

RESEARCH ARTICLE

Lessons from the first dorsal fin in atheriniforms—A new mode of dorsal fin development and its phylogenetic implications

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Abstract

The median fins in extant actinopterygians are the product of millions of years of evolution. During this time, different developmental patterns for the dorsal and anal fins emerged leading to a high variation in median fin morphology and ontogeny. In this study, the development of anal and dorsal fins in atheriniforms is described and its consequences for the current phylogenetic hypothesis are discussed. Developmental series of five atheriniform species were investigated using clearing and staining as well as antibody staining. The skeletal elements of the second dorsal fin and the anal fin emerge in a bidirectional pattern. The first dorsal fin, however, arises separately in front of the second dorsal fin after this one is almost completely formed. The pterygiophores of the first dorsal fin, including the interdorsal pterygiophores, develop from caudal to rostral, but the fin-spines of the first dorsal fin form in the opposite direction. This new mode of fin development has been found in all examined atheriniform species with two dorsal fins. Several morphological characters of atheriniforms, including interdorsal pterygiophores, are also found in one other taxon: the Mugiliformes. Thus, several dorsal fin characteristics may provide evidence for a closer relationship of these two taxa.

KEYWORDS

Atherinomorpha, median fin, Mugiliformes, ontogeny

1 | INTRODUCTION

The emergence of the locomotory system in vertebrates was a key point during their early evolution. Due to the divers remodeling of fins and paired limbs, the locomotory system is predestinated to examine developmental and evolutionary processes in morphogenesis. Especially the unpaired locomotory structures underwent significant remodeling events. Originally deriving as a median fin fold in amphioxus, the fins of hagfish and lampreys already show unsegmented cartilaginous radii (Coates, 1994; Goodrich, 1930; Marinelli & Strenger, 1954a, 1954b; Richardson & Wright, 2003; Starck, 1979; Zhang & Hou, 2004). Median fins, that is, anal, caudal, and dorsal fins, are present in gnathostomes and a median fin fold still occurs in their larval development. Also, new structures such as pterygiophores can be found in extant chondrichthyes and osteichthyes, although it is still unclear if they derived from supraneuralia and infrahaemalia or if they are indeed new

structures (Cope, 1890; Goodrich, 1930; Mabee, 1988; Mabee, Crotwell, Bird, & Burke, 2002; Schmalhausen, 1913; Thacher, 1877). In chondrichthyes, the pterygiophores support strengthening fibers of elastodin called actinotrichia, which are probably formed by the epidermis. In Actinopterygii, actinotrichia are only present in the larval fin folds and in adipose fins (Bender & Moritz, 2013; Kosswig, 1965; Mabee et al., 2002). During the development, the actinotrichia are surrounded by bony parts, thus forming the bilaterally-paired lepidotrichia. The lepidotrichia articulate with the pterygiophores and can be moved by muscles, allowing their erection and adduction as well as lateral inclination in the more advanced taxa (Drucker & Lauder, 2001). Pterygiophores emerge from a single cartilaginous structure and get separated during the development originally resulting in three radials, that is, proximal, medial, and distal radial (Goodrich, 1930; Hilton, 2011). In more derived actinopterygians, one or more radials may get lost by fusion, loss of separation or complete reduction (Hilton, 2011).

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The diversification of the locomotory structures also led to many other modifications resulting in diverse appearances of median fins. Especially within the Actinopterygii, there have been multiple events of reduction and reinvention, mainly of the dorsal fin resulting in different forms and numbers of dorsal fins. Differences in fins can be found in shapes and skeletal structures, but there may also be different forms of development for these fins. Mabee et al. (2002) gave an overview on the different patterns that have been found in median fin development, showing a connection between the fin positioning and the fin patterning of the second dorsal and the anal fin. Focusing on fin supports and fin rays within their development in the dorsal fins and the anal fin, there are three different patterns that occur among actinopterygian fishes: (1) bidirectional: at first, the median structures develop, with new ones adding anterior and posterior to them; (2) anterior to posterior: most anterior structures occur first and new ones are added posterior to them; (3) posterior to anterior: most posterior structures emerge first and new ones develop in front of them. The second dorsal fin and the anal fin often share the same pattern, whereas the first dorsal fin can express a different developmental pattern (Mabee et al., 2002).

Atheriniformes have not been included in the study of Mabee et al. (2002). Therefore, herein we describe the development of the dorsal fins and the anal fin of different species within this taxon. With this information, we test if Atheriniformes fit within the overview given by Mabee et al. (2002). Furthermore, we discuss the origin of interdorsal pterygiophores, which can be found in 8 out of 10 atheriniform families and evaluate our findings in the light of phylogenetic hypotheses of atheriniform interrelationship and intrareationship.

2 | MATERIAL AND METHODS

Developmental series of three atheriniform species (*Marosatherina ladigesi* (Ahl, 1936), *Melanotaenia lacustris* (Munro, 1964) and *Popondichthys furcatus* (Nichols, 1955) and one cyprinodontiform species (*Pachypanchax omalonotus* [Duméril, 1861]) were attained by breeding in aquaria (zoological garden permit HAST/ZOO/05/430/2005 issued by Staatliches Amt für Umwelt und Natur Stralsund). Before storage in 70% ethanol, larvae were euthanized using an overdose of benzocain and afterwards fixated in 4% formalin. Developmental stages and larger individuals of additional species were obtained from the collection of the Deutsches Meeresmuseum (Table 1). Where no ontogenetic series were present, we used small specimens between 50 and 100 mm SL also for larger growing species, like mugilids. The respective specimens, however, hardly differ from the adult condition in terms of position, ossification and relative size. Therefore, we refer to this situation as "adult" in the text, despite the fact that several specimens were not "adult" concerning their gonadal maturation. A total of 246 specimens were examined (Table 1).

Most specimens were cleared and double stained (cartilage blue, calcified structures red) following a protocol based on Dingerkus & Uhler (1977) and Taylor & Van Dyke (1985). Images were taken using a

Leica MZ75 binocular with a Leica DFC425 camera and the software Leica application suite (version 4.3.0).

Immunolabeling of collagen in 23 specimens of *M. ladigesi* was performed following a standard protocol for the DAKO EnVision Flex system (Agilent Technologies, Hamburg, Germany). Collagen tissue was labeled with a CIIC1 primary antibody (Developmental Studies Hybridoma Bank, University of Iowa, Dept. of Biological Science, Iowa City, IA) and an ALEXA 488 secondary antibody (Invitrogen, Thermo Fisher Scientific Inc., Darmstadt, Germany). Additionally, calcified structures were stained using alizarin-red. Results were documented using a Zeiss LSM 510 and the software Zen 2009 and 2012 as well as a Zeiss Axiovert S100 with a Spot Camera 1.4.0 and the software SPOT Advanced 4.0.9.

Afterwards, images of cleared and double stained specimens were processed in Adobe Photoshop CS6. For a higher contrast, all colors were inverted and the background was adjusted to a uniform black. Adobe Illustrator CC was used to assemble all Figures.

3 | RESULTS

3.1 | *Marosatherina ladigesi*

Early developmental stages up to 5.0 mm SL of *M. ladigesi* only show a median fin fold, without any skeletal structures present (Figure 1a). First cartilaginous pterygiophores develop in the ventral trunk region at about 5.5 mm SL (Figure 1b). Before the first fin supports develop dorsally, more pterygiophores of the anal fin are formed anterior and posterior to the already present cartilages. Therefore, the fin supports in the middle are bigger than the ones in the front and in the back. In the dorsal trunk, the second dorsal fin originates first: its first dorsal fin supports appear right above those of the anal fin (Figure 1c). New pterygiophores will be added in the same way as in the anal fin: anterior and posterior to the ones already present. Accordingly, also in the developing second dorsal fin the fin supports in the middle are larger than the ones, which developed later anteriorly and posteriorly. During the formation of these structures, the median fin fold is reduced in the region in front of the developing second dorsal fin as well as between the second dorsal and the caudal fin. In the ventral body part, the fin fold remains only present in the region of the anal fin. More pterygiophores will then develop right in front of the fin supports of the second dorsal fin. From this stage on, new fin supports are added only anterior to the ones already present (Figure 1d,e). The pterygiophores of the second dorsal and the anal fin separate into a proximal and a distal part, starting with the ones in the middle (Figure 1d–f). The pterygiophores in front of and behind them will follow. First fin rays are developing at the median fin supports of the anal and second dorsal fin (Figure 1d). Like the pterygiophores, new fin rays are added anterior and posterior to the ones already present (Figure 1e,f). In the meantime, all pterygiophores in front of the second dorsal fin developed, including the later interdorsal as well as the first dorsal fin pterygiophores. The fin supports in front of the second dorsal fin will not be divided into proximal and distal radials (Figure 1g,h). Afterwards, fin rays emerge at these fin supports too (Figure 1f). The first fin rays develop at the most anterior

