mm SL; Fig. 3F). The ossification of the pterygiophores in all fins proceeds from anterior to posterior. The fin stays that are present posterior to the last pterygiophores of the second dorsal fin and the anal fin are starting to emerge in the largest examined larvae (16.3 mm SL). They develop as cartilaginous outgrowths from the remaining cartilaginous distal tip of the last proximal radial in each fin.

Squamation

First scales appear in larvae of ~6.4 mm SL. The development of squamation begins between the second dorsal fin and the anal fin at the height of the horizontal septum (Fig. 9A). The development of horizontal scale rows proceeds in anterior and posterior directions, and shortly afterwards more scales are added dorsally and ventrally (Fig. 9B). At ~7.6 mm SL, scales start to develop independently from the horizontal rows of the body in front of the pelvic girdle and on top of the head (Fig. 9C, D). By then, the horizontal rows cover the trunk in its full height from the first dorsal fin to the caudal peduncle. Scales in front of the pelvic girdle develop in a few rows towards the pelvic fin and the branchial arches. Scales on the head develop anteriorly covering the frontals, and laterally over the parietal. Also, scales develop posterolaterally, covering the region behind the head and above the pectoral girdle (~8.5 mm). The horizontal rows on the body develop further anteriorly. At ~9.7 mm SL, the body is fully covered in scales (Fig. 9E).

DISCUSSION

SKELETAL DEVELOPMENT OF OTHER MUGILIDS

Herein, the development of aquaculture-reared M. cephalus is described in detail and compared with wild-caught G. argenteus (Rhinomugilinae) and P. macrolepis (Cheloninae), in addition to wild-caught M. cephalus. G. argenteus larvae were available for larval period steps L1–L6, whereas P. macrolepis larvae were available for larval period steps L1–J1. The wild-caught larvae of M. cephalus represent L4–J2.

In general, the development of the postcranial skeleton of all examined species is very similar. However, a significant difference occurs in the development of the vertebral column, because in G. argenteus and P. macrolepis each haemal and neural arch is visibly pre-formed in cartilage. For M. cephalus, we witnessed that only the posterior nine haemal and neural arches are pre-formed in cartilage. We are uncertain whether this observation displays the real developmental mode (i.e. anterior haemal and neural arches are not pre-formed in cartilage) or whether this observation is an artefact attributable to poor cartilage staining in the aquaculture-reared M. cephalus specimens. Nevertheless, the haemal and neural spines develop without separate cartilaginous precursors, by fusion and elongation of the haemal and neural arches, respectively, in both G. argenteus and P. macrolepis. Only the two most posterior haemal and neural spines develop in an autogenous manner. The development of the ribs, the epicentrals and the epipleural resemble the situation in M. cephalus. We were unable to compare the vertebral column and the haemal and neural arch development of wild-caught M. cephalus because the vertebral column was already fully ossified in all specimens. The development of the caudal fin is identical in all three species. In some of the wild-caught M. cephalus larvae we found additional small cartilages in different areas of the caudal skeleton, e.g. between the posterior, autogenous neural spine and the anterior epural or ventral to the distal tip of the posterior, autogenous haemal spine. The paired fins of all three species develop identically. The fusion of two pterygiophores to form the enlarged

anterior pterygiophore in the anal fin has been observed in *G. argenteus* and *P. macrolepis*. The development of the second dorsal fin and the anal fin in *P. macrolepis* resembles the development of *M. cephalus*. The development of the interdorsal pterygiophores and the first dorsal fin could not be witnessed in *G. argenteus* and *P. macrolepis*.

Comparing the wild-caught *M. cephalus* with the aquaculture-reared specimens, there is a difference in size in the larval developmental steps. The wild-caught specimens are ~1 mm smaller at an equal developmental step (i.e. L4, 3.6 vs. 4.6 mm; L5, 4.9 vs. 5.6 mm; L6, 6.8 vs. 7.8 mm). The developmental sequence is principally the same, but the pelvic fin bud appears sooner (in some specimens already in L4) than in the aquaculture-reared larvae.

In sum, the development of the postcranial skeleton of Mugilinae, Rhinomugilinae and Cheloninae is similar, and we hypothesize that at the base of these three subfamilies the skeletal development is pretty much the same as described for *M. cephalus*.

**Implications for adult morphology**

The adult morphology of *M. cephalus* was described in detail by Ghasemzadeh (2015). A detailed discussion on differences from other mugilids and terminology problems is provided by Thieme & Moritz (2020). Most structures of the postcranial skeleton develop as expected, but a few elements are worthy of more detailed discussion. Initially, the autogenous spines of the two posteriormost haemal and neural arches need some attention. In adults, there is almost no visible difference between them and the more anteriorly positioned haemal and neural spines; however, their development differs significantly. Although all other spines ossify directly as a median extension of the fused haemal and neural arches, there are separate cartilaginous precursors to the two posteriormost spines of the haemal and neural arches present in ontogeny. These precursors ossify independently from the haemal and neural arches. In other ovalentarian taxa, such as adrianichthyids, blenniids, cichlids and pomacentrids, but also in a variety of perciform taxa, such as centropomids, percids, scombroids and sparids, neural and/or haemal spines (mostly respective to pu2 and pu3) that support the caudal fin develop autogenously (Peters, 1981; Potthoff et al., 1986, 1987; Fujita, 1992; Potthoff & Tellock, 1993; Roumoundouros et al., 1997, 2001; Ott et al., 2012; Woltering et al., 2018). This connection to the caudal fin might explain the different formation compared with the more anterior haemal and neural spines. This coincides with the autogenous development of the parhypural, the hypurals and the epurals, which are homologized to haemal and neural spines of ural centra, but are modified owing to their specific function (Woltering et al., 2018).

Another crucial structure that needs to be discussed is the compound centrum (cc) of the caudal fin. Ghasemzadeh (2015) named this structure the ‘urostyle’ in *M. cephalus* and described it as a terminal half-centrum that is fused with hyp3 and hyp4. Thieme & Moritz (2020) argued that ‘compound centrum’ is a more adequate term, because ‘urostyle’ is most often used to describe the fusion of pu1 and u1 or of pu1, u1 and u2 (Schultze & Arratia, 2013). Schultze & Arratia (2013) described this problem in detail and pointed out that there is no consistent use of the term ‘urostyle’. As shown above, the cc of *M. cephalus* comprises u1 and u2, whereas pu1 is absent throughout its ontogeny. The upper hypural plate (hyp3 + hyp4) is then fused to the cc (u1 + u2). The term ‘urostyle’ therefore gives a misleading impression of comparability, because one might assume that pu1 is part of the caudal fin in mugilids, although it never develops. A fusion of the cc (u1 + u2) and the parhypural and the lower hypural plate, as stated by Thieme & Moritz (2020) does not occur. Hollister (1937) described a similar caudal fin development for *M. curema*, *Mugil trichodon* Poey, 1875 and *C. saliens* (mentioned as *Mugil brasiliensis*), but was unable to witness a separate u2 in the samples examined. This can be attributed to the small sample size and the short size frame in which the ural centra are still separated.

The intermuscular bones present in *M. cephalus* are described as epineurals in the osteology by Ghasemzadeh (2015). This is in accordance with the proposition made by Patterson & Johnson (1995) and Johnson & Patterson (2001), who state that intermuscular bones lying in the horizontal septum in percomorphs (sensu Johnson & Patterson, 1993) are homologues to epineurals in non-acanthomorphs (sensu Johnson & Patterson, 1993). Based on the development of the intermuscular bones in the horizontal septum in *M. cephalus*, and therefore following the proposition made by Gemballa & Britz (1998), and owing to significant changes in the phylogenetic hypotheses concerning acanthomorphs and percomorphs (Betancur-R et al., 2013, 2017; Hughes et al., 2018), the term ‘epicenters’ seems more appropriate and corresponds to the position of the structures (Fig. 4). Furthermore, the presence of an epipleural in Mugilidae was not stated before. During observation of the development of the ribs in *M. cephalus*, it became obvious that the most anterior rib-like element develops independently from all other ribs and simultaneously as a single unit with the second epicentral. As a result, we regard this structure as an epipleural according to its position within the hypaxial musculature.
The anterior pterygiophore of the anal fin in adult *M. cephalus* is enlarged, and Ghasemzadeh (2015) hypothesized that it represents a fusion of a small anterior pterygiophore and a larger posterior pterygiophore. During development, two distinct cartilages become visible that fuse in later stages (Fig. 8), confirming his hypothesis. Attached to this enlarged pterygiophore are three fin spines in adult *M. cephalus*. The most posterior of the three fin spines is the one serially associated with the posterior pterygiophore that is part of the fusion, attaching to its distal radial. In contrast to the other two fin spines, this one is not a true fin spine, but a spine-like fin ray, because it has segmented hemitrichs that fuse during development. This is similar to the situation in *L. aurata*, in which a spine-like fin ray is present at the same position (Thieme & Moritz, 2020). The second fin spine belongs to the small anterior pterygiophore that is part of the fusion. Only the small anterior fin spine is bare of any supporting structure and should therefore be called a supernumerary spine. Patterson (1992) summarized the presence of supernumerary spines in the anal fin in actinopterygians, stating that there are two supernumerary spines in mugilids and one in atherinomorphs. Based on the work of Richter & Moritz (2017), the two most anterior pterygiophores in atheriniforms fuse during development, and the seemingly supernumerary spine is associated with the most anterior pterygiophore.

Parenti (1981) summarized the anal fin morphology of cyprinodontiforms, stating that fusion of pterygiophores occurs in some species (e.g. *Austrofundulus* Myers, 1932). However, more developmental data are necessary to document the extent of ontogenetic fusion in this taxon. Johnson (1984) inferred two supernumerary spines as the primitive condition in perciforms, which included Ambassidae, Grammatidae, Opistognathidae, Plesiopidae and Pseudochromidae, taxa that are currently part of Ovalentaria (*Plesiopidae* and *Pseudochromidae*). They are also present in Actinopterygian taxa such as *Ambassidae*, *Grammatidae*, *Opistognathidae*, *Plesiopidae* and *Pseudochromidae*. Based on the work of Johnson (1984) and Parenti (1981), preural centrum 1 does not develop, and ural centrum 1 and ural centrum 2 fuse early in ontogeny. Parenti (1993) reported that in the atherinomorphs *Dentatherina* Patten & Ivantsoff, 1983, *Horaichthys* Kulkarni, 1940 and *Phenacostethus* Myers, 1928, pu1 and u1 fuse into a single centrum, whereas u2 remains free, which contrasts with the post-flexion configuration in mugilid larvae. However, Stiassny (1990) showed that pu1 and u1 fuse into a single centrum, whereas u2 remains free, which contrasts with the post-flexion configuration in mugilid larvae. However, Fujita (1992) studied the development of the caudal skeleton of *Oryzias latipes* (Temminck & Schlegel, 1846) and showed that pu1 does not develop as a separate entity from u1. It seems likely that pu1 is not present at any time during development in atherinomorph species, which resembles the ontogeny of mugilids. For the benniid Chasmodes *saburrae* Jordan & Gilbert, 1882, it is stated that only one ural centrum (probably u1) develops during ontogeny (Peters, 1981). Woltering et al. (2018) described the development of Astatotilapia *burtoni* (Günther, 1894) (Cichlidae) and stated that three centra (probably pu1, u1 and u2) are part of the ‘urostyle’. However, they only referred to Sebilia & Andreata (1991), who studied the caudal fin of cichlids based on adult specimens, and did not mention the formation of the compound centrum.

**PHYLOGENETIC COMPARISON**

We compare the development of the postcranial skeleton of *M. cephalus* with available data of atherinomorphs, ovalentarian taxa and, if meaningful, a selection of percomorph taxa (sensu Betancur-R et al., 2017).

In general, the development of the vertebral column in mugilids resembles that in pomacentrids, cichlids and blenniids (Peters, 1981; Potthoff et al., 1987; Woltering et al., 2018). The neural arches develop from anterior to posterior and are pre-formed in cartilage. As discussed above, in *G. argenteus* and *P. macrolepis* the haemal and neural arches are also pre-formed in cartilage. Furthermore, the haemal arches also develop from anterior to posterior. The haemal and neural spines have no separate cartilaginous precursors (with the exception of some posterior ones; see discussion above) and are formed by fusion and elongation of the haemal and neural arches, respectively (Peters, 1981; Potthoff et al., 1987; Woltering et al., 2018). The ossification of the vertebral centra proceeds in an anterior to posterior direction in pomacentrids, cichlids and blenniids. In the pomacentrid *Mi. chrysurus*, there are dorsal and ventral centres of ossification for each vertebral centrum (Potthoff et al., 1987). As in *M. cephalus*, the parapophyses develop in a posterior to anterior direction in *Mi. chrysurus*. A similar developmental pattern of the vertebral column is also found in the centropomid *Centropomus undecimalis* (Bloch, 1798) (Potthoff & Tellock, 1993) and in gempylids, istiophorids and scombrids (Potthoff et al., 1986).

In *M. cephalus*, preural centrum 1 does not develop, and ural centrum 1 and ural centrum 2 fuse early in ontogeny. Parenti (1993) reported that in the atherinomorphs *Dentatherina* Patten & Ivantsoff, 1983, *Horaichthys* Kulkarni, 1940 and *Phenacostethus* Myers, 1928, pu1 and u1 fuse into a single centrum, whereas u2 remains free, which contrasts with the post-flexion configuration in mugilid larvae. However, Stiassny (1990) showed that pu1 and u1 fuse into a single centrum, whereas u2 remains free, which contrasts with the post-flexion configuration in mugilid larvae. However, Fujita (1992) studied the development of the caudal skeleton of *Oryzias latipes* (Temminck & Schlegel, 1846) and showed that pu1 does not develop as a separate entity from u1. It seems likely that pu1 is not present at any time during development in atherinomorph species, which resembles the ontogeny of mugilids. For the benniid *Chasmodes saburrae* Jordan & Gilbert, 1882, it is stated that only one ural centrum (probably u1) develops during ontogeny (Peters, 1981). Woltering et al. (2018) described the development of *Astatotilapia burtoni* (Günther, 1894) (Cichlidae) and stated that three centra (probably pu1, u1 and u2) are part of the ‘urostyle’. However, they only referred to Sebilia & Andreata (1991), who studied the caudal fin of cichlids based on adult specimens, and did not mention the formation of the compound centrum.

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Hence, the composition of the compound centrum of cichlids is still questionable, and the assumption made by Woltering et al. (2018) might be based on the original definition of the term ‘urostyle’. It is possible that in ovalentarian taxa, pu1 is not developed during ontogeny and the compound centrum is composed of two fused ural centra or only a single ural centrum. However, more developmental data are necessary to make a definite statement.

Hyp1 and hyp2, and hyp3 and hyp4, fuse to plates in *M. cephalus*, which is also the case in *Atherina harringtonensis* (Hollister, 1937). In *Oryzias* Jordan & Snyder, 1906, the lower and the upper hypural plates develop as single entities, and single hypurals do not occur during ontogeny (Fujita, 1992). Fujita (1992) and Parenti (1993) hypothesized that multiple hypurals (presumably, hyp3–hyp5) are fused together without developing separate precursors to form the upper hypural plate. Likewise, in *Chasmodes saburrae* the lower and upper hypural plates develop without single hypural precursors, and a separated hyp5 is visible in only a few specimens (Peters, 1981). In *Myxodes viridis* Valenciennes, 1836, (Clidnidae) hyp1–hyp4 and, presumably, hyp5 develop independently during ontogeny, but hyp1, hyp2 and the parhypural fuse early in ontogeny, whereas hyp3 and hyp4 fuse slightly later (see fig. 3 of Zavala-Muñoz et al., 2016; H1 in fig. 3F, G is, in fact, the parhypural). In pomacentrids and cichlids, there is no fusion of hypurals to hypural plates, but the parhypural occasionally fuses to hyp1 distally in *Mi. chrysurus* (Potthoff et al., 1987; Woltering et al., 2018). Based on the phylogenetic hypotheses provided by Betancur-R et al. (2017) and Hughes et al. (2018), there seems to be no clear pattern within Ovalentaria concerning the fusion of hypurals.

The development of the pectoral girdle and fin is generally similar in Mugilidae, Blenniidae, Pomacentridae and Cichlidae (Peters, 1981; Potthoff et al., 1987; Woltering et al., 2018). Data on the development of other ovalentarian taxa are unavailable. In *M. cephalus*, the precursors to the proximal radials emerge much later than in the taxa mentioned above (Peters, 1981; Potthoff et al., 1987; Woltering et al., 2018). This might be a result of a lack of staining but can also be attributed to a shift in the developmental timing. In *Astatotilapia burtoni*, there is a time gap between the emergence of the first distal radial (or propterygium) and the development of the other distal radials, whereas they develop as one series in *M. cephalus* and in *Mi. chrysurus* (Potthoff et al., 1987; Woltering et al., 2018). Compared with the development of the pectoral girdle of *Sander lucioperca* (Linnaeus, 1758), there are many similarities to *M. cephalus* in the series of developing and ossifying structures, except for the appearance of the fin plate, which is again much later in *M. cephalus* (Ott et al., 2012).

The development of the pelvic girdle is similar in mugilids, pomacentrids and cichlids (Potthoff et al., 1987; Woltering et al., 2018). In *Mi. chrysurus*, each basipterygial arm has two ossification centres, lateral and medial, as in *M. cephalus*. However, the anterior basipterygial process ossifies much earlier in *Mi. chrysurus* than in *M. cephalus* and is later incorporated into the internal wing (Potthoff et al., 1987). In several perciform taxa, such as Centropomidae, Percidae and Sparidae, there is a high similarity in the developmental sequence of the pelvic girdle compared with *M. cephalus* (Matsuoka, 1985; Potthoff & Tellock, 1993; Koumoundouros et al., 2001; Sfakianakis et al., 2004, 2005; Ott et al., 2012). In contrast, the first ossification centre to occur is shifted backwards into the middle of the transition from basipterygial arm to basipterygial plate in sparids (Koumoundouros et al., 2001; Sfakianakis et al., 2004, 2005).

The pterygiophores and the fin rays of the second dorsal fin and the anal fin develop bidirectionally and almost simultaneously in *M. cephalus*. The interdorsal pterygiophores presumably develop from posterior to anterior and in series with the pterygiophores of the first dorsal fin. The fin spines of the first dorsal fin emerge almost simultaneously, and we were unable to identify a directional pattern. Mabee et al. (2002) summarized the developmental patterns of the dorsal and anal fins in actinopterygians, including the ovalentarian taxa Cyprinodontiformes and Pomacentridae, in which the second dorsal and the anal fin develop bidirectionally. In Atheriniformes and Beloniformes, the second dorsal fin and the anal fin develop in the same pattern (Noell, 2003; Richter & Moritz, 2017). In the blennid *Chasmodes saburrae*, the second dorsal fin and the anal fin develop from anterior to posterior, a pattern that also seems to be present in cichlids (Peters, 1981; Woltering et al., 2018). In all examined ovalentarian taxa, the second dorsal fin and the anal fin develop simultaneously (Peters, 1981; Potthoff et al., 1987; Mabee et al., 2002; Noell, 2003; Richter & Moritz, 2017; Woltering et al., 2018). Development of the second dorsal and the anal fin in *M. cephalus* is more similar to atherinomorph and pomacentrid development than to blenniid or cichlid development.

The phylogenetic hypothesis by Hughes et al. (2018) that atherinomorphs and cichlids are sister taxa is not supported by these data. A closer relationship between mugilids and atherinomorphs, as proposed by most morphological (Stiassny, 1990, 1993; Johnson & Patterson, 1993) and some molecular studies (Wiley et al., 2000; Chen et al., 2003), is not contradicted by these results. Furthermore, there is at least some evidence that the development of the first dorsal fin
in _M. cephalus_ resembles the development of the first dorsal fin in atheriniforms: the pterygiophores of the first dorsal fin develop in sequence with the interdorsal pterygiophores from posterior to anterior (Richter & Moritz, 2017). This could support a close relationship of these two taxa, but more developmental data of the first dorsal fin in mugilids is still necessary.

The development of the scales in _M. cephalus_ starts sequentially from three centra: (1) anterior to the caudal peduncle, at the level of the horizontal septum; (2) anterior to the pelvic fin; and (3) on top of the head. Based on a review by Sire & Arnulf (1990), the developmental sequence of the squamation in _Planiliza haematocheilus_ (Temminck & Schlegel, 1845) is similar to that in _M. cephalus_. In _C. siliens_, only a single centrum anterior to the caudal peduncle is reported to form at the level of the horizontal septum (Burdak, 1969). In Cyprinodontiformes, Park & Lee (1988) describe three centra for _Kryptolebias marmoratus_ (Poey, 1880): (1) on top of the head; (2) on the caudal peduncle, at the height of the horizontal septum; and (3) on the opercle. In _Poecilia reticulata_ Peters, 1859, Sire & Arnulf (1990) found two centra: (1) on the caudal peduncle; and (2) on the head. In the beloniform _O. latipes_, there are two squamation centra, on the opercle and in the middle of the body, at the height of the horizontal septum (Iwamatsu, 2014). Sire & Arnulf (1990) compared scale development in cichlids, where there can be up to three squamation centra: (1) on the caudal peduncle, at the height of the horizontal septum (in all studied species); (2) on the head; and (3) anterior to the pectoral fin (in _Astatotilapia, Cichlasoma_ and _Hemichromis_). Overall, there seems to be a lot of variation in the number, the place and the sequence of appearance of squamation centra among and within the taxa compared. However, a larger taxon sampling could possibly reveal an underlying phylogenetic pattern.

**CONCLUSION**

A closer examination of the development of _M. cephalus_ clarified questionable characters of adult mullets and revealed several informative details, which might serve in the evaluation of phylogenetic hypotheses. At present, more detailed information from other taxa is missing, incomplete or questionable. Subsequent studies should focus on the developmental patterns and ontogenetic fusions in a comprehensive set of ovalentarian taxa and other perciformian groups. Furthermore, the detailed osteological description provides an overdue basis of comparison for aquaculture-reared mullets, to enable the recognition of malformations and examine them. We conclude that the scientific beauty of a bleak and bland fish, such as the grey mullet, can suddenly emerge by detailed studies on morphology and ontogeny, yielding coherent data on more comprehensive evolutionary hypotheses, or can provide evidence leading to new ideas.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Table S1. Samples used for the growth analysis of Mugil cephalus sorted to their respective developmental step. The mean value and SD for each developmental step are shown. Age is in days post hatching (dph) and length in millimetres (E6–L3 as notochord length and E4–J2 as standard length)
Section 2: Atherinomorpha – Morphological Analyses of a possible Mugiliform Sister-Taxon
Chapter 3. Publication: Development of hexagonal and octagonal scales in *Atherina* (Teleostei, Atheriniformes)

Development of hexagonal and octagonal scales in *Atherina* (Teleostei, Atheriniformes)

Katharina Koch, Philipp Thieme & Timo Moritz*
Deutsches Meeresmuseum, Katharinenberg 14-20, 18439 Stralsund, Germany
*Corresponding author; e-mail: timo.moritz@outlook.com

**Summary:** Species of the genus *Atherina* possess scales with a unique shape for teleosts: they are hexagonal (in *Atherina presbyter*) or octagonal (in *Atherina boyeri* and *Atherina hepsetus*) with the lateral fields curved more or less inwards. Nevertheless, these scales are principally of the cycloid type. Differing shape in scales can be used to distinguish *A. presbyter* from the other two species. Studying the ontogeny of their scales revealed that these unusual scale shapes are not present from the early beginning, but form during ontogeny. The first scales emerging on the larval body have a simple circular shape without any ornamentation.

**Keywords:** Ontogeny, Atherinomorpha, scale ornamentation, circuli

**1. Introduction**

The scales of atheriniform fish, especially their type and shape, have been studied only in very few investigations so far (Bräger & Moritz 2016; Brian & Dyer 2006). The Atheriniformes are a large group comprising about 350 species, which inhabit freshwater, brackish and marine habitats (Nelson, et al. 2016). Bräger & Moritz (2016) were able to show that the shape and type of scales is highly variable within teleosts and respectively also within the Atheriniformes, of which they depicted two species: *Atherina hepsetus* and *Atherinomorus lacunosus*. While scales of fish are generally described as hard, flattened skeletal elements, a whole set of details to this description can be added for teleosts, which have elasmoid scales, as far as the respective taxa have scales at all (Sire & Akimenko 2004; Bräger & Moritz 2016; Zylberberg 2018). Sire & Akimenko (2004) characterized elasmoid scales as “ornamented, thin, lamellar, and collagenous plates located within the upper region of the dermis”. Especially the ornamentations, e.g. cteni, circuli, and radii, of the scales can be seen as a highly variable character complex (Roberts 1993). That is why they can also be used for species identification (Mosher 1969; Casteel 1972; Bräger & Moritz 2016).

In more derived teleosts plenty of modifications evolved. However, the primitive state of scales for teleosts can be described as simple cycloid as defined by Bräger & Moritz (2016). Only little information is available on how scales get
their species specific appearance during growth. Two ways can be hypothesized: first, the typical shape and ornamentation are expressed already in an early stage, or second, the ontogenetic starting point of scales is more or less simple, much resembling between various taxa with specializations forming later during development. A first clue was offered by Pillay (1951), who was able to show that in smaller specimen of four different species of the genus *Mugil*, i.e. *M. cephalus*, *M. corsula*, *M. parvus*, and *M. tade*, less or almost none ornamentations were present in comparison to larger (adult) specimens, which have cteni, more radii and even show a change of shape. Similarly, Sire (1986) found out that in *Hemichromis bimaculatus* additional ornamentations, i.e. circuli, radii, denticles, first emerge during growth, although in this species the principal scale shape stays the same throughout ontogeny.

In this study, we took a closer look to the scales of three species in the genus *Atherina*, as adult members of this genus express a very uncommon polyangular scale shape (Bräger & Moritz 2016). We examined the development of the scales based on different developmental stages and compared them to the scales of adult specimens to describe the origin of their unusual scale shape.

2. Material and methods

The scales used for this study were sampled from specimens stored in the collection of the Deutsches Meeresmuseum, Stralsund, Germany. In total, scales from 22 individuals of at least three species were studied (tab. 1). Scales were sampled from five different body parts, according to the areas described by Bräger & Moritz (2016): C,

<table>
<thead>
<tr>
<th>Species</th>
<th>Reg. Nr.</th>
<th>Specimen Nr.</th>
<th>SL in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Atherina boyeri</em> Risso, 1810</td>
<td>IE/5874</td>
<td>1</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>36.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>45.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>71.3</td>
</tr>
<tr>
<td><em>Atherina leptotus</em> Linnaeus, 1758</td>
<td>IE/5080</td>
<td>1</td>
<td>77.5</td>
</tr>
<tr>
<td></td>
<td>IE/5873</td>
<td>1</td>
<td>58.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>53.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>65.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>74.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>58.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>71.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>63.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>85.5</td>
</tr>
<tr>
<td></td>
<td>IE/5888</td>
<td>1</td>
<td>68.3</td>
</tr>
<tr>
<td><em>Atherina prolitor</em> Cuvier, 1829</td>
<td>IE/6135</td>
<td>1</td>
<td>39.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>32.9</td>
</tr>
<tr>
<td></td>
<td>IE/6157</td>
<td>1</td>
<td>21.5</td>
</tr>
<tr>
<td></td>
<td>IE/9037</td>
<td>1</td>
<td>61.4</td>
</tr>
<tr>
<td></td>
<td>IE/10493</td>
<td>1</td>
<td>75.9</td>
</tr>
<tr>
<td><em>Atherina</em> sp.</td>
<td>IE/14850</td>
<td>1</td>
<td>19.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>20.5</td>
</tr>
</tbody>
</table>
D, F, G, H (fig. 1) in all specimen except the larval stages of *Atherina* sp. Most specimens were cleared and double stained (cartilage blue, bone red) following the protocols of DINGERKUS & UHLER (1977) and TAYLOR & VAN DYKE (1985). Scales from these specimens were taken after staining and stored in 70% ethanol. All other scales were sampled from specimen stored in 70% ethanol. These scales were then stained and stored in a solution of 70% ethanol and alizarin red (0.1 g per 100 ml; ROTH, Germany). Images were taken using a Leica M165C binocular with a Leica DFC 425 camera and dedicated software (LAS 4.9.0, Leica). Images were adapted for contrast, color intensity and white balance and extracted in Adobe Photoshop CC; figures were assembled in Adobe Illustrator CC.

