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

Entwicklung von hexagonalen und oktagonalen Schuppen bei *Atherina* (Teleostei, Atheriniformes)

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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

Zusammenfassung: Die Arten der Gattung *Atherina* besitzen für Teleostier einzigartig geformte Schuppen: sie sind hexagonal (bei *Atherina presbyter*) oder oktagonal (bei *Atheria boyeri* und *Atherina hepsetus*), wobei die lateralen Felder mehr oder weniger deutlich nach innen gebogen sind. Trotzdem sind diese Schuppen prinzipiell Cycloidschuppen. Die unterschiedliche Form der Schuppen erlaubt es, *A. presbyter* von den anderen beiden Arten zu unterscheiden. Das Studium der Schuppenontogenese zeigte, dass die ungewöhnliche Form nicht von Anfang an vorhanden ist, sondern sich im Laufe der Entwicklung bildet. Die ersten Schuppen, die sich auf den Körpern der Larven finden, sind einfache runde Schuppen ohne jegliche Ornamentierung.

Schlüsselwörter: Ontogenese, Atherinomorpha, Schuppenornamentierung, 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. parsia*, 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,

Tab. 1: Studied material; all deposited in the Deutsches Meeresmuseum (DMM).
Tab. 1: Untersuchtes Material; alle Objekte sind im Deutschen Meeresmuseum (DMM) registriert.

Species	Reg. Nr.	Specimen Nr.	SL in mm
Atherina boyeri Risso, 1810	IE/5874	1	32.6
		2	36.3
		3	45.7
		4	71.3
Atherina hepsetus Linnaeus, 1758	IE/5080	1	77.5
	IE/5873	1	58.7
		2	53.4
		3	65.8
		4	74.3
		5	58.3
		6	71.1
		7	63.8
		8	85.5
	IE/5888	1	68.3
Atherina presbyter Cuvier, 1829	IE/6135	1	39.3
		2	32.9
	IE/6157	1	21.5
	IE/9037	1	61.4
	IE/10493	1	75.9
Atherina sp.	IE/14850	1	19.9
		2	20.5

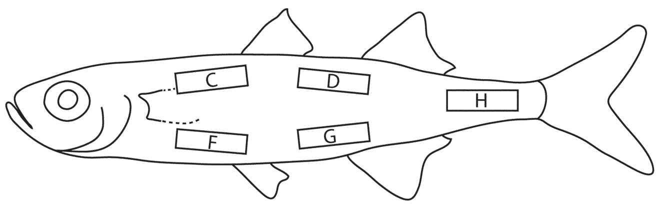

Fig. 1: Schematic drawing of *Atherina presbyter* illustrating the five sampling areas used in this study following BRÄGER & MORITZ (2016).
Abb. 1: Schemazeichnung von *Atherina presbyter,* auf der die fünf Regionen angegeben sind, aus denen Schuppen entnommen wurden wie bei BRÄGER & MORITZ (2016).

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: true 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: true 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)

