Dissertation zur Erlangung des Doktorgrades
an der Fakultät für Geowissenschaften
der Ludwig-Maximilians-Universität München

Gobioids now and then

Statistical investigation of hard part characters of fossil and extant gobies

---

Vorgelegt von
Carolin Gut

21. September 2020

Erstgutachter: Prof. Dr. Bettina Reichenbacher

Zweitgutachter: Prof. Dr. Alexander Nützel

Tag der mündlichen Prüfung: 01. Februar 2021

Statutory declaration and statement

I hereby confirm that my thesis entitled “Gobioids now and then - Statistical investigation of hard part characters of fossil and extant gobies”, is the result of my own original work. Furthermore, I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university and, to the best of my knowledgeand belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the Ludwig-Maximilians-UniversitätMünchen.

München, 17.02.2021

Carolin Gut

Abstract

Gobioidei are usually rather small fish with only few species exceeding the size of 20 cm. They are one of the most diverse groups within the Teleostei. Their sheer diversity and countless adaptations make it possible for them to occur in fresh, brackish or marine waters. Today we count about 270 genera with more than 2200 species distributed all around the globe. Gobioids, or gobies, play an important role in all the different ecosystems that they inhabit – especially in coral reefs. They occupy scientists today due to their radiative and invasive potential. Determining species in gobies can be very difficult because of their potentially high similarities, or their small size. Before the utilization of molecular approaches, the systematics of gobies relied on morphological characters such as the pterygiophore formula or the amount and arrangement of the head sensory papillae. Later molecular studies more or less supported these morphological classifications. However, some families still lack morphological synapomorphies and are so far merely supported by molecular studies like the the Oxudercidae. Today we recognize eight families: Butidae, Eleotridae, Gobiidae, Milyeringidae, Odontobutidae, Oxudercidae, Rhyacichthyidae and Thalasseleotrididae.

In the fossil record, Gobioidei are known since the Eocene. Even though articulated fossil skeletons are a rare find, isolated gobioid otoliths can be high in number and of very good preservation. Therefore, it comes to no surprise that about 70% of all described fossil gobioid species are based only on isolated otoliths. So far more than 100 fossil gobioid species are described with their number still increasing rapidly. Findings of skeletons with otoliths *in situ* are a scarce discovery but an important one, as it links the isolated otoliths to their skeletons. For isolated otoliths species determination can be hard due to their compact shape with only few diagnostic characters. At the beginning of gobioid palaentology, otoliths were preferably assigned to the genus *Gobius* due to lack of extant comparison material and therefore the lack of knowledge about otolith variability in gobioid otoliths. Subjective classifications and a potentially error-prone fossil record was the result. Nowadays, our knowledge of extant gobioid otolith morphology increases and new fossils with otoliths *in situ* are described. Still, an objective support for taxonomical classifications of fossil gobioid otoliths was missing.

In consideration of this, the overall aim of this study is to statistically and quantitatively investigate morphological, morphometrical and meristic data of extant gobioids to explore their variability and their potential for species separation in their skeletons and otoliths. These results will then be applied to a sample set of fossil, presorted isolated gobioid otoliths in order to test objective

support of these erected groups.

This thesis comprises 6 chapters with chapter 2, 4 and 5 already published and 3 as manuscript. Chapter 6 represents hereby the conclusion of this study.

In chapter 2 extant European species of the genera *Gobius* and *Pomatoschistus*, the most species rich genera of Gobiidae and Oxudercidae respectively, are examined using X-rays and their extracted sagittal otoliths. The acquired data (morphometrics, meristics, otolith indices and otolith outline) is statistically evaluated and examined for species separation success. The outcome of the study supported the common assumption that goby otoliths are highly species-specific and therefore of great value for species determination. The best method applied on otoliths for species-separation is the Fourier shape analysis as differences can be subtle. But also, otolith indices, morphometrics and meristics can be of good use for species classifications in the fossil record, like in case of *Pomatoschistus* where this study detected meristic traits that could greatly benefit for species separation in case of skeletal findings.

In chapter 3, the gained knowledge about extant gobioid otoliths is applied to isolated fossil otoliths from two sample sites located in the Vienna Basin. The fossils lived during the Badenian (Middle Miocene, ∼14 Ma). Hereby, the well-preserved otoliths were picked from the samples and were classified using the standard paleontological approach (comparative morphology). The erected groups were tested using Fourier shape analysis and rearranged until the classification success was similar to these of extant gobioid species. Therefore, this study presents for the first time erected gobioid species with objective support based on outline data. Additionally, this study introduces one new genus †*Paromiogobius* nov. (including one new species †*Paromiogobius johnsonae*) and three newly descibed species of *Lesueurigobius* (*L. annajanae* sp. nov., *L. brzobohatyi* sp. nov., *L. harzhauseri* sp. nov.). This suggests that the genus *Lesueurigobius* was more diverse than previously assumed.

In chapter 4 the osteology of the posterior vertebral column and the caudal skeleton of the amphibious mudskippers is examined by using X-rays. This qualitative approach revealed modified caudal vertebrae in many species suggesting an adaptation to their amphibious lifestyle. Modifications were for example the thickening and shortening of caudal rays on the ventral side. Most modified vertebrae occurred in the more terrestrial living species and therefore suggest an adaptive function towards moving and “jumping” on land. The examined aspects were all osteology related and hence provide a great source of information for comparing and assigning fossils of this group.

In chapter 5 the study focuses on extant otoliths of mudskippers. Here the differences of three species were examined in order to improve our understanding of the qualitative variability in these mudskippers. This study showed the atypical shape of *Periophthalmus waltoni* otoliths, which in contrast to other gobioids display a pentagonal shape. As *Ps. waltoni* is the best adapted species to a lifestyle on land it is within reason to assume that different shapes can reflect the adaptation to its amphibious lifestyle. Another reason for special adaptations (especially in syntopically living species like *Ps. waltoni* and *Boleophthalmus dussumieri*) might be for acoustical communication and differentiation outside of the water. As otoliths are hard parts with a good potential to be fossilized, this study provides an interesting insight into otolith morphology of mudskippers and can be of great benefit for studies on fossils of this group.

Zusammenfassung

Gobioidei sind für gewöhnlich kleinere Fische, von denen nur wenige eine Größe von 20 cm überschreiten und sind eine der artenreichsten Gruppen in den Teleostei. Ihre bloße Diversität und ihre unzähligen Adaptionen an ihre Umwelt ermöglichen es ihnen im Süß-, Brack- und Salzwasser vorzukommen. Heute zählen wir ca. 270 Gattungen mit mehr als 2200 Arten, deren Vorkommen sich über den ganzen Globus erstreckt. Gobioids, oder auch Grundeln genannt, spielen eine wichtige Rolle in den verschiedensten Ökosystemen in denen sie leben – vor allem in Korallenriffen. Sie beschäftigen die Wissenschaft heutzutage aufgrund ihres Potentials zur Radiation und ihrer erfolgreichen Invasion neuer Gebiete.

Die Artbestimmung bei den Grundeln kann sich aufgrund ihrer kleinen Größe und großen Ähnlichkeiten als sehr schwierig gestalten. Vor der Einführung von molekularen Methoden basierte die Systematik der Grundeln auf morphologischen Merkmalen, wie die Pterygophoren-Formel oder die Anzahl und Anordnung der Sinnespapillen. Spätere molekulare Untersuchungen bestätigten die morphologischen Klassifizierungen größten Teils, jedoch mangelt es noch in manchen Familien an morphologischen Synapomorphien, welche daher rein molekular gestützt sind, wie zum Beispiel im Fall der Oxudercidae. Soweit sind acht Familien anerkannt: Butidae, Eleotridae, Gobiidae, Milyeringidae, Odontobutidae, Oxudercidae, Rhyacichthyidae und Thalasseleotrididae.

Gobioide sind aus dem Fossilbericht seit dem Eozän bekannt. Auch wenn Funde artikulierter Skelette eher eine Seltenheit darstellen, können freigestellte Otolithen in großer Anzahl und in sehr guter Erhaltung vorkommen. Daher ist es keine Überraschung, dass ca. 70% aller beschriebenen Grundelfossilien allein auf Otolithen basieren. Bisher sind über 100 fossile Arten bekannt und ihre Anzahl steigt noch heute. Skelettfunde mit *in situ* Otolithen sind äußerst selten, jedoch von enormem Wert, da sie die Verbindung zwischen Skelett und losen Otolith herstellen. Eine Artbestimmung bei Grundeln, die allein auf freigestellten Otolithen beruht, gestaltet sich aufgrund der kompakten Otolithenform, welche nur wenige diagnostische Merkmale aufweist, als schwierig. Zu Beginn der paläontologischen Studien zu Gobioiden wurden deren Otolithen bevorzugt der Gattung *Gobius* zugesprochen, da rezentes Vergleichsmaterial und das damit verbundene Wissen über deren Variabilität fehlte. Subjektive Klassifizierungen und ein möglicherweise fehlerbehafteter Fossilbericht waren die Folge. Heute nimmt unser Wissen über die Morphologie rezenter Grundeln stark zu und auch die Anzahl der bekannten Fossilien mit Otolithen *in situ* wächst. Jedoch mangelt es noch immer an einer subjektiven Stütze der Klassifikationen von Grundeln.

In Anbetracht dessen ist das übergreifende Ziel dieser Studie morphologische, morphometrische und meristische Merkmale rezenter Grundeln statistisch und quantitativ zu untersuchen um neue Einblicke in deren Variabilität und deren Potential zur Arttrennung zu erhalten. Hierbei werden Skelett- und Otolithendaten berücksichtigt. Das Resultat wird anschließend in einer Fallstudie auf fossile Otolithen übertragen um deren Klassifizierung statistisch und damit objektiv zu stützen.

Diese Arbeit enthält 6 Kapitel von denen Kapitel 2, 4 und 5 bereits als Publikation vorliegen. Das Kapitel 3 liegt als Manuskipt vor. Das Kapitel 6 stellt das Fazit der Arbeit da.

In Kapitel 2 werden rezente Arten der Gattungen *Gobius* und *Pomatoschistus*, die die artenreichsten Gattungen der Familie Gobiidae bzw. der Familie Oxudercidae in Europa darstellen. Sie werden mit Hilfe von Röntgenaufnahmen und freigestellten Otolithen untersucht. Die erhobenen morphometischen bzw. meristischen Daten, sowie die berechnete Otolithen- Indizes werden statistisch evaluiert und auf ihr Potential zur Arttrennung untersucht. Das Ergebnis der Studie unterstützt die allgemeine Annahme, dass die Otolithen von Grundeln stark artspezifisch und damit sehr wertvoll für die Artbestimmung sind. Das beste Resultat zur Artentrennung erzielte die Methode der Fourier Umrissanalyse, da die Unterschiede der Grundelotolithen sehr subtil sein können. Allerdings haben auch morphometrische und meristische Daten, sowie Otolithen Indizes ihren Nutzen zur fossilen Arttrennung aufgezeigt. Insbesondere bezüglich der Gattung *Pomatoschistus* konnte die Studie den besonderen Wert von meritischen Daten zur Arttrennung herausarbeiten.

In Kapitel 3 wird das gewonnene Wissen über Grundelotolithen auf freigestellte, fossile Otolithen aus zwei Lokalitäten des Wiener Beckens angewandt. Die Fossilien lebten im Badenien (Mittelmiozän, ∼14 Ma). Hierbei wurden die gut erhaltenen Otolithen aus den Proben herausgesucht und nach den klassischen paläontologischen Methoden klassifiziert (vergleichende Morphologie). Die aufgestellten Gruppen wurden mit der Fourier Umrissanalyse untersucht und umgestellt, bis der „classification success“ dem der heutigen Grundeln ähnelte. Damit verzeichnet diese Studie zum ersten Mal aufgestellte, fossile Otolithengruppen, die durch eine objektive Analyse des Umrisses gestützt werden. Zudem beschreibt diese Studie die neue Gattung †*Paromiogobius* nov. (mit der neuen Art †*Paromiogobius johnsonae*) und drei weitere Arten den Gattung *Lesueurigobius* (*L. annajanae* sp. nov., *L. brzobohatyi* sp. nov., *L. harzhauseri* sp. nov.). Dies lässt den Schluss zu, dass die Diversität von *Lesueurigobius* im Badenien höher war, als bisher angenommen.

In Kapitel 4 wird die Osteologie der hinteren Wirbelsäule und das kaudale Skelett von amphibischen Schlammspringern mit Hilfe von Röntgenaufnahmen untersucht. Diese qualitative Herangehensweise zeigte in einigen Arten Modifikationen im kaudalen Skelett, die eine Anpassung an deren amphibischen Lebensweise suggerieren. Solche Modifikationen sind zum Beispiel die Verstärkung und Verkürzung der Kaudal-Strahlen auf der Unterseite. Die am stärksten modifizierten Wirbel traten vor allem in den eher terrestrisch lebenden Arten auf, was auf eine angepasste Funktion zur Fortbewegung und zum „Springen“ an Land hinweist. Da die untersuchten Gesichtspunkte alle osteologiebezogen sind, stellen sie eine hervorragende Quelle von Informationen für den Vergleich von Fossilien dieser Gruppe dar.

In Kapitel 5 liegt der Schwerpunkt der Studie auf den Otolithen der Schlammspringer. Es werden die Unterschiede dreier Arten untersucht um deren qualitative Variation besser zu verstehen. Diese Studie zeigte die untypische Form der Otolithen von *Periophthalmus waltoni* auf, welche im Gegensatz zu anderen Gobioiden eine pentagonale Form aufweisen. Da *Ps. Waltoni* von den Arten am besten an das Leben an Land angepasst ist, liegt die Vermutung nahe, dass diese andersartige Form auf der Anpassung an den amphibischen Lebensstil beruht. Ein anderer Grund für eine spezielle Anpassung (vor allem aufgrund von syntopischen Arten wie *Boleophthalmus dussumieri*) könnte die akustische Kommunikation und die Differenzierung außerhalb des Wassers darstellen. Da Otolithen Hartteile darstellen, die oft im Fossilbericht zu finden sind, eröffnet diese Studie interessante Einblicke in die Otolithen-Morphologie von Schlammspringern und kann damit sehr zu Studien von Fossilien dieser Gruppe oder ähnlichen Untersuchungen beitragen.

Contents

1 Introduction

1.1 General aspects about fishes

What we casually refer to as “fish” is, in reality, hard to define as these creatures come in all sizes, shapes, and colors (Froese & Pauly 2009; Helfmann et al. 2009; Brandl et al. 2018). Fishes display an extraordinary repertoire of adaptations which allows them to occupy all kinds of aquatic and semiaquatic habitats in all latitudes and altitudes from the deep sea to close to the mountain tops where they have to cope with extreme environments and unfavorable conditions like dry seasons (see e.g. Froese & Pauly 2009; Amelio et al. 2007; Helfmann et al. 2009; Li et al. 2013; Milligan et al. 2020). Fishes are the largest group within vertebrates and are distributed all around the globe (Froese & Pauly 2009, Helfmann et al. 2009). Today, there are 78 orders, 597 families $\geq$ 5200 genera and $\geq$ 35,000 species with their number increasing every year (Fricke et al. 2019). With that, they make up more than 50% of all described vertebrate species (Helfman et al. 2009). Fishes can be divided into two distinctive groups the jawless Agnathans (Greek *a* = without, *gnatha* = jaws) and the jawed Gnathostomata (Greek *gnathos* = jaw and *stoma* = mouth) (Helfmann et al. 2009). The Gnathostomata can be subdivided into the Condrichthyans (Greek *condr* = cartilage, and *ichthýs* = fish), and the Osteichthyes (Greek *osteon* = bone and *ichthýs* = fish) due to the material of their skeletons (Nelson et al. 2016). Among the Osteichthyes are the Teleosts (Greek *teleios* = complete, and *osteon* = bone). The Teleosts are the largest group within modern fishes comprising familiar species such as salmons, tuna, pikes, herrings, minnows ect. (Froese & Pauly 2009; Helfman et al. 2009; Fricke et al. 2019). Included in the Teleosts are the Percomorphaceae or Percomorpha (Betancur-R et al. 2017). One of the biggest groups within them are the Gobiiformes with its suborder Gobioidei, latter with more than 2200 species of which about 200 are adapted to life in fresh water (Betancur-R et al. 2017; Fricke et al. 2019).

1.2 Gobioidei *sensu* Betancur-R et al. 2017

Members of Gobioidei occupy highly diverse habitats all around the globe and show an extraordinary array of morphological, ecological and behavioral diversity (Patzner et al. 2011; Nelson et al. 2016; Froese & Pauly 2009). Today there are about 270 recorded genera with more than 2200 species (Fricke et al. 2019). They occur in marine, brackish and fresh waters and are commonly of small size. Most of them are benthic or demersal fish and can display colorful or cryptic patterns which can make detecting and observing them difficult (see Patzner et al. 2011).

Some even show complex symbiotic relationships with shrimps or contribute to the reef population as cleaning fishes (e.g. Patzner et al. 2011; Kohda et al. 2017; Xavier et al. 2019).

1.2.1 Specialists and record holders amongst gobies

As mentioned above gobies are very diverse and can therefore be highly specialized to their habitat (see e.g. Patzner et al. 2011). This led to some outstanding adaptations and abilities:

1) "Smarter" - depending on where you are: Rock pool-dwelling goby species in intertidal zones (e.g. *Bathygobius cocosensis* (Bleeker, 1854) or *Favonigobius lentiginosus* Richardson, 1844) show strong evidence of neural investment in specific regions of the brain, depending on the habitat they are living in (White & Brown 2015). This adaptation facilitates their special learning during high tide; in order to leap to water pools during low tide for safety or to find trapped food (see Aronson 1951; 1971; White & Brown 2015)

2) The smaller the better: Gobies are generally rather small fish, however, some of them are even so miniature in size, that they number among the smallest known vertebrates like *Trimmatom nanus* Winterbottom & Emery, 1981 or *Pandaka pygmaea* Herre, 1927 (see Patzner et al. 2011; Balcombe 2016). This miniaturization in gobies is commonly accompanied by the reduction of squamation, fins, ossified bones, sensory organs and life span (see Herler et al. 2011). It is assumed that size reduction is linked to complex reef habitats as with small size small shelters with low or minimal predation risk are accessible which are unsuitable for larger gobioid or non-gobioid competitors (see Herler et al. 2011).

3) The shrimps' guardian: A well-known adaptation in gobies is the association with burrowing shrimps (see Karplus & Thompson 2011). These so-called guardian gobies (like *Cryptocentrus cinctus* (Herre, 1936) use the burrow of the shrimp as shelter, in return they are looking out for predators. Even though there are no recognizable morphological adaptations known in the goby; this mutual association required communicative and behavioral adaptations (see Karplus & Thompson 2011). This includes the identical diurnal activity rhythm of goby and shrimp or their interspecific communication like the tail flicking of the goby as warning signal for the shrimp in case of arising danger (Preston 1978; Karplus & Thompson 2011).

4) Blind close to the shore: *Typhlogobius californensis* Steindacher, 1879 is a blind goby without pigments, but in contrast to one's expectations it is not living in a deep see or in caves, where

such features are common traits, but in the intertidal region (MacGinitie 1939). A pair of *T. californensis* commonly shares a burrow beneath rocks with a shrimp pair of *Neotrypaea biffari* (Holthuis, 1991) (formerly *Callianassa affinis* Holmes, 1900) along the coast of California (MacGinitie 1939). The lateral line of *T. californensis* displays a "reduced topography", as in contrast to other shrimp associated gobies, *T. californensis* does not leave the dark burrow (MacGinitie 1939; Ahnelt & Scattolin, 2002). Whereas *T. californensis* is completely dependent on the shrimp, the benefit for the shrimp is ambiguous, as *T. californensis* only sometimes fends of invaders and due to its blindness cannot look out for threats from outside the burrow (see MacGinitie 1939).

5) Climbing the waterfall: Species of the genus *Sicyopterus* (e.g. *Sicyopterus stimpsoni* (Gill, 1860)) are known to inhabit steep habitats with high waterfalls on Hawaii (see Cullen et al. 2013). After hatching, juveniles of this species are washed downstream into the ocean, where their larvae will develop for several months until returning to their birthplace (see Schoenfuss & Blob 2013). In order to do so they developed a specialized oral sucking disc, in addition to the typical pelvic sucking disc of gobies, to climb upright waterfalls of 350 m meters high (see Schoenfuss & Blob 2013).

6) The fish on land: One of the most remarkable adaptations can be seen in some species of the mudskippers (within the genera *Boleophthalmus, Periophthalmodon, Periophthalmus, Scartelaos* and *Zappa*), that live an amphibious lifestyle, where they are able to leave the water (Murdy 1989). Mudskippers have conquered mangrove forests and nearby mudflats in tropical and subtropical coastlines (see Jaafar & Murdy 2017). Strong limb-like fins, a modified pectoral girdle and strengthened last caudal vertebrae enable them to move on land and the bucco-opercular cavity keeps their gills moist (see Harris 1960; Pace & Gibb 2009; Jaafar & Murdy 2017; Ghanbarifardi et al. 2020). Also, their skin is part of their respiratory system and is therefore perfused with "systemic arterial blood" (Jaafar & Murdy 2017). Within the mudskippers the most adapted to land is *Periophthalmus waltoni* Koumans, 1941 as it will also stay on land during high tide (see Ishimatsu & Gonzales 2011). It can perform high jumps to escape a predator or to defend its territory against other individuals (see Pace 2017). The locomotion of mudskippers on land is also giving an idea about how stem tetrapods like *Ichthyostega* could have conquered land (e.g. Pierce & Clack 2013; Kawano & Blob 2013).

1.2.2 Taxonomic classification

Gobiiformes are consisting of the suborders Trichonotoidei and Gobioidei with the latter one comprising the group of interest for this thesis (Betancur-R et al. 2017). Currently the Gobioidei – in the following referred to as gobies – comprise eight extant families, namely Rhyacichthyidae, Odontobutidae, Eleotridae, Butidae, Milyeringidae, Thalasseleotrididae, Gobiidae and Oxudercidae (Nelson et al. 2016; Thacker et al. 2015), however, Butidae, Milyeringidae, Oxudercidae (= Gobionellidae), Rhyacichthyidae, and Thalasseleotridae are yet to be examined for their status (Bentacur-R et al. 2017).

1.2.3 Phylogenetic relationships and other issues

Determining species within gobioids using morphological approaches can be very difficult as their appearance can be highly similar and, in many cases, species can only be distinguished by the amount and arrangement of their head sensory papillae (e.g. Miller & Economidis 2004; Pezold & Cage 2002, Kovačić & Šanda 2016). Before the establishment of molecular examination, gobioids were classified according to osteological and phenotypical traits, like the pattern of the sensory papillae (e.g. Miller 1973, 1988). The introduction of molecular based methods revealed new taxonomic groups and shed new light on the evolutionary dynamics in gobioids (e.g. Rüber et al. 2003; Thacker 2009, 2015). However, these methods brought also new questions, as molecular phylogenies often result in ambiguous outcomes, as trees are calculated based on different molecular markers and taxon composition (e.g Thacker 2011, 2013; Agorreta et al. 2013; see Nelson et al 2016). The state of the art suggests that Kurtidae and Apogonidae are sister taxa to Trichonotidae, which again are sister to the Gobioidei (see Thacker 2015; Betancur-R et al. 2017). Even though their phylogenetic relationships among each other and the amount of families are not yet fully resolved in every aspect (Gill & Mooi 2012, Agorreta et al. 2013, Thacker et al. 2019, Betancur-R et al. 2019), they are being used as model organisms to understand evolutionary processes and diversification (e.g. Rüber et al. 2003; Sayer 2005, Agorreta & Rüber 2012).

1.2.4 The fossil record of gobies

Today more than 100 fossil gobioid species are known with 70% of them only based on isolated otoliths (see Nolf 2013). Fossil gobioid skeletons are rather scarce and most of the time only isolated otoliths can be retrieved from outcrops (e.g. Reichenbacher 1993; Brzonohatý et al. 2007;

Nolf 2013). It is thought that due to their small, compact shape and their resistance towards being digested, their chances for being preserved are higher than that of bones (see Nolf 1985, 2013). In very exceptional cases fossils with their otolith still *in situ* are retrieved (e.g. Gierl & Reichenbacher 2015; Schwarzhans et al. 2017). The oldest fossil evidence of a gobioid fish so far are otoliths retrieved from the early Eocene sediments from India (Bajpai & Kapur 2004). The oldest known gobioid skeletons are also of Eocene age with *Gobius*? *praecursor* from Spain (Gaudant 1996; Gaudant & Busquets 1996) and †*Paralates chapelcorneri* formerly known as *Pomatoschistus*? cf. *bleicheri* from England (see Gierl & Reichenbacher 2017). Gobioid fossils remain scarce during the Oligocene, however, increased quite a lot in the middle Miocene (see e.g. Reichenbacher et al. 2018, 2019; Bradić-Milinović et al. 2019; Schwarzhans et al. 2020). So far fossil gobioids are known from North America (e.g. Fitch 1968; Nolf & Stringer 2003), Australia and New Zealand (e.g. McDowall et al. 2006; Schwarzhans et al. 2011) as well as from Eurasia – including Japan – (e.g Aoki 1967; Carnevale et al. 2006; Gaudant & Cavallo 2008 (*Gobius craverii*)); but most of them are known from Europe (e.g. Koken 1884, 1891; Weinfurter 1952; Radwańska 1992; Brzonohatý et al. 2007; Schwarzhans et al. 2017; Reichenbacher et al. 2018, 2019; Bradić-Milinović et al. 2019; Schwarzhans et al. 2020). The amount of described fossil gobioid species rose a lot in recent times and continues to grow (e.g. Schwarzhans et al. 2017, Reichenbacher et al. 2019, Bradić-Milinović et al. 2019; Schwarzhans et al. 2020).

1.3 Otoliths – "The fishes' ear"

Before we go deeper into the topic of gobies it is important to drive our attention to another matter in order to cover the framework of this study: The otoliths.

1.3.1 What is an otolith?

The inner ear – or more precisely the labyrinth system – of a fish usually contains an arrangement of three otoliths namely: sagitta (in the macula sacculi), lapillus (located in the macula utriculi) and asteriscus (in the macula neglecta) (e.g. Popper & Fay 2011). Usually the sagitta is the biggest of them, so also in gobioids (see Campana 2004). Otoliths are calcareous structures that are formed separately from the skeleton with the involvement of structure-influencing proteins (see e.g. Popper 1981; Campana 2004; Hüssy et al. 2020). Lumpreys, sharks and rays, however, lack such otoliths (see Campana 2004). Otoliths play a great but yet not fully understood role in hearing and balance in fish (e.g. Platt & Popper 1981; Popper 1981; Popper & Fay 2011; Schulz-Mirbach et al. 2019).

1.3.2 How does the otolith work?

The otoliths are connected to the inner ear – more precisely to the sensory epithelium – by thousands of hair cells (cilia) which are attached to the sulcus of the otolith (see Popper & Lu 2000; Popper & Fay 2011). With the growth of the fish the number of hair cells increases and in case of damage or loss, there is strong evidence that these cells can be regained (Popper & Fay 2011). As the fish lives in a liquid medium which in its density is not so different from its own body, it requires the denser otoliths that in case of stimulation oscillate at a different amplitude than the body (see Popper & Lu 2000). This vibration sets the hair cells in motion, which transfer the signal to the sensory cells of the inner ear and results in hearing (Popper & Fay 2011). Otoliths are also involved in the sense of balance in a fish and can detect the direction of stimulation as the otolith can vibrate in different directions and intensions (see Riley & Moorman 2000; Schulz-Mirbach et al. 2020). It is hypothesized that the sculpture of a saccular otolith is important for hearing and sound production as there are noticeable size differences in species that are utilizing sound for communication and species that communicate more with coloration (see Paxton 2000; Cruz & Lombarte 2004). The function of the lapillus and asteriscus are largely unknown. It appears that the functionality of the lapillus is, however, essential for survival of fish larvae whereas the sagitta can be spared for sole survival (see Riley & Moorman 2000).

1.3.3 What is it of use to human kind?

The sagitta is commonly used to estimate fish age, growth-rate or spawning intervals which is of interest for fishing industries (see e.g. Campana 2005; Popper & Fay 2011). This information is acquired by examining the pattern and the number of growth-rings in the sagitta which can be incremented in different intensity annually, monthly and even daily (see Campana 2005; Popper & Fay 2011). The most interesting feature of the sagitta for this thesis, however, is its variability amongst species which gives it a species specificness (see e.g. Koken 1884; Nolf 1985; Nolf 2013; Lombarte et al. 2018). In order to determine species, important features of the sagitta (thereafter referred to as otolith) are for example its outline (length/height, projections and rim expression) and its sulcus (shape and orientation) on the medial side (e.g. Tuset et al. 2003; Volpedo & Echeverría 2003; Gierl et al. 2018; Lombarte et al. 2018; Gut et al. 2020). In contrast to the outline which is very valuable to determine species or fish stocks (e.g. Campana & Casselman 1993; Farias et al. 2009; Lombarte et al. 2018), the sulcus in gobioids is considered to be more informative on higher taxonomic levels and possesses a typically shoe sole shape (Gierl et al. 2018). The question why otoliths are so variable between species is yet to be answered; however, it is suggested that this might reflect different evolutionary approaches in fish which basically ended up in the same result to detect and process sounds in different ways, or that the different shapes indeed are different ways of different perception (see Popper & Fay 2011)

1.4 Study significance

Fossil otoliths can occur in high quantities and also in well-preserved conditions (e.g. Reichenbacher 1993; Bratishko et al. 2015; Jost et al. 2015). The assignment to species can be difficult or ambiguous due to their "plain" shape and only slight differences between species and therefore prone to subjective interpretation (see Yu et al. 2014; Lombarte et al. 2018; Gut et al. 2020). As not long-ago extant comparison material was basically not available, many fossil gobioid otoliths were assigned to the genus *Gobius* which, compared to extant *Gobius* members, in many cases do not match. As our knowledge increased, revisions were done (see e.g. Weiler 1950 and Schwarzhans 2017; Weinfurter 1952 and Nolf & Brzobohatý 2009). But even now objective classifications or an objective support of visually assigned fossil groups is still lacking. Therefore, it is important to find a way to reduce human bias in the assignment of fossils to genus or species.

1.5 Aim and outline of this thesis

The aim of this thesis is to test the success of established statistical methods in separating species within certain genera that were securely predetermined morphologically. When enough success is achieved these methods will be applied on fossil otoliths, that in advance were assigned to groups using comparative morphology in order to examine if the achieved separation success is comparable to those of extant species (see Yu et al. 2014; Lombarte et al. 2018) and therefore supporting a fossil group.

Additionally, this thesis will make a small excursion to the osteology and otoliths of mudskippers for a better understanding of hart parts in this group. As these studies focus on hard parts, the obtained information will be of great use for palaeontological studies on mudskippers.

1.6 Author contributions

Chapter 2: Carolin Gut, Jasna Vukić, Radek Šanda, Timo Moritz, Bettina Reichenbacher: Identification of past and present gobies: distinguishing *Gobius* and *Pomatoschistus*

(Teleostei: Gobioidei) species using characters of otoliths, meristics and body morphometry. CG acquired data, designed and conducted the analyses, analysed, interpreted the data, produced drawings, tables and figures and drafted the manuscript. BR contributed the otolith description and their interpretation. JV, RS, TM contributed with the sampling and provision of specimens. All authors contributed to the final manuscript by careful reading and adding suggestions. The Manuscript was published in *Contributions to Zoology* 89 (2020) 282-323.

Chapter 3: Carolin Gut: Shedding new light on fossil gobiid fish otoliths from the Vienna Basin: An exemplary application of Fourier outline analysis and descriptive statistics as taxonomical support. CG carried out all required procedures in order to prosess the samples, analyse the data and draft the manuscript. She hereby wants to thank BR for carefully reading the draft and giving suggestions before implementing it in this thesis.

Chapter 4: Mehdi Ghanbarifardi, Carolin Gut, Zeinab Gholami, Hamid Reza Esmaeili, Christoph Gierl, Bettina Reichenbacher: Osteology of the posterior vertebral column and caudal skeleton of marine amphibious gobies (mudskippers) (Teleostei: Gobioidei). CG aquired X-rays and provided the drawings for the manuscript. All authors contributed to the final manuscript by careful reading and adding suggestions.

Chapter 5: Mehdi Ghanbarifardi, Carolin Gut, Zeinab Gholami, Hamid Reza Esmaeili, Christoph Gierl, Bettina Reichenbacher: Possible link between the structure of otoliths and amphibious mode of life of three mudskipper species (Teleostei: Gobioidei) from the Persian Gulf. CG participated in extracting and measuring the otoliths and conducting the analyses. All authors contributed to the final manuscript by careful reading and adding suggestions.

1.7 References

Agorreta, A., & Rüber, L. (2012). A standardized reanalysis of molecular phylogenetic hypotheses of Gobioidei. *Systematics and Biodiversity*, 10(3), 375-390.

Agorreta, A., San Mauro, D., Schliewen, U., Van Tassell, J. L., Kovacic, M., Zardoya, R., & Rüber, L. (2013). Molecular phylogenetics of Gobioidei and phylogenetic placement of European gobies. *Molecular Phylogenetics and Evolution*, 69(3), 619-633. doi:10.1016/j.ympev.2013.07.017

Ahnelt, H., & Scattolin, G. (2002). The lateral line system of a blind goby, *Typhlogobius californiensis* Steindachner, 1879 (Teleostei: Gobiidae). *Annalen des Naturhistorischen Museums in Wien. Serie B für Botanik und Zoologie*, 11-25.

Amelio, D., Garofalo, F., Brunelli, E., Loong, A. M., Wong, W. P., Ip, Y. K., Tota, B., Cerra, M. C. (2007). Differential NOS expression in freshwater and aestivating *Protopterus dolloi* (lungfish): heart vs kidney readjustments. *Nitric Oxide*, 18(1), 1-10.

Aoki, N. (1967). Some fossil *Gobius* from Japan. *Palaeont. Soc. Japan. Trans. Proc.*, NS(67), 125-128.

Aronson, L. R. (1951). Orientation and jumping behavior in the gobiid fish *Bathygobius soporator. American Museum novitates*; no. 1486.

Aronson, L. R. (1971). Further studies on orientation and jumping behavior in the gobiid fish, *Bathygobius soporator. NYASA*, 188(1), 378-392.

Bajpai, S., & Vivesh, V., Kapur. (2004). Oldest known gobiids from Vastan Lignite Mine (early Eocene), Surat district, Gujarat. *Current Science*, 87(4), 433-435.

Balcombe, J. (2016). *What a fish knows: The inner lives of our underwater cousins*: Scientific American/Farrar, Straus and Giroux.

Betancur-R, R., Wiley, E. O., Arratia, G., Acero, A., Bailly, N., Miya, M., Lecointre, G., Orti, G. (2017). Phylogenetic classification of bony fishes. *BMC Evolutionary Biology*, 17(1), 162.

Bradić-Milinović, K., Ahnelt, H., Rundić, L., & Schwarzhans, W. (2019). The lost freshwater goby fish fauna (Teleostei, Gobiidae) from the early Miocene of Klinci (Serbia). *Swiss Journal of Palaeontology*, 138(2), 285-315.

Brandl, S., Goatley, C., Bellwood, D., & Tornabene, L. (2018). The hidden half: ecology and evolution of cryptobenthic fishes on coral reefs: Cryptobenthic reef fishes. *Biological Reviews*, 93, 1846-1873. doi:10.1111/brv.12423

Bratishko, A., Schwarzhans, W., Reichenbacher, B., Vernyhorova, Y., & Ćorić, S. (2015). Fish otoliths from the Konkian (Miocene, early Serravallian) of Mangyshlak (Kazakhstan): testimony to an early endemic evolution in the Eastern Paratethys. *Paläontologische Zeitschrift*, 89(4), 839-889. doi:10.1007/s12542-015-0274-4

Brzobohatý, R., Nolf, D., & Kroupa, O. (2007). Fish otoliths from the Middle Miocene of Kienberg at Mikulov, Czech Republic, Vienna Basin: their paleoenvironmental and paleogeographic significance. *Bulletin de l'Institut royal des Sciences naturelles de Belgique, Sciences de la Terre*, 77, 167-196.

Campana, S. E. (2004). *Photographic atlas of fish otoliths of the Northwest Atlantic Ocean Canadian special publication of fisheries and aquatic sciences No. 133* (C. P. B. Ed.). Ottawa: NRC Research Press.

Campana, S. E. (2005). Otolith science entering the 21st century. *Marine and freshwater research*, 56(5), 485-495.

Campana, S. E., & Casselman, J. M. (1993). Stock discrimination using otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 50(5), 1062-1083.

Carnevale, G., Caputo, D., & Landini, W. (2006). Late Miocene fish otoliths from the Colombacci Formation (Northern Apennines, Italy): implications for the Messinian 'Lago-mare'event. *Geological Journal*, 41(5), 537-555.

Cruz, A., & Lombarte, A. (2004). Otolith size and its relationship with colour patterns and sound production. *J Fish Biol*, 65(6), 1512-1525.

Cullen, J. A., Maie, T., Schoenfuss, H. L., & Blob, R. W. (2013). Evolutionary novelty versus exaptation: Oral kinematics in feeding versus climbing in the waterfall-climbing Hawaiian goby *Sicyopterus stimpsoni.* PLOS ONE, 8(1), e53274.

Farias, I., Vieira, A. R., Gordo, L. S., & Figueiredo, I. (2009). Otolith shape analysis as a tool for stock discrimination of black scabbardfish, *Aphanopus carbo* Lowe, 1839 (Pisces: Trichiuridae), in Portuguese waters. *Scientia Marina*, 2009, vol. 73S2, p. 47-53.

Fitch, J. E. (1968). *Otoliths and Other Fish Remains from the Timms Point Silt: (early Pleistocene) at San Pedro, California*: Los Angeles County Museum of Natural History.

Fricke, R., Eschmeyer, W. N., & Fong, J. D. (2019). *Species by family/subfamily in the Catalog of Fishes.* [Electronic version]. San Francisco (CA): California Academy of Sciences; 2019. [cited 2019 Oct 21]. In.

Froese, R., & Pauly, D. (2009). *FishBase. World wide web electronic publication.* http://www.fishbase.org.

Gaudant, J. (1996). Mise en évidence des plus anciens Gobioidei (Poissons téléostéens) connus dans le Lutétien inférieur marin de Catalogne (Espagne). *Comptes rendus de l'Académie des sciences. Série 2. Sciences de la terre et des planètes*, 322(1), 71-76.

Gaudant, J., & Busquets, P. (1996). Una ictiofauna de la formación de Vallfogona – Unidad del Cadí (Eoceno marino del dominio prepirenaico catalán). *Batalleria*, 6, 5–18.

Gaudant, J., & Cavallo, O. (2008). The Tortonian-Messini an fish faunas of Piedmont (Italy) and the Adriatic trough: a synthesis dedicated to the memory of Carlo Sturani (1938-1975). *Bollettino della Società Paleontologica Italiana*, 47(2), 177-189.

Ghanbarifardi, M., Gut, C., Gholami, Z., Esmaeili, H. R., Gierl, C., & Reichenbacher, B. (2020). Osteology of the posterior vertebral column and caudal skeleton of marine amphibious gobies (mudskippers)(Teleostei: Gobioidei). *Journal of Applied Ichthyology.* 36(5), 713-721.

Gierl, C., Liebl, D., Šanda, R., Vukić, J., Esmaeili, H. R., & Reichenbacher, B. (2018). What can goby otolith morphology tell us? *Cybium*, 42(4), 349-363.

Gierl, C., & Reichenbacher, B. (2015). A New Fossil Genus of Gobiiformes from the Miocene Characterized by a Mosaic Set of Characters. *Copeia*, 103(4), 792-805. doi:10.1643/ci-14-146

Gierl, C., & Reichenbacher, B. (2017). Revision of so-called *Pomatoschistus* (Gobiiformes, Teleostei) from the late Eocene and early Oligocene. *Palaeontologia Electronica*, 20(2), 1-17.

Gill, A. C., & Mooi, R. D. (2012). Thalasseleotrididae, new family of marine gobioid fishes from New Zealand and temperate Australia, with a revised definition of its sister taxon, the Gobiidae (Teleostei: Acanthomorpha). *Zootaxa*, 3266(1), 41-52.

Gut, C., Vukić, J., Šanda, R., Moritz, T., & Reichenbacher, B. (2020). Identification of past and present gobies: distinguishing *Gobius* and *Pomatoschistus* (Teleostei: Gobioidei) species using characters of otoliths, meristics and body morphometry. *Contributions to Zoology*, 89(3), 282-323.

Harris, V. (1960). On the locomotion of the mudskipper *Periophthalmus koelreu* (Pallas): (Gobiidae). *Proceedings of the Zoological Society of London*, 134(1), 107-135.

Helfman, G., Collette, B. B., Facey, D. E., & Bowen, B. W. (2009). *The diversity of fishes: biology, evolution, and ecology.* Oxford: John Wiley & Sons.

Herler, J., Munday, P. L., & Hernaman, V. (2011). Gobies on Coral Reefs. In R. A. Patzner, J. L. Van Tassell, M. Kovačić, & B. G. Kapoor (Eds.), *The biology of gobies* (pp. 559-607). Enfield: Science Publishers Inc.

Hüssy, K., Limburg, K. E., de Pontual, H., Thomas, O. R. B., Cook, P. K., Heimbrand, Y., Blass, M., Sturrock, A. M. (2020). Trace Element Patterns in Otoliths: The Role of Biomineralization. *Reviews in Fisheries Science & Aquaculture*, 1-33. doi:10.1080/23308249.2020.1760204

Ishimatsu, A., & Gonzales, T. (2011). Mudskippers: front runners in the modern invasion of land. In R. A. Patzner, J. L. Van Tassell, M. Kovačić, & B. G. Kapoor (Eds.), *The biology of gobies* (pp. 609-638). Enfield: Science Publishers Inc.

Jaafar, Z., & Murdy, E. O. (2017). *Fishes out of water: biology and ecology of mudskippers (1 ed.).* Boca Raton: CRC Press.

Jost, J., Kälin, D., Börner, S., Vasilyan, D., Lawver, D., & Reichenbacher, B. (2015). Vertebrate microfossils from the Upper Freshwater Molasse in the Swiss Molasse Basin: Implications for the evolution of the North Alpine Foreland Basin during the Miocene Climate Optimum. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 426, 22-33.

Karplus, I., & Thompson, A. R. (2011). The partnership between gobiid fishes and burrowing alpheid shrimps. In R. A. Patzner, J. L. Van Tassell, M. Kovačić, & B. G. Kapoor (Eds.), *The biology of gobies* (pp. 559-607). Enfield: Science Publishers Inc.

Kawano, S. M., & Blob, R. W. (2013). Propulsive forces of mudskipper fins and salamander limbs during terrestrial locomotion: implications for the invasion of land. *Integrative and comparative biology*, 53(2), 283-294.

Kohda, M., Yamanouchi, H., Hirata, T., Satoh, S., & Ota, K. (2017). A novel aspect of goby-shrimp symbiosis: gobies provide droppings in their burrows as vital food for their partner shrimps. *Marine Biology*, 164(1), 22.

Koken, E. (1884). Über Fisch-Otolithen, insbesondere über diejenigen der norddeutschen Oligocän-Ablagerungen. *Zeitschrift der deutschen geologischen Gesellschaft*, 500-565.

Koken, E. (1891). Neue Untersuchungen an tertiären Fisch-Otolithen. II. *Zeitschrift der deutschen geologischen Gesellschaft*(43), 77-170.

Kovačić, M., & Šanda, R. (2016). A new species of *Gobius* (Perciformes: Gobiidae) from the Mediterranean Sea and the redescription of *Gobius bucchichi*. *J Fish Biol*, 88(3), 1104-1124. doi:10.1111/jfb.12883

Li, Y., Ren, Z., Shedlock, A. M., Wu, J., Sang, L., Tersing, T., Hasegawa, M., Yonezawa, T., Zhong, Y. (2013). High altitude adaptation of the schizothoracine fishes (Cyprinidae) revealed by the mitochondrial genome analyses. *Gene*, 517(2), 169-178.

Lombarte, A., Miletic, M., Kovačić, M., Otero-Ferrer, J., & Tuset, V. (2018). Identifying sagittal otoliths of Mediterranean Sea gobies: Variability among phylogenetic lineages. *J Fish Biol*, 92, 1768-1787. doi:10.1111/jfb.13615.

MacGinitie, G. (1939). The Natural History of the Blind Goby, *Typhlogobius californiensis* Steindachner. *American Midland Naturalist*, 489-505.

McDowall, R., Kennedy, E., Lindqvist, J., Lee, D., Alloway, B., & Gregory, M. (2006). Probable Gobiomorphus fossils from the Miocene and Pleistocene of New Zealand (Teleostei: Eleotridae). *Journal of the Royal Society of New Zealand*, 36(3), 97-109.

Miller, P. (1973). The osteology and adaptive features of *Rhyacichthys aspro* (Teleostei: Gobioidei) and the classification of gobioid fishes. *Journal of Zoology*, 171(3), 397-434.

Miller, P. (1988). New species of *Corcyrogobius*, *Thorogobius* and *Wheelerigobius* from West africa (teleostei: gobiidae). *Journal of Natural History*, 22(5), 1245-1262.

Miller, P. J., & Economidis, P. S. (2004). The freshwater fishes of Europe: Mugilidae, Atherinidae, Atherinopsidae, Blenniidae, Odontobutidae, *Gobiidae 2 (Vol. 8)*: Aula-Verlag.

Milligan, R. J., Scott, E. M., Jones, D. O., Bett, B. J., Jamieson, A. J., O'brien, R., Pereira Costa, S., Rowe, G.T., Ruhl, H. A., Smith Jr, K. L. (2020). Evidence for seasonal cycles in deep-sea fish abundances: a great migration in the deep SE Atlantic? *Journal of Animal Ecology*, 1593-1603. doi:10.1111/1365-2656.13215

Murdy, E. O. (1989). A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae: Oxudercinae). *Records of the Australian Museum*, 1-93.

Nelson, J. S., Grande, T. C., & Wilson, M. V. H. (2016). *Fishes of the World* (5 ed.). New Jersey: John Wiley & Sons, Inc.

Nolf, D. (1985). *Otolithi piscium.* In S. H. P. (Ed.), *Handbook of paleoichthyology* (Vol. 10, pp. 1-145). Stuttgart: Gustav Fischer Verlag.

Nolf, D. (2013). *The diversity of fish otoliths, past and present.* Belgium: Royal Belgian Institute of Natural Sciences.

Nolf, D., & Brzobohatý, R. (2009). Lower Badenian fish otoliths of the Styrian and Lavanttal basins, with a revision of Weinfurter's type material. *Annalen des Naturhistorischen Museums in Wien. Serie A für Mineralogie und Petrographie, Geologie und Paläontologie, Anthropologie und Prähistorie*, 323-355.

Nolf, D., & Stringer, G. L. (2003). *Late Eocene (Priabonian) fish otoliths from the Yazoo Clay at Copenhagen*, Louisiana: Louisiana State University, Louisiana Geological Survey.

Pace, C. (2017). Aquatic and Terrestrial Locomotion. In Z. Jaafar & E. O. Murdy (Eds.), *Fishes out of water: Biology and ecology of Mudskippers.* Boca Raton: CRC Press.

Pace, C., & Gibb, A. C. (2009). Mudskipper pectoral fin kinematics in aquatic and terrestrial environments. *Journal of Experimental Biology*, 212(14), 2279-2286.

Patzner, R. A., Van Tassel, J. L., Kovačić, M., & Kapoor, B. G. eds. (2011). *The Biology of Gobies* (Vol. 2012). Enfield, New Hampshire: Science Publishers.

Paxton, J. R. (2000). Fish otoliths: do sizes correlate with taxonomic group, habitat and/or luminescence? Philosophical Transactions of the Royal Society of London. *Series B: Biological Sciences*, 355(1401), 1299-1303.

Pezold, F., & Cage, B. (2002). A review of the spinycheek sleepers, genus *Eleotris* (Teleostei: Eleotridae), of the Western Hemisphere, with comparison to the West African species. *Tulane Stud. Zool. Bot*, 31(2), 19-63.

Pierce, S. E., Hutchinson, J. R., & Clack, J. A. (2013). Historical perspectives on the evolution of tetrapodomorph movement. *Oxford Academic*, 53(2), 209-223.

Platt, C., & Popper, A. N. (1981). *Fine Structure and Function of the Ear* (W. N. Tavolga, A. N. Popper, & R. R. Fay Eds.). New York: Springer New York.

Popper, A. N. (1981). Comparative scanning electron microscopic investigations of the sensory epithelia in the teleost sacculus and lagena. *Journal of Comparative Neurology*, 200(3), 357-374.

Popper, A. N., & Fay, R. R. (2011). Rethinking sound detection by fishes. *Hearing research*, 273(1-2), 25-36.

Popper, A. N., & Lu, Z. (2000). Structure-function relationships in fish otolith organs. *Fisheries Research*, 46(1), 15-25. doi:https://doi.org/10.1016/S0165-7836(00)00129-6

Preston, J. L. (1978). Communication systems and social interactions in a goby-shrimp symbiosis. *Animal Behaviour*, 26, 791-802.

Radwańska, U. (1992). Fish otoliths the Middle Miocene (Badenian) deposits of southern Poland. *Acta Geologica Polonica*, 42(3-4), 142-238.

Reichenbacher, B. (1993). Mikrofaunen, Paläogeographie und Biostratigraphie der miozänen Brack-und Süßwassermolasse in der westlichen Paratethys unter besonderer Berücksichtigung der Fisch-Otolithen. *Senckenbergiana lethaea*, 73(2), 277-374.

Reichenbacher, B., Filipescu, S., & Miclea, A. (2019). A unique middle Miocene (Sarmatian) fish fauna from coastal deposits in the eastern Pannonian Basin (Romania). *Palaeobiodiversity and Palaeoenvironments*, 99(2), 177-194.

Reichenbacher, B., Gregorová, R., Holcová, K., Šanda, R., Vukić, J., & Přikryl, T. (2018). Discovery of the oldest *Gobius* (Teleostei, Gobiiformes) from a marine ecosystem of Early Miocene age. *Journal of Systematic Palaeontology*, 16(6), 493-513. doi:10.1080/14772019.2017.1313323

Riley, B. B., & Moorman, S. J. (2000). Development of utricular otoliths, but not saccular otoliths, is necessary for vestibular function and survival in zebrafish. *Journal of neurobiology*, 43(4), 329-337.

Rüber, L., Van Tassell, J. L., & Zardoya, R. (2003). Rapid speciation and ecological divergence in the American seven-spined gobies (Gobiidae, Gobiosomatini) inferred from a molecular phylogeny. *Evolution*, 57(7), 1584-1598.

Sayer, M. D. (2005). Adaptations of amphibious fish for surviving life out of water. *Fish and Fisheries*, 6(3), 186-211.

Schoenfuss, H. L., & Blob, R. W. (2003). Kinematics of waterfall climbing in Hawaiian freshwater fishes (Gobiidae): vertical propulsion at the aquatic–terrestrial interface. *Journal of Zoology*, 261(2), 191-205.

Schulz-Mirbach, T., Ladich, F., Mittone, A., Olbinado, M., Bravin, A., Maiditsch, I. P., Melzer, R. R., Krysl, P., Heß, M. (2020). Auditory chain reaction: Effects of sound pressure and particle motion on auditory structures in fishes. *PLOS ONE*, 15(3), e0230578.

Schulz-Mirbach, T., Ladich, F., Plath, M., & Heß, M. (2019). Enigmatic ear stones: what we know about the functional role and evolution of fish otoliths. *Biological Reviews*, 94(2), 457-482.

Schwarzhans, W. (2017). A review of otoliths collected by W. Weiler from the Badenian of Romania and by B. Strashimirov from Badenian equivalents of Bulgaria. *Cainozoic Research*, 17(2), 167-191.

Schwarzhans, W., Ahnelt, H., Carnevale, G., Japundžić, S., Bradić, K., & Bratishko, A. (2017). Otoliths in situ from Sarmatian (Middle Miocene) fishes of the Paratethys. Part III: tales from the cradle of the Ponto-Caspian gobies. *Swiss Journal of Palaeontology*, 136(1), 45-92.

Schwarzhans, W., Brzobohatý, R., & Radwańska, U. (2020). Goby otoliths from the Badenian (middle Miocene) of the Central Paratethys from Czech Republic, Slovakia and Poland: A baseline for the evolution of the European Gobiidae (Gobiiformes; Teleostei). *Bollettino della Società Paleontologica Italiana*, 59(2), 125-173.

Schwarzhans, W., Scofield, R. P., Tennyson, A. J., Worthy, J. P., & Worthy, T. H. (2011). Fish remains, mostly otoliths, from the non-marine early Miocene of Otago, New Zealand. *Acta Palaeontologica Polonica*, 57(2), 319-350.

Thacker, C. (2011). Systematics of Gobiidae. In R. A. Patzner, J. L. Van Tassel, M. Kovačić, R. A. Patzner, & B. G. Kapoor (Eds.), *The biology of gobies* (pp. 129-136). Enfield: Science Publishers Inc.

Thacker, C., E. (2009). Phylogeny of Gobioidei and Placement within Acanthomorpha, with a New Classification and Investigation of Diversification and Character Evolution. *Copeia*, 2009(1), 93-104. doi:10.1643/CI-08-004

Thacker, C. E. (2013). Phylogenetic placement of the European sand gobies in Gobionellidae and characterization of gobionellid lineages (Gobiiformes: Gobioidei). *Zootaxa*, 3619, 369-382.

Thacker, C. E. (2015). Biogeography of goby lineages (Gobiiformes: Gobioidei): origin, invasions and extinction throughout the Cenozoic. *Journal of Biogeography*, 42(9), 1615-1625. doi:doi:10.1111/jbi.12545

Thacker, C. E., Gkenas, C., Triantafyllidis, A., Malavasi, S., & Leonardos, I. (2019). Phylogeny, systematics and biogeography of the European sand gobies (Gobiiformes: Gobionellidae). *Zoological Journal of the Linnean Society*, 185(1), 212-225.

Thacker, C. E., Satoh, T. P., Katayama, E., Harrington, R. C., Eytan, R. I., & Near, T. J. (2015). Molecular phylogeny of Percomorpha resolves *Trichonotus* as the sister lineage to Gobioidei (Teleostei: Gobiiformes) and confirms the polyphyly of Trachinoidei. *Molecular Phylogenetics and Evolution*, 93, 172-179.

Tuset, V.M., Lombarte, A., González, J.A., Pertusa, J.F. & Lorente, M.J. (2003) Comparative morphology of the sagittal otolith in *Serranus* spp. *J. Fish Biol.*, 63 (6), 1491–1504.

Volpedo, A.V., & Echeverría, D.D. (2003) Ecomorphological patterns of the sagitta in fish on the continental shelf off Argentine. *Fish. Res.*, 60 (2–3), 551–560.

Weiler, W. (1950). Die Otolithen aus dem Jungtertiär Süd-Rumäniens: Mittel-Miozän, Torton, Buglow und Sarmat. *Senckenbergiana*, 31(3): 209–258.

Weinfurter, E. (1952). Otolithen aus miozänen Brack-und Süßwasserschichten des Lavanttales in Kärnten. *Sitzungsberichte der Akademie der Wissenschaften in Wien, mathematisch-naturwissenschaftliche Klasse. Abteilung, 1*, 161, 149-172.

White, G. E., & Brown, C. (2015). Microhabitat use affects brain size and structure in intertidal gobies. *Brain, Behavior and Evolution*, 85(2), 107-116.

Xavier, R., Mazzei, R., Pérez-Losada, M., Rosado, D., Santos, J. L., Veríssimo, A., & Soares, M. C. (2019). A risky business? Habitat and social behavior impact skin and gut microbiomes in caribbean cleaning gobies. *Frontiers in microbiology*, 10, 716.

Yu, X., Cao, L., Liu, J., Zhao, B., Shan, X., & Dou, S. (2014). Application of otolith shape analysis for stock discrimination and species identification of five goby species (Perciformes: Gobiidae) in the northern Chinese coastal waters. *Chinese journal of oceanology and limnology*, 32(5), 1060.

Results

The subsequent subchapters follow the style of scientific articles.

2 Identification of past and present gobies: distinguishing *Gobius* and *Pomatoschistus* (Teleostei: Gobioidei) species using characters of otoliths, meristics and body morphometry

Carolin Gut[1], Jasna Vukić[2], Radek Šanda[3], Timo Moritz[4], Bettina Reichenbacher[1,5]

[1]Department of Earth and Environmental Sciences, Ludwig-Maximilians-Universität München, Richard-Wagner Straße 10, 80333 Munich, Germany

[2]Department of Ecology, Faculty of Science, Charles University, Viničná 7, CZ-128 44 Praha, Czech Republic

[3]Department of Zoology, National Museum, Václavské nám. 68, CZ-115 79 Praha, Czech Republic

[4]Deutsches Meeresmuseum, Katharinenberg 14-20, 18439 Stralsund, Germany Institute for Zoology and Evolutionary Research, Friedrich-Schiller-University Jena, Erbertstr. 1, 07743 Jena, Germany

[5]GeoBio-Center, Ludwig-Maximilians-Universität München, 80333 Munich, Germany

2.1 Abstract

Gobies (Gobiidae + Oxudercidae) are among the largest groups of extant marine fishes. Fossils of gobies are abundant since the Miocene, and many species have been reported so far. However, delimitation of fossil goby species is challenging because molecular markers and diagnostic traits such as the disposition of sensory head papillae are lost. This study provides, for the first time, an actualistic framework for the identification of fossil goby species. We focus on characters that can in principle be recognized in fossils, and evaluate their ability to discriminate between extant goby species based on statistical analyses. Using 14 extant species of *Gobius* and seven species of *Pomatoschistus*, we conducted otolith morphometry, elliptic Fourier shape analysis of otoliths using the package 'Momocs', conventional fish morphometry, and meristic counts. In addition, the otoliths of all species are depicted based on SEM images and briefly described. Otolith Fourier shape analysis proved to be most efficient in discrimination of species within both genera, *Gobius* and *Pomatoschistus*. Several characters used in the other approaches also worked well, but the results were variable, and the relative taxonomic significance of particular variables tended to change depending on the species under consideration. We propose otolith shape analysis as a powerful tool to explore ancient goby species diversity when samples with abundant fossil otoliths are present. Overall, the herein presented data will greatly facilitate delimitation of fossil goby species in future studies, and will consequently shed new light on the evolution of goby diversity and biogeography through time.

Keywords Actualistic study; Gobioidei; morphology; otoliths; species discrimination

2.2 Introduction

Gobiidae and Oxudercidae ("gobies" hereafter) are generally small fishes (length < 25 cm), and together they constitute one of the most species-rich groups within the modern bony fishes (Teleostei) (Nelson et al., 2016), with > 1,250 species in the Gobiidae and > 650 species in the Oxudercidae (Fricke et al., 2019). Gobies are globally distributed in diverse marine, brackish and freshwater habitats, and also play important roles in reef ecology (Patzner et al., 2011; Tornabene et al., 2015; Brandl et al., 2018). Many species are highly specialized including close associations with crustacean (Karplus, 1987; Karplus et al., 2011; Kovačić et al., 2016) or as part of the cryptobenthic community (Glavičić et al., 2016; Brandl et al., 2018). The European Gobiidae and Oxudercidae comprise three distinct lineages, which were named *Aphia*-lineage, *Gobius*-lineage, and *Pomatoschistus*-lineage (Agorreta et al., 2013; Thacker, 2015). In the present work, we focus on the name giving genera of the latter two lineages, i.e., *Gobius* Linnaeus, 1758 and *Pomatoschistus* Gill, 1863, which are the most species-rich genera of European gobies (Miller, 1986; Patzner et al., 2011). Their lifestyle is usually benthic or cryptobenthic, with a preference for sandy and rocky substrates (Macpherson & Duarte, 1994; Miller, 2004; Patzner et al., 2011). The genus *Gobius* currently comprises 28 recognized species, which are mainly distributed in the NE Atlantic, the Mediterranean and the Black Sea (Miller, 1986; Patzner et al., 2011; Froese & Pauly, 2019). Notably, two of these species are recent discoveries (Iglésias et al., 2016; Kovačić & Šanda, 2016). Fourteen species are traditionally attributed to the genus *Pomatoschistus*, including three that were only recognized in the last decade (Miller & Šanda, 2008; Engin & Innal, 2017; Engin & Seyhan, 2017; Froese & Pauly, 2019); however, the genus is not monophyletic according to recent phylogenetic studies (Thacker et al., 2019). Species of *Pomatoschistus* mostly occur in coastal habitats along the NE Atlantic, the Baltic Sea, and in some areas of the Mediterranean and the Black Sea (Miller, 1986; Patzner et al., 2011). Identification of the individual species in both genera, *Gobius* and *Pomatoschistus*, relies on coloration patterns, the arrangement of the head sensory organs (papillae), and combinations of various other characteristics such as squamation, morphometric and meristic traits; species determination often turns out to be very difficult, especially between closely related species (Miller, 1974, 1986, 2004; Kovačić & Šanda, 2016; Engin & Seyhan, 2017).

Fossils are the only direct source of information on ancient species diversity and biogeography, and thus provide essential insights into the evolutionary history of taxa. Fossils of gobies are abundant since the early Miocene (23 Ma) and comprise both articulated skeletons (sometimes with otoliths *in situ*) and isolated otoliths (e.g., Weiler, 1963; Radwańska, 1992; Bratishko et al., 2015). Records of fossil otoliths are much more common than discoveries of fossil skeletons, and this explains why about two-thirds of the known fossil goby species have been described solely on the basis of otoliths (Gierl et al., 2018). But the delimitation of a fossil goby species is often problematic because information on the range of variation in skeletal and otolith traits within a respective species and between related taxa is restricted. Furthermore, the characters of the skeleton may vary little between extant goby species (Miller, 1986; Kovačić & Šanda, 2016). This, together with the loss of delicate characters in fossil skeletal material due to taphonomic processes, can lead to underestimation of the number of fossil species (see Gaudant & Quayle, 1988 vs. Gierl & Reichenbacher, 2017; Gaudant, 1998 vs. Bradić-Milinović et al., 2019). Otoliths of gobies have the advantage that they are quite robust and do not experience major loss of informative characters during the fossilisation process. On the other hand, they usually display a high degree of morphological variability, which can make their taxonomic interpretation challenging (Malz, 1978; Nolf, 1985; Jost et al., 2015). In some cases, subtle differences in otolith morphology may allow the identification of several species (Schwarzhans, 2014; Gierl & Reichenbacher, 2015; Reichenbacher et al., 2019), while in others clear differences in otolith morphology may reflect variation in a single species living in different environments (Vasil'eva et al., 2016). The objective of this study is to provide, for the first time, an actualistic framework for the taxonomic value of characters that can in principle be recognized in fossil gobies, i.e., otoliths, body morphometry and meristics. We used 14 extant species of *Gobius* and 7 extant species of *Pomatoschistus* to test the suitability of our approach.

2.3 Material and methods

2.3.1 Samples and datasets

Fourteen species of *Gobius* and seven species of *Pomatoschistus* were collected from sites along the coasts of the Mediterranean and the Baltic Sea. Details of sites and numbers of individuals are provided in Table 1, collection numbers are available in the supplementary Table S1. Specimens were collected with hand nets during scuba diving or with seine nets from the shore and euthanized with an overdose of benzocaine prior to fixation in ethanol. Species identification was based on morphological characters (patterns of sensory papillae and pores, coloration, squamation) using the identification keys of Miller (1986) and Kovačić (2008) as well as the original descriptions for more recently described species (i.e., Kovačić & Šanda, 2016 for *G. incognitus*; Miller & Šanda, 2008 for *P. montenegrensis*). The sampling complied with protocols approved by the responsible governmental authorities (according to the countries where the sampling was conducted). The number of specimens obtained varied from species to species, owing to differences in population sizes or difficulty of access. It is generally recommended that at least 30 specimens per category should be used in a statistical analysis (Kar & Ramalingam, 2013). However, this criterion is an arbitrary one and cannot always be fulfilled in organismic studies, as in the present instance. In such a case, a minimum of seven specimens per species is often considered to be sufficient for a statistical analysis in fish biology. Here, we compiled for each genus a complete dataset containing all collected species, and a reduced dataset including only those species for which at least seven specimens were available. This resulted in four datasets (two for each genus), with 14 and ten species, respectively, in the case of *Gobius*, and seven and five species, respectively, in the case of *Pomatoschistus*. Each dataset was subjected to univariate statistical analyses and assessment of Fourier descriptors. For Linear Discriminant Analysis (LDA), only the reduced datasets were used (see below).