Terminology of scale types, shapes and characteristics used in this study follows BRÄGER & MORITZ (2016). Structure of scales descriptions also follows the latter authors. For the developmental stages, one scale from the C region and one scale from the D region (see fig. 2) are described. For larger specimens (see figs 3, 4) one scale from body region C will be characterized in detail and will be compared to scales from other body areas. *Atherina hepsetus* is not depicted and described in detail, as this information already is available from BRÄGER & MORITZ (2016).

### 3. Results

#### 3.1. *Atherina* sp. (SL = 20.5 mm) (fig. 2a, b)

The examined specimen already has all fins developed and most parts of the internal skeleton are ossified. However, only a few scales were present on the anterior lateral sides of the body, approximately matching body areas C and F. It was still difficult to accurately assign them to one specific body region, as the transition zone between the body areas was very small.

**Type:** cycloid. **Shape:** circular: oval (fig. 2a)/true circular (fig. 2b). **Anterior field:** rounded with smooth margin. **Lateral field:** rounded with smooth margin. **Posterior field:** rounded with smooth margin. **Focus:** not visible. **Circuli:** absent. **Radii:** absent.

#### 3.2. *Atherina* presbyter (SL = 21.5 mm) (fig. 2c, d)

In this developmental stage, scales were present on all body regions, where scales can be found on adult specimen too.

**Type:** cycloid. **Shape:** polygonal: hexagonal: **Anterior field:** flattened or slightly convex with pointed apex and smooth margin. The antero-lateral corners are extended but not pointed. **Lateral field:** flattened to convex. **Posterior field:** rounded with smooth margin. **Focus:** central. **Circuli:** distinct and continuous in the anterior field. **Radii:** absent.

#### 3.3. *Atherina* presbyter (SL = 32.9 mm) (fig. 2e, f)

In this developmental stage, scales were present on all body regions, where scales can be found on adult specimen too.

**Type:** cycloid. **Shape:** polygonal: hexagonal to octagonal. **Anterior field:** flattened (slightly concave) with pointed apex and smooth margin. The antero-lateral corners are extended and strongly pointed. **Lateral field:** concave. **Posterior field:** rounded with smooth margin. **Focus:** central. **Circuli:**
Fig. 3: Scales of *Atherina presbyter* (75.9 mm SL). a Body part C. b Body part D. c Body part E. d Body part G. e Body part H. Scale bar = 500 μm.

Abb. 3: Schuppen von *Atherina presbyter* (75,9 mm SL). a Körperregion C. b Körperregion D. c Körperregion E. d Körperregion H. Maßstab = 500 μm.

distinct and continuous in the anterior field. **Radii:** absent.

The scales of the three developmental stages show certain differences especially in their shape and their anterior fields. The shape changes from oval/true circular to hexagonal and even octagonal. While the anterior field of the scales from *Atherina* sp. is round, it is flattened and tappers off to the middle of the anterior field in the smallest *A. presbyter*. In the larger *A. presbyter* the anterior field is slightly concave and has a pointed apex. The antero-lateral corners are
not visible in the small *Atherina* sp. (fig. 2a, b), but are extended in the only slightly larger *A. presbyter* specimen (fig. 2c, d). However, they are only pointed in the larger specimen (fig. 2e, f).

The outer curvature of the lateral fields develops from round (*Atherina* sp.) to flattened/slightly concave (small *A. presbyter*) into concave (large *A. presbyter*). The posterior field is rounded in all three developmental stages. While the focus is not visible in *Atherina* sp., it is in a central position in both *A. presbyter* specimens. The area of the focus covers a higher portion of the whole scale area in the smaller *A. presbyter* specimen than in the larger one. Around the focus, circuli are present in both examined stages of *A. presbyter*. They have a semicircular shape and are limited to the anterior half of the scale.

3.4. *Atherina presbyter* (SL = 75.9 mm) (fig. 3)

**Type:** Cycloid: true cycloid. **Shape:** Polygonal: Hexagonal. **Anterior field:** flattened with pointed apex and smooth margin. **Lateral field:** flattened, slightly concave. **Posterior field:** rounded end with smooth margin. **Focus:** central. **Circuli:** distinct and continuous in the anterior field. **Radii:** absent

The type and shape of all examined scales of this specimen is true cycloid and hexagonal. The anterior field of the D and G scales is more concave than the anterior field of the C scales, while in the F and H scales the anterior field is flat too. All scales have a pointed apex on the anterior field. Additionally, the antero-lateral corners of all scales are strongly pointed and can protrude (see fig. 3b, c). The lateral fields are mostly flattened (fig. 3c, d), although, in the D and the H scales this field is slightly concave. The overall shape of the posterior field is round and it has a smooth margin in all scales. In the C and the H scales, the posterior field is more angular. The focus is positioned central in all scales and in some scales (fig. 3a, c) a bright spot right on the focus is visible. Circuli can be found on the anterior field as well as the anterior lateral fields in scales from all examined regions. While the circuli on the C scales are more roundish, they are more flat on the F scales. In the other scales, they change from roundish close to the focus to flat/wavy (almost mirroring the outer edge of the anterior field) towards the margin of the anterior field (fig. 3d). Some indistinct markings are present near the margin of the posterior field. Radii cannot be found on any scale.

3.5. *Atherina boyeri* (SL = 71.3 mm) (fig. 4)

**Type:** cycloid: true cycloid. **Shape:** polygonal: octagonal. **Anterior field:** flattened with pointed apex and smooth margin. The antero-lateral corners are extended and strongly pointed. **Lateral field:** strongly concave. **Posterior field:** rounded end with smooth margin. **Focus:** central. **Circuli:** distinct and continuous in the anterior field. **Radii:** absent

For all examined regions and specimen, we found the same scale type and shape. Thus, they are all true cycloid and octagonal. The anterior field of the scales from the regions D, F and G is flattened and has a pointed apex. Scales from region H have a less flattened anterior field, rather being slightly convex. The antero-lateral corners are extended and strongly pointed in all scales from all examined regions. However, sometimes one corner is less pointy than the other one within one scale (fig. 4c). The lateral fields can overall be described as concave. The anterior region is flat, proceeding towards the middle of the scale resulting in a concave middle part. The transition between the lateral field and the posterior field is convex (fig. 4). The posterior field of all examined scales is rounded and their margin is smooth. The ventral and dorsal sides of the posterior field are sometimes asymmetrically (fig. 4b, d). The focus is central. Anterior to the focus, the scales are stained lighter and show a distinct blotch (fig. 4). There are indistinct markings from this point to the apex and possibly to the lateral corners of the anterior field (fig. 4a, c). Circuli can be found on the anterior fields and anterior parts of the lateral field in all scales. They are more roundish in close approximation to the focus. Towards the margin of the lateral field, they adapt to course of the outer line of the anterior field. Some indistinct markings are present near the margin of the posterior field. Radii are absent on all scales.
4. Discussion

4.1. Comparison of adult scales

The scales from members of the genus *Atherina* are unusual in their shape exhibiting a hexagonal or octagonal shape. Scale shapes in other atheriniform species, e.g. *Atherinomorus lacunosus* or *Odontesthes incisa*, are very different (Brian & Dyer 2006; Bräger & Moritz 2016). The scales of *O. incisa* have a square shape and exhibit crenae on the posterior field (Brian &
Fig. 4: Scales of *Atherina boyeri* (71.3 mm SL). a Body part C. b Body part D. c Body part F. d Body part G. e Body part H. Scale bar = 500 μm.

Abb. 4: Schuppen von *Atherina boyeri* (71.3 mm SL). a Körperregion C. b Körperregion D. c Körperregion F. d Körperregion G. e Körperregion H. Maßstab = 500 μm.

Dyer 2006), whereas the scales of *A. lacunosus* are circular to discoidal with circuli as the only ornamentation (Bräger & Moritz 2016). The scales of the herein investigated *Atherina boyeri* and *Atherina presbyter* seem to be quite similar to each other. They are true cycloid and have a polygonal shape, which holds also true for the scales of *Atherina hepsetus* (Bräger & Moritz 2016). However, while scales from *A. presbyter* are hexagonal, the scales from *A. boyeri* and *A. hepsetus* (Bräger & Moritz 2016) are octagonal. The anterior field of the scales of all three spe-
cies is flattened. In *A. presbyter* the scales from body areas D and G are slightly concave, which can also be found in scales from body region A and E in *A. hepsetus* (Bräger & Moritz 2016). The apex of all scales is pointed and there seem to be no distinguishing differences between the three species, as the variation between the scales of one individual/species are too big. While the antero-lateral corners of *A. boyeri* are well extended, these corners do not protrude in *A. presbyter*. The antero-lateral corners of *A. hepsetus* are extended too, however, they are not as strongly pointed as in *A. boyeri*; this is most pronounced in scales from body region D (Bräger & Moritz 2016). These corners mark the transition to the lateral fields in all scales. While in *A. presbyter* the margins of the lateral fields are flat or only slightly concave, the respective margins in *A boyeri* and in *A. hepsetus* are concave. However, the lateral fields of the scales of the latter two species can be divided in three parts: a flat anterior, a concave middle and a convex posterior (transition to the posterior field) part. The posterior fields of all scales can be described as round. The scales from *A. boyeri* and *A. hepsetus* have a bulgy posterior field, because they are extended on the lateral sides. Like the scales from the areas C and H of *A. presbyter*, the posterior field of the scales in *A. hepsetus* are angular, with the posterior apex even being flat in the C, D, E, F and G areas (Bräger & Moritz 2016). The focus of all scales is in central position. Circuli are clearly visible on the anterior field as well as the anterior parts of the lateral fields on all scales of *A. presbyter*, *A. boyeri* and *A. hepsetus* (Bräger & Moritz 2016). Additionally, some indistinct markings are visible on the posterior field, especially near its edge.

The scales of the *A. presbyter* can be easily distinguished from the other two species, because the shape of the lateral fields differs clearly. But, it is not so easy to tell the scales of *A. boyeri* and *A. hepsetus* apart, as their scales look quite similar. Morphometry might be able to discriminate scales on species level, as shown for some clupeids (Bräger et al. 2016; Bräger et al. 2017), but this needs to be proven.

### 4.2 Scale development

Based on the developmental stages it is clear that for *Atherina presbyter* the shape of the scales, which can be seen in adults, is not present in the smallest larvae. It develops gradually with additional ornamentations emerging during growth. While the scale type (true cycloid) does not change, the shape undergoes significant change, i.e. from circular to hexagonal or octagonal respectively. Similar shape changes have not been reported for the cichlid *Hemicromis bimaculatus* (Sire 1986). It might be possible that the specimens available for the latter study have already been too large to follow a change of shape. In the scales of *Atherina* sp. a focus is not visible (Fig. 2a, b). It becomes a visible structure with the emergence of the first circuli (fig. 2c, d). In comparison with Pillay (1951) observations in the genus *Mugil*, we found great differences in the scales of larvae, juveniles and adult specimens too. While the smallest scales examined by Pillay (1951) already showed radii, we did not find any type of ornamentation in the smallest specimens studied herein.

It was not easy to find scales on the smallest larvae and many examined specimen did not even have scales yet. Especially finding the first scales emerging on body area C is difficult. We could not identify a distinct pattern, in which scales emerge, due to little available material and, even more eminent, the highly deciduous scales in larvae of *Atherina* spp. Nevertheless, we are quite confident that the first scales appear on the anterior lateral sides of the larva's bodies. This stands in contrast to the pattern found in the cyprinodontiform *Poecilia reticulata* (Sire & Arnulf 1990). In this species, the first scales emerge on the caudal peduncle and new scales develop in anterior direction. In another cyprinodontiform fish, *Rivulus marmoratus*, there are two developmental centra, one on the head and a second on the caudal peduncle (Park & Lee 1988). From the top of the head, the scales develop in a posterior direction, while the scales on the lateral sides develop in an anterior direction, like they do in *Poecilia reticulata* (Park & Lee 1988; Sire & Arnulf 1990). Although
the Cyprinodontiformes are closely related to the Atheriniformes (Betancur-R. et al. 2017), there seems to be a different pattern within the genus Atherina or maybe even within all Atheriniformes, which has to be investigated in future studies.

4.3. Conclusion

In the genus Atherina scales appear first in ontogeny as circular shaped lacking any ornamentation. Although it is difficult to find larval stages with the first undifferentiated scales, a gradual change during larval growth is visible, showing the transformation to a hexa- or octagonal scale bearing circuli. The slight differences in adult specimen of the examined species are helpful to distinguish at least some species, but seem not to be present or well developed in the larvae and juveniles. The scales of the few other atheriniform species studied so far are very different to the herein studied Atherina species. Therefore, further studies should focus on the scale diversity in atheriniforms, regarding their types, shapes, ornamentations and ontogeny, to get a more complete picture of the characteristics and evolution of scales in this taxon.

Literature


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Structure of the andropodium of the viviparous halfbeak genus *Nomorhamphus* (Atherinomorpha: Beloniformes: Zenarchopteridae), endemic to Sulawesi, Indonesia

Janina Kraemer1,*, Philipp Thieme2, Renny Kurnia Hadiaty2, & Fabian Herder1

**Abstract.** In halfbeaks (Zenarchopteridae), viviparity is known in three of the five genera, including *Nomorhamphus*. During the extremely short copulation, the transfer of spermatozeugmata from the male genital papilla to the female urogenital opening is apparently facilitated by the andropodium, an organ composed of the strongly modified male anterior anal-fin rays. Substructure of the andropodium varies among species, and traits of the modified anal-fin rays have been used as taxonomic characters for species delimitation. The present study examines the microanatomy of the andropodium across 11 of the 12 *Nomorhamphus* species, which are endemic to Sulawesi. Methods applied include contrast-enhanced μCT-imaging and clearing and staining approaches. Similarity in andropodial fin ray traits correlates with general morphology and spatial proximity. Species occurring in sympathy possess similar andropodia; the copulatory organ of *Nomorhamphus rex* is most distinct. In general, andropodial traits allow clear discrimination in most of the species examined, but require careful examination. The supposed incomplete calcification of the modified rays and their resulting flexibility provide arguments against the hypothesis of the andropodium as a true intromittent organ. The structure might rather help to orient the genital papilla in direction of the female genital opening during mating.

**Key words.** *Nomorhamphus*, andropodium, Sulawesi, freshwater halfbeaks, taxonomy, viviparity

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**INTRODUCTION**

Internal fertilisation is a precondition for viviparity, which is characterised by the retention of developing eggs and embryos within the female reproductive tract (Wourms, 1981; Wourms & Lombardi, 1992), and occurs in at least three genera of the halfbeak family Zenarchopteridae (Schindler & Hamlett, 1993; Meisner, 2001; Lovejoy et al., 2004; Aschliman et al., 2005; Reznick et al., 2007). Parts of the anal fin of male Zenarchopteridae are modified to form an organ, the so-called ‘andropodium’ (Brembach, 1976; Fig. 1), which plays an uncertain role during copulation. The closely related freshwater SE-Asian halfbeak genera *Nomorhamphus* and *Dermogenys* share a similar configuration of the andropodium, with the first five to seven anal-fin rays shortened and thickened (Brembach, 1976; Meisner, 2001). The anal fins of *Hemirhamphodon* and *Zenarchopterus* are much less modified, and little is known about the reproductive morphology of the monotypic genus *Tondanichthys* (Anderson & Collette, 1991; Collette, 1995; Meisner, 2001; Tan & Lim, 2013). Though a number of studies have addressed aspects of reproduction in *Nomorhamphus* and *Dermogenys* (Mohr, 1936; Brembach, 1976; Meisner & Burns, 1997a, 1997b; Meisner, 2001; Greven, 2006, 2010), some fundamental questions remained unanswered, including the mechanism of sperm transfer during copulation.

Downing & Burns (1995) studied testis morphology and spermatozeugma formation, and concluded that during copulation sperm bundles are somehow transferred to the female genital pore by the help of the modified anal fin in *Dermogenys* and *Nomorhamphus*. The mating process lasts for several seconds in *Hemirhamphodon* and *Zenarchopterus*, during which the male achieves firm contact to the anal region of the female by clasping the female with its dorsal and anal fin. In *Nomorhamphus* and *Dermogenys*, mating is more rapid and lasts for ca. 40 ms (Kottelat & Lim, 1999; Greven, 2010). In *N. liemi* and *D. pusilla*, a putative copulation is characterised by an extremely quick axial rotation of the male and a forceful push against the female’s anal region (Magyar & Greven, 2007; Greven, 2010).

Brembach (1976) observed that anal-fin rays two to four can be splayed out laterally at an angle of 30°–40°, and hypothesised that they form a groove to direct the sperm bundles to the terminal structure of the second anal-fin ray, which ‘spoons’ the spermatozeugmata into the female genital opening. To achieve a position permitting the contact between
male and female, the andropodium would have to perform a 180°-rotation, as known for the poeciliid gonopodium (Meisner & Burns, 1997a). However, the effective mobility of both genital papilla and anal-fin rays is highly restricted due to the lack of musculature (Greven, 2010). Additionally, the female genital pore is small and a successful conjunction between male and female genital opening within 40 ms appears unlikely (Greven, 2010). Meisner & Burns (1997a) assumed that the andropodium of Nomorhamphus and Dermogenys may primarily serve to orient the genital palp of the male in direction of the female genital pore, similar to the andropodium of goodeids. The cryptoplica, a fleshy sheath forming a duct comprising the modified rays, may prevent the loss of sperm bundles.

Nomorhamphus species inhabit hill-stream habitats and freshwater lakes of Indonesia and the Philippines (Brebach, 1991; Kottelat & Whitten, 1996; Meisner, 2001; Collette, 2004; Kottelat, 2013; Mieszen et al., 2015). With 12 endemic species, Sulawesi is named the hotspot of Nomorhamphus diversity; seven species occur on islands of the Philippines (Meisner, 2001; Huylebrouck et al., 2012, 2014). The structure of the andropodium varies between species and was used by several authors to define species limits within the genus and its sister group Dermogenys (Brebach, 1976, 1991; Meisner & Louie, 2000; Meisner, 2001; Huylebrouck et al., 2012, 2014). Detailed descriptions of the microanatomy of the andropodium were provided by Mohr (1936), Brebach (1976), Meisner (2001) and Huylebrouck et al. (2012). Brebach (1976) introduced the term andropodium, referring to the modified anal fin of the three viviparous genera Hemirhamphodon, Dermogenys, and Nomorhamphus, which was afterwards adopted by all other authors (e.g., Meisner, 2001; Greven, 2010; Huylebrouck et al., 2012, 2014).

The use of the andropodial microanatomy for purposes of taxonomy has remained rather restricted (Brebach, 1991; Meisner, 2001; Huylebrouck et al., 2012, 2014). For example, descriptions and drawings by Brebach (1976, 1991) do not provide enough details for species delimitations. Nevertheless, he recognised differences of the spiculus, a spine-shaped terminal structure of the second anal-fin ray (Fig. 1), in Dermogenys and Nomorhamphus but rated the taxonomic potential of this structure as low. Meisner (2001) noticed that species of Nomorhamphus can be divided into subgroups based on characteristics of the andropodium, particularly shape and segmentation of the spiculus. However, when comparing the drawings and descriptions she presented for different species, it becomes obvious that most of the information given is highly repetitive, and that the spiculus remains the only distinguishing feature. Since her study, three new species were described by Huylebrouck et al. (2012, 2014), who utilised “sub-segments” (minute subdivisions of segments) of the second, third and fourth anal-fin rays that are only visible in cleared and stained specimens as diagnostic traits. The present study compares the ultrastructure of the andropodium in 11 of the 12 species of Nomorhamphus from Sulawesi, and evaluates its taxonomic potential.

MATERIAL AND METHODS
Specimens used in this study are housed in the following museum collections: MZB, Museum Zoologicum Bogoriense, Bogor, Indonesia; WFB, Museum of Wildlife and Fish Biology, University of California, Davis, USA; ZFMK, Zoologisches Forschungsmuseum Alexander Koenig, Leibniz Institute of Animal Biodiversity, Bonn, Germany; ZMH, Zoologisches Museum Hamburg, Hamburg, Germany; ZSM, Zoologische Staatssammlung München, Munich, Germany. Measurements of the standard length (SL) were taken from the tip of the upper jaw to the base of the caudal fin, recorded to the nearest 0.1 mm with a digital calliper. We follow classification resulting from the phylogetic revision of Meisner (2001) and the species descriptions of Huylebrouck et al. (2012, 2014). The species status of N. hageni is highly questionable, as the few fishes left over from the type series are in very poor condition and the type locality is not known with certainty (Weber & de Beaufort, 1922; Mohr, 1936; Brebach, 1991; Meisner, 2001). This taxon was excluded from the present study.

CT scanning and three-dimensional visualisation. In total, 22 male Nomorhamphus, belonging to 11 species (2 specimens per species) were computed tomography (CT) scanned. Prior to scanning, specimens were transferred to plastic tubes containing 80% EtOH solution. The fishes were fixed firmly inside the tube using small pieces of plastic to avoid movements during the scanning process. All specimens were scanned with a 100 kV SkyScan 1272 desk-top X-ray microtomograph (Bruker microCT, Kontich, Belgium). The scanning parameters were 50–60 kV source voltage, 166–200 μA source current, 173–1,039 ms exposure time, camera binning = 2 × 2, frames acquired over 180°, 5–7 μm voxel resolution, filter = Al 0.25 mm, no oversize-scanning. 0.1° angular step size, frame averaging = 6–7, random movement = 15. Digital section reconstruction of the scans to 16 bit tagged image file format (TIFF) image stacks was done without compression by the software NRecon 1.7.1 provided by the SkyScan 1272 system (misalignment compensation = 1.0–5.5, ring artefacts reduction = 4, beam-hardening correction = 8%). Reduction of data size was achieved with DataViewer Version 1.5.4 (Bruker microCT, Kontich, Belgium) by selecting a VOI (Volume of Interest). DrishtiImport Version 2.6.4 was used to convert the data. Volume rendering of the dataset for three-dimensional exploration and visualisation was performed in Drishti Version 2.6.4 (Limaye, 2012).

Clearing and staining. Fourteen male Nomorhamphus representing all species except N. weberi were cleared and double stained with alcian blue for cartilage and alizarin red S for bone following the protocol based on Dingerkus & Uhler (1977) and Taylor & van Dyke (1985). Additional cleared and stained specimens available in the collections of ZFMK and ZMH were also examined.

Light microscopy. Specimens were investigated using an OLYMPUS SZX 12 and an OLYMPUS BX 51 microscope (Olympus Corporation, Tokyo, Japan), for comparison with the results achieved by CT scanning and clearing and staining
Fig. 1. Andropodium of Nomorhamphus rex, ZFMK 44945, 35.0 mm SL, cleared and double stained. Scale bar = 1 mm. Abbreviations: gp, genital papilla; pt1, first anal pterygiophore; sn, spine = spines; sp, spiculus; 1–5, anal-fin rays one to five. The elongate genital papilla (gp) covers the anterior part of the first anal-fin ray (1). Anal-fin rays one to five are modified and considered as andropodium. The second anal-fin ray (2) is most strongly modified with a terminal structure, the tridens flexibilis, consisting of a central spiculus (sp) and two lateral spines (sn). The physa is a pouch-shaped structure located between the third (3) and fourth fin ray (4). In the freshly fixed specimen, the andropodium is covered by a fleshy sheath, the cryptoptica.

Fig. 2. A, B, Andropodium of Nomorhamphus celebensis, ZFMK 49216–49229, 39.4 mm SL. A, cleared and double stained; B, Rendered image. C, D, Andropodium of N. rex, (C) ZFMK 44945, 35.0 mm SL; (D) ZFMK 44944, 41.2 mm SL. C, cleared and double stained; D, rendered image. Scale bar = 1 mm. Arrowheads point to distal part of the second anal-fin ray, the tridens flexibilis.

RESULTS

The Nomorhamphus species from Sulawesi, except for N. rex (Fig. 1), are rather similar in their andropodial traits, but can be distinguished by the substructure of their second anal-fin ray (Figs. 2–6). The andropodium of each species resembles at least one other species. Thus, these 10 species were divided into four groups of morphologically similar andropodia: (1) N. brembachi & N. liemi; (2) N. ebrardtii, N. lanceolatus, & N. sagittarius; (3) N. megarrhamphus & N. weberi; (4) N. celebensis, N. kolonodalensis, & N. towoetii.

Descriptions of andropodia and anal-fin pigmentation

Nomorhamphus brembachi & N. liemi
(Fig. 3)

Pigmentation. N. brembachi: Posterior half of fin with black or brown pigment. N. liemi: Larger concentration of melanophores, anal fin completely black in many males. Both species: cryptoplica and base of fin rays hardly pigmented.

Andropodium. 1st anal pterygiophore thickened, not angled anteriorly. 2nd fin ray with 10–11 segments proximal to paired spines; segments 2/3–10/11 with longitudinal groove in the middle separating these segments into dorsal and ventral part (N. brembachi), with or without three distinct longitudinal rows (N. liemi) without further sub-segments; segment 4/5 or both elongate; spiculus straight, clearly segmented at proximal end, broad tip, distal tip not in contact with tip of third fin ray; distal half of second fin ray curved dorsally
with tip pointing ventrally. Some specimens of *N. liemi* with small geniculus at proximal half of segment 4. 3rd fin ray with segments 3–6 composed of three longitudinal rows, each row approximately 1/3 the height of the respective segment; segments 7 to tip short, tip straight. 4th fin ray with segments 4–5 exhibiting longitudinal groove in the middle separating these segments into dorsal and ventral part (*N. brembachi*) or composed of three longitudinal rows (*N. liemi*); segments 6–7/8 composed of three longitudinal rows, each row approximately 1/3 the height of the respective segment; following segments short. 5th fin ray with segments 2–4 thickened, shortened, approx. half the length of 4th fin ray.

**Nomorhamphus ebrardtii, N. lanceolatus, & N. sagittarius**

(Fig. 4)

**Pigmentation.** Lack of black pigment at posterior anal fin rays, cryptoplica and fin rays with few and small melanophores.

**Andropodium.** 1st anal pterygiophore thickened, not angled anteriorly. 2nd fin ray with 9–10 (7–10 in *N. sagittarius*) segments proximal to spinae; segments 3/4–6/7/8 with a dorsal and ventral row of differently sized sub-segments (squares, rectangles), each sub-segment approximately 1/3
Kraemer et al.: Andropodium of *Nomorhamphus*

Fig. 5. A, B, diagrammatic representation of andropodium of (A) *Nomorhamphus megarrhamphus*, ZMH 7153, 38.3 mm SL and (B) *N. weberi*, ZMH 7970, 41.5 mm SL. C, D, rendered image of andropodium of (C) *N. megarrhamphus*, ZMH 7153, 43.4 mm SL and (D) *N. weberi*. Scale bar = 1 mm. Bone stippled. Arrowheads point to spiculi of *N. megarrhamphus* (A) and *N. weberi* (B) and tips of the third anal-fin ray.

the height of the respective segment; segments 4–5 elongate in large males; spiculus lanceolate, clearly segmented at proximal end, ventrally slightly curved, pointed dorsally at an angle of 45°, distal tips (middle segments in *N. lanceolatus* and *N. sagittarius*) of spiculus and 3rd fin ray in contact. 3rd fin ray slightly constricted longitudinally; segments 2–3 or 3–5 composed of three longitudinal rows, each row approx. 1/3 the height of the respective segment. 4th fin ray with distal half of segment 1 thickened; distal half of ray slightly constricted longitudinally; segments 4–5/6/7 elongate and composed of three longitudinal rows, each row approximately 1/3 the height of the respective segment. 5th fin ray not noticeably thickened (*N. ebrardtii*) or segments 2–5 thickened, (*N. lanceolatus*, *N. sagittarius*).

