Description of body scales of *Myxus elongatus* and *Neomyxus leuciscus* (Teleostei: Mugilidae) including a geometric-morphometric comparison

Beschreibung der Körperschuppen von *Myxus elongatus* und *Neomyxus leuciscus* (Teleostei: Mugilidae) mit einem geometrisch-morphometrischen Vergleich

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Summary: The subfamily Myxinae represents the sister group to all other mugilids (Mugilidae) and comprises the two species *Myxus elongatus* and *Neomyxus leuciscus*. We present, for the first time, detailed descriptions of the body scales for both species. As typical for the family they have quadrilateral, square to rectangular shaped scales with radii and an approximately central focus. Their posterior margin, however, bears a more or less pronounced membranous outgrowth and cteni are absent from lateral body scales. In terms of their gross morphology, the scales of *Myxus elongatus* and *Neomyxus leuciscus* look very much alike, however, they can be distinguished by a more pronounced indentation on the anterior margin in *Neomyxus leuciscus* and a larger membranous outgrowth at the posterior margin in *Myxus elongatus*. Furthermore, geometric morphometry proved to be a valuable method to separate scales of both species. Therefore, body scales of mugilids can provide very helpful information for the identification of species in this otherwise challenging taxon.

Keywords: Myxinae, fish scales, shape variability


Schlüsselwörter: Myxinae, Fischschuppen, Formvariabilität

1. Introduction

The Mugilidae currently comprise about 79 species in 26 genera (FRICKE et al. 2021). Latest genetic analyses subdivide the Mugilidae in four subfamilies: Myxinidae, Mugilinae, Rhinomugilinae, and Cheloninae (XIA et al. 2016). Within the Mugilidae, the Myxinidae are the earliest branching taxon and two extant species are assigned to this subfamily, *Myxus elongatus* and *Neomyxus leuciscus*. The distribution area of *M. elongatus* is restricted to the temperate waters of Australia while *N. leuciscus* is present in central Pacific waters around
southern Japanese and Hawaiian Islands to Samoa (Durand 2015).

Within the Mugilidae, morphological characters show only little variation resulting in difficulties for species discrimination caused by the lack of differences in the mugilid eiconomy (Crosetti & Blaber 2015). However, correct species identification is important for conservation of biodiversity and fisheries management. Therefore, genetic methods, i.e., DNA barcoding, were introduced and have been employed to identify species. This was tested successfully for mugilids (Durand et al. 2017) and is a valuable and important technique for studies on biodiversity. Such methods, though, are not easily available for everybody in the field and may not always be applicable during fishery surveys, to collection material, or in stomach content analyses (Ibáñez & O’Higgins 2011). Therefore, morphological structures that are easily accessible need to be employed to enhance correct identification of mugilids. It was previously shown that scale morphology and scale shape can be useful for species discrimination (Mosher 1969; Ibáñez & Aguirre et al. 2007; Ibáñez & O’Higgins 2011; Bräger et al. 2016; Bräger et al. 2017; Ibáñez et al. 2017; Pacheco-Almanzar et al. 2020). Especially scales from the antero-dorsal flank (termed C-region) have been shown to be useful in morphological and morphometric comparisons (Bräger et al. 2017). Therefore, scales might provide the needed characters to improve identification guides and simplify species identification of mugilids.

Previously, scale characters of mugilid species were only examined in a little number of studies (Jacot 1920; Pillay 1951; Esmaeili et al. 2014; Mussarat-Ul-Ainb et al. 2015; Bräger & Moritz 2016; Theime & Moritz 2020). Additional information on mugilid scales can also be found in some identification guides (e.g. Harrison & Senou 1999; Harrison 2016), which provide data on the scale type found in some species. Scales within the Mugilidae can be either cycloid, e.g. Osteomugil (Pillay 1951; Mussarat-Ul-Ainb et al. 2015), or ctenoid (whole cteni), e.g. Chelon, Planiliza (Pillay 1951; Esmaeili et al. 2014; Mussarat-Ul-Ainb et al. 2015; Theime & Moritz 2020). For scales of Myxinae species, it is stated that M. elongatus has both cycloid and ctenoid scales while N. leuciscus only has cycloid scales (Harrison & Senou 1999). For few species of the genera Chelon, Mugil, Osteomugil, Planiliza, and Rhinomugil, certain characters, e.g. circuli, cteni, etc.,