2.3.2 Methods

Otolith preparation and SEM imaging Fish skulls were opened dorsally under a microscope, and the left and right saccular otoliths ("otolith" hereafter) were removed. Otoliths were incubated in 5% KOH solution for 1h to clean off adherent organic matter, immersed in distilled water for 1-2h, then washed several times with distilled water, and stored in the dry state. All otoliths were mounted and gold-coated for scanning electron microscopy (with either a SEM LEO 1430 VP at

Table 1: Details of the sample sets of the *Gobius* and *Pomatoschistus* species studied.

Species	**Fish specimens**		**Otoliths**	**Locality (N)**	**Region**
	N	**SL ranges (means±SD)**	***N* (left/right)**		
Gobius auratus Risso, 1810	11	26.1–60.2 (37.2±11.9)	17 (11/6)	Croatia: Selce (9); Krk Island, Kamenjak (2)	Adriatic Sea
G. bucchichi Steindachner, 1870	10	44.4–67.3 (48.9±15.1)	20 (10/10)	Croatia: Kraljevica (1); Selce (9)	
G. cobitis Pallas, 1814	11	33.4–80.0 (54.8±7.6)	21 (11/10)	Montenegro: Boka Kotorska Bay, Kostanjica (10); Croatia: Selce (1)	
G. couchi Miller & El-Tawil, 1974	4	32.9–44.3 (38.8±5.2)	8 (4/4)	Croatia: Krk Island, Omišalj (4)	
G. cruentatus Gmelin, 1789	11	78.3–117.0 (101.0±14.1)	21 (11/10)	Croatia: Selce (9), Krk Island, Kamenjak (2)	
G. fallax Sarato, 1889	2	46.0–50.0 (48.0±2.0)	4 (2/2)	Croatia: Unije Island, Cape Maračol (2)	
G. gasteveni Miller, 1974	1	68	2 (1/1)	Spain: Galicia (1)	Atlantic Sea
G. geniporus Valenciennes, 1837	10	63.6–111.6 (90.8±16.6)	14 (10/4)	Montenegro: Boka Kotorska Bay, Kostanjica (4); Croatia: Selce (6)	Adriatic Sea
G. incognitus Kovačić & Šanda, 2016	9	44.0–79.0 (63.0±13.0)	15 (6/9)	Croatia: Pelješac peninsula (9)	
G. kolombatovici Kovačić & Miller, 2000	2	70.7–80.1 (75.4±6.6)	4 (2/2)	Croatia: Krk, Tenki (2)	
G. niger Linnaeus, 1758	10*	40.7–59.3 (49.5±6.6)	20 (10/10)	England: Pilsey Island (10)	Atlantic Sea
G. paganellus Linnaeus, 1758	10	46.8–69.1 (56.2±6.3)	19 (9/10)	Portugal: Ria Formosa lagoons, Faro (10)	
G. roulei de Buen, 1928	10	43.8–57.5 (51.0±4.6)	20 (10/10)	Croatia: Selce (10)	Adriatic Sea
G. vittatus Vinciguerra, 1883	11	17.3–43.2 (30.1±10.4)	21 (10/11)	Croatia: Selce (9); Krk, Kamenjak (2)	
Pomatoschistus knerii (Steindachner, 1861)	10	21.6–27.0 (24.8±1.8)	20 (10/10)	Croatia: Krk Island, PlovanovStol (10)	
P. marmoratus (Risso, 1810)	9	24.8–37.1 (36.9±2.4)	14 (9/5)	Croatia: Selce (7); Albania,Vilunit Lagoon (2)	

Table 1: Details of the sample sets of the *Gobius* and *Pomatoschistus* species studied *(Cont.)*.

Species	**Fish specimens**		**Otoliths**	**Locality (N)**	**Region**
	N	**SL ranges (means±SD)**	***N (left/right)***		
P. microps (Krøyer, 1838)	15	21.7–31.6 (27.9±2.4)	30 (15/15)	Germany: Stralsund (15)	Baltic Sea
P. minutus (Pallas, 1770)	2	48–51.7 (49.9±2.6)	4 (2/2)		
P. montenegrensis Miller & Šanda, 2008	10	10.3–23.7 (20.2±3.9)	19 (10/9)	Albania: Skadar lake, Shegan spring (10)	Adriatic Sea
P. pictus (Malm, 1865)	2	28.4, no data	4 (2/2)	Norway: 4 km S of Brattvåg (2)	Atlantic Sea
P. quagga (Heckel, 1837)	10	26.3–29.9 (28.1±0.9)	20 (10/10)	Croatia: Krk, Kamenjak (10)	Adriatic Sea

Abbreviations: SL, standard length; N, number of fish specimens and otoliths; *ten specimens were used for Fourier analysis, for the other analyses seven specimens were used (see supplementary tables S1–S5).

ZSM Munich, or a HITACHI SU 5000 Schottky FE-SEM at LMU Munich). All otoliths are kept in the Bavarian State collection Palaeontology and Geology under the inventory number 2020 LIV.

Otolith morphometry Ten measurements were taken from the SEM images of the left otolith of each specimen following Gierl et al. (2018) and using ImageJ2 (Rueden et al., 2017). The parameters chosen were maximum length, maximum height, perimeter length, areas of both the whole otolith and the sulcus, as well as the vertical distances from the anterior and posterior ends of the sulcus to the ventral margin of the otolith (Fig. 1c). The measurements were used to calculate 23 otolith variables (according to Gierl et al., 2018), which were multiplied by 1000 for subsequent statistical analyses. The original measurements and the otolith variables derived from them are tabulated for all specimens in supplementary Tables S1 and S2.

Otolith shape analysis On the basis of the SEM images of both right and left otoliths, each otolith image was transformed into a black object on white background using Photoshop CS6 and saved as a JPG file. To conduct elliptic Fourier shape analysis, JPG-files were imported into R using the Momocs software package (Bonhomme et al., 2014). After Momocs had extracted the outlines of the otoliths, the appropriate sequence of commands was implemented to normalize the perimeter outlines in terms of size and orientation, and to estimate the number of harmonics required to attain a cumulative power that corresponded to 99.9% of the shape (24 harmonics in *Gobius* and 20 harmonics in *Pomatoschistus*). Subsequently, an elliptic Fourier analysis was performed, which resulted in the Fourier descriptors (supplementary Table S3). As no significant intraspecific differences in shape were detected between left and right otoliths (MANOVA, $p \leq 0.05$), SEM images of both were included in the subsequent analyses, as has been done in previous work

(Davoodi & Rahimian, 2016).

Body morphometry Digital X-ray images of all fish specimens were acquired prior to otolith dissection, using a Faxitron Bioptics instrument (LLC-Vision NDT version 2.2.5, 45 k.v. and 30 sec) at the SNSB Bavarian State Collection of Zoology, Munich (ZSM). On the basis of these images, standard measurements relating to head-, body- and fin-base proportions (Fig. 1a) were obtained with ImageJ (Rueden et al., 2017), following Liu et al. (2009) and Larson et al. (2001). Measurements included standard length (SL), distances from snout to origins of the first and second dorsal fins (SN/D1, SN/ D2) and anal fin (SN/A); distance from end of second dorsal fin to first dorsal (procurrent) ray of caudal fin (D2C); length of caudal peduncle (CP); body depth at origin of first dorsal fin (B); lengths of the bases of the second dorsal fin (D2b) and anal fin (Ab). All measurements were recorded to the nearest 0.1 mm. Eight variables were calculated by standardizing the measurements based on SL. Raw data and derived variables of all specimens are available in the supplementary Table S4.

Meristic counts Meristic characters were counted based on the X-ray images. They include number of abdominal, caudal, and total vertebrae (last terminal vertebral element was included); number of fin elements in second dorsal fin (D2) and anal fin (A) (the two rays attached to the last pterygiophore were counted as one); number of anal fin pterygiophores inserting in front of haemal spine of first caudal vertebra (AP, Birdsong et al., 1988); and number of dorsal and ventral procurrent rays (Fig. 1b). The number of fin elements (spinous rays) in the first dorsal fin (D1) and the number of branched caudal rays did not contain useful information as no differences were found in either the *Gobius* or the *Pomatoschistus* dataset. Each meristic count was used as a single variable in the statistical analyses. Counts of all specimens are provided in the supplementary Table S5.

Statistical analysis Variables resulting from otolith morphometry and Fourier shape analyses, body morphometry, and meristic characters each served as input for statistical analyses in R (R Core Team, 2019), using the packages Momocs, car, stats, PMCMRplus, MVN and heplot, as well as PAST vers. 3.22 (Hammer et al., 2001). Normal distribution was tested based on the Shapiro-Wilk test ($p > 0.05$ if normal distribution is satisfied). Homogeneity of variances was tested using Levene's test ($p > 0.05$ if homogeneity is satisfied) for the morphometric variables, and the Fligner-Killeen test ($p > 0.05$) for the meristic variables. Normality and homogeneity of the

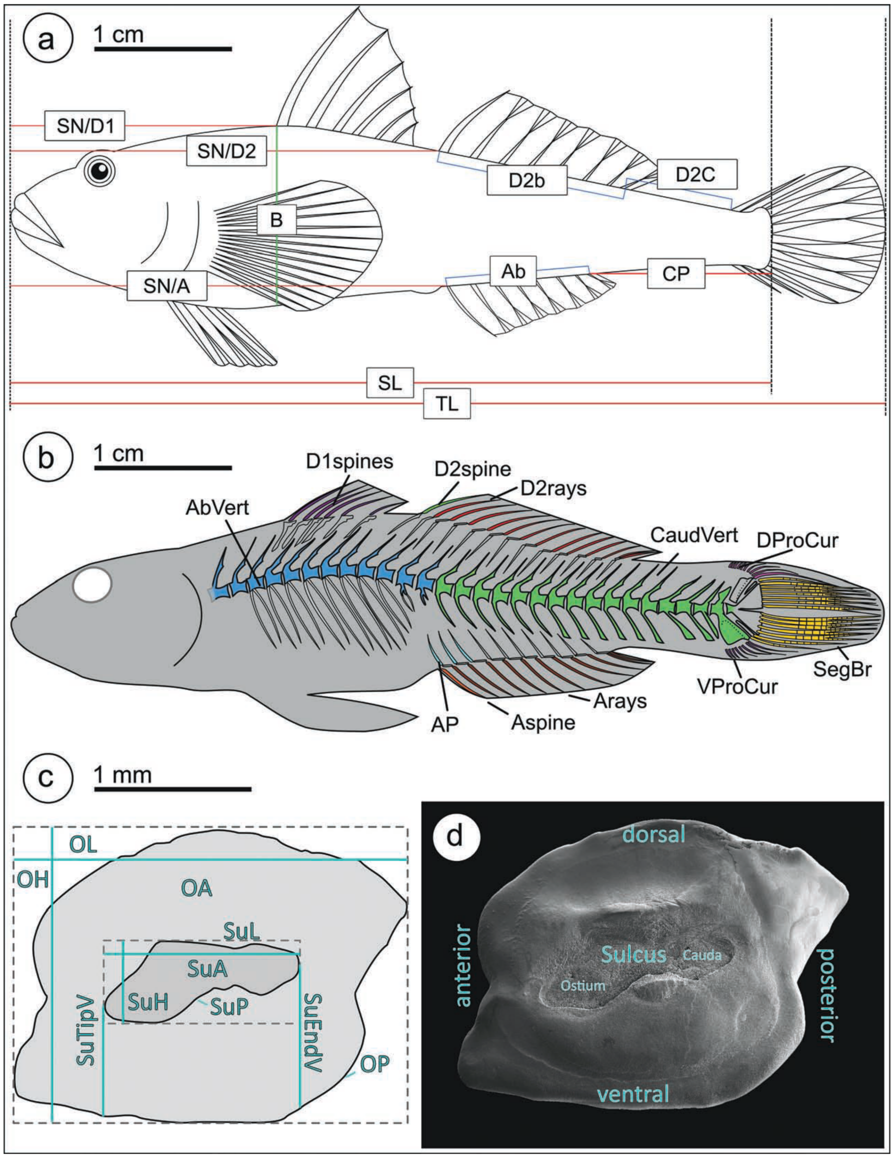


Figure 1: a–b, Schematic drawing of a goby skeleton depicting the standard morphometric (1a) and meristic (1b) characters used in this study; c–d, Schematic drawing (c) and SEM image (d) of a right goby otolith (based on *G. bucchichi*) showing the measured distances and areas (after Gierl et al., 2018) and the established otolith terminology. Colors in a: red, horizontal measurements; blue, measurements along fin bases; green vertical measurements. Abbreviations: (a) Ab, length of anal fin base; B, body depth at origin of first dorsal fin; CP, length of caudal peduncle; D2b, length of second dorsal fin base; D2C, distance between end of second dorsal fin and first dorsal (procurrent) ray of caudal fin; SL, standard length (from snout to begin of caudal fin); SN/A, distance from snout to origin of anal fin; SN/D1, distance from snout to origin of first dorsal fin; SN/D2, distance from snout to origin of second dorsal fin; TL, total length; (b) AbVert, abdominal vertebrae; AP, anal fin pterygiophores inserting in front of haemal spine of first caudal vertebra; Arays, rays of anal fin; CaudVert, caudal vertebrae; D2rays, rays of second dorsal fin; DProCur, dorsal procurrent rays; VProCur, ventral procurrent rays; (c) OA, otolith area; OH, otolith height; OL, otolith length; OP, otolith perimeter; SuA, sulcus area; SuH, sulcus height; SuL, sulcus length; SuP, sulcus perimeter; SuEndV, vertical distance from posterior end of sulcus to ventral margin of otolith; SuTipV, vertical distance from anterior end of sulcus to ventral margin of otolith.

Fourier descriptors were examined with Mardia's test (skewness and kurtosis, $p > 0.05$). One-way analysis of variance (ANOVA, $p \leq 0.05$) was used to evaluate whether any morphometric variable was significant for a species. Some otolith morphometric variables revealed covariance with OL (Spearman, $p \leq 0.05$); in this case one-way analysis of covariance (ANCOVA, $p \leq 0.05$), with otolith length as covariable was applied. If homogeneity was fulfilled, Tukey's HSD post-hoc test ($p \leq 0.05$) was conducted; if homogeneity was not given we carried out Dunnett's T3 post-hoc test ($p \leq 0.05$). A Kruskal-Wallis test ($p \leq 0.05$) with Dunn's post-hoc test ($p \leq 0.05$) was performed to ascertain whether a given meristic value was significant for a species. Furthermore, the otolith and body morphometric datasets, and the meristic variables were each subjected to Principal Component Analysis (based on the individual values per specimen and species). The resulting first four principal components (PC1–4) were inspected for their significance at species level using ANOVA with Tukey's HSD post-hoc test ($p \leq 0.05$). The first two PCs of the Fourier descriptors were used for a multivariate analysis of variance (MANOVA) ($p \leq 0.05$). Based on the reduced datasets for *Gobius* and *Pomatoschistus*, respectively, a LDA was executed based on PC1–4 of the different arrays of variables derived from the otolith and body morphometry (Box's M Test, $p > 0.05$). The maximal separation power of the Fourier descriptors without overfitting the model case of *Pomatoschistus*. Accordingly, the LDA was conducted based on PC1–37 (*Gobius*), and PC1–14 (*Pomatoschistus*) of the Fourier descriptors (Box's M Test, $p > 0.05$). The classification success of each LDA is given based on jack-knifed cross-validation.

Evaluation of characters to discriminate between extant goby species For our quantitative approach, we evaluated the taxonomic value of each variable by calculating its separation success for each species. In other words, we measured from how many of its congeners a given species could be separated based on a certain variable. Thus, 100% separation success means that the variable concerned could separate a species from all of its congeners (included in the respective dataset) and 0% indicates that it could not separate that species from any of its congeners. Moreover, to minimize the impact of potentially coincidental results, we applied the following constraint: A given variable was considered to be taxonomically significant if it discriminated between at least two species, and could also differentiate each of those two species from at least 50% of the congeners (in the respective dataset). Variables that fulfilled this constraint are depicted against a green background in the relevant tables (see below). Variables that failed this test, but were indicative for one species with a success rate $\geq 50\%$ are indicated in italics.

Otolith descriptions High-quality SEM images of otoliths obtained from extant goby species are rarely found in previous publications. Therefore, in addition to the morphometric approaches, SEM images of representative otoliths of each species included in our study are presented and brief descriptions for the otoliths of each species are provided. Otolith terminology follows Nolf (1985) and Lombarte et al. (2018) (see here Fig. 1d).

Abbreviations For otolith morphometry: OA, otolith area; OH, otolith height; OL, otolith length; OP, otolith perimeter; SuA, sulcus area; SuH, sulcus height; SuL, sulcus length; SuP, sulcus perimeter; SuEndV, vertical distance from posterior end of sulcus to ventral margin of otolith; SuTipV, vertical distance from anterior end of sulcus to ventral margin of otolith For body morphometry: Ab, length of anal fin base; B, body depth at origin of first dorsal fin; CP, length of caudal peduncle; D2b, length of second dorsal fin base; D2C, distance between end of second dorsal fin and first dorsal (procurrent) ray of caudal fin; SL, standard length (from snout to begin of branched caudal fin rays); SN/A, distance from snout to origin of anal fin; SN/D1, distance from snout to origin of first dorsal fin; SN/D2, distance from snout to origin of second dorsal fin; TL, total length. All morphometric measurements are indicated in Fig. 1a. For meristics: AbVert, abdominal vertebrae; AP, anal fin pterygiophores inserting in front of haemal spine of first caudal vertebra; Arays, rays of anal fin; CaudVert, caudal vertebrae; D2rays, rays of second dorsal fin; DProCur, dorsal procurrent rays; TotVert, total vertebrae; VProCur, ventral procurrent rays. All meristic characters are depicted in Fig. 1b.

2.4 Results

2.4.1 Species separation in *Gobius*

Otolith morphometry Using the complete dataset (N, 14 species), ten otolith variables (out of 23) were found to be useful for species separation. These variables were significant for a total of eight species (ANCOVA/ANOVA, Table 2a1). The highest separation success was achieved with the variables SuL/SuH (77% for *G. geniporus*) and SuP/SuEndV (77% for *G. paganellus*) (Table 2a1). The five most successful variables were OL/OH, SuL/OP, SuL/SuEndV, SuH/ SuEndV and SuP/SuEndV, but each of these separated no more than three of the 14 included species (Table 2a1). ANOVA based on PC1–4 calculated from the otolith variables successfully discriminated between five species (Table 2b1). *Gobius vittatus* was separated from 69%, the others from 54% of the congeners. Notably, *G. cruentatus*, *G. gasteveni* and *G. roulei*, which had not been separated when the individual otolith variables were used, could now be distinguished from each other (compare Table 2b1 vs. 2a1). When the reduced dataset (N, 10 species) was used, 17 otolith variables (out of 23) were successful in species separation (ANCOVA/ ANOVA, Table 2a2). The same two variables as in the complete dataset (SuL/SuH, SuP/ SuEndV) had maximum separation success and also discriminated the same species (*G. geniporus*, *G. paganellus*), each with increased success (89% vs. 77%). Six further variables were also taxonomically indicative, as each discriminated one or two species from all but two of the congeners (78% success). These variables were OL/OH (*G. incognitus*), OP/OL (*G. cobitis*), SuP/OP (*G. vittatus*), SuL/ OP (*G. auratus*, *G. vittatus*), SuL/OL (*G. auratus*), SuL/OH (*G. incognitus*), and SuL/SuEndV (*G. auratus*) (Table 2a2). With respect to the power of a given variable to discriminate between many species, the two most efficient variables were SuL/SuEndV and SuH/SuEndV; each of these could discriminate between six species (vs. three species when the complete dataset was used). In total, all species could be discriminated in the reduced dataset from $\geq$ 50% of the congeners (Table 2a2). Four species were separated by eight to ten variables (*G. auratus*, *G. geniporus*, *G. paganellus*, *G. vittatus*), four more species were discriminated by four to six variables (*G. cobitis*, *G. incognitus*, *G. niger*, *G. roulei*), and two species (*G. bucchichi*, *G. cruentatus*) by two variables (Table 2a2). Furthermore, the results of ANOVA using PC1–4 calculated from all otolith variables enabled the discrimination of eight species (Table 2b2), vs. five when the complete dataset was analysed in the same way (Table 2b1). Four of these (*G. auratus*, *G. bucchichi*, *G. niger*, *G. paganellus*) had not been separated when the complete dataset was tested. On the other hand, *G.cruentatus* was not separated when PC1-4 were used, although it had been discriminated when the entire dataset was used. Overall, the approach based on PC1–4 was less effective than the analysis of individual otolith variables because it was unable to distinguish between *G. cobitis*, *G. cruentatus* and *G. incognitus* (compare Table 2b2 and 2a2).

Fourier shape analysis of the otoliths Based on PC1 and PC2 of the Fourier descriptors, species separation success was 77–100% and 80–100%, respectively, in the complete and reduced datasets (MANOVA; Table 2c1, c2). *Gobius paganellus* was the only species that could be discriminated from all others with 100% separation success in both datasets. *Gobius cobitis*, *G. cruentatus* and *G. geniporus* were differentiated with 100% success when the complete dataset was used (80% in the reduced dataset), whereas *G. bucchichi* and *G. incognitus* were distinguished with 100% success when the reduced data was analysed (92% and 77% in the complete dataset) (Table

2c1, c2).

Body morphometry When the complete dataset was analysed, four (out of eight) morphometric variables were taxonomically indicative, i.e., SN/D1, SN/ D2, D2b, Ab (ANOVA; Table 2d1). The highest success rates (77%) were achieved with SN/D1 (for *G. gasteveni*) and Ab (for *G. fallax*). The most powerful single variable (with respect to the number of species that can be separated) was Ab, which resolved five species, followed by D2b (four species) (Table 2d1). In total, nine species could be separated based on one, two or three morphometric variables, while five species could not be separated (*G. auratus*, *G. couchi*, *G. cruentatus*, *G. geniporus*, *G. paganellus* (Table 2d1). Using PC1–4 of the variables derived from body morphometry in the ANOVA analysis was less efficient than when the individual variables were used (Table 2e1). Only five species were separated in this case and separation success for a given species varied from 54 to 62% (Table 2e1). *Gobius cruentatus* and *G. geniporus*, which could not be distinguished in the preceding analysis, were now separated. The other three species that could be discriminated were *G. bucchichi*, *G. incognitus* and *G. vittatus*. The results based on the reduced dataset (Table 2d2, e2) were very similar to those obtained from the complete dataset. The same four morphometric variables could separate a species (SN/D1, SN/D2, D2b, Ab), and the individual species were separated with the same success (56–78%; see Table 2d2). As before, most powerful in relation to the number of species that could be separated were Ab (four species) and D2b (five species). With the single exception of *G. roulei* (not separated), the same species as in the complete data were separated (five in total), each based on one to four variables (Table 2d2). When PC1–4 of the morphometric variables were used in the ANOVA, a total of seven species could be separated (Table 2e2). Three of those (*G. roulei*, *G. cruentatus*, *G. geniporus*) had not been separated based on the approach using single morphometric variables (Table 2d2). In contrast, *G. niger* could not be discriminated based on PC1–4 of the morphometric variables (Table 2e2), whereas it was distinguished on the basis of D2b alone (Table 2d2).

Meristic counts None of the individual meristic characters met our criteria for species separation when we used the complete dataset (Kruskal-Wallis; Table 2f1). On the other hand, ANOVA on the basis of PC1–4 of the meristic values distinguished *G. incognitus* (with 92% success), *Gobius fallax* (77%) and *G. geniporus* (62%; see Table 2g1). A similar outcome was obtained based on the reduced dataset. A single character (ventral procurrent rays) separated with 56% success *G.*

geniporus and *G. incognitus* from the others (Table 2f2). ANOVA based on PC1–4 of the meristic variables also separated *G. geniporus* and *G. incognitus* from all others, now with 89% and 100% success, respectively; in addition, *G. cobitis* was now separated (Table 2g2).

Discriminant analyses Each LDA was based on the reduced dataset and the variables used were (i) PC1–4 of the otolith morphometric variables, (ii) PC1–23 of the otolith Fourier descriptors, and (iii) PC1–4 of the body morphometric variables (Fig. 2, Table 3). The first two functions of the LDA based on PC1–4 of the otolith morphometric variables captured 50.5% and 33.5% of the variation, respectively (Fig. 2a). Overall classification success (jack-knifed) was 45.4%; the highest classification success was 60% for both *G. bucchichi* and *G. geniporus*, and the lowest was 18% for *G. cruentatus*, 30% for *G. roulei* and 33% for *G. incognitus* (Table 3a). The scatter plot revealed overlap of all species (Fig. 2a). Three "pairs" of species are tentatively recognizable: *G. vittatus* and *G. auratus*, *G. geniporus* and *G. paganellus*, as well as *G. cruentatus* and *G. cobitis* (Fig. 2a). The members of each pair show some overlap, but only relatively moderate overlap with the other species considered.

The first two functions of the LDA based on PC1–23 of the otolith Fourier descriptors captured 39.1% and 19.1% of the variation, respectively (Fig. 2b). Overall classification success (jack-knifed) of this LDA was 85% (Table 3b). 100% separation success was achieved for *G. cruentatus* and *G. geniporus* (vs. 18% and 60% in the previous LDA), and 95% was obtained for *G. cobitis* (vs. 50% in the previous LDA) (Table 3b). The scatter plot delineates three groups: *Gobius cobitis*, which had broadly overlapped with *G. cruentatus* in the preceding LDA, is now distinct (Fig. 2b). The second group includes *G. geniporus*, *G. niger* and *G. roulei*, and the third one comprises the remaining species (Fig. 2b).

The first two functions of the LDA based on the body morphometric variables accounted for 66.3% and 18.6% of the variation, respectively. Overall classification success (jack-knifed) was 54.6% (Table 3c). Classification success was highest for *Gobius vittatus* and *G. bucchichi*, with 81.8% and 70%, respectively, while the success rates were lowest for *G. auratus*, *G. cobitis* and *G. roulei* (27–30%) (Table 3c). The scatter plot revealed overlap of almost all species, except *G. vittatus*, which is comparatively distinct, and *G. bucchichi* and *G. incognitus*, which overlap with each other, but not or not very much with the remainder (Fig. 2c).

Table 2: Results of the statistical analyses based on the *Gobius* datasets and the indicated variables. The success of a certain variable in separating a species is indicated in % (100% means that the species is separated from all congeners by this variable). Light / dark green color denotes that at least two species were separated by the given variable, and each of those two species from ≥ 50% / ≥ 80% of the congeners (in the respective dataset).

Classification success based on otolith variables (AN(C)OVA, $p \leq 0.05$)																								
	complete dataset														reduced dataset									
Species, N	aur 10	buc 10	cob 11	cou 4	cru 11	fal 2	gas 1	gen 10	inc 6	kol 2	nig 7	pag 9	rou 10	vit 10	aur 10	buc 10	cob 11	cru 11	gen 10	inc 6	nig 7	pag 9	rou 10	vit 10
OL/OH	31	31	38	54	23	15	8	54	62	46	38	46	38	46	33	33	44	33	67	78	44	56	33	56
OP/OL	15	15	62	23	15	0	15	31	38	31	0	15	15	54	22	22	78	44	44	44	11	22	22	67
OP/OH	31	31	31	*62*	38	15	0	38	23	23	23	46	31	38	33	44	33	44	56	33	33	56	56	56
SuP/OP	38	15	15	8	8	0	15	23	62	0	8	46	23	62	44	22	22	11	22	67	11	22	33	78
SuL/OP	62	15	15	8	8	0	23	38	62	0	15	23	23	62	78	22	33	11	56	67	22	22	33	78
SuH/OP	23	23	0	0	15	0	0	15	23	0	0	23	0	0	33	33	0	33	33	33	0	33	0	0
SuTipV/OP	46	15	23	54	38	23	8	31	23	8	23	38	31	54	56	33	22	44	33	22	33	44	44	67
SuEndV/OP	38	38	15	15	23	23	15	46	23	15	46	*54*	38	23	56	56	33	33	44	33	44	67	67	33
SuA/OA	38	15	15	8	23	0	15	15	*54*	0	0	0	0	31	44	22	22	22	22	*56*	0	0	0	33
SuL/OL	*54*	8	23	31	0	0	0	23	23	0	0	15	8	38	78	11	22	11	22	22	0	22	11	56
SuL/OH	38	8	0	38	0	0	0	0	*69*	0	0	0	0	38	44	33	33	44	67	78	67	44	67	44
SuL/SuH	15	15	15	31	15	0	8	77	8	15	23	54	15	31	22	22	22	22	89	22	22	78	22	33
SuL/SuP	0	15	8	15	0	0	0	23	0	0	0	31	0	15	0	22	22	0	33	0	0	33	0	22
SuL/SuTipV	38	23	23	*62*	15	23	23	46	38	15	46	46	46	38	56	22	22	33	56	44	67	56	67	44
SuL/SuEndV	54	23	46	23	23	15	23	54	46	15	23	54	31	38	78	33	56	22	56	56	22	56	44	56
SuH/OL	15	15	23	0	23	0	0	23	0	0	8	23	0	0	22	22	33	33	33	0	11	33	0	0
SuH/OH	31	15	8	0	23	0	0	0	23	0	15	0	0	15	44	33	22	33	0	33	33	0	0	33
SuH/SuP	15	15	15	38	15	0	0	*54*	15	15	15	46	15	46	22	22	22	22	67	11	11	56	22	56
SuH/SuTipV	38	15	15	8	46	0	8	15	23	0	15	8	8	31	56	22	22	56	22	33	22	11	22	44
SuH/SuEndV	54	31	54	38	38	0	31	31	62	0	46	31	46	8	67	44	56	56	44	67	56	44	56	11
SuP/SuTipV	54	31	23	62	23	23	23	38	38	0	38	38	46	38	56	33	22	33	44	44	67	44	67	44
SuP/SuEndV	31	62	62	15	23	23	0	23	23	15	23	77	38	46	44	67	67	33	33	44	33	89	44	56
SuTipV/SuEndV	8	8	8	8	8	15	0	0	8	0	*54*	0	0	8	11	11	11	11	11	11	*100*	11	11	11

a1 a2

Table 2: Results of the statistical analyses based on the *Gobius* datasets and the indicated variables. The success of a certain variable in separating a species (*Cont.*).

	Classification success based on PC1–4 of otolith variables (ANOVA, p ≤ 0.05)																									
		complete dataset														reduced dataset										
	Species	aur	buc	cob	cou	cru	fal	gas	gen	inc	kol	nig	pag	rou	vit	aur	buc	cob	cru	gen	inc	nig	pag	rou	vit	
	PC1	31	46	38	8	38	8	54	23	46	0	31	38	46	69	89	33	33	44	67	44	56	33	67	89	
	PC2	15	23	23	15	54	0	8	54	15	0	15	15	31	38	0	33	22	22	67	22	11	67	22	22	
	PC3	23	23	8	23	54	0	23	54	38	0	8	23	54	23	33	56	22	33	33	22	67	89	56	33	
b1	PC4	8	23	*54*	0	23	0	8	8	23	0	8	23	0	23	44	33	67	22	33	22	33	56	11	11	b2

	Classification success based on Fourier otolith descriptors (MANOVA, $p \leq$ 0.05)																										
		complete dataset														reduced dataset											
	Species	aur	buc	cob	cou	cru	fal	gas	gen	inc	kol	nig	pag	rou	vit	aur	buc	cob	cou	cru	gen	inc	nig	pag	rou	vit	
	N	17	20	20	8	21	4	2	14	15	4	20	19	20	21	17	20	20	8	21	14	15	20	19	20	21	
c1	Fourier descr.	85	92	100	85	100	77	85	100	77	85	85	100	92	85	90	100	80	80	80	80	100	90	100	90	80	c2

	Classification success based on fish morphometric variables (ANOVA, P<0.05)																									
		complete dataset														reduced dataset										
	Species	aur	buc	cob	cou	cru	fal	gas	gen	inc	kol	nig	pag	rou	vit	aur	buc	cob	cru	gen	inc	nig	pag	rou	vit	
	N	11	10	10	4	11	2	1	10	9	2	7	10	10	11	11	10	10	11	10	9	7	10	10	11	
	SN/D1*	38	54	31	38	38	46	77	23	54	0	8	23	0	38	22	67	22	22	22	67	0	22	0	22	
	SN/D2*	15	46	31	0	31	38	54	31	0	54	23	31	0	31	22	78	22	22	22	78	22	22	0	22	
	SN/A*	8	31	31	0	31	0	46	23	0	31	0	23	0	23	11	44	33	44	22	44	0	22	0	22	
	D2C	8	15	23	0	23	0	0	15	23	0	8	8	54	8	11	22	33	33	22	33	11	11	*78*	11	
	CP	8	8	8	8	8	8	8	8	8	8	8	8	8	*100*	11	11	11	11	11	11	11	11	11	*100*	
	B	8	0	8	8	15	0	0	46	0	0	8	15	0	15	11	11	11	22	*56*	0	11	22	0	33	
	D2b*	23	69	46	38	38	0	62	38	23	46	54	38	0	62	22	78	56	44	44	56	56	44	0	78	
d1	Ab*	38	62	62	31	38	77	31	0	46	31	31	8	54	56	44	67	67	44	33	67	33	33	0	56	d2

Table 2: Results of the statistical analyses based on the *Gobius* datasets and the indicated variables. The success of a certain variable in separating a species (*Cont.*).

Classification success based on PC1–4 of fish morphometric variables (ANOVA, $p \leq 0.05$)

	complete dataset														reduced dataset									
Species	aur	buc	cob	cou	cru	fal	gas	gen	inc	kol	nig	pag	rou	vit	aur	buc	cob	cru	gen	inc	nig	pag	rou	vit
PC1	31	54	46	0	31	0	31	23	54	0	31	15	23	46	44	78	56	44	44	78	33	22	33	56
PC2	31	46	31	8	23	8	31	15	54	0	31	31	46	62	33	78	33	33	33	78	33	44	67	56
PC3	23	23	23	15	54	0	0	62	15	0	15	15	23	38	33	33	33	67	78	33	22	22	33	67
PC4	23	23	8	23	46	0	0	46	38	0	8	23	46	23	44	33	11	56	56	78	11	33	56	44

e1 e2

Classification success based on meristic variables (Kruskal-Wallis, $p \leq 0.05$)

	complete dataset														reduced dataset									
Species, N	aur	buc	cob	cou	cru	fal	gas	gen	inc	kol	nig	pag	rou	vit	aur	buc	cob	cru	gen	inc	nig	pag	rou	vit
Variables	11	10	10	4	11	2	1	10	9	2	7	10	10	11	11	10	10	11	10	9	7	10	10	11
TotVert	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CaudVert	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AbVert	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D2rays	8	15	15	8	8	0	0	15	*54*	0	0	8	0	8	22	22	33	11	22	*67*	0	11	11	11
Arays	8	8	0	0	0	0	0	15	38	8	0	8	0	8	22	22	11	0	33	44	0	0	0	33
AP	8	8	*69*	15	8	0	0	15	8	0	8	8	8	8	11	11	*89*	11	22	11	11	11	11	11
DProCur	0	8	8	0	0	0	0	23	38	0	8	8	8	8	0	11	11	0	33	*56*	11	11	11	11
VProCur	8	8	8	0	0	0	0	31	38	8	8	8	0	8	22	22	22	11	56	56	44	11	11	11

f1 f2

Classification success based on PC1–4 of meristic variables (ANOVA, $P<0.05$)

	complete dataset														reduced dataset									
Species	aur	buc	cob	cou	cru	fal	gas	gen	inc	kol	nig	pag	rou	vit	aur	buc	cob	cru	gen	inc	nig	pag	rou	vit
PC1	31	23	46	15	23	77	15	62	92	23	15	23	15	31	33	33	78	22	89	100	22	33	22	33
PC2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PC3	0	0	8	0	0	15	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0
PC4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

g1 g2

Abbreviations: SL, standard length; N, number of fish specimens and otoliths; *ten specimens were used for Fourier analysis, for the other analyses seven specimens were used (see supplementary tables S1–S5).

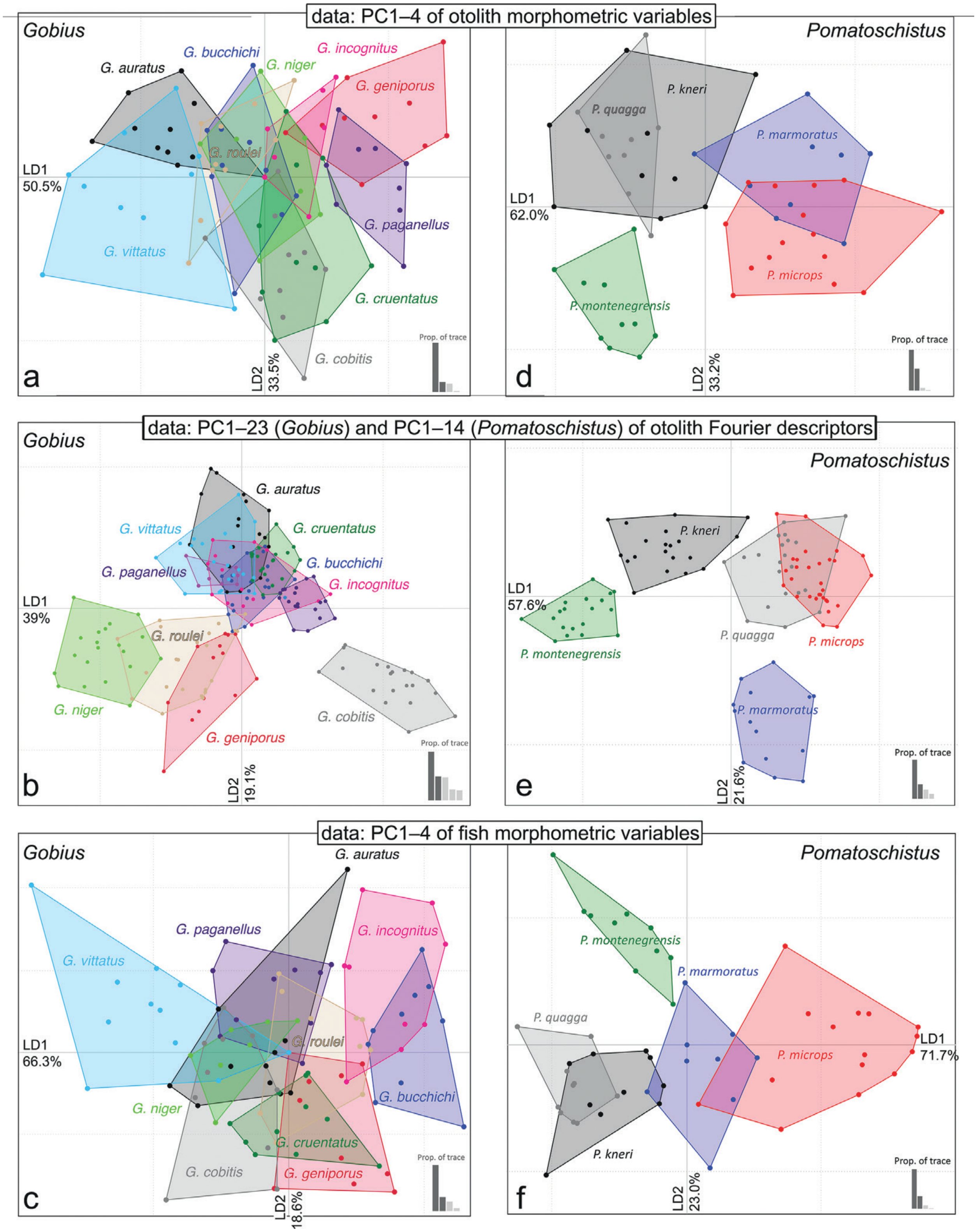


Figure 2: Plots of the discriminant function scores derived from the different arrays of variables (as indicated in the figure) of the studied species of *Gobius* (a–c) and *Pomatoschistus* (d–f). The reduced datasets for *Gobius* and *Pomatoschistus* were used for this analysis. LD1, 2, linear discriminant functions 1 and 2. See Tables 3 and 5 for details and values.

Table 3: Results of the Linear Discriminant Analysis (LDA) using the reduced dataset of *Gobius* and the indicated variables. The numbers in rows are percentages that denote the classification into the species given in columns (correctly classified species are highlighted in grey).

a

Classification success (jack-knifed) based on LDA using PC1–4 of otolith variables

N	Species	*G. auratus*	*G. bucchichi*	*G. cobitis*	*G. cruentatus*	*G. geniporus*	*G. incognitus*	*G. niger*	*G. paganellus*	*G. roulei*	*G. vittatus*
10	*G. auratus*	54.5	18.2	0.0	0.0	0.0	0.0	9.1	0.0	0.0	18.2
10	*G. bucchichi*	0.0	60.0	0.0	20.0	0.0	20.0	0.0	0.0	0.0	0.0
11	*G. cobitis*	0.0	10.0	50.0	20.0	0.0	0.0	20.0	0.0	0.0	0.0
11	*G. cruentatus*	0.0	9.1	27.3	18.2	0.0	9.1	27.3	9.1	0.0	0.0
10	*G. geniporus*	0.0	0.0	0.0	0.0	60.0	10.0	20.0	10.0	0.0	0.0
6	*G. incognitus*	0.0	0.0	0.0	11.1	11.1	33.3	44.4	0.0	0.0	0.0
7	*G. niger*	14.3	0.0	14.3	0.0	0.0	0.0	42.9	14.3	14.3	0.0
9	*G. paganellus*	0.0	0.0	0.0	11.1	22.2	0.0	11.1	55.6	0.0	0.0
10	*G. roulei*	0.0	20.0	0.0	0.0	0.0	10.0	20.0	0.0	30.0	20.0
10	*G. vittatus*	30.0	10.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0

Overall class. success (jack-knifed): 45.4%

b

Classification success (jack-knifed) based on LDA using PC1–23 of Fourier otolith descriptors

N	Species	*G. auratus*	*G. bucchichi*	*G. cobitis*	*G. couchi*	*G. cruentatus*	*G. geniporus*	*G. incognitus*	*G. niger*	*G. paganellus*	*G. roulei*	*G. vittatus*
17	*G. auratus*	88.2	5.9	0.0	0.0	5.9	0.0	0.0	0.0	0.0	0.0	0.0
20	*G. bucchichi*	0.0	75.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0
20	*G. cobitis*	0.0	0.0	95.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0
8	*G. couchi*	0.0	0.0	0.0	75.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0
21	*G. cruentatus*	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
14	*G. geniporus*	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0
15	*G. incognitus*	0.0	0.0	0.0	0.0	0.0	0.0	86.7	0.0	13.3	0.0	0.0
20	*G. niger*	5.0	0.0	0.0	0.0	0.0	0.0	0.0	85.0	0.0	10.0	0.0
19	*G. paganellus*	0.0	5.3	0.0	0.0	0.0	0.0	5.3	0.0	89.5	0.0	0.0
20	*G. roulei*	0.0	4.5	0.0	13.6	0.0	9.1	0.0	0.0	0.0	72.7	0.0
21	*G. vittatus*	4.8	4.8	0.0	19.0	0.0	0.0	0.0	0.0	0.0	0.0	71.4

Overall class. success (jack-knifed): 85.0%

c

Classification success (jack-knifed) based on LDA using PC1–4 of fish morphometric variables

N	Species	*G. auratus*	*G. bucchichi*	*G. cobitis*	*G. cruentatus*	*G. geniporus*	*G. incognitus*	*G. niger*	*G. paganellus*	*G. roulei*	*G. vittatus*
11	*G. auratus*	27.3	0.0	18.2	27.3	0.0	9.1	18.2	0.0	0.0	0.0
10	*G. bucchichi*	0.0	70.0	0.0	0.0	10.0	20.0	0.0	0.0	0.0	0.0
10	*G. cobitis*	10.0	0.0	30.0	10.0	0.0	0.0	40.0	0.0	10.0	0.0
11	*G. cruentatus*	18.2	0.0	0.0	63.6	18.2	0.0	0.0	0.0	0.0	0.0
10	*G. geniporus*	10.0	10.0	0.0	30.0	50.0	0.0	0.0	0.0	0.0	0.0
9	*G. incognitus*	0.0	33.3	0.0	0.0	11.1	55.6	0.0	0.0	0.0	0.0
7	*G. niger*	28.6	0.0	28.6	0.0	0.0	0.0	42.9	0.0	0.0	0.0
10	*G. paganellus*	20.0	0.0	0.0	10.0	0.0	10.0	0.0	60.0	0.0	0.0
10	*G. roulei*	10.0	20.0	10.0	10.0	0.0	10.0	10.0	0.0	30.0	0.0
11	*G. vittatus*	18.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	81.8

Overall class. success (jack-knifed): 51.5%

2.4.2 Species separation in *Pomatoschistus*

Otolith morphometry Five otolith variables (out of 23) were successful in species separation when the complete dataset was used (N, 7 species; ANCOVA/ANOVA; Table 4a1). The maximum classification success for a given species was 67%. OL/ OH and OP/OH were the best variables with respect to the number of species that could be discriminated; OL/OH separated all species except *P. knerii*, and OP/OH discriminated four species (Table 4a1). In total, all species could be separated from one another; *P. marmoratus*, *P. microps* and *P. montenegrensis* each by four variables, and the remainder by a single variable each (Table 4a1). When PC1–4 calculated from the otolith morphometric variables was analysed, the success in species separation declined in the case of *P. microps* (33% vs. 67% in the preceding analysis) and *P. pictus* (0% vs. 50%), but was improved in the case of *P. montenegrensis* (83% vs. 67%; Table 4b1). When the reduced dataset (N, 5 species) was analysed in the same way, most of the otolith morphometric variables contributed to species separation (Table 4a2). Four variables achieved 100% separation success for a given species: SuEndV/OP for *P. marmoratus* and *P. microps*, and SuH/OL, SuH/SuTipV and SuP/SuTipV each for *P. montenegrensis* (Table 4a2). Overall, all species could be separated from $\geq$ 50% of their congeners, and 13–17 variables contributed in each case (Table 4a2). As observed in the complete dataset, the results of ANOVA on the basis of PC1–4 of the otolith morphometric variables discriminated the species less efficiently than when the individual otolith variables were used (compare Table 4b2 vs. 4a2).

Fourier shape analysis of the otoliths When the complete dataset was used, PC1 and PC2 of the Fourier descriptors attained success rates in species separation ranging from 67% (*P. microps*, *P. pictus*) to 100% (*P. knerii*, *P. montenegrensis*, *P. quagga*) (MANOVA; Table 4c1). The values increased to 83–100% per species when the reduced dataset was used for the same analysis (Table 4c2).

Body morphometry Each of the eight morphometric variables contributed to species separation and each species was separated from all others when the complete dataset was used (ANOVA; Table 4d1). 100% separation success was achieved by CP (for *P. minutus*) and Ab (for *P. montenegrensis*). The most efficient variables in relation to the number of species discriminated were Ab (all species), and D2b, D2C and SN/A (six species). *Pomatoschistus quagga* was the only species that could be separated by each morphometric variable from 50–83% of its congeners; the remaining species were

Table 4: Results of the statistical analyses based on the *Pomatoschistus* datasets and the indicated variables. The success of a certain variable in separating a species is indicated in % (100% means that the species is separated from all congeners by this variable). Light / dark green color denotes that at least two species were separated by the given variable, and each of those two species from $\geq$ 50% / $\geq$ 80% of the congeners (in the respective dataset).

Classification success based on otolith variables (AN(C)OVA, p $\leq$ 0.05)

a1

	complete dataset							reduced dataset				
Species, N	kne 10	mar 9	mic 15	min 2	mon 10	pic 2	qua 9	kne 10	mar 9	mic 15	mon 10	qua 9
OL/OH	33	67	67	50	67	50	50	50	75	75	0	50
OP/OL	33	67	33	17	50	0	33	50	75	50	75	0
OP/OH	50	67	67	33	50	33	33	50	75	75	50	50
SuP/OP	17	33	33	0	*50*	0	33	25	50	50	75	50
SuL/OP	0	33	33	0	33	0	33	0	50	50	50	50
SuH/OP	17	17	17	0	*67*	0	17	25	25	25	*100*	25
SuTipV/OP	17	17	17	0	*67*	0	17	25	25	25	*100*	25
SuEndV/OP	33	33	*83*	17	17	0	17	50	100	100	50	50
SuA/OA	17	17	17	0	*50*	0	0	25	25	25	*75*	0
SuL/OL	0	0	0	0	0	0	0	0	50	50	50	50
SuL/OH	0	33	33	0	33	0	33	50	75	75	50	50
SuL/SuH	33	17	33	0	33	0	*50*	75	50	50	50	75
SuL/SuP	17	0	17	0	*50*	0	17	25	0	25	*75*	25
SuL/SuTipV	17	33	17	0	*50*	0	17	50	75	50	75	50
SuL/SuEndV	33	50	67	17	33	0	33	50	75	75	50	50
SuH/OL	17	17	17	0	*67*	0	17	50	25	50	100	25
SuH/OH	17	17	17	0	*83*	17	17	25	25	25	*100*	25
SuH/SuP	33	17	33	0	33	0	*50*	75	50	50	50	75
SuH/SuTipV	17	17	17	0	*67*	0	17	25	50	50	100	25
SuH/SuEndV	17	17	17	0	*67*	0	17	25	25	25	*100*	25
SuP/SuTipV	17	33	17	0	*50*	0	17	50	75	50	100	75
SuP/SuEndV	17	33	*67*	17	33	0	33	50	75	75	50	50
SuTipV/SuEndV	33	33	50	0	50	0	33	50	50	75	75	50

a2

Classification success based on PC1–4 of otolith variables (ANOVA, p $\leq$ 0.05)

b1

	complete dataset							reduced dataset				
Species	kne	mar	mic	min	mon	pic	qua	kne	mar	mic	mon	qua
PC1	50	50	33	50	83	0	67	33	50	33	67	50
PC2	67	33	33	33	33	0	67	50	33	33	33	50
PC3	17	0	17	*50*	17	0	0	0	0	0	0	0
PC4	17	17	33	0	0	0	0	0	17	17	0	0

b2

Classification success based on Fourier otolith descriptors (MANOVA, p $\leq$ 0.05)

c1

	complete dataset							reduced dataset				
Species, N	kne 20	mar 14	mic 30	min 4	mon 19	pic 4	qua 20	kne 20	mar 14	mic 30	mon 19	qua 20
MANOVA	100	83	67	83	100	67	100	100	83	100	100	83

c2

Table 4: Results of the statistical analyses based on the *Pomatoschistus* datasets (*Cont.*).

Classification success based on fish morphometric variables (ANOVA, p ≤ 0.05)

	complete dataset							reduced dataset				
Species, N	kne	mar	mic	min	mon	pic	qua	kne	mar	mic	mon	qua
	10	9	15	2	10	2	10	10	9	15	10	10
SN/D1*	50	17	33	0	50	83	67	50	0	50	25	75
SN/D2	17	33	33	83	33	0	67	0	25	25	25	*75*
SN/A	50	33	67	67	50	50	50	50	25	50	50	75
D2C	50	67	67	50	50	0	50	50	100	100	50	50
CP	33	33	50	100	33	17	67	25	25	50	25	75
B*	50	17	17	0	33	33	83	50	25	25	50	100
D2b	50	50	33	83	83	83	50	50	25	25	75	25
Ab	67	50	67	83	100	83	50	50	25	50	100	25

d1 d2

Classification success based on PC1–4 of fish morph. variables (ANOVA, p ≤ 0.05)

	complete dataset							reduced dataset				
Species	kne	mar	mic	min	mon	pic	qua	kne	mar	mic	mon	qua
PC1	67	50	83	83	100	67	50	75	25	50	50	100
PC2	50	33	50	50	67	17	100	50	50	25	100	75
PC3	50	0	33	33	0	50	33	25	25	*100*	25	25
PC4	33	33	33	67	67	0	33	0	0	0	0	0

e1 e2

Classification success based on meristic variables (Kruskal-Wallis, p ≤ 0.05)

	complete dataset							reduced dataset				
Species, N	kne	mar	mic	min	mon	pic	qua	kne	mar	mic	mon	qua
Variables	10	9	15	2	10	2	10	10	9	15	10	10
TotVert	17	17	17	17	67	67	50	50	25	75	75	75
CaudVert	17	17	17	17	67	0	33	50	25	25	100	50
AbVert	33	17	67	0	33	17	67	50	50	100	50	100
D2rays	17	17	0	33	33	0	0	50	25	0	50	25
Arays	17	0	0	17	*50*	0	17	50	0	25	50	25
AP	17	0	33	0	17	0	33	50	0	50	50	50
DProCur	0	17	17	0	33	0	33	0	25	25	50	50
VProCur	0	0	0	17	33	0	17	0	0	0	25	25

f1 f2

Classification success based on PC1–4 of meristic variables (ANOVA, p ≤ 0.05)

	complete dataset							reduced dataset				
Species	kne	mar	mic	min	mon	pic	qua	kne	mar	mic	mon	qua
PC1	67	50	67	83	83	33	83	75	75	100	100	100
PC2	33	17	17	0	0	0	0	75	25	25	0	25
PC3	0	17	0	*50*	17	0	17	0	0	0	0	0
PC4	0	0	17	0	0	0	17	25	25	75	50	25

g1 g2

separated by three to six variables from their congeners (Table 4d1). Furthermore, each species was separated when PC1–4 of the morphometric variables were analysed (ANOVA; Table 4e1). 100% separation success was obtained for *P. montenegrensis* and *P. quagga* (Table 4e1). Overall, similar results were obtained with the reduced dataset, but some differences are noteworthy (Table 4d2). With the exception of SN/D2, every morphometric variable could be used to separate some species. The reason that SN/D2 was now disqualified lies in the exclusion of *P. minutus* (which had been separated by this variable) from the reduced dataset. Three variables (vs. one in the complete dataset) showed 100% separation success for at least one species: D2C (for *P. marmoratus*, *P. microps*), B (for *P. quagga*) and Ab (for *P. montenegrensis*). Most effective concerning the number of species that could be separated were D2C (all species) and SN/A (four species) (Table 4d2). Apart from *P. marmoratus* (separated by a single variable), each of the included species could be discriminated by five or six variables from their congeners (Table 4d2). When ANOVA was done based on PC1–4 of the morphometric body variables, the separation success was generally similar to that of the previous analysis and all species could be separated (Table 4e2).

Meristic characters Numbers of total and abdominal vertebrae contributed to species separation with moderate success (50–67%) when the complete dataset was employed (Table 4f1). *Pomatoschistus microps*, *P. montenegrensis* and *P. pictus* could be separated by either the total or the abdominal vertebrae count, while *P. quagga* was discriminated by both of these counts (Table 4f1). ANOVA using PC1–4 of the meristic counts showed improved results, as PC1 separated all species apart from *P. pictus*, with highest separation success (83%) for *P. minutus*, *P. montenegrensis*, and *P. quagga* (Table 4g1). In the reduced dataset, seven meristic characters contributed to species separation (vs. two in the complete dataset), with an improved overall separation success (50–100% vs. 50–67%) (Table 4f2). The caudal and the abdominal vertebrae count separated *P. montenegrensis* and *P. microps*/*P. quagga*, respectively, from all other species. The latter was most efficient in relation to the number of species that could be separated (all five species) (Table 4f2). In total, all five species could be separated: *P. knerii*, *P. montenegrensis* and *P. quagga* by six, seven and five variables, respectively, and *P. marmoratus* and *P. microps* by one and three variables, respectively (Table 4f2). ANOVA using PC1–4 of the meristic values separated each species from 75–100% of its congeners, with 100% success for *P. microps*, *P. montenegrensis* and *P. quagga* (Table 4g2).

Discriminant analyses As for *Gobius*, each LDA was based on the reduced dataset. The variables used as input were (i) PC1–4 of the otolith morphometric variables, (ii) PC1–14 of the Fourier descriptors, and (iii) PC1–4 of the body morphometric variables (Table 5). The first two functions of the LDA based on PC1–4 of the otolith morphometric variables explained 62.0% and 33.2% of the variation, respectively (Fig. 2d). Overall classification success (jack-knifed) of the LDA was 71.1%. The highest classification success was achieved for *P. montenegrensis* (90%) and *P. microps* (87%), while the success rates were between 56 and 60% for *P. knerii*, *P. marmoratus* and *P. quagga* (Table 5a). The scatter plot depicts three groups (Fig. 2d). The first consists of *P. montenegrensis* and is completely distinct. The second and third groups include *P. microps*/*P. marmoratus* and *P. knerii*/*P. quagga*; the members of each group overlap with each other, but very little or not at all with the others (Fig. 2d). The first two functions of the LDA based on PC1–14 of the Fourier descriptors captured 57.6% and 21.6% of the variance, respectively. Overall classification success (jack-knifed) was 96%, with 100% success for *P. microps* and *P. quagga*, >90% success for the remainder (Table 5b). The corresponding scatter plot depicts *P. knerii*, *P. marmoratus*, and *P. montenegrensis* as separate groups, while *P. microps* and *P. quagga* overlap (Fig. 2e). The first two functions of the LDA based on the body morphometric variables account for 71.7% and 23% of the variation, respectively. Overall classification success (jackknifed) was 75.9%, with 90% success for *P. montenegrensis* and *P. quagga*, and 67–70% success for the remainder (Table 5c). The success in separation is also seen in the scatter plot, which reveals that each species is relatively well separated, although some overlap is seen (Fig. 2f).

2.4.3 Summary of the results based on the statistical analyses

A comparison of the results based on the four methodological approaches (otolith morphometry, otolith Fourier shape analysis, body morphometry, meristic counts) reveals that Fourier shape analysis of otoliths is the most powerful species discriminator of all the methods tested here (Table 6). A plot of the relative discriminatory power of the individual variables used in the other three approaches indicates that rates of success in species separation vary, and implies that some characters have more taxonomic significance than others (Table 7). For example, none of the individual otolith variables had the power to separate all of the species of *Gobius* or *Pomatoschistus* with good success (i.e., with $\geq$75%; Table 7). While some variables (SuTipV/ OP, SuH/SuEndV) were capable (albeit with moderate success) of separating some of the species of *Gobius* from the others, these same variables failed to discriminate between species of *Pomatoschistus* spp.,

Table 5: Results of the Linear Discriminant Analysis (LDA) using the reduced dataset of *Pomatoschistus* and the indicated variables. The numbers in rows are percentages that denote the classification into the species given in columns (correctly classified species are highlighted in grey).

a

Class. success (jack-knifed) based on LDA using PC1–4 of otolith variables

N	Species	*P. kneri*	*P. marmoratus*	*P. microps*	*P. montenegrensis*	*P. quagga*
10	*P. kneri*	60.0	10.0	0.0	0.0	30.0
9	*P. marmoratus*	11.0	56.0	33.0	0.0	0.0
15	*P. microps*	0.0	13.0	87.0	0.0	0.0
10	*P. montenegrensis*	10.0	0.0	0.0	90.0	0.0
9	*P. quagga*	33.0	0.0	0.0	11.0	56.0

Overall class. success (jack-knifed): 71.1%

b

Class. success (jack-knifed) based on LDA using PC1–14 of Fourier otolith descr.

N	Species	*P. kneri*	*P. marmoratus*	*P. microps*	*P. montenegrensis*	*P. quagga*
20	*P. kneri*	90.0	0.0	0.0	0.0	10.0
14	*P. marmoratus*	0.0	92.9	7.1	0.0	0.0
30	*P. microps*	0.0	0.0	100.0	0.0	0.0
19	*P. montenegrensis*	5.3	0.0	0.0	94.7	0.0
20	*P. quagga*	0.0	0.0	0.0	0.0	100.0

Overall class. success (jack-knifed): 96%

c

Class. success (jack-knifed) based on LDA using PC1–4 of fish morph. variables

N	Species	*P. kneri*	*P. marmoratus*	*P. microps*	*P. montenegrensis*	*P. quagga*
10	*P. kneri*	70.0	20.0	0.0	0.0	10.0
9	*P. marmoratus*	11.1	66.7	0.0	11.1	11.1
15	*P. microps*	0.0	33.3	66.7	0.0	0.0
10	*P. montenegrensis*	0.0	10.0	0.0	90.0	0.0
10	*P. quagga*	10.0	0.0	0.0	0.0	90.0

Overall class. success (jack-knifed): 75.9%

and vice versa (Table 7). Furthermore, a single variable could be indicative for a certain species, but be unable to reliably discriminate its congeners. An example is SuL/SuH, which achieved good separation success only for *G. geniporus* (89%) and *G. paganellus* (78%) (reduced dataset, Table 2a2). A further outcome was that the number of species that could be separated from their congeners with a success of ≥50% declined when PC1–4 of the otolith or the morphometric variables were analysed instead of the individual variables (Table 6, complete datasets). Moreover, the number of specimens employed in the statistical analyses did not necessarily influence their separation success. Examples are *G. fallax* (represented with only two specimens) and *G. gasteveni* (one specimen), which were well separated from their congeners using body morphometry, whereas *G. paganellus* and *G. roulei* (ten specimens each) could not be separated based on the same approach (Table 2d1). On the other hand, as already pointed out by Lombarte et al. (2018), the number of species used in the statistical analysis could have an influence on the results, insofar

Table 6: Summary of the taxonomic significance of the different groups of variables (indicated by colors). Numbers and percentages in rows indicate how many species were separated from $\geq$ 50% of their congeners in the dataset given in the columns.

Statistical analysis	*Gobius* datasets		*Pomatoschistus* datasets	
	complete (N, 14)	reduced (N, 10)	complete (N, 7)	reduced (N, 5)
Otolith variables (AN(C)OVA)	8 (57%)	All	All	All
PC1–4 of otolith variables (ANOVA)	5 (36%)	7 (70%)	5 (71%)	4 (80%)
PC1–4 of otolith variables (LDA)	–	6 (60%)	–	All
Fourier otolith shape analysis (MANOVA)	All	All	All	All
Fourier otolith shape analysis (LDA)	–	All	–	All
Fish morph. variables (ANOVA)	9 (64%)	5 (50%)	All	All
PC1–4 of fish morph. variables (ANOVA)	5 (36%)	7 (70%)	All	All
PC1–4 of fish morph. variables (LDA)	n.a.	6 (60%)	n.a.	All
Meristic variables (Kruskal-Wallis)	0	2 (20%)	4 (57%)	All
PC1–4 of meristic variables (ANOVA)	3 (21%)	3 (30%)	6 (86%)	All

Abbreviations: All, all species were separated; N, numbers of species; –, not applicable. See Tables 2–5 for details. AN(C)OVA means that ANOVA or, if covariance was detected, ANCOVA was used; LDA, Linear Discriminant Analysis.

as species separation becomes more successful when the number of species considered decreases, and less successful when the number of species increases. This phenomenon appears to be related to growing spread (variance) after inserting values of additional species. In other words, the additional values increased the overall variance of the variables, which finally blurs the information of a certain variable that would otherwise be characteristic for an individual species. This likely explains why in our study the success of a given variable in species separation generally increased when the reduced datasets of *Pomatoschistus* and *Gobius* were used (Tables 6, 7).

2.4.4 Descriptions of the otoliths

For the detailed descriptions of the shapes of all sagittal otoliths, we have divided our dataset in four groups, i.e., (i) the otoliths of the *G. auratus* species complex (*sensu* Miller & El-Tawil, 1974; Herler et al., 2005) (Fig. 3), (ii) the otoliths of *Gobius* species that could be identified based on otolith variables (Fig. 4), (iii) the otoliths of *Gobius* species that could not be identified based on otolith variables (Fig. 5), and (iv) the otoliths of the species of *Pomatoschistus* (Fig. 6). The descriptions refer to the mesial side of the otoliths, which, as in all saccular otoliths of teleost fish, bears the sulcus and most relevant characters. In all studied otoliths, the sulcus acusticus is slightly inclined and reveals the 'shoe sole-like' shape that is typical for most Gobioidei (Nolf, 1985, 2013; Gierl et al., 2018; Lombarte et al., 2018). The cauda is slightly or clearly shorter than the ostium and the ostium is anteriorly tapering or pointed (Fig. 3–6). The sulcus of all species is covered by a microcrystalline texture that is usually smooth, with exceptions in *G. niger* and *G. roulei* (presence of some flat bumps; Fig. 5g–l), and *G. cobitis*, *G. incognitus* and *G. paganellus* (lack of this texture

Table 7: Summary of the taxonomic significance of the different groups of variables (indicated by colors). Numbers and percentages in rows indicate how many species were separated from ≥ 50% of their congeners in the dataset given in the columns.