TABLE 1 Ontogenetic series and stages of taxa used in this study

Order and family	Species	Number	Size range	Catalogue number
Atheriniformes				
Atherinopsidae	<i>Membras martinica</i> (Valenciennes, 1835)	1	49.9	DMM-IE/11398
	<i>Menidia conchorum</i> (Hildebrandt & Ginsburg, 1927)	1	64.7	DMM-IE/11399
Atherinidae	<i>Atherina</i> sp.	15	7.1–17.7	DMM-IE/11385
	<i>Atherina</i> sp.	2	10.5–11.9	DMM-IE/11425
	<i>Atherina boyeri</i> (Risso, 1810)	5	14.8–19.7	DMM-IE/11384
	<i>Atherina boyeri</i>	1	72	DMM-IE/11401
	<i>Atherina hepsetus</i> (Linnaeus, 1758)	1	32.9	DMM-IE/11378
	<i>Atherina hepsetus</i>	1	37	DMM-IE/11405
	<i>Atherina presbyter</i> (Cuvier, 1829)	17	8.4–13.5	DMM-IE/11386
	<i>Atherina presbyter</i>	16	8.1–12.5	DMM-IE/11387
	<i>Atherina presbyter</i>	15	11.7–18.8	DMM-IE/11391
	<i>Atherina presbyter</i>	2	12.0–16.2	DMM-IE/11426
Bedotiidae	<i>Bedotia geayi</i> (Pellegrin, 1907)	13	6.3–17.0	DMM-IE/11396
	<i>Bedotia geayi</i>	7	6.1–8.2	DMM-IE/11397
	<i>Bedotia geayi</i>	4	6.5–13.4	DMM-IE/11411
Melanotaeniidae	<i>Iriatherina weneri</i> (Meinken, 1974)	1	29.1	DMM-IE/11395
	<i>Iriatherina weneri</i>	1	32.5	DMM-IE/11407
	<i>Melanotaenia lacustris</i> (Munro, 1964)	8	3.2–14.0	DMM-IE/11375
	<i>Melanotaenia lacustris</i>	8	7.6–12.3	DMM-IE/11376
	<i>Melanotaenia lacustris</i>	2	12.2–13.4	DMM-IE/11379
	<i>Melanotaenia lacustris</i>	7	6.7–14.8	DMM-IE/11414
Pseudomugilidae	<i>Popondichthys furcatus</i> (Nichols, 1955)	12	4.9–14.3	DMM-IE/11394
	<i>Popondichthys furcatus</i>	1	25.4	DMM-IE/11409
	<i>Popondichthys furcatus</i>	3	5.9–7.4	DMM-IE/11412
	<i>Pseudomugil paskai</i> (Allen & Ivantsoff, 1986)	1	28.8	DMM-IE/11380
	<i>Pseudomugil signifer</i> (Kner, 1866)	2	20.7–23.9	DMM-IE/11408
Telmatherinidae	<i>Marosatherina ladigesi</i> (Ahl, 1936)	13	4.6–12.4	DMM-IE/11388
	<i>Marosatherina ladigesi</i>	8	5.8–10.1	DMM-IE/11389
	<i>Marosatherina ladigesi</i>	9	3.4–11.2	DMM-IE/11390
	<i>Marosatherina ladigesi</i>	1	47.5	DMM-IE/11402
	<i>Marosatherina ladigesi</i>	6	4.1–8.9	DMM-IE/11413
	<i>Marosatherina ladigesi</i>	13	4.8–11.0	DMM-IE/11415
Cyprinodontiformes				
Aplocheilidae	<i>Pachypanchax omalonotus</i> (Duméril, 1861)	17	4.4–14.4	DMM-IE/11392
	<i>Pachypanchax omalonotus</i>	5	5.9–13.8	DMM-IE/11393
	<i>Pachypanchax omalonotus</i>	1	46.5	DMM-IE/11403
	<i>Pachypanchax omalonotus</i>	4	5.6–6.3	DMM-IE/11410
Mugiliformes				
Mugilidae	<i>Mugil</i> sp.	5	15.8–21.3	DMM-IE/11371
	<i>Chelon</i> sp.	5	18.7–24.9	DMM-IE/11372
	<i>Liza</i> sp.	1	36.7	DMM-IE/11377
	<i>Liza</i> sp.	1	36.6	DMM-IE/11404
	<i>Liza aurata</i> (Risso, 1810)	5	20.7–24.8	DMM-IE/11373
	<i>Liza aurata</i>	2	27.1–28.7	DMM-IE/11374
	<i>Liza aurata</i>	1	28.1	DMM-IE/11406
	<i>Rhinomugil corsula</i> (Hamilton, 1822)	1	79.9	DMM-IE/11370
	<i>Rhinomugil corsula</i>	1	63.8	DMM-IE/11400

Size is given as notochord length (NL) for small larvae and standard length (SL) for specimens after notochord flexion (in mm).

fin support; new ones are added posterior to them. However, not all fin supports will indeed support a fin ray. Between the first and the second dorsal fin, some pterygiophores will remain as interdorsal pterygiophores without a fin ray. These fin supports may alter their shape during development (Figure 1f,g). In adult specimens, the shape of interdorsal pterygiophores is constant within as well as between the

examined specimens of *M. ladigesi* (Figure 1h). They can be described as bar-like structures (Figure 1f–h). In the meantime, the first proximal radial of the anal fin fuses with the second proximal radial (Figure 1h). In addition, the proximal radial of the last pterygiophore of the anal and second dorsal fin gains a cartilaginous attachment on its dorsocaudal end (Figure 1g). The distal radials of these pterygiophores each support

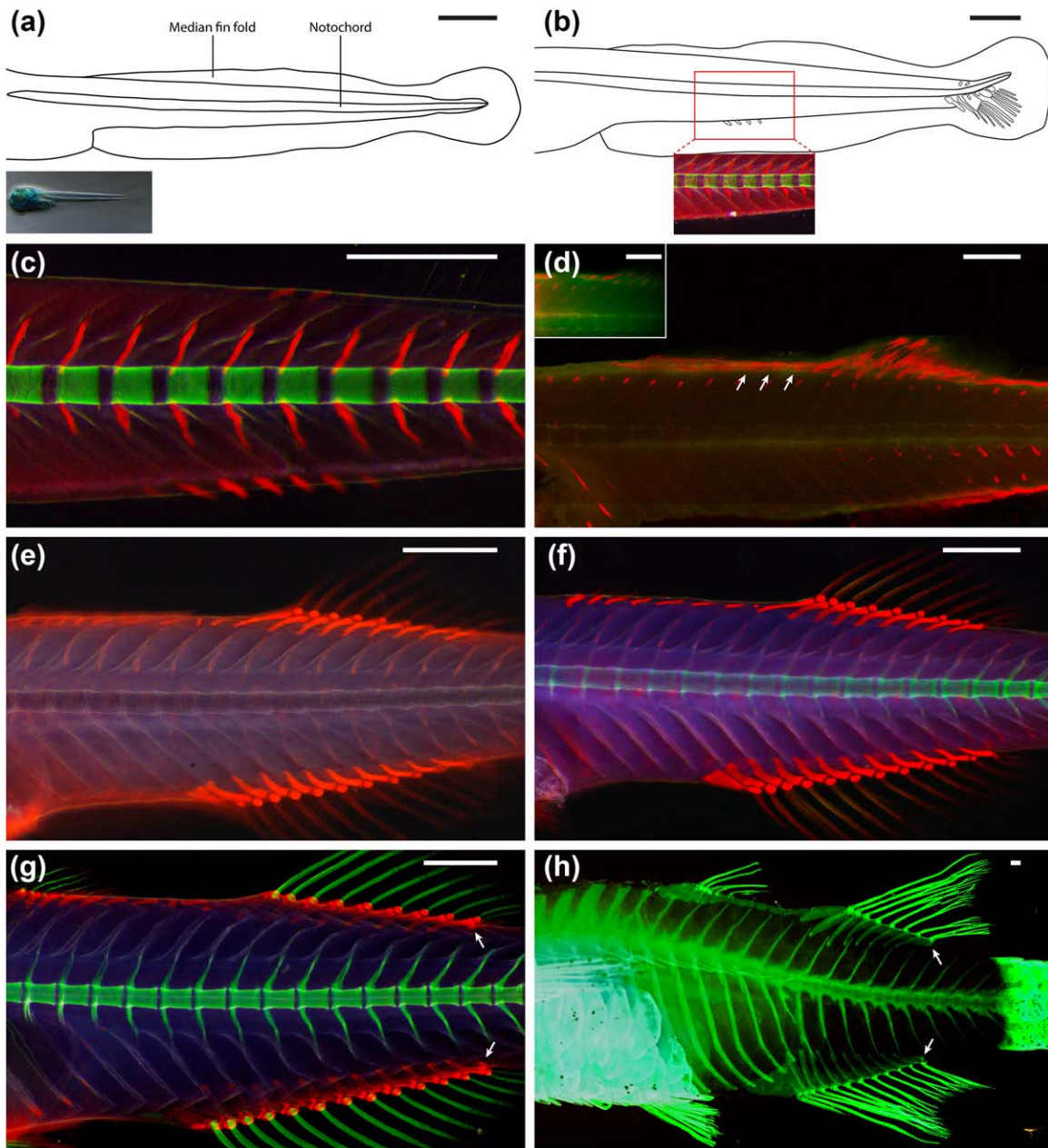


FIGURE 1 *Marosatherina ladiges*, developmental series. (a) NL = 4.1 mm; (b) SL = 6.1 mm; (c) SL = 6.6 mm; (d) SL = 5.5 mm, antibody staining (insert: fluorescence microscopy), arrows indicate the developing first dorsal pterygiophores in front of the second dorsal fin; (e) SL = 7.1 mm; (f) SL = 8.1 mm; (g) SL = 8.9 mm, arrows indicate attachments to most posterior anal and second dorsal fin pterygiophore; (h) SL = 47.5 mm, arrows display attachments to most posterior anal and second dorsal fin pterygiophore; scale bars = 500 μ m

two fin rays (Figure 1g,h). In adult specimens, the lepidotrichia of the first dorsal fin are fin spines as is the first lepidotrichia of the anal and the second dorsal fin (Figure 1h). In addition, only the pterygiophores of the anal and second dorsal fin are separated in proximal and distal radials, the fin supports of the first dorsal fin and the interdorsal pterygiophores are just proximal radials (Figure 1h).

3.2 | Other atheriniform fishes

To determine if the fin development pattern found in *M. ladiges* applies to all atheriniform fishes, more developmental series were examined.

3.2.1 | *Melanotaenia lacustris*

As seen in *M. ladiges* a median fin fold is also present in young larvae of *M. lacustris*. First cartilaginous structures then arise in the ventral trunk. These unseparated pterygiophores first appear in the middle of the trunk; more are added anterior and posterior to them. Like in *M. ladiges* the development of pterygiophores in the dorsal body starts later. Again, first fin supports occur in the middle of the trunk and more are added anterior and posterior to them (Figure 2a). When most pterygiophores of the anal and second dorsal fin are present, first separations of the pterygiophores can be seen. Also, fin rays start developing at the middle fin supports (Figure 2b). In front of the pterygiophores of the