***Type*:** cycloid: true 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*:**

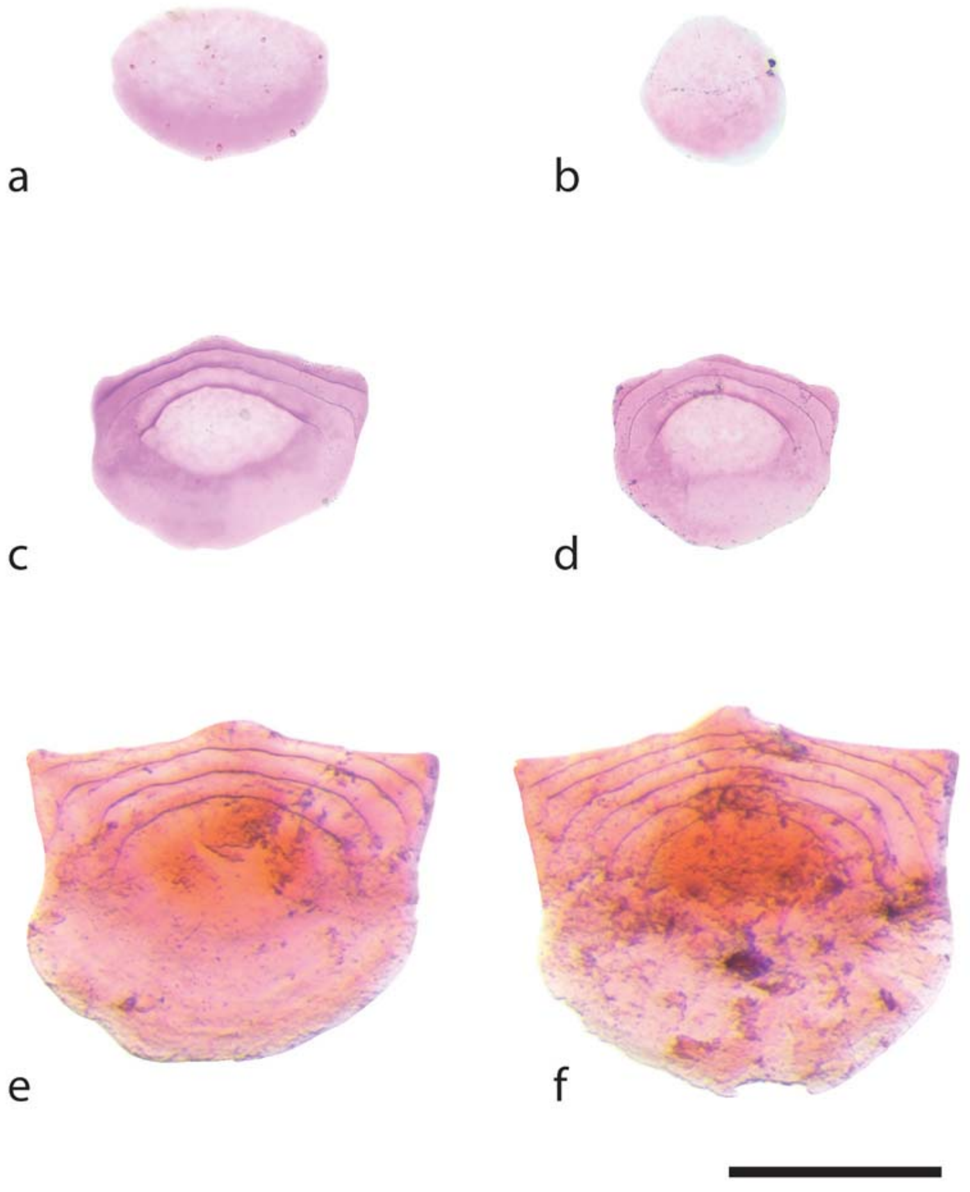

Fig. 3: Scales of *Atherina presbyter* (75.9 mm SL). **a** Body part C. **b** Body part D. **c** Body part F. **d** Body part G. **e** Body part H. Scale bar = 500 µm.
Abb. 3: Schuppen von *Atherina presbyter* (75,9 mm SL). **a** Körperregion C. **b** Körperregion D. **c** Körperregion F. **d** Körperregion 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, e). 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, e). 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.