Type of variables	Variables	*Gobius* spp.		*Pomatoschistus* spp.	
		complete dataset	reduced dataset	complete dataset	reduced dataset
Otolith morphometry	OL/OH	△	△	▲	▲
	OP/OL	△	△	△	▲
	OP/OH		△	▲	▲
	SuP/OP	△	△		▲
	SuL/OP	△	△		▲
	SuH/OP				
	SuTipV/OP	△	△		
	SuEndV/OP		△		▲
	SuA/OA				
	SuL/OL		△		▲
	SuL/OH		△		▲
	SuL/SuH	△	△		▲
	SuL/SuP				
	SuL/SuTipV		△		▲
	SuL/SuEndV	△	△	△	▲
	SuH/OL				▲
	SuH/OH				
	SuH/SuP		△		▲
	SuH/SuTipV		△		▲
	SuH/SuEndV	△	△		
	SuP/SuTipV	△	△		▲
	SuP/SuEndV	△	△		▲
	SuTipV/SuEndV			△	▲
Fish morphometry	SN/D1	△	△	▲	▲
	SN/D2	△	△	△	
	SN/A			▲	▲
	D2C			▲	▲
	CP			▲	△
	B			△	▲
	D2b	△	△	▲	△
	Ab	△	△	▲	▲
Meristics	TotVert			△	▲
	CaudVert				▲
	AbVert			△	▲
	D2rays				△
	Arays				△
	AP				▲
	DProCur				△
	VProCur		△		

△ , at least two species were separated from ≥ 50% of their congeners; ▲ , ≥ 50% of the species included were separated from ≥ 50% of the congeners. See methods for abbreviations of variables.

along the dorsal part of the ostium; Fig. 4a–c, h–k). Some irregularities of the sulcus shape can occur within a species: one specimen of *G. couchi* showed a triangular extension of the dorsal ostial margin (Fig. 3f), and one specimen of *G. fallax* revealed an irregular shape of the ostium (Fig. 3h). In the otoliths of *Pomatoschistus*, the shape of the sulcus is generally somewhat variable (Fig. 6). Additionally, the size of the cauda may be reduced in *P. knerii* (Fig. 6a–d), *P. marmoratus* (Fig. 6e–h) and *P. microps* (Fig. 6i–l), and is always reduced in *P. montenegrensis* (Fig. 6n–p) and *P. quagga* (Fig. 6r–t). A well defined, rounded or elongate, thick crista inferior is visible at the beginning of the cauda in almost all species of *Gobius* and *Pomatoschistus* ('subcaudal iugum' sensu Schwarzhans, 2014). The shape and strength of the crista inferior can vary between and also within a species (Fig. 3–6). In otoliths of *Pomatoschistus* that possess a reduced cauda, the crista inferior reaches almost the end of the cauda (*P. knerii*, *P. marmoratus*, *P. microps*; Fig. 6a–l), or even continues beyond (*P. montenegrensis*, *P. quagga*;Fig. 6n–p, r–t).

The *Gobius auratus* species complex (Fig. 3) The otolith shape in all species of the *G. auratus* species complex is approximately rhomboidal, with the highest shape seen in *G. couchi* (Fig. 3d–f), and the longest in *G. kolombatovici* (Fig. 3k, l). All species display a prominent posterodorsal projection at the posterior margin and slight differences in the shape of this projection occur between the species: it is blunt in *G. auratus* (Fig. 3a–c) and *G. fallax* (Fig. 3g, h), slightly tapering in *G. couchi* (Fig. 3d–l), short and truncated in *G. gasteveni* (Fig. 3i, j), and broadly rounded in *G. kolombatovici* (Fig. 3k, l). The further posterior margin is separated from the posterodorsal projection by a clear incision or notch; it is slightly rounded in *G. auratus* and *G. fallax*, and relatively straight in the remainders. The ventral margin is slightly curved in *G. couchi* and *G. gasteveni*, in the others it is relatively straight. A well developed preventral projection occurs in *G. fallax*, *G. gasteveni*, *G. kolombatovici* and, to a lesser extent, also in *G. couchi.* In *G. auratus* no clear preventral projection is present, but the ventral margin ends in a sharp point. The anterior margin is usually concave or slightly incised in the middle in all species. A small predorsal projection can be developed, but its size and shape is variable within and between the species. It seems that otoliths of smaller specimens tend to have such a projection, while those of larger specimens do not (see Fig. 3). The dorsal margin is rounded and can bear some lobes or crenulations; only in *G. kolombatovici* it is relatively flat. A median tip of the dorsal margin is visible in *G. auratus* and *G. couchi.*

***Gobius* species that could be identified based on otolith variables (Fig. 4)** Six species could be recognized with good success based on their otolith variables (Table 2a), i.e., *G. auratus* (described above), *G. cobitis*, *G. geniporus*, *G. incognitus*, *G. paganellus* and *G. vittatus*. Of those, the otoliths of *G. incognitus* (Fig. 4g–i) and *G. vittatus* (Fig. 4m–p) display a rhomboidal shape, while the otoliths of *G. cobitis* (Fig. 4a–c), *G. geniporus* (Fig. 4d–f), and *G. paganellus* (Fig. 4j–l) are more rectangular. The otoliths of all five species are usually longer than high except some otoliths of *G. vittatus*, which are approximately quadratic. All five species possess a prominent posterodorsal projection on the posterior margin, which differs slightly between them: it is blunt, slightly to distinctly crenulated and mostly facing upwards (dorsally) in *G. cobitis* (Fig. 4a–c) and *G. paganellus* (Fig. 4j–l); it is slightly pointed and mostly facing upwards in *G. incognitus* (Fig. 4g–i); it is of triangular-rounded shape in *G. vittatus* (Fig. 4m–p); and it is broadly rounded in *G. geniporus* (Fig. 4d–f). The posterior margin beneath the projection is relatively straight in all five species and can bear slight crenulations, only in *G. vittatus* it is always smooth. The ventral margin is faintly curved in *G. incognitus* and *G. vittatus*, and relatively straight in *G. cobitis*, *G. geniporus*, and *G. paganellus*. A clear preventral projection occurs only in some otoliths of *G. cobitis* (Fig. 4c) and *G. paganellus* (Fig. 4k, l). In both *G. incognitus* and *G. vittatus* the anterior end of the ventral margin is relatively sharp and pointed. The anterior margin is straight in *G. cobitis*, concave in *G. geniporus*, oblique in *G. incognitus* and *G. paganellus*, and straight with a clear incision in the middle in *G. vittatus*. A predorsal projection occurs only in *G. geniporus* and *G. vittatus*, it is sharp in *G. geniporus*, while it is rounded in *G. vittatus*. A relatively flat dorsal margin occurs in *G. incognitus* and *G. paganellus*; the dorsal margin is regularly rounded in the other three species. A median dorsal tip is usually visible in *G. vittatus* and in some otoliths of *G. incognitus*. A smooth dorsal margin occurs in *G. vittatus*, in the other species the dorsal margin can bear lobes or crenulations.

***Gobius* species that could not be identified based on otolith variables (Fig. 5)** The otoliths of *G. bucchichi*, *G. cruentatus*, *G. niger*, and *G. roulei* could not be separated based on the individual otolith variables (Table 2a). Their otoliths are longer than high, with a clear rhomboidal shape in *G. bucchichi* (Fig. 5a–c), and a rectangular to rhomboidal shape in *G. cruentatus* (Fig. 5d–f), *G. niger* (Fig. 5g–i), and *G. roulei* (Fig. 5j–l). The otoliths of all four species possess a clear posterodorsal projection, which reveals slight differences between the species: it is rounded to slightly pointed in *G. bucchichi*; it is rounded-to-squared in *G. cruentatus*; and it is broad,

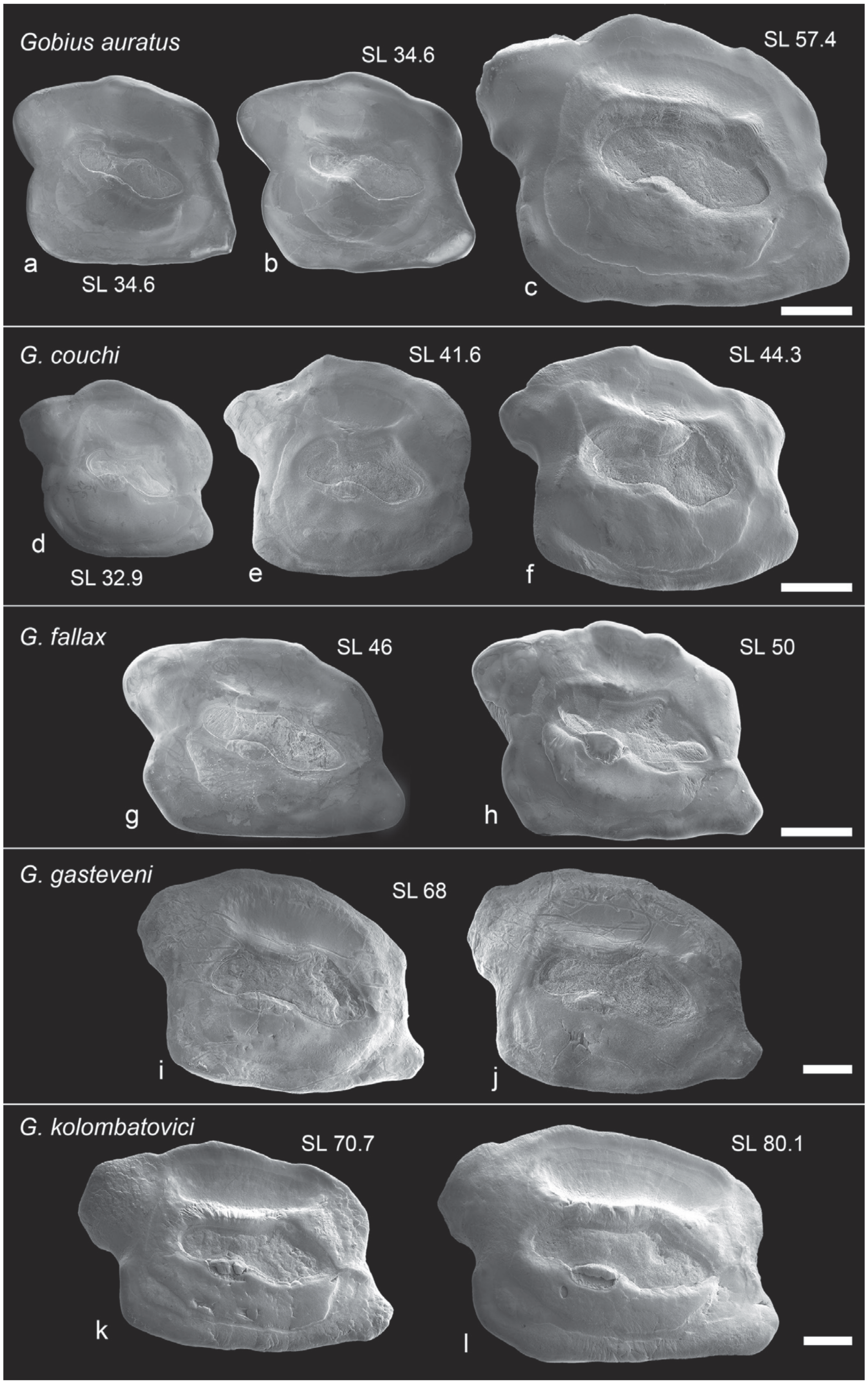


Figure 3: Otoliths (mesial view) of the species of the *Gobius auratus* complex, i.e. *G. auratus* (a: Selce, 6l; b: Selce, 5l; c: Krk, 2r mirrored), *G. couchi* (d–f: Krk, 2l, 3r mirrored, 1l), *G. fallax* (g, h: Unije Island, 2r mirrored, 1l), *G. gasteveni* (i, j: Galicia, 1l, 1r mirrored) and *G. kolombatovici* (k, l: Krk, 1l, 2l). Numbers following the localities refer to the fish specimen from which the otolith was extracted; l, left otolith; r, right otolith, mirrored for better comparison. SL denotes the standard length (in mm) of the corresponding fish specimen. Scale bars: 0.5 mm. All figured otoliths are kept in the Bavarian State Collection (collection number SNSB-BSPG 2020 LIV).

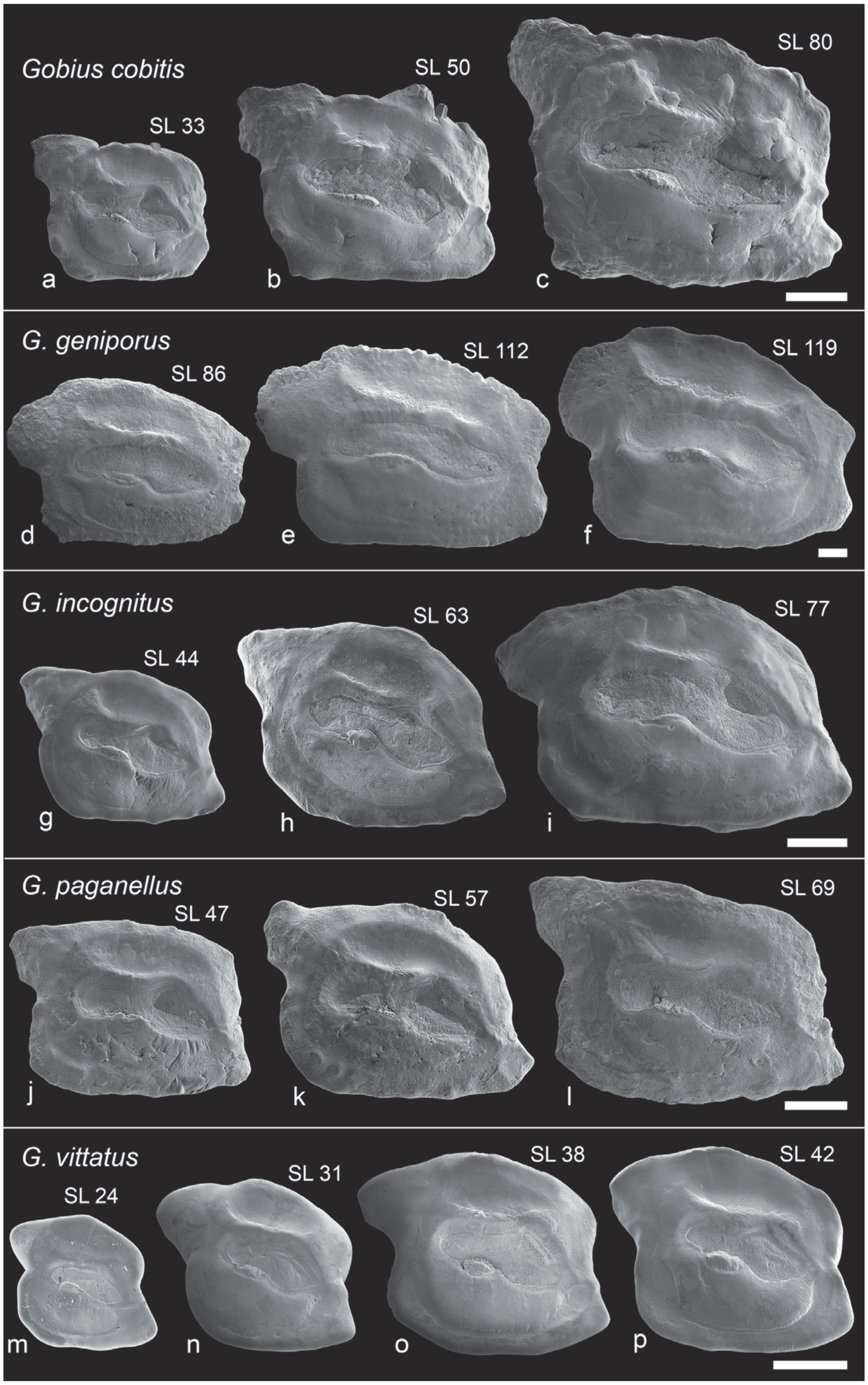


Figure 4: Otoliths (mesial view) of *Gobius cobitis* (a–c: Montenegro, 7l, 4l, 1l), *Gobius geniporus* (d: Montenegro, 3l; e, f: Selce, 2l, 'medium'), *Gobius incognitus* (g–i: Pelješac Peninsula, J1914l, J1910r mirrored, J1906l), *Gobius paganellus* (j–l: Galicia, 1l, 6l, 8l) and *Gobius vittatus* (m, Selce, 2l; n, o, Krk, Krk, 1l; 2l; p: Selce 2l). Numbers following the localities refer to the fish specimen from which the otolith was extracted; l, left otolith; r, right otolith, mirrored for better comparison. SL denotes the standard length (in mm) of the corresponding fish specimen. Scale bars: 0.5 mm. All figured otoliths are kept in the Bavarian State Collection (collection number SNSB-BSPG 2020 LIV).

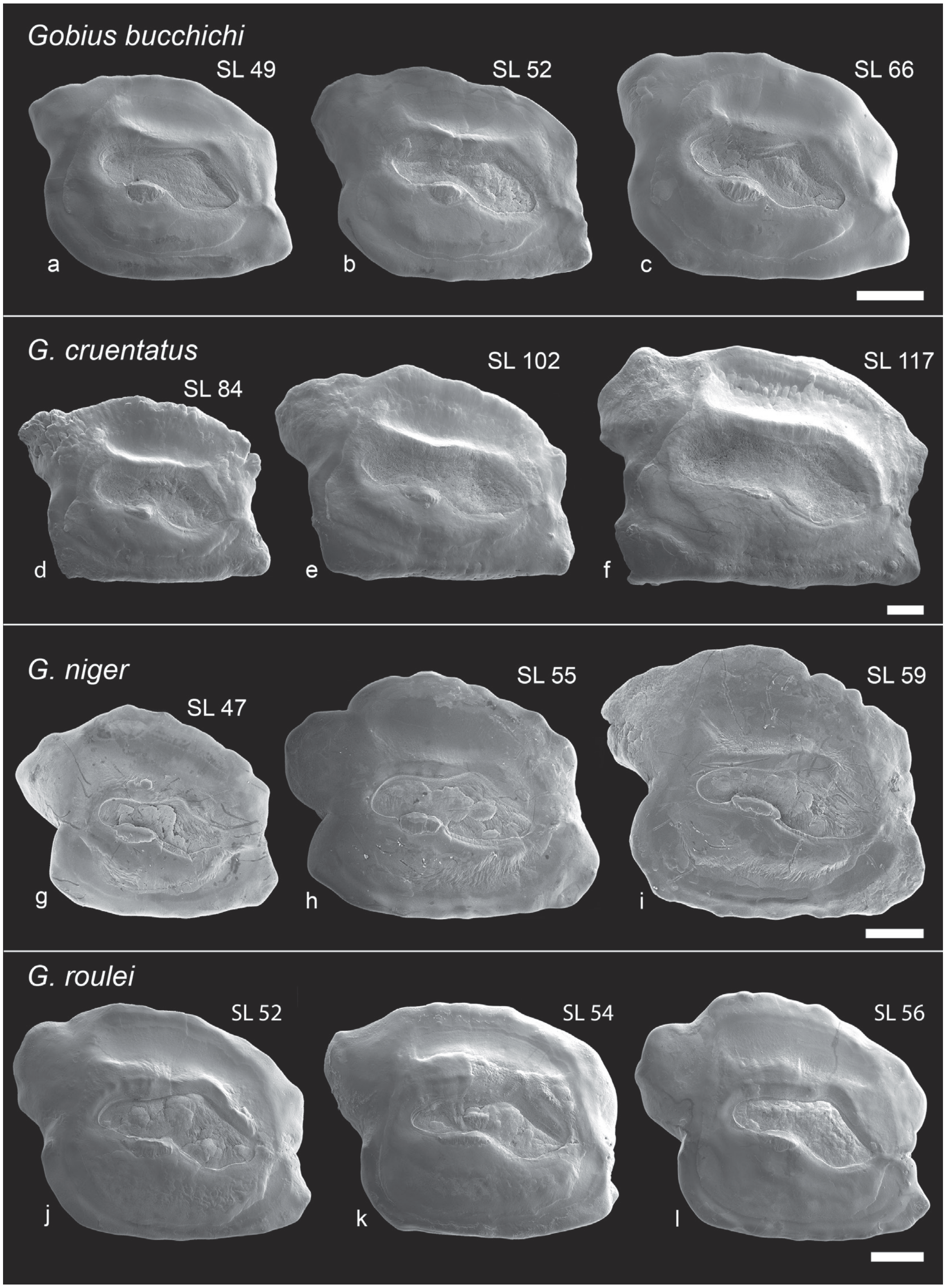


Figure 5: Otoliths (mesial view) of *Gobius bucchichi* (a–c: Selce, 3l, 1l, 4l), *G. cruentatus* (d–f: Selce, 2l, 3l, 8l), *G. niger* (g–i: Pilsey Island, 2l, 6l, 5l) and *G. roulei* (j–l: Selce, 2l, 1l, 3l). Numbers following the localities refer to the fish specimen from which the otolith was extracted; l, left otolith. SL denotes the standard length (in mm) of the corresponding fish specimen. Scale bars: 0.5 mm. All figured otoliths are kept in the Bavarian State Collection (collection number SNSB-BSPG 2020 LIV).

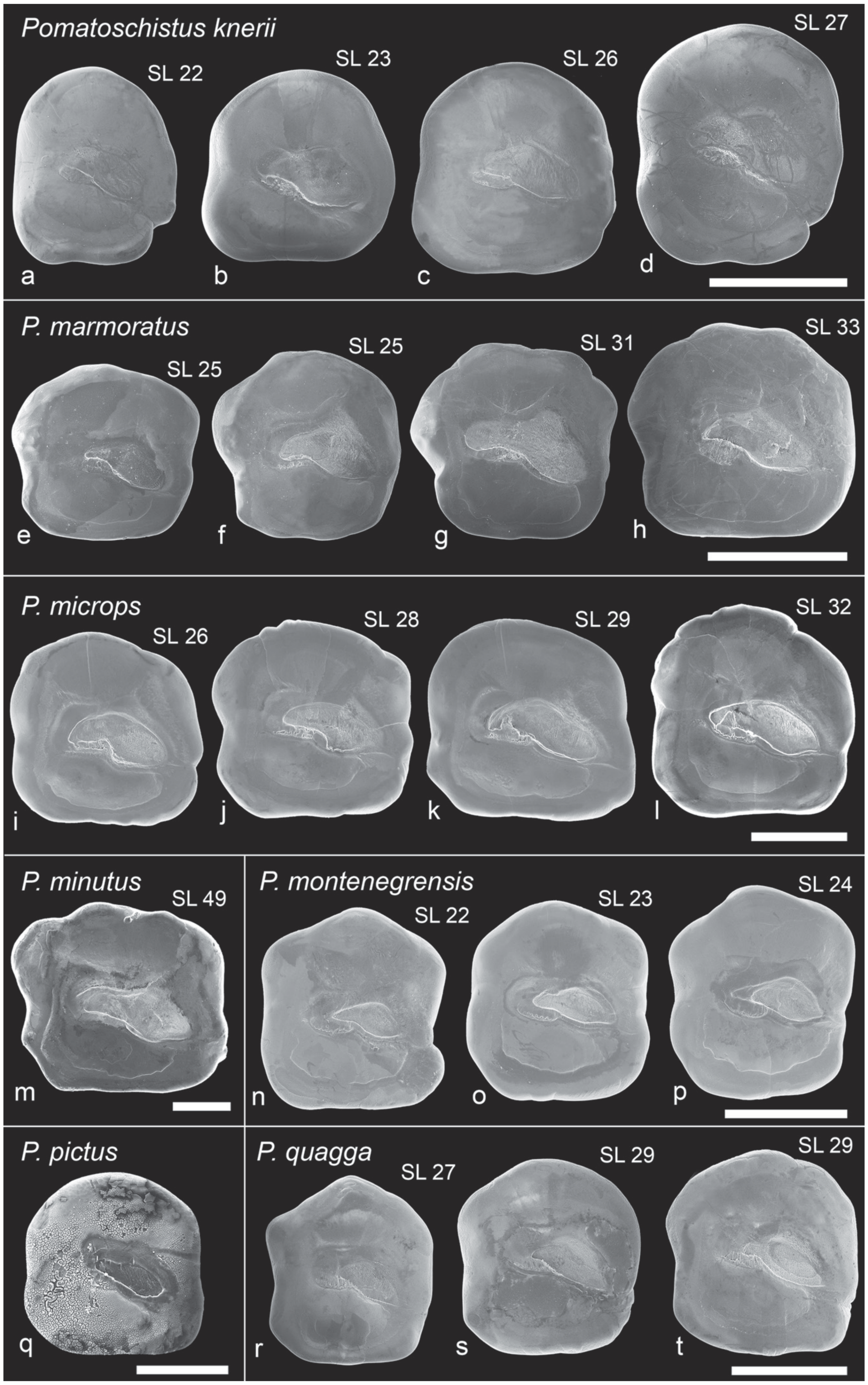


Figure 6: Otoliths (mesial view) of *P. knerii* (a–d: Krk, 1r, 2r, each mirrored, 9l, 4r mirrored), *Pomatoschistus marmoratus* (e–h: Selce, 1r, 2r, 3r, each mirrored, 4l), *P. microps* (i–l: Stralsund, 8l, 5l, 6l, 14l), *P. minutus* (m: Stralsund, 1l), *P. montenegrensis* (n–p: Skadar lake, 8l, 9l, 6l), *P. pictus* (m: Norway, 2r mirrored), and *P. quagga* (r–t: Krk, 2l, 8r mirrored, 4l). Numbers following the localities refer to the fish specimen from which the otolith was extracted; l, left otolith; r, right otolith, mirrored for better comparison. SL denotes the standard length (in mm) of the corresponding fish specimen. Scale bars: 0.5 mm. All figured otoliths are kept in the Bavarian State Collection (collection number SNSB-BSPG 2020 LIV).

mostly rounded and sometimes slightly lobed in *G. niger* and *G. roulei.* The posterior margin beneath the projection and also the posteroventral angle are clearly (*G. bucchichi*) or slightly (*G. roulei*) rounded. In *G. cruentatus* the posterior margin is straight and can be weakly crenulated, in *G. niger* it is straight or curved; the posteroventral angle is mostly angular in these two species. The ventral margin is straight in *G. cruentatus*, and faintly curved in the other three species. A weak preventral projection occurs in some otoliths of *G. cruentatus* (Fig. 5d) and *G. roulei* (Fig. 5j–l). The anterior margin is mostly concave, with an incision in the middle part. An angular predorsal angle is typical for *G. bucchichi*, *G. cruentatus* and *G. niger*; in *G. roulei* a rounded predorsal "angle" can occur (Fig. 5k, l). The dorsal margin is mostly slightly undulated; it is gently inclined in *G. bucchichi*, slightly rounded in *G. cruentatus*, clearly rounded in *G. roulei*, and strongly rounded in *G. niger*.

The otoliths of the *Pomatoschistus* species (Fig. 6) The otoliths of *Pomatoschistus* are usually rounded to square, but reveal a relatively high intraspecific variation regarding the details of their contours (Fig. 6). The otoliths of *P. knerii* (Fig. 6a–d), *P. montenegrensis* (Fig. 6n–p) and *P. quagga* (Fig. 6r–t) are slightly higher than long, the otoliths of *P. minutus* reveal a relatively rectangular shape (Fig. 6m), and those of *P. marmoratus* (Fig. 6e–h) and *P. microps* (Fig. 6i–l) are nearly quadratic. A posterodorsal projection can be present or not in *P. marmoratus*, *P. microps* and *P. minutus*, it is lacking in *P. knerii*, *P. microps* and *P. pictus*, and it is usually not present or replaced by a broad bulge in *P. montenegrensis* and *P. quagga.* The further posterior margin is relatively straight and slightly or clearly incised approximately in the middle; the posteroventral angle is rounded, angular or oblique. The ventral margin is straight or faintly curved in all species, no preventral projection is present. The anterior margin is straight, oblique or rounded, an incision in the middle may be present or not. An angular predorsal angle may occur in all species, except in *P. knerii.* The rounded dorsal margin is slightly lobed or crenulated in *P. marmoratus*, *P. microps* and *P. minutus.* It is smooth in *P. knerii*, *P. pictus*, *P. montenegrensis* and *P. quagga*; a clear median tip can occur in the latter two (Fig. 6n, p, r).

2.5 Discussion

The objective of this study was to evaluate the ability of characters of otoliths, body morphometric traits and meristic counts, all of which can be preserved in fossils, to discriminate species from their congeners.

2.5.1 Evaluation of otolith morphometry and otolith shape analysis

The morphology of the sagittal otolith is generally considered species-specific. This view goes back to the pioneering work of Koken (1884, 1891) and has since been demonstrated in numerous studies dealing with many different fish groups (Nolf, 1985; Campana, 2004; Tuset et al., 2008). Besides genetic and ontogenetic factors, also environmental parameters can influence the overall otolith shape (Tuset et al., 2003; Volpedo & Echeverría, 2003; Vignon & Morat, 2010). Regarding otolith morphometry, the varying results from different statistical analysis (Table 6), and the fact that the taxonomic significance of a certain otolith variable depends on the species under consideration are consistent with previous work on the taxonomic value of otolith morphometry (e.g., Reichenbacher & Reichard, 2014; Avigliano et al., 2016; Gierl et al., 2018). In the context of samples of fossil goby otoliths and attempts to detect the number of species represented, it therefore may be worth considering all otolith variables at the outset of the study. Otolith shape analysis is an established method for stock or population discrimination in fisheries research (Campana & Casselmann, 1993; Mérigot et al., 2007; Stransky et al., 2008; Libungan et al., 2015), but not commonly used for species discrimination. But for gobies, several studies are available that have analysed the differences between species: Elliptic Fourier analysis was conducted by Lord et al. (2012) to analyse three sympatric species of *Sicyopterus*, by Bani et al. (2013) to examine three sympatric Caspian species (one species of *Neogobius*, two of *Ponticola*, by Davoodi & Rahimian (2016) for three sympatric Caspian species of *Neogobius*, and by Yu et al. (2014) for the study of five species representing five different genera from northern Chinese coastal waters. Moreover, Lombarte et al. (2018) used wavelet analysis to examine 25 Mediterranean goby species distributed in 14 genera (including ten species of *Gobius* and two species of *Pomatoschistus*). In each of these studies, the authors found that the species included could be reliably discriminated based on their otoliths, but that the classification success varied among species. Our study largely confirms these results. In addition, it significantly advances our knowledge of the interspecific differences between goby otoliths within a given genus, as we focused on the discrimination of individual species from their congeners. Moreover, like Bani et al. (2013) and Yu et al. (2014) we found that otolith Fourier shape analysis works more effectively in goby species separation than otolith morphometry. This appears to be related to the circumstance that otolith shape differences between goby species can be very subtle (see Figs. 3–6), and in such cases cannot easily (or not at all) be quantified using single otolith variables (see Yu et al., 2014; Bonhomme et al., 2014; Caillon et al., 2018). The high discriminatory power of the otolith Fourier shape analysis makes this method a promising tool to examine ancient species diversity when abundant and well preserved fossil otoliths are present.

2.5.2 Evaluation of body morphometry

Body morphometry in fish can be greatly influenced by the demands of swimming, and associated factors like habitat size or presence/absence of predators and competitors (Gholami et al., 2015 and references therein). Therefore, body morphometry is often not considered to be relevant for species identification. Also, the taxonomic keys to the species of *Gobius* and *Pomatoschistus* provided in Miller (1986) do not include morphometric characters. On the other hand, body morphometry can be very efficient in detecting differences between populations or stocks (e.g., Haddon & Willis, 1995; Turan, 2004; Cheng et al., 2005; Buj et al., 2008). In our study, some body morphometric variables showed a high degree of separation success among species of both *Gobius* and *Pomatoschistus* (Tables 2d, 4d, 7); but only one population per species was available. Addition of further populations could reveal intraspecific differences in some of these variables, and eventually render them unsuitable for species separation. However, fossil finds are usually restricted to a single location, and often to a certain timeframe. Perhaps the most famous example is the locality of the fossil Lagerstätte Monte Bolca (c. 50 Ma; Marramà et al., 2016 and references therein). Examples of fossil locations bearing several goby fossils include Illerkirchberg in Germany (c. 17 Ma; Gierl & Reichenbacher, 2015), Klinci in Serbia (c. 16–19 Ma; Bradić-Milinović et al., 2019) and Oran in Algeria (c. 5–6 Ma; Arambourg, 1927). Our results indicate that measurements of SN/D1, SN/D2, D2b, and Ab are well suited to evaluating the ancient goby species diversity found in such singular localities.

2.5.3 Evaluation of the meristic counts

Meristic traits are usually estimated to work better than morphometry in species separation, but meristic counts among gobies are often very similar (Birdsong et al., 1988). In our study, the meristic values had little or no discriminatory power within *Gobius*, whereas they worked more efficiently for *Pomatoschistus*, especially when the reduced dataset was used (Tables 6, 7). It is possible that the use of only five species in the reduced *Pomatoschistus* dataset contributed to the greater separation success in that case (see above and Lombarte et al., 2018). However, the most conspicuous meristic character that distinguishes *Pomatoschistus* from *Gobius* is the elongated vertebral column, usually comprising 30–31 vertebrae (vs. 28 in *Gobius*) (Miller, 1986; supplementary Table S3). An elongated body form can, among other things, improve swimming abilities, affect feeding habits, and promote adaptation to specialized microhabitats (Claverie & Wainwright, 2014). An example among the Oxudercidae is *Luciogobius*, whose adaptive radiation into interstitial microhabitats was facilitated by marked body elongation (Yamada et al., 2009). Thus, it is possible that species of *Pomatoschistus* can be more readily separated from each other based on meristic counts than species of *Gobius* because they are adapted to more specific microhabitats. However, as data on microhabitats such as distinctive grain sizes, or abundance of certain algae, or presence of distinct shell sizes for spawning, are relatively limited for species of *Pomatoschistus* and *Gobius* (Miller, 1986; Nellbring, 1993; Wilkins & Myers, 1992; Kovačić & Šanda, 2016), this issue cannot be resolved at present. In the context of fossils, our results indicate that shared meristic characters among fossil gobies need not necessarily indicate the presence of only a single species, as has been proposed in some previous work (e.g., Gaudant & Quayle, 1988; Gaudant, 1998; Reichenbacher et al., 2007).

2.5.4 Comparisons of the otolith morphology of *Gobius* with previous works

Gobius auratus, G. geniporus, G. paganellus and *G. vittatus* could be separated by many otolith variables (Table 8) and revealed also characteristic shapes based on the SEM im-ages (Figs. 3a–c, 4d–f, j–p). *Gobius vittatus* could also be separated from its congeners based on two body morphometric variables, but no body morphometric variable separated the other three species from one another. Previous studies have demonstrated clear links between otolith shape and environmental parameters (e.g., Volpedo & Echeverría, 2003; Lombarte et al., 2010; Vignon & Morat, 2010; Avigliano et al., 2016). Based on these works it can be hypothesised that the specific otolith morphometry of *G. auratus, G. geniporus, G. paganellus* and *G. vittatus* reflects particular habitat preferences, e.g., for a certain water depth or temperature or a specific type of substrate and diet. A review of the literature on the four species under discussion provides some support for such a relationship.

(i) Herler et al. (2005) investigated the habitat use of *G. auratus* and *G. fallax* in the northern Adriatic Sea. The authorsfound that both species prefer water depths greater than 8 m, but that *G. auratus* clearly differed from *G. fallax* in its preference for more inclined substrates (>30–90°).

(ii) *Gobius geniporus* inhabits solely sandy bottoms and does not consume algae; both traits are unusual for *Gobius species* (Zander, 2011).

(iii) The rock goby *G. paganellus* is an intertidal-to-inshore, epibenthic species that lives under stones or on sheltered rocky shores with abundant algal cover (Miller, 1986; Zander, 2011). In their study on the composition of fish assemblages in six different macrohabitats in the Gulf of Trieste, Bonaca & Lipej (2005) found *G. paganellus* to be associated with a specific assemblage of algae (Wrangelia and Padina), while *G. cobitis, G. cruentatus and G. fallax* each occurred in all or almost all of the macrohabitats studied. It thus appears that *G. paganellus* has specific habitat preferences.

(iv) The striped goby *G. vittatus* differs phenotypically from its congeners in having a broad, black, longitudinal band that extends along its head and body (Miller, 1986). It is found offshore on coralline substrates at depths of 5 to 34 (exceptionally 42) m, with a preference for moderately to steeply inclined bottoms composed of both rock and sand (Miller, 1986; Kovačić, 2007; Kovačić & Arko Pijevac, 2008). Its characteristic broad stripe may reflect an adaptation to some additional aspect(s) of its habitat.

Table 8: Comparison of the species classification success in the reduced dataset of *Gobius* based on the different groups of variables (indicated by colors) and different statistical analyses. Bold numbers in rows indicate how many individual variables had separated the species given in the columns from $\geq$ 50% of the congeners (see Table 2a2, d2, f2 for details). Percentages in rows represent the success of the respective multivariate analyses in separating the species given in the columns (100% means that the species is separated from all congeners by this variable; see Table 2c2 and Table 3 for details). Species are sorted by the maximum number of otolith variables that account for their separation from $\geq$ 50% of the congeners.

G. vittatus	*G. auratus*	*G. paganellus*	*G. geniporus*	*G. incognitus*	*G. cobitis*	*G. roulei*	*G. niger*	*G. bucchichi*	*G. cruentatus*	
10**	9***	8**	8*	6**	4*	6	4	2	2	Individual otolith variables (AN(C)OVA)
50%	54%	56%	60%	33%	50%	30%	43%	60%	18%	PC1–4 of otolith variables (LDA)
80%	90%	100%	80%	100%	80%	90%	90%	100%	80%	Fourier otolith descriptors (MANOVA)
71%	88%	89%	100%	87%	95%	73%	85%	75%	100%	PC1–23 of Fourier otolith descriptors (LDA)
2*	0	0	0	4*	2	0	1	4**	0	Individual fish morphometric variables (ANOVA)
82%	27%	60%	50%	56%	30%	30%	43%	70%	64%	PC 1-4 of fish morphometric variables (LDA)
0	0	0	1	1	0	0	0	0	0	Meristic variables (Kruskal-Wallis)

Abbreviations: *, **, *** indicates that one, two or three singular variables separated the species given in the columns from 78–89% of the congeners. AN(C)OVA means that ANOVA or, if covariance was detected, ANCOVA was used; LDA, Linear Discriminant Analysis.

In the *Pomatoschistus* datasets, otolith morphometry was most significant for *P. montenegrensis* (Table 9). In this case too, a relationship to environmental factors could be proposed, as *P. montenegrensis* is one of the few freshwater species in the genus *Pomatoschistus* (Šanda & Kovačić, 2009). Alternatively phylogenetic factors may play a role as there is some evidence that *P.*

Table 9: Comparison of the species classification success in the reduced dataset of *Pomatoschistus* based on the different groups of variables (indicated by colors) and different statistical analyses. Bold numbers in rows indicate how many individual variables had separated the species given in the columns from ≥50% of the congeners (see Table 4a2, d2, f2 for details). Percentages in rows represent the success of the respective multivariate analyses in separating the species given in the columns (100% means that the species is separated from all congeners by this variable; see Table 4c2 and Table 5 for details). Species are sorted by the maximum number of otolith variables that account for their separation from ≥50% of the congeners.

P. mon	*P. mic*	*P. kne*	*P. qua*	*P. mar*	
16***	17*	13	14	16*	Individual otolith variables (AN(C)OVA)
90%	87%	60%	56%	56%	PC1–4 of otolith variables (LDA)
100%	100%	100%	83%	83%	Fourier otolith descriptors (MANOVA)
95%	100%	90%	100%	93%	PC1–14 of Fourier otolith descriptors (LDA)
5*	5*	6	5*	1*	Individual fish morphometric variables (ANOVA)
90%	67%	70%	90%	67%	PC 1-4 of fish morphometric variables (LDA)
7*	3*	6	5*	1	Meristic variables Kruskal-Wallis

Abbreviations: *, **, *** indicates that one, two or three singular variables separated the species given in the columns from 78–89% of the congeners. AN(C)OVA means that ANOVA or, if covariance was detected, ANCOVA was used.

montenegrensis actually might belong to a different genus, i.e. *Ninnigobius* (see Miller & Šanda, 2008; Freyhof, 2011; Thacker et al., 2019). Regarding fossil otoliths, it is obvious that some otolith-based species of *Gobius* are easier to recognize than others. Examples are *G. rostratus* from the lower Miocene of the Mainz Basin, Germany (easily recognizable by its prolonged preventral projection; see Weiler, 1963: Figs. 190–198), *Ponticola zosimovichi* from the middle Miocene of Kazakhstan (well identifiable due to its strongly bulged an-terodorsal margin; see Bratishko et al., 2015: Figs. 9–21), and several others. The results of our studies tentatively indicate that such distinctive otolith shapes may be related to specific environmental niches that these ancient species occupied. A further factor that could potentially influence otolith shape but cannot be evaluated yet is intraspecific communication based on acoustic signals. Experimental studies have revealed that gobies are capable of producing multiple sounds (Malavasi et al., 2008; Polgar et al., 2011; Parmentier et al., 2013; Horvatić et al., 2016), and acoustic courtship signals were demonstrated to be relevant for mating success in the painted goby *Pomatoschistus pictus* (Amorim et al., 2013). However, additional studies will be necessary before such a link between acoustic communication and otolith morphology can be seriously considered (see Popper et al., 2005).

2.6 Conclusions

The outcome of this study significantly advances our knowledge of the interspecific differences between goby otoliths within a given genus. In agreement with previous work, we have shown that goby otoliths show a high species-specificity and are useful for taxonomic studies concerning this taxon. As the interspecific differences can be very delicate, the otolith shape analysis is a particular valuable tool for species identification. Studies of past goby species diversity using fossil otoliths may greatly benefit from the use of both singular otolith variables and elliptic Fourier shape analysis, but also traditional comparative morphology remains useful. Moreover, we uncovered certain body and fin proportions, and in the case of *Pomatoschistus* also meristic traits suitable to unravel species diversity when fossil skeletons are preserved. Notably, shared meristic characters among fossil gobies do not necessarily indicate that only a single species is present, as has been proposed in some previous works. The outcome of our study provides a new basis for the delimitation of fossil goby species in future studies, and will consequently help to better understand the evolution of goby diversity and biogeography through time.

2.7 Acknowledgement

We are grateful to Marcelo Kovačić (NHM Rijeka) for his long term support in investigation of the gobies of the Northern Adriatic. For providing technical assistance and access to specimens from the SNSB-ZSM collection we thank Dirk Neumann and Ulrich Schliewen, respectively (both SNSB-ZSM, Munich, Germany). We thank James Maclaine (NHM London) for providing samples of *G. niger*, and we are grateful to Denik Ulqini (Skhodra University) for help in obtaining *P. montenegrensis*. The EU FP7 project ASSEMBLE at CCMar/Centre of Marine Sciences of Algarve, Faro, Portugal, provided the opportunity to obtain samples of *G. paganellus*. We thank Inda Brinkmann, Dorothea Liebl and Zeinab Gholami (LMU Munich) for otolith preparation, measurements of otoliths and fish specimens and conducting meristic counts. BR acknowledges funding for this project from the Deutsche Forschungsgemeinschaft (RE- 1113/20), RS was supported by the Ministry of Culture of the Czech Republic (DKRVO 2019- 2023/6.III.a National Museum, 00023272) and JV by institutional resources of the Ministry of Education, Youth, and Sports of the Czech Republic. Finally, we gratefully acknowledge the constructive and detailed review comments of three anonymous reviewers.

2.8 References

Agiadi, K., Koskeridou, E., Triantaphyllou, M., Girone, A. & Karakitsios, V. (2013) Fish otoliths from the Pliocene Heraklion Basin (Crete Island, Eastern Mediterranean). *Geobios*, 46 (6), 461–472.

Agiadi, K., Girone, A., Koskeridou, E., Moissette, P., Cornee, J.-J. & Quillévéré, F. (2018) Pleistocene marine fish invasions and paleoenvironmental reconstructions in the eastern Mediterranean. *Quat. Sci. Rev.*, 196, 80–99.

Agiadi, K., Vasileiou, G., Koskeridou, E., Moisette, P. & Cornée, J.-J. (2019) Coastal fish otoliths from the early Pleistocene of Rhodes (eastern Mediterranean). *Geobios*, 55, 1–15.

Agorreta, A., San Mauro, D., Schliewen, U., Van Tassell, J.L., Kovačić, M., Zardoya, R. & Rüber, L. (2013) Molecular phylogenetics of Gobioidei and phylogenetic placement of European gobies. *Mol. Phylogenet. Evol.*, 69 (3), 619–633

Amorim, M.C.P., Pedroso, S.S., Bolgan, M., Jordão, J.M., Caiano, M. & Fonseca, P.J. (2013) Painted gobies sing their quality out loud: acoustic rather than visual signals advertise male quality and contribute to mating success. *Funct. Ecol.*, 27 (2), 289–298.

Arambourg, C. (1927) Les poissons fossiles d'Oran. *Matériaux pour la Carte géologique d'Algérie, 1re série, Paléontologie*, 6, 1–291.

Avigliano, E., Jawad, L.A. & Volpedo, A.V. (2016) Assessment of the morphometry of saccular otoliths as a tool to identify triplefin species (Tripterygiidae). *J. Mar. Biolog. Assoc. U.K.*, 96 (5), 1167–1180.

Bani, A., Poursaeid, S. & Tuset, V.M. (2013) Comparative morphology of the sagittal otolith in three species of south Caspian gobies. *J. Fish Biol.*, 82 (4), 1321–1332.

Birdsong, R.S., Murdy, E.O. & Pezold, F.L. (1988) A study of the vertebral column and median fin osteology in gobioid fishes with comments on gobioid relationships. *Bull. Mar. Sci.*, 42 (2), 174–214.

Bonaca, M.O. & Lipej, L. (2005) Factors affecting habitat occupancy of fish assemblage in the Gulf of Trieste (Northern Adriatic Sea). *Mar. Ecol.*, 26 (1), 42–53.

Bonhomme, V., Picq, S., Gaucherel, C. & Claude, J. (2014) Momocs: outline analysis using R. *J. Stat. Softw.*, 56 (13), 1–24.

Bradić-Milinović, K., Ahnelt, H., Rundić, L. & Schwarzhans, W. (2019) The lost freshwater goby fish fauna (Teleostei, Gobiidae) from theearly Miocene of Klinci (Serbia). *Swiss J. Palaeontol.*, 138 (2), 285–315.

Brandl, S.J., Goatley, C.H.R., Bellwood, D.R. & Tornabene, L. (2018) The hidden half: ecology and evolution of cryptobenthic fishes on coral reefs. *Biol. Rev.*, 93 (4), 1846–1873.

Bratishko, A., Schwarzhans, W., Reichenbacher, B., Vernyhorova, Y. & Ćorić, S. (2015) Fish otoliths from the Konkian (Miocene, early Serravallian) of Mangyshlak (Kazakhstan): testimony to an early endemic evolution in the Eastern Paratethys. *Paläontol. Z.*, 89 (4), 839–889.

Buj, I., Podnar, M., Mrakovčić, M., Ćaleta, M., Mustafić, P., Zanella, D. & Marčić, Z. (2008) Morphological and genetic diversity of *Sabanejewia balcanica* in Croatia. *Folia zool*, 57 (1–2), 100–110.

Caillon, F., Bonhomme, V., Möllmann, C. & Frelat, R. (2018) A morphometric dive into fish diversity. *Ecosphere*, 9 (5), e02220.

Campana, S.E. & Casselman, J.M. (1993) Stock discrimination using otolith shape analysis. *Can. J. Fish. Aquat. Sci.*, 50 (5), 1062–1083.

Campana, S.E. (2004) Photographic Atlas of Fish Otoliths of the Northwest Atlantic Ocean. *NRC Research Press*, Ottawa, Ontario.

Cheng, Q.-Q., Lu, D.-R. & Ma, L. (2005) Morphological differences between close populations discernible by multivariate analysis: a case study of genus *Coilia* (Teleostei: Clupeiforms). *Aquat. Living Resour.*, 18 (2), 187–192.

Claverie, T. & Wainwright, P.C. (2014) A morphospace for reef fishes: elongation is the dominant axis of body shape evolution. *PloS ONE*, 9 (11), e112732.

Davoodi, P. & Rahimian, H. (2016) Identifying *Neogobius* species from the southern Caspian Sea by otolith shape (Teleostei: Gobiidae). *Zootaxa*, 4205 (1), 81–86.

Engin, S. & İnnal, D. (2017) A new species of *Pomatoschistus* (Teleostei: Gobiidae) from Southern Anatolia. *Zool. Middle East*, 63 (4), 316–324.

Engin, S. & Seyhan, D. (2017) A new species of *Pomatoschistus* (Teleostei, Gobiidae): the Mediterranean's smallest marine fish. *J. Fish Biol.*, 91 (4), 1208–1223.

Freyhof, J. (2011) Diversity and distribution of freshwater gobies from the Mediterranean, Black and Caspian Seas. In: R.A. Patzner, J.L. Van Tassell, M. Kovačić & B.G. Kapoor (Eds) *The Biology of Gobies*, pp. 279–288. Science Publishers Inc., Enfield, NH.

Fricke, R., Eschmeyer, W.N. & Fong, J.D. (2019) Species by Family/Subfamily. http://researcharchive.calacademy.org/research/ichthyology/ catalog/SpeciesByFamily. Accessed February 2019.

Froese, R. & Pauly, D. Eds (2019) *FishBase. World Wide Web electronic publication.* www.fishbase.org, version (11/2019), Accessed February 2019.

Gaudant, J. & Quayle, W.J. (1988) New palaeontological studies on the Chapelcorner fish bed (Upper Eocene, Isle of Wight). *Bull. British Mus. Nat. Hist. (Geol.)*, 44 (1), 15–39.

Gaudant, J. (1998) L'ichthyofaune des eaux continentales miocènes de Serbie (Yougoslavie): une révision. *Neues Jahrb. Geol. Palaontol. Abh.*, 207 (1), 107–123.

Gholami, Z., Esmaeili, H.R., Erpenbeck, D. & Reichenbacher, B. (2015) Genetic connectivity and phenotypic plasticity in the cyprinodont *Aphanius farsicus* from the Maharlu Basin, south-western Iran. *J. Fish Biol.*, 86, 882–906.

Gierl, C. & Reichenbacher, B. (2015) A new fossil genus of Gobiiformes from the Miocene characterized by a mosaic set of characters. *Copeia*, 103 (4), 792–805.

Gierl, C. & Reichenbacher, B. (2017) Revision of so-called *Pomatoschistus* (Gobiiformes, Teleostei) from the late Eocene and early Oligocene. *Palaeontol. Electronica*, 20.2.33A, 1–17.

Gierl, C., Liebl, D., Šanda, R., Vukić, J., Esmaeili, H.R. & Reichenbacher, B. (2018) What can goby otolith morphology tell us? *Cybium*, 42 (4), 349–363.

Glavičić, I., Paliska, D., Soldo, A. & Kovačić, M. (2016) A quantitative assessment of thecryptobenthic fish assemblage at deep littoral cliffs in the Mediterranean. *Sci. Mar.*, 80 (3), 329–337.

Haddon, M. & Willis, T.J. (1995) Morphometric and meristic comparison of orange roughy (*Hoplo-stethus atlanticus*: Trachichthyidae) from the Puysegur Bank and Lord Howe Rise, New Zealand, and its implications for stock structure. *Mar. Biol.*, 123 (1), 19–27.

Hammer, Ø., Harper, D.A.T., & Ryan, P.D. (2001) PAST: Paleontological statistics software pack-age for education and data analysis. *Palaeontologia Electronica*, 4 (1), 1–9.

Herler, J., Patzner, R. & Sturmbauer, C. (2005) A preliminary revision of the *Gobius auratus* species complex with redescription of *Gobius auratus*. *J. Nat. Hist.*, 39 (14), 1043–1075.

Horvatić, S., Cavraro, F., Zanella, D. & Malavasi, S. (2016) Sound production in the Ponto-Caspian goby *Neogobius fluviatilis* and acoustic affinities within the *Gobius* lineage: implications for phylogeny. *Biol. J. Linn. Soc. Lond.*, 117 (3), 564–573.

Iglésias, S.P., Frotté, L. & Sellos, D.Y. (2016) *Gobius salamansa*, a new species of goby (Gobiidae) from the Cape Verde Islands supported by a unique cephalic lateral line system and DNA barcoding. *Ichthyol. Res.*, 63 (3), 356–369.

Jost, J., Kälin, D., Börner, S., Vasilyan, D., Lawver, D. & Reichenbacher, B. (2015) Vertebrate microfossils from the Upper Freshwater Molasse in the Swiss Molasse Basin: Implications for the evolution of the North Alpine Foreland Basin during the Miocene Climate Optimum. *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 426, 22–33.

Kar, S.S. & Ramalingam, A. (2013) Is 30 the magic number? Issues in sample size estimation. *Nat. J. Comm. Medic.*, 4 (1), 175–179.

Karplus, I. (1987) The association between gobiid fishes and burrowing alpheid shrimps. *Oceanogr. Mar. Biol.*, 25, 507–562.

Karplus, I. & Thompson, A.R. (2011) The partnership between gobiid fishes and burrowing alpheid shrimps. In: R.A. Patzner, J.L. Van Tassell, M. Kovačić & B.G. Kapoor (Eds) *The Biology of Gobies*, pp. 559–607. Science Publishers Inc., Enfield, NH.

Koken, E. (1884) Über Fisch-Otolithen, insbesondere über diejenigen der norddeutschen Oligocän-Ablagerungen. *Z. dt. geol. Ges.*, 36, 500–565.

Koken, E. (1891) Neue Untersuchungen an tertiären Fisch-Otoloithen II. *Z. dt. geol. Ges.*, 43, 77–170.

Kovačić, M. (2007) Reproductive biology of the striped goby, *Gobius vittatus* (Gobiidae) in the northern Adriatic Sea. *Sci. Mar.*, 71 (1), 145–151.

Kovačić, M. (2008) The key for identification of Gobiidae (Pisces: Perciformes) in the Adriatic Sea. *Acta Adriat.*, 49 (3), 245–254.

Kovačić, M. & Arko Pijevac, M. (2008) Habitat preferences, distribution, and abundance of *Gobius vittatus* (Gobiidae) in the Kvarner area (northern Adriatic Sea). *Vie Milieu*, 58 (1), 39–45.

Kovačić, M., Bogorodsky, S.V. & Mal, A.O. (2016) A new species of *Coryogalops* (Perciformes: Gobiidae) and the first adult record of *Feia nympha* from the Red Sea. *Zootaxa*, 4097 (3), 341–352.

Kovačić, M., & Šanda, R. (2016) A new species of *Gobius* (Perciformes: Gobiidae) from the Mediterranean Sea and the redescription of *Gobius bucchichi. J. Fish Biol.*, 88 (3), 1104–1124.

Larson, H.K., Murdy, E.O. & Gill, A.C. (2001) Suborder Gobioidei. In: K.E. Carpenter, & V.H. Niem (Eds) *FAO Species Identification Guide for Fishery Purposes. The Living Marine Resources of the Western Central Pacific. Volume 6. Bony Fishes Part 4 (Labridae to Latimeriidae), Estuarine Crocodiles, Sea Turtles, Sea Snakes and Marine Mammals*, pp. 3574–3609. FAO, Rome.

Libungan, L.A., Óskarsson, G.J., Slotte, A., Jacobsen, J.A. & Pálsson, S. (2015) Otolith shape: a population marker for Atlantic herring *Clupea harengus. J. Fish Biol.*, 86 (4), 1377–1395.

Lin, C.-H., Girone, A. & Nolf, D. (2015) Tortonian fish otoliths from turbiditic deposits in Northern Italy: Taxonomic and stratigraphic significance. *Geobios*, 48 (3), 249–261.

Liu, H.T.H., Ahnelt, H., Balma, G.A.C. & Delmastro, G.B. (2009) First record of *Gobius roulei* (Gobiidae) in the Ligurian Sea. *Cybium*, 33 (3), 253–254.

Lombarte, A., Palmer, M., Matallanas, J., Gómez- Zurita, J. & Morales-Nin, B. (2010) Ecomorphological trends and phylogenetic inertia of otolith sagittae in Nototheniidae. *Environ. Biol. Fishes*, 89 (3–4), 607–618.

Lombarte, A., Miletić, M., Kovačić, M., Otero-Ferrer, J.L. & Tuset, V.M. (2018) Identifying sagittal otoliths of Mediterranean Sea gobies: variability among phylogenetic lineages. *J. Fish Biol.*, 92 (6), 1768–1787.

Lord, C., Morat, F., Lecomte-Finiger, R. & Keith, P. (2012) Otolith shape analysis for three *Sicyopterus* (Teleostei: Gobioidei: Sicydiinae) species from New Caledonia and Vanuatu. *Environ. Biol. Fishes*, 93 (2), 209–222.

Macpherson, E. & Duarte, C.M. (1994) Patterns in species richness, size, and latitudinal range of East Atlantic fishes. *Ecography*, 17 (3), 242–248.

Malavasi, S., Collatuzzo, S. & Torricelli, P. (2008) Interspecific variation of acoustic signals in Mediterranean gobies (Perciformes, Gobiidae): comparative analysis and evolutionary outlook. *Biol. J. Linn. Soc. Lond.*, 93 (4), 763–778.

Malz, H. (1978) Vergleichend-morphologische Untersuchungen an aquitanen Fisch-Otolithen aus dem Untergrund von Frankfurt am Main. *Senckenbergiana lethaea,* 59 (4/6), 441–481.

Marramà, G., Bannikov, A.F., Tyler, J.C., Zorzin, R. & Carnevale, G. (2016) Controlled excavations in the Pesciara and Monte Postale sites provide new insights about the palaeoecology and taphonomy of the fish assemblages of the Eocene Bolca Konservat-Lagerstätte, Italy. *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 454, 228–245.

Mérigot, B., Letourneur, Y. & Lecomte-Finiger, R. (2007) Characterization of local populations of the common sole *Solea solea* (Pisces, Soleidae) in the NW Mediterranean through otolith morphometrics and shape analysis. *Mar. Biol.*, 151 (3), 997–1008.

Miller, P.J. (1974) A new species of Gobius (Teleostei: Gobiidae) from the western English Channel, with a key to related species in the British and Irish fauna. *J. Zool.*, 174 (4), 467–480.

Miller, P.J. & El-Tawil, M.Y. (1974) A multidisciplinary approach to a new species of *Gobius* (Teleostei: Gobiidea) from southern Cornwall. *J. Zool.*, 174 (4), 539–574.

Miller, P.J. (1986) Gobiidae. In: P.J.P. Whitehead, M.- L. Bauchot, J.-C. Hureau, J. Nielsen & E. Tortonese (Eds) *Fishes of the North-Eastern Atlantic and the Mediterranean* (FNAM), pp. 1019–1085. UNESCO, Paris.

Miller, P.J. Ed (2004) *The Freshwater Fishes of Europe. Vol. 8/II. Gobiidae 2.* AULA-Verlag, Wiebelsheim.

Miller, P.J. & Šanda, R. (2008) A new West Balkanian sand-goby (Teleostei: Gobiidae). *J. Fish Biol.*, 72 (1), 259–270.

Nellbring, S. (1993) Spawning of two *Pomatoschistus* species (Gobiidae) in relation to nest availability and depth: A field experiment. *Neth. J. Sea Res.*, 31 (2), 173–179.

Nelson, J.S., Grande, T.C. & Wilson, M.V.H. (2016) *Fishes of the World*, fifth edition. John Wiley & Sons, inc., Hoboken, New Jersey.

Nolf, D. (1985) *Handbook of Paleoichthyology, Volume 10, Otolithi piscium.* Verlag Dr. Friedrich Pfeil, München.

Nolf, D. (2013) *The Diversity of Fish Otoliths, Past and Present.* Royal Belgian Institute of Natural Sciences, Brussels.

Parmentier, E., Kéver, L., Boyle, K., Corbisier, Y.E., Sawelew, L. & Malavasi, S. (2013) Sound production mechanism in *Gobius paganellus* (Gobiidae). *J. Exp. Biol.*, 216 (17), 3189–3199.

Patzner, R.A., Van Tassell, J.L., Kovačić, M. & Kapoor, B.G. Eds,(2011) *The Biology of Gobies.* Science Publishers Inc., Enfield, NH.

Polgar, G., Malavasi, S., Cipolato, G., Georgalas, V., Clack, J.A. & Torricelli, P. (2011) Acoustic Communication at the Water's Edge: Evolutionary Insights from a Mudskipper. *PloS ONE*, 6 (6), e21434.

Popper, A.N., Ramcharitar, J. & Campana, S.E. (2005) Why otoliths? Insights from inner ear physiology and fisheries biology. *Mar. Freshw. Res.*, 56 (5), 497–504.

R Core Team (2019) *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, Austria.

Radwańska, U. (1992) Fish otoliths in the Middle Miocene (Badenian) deposits of southern Poland. *Acta Geol. Pol.*, 42 (3–4), 141–328.

Reichenbacher, B. & Cappetta, H. (1999) First evidence of an early Miocene marine teleostean fish fauna (otoliths) from La Paillade (Montpellier, France). *Palaeovertebrata*, 28 (1), 1–46.

Reichenbacher, B., Gaudant, J. & Griessemer, T.W. (2007) A late Burdigalian gobiid fish, *Gobius brevis* (AGASSIZ, 1839), in the Upper Hydrobia Beds in the middle Upper Rhine Graben (W-Germany). *Paläontol.* Z., 81 (4), 365–375.

Reichenbacher, B. & Reichard, M. (2014) Otoliths of five extant species of the annual killifish *Nothobranchius* from the East African savannah. *PLoS ONE*, 9 (11), e112459.

Reichenbacher, B., Filipescu, S. & Miclea, A. (2019) A unique middle Miocene (Sarmatian) fish fauna from coastal deposits in the eastern Pannonian Basin (Romania). *Paleobiodivers. Paleoenviron.*, 99 (2), 177–194.

Rueden, C.T., Schindelin, J., Hiner, M.C., DeZonia, B.E., Walter, A.E., Arena, E.T. & Eliceiri, K.W. (2017) ImageJ2: ImageJ for the next generation of scientific image data. *BMC bioinformatics*, 18 (1), 529.

Šanda, R. & Kovačić, M. (2009) Freshwater gobies in the Adriatic drainage basin of the Western Balkans. *Ann. Ser. Hist. Nat.*, 19 (1), 1–10.

Schwarzhans, W. (2014) Otoliths from the middle Miocene (Serravallian) of the Karaman Basin, Turkey. *Cainozoic Research*, 14 (1), 35–69.

Stransky, C., Murta, A.G., Schlickeisen, J. & Zimmermann, C. (2008) Otolith shape analysis as a tool for stock separation of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean. *Fish. Res.*, 89 (2), 159–166.

Thacker, C.E. (2015) Biogeography of goby lineages (Gobiiformes: Gobioidei): origin, invasions and extinction throughout the Cenozoic. *J. Biogeogr.*, 42 (9), 1615–1625.

Thacker, C.E., Gkenas, C., Triantafyllidis, A., Malavasi, S. & Leonardos, I. (2019) Phylogeny, systematics and biogeography of the European sand gobies (Gobiiformes: Gobionellidae). *Zool. J. Linn.* Soc., 185 (1), 212–225.

Tornabene, L., Valdez, S., Erdmann, M. & Pezold, F. (2015) Support for a 'Center of Origin' in the Coral Triangle: Cryptic diversity, recent speciation, and local endemism in a diverse lineage of reef fishes (Gobiidae: *Eviota*). *Mol. Phylogenet. Evol.*, 82, 200–210.

Turan, C. (2004) Stock identification of Mediterranean horse mackerel (*Trachurus mediterraneus*) using morphometric and meristic characters. *ICES J. Mar. Sci.*, 61 (5), 774–781.

Tuset, V.M., Lombarte, A., González, J.A., Pertusa, J.F. & Lorente, M.J. (2003) Comparative morphology of the sagittal otolith in *Serranus* spp. *J. Fish Biol.*, 63 (6), 1491–1504.

Tuset, V.M., Lombarte, A. & Assis, C.A. (2008) Otolith atlas for the western Mediterranean, north and central eastern Atlantic. *Sci. Mar.*, 72 (S1), 7–198.

Vasil'eva, E.D., Schwarzhans, W.W., Medvedev, D.A. & Vasil'ev, V.P. (2016) Cryptic species of Ponto- Caspian bighead goby of the genus *Ponticola* (Gobiidae). *J. Ichthyol.*, 56 (1), 1–18.

Vignon, M. & Morat, F. (2010) Environmental and genetic determinant of otolith shape revealed by a non-indigenous tropical fish. *Mar. Ecol. Prog. Ser.*, 411, 231–241.

Volpedo, A.V. & Echeverría, D.D. (2003) Ecomorphological patterns of the sagitta in fish on the continental shelf off Argentine. *Fish. Res.*, 60 (2–3), 551–560.

Weiler, W. (1963) Die Fischfauna des Tertiärs im oberrheinischen Graben, des Mainzer Beckens, des unteren Maintals und der Wetterau, unter besonderer Berücksichtigung des Untermiozäns. *Abh. Senckenb. Nat.-forsch. Ges.*, 504, 1–75.