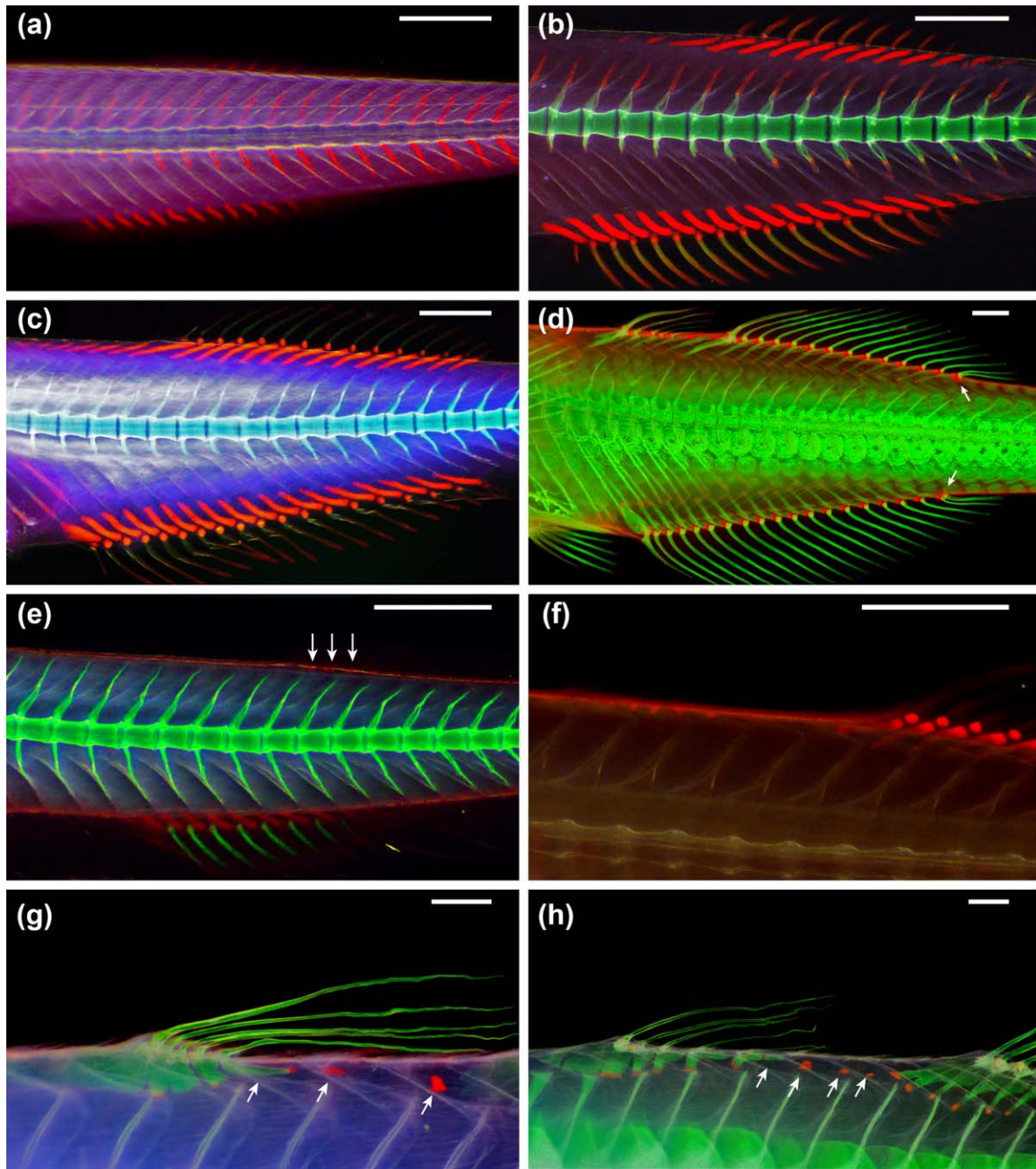


FIGURE 2 Developmental series of *Melanotaenia lacustris* (a–d) and *Popondichthys furcatus* (e–g). (a) SL = 6.7 mm; (b) SL = 8.2 mm; (c) SL = 8.8 mm; (d) SL = 14.8 mm, arrows indicate attachments to the most posterior pterygiophore of the anal and second dorsal fin; (e) SL = 5.9 mm, arrows display developing pterygiophores in the dorsal trunk; (f) SL = 7.4 mm; (g) SL = 25.4 mm, arrows indicate ossified (green) or cartilaginous (red) interdorsal pterygiophores; (h) *Pseudomugil signifer*, SL = 23.9 mm, arrows indicate ossified (green) or cartilaginous (red) interdorsal pterygiophores; scale bars = 500 μm

second dorsal fin more fin supports, that is, interdorsal as well as first dorsal pterygiophores, appear, developing from posterior to anterior (Figure 2b,c). Afterwards, fin rays are formed at the anterior pterygiophores mostly leaving two fin supports without fin rays, the interdorsal pterygiophores (Figure 2d). In *M. lacustris*, the first proximal radial of the anal fin fuses with the proximal radial of the second pterygiophore, like in *M. ladigesii*. But additionally, the first two proximal radials of the second dorsal fin also fuse in *M. lacustris* (Figure 2d). In some individuals, also the third and fourth proximal radial fuse. As already described for

M. ladigesii, the proximal radials of the last pterygiophores of the anal and the second dorsal fin have a cartilaginous attachment, which in some specimens is even ossified (Figure 2d). In adult specimens, the lepidotrichia of the first dorsal fin are fin spines, as is the first lepidotrichia of the anal and the second dorsal fin. The interdorsal pterygiophores and the first dorsal fin pterygiophores are not divided (Figure 2d).

3.2.2 | *Popondichthys furcatus*

Again, young larvae present a median fin fold. The first cartilaginous structures in the dorsal trunk occur after first pterygiophores are

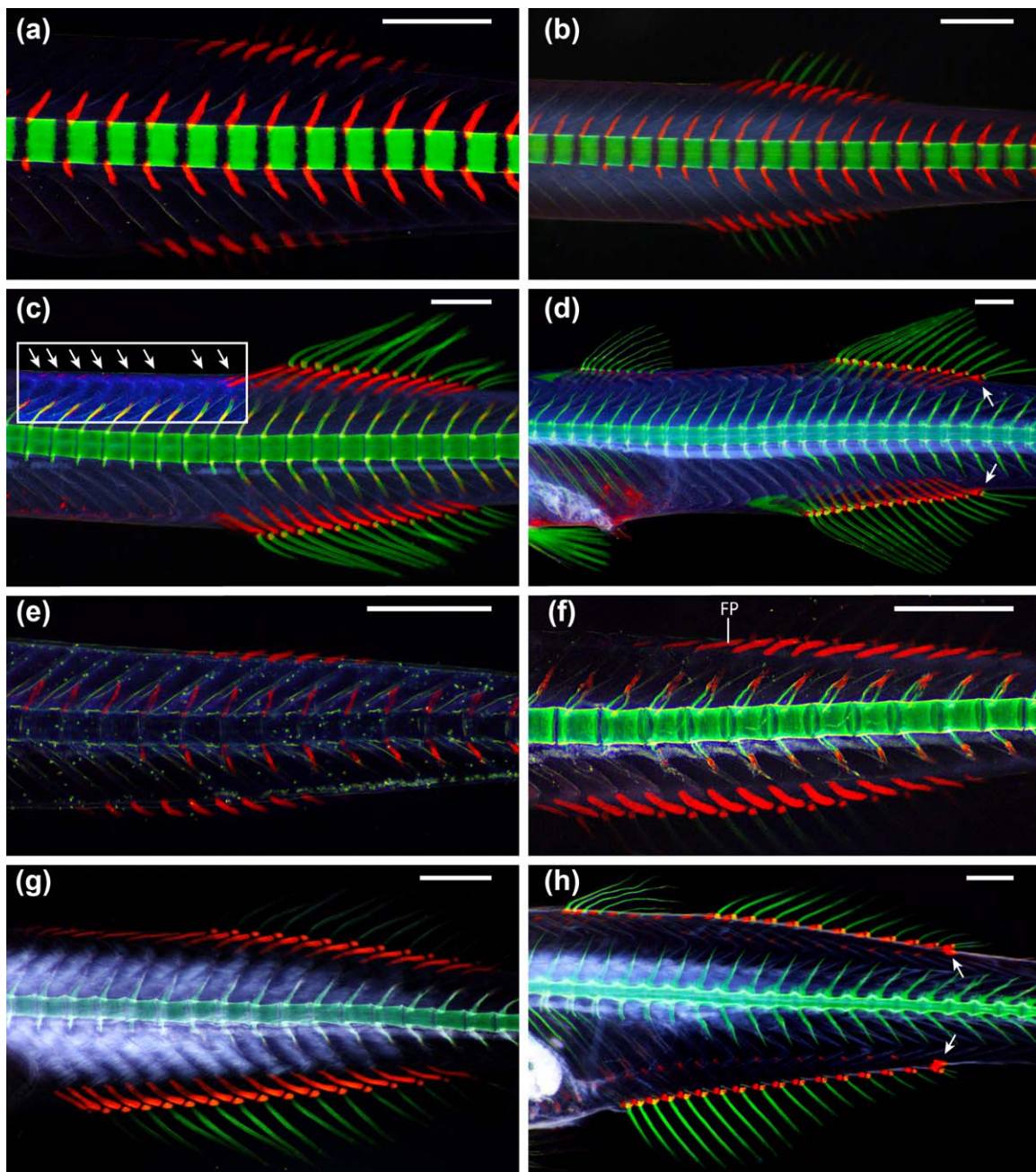


FIGURE 3 Developmental series of *Atherina* sp. (a–d) and *Bedotia geayi* (e–h). (a) SL = 10.5 mm; (b) SL = 11.9 mm; (c) SL = 12.0 mm, Insert displays section with enhanced contrast, arrows point to developing first dorsal fin complex pterygiophores; (d) SL = 16.2 mm; (e) SL = 6.5 mm; (f) SL = 7.5 mm; FP, first second dorsal pterygiophore; (g) SL = 9.0 mm; (h) SL = 13.4 mm, arrows indicate attachments to most posterior anal and second dorsal fin pterygiophore; scale bars = 500 μ m

present in the ventral trunk (Figure 2e). Later emerging fin supports are added anterior and posterior in both, the anal and the second dorsal fin. After these pterygiophores are separated in proximal and distal radials and first fin rays are developed (in the same pattern as the pterygiophores occurred), cartilaginous structures emerge in front of the second dorsal fin (Figure 2f). In contrast to *M. ladiges* and *M. lacustris*, there is a rather big gap between these structures and the second dorsal fin (Figure 2f). However, they still develop from posterior to anterior. The fin rays of the first dorsal fin develop from anterior to posterior. In adult specimens, the remains of two not ossified interdor-

sal fin supports follow one ossified and bar-like interdorsal pterygiophore (Figure 2g). *Pseudomugil signifer* a closely related species to *P. furcatus* also shows the remains of interdorsal pterygiophores. Also, one ossified interdorsal pterygiophore is present in adult specimens of *P. signifer* (Figure 2h). Specimens of both species have an attachment at the proximal radials of the last pterygiophores of the anal and second dorsal fin, as already described in *M. ladiges* and *M. lacustris*. And, just like the two species mentioned before, adult specimens of *P. furcatus* and *P. signifer* have fin spines in the first dorsal fin, but only fin rays in anal and second dorsal fin. The pterygiophores in front of the second