Tab. 1: Specimens examined in this study. Abbreviations: AM – Australian Museum; DMM – Deutsches Meeresmuseum; ZSM – Zoologische Staatssammlung München.

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Species</th>
<th>Collection-ID</th>
<th>Number of specimens</th>
<th>Scales used for G &amp; M analysis</th>
<th>Standard length</th>
<th>Collection region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myxinae</td>
<td>Myxus elongatus</td>
<td>AM-1.41833-001</td>
<td>1</td>
<td>3</td>
<td>18.6 cm</td>
<td>Australia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AM-1.42993-001</td>
<td>5</td>
<td>17</td>
<td>12.0-19.0 cm</td>
<td>Australia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AM-1.43213-001</td>
<td>3</td>
<td>8</td>
<td>11.2-14.5 cm</td>
<td>Australia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AM-1.44820-005</td>
<td>2</td>
<td>6</td>
<td>5.0-10.0 cm</td>
<td>Australia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMM IE/16607</td>
<td>1</td>
<td>0</td>
<td>3.8 cm</td>
<td>Australia</td>
</tr>
<tr>
<td>Novaculidae</td>
<td>novaculinae</td>
<td>AM-1.22124-003</td>
<td>4</td>
<td>8</td>
<td>10.6-12.2 cm</td>
<td>French Polynesia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AM-1.34510-018</td>
<td>1</td>
<td>3</td>
<td>17.5 cm</td>
<td>French Polynesia</td>
</tr>
<tr>
<td>Cheloninae</td>
<td>Planiliza macroptera</td>
<td>DMM IE/12323</td>
<td>1</td>
<td>3</td>
<td>15.9 cm</td>
<td>Taiwan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZSM 23449</td>
<td>2</td>
<td>7</td>
<td>8.6-10.7 cm</td>
<td>Eritrea</td>
</tr>
</tbody>
</table>
and radii, were described (JACOT 1920; PILLAY 1951; ESMAEILI et al. 2014; MUSSARAT-UL-AINB et al. 2015; BRÄGER & MORITZ 2016; THIEME & MORITZ 2020), however, no further details are known of Myxinae scales.

Scale shape was previously studied in some Mugil species as well as Chelon labrosus and Chelon saliens (IBÁÑEZ-AGUIRRE et al. 2006; IBÁÑEZ AGUIRRE et al. 2007; IBÁÑEZ & O’HIGGINS 2011; IBÁÑEZ et al. 2017) focussing on genus, species and population discrimination. So far, no study addressed the scales of Myxinae in this context.

Therefore, the goal of this study is to describe the scale morphology of M. elongatus and N. leuciscus, the sole representatives of the earliest branching subfamily within the Mugilidae. Furthermore, geometric morphometric analysis is applied to study differences in the shape of the scales and to test if scale shape is suitable for species discrimination within the Myxinae.

2. Material and Methods

The scales used in this study were sampled from specimens stored in the collections of the Australian Museum (AM), the Deutsches Meeresmuseum (DMM), and the Zoologische Staatssammlung München (ZSM). Scales of all body areas were investigated from 20 individuals representing three species (tab. 1). From each specimen, scale samples for detailed analysis were taken from the antero-dorsal flank representing body region C (according to BRÄGER & MORITZ 2016; fig. 1). Additional samples were taken from further body regions (fig. 1) of M. elongatus (AM-I.41833-001 and DMM IE/16697) and of Neomyxus leuciscus (AM-I.34510-018). The terminology for the description of the scales follows BRÄGER & MORITZ (2016) and is based on scales from body region C. Scales were stained in a 0.5% KOH solution with Alizarin-red. Afterwards, pictures of each scale were taken with a Leica M205 FCA stereomicroscope with an attached Leica DMC6200 camera operated

Fig. 1: Schematic drawing of Myxus elongatus showing the five sampling areas (C, D, F, G, H, I, J, K) following BRÄGER et al. (2016).