**Nomorhamphus megarrhamphus & N. weberi**

(Fig. 5)

**Pigmentation.** Lack of black pigment at posterior anal fin rays, few and small melanophores at cryptoplica and base of fin rays.

**Andropodium.** All males investigated are lacking a physa and the cryptoplica is weakly developed, covering only a small basal part of the andropodial fin rays. The anal-fin rays forming the andropodium appear less curved and modified compared to other species. 1st anal pterygiophore thickened, angled anteriorly. 2nd fin ray with 9–11 segments proximal to spinae; segments 3–5 broader than all other segments of the 2nd fin ray. *N. megarrhamphus*: spiculus clearly segmented at proximal end, short and thin, dorsal
portion slightly curved, in contact with distal segments of 3rd fin ray. *N. weberi*: sickle-shaped spiculus, not clearly segmented, pointed ventrally, not in contact with tip of 3rd fin ray. 3rd fin ray with segments 2/3–6/7 composed of three longitudinal rows, each row approximately 1/3 the height of the respective segment. 4th fin ray approximately as broad as 2nd fin ray. *N. weberi*: segments 3–5 composed of three longitudinal rows, each row approximately 1/3 the height of the respective segment. 5th fin ray thin, not noticeably thickened.

**Nomorhamphus rex**

(Fig. 6A, C)

**Pigmentation of anal fin.** Distal tips of anal-fin rays greyish or black in most males; range and intensity of pigmentation varies between individuals; cryptoplica and fin rays slightly pigmented, small melanophores.

**Andropodium.** 1st anal pterygiophore thickened, not angled anteriorly. 2nd fin ray with 3–4 segments proximal to spinae; segment 3 or 4 greatly elongate, approximately half the length of the entire ray, with an irregular dorsal and
ventral row of small sub-segments (squares and rectangles) of different sizes, each sub-segment approximately 1/3 the height of segment 3 or 4, number of sub-segments variable; spiculus sickle-shaped, clearly segmented, short, upright position and curved (degree of curvature varies between specimens), its proximal and middle segments in contact with the distal tip of the third anal-fin ray. 3rd fin ray with segment 3 greatly elongate, approximately half the length of the entire ray, constricted longitudinally, composed of three longitudinal rows, each row approximately 1/3 the height of the respective segment; segments 4 to tip short, tip nearly straight. 4th fin ray with segment 4 composed of three longitudinal rows, greatly elongate, following segments short. 5th fin ray thickened in larger males.

**Nomorhamphus celebensis, N. kolonodalensis, & N. towoetii** (Fig. 6B, D)

**Pigmentation.** Cryptopica and fin rays slightly or not pigmented. *N. celebensis*: anal fin completely dusky and without distinctly black tips, higher concentration of melanophores compared to *N. kolonodalensis*. *N. kolonodalensis*: distal tips of anal-fin rays distinctly black; in smaller males only dorsal tips, in larger males dorsal 2/3. *N. towoetii*: distal tips of anal-fin rays with diffuse grey or black pigmentation or anal-fin completely dusky.

**Andropodium.** 1st anal pterygiophore thickened, not angled anteriorly. 2nd fin ray with 9/10–11 segments proximal to spinia. *N. kolonodalensis*: segments 7–8 in some specimens with dorsal and ventral row of irregular sub-segments (≥ 1, squares, rectangles), each segment approximately 1/3 the height of segment 7 or 8; spiculus only segmented at proximal end (*N. celebensis*) or spiculus clearly segmented (*N. kolonodalensis*), dorsal portion slightly curved or straight, not thickened, elongate in large males, not in contact to tip of 3rd anal fin ray. 3rd fin ray with segments 3/4–5/6/7 composed of three longitudinal rows, each row approximately 1/3 the height of the respective segment; tip of ray 3 nearly straight, parallel to spiculus. 4th fin ray: *N. kolonodalensis*: distal half thin and dorsal portion curved; segments 5–6/7 composed of three longitudinal rows, each row approximately 1/3 the height of the respective segment. 5th fin ray with segments 2–4 slightly thickened in *N. celebensis*, not thickened in *N. kolonodalensis* and *N. towoetii*.

**Differences between populations of Nomorhamphus towoetii.** Balambano River (ZFMK 49297–49299) – 2nd fin ray: spiculus straight, clearly segmented, contacts distal tip of 3rd fin ray. 3rd fin ray with segments 3–6 composed of three longitudinal rows, each row approximately 1/3 the height of the respective segment. 4th fin ray with segments 4–7 composed of three longitudinal rows, each row approximately 1/3 the height of the respective segment. Southeast of Lake Matano (ZFMK 48960–49000) – 2nd fin ray: segment 9 with dorsal and ventral row of irregular sub-segments (≥ 1, squares, rectangles), each segment approximately 1/3 the height of segment 9, spiculus straight, clearly segmented, no contact to tip of 3rd fin ray. 3rd fin ray with segments 2–6/7 composed of three longitudinal rows, each row approximately 1/3 the height of the respective segment. 4th fin ray with segments 5–7 composed of three longitudinal rows, each row approximately 1/3 the height of the respective segment.

North of Lake Poso (ZFMK 49177–49215); North of Lake Matano (ZFMK 49119–49146) – 2nd fin ray with spiculus straight, no contact to tip of 3rd fin ray but tips close.

**Microanatomy of the andropodium.** The first segment of the first anal-fin ray has a characteristic shape. It comprises approximately half the length of the total fin ray, is slightly constricted longitudinally and a groove in the middle of the segment gives the appearance of a longitudinal fissure (Fig. 1). The number of segments contained in the first fin ray is intraspecifically highly variable. Larger males tend to have more segments than smaller males. The second fin ray usually lacks a distinct genusicus as present in Dermogenys spp., but in some specimens of *N. liemi* a small, geniculus-like “knee” is present (Meisner, 2001: Fig. 3B). The second fin ray comprises segments of different length, the size of these segments varies from proximal to distal, with the smallest segment situated prior to the basal segment of the spinae. *Nomorhamphus rex* is considered to be an exception, as the third or fourth segment is greatly elongate, followed by a minute segment prior to the spines (Figs. 1, 6A). The andropodium of *N. rex* is unique and can be distinguished from all others due to its low number of segments prior to the spines and the pronounced, sickle-shaped spiculus (Fig. 6A, C).

Six out of 11 species considered (*N. ebrardtii, N. kolonodalensis, N. lanceolatus, N. rex, N. sagittarius, some populations of *N. towoetii*) possess irregular dorsal and ventral rows of small sub-segments in the central part of the second fin ray. In all species investigated, two grooves in the segments of the third and/or fourth fin ray give the appearance of segments composed of three longitudinal rows. The presence or absence of sub-segments in the second fin ray and longitudinal rows in the third and fourth fin ray is characteristic for each species, although number of sub-segments and expansion are slightly varying between the individuals. In the first and fifth fin ray, neither such modifications are present. The distal segments of fin rays 1 to 5 are flexible in the vast majority of investigated specimens.

**ARTIFICIAL KEY BASED ON ANDROPODIAL CHARACTERS**

For distinguishing the *Nomorhamphus* species of Sulawesi, this identification key is mainly based on features of the second anal-fin ray with emphasis on the spiculus. Five of 11 species are not clearly definable by andropodial features alone; these species are: *N. celebensis, N. kolonodalensis, N. lanceolatus, N. sagittarius,* and *N. towoetii.*
1. Pterygiophore angled anteriorly, physa absent, cryptoplica poorly developed, lack of black pigment at posterior anal-fin rays...

2. Pterygiophore not angled anteriorly, physa present, cryptoplica well developed in mature males

3. Spiculus clearly segmented at proximal end, short, not thickened, only slightly curved, contacts tip of 3rd fin ray

4. Spiculus clearly segmented, sickle-shaped, strongly curved, tip points ventrally, not in contact with tip of 3rd fin ray

5. Spiculus elongate, middle segments contact distal tip of 3rd fin ray

6. Spiculus elongate, distal tip contacts distal tip of 3rd fin ray

7. Spiculus elongate, segments 2-3'-7-10/11 with longitudinal groove separating segments into dorsal and ventral part

DISCUSSION

In Nomorhamphus, andropodial traits vary conspicuously with geographic proximity, and are consistent throughout morphologically similar groups of species that occur in sympatry (e.g., N. brembachi and N. liemii compared to N. rex). Species with similar andropodial traits tend to share a similar overall gestalt, and are often restricted to the same habitat (e.g., N. megarrhamphus and N. weberi). This pattern seems to be more common in Nomorhamphus than in Dermogenys, a genus with a much broader distribution throughout fresh and brackish waters of Southeast Asia (Meisner, 2001). Dermogenys species can be defined easily by the shape of their andropodium, as males of allopatric species show remarkable differences in the microanatomy of their anal fin (Meisner, 2001).

Meisner (2001) described the spiculus of N. brembachi and N. liemii, two morphologically similar species from the highland of Maros, as thick, short and laterally expanded. In this study, the spiculus was found to be segmented at its proximal end and lacking the contact to the tip of the third anal-fin ray. As mentioned by Meisner (2001), the dorsal portions of anal-fin rays two to four are curved in their distal half, which gives the andropodium a distinctly curved shape.

Huylebrouck et al. (2014) described two species from Sulawesi Tenggara with a lanceolate spiculus, which show remarkable similarities to N. ebrardtii, whose spiculus was previously reported to be elongate, curved ventrally and in contact to the third anal-fin ray (Meisner, 2001). Nomorhamphus lanceolatus can be distinguished by its shorter lower jaw and the black fin pigmentation, which is absent in N. sagittarius and N. ebrardtii (Huylebrouck et al. 2014). Nomorhamphus sagittarius has a deeper body and an elongate lower jaw compared to N. ebrardtii.

Nomorhamphus megarrhamphus and N. weberi, both endemic to the freshwater lakes To wuti and Matano, are characterised by a slender, elongate body and an extremely elongate lower jaw (Meisner, 2001). The findings of Brembach (1991), who described their cryptoplica as weakly developed and the absence of a physa, a pouch-shaped structure located between the third and fourth anal-fin ray, were confirmed in the present study. Brembach (1991) assumed the physa to be involved in sperm-storage, but its absence in mature males questions this hypothesis, as it seems not to be necessary for successful mating (Brembach, 1976, 1991). Another peculiarity of the two lake-dwelling species is their first anal pterygiophore, which is angled anteriorly at a sharper angle compared to all other species. Nomorhamphus weberi differs from N. megarrhamphus in having a sickle-shaped and unsegmented spiculus.

Nomorhamphus celebensis, N. kolonodalensis, and N. towoetti possess a relatively unspecic andropodium and are difficult to identify by this structure alone. Common to both is the straight spiculus, which is not thickened but elongate in large males (Brembach, 1991; Meisner & Louie, 2000; Meisner, 2001). It is distinctly segmented in most specimens of N. kolonodalensis and N. towoetti, but differs in N. celebensis in that only the proximal end is segmented. The spiculus is more or less parallel to the tip of the third anal-fin ray. In N. towoetti, substantial differences between four populations were recorded, mainly concerning the composition of the third and fourth anal-fin ray. Nomorhamphus towoetti is widely distributed in the Malili Lakes region and the Lake Poso region (Meisner, 2001) with some populations completely isolated from each other, favouring a high variability in morphological traits. In contrast, variability within andropodial features was absent in species with a small distribution, such as N. lanceolatus and N. brembachi.

Taxonomic relevance of the modified anal-fin rays. Brembach (1991) clearly underestimated the taxonomic potential of the andropodium in Nomorhamphus when stating that it offers no utility for species delimitation. Nevertheless, careful observations of the anal fin on the basis of cleared and stained specimens were not adequate to clearly distinguish all of the 11 species investigated. Thus, further parameters such as fin pigmentation and jaw length should be incorporated. Nomorhamphus rex has an andropodium unique among all Nomorhamphus described so far (Huylebrouck et al., 2012). It mainly differs from the andropodium of other species in that the number of segments proximal to the spines is greatly reduced to three or four remaining segments versus
seven or more in other *Nomorhamphus*. Thus, the condition present in *N. rex* is considered as a derived state, potentially originating from the fusion of a higher number of segments.

**Function of the andropodium.** In most of the rendered images, the tips of the first and second anal-fin rays, including the spiculus, were not visible, although the resolution was high (Fig. 2B, D). Accordingly, the fin rays of all cleared and stained specimens were unstained at their tips or stained with alcian blue exclusively (Fig. 2A, C). In detail, the last few segments of the first fin ray, at least the distal tip of the spiculus and the tip of the third fin ray appeared to be not completely ossified or to possess very thin ossifications that might have been decalcified during the staining process. Similar results were obtained in the studies by Brembach (1976) and Meisner (2001). Given that the modified rays somehow support the transfer of spermatozeugmata or even serve as a true copulatory organ, which is inserted into the female genital opening, the distal parts of the fin rays would have to be stiffened to enable the physical contact. Obviously, this is not the case in *Nomorhamphus*, as the distal segments of fin rays one to five are flexible in most specimens examined.

Additionally, the limited movability of the modified anal-fin rays and the rapidity of the mating process contradict the hypothesis of the andropodium having the function of a true intromittent organ (Brembach, 1976; Greven, 2010; Kelly & Moore, 2016). The greatly elongate genital papilla is more likely to enable the physical contact, whereas the cryptopilica and the modified anal-fin rays could help to orient the genital papilla in the direction of the female during mating, similar to the andropodium of goodeids (Meisner & Burns, 1997a). The limited movability of the genital papilla is probably compensated by the strongly modified anal-fin rays, conducting the spermatozeugmata in the right direction. In contrast, the halfbeak genera *Hemirhamphodon* and *Zenarchopterus* possess a less modified andropodium, as their muscular genital papilla meets the criteria of a true copulatory organ (Greven, 2010). Furthermore, the functional differences of the modified rays are minute between the species, as they are mainly restricted to the shape of the spiculus. According to Arnegist (1997), in animals exhibiting internal fertilisation, genital morphology of the males usually differs markedly among the species, even if these are quite similar in general morphology. As halfbeaks of the genus *Nomorhamphus* look indeed very similar, and some do occur in sympatry, hybridisation might be avoided by rapid evolution of genitalia, given that females are internally fertilised by the andropodium. The absence of such pronounced differences in male andropodial morphology within the genus weakens the hypothesis of the direct transfer of spermatozeugmata by the modified anal-fin rays.

**Conclusions.** For determining species-limits within *Nomorhamphus* and its sister clade, general external morphology in combination with the detailed examination of andropodium is useful. Not all species of *Nomorhamphus* endemic to Sulawesi can be determined by andropodial features alone, but the substructure of the modified anal-fin rays allows the classification of distinct groups, exhibiting similar morphology and distributional patterns. The use of this trait is highly recommended, as traditional meristic characters such as counting of scales and fin rays are less informative for morphologically similar species. Besides other findings, the indicated incomplete calcification of the modified fin rays and the resulting flexibility of the whole structure weaken the hypothesis of the andropodium serving as a true intromittent organ.

**Material examined.** *Nomorhamphus brembachi* – ZMH 7165, holotype, male, 37.6 mm SL; Indonesia, Sulawesi, Southeastern highlands of South-Sulawesi, mountain stream near village Longron, coll. D. Vogt, May 1978; ZMH 7166, paratype, 1 male, 37.0 mm SL; Indonesia, same data as ZMH 7165; MZB 14450, 2 males, 46.0–48.2 mm SL (CT scanned); Indonesia, Sulawesi Selatan, Simbang, Maros, Ta’deang River, destination Samanggi, coll. R. K. Hadiaty, 29 July 2007. Paratypes of *N. ravnaki ravnaki*: ZHM 7159, 1 male, 55.3 mm SL; ZMH 7160, 4 males, 33.7–39.0 mm SL; Indonesia, Sulawesi, highlands of Maros, Ban Timurung, coll. M. Brembach, August 1978. Paratypes of *N. ravnaki australis*: ZMH 7162, 1 male, 55.2 mm SL; ZMH 7163, 14 males, 32.9–56.5 mm SL (1, 33.2 mm SL, cleared and double stained [C&S]); Indonesia, Sulawesi, highlands of Maros, mountain stream near Bossolo, coll. D. Vogt, July 1979. Paratypes of *N. sanussi*: ZHM 7615, 1 male, 44.4 mm SL; ZMH 7616, 10 males, 38.3–49.3 mm SL (1, 40.3 mm SL, C&S); Indonesia, South-Sulawesi, mountain stream near to Segoya, coll. M. Brembach, August 1978.

*Nomorhamphus celebensis* – ZFMK 49216–49229, 5 males, 28.8–46.5 mm SL (1, 40.7 mm SL, CT scanned; 1, 39.4 mm SL, C&S); Indonesia, Sulawesi, stream crossing road from Tentena southward along East-shore of Lake Poso, 01°48’55.1’S 120°38’03.6’E, coll. F. Herder & B. Stelbrink, 9 September 2012; ZFMK 49230–49231, 2 males, 34.1–36.3 mm SL (1, 36.3 mm SL, CT scanned); Indonesia, Sulawesi, Poso river, 01°45.480’S 120°38.738’E, coll. F. Herder et al., 10 September 2012; ZFMK 49232–49235, 1 male, 34.9 mm SL; Indonesia, Sulawesi, Poso river, 01°45.480’S 120°38.738’E, coll. F. Herder et al., 10 September 2012; ZFMK 49293–49296, 2 males, 38.6–42.3 mm SL; Indonesia, Sulawesi, Poso-mountains near Tentena, Saloupa-waterfall, 01°45.146’S 120°32.498’E, coll. H.-G. Evers et al., 26 September 2010.

*Nomorhamphus ebrardtii* – ZFMK 49156–49176, 15 males, 42.3–46.7 mm SL (1, 43.0 mm SL, CT scanned; 1, 40.0 mm SL, C&S); Indonesia, Sulawesi Selatan, stream on Mallili Road, 2°38.161’S 121°12.920’E, coll. F. Herder et al., 4 May 2004; ZFMK 49287–49292, 2 males, 35.6–36.2 mm SL (1, 36.2 mm SL, CT scanned); Indonesia, Sulawesi Selatan, stream on Mallili Road, 2°38.161’S 121°12.920’E, coll. F. Herder et al., 4 May 2004.

*Nomorhamphus kolonodalensis* – ZFMK 48876–48944, 26 males, 25.9–44.0 mm SL (1, 36.1 mm SL, C&S); Indonesia, Sulawesi Selatan, near Nuha, 2°25.356’S 121°21.426’E, coll. J. Pfander & J. Schwarzer, 6 December 2002; ZFMK
Nomorhamphus lanceolatus – MZB 21299, holotype, male, 40.8 mm SL; Indonesia, Sulawesi, Southeast Sulawesi Province, Regency of Kolaka Utara, Wawolamo River, near the bridge on the road, between Kolaka and Kendari, 04°02.516'S 121°42.408'E, coll. R. K. Hadiyat et al., 8 July 2011; MZB 21300, paratypes, 3 males, 30.2–43.0 mm SL (1, 30.2 mm SL, CT scanned); same data as MZB 21299; ZFMK 49526–49529, paratypes, 2 males, 33.7–34.8 mm SL (1, 34.8 mm SL, C&S); same data as MZB 21299; ZMH 25920–25921, paratype, 1 male, 33.6 mm SL; same data as MZB 21299; WFB 3125–3128, paratypes, 2 males, 32.3–33.4 mm SL (1, 32.3 mm SL, CT scanned); same data as MZB 21299.

Nomorhamphus liemi – MZB 14473, 3 males, 41.1–47.5 mm SL (2, 41.1–47.5 mm SL, CT scanned); Indonesia, Sulawesi Selatan, Maros, well of Lampisip, coll. R. K. Hadiyat, 19 July 2007. Paratype of N. liemi liemi: ZMH 7618, 1 male, 40.3 mm SL; Indonesia, Sulawesi Selatan, highlands of Maros near Malawa, coll. D. Vogt, August 1978. Paratypes of N. liemi snijdersi: ZMH 7156, 1 male, 48.3 mm SL; Indonesia, Sulawesi Selatan, highlands of Maros near Malawa, coll. D. Vogt, August 1978; ZMH 7157, 14 males, 36.6–49.9 mm SL (1, 36.6 mm SL, C&S); Indonesia, South-Sulawesi, highlands of Maros near Malawa, coll. D. Vogt, August 1978.

Nomorhamphus megarrhamphus – ZMH 7152, paralecotype, 1 male, 48.3 mm SL; Indonesia, Sulawesi, Lake Towuti, coll. D. Vogt, July 1981; ZMH 7153, paratypes, 24 males, 37.4–47.9 mm SL (2, 43.4–44.0 mm SL, CT scanned; 1, 38.3 mm SL, C&S); same data as ZMH 7152.

Nomorhamphus rex – ZFMK 44944–44955, paratypes, 5 males, 28.9–41.5 mm SL (1, 41.2 mm SL, CT scanned; 1, 35.0 mm SL, C&S); Indonesia, Sulawesi, South Sulawesi Province, Wewu River, a headwater of the Cerekang River drainage west of Lake Matano, tributary at Village Laroeha, small river a few hundreds meters upstream of the main river, 2°28.226'S, 121°04.125'E, coll. F. Herder & R. K. Hadiyat, 4 May 2004; ZSM 41743a–d, paratypes, 2 males, 29.2–31.1 mm SL; same data as ZFMK 44944–44955; ZFMK 44956–44961, paratypes, 2 males, 35.7–36.8 mm SL; Indonesia, Sulawesi, South Sulawesi Province, Toleto River at the village of Toleto, at a truck washing place about 150 m upstream of a large river bridge at the road to Wasaponda, 2°31.664'S, 121°06.726'E, coll. F. Herder & R. K. Hadiyat, 4 May 2004; ZFMK 44962–44968, 3 males, 38.0–42.5 mm SL (1,42.5 mm SL, CT scanned; C&S); Indonesia, Sulawesi Selatan, Tana Toraja, in a clearwater pool at an excavation of a small river with unknown name near the village of Tilang, 3°02.126'S, 119°53.232'E, H.-G. coll. Evers et al., 24 September 2010.

Nomorhamphus sagittarius – MZB 21301, holotype, male, 42.8 mm SL; Indonesia, Sulawesi, Southeast Sulawesi Province, Regency of Kolaka Utara, District Kolaka, Village Ulunggolokka, Mangolo River, coll. R. K. Hadiyat et al., 5 July 2011; MZB 21302, paratype, 1 male, 30.5 mm SL; same data as MZB 21301; MZB 21303, paratypes, 2 males, 33.4–37.1 mm SL; Indonesia, Sulawesi, Mangolo River, about 500 m away from locality of holotype, 03°58.566'S 121°34.055'E, coll. R. K. Hadiyat et al., 5 July 2011; MZB 21304, paratypes, 2 males, 38.0–38.3 mm SL; Indonesia, Sulawesi, Regency of Kolaka Utara, District Kolaka, Village Ulunggolokka, TawoTawo River, 03°59.088'S 121°33.455'E, coll. R. K. Hadiyat et al., 7 July 2011; ZFMK 49530, paratype, 1 male, 37.2 mm SL (CT scanned); same data as MZB 21302; ZFMK 49532, paratype, 1 male, 36.7 mm SL (C&S); same data as MZB 21303; ZFMK 49534, paratype, 1 male, 38.9 mm SL; same data as MZB 21304; ZFMK 49536, paratype, 1 male, 39.7 mm SL; same data as MZB 21305; ZMH 25922, paratype, 1 male, 38.1 mm SL; same data as MZB 21304; ZMH 25924, paratype, 1 male, 32.3 mm SL; same data as MZB 21305; WFB 3129, paratype, 1 male, 39.3 mm SL (CT scanned); same data as MZB 21304; WFB 3131, paratype, 1 male, 33.3 mm SL; same data as MZB 21305; MZB 20443, 1 male, 35.2 mm SL (C&S); same data as MZB 21304; MZB 20452, 1 male, 41.2 mm SL (C&S); same data as MZB 21305.

Nomorhamphus towoetii – ZFMK 49254–49281, 8 males, 25.6–40.0 mm SL; Indonesia, Sulawesi, Saluro River, Petea tributary, 02°31.842'S 121°30.006'E, coll. F. Herder, 2 November 2002; ZFMK 49283–49286, 1 male, 40.1 mm SL; Indonesia, Sulawesi, coll. F. Herder, November 2002; ZFMK 48960–49000, 15 males, 25.5–41.6 mm SL (1, 30.5 mm SL, CT scanned); 1, 36.8 mm SL, C&S); Indonesia, Sulawesi, Poso River, 02°32.434'S 121°33.520'E, coll. F. Herder et al., 7 November 2002; ZFMK 49001–49045, 13 males, 29.5–45.9 mm SL; Indonesia, Sulawesi, 02°32.275'S 121°32.281'E, coll. F. Herder et al., 7 November 2002; ZFMK 49046–49077, 15 males, 31.0–44.2 mm SL; Indonesia, Sulawesi, 02°32.243'S 121°31.818'E, coll. F. Herder et al., 7 November 2002; ZFMK 49119–49146, 5 males, 39.4–45.0 mm SL (1, 40.2 mm SL, C&S); Indonesia, Sulawesi, 02°25.320'S 121°13.509'E, coll. F. Herder & R. K. Hadiyat, 4 November 2006; ZFMK 49078–49102, 7 males, 28.3–47.9 mm SL; Indonesia, Sulawesi, Lampaesu River, 02°35.358'S 121°40.440'E, coll. F. Herder, 13 November 2006; ZFMK 49147–49155, 2 males, 39.7–44.4 mm SL; Indonesia, Sulawesi, 02°25.320'S 121°13.509'E, coll. F. Herder & R. K. Hadiyat, 14 June 2010; ZFMK 49297–49299, 2 males, 37.6–41.2 mm SL (1, 37.6 mm SL, C&S); Indonesia, Sulawesi, 02°37.899'S 121°12.995'E, Balambano River, coll. H.-G. Evers et al., 28 September 2011; ZFMK 49177–49215, 17 males, 26.9–35.8 mm SL (1, 37.6 mm SL, CT scanned; 1,
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LITERATURE CITED


Fusion Confusion: The development of the caudal fin skeleton reveals multiple convergent fusions within Atherinomorpha

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Philipp Thieme\textsuperscript{1,2*}, Peter Warth\textsuperscript{3}, and Timo Moritz\textsuperscript{1,2}

\textsuperscript{1}Deutsches Meeresmuseum, Katharinenberg 14–20, 18439 Stralsund, Germany

\textsuperscript{2}Institut für Zoologie und Evolutionsforschung, Friedrich-Schiller-Universität Jena, Erbertstraße 1, 07743 Jena, Germany

\textsuperscript{3}Staatliches Museum für Naturkunde Stuttgart, Rosenstein 1, 70191 Stuttgart, Germany

* Correspondence: phil.thieme2016@gmail.com
Abstract

**Background:** The caudal fin of teleosts is a highly diverse morphological structure and a valuable source of information for comparative analyses. Within the Atherinomorpha a high variation of conditions of the caudal fin skeleton can be found. These range from complex but basal configurations to simple yet derived configurations. When comparing atherinomorph taxa, it is often difficult to decide on the homology of skeletal elements if only considering adult specimens. However, observing the development of caudal fin skeletons allows to evaluate complex structures, reveal homologies and developmental patterns, and even reconstruct the grundplan of the examined taxa.

**Results:** The development of the caudal fin skeleton was studied in different atheriniform, beloniform and cyprinodontiform species using cleared and stained specimens. Subsequently the development was compared to find similarities and differences in terms of 1) which structures are formed and 2) which structures fuse during ontogeny. For many structures, i.e. the parhypural, the epural(s), the haemal and neural spines of the preural centra and the uroneural, there were either no or only minor differences visible between the three taxa. However, the development of the hypurals revealed a high variation of fusions within different taxa which partly occurred independently in atheriniforms, beloniforms and cyprinodontiforms. Moreover, comparing the development of the ural centra exposed two ways of formation of the compound centrum: 1) in atheriniforms and *Oryzias* two ural centra develop that fuse during ontogeny while 2) in cyprinodontiforms and other beloniforms only a single ural centrum is formed during ontogeny.