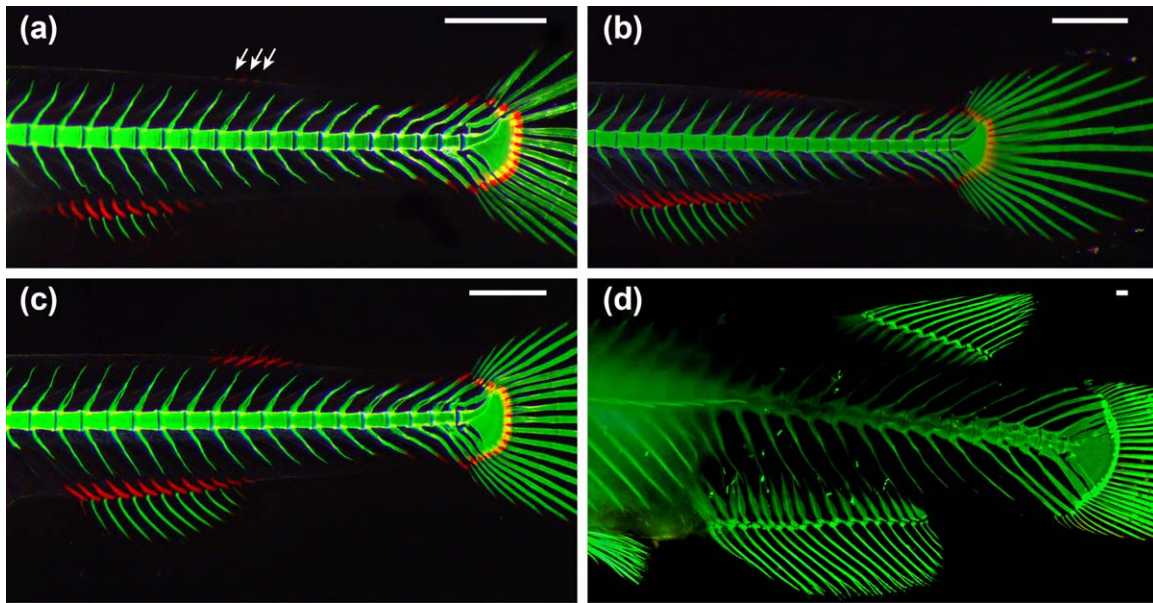


FIGURE 4 *Pachypanchax omalonotus*, developmental series. (a) SL = 5.6 mm, arrows show developing dorsal fin pterygiophores; (b) SL = 6.0 mm; (c) SL = 6.3 mm; (d) SL = 46.5 mm; scale bars = 500 μm

dorsal fin are not separated, although very few specimens occasionally do have a distal radial at different pterygiophores.

3.2.3 | *Atherina* sp

The median fin fold is present in larvae. The pterygiophores of the anal and the second dorsal fin occur at about the same time, the ones of the anal fin a little bit sooner. New fin supports are added anterior and posterior in both fins (Figure 3a). After actinotrichia developed, starting at the middle fin supports in a bidirectional pattern (Figure 3a), lepidotrichia develop, surrounding the already present actinotrichia (Figure 3b). The pterygiophores of the anal and the second dorsal fin separate in proximal and distal radials. When all parts of the second dorsal fin are present, more fin supports, that is, interdorsal as well as first dorsal pterygiophores, develop in front of it in anterior direction. (Figure 3c, white box). The fin rays of the first dorsal fin emerge after all fin supports in front of the second dorsal fin are present. They develop from anterior to posterior starting at the most anterior pterygiophore. At several fin supports between the first and second dorsal fin, no fin rays develop (Figure 3d). In adult specimens (Figure 3d), the first proximal radial of the anal fin is fused with the proximal radial of the second pterygiophore in the anal fin. Also, the last fin supports of the anal and second dorsal fin have a proximal attachment. The last fin ray of both fins is rather placed on this attachment than it is to the distal radial of the last fin support. The lepidotrichia of the first dorsal fin are fin spines, the ones of the anal and second dorsal fin are fin rays with the exception of the first, which are also fin spines (Figure 3d).

3.2.4 | *Bedotia geayi*

Like in all previously described species, a median fin fold is also present in larvae of *Bedotia geayi*. In addition, first pterygiophores in the anal fin emerge in the middle of the ventral trunk before fin supports of the second dorsal fin develop in the dorsal trunk above the ones in the anal

fin (Figure 3e). Newly developing pterygiophores are positioned anterior or posterior to the ones already present. The pterygiophores are separated in proximal and distal radials. At the same time, actinotrichia develop at the fin supports and are later surrounded by lepidotrichia (Figure 3f). These developmental events follow the same bidirectional pattern as the development of the pterygiophores. In front of the fin supports of the second dorsal fin, more pterygiophores, that is, interdorsal as well as first dorsal pterygiophores, emerge (Figure 3f). More fin supports are added anteriorly. The first lepidotrichia of the first dorsal fin develop at the most anterior of these fin supports, before more are added in caudal direction (Figure 3g). After all lepidotrichia of the first dorsal fin are in place, only a few interdorsal pterygiophores without lepidotrichia are left (Figure 3h). In adult specimens, the same characters which were found in the other species are present: fin spines in the first dorsal fin as well as in the anal and second dorsal fin (only the first lepidotrichia); fused first proximal radial (with the second proximal radial) in the anal fin; posterior attachment to the last proximal radial in the anal and second dorsal fin (the additional fin ray is placed on the distal radial); pterygiophores in front of the second dorsal fin are not separated in proximal and distal radials (Figure 3h).

3.3 | Outgroup species

To distinguish the fin-developing pattern of atheriniform fishes from the ones of their sistergroup and to make sure, interdorsal pterygiophores do not show up in the development of them, *P. omalonotus* (Cyprinodontiformes, Aplocheilidae) was examined.

Just like in all studied atheriniform fishes, a median fin fold is present in the larvae. First fin supports develop in the middle of the ventral trunk in a bidirectional pattern. Also, the first lepidotrichia develop at the fin supports of the anal fin, also in a bidirectional pattern (Figure 4a). After the first fin rays are present, first fin supports occur in the

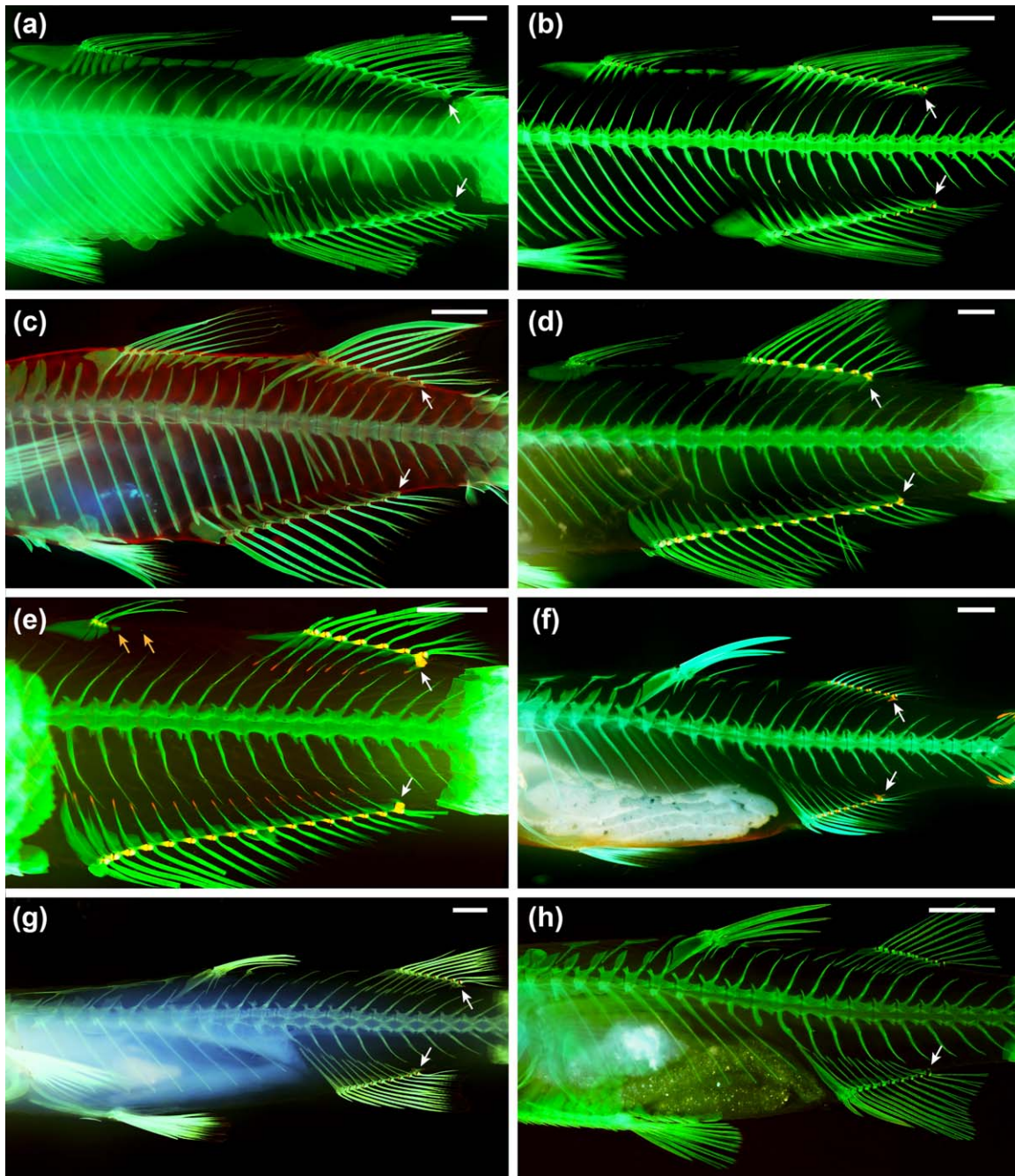


FIGURE 5 Anal and dorsal fins of atheriniforms (a–e) and mugiliforms (f–h); white arrows indicate attachments to most posterior anal and second dorsal fin pterygiophore (a) *Atherina boyeri*, SL = 72.0 mm; (b) *Atherina hepsetus*, SL = 37.0 mm; (c) *Iriatherina weneri*, SL = 32.5 mm; (d) *Menidia conchorum*, SL = 64.7 mm; (e) *Membras martinica*, SL = 49.9 mm, orange arrows point on interdorsal pterygiophores; (f) *Mugil* sp., SL = 36.6 mm; (g) *Rhinomugil corsula*, SL = 63.8 mm; (h) *Liza aurata*, SL = 28.1 mm; scale bars = 2 mm

dorsal trunk. They are located behind the anal fin pterygiophores (Figure 4a,b). Afterwards, new fin supports are added in the dorsal trunk anterior and posterior to the ones already present (Figure 4b,c). Lepidotrichia develop at the dorsal fin supports from the middle to both sides (Figure 4c). The pterygiophores of both fins separate in proximal and distal radials. There are no indications on structures in front of the dorsal fin (Figure 4d). In contrast to the atheriniform species, there are no attachments to the last pterygiophores in either fin, nor are there two fin rays at the last fin supports (Figure 4d).