Fig. 2: Schematic drawing of a mugilid scale showing the seven landmarks used for geometric morphometric analysis. Dotted lines separate the fields of the scales. Asterisks indicate the area of the scale that is in contact with the environment. The focus is indicated by landmark 7.


Fig. 3: Scales of *Myxus elongatus* (AM-I.41833, SL = 18.6 cm) from the five sampled body regions. Scale bar: 2 mm.

Abb. 3: Schuppen von *Myxus elongatus* (AM-I.41833, SL = 18.6 cm) von den fünf beprobten Körperregionen. Maßstab: 2 mm.

with the software Leica Application Suite (version: 3.6.0.20104). Image processing was done in Adobe Photoshop and figure plates were assembled in Adobe Illustrator. Plates are numbered based on the body region each scale was sampled from.

Geometric morphometric analysis was performed for C-scales of the examined specimens (tab. 1). First, pictures were merged in one file and then randomly ordered using tspUtil (version: 1.78). Afterwards, landmarks were placed on seven positions (according to Ibáñez Aguirre
et al. 2007; fig. 2) using the software tpsDig2 (version: 2.31). The landmark data was analysed in RStudio (version: 1.3.1093; R version: 4.0.4) utilizing the software package Geomorph (version: 4.0.0) and Morpho (version: 2.8). Two datasets were analysed: one containing the landmarks of *M. elongatus* and *N. leuciscus* scales and the other containing the scales of one outgroup species, *Planiliza macrolepis*, and of *M. elongatus* and *N. leuciscus*. First, Generalized Procrustes analysis was performed to obtain shape variables by removing non-shape differences of the landmark configurations, i.e., translation, rotation, and rescaling. Then, canonical variates analysis (CVA) was performed to describe differences in scale shape in the tested species and to determine the number of specimens correctly assigned to each species. Furthermore, a principal component analysis (PCA) was performed to evaluate if scale shape is suitable for species discrimination within the Myxinae.

3. Results

3.1. *Myxus elongatus* (figs 3, 4)

**Type:** cycloid; true cycloid. **Shape:** quadrilateral: square to rectangular. **Anterior field:** flattened but slightly waved with striated margin. **Lateral field:** flattened. **Posterior field:** rounded end with membranous margin. **Focus:** central. **Circuli:** distinct, discontinuous in the anterior field and continuous in the lateral and posterior field. **Radii:** primary and secondary radii are present in the anterior field in parallel to radial orientation.

The type of scale varies depending on the body region. Scales in the dorsal and lateral body regions, as well as, scales dorsally on the head are true cycloid (fig. 3). Scales in the ventral body region, antero-ventrally to the pectoral fin, as well as, laterally and ventrally on the head are ctenoid with whole cteni (fig. 4). The shape of the scales varies slightly between the examined body regions. Scales from body regions C, D and G are square (fig. 3C, D, G), while scales from body regions F and H have a more rectangular shaped (fig. 3F, H). The anterior field has a shallow indentation in the middle which is most pronounced in C- and F-scales. The lateral fields of some scales can be slightly convex (e.g. fig. 3D, G). The membranous margin of the posterior field shows irregular indentations. Circuli in the anterior field are discontinuous due to radii and are much denser than in the other fields. In the posterior
field, the circuli are sometimes discontinuous and irregular (fig. 3F). Some scales show a canal running through the focus independent from the body region (fig. 3C).