**Conclusions:** We were able to reconstruct the grundplan of the developmental pattern of the caudal fin skeleton of the Atheriniformes, Beloniformes and Cyprinodontiformes as well as their last common ancestors. We found two developmental modes of the compound centrum within the Atherinomorpha, i.e. the fusion of two developing ural centra in atheriniforms and beloniforms and the development of only one ural centrum in cyprinodontiforms. Further differences and similarities for the examined taxa are discussed, resulting in the hypothesis that the grundplan of the caudal fin development of atherinomorphs is very much alike to that of atheriniforms.

**Keywords**

Ontogeny, Atheriniformes, Beloniformes, Cyprinodontiformes, Compound centrum, Hypural plate, Morphology
Background

Compared to other fish-like vertebrates, teleosts have a highly specialized caudal fin and, starting from a common bauplan, the caudal fin skeleton evolved a high morphological diversity within Teleostei [1-3]. Sometimes the morphological diversity is even very high within certain teleostean taxa, e.g. in Osteoglossomorpha [4] or in Paracanthopterygii [5]. Morphological studies on phylogenetic relationships of teleosts therefore often used the caudal fin skeleton as a rich source of information [6-9]. Also within the Atherinomorpha, comprising the Atheriniformes, Beloniformes and Cyprinodontiformes [10], an immense variety is present, ranging from a presumably basal condition, with distinct hypurals, e.g. in Odontesthes bonariensis [1: Fig. 168], to taxa in which most of the caudal fin skeleton is fused to one large compound structure, e.g. in Hypsolebias trilineatus [11: Fig. 3]. The evolution of the caudal fin skeleton within atherinomorphs however is not well understood and requires further study. Especially since in the light of current phylogenetic hypotheses, fusions and losses of different elements have to be considered to have happened multiple times independently within the group.

The monophyly of the Atherinomorpha is widely accepted and was first suggested almost 60 years ago, based on various character similarities, e.g. absence of pharyngobranchial 1 and attachment of Baudelot’s ligament to the basicranium [10], which are both shared by other taxa. In subsequent morphological phylogenetic analyses, the monophyly of atherinomorphs was confirmed multiple times [12-21] and synapomorphies such as “rostral cartilage decoupled from premaxilla” or “the absence of the third, fourth and fifth infraorbital” have been proposed [17, pp. 20-21]. Many, especially recent, molecular-genetic analyses also support the close relationship of atheriniforms, beloniforms and cyprinodontiforms [22-28]. In the past decades only few studies questioned the monophyly of Atherinomorpha by including representatives of other taxa, i.e. mugilids, cichlids, blennids and gobiesocids, though mostly with little support [29-32]. In the latest studies all these taxa are among Atherinomorpha and many other taxa positioned in the Ovalentaria [22-24]. The relationships within Ovalentaria presently remain largely unresolved, complicating outgroup comparisons for atherinomorph characters.

Within the Atherinomorpha, the Atheriniformes are considered to be the basal branching taxon while the Beloniformes and Cyprinodontiformes form a sister-clade and are regarded more derived [17, 19, 22, 25, 27, 28]. This view is challenged by recent studies based
on large molecular-genetic datasets: Betancur-R R, Wiley EO, Arratia G, et al. [23] and Hughes LC, Orti G, Huang Y, et al. [24] proposed that beloniforms are the basal branching taxon within atherinomorphs and atheriniforms and cyprinodontiforms are more derived sister taxa. However, morphological characters clearly support the basal position of atheriniforms which in many character complexes show the more basal condition, while beloniforms and cyprinodontiforms share reduced or fused conditions, e.g. further reduction of infraorbitals or the absence of the first pharyngobranchial, that are regarded more derived [10, 13, 14, 16, 17, 19, 20]. The caudal skeleton of atherinomorphs however is not understood well enough to draw an evolutionary scenario in the light of this phylogenetic framework. We therefore analysed the caudal skeleton of several ovalentarian taxa for comparison and especially the development of the caudal skeleton in representatives of Beloniformes, Cyprinodontiformes and Atheriniformes.

Developmental morphology is a powerful method to infer homology of elements and uncover characters [e.g. 33]. In the present study, we investigated the development of the caudal fin skeleton within all subgroups of atherinomorphs allowing detailed evaluation of the complicated caudal fin complexes found in several adult atherinomorph taxa. The results allow to trace the evolution of caudal fin development within this taxon, revealing homologous and convergent developmental patterns, and allowing to reconstruct the grundplan of the Atherinomorpha and its comprising taxa Atheriniformes, Beloniformes and Cyprinodontiformes.

**Material and Methods**

**Larval rearing and sampling**

Fish larvae of the species *Aplocheilus lineatus*, *Epiplatys annulatus*, *Glossolepis incisus*, *Oryzias woworae*, *Poropanchax normani* and *Pseudomugil furcatus* were reared at the facilities of the Deutsches Meeresmuseum in Stralsund, Germany. Fertilized eggs were collected constantly once per week from spawning mops, which were placed in each species tank respectively, and raised at room temperature (23-24 °C) and consistent water conditions of 400-500 µS and pH 7.2-7.5. For *A. lineatus*, *E. annulatus*, *O. woworae* and *Po. normani* first samples were taken before hatching occurred and there the eggshell was removed before further steps proceeded. All sampled specimens were euthanised using a benzocain-solution (Ethyl p-Amino Benzoate, E-1501, Sigma Aldrich, MO, USA). Afterwards they were fixed in 4% formaldehyde.
Specimens of *Hyporhamphus cf. limbatus* were sampled with a 500 μm mesh plankton net in mangrove creek channels in the Persian Gulf. The net was towed from a small boat at low speed for 5 to 10 minutes per tow. Larvae were immediately fixed in formaldehyde and later transferred to 70 % ethanol for long term storage at the Phyletisches Museum, Jena.

**Clearing and staining**

Specimens examined in this study were either reared (as stated above) at the Deutsches Meeresmuseum, taken from the ichthyological collection of the Deutsches Meeresmuseum, Stralsund (Table 1) or taken from the collection of the Phyletisches Museum, Jena (Table 1). Clearing and staining of the specimens principally followed the protocols of Dingerkus G and Uhler LD [34], Schnell NK, Konstantinidis P and Johnson GD [35] and Taylor WR and Van Dyke GC [36]. Reared embryos and larvae were transferred to 100% ethanol after fixation using an ascending ethanol series (30%, 50%, 70%). Collection material, which was stored in 70% ethanol, was directly transferred to 100% ethanol for clearing and staining. Afterwards specimens were stained for cartilage using an Alcian blue solution (2 parts glacial acetic acid and 8 parts 100% ethanol with 0.04 g/100 ml Alcian blue powder). Specimens were placed in this solution until the distal radials of the anal pterygiophores were stained distinctly blue, which took up to 3h for embryos and larvae and up to 16h for adults.

Before clearing, the specimens were put back into 100% ethanol and then transferred in a borate-solution (65% to 35% saturated borate solution/distilled water) via a descending ethanol series (70%, 50%, 30%). A trypsin solution was used for clearing (0.0375 g trypsin powder [1000-2000 BAEE units/mg, Sigma Aldrich, MO, USA] per 100ml diluted borax solution) of the specimens. Depending on size, it took up to 8h for embryos and larvae to clear, while adults took up to 5days. For bleaching, the specimens were placed in a 0.5% KOH solution to which 0.05 ml 30% H$_2$O$_2$ was added per 100 ml. After pigments were removed, the specimens were transferred into an Alizarin red solution (0.01 g Alizarin red powder per 100 ml 0.5% KOH) for bone staining. Lastly, the specimens were transferred into 1:2, 1:1 and 2:1 solutions of 100% glycerol to 0.5% KOH before being placed in 86.5% glycerol for documentation and storage.
Table 1: List of specimens from the Deutsches Meeresmuseum (DMM, if not indicated otherwise), Phyletisches Museum (PMJ) and Zoologisches Forschungsmuseum Alexander König (ZFMK) examined during this study. Length as Standard Length (SL) and as Notochord Length (NL, indicated by asterisk).

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**Imaging and documentation**

For documentation of the caudal fin development, embryos and larvae were photographed using a Leica M205 FCA with an attached Leica DMC6200 camera operated with the software Leica Application Suite (version: 3.6.0.20104). Additionally,
specimens of *Glossolepis incisus*, *Oryzias woworae* and *Poropanchax normani* were imaged using fluorescent light making use of the autofluorescent properties of Alizarin red. Adult specimens were photographed using a Canon EOS 80D with a Canon MP-E 65mm objective. Images were processed, without altering any morphological structures, and drawings were produced using Adobe Photoshop (version: 22.0.0). Figure plates were assembled in Adobe Illustrator (version: 25.0).

**Terminology**

The terminology used in this study in general follows the definitions given in Schultze H-P and Arratia G [8]. Differing from the former, we herein define the compound centrum as the most posterior vertebral centrum to which the lower and upper hypurals and the parhypural are connected (either fused to the vertebral centrum or articulating with it). The term does not infer any homology of the structure across taxa. Numbering of elements, e.g. the epurals, does not necessarily reflect the assumption of homology across taxa or an attribution to a certain body segment.

**Results**

**Atheriniformes: Glossolepis incisus**

The caudal fin skeleton of *G. incisus* (Fig. 1a) comprises the compound centrum (CC) and the preural centra 2, 3, 4, and 5 (PU 2-5) as well as the associated dorsal (except PU5) and ventral elements. Fused to each of the PU are a neural arch dorsally and a haemal arch ventrally with an elongated neural (NS) and a haemal spine (HS) respectively. The NS of PU2 is exceptional, as it is only about one third of the length of the other neural spines in the caudal region. The HS of PU2 is slightly broadened in lateral view. The shape of the CC is characterized by a half-hourglass shaped anterior portion and an upward-pointing posterior cone-like portion. The PH and the LHP are almost completely fused, with only a small gap remaining proximally, where they approach the CC. While the proximal part of the PH articulates with the anterior portion of the CC, the LHP is firmly fused to the CC posteriorly. Posterodorsally, hypural (HYP) 3, HYP4 and HYP5 articulate to the CC. HYP4 and HYP5 are fused along a well visible margin. Membranous extensions of the CC overlap the anterior HYP3 and HYP4 laterally. A reduced neural arch is fused dorsally to the anterior portion of the CC. One pair of uroneurals (UN) is present dorsally to the posterior portion of the CC and overlaps with HYP5.
laterally. Between the NS of PU3 and HYP5, two epurals (EU) are present. Between the distal tips of the HS of PU2 and PU3 the inter-haemal cartilage (IHC) 3 is present.

The development of the caudal fin skeleton of *Glossolepis incisus* starts with the appearance of cartilaginous precursors to the PH and HYP1, HYP2, HYP3 and HYP4 (Fig. 2a). At this stage already three principal fin rays are distinguishable. While the first vertebral centra start to ossify from anterior to posterior, the haemal arches and neural arches develop beforehand in the same direction. However, the neural arches develop slightly after the haemal arches. The haemal arch of PU2 emerges after the PH and HYP1-4 developed (Fig. 2b). Shortly after their appearance, the cartilaginous HYP1 and HYP2 fuse distally and later also proximally, forming the LHP (Fig. 2b). Proximal within the LHP a foramen persists. Five ventral and five dorsal principal fin rays can be distinguished. The cartilaginous precursors of EU1 and EU2 as well as the neural arch of PU2 develop next (Fig. 2b). Flexion of the notochord starts only after the development of these structures. Between the distal tips of the haemal arch of PU2 the associated haemal spine develops autogenous as a small cartilage. During ontogeny it enlarges gradually in ventral direction. The cartilaginous precursor to HYP5 appears dorsal to HYP4 (Fig. 2c). A cartilaginous connection between the proximal tip of the PH and the LHP is established. Also, the PH fuses distally to the LHP (Fig. 2c). Antero-dorsally to the LHP an ossification centre develops around the ventral surface of the notochord (Fig. 2c). This ossification centre represents ural centrum (UC) 1 and subsequently grows dorsally. Opposite to the first ossification centre on the notochord another one emerges and both grow towards each other to form a full centrum (Fig. 2d). There are now seven ventral and seven dorsal principal fin rays present. The vertebral centrum of PU2 forms next. First, an ossification centre emerges ventrally and later also dorsally. Anterior to HYP3 and HYP4
a ventral and a dorsal ossification centre develop around the notochord representing UC2. These ossifications also grow towards each other to form a full vertebral centrum (Fig. 3a). Ossification of the hypurals first start at the antero-dorsal portion of HYP1 (Fig. 2d). While HYP1 then gradually ossifies from anterior to posterior, ossification sites appear in all other hypurals and the PH and they too ossify from anterior to posterior (Fig. 2e). Anterior to UC2 the paired uroneural develops and then elongates in ventral and dorsal direction. The autogenous haemal spine of PU2 also ossifies in this stage. Anterior to its distal tip a cartilage emerges, the IHC3. The epurals start to ossify from the middle to the tips. The margins of the two ural centra get close together and fusion of these two centra starts (Fig. 2f). UC2 then gets shorter and a CC is formed (Fig. 2f, 3b). HYP4 and HYP5 first fuse distally, then proximal so that a foramen is formed, which later is reduced due to complete fusion of the two hypurals. The boundaries of each hypural nevertheless remain visible even in adults (Fig. 1a). The LHP starts to fuse to the CC. Proximal on the PH the parapophysis develops. The proximal cartilaginous part of the PH, connecting it to the LHP gets reduced and the PH grows proximally around the CC to which it then articulates. After all structures of the caudal fin skeleton

Figure 2: Development of caudal fin skeleton of the atheriniform Glossolepis incisus (DMM IE/16585). a) NL = 4.86 mm; b) SL = 6.13 mm; c) SL = 6.56 mm; d) SL = 6.48 mm; e) SL = 9.50 mm; f) SL = 15.01 mm. Abbreviations: CC, compound centrum; EU, epural; FR, fin ray; HA, haemal arch; HS, haemal spine; HYP, hypural; IHC, inter-haemal spine cartilage; LHP, lower hypural plate; NA, neural arch; NO, notochord; NS, neural spine; PH, parhypural; PU, preural centrum; UC, ural centrum; UN, uroneural. Scale bar: 200 μm.
are developed and most of them are ossified, the CC shrinks relative to the other elements, as the dorsal/posterior portion is reduced to a short upwards-directed horn. The uroneural grows dorsally and overlies the HYP4 and HYP5 laterally. From the CC a triangular outgrowth is formed, which covers the proximal margin of HYP3 laterally (Fig. 1a).

Alongside *Glossolepis incisus* other atheriniform species were examined: *Atherina presbyter*, *Bedotia geayi*, *Iriatherina werneri*, *Leuresthes tenuis*, *Marosatherina ladigesi*, *Melanotaenia lacustris*, *Pseudomugil furcatus*. The development of the caudal fin skeleton in these taxa is very similar to that found in *G. incisus*. The closely related melanotaeniid *M. lacustris* shows no differences in the development while in the other melanotaeniid *I. werneri* HYP3 distally fuses to HYP4 very late in ontogeny. During the ontogeny of the telmatherinid species *M. ladigesi* the PH does not fuse to the LHP and remains separated from the CC in adult specimens. HYP3, HYP4 and HYP5 stay separate and do not fuse to the CC, too. The development in the pseudomugilid *P. furcatus*, shows remarkable differences to that of *G. incisus*: HYP1 and HYP2 do not develop separately but form the LHP from the beginning on; the PH develops as a portion of this LHP and is only distinguishable from it by a small proximal notch (Fig. 4a); HYP3 and HYP4 develop as separate structures but the cartilages almost immediately

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Figure 3: Alizarin-red autofluorescence showing the development of the ural centra of *Glossolepis incisus* (a, b), *Oryzias woworae* (c, d) and *Poropanchax normani* (e, f). a) DMM IE/16585, SL = 7.76 mm; b) DMM IE/16585, SL = 15.01 mm; c) DMM IE/16587, SL = 3.78 mm; d) DMM IE/16587, SL = 7.28 mm; e) DMM IE/16586, SL = 3.32 mm; f) DMM IE/16586, SL = 3.43 mm. Abbreviations: CC, compound centrum; UC, ural centrum. Scale bar: 200 μm.
Figure 4: Developmental stages of additional atheriniform (a-d), beloniform (e, f) and cyprinodontiform (g-j) species. a) *Bedotia geayi*, DMM IE/16583, NL = 4.86 mm; b) *B. geayi*, DMM IE/16583, SL = 10.49 mm; c) *Pseudomugil furcatus*, DMM IE/16582, SL = 3.38 mm; d) *P. furcatus*, DMM IE/16582, SL = 5.89 mm; e) *Oryzias sinensis*, DMM IE/16499 SL = 13.86 mm; f) *Belone belone*, DMM IE/16512 SL = 25.27 mm; g) *Aplocheilus lineatus*, DMM IE/16584, SL = 3.72 mm; h) *A. lineatus*, DMM IE/16584, SL = 6.81 mm; i) *Aphyosemion striatum*, DMM IE/16581, SL = 2.93 mm; j) *Epilatys annulatus*, DMM IE/16588 SL = 3.19 mm. Abbreviations: CC, compound centrum; EO, extra caudal ossicle; EU, epural; HA, haemal arch; HS, haemal spine; HYP, hypural; IHC, inter-haemal spine cartilage; LHP, lower hypural plate; NA, neural arch; NO, notochord; NS, neural spine; PH, parhypural; PU, preural centrum; UC, ural centrum; UHP, upper hypural plate; UN, uroneural. White arrows indicate duplicated NA and NS. Scale bar: 200 μm.
fuse to form the upper hypural plate (UHP); during the ossification of the LHP and the UHP both fuse onto the developing centrum anterior to each of them (Fig 4b). No HYP5 is developed during ontogeny. The bedotiid *B. geayi* retains separated HYP3, HYP4 and HYP5 during ontogeny and the fusion of HYP1 and HYP2 to form the LHP happens very late in ontogeny during the ossification of these structures. In the atherinid *A. presbyter* the PH develops separated from the LHP and does not fuse to it. Also, HYP3, HYP4 and HYP5 remain separated and do not fuse to the CC. Similar, during the development of the atherinopsid *L. tenuis* the PH remains separated from the LHP and HYP3, HYP4 and HYP5 do not fuse.

**Beloniformes: Oryzias woworae**

In adult specimens of *Oryzias woworae* (Fig. 1b) the caudal fin skeleton comprises PU2, PU3, PU4 and the CC. Fused to each PU are a neural arch with an elongated NS and a haemal arch with an elongated HS. The haemal spine of PU2 is enlarged. The CC is characterized by its shape: the anterior portion is shaped like a half hourglass centrum, while the posterior portion is similar to an upwards-pointing cone. Ventrally the PH articulates to the CC. Postero-ventrally the LHP is fused to the CC and posteriorly the UHP is fused to the CC. A reduced neural arch is present on the CC dorsally. Antero-dorsally to the UHP two EU are present. Additionally, between the HS of PU2 and the HS of PU3 an extra caudal ossicle is present (EO). Between the distal tip of the EO and the distal tip of the HS of PU2 the IHC3 is present (not stained in Fig. 1b).

The first elements of the caudal fin skeleton to develop in *Oryzias woworae* are the cartilaginous precursors to the LHP and UHP which appear only after the flexion of the notochord has started (Fig. 5a). No separate HYP1 or HYP2 and no separate HYP3, HYP4 or HYP5 are visible during development at any time. The next structures to emerge are the cartilaginous PH, which is separated from the notochord and the LHP, the cartilaginous haemal arch of PU2 as well as the cartilaginous EU2 (Fig. 5b). Two ossification centres ventral to the notochord and anterior to the LHP and the UHP, respectively, develop, which represent UC 1 and UC2 (Fig. 5b). The centra of PU2 and PU3 are formed in sequence with the rest of the vertebral centra and emerge slightly after the ural centra, which ossify around the notochord from ventral to dorsal (Fig. 3c, 5c). The ossification of the hypural plates starts after the formation of the ural centra and the plates directly fuse to their anterior centrum (Fig. 5c, d). The haemal spine of PU2 develops as an autogenous cartilage between the tips of the respective haemal arch halves. The neural arch of PU2 develops shortly after the development of the
Figure 5: Development of caudal fin skeleton of the beloniform *Oryzias woworae* (DMM IE/16587). a) NL = 2.77 mm; b) SL = 3.48 mm; c) SL = 3.84 mm; d) SL = 3.91 mm; e) SL = 5.79 mm; f) SL = 6.54 mm. Abbreviations: CC, compound centrum; EO, extra caudal ossicle; EU, epural; FR, fin ray; HA, haemal arch; HS, haemal spine; IHC, inter-haemal spine cartilage; LHP, lower hypural plate; NA, neural arch; NO, notochord; PH, parhypural; PU, preural centrum; UC, ural centrum; UHP, upper hypural plate. Scale bar: 200 μm.

Figure 6: Development of the caudal fin skeleton of the beloniform *Hyporhamphus cf. limbatus*. a) SL = 3.65 mm; b) SL = 7.23 mm; c) SL = 10.15 mm; d) SL = 11.91 mm. Abbreviations: EU, epural; FR, fin ray; HA, haemal arch; HP, hypural plate; HS, haemal spine; LHP, lower hypural plate; NA, neural arch; NO, notochord; PH, parhypural; PU, preural centrum; UC, ural centrum; UHP, upper hypural plate. Scale bar: 200 μm.
centrum is completed (Fig. 5d). Postero-dorsally to UC2 cartilaginous cells develop at the tip of the notochord. These cells are distinct from the rest of the notochord and later ossify as part of UC2. The parhypural ossifies and the haemal spine of PU2, which is proximally surrounded by the haemal arch of PU2, also starts ossifying and fuses to the haemal arch (Fig. 5e). EU2 has grown and starts ossifying, too. Anterior to it, EU1 emerges a small cartilage and ventrally a cartilage develops anterior to the distal tip of the haemal spine of PU2, representing the precessor of the extra caudal ossicle (EO; Fig. 5e). On UC1 a neural arch develops and both ural centra grow towards each other. Then, they start fusing to form a CC (Fig. 3d, 5f), where the margins of the two UC remain visible. Both, EU1 and the EO, have grown and start to ossify. Between the EO and the haemal spine of PU2 the inter-haemal spine cartilage 3 develops. In the further course of ontogeny, the CC shrinks as mostly the posterior portion is reduced in length. The PH grows towards the ventro-lateral margin of the CC and articulates to it. A tiny parapophysis develops on the proximal part of the PH.

Additional to Oryzias woworae, late developmental stages of Belone belone and Oryzias sinensis as well as an ontogenetic series of Hyporhamphus cf. limbatus were available for examination. While the specimens of O. sinensis suggest a similar development of the caudal fin structures as in O. woworae (Fig. 4e), the ontogenetic series of H. limbatus indicates some differences compared to the development of O. woworae. In preflexion larvae, single hypurals (i.e. HYP1, HYP2, HYP3 and HYP4) develop which in flexion and postflexion stages fuse to form the LHP and UHP (Fig. 6a, b). HYP5 develops after the ossification of the other hypurals has begun. After UC1 has emerged, UC2 develops from a dorsal ossification center only, which is in close contact to UC1 (Fig. 6b). Afterwards UC2 grows dorsally towards the tip of the notochord and posteriorly towards the UHP (Fig. 6c), while fusing with UC1 anteriorly. When UC2 has fully surrounded the notochord it also fuses with UC1 posteriorly (Fig. 6d). There are no traces of the margins of the two UC left after the fusion is completed. Additionally, an UN develops which in course of ontogeny enlarges and obtains a triangular shape (Fig. 6c, d). Three EU develop in H. limbatus of which the most anterior one develops temporally shifted after the other two (Fig. 6b, c). During the development of the PH a cartilaginous connection between the proximal tip of the PH and the LHP is present. In B. belone five hypurals develop also before HYP1 and HYP2 fuse to form the LHP while HYP3, HYP4 and HYP5 remain separate (Fig. 4f). The CC has already formed in the examined larval stages. Based on the shape of the CC in these larvae compared to H. limbatus it can be assumed that U1 and U2 developed and fused. An EO does not develop in H. limbatus and B. belone.
Cyprinodontiformes: Poropanchax normani

The caudal fin skeleton of adult Poropanchax normani (Fig. 1c) comprises three preural vertebra (PU2, PU3 and PU4) and the CC. Fused to each PU are a neural arch and a haemal arch which each have elongated unpaired spines. The shape of the CC is characterized by an anterior portion that is formed like a half hourglass and a posterior portion that can best be described as an upward-pointing cone. Ventrally the PH articulates to the CC and posteriorly one large hypural plate (HP), which has a characteristic foramen in its anterior middle portion, is fused to the CC. A small uroneural is fused to the CC dorsally. Above the CC there is one EU. In adult specimens there are 5 lower and 5 upper principal caudal fin rays and 6-7 ventral and 6 dorsal procurrent fin rays.

The development of skeletal structures in the caudal fin of Poropanchax normani begins after the flexion of the notochord started. First elements to emerge are the cartilaginous hypurals that represent the LHP and the UHP. Anterior to the LHP a separated cartilage, the PH, develops (Fig. 7a). These structures then grow and first fin rays develop (at the beginning two associated with each hypural plate). The hypural plates grow towards each other.
proximally and distally and fuse, leaving a foramen in their middle (Fig. 7b). The neural and haemal arches of PU2 to PU4 form in series with the anterior arches in cartilage. An epural emerges dorsally opposite to the parhypural (Fig. 7c). Between the distal tips of the most posterior neural and haemal arches additional cartilaginous elements are present, representing autogenous neural and haemal spines. On the dorsal side of the notochord, anterior to the hypural plate, an ossification centre emerges, which signalizes the development of the ural centrum (Fig. 3e, 7c). While the caudal tip of the notochord shortens, the hypural plate grows dorsally filling the resulting space. Opposite to the first ossification centre, ventral on the notochord, another ossification centre develops (Fig. 7d). Both grow towards each other to form the ural centrum (Fig. 3f). The vertebra centra ossify from anterior to posterior and the centra of PU2 to PU4 develop last in this sequence. The hypural plate ossifies very fast from anterior to posterior (Fig. 7d, e). The cartilages between the neural and haemal arches of the posterior centra grow distally and form elongated neural and haemal spines (Fig. 7e). The neural and haemal arches ossify and fuse to the respective centra (Fig. 7e, f). Dorsally on the ural centrum the paired uroneural develops which later additionally fuses to the hypural plate. The neural and haemal spines as well as the parhypural and epural ossify last. The autogenous neural and haemal spines fuse to their respective arch while ossifying (Fig. 7f). The PH grows dorsally towards the ural centrum and articulates to it (Fig. 1c). A parapophysis develops proximo-lateral on the PH. The foramen in the hypural plate remains but gets smaller during growth. The ural centrum shortens posteriorly resulting in a half-centrum anteriorly and a dorsally pointing cone posteriorly (Fig. 1c).

Alongside Poropanchax normani other cyprinodontiform species were examined: Aplocheilus lineatus, Epiplatys annulatus, Aphyosemion striatum and Pachypanchaxomalontus. The caudal fin development in cyprinodontiforms in general is very similar. In the aplocheilid A. lineatus the LHP develops as one entity but HYP3 and HYP4 develop separately before fusing later in development. No HYP5 and no uroneural get developed though. In late developmental stages of the second examined aplocheilid P. oمالونتوس the LHP and the UHP (unclear if HYP3 and HYP4 develop separately) fuse to form one large HP. The development of the caudal fin skeleton in the nothobranchids Epiplatys annulatus and Aphyosemion striatum is similar. The LHP and UHP develop as single entities respectively and then fuse anteriorly. The single ural centrum that develops, first appears centered anterior to the LHP and UHP. The development of a pair of uroneurals is not observed.
Discussion

**Atheriniform caudal fin development**

The development of the caudal fin skeleton is largely consistent throughout the analysed atheriniforms. In most of the examined species five hypurals develop as separate entities. The LHP is then formed by fusion of HYP1 and HYP2. The upper hypurals (HYP3, HYP4 and HYP5) show different grades of fusion in different species, e.g. HYP4 and HYP5 fuse in *G. incisus* and HYP3 and HYP4 fuse in *I. werneri* as well as in *Atherina harringtonensis* [37]. An exception is *Pseudomugil furcatus* in which two hypural plates (LHP and UHP) are present but no separate hypurals develop as individual entities during any stage of ontogeny.