3.4 | Comparison of adult specimens

Median fin structures (anal and dorsal fins) of eight adult specimens of eight different species representing the Atheriniformes (*Atherina boyeri*, *Atherina hepsetus*, *Iriatherina weneri*, *Menidia conchorum*, *Membras martinica*) and Mugiliformes (*Mugil* sp., *Rhinomugil corsula*, *Liza aurata*) were compared (Figure 5).

In all examined adult specimens, the pterygiophores of the anal fin are separated in proximal and distal radials. The first proximal radial is

widened in the direction of the body axis. However, in *A. boyeri* (Figure 5a) and *A. hepsetus* (Figure 5b), this radial is more broadened than it is in the other atheriniform species. The first proximal radials in the mugiliform species are only slightly broadened. At the last proximal radial, all species show an attachment. This is completely fused to the radial in both *Atherina* species and more recognizable as added structure in *I. weneri* (Figure 5c) and *M. conchorum* (Figure 5d). In *M. martinica* (Figure 5e), this attachment looks like an additional proximal radial, which is only fused slightly to the radial in front of it. *Mugil* sp. (Figure 5f) and *R. corsula* (Figure 5g) also have an attachment at the last proximal radial. In *L. aurata* (Figure 5h), there is a single ossified and a ball-like structure behind the last pterygiophore, not fused to the radial in front of it. All species have a fin spine located at the first proximal radial. Additionally, *Mugil* sp., *R. corsula*, and *L. aurata* (Figure 5f–h) have a second fin spine located at this first proximal radial. All distal radials support fin rays in the examined species, with the exception of the first distal radials in *Mugil* sp. and *R. corsula* where they support a fin spine. There also is a second fin ray located at the last pterygiophore. However, this one is not supported by a distal radial, but by the last proximal radial instead.

Just like in the anal fins, the pterygiophores in the second dorsal fins are all separated in proximal and distal radials. In all atheriniform species, the first proximal radial is widened and supports a fin spine. The mugiliform species do not have widened proximal radials. All examined species, with the exception of *L. aurata*, have an attachment to the last proximal radial, just like in the anal fin. In *A. boyeri*, *A. hepsetus*, and *I. weneri* (Figure 5a–c) the proximal radial and the attachment build a solid complex. Whereas, in the other species, especially in *M. conchorum* and *M. martinica* (Figure 5d,e), the attachment looks like a separate pterygiophore, which is connected to the proximal radial only by a small cartilaginous bridge. All distal radials support one fin ray, except for the first distal radial in *Mugil* sp. and *R. corsula* (Figure 5f,g), where it supports a fin spine. Like in the anal fin, there are two fin rays at the last pterygiophore. The posterior one is not attached to the last distal radial, but rather orientated at the last proximal radial.

Additionally to the second dorsal fin, all examined species have a first dorsal fin as well as interdorsal pterygiophores. Moreover, all mugiliform species have three supraneurals in front of the first dorsal fin (Figure 5f–h). The pterygiophores of the first dorsal fins are not separated in proximal and distal radials: the first pterygiophore is extended anteriorly in all species. In *Mugil* sp., *R. corsula*, and *L. aurata* (Figure 5f–h) the last pterygiophore of the first dorsal fin is shifted posteriorly leaving a small gap between it and the pterygiophores in front of it. In all species examined, only fin spines are present in the first dorsal fin. In *A. boyeri*, *A. hepsetus*, *I. weneri*, and *M. conchorum* (Figure 5a–d), there is one more fin spine present than pterygiophores. The first pterygiophore supports two of them. Between the first and second dorsal fin, interdorsal pterygiophores are present. They are not separated in proximal or distal radials. In *A. boyeri* and *A. hepsetus* (Figure 5a,b), they can be described as bar-like structures connecting both dorsal fins. In *A. hepsetus*, the last interdorsal pterygiophores shift from a bar-like shape to a more fin-support-like shape. In *Iriatherina* (Figure 5c), the interdorsal pterygiophores have a fin-support-like shape and connect

both dorsal fins. In *Menidia* (Figure 5d), they are bar-like and smaller than they are in *A. boyeri* and *A. hepsetus*, even losing size from anterior to posterior. There is a small gap between the last interdorsal pterygiophore and the second dorsal fin. In *Membras* (Figure 5e), there is one bar-like interdorsal pterygiophore followed by a ball-like interdorsal pterygiophore. Between these two structures, there is a small gap. There is a large gap between the last interdorsal pterygiophore and the second dorsal fin. All examined mugiliform species have fin-support-like interdorsal pterygiophores that are arranged in even distances to each other and to both dorsal fins (Figure 5f–h).

4 | DISCUSSION

4.1 | Dorsal fin developmental pattern

All atheriniform species show the same developmental pattern in their dorsal fins. First, the median pterygiophores of the second dorsal fin develop. More fin supports are added anterior and posterior to them. Afterwards, the median pterygiophores are separated in proximal and distal radials and first lepidotrichia emerge at the distal radials. The anal fin structures develop following the same pattern, but development starts slightly before the one of the second dorsal fin. In front of the anteriormost pterygiophore of the second dorsal fin, more fin supports emerge in posterior to anterior direction. They include the later interdorsal as well as the first dorsal fin pterygiophores, which together form a developmental unit. Afterwards, lepidotrichia develop at these pterygiophores starting anteriorly. But, not all pterygiophores in front of the second dorsal fin will finally support lepidotrichia: a species-dependent number of pterygiophores will persist without fin rays. Since interdorsal and first dorsal pterygiophores are a developmental unit, the interdorsal pterygiophores of atheriniforms should be considered part of their first dorsal fin. Thus, we will refer to the set of interdorsal pterygiophores and the first dorsal fin as first dorsal fin complex. The pterygiophores of the first dorsal fin complex are not divided. This pattern is consistent with the one found in *I. weneri*, also an atheriniform species (Enkelmann & Moritz, 2014). In *P. furcatus*, the pattern is slightly altered caused by a gap between the pterygiophores of the second dorsal fin and the newly developing pterygiophores anterior to them.

Closely related taxa such as the Cyprinodontiformes and the Beloniformes lack a first dorsal fin (Nelson, Grande, & Wilson, 2016). There are also no additional structures in front of their single dorsal fin that could be corresponding to the interdorsal pterygiophores in atheriniforms (Figure 4; Noell, 2003). However, the developmental pattern of this single dorsal fin as well as of the anal fin found in *Pachypanchax omalonontus*, a cyprinodontiform species, is consistent with the atheriniform developmental pattern (Figure 4). For the other group within the Atherinomorpha, the Beloniformes, there are no ontogenetic descriptions for the median fins available yet. However, the results published by Noell (2003) imply that the anal and dorsal fin developmental pattern of Beloniformes is also consistent with the other two groups. Mabee et al. (2002) categorized the developmental pattern, now found in the anal and (second) dorsal fin of atheriniform fish, as

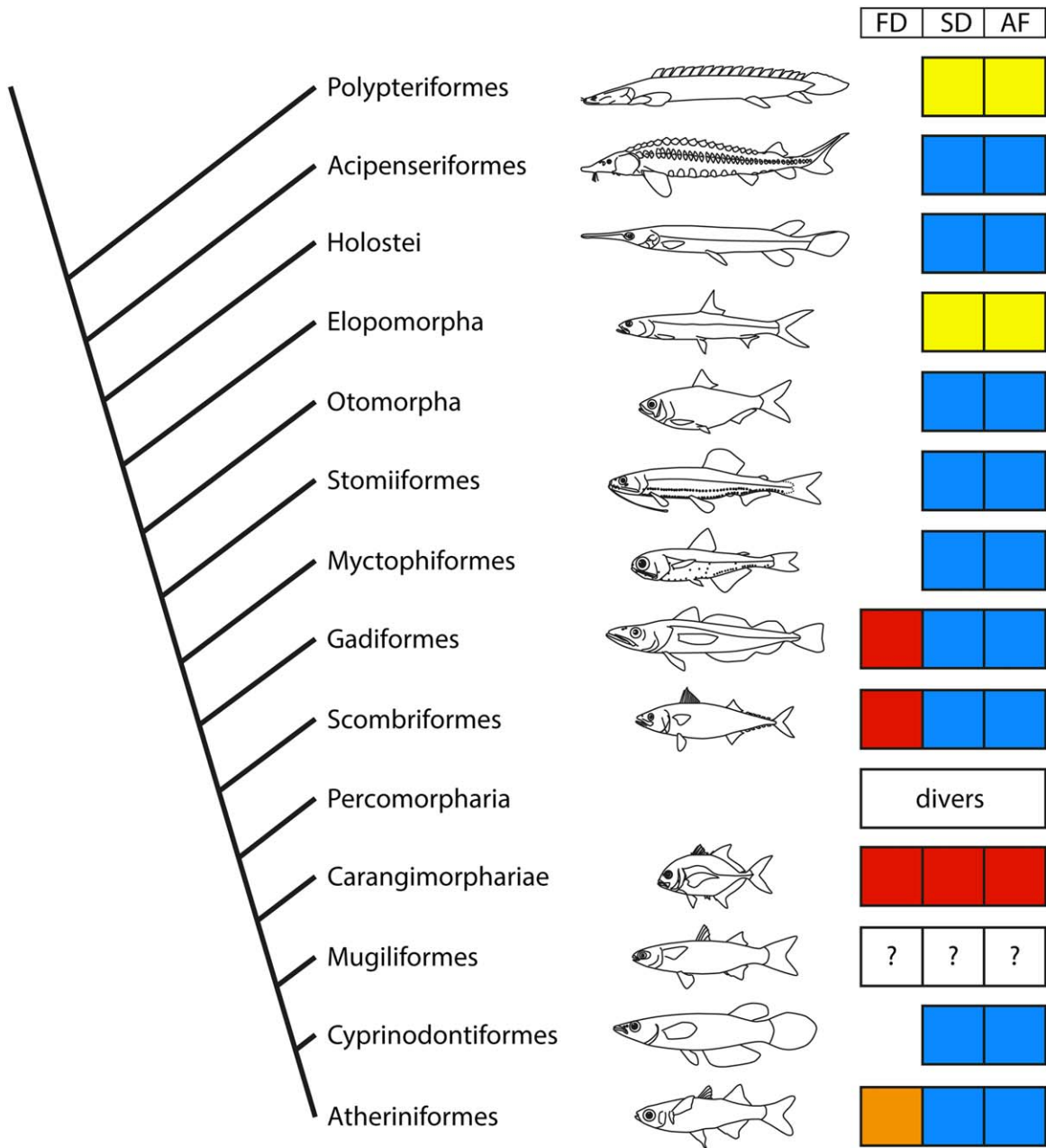


FIGURE 6 Overview on the developmental patterns of the first (FD) and second dorsal fin (SD) as well as the anal fin (AF) of different actinopterygian taxa (Mabee et al., 2002) and atheriniforms based on a simplified phylogeny adapted from “The Tree of Life and a New Classification of Bony Fishes,” by R. Betancur-R et al., , PLoS Currents, 5. Yellow, formation from posterior to anterior; blue, bidirectional formation; red, formation from anterior to posterior; orange, pterygiophore’ formation from posterior to anterior and lepidotrichia formation from anterior to posterior. Within the divers Percomorpharia several developmental modes are known. For Mugiliformes, no information are available yet