Wilkins, H.K.A. & Myers, A.A. (1992) Microhabitat utilisation by an assemblage of temperate Gobiidae (Pisces: Teleostei). *Mar. Ecol. Prog. Ser.*, 90 (2), 103–112.

Yamada, T., Sugiyama, T., Tamaki, N., Kawakita, A. & Kato, M. (2009) Adaptive radiation of gobies in the interstitial habitats of gravel beaches accompanied by body elongation and excessive vertebral segmentation. *BMC Evol. Biol.*, 9, 1–14.

Yu, X., Cao, L., Liu, J., Zhao, B., Shan, X. & Dou, S. (2014) Application of otolith shape analysis for stock discrimination and species identification of five goby species (Perciformes: Gobiidae) in the northern Chinese coastal waters. *Chin. J. Oceanol. Limnol.*, 32, 1060–1073.

Zander, C.D. (2011) Gobies as predator and prey. In: R.A. Patzner, J.L. Van Tassell, M. Kovačić, & B.G. Kapoor (Eds) *The Biology of Gobies*, pp. 291–344. Science Publishers Inc., Enfield, NH.

3 Shedding new light on fossil gobiid fish otoliths from the Vienna Basin: An exemplary application of Fourier outline analysis and descriptive statistics as taxonomical support

Carolin Gut[1]

[1]Department of Earth and Environmental Sciences, Ludwig-Maximilians-Universität München, Richard-Wagner Straße 10, 80333 Munich, Germany

3.1 Abstract

Gobioid fishes are one of the most diverse groups within the Teleostei and considered to be abundant since the Miocene (ca. 23 Ma). Due to the rarity of articulated skeletons, most fossil gobioid species are based on isolated otoliths (sagittae). Despite the species specificity of gobioid otoliths, species determination can be challenging as subtle morphological differences are difficult to evaluate in their taxonomic significance. This study represents a re-evaluation of fossil gobioid otoliths from two middle Miocene (Badenian) localities (Kienberg, Vöslau) of the Vienna Basin (Austria). Utilizing elliptic Fourier shape analysis and otolith-indices showed statistical support of visually predetermined taxonomic groups. A new index (OSH/TED) acquired by the ratio of the newly introduced measure distances OSH (ostial sulcus height) and TED (sulcus-tip to sulcus-end diagonal) is introduced as new tool for sulcus-comparisons. The results justify the introduction of a new genus (†*Paromiogobius* gen. nov., represented by one species: †*P. johnsonae* gen. et sp. nov.) and further new *Lesueurigobius* species, i.e. *L. annajanae* sp. nov., *L. brzobohatyi* sp. nov. and *L. harzhauseri* sp. nov. The distinct species assemblages of the localities reflect differences in water depth. Seven species were described from Kienberg, a shallow marine near-shore habitat according to previous works. The further offshore locality Vöslau shows with three species less diversity. The described new species add to the diversity known from the Miocene and support the assumption of an increasing gobioid fish diversity during the Miocene.

Keywords Gobioid otoliths • middle Miocene • Vienna Basin • Central Paratethys • Fourier analysis

3.2 Introduction

Fossil remains of teleost fishes usually comprise isolated bones, scales, teeth and otoliths, as articulated skeletons are rather scarce and skeletons with otoliths *in situ* an exception. If otoliths are present, it is usually the sagitta (the largest of the three otolith types in most teleost species), which is preserved, at least in marine sediments (Koken 1884; Nolf 2013).

Gobioid fishes represent today one of the largest groups within the bony fishes (Nelson et al. 2016). Fossil otoliths (sagittae) of gobioids are usually very abundant in shallow marine deposits of Miocene or younger age (see Nolf 1985, 2013 for reviews). Due to their abundant fossil record they can serve as a valuable group for research in palaeodiversity, palaeoecology and evolutionary history – especially since the Miocene (see Nolf 2013; Schwarzhans 2014; Reichenbacher et al. 2019; Schwarzhans et al. 2020).

Nevertheless, determining gobioid species based solely on otoliths is challenging due to the presence of only few diagnostic characters and great variability in their morphological expression (see Malz 1978; Brzobohatý 1994; Schwarzhans 2014). In addition, the limited availability of extant comparison material has led to a confusing fossil gobioid otolith record, especially in older works (see comments in Nolf 1985 and Bratishko et al. 2015). This has now been gradually improved by new studies of articulated gobioid fish skeletons with otoliths preserved *in situ* (e.g. Brzobohatý et al. 2003; Reichenbacher et al. 2007; Gierl & Reichenbacher 2015; Schwarzhans et al. 2017; Bradić-Milinović et al. 2019). Furthermore, studies on otoliths of recent gobioid fishes, as well as a growing methodological tool-kit dealing with otolith shape analysis contributed to a better understanding of otolith variation within and between gobioid species (e.g. Yu et al. 2014; Lombarte et al. 2018; Gut et al. 2020).

The middle Miocene Vienna Basin with its well-known geological history, its great amount of biostratigraphic data and its fossil-rich sediments (see below for references) represents a suitable locality to study ancient gobioid diversity. The objective of this study is to examine fossil gobioid otoliths from the middle Badenian (= upper Langhian) of the Vienna Basin. The samples originate from the sites Vöslau, which is the type locality of the Badenian (see Cicha 1975; Papp & Steininger 1978; Rögl et al. 2008), and Kienberg (= Mikulov; see Schultz et al. 2010). These outcrops have provided numerous otoliths during earlier works, which were stored in the Natural History Museum in Vienna. For this study 140 and 63 well-preserved gobioid otoliths were examined from Kienberg and Vöslau respectively. The specific aim was to determine their species diversity using Fourier outline analysis and descriptive statistics to support species delimitation.

3.3 Geological setting and study site

During the middle Miocene stage of the Badenian (c. 16.3–12.8 Ma), the Vienna Basin was part of the Central Paratethys, which was a mostly shallow sea, north of the former Tethys, with an established connection to the Mediterranean Sea at that time (e.g. Rögl 1998; Kováč et al. 2017; Harzhauser et al. 2018). Around 14 Ma it is believed that the "Miocene Climate Optimum" came to an end and the "Middle Miocene Climate Transition" followed (Hohenegger et al. 2014, see Fig. 1b). As a result, a change from a "warm water fauna" to a "slightly cooler but still warm water" fauna has been observed in the composition of benthic foraminifera species (Hohenegger et al. 2008; Hohenegger et al. 2014).

The study site Kienberg (=Mikulov) is located at the NW margin of the Vienna Basin (see Schultz et al. 2010), while the site Vöslau (=Baden Sooss) is located near the western border of the Southern Vienna Basin. The age of both localities is middle Badenian (∼14 Ma) based on biostratigraphic examinations on foraminifers and calcareous nannoplankton (Hohenegger et al. 2007; Kováč et al. 2017) (Fig. 1a). Kienberg represents a shallow marine regime (∼60 m) close to the former shore (see Schultz et al. 2010), whereas Vöslau is assumed to represent a locality with greater depths (50—100 m) further offshore (see Papp & Steininger 1978; Rögl et al. 2008).

Previous work on the gobioid otolith fauna of Vöslau was conducted by Schubert (1906). Schubert (1906) recognized "*Otolithus* (*Gobius*) *vicinalis*" (Koken, 1891), "*Otolithus* (*Gobius*) *pretiosus*" Procházka, 1893, which, according to Nolf (1981, 2013) represents a collection of multiple species and according to Schwarzhans et al. (2020) should be considered as *nomen dubium* until the type specimen is retrieved. Schubert (1906) additionally described "*Otolithus* (*Gobius*) *intimus*" Procházka, 1893, which was partly reassigned to *Thorogobius iucundus* by Schwarzhans (2014); and Schwarzhans et al. (2020) considers the name used today "*Gobius intimus*" as *nomen dubium* until new material is retrieved or the type specimen rediscovered. Furthermore, Schubert (1906) described "*Otolithus* (*Gobius*) *telleri*" Schubert, 1906, which was reassigned to *Deltentosteus telleri* by Radwańska (1992). The otoliths from Kienberg have been examined by Brzobohatý et al. (2007). Among the gobioids he recognized *Deltentosteus telleri, "Gobius multipinnatus", Lesueurigobius* aff. *vicinalis, Oxyurichthys* sp. and *Priolepis* sp. However, the true otoliths of *"G. multipinnatus"* are unknown (see Gierl & Reichenbacher 2015; Schwarzhans et al. 2020)

Institutional abbreviations used: NHMW, Naturhistorisches Museum Wien (Museum of Natural

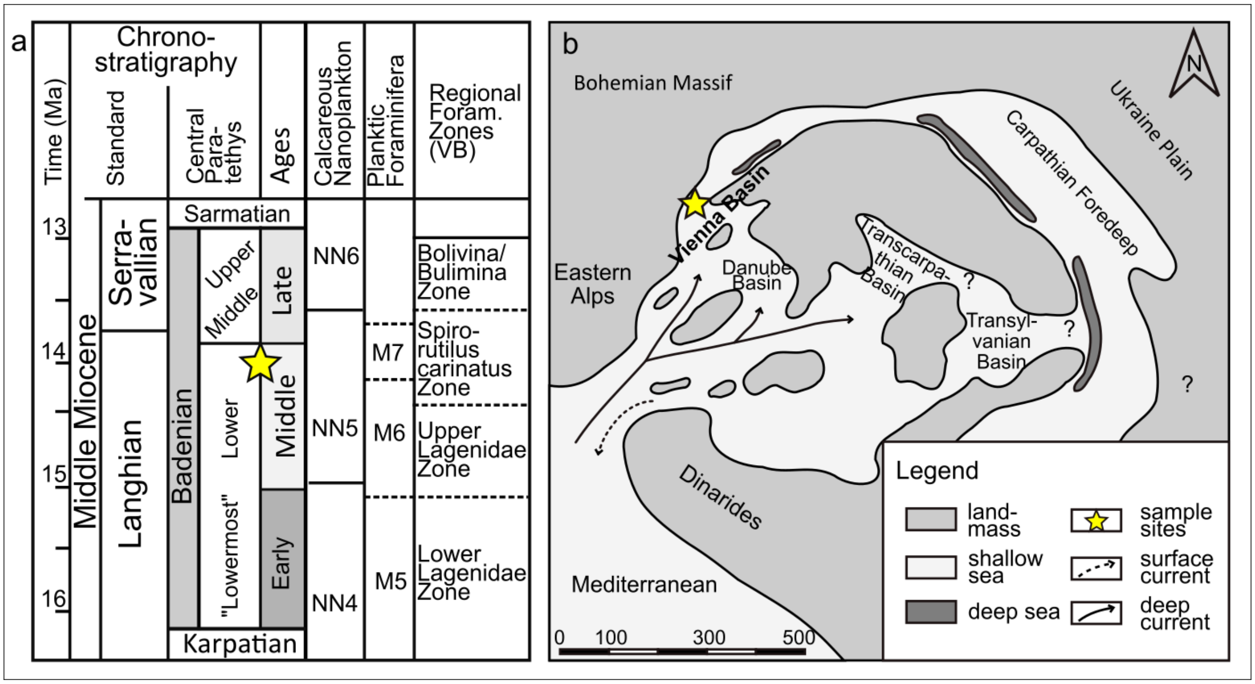


Figure 1: **a** Chronological chart and **b** paleogeographic map of the Central Paratethys during the middle Badenian edited after Schultz et al. (2010), Hohenegger et al. (2014), and Kováč et al. (2017). Stars are indicating the geographical and chronological position of the samples.

History of Vienna).

3.4 Material and methods

The samples of Kienberg and Vöslau were provided by the collection of the Natural History Museum of Vienna (inventory numbers NHMW 2006z0345/000x for Kienberg, NHMW 1995/0023/000x for Vöslau). The Kienberg specimens originate from sample "S14" described in Schultz et al. (2010). The entire gobioid otolith material comprised 500–600 otoliths. Based on visual inspection, only well preserved otoliths (Kienberg 140; Vöslau 63) were selected for this study. Criteria of selection were a completely preserved outline, a well recognizable sulcus and a clearly defined ventral furrow. All otoliths fulfilling these criteria (totally 203) were considered for this study.

The selected otoliths were visually compared to one another using a stereomicroscope (LeicaEZ4) and sorted into putative taxonomic groups based on outline, projections, angles and sulcus shape. They were fixated on elastic rubber and photographed in medial view using a Keyence digital microscope VHX 5000 of the SNSB-Bavarian State Collection Palaeontology and Geology. Group-representative otoliths were selected for SEM imaging, coated with gold and imaged using the HITACHI SU 5000 Schottky FE-SEM at the Department of Earth and Environmental Sciences, Ludwig-Maximilians University (LMU) Munich.

For Fourier shape analysis all SEM images and the digital images of remaining specimens were turned into binary pictures with a black object on white background, saved as ".jpg" and loaded into R (R Development Core Team, 2008) using the package MOMOCS (version 1.2.9; Bonhomme et al. 2014). The results revealed that 22 harmonics explained 99.9% of shape variation between the groups. Significance of group mean values were tested with the MANOVA function ($p \leq 0.05$) implemented in MOMOCS. A threshold of 72% classification success was set as a standard, as this value corresponds to the minimal classification success among otoliths of extant gobioid species based on shape analysis (Lombarte et al. 2018 (72.1%)). If the classification success was $\leq$72% the result was discarded and the otolith groups were newly arranged, while keeping similarities that are undetectable by the method like structures in the sulcus, dorsal margin and the ventral line in mind. Only when the MANOVA results indicated an overall classification success of >72%, the newly arranged groups were accepted. Further rearrangements based on the same criteria were processed until the overall classification success for the entire dataset could not be increased further without fusing otolith groups with clear differences of their sulcus details. The resulting groups were then treated as suggestions for species (for details see discussion). For detecting shared taxonomic groups between Kienberg and Vöslau, visually similar groups were tested against each other utilizing again MANOVA ($p \leq 0.05$).

As the Fourier outline analysis considers only the otolith outline, the sulcus shape was examined separately. Based on the groups that were confirmed by MANOVA, the following measurements were taken following Gierl et al. (2018): otolith height (OH), otolith length (OL), sulcus height (SuH), sulcus length (SuL), sulcus tip (SuTip) and sulcus end (SuEnd). Out of these the OL/OH, SuH/SuL and SuTip/SuEnd indices were acquired. In addition, two measurements are newly introduced: TED ("tip of sulcus to end of sulcus diagonal"), which connects the starting points of sulcus tip and sulcus end. And OSH ("ostial sulcus height"), which is an orthogonal linear projection upon TED, with each end defined by a TED-parallel tangent, that touches the ostium dorsally and ventrally, respectively (see Fig. 2b). Measurements were taken utilizing ImageJ2 (Rueden et al. 2017). Out of these two measurements the new index OSH/TED was calculated. The number of measured specimens for index calculation can differ from the stated number used for Fourier shape analysis as in cases where the sulcus was not unambiguously depicted, no index measurements were acquired due to comparability between the violin plots of OSH/TED and OL/OH. Otolith nomenclature and distances of measurements of OL, OH, TED and OSH are given in Fig. 2.

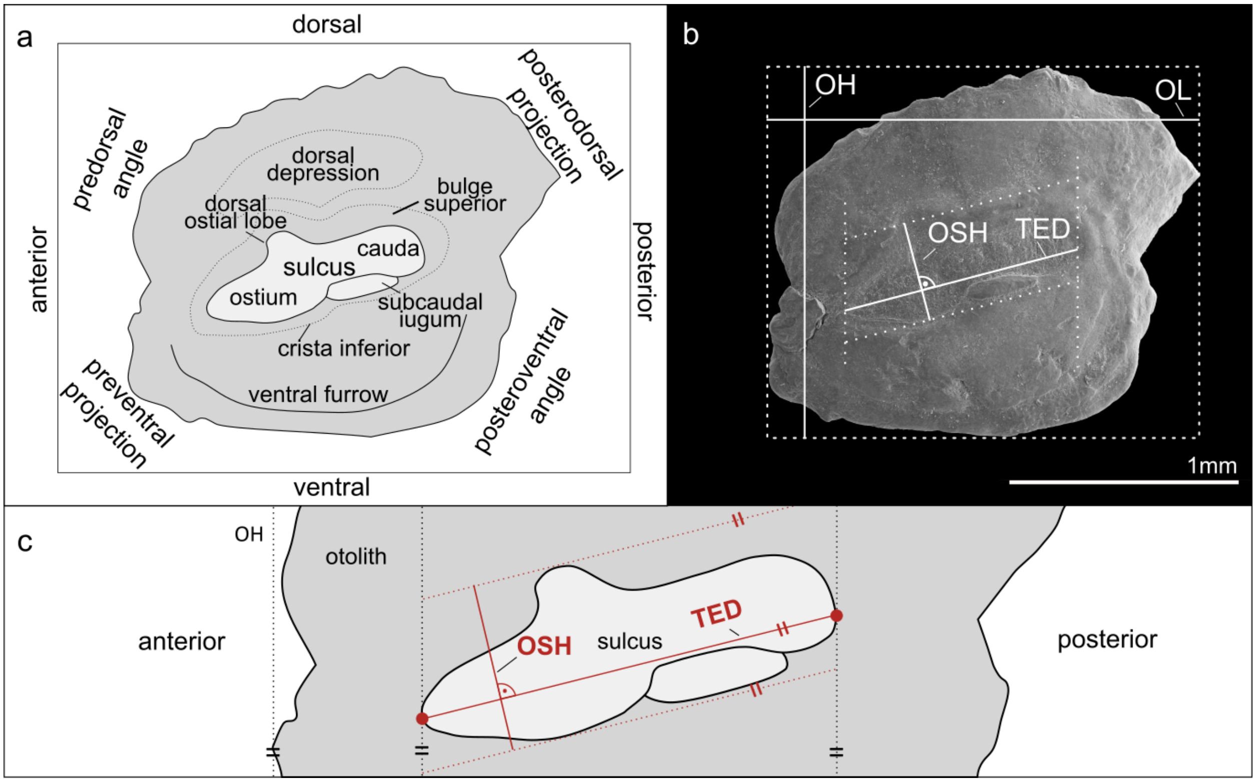


Figure 2: **a** Otolith nomenclature following Nolf (1985), Schwarzhans (2014) and Lombarte et al. (2018); **b** measurements following Gierl et al. (2018), conducted on mirrored *Gobius mustus*, Kienberg (L). Measurement abbreviations: OL, otolith length; OH, otolith height; TED, sulcus tip to sulcus end diagonal; OSH, ostial sulcus height. **c** Detailed schemata for constructing TED and OSH on a closeup of *G. mustus*.

Otolith nomenclature follows Nolf (1985); Tuset et al. (2008); Gierl et al. (2013); Schwarzhans (2014) and Lombarte et al. (2018). The term “bulge superior” is introduced for describing the thickened structure separating the dorsal depression from the dorsal part of the sulcus.

For descriptive comparison the different indices were respectively plotted as a violin plot. The jitter function was used, that added random noise in order to scale down datapoint overlaps. Within a group a tolerance of 0.2 from the median was allowed before the group was considered heterogeneous.

The recognized groups of this study were considered to represent a species (see also discussion section). They are described and compared to previously described species in the systematic section of this paper.

All photographs and .jpg images in this study were prepared using Photoshop CS6 (version 13.0). Plots were all generated in R (version 3.6.3). Vector graphics were compiled or edited using Inkscape (version 0.92).

3.5 Results

OH/OL and OSH/TED turned out to be very useful in describing the sulcus, as in contrast to other indices (like SuL/SuH, SuTip/SuEnd), it is less influenced by the rotation of the otolith or the inclination of the sulcus. Due to this, the less reliable data of SuL/SuH, SuTip/SuEnd is not shown in this study. As most retrieved fossil otoliths display an OL/OH <1, they can be easily distinguished from modern *Gobius* otoliths (OL/OH >1). Additional similarities of the fossil otoliths of this study like the sulcus, the subcaudal iugum ect. can be observed in genera like *Pomatoschistus* or *Lesueurigobius*.

The new approach presented herein supports the presence of seven species in the sample of Kienberg (MANOVA, $p \leq 0.05$, Fig. 3a). *Lesueurigobius harzhauseri* sp. nov. is the dominating species in this set (49.6%) and †*Paromiogobius johnsonae* gen. et sp. nov. is the rarest (3.5%) (Fig. 4a). The sample from Vöslau comprises three species (Fig. 3b) with *Lesueurigobius magniiugis* Schwarzhans, 2017 being the most abundant (71.4%) and *L. annajanae* sp. nov. the rarest (6.3%, Fig. 4a). The violin plots of OL/OH and OSH/TED give additional support to these groups as their outline and sulcus proportions are considered to be intraspecifically homogenous, as no great deviations from the median (≤ 0.2) were detected (Fig. 5). The violin plot additionally shows the consistent differences in OH/OL between lineages, as the *Gobius* lineage (represented in this study by *Gobius* and *Thorogobius*) show higher OH/OL values than the members of the *Aphia* lineage (represented in this study by *Lesueurigobius* and probably †*Paromiogobius*).

Figure 3: Results of the MANOVA analysis based on elliptic Fourier descriptors. 1, significant difference between groups (p≤0.05); 0, no significant difference between groups (p>0.05) with # being the absolute number of specimens.

a Groups of Kienberg

MANOVA (p≤0.05) Total#: 140

Overall class. success[%]	22 harmonics	*G. mus*	*L. ann*	*L. brz*	*L. har*	*L. mag*	*P. joh*	*T. iuc*	%	#
85.71	*G. mus*		1	1	1	1	1	1	100.0	6
	L. ann			1	1	0	0	1	66.7	9
	L. brz				1	1	1	1	100.0	11
	L. har					1	1	1	100.0	70
	L. mag						0	1	66.7	9
	P. joh							1	66.7	5
	T. iuc								100.0	30

b Groups of Vöslau

MANOVA (p≤0.05) Total#: 63

Overall class. success[%]	22 harmonics	*L. ann*	*L. harz*	*L. mag*	%	#
100	*L. ann*		1	1	100.0	4
	L. harz			1	100.0	14
	L. mag				100.0	45

c Combined groups of Kienberg and Vöslau

MANOVA (p≤0.05) Total#: 203

Overall class. success[%]	22 harmonics	*G. mus*	*L. ann*	*L. brz*	*L. har*	*L. mag*	*P. joh*	*T. iuc*	%	#
85.71	*G. mus*		1	1	1	1	1	1	100.0	6
	L. ann			1	1	0	0	1	66.7	13
	L. brz				1	1	1	1	100.0	11
	L. har					1	1	1	100.0	84
	L. mag						0	1	66.7	54
	P. joh							1	66.7	5
	T. iuc								100.0	30

d Visually similar groups of Kienberg and Vöslau

MANOVA (p≤0.05)

22 harmonics	Kienberg	Vöslau	p-Value	#K	#V
	L. ann (K)	*L. ann* (V)	0.33451	9	4
	L. harz (K)	*L. harz* (V)	1E-05	70	14
	L. mag (K)	*L. mag* (V)	0.07815	9	45

The two sample sites are clearly distinguishable from one another, due to their species composition and the amount of their representatives (see Fig. 4a). An exception here is *Lesueurigobius annajanae* sp. nov., which was observed in almost equal percentages at both localities.

Comparing the species composition between Kienberg and Vöslau, the MANOVA outcome indicates the presence of two shared species; namely *Lesueurigobius annajanae* sp. nov. and *L. magniiugis* (Fig. 3d). *Lesueurigobius harzhauseri* sp. nov. from Vöslau (V) is classified as significantly different from *L. harzhauseri* sp. nov. from Kienberg (K) (MANOVA (p≤0.05). However, visual inspection shows that all otoliths of *L. harzhauseri* sp. nov. (V) share strong similarities with many individuals of *L. harzhauseri* sp. nov. (K) and that *L. harzhauseri* sp. nov. (V) seems to represent a morphological subset of *L. harzhauseri* sp. nov. (K). Due to their resemblance, they are considered here as a single species. This observation is also supported by the OSH/TED index, which shows a common distribution of sulcus shapes across the specimens from Kienberg and Vöslau (see Fig. 5b). Consequently, the three species recognized from Vöslau in this study can also be found in Kienberg.

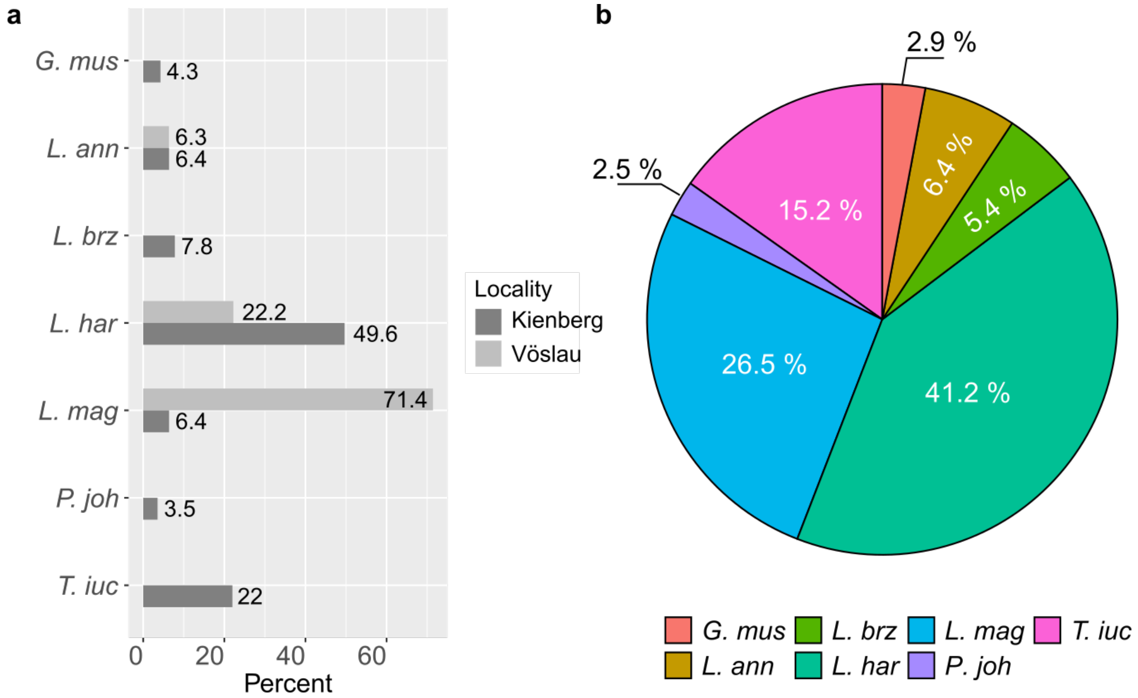


Figure 4: **a** Barplot showing the quantitative species distribution within and between sample sites [%]. **b** Pie chart showing the overall quantitative species distribution across sample sites [%].

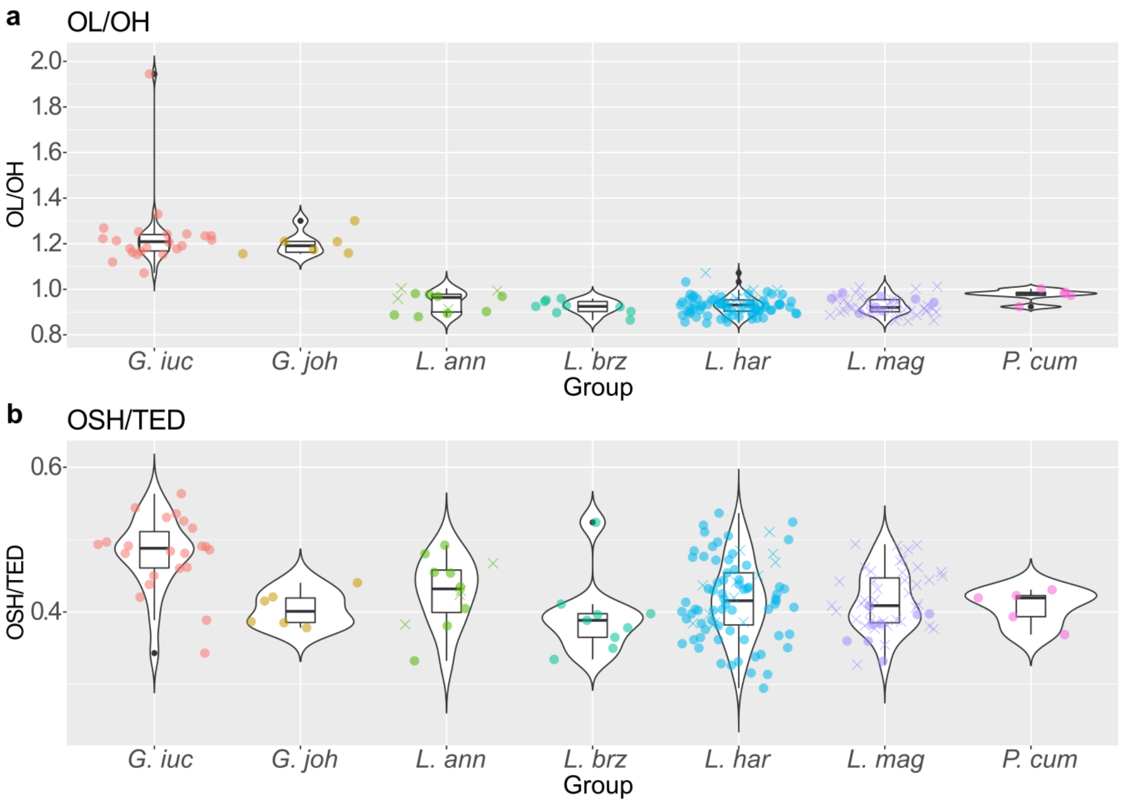


Figure 5: Violinplot showing the distribution of **a** OL/OH and **b** OSH/TED across MANOVA supported groups ($p \leq 0.05$) with an applied random left and right jitter for improved overview. Integrated boxplots show the median (line in box), with 25th and 75th percentiles (bottom and top of the box respectively).

Regarding the entire material of this study, *Lesueurigobius harzhauseri* sp. nov. is the most common goby species (41.2%) with its highest distribution detected in Kienberg (Fig. 4b). With 2.5%, †*Paromiogobius johnsonae* gen. et sp. nov. is the rarest species over the whole sample set, closely followed by *Gobius mustus* Schwarzhans, 2014 with 2.9%, both only known from Kienberg.

3.6 Systematic Paleontology

If not indicated otherwise the following descriptions are valid for every otolith in this study: the dorsal margin is convexly ascending with the maximum height located behind the midline of the otolith. The incision of the anterior margin is positioned below the incision of the posterior margin. The ventral margin is straight or slightly convex.

Order **Gobiiformes** Betancur-R et al., 2017
Suborder **Gobioidei** Jordan and Evermann, 1896
Family **Gobiidae** Cuvier, 1816

Remark. According to phylogenetic studies (e.g. Agorreta et al. 2013; Thacker 2013, 2015) the family Gobiidae, can be distinguished in two major clades, which were also accepted in Nelson et al. (2016). This study follows the nomenclature suggested by Nelson et al. (2016) and refers to the two clades as Gobiidae and Oxudercidae.

Genus *Gobius* Linnaeus, 1758

Type species. Gobius niger Linnaeus, 1758
Gobius mustus Schwarzhans, 2014
Figure 6 a–c

*1906 *Otolithus* (*Gobius*) *pretiosus* Proch. – Schubert: p. 65, pl. 6, fig. 31 (non figs. 29-30).
1943 *Gobius pretiosus* Prochazka – Weiler: p. 231, pl. 4, fig. 25 (non pl.4, fig. 27; non pl. 8, fig. 62).
1966 *Gobius multipinnatus* (H. von Meyer) – Śmigielska: p. 262, pl. 18, fig. 12 (non figs 13-16).
1973 *Gobius praetiosus* Procházka – Śmigielska: p. 23, pl. 4, figs. 6-7 (non figs. 3-5).
1981 *Gobius*? *pretiosus* Koken (sic!) – Rado: pl. 3, fig. 8 (non figs. 9, 12).
1989 ?*Gobius* cf. *multipinnatus* (H. v. Meyer, 1852) – Brzobohatý: p. 44, pl.3, fig. 10.
?2007 *Gobius multipinnatus* (von Mayer, 1852) – Brzobohatý et al.: p. 195, pl. 8, fig. 6, 8 (non fig. 7).
2014 *Gobius mustus* n. sp. – Schwarzhans: p. 52, pl. 8, figs. 4-16.
2015 "*Gobius multipinnatus*" (H. v. Meyer) *sensu* Brzobohatý et al., 2007 – Gierl and Reichenbacher: p. 801, figs. 6A, 6B (?), 6C.
2017 *Gobius frici* Procházka, 1900 – Schwarzhans: p. 175, pl. 3, fig. 5.
2017 *Gobius mustus* Schwarzhans, 2014 – Schwarzhans, Ahnelt, Carnevale, Japundžić, Bradić and Bratishko: p. 60, Fig. 7g-h.
2020 *Gobius mustus* Schwarzhans, 2014 – Schwarzhans et al.: p. 145, pl. 4, figs. 1-5.

Material. Six sagittae, Kienberg (NHMW 2006z0345/0027).

Locality and horizon. Vineyard (W slope of Kienberg hill) at Kienberg, sample S14, upper part of the Hrušky Formation (fine-grained calcareous sands of yellowish color) (see Schultz et al. 2010). Middle Miocene, upper Langhian, middle Badenian.

Measurements (n=6). OL = 1.5–3, OL/OH = 1.2–1.3 (1.2±0.05).

Description. Approximately rectangular otoliths with a slightly rhomboidal shaped outline. The ascending dorsal margin is commonly recognizably crenated. The posterodorsal projection expresses a pointy sharp, rounded or flattened tip that is directed horizontally or subhorizontally. Both, the posterior and anterior margin are crenated and have a relatively deep V-shaped incision (∼90°). The posteroventral angle is rounded, the ventral margin faintly convex. There is no or a small preventral projection. The predorsal angle is slightly projecting and broadly rounded. The dorsal depression is shallow and bean shaped. The bulge superior is well defined but thin, it follows most of the sulcus outline. The ventral line is clearly defined and smooth but can be crenated. The subcaudal iugum is prominent, narrow and boarders about $\frac{3}{4}$ of the caudal length. The sulcus is relatively elongate. The cauda and ostium are similar in length but can exceed the ostium and widens towards its end. The ostium is rounded ventrally and possesses a pointy tip. The dorsal ostial lobe is wide and rounded and slightly inclined anteriorly.

Discussion. With its rectangular shape, and its well-developed predorsal angle and posterodorsal projection this species is conforming to the depicted specimens in Schwarzhans et al. (2020, pl. 4, figs. 1-5).

The literature dealing with the Badenian reports the commonly occurring but not yet renamed species formerly known as "*Gobius multipinnatus*" (H. v. Meyer, 1851) and "*G. pretiosus*" Procházka, 1893 (see Gierl & Reichenbacher 2015 for summary on this topic). Many studies list "*G. multipinnatus*" from the Central Paratethys. For example Brzobohatý et al. (2007) reports "*G. multipinnatus*" otoliths from Kienberg, however, the specimens examined differ from the *in situ* otoliths of the "*G. multipinnatus*" (now *Eleogobius gaudanti*) reported by Weiler (1955) in many aspects (sulcus shape, posterodorsal projection, subcaudal iugum), making this assignment doubtful and the actual *G. multipinnatus* remains unidentified (see Gierl & Reichenbacher 2015 for details).

Comparing *G. mustus* to otoliths described as "*G. multipinnatus*" from the Central Paratethys by Śmigielska (1966), Brzobohatý (1989) and Brzobohatý et al. (2007) reveals many similarities. Schwarzhans et al. (2020) recently reassigned specimens from Śmigielska (1966) and putatively specimens of Brzobohatý et al. (2007). "*G. multipinnatus*" specimens depicted in Brzobohatý et al. (2007, pl. 8, fig. 6, 8) have a similar general outline especially in their posterodorsal projection. Strong resemblances in Brzobohatý et al. (2007, pl. 8, fig. 8) can be seen in the elongated sulcus and the small and narrow subcaudal iugum. Therefore, this study agrees with Schwarzhans et al. (2020) and assigns the two specimens of "*G. multipinnatus*" depicted in Brzobohatý et al. (2007) to *G. mustus*.

Depictions of "*G.* cf. *multipinnatus*" in Brzobohatý (1989; pl. 3, figs.9-12) differ in the clearly shorter sulcus (Brzobohatý 1989; pl. 3, figs.9, 11 and 12). However, a strong resemblance to *G. mustus* is recognizable in depiction 10, which is very similar in its outline shape, sulcus expression, subcaudal iugum and the morphology of the dorsal ostial lobe. Therefore, this study also assigns the depicted "*G.* cf. *multipinnatus*" in Brzobohatý (1989; pl. 3, figs. 10) to *G. mustus*.

The problematic of "*G. multipinnatus*" cannot be fully resolved in this study, however, many specimens of the formerly known "*G. multipinnatus*" could be assigned to *G. mustus*. Remaining specimens will have to be examined also for their affinities to the highly debated "*G. pretiosus*" and assigned to their proper species based on future studies. At the moment "*G. pretiosus*" remains due to its ambiguous diagnostic drawing (see Procházka 1893) a *nomen dubium* (see Gierl& Reichenbacher 2015, Schwarzhans et al. 2020). The discussion about "*G. pretiosus*" remains open, as this study did not find similarities between "*G. pretiosus*" and the retrieved samples.

Occurrences. Kienberg, Czech Republic, Middle Miocene (Badenian): this study, Brzobohatý et al. (2007), Gierl & Reichenbacher (2015), Schwarzhans et al. (2020). Romania; Langhian (Badenian): Rado (1981). Romania, Middle Miocene (Badenian): Schwarzhans (2017). Romania, Miocene: Weiler (1943). Austria; Lower Eggenburgian?: Brzobohatý (1989). Austria, Miocene: Schubert (1906). Southern Poland, Middel Miocene (Badenian): Radwańska (1992). Poland, Tortonian: Śmigielska (1966, 1973). Karaman Basin, Turkey, Middle Miocene (Serravallian): Schwarzhans (2014). Turkey, Serravallian: Schwarzhans et al. (2017).

Genus *Lesueurigobius* Whitley, 1950

Type species. Gobius suerii Risso, 1810

Remark. Several fossil species of *Lesueurigobius* have previously been described from the Miocene, i.e. *Gobius laevis* (Weiler, 1942; reassigned to *Lesueurigobius* by Schwarzhans 2010), *L. magniiugis* (in Schwarzhans et al. 2017, 2020), *L. suerii* (Risso, 1810) (in Schwarzhans 2014), *L. vicinalis* (Koken, 1891) (in e.g. Schubert 1906), *Lesueurigobius* sp. (in Schwarzhans 2010). Among those, most commonly reported from the Paratethys and Mediterranean is *Lesueurigobius vicinalis* and has also been described from Vöslau (Schubert 1906; Radwańska 1992; Koken, 1891). However, *L. vicinalis* comprises a variable group of otoliths and therefore has been interpreted as species complex that may include two or three closely related species (Reichenbacher & Cappetta 1999). The unique characters of otoliths of *L. vicinalis* according to Koken (1891) are the even curvature, deep dorsal depression, well defined ventral furrow and a smooth surface. A further inspection of the holotype of *L. vicinalis* reveals an almost squared otolith (OL/OH = 0.95) (see also Fig. 7f or Schwarzhans et al. 2020, pl. 2, fig. 5). Its dorsal margin is rounded and shows a notch just before the middle of the dorsal margin. The posterodorsal projection is rounded and slightly flattened, the posterior margin possesses an U-shaped incision, the posteroventral angle is broadly rounded and slightly more extending than the posterodorsal projection, the ventral margin is slightly convex, and a projecting preventral projection is missing. The anterior margin possesses a shallow (>120°) incision. The predorsal transition is rounded and slightly bulged. The dorsal depression is bean shaped and distinct. The bulge superior is narrow and well recognizable.

The dorsal ostial lobe is pointy and not tilted. The ventral furrow is smooth and well defined. The subcaudal iugum is not well preserved but appears to be elongated and narrow. The sulcus displays the typical shoe sole shape of gobioid otoliths and is placed centrally. The sulcus is clearly deepened, the length of the cauda slightly exceeds the length of the ostium (4:3). The junction between cauda and ostium is clearly visible. The cauda is noticeably bended upwards. The ostium possesses a well-rounded ventral side that ends in a sharp edge at the junction. The posterior- and ventral side of the ostium is well rounded and appears to be slightly pointing dorsally.

Lesueurigobius annajanae sp. nov.
Figure 6 d–i
Etymology. In honor of the author's two second cousins Anna and Jana.

Holotype. Left sagitta (NHMW 2006z0345/0019).

Paratypes. Three left sagitta (NHMW 2006z0345/0020, NHMW 2006z0345/0021, NHMW 1995/00230008). Two right sagitta (NHMW 1995/00230009, NHMW 1995/00230010).

Further material. Six sagitta, Kienberg (NHMW 2006z0345/0028) and one sagitta, Vöslau (NHMW 1995/002300014).

Locality and horizon. Vineyard (W slope of Kienberg hill) at Kienberg, sample S14, upper part of the Hruška Formation (fine-grained calcareous sands of yellowish color) (see Schultz et al. 2010). Middle Miocene, upper Langhian, middle Badenian.

Diagnosis. Voluminous, almost square or rectangular otolith. Depression in dorsal margin. Shallow dorsal depression. Subcaudal iugum present and bean shaped. Otolith height increases from anterior to posterior.

Measurements (n = 12). OL = 1.4–2.8; OL/OH = 0.9–1 (0.94±0.05).

Description. Voluminous otoliths of almost quadratic shape. The dorsal margin shows a concave curvature, which in some specimens, is developed to great extends. The posterodorsal projection is short, flattened and faces dorsally or horizontally. The incision of the posterior margin is smooth but V-shaped (>120°). The posteroventral angle is rounded or flattened. The preventral projection is broad, smooth but can be flattened in some specimens. The anterior margin is smoothly concave with an U-shaped incision (>120°). The predorsal angle is broadly rounded and slightly exceeding the preventral projection.

The dorsal depression is characterized by a remarkably shallow oval imprint. The bulge superior is well defined with a noticeable thinning at the ostium-cauda-intersection. The ventral furrow is well defined and can be slightly lobed. The subcaudal iugum, if present, is of elliptical shape and of moderate size.

The sulcus expresses the gobioid shoe sole shape but can be slightly elongated in some specimens. It is placed centrally and can be slightly inclined. The sulcus is deeply impressed and overall well defined. The ostium is larger than the cauda and no widening or bending can be seen in the cauda towards its posterior end. The ostium displays a remarkably rounded ventral side and a pointy anterior tip. The dorsal ostial lobe is broadly rounded and not inclined.

Discussion. According to the MANOVA ($p \leq 0.05$) great similarities in the outline can be seen in *Lesueurigobius magniiugis* and †*Paromiogobius johnsonae* gen. et sp. nov. However, visual inspection could not confirm this similarity, as the two groups differ from *L. annajanae* sp. nov. in the sculpture of the otolith margins (crenated in †*P. johnsonae* gen. et sp. nov. and lobed in *L. magniiugis*) as well as in the volumes of posterodorsal projections and predorsal angle (pointy posterodorsal projection and predorsal angle in †*P. johnsonae* gen. et sp. nov.; posterodorsal projection less in volume and predorsal angle not projecting in *L. magniiugis*). The analysis outcome might be a result due to the small sample size of *L. annajanae* sp. nov. and †*P. johnsonae* gen. et sp. nov. For further similarities between groups within the sample set see the discussion of *L. magniiugis*. The otolith with the closest resemblance to *L. annajanae* sp. nov. is the extant and fossil *Lesueurigobius suerii* in Lombarte et al. (2006) and Schwarzhans et al. (2020, pl. 2, fig. 4). The similarities are the squared voluminous shape, the noticeable dorsal depression and the general and sometimes elongated shoe sole like sulcus. However, great differences can be seen in the posterodorsal projection of *L. suerii* which in contrast to *L. annajanae* sp. nov. is rounded or noticeably pointy but never flattened (see Schwarzhans 2014, Plate 11, figs. 1-3). Also, the orientation of the posterodorsal projection in *L. annajanae* sp. nov. is generally directed upwards, whereas in *L. suerri* the posterodorsal projection faces horizontally and appears to be less voluminous. *L. annajanae* sp. nov. also resembles very vaguely a specimen of *Gobius rudis* Schwarzhans (2010; Plate 102, 4b) but differs despite of the depression in the dorsal rim strongly from it.

Occurrences. Kienberg and Vöslau, Middle Miocene (Badenian).

Lesueurigobius brzobohatyi sp. nov.
Figure 6 j–l

1992 "genus *Gobiidarum*" sp. 2 – Radwańska: p. 291, pl. 35, Fig. 9 (non fig. 10).

Etymology. In honor of Prof. RNDr. Rostislav Brzobohatý, Muni Masaryk University, Czech Republic, for his great contributions to our knowledge of gobiid otoliths.

Holotype. Left sagitta (NHMW 2006z0345/0010).

Paratypes. Two left sagitta (NHMW 2006z0345/0011, NHMW 2006z0345/0012).

Further material. Eight sagitta, Kienberg, (NHMW 2006z0345/0025).

Locality and horizon. Vineyard (W slope of Kienberg hill) at Kienberg, sample S14, upper part of the Hrušky Formation (fine-grained calcareous sands of yellowish color) (see Schultz et al. 2010). Middle Miocene, upper Langhian, middle Badenian.

Diagnosis. Rectangular or almost squared otolith. Rounded dorsal margin with flattened lobes. Narrow sulcus with narrow cauda. Otolith with shallow dorsal depression. Posterodorsal projection weakly projecting.

Measurements (n=9). OL = 1.5–1.9; OL/OH = 0.9–1 (0.92±0.03).

Description. A rectangular or almost squared otolith with lobed and/or flattened dorsal margin. The posterodorsal projection is rounded, slightly projecting with an ambiguous transition to the dorsal rim and facing dorsally. The posterior margin is broadly V- to U-shaped ($\geq 90°$) with a convex

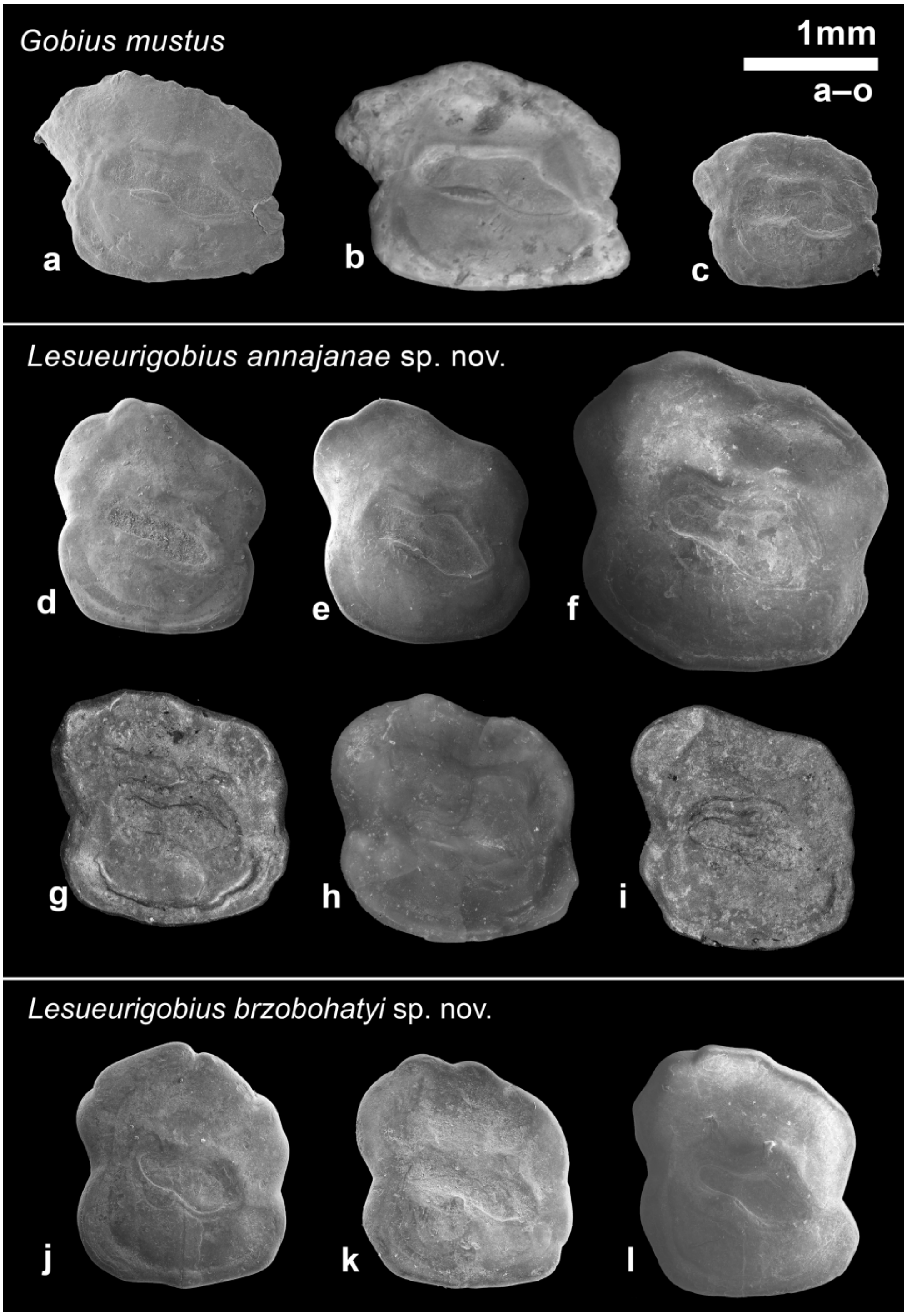


Figure 6: **a–c** *Gobius mustus* Schwarzhans, 2014; **a** specimen 2006z0345/0016, Kienberg, mirrored; **b** specimen 2006z0345/0017, Kienberg, also depicted in Gierl & Reichenbacher (2015, Fig. 6B); **c** specimen 2006z0345/0018, Kienberg. **d–i** *Lesueurigobius annajanae* sp. nov.; **d** paratype, 2006z0345/0021, Kienberg, mirrored; **e** paratype, 2006z0345/0020, Kienberg, mirrored; **f** holotype, 2006z0345/0019, Kienberg; **g** paratype, 1995/00230008, Vöslau; **h** paratype, 1995/00230009, Vöslau; **i** paratype, 1995/00230010, Vöslau. **j–l** *L. brzobohatyi* sp. nov.; **j** holotype, 2006z0345/0010, Kienberg; **k** paratype, 2006z0345/0011, Kienberg; **l** paratype, 2006z0345/0012, Kienberg.

and a concave side. The posteroventral angle is broadly rounded and slightly bended downwards or facing horizontally. The preventral projection is flattened or rounded. The anterior margin is smooth showing a V-shaped incision (≥90°) with one straight and one convex side. The predorsal angle is weakly projecting and can display a lobed sculpture of the margin in some cases.

A dorsal impression is barely expressed or missing. The bulge superior is moderately expressed but partially ambiguous in shape. The ventral furrow is well defined and smooth. The subcaudal iugum is drop shaped but subtle in expression.

The typical gobiid sulcus is located in the center of the otolith. It is short, shallow and slightly inclined. The cauda is almost as large as the ostium. The cauda is bended upwards prominently and is commonly rather narrow. The ostium is ventrally broadly convex without clear peak but can also show the common rhomboidal expression in some species. The margin of the dorsal ostial lobe is ambiguous but shows a wide rounded peak.

Discussion. *Lesueurigobius brzobohatyi* sp. nov. resembles the extant *Lesueurigobius friesii* Malm 1847 (Nolf 2013; Lombarte et al. 2006 (sample site Rijeka); Schwarzhans et al. 2020 (pl. 2, fig. 1), from which it differs in the lower length-height-ratio (0.92 vs. ∼1 (Lombarte et al. 2006) vs. 0.96 (Schwarzhans et al. 2020)) and flattened/broader granulation. It also differs in having a broader ostial lobe and a noticeably shallow dorsal depression.

Occurrences. Kienberg; Middle Miocene (Badenian): this study. Southern Poland (Badenian): Radwńska (1992).

Lesueurigobius harzhauseri sp. nov.

Figure 7 a–e

1994 ? *Gobius* sp. 2 – Brzobohatý: p. 93, pl. 7, figs. 11-13.
2007 ? *Lesueurigobius* aff. *vicinalis* (Koken, 1891) – Brzobohatý et al.: p. 195, figs. 13-14 (non fig. 12).

Etymology. In honor of Priv.-Doz. Mag. Dr. Harzhauser, University of Vienna, Austria, in recognition of his numerous contributions to our understanding of invertebrates and their implications in Neogene and Paleogene sediments.

Holotype. Right sagitta (NHMW 2006z0345/0006).

Paratypes. Three left sagitta (NHMW 2006z0345/0004, NHMW 2006z0345/0005, NHMW 1995/00230003). One right sagitta (NHMW 1995/00230003).

Further material. 67 sagitta, Kienberg, (NHMW 2006z0345/0023) and 10 sagitta, Vöslau, (NHMW 1995/002300012).

Locality and horizon. Vineyard (W slope of Kienberg hill) at Kienberg, sample S14, upper part of the Hrušky Formation (fine-grained calcareous sands of yellowish color) (see Schultz et al. 2010). Middle Miocene, upper Langhian, middle Badenian.

Diagnosis. Rounded otolith with smooth outline. Shallow dorsal depression. Subcaudal iugum present and bean shaped. Posterodorsal projection not strongly developed. Posteroventral angle weakly projecting.

Measurements ($n = 81$). $OL = 1.2–2.6$; $OL/OH = 0.9–1.1$ (0.93 ± 0.04).

Description. The otolith displays an overall rectangular to rounded shape ($OL/OH<1$). The dorsal margin is smooth to broadly lobed. The short, smooth and broad posterodorsal projection is rounded or flattened and faces commonly dorsally or horizontally. The posterior margin is incised (>120°). The posteroventral angle can be rounded, angular or slightly flattened. If the preventral projection is moderately projecting, it can display a smooth or a rounded concavity. The anterior margin shows a shallow to widely U-shaped (>120°) incision. The predorsal angle is smooth, broadly rounded and facing horizontally or slightly ventrally.

The dorsal depression is prominent and oval shaped. The bulge superior is thick rimmed and curved. The ventral furrow is equally curved and moderately prominent. The subcaudal iugum is oval shaped and boarders most of the cauda length as well as the cauda-ostium junction. The sulcus is showing a slightly elongated version of the typical gobioid shape. It is well defined and rather deep. The ostium shows an elongated, roundish anterior peak with the dorsal ostial lobe being broad, smooth and round.

Discussion. According to the MANOVA ($p \leq 0.05$) *Lesueurigobius harzhauseri* sp. nov. shares some similarities with †*Paromiogobius johnsonae* gen. et sp. nov., especially in its overall curved outline. *L. harzhauseri* sp. nov., however, differs noticeably from †*P. johnsonae* gen. et sp. nov. in its up facing short posterodorsal projection. The dorsal rim in *L. harzhauseri* sp. nov. is smoother than in †*P. johnsonae* gen. et sp. nov. and shows frequently a straight expression, whereas in †*P. johnsonae* gen. et sp. nov. the dorsal rim is more convex and lobed. Differences can also be detected in the sulcus as the cauda in †*P. johnsonae* gen. et sp. nov. tends to be more elongated than in *L. harzhauseri* sp. nov.

Outside the sample set the *L. harzhauseri* sp. nov. otoliths share many common features with *Lesueurigobius friesii* (Nolf 2013; Lombarte et al. 2006 sample site Rijeka) which is also known as fossil from Italy (Pleistocene; see Nolf 2013). It shares mainly its overall expression, the dorsal rim and the sulcus position and shape. Slight differences can be seen in the preventral projection which in extant *L. friesii* does not show a pointed, up-bended shape. Also, the dorsal ostial lobe in *L. friesii* is not as well developed as in *L. harzhauseri* sp. nov.

L. harzhauseri sp. nov. also shares some similarities to the variable fossil species *Lesueurigobius vicinalis* (see Fig. 7f). However, in time so many different specimens have been assigned to this species that it is hard to find a common set of characters. The biggest resemblance to *L. harzhauseri* sp. nov. is seen in specimens of Radwańska (1992; Plate 34, figs. 3-6). However, the OL/OH in *L. vicinalis* do not match the higher otoliths of *L. harzhauseri* sp. nov. Also, the otolith margins do not quite fit those of *L. harzhauseri* sp. nov., as they are smoother in their outline with smoother incisions. Additionally, the marginal incisions differ in their shape intensity. Also, as *L. harzhauseri* sp. nov. is not noticably smooth and lacks a deep impression of the dorsal depression, this otolith is not considered to be a member of *L. vicinalis*.

Occurrences. Kienberg, Middle Miocene (Badenian): this study, ?Brzobohatý et al. (2007). ?Gainfarn, Middle Miocene (Badenian): Brzobohatý (1994).

Lesueurigobius magniiugis Schwarzhans, 2017

Figure 7 g–l

1994 ? *Gobius* sp. 1 – Brzobohatý: p. 93, pl. 7, figs. 5-7.
2017 *Lesueurigobius magniiugis* n. sp. – Schwarzhans: pl. 3, fig. 6.
2020 *Lesueurigobius magnijugis* Schwarzhans, 2017 – Schwarzhans et al.: pl. 2, figs. 13-17.

Material. Twelve sagitta, Kienberg, (NHMW 2006z0345/0024) 42 sagitta, Vöslau, (NHMW

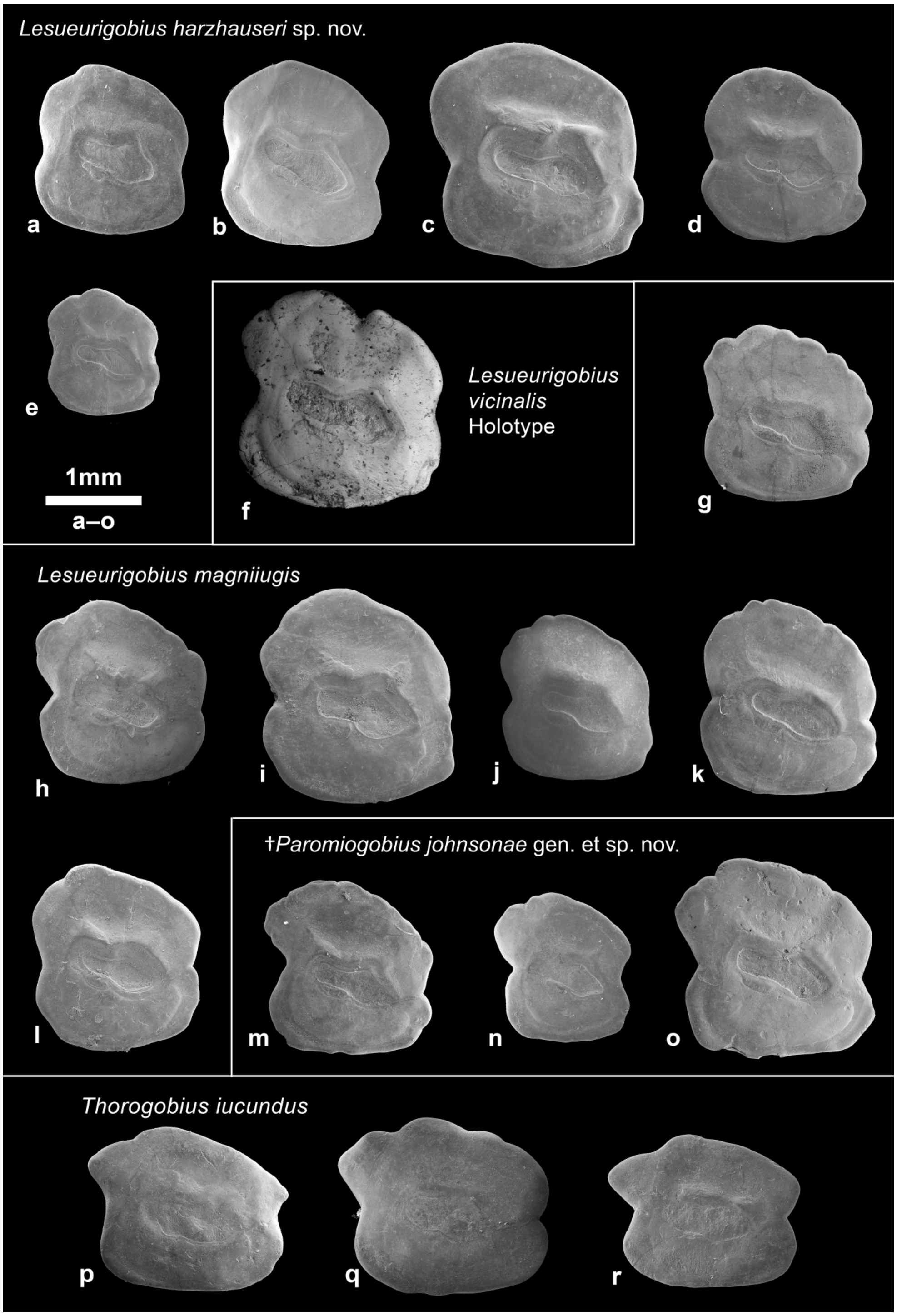


Figure 7: **a–e** *Lesueurigobius harzhauseri* sp. nov.; **a** paratype, 2006z0345/0004, Kienberg, mirrored; **b** paratype, 2006z0345/0005, Kienberg, mirrored; **c** paratype, 1995/00230003, Vöslau; **d** paratype, 1995/00230004, Vöslau, mirrored; **e** paratype, 2006z0345/0006, Kienberg. **f** *L. vicinalis* (Koken 1891), holotype, SMF P2438; **g–l** *L. magniiugis*; **g** specimen 2006z0345/0007, Kienberg; **h** specimen 1995/00230006, Vöslau; **i** specimen 1995/00230005, Vöslau, mirrored; **j** specimen 2006z0345/0009, Kienberg; **k** specimen 2006z0345/0008, Kienberg; **l** specimen 1995/00230007, Vöslau, mirrored. **m–o** †*Paromiogobius johnsonae* gen. et sp. nov.; **m** holotype, 2006z0345/0001, Kienberg; **n** paratype, 2006z0345/0002, Kienberg, mirrored; **o** 2006z0345/0003, Kienberg; **p-r** *Thorogobius iucundus* Schwarzhans 2014; **p** specimen 2006z0345/0013, Kienberg; **q** specimen 2006z0345/0014, Kienberg, mirrored. **r** specimen 2006z0345/0015, Kienberg, mirrored.

1995/002300013).

Locality and horizon. Vineyard (W slope of Kienberg hill) at Kienberg, sample S14, upper part of the Hrušky Formation (fine-grained calcareous sands of yellowish color) (see Schultz et al. 2010). Middle Miocene, upper Langhian, middle Badenian.

Diagnosis. Otolith with consistent, curved transition from anterior rim to dorsal rim. Otolith height increasing towards the posterior. Posterodorsal projection weakly projecting.

Measurements (n = 47). OL = 1.4–2.6; OL/OH = 0.9–1 (0.93±0.04).

Description. The otolith shows a smoothly bended transition from dorsal margin to anterior margin, but can show square-shaped tendencies in its outline. The dorsal margin is lobed or almost smooth. The posterodorsal projection is flattened, lobed or rounded, facing upwards. The posterior margin is smooth with a V-shaped incision ($\geq$120°). The posteroventral angle is smooth, broad and flattened. The preventral projection is lobed with a U-shaped incision at the bottom line, generally facing ventrally. The anterior margin is smooth and lobed in some specimens with a V-shaped incision ($\sim$90°) and convex sides. The predorsal angle is lobed with an ambiguous transition to the anterior margin.

The dorsal depression is remarkably shallow or not expressed. Also, the bulge superior is only weakly present. The ventral furrow is weakly expressed and can be partially ambiguous. The subcaudal iugum is narrow, bean shaped and boarders most of the cauda. The sulcus can be elongated but follows the typical gobioid shoe sole shape. It is located in the center and is slightly inclined in some specimens. The cauda is prominently long without widening towards the tip; however, it is sometimes inclined posteriorly. The ostium is of common gobioid shape. The dorsal ostial lobe's peak is rounded and no remarkable inclination is recognizable.

Discussion. According to the MANOVA ($p \leq 0.05$) *Lesueurigobius magniiugis* is not separable from *L. annajanae* sp. nov. and †*Paromiogobius johnsonae* gen. et sp. nov. However, it is distinguishable from †*Paromiogobius johnsonae* gen. et sp. nov. in its slightly broader and more compact overall shape. Another noticeable difference can be seen in the weaker incised anterior and commonly straight rim. Also, the posterodorsal projection is noticeably weaker expressed in

L. magniiugis than in †*P. johnsonae* gen. et sp. nov. In its compact shape *L. magniiugis* also shows similarities to *L. annajanae* sp. nov., however, can be separated from it by its convex dorsal margin and rounder predorsal angle. Additionally, the posterodorsal projection of *L. magniiugis* is less developed in mass compared to *L. annajanae* sp. nov.
The morphology of *L. magniiugis* is conform to those of the depicted otoliths in Schwarzhans et al. (2020, pl. 2, figs. 13-17). They share a higher than wide outline, the short but well developed subcaudal iugum and an ambiguous transition of predorsal angle to dorsal rim.