In all examined atheriniforms (i.e. *Atherina presbyter, Bedotia geayi, Iriatherina werneri, Leuresthes tenuis, Marosatherina ladigesi, Melanotaenia lacustris, Pseudomugil furcatus*), we observed two separate ural centra in late flexion to early postflexion larval stages. These initially separated centra fuse in later stages to form the CC. This was also reported by Parenti LR [16], who described that in developmental stages of *Phenacostethus* and *Dentatherina* two ural centra are present. Two studies, on the development of the caudal skeleton in atheriniforms i.e. *Atherina harringtonensis* [37] and *Leuresthes tenuis* [38] did not report this detail specifically. Neither the depicted specimens of *Atherina harringtonensis* nor the description [37] give information on the presence of two separate ossifications. This may be owed to the relatively short frame during development in which the separate ossification centers are observable and the limited material available. However, the stipplings in the drawing of the latter study [38: Fig. 1] indicate the formation of two separate UC, thereby supporting our findings. We therefore conclude that the presence of two separate ural centra during ontogeny is a general atheriniform character. Parenti LR [16] assumed that PU1 and UC1 fuse into the anterior of these centra. In our examined specimens there was no sign of developing PU1 and we conclude that PU1 is never developed.

The PH develops as an autogenous cartilage that initially has no connection to the notochord/UC1/CC or HYP1/LHP. During development a common cartilaginous base is formed that connects the PH and the LHP proximally and further articulates both structures to the notochord and subsequently to UC1 and then to the CC. This cartilage is later reduced and the PH is separated from the LHP again and articulates with the CC. In few species the PH fuses to the LHP distally, i.e. *G. incisus*. After the reduction of the cartilage connecting the PH and the LHP, the latter fuses to the CC (or UC1) in all examined species and also in *A. harringtonensis* [37].
Beloniform caudal fin development

The herein documented development of the caudal fin skeleton of *Oryzias woworae* is consistent with that of *Oryzias latipes* as described by Fujita [39]. Despite the availability of several smaller specimens, we could not find separate HYP1 and HYP2 and suspect that the LHP of *Oryzias* is a product of evolutionary fusion of HYP1 and HYP2. An evolutionary fusion of HYP1 and HYP2 therefore seems present in Adrianichthyidae. In the hemiramphid *Hyporhamphus cf. limbatus* HYP1 and HYP2 develop as separate entities before they fuse to form the LHP and we suspect a similar development in *Belone belone* (Belonidae), and *Hyporhamphus sajori* (Hemiramphidae), where HYP1 and HYP2 are already fused distally in the examined specimen [39, 40]. In *Cypselurus doederleini* (Exocoetidae) [41] and *Cololabis saira* [42] a LHP that formed by the fusion of HYP1 and HYP2 was reported, but at hatching the LHP was already formed and it is unclear if HYP1 and HYP2 develop separately before. The character state in the grundplan of beloniforms is therefore debatable. In the evolutionary framework of Atherinomorpha either two evolutionary fusions of HYP1 and HYP2 must have occurred (stem groups of Cyprinodontiformes and Adrianichthyidae) or one evolutionary fusion in the stem group of the Cyprinodontea and a subsequent separation in Belonoidei. We believe that the evolution of such a fusion is more likely than an evolutionary separation with a developmental fusion. We therefore consider the developmental pattern of separately developing HYP1 and HYP2 and a subsequent fusion during development, as shown for *Hyporhamphus cf. limbatus*, as part of the grundplan of Beloniformes.

The components of the UHP of *Oryzias* are not that easy to conclude as it could either comprise HYP3, HYP4 and HYP5 or only HYP3 and HYP4, which would include the presumption that HYP5 is reduced [39]. In the belonids *B. belone* and *Cololabis saira* and the hemiramphids *H. sajori* and *H. limbatus* HYP3, HYP4 and HYP5 develop separately and HYP3 and HYP4 fuse to form the UHP [40, 42]. In the exocoetid *C. doederleini* the UHP is already present at hatching and its components remain unclear [41], while in another exocoetid, *Parexocoetus mento*, two upper hypurals, presumably HYP3 and HYP4, are present and fuse to form the UHP [43]. HYP5 is not developed in either of these two taxa. It seems likely that the UHP in *Oryzias* is a product of fusion of these HYP3 and HYP4 and that HYP5 is completely reduced.

The CC in all examined *Oryzias* species is a product of the fusion of UC1 and UC2. While Fujita [39] assumed that PU1 is part of the anterior ural centrum, we inferred it to comprise only UC1, as there are no signs of the occurrence of a separate PU1 during ontogeny. In *C.
saira, Cy. doederleini and H. sajori only one UC supposedly develops [40, 42]. However, studying the development of H. limbatus we found two UC which fuse to form the CC. This contradicts these previous results and at least supports the assumption that in hemiramphids two UC are present during development. Comparing the late developmental stages of B. belone to the H. limbatus, it seems possible that the CC is also the product of fusion of UC1 and UC2. However, the developmental data for C. saira contradicts this assumption, leaving the presence of two UC at the evolutionary base of the belonids in question. The condition in the grundplan of the Beloniformes, however, still seems to be the presence of two ural centra as the reduction of one centrum or the evolutionary fusion of both centra seems more likely than the resurgence of one centrum within two families of beloniforms.

The development of other caudal fin skeleton structures is similar in Oryzias and the other studied beloniform species [39-43]. Exceptions are the development of an UN as well as the presence of a third EU which both are not present in adrianichthyids but in all other beloniforms [39-44]. The development of an extra caudal ossicle is restricted to Adrianichthyidae and is an autapomorphy of this family [39, 44].

**Cyprinodontiform caudal fin development**

A variation in the pattern of hypural formation was observed among the cyprinodontiform species studied in here. While in Aplocheilus lineatus the LHP and HYP3 and HYP4 develop, only two separated elements, the LHP and UHP, develop in Aphyosemion striatum, Epiplatys annulatus and Poropanchax normani. For Fundulus xenicus it is reported that only a single hypural plate develops [45]. In none of the examined species HYP5 is present during any point of ontogeny. It can be assumed that in the grundplan of cyprinodontiforms HYP5 was already reduced and that HYP3 and HYP4 developed as separated entities, much like in Aplocheilus lineatus. A common feature of cyprinodontiform development is the development of only one ural centrum, which emerges centred anterior to the LHP and UHP/HYP3 & HYP4. An uroneural, which is present in Poropanchax normani, however, does not develop in the other examined species.

**Grundplan of the caudal fin skeleton in Atherinomorpha**

The independent development of the lower hypurals (HYP1 and HYP2) is a shared character of atheriniform species [37, 38] and beloniform species [39-42]. In these taxa HYP1 and HYP2 fuse to form the LHP during ontogeny (Fig. 8). In the examined adrianichthyids
and cyprinodontiforms the LHP seemingly develops without prior separated hypurals. As we concluded that in beloniforms HYP1 and HYP2 develop separately which is also the case in atheriniforms, the evolutionary fusion of HYP1 and HYP2 evolved parallel in adrianichthyids and at the base of the cyprinodontiforms (Fig. 8). In the grundplan of the Atherinomorpha HYP1 and HYP2 develop separately and fuse later in ontogeny. A difference in the way the LHP is developed is not visible between adult atheriniforms (Fig. 9a-d) [1, 11, 12, 14-16, 18, 20, 21, 46-50], most adult beloniforms (Fig. 9e,f,h) [1, 11, 14, 16, 51] and the adult cyprinodontiforms where the LHP and UHP are not fused (Fig. 9i-l) [11, 14, 52]. In adult specimens of the beloniform *B. belone* a foramen in the LHP still indicates the fusion of two formerly separated bones (Fig. 9g).

Figure 8: Scheme of the evolution of the caudal fin skeleton development within Atherinomorpha, showing hypothetical developmental stages with all developing structures presented for extant taxa and the reconstructed grundplan of atherinomorphs. Adult caudal fin skeletons are presented for extant taxon groups. For extant taxa hypothetical developmental stages show all developing elements. Colour code: CC, grey gradient; EO, pink; EU, dark blue; HA, grey-brown; HS, light grey-brown; HYP1 & HYP2 / LHP, yellow; HYP3 & HYP4 / UHP, orange; HYP5, red; IHC3, mint NA, brown; NO, white; NS, light brown; PH, light blue; PU, light grey; UC1, dark grey; UC2, semi-light grey; UC, grey; UN, violet.
The upper hypurals (HYP3, HYP4 and HYP5) develop separately at the base of atheriniforms and at the base of beloniforms. At the base of the cyprinodontiforms presumably only two upper hypurals (HYP3 and HYP4) develop. We conclude that in the grundplan of the Atherinomorpha three upper hypurals get developed and that the reduction of HYP5 occurred at the base of the Cyprinodontiformes (Fig. 8). In adult specimens the separated upper hypurals are still visible in many atheriniform taxa (Fig. 9a-c) [20, 47]. In very few adult beloniforms there are also separated upper hypurals present, i.e. B. belone and Tylosurus crocodilus [1]. In zenarchopterids and exocoetids HYP3 and HYP4 are fused to a UHP (in many species only partially) and in some species HYP5 is also fused to the UHP [51]. In the scomberesocid Cololabis saira HYP5 remains separated from the UHP [1, 42]. In adrianichthyids HYP5 seems to be reduced [1, 11]. In most cyprinodontiforms an UHP is present, which is composed of HYP3 and HYP4. Exceptions are for example Aplocheilus lineatus and Epiplatys steindachneri where HYP3 and HYP4 remain separated [11]. No HYP5 is distinguishable in any cyprinodontiform species.

A common ontogenetic character of atheriniforms and beloniforms is the development of two ural centra which fuse to form the compound centrum during ontogeny. In cyprinodontiforms only one ural centrum is developed. In the grundplan of the Atherinomorpha two ural centra are developed and fuse to form the compound centrum (Fig. 8). We cannot be sure if the one ural centrum that is developed in cyprinodontiform species is the result of evolutionary fusion of both or due to the reduction of either UC1 or UC2. The position of the developing ural centrum, centred anterior to the LHP and UHP, would support the first case, as in atheriniforms and beloniforms UC1 and UC2 develop anterior to the lower hypurals and upper hypurals respectively. The fusion of the two UC could be expected to develop in an intermediate state. If the second case applies, it would be almost impossible to homologize the developing ural centrum with either UC1 or UC2 in atheriniforms and beloniforms. In adult specimens of all three taxa, the compound centrum of atheriniforms and beloniforms and the ural centrum of cyprinodontiforms are not distinguishable by their shape, which can be described as an anterior half centrum and a posterior upward-pointing cone (Fig. 9). This would also support the hypothesis that the ural centrum of cyprinodontiforms is the result of evolutionary fusion. A PU1 is neither developed separately in any of our examined species nor in any of the previously studied species [37-42, 45]. Although it was hypothesised by some of these authors that PU1 is part of the CC in some species, we found
no evidence that would support this hypothesis.

Further similarities of atheriniforms, beloniforms and cyprinodontiforms which are also part of the grundplan of the Atherinomorpha include the autogenous development of the parhypural and the epurals as well as the autogenous development of at least the haemal and neural spines of PU2-PU5 (Fig. 8).

Figure 9: Adult caudal fin skeleton of atheriniforms (a-d), beloniforms (e-h) and cyprinodontiforms (i-l). a) Membras martinica, DMM IE/11398 SL = 49.0 mm; b) Atherina hepsetus, DMM IE/16510 SL = 70.32 mm; c) Bedotia geayi, DMM IE/16309 SL = 63.13 mm; d) Pseudomugil furcatus, DMM IE/16311 SL = 41.14 mm; e) Dermogenys siamensis, DMM IE/16502 SL = 27.15 mm; f) Nomorhamphus kolonodalensis, ZFMK 49237-53, SL = 29.08 mm; g) Belone belone, DMM IE/16519 SL = 84.72 mm; h) Xenentodon cancila, DMM IE/16509 SL = 95.66 mm; i) Aplocheilus lineatus, DMM IE/… SL = 42.08 mm; j) Ameca splendidus, DMM IE/16535 SL = 37.91 mm; k) Anableps macrolepis, DMM IE/14934 SL = 68.4 mm; l) Aphyosemion bitaeniatum, DMM IE/16522 SL = 20.93 mm. Abbreviations: CC, compound centrum; EU, epural; HP, hypural plate; HYP, hypural; IHC, inter-haemal spine cartilage; INC, inter-neural spine cartilage; LHP, lower hypural plate; PH, parhypural; PU, preural centrum; UHP, upper hypural plate; UN, uroneural. White arrows indicate duplicated NA and NS. Scale bar = 1 mm.
To recap, the grundplan of the caudal fin development of the Atherinomorpha includes: 1) the development of five hypurals of which HYP1 and HYP2 fuse to form the LHP; 2) the development of two ural centra which fuse to form the CC; 3) the absence of PU1 during ontogeny, 4) the development of an autogenous PH; the development of autogenous HS and NS of at least PU2 to PU5; 5) the development of two autogenous EU and 6) the development of IHC3 (Fig. 8).

**Comparison to ovalentarian taxa**

The Atherinomorpha have been considered a monophyletic group almost throughout the last 60 years [10, 12, 13, 16, 17, 19, 20, 22-28, 53] but their phylogenetic position within Percomorphaceae remains uncertain and many taxa were proposed as their sister-taxon or otherwise closely related. Betancur-R, Broughton RE, Wiley EO, *et al.* [22] and Betancur-R, Wiley EO, Arratia G, *et al.* [23] provide convincing evidence for the Atherinomorpha as part of the Ovalentaria. Support values for ovalentarian intrarelationships are for most cases quite low. Possible sister-taxon relationships previously suggested for atherinomorphs and still congruent with the recent molecular investigations include the Mugilidae [13, 19, 20, 25, 27], the Blennioidei and Gobiesocidae [26], the Cichlidae, Embiotocidae and Pomacentridae [28], while recent studies suggest that the Cichlidae [24] or the group comprising Cichlidae, Polycentridae and Pholidichthyidae [22, 23] are more closely related to the Atherinomorpha.

For many of the contemplable taxa studies on the development of the caudal fin are scarce or missing. For blenniids [54], cichlids [55, 56] and clinids [57, 58] some ontogenetic data, and for mugilids [59] and pomacentrids [60] detailed description are available. Similarities between the caudal fin development of these taxa and the Atherinomorpha include autogenous development of the parhypural and the epurals, the autogenous development of some haemal and neural spines of the preural centra (i.e. PU2 and PU3 in mugilids and at least PU2 and PU3 in blenniids, cichlids and pomacentrids) [56, 57, 59, 60]. In the cichlids examined for this study (*Amatitlania nigrofasciata*, *Geophagus* sp., *Hemichromis bimaculatus*), the HS and NS of PU2 and PU3 develop autogenous. A cartilaginous bridge connects the proximal tip of the PH to the proximal tip of HYP1 during ontogeny in atheriniforms. Such a connection is also present in cichlids, clinids, mugilids and pomacentrids [54, 56, 59, 60] suggesting that at the base of the Atherinomorpha such a connection was present and got reduced within beloniforms and cyprinodontiforms.
At the base of the Atherinomorpha five hypurals are present during development and HYP1 and HYP2 fuse to form the LHP. Five hypurals also can be seen during ontogeny in cichlids, some clinids, e.g., *Clinus cottoides*, mugilids and pomacentrids [56, 57, 59, 60]. While in cichlids no hypural-fusion occurs and the hypurals remain separated in adults, HYP1 and HYP2 fuse to form the LHP in clinids, mugilids and pomacentrids. In clinids this fusion occurs early in development and additionally the PH fuses to the LHP. The fusion of the lower hypurals to form the LHP could be a character that positions the clinids, mugilids and pomacentrids closer to the Atherinomorpha. Fusion of the upper hypurals happens in clinids and mugilids, where HYP3 and HYP4 fuse to form the UHP. Although such a fusion occurs in beloniforms and cyprinodontiforms too, it seems likely that this trait evolved independently within the atherinomorphs and clinids/mugilids based on the well supported monophyly of the Atherinomorpha. In blenniids, the LHP and UHP develop without separated hypural-precursors [54]. Apparently, this is also a separately acquired character in blenniids and cyprinodontiforms.

The compound centrum in atherinomorphs develops by fusion of UC1 and UC2. Within the Ovalentaria a similar development is only known from mugilids, where UC1 emerges anterior to the lower hypurals and UC2 anterior to the upper hypurals and both fuse and later form a CC with an identical shape to the CC of atherinomorphs [59]. In the other previously studied ovalentarian taxa, only one elongated ural centrum develops that covers the notochord from the beginning of the PH almost to the caudal tip of the notochord [54-58, 60]. During ontogeny this centrum also shortens and in adults has a similar shape as in atherinomorphs and mugilids [1]. The similar development in atherinomorphs and mugilids could indicate a closer relationship of these taxa or a shared plesiomorphic character absent in the remaining ovalentarians. The development of the ural centrum in the other taxa in contrast raises the question if this is the result of evolutionary fusion of two previously separated centra or if one ural centrum got reduced and the remaining centrum elongated and took the formers place. The connection of these two developmental modes remains unanswered for now and needs more detailed developmental studies of a variety of ovalentarian taxa to be answered with more certainty.
Conclusion

At the base of atheriniforms and beloniforms five hypurals develop of which hypural 1 and hypural 2 fuse to form the lower hypural plate, while only the lower hypural plate and two upper hypurals develop at the base of cyprinodontiforms. The development of the compound centrum is very alike in atheriniforms and *Oryzias* as two ural centra develop and fuse to form the compound centrum, whereas in the other studied beloniforms and in cyprinodontiforms only one centrum develops. The reduction of one centrum or the evolutionary fusion of the two centra must have occurred independently within beloniforms and in cyprinodontiforms based on the accepted phylogenetic relationships within atherinomorphs. The grundplan of the Atherinomorpha is very alike to the grundplan of the Atheriniformes. Comparing the caudal fin development of atherinomorphs to that of other ovalentarian taxa, we found most similarities with mugilids, which develop five separated hypurals of which hypural 1 and hypural 2 fuse, two ural centra, which fuse, and an autogenous parhypural that is connected to hypural 1 by a cartilaginous bridge.

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Authors contributions

PT and TM designed the study. All authors contributed to manuscript writing and collected specimens. PT prepared the specimens, took images and made drawings of the specimens.

Ethics approval and consent to participate

Experimental procedures used in this study were in compliance with national guidelines.
Availability of data and materials

All specimens examined in this study are included in the Material and Methods section of this publication. Raw images used for drawings are available upon request from the first author.

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Section 3: Phylogenetic Relationships of the Mugiliformes
REVIEWING MORPHOLOGICAL CHARACTERS IN LIGHT OF NEW MOLECULAR PHYLOGENIES – THE CAUDAL FIN SKELETON OF OVALENTARIAN TAXA

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Philipp Thieme¹², Nalani K. Schnell³, Kerryn Parkinson⁴, and Timo Moritz¹²

¹Deutsches Meeresmuseum, Katharinenberg 14–20, 18439 Stralsund, Germany
²Institute of Zoology and Evolutionary Research, Friedrich-Schiller-University Jena, Ebertstrasse 1, 07743 Jena, Germany
³Institut Systématique Evolution Biodiversité (ISYEB), Muséum national d’Histoire naturelle, CNRS, Sorbonne Université, EPHE, Station Marine de Concarneau, Place de la Croix, 29900 Concarneau, France
⁴Australian Museum Research Institute, Australian Museum, 1 William Street, Sydney, NSW 2010, Australia

*Correspondence to be sent to: Deutsches Meeresmuseum, Katharinenberg 14–20, 18439 Stralsund, Germany; Email: phil.thieme2016@gmail.com
ABSTRACT

The Ovalentaria is a taxon of teleosts which has only recently been proposed on the basis of molecular-genetic analyses. Multiple, previously widely-separated fish families are assembled with this taxon. For the first time, the Ovalentaria are analysed using a comparative morphological approach. The caudal fin skeleton of 355 species covering all 48 ovalentarian families are examined in cleared and stained specimen, µCT datasets and x-ray images. A total of 38 morphological characters are evaluated and used for ancestral character state reconstructions and phylogenetic analyses. Results provided phylogenetic hypotheses for the relationships of ovalentarian taxa and a grundplan of the caudal fin skeleton of the last common ancestor of all Ovalentaria. An evolutionary trend towards the reduction of skeletal elements in the caudal fin can be observed. Furthermore, a connection between the evolution of the caudal skeleton and the modes of locomotion found in ovalentarian taxa is discussed. The phylogenetic analyses provided convincing topologies that are highly in agreement with molecular-genetic based hypotheses.

KEYWORDS

Teleostei, comparative anatomy, ancestral character state reconstruction, Cichlidae, Atherinomorpha

INTRODUCTION

Within the past two decades, our knowledge of the phylogenetic systematic of actinopterygians got redefined in many parts due to comprehensive molecular-genetic analyses. The amount of data available and processable increased constantly (Miya, et al. 2003, Mabuchi, et al. 2007, Setiamarga, et al. 2008, Wainwright, et al. 2012, Betancur-R, et al. 2013, Betancur-R, et al. 2017). Such studies provided us with phylogenies covering all major actinopterygian clades. In the past, morphology was used to reconstruct phylogenetic relationships and the respective character evolution was discussed at the same time (e.g., Johnson and Patterson 1993, Dyer and Chernoff 1996, Stiassny, et al. 1996). Since the mid-2010s, mostly genetic data is used to analyse phylogenetic relationships of actinopterygians and detailed discussions of morphological data has become more and more rare. Often only morphological examples are provided as discussion starters (e.g., Wainwright, et al. 2012,
Verma, et al. 2019). In many cases comprehensive molecular-based phylogenies propose relationships that have never been considered with morphological data before and thereafter stimulate new comparative studies in order to test those relationships (e.g., Lavoué, et al. 2008, Near, et al. 2012, Wainwright, et al. 2012). There are several advantages of using molecular-genetic analyses as a base for new comparative morphological studies: 1) molecular data can provide a foundation for the reconstruction of the evolution of morphological structures and characters, and 2) phylogenetic hypotheses can be reviewed in the light of...
comparative morphological data. Many actinopterygian character complexes are suitable for such studies, e.g., the pectoral girdle, the median fins, or the caudal fin, or a combination of those different complexes.

Investigations of the caudal fin seem to be a promising starting point as the evolution of this character complex had a major influence on diversity of teleost fishes (Lauder and Liem 1983, Flammang and Lauder 2009). This was promoted by the evolutionary transition from a heterocercal towards a homocercal caudal fin, an apomorphic character of the Teleostei (Goodrich 1930, Patterson 1973, Metscher and Ahlberg 2001). Diverse locomotory abilities evolved (Lauder 1989, 2000, Flammang and Lauder 2009) which resulted in manifold shapes (Nelson, et al. 2016) and influenced the diversification of teleosts to the most speciose vertebrate taxon (Schultze and Arratia 2013, Nelson, et al. 2016, Desvignes, et al. 2018).


Combining the results of recent molecular-based phylogenetic studies with a morphological analysis of the caudal skeleton seems to be the logical step to advance the knowledge on teleost evolutionary history. An exemplary taxon suited for such an approach are the Ovalentaria. This assemblage represents many taxa that previously were regarded to be only distantly related within percomorphs s.l. (Nelson 2006), which is the reason why there are no comparative morphological analyses available yet covering them. This presents an opportunity to evaluate the caudal fins of these taxa in the light of a new phylogenetic hypothesis.

The taxon Ovalentaria was first proposed by Wainwright, et al. (2012) based on DNA sequence data from 10 nuclear loci. The taxon was retrieved again in subsequent analyses (Betancur-R, et al. 2013, Betancur-R, et al. 2017, Hughes, et al. 2018). The Ovalentaria comprise about 42 (Nelson, et al. 2016) to 48 (Fricke, et al. 2020) families. However, the monophyly of some of these families (e.g., Grammatidae, Labrisomidae) was questioned in these analyses (Wainwright, et al. 2012, Betancur-R, et al. 2013, Betancur-R, et al. 2017). Of the taxa
comprised within the Ovalentaria (Fig. 1), the Atherinomorpha and Mugiliformes were previously thought to be closely related and possible sister-taxa to the Percomorpha, while all other taxa were assembled in the Perciformes, without a settled classification (Nelson 2006). The taxon Ovalentaria is well supported by molecular data, however, relationships of ovalentarian taxa remain unresolved as support values for many nodes are low (Wainwright, et al. 2012, Betancur-R, et al. 2013, Betancur-R, et al. 2017, Hughes, et al. 2018).

For the first time, the phylogenetic relationships of the just recently proposed Ovalentaria are analysed using morphological data. The aims of this study are 1) to compare the caudal fin skeleton of ovalentarian taxa based on detailed descriptions, 2) to reconstruct the evolution of the caudal fin skeleton within the Ovalentaria based on the phylogenetic hypothesis provided by Betancur-R, et al. (2017), and 3) to use caudal fin characters to construct a phylogenetic hypothesis based only on morphological data. The agreements and discrepancies between the molecular-based and morphology-based topologies are discussed in light of the evolution of the caudal fin.

**Material & Methods**

*Taxonomic Sampling and morphological analysis*

A total of 355 species were examined and/or reviewed in this study (online Appendix 1). Examined specimen were taken from the collections of the Australian Museum (AM), the Deutsches Meeresmuseum (DMM), the Florida Museum of natural history (FLMNH), the Muséum national d’Histoire naturelle (MNHN), the Phyletisches Museum Jena (PMJ), and the Zoologische Staatssammlung München (ZSM). Cleared and stained specimen, x-ray images as well as μCT-Scans of the examined specimens were evaluated. Additionally, further specimens were cleared and double stained (bone stained in red and cartilage in blue) during this study principally following the protocols by Dingerkus and Uhler (1977) and Taylor and Van Dyke (1985). Larval specimen examined in this study were cleared and stained following the protocol by Schnell, et al. (2016). Pictures of the caudal fin skeleton were taken either with a Canon EOS 80D and a Canon MP-E 65mm objective, an Axiocam microscope camera attached to a ZEISS Discovery V20 stereomicroscope, or a Leica M205 Stereoscope with a DMC 4500 camera. Afterwards, pictures were processed using Adobe Photoshop and Zeiss ZEN software and plates were assembled using Adobe Illustrator. Species reviewed in this study were taken from various literature sources (online Appendix 1).
Thirty-eight characters of the caudal fin skeleton were evaluated for each species during this study (online Appendix 1). Based on the character states found, a grundplan was reconstructed for each family to be used for morphological description, ancestral characters state reconstruction and phylogenetic analyses (online Appendix 2).

**Ancestral character state reconstruction**

Ancestral character reconstruction was performed in RStudio using the packages ape (Paradis and Schliep 2019), Geiger (Pennell, et al. 2014), and phytools (Revell 2012) using the phylogenetic tree provided by Betancur-R, et al. (2017) as basis for the analysis. First, the phylogenetic tree provided by Betancur-R, et al. (2017) was trimmed to only the Ovalentaria and the outgroup Polymixia. Then all ovalentarian taxa were further trimmed to family level except atheriniforms and cyprinodontiforms, which were reduced to their last common ancestor, as the provided tree did not represent the full diversity of families of these taxa. Afterwards, the best-fitting model for each character was determined using a customized script mainly employing the functions fitMk (phytools) and fitDiscrete (Geiger). Ancestral character state estimation was performed using the make.simmap function (phytools) and a modified version of the describe.simmap function (phytools). For plotting trees, the packages ape (Paradis and Schliep 2019) and ggtree (Yu, et al. 2017) were used.