bidirectional. The developmental pattern of the first dorsal fin complex in atheriniform fish was not found in any other examined taxa before: an opposed development with pterygiophores emerging from posterior to anterior and lepidotrichia in contrary direction.

Most other atherinomorph taxa with one dorsal fin also showed the bidirectional developmental pattern for the anal and dorsal fin (Figure 6). A posterior to anterior pattern for both, the anal and dorsal fin, was reported so far only for Polypteriformes (Bartsch & Gemballa, 1992) and Elopomorpha (Bartsch & Gemballa, 1992;

Leiby, 1979). Considering the taxa with two dorsal fins, some Percomorpharia and Carangimorpharia show different developmental patterns in the anal and the second dorsal fin. For the development of the first dorsal fin, only two patterns are known: anterior to posterior (Gadiformes and most Scombriformes) and bidirectional (most Carangimorpharia and most Percomorpharia). For the mugiliformes, no data on fin development is available yet. Therefore, the Atherinomorpharia equal most other teleosts in terms of anal and second dorsal fin developmental pattern. These results also support

the hypothesis offered by Mabee et al. (2002) that those two fins should be considered a developmental module. However, the Atheriniformes show an exclusive pattern in regard to the development of the first dorsal fin.

4.2 | Comparison of atheriniform dorsal fins

Considering the elements highlighted above, there are five characters within the dorsal fin complex to be compared and discussed: (1) The lepidotrichia of the first dorsal fin; (2) The first lepidotrichia of the second dorsal fin; (3) The most posterior fin-support of the second dorsal fin; (4) Radials of the pterygiophores of the first dorsal fin complex; (5) Shape of the interdorsal pterygiophores.

4.2.1 | First dorsal fin lepidotrichia

The lepidotrichia of the first dorsal fin of all herein examined atheriniforms are fin spines (Figures 1–5), that is, they show no segmentation and/or ramification. This holds true for most other atheriniform species (Dyer, 2006; Dyer & Chernoff, 1996; Herre, 1944; Ivantsoff & Ivantsoff, 1996, 1997; Ivantsoff & Saeed, 1987; Jordan & Starks, 1902; Parenti, 1993). Phallostethid species on the other hand have a reduced or absent first dorsal fin. If a first dorsal fin is present at all, the lepidotrichia can be either fin spines or thickened fin rays (Myers, 1935; Nelson et al., 2016). In most examined atheriniform species, two fin spines are located at the most anterior first dorsal fin pterygiophore (Figure 7). No clear information is available for *Dentatherina merceri* (Ivantsoff et al., 1987) and species of the family Phallostethidae. This condition seems not to be the result of a fusion of two fin spines supporting pterygiophores but rather a non-serial association of the first fin spine with the first pterygiophore (e.g., Figures 1f, 2d, and 3h).

4.2.2 | First lepidotrichia of the second dorsal fin

This character can occur in two different states, namely as a fin spine or a fin ray. The widespread condition in atheriniforms except for Bedotiidae, Pseudomugilidae, Telmatherinidae, and Phallostethidae is that a fin spine precedes the lepidotrichia of the second dorsal fin (Figure 7; Dyer & Chernoff, 1996; Nelson et al., 2016). In bedotiids, pseudomugilids, and telmatherinids, all lepidotrichia of the second dorsal are fin rays (Dyer & Chernoff, 1996; Ivantsoff, Ivantsoff, & Kottelat, 1998; Saeed & Ivantsoff, 1991). According to most authors, only two of four phallostethid genera, *Gulaphallus* and *Neostethus*, have a fin spine in the second dorsal fin (Bailey, 1936; Eschmeyer, Fricke, & van der Laan, 2017; Herre, 1939; Myers, 1928, 1935, 1937). Hubbs (1944), however, mentioned that he was not able to find fin spines in the second dorsal fin of any of the both genera. In two species (*Rhadinocentrus ornatus* and *Caimsichthys rhombosomoides*) of the family Melanotaenidae the fin spine is reduced, too, and they differ from all other examined melanotaenid species in this regard (Ivantsoff & Ivantsoff, 1996, 1997). In Campanella et al. (2015), *R. ornatus* takes a basal position in melanotaenids and *C. rhombosomoides* is not even placed within the melanotaenids but rather represents the sister-group of pseudomugilids and telmatherinids (Figure 7). Therefore, it is possible that only in *R. ornatus* an additional reduction of the fin spine took place, whereas the reduction in *C. rhombosomoides* represents a putative synapomorphy with pseudomugilids and telmatherinids.

4.2.3 | Most-posterior pterygiophore of the second dorsal fin

In atheriniforms, the last pterygiophore of the second dorsal fin has an attachment (Figure 5a–e). This attachment is not supporting a fin ray. Instead, two fin rays are coupled to the distal radial of the last pterygiophore. Although in some species, for example, *A. hepsetus* (Figure 5b) or *M. martinica* (Figure 5e), it remains unclear if both fin rays are indeed associated with the last distal radial, or if the last one is rather positioned above the cartilaginous bridge that connects the last pterygiophore to its attachment. In atherinopsids, this attachment is an additional ossified proximal radial that is connected to the last fin-ray-supporting pterygiophore via a cartilaginous bridge (Figure 5d,e; Dyer & Chernoff, 1996). Dyer and Chernoff (1996) mentioned that *Iso* shares the same character state as atherinopsids. Furthermore, they state that only in *Notocheirus* such an attachment is not present (Dyer & Chernoff, 1996). All other atheriniforms share a character state in which only a cartilaginous attachment or attached plate is present posterior to the last pterygiophore (Figure 5a–c; Dyer & Chernoff, 1996). Therefore, it seems most likely that the proximal radial-like attachment is reduced in more derived atheriniforms.

4.2.4 | Radials of the pterygiophores of the first dorsal fin complex

In all atheriniform species examined, the pterygiophores in front of the second dorsal fin are not separated (e.g., Figures 1g and 5a–c). However, in four species, some specimens with a fully developed but not completely ossified first dorsal fin, have distal radials at the first pterygiophore (*M. lacustris*, *P. furcatus*, and *P. signifer*) and/or second most anterior pterygiophores (*M. lacustris* and *B. geayi*). This could only be observed after the alizarin-red dye got lost (washed out) in cleared and stained specimens. The formerly stained lepidotrichia got transparent and allowed to see the enclosed distal radials. In phallostethids, the fin supports of the first dorsal fin, if one is present, are fused to a plate-like structure (Bailey, 1936; Herre, 1939). Further studies on phallostethids must decide if more than one radial and maybe interdorsal pterygiophores are present in the development of this structure.

4.2.5 | Shape of interdorsal pterygiophores

Interdorsal pterygiophores can be found in all atheriniform species except phallostethids and *Iso* (Figure 7; Parenti, 1993, Saeed, Ivantsoff, & Ivantsoff, 2005). Phallostethids do not have interdorsal pterygiophores whether a first dorsal fin with fused pterygiophores is present or not (Figure 7; Bailey, 1936; Herre, 1939; Myers, 1937). In all other atheriniforms, interdorsal fin supports can be categorized, according to their shape, in three groups: fin support-like, bar-like or ball-like. It is possible that more than one state is present in one species. Additionally, a gap between the interdorsal pterygiophores and the second dorsal fin can be present. In Atherinopsidae for example, *Labidesthes sicculus* (Parenti, 1993) and *M. martinica* have bar-like interdorsal pterygiophores (Figure 7). *Membras martinica*, a closely related species (Campanella et al., 2015), has two bar-like pterygiophores followed by a ball-like interdorsal pterygiophore and caudally of it a large gap until