Occurrences. Kienberg and Vöslau, Middle Miocene (Badenian): this study, Brzobohatý (1994), Niskowa, Early Badenian: Schwarzhans et al. (2020). Týnec, Late Badenian: Schwarzhans et al. (2020).

Genus †*Paromiogobius* nov.

Type species. †*Paromiogobius johnsonae* gen. et sp. nov.
Etymology. From the greek word "*paromios*" for "similar" due to its resemblance to species of *Gobius.*

Diagnosis. A fossil genus based on moderately sized otoliths, probably belonging to the family Gobiidae. They can display an OL/OH of 0.9–1.0. The overall shape is curvy with a projecting preventral projection, exceeding commonly the predorsal angle. The sulcus shows the common shoe sole shape with a narrowed cauda-ostium junction. The sulcus possesses a well-defined dorsal ostial lobe. The subcaudal iugum is present and boarding most of the cauda.

Discussion. So far †*Paromiogobius* nov. is monospecific. The composition of a curved outline with arching dorsal rim and regular lobing, projecting of preventral and posterodorsal projection and the moderately well impressed dorsal depression differs from all fossil and extant European gobioid fossils (e.g. Nolf 2013; Lombarte et al. 2006; Gut et al. 2020). A similar curved outline can be seen in the otolith of *Amblyeleotris radwanskaae* Schwarzhans 2010 (see Schwarzhans 2010, pl. 100, fig. 2; Nolf 2013, pl. 319), however, *A. radwanskaae* is noticeably higher than wide with the preventral projection being only weakly expressed. Additionally, its posteroventral angle is weakly developed and almost vertical. So also, in other extant species of *Amblyeleotris* (*A. aurora* Polunin & Lubbock, 1977, *A. steinitzi* Klausewitz, 1974; see Lombarte et al. 2006) where the curved outline is not as dominant. Therefore, †*Paromiogobius* nov. is considered to be not a part of this genus. A similar lobing is known from the genus *Deltentosteus* (e.g. *D. quadrimaculatus* Valenciennes, 1837, *D. collonianus* Risso, 1820; see Lombarte et al. 2006), or *Lesueurigobius* (e.g. *L. sanzi*, *L. friesii*; see Lombarte et al. 2006), however, in both species neither the overall shape, their OL/OH nor the sulcus expression resemble †*Paromiogobius* nov. in any way.

The otolith morphology of †*Paromiogobius* nov. resembles more otoliths from species of the extant *Aphia*-lineage (*Aphia*, *Lesueurigobius*) and due to its well developed sulcus and higher than wide proportions (OL/OH≤1) expecially *Lesueurigobius* than members of the *Pomatoschistus*-lineage (e.g. *Buenia*, *Deltentosteus*, *Economidichthys*); (see Thacker 2015, see Lombarte et al. 2006; Lombarte et al. 2018; Schwarzhans et al. 2020), which are most of the times more rounded or squared in shape. Therefore, †*Paromiogobius* nov. is assumed to belong more likely to the *Aphia*-lineage.

†*Paromiogobius johnsonae* gen. et sp. nov.

Figure 7 m–o

2020 *Lesueurigobius vicinalis* (Koken, 1891) – Schwarzhans et al.: p. 141, pl. 2, figs. 8-10. (non fig. 6,7,11,12)

Etymology. In honor of the mathematical genius Katherine G. Johnson (*26.08.1918 †24.02.2020) for her great contributions at NASA and her impact on equality in science, work life and society.

Holotype. Left sagitta (NHMW 2006z0345/0001).

Paratypes. Right sagitta (NHMW 2006z0345/0002), left sagitta (NHMW 2006z0345/0003).

Further material. Two sagitta, Kienberg, (NHMW 2006z0345/0022).

Locality and horizon. Vineyard (W slope of Kienberg hill) at Kienberg, sample S14, upper part of the Hrušky Formation (fine-grained calcareous sands of yellowish color) (see Schultz et al. 2010). Middle Miocene, upper Langhian, middle Badenian.

Diagnosis. Otolith with strongly ascending dorsal rim that arches behind midline. Dorsal rim weakly lobed. The dorsal depression is moderately impressed. Subcaudal iugum narrow and elongated.

Measurements (n = 5). OL = 1.4–2.1; OL/OH = 0.9–1 (0.97±0.03).

Description. The otolith displays a dorsally arching, curved outline with granulated rims of varying intensity. The peak of the dorsal rim is clearly defined but can be flattened in some species. The posterodorsal projection faces ventrally or horizontally with a pointy or slightly flattened peak. The posterior margin is incised in a trapezial or V-shape (>90°). The posteroventral angle and the posterodorsal projection are almost equally projecting. However, the posteroventral angle is rounder than the posterodorsal projection and is more voluminous in shape. The preventral projection is bended upwards or barely present and can exceed the dorsal projection slightly. The anterior margin is incised in a V-shape (∼90°). The predorsal angle is located lower than the posterodorsal projection and rounded, pointing horizontally or slightly dorsally.

The dorsal depression is elliptical with a wide depression. The bulge superior is prominently to moderately expressed. The ventral furrow is well defined and equally bent with a tighter curve on the anterior side. The subcaudal iugum is bean or waterdrop shaped and narrows towards the posterior side. The sulcus is well defined, located in the lower center of the otolith and in some cases slightly inclined. The cauda is slightly shorter than the ostium, long and rounded without much widening posteriorly. The ostium peaks anteriorly with its anterior margin slightly convex. The dorsal ostial lobe is a smooth rounded peak without noticeable inclination.

Discussion. According to the MANOVA ($p \leq 0.05$) high similarities of †*Paromiogobius johnsonae* gen. et sp. nov. to other erected groups of this study can be seen in *Lesueurigobius magniiugis* and *L. annajanae* sp. nov. For detailed comparison see the discussion sections of the respective species.

The otoliths of †*P. johnsonae* gen. et sp. nov. share some similarities with *Proterorhinus vasilievae* Schwarzhans et al. (2015, Fig. 8, figs. 2-5) of late Badenian/early Serravallian age from Serbia. The most striking resemblance between these groups is the overall curved outline. Also, the OL/OH ratio is similar. However, the sculpture of the otolith margins of †*P. johnsonae* gen. et sp. nov. and *Pr. vasilievae* differs noticeably. The margin sculpture in *Pr. vasilievae* is pictured to be smooth, or irregular, whereas the sculpture of the margin in †*P. johnsonae* gen. et sp. nov. is quite regularly lobed or almost smooth. Another difference in *Pr. vasilievae* to †*P. johnsonae* gen. et sp. nov. is the dominating almost vertical anterior rim. Therefore, an assignment to this extant genus should be considered cautiously and a fossil genus is introduced.

The depicted specimens of *Lesueurigobius vicinalis* in Schwarzhans et al. (2020, pl. 2, figs. 8-10) share stronger similarities to †*P. johnsonae* gen. et sp. nov. than to the *L. vicinalis* holotype due to their overall shape, their shape and size of the subcaudal iugum and the shape of their preventral projection . Therefore, this study reassigns these specimens to the species †*P. johnsonae* gen. et sp. nov.

Occurrences. Kienberg; Middle Miocene (Badenian): this study. Kortynica, Hodonín; Middle Miocene (Badenian) and Holíč; early Sarmatian: Schwarzhans et al. 2020.

Genus ***Thorogobius*** Miller, 1969

Type species. *Gobius ephippiatus* Lowe, 1839

Thorogobius iucundus Schwarzhans, 2014

Figure 7 p–r

*1906	*Otolithus* (*Gobius*) *intimus* Prochazka, 1893 – Schubert: pl. 20, figs. 35, 37 (non Fig. 36).
1992	*Gobius* sp. – Radwańska: p. 289, text-Fig. 144, pl. 34, figs 7-9.
1994	*Gobius* sp. 6 – Brzobohatý: p. 91, pl. 6, figs 17-20.
2007	*Priolepis* sp. – Brzobohatý et al.: p. 195, pl. 8, figs 9-10.
?2009	genus *Gobiidarum* sp. 2 – Nolf and Brzobohatý: p. 353, pl.7, Fig. 10.
2010	*Thorogobius intimus* (Prochazka, 1893) – Schwarzhans: p. 263, pl. 106, figs. 11-13.
2014	*Thorogobius iucundus* n. sp. – Schwarzhans: p. 61, pl. 13, figs. 3-13.
2015	“Otoliths from Kienberg“ – Gierl and Reichenbacher: p. 801, Fig. 6D-G.
2020	*Thorogobius iucundus* Schwarzhans, 2014 – Schwarzhans et al.: p. 148, pl. 5, figs. 7-13.

Material. Six otoliths, Kienberg (NHMW 2006z0345/0026).

Description. The shape of *Thorogobius iucundus* is mostly rectangular. The dorsal margin is slightly convex and smoothly lobed. The posterodorsal projection is prominent, smooth and pointy. The posterior margin shows a V-shaped incision (≥120°), otherwise it is straight. The posteroventral angle is broadly rounded or slightly pointy facing horizontally or slightly upwards. The preventral projection is broadly rounded. The anterior margin is smooth and slightly convex, it has a V-shaped incision (∼90°). The predorsal angle is smooth, short and widely rounded.

The dorsal depression is shallow and elliptical. The bulge superior is prominent and thins out at the dorsal ostial lobe. The ventral furrow is weakly or not expressed. The subcaudal iugum is weakly expressed and can also be absent. The sulcus is located in the middle of the otolith, slightly inclined and relatively shallow. Its overall shape is short and broad but the common shoe sole shape of the Gobiidae is recognizable. The short cauda is almost as broad as the ostium and widens towards the end. The ostium is slightly elongated with a well-defined rim on the ventral side which in some cases possesses a pointy ventral peak. The pointy dorsal ostial lobe is wide, U-shaped (≥120°) and does not exceed the cauda dorsally.

Measurements (n=21). OL=1.4–2.5; OL/OH = 1.1–1.3 (1.20±0.06).

Discussion. These otoliths conform to the description of *Thorogobius iucundus* from the middle Miocene (Serravallian) of the Karaman Basin (Schwarzhans 2014) and the depicted specimens (Badenian) from Poland, Ukraine and Czech Republic (Schwarzhans et al. 2020). Images of extant species of this genus are available from the AFORO database (Lombarte et al. 2006, *Thorogobius macrolepis* (Kolombatović, 1891)), Schwarzhans (2014, pl. 13, figs. 1, 2, *Thorogobius angolensis* (Norman, 1935)) and from Schwarzhans et al. (2020, pl. 5, fig. 1, *Thorogobius macrolepis*, fig. 2, *T. ephippiatus* (Lowe, 1839), fig. 3, *T. rofeni* Miller, 1988, fig.4, *T. angolensis*, fig. 5 *T. laureatus* Sauberer, Iwamoto & Ahnelt, 2018, fig. 6, *T. alvheimi* Sauberer, Iwamoto & Ahnelt, 2018). *Thorogobius* shows in many cases a narrow and elongated sulcus. However, a relatively broad and rounded sulcus like in *Thorogobius iucundus* can be seen in *T. alvheimi.*

Occurrences. Kienberg, Czech Republic, Middle Miocene (Badenian): this study, Brzobohatý (1994, as "*Gobius* sp. 6"), Brzobohatý et al. (2007, as "*Priolepis* sp."). Karaman Basin, Turkey, Middle Miocene (Serravallian): Schwarzhans (2014). Wetzelsdorf, Austria, Middle Miocene (Badenian) and Borský Svätý Mikulás, Slovakia (Badenian): Schwarzhans (2010, as "*Thorogobius intimus*"). Southern Poland, Middle Miocene (Badenian): Radwańska (1992, as "*Gobius* sp."). Čáry, Korytnica, Weglinek, Poland, Kúty, Ukranie, Hrŭsky, Lužice, Czech Republic, Middle Miocene (Badenian): Schwarzhans et al. (2020).

3.7 Discussion

A bias by considering only excellently preserved otoliths in this study cannot be excluded. Nevertheless, visual inspection of discarded otoliths suggests no grave changes in the abundance ratios reflected by the excellently preserved otoliths.

3.7.1 The methodology of erecting fossil gobioid species

Gobioid sagitta are easily recognizable due to their shoe sole shaped sulcus (Nolf 2013; Gierl et al. 2018; Lombarte et al. 2018). Differentiating species is, however, much more difficult due to only a few distinguishing characteristics and their intraspecific variability in morphological expressions (see Malz 1978; Schwarzhans 2014; Gut et al. 2020). For these reasons, distinguishing gobioid species, especially in the fossil record requires an experienced eye and a vast knowledge of modern gobioid otoliths. Of great calibration help is the growing record of *in situ* otoliths (see e.g. Gierl & Reichenbacher 2015; Schwarzhans et al. 2017, Bradić-Milinović et al. 2019). Nevertheless, the visual comparison of similar isolated otoliths is prone to subjective influences.

The outline analysis of otoliths is vastly used for stock discrimination (e.g. Campana & Casselman 1993; Farias et al. 2009) and has proven to be a great tool for species differentiation in gobioids (e.g. Lombarte et al. 2018; Gut et al. 2020). This study for the first time applies this method on fossil gobioid otoliths as objective support for the reported species. Even though this approach reduces the subjective influence of the taxonomist, it is not a guarantee to detect all species accurately, as it only considers the outline of the otoliths. Due to this, the shape analysis is suggested to be a great tool for reducing subjective influence, however, it works better in combination with an experienced taxonomist.

3.7.2 Diversity of today's gobiid communities and resulting implications for Kienberg and Vöslau

Extant gobiid species are known to inhabit preferably shallow marine waters (e.g. Miller 2004; Patzner et al. 2011). In such areas gobiid species diversity is generally higher than in deeper waters (see Miller 2004; Brandl et al. 2018). The here studied otoliths revealed a higher diversity at Kienberg (seven gobiid species) than at Vöslau (three gobiid species). This indicates that the preference to occupy shallow habitats in modern gobiid species is congruent with the observed species diversity in Kienberg (seven described species; 60 m deep (Schultz et al. 2010)) and

Vöslau (three described species; 200 m deep (Rögl et al. 2008)). The differences in abundance of species between sample sites may reflect habitat preferences. Schwarzhans et al. (2020) observed *L. magniiugis* from a near-shore environment within the Carpathian range, however, *L. magniiugis* is observed to be a rare species at this locality. Schwarzhans et al. (2020) therefore considers it to be a shallow water-species within *Lesueurigobius*. The rare abundance of *L. magniiugis* in shallow waters is congruent to the findings of this study as *L. magniiugis* is also recognized in Kienberg, however, its abundance is greater in the deeper waters of Vöslau. This might support the idea that this species actually might have preferred deeper waters but also occupied near-shore habitats. Such preferences of extant gobiid species are known, e.g. in *Lesueurigobius friesii*, which occurs in depths of 10-350 m, but seems to be most abundant at depths between 40-150 m (see Wheeler & Stebbing 1978, Nash 1982, Froese & Pauly 2019).

Today, 70 extant species of gobies are known from the Mediterranean Sea (Froese & Pauly 2009). High species diversity is known from the Adriatic Sea (46 species, see Kovačić et al. 2012), with 17 species alone from Crete (Kovačić et al. 2011). Additionally, goby species can also occupy microhabitats that differ only slightly in depth, substrate composition or inclination (Wilkins & Myers 1992; Herler & Patzner 2005; Kovačić & Šanda 2016; Gertzen et al. 2016; Brandl et al. 2018). Thus, a number of seven species from a single site (Kienberg), as revealed in this study, appears to be plausible. Also, Schwarzhans et al. (2020, Fig. 4) schematically noted multiple species co-occurring at a single locality, with for example seven species co-occuring close to the shore in an assumed seagrass environment and three species living together further off the shore in a deep-water terrain.

3.7.3 Known intraspecific variability from extant gobies

The sagitta in extant gobiids can be quite variable within a species (Nolf 1985, 2013; Gut et al. 2020). On the other hand, only slight differences in the sagitta may be present between species (Yu et al. 2014; Lombarte et al. 2018; Gut et al. 2020). Many intraspecific character variations like the projection intensity or the orientation of the preventral angle, the posterodorsal projection or the posteroventral angle observed in extant otoliths (see Nolf 2013, Lombarte et al. 2018; Gut et al. 2020; Sadeghi et al. 2020) can be also seen in the fossil groups, including the groups of this study. Concerning extant *Lesueurigobius*, the AFORO database of Lombarte et al. (2006) reveals that the sagitta shape of *L. friesii* differs strongly between populations from different geographic areas. For example, specimens collected in Croatia display a smooth and rounded outline, whereas specimens from Spain possess a flattened and sculptured dorsal rim (see Lombarte et al. 2006).

A recent study has shown that the gobioid *Istigobius ornatus* (Rüppell, 1830) from heterogeneous habitats could be successfully separated by their otolith shape (Sadeghi et al. 2020). The authors showed that differences in otolith shape between populations are increasing with their geographic distance between populations. However, a geographic variation within the sample sets of Kienberg and Vöslau is rather unlikely as between the localities any evidence of species isolation is missing so far.

The local species diversity observed in this study could have also been affected by the phenomenon of time-averaging, where local organic remains accumulate over a considerable period of time as the sedimentation rate usually is significantly slower than the change of generations and hence remains of multiple specimens and species can occur in a common layer, without necessarily having lived at the same time (see Kidwell 1998).

The sedimentation rate for the deposits at Kienberg and Vöslau is unknown as sedimentation rates during the Badenian are difficult to estimate due to the absence of reliable chronostratigraphic markers (Paulissen et al. 2011). However, Paulissen et al. (2011) estimate for the Vienna Basin a sedimentation rate of 0.43 m/kyr during the early Sarmatian. Using this estimation and a sampled layer thickness of a few centimeters in Kienberg (see Schultz et al. 2010) suggests a rather short duration of 0.2 kyr for the sampled layer to accumulate. This short period of time renders a big effect of time averaging unlikely.

Nevertheless, genetic changes are believed to affect local parts of the otolith (Vignon & Morat 2010). Sadeghi et al. (2020) demonstrated on *Istigobius ornatus* that slight differences in the posterodorsal projection and/or preventral projection can be sufficient for population differentiation in gobiids. However, despite the relatively fast substitution rate in the genome of extant gobiids and its local influence on otolith shape, a big impact on shape changes over this short period of 0.2 kyr seems to be possible but unlikely (see Vignon & Morat 2010; Rüber & Agorreta 2011; Akihito et al. 2016).

An external factor that influences the overall shape, is the environment itself, by different temperatures, oxygen or food availability (see e.g. Morales-Nin 2000; Campana 2005; Vignon & Morat 2010). The temperature might have influenced otolith shape in the samples as during the Badenian after the "Miocene climate optimum" a general cooling trend is recognizable (see Piller et al. 2007). This trend is also manifested in the fossil report of thermophile molluscs and gadoids (see e.g. Brzobohatý et al. 2007). This might have affected gobiid otolith growth, however, these overall changes were gradually over a time period of 3—4 Ma years (see Piller et al. 2007), a study on local climatic fluctuations during the rather short geological time of the middle Badenian and therefore an estimation on how great such influences could have been are missing.

3.7.4 Gobiid otoliths today and in the past

The oldest gobioid fossil otoliths are known from the Eocene (Ypresian) from India (Bajpai & Kapur 2004). Today, most otolith based fossil species are described from Europe with the numbers of described species having increased drastically from 30 (Nolf 1985) to >80 (e.g. Nolf 2013; Schwarzhans et al. 2017; Reichenbacher et al. 2019; Bradić-Milinović et al. 2019; Schwarzhans et al. 2020). A detailed overview of previously described fossil gobiid species from the Miocene of the Central Paratethys is given in Schwarzhans et al. (2020, Fig. 5).

A noticeable difference between the fossil gobiid otoliths from Vöslau and Kienberg and the otoliths of present-day European gobiids (Lombarte et al. 2018, Gut et al. 2020) is the occurrence of a OL/OH ratio <1.0 in the fossil assemblage, which is dominated by *Lesueurigobius* (vs. >1.0 in the extant species). Today, the most specious rich gobiid genus in Europe is *Gobius* (see Miller 1996; Kovačić et al. 2012). Its sagittae are longer than high (e.g. Lombarte et al. 2018; Gut et al. 2020), whereas in several other European gobiid genera the sagitta shapes are squared or higher than long (examples are *Aphia* (Lombarte et al. 2006; Lombarte et al. 2018, Fig. 2a), *Buenia* (Lombarte et al. 2006; Lombarte et al. 2018, Fig 1f), *Lesueurigobius* (e.g. Lombarte et al. 2006; Lombarte et al. 2018, Fig. 2b,c), *Zebrus* (Lombarte et al. 2006; Lombarte et al. 2018, Fig 4e)). This supports the classification of most species in this study as non *Gobius*, even though many gobiid species were assigned in classic taxonomy to this genus, due to lacking extant material for comparison (e.g. Koken 1891; Procházka 1900).

Today only five *Lesueurigobius* species are known with all of them occuring in Europe and at the coasts of the Eastern Atlantic (Foese & Pauly 2019). This amount is clearly outnumbered by the extant genus *Gobius* which is represented by 28 species (see Fricke et al. 2019). Despite of being noticeably more diverse in the Miocene, *Lesueurigobius* also used to occur in remarkably higher quantities than *Gobius* (e.g. Reichenbacher & Cappetta 1999; Brzobohatý et al. 2007).

According to the current state of the art, eight *Gobius* and six *Lesueurigobius* species are known from the Central Paratethys during the Badenian (see Fig. 8). Even though in the Badenian *Gobius* was represented by two species more than *Lesueurigobius*, the discrepancy in diversity of these genera is neglectable compared to the diversity observed today. A factor that might have influenced the quantities of *Lesueurigobius* at Kienberg or Vöslau might have been the sandy substrate which is preferred by *Lesueurigobius*, whereas most *Gobius* species favor to inhabit gravel bottoms (see Miller 2004; Froese & Pauly 2019; Papp & Steininger 1978; Schultz et al. 2010). This might have given *Lesueurigobius* better and more nesting opportunities than *Gobius*.

Species of *Lesueurigobius* today occupy deeper shelf environments, most of them between depths of 50 m to 100 m (Froese & Pauly 2019; see also Schwarzhans et al. 2020), which suits the conditions given in Kienberg or Vöslau. Even though also some *Gobius* species are known from these depths, most extant European representatives occupy habitats of only a few meters depth (<50 m; Miller 2004; Froese & Pauly 2019) making Kienberg and Vöslau during the Miocene potentially better habitats for *Lesueurigobius* than *Gobius*.

The high amount of fossil *Lesueurigobius* species might also be explained by unknown associations in *Gobius* and/or *Lesueurigobius* to other species, like crustaceans, plants or corals, that might have helped *Lesueurigobius* to expand, as it is known from gobiids today (e.g. Herler et al. 2011; Tornabene et al. 2013). It is also suggested that diet overlap in gobies and other fishes or the use of spacial resources have great influences on the spacial distribution of a gobioid species (see Hobbs & Munday 2004; Burkett & Jude 2015). *Lesueurigobius* might have had an unknown advantage in using and therefore occupying habitats.

The "variability" in *Lesueurigobius vicinalis*:

The genus *Lesueurigobius* is probably known from the Central Paratethys since the late Oligocene ("aff. *Lesueurigobius*"; Nolf & Brzobohatý 1994). The oldest specimens with an assigned species name (*L. vicinalis*) were retrieved from the Aquitanian (early Miocene) of La Paillade in South France (Reichenbacher & Cappetta 1999). Reported nominal *Lesueurigobius* species of Badenian age are *L. bicornutus* (Lin, Girone & Nolf, 2015), *L. magniiugis* and *L. vicinalis* (see Schwarzhans et al. 2020, Fig. 5).

L. vicinalis was reported from the Central Paratethys in numerous studies (e.g. Schubert 1906; Radwańska 1992; Brzobohatý et al. 2003; Schwarzhans et al. 2020) but is also reported from the Mediterranean, the Aquintine Basin and the North Sea Basin (e.g. Nolf & Steurbaut 1979; Reichenbacher & Cappetta 1999; Schwarzhans 2010). According to the original description, unique characters of *L. vicinalis*' otoliths are their even curvature, deep dorsal depression, well defined ventral furrow and their smooth surface (Koken 1891). This description by Koken (1891), however, is not exclusively valid for *L. vicinalis*. Additionally, *L. vicinalis* was interpreted as species complex of multiple, closely related species (see Reichenbacher & Capetta 1999), because of a great variability in its otoliths. This led to a wide morphological range of *L. vicinalis* in the literature (see e.g. Śmigielska 1973: pl. IV, figs. 1-2 vs. Reichenbacher & Cappetta 1999: pl. 3, figs. 1-5 vs. Schwarzhans 2010: pl. 105, figs. 10-14 vs. Nolf 2013: pl. 322 vs. Schwarzhans et al. 2020: pl. 2, figs. 5-12). Table 1 summarizes the differences between the *L. vicinalis* holotype (see Fig. 7f or Nolf 2013; Schwarzhans et al. 2020) and previously described representatives, as well as the differences to the new *Lesueurigobius* species of this study.

These noticeable differences in Table 1 between the *L. vicinalis* holotype and further described specimens of this species, indicate not only a hight variablitiy of this species but a possible heterogeneity.

During the middle Badenian *Lesueurigobius* was solely represented by the species *L. vicinalis* in the literature (see Fig. 8). The presence of *L. magniiugis* and *L. bicornutus* in the middle Badenian can be assumed as it was described from the early and late Badenian (see Schwarzhans et al. 2020). This study fills now the gap in the record of *L. magniiugis* from the middle Badenian, whereas *L. bicornutus* has not been explicitly reported from this time period, yet (see Fig. 8). Additionally, this study assigns now *L. annajanae* sp. nov., *L. brzobohatyi* sp. nov. and *L. harzhauseri* sp. nov. to this genus. These new species and the assumed heterogeneity of *L. vicinalis* suggest that the diversity of *Lesueurigobius* could have been higher than previously assumed.

Table 1: Differences between described *Lesueurigobius vicinalis* specimens from the literature, new *Lesueurigobius* species and the *L. vicinalis* holotype.

Subject of comparison	pl. figs.	Differences to *L. vicinalis* holotype (e.g. Fig. 7f)
Śmigielska (1973)	pl. IV, figs. 1-2	no even curvature due to their edgy posterodorsal projection
Reichenbacher & Cappetta (1999)	pl. 3, figs. 1-5	dorsal rim without incision; posterior margin barely incised
Schwarzhans (2010)	pl. 105, figs. 10-13	lack of the even curvature
	pl. 105, figs. 10, 13, 14	the deep dorsal depression
Schwarzhans et al. (2020)	pl. 2, figs. 6, 8	specimens differ in projecting reventral projection
	pl. 2, figs. 9,10	projecting posteroventral angle
	pl. 2, figs. 7,11,12	voluminous posteroventral projection
L. annajanae sp. nov.	Fig. 6, figs. d-i	more volominous in the posterodorsal projection and predorsal angle; posterodorsal projection exceeding posteroventral angle
L. brzobohatyi sp. nov.	Fig. 6, figs. j-l	OL/OH<1, dorsal area barely impressed
L. harzhauseri sp. nov.	Fig. 7, figs. a-e	OL/OH<1, wider and longer ostium

The lacking *Lesueurigobius vicinalis* and *Deltentosteus telleri*:

Surprisingly commonly occurring species in the Central Paratethys from the Badenian like

Deltentosteus telleri or *Lesueurigobius vicinalis* (Radwańska 1992; Brzobohatý 1994; Brzobohatý et al. 2007, Schwarzhans et al. 2020) were not discovered in the sample set. Schubert (1906, pl. 6, figs. 32-24) described "*Otolithus* (*Gobius*) *vicinalis*", from Vöslau, however, our study lacks similar otoliths to those described by Schubert (1906), mainly due to lower OL/OH ratios. This phenomenon of *L. vicinalis* missing in the samples of Vöslau could be a sampling effect as the completeness of the examined sample is unknown.

In regard of Kienberg Brzobohatý et al. (2007) states the occurrence of *D. telleri* and a *Lesueurigobius* aff. *vicinalis*. The latter group was assigned later to *L. vicinalis* by Schwarzhans (2010). However, considering the overall expression of the outline in these specimens and their sulcus shape with an elongated, narrow subcaudal iugum in two of the three depicted specimens, they are considered to be more similar to *Lesueurigobius harzhauseri* sp. nov. than to *L. vicinalis* (see discussion in the systematic section of this species).

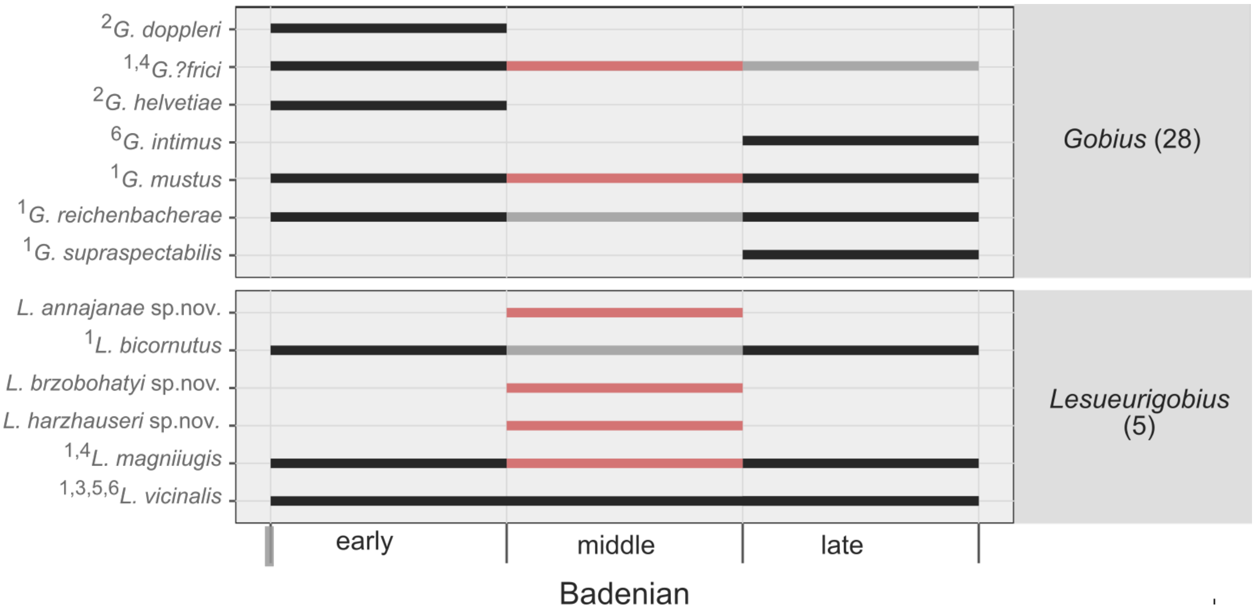
Table 2: Previous work on otoliths in the Central Paratethys used for the construction of Fig. 8, listed chronologically according to publication date.

Authors	Index	Study area
Schwarzhans et al. (2020)	1	Czech Republic, Slovakia, Poland
Mandic et al. (2019)	2	Croatia
Brzobohatý & Nolf (2018)	3	Czech Republic
Schwarzhans (2017)	4	Romania
Radwańska (1992)	5	Poland
Nolf (1981)	6	Austria, Revision Schubert Material

3.8 Conclusion

For the first time statistical approaches were used to test support for presorted fossil groups of isolated sagittal otoliths. In this study a total of seven species, with all seven species occurring in Kienberg and three of them also detected in Vöslau were presented. Four of them were described as new species namely *Lesueurigobius annajanae* sp. nov., *L. brzobohatyi* sp. nov., *L. harzhauseri* sp. nov., †*Paromiogobius johnsonae* gen. et sp. nov. Therefore, it can be assumed that the genus *Lesueurigobius* was more diverse in the Miocene than previously known. The described species abundancy and diversity reflects the different habitats of Kienberg and Vöslau, with Kienberg representing a shallow coastal area (∼60 m) which, like known in extant gobies, displays a higher diversity than further off shore areas like Vöslau (∼50—100 m). Both localities appear to have been more favorable to *Lesueurigobius* than to *Gobius*, probably due to their respective depth and

Figure 8: Timeline for fossil otoliths of the genera *Gobius* and *Lesueurigobius* reported from the Central Paratethys occurring during the Badenian. Superscripted numbers indicate the concerning literature (see Table 2). Red: middle Badenian samples from Kienberg and Vöslau (this study), black: record from literature data, grey: possible record, no literature data. International stratigraphy according to the International Commission on Stratigraphy (httpa://stratigraphy.org; 2020). Stratigraphy of the Central Paratethys follows Piller & Harzhauser (2005) and Hohenegger et al. (2014).



substrate.

3.9 Acknowledgements

I am grateful to the Natural History Museum of Vienna for providing the samples. Special thanks go to Mathias Harzhauser (Natural History of Vienna) and Ursula Göhlich (Natural History Museum of Vienna) for their valuable information on sample locality and history. Many thanks go also to Prof. Dr. Bettina Reichenbacher for her numerous, valuable and detailed comments on this work. I also want to thank PD Dr. Mike Reich for granting me access to the digital microscope and Bork Ilsemann for his kind and detailed introduction (both Ludwig-Maximilians University of Munich). Thank you also to Baran Karapunar for his experienced advice (Ludwig-Maximilians University of Munich).

Declarations

Funding (Not applicable)

Conflicts of interest/Competing interests (The authors declare that there is no conflict of interest.)

Ethics approval (Not applicable)

Availability of data and material (The pictures used for acquiring data are stored at Ludwig-Maximilians University in Munich and can be transferred by contacting the corresponding author. Otolith measurements and calculated otolith indices are attached as supplementary file Online Resource 1.)

3.10 References

Agorreta, A., San Mauro, D., Schliewen, U., Van Tassell, J. L., Kovacic, M., Zardoya, R., & Rüber, L. (2013). Molecular phylogenetics of Gobioidei and phylogenetic placement of European gobies. *Molecular Phylogenetics and Evolution*, 69(3), 619-633. doi:10.1016/j.ympev.2013.07.017

Akihito, Akishinonomiya, F., Ikeda, Y., Aizawa, M., Nakagawa, S., Umehara, Y., Yonezawa, T., Mano, S., Hasegawa, M., Nakabo, T., Gojobori, T. (2016). Speciation of two gobioid species, *Pterogobius elapoides* and *Pterogobius zonoleucus* revealed by multi-locus nuclear and mitochondrial DNA analyses. *Gene*, 576(2), 593-602.

Bajpai, S., & Vivesh, V., Kapur. (2004). Oldest known gobiids from Vastan Lignite Mine (early Eocene), Surat district, Gujarat. *Current Science*, 87(4), 433-435.

Bonhomme, V., Picq, S., Gaucherel, C., & Claude, J. (2014). Momocs: Outline Analysis Using R. *Journal of Statistical Software*, 56(13), 1-24.

Bradić-Milinović, K., Ahnelt, H., Rundić, L., & Schwarzhans, W. (2019). The lost freshwater goby fish fauna (Teleostei, Gobiidae) from the early Miocene of Klinci (Serbia). *Swiss Journal of Palaeontology*, 138(2), 285-315.

Brandl, S., Goatley, C., Bellwood, D., & Tornabene, L. (2018). The hidden half: ecology and evolution of cryptobenthic fishes on coral reefs: Cryptobenthic reef fishes. *Biological Reviews*, 93, 1846-1873. doi:10.1111/brv.12423

Bratishko, A., Schwarzhans, W., Reichenbacher, B., Vernyhorova, Y., & Ćorić, S. (2015). Fish otoliths from the Konkian (Miocene, early Serravallian) of Mangyshlak (Kazakhstan):

testimony to an early endemic evolution in the Eastern Paratethys. *Paläontologische Zeitschrift*, 89(4), 839-889. doi:10.1007/s12542-015-0274-4

Brzobohatý, R. (1989). Die untermiozäne Otolithenfauna von Maigen bei Eggenburg, Niederösterreich. *Annalen des Naturhistorischen Museums in Wien. Serie A für Mineralogie und Petrographie, Geologie und Paläontologie, Anthropologie und Prähistorie*, 90, 21-47.

Brzobohatý, R. (1994). Die Fischotolithen des Badenien von Gainfarn, Niederösterreich (Mittelmiozän, Wiener Becken). *Annalen des Naturhistorischen Museums in Wien. Serie A für Mineralogie und Petrographie, Geologie und Paläontologie, Anthropologie und Prähistorie*, 96, 67-93.

Brzobohatý, R., & Nolf, D. (2018). Revision of the Middle Badenian fish otoliths from the Carpathian Foredeep in Moravia (Middle Miocene, Czech Republic). *Cybium*, 42(2), 143-167.

Brzobohatý, R., Nolf, D., & Kroupa, O. (2007). Fish otoliths from the Middle Miocene of Kienberg at Mikulov, Czech Republic, Vienna Basin: their paleoenvironmental and paleogeographic significance. *Bulletin de l'Institut royal des Sciences naturelles de Belgique, Sciences de la Terre*, 77, 167-196.

Brzobohatý, R., Reichenbacher, B., & Gregorová, R. (2003). Teleostei (otoliths, skeletons with otoliths in situ) from the Karpatian of the Central Paratethys. In R. Brzobohatý, I. Cicha, M. Kováč, & F. Rögl (Eds.), *The Karpatian, a Lower Miocene stage of the Central Paratethys* (pp. 265-279). Brno: Masaryk University.

Burkett, E. M., & Jude, D. J. (2015). Long-term impacts of invasive round goby *Neogobius melanostomus* on fish community diversity and diets in the St. Clair River, Michigan. *Journal of Great Lakes Research*, 41(3), 862-872.

Campana, S. E. (2005). Otolith science entering the 21st century.*Marine and freshwater research*, 56(5), 485-495.

Campana, S. E., & Casselman, J. M. (1993). Stock discrimination using otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 50(5), 1062-1083.

Cicha, I. (1975). *Biozonal division of the Upper Tertiary basins of the Eastern Alps and West Carpathians: International Union of Geological Sciences, Commission on Stratigraphy,* *Subcommission on Neogene Stratigraphy, Gerional Committee on Mediterranean Neogene Stratigraphy; proceedings of the VIth congress, Bratislava, September 1975*: Geological Survey.

Farias, I., Vieira, A. R., Gordo, L. S., & Figueiredo, I. (2009). Otolith shape analysis as a tool for stock discrimination of black scabbardfish, *Aphanopus carbo* Lowe, 1839 (Pisces: Trichiuridae), in Portuguese waters. *Scientia Marina*, 73S2, 47-53. doi:10.3989/scimar.2009.73s2047

Fricke, R., Eschmeyer, W. N., & Fong, J. D. (2019). Species by family/subfamily in the Catalog of Fishes. [Electronic version]. San Francisco (CA): California Academy of Sciences; 2019. [cited 2019 Oct 21]. In.

Froese, R., & Pauly, D. (2009). *FishBase. World wide web electronic publication.* http://www.fishbase.org.

Gertzen, S., Fidler, A., Kreische, F., Kwabek, L., Schwamborn, V., & Borcherding, J. (2016). Reproductive strategies of three invasive Gobiidae co-occurring in the Lower Rhine (Germany). *Limnologica*, 56, 39-48.

Gierl, C., Liebl, D., Šanda, R., Vukić, J., Esmaeili, H. R., & Reichenbacher, B. (2018). What can goby otolith morphology tell us? *Cybium*, 42(4), 349-363.

Gierl, C., & Reichenbacher, B. (2015). A New Fossil Genus of Gobiiformes from the Miocene Characterized by a Mosaic Set of Characters. *Copeia*, 103(4), 792-805. doi:10.1643/ci-14-146

Gierl, C., Reichenbacher, B., Gaudant, J., Erpenbeck, D., & Pharisat, A. (2013). An Extraordinary Gobioid Fish Fossil from Southern France. *PLOS ONE*, 8(5), e64117. doi:10.1371/journal.pone.0064117

Gut, C., Vukić, J., Šanda, R., Moritz, T., & Reichenbacher, B. (2020). Identification of past and present gobies: distinguishing *Gobius* and *Pomatoschistus* (Teleostei: Gobioidei) species using characters of otoliths, meristics and body morphometry. *Contributions to Zoology*, 89(3), 282-323.

Harzhauser, M., Grunert, P., Mandic, O., Lukeneder, P., Gallardo, Á. G., Neubauer, T. A., Carnevale, G., Landau, B. M., Sauer, R., Strauss, P. (2018). Middle and late Badenian palaeoenvironments in the northern Vienna Basin and their potential link to the Badenian Salinity Crisis. *Geologica Carpathica*, 69(2), 149-168.

Herler, J., Munday, P. L., & Hernaman, V. (2011). Gobies on Coral Reefs. In R. A. Patzner, J. L. Van Tassell, M. Kovačić, & B. G. Kapoor (Eds.), *The biology of gobies* (pp. 559-607). Enfield: Science Publishers Inc.

Herler, J., & Patzner, R., A. (2005). Spatial segregation of two common Gobius species (Teleostei: Gobiidae) in the northern Adriatic Sea. *Marine Ecology*, 26(2), 121-129. doi:doi:10.1111/j.1439-0485.2005.00045.x

Hobbs, J.-P. A., & Munday, P. L. (2004). Intraspecific competition controls spatial distribution and social organisation of the coral-dwelling goby *Gobiodon histrio*. *Marine Ecology Progress Series*, 278, 253-259.

Hohenegger, J., Andersen, N., Baldi, K., Coric, S., Pervesler, P., Rupp, C., & Wagreich, M. (2008). Paleoenvironment of the Early Badenian (Middle Miocene) in the southern Vienna Basin (Austria)-multivariate analysis of the Baden-Sooss section. *Geologica Carpathica*, 59(5), 461-488.

Hohenegger, J., Ćorić, S., Khatun, M., Pervesler, P., Rögl, F., Rupp, C., Selge, A., Uchman, A., Wagreich, M. (2007). Cyclostratigraphic dating in the Lower Badenian (Middle Miocene) of the Vienna Basin (Austria): the Baden-Sooss core. *International Journal of Earth Sciences*, 98(4), 915-930.

Hohenegger, J., Ćorić, S., & Wagreich, M. (2014). Timing of the middle miocene Badenian stage of the central Paratethys. *Geologica Carpathica*, 65(1), 55-66.

Kidwell, S. M. (1998). Time-averaging in the marine fossilrecord: Overview of strategies and uncertainties. *Geobios*, 30(7), 977-995.

Koken, E. (1884). Über Fisch-Otolithen, insbesondere über diejenigen der norddeutschen Oligocän-Ablagerungen. *Zeitschrift der deutschen geologischen Gesellschaft*, 500-565.

Koken, E. (1891). Neue Untersuchungen an tertiären Fisch-Otolithen. II. *Zeitschrift der deutschen geologischen Gesellschaft*, 43, 77-170.

Kováč, M., Hudáčková, N., Halásová, E., Kováčová, M., Holcová, K., Oszczypko-Clowes, M., Bládi, K., Less, G., Nagymarosy, A., Ruman, A., Klučiar & T., Jamrich, M. (2017). The Central Paratethys palaeoceanography: a water circulation model based on microfossil proxies, climate, and changes of depositional environment. *Acta Geologica Slovaca*, 9(2), 75-114.

Kovačić, M., Miletić, M., & Papageorgiou, N. (2011). A first checklist of gobies from Crete with ten new records. *Cybium, 35*(3), 245-253.

Kovačić, M., & Šanda, R. (2016). A new species of *Gobius* (Perciformes: Gobiidae) from the Mediterranean Sea and the redescription of *Gobius bucchichi*. *J Fish Biol*, 88(3), 1104-1124. doi:10.1111/jfb.12883

Kovačić, M., Šanda, R., Kirinčić, M., & Zanella, D. (2012). Geographic distribution of gobies (Gobiidae) in the Adriatic Sea with thirteen new records for its southern part. *Cybium*, 36(3), 435-445.

Lin, C.-H., Brzobohatý, R., Nolf, D., & Girone, A. (2017). Tortonian teleost otoliths from northern Italy: taxonomic synthesis and stratigraphic significance. *European Journal of Taxonomy*(322).

Lombarte, A., Chic, Ò., Parisi-Baradad, V., Olivella, R., Piera, J., & García-Ladona, E. (2006). A web-based environment for shape analysis of fish otoliths. The AFORO database. *Scientia Marina*, 70(1), 147-152.

Lombarte, A., Miletic, M., Kovačić, M., Otero-Ferrer, J., & Tuset, V. (2018). Identifying sagittal otoliths of Mediterranean Sea gobies: Variability among phylogenetic lineages. *J Fish Biol*, 92, 1768-1787. doi:10.1111/jfb.13615

Malz, H. (1978). Vergleichend-morphologische Untersuchungen an aquitanen Fisch-Otolithen aus dem Untergrund von Frankfurt am Main. *Senckenbergiana lethaea*, 59(4/6), 441-481.

Mandic, O., Hajek-Tadesse, V., Bakrač, K., Reichenbacher, B., Grizelj, A., & Miknić, M. (2019). Multiproxy reconstruction of the middle Miocene Požega palaeolake in the Southern Pannonian Basin (NE Croatia) prior to the Badenian transgression of the Central Paratethys Sea. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 516, 203-219.

Miller, P. (1996). *The functional ecology of small fish: some opportunities and consequences.* Paper presented at the Symposia of the Zoological Society of London.

Miller, P. J. (2004). *The Freshwater Fishes of Europe* (Vol. 8/II). Wiebelsheim: AULA-Verlag.

Morales-Nin, B. (2000). Review of the growth regulation processes of otolith daily increment formation. *Fisheries Research*, 46(1-3), 53-67.

Nash, R. D. M. (1982). The biology of Fries' goby, *Lesueurigobius friesii* (Malm), in the Firth of Clyde, Scotland, and a comparison with other stocks. *J Fish Biol*, 21(1), 69-85.

Nelson, J. S., Grande, T. C., & Wilson, M. V. H. (2016). *Fishes of the World* (5 ed.). New Jersey: John Wiley & Sons, Inc.

Nolf, D. (1981). Revision des types d'otolithes de poissons fossiles decrits par R. Schubert. *Verhandlungen der Geologischen Bundesanstalt, 1981*(2), 133-183.

Nolf, D. (1985). Otolithi piscium. In S. H. P. (Ed.), *Handbook of paleoichthyology* (Vol. 10, pp. 1-145). Stuttgart: Gustav Fischer Verlag.

Nolf, D. (2013). *The diversity of fish otoliths, past and present.* Belgium: Royal Belgian Institute of Natural Sciences.

Nolf, D., & Brzobohatý, R. (1994). Fish otoliths from the Late Oligocene (Eger and Kiscell Formations) in the Eger area (northeastern Hungary). *Bulletin de l'Institut royal des Sciences naturelles de Belgique, Sciences de la Terre*, 64, 225-252.

Nolf, D., & Steurbaut, E. (1979). Les otolithes de téléostéens des Faluns sallomaciens d'Orthez et de Sallespisse (Miocène moyen d'Aquitaine méridionale, France). *Palaeontographica Abteilung A, 164(1-3)*, 1-23.

Papp, A., & Steininger, F. (1978). Holostratotypus: Baden-Sooss. *M4-Badenien (Moravien, Wielicien, Kosovien). Chronostratigraphie und Neostratotypen*, 6, 158-168.

Patzner, R. A., Van Tassel, J. L., Kovačić, M., & Kapoor, B. G. (2011). *The Biology of Gobies* (Vol. 2012). USA: Science Publishers.

Paulissen, W., Luthi, S., Grunert, P., Ćorić, S., & Harzhauser, M. (2011). Integrated high-resolution stratigraphy of a Middle to Late Miocene sedimentary sequence in the central part of the Vienna Basin. *Geologica Carpathica*, 62(2), 155-169.

Piller, W. E., Harzhauser, M., & Mandic, O. (2007). Miocene Central Paratethys stratigraphy–current status and future directions. *Stratigraphy*, 4, 151-168.

Procházka, V. J. (1893). Das Miocaen von Seelowitz in Mähren und dessen Fauna. *Rozpravy Ceské Akademie Cisare Frantiska Josefa pro Vedy Slovesnost a Umeni v Praze, 2*(24), 1-90.

Procházka, V. J. (1900). *Das ostböhmische Miocaen* (Vol. 2). Prague.

Rado, G. (1981). Otolite din depozitele langhiene (Badenian inferior) de la Lapugiul de Sus. *Analele Universitatii Bucu-resti, Série Stiintele Naturii, Géologie-Gegrafie*, 30, 29-49.

Radwańska, U. (1992). Fish otoliths the Middle Miocene (Badenian) deposits of southern Poland. *Acta Geologica Polonica*, 42(3-4), 142-238.

Reichenbacher, B., & Cappetta, H. (1999). First evidence of an early Miocene marine teleostean fish fauna (otoliths) from La Paillade (Montpellier, France). *Palaeovertebrata*, 28, 1-46.

Reichenbacher, B., Filipescu, S., & Miclea, A. (2019). A unique middle Miocene (Sarmatian) fish fauna from coastal deposits in the eastern Pannonian Basin (Romania). *Palaeobiodiversity and Palaeoenvironments*, 99(2), 177-194.

Reichenbacher, B., Gaudant, J., & Griessemer, T. W. (2007). A late Burdigalian gobiid fish, *Gobius brevis* (Agassiz, 1839), in the Upper Hydrobia Beds in the middle Upper Rhine Graben (W-Germany). *Paläontologische Zeitschrift*, 81(4), 365-375. doi:10.1007/bf02990249

Rögl, F. (1998). Paratethys Oligocene–Miocene stratigraphic correlation. *Abhandlungen der senckenbergischen naturforschenden Gesellschaft*, 549, 3-7.

Rögl, F., Ćorić, S., Harzhauser, M., Jimenez-Moreno, G., Kroh, A., Schultz, O., Wessely, G., Zorn, I. (2008). The Middle Miocene Badenian stratotype at Baden-Sooss (Lower Austria). *Geologica Carpathica*, 59(5), 367-374.

Rüber, L., & Agorreta, A. (2011). Molecular systematics of gobioid fishes. In R. A. Patzner, J. L. Van Tassell, M. Kováčić, & B. G. Kapoor (Eds.), *The biology of gobies.* (pp. 23-50). Enfield, NH: Science Publishers Inc.

Rueden, C., T, Schindelin, J., & Hiner, M., C. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*, 18(529). doi:10.1186/s12859-017-1934-z

Sadeghi, R., Esmaeili, H. R., Zarei, F., & Reichenbacher, B. (2020). Population structure of the ornate goby, *Istigobius ornatus* (Teleostei: Gobiidae), in the Persian Gulf and Oman Sea as determined by otolith shape variation using ShapeR. *Environmental Biology of Fishes*, 103, 1217–1230.

Schubert, R. J. (1906). Die Fischotolithen des österr.-ungar. Tertiärs. III. *Jahrbuch der Kaiserlich-Königlichen Geologischen Reichsanstalt*, 55, 623-706.

Schultz, O., Brzobohatý, R., & Kroupa, O. (2010). Fish teeth from the Middle Miocene of Kienberg at Mikulov, Czech Republic, Vienna Basin. *Annalen des Naturhistorischen Museums in Wien. Serie A für Mineralogie und Petrographie, Geologie und Paläontologie, Anthropologie und Prähistorie*, 112, 489-505.

Schwarzhans, W. (2010). *The otoliths from the Miocene of the North Sea Basin.* Leiden: Backhuys Publishers.

Schwarzhans, W. (2014). Otoliths from the middle Miocene (Serravallian) of the Karaman Basin, Turkey. *Cainozoic Research*, 14(1), 35-69.

Schwarzhans, W. (2017). A review of otoliths collected by W. Weiler from the Badenian of Romania and by B. Strashimirov from Badenian equivalents of Bulgaria. *Cainozoic Research*, 17(2), 167-191.

Schwarzhans, W., Ahnelt, H., Carnevale, G., Japundžić, S., Bradić, K., & Bratishko, A. (2017). Otoliths in situ from Sarmatian (Middle Miocene) fishes of the Paratethys. Part III: tales from the cradle of the Ponto-Caspian gobies. *Swiss Journal of Palaeontology*, 136(1), 45-92.

Schwarzhans, W., Bradić, K., & Rundić, L. (2015). Fish-otoliths from the marine-brackish water transition from the Middle Miocene of the Belgrade area, Serbia. *Paläontologische Zeitschrift*, 89(4), 815-837.

Schwarzhans, W., Brzobohatý, R., & Radwańska, U. (2020). Goby otoliths from the Badenian (middle Miocene) of the Central Paratethys from Czech Republic, Slovakia and Poland: A baseline for the evolution of the European Gobiidae (Gobiiformes; Teleostei). *Bollettino della Società Paleontologica Italiana*, 59(2), 125-173.

Śmigielska, T. (1966). Otoliths of fishes from the Tortonian of Southern Poland. *Annales Societatis Geologorum Poloniae*, 36(3), 205-275.

Śmigielska, T. (1973). Fish otolithes from the Lower Tortonian deposits at Niskowa near Nowy Sacz. *Annales Societatis Geologorum Poloniae*, 43(1), 3-40.

Thacker, C. E. (2013). Phylogenetic placement of the European sand gobies in Gobionellidae and characterization of gobionellid lineages (Gobiiformes: Gobioidei). *Zootaxa*, 3619, 369-382.

Thacker, C. E. (2015). Biogeography of goby lineages (Gobiiformes: Gobioidei): origin, invasions and extinction throughout the Cenozoic. *Journal of Biogeography*, 42(9), 1615-1625. doi:doi:10.1111/jbi.12545

Tornabene, L., Ahmadia, G. N., Berumen, M. L., Smith, D. J., Jompa, J., & Pezold, F. (2013). Evolution of microhabitat association and morphology in a diverse group of cryptobenthic coral reef fishes (Teleostei: Gobiidae: Eviota). *Molecular Phylogenetics and Evolution*, 66(1), 391-400.

Tuset, V. M., Lombarte, A., & Assis, C. A. (2008). Otolith atlas for the western Mediterranean, north and central eastern Atlantic. *Scientia Marina*, 72(S1), 7-198.

Vignon, M., & Morat, F. (2010). Environmental and genetic determinant of otolith shape revealed by a non-indigenous tropical fish. *Marine Ecology Progress Series*, 411, 231-241.

Weiler, W. (1955). Untersuchungen an der Fischfauna von Unter- und Oberkirchberg bei Ulm vornehmlich an Hand von Otolithen in situ. *Paläontologische Zeitschrift*, 29(1-2), 88-102.

Weiler, W. (1963). Die Fischfauna des Tertiärs im oberrheinischen Graben, des-Mainzer Beckens, des unteren Maintals und der Wetterau,-unter besonderer Berücksichtigung des Untermiozäns. *Abhandlungen der senckenbergischen naturforschenden Gesellschaft*, 504, 1-75.

Wheeler, A., & Stebbing, P. (1978). *Key to the fishes of Northern Europe; a guide to the identification of more than 350 species.* London: Warne.

Wilkins, H. K., & Myers, A. A. (1992). Microhabitat utilisation by an assemblage of temperate Gobiidae (Pisces: Teleostei). *Marine ecology progress series. Oldendorf*, 90(2), 103-112.

Yu, X., Cao, L., Liu, J., Zhao, B., Shan, X., & Dou, S. (2014). Application of otolith shape analysis for stock discrimination and species identification of five goby species (Perciformes: Gobiidae) in the northern Chinese coastal waters. *Chinese journal of oceanology and limnology*, 32(5), 1060.

4 Osteology of the posterior vertebral column and caudal skeleton of marine amphibious gobies (mudskippers) (Teleostei: Gobioidei)

Mehdi Ghanbarifardi[1], Carolin Gut[2], Zeinab Gholami[2], Hamid Reza Esmaeili[2,3], Christoph Gierl[2], Bettina Reichenbacher[2,4]

[1]Department of Biology, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan, Iran

[2]Department of Earth and Environmental Sciences, Ludwig-Maximilians-Universität München, Richard-Wagner Straße 10, 80333 Munich, Germany

[3]Zoology Section, Department of Biology, School of Science, Shiraz University, Shiraz, Iran

[4]GeoBio-Center, Ludwig-Maximilians-Universität München, 80333 Munich, Germany

4.1 Abstract

Mudskippers are amphibious gobies (Teleostei: Gobioidei, Oxudercinae) that have served as models for the specialised physiology and behaviour of fishes out of water. In this study, a comparative analysis of the posterior vertebral column and the caudal skeleton of ten mudskipper species was conducted on the basis of X-ray imaging. The species considered were *Apocryptes bato, Apocryptodon madurensis, Boleophthalmus dussumieri, Oxuderces dentatus, Periophthalmodon freycineti, Pn. schlosseri, Periophthalmus novemradiatus, Ps. waltoni, Pseudapocryptes borneensis, and Scartelaos tenuis.* For the osteological description the new term 'modified caudal vertebra' is used for all those vertebrae that display visibly modified neural and/or haemal spines compared to the spines of a 'usual' caudal vertebra, but are not involved in the support of caudal rays. The results reveal that the most terrestrial forms (*Pn. freycineti, Pn. schlosseri, Ps. novemradiatus, Ps. waltoni*) possess distinct traits that are seldom found in the other species. Among these features are (a) the existence of at least two modified caudal vertebrae (also present in S. tenuis), (b) a particularly close, dovetailing association between the neural spines of the preural vertebrae two and three (restricted to *Ps. novemradiatus* and *Ps. waltoni*), and (c) thickening and shortening of the ventralmost principal caudal rays (also present in *B. dussumieri* and *S. tenuis*). These findings support the idea that the posterior caudal vertebrae and caudal skeleton of the mentioned species are modified to enhance locomotion on land. Moreover, a relationship between character development and degree of terrestrial adaptation is probable, as all three traits are most pronounced in *Ps. waltoni*, which correlates with its strikingly high level of adaptation to amphibious life. A further aspect of this study is that the newly recognized skeletal structures have good fossilization potential and could therefore facilitate recognition of fossil species of mudskippers, which are currently unknown.

Keywords amphibious goby, Gobiidae, osteology, terrestrial adaptation

4.2 Introduction

Mudskippers are small to moderately sized amphibious fishes that belong to the Gobiidae *sensu* Gill & Mooi, 2012 and constitute the subfamily Oxudercinae (Murdy, 1989; Murdy & Jaafar, 2017). Mudskippers are widely distributed in coastal mangrove and mudflat areas in the tropics, with the exception of the New World (Hoese, 1984; Murdy, 1989; Parenti & Jaafar, 2017). They are characterized by specialised behaviours and the ability to use their limb-like pectoral fins for locomotion on land (Harris, 1960; Sayer, 2005; Jaafar & Murdy, 2017 and references therein). The members of ten genera are usually referred to as mudskippers, i.e. *Apocryptes* Valenciennes, 1837 (represented with one species), *Apocryptodon* Bleeker, 1874 (three species), *Boleophthalmus* Valenciennes, 1837 (six species), *Oxuderces* Eydoux & Souleyet, 1850 (two species), *Parapocryptes* Bleeker, 1874 (two species), *Periophthalmodon* Bleeker, 1874 (three species), *Periophthalmus* Bloch & Schneider, 1801 (19 species), *Pseudapocryptes* Bleeker, 1874 (two species), *Scartelaos* Swainson, 1839 (four species), and *Zappa* Murdy, 1989 (one species) (see Murdy & Jaafar, 2017). Of these, only members of four genera spend time on land as part of their daily life cycle, namely *Boleophthalmus, Periophthalmodon, Periophthalmus* and *Scartelaos* (Murdy, 1989). Their species can easily move about on muddy or moist surfaces and excavate burrows in the mud; some are even able to climb rocks, mangrove roots or stems, and all share several anatomical, physiological and sensorial specialisations (e.g. Michel, Adriaens, Aerts, Dierick, & Van Wassenbergh, 2014; Murdy, 1989; Pace, 2017; Schöttle, 1931; You et al., 2018; Zander, 2011). *Scartelaos* is the most aquatic (or least terrestrial) of the four genera, while *Periophthalmus* is the most terrestrial genus (Ishimatsu & Gonzales, 2011; Murdy, 1989; Polgar & Crosa, 2009).

A comprehensive investigation of the morphological traits of the Oxudercinae has been provided by Murdy (1989). In addition, there are many works dealing with the specific adaptations of the four mudskipper genera that are regularly amphibious (see Jaafar & Murdy, 2017 and references therein). However, the vertebral column and the caudal skeleton of mudskippers have received relatively little attention and were studied mainly within broader taxonomic contexts (Birdsong, Murdy, & Pezold, 1988; Fujita, 1990). Limited data, mostly restricted to the caudal skeleton, is solely available for three mudskipper species, i.e. *Boleophthalmus pectinirostris* (Linnaeus, 1758) (Fujita, 1990: fig. 502), *Ps. barbarous* (Linnaeus, 1766) (Harris, 1960: fig.4, as *Ps. koelreuteri*) and *Ps. kalolo* Lesson, 1831 (Fujita, 1990: fig. 499, as *Ps. vulgaris*). The objective of this study is to gain a better knowledge of the posterior vertebral column and the caudal skeleton of mudskippers. These structures are of central importance for locomotion in aquatic vertebrates (Lauder, 1989). For mudskippers, the caudal skeleton is also important for locomotion on land (Harris, 1960; Pace, 2017; Swanson & Gibb, 2004). The specific aim was to examine if certain traits can be detected that may be associated to adaptations to terrestrial locomotion.

4.3 Material and methods

4.3.1 Species studied

The material comprises 10 oxudercine species representing eight genera, i.e. *Apocryptes bato* (Hamilton, 1822), *Apocryptodon madurensis*(Bleeker, 1849), *Boleophthalmus dussumieri* Valenciennes, 1837, *Oxuderces dentatus* Eydoux & Souleyet, 1850, *Pn. Freycineti* (Quoy & Gaimard, 1824), *Pn. Schlosseri* (Pallas, 1770), *Ps. novemradiatus* (Hamilton, 1822), *Ps. waltoni* Koumans, 1955, *P. borneensis* (Bleeker, 1855), and *S. tenuis* (Day, 1876) (see Table 1 for details). Species identification was verified in each case based on the speciesdiagnostic characters, the sampling location and further information provided in Murdy (1989). A specimen of *Gobius niger* Linnaeus, 1758 from the NE Atlantic Sea (collection of the National Museum Prague, Czech Republic, number NMP6V 146072) was used as a representative species of the 'gobiine-like' Gobiidae *sensu* Agorreta et al. (2013). Digital X-ray images were taken with a Faxitron Bioptics (LLC-Vision NDT version 2.2.5, 45 k.v. and 30 s) housed in the State Zoological Collection, Munich for all specimens and served as basis for the osteological study.

4.3.2 Study of osteology

Fig. 1 provides an overview of the vertebral column and caudal skeleton, the osteological terminology follows Schultze and Arratia (2013). The vertebral column comprises an abdominal and a caudal part (Fig. 1a). Each vertebra consists of a centrum, and, except the last vertebra (= terminal centrum, TC), a neural arch and a neural spine (Fig. 1a, b). Abdominal vertebrae, except

Table 1: List of studied species and locality data. N, number of specimens.