**Phylogenetic analysis**

The compiled data was analysed with parsimony, maximum likelihood and Bayesian inference approaches. *Polymixia* was chosen as outgroup for all analyses. With each approach three taxon sets were analysed with the first including Atheriniformes, Cyprinodontiformes and all beloniform families, the second including Atheriniformes, Beloniformes and Cyprinodontiformes, and the third grouping these taxa together as a single taxon, the Atherinomorpha.

The parsimony analyses were conducted with TNT v1.5 (Goloboff, et al. 2008, Goloboff and Catalano 2016). Heuristic searches were carried out with Traditional search (Wagner trees: 500 replicates and TBR: 350 replications) and New Technology search algorithms (RAS: 5000 additive sequences, Sectorial Search: RSS + CSS [500 rounds] with minimal sector size 5, Ratchet: 500 iterations, Drift: 500 cycles and Tree fusing: 20 rounds). Node supports were calculated as Bremer supports as well as Bootstrap and symmetric resampling (1,000 replica-
tions using Traditional search) with support values given as frequency differences (Goloboff, et al. 2003).

Maximum likelihood analyses were performed in iqtree2 (Minh, et al. 2020). First, the ModelFinder algorithm (Kalyaanamoorthy, et al. 2017) was used to select the optimal model for the subsequent phylogenetic estimation process. Second, the maximum likelihood phylogenetic analysis was run including the ultrafast bootstrap approximation (Hoang, et al. 2017) with 100,000 bootstrap replicates to compute branch support values.

Bayesian inferences analyses were conducted in Mr.Bayes3.2.7a (Ronquist, et al. 2012) employing the CIPRES Science Gateway (Miller, et al. 2012). The Mkv model (with rates of the character evolution model set to a Log-normal distribution) with one partition was run under the following settings: four separated runs each with one cold and five heated chains, three swaps and heated chain temperature set to 0.09; burn-in fraction set at 0.25 for 10^7 generations sampled every 1,000 generations. The function ‘run BEAGLE’ in CIPRES was activated for the analyses (Ayres, et al. 2012). The consensus topology was calculated under the majority rule together with the posterior probabilities of each node.

Terminology

The terminology of the caudal fin skeletal elements generally follows Schultze and Arratia (2013) and (Fujita 1989). In the following, terms are shortly defined or, if differing from the above, explained in detail:

Compound centrum (CC): most posterior vertebra to which the lower and upper hypurals are connected (articulated or fused); defined by its shape which is given by an anterior portion that resembles a half centrum and a posterior portion which is cone-shaped and can be bent upwards. The compound centrum is not a phylogenetically defined term as the development of this structure underlies a high variation between taxa (either only one ural centrum develops or two ural centra develop which eventually fuse) but still results in similar adult morphologies. Therefore, it is an anatomical term not implying homology between taxa.

Hypural diastema (Dia): space between hypural 2 and hypural 3.

Epural (Ep): detached neural spine previously associated with neural arch of preural or ural centrum. When several epurals are present, these are numbered from anterior to posterior. Numbers do not imply homology.
Haemal arch (HA): ventral attachment to caudal vertebra enclosing the arteria caudalis.

Haemal spine (HS): spine-like, ventral extension of the fused tips of the left and right halves of the haemal arch or cartilaginous preformed element which fuses to the tips of the haemal arch during ontogeny.

Hypurapophysis (HU): attachment site for the hyperchordal longitudinalis muscles bilaterally on the parhypural.

Hypural (Hyp): modified haemal spine without haemal arch that is associated to an ural centrum or the compound centrum (either articulated or fused).

Inter-haemal spine cartilage (IHC): cartilaginous element posterior to tip of the respective haemal spine (indicated by respective number). Sometimes in close proximity to the following haemal spine.

Inter-neural spine cartilage (INC): cartilaginous element posterior to tip of the respective neural spine (indicated by respective number). Sometimes in close proximity to the following neural spine.

Lower hypural plate (LHP): fused hypural 1 and hypural 2.

Neural arch (NA): dorsal attachment to vertebra enclosing the neural chord.

Neural spine (NS): spine-like, dorsal extension of the fused tips of the left and right halves of the of the left and right halves of the neural arch or cartilaginous preformed element which fuses to the tips of the neural arch during ontogeny. A reoccurring character state of the neural spine of preural centrum 2 is that it is shortened. While the normal length of this neural spine will herein be defined as at least as long as the neural spine of first non-preural neural spine, shortening can occur in two states: short (>50% normal length) or truncated (<50% normal length).

Parhypural (PH): haemal arch and haemal spine or only haemal spine anterior to Hyp1. The haemal arch of the PH, if present, provides the exit point of the arteria caudalis.

Preural centrum (PU): vertebral centrum anterior to the ural centra/compound centrum which supports caudal fin rays with its haemal and/or neural spines. Preural centra are counted from posterior to anterior. Preural centrum 1 is the most posterior preural centrum and, if present, supports the parhypural.

Upper hypural plate (UHP): fused hypural 3 and hypural 4. Hypural 5 can additionally be included into the upper hypural plate.

Ural centrum (UC): centrum at the posterior end of the vertebral column characterized
by missing haemal arch and supporting hypurals ventrally.

Uroneural (UN): paired, elongated bones dorsal to the ural centra/compound centrum and dorso-lateral to the notochord; evolutionary derived from ural neural arches.

**RESULTS**

*Morphology of the caudal fin skeleton*

In the following, the composition of the caudal fin skeleton of ovalentarian families will be presented. The grundplan of each family is inferred from examined and reviewed species (online Appendix 1). A summary of the grundplan is provided and variations found in species from the respective family are reported. References used for each family are additionally provided at the end of the respective paragraph.

**Atheriniformes**

*Atherinopsidae* — CC + PU2 + PU3 + PU4 + PU5 contribute to the caudal fin (Fig. 2a); CC contains UC1 + UC2 [CC (UC1 + UC2)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 separate, Hyp3 + Hyp4 articulate with CC; Hyp5 present; Hyp5 not fused to Hyp4 nor to CC; PH articulates with CC, not fused to LHP; HU present on PH, HU splint-like and directed posteriorly; UN1 fused to CC; two Ep present; HA of PU2 and HA of PU3 fused to respective centrum; HSP of PU2 + PU3 + PU4 autogenous; NS of PU2 truncated; IHC2 + IHC3 + IHC4 + IHC5 present, INC3 + INC4 present.

The ontogeny of *Leuresthes tenuis* show two ural centra (U1 + U2) in early ontogeny that fuse later to form the CC (Valdez-Moreno and Vásquez-Yeomans 2001, Thieme, et al. submitted). In *Menidia conchorum* Hyp3 + Hyp4 are fused to form a UHP (Fig. 2a), which is fused to the CC, and Hyp5 is fused to the UHP. The number of IHC and INC is reduced in *Leuresthes tenuis, Membras martinica* (both only IHC3) and *Menidia conchorum* (only IHC4).


*Atherionidae* — CC + PU2 + PU3 + PU4 + PU5 contribute to the caudal fin; components of CC unknown [CC (?)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP articulates with CC; Hyp5 present, Hyp5 fused to CC; PH articulates with CC, not fused to LHP; HU present on PH, HU splint-like, short (not reaching the posterior...
Figure 2: Caudal fin skeleton of cleared and stained specimens of atheriniform families: a) Atherinopsidae – *Menidia conchorum* (DMM IE/11399, SL = 65.51mm); b) Phallostethidae – *Gulaphallus mirabilis* (MNHN 2020 0379, SL = 24.20mm); c) Atherinidae – *Atherina boyeri* (DMM IE/16473, SL = 63.87mm); d) Bedotiidae – *Bedotia geayi* (DMM IE/15880, SL = 78.11mm); e) Melanotaenidae – *Glossolepis incisus* (DMM IE/12202, SL = 45.73mm); f) Melanotaenidae – *Iriatherina werneri* (DMM IE/11407, SL = 30.36mm); g) Telmatherinidae – *Marosatherina ladigesi* (DMM IE/11011, SL = 35.48mm); h) Pseudomugilidae – *Pseudomugil furcatus* (DMM IE16311, SL = 29.41mm). Abbreviations: CC, compound centrum; Dia, hypural diastema; Ep, epural; IHC, inter-haemal spine.
border of the PH) and pointing posteriorly; UN1 present, UN1 fused to Hyp5; two Ep present; HA of PU2 + PU3 fused to respective centrum; HSP of PU2 + PU3 + PU4 autogenous; NS of truncated; IHC3 + IHC4 present.


Phallostethidae — CC + PU2 + PU3 + PU4 + PU5 contribute to the caudal fin (Fig. 2b); CC contains UC1 + UC2 [CC (UC1 + UC2)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; Hyp5 either fused into UHP or absent; PH articulates with CC, not fused to LHP; UN1 present, UN1 fused to CC; two Ep present; HA of PU2 + HA of PU3 fused to respective centrum; HSP of PU2 autogenous; NS of PU2 normal length; IHC3 + IHC4 + IHC5+ IHC6 present, INC2 + INC3 + INC4 present.

In Neostethus lankesteri and Phenacostethus smithii PU6 also contributes to the caudal fin. Parenti (1984) did not report a NS on PU2 for Phallostethus dunckeri. In Gulaphallus mirabilis there are additional IHC6, INC5 and INC6 present (Fig. 2b).


Isonidae — CC + PU2 + PU3 + PU4 + PU5 contribute to the caudal fin; components of the CC unknown [CC (?)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP articulates with CC; Hyp5 present, Hyp5 fused to CC; PH fused to CC and to LHP; HU present on PH, HU splint-like and directed posteriorly; UN1 present, UN1 fused to Hyp5; one Ep present; HA of PU2 and HA of PU3 fused to respective centrum; HSP of PU2 + PU3 + PU4 autogenous; NS of PU2 truncated; IHC3 present, INC absent.

Fujita (1990) reported that Hyp5 is part of the UHP and not fused to the CC in Iso sp. Additionally, UN1 is fused to the CC.


Atherinidae — CC + PU2 + PU3 + PU4 + PU5 contribute to the caudal fin (Fig. 2c); CC contains UC1 + UC2 [CC (UC1 + UC2)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 not fused, Hyp3 + Hyp4 articulate with CC; Hyp5 present, Hyp5 not fused to CC

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cartilage; INC, inter-neural spine cartilage; HA, haemal arch; HS, haemal spine; HU, hypurapophysis; Hyp, hypural; LHP, lower hypural plate; NA, neural arch; NS, neural spine; PH, parhypural; PU, preural centrum; UH, upper hypural plate; UN, uroneural. Scale bar = 1mm.
nor to Hyp4; PH articulates with CC, not fused to LHP; HU present on PH, HU splint-like and directed postero-dorsally; UN1 present, UN1 fused to CC; 2 Ep present; HA of PU2 and HA of PU3 fused to respective centrum; HSP of PU2 + PU3 + PU4 autogenous; NS of PU2 truncated; IHC3 + IHC4 present, INC absent.

In many atherinid taxa Hyp3 and Hyp4 are fused to form the UHP (i.e., *Atherina harringtonensis*, *Atherinomorus vaigiensis*, *Doboatherina bleekeri*, *Hypoatherina barnesi*). The HU of *Craterocephalus amniculus* and *Craterocephalus eyresii* is shorter than in other atherinid species. The uroneural of *D. bleekeri*, *H. barnesi*, *Kestratherina esox* is additionally fused to Hyp5.


**Bedotiidae** — CC + PU2 + PU3 + PU4 contribute to the caudal fin (Fig. 2d); CC contains UC1 + UC2 [CC (UC1 + UC2)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 separate, Hyp3 and Hyp4 fused to CC; Hyp5 present, Hyp5 articulates with CC, Hyp5 not fused to Hyp4; PH articulates with CC, not fused to LHP; HU present on PH, HU splint-like and directed posteriorly; UN1 present, UN1 fused to CC; two Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 autogenous; NS of PU2 truncated; IHC3 + IHC4 present, INC absent.

In *Rheocles alaotrensis* Hyp4 and Hyp5 are fused. In the genus *Rheocles* the PH is partially or completely fused to the LHP. In *Bedotia geayi* (Fig. 2d), *R. derhami*, *R. lateralis*, *R. vatosoa* IHC4 is absent and in *R. pellegrini* IHC3 + IHC4 are absent. INC3 is present in *R. wrightae*.


**Melanotaeniidae** — CC + PU2 + PU3 + PU4 contribute to the caudal fin (Fig. 2e, f); CC contains UC1 + UC2 [CC (UC1 + UC2)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 separate, Hyp3 and Hyp4 articulate with CC; Hyp5 present, Hyp5 articulates with CC, Hyp5 fused to Hyp4; PH articulates with CC, PH fused to LHP; HU present on PH, HU splint-like and directed posteriorly; UN1 present, UN1 not fused to CC or upper hypurals; two Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2-PU5 autogenous; NS of PU2 truncated; IHC3 present, INC absent.

In some species Hyp2 and Hyp3 grow towards each other anteriorly narrowing the
Dia, i.e., *Glossolepis incisus* (Fig. 2e), *Melanotaenia lacustris*, *Melanotaenia nigrans*, *Pelangia mbutaensis*). Hyp3 + Hyp4 are fused to form the UHP in *P. mbutaensis* and *Rhadinocentrus ornatus*. In *Chilatherina axelrodi* Hyp5 is not fused to Hyp4. The upper hypurals (Hyp3-Hyp5) are fused to the CC in *R. ornatus*. The UN1 is fused to the CC in some species, i.e., *G. incisus*, *Iriatherina werneri* (Fig. 2f), *M. lacustris*, *P. mbtuaensis*, *R. ornatus*. In *M. nigrans* one Ep is absent. IHC3 is absent in *C. axelrodi* while IHC4 is present in *I. werneri* and *M. lacustris*. Based on the latest molecular-genetic hypothesis, the species *Cairnsichthys rhombosomoides* is no longer considered a melanotaeniid as it is seemingly closer related to telmatherinids and pseudomugilids (Campanella, et al. 2015). It differs from melanotaeniid species in having the PH separated from the LHP and missing IHC3.


*Telmatherinidae* — CC + PU2 + PU3 + PU4 contribute to the caudal fin (Fig. 2g); CC contains UC1 + UC2 [CC (UC1 + UC2)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 separate, Hyp3 + Hyp4 articulate with CC; Hyp5 present, Hyp5 articulates with CC, Hyp5 not fused to Hyp4; PH articulates with CC, PH not fused to LHP; HU present on PH, HU splint-like and directed posteriorly; UN1 present, UN1 not fused to CC or upper hypurals; two Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 + PU3 autogenous; NS of PU2 truncated; IHC3 + IHC4 present, INC3 + INC4 present.

In half of the examined specimen of *Marosatherina ladigesi* Hyp4 and Hyp5 are fused. No IHC or INC are reported for *K. helodes*.


*Pseudomugilidae* — CC + PU2 + PU3 + PU4 contribute to the caudal fin (Fig. 2h); CC contains UC1 + UC2 [CC (UC1 + UC2)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP articulates with CC; Hyp5 absent; PH articulates with CC, PH part of the LHP; HU present on PH, HU spur and directed laterally; UN1 present, UN1 fused to UHP and CC; two Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 + PU3 autogenous; NS of PU2 truncated; IHC3 + IHC4 present, INC3 + INC4 present.

In *Pseudomugil furcatus* the PH develops as part of the LHP (Thieme, et al. submitted). In *P. majusculus* and some specimen of *P. signifer* the PH is separated from the LHP (Saeed,
et al. 1989), which indicates the individual development of the PH. Therefore, it is possible that in the grundplan of pseudomugilids the PH still develops as an individual element. In *P. tenellus* only IHC3 is present.

**Dentatherinidae** — CC + PU2 + PU3 + PU4 contribute to the caudal fin; CC probably contains UC1 + UC2 [CC (UC1 + UC2)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP articulates with CC; Hyp5 present, Hyp5 articulates with CC, Hyp5 not fused to UHP; PH articulates with CC, PH not fused to LHP; HU present on PH, HU directed antero-ventrally; UN1 present, UN1 fused to Hyp5; one Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 autogenous; NS of PU2 truncated, IHC and INC absent.

References: Patten and Ivantsoff (1983).

**Beloniformes**

**Adrianichthyidae** — CC + PU2 + PU3 + PU4 contribute to the caudal fin (Fig. 3a); CC contains UC1 + UC2 [CC (UC1 + UC2)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; Hyp5 absent; PH articulates with CC, PH not fused to LHP; HU present on PH, HU stout dorsal projection; UN1 present, UN1 fused to CC; two Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 autogenous; NS of PU2 normal length; IHC3 present, INC absent; extra caudal ossicle (EO) present between HSP of PU2 and PH.

In *Oryzias sinensis* and *O. woworae* PU4 does not contribute to the caudal fin (Fig. 3b). And in *O. sarasinorum* only one Ep is present.


**Zenarchopteridae** — CC + PU2 + PU3 contribute to the caudal fin (Fig. 3d); components of CC unknown [CC (?)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, Hyp3 + Hyp4 fused CC; Hyp5 fused to Hyp4 and CC; PH fused to CC, PH fused to LHP; UN1 present, UN1 fused to CC; three Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 + PU3 autogenous; NS of PU2 truncated; IHC3 present, INC absent.

In *Hemirhamphodon phaiosoma* PU4 additionally contributes to the caudal fin (Fig. 3c). In many zenarchopterid species, the LHP and UHP are grown towards each other, resulting in a narrowed Dia. In *H. kuekenthali* the LHP and UHP are even partially fused. In *Nomorhamphus* (except *N. kolonodalensis* and *N. viviparus*) Hyp5 is not distinguishable from the UHP anymore. Based on the fusion of Hyp5 to the UHP and the CC in other zenarchopterids, it is...
highly plausible that Hyp5 is fused to the UHP in these species. Meisner (2001) reported that in the zenarchopterids (H. kuekenthali, N. viviparus, Tondanichthys kottelati and Zenarchopterus rasori) the PH articulates with the CC. The PH is not fused to the LHP in Tondanichthys kottelati and Zenarchopterus rasori. UN1 is fused only to the UHP in T. kottelati and Z. rasori and fused to the CC and the UHP in Nomorhamphus and H. kuekenthali. Additionally, IHC4 is present in D. pusilla, N. lanceolatus, N. liemi and N. megarrhamphus.


**Hemiramphidae** — CC + PU2 + PU3 + PU4 contribute to the caudal fin (Fig. 3e); CC contains UC1 + UC2 [CC (UC1 + UC2)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP not fused to CC; LHP and UHP grown towards each other posteriorly; Hyp5 present, Hyp5 articulates with CC, Hyp5 not fused to UHP; PH articulates with CC, PH not fused to LHP; HU present on PH, HU broad lateral projection; UN1 present, UN1 fused to CC, UN1 expanded antero-dorsally; three Ep present; HA of PU2 and PU3 fused to centrum; HSP of PU2-PU6 autogenous; NS of PU2 truncated; IHC and INC absent.

The elements of the caudal skeleton are in general expanded in the medial axis, which is particularly visible in the enlarged NA, NS, HA and HS (Fig. 3e). In an examined specimen of *Hyporhamphus limbatus* Hyp5 is fused to the UHP (Fig. 3e) Rosen (1964) depicted that Hyp5 is fused to the CC in Chriodorus ahterinoides. In *Hemiramphus brasiiliensis* the PH is fused to the CC. Due to this fusion the HU is positioned lateral to the CC, however, we still regard it to be part of the PH and to be one result of this fusion. Fujita (1990) reported that in *Hyporhamphus sajori* the HA of PU2 articulates with the centrum, but the drawings of Lee, et al. (2001) suggest that these elements are fused, which would correspond to all other examined hemiramphids. In *Hyporhamphus picarti* IHC3 is absent, and in *H. limbatus* IHC4 is present.


**Exocoetidae** — CC + PU2 + PU3 + PU4 + PU5 + PU6 contribute to the caudal fin; CC probably contains 1UC [CC (UC1/UC2?)]; LHP present, LHP articulates with CC; UHP present, UHP articulates with CC; Hyp5 absent; PH fused to CC, PH not fused to LHP; HU present lateral on CC due to fusion of PH and CC, directed laterally and posteriorly; UN1 present, UN1 fused to CC and extremely enlarged; three Ep present; HA of PU2 and PU3 fused to respec-
The elements of the caudal skeleton are in general expanded which is particularly visible in the enlarged NA, NS, HA and HS. Ontogenetic data from *Cypselurus doederleini* suggest that only one UC develops (Dasilao and Yamaoka 1998b). In small specimens of *Parexocoethus brachypterus* and *C. doederleini* the PH still articulates with the CC.


**Belonidae** — CC + PU2 + PU3 contribute to the caudal fin (Fig. 3f); CC probably contains UC1 + UC2 [CC (UC1 + UC2)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP articulates with CC; Hyp5 present, Hyp5 not fused to UHP or CC; PH articulates with CC, PH not fused to LHP; HU present on PH, HU stout lateral projection; UN1 present, UN1 fused to CC, UN1 antero-dorsally expanded; three Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 + PU3 autogenous; NS of PU2 truncated; IHC3 present, INC absent.

The elements of the caudal skeleton are in general expanded which is particularly visible in the enlarged NA, NS, HA and HS (Fig. 3f). In *Tylosurus crocodilus* and a few *Belone belone* specimen Hyp3 and Hyp4 are separate (Fig. 3f). In some *B. belone* specimen and in *Potamorrhaphus guianensis* there are only two Ep present. IHC3 is absent in *B. belone*.


**Cyprinodontiformes**

**Aplocheilidae** — CC + PU2 + PU3 + PU4 + PU5 contribute to the caudal fin (Fig. 4a); CC probably contains 1UC [CC (UC1/UC2)]; LHP present; Hyp3 + Hyp4 fused to form UHP; LHP + UHP fused to form large HP, HP fused to CC, Dia absent; Hyp5 absent; PH articulates with CC, PH not fused to LHP; HU present on PH, HU triangularly extended dorsally; UN1 present, UN1 fused to CC and HP; one Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2-PU5 autogenous; NS of PU2 normal length; IHC and INC absent.

In some juvenile specimen of *Aplocheilus lineatus* LHP, Hyp3 and Hyp4 are separate and not fused.

Nothobranchiidae — CC + PU2 + PU3 + PU4 contribute to the caudal fin (Fig. 4b); components of CC unknown [CC (?)]; LHP present, LHP fused to CC; Hyp3 + Hyp4 separate, Hyp3 + Hyp4 fused to CC; Hyp5 absent; PH articulates with CC, PH not fused to LHP; HU absent; UN1 present, UN1 fused to CC and UHP; one Ep present; HA of PU2 and PU3 fused to respec-
Hyp3 and Hyp4 are fused in the majority of the examined nothobranchiid species to form the UHP, i.e., Aphyosemion, Epilatys spilargyreius, E. annulatus, Foerschichthys, Fundulopanchax, Nothobranchius, Pronothobranchius. In some of these species LHP and UHP are also fused resulting in a large HP and a completely reduced Dia, i.e., Foerschichthys, Fundulopanchax and Nothobranchius) while in Pronothobranchius only a partial fusion is observed which leads to a narrowed Dia. In the genera Epilatys (Fig. 4b), Foerschichthys and Fundulopanchax the PH is separate from the CC.


Rivulidae — CC + PU2 + PU3 + PU4 contribute to the caudal fin; components of CC unknown [CC (?)]; LHP present, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; Hyp5 absent; PH separate from CC, PH not fused to LHP; HU absent; UN1 present, UN1 fused to CC and UHP; one Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2-PU4 autogenous; NS of PU2 normal length; IHC and INC absent.

In Hypsolebias trilineatus and Spectrolebias semiocellatus LHP and UHP are fused to form a large HP. As a result of this fusion the Dia is absent. In Anablepsoides bahianus no UN is present.


Profundulidae — CC + PU2 + PU3 + PU4 contribute to the caudal fin; components of CC unknown [CC (?)]; LHP present, UHP present; LHP + UHP fused to form large HP, HP fused to CC, Dia absent; Hyp5 absent; PH articulates with CC, PH not fused to LHP; HU present on PH; HU short and directed postero-dorsally; presence of UN1 uncertain; one Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2-PU4 autogenous; NS of PU2 normal length; IHC and INC absent.

Goodeidae — CC + PU2 + PU3 + PU4 + PU5 + PU6 contribute to the caudal fin (Fig. 4c); components of CC unknown [CC (?)]; HP present, HP fused to CC; Hyp5 absent; PH articulates with CC, PH not fused to HP; HU present on CC; UN1 present, UN1 fused to CC and HP; 1 Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 + PU3 autogenous; NS of PU2 normal length; IHC and INC absent.
**Fundulidae** — CC + PU2 + PU3 + PU4 + PU5 contribute to the caudal fin (Fig. 4d); components of CC unknown [CC (?)]; LHP present; UHP present; LHP and UHP fused to form large HP, HP fused to CC, Dia absent; Hyp5 absent; PH articulates to CC, PH not fused to LHP; HU present on PH, HU directed dorsally (lateral to CC); UN1 present, UN1 fused to CC and HP; UN2 absent; one Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2-PU5 autogenous; NS of PU2 normal length; IHC4 + IHC5 + IHC6 present, INC4 + INC5 + INC6 present.

In the examined specimen of *Fundulus cf. jenkinsi* LHP and UHP are not completely fused, they are separate anteriorly and fused posteriorly. Further, at this early stage during ontogeny, UN1 can be distinguished from the developing NA of the CC. In *Fundulus cf. similis* IHC4 and INC4 (Fig. 4d), in *Fundulus sciadicus* IHC3 + IHC4 and INC3, and in *Lucania parva* IHC3 + IHC4 and INC3 + INC4 are present.


**Valenciidae** — CC + PU2 + PU3 + PU4 contribute to the caudal fin; components of CC unknown [CC (?)]; LHP present; UHP present; LHP + HUP fused to form large HP, HP fused to CC; Hyp5 absent; PH articulates with CC, PH not fused to HP; HU present on PH; presence of UN1 uncertain; one Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2-PU3 autogenous; NS of PU2 normal length; IHC4 present. INC3 + INC4 present.

In the specimen depicted in Costa (2012: Fig 1C) an extra ossified structure is present in between the distal tips of HSPU2 and HSPU3.

References: (Costa 2012).

**Cyprinodontidae** — CC + PU2 + PU3 + PU4 contribute to the caudal fin; components of CC unknown [CC (?)]; large HP present, HP fused to CC, Dia absent; Hyp5 absent; PH separate from CC, PH not fused to HP; HU absent; UN1 present, UN1 fused to CC and HP; one Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 + PU3 autogenous; NS of PU2 normal length; IHC3 + IHC4 + IHC5 present, INC3 + INC4 + INC5 present.


**Aphaniidae** — CC + PU2 + PU3 + PU4 contribute to the caudal fin; components of CC unknown [CC (?)]; LHP present; UHP present; LHP + UHP fused to form large HP, HP fused
to CC, Dia absent; Hyp5 absent; PH separate from CC, PH not fused to HP; presence of HU uncertain; UN1 present, UN1 fused to CC and HP; one Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2-PU4 autogenous; NS of PU2 normal length; IHC4 + IHC5 present.


**Procatopodidae** — CC + PU2 + PU3 + PU4 contribute to the caudal fin (Fig. 4e); CC contains one UC [CC (UC1/UC2?)]; LHP present; UHP present; LHP + UHP fused to form large HP, HP fused to CC, Dia absent; Hyp5 absent; PH articulates with CC, PH not fused to HP; HU present on PH; UN1 present, UN1 fused to CC and HP; UN1 anteriorly with lateral projection; one Ep present; HA of PU2 and PU3 fused to respective CC; HSP of PU2-PU4 autogenous; NS of PU2 normal length; IHC3 + IHC4 + IHC5 present, INC3 + INC4 + INC5 present.