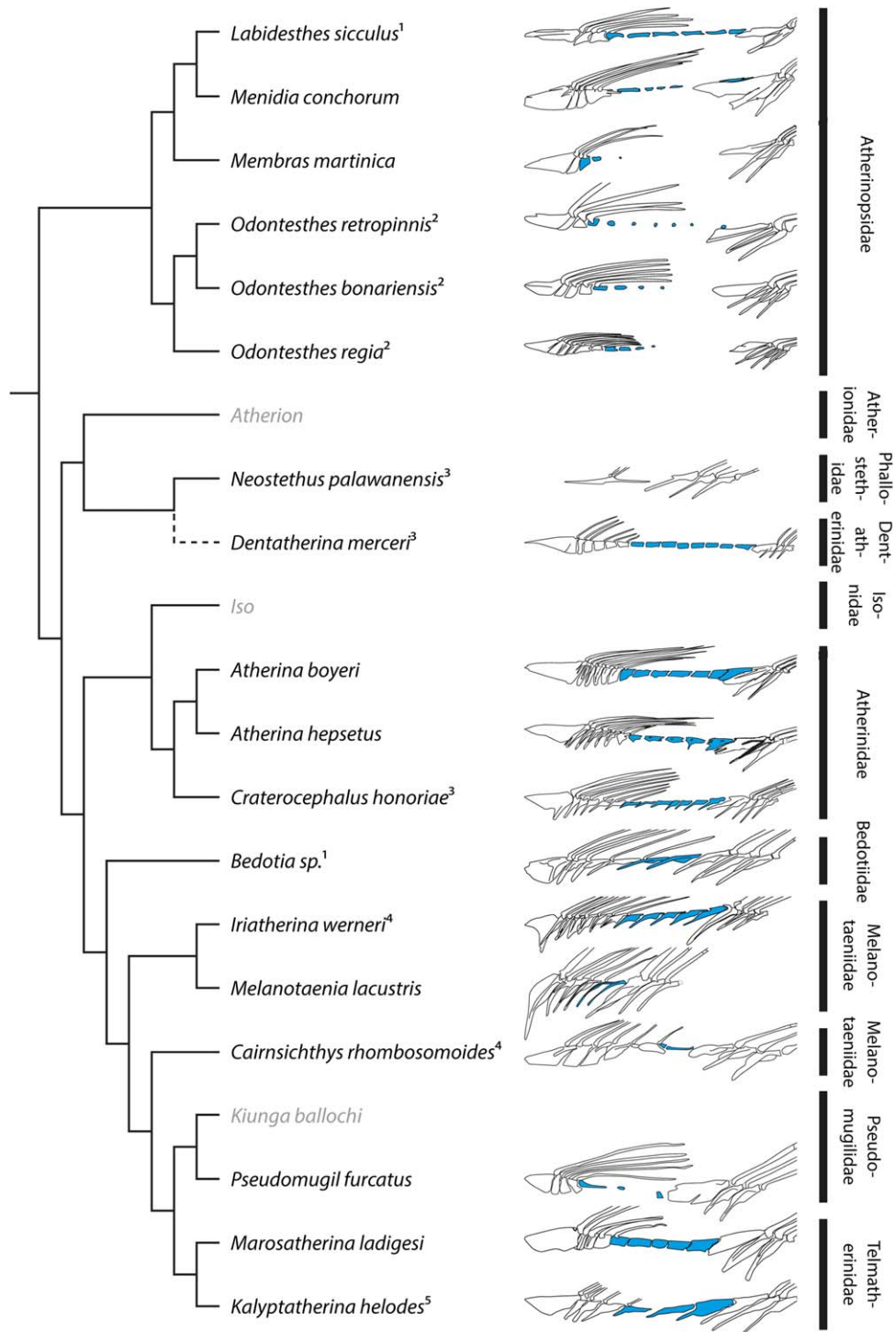


FIGURE 7 Phylogeny of atheriniform families based on adapted from “Multi-Locus Fossil-Calibrated Phylogeny of Atheriniformes (Teleostei, Ovalentaria),” by D. Campanella et al., 2015, *Molecular Phylogenetics and Evolution*, 86, pp. 8–23 and “Fishes of the World (5th ed.)” by J. S. Nelson et al., 2016, Hoboken, New Jersey: Wiley with depictions of the dorsal fins of selected species. No illustrations were available for *Atherion*, *Iso*, and *Kiunga*. Blue, Interdorsal pterygiophores. (1) Adapted from “Relationships of Atherinomorph Fishes (Teleostei),” by L. R. Parenti, 1993, *Bulletin of Marine Science*, 52, pp. 170–196; (2) adapted from “Systematic Revision of the South American Silversides (Teleostei, Atheriniformes),” by B. S. Dyer, 2006, *Biocell*, 30, pp. 69–88; (3) adapted from “Systematic Position of the Family Dentatherinidae in Relationship to Phallostethidae and Atherinidae,” by W. Ivantsoff & B. Saeed, 1987, *Copeia*, 1987, pp. 649–658; (4) adapted from “Descriptive Anatomy of *Cairnsichthys rhombosomoides* and *Iriatherina wernerii* (Teleostei: Atheriniformes), and a Phylogenetic Analysis of Melanothaeniidae,” by A. Ivantsoff & W. Ivantsoff, 1997, *Ichthyological Exploration of Freshwaters*, 8, pp. 107–150; (5) adapted from “*Kalyptatherina*, the First Telmatherinid Genus Known Outside of Sulawesi,” by B. Saeed & W. Ivantsoff, 1991, *Ichthyological Exploration of Freshwaters*, 2, pp. 227–238

the origin of the second dorsal fin (Figure 7). The gap probably resembles a space where interdorsal pterygiophores were present in ancestor species, but are reduced now. In *Odontesthes*, a similar situation is present (Figure 7). *Odontesthes retropinnis* still has mostly ball-like interdorsal pterygiophores, only the first one is bar-like, which cover most of the space between the first and the second dorsal fin. The gaps between these pterygiophores are large and indicate a reduction of a structure that previously covered a larger space. In *O. bonariensis* and *O. regia*, the first two or three interdorsal pterygiophores are bar-like; the gap between them is smaller than the gap between the last bar-like and the following ball-like structures (Figure 7). Additionally, a large gap between the interdorsal pterygiophores and the second dorsal fin is present. In all other examined atheriniform taxa with interdorsal pterygiophores, no ball-like structures (except *P. furcatus*) and gaps toward the second dorsal fin occur (Figure 7). In *D. merceri*, bar-like interdorsal pterygiophores are present (Ivantsoff & Saeed, 1987). Formerly classified as phallostethids (Ivantsoff & Ivantsoff, 1997; Nelson, 2006), *D. merceri* is now placed in the monotypic family Dentatherinidae, which possibly is the sister taxon to phallostethids (Figure 7; Ivantsoff, 1999; Nelson et al., 2016). Therefore, it seems likely that reduction of interdorsal pterygiophores in phallostethids and isonids occurred independently. This hypothesis is further supported by the presence of bar-like interdorsal fin supports in the family Atherinidae, which is the sister taxon to the Isonidae (Figure 7; Campanella et al., 2015). The interdorsal pterygiophores in *Craterocephalus honoriae*, also a representative of the Atherinidae, resemble reduced fin support-like interdorsal pterygiophores (Ivantsoff & Saeed, 1987). In *A. hepsetus*, some of the bar-like interdorsal fin-supports feature a ventral outgrowth similar to fin support-like interdorsal pterygiophores (Figure 7). In bedotiids and melanotaenids, the interdorsal pterygiophores are fin support-like structures (Figure 7; Ivantsoff & Ivantsoff, 1997; Parenti, 1993). *Caimsichthys rhombosomoides*, does not have fin support-like pterygiophores like "other melanotaeniids" but rather bar-like interdorsal pterygiophores (Figure 7; Ivantsoff & Saeed, 1987). These are more similar to the structures found in pseudomugilids, for example, *Pseudomugil furcatus*, or telmatherinids (Figure 7) and would therefore support the view, that *C. rhombosomoides* is indeed more closely related to the latter two families (Campanella et al., 2015). Saeed, Ivantsoff, and Allen (1989) remarked that no interdorsal pterygiophores are present in *Kiunga* and *Pseudomugil*. In the description of the genus *Kiunga*, Allen (1983) described that one interdorsal pterygiophore is present. Furthermore, in *Pseudomugil pellucides* and *Pseudomugil novaeguineae* (Allen, 1998) as well as *Pseudomugil reticulatus* (Ivantsoff, Shepherd, & Allen, 1997) interdorsal pterygiophores are also present. In *P. furcatus* and *P. signifer*, interdorsal pterygiophores are present (Figure 2g,h). However, most of them, except for the most anterior interdorsal pterygiophore, seem to be reduced to ball-like structures. Since our specimens examined in both species were not adult, the adult situation cannot be described. In Telmatherinidae, slightly reduced fin support-like interdorsal pterygiophores are present in *Kalyptatherina helodes* (Saeed & Ivantsoff, 1991), whereas bar-like structures can be found in *M. ladigesi* (Figure 7).

It is not possible to outline the original state of interdorsal pterygiophores in atheriniforms based on the present phylogenetic hypothesis (Campanella et al., 2015). But, it seems certain that bar-like as well as ball-like fin supports are reduced character states in comparison to fin support-like structures like those in *I. werneri* or *M. lacustris*. Also, gaps between interdorsal pterygiophores and the second dorsal fin are most likely the result of the reduction and/or loss of interdorsal fin supports during evolution, partly maybe still during ontogeny. Therefore, it seems likely that in the basal condition fin-support-like pterygiophores linked both dorsal fins.

4.3 | Relationship between Atherinomorpha and Mugiliformes

The interdorsal pterygiophores are a common character of all atheriniform taxa, with the exception of phallostethids. The Beloniformes as well as the Cyprinodontiformes cannot share this trait as they have no first dorsal fin. There are also no other structures in front of the second dorsal fin in these taxa. Together the Atheriniformes and their sister group consisting of Beloniformes and Cyprinodontiformes form a monophyletic group, the Atherinomorpha (Figure 8; Betancur-R et al., ; Campanella et al., 2015; Rosen & Parenti, 1981). Therefore, interdorsal pterygiophores either are a character existing in all Atherinomorpha and are reduced in Beloniformes and Cyprinodontiformes, or are a new trait only present in Atheriniformes (Stiassny, 1990, 1993). Another group that also has interdorsal pterygiophores are the Mugiliformes, which already were positioned as sister group to the Atheriniformes or Atherinomorpha in different phylogenies (Figure 8a,b; Johnson & Patterson, 1993; Sparks & Smith, 2004; Stiassny, 1990; Wiley, Johnson, & Dimmick, 2000). The Atherinomorpha and Mugiliformes share even more morphological traits. According to Stiassny (1990), there are at least three derived characters in the pharyngeal region namely: (1) a divided pharyngocleithralis externus, (2) the morphology of the pharyngohyoideus muscle and its tendon, and (3) an extremely similar configuration of the levators externi and interni. Also the anterior neural arches are expanded in Mugiliformes as well as Atherinomorpha (Stiassny, 1990). The functional independence of the pharyngeal musculature characters can be doubted, which indicates a phylogenetic independent origin of these characters (Stiassny, 1990). Nevertheless, there are more characters both groups share: a reduced supracleithrum without a sensory canal, a reduced marginal pectoral ray, a posteriorly orientated dorsal cleithral process and an enlarged abductor profundus muscle (Stiassny, 1993). Stiassny (1990, 1993) did not mention the interdorsal pterygiophores in her studies. First, Parenti (1993) compared the interdorsal pterygiophores of the mugilids and atheriniforms. She concluded, that they evolved independently in both groups due to the almost complete dorsal fin in bedotiids which, according to her opinion, would represent the most basal condition in atheriniforms (Parenti, 1993). A continuous development of one dorsal fin, like Parenti (1993) apparently has assumed, could not be supported with the herein presented data (Figure 6). This indicates that the amount of supposed lepidotrichia which got lost is no argument for an independent origin of interdorsal pterygiophores in mugilids and atheriniforms.