Species	Drainage and locality	Museum codes	N
Apocryptes bato (Hamilton, 1822)	SMF 1736: Ganges, Bengalen, Asia (1847) SMF 23715-1,2: Hooghly-river, Bengalen, SSW Calcutta, S India, 88° 5’ E, 21° 55’ N (1.10.1988)	SMF 1736, 23715-1, -2	3
Apocryptodon madurensis (Bleeker, 1849)	ZMB 6510: Singapore (1862) ZMB 7637: Manila, Philippines (1862)	ZMB 6510, 7637	2
Boleophthalmus dussumieri Valenciennes, 1837	Persian Gulf, Khorramshahr, Iran 48° 11’ 41.13” E, 30° 26’7.11” N	ZM-CBSU 4, 6-9, 11	6
Oxuderces dentatus Eydoux & Souleyet, 1850	East China Sea; Fuckwing/Fuqing, Fujian Shng/Fukien (1893)	SMNS 3410-1, -2, - 3	3
Periophthalmodon freycineti (Quoy & Gaimard, 1824)	Airdhills, Gulf District, Papua New Guinea, 144° 21’20.00” E, 7° 26’ 50.00” S	ZSM-PIS 19649, 19650	2
Periophthalmodon schlosseri (Pallas, 1770)	Andaman Sea, Indian Ocean, Thailand	ZSM-PIS 023806	1
Periophthalmus novemradiatus (Hamilton, 1822)	Ganges, Calcutta, India	ZMB 5987-1, -2(cf.)	2
Periophthalmus waltoni Koumans, 1941	Persian Gulf, Heleh Wetland, Iran 50° 40’ 43.38” E, 29° 14’ 55.95” N	ZM-CBSU 92, 93, 95, 96, 101, 1716, 1727	5
Pseudapocryptes borneensis (Bleeker, 1855)	Timor, Indonesia (1874-1876)	ZMB 9931	1
Scartelaos tenuis (Day, 1876)	Persian Gulf, Heleh Wetland, Iran 50° 40’ 43.38” E, 29° 14’ 55.95” N	ZM-CBSU 82, 84, 85, 86, 89	7

Note: Institutional abbreviations: SMF, Senckenberg Forschungsinstitut und Naturmuseum Frankfurt am Main, Germany; SMNS, Staatliches Museum für Naturkunde Stuttgart, Germany; ZMB, Museum für Naturkunde Berlin, Germany; ZM-CBSU, Zoological Museum of Shiraz University, Biology Department, Iran; ZSM-PIS, Zoologische Staatssammlung München (Bavarian State Collection of Zoology), Germany.

the first one or two, are associated with ribs; caudal vertebrae (except TC) are characterized by the presence of a closed haemal arch and a haemal spine (Fig. 1a, b). Following (Schultze and Arratia (2013), solely the penultimate and antepenultimate caudal vertebrae are designated here as preural vertebrae (PU) as their spines contribute to the support of the caudal rays. The penultimate caudal vertebra represents PU2 and the antepenultimate one is PU3 (Fig. 1b). The usual condition in gobioids is that PU2 possesses an expanded haemal spine (which supports caudal rays, see (Fig. 1b), but a shortened neural spine (see Fujita, 1990). Also the neural and/or haemal spine of PU3 are visibly differentiated from a 'usual' caudal vertebra - i.e. more elongated, or more expanded, or more bent - and the distaltips of the PU3-spines are associated with (or near to) the caudal cartilage that supports the dorsal and ventral procurrent caudal rays (see Fujita, 1990). Further elements of the caudal skeleton of gobiids are two large hypural plates (Hy1+2, Hy3+4), an autogenous small hypural plate (Hy5), a parhypural (Php) and one or two epural bones (Ep1, 2) (Fig. 1b). The hypural plates and the parhypural support the principal caudal rays and the epural bones are associated with the support of the dorsal procurrent caudal rays (Fig. 1b). For the purpose of this study, we use as additional term 'modified caudal vertebra' (MC), which is numbered from the posteriormost to the anteriormost (like the preural vertebrae). This term is used for each vertebra that has, like a preural vertebra, a visible modification of its neural and/or haemal spine in comparison to a 'usual' caudal vertebra, but differs from a preural vertebra as it is not involved in caudal ray support (Fig. 1b). The same terminology and definition are used in Charmpila et al. (in press).

4.4 Results

Representative X-ray images of each species, with depictions of their preural and modified caudal vertebrae, are provided in the Appendix Figures A1 and A2.

4.4.1 The posteriormost vertebral column

The presence of two preural vertebrae (PU2, PU3), as seen in *Gobius niger* (Fig. 2a), and the absence of modified caudal vertebrae in the definition of the present study can be considered as the usual configuration in the Gobiidae; it also occurs in *As. bato, O. dentatus*, and *An. madurensis* (Fig. 2b-d, Appendix: Fig. A1). The addition of a single modified caudal vertebra (MC1) is seen in the posterior vertebral column of *P. borneensis* and *B. dussumieri*(Fig. 2e, Fig. 3a, Appendix: Fig. A1). Note that *B. dussumieri* exhibits a neural spine of MC1 that is not only slightly broadened

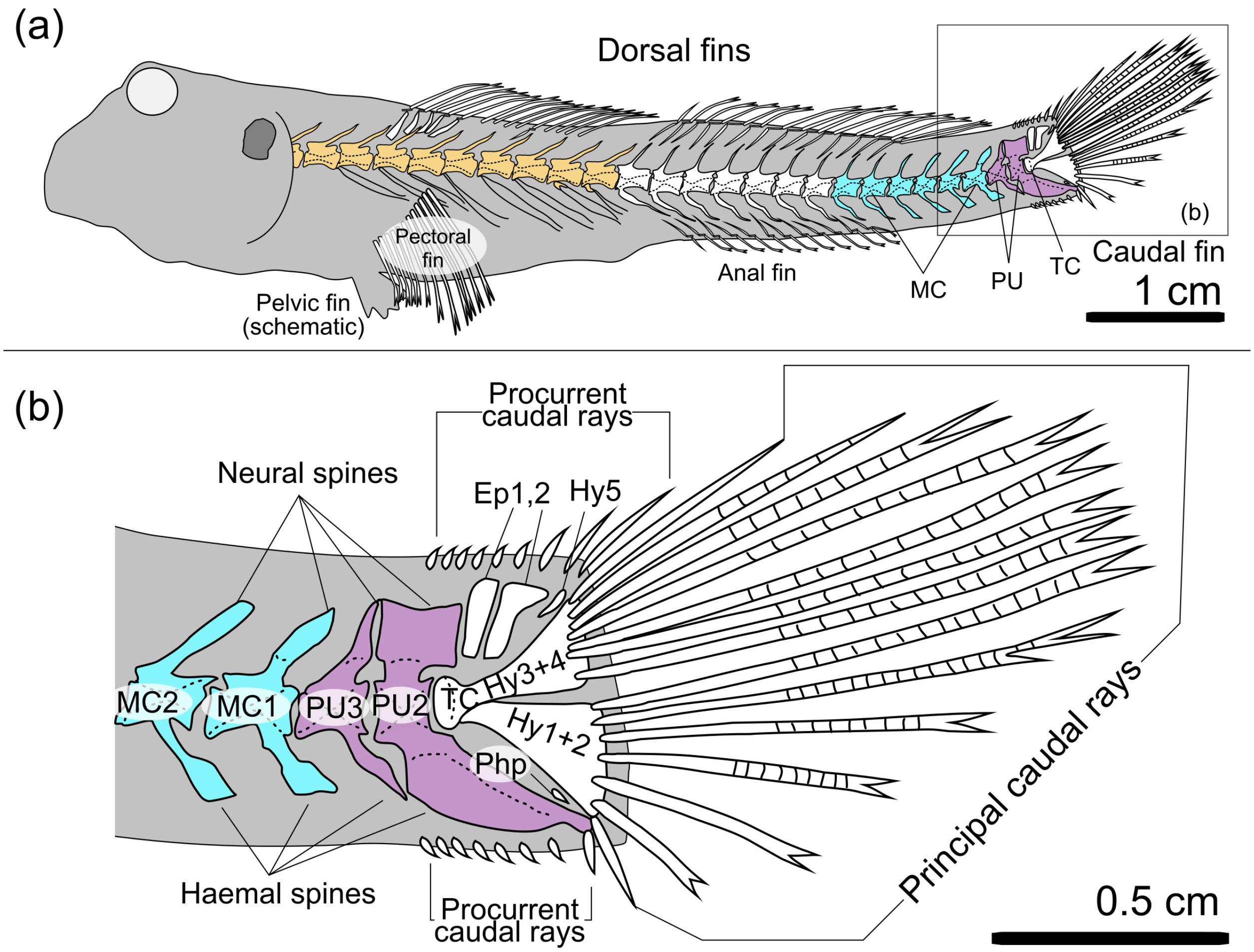


Figure 1: (a) Schematic osteology based on the mudskipper species *Ps. waltoni* showing the abdominal (light orange) and caudal vertebral column (light blue indicates a modified caudal vertebra, purple depicts a preural vertebra, no colour shading is a 'usual' caudal vertebra). (b) Close-up of posteriormost vertebrae and caudal skeleton. Abbreviations: Ep, epural; Hy, hypural plate; MC, modified caudal vertebra; Php, parhypural; PU, preural vertebra; TC, terminal centrum.

(as in *P. borneensis*), but also relatively closer positioned to the neural spine of PU3 (Fig. 3a). Two modified caudal vertebrae (MC1, MC2) occur in *S. tenuis* (Appendix: Fig. A1) and *Pn. freycineti* (Appendix: Fig. A2), and 3-6 modified caudal vertebrae are recorded in *Pn. schlosseri* (MC1–MC3, Appendix: Fig. A2), *Ps. novemradiatus* (MC1–MC5, Appendix: Fig. A2) and *Ps. waltoni* (MC1–MC6, Appendix: Fig. A2). Furthermore, the shape, size and inclination of the neural and haemal spines of PU2 and PU3 revealed clear differences between the species (Fig. 2-4), of which the details are specified in Table 2. Most noticeable is a particular close association between the neural spines of PU2 and PU3 in *Ps. novemradiatus* (Fig. 2) and *Ps. waltoni* (Fig. 3e, 4i): the PU2 spine has a convex anterior margin that dovetails with the concave posterior margin of the PU3 spine.

4.4.2 Caudal skeleton

The configuration of the hypural plates is largely the same in all ten species (Figs. 2, 3, Appendix: Figures A1 and A2). There are two large hypural plates (Hy1+2, Hy3+4), approximately equal in size and separated by a gap; HY3+4 is fused to the terminal centrum. Hypural plate 5 (Hy5) is a small, elongate or needle-shaped autogenous bone. Two relatively robust epural bones are present in all species. The parhypural is slender and not connected to the terminal centrum. In *P. borneensis* (Fig. 2e), *S. tenuis* (Fig. 2f), *Pn. Freycineti* (Fig. 3b),*Pn. Schlosseri* (Fig. 3c), *Ps. novemradiatus* (Fig. 3d) and *Ps. waltoni* (Fig. 3e), the parhypural is distinctly smaller than in the other species. The principal caudal rays are relatively uniform in *As. Bato* (Fig. 2b), *An. Madurensis* (Fig. 2d), *O. dentatus* (Fig.2c) and *P. borneensis* (Fig. 2e). In contrast, the principal caudal rays show an asymmetrical configuration in *B. dussumieri* (Fig. 3a), *Pn. freycineti* (Fig. 3b), *Pn. schlosseri* (Fig. 3c), *Ps. novemradiatus* (Fig. 3d), *Ps. waltoni* (Fig. 3e) and *S. tenuis* (Fig. 2f), with thickened and shortened ventralmost rays compared to their dorsal counterparts. This is most pronounced in *Ps. waltoni*, and least developed in *S. tenuis*.

4.5 Discussion

Several of the studied mudskipper species displayed at least one modified caudal vertebra (versus not present in other gobiids), with highest numbers in *Pn. schlosseri, Ps. novemradiatus*, and *Ps. waltoni* (Table 2). Modified caudal vertebrae may provide extra robustness to the posterior vertebral column, and thus help support and stabilize the body when the fish moves on land. This would be in line with the occurrence of the highest counts of modified caudal vertebrae in *Pn.*

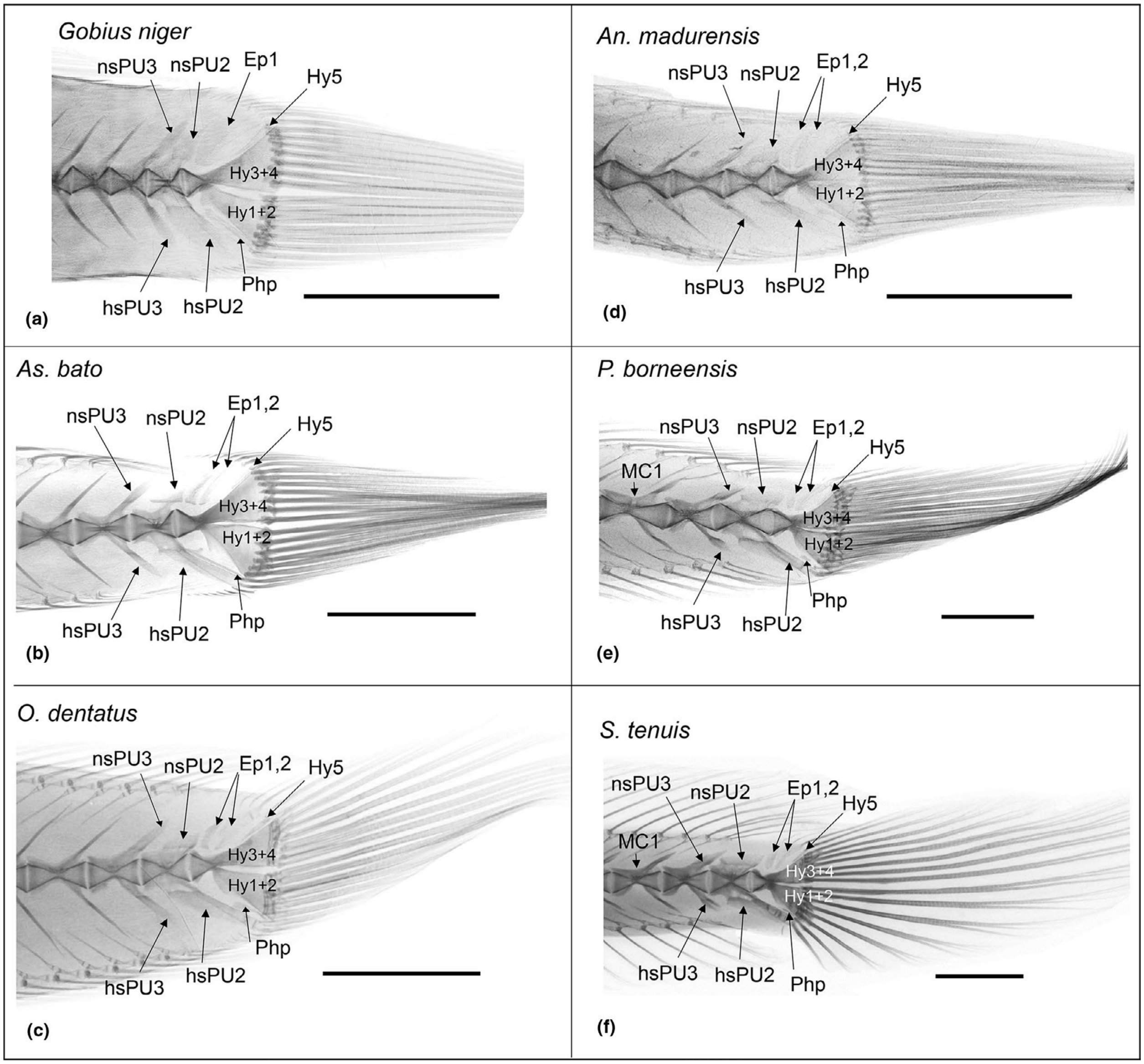


Figure 2: (X-ray images showing the posteriormost vertebral column and the caudal skeleton of a typical gobiid (a) and of the mudskipper species *Apocryptes bato* (b), *Oxuderces dentatus* (c), *Apocryptodon madurensis* (d), *Pseudapocryptes borneensis* (e), and *Scartelaos tenuis* (f). Collection numbers of specimens: a, NMP6V 146072; b, SMF 23715-2; c, SMNS 3410-1; d, ZMB 7637; e, ZMB 9931; f, ZM-CBSU 89. Abbreviations: Ep, epural; hs, haemal spine; Hy, hypural plate; MC, modified caudal vertebra; ns, neural spine; Php, parhypural; PU, preural vertebra. Institutional abbreviations as in Table 1; NMP, National Museum Prague. Scale bars = 5 mm

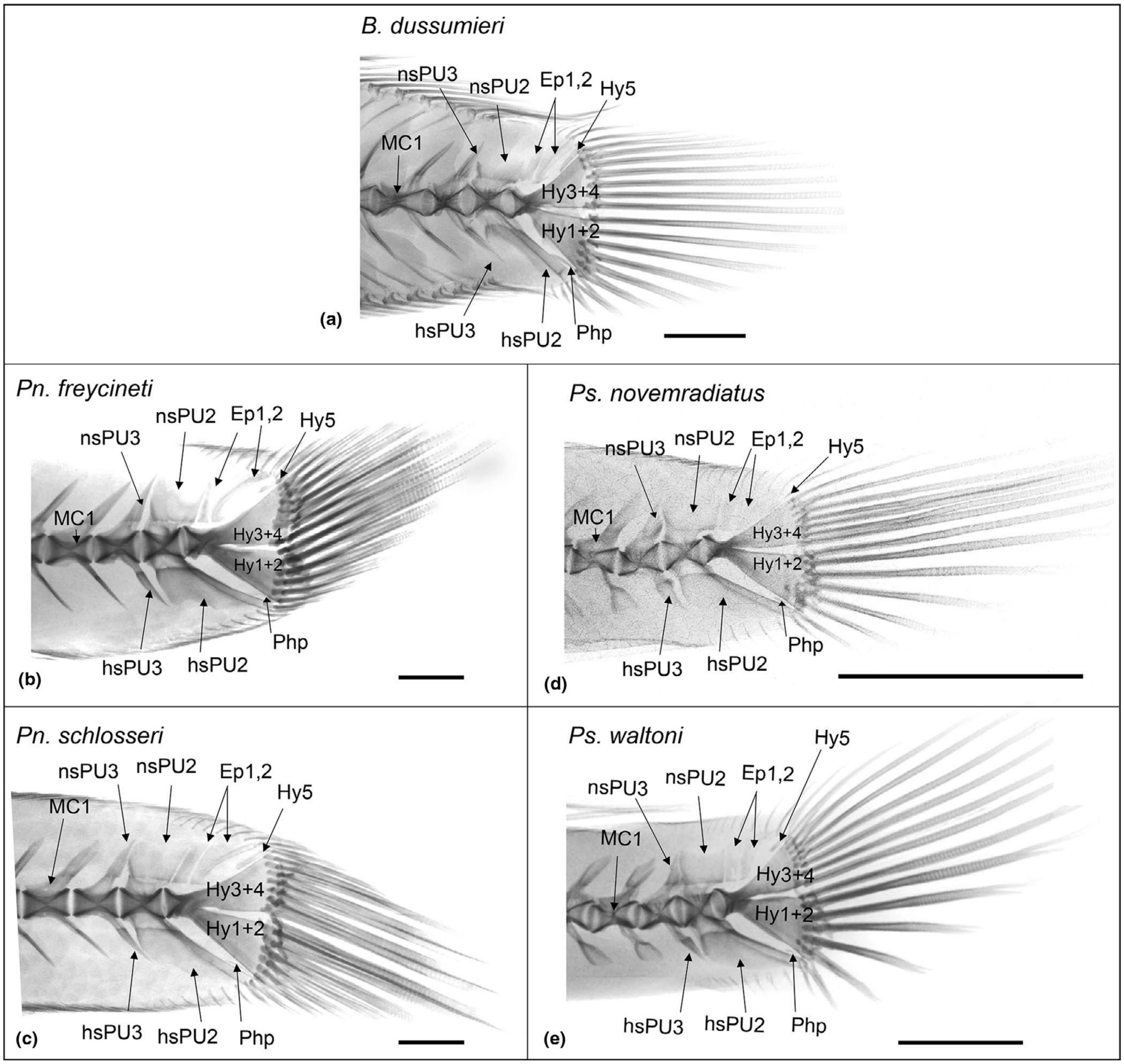


Figure 3: X-ray images showing the posteriormost vertebral column and the caudal skeleton of the mudskipper species *B. dussumieri* (a), *Periophthalmodon freycineti* (b), *Pn. schlosseri* (c), *Periophthalmus novemradiatus* (d), and *Ps. waltoni* (e). Collection numbers of specimens: a, ZM-CBSU 8; b, ZSM-PIS-019650; c, ZSM-PIS-023806; d, ZMB 5987–1; e, ZM-CBSU 96. Abbreviations for bones as in Fig. 2. Institutional abbreviations as in Table 1. Scale bars =5 mm

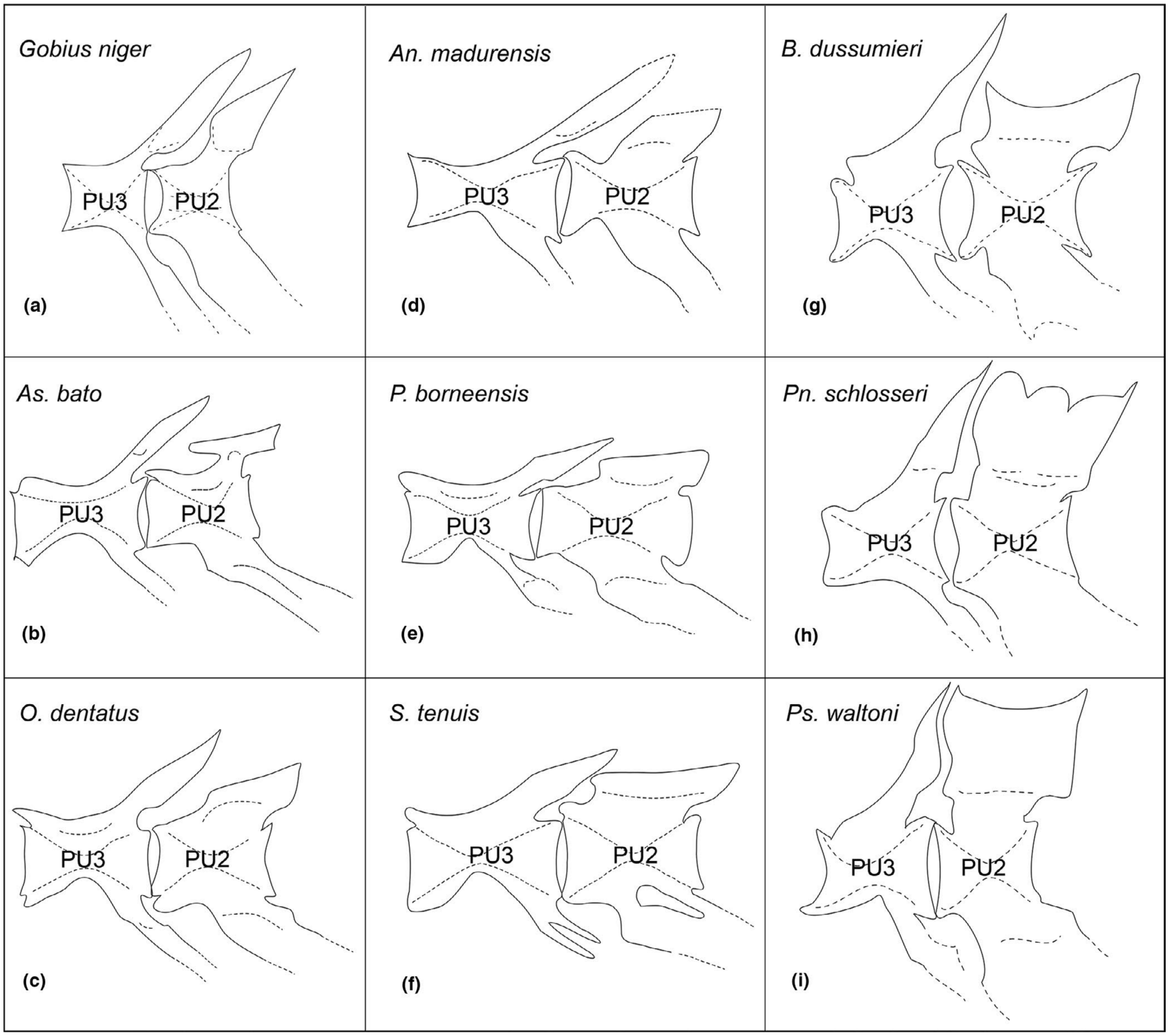


Figure 4: Schematic depiction of the preural vertebrae (PU) of the specimens shown in Figures 2 and 3 (not to scale) to show the disparity of the neural spine between the species

Table 2: Summary of the distinctive traits of the posteriormost vertebral column, caudal fin skeleton and caudal rays of the studied mudskippers; species are arranged (from left to right) according to the increase in specific characters.

Characters		*Gobius niger* (for comparison)	Studied mudkipper species									
			As. bato	*O. dentatus*	*An. madurensis*	*P. borneensis*	*S. tenuis*	*B. dussumieri*	*Pn. freycineti*	*Pn. schlosseri*	*Ps. novemradiatus*	*Ps. waltoni*
Presence of preural vertebrae		x	x	x	x	x	x	x	x	x	x	x
Presence of modified caudal vertebrae	MC1					(x)	x	x	x	x	x	x
	MC2						(x)		(x)	x	x	x
	MC3									x	x	x
	MC4 and MC5										x	x
	MC6											(x)
Shape neural spine PU2 (versus PU3)	Widened and pointed	(x)	(x)	(x)								
	Widened and truncated				(x)	x	x	x	x	x	x	x
	Elevated								x	x	x	x
Shape haemal spine PU2 (versus PU3)	Slightly elliptic	x										
	Plank-like		x	x	x	x	x	x				
	Fan-shaped					(x)	(x)		x	x	x	x
Proximity neural spines PU2 - PU3	Distance in-between	x	x	x	x	x	x					
	Very close							x	x	x	x	x
	Dovetailing										x	x
Dip neural spine PU3	Inclined	x	x	x	x	x	x					
	Almost upright							x	x	x	x	x
Parhypural	Long	x	x									
	Medium-sized or short			x	x	x		x				
	tiny					x	x		x	x	x	x
Principal caudal rays	Relatively uniform	x	x	x	x	x						
	Ventralmost rays thickened and shortened						x	x	x	x	x	x

schlosseri, Ps. novemradiatus, and *Ps. waltoni*, as these species are among the most amphibious of all oxudercine species (Murdy, 1989; Polgar et al., 2017; Polgar, Sacchetti, & Galli, 2010; Takita, Larson, & Ishimatsu, 2011). The dovetailing configuration of the neural spines of PU2 and PU3 seen in the two studied species of *Periophthalmus* (Figure 4h, i) is also visible in the drawings of the caudal skeleton of *Ps. kalolo* by Fujita (1990: fig. 499) and *Ps. barbarous* by Harris (1960: fig. 4). Harris (1960) explained it as an adaptation that enables the fish to execute powerful terrestrial 'jumps'('skipping' according to Harris, 1960, because the animal uses its tail to propel its body into the air, rather than the hind limbs, as in true jumping). Accordingly, the very close association between the neural spines of PU2 and PU3 represents a strengthening of these vertebrae, to withstand the strong forces that act upon them during skipping. That it is solely present in species of *Periophthalmus* correlates well with their high levels of adaptation to amphibious life (see Polgar et al., 2017). A thickening and shortening of the ventralmost principal caudal rays was previously noted only for *Periophthalmus* (Harris, 1960; Murdy, 1989). It has been confirmed here for *Ps. novemradiatus* and *Ps. waltoni* and was also recorded for *Pn. freycineti, Pn. schlosseri, B. dussumieri* and *S. tenuis* (Figs. 2f, 3a-e). The ventral portion of the caudal fin supports the body when the fish moves about on land or performs terrestrial jumps, e.g. during courtship or as an escape response (Pace, 2017; Zander, 2011), and the strengthening of the ventralmost principal caudal rays is an adaptation that supports these modes of locomotion (Harris, 1960). That the thickening and shortening of these rays is most pronounced in *Ps. waltoni* and least developed in *S. tenuis* correlates well with their relative levels of adaptation to amphibious life (see Polgar et al., 2017). A further aspect of this study is that the newly recognized traits all relate to hard parts that can easily fossilize. At present, no fossil mudskippers are known, although they probably have a long evolutionary history (Polgar et al., 2014). The improved knowledge of their skeletal structures can also facilitate the identification of mudskipper fossils that may come to light in the future.

4.6 Acknowledgements

For facilitating access to specimens we are grateful to Dr. Ulrich Schliewen and Dirk Neumann (SNSB-Bavarian State Collection of Zoology, Munich, Germany), Dr. Peter Bartsch and Edda Aßel (Museum für Naturkunde Berlin, Germany), Dr. Friedhelm Krupp and Dr. Moritz Sonnewald (Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt, Germany), Dr. Stefan Merker and Dagmar Beermann (Staatliches Museum für Naturkunde Stuttgart, Germany). We thank Dr. Radek Šanda (National Museum Praha, Czech Republic) and Dr. Jasna Vukić (Charles University, Praha, Czech Republic) for personally delivering the specimen of *Gobius niger.* Dirk Neumann (ZSM) kindly conducted the X-rays of *Pn. freycineti* and *Pn. schlosseri.* The fieldworks in Iran were financially supported by Shiraz University and University of Sistan and Baluchestan. We acknowledge Mr. Abdulwahed Pehpouri (Mashad, Iran) for logistical help with field trip and accommodation. HRE thanks DAAD (German Academic Exchange Service) for financial support to visit Ludwig-Maximilians-Universität München to undertake the present study. The manuscript benefited greatly from the constructive comments of Dr. Gianluca Polgar (Western Kentucky University, USA) and two anonymous reviewers. Finally, we thank Dr. Paul Hardy (Düsseldorf, Germany) for critical reading of the manuscript. The authors have no conflict of interest to declare.

4.7 References

Agorreta, A., San Mauro, D., Schliewen, U., Van Tassell, J. L., Kovačić, M., Zardoya, R., & Rüber, L. (2013). Molecular phylogenetics of gobioidei and phylogenetic placement of European gobies. *Molecular Phylogenetics and Evolution*, 69(3), 619–633. https://doi.org/10.1016/j.ympev.2013.07.017

Birdsong, R. S., Murdy, E. O., & Pezold, F. L. (1988). A study of the vertebral column and median fin osteology in gobioid fishes with comments on gobioid relationships. *Bulletin of Marine Science*, 42(2), 174–214.

Charmpila, E. A., Teimori, A., Freyhof, J., Weissenbacher, A., & Reichenbacher, B. New osteological and morphological data of four species of *Aphaniops* (Teleostei; Aphaniidae). *Journal of Applied Ichthyology.* (in review).

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

Gill, A. C., & Mooi, R. D. (2012). Thalasseleotrididae, new family of marine gobioid fishes from New Zealand and temperate Australia, with a revised definition of its sister taxon, the Gobiidae (Teleostei: Acanthomorpha). *Zootaxa*, 3266(1), 41–52. https://doi.org/10.11646 / zoota xa.3266.1.3

Harris, V. A. (1960). On the locomotion of the mudskipper *Periophthalmus koelreuteri* (Pallas): (Gobiidae). *Proceedings of the Zoological Society of London*, 134(1), 107–135. https://doi.org/10.1111/j.1469-7998.1960. tb059 21.x

Hoese, D. F. (1984). Gobioidei: Relationships. In H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, J. A. W. Kendall & S. L. Richardson (Eds.), *Ontogeny and systematics of fishes* (pp. 588– 591). Gainesville, Florida: American Society of Ichthyologists and Herpetologists.

Ishimatsu, A., & Gonzales, T. T. (2011). Mudskippers: Front runners in the modern invasion of land. In R. A. Patzner, J. L. Van Tassell, M. Kovačić, & B. G. Kapoor (Eds.), *The biology of gobies* (pp. 609–638). Enfield, NH: Science Publishers Inc.

Jaafar, Z., & Murdy, E. O. (Eds.) (2017). *Fishes out of water: Biology and ecology of Mudskippers*, CRC Marine Sciences Series, 1st ed. Boca Raton, FL: Taylor & Francis.

Lauder, G. V. (1989). Caudal fin locomotion in ray-finned fishes: Historical and functional analyses. *American Zoologist*, 29(1), 85–102. https:// doi.org/10.1093/icb/29.1.85

Michel, K. B., Adriaens, D., Aerts, P., Dierick, M., & Van Wassenbergh, S. (2014). Functional anatomy and kinematics of the oral jaw system during terrestrial feeding in *Periophthalmus barbarus*. *Journal of Morphology*, 275(10), 1145–1160. https://doi.org/10.1002/jmor.20291

Murdy, E. O. (1989). A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae: Oxudercinae). *Records of the Australian Museum, Supplements*, 11, 1–93. https://doi.org/10.3853/ j.0812-7387.11.1989.93

Murdy, E. O., & Jaafar, Z. (2017). Taxonomic and systematic review. In Z. Jaafar, & E. O. Murdy (Eds.), *Fishes Out of Water: Biology and Ecology of Mudskippers* (pp. 1–36). Boca Raton, FL: Taylor & Francis Group.

Pace, C. (2017). Aquatic and terrestrial locomotion. In Z. Jaafar, & E. O. Murdy (Eds.), *Fishes out of water: Biology and ecology of mudskippers* (pp. 386–414). Boca Raton, FL: Taylor & Francis Group.

Parenti, L. R., & Jaafar, Z. (2017). *The Natural Distribution of Mudskippers.* In Z. Jaafar, & E. O. Murdy (Eds.), Fishes out of water: Biology and ecology of mudskippers (pp. 37-68). Boca Raton, FL: Taylor & Francis Group.

Polgar, G., & Crosa, G. (2009). Multivariate characterisation of the habitats of seven species of Malayan mudskippers (Gobiidae: Oxudercinae). *Marine Biology*, 156(7), 1475–1486. https://doi.org/10.1007/s00227-009-1187-0

Polgar, G., Ghanbarifardi, M., Milli, S., Agorreta, A., Aliabadian, M., Esmaeili, H. R., & Khang, T. F. (2017). Ecomorphological adaptation in three mudskippers (Teleostei: Gobioidei: Gobiidae) from the Persian Gulf and the Gulf of Oman. *Hydrobiologia*, 795(1), 91–111. https://doi. org/10.1007/s1075 0-017-3120-8

Polgar, G., Sacchetti, A., & Galli, P. (2010). Differentiation and adaptive radiation of amphibious gobies (Gobiidae: Oxudercinae) in semi-terrestrial habitats. *Journal of Fish Biology*, 77(7), 1645–1664. https:// doi.org/10.1111/j.1095-8649.2010.02807.x

Polgar, G., Zane, L., Babbucci, M., Barbisan, F., Patarnello, T., Rüber, L., & Papetti, C. (2014). Phylogeography and demographic history of two widespread Indo-Pacific mudskippers (Gobiidae: *Periophthalmus*). *Molecular Phylogenetics and Evolution*, 73, 161–176. https://doi. org/10.1016/j.ympev.2014.01.014

R Development Core Team. (2008). R: A Language and Environment for Statistical Computing: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Sayer, M. D. J. (2005). Adaptations of amphibious fish for surviving life out of water. *Fish and Fisheries*, 6(3), 186–211. https://doi. org/10.1111/j.1467-2979.2005.00193.x

Schöttle, E. (1931). Morphologie und physiologie der Atmung bei wasser-, schlamm-, und landlebenden Gobiiformes. *Zeitschrift Für Wissenschaftliche Zoologie*, 140, 1–114.

Schultze, H.-P., & Arratia, G. (2013). *The caudal skeleton of basal teleosts, its conventions, and some of its major evolutionary novelties in a temporal dimension.* In G. Arratia, H.-P. Schultze, & M. V. H. Wilson (Eds.), Mesozoic Fishes 5 – Global Diversity and Evolution, Vol. 5 (pp.187–246). München: Verlag Dr. Friedrich Pfeil.

Swanson, B. O., & Gibb, A. C. (2004). Kinematics of aquatic and terrestrial escape responses in mudskippers. *The Journal of Experimental Biology*, 207(23), 4037–4044. https://doi.org/10.1242/jeb.01237

Takita, T., Larson, H. K., & Ishimatsu, A. (2011). The natural history of mudskippers in northern Australia, with field identification characters. *Beagle: Records of the Museums and Art Galleries of the Northern Territory*, 27, 189–204.

You, X., Sun, M., Li, J., Bian, C., Chen, J., Yi, Y., Yu, H., & Shi, Q. (2018). Mudskippers and their genetic adaptations to an amphibious lifestyle. *Animals*, 8(2), 24. https://doi.org/10.3390/ani80 20024

Zander, C. D. (2011). *Morphological adaptations to special environments of gobies.* In R. A. Patzner, J. L. Van Tassell, M. Kovačić, & B. G. Kapoor (Eds.), The Biology of Gobies (pp. 345–366). Enfield, NH: Science Publishers Inc.

5 Possible link between the structure of otoliths and amphibious mode of life of three mudskipper species (Teleostei: Gobioidei) from the Persian Gulf

Mehdi Ghanbarifardi[1], Carolin Gut[2], Zeinab Gholami[2], Hamid Reza Esmaeili[2,3], Christoph Gierl[2], Bettina Reichenbacher[2,4]

[1]Department of Biology, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan, Iran

[2]Department of Earth and Environmental Sciences, Ludwig-Maximilians-Universität München, Richard-Wagner Straße 10, 80333 Munich, Germany

[3]Zoology Section, Department of Biology, School of Science, Shiraz University, Shiraz, Iran

[4]GeoBio-Center, Ludwig-Maximilians-Universität München, 80333 Munich, Germany

5.1 Abstract

The otoliths of three mudskipper species from the Persian Gulf that are characterised by different degrees of amphibious adaptation were examined using SEM imaging and otolith morphometry. *Scartelaos tenuis*, which is the most aquatic of the three species, has rhomboid to rectangular otoliths that conform to the general otolith type of many marine gobiid species. The otoliths of *Periophthalmus waltoni*, which is one of the most amphibious mudskipper species, are pentagonal-shaped and deviate from the usual otolith type of the Gobiidae by the absence of a posterodorsal projection and deep ostial lobes. *Boleophthalmus dussumieri* is intermediate with respect to its amphibious adaptation between *S. tenuis* and *P. waltoni*, and the shape of its otoliths is intermediate between the otoliths of those two species. Otolith morphometry related to sulcus measurements more clearly separated *P. waltoni* from *B. dussumieri*, than *S. tenuis* from either *P. waltoni* or *B. dussumieri*. As *P. waltoni* and *B. dussumieri* can occur syntopically and display similar behaviours, the morphometric disparity of their sulcus proportions might be related to efficient intraspecific communication among neighbouring species.

Keywords: Amphibious goby; amphibious adaptation; otolith morphometry; intraspecies communication

5.2 Introduction

Mudskippers are amphibious fishes that form part of the *Periophthalmus* lineage within the 'gobionelline-like Gobiidae' among the Gobioidei (Agorreta et al., 2013) and are widely distributed in coastal mangrove and mudflat areas in the tropics (Hoese, 1984; Murdy, 1989; Parenti & Jaafar, 2017). They are characterised by specialised behaviours, including the construction of nests and burrows, defence of territories and performance of courtship displays, and the ability to use their limb-like pectoral fins for locomotion on land (Harris, 1960; Sayer, 2005; Pace, 2017). However, otoliths of mudskippers are poorly known and whether the otoliths reveal any interrelationship to the unique mode of life of these fish has not yet been explored.

Otoliths are aragonitic structures located in the inner ear of a fish where they are arranged in three pairs referred to as sagittae (otoliths of the sacculus), lapilli (otoliths of the utriculus) and asterisci (otoliths of the lagena), respectively (Nolf 1985; Schulz-Mirbach & Reichenbacher, 2006). Otoliths develop independently of the skeleton and contribute to the senses of hearing and balance (Popper, 1981; Campana & Neilson, 1985; Popper & Lu, 2000). In most teleosts, the sagitta is the largest otolith, while the lapillus and the asteriscus are relatively small structures (Assis, 2003). The morphology of the sagitta represents a meaningful taxonomic tool for species identification (Tuset, Lombarte, & Assis, 2008; Nolf, 2013; Reichenbacher & Reichard, 2014), although environmental factors are known to influence its shape and size (Lombarte & Lleonart, 1993; Volpedo & Echeverría, 2003; Vignon & Morat, 2010). The specific morphology of the sagitta appears to be associated with functional demands made on the sensory system of the inner ear by the ecological niches occupied by individual species (Popper, Ramcharitar, & Campana, 2005; Lombarte, Palmer, Matallanas, Gómez-Zurita, & Morales-Nin, 2010) and also by intraspecific acoustic communication (Ramcharitar, Higgs, & Popper, 2001; Ramcharitar, Deng, Ketten, & Popper, 2004; Schulz-Mirbach, Ladich, Plath, Metscher, & Hess, 2014).

Photos of otoliths, but no descriptions, have been provided for three mudskipper species, namely *Boleophthalmus pectinirostris* (Linnaeus, 1758) (see Nanami, 2017: fig. 4.1), *Periophthalmodon schlosseri* (Pallas, 1770) (see Sarimin, Ghaffar, & Mohamed, 2009: fig. 2) and *Periophthalmus kalolo* Lesson, 1831 (see Rivaton & Bourret, 1999: pl. 59, figs. 5–6, as P. vulgaris). The focus of this study is to investigate otolith morphology and differentiation among three mudskipper species that are restricted to the Persian Gulf and the adjoining coasts of Pakistan and western India (Figure 1), i.e. *Boleophthalmus dussumieri* Valenciennes, 1837, *Periophthalmus waltoni* Koumans, 1955 and *Scartelaos tenuis* (Day, 1876). The three species are characterised by distinctive body shapes and body surface-to-volume ratios (Polgar et al., 2017). *Periophthalmus waltoni* has a sub-cylindrical body shape and is well adapted to semi-terrestrial habitats, the elongate shape of *S. tenuis* is indicative for a mostly aquatic environment, and *B. dussumieri* is intermediate between the two others in both respects (Clayton & Snowden, 2000; Polgar et al., 2017). The different degrees of amphibious adaptation are also reflected in their behaviours and relative mobilities when they are on land. *Boleophthalmus dussumieri* and *S. tenuis* largely depend on undulatory motions of the whole body, and they cannot easily raise their bodies off the substrate, whereas *P. waltoni* relies much less on undulating movements and can easily raise its body off the substrate (Polgar et al., 2017). When the three species occur syntopically, their burrows are clearly separated within the intertidal zone, with burrows of *S. tenuis* being located lowest and those of *P. waltoni* highest on the tidal flat (Clayton, 1993; Polgar et al., 2017).

Photos, descriptions and otolith morphometry for the three species from the Persian Gulf to which the present study refers have been provided by Gierl, Liebl, Šanda, Vukić, Esmaeili, & Reichenbacher (2018) within a broader taxonomic context, but not considering the specific adaptation of each species. The objective of our study was to scrutinise the morphological and morphometric differences between the otoliths of *B. dussumieri, P. waltoni* and *S. tenuis* and to discuss whether any of the otolith differences might be linked to the different degrees of amphibious adaptation of the three species or other specialisations.

5.3 Material and Methods

Material. The individuals of *Boleophthalmus dussumieri, Periophthalmus waltoni* and *Scartelaos tenuis* were collected with hand nets at low tide from coastal mudflat and mangrove sites along the Persian Gulf in southern Iran. The specimens of *S. tenuis* and *P. waltoni* were obtained from the Heleh wetland, and those of *B. dussumieri* from Khorramshahr (Figure 1, Table 1). All specimens were fixed in ethanol. All aspects of the sampling complied with protocols approved by the Ethics Committee of the Biology Department at Shiraz University and adhered to the prevailing legal requirements in Iran (SU-9330207).

Otolith study. Fish skulls were opened dorsally under a microscope and left and right saccular otoliths (sagittae) were removed. Otoliths were incubated in 5% KOH solution for 1 h to clean off adherent organic matter, immersed in distilled water for 1-2 h, then washed several times with distilled water, dried and stored. All otoliths were mounted and gold-coated for scanning

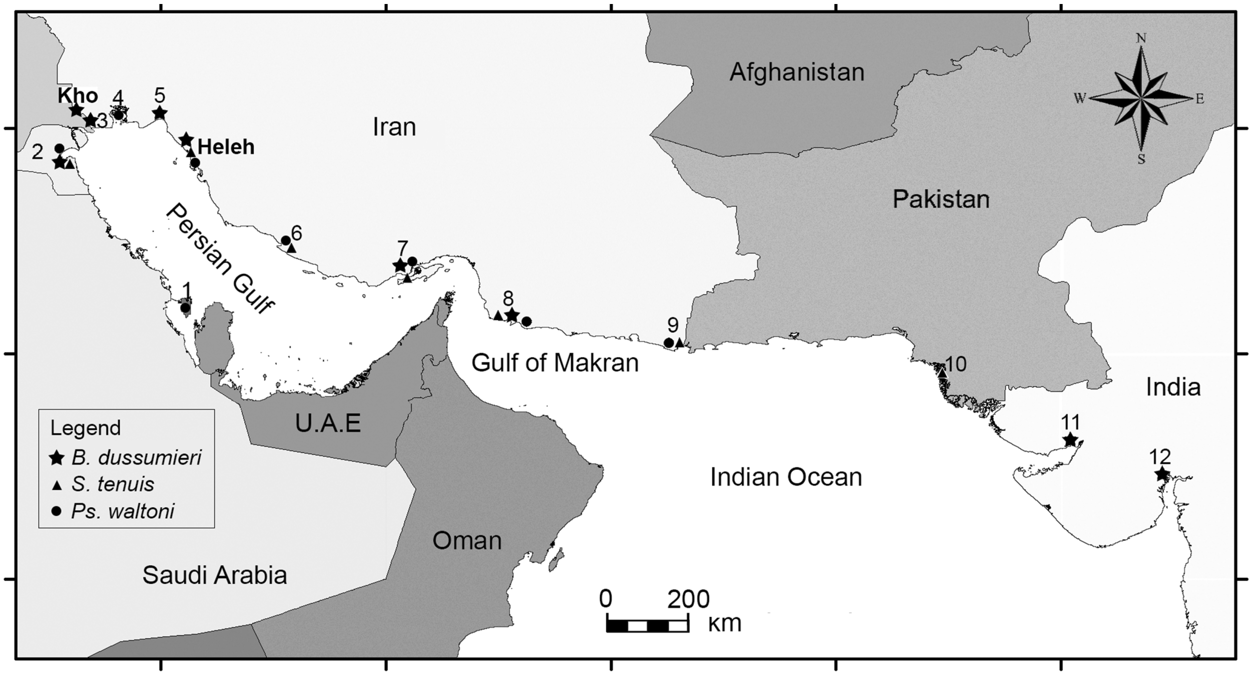


Figure 1: Distribution of *Boleophthalmus dussumieri*, *Scartelaos tenuis* and *Periophthalmus waltoni*. The specimens of *B. dussumieri* used in our study were collected at Khorramshar (Kho), those of *Ps. waltoni* and *S. tenuis* at Heleh. References: Murdy (1989), Bishop (2003), Ghan-barifardi & Malek (2007), Ghanbarifardi, Aliabadian, Esmaeili, & Polgar (2014), Ghanbarifardi, Esmaeili, Gholami, Aliabadian, & Reichenbacher (2016), Ghanbarifardi, Aliabadian, & Esmaeili (2018a), Ghanbarifardi, Aliabadian, & Esmaeili (2020).

electron microscopy (SEM LEO 1430 VP at ZSM Munich). On the basis of the SEM images, otolith descriptions and measurements were conducted. The otolith shape was described using the terminology of Lombarte, Miletić, Kovačić, Otero-Ferrer, and Tuset, (2018), further otolith terminology followed established conventions (Figure 2a). Otolith morphometry was conducted according to Gierl et al. (2018) and included 10 measurements (Figure 2b), i.e. otolith area (OA), otolith height (OH), otolith length (OL), otolith perimeter (OP), sulcus area (SuA), sulcus height (SuH), sulcus length (SuL), sulcus perimeter (SuP), distance from sulcus end to ventral margin (SuEndV), and distance from sulcus tip to ventral margin (SuTipV). The measurements were used to calculate a total of 23 variables, i.e. OL/OH, OP/OL, OP/OH, SuA/OA, SuP/OP, SuP/SuTipV, SuP/SuEndV, SuL/OL, SuL/OH, SuL/SuH, SuL/SuTipV, SuL/SuEndV, SuL/OP, SuL/SuP, SuH/OL, SuH/OH, SuH/SuTipV, SuH/SuEndV, SuH/OP, SuH/SuP, SuTipV/OP, SuTipV/SuEndV, and SuEndV/OP. The value of each variable was multiplied by 1000 to facilitate further analyses (raw data given in Supplementary Table S1). Standard statistical analyses were conducted based on PASW 26.00 (SPSS, 2019) and the program R (R Core Team, 2019) with its packages car, corrplot and stats. Normal distribution of otolith variables for each species was

Table 1: Material used for this study. N, number of specimens; SL, standard length of fish specimen; SD, standard deviation; ZM-CBSU, Zoological Museum of Shiraz University, Biology Depart-ment.

Species	**N**	**SL (cm) range (mean ±SD)**	**Locality collection date**	**Collection codes**
Boleophthalmus dussumieri Valenciennes, 1837	6	5.7–13.7 (11.3±3.0)	Persian Gulf, Khorramshahr, Iran; 48°11'E, 30°26'N (9.iv.2015)	ZM-CBSU 4, 6–9, 11
Periophthalmus waltoni Koumans, 1941	7	6.8 – 9.7 (8.2 ± 1.1)	Persian Gulf, Heleh Wetland, Iran; 50°40'E 29°14'N (11.xii.2015)	ZM-CBSU 92, 93, 95, 96, 101, 1716, 1727
Scartelaos tenuis (Day, 1876)	5	5.8 – 8.9 (7.7 ± 1.4)		ZM-CBSU 82, 84, 85, 86, 89

examined using the Shapiro-Wilk test ($P>0.05$), which indicated that, with few exceptions, all variables were normally distributed. Covariance between otolith variable and otolith length was tested using Pearson and Spearman tests ($P<0.05$); in case of covariance, otolith length was added as covariate for further analyses. Homogeneity of variances was examined using Levene's test ($P>0.05$). One-way ANOVA or ANCOVA (in case of covariance) with post-hoc tests ($P<0.05$) was used to test the significance of individual otolith variables in discriminating a species. In the case of homogeneity, Tukey HSD post-hoc test was applied, otherwise Dunnett T3 post-hoc test was carried out.

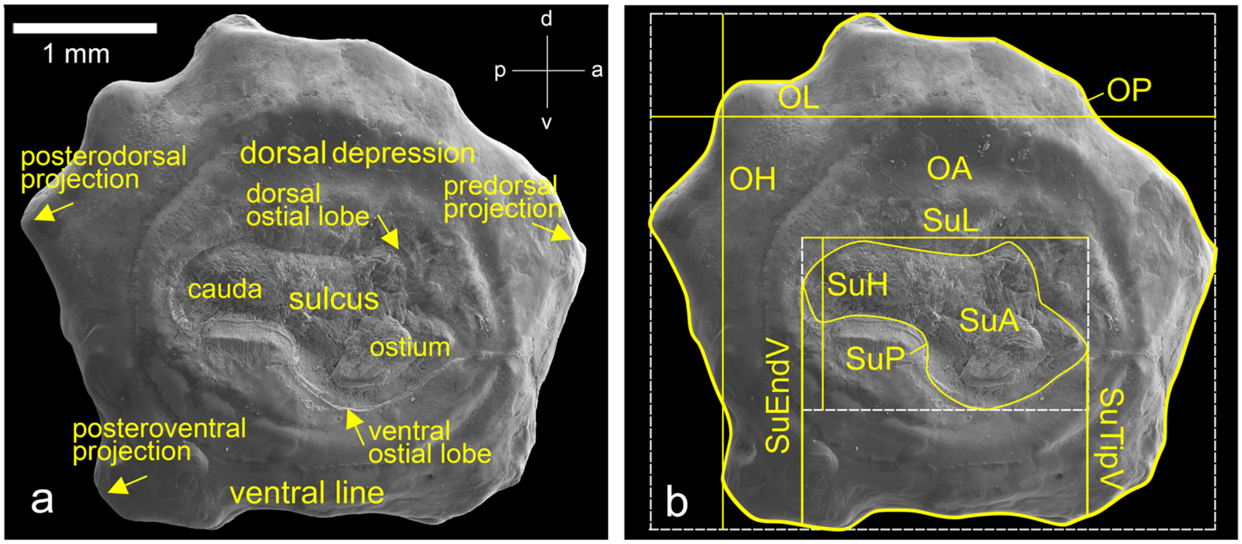


Figure 2: (a) Left otolith (sagitta) of *Periophthalmus waltoni* in medial view (ZM-CBSU 93, SEM image) showing the otolith terminology used (after Nolf, 1985 and Schwarzhans, 2014, slightly modified). (b) Measured distances and areas (after Gierl et al., 2018). Abbreviations: OA, otolith area; OH, otolith height; OL, otolith length; OP, otolith perimeter; SuA, sulcus area; SuH, sulcus height; SuL, sulcus length; SuP, sulcus perimeter; SuEndV, distance from sulcus end to ventral margin; SuTipV, distance from sulcus tip to ventral margin.

5.4 Results

The otoliths of the three species from the Persian Gulf differ from each other in their characteristic overall outline. The otoliths of *B. dussumieri* are rhomboid (Figure 3d–f), those of *P. waltoni* are pentagonal (Figure 3g–i), and the otolith shape of *S. tenuis* is rhomboid to rectangular (Figure 3a–c). The dorsal margin is slightly or distinctively crenulated in *B. dussumieri* and *S. tenuis* or lobed (*B. dussumieri, P. waltoni*). The dorsal margins of the otoliths of *B. dussumieri* and *P. waltoni* are symmetrically curved, while the otoliths of *S. tenuis* display a sloping dorsal margin. A prominent posterodorsal projection occurs in the otoliths of *B. dussumieri* and *S. tenuis*; it is straight in the former and dorsally directed in the latter (Figure 3a–f). In *P. waltoni* an edge (Figure 3g, i) or a bulge (Figure 3h) is found at the position where the posterodorsal projection would be expected. A predorsal projection is visible in the otoliths of *P. waltoni* and *S. tenuis*, but not in *B. dussumieri* (Figure 3). The otoliths of all species display an elongate dorsal depression and a well developed ventral line extending upwards to the dorsal depression (Figure 3). Also the sulcus is largely similar between the three species, it has the shoe-sole like shape that is generally typical for gobiid otoliths, with the ostium being inclined anteroventrally. The position of the sulcus is supramedian in *B. dussumieri* (Figure 3d–f), and median in both *P. waltoni* (Figure 3g–i) and *S. tenuis* (Figure 3a–c). In all three species, the dorsal and ventral margins of the ostium are strongly curved, forming distinctive dorsal and ventral ostial lobes (Figures 2a, 3). There is some variability in the shape of the ostial lobes both within and between the three species. *Periophthalmus waltoni* generally displays a deeper curved ventral ostial lobe than is seen in *B. dussumieri* and *S. tenuis* (Figure 3). The otolith dissimilarities are additionally confirmed by significant differences of OL/OH, SuL/SuH, SuH/OH, SuH/SuP and SuH/SuTipV between the three species (Figure 4). OL/OH is highest in *S. tenuis* (mean 1.2±0.05 vs. 1.1±0.05 in *B. dussumieri* and *P. waltoni*, Suppl. Table S1, see also Figure 4a), which is consistent with the more rectangular outline of *S. tenuis* (Figure 3a–c). SuL/SuH is similar in *P. waltoni* and *S. tenuis* (mean 1.9±0.3 and 1.7±0.1, respectively, Suppl. Table S1, see also Figure 4b), but clearly higher in *B. dussumieri* (mean 2.2±0.3). SuH/OH, SuH/SuP, and SuH/SuTipV had similar values in *P. waltoni* and *S. tenuis*, but were significantly smaller in *B. dussumieri* (Suppl. Table S1, see also Figure 4c–e). Overall, four variables (SuL/SuH, SuH/OH, SuH/SuP, SuH/SuTipV) separated *B. dussumieri* vs. *P. waltoni*, two (OL/OH, SuH/SuTipV) discriminated between *B. dussumieri* and *S. tenuis*, while a single variable (OL/OH) distinguished *P. waltoni* from *S. tenuis* (Table 2).

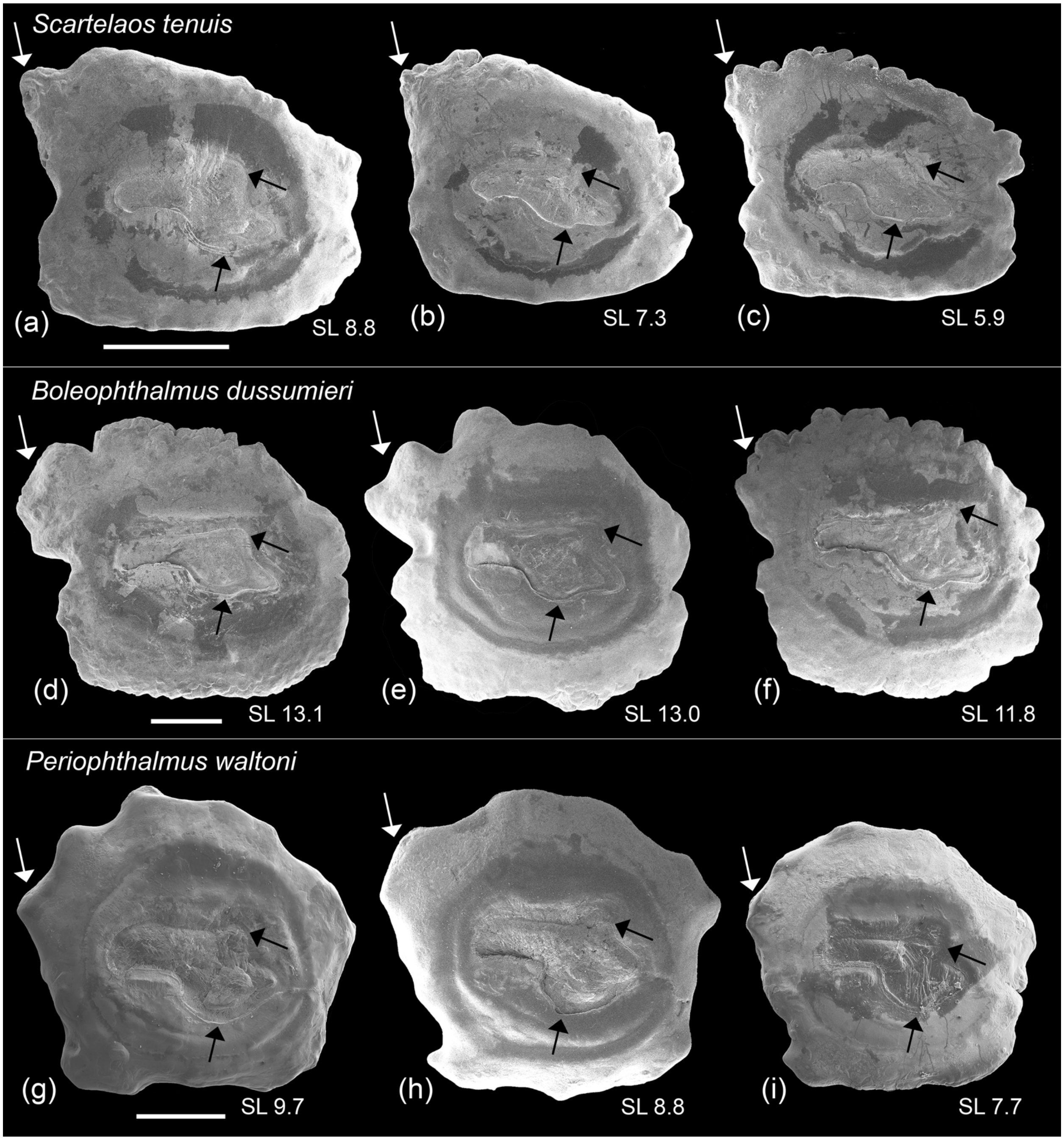


Figure 3: Morphology of the otoliths of the three species from the Persian Gulf (SEM images, medial view, left sagittae). Black arrows indicate the ventral and dorsal ostial lobe, white arrows designate the posterodorsal projection. SL refers to standard length (in cm) of the specimen from which the otolith was extracted. Collection numbers: (a), ZM-CBSU 89; (b), ZM-CBSU 84; (c), ZM-CBSU 86; (d), ZM-CBSU 07; (e), ZM-CBSU 09; (f), ZM-CBSU 08; (g), ZM-CBSU 93; (h), ZM-CBSU 92; (i), ZM-CBSU 96; Scale bars = 1 mm.

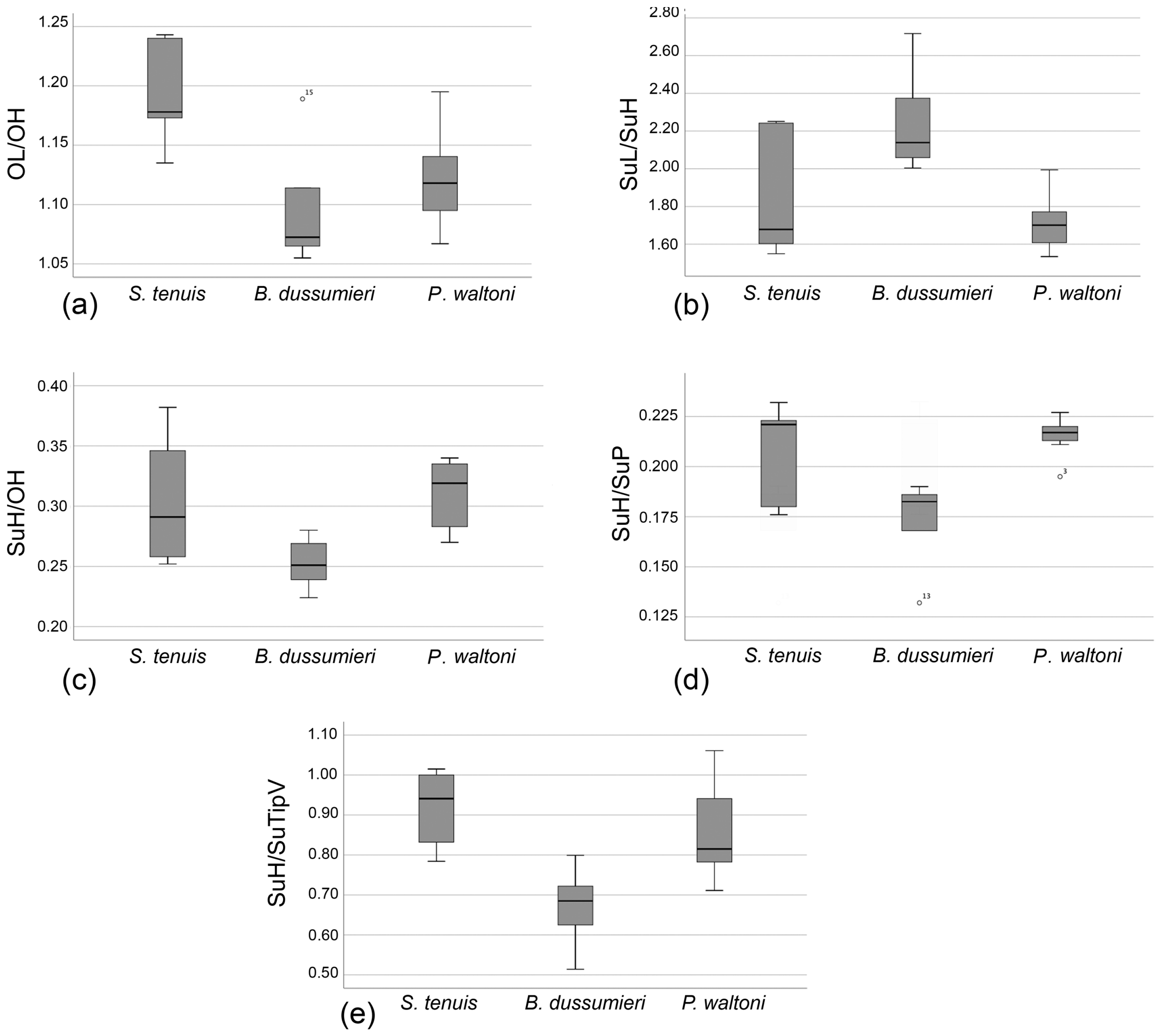


Figure 4: Box plots of the five otolith variables that differ significantly between the studied species showing the median (line within the box), the 25th and 75th percentiles, the variance, and the data range (y-axis) of the respective otolith variable. OL/OH, ratio otolith length and otolith height; SuL/SuH, ratio sulcus length and sulcus height; SuH/OH, ratio sulcus height and otolith height; SuH/SuP, ratio sulcus height and sulcus perimeter; SuH/SuTipV, ratio sulcus height and distance from sulcus tip to ventral margin. Open circles refer to outliers within the 100th percentile. See Table 2 for significance of differences.

Table 2: Differences of the otolith variables between the three mudskipper species from the Persian Gulf based on One-way ANOVA ($P<0.05$) with Tukey HSD (indicated with superscript T) and Dunnett T3 (superscript D) post-hoc tests, depending on homogeneity of variances (Levene's test, $P>0.05$). * significant difference; - no significant difference.