In *Lamprichthys tanganicanus* PU5 also contributes to the caudal fin. In *Micropanchax hutereauli* INC3 is additionally present.


**Anablepidae** — CC + PU2 + PU3 + PU4 + PU5 + PU6 + PU7 contribute to the caudal fin; components of CC unknown [CC (?)]; LHP present, LHP fused to CC; UHP present, UHP fused to CC; LHP + UHP partially fused, Dia narrowed; Hyp5 absent; PH articulates with CC, PH not fused to LHP; HU present on PH, HU splint-like and directed postero-dorsally; UN1 present, UN1 fused to CC and UHP; UN1 anteriorly with lateral projection; one Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2-PU4 autogenous; NS of PU2 normal length; IHC6 + IHC7 + IHC8 present, INC6 + INC7 present.


**Poecilidae** — CC + PU2 + PU3 + PU4 + PU5 contribute to the caudal fin (Fig. 4f); CC probably contains one UC [CC (UC1/UC2?)]; LHP present; UHP present; LHP + UHP fused to form large HP, HP fused to CC, Dia absent; Hyp5 absent; PH articulates with CC, PH not fused to HP; HU present on PH, HU triangular dorsal projection; UN1 present, UN1 fused to CC and HP; UN1 with lateral projection; one Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2-PU4 autogenous; NS of PU2 normal length; IHC4 + IHC5 present, INC4 + INC5 present.
In *Pamphorichthys hollandi*, *Poecilia mexicana* (Fig. 4f) and *Po. formosa* the LHP and the UHP are in close contact but do not fused. Costa (1996) depicted the PH fused to CC and HP in *Fluviphylax zonatus* and *Pa. hollandi*. We were not able to observe this in any other poecilid. In *Neoheterandria elegans* and *Pa. hollandi* the HU is absent. IHC3 and INC3 are absent in *Tomerus gracilis*, while INC3 is missing in *F. zonatus*. IHC2 is present in *Gambusia affinis*, *N.*
affinis, Po. bifurcate, and Po. picta. An additional IHC6 is present in Po. mexicana (Fig. 6f) and Po. sphenops, and INC6 is additionally present in Po. sphenops.


Cichliformes

Cichlidae — CC + PU2 + PU3 contribute to the caudal fin (Fig. 5a); CC presumably contains one UC [CC (UC1/UC2?)]; Hyp1 + Hyp2 separated, Hyp1 + Hyp2 articulate with CC; Hyp3 + Hyp4 separated, Hyp3 + Hyp4 articulate with CC; Hyp5 articulates with CC, Hyp5 not fused to Hyp4; PH articulates with CC, PH not fused to Hyp1; HU present on PH, HU splint-like and directed postero-dorsally; UN1 present, UN1 not fused to CC or Hyp; two Ep present; HA of PU2 articulates with centrum, HA of PU3 fused to centrum; HSP of PU2 + PU3 autogenous; NS of PU2 truncated; IHC2 + IHC3 + IHC4 present, INC4 present.

The anterior margins of Hyp2 and Hyp3 are close together in Astronotus ocellatus, Cichla ocellaris, and Oreochromis niloticus which results in a narrowed Dia. In two of the examined species, i.e., Crenicichla saxatilis and Pterophyllum scalare, Hyp3 and Hyp4 are fused to form the UHP which is fused to the CC. The HU is elongated in Tilapia sparrmanii, shortened in Amatitlania nigrofasciata, A. ocellatus, Geophagus brasiliensis, and P. scalare and reduced to a ridge in Apistogramma steindachneri, Cichlasoma portalagrense, Crenicichla, and Mesonauta guyanae. In P. scalare and Apistogramma steindachneri the HA of PU2 is fused to the centrum.


incertae sedis

Pholidichthyidae — CC forms the caudal fin skeleton (Fig. 5b); components of CC unknown [CC (?)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; presence of Hyp5 questionable (either part of UHP or absent); PH fused to CC, PH fused to LHP; HU present on the PH, HU directed laterally, HU very short; UN1 absent; Ep absent; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 + PU3 autogenous, HSP of PU3 not connected to HA; NS of PU2 normal length, IHC and INC absent.

Polycentridae — CC + PU2 + PU3 contribute to the caudal fin (Fig. 5c); components of CC unknown [CC (?)]; Hyp1 + Hyp2 separate, Hyp1 + Hyp2 articulate with CC; Hyp3 + Hyp4
separate, Hyp3 + Hyp4 fused to CC; Hyp5 present, Hyp5 not fused to Hyp4 nor to CC, Hyp5 shortened; PH articulates with CC, PH not fused to Hyp1; HU present on PH, HU directed laterally, HU short; UN1 present, UN1 not fused to CC nor to Hyp; one Ep present; HA of PU2 articulates with centrum, HA of PU3 fused to centrum; HSP of PU2 and PU3 autogenous; NS of PU2 normal length; IHC3 + IHC4 present, INC3 + INC4 present.

Small specimen of *Polycentropsis abbreviata* suggest that only one UC is developed during ontogeny based on similarities to cichlids and pomacentrids which in comparable ontogenetic stages exhibit very alike morphologies. The general caudal fin composition of polycentrids is very steady. In *Monocirrhus polyacanthus* Hyp5 is severely shortened and Hyp2 and Hyp3 grow towards each other leaving only a narrowed and small Dia. Otherwise, different combinations of reduced IHC and INC are observable.


**Ambassidae** — CC + PU2 + PU3 contribute to the caudal fin (Fig. 5d); components of CC unknown [CC (?)]; Hyp1 + Hyp2 fused to form LHP, LHP articulates with CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; Hyp5 present, Hyp5 not fused to UHP nor to CC; PH articulates with/fused to CC, not/fused to LHP/Hyp1; HU present on PH, HU splint-like directed postero-dorsally, HU reaches UN1 present, UN1 not fused to CC nor to UHP; three Ep present; HA of PU2 articulates with centrum, HA of PU3 fused to centrum; HSP of PU2 autogenous; NS of PU2 truncated; IHC2 + IHC3 + IHC4 present, INC3 + INC4 present.

The LHP is fused to the CC in *Parambassis* and *Gymnochanda*. Only two Ep are present *Ambassis dussumieri* (Fig. 5d) and one of the examined specimens of *Parambassis siamensis*. The HA of PU2 is fused to the centrum in *Parambassis* and *Gymnochanda*.

**Pomacentridae** — CC + PU2 + PU3 contribute to the caudal fin (Fig. 5e); CC probably contains 1UC [CC (UC1/UC2?)]; Hyp1 + Hyp2 separate, Hyp1 + Hyp2 articulate with CC; Hyp3 + Hyp4 separate, Hyp3 + Hyp4 articulate with CC; Hyp5 present, Hyp5 not fused to Hyp4 nor to CC, Hyp5 shortened; PH articulates with CC, not fused to Hyp1; HU present on PH, HU directed postero-dorsally, HU thin and elongated; UN1 present, UN1 fused to CC, UN1 enlarged; three Ep present; HA of PU2 and HA of PU3 articulate with respective centrum; HSP of PU2 and PU3 autogenous; NS of PU2 truncated; IHC2 + IHC3 + IHC4 present, INC3 + INC4 present.
During the ontogeny of *Amphiprion ocellaris* one elongated UC develops anterior to the hypurals. It is not distinguishable if this UC is representing UC1, UC2 or a product of evolutionary fusion of these two UC. In some species the PH and Hyp1 (i.e., *Am. frenatus, Am. ocellaris* and *Pomachromis richardsoni*), Hyp1 and Hyp2 (i.e., *Abudefduf sexfasciatus, Am. frenatus, Am. ocellaris* and *P. richardsoni*), Hyp3 and Hyp4 (i.e., *Ab. sexfasciatus, Am. frenatus, Am. ocellaris, Dascyllus aruanus* and *P. richardsoni*) or/and Hyp4 and Hyp5 (i.e., *Am. ocellaris*) are fused. Hyp2 and Hyp3 (or the LHP and UHP, if present) grow towards each other in some species, resulting in a narrowed Dia. The Dia is still large in *Ab. bengalensis, Ab. sexfasciatus, Ab. sordidus, Chrysiptera rex* and *Plectroglyphidodon leucozonus* but narrowed in *Amphiprion, Chromis, Pomacentrus coelestis* and *Pomacentrus rhodonotus*. In many species Hyp5 is further shortened (i.e., *Ab. vaigiensis, Ab. sordidus, Ab. sexfasciatus, Cheilopriion labiatus, Chromis chromis, Chry. leucopoma, D. aruanus, Pl. leucozonus, Pomacentrus, Stegastes nigricans*). Two instead of three Ep are present in *Pl. leucozonus*. In many species INC3 is absent.


*Embiotocidae — CC + PU2 + PU3 contribute to the caudal fin; components of the CC unknown [CC (?)]; Hyp1 + Hyp2 + Hyp3 and Hyp4 articulate with CC, Hyp2 and Hyp3 closely together, Dia small; Hyp5 present, Hyp5 shortened, Hyp5 not fused to UHP; PH articulates with CC, PH not fused to Hyp1; HU present on PH; UN1 present, UN1 enlarged; three Ep present; HA of PU2 articulates with centrum, HA of PU3 fused to centrum; HSP of PU2 autogenous; NS of PU2 short; IHC2 + IHC3 + IHC4 present, INC3 + INC4 present.


*Mugiliformes*

*Mugilidae — CC + PU2 + PU3 contribute to the caudal fin (Fig. 5f); CC contains U1 and U2 [CC (U1+U2)]; Hyp1 + Hyp2 fused to form LHP, LHP articulates with CC; Hyp3 + Hyp4 fused to CC; Hyp5 present, Hyp5 not fused to Hyp4 nor to CC; PH articulates with CC, not fused to LHP; HU present on PH, HU splint-like and directed postero-dorsally; UN1 present, UN1 not fused to CC nor to Hyp5 or UHP; two Ep present; HA of PU2 articulates with centrum, HA of PU3 fused to centrum; HSP of PU2 + PU3 autogenous; NS of PU2 truncated; IHC2 + IHC3 + IHC4 + INC4 present.

Hyp3 and Hyp4 are separate in *Aldrichetta, Gracilimugil, Myxus* and *Trachystoma*. The
NS of PU2 is only short in *Mugil curema* and *M. incilis*. In *Paramugil* species only INC3 and INC4 are present.


*incertae sedis*

Congrogadidae — CC + PU2 contribute to the caudal fin (Fig. 6a); components of the CC unknown [CC (?)]; Hyp1 + Hyp2 fused to form LHP, LHP articulates with CC; Hyp3 and Hyp4 fused to form UHP, UHP fused to CC, Dia absent; Hyp5 either fused into UHP or absent; PH articulates with CC, fused to LHP; HU absent; UN1 present, fused to CC and UHP; one Ep present; HA of PU2 and HA of PU3 fused to respective centrum; HSP of PU2 autogenous; NS of PU2 normal length; no IHC or INC present.

_Plesiopidae_ — CC + PU2 + PU3 contribute to the caudal fin (Fig. 6b); components of CC unknown [CC (?)]; Hyp1 + Hyp2 fused to form LHP, LHP articulates with CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; Hyp5 present, Hypm5 shortened, Hyp5 not fused to UHP; PH articulates with CC, not fused to LHP; HU present, HU splint-like and directed postero-dorsally; UN1 present, UN1 fused to CC, not fused to Hyp5 or UHP; three Ep present; HA of PU2 articulates with centrum, HA of PU3 fused to centrum; HSP of PU2 + PU3 autogenous; NS of PU2 truncated; IHC2 + IHC3 + IHC4 present, INC4 present.

The length of Hyp5 varies between the plesiopid species: in *Belonepterygion fasciola-tum*, *Beliops xanthokrossos*, *Plesiops coeruleolineatus*, and *Trachinops noarlungae* Hyp5 is severely shortened and in _Acanthoplesiops_ Hyp5 is reduced to a small spur. The HU is elongated in _P. coeruleolineatus_ and _B. fasciolatum_, while it is short in _Acanthochilinus fuscus_, _Acanthochilinus littoreus_ and _Beliops xanthokossos_ and completely absent in _Acanthoplesiops psilogaster_. The UN is additionally fused to Hyp5 in _Acanthochilinus littoreus_, *Steeneichthys plesiopsus* and _T. noarlungae_. In _Plesiops_ sp. (Fig. 6b) and _S. plesiopsus_ the two posterior epurals are fused. The NS of PU2 is present in its complete length in _Acanthoplesiops psilogaster_ while in _T. noarlungae_ this NS is short. In _Plesiops_ sp. IHC4 is absent, but INC3 is present (Fig. 6b).

Grammatidae — CC + PU2 + PU3 contribute to the caudal fin (Fig. 6c); components of the CC unknown [CC (?)]; Hyp1 + Hyp2 fused to form LHP, LHP articulates with CC; Hyp3 + Hyp4 fused to form UHP, UHP articulates with CC; Hyp5 present, Hyp5 not fused to UHP nor CC, Hyp5 shortened; PH articulates with CC, fused to LHP; HU present on PH, HU splint-like.
directed postero-dorsally; UN1 present, UN1 not fused to CC nor to UHP; two Ep present; HA of PU2 and PU3 articulate with respective centrum; HSP of PU2 autogenous; NS of PU2 truncated; IHC2 + IHC3 + IHC4 present, INC4 present.

In the genus *Lipogramma* Hyp5 is severely shortened. In contrast, UN1 in *Gramma* is fused to the CC and the UHP (Fig. 6c).

*Pseudochromidae* — CC + PU2 + PU3 contribute to the caudal fin (Fig. 6d); components of CC unknown [CC (?)]. Hyp1 + Hyp2 fused to form LHP, LHP articulates with CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; LHP and UHP contact each other anterior; Hyp5 present, Hyp5 not fused to UHP or CC, Hyp5 severely shortened; PH articulates with CC, PH not fused to LHP; HU present on PH; UN1 present, UN1 fused to CC; three Ep present; HA of PU2 articulates with centrum, HA of PU3 fused to centrum; HSP of PU2 and PU3 autogenous; NS of PU2 truncated, outgrowth of membrane bone dorsal to NA; IHC2 + IHC3 + IHC4 present, INC3 + INC4 present.

In many species the PH is fused to the LHP (i.e., *Amsichthys knighti*, *Chlidichthys johnvoelckeri*, *Lubbockichthys* sp., *Pectinochromis lubbocki*, *Pseudochromis aldabraensis* and in the genus *Pseudoplesiops*). In *C. johnvoelckeri*, *Lubbockichthys* sp., *Pe. lubricoki*, *Ps. aldabraensis* and *Pseudoplesiops* UN1 is additionally fused to the UHP (Fig. 6d). The HA of PU2 is fused to the centrum in *A. knighti*, *Lubbockichthys* sp., *Pe. lubbocki*, *Ps. aldabraensis* and *Pseudoplesiops*. Two epurals instead of three were observed in *A. knighti*, *Lubbockichthys* sp., *Pseudoplesiops howensis* and *Pseudoplesiops typus*.


*Opistognathidae* — CC + PU2 + PU3 contribute to the caudal fin (Fig. 6e); components of CC unknown [CC (?)]. Hyp1 + Hyp2 fused to form LHP, LHP articulates with CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; Hyp5 present, Hyp5 severely shortened, Hyp5 not fused to UHP nor CC; PH articulates with CC, PH fused to LHP; HU present on PH, HU splint-like directed postero-dorsally; UN1 fused to CC, not fused to Hyp5 or UHP; three Ep present; HA of PU2 articulates with centrum; HSP of PU2 autogenous; NS of PU2 truncated; IHC3 present, INC3 present.

In an examined juvenile specimen of *Stalyx* sp. an elongated UC was observed, which is similar to the condition found in cichlids. We hypothesize that in opistognathids too, only
one UC develops and forms the CC. In *Opistognathus aurifrons* IHC4 + INC4 are additionally present (Fig. 6e).


**Gobiesociformes**

*Gobiesocidae* — CC + PU2 contribute to the caudal fin (Fig. 6f); components of CC unknown [CC (?)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; LHP and UHP partially fused, Dia narrowed; Hyp5 not distinguishable (fused to UHP or absent); PH separate from CC, not fused to LHP; HU absent; UN1 present, UN1 fused to CC; one Ep present; HA of PU2 and HA of PU3 fused to respective centrum; HSP of PU2 autogenous; NS of PU2 normal length, IHC and INC absent.

Due to the fusion of the LHP and the UHP the diastema in *Apletodon dentatus*, *Diplecogaster bimaculatus* (Fig 6.f) is small, while in *Gobiesox funebris* and *Lepadogaster candolei* a complete Dia is present. Vaz and Hilton (2020) showed no PH nor an Ep for *G. strumosus* (cartilaginous elements not stained), while Rosen and Patterson (1969) reported no Ep for *G. funebris*. INC3 is present in *L. candolei* while IHC2, IHC3 and INC3 are present in *L. lepadogaster*.


**Blenniiformes**

*Tripterygiidae* — CC + PU2 + PU3 contribute to the caudal fin (Fig. 7a); components of CC unknown [CC (?)]; Hyp1 + Hyp2 fused to form LHP, LHP articulates with CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; Hyp5 not fused to UHP nor to CC, Hyp5 small; PH articulates with CC, fused to LHP; HU present on PH, splint-like and directed postero-dorsally; UN1 present, UN1 fused to CC; two Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 autogenous; NS of PU2 normal length, IHC and INC absent.

*Enneanectes carminalis* and *Norfolkia brachylepis* have three Ep. In *E. carminalis* IHC3 and IHC4 are present. There are no IHC or INC reported for other tryperigid species.


*Blenniidae* — CC + PU2 + PU3 contribute to the caudal fin (Fig. 7b); CC presumably contains 1UC [CC (UC1/UC2?)]; Hyp1 + Hyp2 fused to form LHP, LHP articulates with CC; Hyp3
+ Hyp4 fused together to form UHP, UHP fused to CC; Hyp5 present, Hyp5 not fused to UHP nor to CC, Hyp5 shortened; PH articulates with CC, fused to LHP; HU on PH, HU splint-like and directed postero-dorsally, HU shortened; UN1 present, UN1 fused to CC and UHP; two Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 autogenous; NS of PU2 normal length; IHC2 + IHC3 + IHC4 (connected) present, INC2 + INC3 + INC4 (connected) present.
Developmental data of *Enchelyurus brunneolus* suggests that only one UC develops during ontogeny (Watson 1987). In *Apidontus taeniatius* and *Plagiotremus tapeinosoma* the LHP has dorsal outgrowth while the UHP has a ventral outgrowth which results in a small Dia. In *Apidontus taeniatius, Plagiotremus tapeinosoma, Chasmodes bosquianus* no Hyp is present. Watson (1987) reported a small cartilage dorsal to the UHP in his developmental stages of *E. brunneolus* but interpreted it as a radial cartilage rather than Hyp5. Since Hyp5 is present in most other examined blenniids at the same position, it seems reasonable to interpret this structure to be Hyp5. The PH is separate from the LHP in *Ecsenius bicolor*. In *C. bosquianus* and *E. brunneolus* the LHP and UHP are partially fused. Only one Ep is present in *A. taeniatius, C. bosquianus, Parablennius yatabei, Petroscirtes breviceps*, and *P. tapeinosoma*. The NS of PU2 is short in *Istiblennius enosimae, Parablennius*, and *Entomacrodus nigricans*.


*Clinidae* — CC + PU2 + PU3 contribute to the caudal fin (Fig. 7c); CC contains one UC [CC (UC1/UC2?)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; LHP and UHP partially fused, Dia narrowed but still large; Hyp5 present, Hyp5 very short, Hyp5 not fused to UHP; PH fused to CC, fused to LHP; HU absent; UN1 present, UN1 fused to CC and UHP; two Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 autogenous; NS of PU2 normal length; IHC2 + IHC3 present, INC absent.

The developmental data of *Clinus cottoides* and *Myxodes viridis* suggest that only one UC develops during ontogeny and is eventually reduced in size to form the CC (Fishelson and Gon 2009, Zavala-Muñoz, et al. 2016). Due to the fusion of the LHP and UHP, the diastema is narrowed in all clinids and is even more narrowed in *Heteroclinus heptaeolus* and *Ericentrus rubrus*. While Hyp5 is present in some *Heteroclinus* species and in *Cristiceps australis*, Hyp5 is not distinguishable in the other examined species and developmental data of *Myxodes viridis* indicates that no Hyp5 is developed in this species. IHC3 was only observable in *Heteroclinus*.


*Labrisomidae* — CC + PU2 + PU3 contribute to the caudal fin (Fig. 7d, e); CC probably contains one UC [CC (UC1/UC2?)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; Hyp5 present, Hyp5 not fused to UHP nor to CC,
HS very short; PH articulates with CC, fused to LHP; HU absent; UN1 present, UN1 fused to CC and to UHP; two Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 + PU3 autogenous; NS of PU2 truncated; IHC2 + IHC3 + IHC4 present, INC3 + INC4 present.

A juvenile specimen of *Paraclinus marmoratus* indicates that only one UC is developed and forms the CC as it very much resembles the developmental shape of the CC in blennids. In *P. altivelis* a HU is present on the PH and INC4 is missing (Fig. 7e).

*Chaenopsidae* — CC + PU2 + PU3 contribute to the caudal fin (Fig. 7f); components of the CC unknown [CC (?)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; LHP and UHP partially fused, Dia narrowed; Hyp5 present, Hyp5 very small, Hyp5 not fused to UHP; PH fused to CC, PH fused to LHP; HU absent; UN1 absent; one Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 + PU3 autogenous; NS of PU2 normal length; IHC2 and IHC3 + IHC4 (connected) present, INC2 + INC3 + INC4 (connected) present.

In *Neoclinus bryope* the LHP and PH articulate with the CC. Also, the LHP and UHP are not fused and the Dia is not narrowed. There are two Ep present in *N. bryope*. In *Mccoskerichthys sandae* Hyp5 is not distinguishable but it seems likely that it is part of the UHP as this plate is larger in *M. sandae* than in the other examined species and occupies the space where Hyp5 is expected to be. There are no IHC4 and INC4 present in *M. sandae*.


*Dactyloscopidae* — CC + PU2 contribute to the caudal fin; components of CC unknown [CC (?)]; Hyp1 + Hyp2 fused to form LHP, LHP fused to CC; Hyp3 + Hyp4 fused to form UHP, UHP fused to CC; LHP and UHP partly fused anteriorly; Hyp5 not distinguishable (either fused to UHP or absent); PH fused to CC and to LHP; HU absent; UN1 present, UN1 fused to CC and to UHP; two Ep present; HA of PU2 and PU3 fused to respective centrum; HSP of PU2 autogenous; NS of PU2 normal length; no IHC or INC present.

While the UN is easily distinguished in *Gillellus semicinctus* and *Leurochilus acon*, its dorsal portion is very much reduced in the other examined species. In *Dactylagnus mundus* and *Gillellus semicinctus* only one Ep is present.

Ancestral character state reconstruction

The ancestral character state reconstructions (Fig. 8 and online Appendix 3) based on the phylogenetic tree provided by Betancur-R, et al. (2017) revealed several characters that support the Atherinomorpha as a clade as well as the clade including Lipogramma up to the Blenniimorphae (sensu Betancur-R, et al. 2017), or subgroups of it. The Atherinomorpha are supported by five characters likely present in their last common ancestor: (1) three preural centra support the caudal fin, (2) fusion of the LHP with the CC, (3) fusion of UN1 with the CC, (4) fusion of the HA of PU2 with the respective centrum and (5) absence of IHC2. Additionally, two characters, Hyp3 and Hyp4 not fused to CC and a full length Hyp5, would be shared by a last common ancestor of atherinomorphs and cichlids. The taxon including Grammatidae, Plesiopidae, Pseudochromidae, Opistognathidae and the Blenniimorphae are supported by a severely shortened Hyp5 and the fusion of the PH with the LHP. Furthermore, a last common ancestor of the Blenniimorphae shares the fusion of the HA of PU2 with the respective centrum, which seemingly evolved convergently in Atherinomorpha. The Blenniiformes without Tripterygiidae share the fusion of UN1 with the CC and the UHP. A common ancestor of the taxa Chaenopsidae, Clinidae, Dactyloscopidae, and Labrisomidae should have the HU missing, and the LHP fused to the CC, the latter seemingly evolved independently in Atherinomorpha.

The ancestral character state reconstruction also provides a possible grundplan for the last common ancestor of all ovalentarian taxa. However, not for all characters an explicit state can be identified. This last common ancestor probably had two preural centra incorporated in their caudal fin skeleton. The lower hypurals were fused and formed a lower hypural plate while the upper hypurals were separated. The parhypural and the lower hypural plate articulated with the respective ural centrum or compound centrum. A short hypural 5 was present. A diastema separated the lower hypural plate from the upper hypurals. One uro- neural was present, which was not fused to the compound centrum or the upper hypurals. The haemal arch of preural centrum 2 was not fused to the respective centrum but the haemal arch of preural centrum 3 was. The neural spine of preural centrum was very short. Two epurals were present just as interhaemal cartilages 2, 3, and 4.

Phylogenetic Analysis

The results of the different phylogenetic analyses (Fig. 9 and online Appendix 4) dif-
ferred greatly based on the restrictions provided by the respective data sets. The data set containing Atheriniformes, Cyprinodontiformes and all families of the Beloniformes resulted in derived Beloniformes with Oryzias as their most basal taxon and the Atheriniformes clustering within this group as sister taxon to belonids, exocoetids and hemiramphids. The Cyprinodontiformes were retrieved rather distant as sister-taxon to the Labrisomidae. The derived position of the beloniforms in this analysis can be due to the assumption that Oryzias is the most basal taxon (which is in congruence with other phylogenetic analyses) and represents the basal character state conditions of beloniforms. However, the ancestral character state reconstruction showed that Oryzias has many derived character states and may not ideally represent the ancestral character states of beloniforms. Further, the Atheriniformes clustering within the Beloniformes can also be seen as an indicator that the assumptions in the phylogenetic analyses may be incorrect.

Using a slightly altered data set summarizing all beloniform families as one
unit, i.e., Beloniformes, provided a much different result. Atheriniformes and Beloniformes were retrieved as sister taxa and occupied a much more basal position within the Ovalentaria closely to the Cichlidae in the resulting phylogenetic tree. The Cyprinodontiformes again were retrieved as sister-taxon to the Labrisomidae. While the Cyprinodontiformes cluster with atheriniforms and beloniforms in many other phylogenetic analyses, it seems as if the derived caudal fin morphology of cyprinodontiforms has a big influence on the results of the herein conducted analyses.

As many other studies provided convincing results for a close relationship of atheriniforms, beloniforms and cyprinodontiforms (Nelson, et al. 2016) and the close relationship of atheriniforms and beloniforms retrieved herein, the third data set summarized these three taxa as Atherinomorpha. The phylogenetic analyses using maximum parsimony (MP), maximum like-
likelihood (ML) and Bayesian inference (BI) analyses resulted in overall similar phylogenetic hypotheses with this set of data (Fig. 9). Although the support values provided by these analyses are only moderate, this can be attributed to the rather small set of characters used. Minor differences in the topologies of the phylogenetic trees still exist as the Embiotocidae, the basal taxon in ML and BI analyses, is positioned above basal Atherinomorphs and Cichlidae with MP. Further, the Opistognathidae and Plesiopidae are positioned more derived compared to the monophyletic Grammatidae in MP while they are positioned more basal in ML and BI. One remarkable result retrieved by all three methods is the position of Gobiesocidae, Congrogadidae and *Pholidichthys* as the most derived taxa.

**DISCUSSION**

*Evolutionary trends*

The manifold caudal fin shapes that can be found in ovalentarian taxa (Fig. 1), is surpassed by the different compositions of the caudal fin skeletons in this clade. Many authors presented detailed descriptions of caudal fins for numerous ovalentarian taxa (e.g., Fujita 1990), but none of them compared these taxa in a phylogenetic context, yet. When doing so, the caudal fin skeletons revealed different evolutionary trends that are observable within the Ovalentaria.