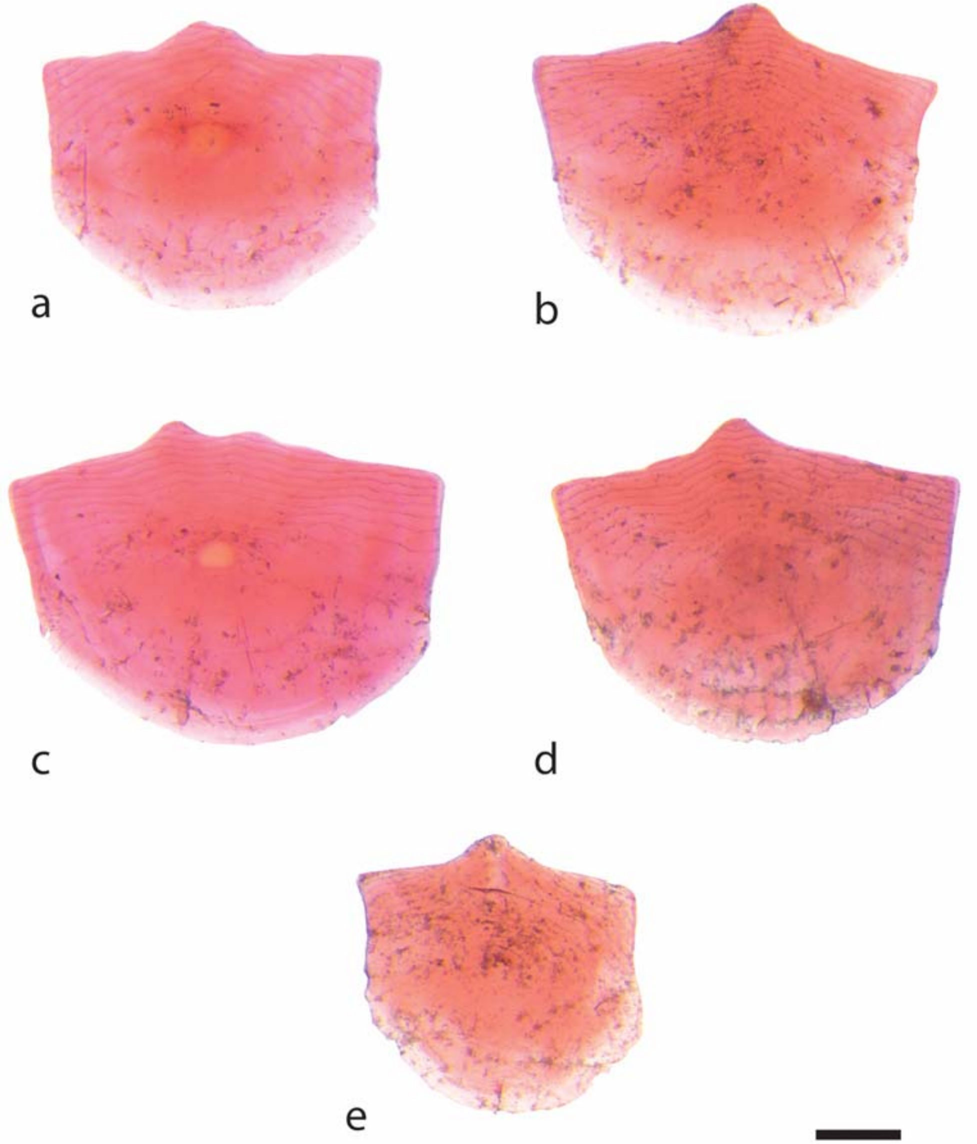

Fig. 3: Scales of *Atherina presbyter* (75.9 mm SL). **a** Body part C. **b** Body part D. **c** Body part F. **d** Body part G. **e** Body part H. Scale bar = 500 µm.
Abb. 3: Schuppen von *Atherina presbyter* (75,9 mm SL). **a** Körperregion C. **b** Körperregion D. **c** Körperregion F. **d** Körperregion H. Maßstab = 500 µm.

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 *Odonthestes 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 &

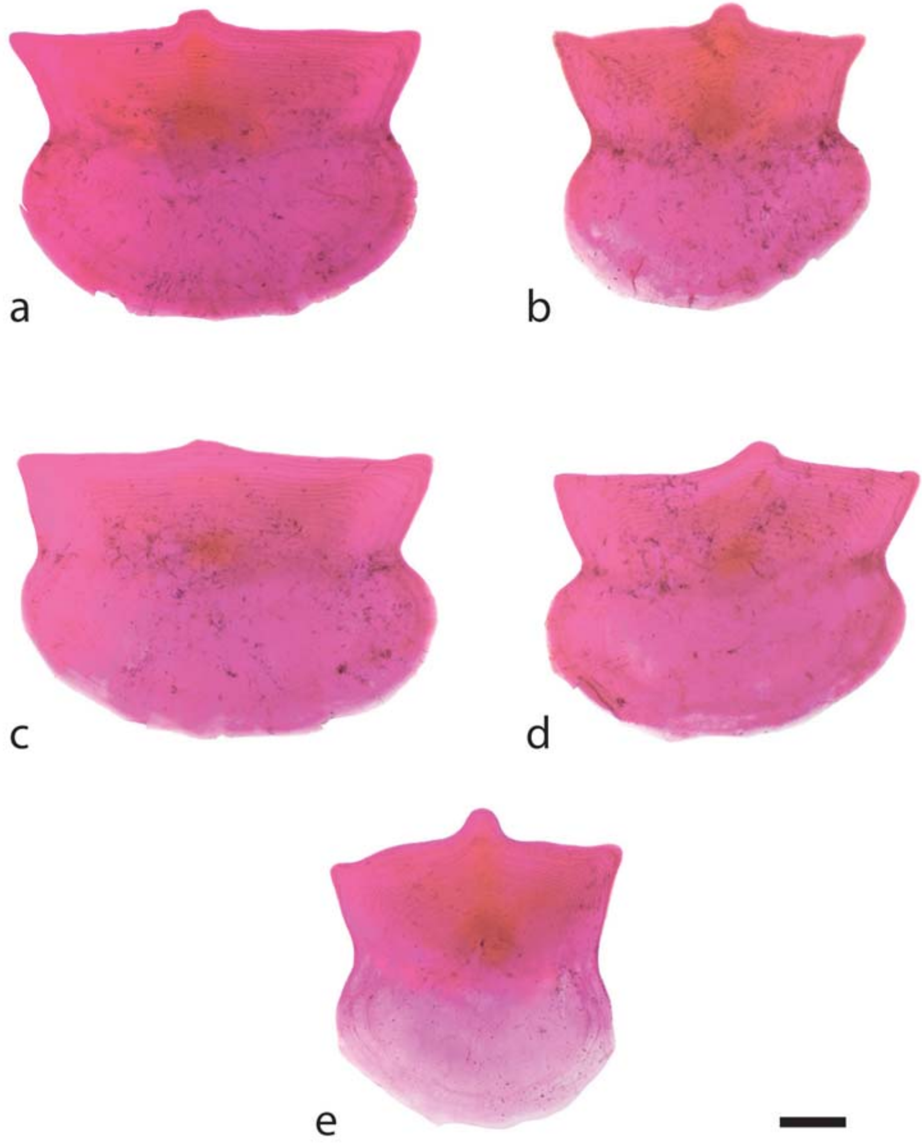

Fig. 4: Scales of *Atherina boyeri* (71.3 mm SL). **a** Body part C. **b** Body part D. **c** Body part F. **d** Body part G. **e** Body part H. Scale bar = 500 µm.
Abb. 4: Schuppen von *Atherina boyeri* (71,3 mm SL). **a** Körperregion C. **b** Körperregion D. **c** Körperregion F. **d** Körperregion 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 species 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 *Hemichromis 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 specimen 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 larvae'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.

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