	B. dussumieri vs. *S. tenuis*	*B. dussumieri* vs. *P. waltoni*	*S. tenuis* vs. *P. waltoni*
OL/OH (otolith length / otolith height)^{T}	*	-	*
SuL/SuH (sulcus length / sulcus height)^{D}	-	*	-
SuH/OH (sulcus height / otolith height)^{D}	-	*	-
SuH/SuP (sulcus height / sulcus perimeter)^{D}	-	*	-
SuH/SuTipV (sulcus height / distance from sulcus tip to ventral margin)^{T}	*	*	-

5.5 Discussion

The overall shape of otoliths of gobionelline-like Gobiidae (*sensu* Agorreta et al., 2013) is typically rhomboid to rectangular or rhomboid to quadratic (Nolf, 2013; Gierl et al., 2018; Lombarte et al., 2018). Among the three mudskipper species from the Persian Gulf, only the otoliths of *S. tenuis* (rhomboid to rectangular) conform to this pattern, and this species likewise resembles the vast majority of gobiids in having a predominantly aquatic lifestyle (see Polgar et al., 2017). The pentagonal shape of the otoliths in *P. waltoni* can be interpreted as derived because it is clearly atypical among gobiids, and may be correlated with the unusual semi-terrestrial habitat favoured by this species (see Polgar et al., 2017). *Boleophthalmus dussumieri*, on the other hand, possesses otoliths 'intermediate' between those of *S. tenuis* and *P. waltoni*, and also its habitat has been shown to be somewhat intermediate between that of *S. tenuis* and *P. waltoni* (see Polgar et al., 2017). It thus appears that the different otolith shapes of the three species reflect their different degrees of amphibious adaptation. However, the role of other factors such as water depth, communication or phylogenetic distance cannot be excluded and studies on the otoliths of additional mudskipper species are necessary to confirm our suggestion. While the pentagonal otolith shape of *P. waltoni* (Figure 3g–i) is strikingly different from the rectangular otolith shape of *S. tenuis* (Figure 3a–c), otolith morphometry more clearly separates *P. waltoni* from *B. dussumieri*, than *S. tenuis* from either *P. waltoni* or *B. dussumieri* (Figure 4, Table 2). Notably, the separating otolith variables all deal with the sulcus, namely sulcus length (SuL), height (SuH), perimeter (SuP), and the vertical distance from the tip of the sulcus to the ventral margin (SuTipV). The sulcus is the only part of the otolith inner surface that is directly in contact with the sensory hair bundles of the vestibular sensory organs of the inner ear (Fekete, 2003; Schulz-Mirbach et al., 2014). Thus it can be assumed that sulcus morphology more directly reflects the demands of hearing and equilibrium than the remaining otolith surface. Previous works have demonstrated that both *P. waltoni* and *B. dussumieri* are strongly territorial; adult specimens of *P. waltoni* may establish several burrows within an area of 2-3 m2, and large individuals of *B. dussumieri* each live in mud-walled sections on tidal mudflats (Clayton & Vaughan, 1988; Clayton, 1993; Clayton & Snowden, 2000). When both species occur in the same locality, they usually set up their burrows along a tidal gradient (Polgar et al., 2017). It seems logical to assume that neighbouring species with similar behaviours must develop a very efficient mode of intraspecific communication; for example, to defend their burrows, or during courtship displays. If this were the case for *P. waltoni* and *B. dussumieri*, their species-specific communication skills might well be reflected in the morphometric differences between the sulcus of their respective otoliths.

5.6 Supplementary Material

Supplementary material (Table S1) is given as a Supplementary Annex, which is available via the "Supplementary" tab on the article's online page (http://dx.doi.org/10.1080/09397140.2020.1805140).

5.7 Funding

The fieldworks in Iran were financially supported by Shiraz University and University of Sistan and Baluchestan. HRE thanks DAAD (German Academic Exchange Service) for financial support to visit BR at the Ludwig-Maximilians-Universität München (Munich, Germany) to undertake the present study.

5.8 Acknowledgements

We acknowledge Mr. Abdulwahed Pehpouri (Qeshm Island, Iran) for logistical help with field trip and accommodation. The manuscript benefited greatly from the constructive comments of Dr. Gianluca Polgar (Western Kentucky University, USA), Dr. Martin Dohrmann (LMU Munich, Germany), and Dr. Michael Krings (SNSB-Bavarian State Collection Palaeontology and Geology, Munich, Germany). Finally, we thank Dr. Paul Hardy (Düsseldorf, Germany) for critical reading of the manuscript, and the two reviewers for their valuable comments. Disclosure statement No potential conflict of interest was reported by the authors.

5.9 References

Agorreta, A., San Mauro, D., Schliewen, U., Van Tassell, J. L., Kovačić, M., Zardoya, R., & Rüber, L. (2013). Molecular phylogenetics of Gobioidei and phylogenetic placement of European gobies. *Molecular Phylogenetics and Evolution, 69*, 619–633.

Assis, C. A. (2003). The lagenar otoliths of teleosts: their morphology and its application in species identification, phylogeny and systematics. *Journal of Fish Biology, 62*, 1268–1295.

Bishop, J. M. (2003). History and current checklist of Kuwait's ichthyofauna. *Journal of Arid Environments, 54*, 237–256.

Campana, S. E., & Neilson, J. D. (1985). Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences, 42*, 1014–1032.

Clayton, D. A. (1993). Mudskippers. *Oceanography and Marine Biology: An annual review, 31*, 507–577.

Clayton, D. A., & Snowden, R. (2000). Surface activity in the mudskipper, *Periophthalmus waltoni* Koumans 1941 in relation to prey activity and environmental factors. *Tropical Zoology, 13*, 239–249.

Clayton, D. A., & Vaughan, T. C. (1988). Ethogram of *Boleophthalmus boddarti* (Pallas) (Teleostei, Gobiidae), a mudskipper found on the mudflats of Kuwait. *The Journal of the University of Kuwait (Science), 15*, 115–140.

Fekete, D. M. (2003). Rocks that roll zebrafish. *Science, 302*(5643), 241–242.

Ghanbarifardi, M., Aliabadian, M., & Esmaeili, H. R. (2018). Phylogeography of Walton's Mudskipper, *Periophthalmus waltoni* Koumans, 1941 (Perciformes: Gobiidae), from the Persian Gulf and Gulf of Oman. *Zoology in the Middle East, 64*, 207–218.

Ghanbarifardi, M., Aliabadian, M., & Esmaeili, H. R. (2020). Shape variation of the Indian Ocean slender mudskipper, . *Scartelaos tenuis* (Day, 1876) from the Persian Gulf and Sea of Oman (Gobioidei: Gobiidae). *Iranian Journal of Fisheries Science, 19*, 612–622.

Ghanbarifardi, M., Aliabadian, M., Esmaeili, H. R., & Polgar, G. (2014). Morphological divergence in the Walton's Mudskipper, . *Periophthalmus waltoni* Koumans, 1941, from the Persian Gulf and Gulf of Oman (Gobioidei: Gobiidae). *Zoology in the Middle East, 60*, 133–143.

Ghanbarifardi, M., Esmaeili, H. R., Gholami, Z., Aliabadian, M., & Reichenbacher, B. (2016). Molecular phylogeny of three mudskippers (Gobiidae) from the Persian Gulf and Gulf of Oman (Makran). *Journal of Applied Ichthyology, 32*, 416–420.

Ghanbarifardi, M., & Malek, M. (2007). Permanent intertidal fish from the Persian Gulf and Gulf of Oman, Iran. *Iranian Journal of Animal Biosystematics, 3*, 1–14.

Gierl, C., Liebl, D., Šanda, R., Vukić, J., Esmaeili, H. R., & Reichenbacher, B. (2018). What can goby otolith morphology tell us? *Cybium, 42*, 349–363.

Harris, V. A. (1960). On the locomotion of the mud-skipper *Periophthalmus koelreuteri* (Pallas): (Gobiidae). *Proceedings of the Zoological Society of London, 134*, 107–135.

Hoese, D. F. (1984). Gobioidei: relationships. Pp. 588–591. In: H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, J. Kendall, A. W., & S. L. Richardson (Eds.), *Ontogeny and systematics of fishes.* Gainesville, Florida: American Society of Ichthyologists and Herpetologists.

SPSS (2019). *IBM SPSS Statistics for Macintosh, Version 26.0.* www-01.ibm.com/software/analytics/spss/products/statistics.

Lombarte, A., & Lleonart, J. (1993). Otolith size changes related with body growth, habitat depth and temperature. *Environmental Biology of Fishes, 37*, 297–306.

Lombarte, A., Miletić, M., Kovačić, M., Otero-Ferrer, J. L., & Tuset, V. M. (2018). Identifying sagittal otoliths of Mediterranean Sea gobies: variability among phylogenetic lineages. *Journal of Fish Biology, 92*, 1768–1787.

Lombarte, A., Palmer, M., Matallanas, J., Gómez-Zurita, J., & Morales-Nin, B. (2010). Ecomorphological trends and phylogenetic inertia of otolith sagittae in Nototheniidae. *Environmental Biology of Fishes, 89*, 607–618.

Murdy, E. O. (1989). A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae: Oxudercinae). *Records of the Australian Museum, Supplements, 11*, 1–93.

Nanami, A. (2017). Age and growth. Pp. 175–216. In: Z. Jaafar & E. O. Murdy (Eds), *Fishes out of water: biology and ecology of mudskippers.* London: Taylor & Francis Group.

Nolf, D. (1985). *Handbook of paleoichthyology, Volume 10. Otolithi piscium.* Munich: Verlag Dr. Friedrich Pfeil.

Nolf, D. (2013). *The diversity of fish otoliths, past and present.* Brussels: Royal Belgian Institute of Natural Sciences.

Pace, C. (2017). Aquatic and terrestrial locomotion. Pp. 386–414. In: Z. Jaafar & E. O. Murdy (Eds), *Fishes out of water: biology and ecology of mudskippers.* London: Taylor & Francis Group.

Parenti, L. R., & Jaafar, Z. (2017). The natural distribution of mudskippers. Pp. 37–68. In: Z. Jaafar & E. O. Murdy (Eds), *Fishes out of water: biology and ecology of mudskippers.* London: Taylor & Francis Group.

Polgar, G., Ghanbarifardi, M., Milli, S., Agorreta, A., Aliabadian, M., Esmaeili, H. R., & Khang, T. F. (2017). Ecomorphological adaptation in three mudskippers (Teleostei: Gobioidei: Gobiidae) from the Persian Gulf and the Gulf of Oman. *Hydrobiologia, 795*, 91–111.

Popper, A. N. (1981). Comparative scanning electron microscopic investigations of the sensory epithelia in the teleost sacculus and lagena. *Journal of Comparative Neurology, 200*, 357–374.

Popper, A. N., & Lu, Z. (2000). Structure-function relationships in fish otolith organs. *Fisheries Research, 46*, 15–25.

Popper, A. N., Ramcharitar, J., & Campana, S. E. (2005). Why otoliths? Insights from inner ear physiology and fisheries biology. *Marine & Freshwater Research, 56*, 497–504.

R Core Team (2019). *R: A language and environment for statistical computing.* Version 3.6.1. R Foundation for Statistical Computing. www.R-project.org.

Ramcharitar, J., Higgs, D. M., & Popper, A. N. (2001). Sciaenid inner ears: A study in diversity. *Brain Behavior and Evolution, 58*, 152–162.

Ramcharitar, J. U., Deng, X., Ketten, D., & Popper, A. N. (2004). Form and function in the unique inner ear of a teleost: The silver perch (*Bairdiella chrysoura*). *The Journal of Comparative Neurology, 475*, 531–539.

Reichenbacher, B., & Reichard, M. (2014). Otoliths of five extant species of the annual killifish *Nothobranchius* from the East African savannah. *PLOS ONE, 9*(11), e112459.

Rivaton, J., & Bourret, P. (1999). Les otolithes des poissons de l'Indo-Pacifique. *Documents Scientifiques et Techniques, II2.* Volume spécial, 7–378. Nouméa, New Caledonia: Institut de Recherche pour le Développement.

Sarimin, A. S., Ghaffar, M. A., & Mohamed, C. A. R. (2009). Variation of Ca, Sr, Ba and Mg in the otolith of mudskipper in West Coast of Peninsular Malaysia. *Pakistan Journal of Biological Sciences, 12*, 231–238.

Sayer, M. D. J. (2005). Adaptations of amphibious fish for surviving life out of water. *Fish and Fisheries, 6*, 186–211.

Schulz-Mirbach, T., Ladich, F., Plath, M., Metscher, B. D., & Hess, M. (2014). Are accessory hearing structures linked to inner ear morphology? Insights from 3D orientation patterns of ciliary bundles in three cichlid species. *Frontiers in Zoology, 11, 25*

Schulz-Mirbach, T., & Reichenbacher, B. (2006). Reconstruction of Oligocene and Neogene freshwater fish faunas – an actualistic study on cypriniform otoliths. *Acta Palaeontologica Polonica, 51*, 283–304.

Schwarzhans, W. (2014). Otoliths from the middle Miocene (Serravallian) of the Karaman Basin, Turkey. *Cainozoic Research, 14*, 35–69.

Tuset, V. M., Lombarte, A., & Assis, C. A. (2008). Otolith atlas for the western Mediterranean, north and central eastern Atlantic. *Scientia Marina, 72*(S1), 7–198.

Vignon, M., & Morat, F. (2010). Environmental and genetic determinant of otolith shape revealed by a non-indigenous tropical fish. *Marine Ecology Progress Series, 411*, 231–241.

Volpedo, A. V., & Echeverría, D. D. (2003). Ecomorphological patterns of the sagitta in fish on the continental shelf off Argentine. *Fisheries Research, 60*, 551–560.

6 Conclusion

Taking into account the sheer amount of gobioid species known today (>2200 species; Fricke et al. 2019) and the fact that only a few of these have been examined from an osteological point of view (e.g. Birdsong 1975; Herler et al. 2006), it is clear that a lot of insights into hard parts in gobies remain to be explored. A more comprehensive description and quantitative evaluation of hard parts could be of great value for future paleontological and evolutionary studies.

In order to contribute to the growing knowledge of hard parts in gobies, Gut et al. (2020) examined hard parts of extant gobioid species belonging to *Gobius* and *Pomatoschistus*, which represent the two most diverse genera in Europe. The study focused on skeletal (morphometrics, meristics) and otolith characteristics (otolith indices, outline analysis). Its main goal was to test the value of hard parts for species separation by exploring their variation and their statistical evaluation.
The results of the study showed that meristic characters, especially in *Pomatoschistus*, can be of great benefit for species separation. Morphometrics also showed a good potential for distinguishing species in both *Gobius* and *Pomatoschistus.* Otolith indices revealed a good separation success in some *Gobius* and some *Pomatoschistus* species. However, no method was as successful as the Fourier outline analysis. This study provided new data on extant gobioid species and demonstrated the great value of hard parts for species separation. With this Gut et al. (2020) can be a great reference for character variability and species separation potential. Along with future studies in this field it can contribute to the understanding of fossil gobioid diversity and therefore be very helpful for comprehending the evolutionary history of gobioids.

Since over a century, palaeontologists have been using the species specificness of otoliths to describe new species in fish, as these are one of the most common remains regarding these animals (e.g. Koken 1884; Weinfurter 1952; Reichenbacher et al. 2019; Schwarzhans et al. 2020). The assignment of different fossil gobioid species to the genus *Gobius* was common for a long time as data on hard parts of extant fishes used to be still scarce (e.g. Nolf 1985). The knowledge about gobioids started improving, as extant material was examined and compiled in greater amounts (e.g. Campana 2004; Lombarte et al. 2006; Nolf 2013). With this it became clear, that many ancient assignments and classifications of isolated fossil otoliths were doubtful. High similarities, few diagnostic characters and unknown variation in gobioid otoliths are the main reasons that make their evaluation difficult and their assignment prone to subjective influence. In order to reduce this subjective influence, Gut (manuscript) applied the gained knowledge about variation and the separation success utilizing Fourier outline analysis from Gut et al. (2020) on a sample set of grouped fossil, gobioid otoliths from the middle Miocene (∼14 Ma). The groups were tested and rearranged until the classification success of modern species was met.

Due to the fact that the outline analysis did not consider , by definition, important surface structures like the sulcus, Gut (manuscript) introduced the distances of measurement OSH and TED because they showed higher accuracy than the previously used indices, which were influenced by the rotation of the otolith and the inclination of the sulcus.

The newly introduced index (OSH/TED) supports the assumption of genera, as according to Gierl et al. (2018) genera or higher taxonomical levels can be distinguished by their sulcus shape and used as a reference for undetected inhomogeneity by the Fourier analysis within a group. Apart from that, this study introduced one new genus and four new species. Hereby, this study came to the conclusion that the species diversity of *Lesueurigobius* during the middle Badenian was higher than previously known.

All in all, Gut (manuscript) introduced a new application of the Fourier shape analysis for testing the assignment of fossil, isolated otoliths to species. In that way, Gut (manuscript) showed that an objective support can be given to presorted taxonomical groups as a feedback for the taxonomist and contributed to reduce the subjective influence, shedding new light on the fossil record of gobioids.

Ghanbarifardi et al. (2020a) took a closer look at the osteology of mudskippers (Teleostei: Gobioidei), which are considered to be model species for physiological and behavioral adaptations for their amphibious lifestyle (Pierce & Clack 2013; Kawano & Blob 2013). Even though the morphological traits of mudskippers have been examined thoroughly by Murdy (1989), the investigation of the caudal skeleton and the vertebral column did not receive as much attention as the other parts. As these parts could also be utilized for species separation in specific cases (see Gut et al. 2020) Ghanbarifardi et al. (2020a) examined them in greater detail. The study revealed three main different characters in more terrestrial species that rarely occur in the other examined mudskipper species: the modified caudal vertebrae, a close dovetailing association of neural spines and preural vertebrae 2 and 3 or short and thick principal caudal rays on the ventralmost side of the mudskipper. These results could indicate that the posterior caudal vertebrae and the caudal skeleton are modified in more terrestrial mudskipper species to facilitate their locomotion on land. As Ghanbarifardi et al. (2020a) focused on hard part characters in mudskippers, future paleontological studies can benefit from it as a source for species separation or as an initial point for estimations regarding adaptation degrees of fossil mudskippers to amphibious lifestyles.

Ghanbarifardi et al. (2020b) examined furthermore the otoliths of three mudskipper species that are known for their differing degrees in amphibious adaptation (e.g. Harris, 1960; Sayer, 2005; Pace, 2017). The most aquatic species, *Scartelaos tenuis*, possesses a rhomboid to rectangular outline, which is common in marine gobiid species. *Periophthalmus waltoni*, the most terrestrial species, differs from this common shape as it possesses a pentagonal shaped otolith. The moderately amphibious species *Boleophthalmus dussumieri* displays an intermediate otolith outline between a rhomboidal and pentagonal shape. This might indicate that the otolith shape is an adaptation to different degrees of amphibious lifestyle, since out of water hearing conditions on the shore are different from under water. Ghanbarifardi et al. (2020b) noted that using characters related to the sulcus, separated *P. waltoni* statistically better from the moderately terrestrial *B. dussumieri* than from the more aquatic *S. tenuis*, which is distinctive in its outline. This might be due to the required differentiation in (frequential) hearing of the two species, as *P. waltoni* and *B. waltoni* are syntopical and in terms of e.g. reproduction have to be able to distinguish one another. Through this examination of mudskipper otoliths, Ghanbarifardi et al. (2020b) contributed to the knowledge of otolith diversity and can be of great assistance to future paleontological studies of mudskippers as otoliths are a likely part of the fish to fossilize.

In conclusion, this thesis along with its four studies constitutes a qualitative and quantitative evaluation of hard parts of extant gobioid species and introduces a new application of the Fourier shape analysis on fossil, isolated otoliths. In that way, it is meant to contribute to the future exploration of gobioid hard parts and their variation and to reveal new potentials for species separation.

6.1 References

Birdsong, R. S. (1975). The osteology of Microgobius signatus Poey (Pisces: Gobiidae), with comments on other gobiid fishes. Bulletin of the Florida State Museum. *Biological sciences*, 19(3), 135–187.

Campana, S. E. (2004). *Photographic atlas of fish otoliths of the Northwest Atlantic Ocean Canadian special publication of fisheries and aquatic sciences No. 133* (C. P. B. Ed.). Ottawa: NRC Research Press.

Fricke, R., Eschmeyer, W. N., & Fong, J. D. (2019). Species by family/subfamily in the Catalog of Fishes. [Electronic version]. San Francisco (CA): California Academy of Sciences; 2019. [cited 2019 Oct 21]. In.

Ghanbarifardi, M., Gut, C., Gholami, Z., Esmaeili, H. R., Gierl, C., & Reichenbacher, B. (2020a). Osteology of the posterior vertebral column and caudal skeleton of marine amphibious gobies (mudskippers)(Teleostei: Gobioidei). *Journal of Applied Ichthyology*, 36(5), 713-721.

Ghanbarifardi, M., Gut, C., Gholami, Z., Esmaeili, H. R., Gierl, C., & Reichenbacher, B. (2020b). Possible link between the structure of otoliths and amphibious mode of life of three mudskipper species (Teleostei: Gobioidei) from the Persian Gulf. *Zoology in the Middle East*. 10.1080/09397140.09392020.01805140.

Gierl, C., Liebl, D., Šanda, R., Vukić, J., Esmaeili, H. R., & Reichenbacher, B. (2018). What can goby otolith morphology tell us? *Cybium*, 42(4), 349-363.

Gut, C. (manuskript). Shedding new light on fossil gobiid fish otoliths from the Vienna Basin: An exemplary application of Fourier outline analysis and descriptive statistics as taxonomical support.

Gut, C., Vukić, J., Šanda, R., Moritz, T., & Reichenbacher, B. (2020). Identification of past and present gobies: distinguishing *Gobius* and *Pomatoschistus* (Teleostei: Gobioidei) species using characters of otoliths, meristics and body morphometry. *Contributions to Zoology*, 89(3), 282-323.

Harris, V. (1960). On the locomotion of the mudskipper *Periophthalmus koelreu* (Pallas): (Gobiidae). *Proceedings of the Zoological Society of London*, 134(1), 107-135.

Herler, J., Hilgers, H., & Patzner, R. A. (2006). Osteology and dentition of two Mediterranean *Gobius* species (Teleostei, Gobiidae). *Italian Journal of Zoology*, 73(2), 105-115.

Kawano, S. M., & Blob, R. W. (2013). Propulsive forces of mudskipper fins and salamander limbs during terrestrial locomotion: implications for the invasion of land. *Integrative and comparative biology*, 53(2), 283-294.

Koken, E. (1884). Über Fisch-Otolithen, insbesondere über diejenigen der norddeutschen Oligocän-Ablagerungen. *Zeitschrift der deutschen geologischen Gesellschaft*, 500-565.

Lombarte, A., Chic, Ò., Parisi-Baradad, V., Olivella, R., Piera, J., & García-Ladona, E. (2006). A web-based environment for shape analysis of fish otoliths. The AFORO database. *Scientia Marina*, 70(1), 147-152.

Murdy, E. O. (1989). A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae: Oxudercinae). *Records of the Australian Museum*, 1-93.

Nolf, D. (1985). *Otolithi piscium.* Handbook of paleoichthyology. Stuttgart: Gustav Fischer Verlag.

Nolf, D. (2013). *The diversity of fish otoliths, past and present.* Belgium: Royal Belgian Institute of Natural Sciences.

Pace, C. (2017). Aquatic and Terrestrial Locomotion. In Z. Jaafar & E. O. Murdy (Eds.), *Fishes out of water: Biology and ecology of Mudskippers.* Boca Raton: CRC Press.

Pierce, S. E., Hutchinson, J. R., & Clack, J. A. (2013). Historical perspectives on the evolution of tetrapodomorph movement. *Oxford* Academic, 53(2), 209-223.

Reichenbacher, B., Filipescu, S., & Miclea, A. (2019). A unique middle Miocene (Sarmatian) fish fauna from coastal deposits in the eastern Pannonian Basin (Romania). *Palaeobiodiversity and Palaeoenvironments*, 99(2), 177-194.

Sayer, M. D. (2005). Adaptations of amphibious fish for surviving life out of water. *Fish and Fisheries*, 6(3), 186-211.

Schwarzhans, W., Brzobohatý, R., & Radwańska, U. (2020). Goby otoliths from the Badenian (middle Miocene) of the Central Paratethys from Czech Republic, Slovakia and Poland: A baseline for the evolution of the European Gobiidae (Gobiiformes; Teleostei). *Bollettino della Società Paleontologica Italiana*, 59(2), 125-173.

Weinfurter, E. (1952). Otolithen aus miozänen Brack-und Süßwasserschichten des Lavanttales in Kärnten. *Sitzungsberichte der Akademie der Wissenschaften in Wien, mathematisch-naturwissenschaftliche Klasse. Abteilung,1*, 161, 149-172.

Appendices

Appendix for chapter 2

Table S1: List of all specimens used in the manuscript, their collection numbers, and raw data of otolith measurements (mm for distances, square mm for areas); for details of localities see Table 1. NMP, National Museum Praha; ZSM, Zoological State Collection, Munich (SNSB). OA, otolith area; OH, otolith height; OL, otolith length; OP, otolith perimeter; SuA, sulcus area; SuH, sulcus height; SuL, sulcus length; SuP, sulcus perimeter; SuEndV, vertical distance from posterior end of sulcus to ventral margin of otolith; SuTipV, vertical distance from anterior end of sulcus to ventral margin of otolith.

collection number	Species	specimen number	locality	OL	OH	OP	OA	SuL	SuH	SuP	SuA	SuTipV	SuEndV
NMP6V 146082	*Gobius auratus*	1	Selce	left otolith was lost									
NMP6V 146083	*Gobius auratus*	2	Selce	1.568	1.306	5.130	1.604	0.743	0.315	1.806	0.132	0.502	0.712
NMP6V 146084	*Gobius auratus*	3	Selce	1.590	1.279	5.129	1.585	0.691	0.328	1.702	0.124	0.474	0.707
NMP6V 146085	*Gobius auratus*	4	Selce	1.270	1.136	4.220	1.115	0.538	0.241	1.307	0.067	0.450	0.604
NMP6V 146086	*Gobius auratus*	5	Selce	1.687	1.418	5.501	1.832	0.764	0.315	1.850	0.129	0.576	0.761
NMP6V 146087	*Gobius auratus*	6	Selce	1.574	1.339	5.235	1.696	0.671	0.321	1.646	0.095	0.542	0.727
NMP6V 146088	*Gobius auratus*	7	Selce	1.733	1.422	5.632	1.915	0.786	0.382	1.934	0.159	0.506	0.785
NMP6V 146089	*Gobius auratus*	8	Selce	1.414	1.258	4.802	1.416	0.651	0.293	1.569	0.106	0.488	0.658
NMP6V 146090	*Gobius auratus*	9	Selce	1.179	1.096	4.019	1.008	0.478	0.238	1.217	0.067	0.443	0.556
NMP6V 146091	*Gobius auratus*	1	Krk	2.483	1.924	7.964	3.726	1.103	0.561	2.793	0.349	0.699	1.076
NMP6V 146092	*Gobius auratus*	2	Krk	2.482	1.946	7.941	3.674	1.120	0.549	2.825	0.367	0.718	1.007
NMP6V 146093	*Gobius bucchichi*	1	Kraljevica	2.179	1.671	6.711	2.815	1.124	0.556	2.822	0.333	0.583	0.921
NMP6V 146094	*Gobius bucchichi*	1	Selce	2.060	1.616	6.548	2.612	1.059	0.506	2.655	0.267	0.559	0.852
NMP6V 146095	*Gobius bucchichi*	2	Selce	1.996	1.501	6.349	2.308	0.972	0.491	2.472	0.285	0.561	0.813
NMP6V 146096	*Gobius bucchichi*	3	Selce	1.969	1.536	6.200	2.392	0.975	0.447	2.434	0.239	0.543	0.819
NMP6V 146097	*Gobius bucchichi*	4	Selce	2.226	1.694	7.006	3.016	1.162	0.524	2.916	0.326	0.591	0.918
NMP6V 146098	*Gobius bucchichi*	5	Selce	2.510	1.904	7.872	3.355	1.236	0.564	3.058	0.392	0.648	0.886
NMP6V 146099	*Gobius bucchichi*	6	Selce	2.094	1.583	6.848	2.545	1.047	0.464	2.583	0.271	0.545	0.787
NMP6V 146100	*Gobius bucchichi*	7	Selce	2.417	1.939	7.878	3.662	1.293	0.698	3.306	0.476	0.607	0.876
NMP6V 146101	*Gobius bucchichi*	8	Selce	2.653	1.991	8.231	4.044	1.406	0.645	3.427	0.510	0.618	0.914
NMP6V 146102	*Gobius bucchichi*	9	Selce	2.014	1.372	6.031	2.030	0.827	0.357	2.054	0.182	0.521	0.750
NMP6V 146103	*Gobius cobitis*	1	Montenegro	3.546	2.713	12.015	7.035	1.836	0.620	4.258	0.611	0.836	1.197
NMP6V 146104	*Gobius cobitis*	2	Montenegro	1.565	1.260	5.192	1.570	0.794	0.406	2.023	0.163	0.443	0.652
NMP6V 146105	*Gobius cobitis*	3	Montenegro	1.560	1.191	5.091	1.473	0.757	0.313	1.869	0.132	0.421	0.607
NMP6V 146106	*Gobius cobitis*	4	Montenegro	2.124	1.646	7.348	2.855	1.154	0.551	2.846	0.353	0.595	0.804
NMP6V 146107	*Gobius cobitis*	5	Montenegro	1.544	1.304	5.362	1.630	0.854	0.393	2.154	0.181	0.411	0.649
NMP6V 146108	*Gobius cobitis*	6	Montenegro	2.771	2.239	9.404	4.775	1.596	0.748	4.039	0.680	0.732	1.108
NMP6V 146109	*Gobius cobitis*	7	Montenegro	1.480	1.221	5.055	1.454	0.796	0.326	1.945	0.146	0.430	0.613
NMP6V 146110	*Gobius cobitis*	8	Montenegro	2.254	1.738	7.646	3.180	1.254	0.529	3.137	0.367	0.585	0.842
NMP6V 146111	*Gobius cobitis*	9	Montenegro	2.178	1.802	7.507	3.050	1.157	0.479	2.829	0.314	0.596	0.862
NMP6V 146112	*Gobius cobitis*	10	Montenegro	2.383	1.945	8.669	3.662	1.315	0.592	3.174	0.413	0.652	0.924
NMP6V 146113	*Gobius cobitis*	1	Selce J850	2.098	1.584	6.991	2.654	1.150	0.513	2.928	0.355	0.554	0.798
NMP6V 146114	*Gobius couchi*	1	Krk	2.129	1.746	6.774	2.765	0.980	0.528	2.544	0.284	0.692	0.847
NMP6V 146115	*Gobius couchi*	2	Krk	1.358	1.246	4.632	1.305	0.580	0.325	1.504	0.098	0.466	0.636
NMP6V 146116	*Gobius couchi*	3	Krk	1.700	1.582	5.936	2.167	0.820	0.381	2.027	0.166	0.558	0.759
NMP6V 146117	*Gobius couchi*	4	Krk	1.574	1.336	5.286	1.575	0.714	0.391	1.857	0.139	0.482	0.688
NMP6V 146118	*Gobius cruentatus*	1	Selce	3.493	2.431	10.998	6.726	1.675	0.683	4.116	0.607	0.856	1.232
NMP6V 146119	*Gobius cruentatus*	2	Selce	3.516	2.614	11.595	7.116	1.788	0.824	4.419	0.819	0.863	1.288

NMP6V 146120	*Gobius cruentatus*	3	Selce	4.150	2.990	12.989	9.565	2.171	0.947	5.265	1.067	0.988	1.638
NMP6V 146121	*Gobius cruentatus*	4	Selce	left otolith was lost									
NMP6V 146122	*Gobius cruentatus*	5	Selce	4.096	3.106	13.469	10.147	2.177	1.122	5.611	1.354	1.004	1.571
NMP6V 146123	*Gobius cruentatus*	6	Selce	4.421	3.415	14.709	12.381	2.370	1.037	5.761	1.416	1.081	1.583
NMP6V 146124	*Gobius cruentatus*	7	Selce	4.087	3.213	13.199	10.491	2.219	1.071	5.591	1.445	1.094	1.512
NMP6V 146125	*Gobius cruentatus*	8	Selce	4.575	3.376	15.185	12.681	2.677	1.301	6.749	2.016	1.116	1.612
NMP6V 146126	*Gobius cruentatus*	9	Selce	4.498	3.382	15.060	12.950	2.693	1.093	6.603	1.886	1.152	1.559
NMP6V 146127	*Gobius cruentatus*	1	Krk	3.558	2.703	10.666	6.700	1.725	0.725	3.884	0.586	0.819	1.203
NMP6V 146128	*Gobius cruentatus*	2	Krk	3.500	2.657	11.711	7.139	1.757	0.900	4.189	0.802	0.764	1.329
NMP6V 146129	*Gobius fallax*	1	Croatia	2.087	1.581	6.969	2.509	1.040	0.422	2.536	0.253	0.559	0.866
NMP6V 146130	*Gobius fallax*	2	Croatia	left otolith was lost, right otolith was used									
NMP6V 146131	*Gobius gasteveni*	1	Galicia	3.020	2.264	9.378	5.091	1.594	0.721	4.044	0.676	0.767	1.048
NMP6V 146132	*Gobius geniporus*	1	Montenegro	5.408	3.565	16.886	14.769	2.973	0.991	6.986	1.709	1.286	1.656
NMP6V 146133	*Gobius geniporus*	2	Montenegro	4.029	2.792	12.217	8.535	1.779	0.786	4.240	0.761	0.958	1.356
NMP6V 146134	*Gobius geniporus*	3	Montenegro	4.331	3.053	13.397	10.152	2.425	1.016	5.864	1.394	1.008	1.406
NMP6V 146135	*Gobius geniporus*	4	Montenegro	4.463	2.978	13.835	10.380	2.478	0.880	5.825	1.227	1.020	1.429
NMP6V 146136	*Gobius geniporus*	1	Selce	3.222	2.336	9.818	5.748	1.743	0.632	4.190	0.597	0.792	1.166
NMP6V 146137	*Gobius geniporus*	2	Selce	4.855	3.513	15.124	13.050	2.310	0.891	5.445	1.149	1.210	1.647
NMP6V 146138	*Gobius geniporus*	3	Selce	5.315	3.654	16.662	15.285	3.031	1.020	7.181	1.857	1.406	1.799
NMP6V 146139	*Gobius geniporus*	small	Selce	4.923	3.408	14.781	13.112	2.732	0.811	6.207	1.244	1.128	1.574
NMP6V 146140	*Gobius geniporus*	medium	Selce	5.297	3.881	16.267	15.901	2.975	1.151	6.919	1.796	1.297	1.872
NMP6V 146141	*Gobius geniporus*	large	Selce	5.588	4.186	17.318	18.254	3.277	1.175	7.920	2.210	1.341	1.963
NMP6V 146142	*Gobius incognitus*	J1904l	Croatia	2.616	1.936	8.294	3.907	1.403	0.632	3.613	0.526	0.600	0.965
NMP6V 146143	*Gobius incognitus*	J1906l	Croatia	3.059	2.075	9.004	4.598	1.625	0.695	4.030	0.669	0.752	1.044
NMP6V 146144	*Gobius incognitus*	J1907l	Croatia	left otolith was lost									
NMP6V 146145	*Gobius incognitus*	J1910l	Croatia	left otolith was lost									
NMP6V 146146	*Gobius incognitus*	J1911l	Croatia	1.773	1.276	5.444	1.724	0.924	0.429	2.308	0.200	0.420	0.694
NMP6V 146147	*Gobius incognitus*	J1912l	Croatia	2.979	2.005	8.746	4.566	1.714	0.793	4.301	0.754	0.711	1.030
NMP6V 146148	*Gobius incognitus*	J1913l	Croatia	2.279	1.640	6.761	2.778	1.217	0.475	2.964	0.306	0.557	0.744
NMP6V 146149	*Gobius incognitus*	J1914l	Croatia	1.751	1.324	5.530	1.786	0.877	0.369	2.113	0.140	0.470	0.685
NMP6V 146150	*Gobius incognitus*	J1915l	Croatia	left otolith was lost									
NMP6V 146151	*Gobius kolombatovici*	1	Krk	3.311	2.220	9.718	5.507	1.485	0.594	3.606	0.518	0.804	1.089
NMP6V 146152	*Gobius kolombatovici*	2	Krk	3.589	2.498	10.820	6.998	1.777	0.680	4.293	0.700	0.865	1.188
NMP6V 146072	*Gobius niger*	1	Pilsey Island	2.266	1.817	7.203	3.291	1.207	0.471	2.963	0.344	0.615	0.782
NMP6V 146074	*Gobius niger*	2	Pilsey Island	2.479	1.950	7.536	3.544	1.217	0.499	2.981	0.330	0.694	0.850
NMP6V 146075	*Gobius niger*	3	Pilsey Island	2.623	2.025	8.561	4.045	1.351	0.554	3.265	0.426	0.664	0.883
NMP6V 146076	*Gobius niger*	4	Pilsey Island	2.685	2.172	8.888	4.634	1.239	0.562	3.049	0.408	0.966	1.013
NMP6V 146077	*Gobius niger*	5	Pilsey Island	3.121	2.649	10.614	6.373	1.538	0.681	3.833	0.562	0.831	1.141
NMP6V 146078	*Gobius niger*	6	Pilsey Island	2.662	2.077	8.659	4.210	1.365	0.568	3.380	0.494	0.736	0.897
NMP6V 146079	*Gobius niger*	7	Pilsey Island	2.278	1.834	7.304	3.057	1.033	0.441	2.527	0.265	0.644	0.849
NMP6V 146080	*Gobius niger*	8	Pilsey Island										
NMP6V 146081	*Gobius niger*	9	Pilsey Island	only used for Fourier									
NMP6V 146073	*Gobius niger*	10	Pilsey Island										
NMP6V 146153	*Gobius paganellus*	1	Faro	1.982	1.425	6.365	2.269	0.973	0.380	2.342	0.216	0.490	0.738

NMP6V 146154	*Gobius paganellus*	2	Faro	2.187	1.557	7.071	2.703	1.277	0.518	3.163	0.340	0.462	0.721
NMP6V 146155	*Gobius paganellus*	3	Faro	left otolith was lost									
NMP6V 146156	*Gobius paganellus*	4	Faro	2.255	1.638	7.436	2.867	1.157	0.456	2.771	0.309	0.581	0.837
NMP6V 146157	*Gobius paganellus*	5	Faro	2.167	1.563	7.007	2.813	1.163	0.388	2.778	0.248	0.519	0.720
NMP6V 146158	*Gobius paganellus*	6	Faro	2.232	1.577	6.839	2.690	1.064	0.398	2.627	0.236	0.510	0.774
NMP6V 146159	*Gobius paganellus*	7	Faro	2.237	1.630	7.173	2.817	1.147	0.416	2.734	0.316	0.587	0.750
NMP6V 146160	*Gobius paganellus*	8	Faro	2.590	1.829	7.992	3.503	1.590	0.641	3.056	0.328	0.610	0.785
NMP6V 146161	*Gobius paganellus*	9	Faro	2.438	1.704	7.746	3.285	1.248	0.468	2.974	0.305	0.511	0.757
NMP6V 146162	*Gobius paganellus*	10	Faro	2.417	1.640	7.502	2.977	1.173	0.444	2.856	0.287	0.588	0.711
NMP6V 146163	*Gobius roulei*	1	Selce	2.909	2.226	9.150	5.143	1.427	0.528	3.347	0.411	0.843	1.048
NMP6V 146164	*Gobius roulei*	2	Selce	2.906	2.249	9.157	5.070	1.462	0.721	3.626	0.579	0.840	1.048
NMP6V 146165	*Gobius roulei*	3	Selce	2.892	2.355	9.237	5.109	1.354	0.631	3.343	0.454	0.792	1.101
NMP6V 146166	*Gobius roulei*	4	Selce	2.613	2.097	8.338	4.394	1.309	0.630	3.240	0.436	0.716	1.061
NMP6V 146167	*Gobius roulei*	Gr1	Selce	2.907	2.254	9.070	5.005	1.394	0.581	3.337	0.445	0.802	1.089
NMP6V 146168	*Gobius roulei*	Gr2	Selce	1.961	1.687	6.600	2.550	0.985	0.468	2.403	0.219	0.567	0.840
NMP6V 146169	*Gobius roulei*	Gr3	Selce	2.056	1.792	7.137	2.835	0.995	0.493	2.533	0.271	0.639	0.859
NMP6V 146170	*Gobius roulei*	Gr4	Selce	1.831	1.534	5.878	2.260	1.007	0.440	2.409	0.232	0.524	0.825
NMP6V 146171	*Gobius roulei*	Gr5	Selce	2.766	2.098	8.643	4.566	1.439	0.619	3.472	0.481	0.705	1.068
NMP6V 146172	*Gobius roulei*	Gr6	Selce	2.480	1.990	7.727	3.840	1.355	0.661	3.367	0.445	0.741	1.014
NMP6V 146173	*Gobius vittatus*	1	Selce	left otolith was lost									
NMP6V 146174	*Gobius vittatus*	2	Selce	1.023	0.942	3.506	0.746	0.454	0.233	1.130	0.047	0.328	0.482
NMP6V 146175	*Gobius vittatus*	3	Selce	0.748	0.763	2.668	0.454	0.301	0.149	0.729	0.023	0.303	0.372
NMP6V 146176	*Gobius vittatus*	4	Selce	0.872	0.889	3.194	0.639	0.365	0.212	0.937	0.031	0.316	0.478
NMP6V 146177	*Gobius vittatus*	5	Selce	1.806	1.634	6.076	2.285	0.874	0.454	2.267	0.174	0.540	0.817
NMP6V 146178	*Gobius vittatus*	6	Selce	0.762	0.792	2.697	0.489	0.389	0.193	0.956	0.035	0.286	0.416
NMP6V 146179	*Gobius vittatus*	7	Selce	1.675	1.441	6.107	1.873	0.772	0.341	1.927	0.135	0.507	0.705
NMP6V 146180	*Gobius vittatus*	8	Selce	left otolith was lost, right otolith was used									
NMP6V 146181	*Gobius vittatus*	9	Selce	left otolith was lost, right otolith was used									
NMP6V 146182	*Gobius vittatus*	1	Krk	1.420	1.192	4.628	1.263	0.599	0.306	1.476	0.100	0.468	0.639
NMP6V 146183	*Gobius vittatus*	2	Krk	1.770	1.423	5.668	1.893	0.818	0.389	2.054	0.177	0.492	0.730
NMP6V 146184	*Pomatoschistus kneri*	1	Krk	0.612	0.732	2.289	0.362	not clear	not clear	not clear	not clear	not clear	not clear
NMP6V 146185	*Pomatoschistus kneri*	2	Krk	0.724	0.710	2.475	0.423	0.363	0.216	0.978	0.048	0.353	0.194
NMP6V 146186	*Pomatoschistus kneri*	3	Krk	0.822	0.842	2.861	0.550	0.334	0.195	0.874	0.035	0.418	0.257
NMP6V 146187	*Pomatoschistus kneri*	4	Krk	0.739	0.874	2.816	0.544	0.367	0.227	0.956	0.043	0.447	0.282
NMP6V 146188	*Pomatoschistus kneri*	5	Krk	0.680	0.747	2.494	0.414	0.299	0.200	0.815	0.031	0.394	0.263
NMP6V 146189	*Pomatoschistus kneri*	6	Krk	0.816	0.822	2.751	0.530	0.418	0.233	1.070	0.044	0.453	0.271
NMP6V 146190	*Pomatoschistus kneri*	7	Krk	0.733	0.812	2.654	0.483	0.264	0.170	0.683	0.023	0.422	0.293
NMP6V 146191	*Pomatoschistus kneri*	8	Krk	0.777	0.843	2.795	0.531	0.322	0.192	0.855	0.033	0.405	0.258
NMP6V 146192	*Pomatoschistus kneri*	9	Krk	0.742	0.772	2.642	0.475	0.342	0.194	0.878	0.041	0.383	0.296
NMP6V 146193	*Pomatoschistus kneri*	10	Krk	0.727	0.766	2.656	0.436	0.270	0.206	0.769	0.030	0.413	0.207
NMP6V 146194	*Pomatoschistus marmoratus*	1	Selce	1.128	1.060	3.856	1.002	0.519	0.256	1.318	0.062	0.529	0.310
NMP6V 146195	*Pomatoschistus marmoratus*	2	Selce	1.172	1.131	3.937	1.036	0.579	0.317	1.495	0.100	0.523	0.266
NMP6V 146196	*Pomatoschistus marmoratus*	3	Selce	1.228	1.169	4.177	1.179	0.716	0.353	1.826	0.134	0.574	0.354
NMP6V 146197	*Pomatoschistus marmoratus*	4	Selce	1.367	1.291	4.590	1.461	0.621	0.346	1.576	0.119	0.599	0.464

NMP6V 146198	*Pomatoschistus marmoratus*	5	Selce	1.353	1.243	4.565	1.372	0.639	0.344	1.684	0.100	0.558	0.336
NMP6V 146199	*Pomatoschistus marmoratus*	6	Selce	1.120	1.142	3.895	1.036	0.494	0.283	1.302	0.078	0.569	0.334
NMP6V 146200	*Pomatoschistus marmoratus*	7	Selce	1.446	1.254	4.588	1.436	0.721	0.344	1.850	0.147	0.629	0.470
NMP6V 146201	*Pomatoschistus marmoratus*	2	Albania	1.101	0.992	3.985	0.892	0.497	0.275	1.296	0.073	0.525	0.290
NMP6V 146202	*Pomatoschistus marmoratus*	3	Albania	0.981	0.914	3.416	0.736	0.421	0.243	1.108	0.054	0.492	0.320
collection of BR	*Pomatoschistus microps*	2	Stralsund	1.079	0.963	3.681	0.867	0.581	0.291	1.544	0.095	0.414	0.326
collection of BR	*Pomatoschistus microps*	3	Stralsund	1.031	1.012	3.634	0.871	0.419	0.212	1.073	0.051	0.490	0.387
collection of BR	*Pomatoschistus microps*	4	Stralsund	1.077	1.060	3.782	0.930	0.467	0.241	1.159	0.059	0.489	0.268
collection of BR	*Pomatoschistus microps*	5	Stralsund	1.043	1.041	3.753	0.911	0.469	0.259	1.228	0.068	0.514	0.381
collection of BR	*Pomatoschistus microps*	6	Stralsund	1.105	1.035	3.821	0.965	0.573	0.317	1.590	0.098	0.480	0.373
collection of BR	*Pomatoschistus microps*	7	Stralsund	1.142	1.105	4.034	1.055	0.511	0.284	1.368	0.073	0.497	0.372
collection of BR	*Pomatoschistus microps*	8	Stralsund	1.022	1.008	3.598	0.859	0.515	0.286	1.421	0.086	0.443	0.340
collection of BR	*Pomatoschistus microps*	9	Stralsund	1.117	1.069	3.854	0.986	0.579	0.275	1.463	0.086	0.494	0.354
collection of BR	*Pomatoschistus microps*	10	Stralsund	0.937	0.969	3.324	0.722	0.450	0.216	1.210	0.054	0.420	0.317
collection of BR	*Pomatoschistus microps*	11	Stralsund	1.132	1.078	3.882	0.988	0.581	0.262	1.537	0.084	0.506	0.366
collection of BR	*Pomatoschistus microps*	12	Stralsund	1.019	1.030	3.562	0.847	0.418	0.216	1.089	0.047	0.448	0.341
collection of BR	*Pomatoschistus microps*	13	Stralsund	1.089	1.018	3.764	0.902	0.392	0.200	1.052	0.041	0.409	0.313
collection of BR	*Pomatoschistus microps*	14	Stralsund	1.059	1.098	3.829	0.963	0.555	0.280	1.479	0.081	0.521	0.332
collection of BR	*Pomatoschistus microps*	15	Stralsund	1.089	1.055	3.818	0.948	0.574	0.255	1.476	0.083	0.467	0.375
collection of BR	*Pomatoschistus microps*	16	Stralsund	1.133	1.066	3.858	0.976	0.476	0.244	1.207	0.054	0.509	0.345
collection of BR	*Pomatoschistus minutus*	1	Stralsund	1.875	1.626	6.350	2.532	0.919	0.404	2.242	0.218	0.730	0.482
collection of BR	*Pomatoschistus minutus*	17	Stralsund	1.711	1.579	5.781	2.244	0.793	0.453	2.072	0.178	0.762	0.438
NMP6V 146203	*Pomatoschistus montenegrensis*	1	Skadar Lake	0.731	0.818	2.756	0.494	0.307	0.138	0.745	0.024	0.412	0.319
NMP6V 146204	*Pomatoschistus montenegrensis*	2	Skadar Lake	0.609	0.696	2.277	0.360	0.215	0.125	0.563	0.018	0.327	0.267
NMP6V 146205	*Pomatoschistus montenegrensis*	3	Skadar Lake	0.690	0.768	2.599	0.455	0.234	0.114	0.576	0.017	0.367	0.291
NMP6V 146206	*Pomatoschistus montenegrensis*	4	Skadar Lake	0.802	0.855	2.860	0.566	0.324	0.153	0.798	0.029	0.399	0.331
NMP6V 146207	*Pomatoschistus montenegrensis*	5	Skadar Lake	0.607	0.718	2.411	0.389	0.261	0.151	0.673	0.023	0.356	0.307
NMP6V 146208	*Pomatoschistus montenegrensis*	6	Skadar Lake	0.794	0.870	2.899	0.548	0.362	0.185	0.900	0.037	0.427	0.280
NMP6V 146209	*Pomatoschistus montenegrensis*	7	Skadar Lake	0.648	0.721	2.366	0.384	0.278	0.134	0.680	0.021	0.356	0.253
NMP6V 146210	*Pomatoschistus montenegrensis*	8	Skadar Lake	0.753	0.826	2.772	0.529	0.271	0.138	0.674	0.022	0.379	0.305
NMP6V 146211	*Pomatoschistus montenegrensis*	9	Skadar Lake	0.752	0.820	2.760	0.520	0.328	0.147	0.794	0.030	0.411	0.312
NMP6V 146212	*Pomatoschistus montenegrensis*	10	Skadar Lake	0.723	0.799	2.693	0.490	0.285	0.125	0.681	0.018	0.372	0.290
ZSM-PIS-043983	*Pomatoschistus pictus*	1	Norway	1.099	1.018	3.667	0.942	0.548	0.263	1.369	0.075	0.524	0.346
ZSM-PIS-043983	*Pomatoschistus pictus*	2	Norway	0.989	1.004	3.675	0.853	0.372	0.235	1.948	0.040	0.499	0.359
NMP6V 146213	*Pomatoschistus quagga*	1	Krk	0.717	0.790	2.666	0.461	0.293	0.192	0.790	0.032	0.397	0.264
NMP6V 146214	*Pomatoschistus quagga*	2	Krk	0.728	0.824	2.658	0.484	0.265	0.201	0.744	0.030	0.398	0.256
NMP6V 146215	*Pomatoschistus quagga*	3	Krk	0.769	0.846	2.822	0.536	0.352	0.197	0.902	0.040	0.416	0.277
NMP6V 146216	*Pomatoschistus quagga*	4	Krk	0.848	0.896	3.051	0.625	0.368	0.195	0.931	0.041	0.448	0.309
NMP6V 146217	*Pomatoschistus quagga*	5	Krk	0.810	0.903	3.058	0.615	0.341	0.219	0.917	0.042	0.437	0.269
NMP6V 146218	*Pomatoschistus quagga*	6	Krk	left otolith was lost									
NMP6V 146219	*Pomatoschistus quagga*	7	Krk	0.821	0.860	2.994	0.580	0.249	0.241	0.773	0.032	0.420	0.252
NMP6V 146220	*Pomatoschistus quagga*	8	Krk	0.780	0.864	2.869	0.564	0.332	0.211	0.863	0.030	0.436	0.259
NMP6V 146221	*Pomatoschistus quagga*	9	Krk	0.841	0.900	3.121	0.633	0.317	0.222	0.852	0.039	0.453	0.308
NMP6V 146222	*Pomatoschistus quagga*	10	Krk	0.821	0.880	3.031	0.599	0.366	0.233	0.982	0.038	0.436	0.270

Table S2: Values of otolith variables; specimen numbers as in Suppl. Data 1; l and r refers to left and right otolith. OA, otolith area; OH, otolith height; OL, otolith length; OP, otolith perimeter; SuA, sulcus area; SuH, sulcus height; SuL, sulcus length; SuP, sulcus perimeter; SuEndV, vertical distance from posterior end of sulcus to ventral margin of otolith; SuTipV, vertical distance from anterior end of sulcus to ventral margin of otolith.

Species	specimen number	locality	OL/ OH	OP/OL	OP/ OH	SuP/ OP	SuL/ OP	SuH/ OP	SuTipV/ OP	SuEndV/ OP	SuA/ OA	SuL/ OL
Gobius auratus	2l	Selce	1.20	3.27	3.93	0.35	0.14	0.06	0.10	0.14	0.08	0.47
Gobius auratus	3l	Selce	1.24	3.23	4.01	0.33	0.13	0.06	0.09	0.14	0.08	0.43
Gobius auratus	4l	Selce	1.12	3.32	3.71	0.31	0.13	0.06	0.11	0.14	0.06	0.42
Gobius auratus	5l	Selce	1.19	3.26	3.88	0.34	0.14	0.06	0.10	0.14	0.07	0.45
Gobius auratus	6l	Selce	1.18	3.33	3.91	0.31	0.13	0.06	0.10	0.14	0.06	0.43
Gobius auratus	7l	Selce	1.22	3.25	3.96	0.34	0.14	0.07	0.09	0.14	0.08	0.45
Gobius auratus	8l	Selce	1.12	3.40	3.82	0.33	0.14	0.06	0.10	0.14	0.07	0.46
Gobius auratus	9l	Selce	1.08	3.41	3.67	0.30	0.12	0.06	0.11	0.14	0.07	0.41
Gobius auratus	1l	Krk	1.29	3.21	4.14	0.35	0.14	0.07	0.09	0.14	0.09	0.44
Gobius auratus	2l	Krk	1.28	3.20	4.08	0.36	0.14	0.07	0.09	0.13	0.10	0.45
Gobius bucchichi	1l	Kraljevica	1.30	3.08	4.02	0.42	0.17	0.08	0.09	0.14	0.12	0.52
Gobius bucchichi	1l	Selce	1.27	3.18	4.05	0.41	0.16	0.08	0.09	0.13	0.10	0.51
Gobius bucchichi	2l	Selce	1.33	3.18	4.23	0.39	0.15	0.08	0.09	0.13	0.12	0.49
Gobius bucchichi	3l	Selce	1.28	3.15	4.04	0.39	0.16	0.07	0.09	0.13	0.10	0.50
Gobius bucchichi	4l	Selce	1.31	3.15	4.14	0.42	0.17	0.07	0.08	0.13	0.11	0.52
Gobius bucchichi	5l	Selce	1.32	3.14	4.13	0.39	0.16	0.07	0.08	0.11	0.12	0.49
Gobius bucchichi	6l	Selce	1.32	3.27	4.33	0.38	0.15	0.07	0.08	0.11	0.11	0.50
Gobius bucchichi	7l	Selce	1.25	3.26	4.06	0.42	0.16	0.09	0.08	0.11	0.13	0.53
Gobius bucchichi	8l	Selce	1.33	3.10	4.13	0.42	0.17	0.08	0.08	0.11	0.13	0.53
Gobius bucchichi	9l	Selce	1.47	2.99	4.40	0.34	0.14	0.06	0.09	0.12	0.09	0.41
Gobius cobitis	1l	Montenegro	1.31	3.39	4.20	0.43	0.17	0.08	0.08	0.12	0.14	0.58
Gobius cobitis	2l	Montenegro	1.24	3.32	4.12	0.39	0.15	0.08	0.09	0.13	0.10	0.51
Gobius cobitis	3l	Montenegro	1.31	3.26	4.27	0.37	0.15	0.06	0.08	0.12	0.09	0.49
Gobius cobitis	4l	Montenegro	1.29	3.46	4.46	0.39	0.16	0.07	0.08	0.11	0.12	0.54
Gobius cobitis	5l	Montenegro	1.18	3.47	4.11	0.40	0.16	0.07	0.08	0.12	0.11	0.55
Gobius cobitis	6l	Montenegro	1.24	3.39	4.41	0.42	0.16	0.07	0.08	0.11	0.13	0.55
Gobius cobitis	7l	Montenegro	1.21	3.42	4.14	0.38	0.16	0.06	0.09	0.12	0.10	0.54
Gobius cobitis	8l	Montenegro	1.30	3.39	4.40	0.41	0.16	0.07	0.08	0.11	0.12	0.56
Gobius cobitis	9l	Montenegro	1.21	3.45	4.17	0.38	0.15	0.06	0.08	0.11	0.10	0.53
Gobius cobitis	10l	Montenegro	1.23	3.64	4.46	0.37	0.15	0.07	0.08	0.11	0.11	0.55
Gobius couchi	1l	Krk	1.22	3.18	3.88	0.38	0.14	0.08	0.10	0.13	0.10	0.46
Gobius couchi	2l	Krk	1.09	3.41	3.72	0.32	0.13	0.07	0.10	0.14	0.08	0.43
Gobius couchi	3l	Krk	1.07	3.49	3.75	0.34	0.14	0.06	0.09	0.13	0.08	0.48
Gobius couchi	4l	Krk	1.18	3.36	3.96	0.35	0.14	0.07	0.09	0.13	0.09	0.45
Gobius cruentatus	1l	Selce	1.44	3.15	4.52	0.37	0.15	0.06	0.08	0.11	0.09	0.48

Gobius cruentatus	2l	Selce	1.35	3.30	4.44	0.38	0.15	0.07	0.07	0.11	0.12	0.51
Gobius cruentatus	3l	Selce	1.39	3.13	4.34	0.41	0.17	0.07	0.08	0.13	0.11	0.52
Gobius cruentatus	5l	Selce	1.32	3.29	4.34	0.42	0.16	0.08	0.07	0.12	0.13	0.53
Gobius cruentatus	6l	Selce	1.29	3.33	4.31	0.39	0.16	0.07	0.07	0.11	0.11	0.54
Gobius cruentatus	7l	Selce	1.27	3.23	4.11	0.42	0.17	0.08	0.08	0.11	0.14	0.54
Gobius cruentatus	8l	Selce	1.36	3.32	4.50	0.44	0.18	0.09	0.07	0.11	0.16	0.59
Gobius cruentatus	9l	Selce	1.33	3.35	4.45	0.44	0.18	0.07	0.08	0.10	0.15	0.60
Gobius cruentatus	1l	Krk	1.32	3.00	3.95	0.36	0.16	0.07	0.08	0.11	0.09	0.48
Gobius cruentatus	2l	Krk	1.32	3.35	4.41	0.36	0.15	0.08	0.07	0.11	0.11	0.50
Gobius fallax	1l	Croatia	1.32	3.34	4.41	0.36	0.15	0.06	0.08	0.12	0.10	0.50
Gobius fallax	2r	Croatia	1.36	3.11	4.23	0.43	0.17	0.08	0.07	0.14	0.12	0.54
Gobius gasteveni	1l	Galicia	1.33	3.11	4.14	0.43	0.17	0.08	0.08	0.11	0.13	0.53
Gobius geniporus	1l	Montenegro	1.52	3.12	4.74	0.41	0.18	0.06	0.08	0.10	0.12	0.55
Gobius geniporus	2l	Montenegro	1.44	3.03	4.38	0.35	0.15	0.06	0.08	0.11	0.09	0.44
Gobius geniporus	3l	Montenegro	1.42	3.09	4.39	0.44	0.18	0.08	0.08	0.10	0.14	0.56
Gobius geniporus	4l	Montenegro	1.50	3.10	4.65	0.42	0.18	0.06	0.07	0.10	0.12	0.56
Gobius geniporus	1l	Selce Jan.17	1.38	3.05	4.20	0.43	0.18	0.06	0.08	0.12	0.10	0.54
Gobius geniporus	2l	Selce Jan.17	1.38	3.12	4.31	0.36	0.15	0.06	0.08	0.11	0.09	0.48
Gobius geniporus	3l	Selce Jan.17	1.45	3.13	4.56	0.43	0.18	0.06	0.08	0.11	0.12	0.57
Gobius geniporus	small	Selce May16	1.44	3.00	4.34	0.42	0.18	0.05	0.08	0.11	0.09	0.55
Gobius geniporus	medium	Selce May16	1.36	3.07	4.19	0.43	0.18	0.07	0.08	0.12	0.11	0.56
Gobius geniporus	large	Selce May16	1.33	3.10	4.14	0.46	0.19	0.07	0.08	0.11	0.12	0.59
Gobius incognitus	J1904l	Croatia	1.35	3.17	4.28	0.44	0.17	0.08	0.07	0.12	0.13	0.54
Gobius incognitus	J1906l	Croatia	1.47	2.94	4.34	0.45	0.18	0.08	0.08	0.12	0.15	0.53
Gobius incognitus	J1911l	Croatia	1.39	3.07	4.27	0.42	0.17	0.08	0.08	0.13	0.12	0.52
Gobius incognitus	J1912l	Croatia	1.49	2.94	4.36	0.49	0.20	0.09	0.08	0.12	0.17	0.58
Gobius incognitus	J1913l	Croatia	1.39	2.97	4.12	0.44	0.18	0.07	0.08	0.11	0.11	0.53
Gobius incognitus	J1914l	Croatia	1.32	3.16	4.18	0.38	0.16	0.07	0.08	0.12	0.08	0.50
Gobius kolombatovici	1l	Krk	1.49	2.94	4.38	0.37	0.15	0.06	0.08	0.11	0.09	0.45
Gobius kolombatovici	2l	Krk	1.44	3.01	4.33	0.40	0.16	0.06	0.08	0.11	0.10	0.50
Gobius niger	1l	Pilsey Island	1.25	3.18	3.96	0.41	0.17	0.07	0.09	0.11	0.10	0.53
Gobius niger	2l	Pilsey Island	1.27	3.04	3.86	0.40	0.16	0.07	0.09	0.11	0.09	0.49
Gobius niger	3l	Pilsey Island	1.30	3.26	4.23	0.38	0.16	0.06	0.08	0.10	0.11	0.51
Gobius niger	4l	Pilsey Island	1.24	3.31	4.09	0.34	0.14	0.06	0.11	0.11	0.09	0.46
Gobius niger	5l	Pilsey Island	1.18	3.40	4.01	0.36	0.14	0.06	0.08	0.11	0.09	0.49
Gobius niger	6l	Pilsey Island	1.28	3.25	4.17	0.39	0.16	0.07	0.08	0.10	0.12	0.51
Gobius niger	7l	Pilsey Island	1.24	3.21	3.98	0.35	0.14	0.06	0.09	0.12	0.09	0.45
Gobius paganellus	1l	Faro	1.39	3.21	4.47	0.37	0.15	0.06	0.08	0.12	0.10	0.49
Gobius paganellus	2l	Faro	1.40	3.23	4.54	0.45	0.18	0.07	0.07	0.10	0.13	0.58
Gobius paganellus	4l	Faro	1.38	3.30	4.54	0.37	0.16	0.06	0.08	0.11	0.11	0.51
Gobius paganellus	5l	Faro	1.39	3.23	4.48	0.40	0.17	0.06	0.07	0.10	0.09	0.54
Gobius paganellus	6l	Faro	1.42	3.06	4.34	0.38	0.16	0.06	0.07	0.11	0.09	0.48
Gobius paganellus	7l	Faro	1.37	3.21	4.40	0.38	0.16	0.06	0.08	0.10	0.11	0.51
Gobius paganellus	8l	Faro	1.42	3.09	4.37	0.38	0.20	0.08	0.08	0.10	0.09	0.61