3.2. Neomyxus leuciscus (fig. 5)

The type of all scales is true cycloid. The shape of the scales varies between the examined body regions. While scales from body regions C, D, F, and G are square (fig. 5C, D, F, G), scales from body region H are rectangular (fig. 5H). The anterior field has an indentation in the middle which is very pronounced in C-, D-, and H-scales (fig. 5C, D, H). However, there is some variance in the depth and in the distinction of the indentation (fig. 5C, D). The lateral fields can be slightly convex (fig. 5F). The posterior field is rounded. In H-scales the posterior field has a pointed tip (fig. 5H). A very small and indistinct membranous margin surrounds the posterior margin. Circuli in the anterior field are discontinuous due to radii and are much denser than in the other fields. In the posterior field, the circuli are sometimes discontinuous and irregular (fig. 5D, H). Some scales show a canal running through the focus independent from the body region (fig. 5C).

### 3.3. Morphometric analysis

The CVA of the data set including *Myxus elongatus*, *Neomyxus leuciscus*, and *Planiliza macrolepis* resulted in canonical variate 1 (CV 1) describing 75.9% and canonical variate 2 (CV 2) describing 24.1% of total variance between the genera (fig. 6). Three distinct clusters, each representing one species, are observable. Scales from *M. elongatus* and *N. leuciscus* principally differ along the axis of CV 2, while *M. elongatus* and *P. macrolepis* differ along the axis of CV 1 (fig. 6). The cross-validated classification resulted in 98.2% of all scales being correctly classified. All *N. leuciscus* and *P. macrolepis* scales were correctly classified while 2.9% of *M. elongatus* scales were erroneously classified as *N. leuciscus* scales.

The PCA comparing scales from *M. elongatus* and *N. leuciscus* showed that scale shape of both species mainly differs along principal component 1 (fig. 7B). Pattern of shape change along

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**Fig. 6:** Results from the CVA comparing scale shapes of three mugilid species. Ellipses show 90% confidence interval for each species respectively.

**Abb. 6:** Ergebnisse der kanonischen Variablenanalyse mit dem Vergleich der Schuppenform von drei Meeräschen. Ellipsen zeigen das 90-%-Vertrauensintervall für jede Art.
principal component 1 is influenced by ante-ro-ventral and antero-dorsal displacement of the dorsal and ventral corners of the anterior field, as well as postero-ventral and postero-dorsal displacement of the dorsal and ventral corners of the posterior field (fig. 7D). Similarly, computed mean shapes of *M. elongatus* and *N. leuciscus* differ in the relative position of the corners of the anterior and posterior field (fig. 7C).

4. Discussion

The scales of the respective lateral body regions of *Myxus elongatus* and *Neomyxus leuciscus* are very similar and share many features (fig. 3, 5). These scales are true cycloid with flattened anterior and lateral fields and rounded posterior fields. The focus is positioned central, but can be slightly displaced posteriorly in *N. leuciscus*. In scales of both species circuli are present which are disrupted in the anterior field by primary and secondary radii. These characters can be adopted as characteristics for the earliest branching subfamily within the Mugilidae, the Myxinae, for which *M. elongatus* and *N. leuciscus* are the only two representatives (Durand et al. 2012; Xia et al. 2016). However, there are also two scale characters that differ greatly between *M. elongatus* and *N. leuciscus* and can be used to distinguish the two species based on their scales. 1) Scales from *N. leuciscus* have a distinct and pronounced indentation in the anterior field, while scales of *M. elongatus* only have a minor indentation. 2) The membranous margin of the posterior field is much more protruding in *M. elongatus* than in *N. leuciscus*. Furthermore, *M. elongatus* possesses cycloid and ctenoid scales (fig. 4). While most scales, including all of the body flanks, are cycloid, there are ctenoid scales in certain body regions, i.e., the ventral body.