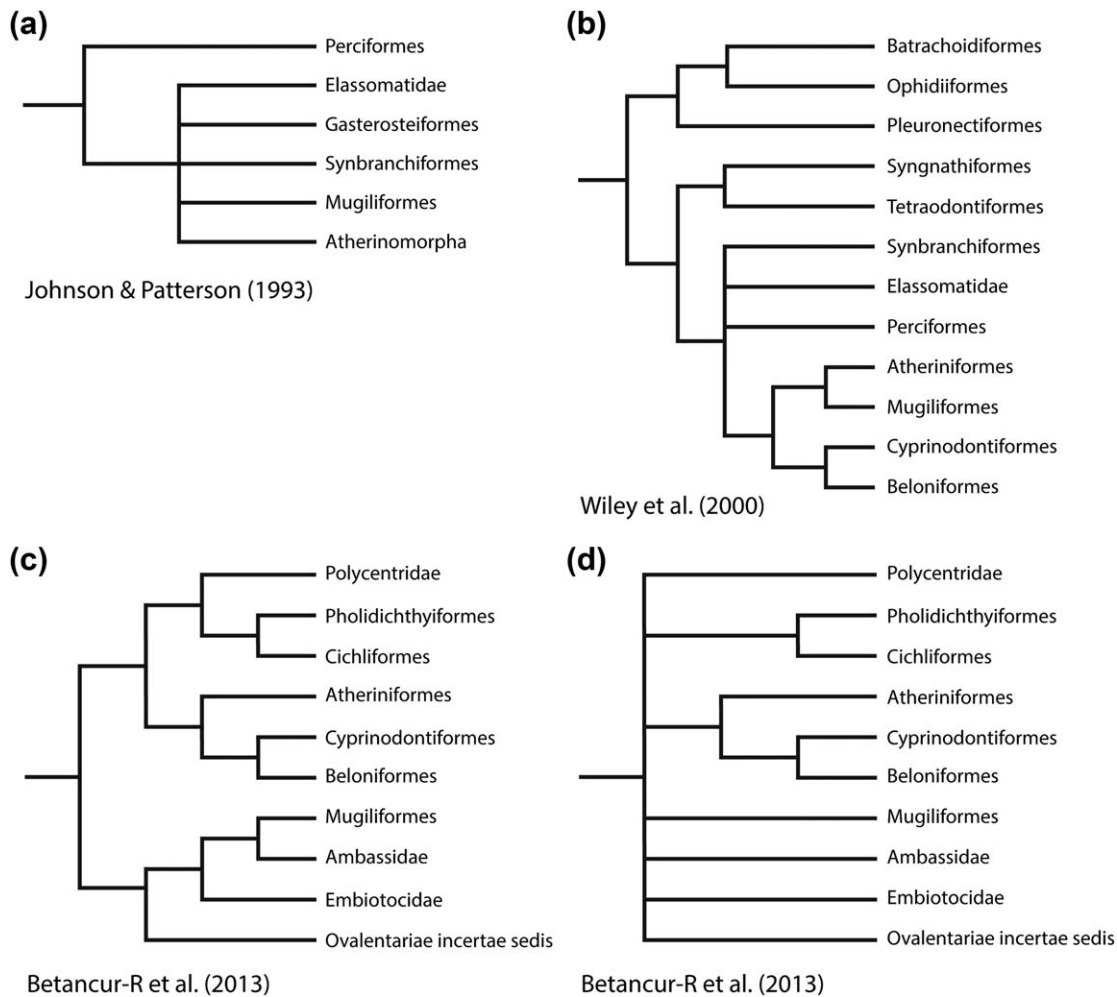


FIGURE 8 Hypophysis of atherinomorph interrelationships: (a) adapted from “Percomorph Phylogeny: A Survey of Acanthomorphs and a New Proposal,” by D. G. Johnson & C. Patterson, 1993, *Bulletin of Marine Science*, 52, pp. 554–626; (b) adapted from “The Interrelationships of Acanthomorph Fishes: A Total Evidence Approach Using Molecular and Morphological Data,” by E. O. Wiley et al., 2000, *Biochemical Systematics and Ecology*, 28, 319–350; (c) adapted from “The Tree of Life and a New Classification of Bony Fishes,” by R. Betancur-R et al., *PLoS Currents*, 5; (d) adapted from “The Tree of Life and a New Classification of Bony Fishes,” by R. Betancur-R et al., *PLoS Currents*, 5, all nodes under 75% bootstrap value collapsed

However, interdorsal pterygiophores are not only found in mugilids and atheriniforms, but also in some gadiforms like *Muraenolepis marmoratus* (Markle, 1989) and some Sphyaenidae like *Sphyaena sphyaena* (personal observation). Due to their phylogenetic position (Betancur-R et al.,), there seems to be no evolutionary connection between these structures and those found in mugilids and atheriniforms. The interdorsal fin supports in mugilids resemble the fin supports of the first dorsal fin in the respective species (Figure 5). The examined mugilid species cover three of four subfamilies that were introduced by Xia, Durand, and Fu (2016). No species of the most basal subfamily, the Myxinae, was evaluated, but it can be assumed that interdorsal pterygiophores are a character shared by all mugilids. The interdorsal fin supports of some atheriniforms like *B. geayi* or *I. wernerii* are quite similar to the ones of the mugilid species (Figure 5). A common origin of the character “interdorsal pterygiophores” shared by mugilids and atheriniforms should be considered and the character added to the list of possible apomorphies of both groups. However,

more information are required, for example, a description of mugilid dorsal fin ontogeny. Additionally, other characters could also be included in the discussion about their relationship: Mugilids and atheriniforms do have single radial pterygiophores in front of the second dorsal fin (Figure 5). Also, a fin spine precedes the fin rays in the second dorsal fin in the examined adult specimens except for *L. aurata* (Figure 5). Another character that is also discussed herein is the presence of attachments at the last dorsal and anal pterygiophore. In basal atheriniforms, for example, *M. martinica*, these attachments occur to be ossified proximal radials that are fused to the last pterygiophore via a cartilaginous bridge (Figure 5e). In the examined mugilids, it appears as if the attachments are also proximal radials that are slightly modified and connected to the last pterygiophore through a cartilaginous bridge (Figure 5f,g). In *L. aurata*, only a separated and ossified structure is present behind the last anal pterygiophore (Figure 5h). Dyer and Chernoff (1996) also discussed this character and even considered the proximal radial-like attachment (in this study) to be the last

pterygiophore fused to the penultimate pterygiophore. Potthoff (1975) did mention a similar condition in *Thunnus atlanticus*, considering it to be a fourth radial or “end piece”/“stay,” following Weitzman’s (1962) description of *Brycon meeki*, rejecting the idea of it being part of an additional pterygiophore. As seen in Figure 5d–g, it is more probable that the attachment at the last pterygiophores are reduced and fused parts of a formerly extra pterygiophore. Furthermore, both, mugilids and atheriniforms, have at least one fin spine at the first anal pterygiophore. In atheriniforms one fin spine attaches to the first proximal radial and a fin ray to the corresponding distal radial (Figure 5a–e). During development, the first anal proximal radial (sometimes the whole pterygiophore) fuses with the second anal proximal radial resulting in the “first” anal pterygiophore in adult atheriniforms (e.g., Figures 1g,h and 3g,h). In adult mugilids, two fin spines are connected to the first proximal radial of the anal fin (Figure 5f–h). It is possible that this is the result of a fusion of two or more pterygiophores or single radials, too. This hypothesis could be tested by studying the development of mugilid median fins. Conversely, some traits that were used to refute a phylogenetic relationship between mugilids and atherinomorphs need to be revised in view of new phylogenetic hypothesis (Betancur-R et al.,). Especially three characters concerning the pectoral girdle need to be discussed in this context. It was assumed, that mugilids are related to some more derived “percomorphs” due to (1) a suture in the posterior field of the connected pectoral girdle halves, (2) a ligament connecting the two parts of the pectoral girdle and (3) a ventrally shifted anterior processus (Stiassny, 1990, 1993; Stiassny & Moore, 1992). Species with similar traits can be found in the more basal Holocentridae as well as in more derived percomorph taxa such as *Sebastes* sp. and *Morone* sp. (Stiassny, 1990, 1993; Stiassny & Moore, 1992). Based on the phylogenies of Betancur-R et al. () and Nelson et al. (2016), the Holocentridae are no longer considered percomorphs and the Scorpaeniformes (i.e., *Sebastes* sp.) and Moroniformes (i.e., *Morone* sp.) are included in a group called Percomorpharia. Mugilids and atherinomorphs, conversely, are part of the Ovalentariae (Figure 8c, sensu Betancur-R et al.,). It can be assumed that characters (1) and (2) originated earlier in the stem-group of the Euacanthomorpha (Betancur-R et al., ; Stiassny & Moore, 1992). Additionally, character (3) seems to have occurred in the Acanthomorpha first and originated convergent in only one other group, the Zeiformes, which are part of the Paracanthomorpha. Therefore, mugilids and atherinomorphs as members of the Percomorphacea (Betancur-R et al.,) also should share these characters. The different situation in atherinomorphs has to be interpreted as a secondary reduction in the light of present phylogenetic hypothesis. Hence, all three characters mentioned above do not account against a possible relationship between mugilids and atherinomorphs. On the contrary, Stiassny (1993) mentioned another character in the pelvic girdle that both these groups do share: there is a ligament attached to the lateral processus, which proceeds to a pleural rib in atherinomorphs and to the postcleithrum in mugilids. The changed insertion point of the ligament in atherinomorphs can be explained as a derived character state in comparison to the ones in mugilids. Also, the character state found in mugilids can be interpreted as a transitioning state between

the one found in acanthomorphs, where the ligament attaches directly at the pelvic girdle and proceeds to the postcleithrum, and the one found in atherinomorphs (Parenti, 1993; Stiassny, 1993; Stiassny & Moore, 1992).

The most recent phylogenetic study (Figure 8c; Betancur-R et al.,) cannot give an accurate phylogenetic position of the mugilids, that is, a sister-group relation of Atherinomorpha and Mugiliformes cannot be rejected on the presented data (Figure 8d). Therefore, the previously mentioned characters do depict a solid foundation to assume, that the Mugiliformes are indeed the sister-group to the Atherinomorpha. Developmental studies on mugilids will provide further information on this issue supporting or declining the herein stated hypothesis.

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