1) Reduction of the overall number of hypural elements (Fig. 8a). First, hypurals 1 and 2 fuse to form a lower hypural plate. While in few ovalentarian taxa, i.e., Cichlidae, Polycentridae and Pomacentridae, these two elements remain separated, they are fused in all other taxa. Developmental data suggests that the tendency of fusion is reflected by the time it occurs during ontogeny. While in more basal ovalentarian taxa, e.g., mugilids, atheriniforms and beloniforms, the single hypurals are still preformed as separate cartilages and then fuse (Valdez-Moreno and Vásquez-Yeomans 2001, Thieme, et al. 2020, Thieme, et al. submitted), in more derived taxa, e.g., blenniids and clinids, the lower hypural plate already develops from a single cartilaginous element (Peters 1981, Fishelson and Gon 2009). Also, within the Pomacentridae the fusion of the lower hypurals evolved independently emphasizing the tendency of the reduction of the amount of hypural elements (Fujita 1990). Second, a similar trend is observable for hypurals 3 and 4, which are also fused in the majority of ovalentarian taxa (Fig. 8a). While these elements remain separate in atheriniforms, cichlids, embiotocids, polycentrinds, and pomacentrids, this strengthens the hypothesis that hypurals tend to fuse,
as it requires several independent acquisitions of this feature. Third, the fifth hypural is reduced in size in different ovalentarian taxa or even absent, e.g., Congrogadidae, Cyprinodontiformes, Dactyloscopidae, and Gobiesocidae (Fig. 8a). A shortened hypural 5 is observable in many taxa, but within the taxon assemblage including grammatids, pseudochromids, gobiids, and blenniids, it is severely shortened and missing in two of the included families, i.e., dactyloscopids and gobiids. The overall reduction of the number of hypural elements results in less flexible and stiffer hypural plates. This is further emphasized by the fusion of the lower and upper hypural plate and the fusion of the parhypural to the lower hypural plate which is observable in some taxa (Fig. 8a).

2) Fusion of the hypurals to the compound centrum. Both the lower hypurals, respectively the lower hypural plate, and the upper hypurals, respectively the upper hypural plate, tend to fuse to the compound centrum. The fusion of the lower hypural plate with the compound centrum is observable in atherinomorphs and the Blenniiformes (without the blenniids) as well as pholidichthyids and gobiids (Fig. 8b). The fusion of the upper hypurals with the compound centrum is present in almost all ovalentarian taxa except atheriniforms, cichlids, embiotocids, and the beloniform families Belonidae, Exocoetidae and Hemiramphidae. The fusion of the hypural elements with the compound centrum results in a stiffened caudal fin complex.

3) Transition from a forked fin shape to a rounded fin shape (Fig. 8c). Only few taxa within the Ovalentaria retain a forked caudal fin, i.e., Ambassidae, Atheriniformes, Beloniformes, Embiotocidae, Mugilidae, and Pomacentridae. Within the Atheriniformes and Beloniformes many species have already altered caudal fin shapes, e.g., many melanotaeniids have lunate caudal fins and zenarchopterids have rounded caudal fins.

The illustrated evolutionary trends correlate with the locomotion types employed by the different ovalentarian taxa. While the locomotion of fishes is well-investigated (e.g., Webb 1984, Lauder 1989, Webb 1994, Sfakiotakis, et al. 1999, Lauder 2000, Drucker and Lauder 2002, Flammang and Lauder 2009, Lauder 2015), the influence of the caudal fin skeleton on locomotion and vice versa was only considered by Gosline (1997). Herein we shortly want to introduce two examples on the likely interaction between caudal fin skeleton and the locomotion of the respective taxa.

1) Mugilids can be best described as generalists in terms of their locomotion. They use a combination of body and caudal fin propulsion where a propulsive wave is created
with the caudal fin which is then passed through the trunk, and median and paired fin propulsion, where a propulsive force is produced with the median or paired fins, primarily with the pectoral fins (Webb 1984, 1994, Sfakiotakis, et al. 1999). Such a combination gives them fairly well cruising and accelerating abilities but still allows for good manoeuvrability (Webb 1984). The caudal fin supports all three of these swimming modes by creating a propulsive wave that reaches halfway through the trunk, this is called carangiform motion: intermediate state between full body undulation and caudal peduncle oscillation (Sfakiotakis, et al. 1999). Required for such a locomotion is a certain degree of movability of the skeletal elements of the caudal fin and at the same time some stiffness in the caudal fin skeleton as the generated forward forces need to be counteracted (Gosline 1997). In mugilids this is achieved by the fusion of hypurals 1 and 2 as well as hypurals 3 and 4 which are also fused to the compound centrum. Furthermore, the shape of the caudal fin is forked. This creates a moderate stiffness in the caudal fin but still allows for a lateral movement of the lower and upper fin lobes.

2) Blenniids are more specialized in their locomotion. In their bottom dwelling lifestyle, they mainly use median and paired fin propulsion and their caudal fin is primarily used for manoeuvring. The caudal fin serves as an elongation of the body that on one hand generates an undulatory motion and on the other supports the undulatory movement of the median fins. The rounded shape of the caudal fin fits this type of locomotion as projecting appendages are reduced (Webb 1994). Further, the caudal fin skeleton needs neither flexible elements which allow for a high degree of independent movement of the lower and upper fin elements nor stiffened elements to counteract strong forces. However, in blenniids single skeletal elements get reduced by fusion, i.e., hypurals 1 and 2, hypurals 3 and 4, upper hypural plate to compound centrum or are reduced in size, i.e., hypural 5 (Fig. 8a). This seems necessary as it stiffens the caudal peduncle, reduces independent movements of the caudal fin, and, therefore, results in a direct prolongation of the horizontal axis of the vertebral column up to the posterior tip of the caudal fin.

The influence of the caudal fin skeleton on the mode of locomotion and vice versa obviously is well-understudied. The two examples explained above already emphasize the missed opportunities in not examining the skeleton when analysing the locomotion of fish. Combining these two fields of study can give new insights into the evolution of modes of locomotion within different teleost taxa and simultaneously reveal associated changes in the caudal fin skeleton.
Grundplan of the ovalentarian caudal fin skeleton

The reconstruction of the grundplan of the Ovalentaria provides an overview of possible character states in the last common ancestor of ovalentarian taxa. In general, the states of most characters are similar to that of the chosen outgroup, Polymixia. However, few characters states seem questionable although their reconstructed probabilities are unambiguous, e.g., fusion of hypural 1 and hypural2. Regarding this specific character, one would assume that these two elements were separate in the last common ancestor of all ovalentarian taxa as they remain separate in some more basal taxa, i.e., Cichlidae, Embiotocidae, Pomacentridae, Pomacanthidae, as well as in the outgroup taxon. Furthermore, developmental data shows that even in some of the more basal taxa, in which these hypurals are fused in adults, they develop separately during ontogeny (Hollister 1937a, Potthoff, et al. 1987, Valdez-Moreno and Vásquez-Yeomans 2001, Woltering, et al. 2018, Thieme, et al. 2020, Thieme, et al. submitted). However, the underlying phylogenetic hypothesis has very low support values for the basal nodes within the Ovalentaria and, therefore, the topology of the phylogenetic tree needs to be questioned (Betancur-R, et al. 2017). For the described examples a slightly altered topology might change the results of the character state reconstruction. For other characters, the topology of the basal taxa seems to have less impact.

Phylogenetic relationships of ovalentarian taxa

This is the first time, morphology based phylogenetic analyses including all ovalentarian taxa has been performed. The taxon assemblage proposed by molecular-genetic data comprises between 42 and 48 families, depending on author (Nelson, et al. 2016, Fricke, et al. 2020), which previously were widely scattered within the Percomorpha. Hence, no study analyzed this specific composition of taxa with morphological data before. Herein, 48 families were examined, of which all atheriniform and all cyprinodontiform families were condensed as Atheriniformes and Cyprinodontiformes respectively as their monophyly was confirmed by both, morphological and molecular-genetic data (e.g., Dyer and Chernoff 1996, Hertwig 2008, Campanella, et al. 2015, Pohl, et al. 2015, Betancur-R, et al. 2017).

The phylogenetic reconstructions based on characters from the caudal fin skeleton resulted in overall congruent typologies (Fig. 9). Furthermore, there is also a high congruence between the herein presented results and recent phylogenetic hypothesis based on molecular-genetic data (Wainwright, et al. 2012, Betancur-R, et al. 2017, Hughes, et al. 2018). A dif-
ference revealed by Bayesian inference and maximum likelihood approaches is the position of the Embiotocidae as the most basal ovalentarian taxon. Species in this family have a very similar caudal fin skeleton as the chosen outgroup Polymixia. A character that may affect the retrieved position of embiotocids is the hypural diastema. However, its character state in both taxa should be regarded as derived which is why the results retrieved with the maximum parsimony approach, may be considered more accurate (Fig. 9c). There, the Embiotocidae were in a rather basal position after the splits of Atherinomorpha and Cichlidae, which is inversely the results from the other analyses. An important similarity between molecular and morphological phylogenetic analyses is the retrieved position of Atherinomorpha and Cichlidae at the base of the Ovalentaria. Although molecular data suggested that pholidichthyids and polycentrinds are more closely related to cichlids than atherinomorphs, Bayesian inference and maximum likelihood provide some evidence for a close relationship of cichlids and polycentrinds (Fig. 9a, b). Within the molecular-based phylogenetic hypotheses the position of the Ambassidae, Mugilidae, and Pomacentridae is uncertain. However, they are positioned rather basal which is consistent with morphology-based topologies. A close relationship of mugilids and atheriniforms, which was proposed on the basis of morphological (Stiassny 1990, 1993) as well as molecular data (Dettai and Lecointre 2005), is not supported by the caudal fin morphology. As closer relationship of ambassids and mugilids as proposed by Wainwright, et al. (2012) and Hughes, et al. (2018) is retrieved by Bayesian inference analysis (sister-taxa) as well as maximum likelihood and maximum parsimony (consecutive taxa). Further taxa, i.e., Plesiopidae, Pseudochromidae, Opistognathidae and Grammatidae, were retrieved in similar positions within both, molecular-based and morphology-based, analyses. While molecular data suggests that the two grammatid genera Gramma and Lipo-gramma are only distantly related, which results in paraphyletic Grammatidae (Betancur-R, et al. 2013, Betancur-R, et al. 2017), the morphological data supports monophyletic Grammatidae (Fig. 9). The Blenniiformes were also retrieved in the morphological analyses. Within this taxon assemblage the basal positions of tripterygiids and blenniiids is supported, however, further taxon relationships differ between molecular-based and morphology-based results. The phylogenetic hypothesis provided by Betancur-R, et al. (2017) suggested that the Chaenopsidae and Labrisomidae are paraphyletic taxa. These findings are also supported by morphological data as Neoclinus is retrieved basal within the Blenniiformes while the remaining chaenopsids are positioned more derived. The two subgroups of labrisomids were retrieved as consecutive taxa.
Three of the analyzed taxa, i.e., Congrogadidae, Gobiesocidae, and Pholidichthyidae, were positioned completely differently within the herein present phylogenetic topologies than in the molecular-based phylogenetic trees. They are the most derived taxa and are positioned within the Blenniimorphae. This is explainable based on the composition of their caudal fin skeleton. Many characters of their caudal fin skeletons have derived character states, similar to the ones present in the Dactyloscopidae, which determine their resulting position within the phylogenetic tree (Fig. 8, 9). However, in molecular-based phylogenies, the Pholidichthyidae are positioned as sister-taxon to the Cichlidae, the Congrogadidae are closely related to the Pomacentridae, and the Gobiesocidae take a basal position within the Blenniimorphae (Wainwright, et al. 2012, Betancur-R, et al. 2017). The derived state of the caudal fin skeleton in these taxa could be the results of independent adaptations to similar functional and locomotory needs and the occupation of similar habitats which require such adaptations. Therefore, in the morphological analysis convergent similarities reflecting their swimming behavior, i.e., distinct reductions and fusions, are not unlikely to outmatch the phylogenetic signal. However, combining characters from additional morphological complexes may help in retrieving more meaningful results for the phylogenetic position of congroagadids, gobiesocids and pholidichthyids.

**Conclusion**

The present study shows that recent molecular-genetic phylogenies can contribute to new hypotheses in the evolution of morphological structures, while morphological data can be employed to independently test molecular-genetic findings. Overall, both approaches work well together and can lead to new insights in the evolution of fish diversity. The phylogenetic analyses of the Ovalentaria using a restricted data set covering only one morphological complex, the caudal fin skeleton, resulted in similar topologies as proposed by molecular data, which on the one hand supports these findings and on the other hand demonstrates the power of morphological data. The well-known disadvantage, the time-consuming data acquisition, of morphological analyses should not be considered an obstacle as the results from such analyses bring forth multiple new hypotheses useful in various disciplines, e.g., anatomy, evo-devo, functional morphology, phylogenetics, and many other disciplines.
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Discussion

Insights on the Osteology of Grey Mullets

In Chapter 1 and Chapter 2, the osteology of one species, *Liza aurata*, was described in detail and the development of the postcranial skeleton of *Mugil cephalus* was presented. The overall results indicate that the skeleton of mugiliforms is relatively similar between different species. However, some anatomical structures show differences which may be useful for analysing the intrarelationships of mugiliforms. In the following a selection of these characters shall be discussed on the basis on the most recent phylogenetic hypothesis of mugiliform intrarelationships (Fig. 4; Xia et al. 2016).

The skull is the most complex osteological structure found in the teleost body with numerous single bones that are interconnected in various ways, e.g., fixed sutures, different joints, overlay, or connected via cartilage. It is also the complex in which multiple differences between *Liza aurata* and *Mugil cephalus* are noticeable, e.g., connection of basisphenoid and prootic, shape of the metapterygoid, position of the nasal, or the composition of the branchial arches (Chapter 1). Likely to its complexity, the skull is also the least investigated structure in mugiliforms. Therefore, for a subsequent comparative analysis between multiple mugiliform taxa there is presently not enough data published. Other characters like the pelvic girdle, the median fins, and the caudal fin were studied in more mugiliform species allowing for a more detailed discussion of their potential relationships.

The pelvic girdle of mugiliforms comprises two basipterygia each having an anterior basipterygial arm and a posterior basipterygial plate. The pelvic girdle is antero-dorsally inclined with its anterior tips positioned underneath the coracoids. In *Liza aurata* the basipterygial plates interdigitate medial which ensures a solid connection of both halves of the pelvic girdle. In other Cheloninae genera, i.e., *Chelon*, *Planiliza*, and *Oedalechilus*, the basipterygial plates are also interdigitated (personal observation). The same connection can be found in the basal Myxinae, e.g, *Myxus elongatus*. In the genus *Mugil* the medial border of the basipterygial plates are thickened but only contact each other without interdigitation (Ghasemzadeh 2015b, personal observation). In other Mugilinae genera the situation is different. There, a similar condition as in *Cheloniae* and *Myxinae* can be observed (Ghasemzadeh 1998). In different
Rhinomugilinae genera, i.e., *Aldrichetta*, *Gracilimugil*, *Paramugil*, and *Rhinomugil*, the basipterygial plates interdigitate with each other (personal observation). However, in *Squalomugil nasutus* and *Ellochelon vaigiensis* different conditions are present. While in *S. nasutus* a medial process from each basipterygial plate reaches towards the opposing basipterygial plate connecting both pelvic girdle halves, in *E. vaigiensis* the basipterygial plates are well separated without any connection (Ghasemzadeh 1998). The basal character state for the connection of the basipterygial plates in mugiliforms seems to be the interdigitation of both elements. Twice, once at the base of the genus *Mugil* and once in *E. vaigiensis*, this type of connection was independently dissolved, while only in *E. vaigiensis* both halves became separated. The particular connection of the pelvic girdle halves found in *S. nasutus* is seemingly only present in this single species and is a characteristic trait of *S. nasutus*.

The composition of the caudal fin skeleton of most mugiliforms is very similar to that found in *L. aurata* and *M. cephalus*. A compound centrum, formed by fusion of two ural centra (Chapter 2), is present to which a parhypural and a lower hypural plate articulate. Dorsally, an upper hypural plate, which is fused to the compound centrum and hypural 5, is present (Chapter 1). Only in few mugiliform species, i.e., *Aldrichetta forsteri*, *Cestraeus plicatilis*, *Cestraeus oncyrhynchus*, *Gracilimugil argenteus*, *Myxus elongatus*, and *Trachystoma petardi* hypural 3 and hypural 4 are not fused to form the upper hypural plate (Chapter 6, Ghasemzadeh 1998). Developmental stages of *M. cephalus* and *G. argenteus* showed that in both species, both hypural 3 and hypural 4 are separately preformed in cartilage before they fused during ossification in *M. cephalus* and remain separated in *G. argenteus* (Chapter 2). Due to the close relationship of *A. forsteri*, *G. argenteus* and *T. petardi* it seems reasonable that their last common ancestor would have shown the same character state. Based on the principle of Ockham’s razor the most parsimonious assumption would be that in the grundplan of mugiliforms, hypural 3 and hypural 4 fuse during ontogeny to form the hypural plate. During evolution, however, the fusion of these two elements was inhibited once, within the Myxiniae, in *Myxus elongatus*, another time, within the Mugilinae, in the genus *Cestraeus* and a third time, within the Rhinomugilinae, in the last common ancestor of *Aldrichetta*, *Gracilimugil*, and *Trachystoma*. This is an independently gained synapomorphy for each of these sets of taxa.
These two examples show that the skeleton of mugiliforms provides useful characters for the analyses of the phylogenetic intrarelationships of mugiliform species. Furthermore, they can be used to characterize genera and species with anatomical data. However, the data available for different mugiliform taxa is not yet sufficient to use it for a comprehensive phylogenetic analysis or to propose apomorphies for certain clades.

**Phylogenetic Relationship of Mugiliformes and Atherinomorpha**

The phylogenetic position of mugiliforms has been discussed for a long time and by various authors (e.g., Starks 1899; Berg 1940; Gosline 1962). Neither morphological approaches (e.g., Gosline 1971; Stiassny 1990; Johnson and Patterson 1993; Stiassny 1993) nor molecular-genetic analyses (e.g., Chen et al. 2003; Dettai and Le-
cointre 2005; Setiamarga et al. 2008; Wainwright et al. 2012; Betancur-R et al. 2017) yielded satisfying results. A closer relationship of mugiliforms and atherinomorphs (especially basal atheriniforms) seemed conceivable due to their resemblance in body form and fin placements. Three very detailed morphological studies suggested different characters supporting such a relationship: Johnson and Patterson (1993) grouped both taxa within their Smegmamorpha based on one shared character, i.e., configuration of first vertebra and its intermuscular bone. Stiassny (1990) proposed four characters that support a closer relationship of only mugiliforms and atherinomorphs. In a later study (Stiassny 1993), she reviewed these characters and had to abandon one while suggesting four newly found shared features. Of the seven characters proposed, three are associated to the musculature of the pharyngeal system, i.e., subdivision of pharyngocleithralis muscle and separation of the levator externus 1 from the levator externus 2-4, as well as the pelvic girdle, i.e., extensive abductor profundis muscle. The other four characters deal with skeletal structures of the vertebral column, i.e., expanded anterior neural arches, and the pectoral girdle, i.e., supracleithrum reduced and without sensory canal, marginal pectoral ray reduced to spur, and posterior directed dorsal cleithral process. While these characters support a close relationship of mugiliforms and atherinomorphs when compared to paracanthopterygians and a selection of acanthomorph species, it remains doubtful if they can withstand a closer analysis using the whole set of ovalentarian taxa. The recently proposed Ovalentaria assembles many taxa previously widely spread within percomorphs (Nelson 2006; Wainwright et al. 2012; Betancur-R et al. 2017). In Stiassny’s (1993) study only two ovalentarian taxa besides mugiliforms and atherinomorphs were examined: the ambassid *Ambassis urotaenia* and the cichlid *Coptodon zillii*. Furthermore, based on the type of preparation of these specimens, it was not possible to fully analyse the musculature of *A. urotaenia* (cleared and stained specimen) and it seems improbable that detailed skeletal features of *C. zillii* (alcohol specimen) were examined.

After analysing the proposed skeletal features in various ovalentarian taxa (personal observations), only the reduced marginal spur remains as a character shared by only mugiliforms and atherinomorphs. Expanded neural arches are also present in other ovalentarian taxa, i.e., Chaenopsidae, Labrisomidae, Opistognathidae, Pholidichthyidae, Polycentridae, and Pomacentridae. However, in most of these taxa the expansion is not as pronounced as in mugiliforms or atherinomorphs. The second
skeletal character, i.e., reduced supracleithrum without sensory canal, is even questionable for mugiliforms. Although the supracleithrum in mugiliforms does not bear a sensory canal or groove, it is debatable if it should be categorized as ‘reduced’. While the supracleithrum in atherinomorph taxa can be considered very small in proportion to the posttemporal or is even absent in some taxa, i.e., Adrianichthys, it is considerable larger in comparison to the posttemporal in mugiliforms (Chapter 1, Stiassny 1993, personal observation). A similar size ratio of supracleithrum and posttemporal as in mugiliforms can be found in some ovalentarian taxa, e.g., Pomacentridae and Tripterygiidae (personal observation). In Ambassidae the supracleithrum is still large, but it also lacks the sensory canal. The posterior directed dorsal cleithral process can also be found in Blenniidae, Chaenopsidae, Cichlidae, Grammatidae, Plesiopidae and Pomacentridae. Again, in some of these species the posterior direction is not as pronounced as in mugiliforms or atherinomorphs.

Although only one character proposed by Stiassny (1990, 1993) did withstand this provisional test with various ovalentarian taxa, the resemblance of mugiliforms and atherinomorphs (particularly basal atheriniforms) still seems to be an intriguing reason to assume a closer relationship. Especially the positioning of the two dorsal fins and the presence of interdorsal pterygiophores could be a good indication for that hypothesis (Richter and Moritz 2017). However, there are multiple differences in the composition of these characters between mugiliforms and atherinomorphs. First off, in atheriniform taxa, a varying number of fin spines is present (e.g., Saeed et al. 1989; Ivantsoff and Crowley 1991; Parenti 1993; Richter and Moritz 2017), whereas there is a definite number of four enlarged fin spines present in all mugiliform species (Chapter 1, Nelson et al. 2016). Furthermore, the shape and number of interdorsal pterygiophores is different in both taxa. Just like the first dorsal fin rays, the number and also the shape of the bones vary greatly among atheriniform species (Richter and Moritz 2017), while there are always three interdorsal pterygiophores present in mugiliforms resembling the pterygiophores of the second dorsal fin (personal observation, Chapter 1). Lastly, in atheriniform species, the first fin ray of the second dorsal fin is spiny, while in mugiliforms there are no spiny fin rays present in the second dorsal fin. Developmental data of the dorsal fins may help strengthen a closer relationship of mugiliforms and atheriniforms: In the latter taxon the interdorsal pterygiophores and the pterygiophores of the first dorsal fin develop in series from posterior to anterior.
and the associated spiny fin rays develop from anterior to posterior afterwards (Rich-
ter and Moritz 2017). In mugiliforms a similar pattern was observed as interdorsal and
first dorsal fin pterygiophores presumably develop in series from posterior to anterior
(Chapter 2). The development of the fin spines, however, did not follow a specific di-
rectional pattern in mugiliforms.

The development of the caudal fin skeleton revealed an interesting character
that mugiliforms and atherinomorphs share: fusion of two ural centra to form the com-
pound centrum (Chapter 2 and Chapter 5). However, due to a lack of comparative
data of multiple ovalentarian taxa as well as the presence of two ural centra in many
non-ovalentarian acanthomorphs, it needs to be assumed that this is a plesiomorphic
character state. Further similarities in the caudal fin skeleton and its development for
example comprise the development of five single hypurals of which at least the lower
two fuse to form a plate, the presence of two epurals, as well as the development of a
miniature neural spine close to the neural arch of preural centrum 2 (Chapter 2, Chap-
ter 5 and Chapter 6). But again, these characters too are most likely plesiomorphic as
they are also present in other ovalentarian taxa (e.g., Potthoff et al. 1987; Woltering
et al. 2018).

Overall, mugiliform and atherinomorph taxa have various characters in common,
e.g., development of the second dorsal fin and the anal fin, a lower hypural plate in
the caudal fin skeleton, and broadened anterior neural arches, however, most of them
need to be considered plesiomorphic as they are also present in other ovalentarian
taxa. Other traits, like the interdorsal pterygiophores, probably evolved convergently
which could explain the different shapes and different numbers found in mugiliforms
and atheriniforms. A close relationship of mugiliforms and atherinomorphs may not be
ruled out, but both morphological and molecular data do also not implicitly support it.

**Phylogenetic Position of Mugiliformes within the Ovalentaria**

Recently various molecular-genetic analyses positioned the Mugiliformes within
the taxon Ovalentaria (Wainwright et al. 2012; Betancur-R et al. 2013; Betancur-R et
al. 2017; Hughes et al. 2018). While the Atherinomorpha are also part of this taxon, the
Mugiliformes were not retrieved as their sister-taxon. Rather the Ambassidae (Betan-
Betancur-R et al. (2017) were proposed as possible sister-taxa to the Mugiliformes, however with very low support values. The phylogenetic analyses using caudal fin skeleton characters also retrieved the Ambassidae as the closest related taxon to the Mugiliformes (Chapter 6). The two taxa share the presence of an upper hypural plate which is fused to the compound centrum and an unfused uroneural among other characters. However, many of the caudal fin characters are either plesiomorphic or also evolved convergently in other ovalentarian taxa. Comparing other osteological features of ambassids and mugiliforms revealed that they have more characters in common. For example, the pelvic girdle is anteriorly inclined with the medial portions of the basipterygial plates enlarged and contacting each other (similar to Mugil) and a ligament connects the ventral postcleithrum to the basipterygial arm. Furthermore, the two anterior-most pterygiophores of the anal fin are also fused in ambassids and three fin spines are present in the anal fin with similar articulations as in mugiliforms. These few characters present in mugiliforms and ambassids might emerge as apomorphies, however, a much more detailed analysis including all ovalentarian taxa and various morphological structures is need to reveal the most likely sister-taxon to the Mugiliformes.

Figure 12: Cleared and stained specimen of A) the mugiform Chelon auratus (DMM IE/6620, SL = 39.29mm), and B) the ambassid Gymnochanda ploegi (DMM IE/15773, SL = 28.88mm).
Outlook

The herein presented studies provide new data on the osteological features of mugiliforms, their development, and their value for phylogenetic analyses. The first section showed the need of developmental studies to understand and correctly interpret the adult morphology of different taxa. In section two, the comparison of such developmental data revealed different developmental modes resulting in similar adult morphologies, reiterating the importance of developmental studies. The comparison of characters from the caudal fin skeleton in the last section retrieved similar results as extensive molecular-genetic analyses, although based only on a small amount of morphological data. This provides evidence on the significance of incorporating morphological data in phylogenetic analyses as well as information gained from developmental studies for the understanding of evolutionary processes and phylogenetic relationships.

Based on the provided data in section one, a comparative morphological study including all mugiliform genera can bring forth characterizing traits for these genera or the proposed subfamilies. Furthermore, in section two it was shown that different developmental aspects found in atherinomorphs are unknown in many ovalentarian taxa. Following that up, future analyses should focus on certain aspects such as the developmental of the compound centrum in the Ovalentaria or the general development of scales in various taxa. Although the results in section three provided new insights on the phylogenetic position of mugiliforms, it is still not sufficiently resolved. A broader morphological analysis incorporating more skeletal characters from various body parts will help to achieve a better resolution in phylogenetic reconstructions. Last but not least, section three also showed that the functional aspects of the caudal fin skeleton are largely unknown. The influences that variations in the caudal fin skeletons have on function of the caudal fin should therefore be investigated in a larger set of actinopterygians.
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Appendix

All supplementary files are provided on the appended CD

Ort, Datum

Philipp Thieme