**Fig. 7:** Results from PCA comparing scale shape of Myxinae species. Scale shape differences along PC 2 (A), between the means of *Myxus elongatus* and *Neomyxus leuciscus* (C), and along PC 1 (D) are visualized using thin plate spine transformation grids.

**Abb. 7:** Ergebnisse der PCA mit dem Vergleich der Schuppenform der Myxinae-Arten. Unterschiede der Schuppenform entlang PC 2 (A), zwischen den berechneten Mittelwerten von *Myxus elongatus* und *Neomyxus leuciscus* (C), und entlang PC 1 (D) werden durch die Thin Plate Spine Transformations Raster visualisiert.
side, antero-ventrally to the pectoral girdle, and laterally and ventrally on the head. In *N. leuciscus* only cycloid scales are present.

Cycloid scales are also present in the genus *Osteomugil* (Rhinomugilinae) while the scales of other mugilid genera, i.e., *Chelon*, *Mugil*, *Planiliza*, and *Rhinomugil*, are ctenoid (Pillay 1951; Esmaeili et al. 2014; Mussarat-Ul-Ainb et al. 2015). Harrison & Senou (1999) reported a combination of cycloid and ctenoid scales, similar to *M. elongatus*, for the genera *Aldrichetta*, *Gracilimugil*, *Osteomugil*, and *Paramugil*, which all belong to the subfamily Rhinomugilinae. Scale shape within mugilids can generally be described as quadrilateral, however, scales of *Osteomugil speigleri* vary in their shape and some may be categorized as intermediate (Pillay 1951; Esmaeili et al. 2014; Mussarat-Ul-Ainb et al. 2015; Bräger & Moritz 2016). Another character common in mugilid scales is the presence of primary and secondary radii in the anterior field, which disrupt the otherwise continuous circuli. A posterior field with a membranous margin similar to that found in *M. elongatus* and *N. leuciscus* is present in species of the genus *Osteomugil* (Esmaeili et al. 2014). However, in this genus the membranous margin is much more protruding posteriorly in comparison to *M. elongatus* and *N. leuciscus* and has incisions occurring in constant distances.

The results of the geometric morphometric analysis showed that scale shape is useful to discriminate between *M. elongatus* and *N. leuciscus*. Both species were well separated in the PCA and warp grids provided a visualization of differences in scale shape between them (fig. 7). The CVA additionally showed that scales from species of the subfamily Myxinae distinctly differ from the scales of *Planiliza macrolepis*, a species of the subfamily Cheloninae (fig. 6). This indicates that scale shape may be useful to also discriminate between mugilid subfamilies and may also between mugilid genera. Ibáñez Aguirre et al. (2007) were able to successfully discriminate the mugilid genera *Mugil*, *Chelon*, and *Liza* using the same landmarks used in the present study, supporting this hypothesis.

5. Conclusions

Both, scale morphology and scale shape, can be used to successfully identify scales within the mugilid subfamily Myxinae. Scales of *Mylxus elongatus* and *Neomyxus leuciscus* differ in the indentation in their anterior fields, the protrusion of the membranous margin of the posterior field as well as the relative positions of the corners of the anterior and posterior fields. These characters can be employed in identification guides to easily distinguish both species. Compared to other mugilid species, these characters seem to be useful, too. However, further studies on scale morphology and scale shape of mugilid species from the Western Pacific region need to be done to determine which characters can be incorporated into identification guides to not only distinguish *M. elongatus* and *N. leuciscus* but to discriminate between other mugilid genera and species.

Acknowledgement

We thank the Gesellschaft für Ichthyologie e.V. for supporting this project by covering travel expenses of PT for a research stay at the Australian Museum. We thank Amanda Hay, Sally Readers and Kerryn Parkinson from the Australian Museum for providing access to the examined specimen and support in data acquisition. We thank Jane Köpp for photographing fish scales. We are thankful to two anonymous reviewers who helped to improve the manuscript.

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Received: 03.06.2021
Accepted: 03.08.2021
Published: 20.08.2021