Gobius paganellus	9l	Faro	1.43	3.18	4.55	0.38	0.16	0.06	0.07	0.10	0.09	0.51
Gobius paganellus	10l	Faro	1.47	3.10	4.57	0.38	0.16	0.06	0.08	0.09	0.10	0.49
Gobius roulei	1l	Selce	1.31	3.15	4.11	0.37	0.16	0.06	0.09	0.11	0.08	0.49
Gobius roulei	2l	Selce	1.29	3.15	4.07	0.40	0.16	0.08	0.09	0.11	0.11	0.50
Gobius roulei	3l	Selce	1.23	3.19	3.92	0.36	0.15	0.07	0.09	0.12	0.09	0.47
Gobius roulei	4l	Selce	1.25	3.19	3.98	0.39	0.16	0.08	0.09	0.13	0.10	0.50
Gobius roulei	Gr1l	Selce	1.29	3.12	4.02	0.37	0.15	0.06	0.09	0.12	0.09	0.48
Gobius roulei	Gr2l	Selce	1.16	3.37	3.91	0.36	0.15	0.07	0.09	0.13	0.09	0.50
Gobius roulei	Gr3l	Selce	1.15	3.47	3.98	0.35	0.14	0.07	0.09	0.12	0.10	0.48
Gobius roulei	Gr4l	Selce	1.19	3.21	3.83	0.41	0.17	0.07	0.09	0.14	0.10	0.55
Gobius roulei	Gr5l	Selce	1.32	3.12	4.12	0.40	0.17	0.07	0.08	0.12	0.11	0.52
Gobius roulei	Gr6l	Selce	1.25	3.12	3.88	0.44	0.18	0.09	0.10	0.13	0.12	0.55
Gobius vittatus	2l	Selce	1.09	3.43	3.72	0.32	0.13	0.07	0.09	0.14	0.06	0.44
Gobius vittatus	3l	Selce	0.98	3.57	3.50	0.27	0.11	0.06	0.11	0.14	0.05	0.40
Gobius vittatus	4l	Selce	0.98	3.66	3.59	0.29	0.11	0.07	0.10	0.15	0.05	0.42
Gobius vittatus	5l	Selce	1.11	3.36	3.72	0.37	0.14	0.07	0.09	0.13	0.08	0.48
Gobius vittatus	6l	Selce	0.96	3.54	3.41	0.35	0.14	0.07	0.11	0.15	0.07	0.51
Gobius vittatus	7l	Selce	1.16	3.65	4.24	0.32	0.13	0.06	0.08	0.12	0.07	0.46
Gobius vittatus	8r	Selce	1.28	3.18	4.06	0.38	0.15	0.08	0.09	0.12	0.13	0.48
Gobius vittatus	9r	Selce	1.21	3.19	3.87	0.37	0.15	0.07	0.10	0.14	0.09	0.49
Gobius vittatus	1l	Krk	1.19	3.26	3.88	0.32	0.13	0.07	0.10	0.14	0.08	0.42
Gobius vittatus	2l	Krk	1.24	3.20	3.98	0.36	0.14	0.07	0.09	0.13	0.09	0.46
Pomatoschistus kneri	1l	Krk	0.84	3.74	3.13	not clear, not measured						
Pomatoschistus kneri	2l	Krk	1.02	3.42	3.49	0.40	0.15	0.09	0.08	0.14	0.11	0.50
Pomatoschistus kneri	3l	Krk	0.98	3.48	3.40	0.31	0.12	0.07	0.09	0.15	0.06	0.41
Pomatoschistus kneri	4l	Krk	0.85	3.81	3.22	0.34	0.13	0.08	0.10	0.16	0.08	0.50
Pomatoschistus kneri	5l	Krk	0.91	3.67	3.34	0.33	0.12	0.08	0.11	0.16	0.07	0.44
Pomatoschistus kneri	6l	Krk	0.99	3.37	3.35	0.39	0.15	0.08	0.10	0.17	0.08	0.51
Pomatoschistus kneri	7l	Krk	0.90	3.62	3.27	0.26	0.10	0.06	0.11	0.16	0.05	0.36
Pomatoschistus kneri	8l	Krk	0.92	3.60	3.32	0.31	0.12	0.07	0.09	0.15	0.06	0.41
Pomatoschistus kneri	9l	Krk	0.96	3.56	3.42	0.33	0.13	0.07	0.11	0.14	0.09	0.46
Pomatoschistus kneri	10l	Krk	0.95	3.65	3.47	0.29	0.10	0.08	0.08	0.16	0.07	0.37
Pomatoschistus marmoratus	1l	Selce	1.06	3.42	3.64	0.34	0.13	0.07	0.08	0.14	0.06	0.46
Pomatoschistus marmoratus	2l	Selce	1.04	3.36	3.48	0.38	0.15	0.08	0.07	0.13	0.10	0.49
Pomatoschistus marmoratus	3l	Selce	1.05	3.40	3.57	0.44	0.17	0.08	0.08	0.14	0.11	0.58
Pomatoschistus marmoratus	4l	Selce	1.06	3.36	3.56	0.34	0.14	0.08	0.10	0.13	0.08	0.45
Pomatoschistus marmoratus	5l	Selce	1.09	3.37	3.67	0.37	0.14	0.08	0.07	0.12	0.07	0.47
Pomatoschistus marmoratus	6l	Selce	0.98	3.48	3.41	0.33	0.13	0.07	0.09	0.15	0.08	0.44
Pomatoschistus marmoratus	7l	Selce	1.15	3.17	3.66	0.40	0.16	0.07	0.10	0.14	0.10	0.50
Pomatoschistus marmoratus	2l	Albania	1.11	3.62	4.02	0.33	0.12	0.07	0.07	0.13	0.08	0.45
Pomatoschistus marmoratus	3l	Albania	1.07	3.48	3.74	0.32	0.12	0.07	0.09	0.14	0.07	0.43
Pomatoschistus microps	2l	Stralsund	1.12	3.41	3.82	0.42	0.16	0.08	0.09	0.11	0.11	0.54
Pomatoschistus microps	3l	Stralsund	1.02	3.52	3.59	0.30	0.12	0.06	0.11	0.13	0.06	0.41
Pomatoschistus microps	4l	Stralsund	1.02	3.51	3.57	0.31	0.12	0.06	0.07	0.13	0.06	0.43

Pomatoschistus microps	5l	Stralsund	1.00	3.60	3.60	0.33	0.12	0.07	0.10	0.14	0.07	0.45
Pomatoschistus microps	6l	Stralsund	1.07	3.46	3.69	0.42	0.15	0.08	0.10	0.13	0.10	0.52
Pomatoschistus microps	7l	Stralsund	1.03	3.53	3.65	0.34	0.13	0.07	0.09	0.12	0.07	0.45
Pomatoschistus microps	8l	Stralsund	1.01	3.52	3.57	0.39	0.14	0.08	0.09	0.12	0.10	0.50
Pomatoschistus microps	9l	Stralsund	1.04	3.45	3.60	0.38	0.15	0.07	0.09	0.13	0.09	0.52
Pomatoschistus microps	10l	Stralsund	0.97	3.55	3.43	0.36	0.14	0.06	0.10	0.13	0.07	0.48
Pomatoschistus microps	11l	Stralsund	1.05	3.43	3.60	0.40	0.15	0.07	0.09	0.13	0.09	0.51
Pomatoschistus microps	12l	Stralsund	0.99	3.50	3.46	0.31	0.12	0.06	0.10	0.13	0.05	0.41
Pomatoschistus microps	13l	Stralsund	1.07	3.46	3.70	0.28	0.10	0.05	0.08	0.11	0.05	0.36
Pomatoschistus microps	14l	Stralsund	0.96	3.62	3.49	0.39	0.15	0.07	0.09	0.14	0.08	0.52
Pomatoschistus microps	15l	Stralsund	1.03	3.51	3.62	0.39	0.15	0.07	0.10	0.12	0.09	0.53
Pomatoschistus microps	16l	Stralsund	1.06	3.40	3.62	0.31	0.12	0.06	0.09	0.13	0.06	0.42
Pomatoschistus minutus	1l	Stralsund	1.15	3.39	3.91	0.35	0.14	0.06	0.08	0.11	0.09	0.49
Pomatoschistus minutus	17l	Stralsund	1.08	3.38	3.66	0.36	0.14	0.08	0.08	0.13	0.08	0.46
Pomatoschistus montenegrensis	1l	Skadar Lake	0.89	3.77	3.37	0.27	0.11	0.05	0.12	0.15	0.05	0.42
Pomatoschistus montenegrensis	2l	Skadar Lake	0.88	3.74	3.27	0.25	0.09	0.05	0.12	0.14	0.05	0.35
Pomatoschistus montenegrensis	3l	Skadar Lake	0.90	3.77	3.38	0.22	0.09	0.04	0.11	0.14	0.04	0.34
Pomatoschistus montenegrensis	4l	Skadar Lake	0.94	3.57	3.35	0.28	0.11	0.05	0.12	0.14	0.05	0.40
Pomatoschistus montenegrensis	5l	Skadar Lake	0.85	3.97	3.36	0.28	0.11	0.06	0.13	0.15	0.06	0.43
Pomatoschistus montenegrensis	6l	Skadar Lake	0.91	3.65	3.33	0.31	0.12	0.06	0.10	0.15	0.07	0.46
Pomatoschistus montenegrensis	7l	Skadar Lake	0.90	3.65	3.28	0.29	0.12	0.06	0.11	0.15	0.05	0.43
Pomatoschistus montenegrensis	8l	Skadar Lake	0.91	3.68	3.36	0.24	0.10	0.05	0.11	0.14	0.04	0.36
Pomatoschistus montenegrensis	9l	Skadar Lake	0.92	3.67	3.37	0.29	0.12	0.05	0.11	0.15	0.06	0.44
Pomatoschistus montenegrensis	10l	Skadar Lake	0.90	3.72	3.37	0.25	0.11	0.05	0.11	0.14	0.04	0.39
Pomatoschistus pictus	1l	Norway	1.08	3.34	3.60	0.37	0.15	0.07	0.09	0.14	0.08	0.50
Pomatoschistus pictus	2l	Norway	0.99	3.72	3.66	0.26	0.10	0.06	0.10	0.14	0.05	0.38
Pomatoschistus quagga	1l	Krk	0.91	3.72	3.37	0.30	0.11	0.07	0.10	0.15	0.07	0.41
Pomatoschistus quagga	2l	Krk	0.88	3.65	3.23	0.28	0.10	0.08	0.10	0.15	0.06	0.36
Pomatoschistus quagga	3l	Krk	0.91	3.67	3.34	0.32	0.12	0.07	0.10	0.15	0.07	0.46
Pomatoschistus quagga	4l	Krk	0.95	3.60	3.41	0.31	0.12	0.06	0.10	0.15	0.07	0.43
Pomatoschistus quagga	5l	Krk	0.90	3.78	3.39	0.30	0.11	0.07	0.09	0.14	0.07	0.42
Pomatoschistus quagga	7l	Krk	0.95	3.65	3.48	0.26	0.08	0.08	0.08	0.14	0.06	0.30
Pomatoschistus quagga	8l	Krk	0.90	3.68	3.32	0.30	0.12	0.07	0.09	0.15	0.05	0.43
Pomatoschistus quagga	9l	Krk	0.93	3.71	3.47	0.27	0.10	0.07	0.10	0.15	0.06	0.38
Pomatoschistus quagga	10l	Krk	0.93	3.69	3.44	0.32	0.12	0.08	0.09	0.14	0.06	0.45
Species	specimen number	locality	SuL/ OH	SuL/ SuH	SuL/ SuP	SuL/ SuTipV	SuL/ SuEndV	SuH/ OL	SuH/ OH	SuH/ SuP	SuH/ SuTipV	
Gobius auratus	2l	Selce	0.57	2.36	0.41	1.48	1.04	0.20	0.24	0.17	0.63	
Gobius auratus	3l	Selce	0.54	2.11	0.41	1.46	0.98	0.21	0.26	0.19	0.69	
Gobius auratus	4l	Selce	0.47	2.23	0.41	1.20	0.89	0.19	0.21	0.18	0.54	
Gobius auratus	5l	Selce	0.54	2.43	0.41	1.33	1.00	0.19	0.22	0.17	0.55	

Gobius auratus	6l	Selce	0.50	2.09	0.41	1.24	0.92	0.20	0.24	0.20	0.59
Gobius auratus	7l	Selce	0.55	2.06	0.41	1.55	1.00	0.22	0.27	0.20	0.75
Gobius auratus	8l	Selce	0.52	2.22	0.41	1.33	0.99	0.21	0.23	0.19	0.60
Gobius auratus	9l	Selce	0.44	2.01	0.39	1.08	0.86	0.20	0.22	0.20	0.54
Gobius auratus	1l	Krk	0.57	1.97	0.39	1.58	1.03	0.23	0.29	0.20	0.80
Gobius auratus	2l	Krk	0.58	2.04	0.40	1.56	1.11	0.22	0.28	0.19	0.76
Gobius bucchichi	1l	Kraljevica	0.67	2.02	0.40	1.93	1.22	0.26	0.33	0.20	0.95
Gobius bucchichi	1l	Selce	0.66	2.09	0.40	1.89	1.24	0.25	0.31	0.19	0.91
Gobius bucchichi	2l	Selce	0.65	1.98	0.39	1.73	1.20	0.25	0.33	0.20	0.88
Gobius bucchichi	3l	Selce	0.63	2.18	0.40	1.80	1.19	0.23	0.29	0.18	0.82
Gobius bucchichi	4l	Selce	0.69	2.22	0.40	1.97	1.27	0.24	0.31	0.18	0.89
Gobius bucchichi	5l	Selce	0.65	2.19	0.40	1.91	1.40	0.22	0.30	0.18	0.87
Gobius bucchichi	6l	Selce	0.66	2.26	0.41	1.92	1.33	0.22	0.29	0.18	0.85
Gobius bucchichi	7l	Selce	0.67	1.85	0.39	2.13	1.48	0.29	0.36	0.21	1.15
Gobius bucchichi	8l	Selce	0.71	2.18	0.41	2.28	1.54	0.24	0.32	0.19	1.04
Gobius bucchichi	9l	Selce	0.60	2.32	0.40	1.59	1.10	0.18	0.26	0.17	0.69
Gobius cobitis	1l	Montenegro	0.71	2.13	0.40	2.18	1.44	0.27	0.33	0.19	1.02
Gobius cobitis	2l	Montenegro	0.63	1.96	0.39	1.79	1.22	0.26	0.32	0.20	0.92
Gobius cobitis	3l	Montenegro	0.64	2.42	0.41	1.80	1.25	0.20	0.26	0.17	0.74
Gobius cobitis	4l	Montenegro	0.70	2.09	0.41	1.94	1.44	0.26	0.33	0.19	0.93
Gobius cobitis	5l	Montenegro	0.65	2.17	0.40	2.08	1.32	0.25	0.30	0.18	0.96
Gobius cobitis	6l	Montenegro	0.73	2.24	0.39	2.08	1.44	0.24	0.32	0.18	0.93
Gobius cobitis	7l	Montenegro	0.65	2.44	0.41	1.85	1.30	0.22	0.27	0.17	0.76
Gobius cobitis	8l	Montenegro	0.72	2.37	0.40	2.14	1.49	0.23	0.30	0.17	0.90
Gobius cobitis	9l	Montenegro	0.64	2.42	0.41	1.94	1.34	0.22	0.27	0.17	0.80
Gobius cobitis	10l	Montenegro	0.68	2.22	0.41	2.02	1.42	0.25	0.30	0.19	0.91
Gobius couchi	1l	Krk	0.56	1.86	0.39	1.42	1.16	0.25	0.30	0.21	0.76
Gobius couchi	2l	Krk	0.47	1.78	0.39	1.24	0.91	0.24	0.26	0.22	0.70
Gobius couchi	3l	Krk	0.52	2.15	0.40	1.47	1.08	0.22	0.24	0.19	0.68
Gobius couchi	4l	Krk	0.53	1.83	0.38	1.48	1.04	0.25	0.29	0.21	0.81
Gobius cruentatus	1l	Selce	0.69	2.45	0.41	1.96	1.36	0.20	0.28	0.17	0.80
Gobius cruentatus	2l	Selce	0.68	2.17	0.40	2.07	1.39	0.23	0.32	0.19	0.95
Gobius cruentatus	3l	Selce	0.73	2.29	0.41	2.20	1.33	0.23	0.32	0.18	0.96
Gobius cruentatus	5l	Selce	0.70	1.94	0.39	2.17	1.39	0.27	0.36	0.20	1.12
Gobius cruentatus	6l	Selce	0.69	2.29	0.41	2.19	1.50	0.23	0.30	0.18	0.96
Gobius cruentatus	7l	Selce	0.69	2.07	0.40	2.03	1.47	0.26	0.33	0.19	0.98
Gobius cruentatus	8l	Selce	0.79	2.06	0.40	2.40	1.66	0.28	0.39	0.19	1.17
Gobius cruentatus	9l	Selce	0.80	2.46	0.41	2.34	1.73	0.24	0.32	0.17	0.95
Gobius cruentatus	1l	Krk	0.64	2.38	0.44	2.11	1.43	0.20	0.27	0.19	0.89
Gobius cruentatus	2l	Krk	0.66	1.95	0.42	2.30	1.32	0.26	0.34	0.21	1.18
Gobius fallax	1l	Croatia	0.66	2.46	0.41	1.86	1.20	0.20	0.27	0.17	0.75
Gobius fallax	2r	Croatia	0.73	2.10	0.41	2.31	1.24	0.26	0.35	0.19	1.10
Gobius gasteveni	1l	Galicia	0.70	2.21	0.39	2.08	1.52	0.24	0.32	0.18	0.94
Gobius geniporus	1l	Montenegro	0.83	3.00	0.43	2.31	1.80	0.18	0.28	0.14	0.77

Gobius geniporus	2l	Montenegro	0.64	2.26	0.42	1.86	1.31	0.20	0.28	0.19	0.82
Gobius geniporus	3l	Montenegro	0.79	2.39	0.41	2.41	1.72	0.23	0.33	0.17	1.01
Gobius geniporus	4l	Montenegro	0.83	2.82	0.43	2.43	1.73	0.20	0.30	0.15	0.86
Gobius geniporus	1l	Selce Jan.17	0.75	2.76	0.42	2.20	1.49	0.20	0.27	0.15	0.80
Gobius geniporus	2l	Selce Jan.17	0.66	2.59	0.42	1.91	1.40	0.18	0.25	0.16	0.74
Gobius geniporus	3l	Selce Jan.17	0.83	2.97	0.42	2.16	1.68	0.19	0.28	0.14	0.73
Gobius geniporus	small	Selce May16	0.80	3.37	0.44	2.42	1.74	0.16	0.24	0.13	0.72
Gobius geniporus	medium	Selce May16	0.77	2.58	0.43	2.29	1.59	0.22	0.30	0.17	0.89
Gobius geniporus	large	Selce May16	0.78	2.79	0.41	2.44	1.67	0.21	0.28	0.15	0.88
Gobius incognitus	J1904l	Croatia	0.72	2.22	0.39	2.34	1.45	0.24	0.33	0.17	1.05
Gobius incognitus	J1906l	Croatia	0.78	2.34	0.40	2.16	1.56	0.23	0.33	0.17	0.92
Gobius incognitus	J1911l	Croatia	0.72	2.15	0.40	2.20	1.33	0.24	0.34	0.19	1.02
Gobius incognitus	J1912l	Croatia	0.85	2.16	0.40	2.41	1.66	0.27	0.40	0.18	1.12
Gobius incognitus	J1913l	Croatia	0.74	2.56	0.41	2.18	1.64	0.21	0.29	0.16	0.85
Gobius incognitus	J1914l	Croatia	0.66	2.38	0.42	1.87	1.28	0.21	0.28	0.17	0.79
Gobius kolombatovici	1l	Krk	0.67	2.50	0.41	1.85	1.36	0.18	0.27	0.16	0.74
Gobius kolombatovici	2l	Krk	0.71	2.61	0.41	2.05	1.50	0.19	0.27	0.16	0.79
Gobius niger	1l	Pilsey Island	0.66	2.56	0.41	1.96	1.54	0.21	0.26	0.16	0.77
Gobius niger	2l	Pilsey Island	0.62	2.44	0.41	1.75	1.43	0.20	0.26	0.17	0.72
Gobius niger	3l	Pilsey Island	0.67	2.44	0.41	2.03	1.53	0.21	0.27	0.17	0.83
Gobius niger	4l	Pilsey Island	0.57	2.21	0.41	1.28	1.22	0.21	0.26	0.18	0.58
Gobius niger	5l	Pilsey Island	0.58	2.26	0.40	1.85	1.35	0.22	0.26	0.18	0.82
Gobius niger	6l	Pilsey Island	0.66	2.40	0.40	1.85	1.52	0.21	0.27	0.17	0.77
Gobius niger	7l	Pilsey Island	0.56	2.34	0.41	1.60	1.22	0.19	0.24	0.17	0.68
Gobius paganellus	1l	Faro	0.68	2.56	0.42	1.99	1.32	0.19	0.27	0.16	0.78
Gobius paganellus	2l	Faro	0.82	2.47	0.40	2.76	1.77	0.24	0.33	0.16	1.12
Gobius paganellus	4l	Faro	0.71	2.54	0.42	1.99	1.38	0.20	0.28	0.16	0.78
Gobius paganellus	5l	Faro	0.74	3.00	0.42	2.24	1.62	0.18	0.25	0.14	0.75
Gobius paganellus	6l	Faro	0.67	2.67	0.41	2.09	1.37	0.18	0.25	0.15	0.78
Gobius paganellus	7l	Faro	0.70	2.76	0.42	1.95	1.53	0.19	0.26	0.15	0.71
Gobius paganellus	8l	Faro	0.87	2.48	0.52	2.61	2.03	0.25	0.35	0.21	1.05
Gobius paganellus	9l	Faro	0.73	2.67	0.42	2.44	1.65	0.19	0.27	0.16	0.92
Gobius paganellus	10l	Faro	0.72	2.64	0.41	1.99	1.65	0.18	0.27	0.16	0.76
Gobius roulei	1l	Selce	0.64	2.70	0.43	1.69	1.36	0.18	0.24	0.16	0.63
Gobius roulei	2l	Selce	0.65	2.03	0.40	1.74	1.40	0.25	0.32	0.20	0.86
Gobius roulei	3l	Selce	0.57	2.15	0.41	1.71	1.23	0.22	0.27	0.19	0.80
Gobius roulei	4l	Selce	0.62	2.08	0.40	1.83	1.23	0.24	0.30	0.19	0.88
Gobius roulei	Gr1l	Selce	0.62	2.40	0.42	1.74	1.28	0.20	0.26	0.17	0.72
Gobius roulei	Gr2l	Selce	0.58	2.10	0.41	1.74	1.17	0.24	0.28	0.19	0.83
Gobius roulei	Gr3l	Selce	0.56	2.02	0.39	1.56	1.16	0.24	0.27	0.19	0.77
Gobius roulei	Gr4l	Selce	0.66	2.29	0.42	1.92	1.22	0.24	0.29	0.18	0.84
Gobius roulei	Gr5l	Selce	0.69	2.32	0.41	2.04	1.35	0.22	0.30	0.18	0.88
Gobius roulei	Gr6l	Selce	0.68	2.05	0.40	1.83	1.34	0.27	0.33	0.20	0.89
Gobius vittatus	2l	Selce	0.48	1.95	0.40	1.38	0.94	0.23	0.25	0.21	0.71

Gobius vittatus	3l	Selce	0.39	2.02	0.41	0.99	0.81	0.20	0.20	0.20	0.49
Gobius vittatus	4l	Selce	0.41	1.72	0.39	1.16	0.76	0.24	0.24	0.23	0.67
Gobius vittatus	5l	Selce	0.53	1.93	0.39	1.62	1.07	0.25	0.28	0.20	0.84
Gobius vittatus	6l	Selce	0.49	2.02	0.41	1.36	0.94	0.25	0.24	0.20	0.67
Gobius vittatus	7l	Selce	0.54	2.26	0.40	1.52	1.10	0.20	0.24	0.18	0.67
Gobius vittatus	8r	Selce	0.62	1.93	0.40	1.62	1.25	0.25	0.32	0.21	0.84
Gobius vittatus	9r	Selce	0.59	2.20	0.41	1.48	1.12	0.22	0.27	0.19	0.67
Gobius vittatus	1l	Krk	0.50	1.96	0.41	1.28	0.94	0.22	0.26	0.21	0.65
Gobius vittatus	2l	Krk	0.57	2.10	0.40	1.66	1.12	0.22	0.27	0.19	0.79
Pomatoschistus kneri	1l	Krk	not clear, not measured								
Pomatoschistus kneri	2l	Krk	0.51	1.68	0.37	1.87	1.03	0.30	0.30	0.22	1.11
Pomatoschistus kneri	3l	Krk	0.40	1.71	0.38	1.30	0.80	0.24	0.23	0.22	0.76
Pomatoschistus kneri	4l	Krk	0.42	1.62	0.38	1.30	0.82	0.31	0.26	0.24	0.80
Pomatoschistus kneri	5l	Krk	0.40	1.50	0.37	1.14	0.76	0.29	0.27	0.25	0.76
Pomatoschistus kneri	6l	Krk	0.51	1.79	0.39	1.54	0.92	0.29	0.28	0.22	0.86
Pomatoschistus kneri	7l	Krk	0.33	1.55	0.39	0.90	0.63	0.23	0.21	0.25	0.58
Pomatoschistus kneri	8l	Krk	0.38	1.68	0.38	1.25	0.79	0.25	0.23	0.22	0.74
Pomatoschistus kneri	9l	Krk	0.44	1.76	0.39	1.16	0.89	0.26	0.25	0.22	0.66
Pomatoschistus kneri	10l	Krk	0.35	1.31	0.35	1.30	0.65	0.28	0.27	0.27	1.00
Pomatoschistus marmoratus	1l	Selce	0.49	2.03	0.39	1.67	0.98	0.23	0.24	0.19	0.83
Pomatoschistus marmoratus	2l	Selce	0.51	1.83	0.39	2.18	1.11	0.27	0.28	0.21	1.19
Pomatoschistus marmoratus	3l	Selce	0.61	2.03	0.39	2.02	1.25	0.29	0.30	0.19	1.00
Pomatoschistus marmoratus	4l	Selce	0.48	1.79	0.39	1.34	1.04	0.25	0.27	0.22	0.75
Pomatoschistus marmoratus	5l	Selce	0.51	1.86	0.38	1.90	1.15	0.25	0.28	0.20	1.02
Pomatoschistus marmoratus	6l	Selce	0.43	1.75	0.38	1.48	0.87	0.25	0.25	0.22	0.85
Pomatoschistus marmoratus	7l	Selce	0.57	2.10	0.39	1.53	1.15	0.24	0.27	0.19	0.73
Pomatoschistus marmoratus	2l	Albania	0.50	1.81	0.38	1.71	0.95	0.25	0.28	0.21	0.95
Pomatoschistus marmoratus	3l	Albania	0.46	1.73	0.38	1.32	0.86	0.25	0.27	0.22	0.76
Pomatoschistus microps	2l	Stralsund	0.60	2.00	0.38	1.78	1.40	0.27	0.30	0.19	0.89
Pomatoschistus microps	3l	Stralsund	0.41	1.98	0.39	1.08	0.86	0.21	0.21	0.20	0.55
Pomatoschistus microps	4l	Stralsund	0.44	1.93	0.40	1.74	0.95	0.22	0.23	0.21	0.90
Pomatoschistus microps	5l	Stralsund	0.45	1.81	0.38	1.23	0.91	0.25	0.25	0.21	0.68
Pomatoschistus microps	6l	Stralsund	0.55	1.81	0.36	1.54	1.19	0.29	0.31	0.20	0.85
Pomatoschistus microps	7l	Stralsund	0.46	1.80	0.37	1.37	1.03	0.25	0.26	0.21	0.76
Pomatoschistus microps	8l	Stralsund	0.51	1.80	0.36	1.52	1.17	0.28	0.28	0.20	0.84
Pomatoschistus microps	9l	Stralsund	0.54	2.10	0.40	1.63	1.17	0.25	0.26	0.19	0.78
Pomatoschistus microps	10l	Stralsund	0.46	2.09	0.37	1.42	1.07	0.23	0.22	0.18	0.68
Pomatoschistus microps	11l	Stralsund	0.54	2.22	0.38	1.59	1.15	0.23	0.24	0.17	0.71
Pomatoschistus microps	12l	Stralsund	0.41	1.93	0.38	1.23	0.93	0.21	0.21	0.20	0.63
Pomatoschistus microps	13l	Stralsund	0.39	1.96	0.37	1.25	0.96	0.18	0.20	0.19	0.64
Pomatoschistus microps	14l	Stralsund	0.51	1.98	0.38	1.67	1.07	0.26	0.25	0.19	0.84
Pomatoschistus microps	15l	Stralsund	0.54	2.25	0.39	1.53	1.23	0.23	0.24	0.17	0.68
Pomatoschistus microps	16l	Stralsund	0.45	1.95	0.39	1.38	0.93	0.22	0.23	0.20	0.71
Pomatoschistus minutus	1l	Stralsund	0.57	2.28	0.41	1.91	1.26	0.22	0.25	0.18	0.84

Pomatoschistus minutus	17l	Stralsund	0.50	1.75	0.38	1.81	1.04	0.26	0.29	0.22	1.03
Pomatoschistus montenegrensis	1l	Skadar Lake	0.38	2.22	0.41	0.96	0.75	0.19	0.17	0.19	0.43
Pomatoschistus montenegrensis	2l	Skadar Lake	0.31	1.72	0.38	0.81	0.66	0.21	0.18	0.22	0.47
Pomatoschistus montenegrensis	3l	Skadar Lake	0.30	2.05	0.41	0.80	0.64	0.17	0.15	0.20	0.39
Pomatoschistus montenegrensis	4l	Skadar Lake	0.38	2.12	0.41	0.98	0.81	0.19	0.18	0.19	0.46
Pomatoschistus montenegrensis	5l	Skadar Lake	0.36	1.73	0.39	0.85	0.73	0.25	0.21	0.22	0.49
Pomatoschistus montenegrensis	6l	Skadar Lake	0.42	1.96	0.40	1.29	0.85	0.23	0.21	0.21	0.66
Pomatoschistus montenegrensis	7l	Skadar Lake	0.39	2.07	0.41	1.10	0.78	0.21	0.19	0.20	0.53
Pomatoschistus montenegrensis	8l	Skadar Lake	0.33	1.96	0.40	0.89	0.72	0.18	0.17	0.20	0.45
Pomatoschistus montenegrensis	9l	Skadar Lake	0.40	2.23	0.41	1.05	0.80	0.20	0.18	0.19	0.47
Pomatoschistus montenegrensis	10l	Skadar Lake	0.36	2.28	0.42	0.98	0.77	0.17	0.16	0.18	0.43
Pomatoschistus pictus	1l	Norway	0.54	2.09	0.40	1.58	1.05	0.24	0.26	0.19	0.76
Pomatoschistus pictus	2l	Norway	0.37	1.58	0.39	1.03	0.75	0.24	0.23	0.25	0.65
Pomatoschistus quagga	1l	Krk	0.37	1.53	0.37	1.11	0.74	0.27	0.24	0.24	0.73
Pomatoschistus quagga	2l	Krk	0.32	1.32	0.36	1.04	0.67	0.28	0.24	0.27	0.79
Pomatoschistus quagga	3l	Krk	0.42	1.79	0.39	1.27	0.85	0.26	0.23	0.22	0.71
Pomatoschistus quagga	4l	Krk	0.41	1.89	0.40	1.19	0.82	0.23	0.22	0.21	0.63
Pomatoschistus quagga	5l	Krk	0.38	1.56	0.37	1.27	0.78	0.27	0.24	0.24	0.81
Pomatoschistus quagga	7l	Krk	0.29	1.03	0.32	0.99	0.59	0.29	0.28	0.31	0.96
Pomatoschistus quagga	8l	Krk	0.38	1.57	0.38	1.28	0.76	0.27	0.24	0.24	0.81
Pomatoschistus quagga	9l	Krk	0.35	1.43	0.37	1.03	0.70	0.26	0.25	0.26	0.72
Pomatoschistus quagga	10l	Krk	0.42	1.57	0.37	1.36	0.84	0.28	0.26	0.24	0.86

Species	specimen number	locality	SuH/ SuEndV	SuP/ SuTipV	SuP/ SuEndV	SuTipV/SuEndV
Gobius auratus	2l	Selce	0.28	3.60	5.05	0.71
Gobius auratus	3l	Selce	0.29	3.59	5.08	0.67
Gobius auratus	4l	Selce	0.31	2.90	4.81	0.75
Gobius auratus	5l	Selce	0.25	3.21	4.22	0.76
Gobius auratus	6l	Selce	0.28	3.04	4.18	0.75
Gobius auratus	7l	Selce	0.28	3.82	4.87	0.64
Gobius auratus	8l	Selce	0.31	3.22	4.89	0.74
Gobius auratus	9l	Selce	0.36	2.75	4.94	0.80
Gobius auratus	1l	Krk	0.21	4.00	3.71	0.65
Gobius auratus	2l	Krk	0.22	3.93	3.91	0.71
Gobius bucchichi	1l	Kraljevica	0.28	4.84	5.26	0.63
Gobius bucchichi	1l	Selce	0.29	4.75	5.57	0.66
Gobius bucchichi	2l	Selce	0.30	4.41	5.42	0.69
Gobius bucchichi	3l	Selce	0.28	4.48	5.47	0.66
Gobius bucchichi	4l	Selce	0.26	4.93	5.37	0.64
Gobius bucchichi	5l	Selce	0.25	4.72	5.33	0.73

Gobius bucchichi	6l	Selce	0.28	4.74	6.02	0.69
Gobius bucchichi	7l	Selce	0.33	5.45	6.22	0.69
Gobius bucchichi	8l	Selce	0.27	5.55	6.07	0.68
Gobius bucchichi	9l	Selce	0.24	3.94	5.26	0.69
Gobius cobitis	1l	Montenegro	0.68	5.52	3.65	0.66
Gobius cobitis	2l	Montenegro	0.62	4.57	3.10	0.68
Gobius cobitis	3l	Montenegro	0.52	4.44	3.08	0.69
Gobius cobitis	4l	Montenegro	0.69	4.78	3.54	0.74
Gobius cobitis	5l	Montenegro	0.61	5.24	3.32	0.63
Gobius cobitis	6l	Montenegro	0.64	5.29	3.67	0.69
Gobius cobitis	7l	Montenegro	0.53	4.52	3.17	0.70
Gobius cobitis	8l	Montenegro	0.63	5.36	3.73	0.69
Gobius cobitis	9l	Montenegro	0.56	4.75	3.28	0.69
Gobius cobitis	10l	Montenegro	0.64	4.87	3.44	0.71
Gobius couchi	1l	Krk	0.29	3.68	4.34	0.82
Gobius couchi	2l	Krk	0.38	3.23	5.07	0.73
Gobius couchi	3l	Krk	0.30	3.63	4.79	0.74
Gobius couchi	4l	Krk	0.36	3.85	5.60	0.70
Gobius cruentatus	1l	Selce	0.16	4.81	3.90	0.69
Gobius cruentatus	2l	Selce	0.18	5.12	3.98	0.67
Gobius cruentatus	3l	Selce	0.14	5.33	3.25	0.60
Gobius cruentatus	5l	Selce	0.17	5.59	3.56	0.64
Gobius cruentatus	6l	Selce	0.15	5.33	3.37	0.68
Gobius cruentatus	7l	Selce	0.17	5.11	3.38	0.72
Gobius cruentatus	8l	Selce	0.18	6.05	3.75	0.69
Gobius cruentatus	9l	Selce	0.16	5.73	3.68	0.74
Gobius cruentatus	1l	Krk	0.60	4.74	3.23	0.68
Gobius cruentatus	2l	Krk	0.68	5.48	3.15	0.57
Gobius fallax	1l	Croatia	0.49	4.54	2.93	0.65
Gobius fallax	2r	Croatia	0.59	5.69	3.04	0.53
Gobius gasteveni	1l	Galicia	0.23	5.27	5.03	0.73
Gobius geniporus	1l	Montenegro	0.11	5.43	3.28	0.78
Gobius geniporus	2l	Montenegro	0.14	4.43	3.26	0.71
Gobius geniporus	3l	Montenegro	0.17	5.82	4.14	0.72
Gobius geniporus	4l	Montenegro	0.14	5.71	4.00	0.71
Gobius geniporus	1l	Selce Jan.17	0.17	5.29	4.54	0.68
Gobius geniporus	2l	Selce Jan.17	0.11	4.50	2.73	0.73
Gobius geniporus	3l	Selce Jan.17	0.11	5.11	2.84	0.78
Gobius geniporus	small	Selce May16	0.52	5.50	3.94	0.72
Gobius geniporus	medium	Selce May16	0.61	5.33	3.70	0.69
Gobius geniporus	large	Selce May16	0.60	5.91	4.03	0.68
Gobius incognitus	J19041	Croatia	0.65	6.02	3.74	0.62
Gobius incognitus	J19061	Croatia	0.67	5.36	3.86	0.72
Gobius incognitus	J19111	Croatia	0.62	5.50	3.33	0.61

Gobius incognitus	J1912l	Croatia	0.77	6.05	4.18	0.69
Gobius incognitus	J1913l	Croatia	0.64	5.32	3.98	0.75
Gobius incognitus	J1914l	Croatia	0.54	4.50	3.08	0.69
Gobius kolombatovici	1l	Krk	0.55	4.49	3.31	0.74
Gobius kolombatovici	2l	Krk	0.57	4.96	3.61	0.73
Gobius niger	1l	Pilsey Island	0.60	4.82	3.79	0.79
Gobius niger	2l	Pilsey Island	0.59	4.29	3.51	0.82
Gobius niger	3l	Pilsey Island	0.63	4.92	3.70	0.75
Gobius niger	4l	Pilsey Island	0.55	3.16	3.01	0.95
Gobius niger	5l	Pilsey Island	0.60	4.61	3.36	0.73
Gobius niger	6l	Pilsey Island	0.63	4.59	3.77	0.82
Gobius niger	7l	Pilsey Island	0.52	3.92	2.98	0.76
Gobius paganellus	1l	Faro	0.26	4.78	6.48	0.66
Gobius paganellus	2l	Faro	0.33	6.85	9.50	0.64
Gobius paganellus	4l	Faro	0.24	4.77	5.70	0.69
Gobius paganellus	5l	Faro	0.25	5.35	7.43	0.72
Gobius paganellus	6l	Faro	0.23	5.15	6.66	0.66
Gobius paganellus	7l	Faro	0.25	4.66	6.21	0.78
Gobius paganellus	8l	Faro	0.82	5.01	3.89	0.78
Gobius paganellus	9l	Faro	0.25	5.82	7.69	0.68
Gobius paganellus	10l	Faro	0.26	4.86	6.83	0.83
Gobius roulei	1l	Selce	0.50	3.97	3.19	0.80
Gobius roulei	2l	Selce	0.69	4.32	3.46	0.80
Gobius roulei	3l	Selce	0.57	4.22	3.04	0.72
Gobius roulei	4l	Selce	0.59	4.53	3.05	0.67
Gobius roulei	Gr1l	Selce	0.53	4.16	3.06	0.74
Gobius roulei	Gr2l	Selce	0.56	4.24	2.86	0.68
Gobius roulei	Gr3l	Selce	0.57	3.96	2.95	0.74
Gobius roulei	Gr4l	Selce	0.53	4.60	2.92	0.64
Gobius roulei	Gr5l	Selce	0.58	4.92	3.25	0.66
Gobius roulei	Gr6l	Selce	0.65	4.54	3.32	0.73
Gobius vittatus	2l	Selce	0.47	3.45	7.15	0.68
Gobius vittatus	3l	Selce	0.54	2.41	6.47	0.81
Gobius vittatus	4l	Selce	0.51	2.97	6.20	0.66
Gobius vittatus	5l	Selce	0.31	4.20	5.14	0.66
Gobius vittatus	6l	Selce	0.61	3.34	8.04	0.69
Gobius vittatus	7l	Selce	0.29	3.80	5.39	0.72
Gobius vittatus	8r	Selce	0.65	4.09	3.16	0.77
Gobius vittatus	9r	Selce	0.51	3.59	2.71	0.75
Gobius vittatus	1l	Krk	0.34	3.15	4.94	0.73
Gobius vittatus	2l	Krk	0.30	4.17	5.72	0.67
Pomatoschistus kneri	1l	Krk	not clear, not measured			
Pomatoschistus kneri	2l	Krk	0.61	5.04	2.77	0.55
Pomatoschistus kneri	3l	Krk	0.47	3.40	2.09	0.61

Pomatoschistus kneri	4l	Krk	0.51	3.39	2.14	0.63
Pomatoschistus kneri	5l	Krk	0.51	3.10	2.07	0.67
Pomatoschistus kneri	6l	Krk	0.51	3.95	2.36	0.60
Pomatoschistus kneri	7l	Krk	0.40	2.33	1.62	0.70
Pomatoschistus kneri	8l	Krk	0.47	3.31	2.11	0.64
Pomatoschistus kneri	9l	Krk	0.51	2.97	2.29	0.77
Pomatoschistus kneri	10l	Krk	0.50	3.71	1.86	0.50
Pomatoschistus marmoratus	1l	Selce	0.48	4.25	2.49	0.58
Pomatoschistus marmoratus	2l	Selce	0.61	5.62	2.86	0.51
Pomatoschistus marmoratus	3l	Selce	0.61	5.16	3.18	0.62
Pomatoschistus marmoratus	4l	Selce	0.58	3.40	2.63	0.77
Pomatoschistus marmoratus	5l	Selce	0.62	5.01	3.02	0.60
Pomatoschistus marmoratus	6l	Selce	0.50	3.90	2.29	0.59
Pomatoschistus marmoratus	7l	Selce	0.55	3.94	2.94	0.75
Pomatoschistus marmoratus	2l	Albania	0.52	4.47	2.47	0.55
Pomatoschistus marmoratus	3l	Albania	0.49	3.46	2.25	0.65
Pomatoschistus microps	2l	Stralsund	0.70	4.74	3.73	0.79
Pomatoschistus microps	3l	Stralsund	0.43	2.77	2.19	0.79
Pomatoschistus microps	4l	Stralsund	0.49	4.32	2.37	0.55
Pomatoschistus microps	5l	Stralsund	0.50	3.22	2.39	0.74
Pomatoschistus microps	6l	Stralsund	0.66	4.26	3.31	0.78
Pomatoschistus microps	7l	Stralsund	0.57	3.68	2.75	0.75
Pomatoschistus microps	8l	Stralsund	0.65	4.18	3.21	0.77
Pomatoschistus microps	9l	Stralsund	0.56	4.13	2.96	0.72
Pomatoschistus microps	10l	Stralsund	0.51	3.82	2.88	0.75
Pomatoschistus microps	11l	Stralsund	0.52	4.20	3.04	0.72
Pomatoschistus microps	12l	Stralsund	0.48	3.19	2.43	0.76
Pomatoschistus microps	13l	Stralsund	0.49	3.36	2.57	0.77
Pomatoschistus microps	14l	Stralsund	0.54	4.45	2.84	0.64
Pomatoschistus microps	15l	Stralsund	0.55	3.94	3.16	0.80
Pomatoschistus microps	16l	Stralsund	0.48	3.50	2.37	0.68
Pomatoschistus minutus	1l	Stralsund	0.55	4.65	3.07	0.66
Pomatoschistus minutus	17l	Stralsund	0.60	4.73	2.72	0.58
Pomatoschistus montenegrensis	1l	Skadar Lake	0.33	2.34	1.81	0.77
Pomatoschistus montenegrensis	2l	Skadar Lake	0.38	2.11	1.72	0.82
Pomatoschistus montenegrensis	3l	Skadar Lake	0.31	1.98	1.57	0.79
Pomatoschistus montenegrensis	4l	Skadar Lake	0.38	2.41	2.00	0.83
Pomatoschistus montenegrensis	5l	Skadar Lake	0.42	2.19	1.89	0.86
Pomatoschistus montenegrensis	6l	Skadar Lake	0.43	3.21	2.11	0.66
Pomatoschistus montenegrensis	7l	Skadar Lake	0.38	2.69	1.91	0.71
Pomatoschistus montenegrensis	8l	Skadar Lake	0.36	2.21	1.78	0.80
Pomatoschistus montenegrensis	9l	Skadar Lake	0.36	2.54	1.93	0.76
Pomatoschistus montenegrensis	10l	Skadar Lake	0.34	2.35	1.83	0.78
Pomatoschistus pictus	1l	Norway	0.50	3.95	2.61	0.66

Pomatoschistus pictus	2l	Norway	0.47	2.64	1.90	0.72
Pomatoschistus quagga	1l	Krk	0.48	2.99	1.99	0.66
Pomatoschistus quagga	2l	Krk	0.51	2.91	1.87	0.64
Pomatoschistus quagga	3l	Krk	0.47	3.26	2.17	0.67
Pomatoschistus quagga	4l	Krk	0.44	3.01	2.08	0.69
Pomatoschistus quagga	5l	Krk	0.50	3.41	2.10	0.62
Pomatoschistus quagga	7l	Krk	0.57	3.07	1.84	0.60
Pomatoschistus quagga	8l	Krk	0.48	3.33	1.98	0.59
Pomatoschistus quagga	9l	Krk	0.49	2.77	1.88	0.68
Pomatoschistus quagga	10l	Krk	0.53	3.64	2.25	0.62

,

Table S3:

Due to the volumes of the tables and the great variance in amount of digits the Fourier descriptors are given in electronical form or can be downloaded at “https://brill.figshare.com/articles/Identification_of_past_and_present_gobies_distinguishing_Gobius_and_Pomatoschistus_Teleostei_Gobioidei_species_using_characters_of_otoliths_meristics_and_body_morphometry/11994000”.

Table S4: Raw data of the fish measurements (in mm) and standardized fish morphometric variables (expressed in % of SL, bold-faced); for details of localities see Table 1. Ab, length of anal fin base; B, body depth at origin of first dorsal fin; CP, length of caudal peduncle; D2b, length of second dorsal fin base; D2C, distance between end of second dorsal fin and first dorsal (procurrent) ray of caudal fin; SL, standard length (from snout to begin of caudal fin); SN/A, distance from snout to origin of anal fin; SN/D1, distance from snout to origin of first dorsal fin; SN/D2, distance from snout to origin of second dorsal fin.

Sp.	Sp.Nr.	locality	SL	SN/D1	%SN/D1	SN/D2	%SN/D2	SN/A	%SN/A	D2C	% D2C	CP	%CP	B	%B	D2b	%D2b	Ab	%Ab
G. auratus	1	Selce	41.6	14.5	34.8	21.8	52.4	24.0	57.7	5.9	14.1	7.7	18.6	7.4	17.7	11.8	28.3	10.3	24.7
G. auratus	2	Selce	32.7	11.4	34.8	17.9	54.6	18.9	57.9	4.5	13.8	7.0	21.3	5.9	18.1	9.2	28.2	6.6	20.1
G. auratus	3	Selce	33.1	11.7	35.4	17.9	54.1	19.2	57.9	4.5	13.6	6.5	19.6	5.7	17.2	9.6	29.1	7.6	22.9
G. auratus	4	Selce	26.5	9.6	36.2	14.3	54.0	15.5	58.3	3.8	14.3	5.1	19.2	4.8	17.9	7.6	28.6	5.6	21.0
G. auratus	5	Selce	34.6	12.5	36.2	18.8	54.4	20.2	58.4	4.3	12.4	6.4	18.4	6.2	18.0	10.2	29.4	8.0	23.2
G. auratus	6	Selce	34.6	12.6	36.3	18.9	54.5	20.2	58.2	4.6	13.4	6.7	19.4	6.1	17.6	10.2	29.3	7.8	22.5
G. auratus	7	Selce	36.3	13.6	37.4	20.1	55.3	21.7	59.8	5.3	14.6	7.4	20.3	6.1	16.9	9.4	25.9	7.2	19.9
G. auratus	8	Selce	30.3	10.9	35.9	16.4	54.2	17.8	58.8	3.9	13.0	5.6	18.5	5.3	17.4	8.9	29.2	6.9	22.7
G. auratus	9	Selce	26.1	10.0	38.2	14.7	56.2	15.5	59.3	3.7	14.1	5.0	19.2	4.6	17.8	6.9	26.3	5.8	22.1
G. auratus	1	Krk	60.2	18.7	31.0	30.4	50.5	32.5	53.9	8.1	13.4	12.8	21.3	12.9	21.4	19.6	32.6	14.9	24.8
G. auratus	2	Krk	57.4	20.1	34.9	31.7	55.2	34.0	59.2	7.4	12.8	10.5	18.2	11.2	19.4	16.8	29.2	12.9	22.4
G. bucchichi	1	Kraljevica	54.6	18.2	33.3	28.6	52.3	31.2	57.2	6.4	11.8	9.8	17.9	8.4	15.3	17.8	32.6	13.5	24.7
G. bucchichi	1	Selce	51.7	16.8	32.5	27.2	52.6	29.5	57.0	6.3	12.2	9.7	18.8	8.5	16.5	16.3	31.6	12.4	24.0
G. bucchichi	2	Selce	50.5	16.1	31.8	26.5	52.4	28.7	56.8	6.2	12.3	10.0	19.8	7.5	14.8	16.3	32.2	11.5	22.8
G. bucchichi	3	Selce	49.0	15.6	31.9	25.6	52.3	27.1	55.4	5.6	11.4	9.6	19.6	8.4	17.1	16.0	32.6	12.9	26.3
G. bucchichi	4	Selce	65.6	21.2	32.4	33.5	51.1	35.4	54.1	7.8	11.9	12.6	19.3	9.1	13.9	21.5	32.8	17.5	26.7
G. bucchichi	5	Selce	67.3	21.1	31.3	34.1	50.6	36.5	54.2	8.5	12.6	13.4	19.9	9.7	14.4	22.3	33.1	17.4	25.8
G. bucchichi	6	Selce	49.6	14.6	29.4	24.6	49.5	27.4	55.2	6.5	13.1	9.7	19.5	8.5	17.1	16.8	33.8	12.6	25.4
G. bucchichi	7	Selce	54.2	17.7	32.7	28.3	52.2	31.2	57.5	5.8	10.7	9.0	16.6	7.6	14.0	18.2	33.6	14.3	26.3
G. bucchichi	8	Selce	61.5	20.7	33.6	32.5	52.9	35.4	57.5	7.6	12.3	11.1	18.1	8.6	14.0	19.0	30.9	15.0	24.4
G. bucchichi	9	Selce	44.4	14.2	32.0	23.1	51.9	24.6	55.5	5.2	11.7	8.7	19.5	7.1	15.9	14.4	32.4	11.0	24.8
G. cobitis	1	Montenegro	80.0	28.3	35.3	43.9	54.8	48.4	60.5	10.6	13.3	16.4	20.5	13.7	17.1	22.2	27.7	15.1	18.9
G. cobitis	2	Montenegro	34.2	12.0	35.0	18.6	54.6	20.1	59.0	4.6	13.6	7.1	20.8	6.0	17.5	9.5	27.9	6.9	20.3
G. cobitis	3	Montenegro	33.7	11.9	35.4	18.8	55.7	20.2	60.0	5.2	15.3	7.4	22.0	5.0	14.8	9.3	27.7	6.2	18.4
G. cobitis	4	Montenegro	49.8	17.9	35.9	26.9	54.1	29.9	60.0	10.0	20.1	6.5	13.0	8.7	17.5	13.6	27.3	10.1	20.2
G. cobitis	5	Montenegro	37.2	13.6	36.6	20.3	54.7	22.6	60.9	4.2	11.3	7.1	19.2	5.6	15.1	10.7	28.7	7.4	19.9
G. cobitis	6	Montenegro	48.5	17.2	35.5	26.1	53.8	29.0	59.8	5.9	12.2	9.6	19.7	7.9	16.2	14.4	29.6	10.1	20.7
G. cobitis	7	Montenegro	33.4	11.8	35.3	18.2	54.3	20.0	59.9	4.5	13.3	7.2	21.4	5.7	17.1	9.3	27.7	6.3	18.8
G. cobitis	8	Montenegro	56.5	19.1	33.8	30.3	53.5	33.5	59.3	7.3	13.0	12.2	21.6	10.2	18.1	16.1	28.4	10.7	19.0
G. cobitis	9	Montenegro	53.6	18.5	34.5	28.4	53.0	31.6	59.0	7.3	13.6	10.8	20.1	9.5	17.8	15.6	29.2	11.1	20.8
G. cobitis	10	Montenegro	61.9	22.9	36.9	34.0	55.0	37.6	60.7	7.9	12.8	12.4	20.0	11.0	17.8	16.5	26.7	12.1	19.5
G. couchi	1	Krk	44.3	14.7	33.1	23.9	54.1	26.0	58.6	5.3	12.0	8.6	19.3	7.9	17.9	13.2	29.7	10.3	23.3
G. couchi	2	Krk	32.9	10.9	33.2	17.4	53.0	19.0	57.7	4.7	14.2	6.3	19.2	6.1	18.7	9.9	30.2	7.3	22.1
G. couchi	3	Krk	41.6	13.2	31.7	21.3	51.1	23.1	55.5	5.6	13.5	8.9	21.3	6.9	16.5	12.9	31.0	9.6	23.1
G. couchi	4	Krk	36.2	11.7	32.2	18.4	50.9	20.3	56.0	5.3	14.6	8.2	22.6	7.6	21.0	10.9	30.1	8.3	23.0
G. cruentatus	1	Selce	78.3	27.2	34.8	43.6	55.6	47.6	60.8	8.4	10.7	12.8	16.4	13.2	16.8	24.9	31.8	19.6	25.0
G. cruentatus	2	Selce	84.4	31.7	37.5	47.6	56.4	51.0	60.4	9.6	11.4	15.6	18.5	15.3	18.1	23.8	28.1	17.8	21.1

G. cruentatus	3	Selce	102.3	35.0	34.2	55.9	54.7	60.5	59.2	12.1	11.8	18.8	18.4	15.7	15.3	30.8	30.1	23.0	22.4
G. cruentatus	4	Selce	89.4	32.1	35.9	50.7	56.7	55.3	61.8	10.8	12.0	15.5	17.3	16.9	18.9	25.6	28.7	19.1	21.4
G. cruentatus	5	Selce	98.1	35.8	36.5	54.3	55.4	58.9	60.0	11.6	11.8	17.7	18.0	15.4	15.7	28.5	29.0	22.0	22.5
G. cruentatus	6	Selce	110.5	37.8	34.2	59.4	53.7	66.9	60.6	13.0	11.8	19.0	17.2	21.7	19.6	33.2	30.1	25.0	22.7
G. cruentatus	7	Selce	100.1	36.4	36.3	55.7	55.7	61.4	61.3	13.0	13.0	18.4	18.4	18.3	18.3	28.5	28.5	20.5	20.5
G. cruentatus	8	Selce	116.9	41.5	35.5	63.8	54.6	72.3	61.8	13.3	11.4	21.6	18.5	20.3	17.4	34.0	29.1	24.6	21.1
G. cruentatus	9	Selce	117.0	38.5	32.9	61.8	52.8	69.9	59.7	16.7	14.2	20.3	17.4	21.1	18.1	36.5	31.2	26.1	22.3
G. cruentatus	1	Krk	89.1	31.6	35.4	49.1	55.1	53.0	59.4	10.4	11.7	17.4	19.5	15.1	17.0	26.0	29.2	18.9	21.3
G. cruentatus	2	Krk	86.0	29.7	34.5	47.1	54.7	50.6	58.8	10.2	11.8	16.2	18.8	15.8	18.4	25.2	29.3	20.5	23.8
G. fallax	1	Croatia	50.0	16.0	32.0	26.0	52.0	28.0	56.0	6.0	12.0	9.0	18.0	9.0	18.0	17.0	34.0	12.0	24.0
G. fallax	2	Croatia	46.0	15.0	32.6	24.0	52.2	27.0	58.7	6.0	13.0	10.0	21.7	8.0	17.4	13.0	28.3	9.0	19.6
G. gasteveni	1	Galicia	68.0	18.5	27.2	35.0	51.5	37.8	55.6	8.2	12.0	12.4	18.3	13.9	20.4	22.7	33.4	18.7	27.6
G. geniporus	1	Montenegro	104.1	35.7	34.3	56.5	54.3	60.4	58.1	not clear		18.2	17.5	19.7	18.9	32.4	31.1	25.5	24.5
G. geniporus	2	Montenegro	78.2	26.8	34.3	42.7	54.5	45.5	58.2	9.8	12.5	13.6	17.4	15.3	19.6	23.2	29.6	18.9	24.2
G. geniporus	3	Montenegro	86.3	30.8	35.7	48.2	55.8	50.7	58.8	10.1	11.7	15.4	17.8	16.8	19.5	25.8	29.9	20.3	23.5
G. geniporus	4	Montenegro	89.9	30.6	34.0	48.8	54.2	51.9	57.7	10.2	11.3	16.0	17.8	17.1	19.1	27.4	30.5	21.8	24.3
G. geniporus	1	Selce	63.6	21.0	33.0	33.8	53.2	36.3	57.2	8.2	12.9	11.5	18.1	10.4	16.4	19.9	31.4	15.7	24.6
G. geniporus	2	Selce	101.8	34.9	34.3	55.8	54.8	60.9	59.8	10.2	10.0	16.4	16.2	14.9	14.7	31.9	31.4	24.2	23.8
G. geniporus	3	Selce	111.6	39.9	35.8	61.5	55.1	67.0	60.0	12.6	11.2	18.6	16.7	16.6	14.9	34.7	31.1	25.9	23.2
G. geniporus	small	Selce	107.4	39.3	36.6	59.3	55.2	65.8	61.2	14.2	13.2	18.5	17.2	15.6	14.5	30.3	28.2	23.7	22.1
G. geniporus	medium	Selce	118.9	41.6	34.9	64.8	54.5	71.0	59.7	13.8	11.6	20.6	17.3	17.8	14.9	36.3	30.5	27.0	22.7
G. geniporus	large	Selce	129.7	44.3	34.2	70.0	54.0	76.4	58.9	15.2	11.7	20.8	16.1	18.4	14.2	39.6	30.5	32.8	25.3
G. incognitus	1904	Croatia	71.0	21.0	29.6	35.0	49.3	39.0	54.9	9.0	12.7	13.0	18.3	14.0	19.7	24.0	33.8	20.0	28.2
G. incognitus	1906	Croatia	77.0	26.0	33.8	40.0	51.9	45.0	58.4	10.0	13.0	14.0	18.2	12.0	15.6	24.0	31.2	18.0	23.4
G. incognitus	1907	Croatia	65.0	21.0	32.3	33.0	50.8	37.0	56.9	8.0	12.3	11.0	16.9	12.0	18.5	21.0	32.3	17.0	26.2
G. incognitus	1910	Croatia	63.0	19.0	30.2	31.0	49.2	33.0	52.4	8.0	12.7	13.0	20.6	11.0	17.5	21.0	33.3	17.0	27.0
G. incognitus	1911	Croatia	45.0	15.0	33.3	23.0	51.1	24.0	53.3	6.0	13.3	9.0	20.0	8.0	17.8	14.0	31.1	11.0	24.4
G. incognitus	1912	Croatia	79.0	25.0	31.6	40.0	50.6	45.0	57.0	9.0	11.4	15.0	19.0	13.0	16.5	26.0	32.9	19.0	24.1
G. incognitus	1913	Croatia	56.0	17.0	30.4	28.0	50.0	30.0	53.6	8.0	14.3	12.0	21.4	10.0	17.9	18.0	32.1	14.0	25.0
G. incognitus	1914	Croatia	44.0	14.0	31.8	22.0	50.0	24.0	54.5	6.0	13.6	9.0	20.5	7.0	15.9	13.0	29.5	11.0	25.0
G. incognitus	1915	Croatia	69.0	22.0	31.9	36.0	52.2	38.0	55.1	7.0	10.1	13.0	18.8	12.0	17.4	23.0	33.3	17.0	24.6
G. kolombatovici	1	Krk	70.7	25.3	35.8	38.3	54.2	41.9	59.3	8.8	12.5	13.7	19.4	11.8	16.7	20.1	28.5	15.6	22.1
G. kolombatovici	2	Krk	80.1	27.4	34.2	42.6	53.2	46.2	57.8	9.4	11.8	14.8	18.5	13.1	16.4	23.2	28.9	19.5	24.3
G. niger	1	Pilsey Island	43.1	15.3	35.6	24.2	56.0	25.6	59.4	5.9	13.8	9.0	20.9	7.6	17.5	11.5	26.8	8.5	19.6
G. niger	2	Pilsey Island	46.0	15.8	34.3	25.8	56.2	26.7	58.0	6.2	13.5	8.8	19.2	8.6	18.8	12.8	27.8	9.2	19.9
G. niger	3	Pilsey Island	50.7	19.1	37.7	27.8	54.9	30.9	60.9	6.8	13.3	9.9	19.5	8.3	16.3	13.8	27.3	10.4	20.6
G. niger	4	Pilsey Island	52.7	17.4	33.1	27.7	52.6	29.6	56.2	7.0	13.3	10.3	19.5	9.2	17.5	15.0	28.4	12.3	23.4
G. niger	5	Pilsey Island	59.3	17.8	30.0	31.7	53.4	33.7	56.8	8.3	14.0	11.7	19.7	9.7	16.4	16.4	27.6	12.0	20.2
G. niger	6	Pilsey Island	54.2	18.2	33.6	29.4	54.3	31.4	58.0	7.3	13.4	10.8	19.9	9.4	17.4	15.2	28.1	11.2	20.7
G. niger	7	Pilsey Island	40.7	14.1	34.7	22.6	55.5	24.1	59.3	5.4	13.2	8.7	21.3	7.1	17.6	11.5	28.2	8.4	20.7
G. paganellus	1	Faro	46.8	16.3	34.9	25.3	54.1	27.6	59.1	5.0	10.8	9.0	19.2	9.1	19.5	14.1	30.2	10.4	22.2
G. paganellus	2	Faro	54.5	19.1	35.1	29.1	53.5	31.6	58.1	7.2	13.3	12.2	22.3	11.6	21.3	15.6	28.6	12.2	22.3
G. paganellus	3	Faro	53.0	18.7	35.4	29.2	55.2	31.5	59.4	6.5	12.3	10.5	19.8	11.5	21.6	15.4	29.0	10.6	20.0
G. paganellus	4	Faro	54.5	18.0	33.1	28.9	53.0	31.3	57.4	6.8	12.4	11.0	20.3	11.5	21.2	16.8	30.8	12.3	22.7

G. paganellus	5	Faro	52.6	16.9	32.1	27.6	52.4	30.0	57.0	6.4	12.2	10.8	20.5	10.4	19.7	17.0	32.3	11.8	22.5
G. paganellus	6	Faro	57.3	20.0	34.8	31.0	54.1	33.4	58.2	7.7	13.5	12.3	21.5	12.0	21.0	16.7	29.1	11.5	20.2
G. paganellus	7	Faro	53.1	18.3	34.4	29.0	54.7	31.3	59.0	6.8	12.9	11.0	20.6	10.4	19.5	15.5	29.2	10.7	20.2
G. paganellus	8	Faro	69.2	23.9	34.5	37.2	53.8	40.0	57.8	7.1	10.3	13.4	19.4	14.9	21.6	21.4	30.9	15.6	22.5
G. paganellus	9	Faro	63.7	22.4	35.1	33.9	53.3	36.7	57.6	7.9	12.3	13.5	21.2	12.8	20.2	19.3	30.2	14.3	22.4
G. paganellus	10	Faro	57.9	20.1	34.7	30.8	53.2	32.2	55.6	6.7	11.6	11.8	20.5	11.9	20.6	18.1	31.2	13.9	24.0
G. roulei	1	Selce	53.9	19.4	35.9	29.8	55.2	32.3	59.9	7.1	13.1	10.0	18.6	9.1	16.9	15.5	28.7	11.7	21.7
G. roulei	2	Selce	52.0	19.0	36.6	29.0	55.8	31.1	59.8	6.5	12.6	9.9	19.0	7.4	14.3	15.0	28.8	11.2	21.5
G. roulei	3	Selce	56.2	18.9	33.7	28.9	51.5	32.0	57.0	7.4	13.2	11.0	19.6	9.1	16.3	17.9	31.8	13.8	24.6
G. roulei	4	Selce	54.4	18.1	33.2	27.9	51.2	30.9	56.7	7.4	13.6	10.6	19.5	9.2	16.9	15.6	28.7	13.1	24.1
G. roulei	Gr1	Selce	57.5	19.7	34.3	30.8	53.6	32.7	56.9	7.1	12.4	10.8	18.8	7.8	13.6	17.6	30.6	13.9	24.2
G. roulei	Gr2	Selce	43.8	11.6	26.4	18.1	41.2	19.2	43.9	4.9	11.2	7.3	16.6	4.9	11.1	10.2	23.2	7.4	17.0
G. roulei	Gr3	Selce	49.9	13.2	26.5	19.5	39.1	22.9	45.8	5.7	11.4	9.0	18.1	7.8	15.6	10.9	21.9	7.4	14.8
G. roulei	Gr4	Selce	46.4	12.4	26.7	19.4	41.9	21.0	45.2	4.8	10.4	8.2	17.6	4.7	10.1	10.9	23.4	8.0	17.3
G. roulei	Gr5	Selce	49.5	16.6	33.5	26.5	53.4	28.1	56.7	6.1	12.4	9.9	20.0	8.1	16.3	15.7	31.8	12.2	24.7
G. roulei	Gr6	Selce	46.3	16.6	35.8	25.1	54.1	27.0	58.2	6.2	13.3	10.6	22.8	7.2	15.5	14.1	30.4	10.5	22.6
G. vittatus	1	Selce	19.6	7.1	36.3	10.6	54.1	11.3	57.7	3.4	17.5	4.3	21.9	3.6	18.3	5.1	25.9	3.7	18.8
G. vittatus	2	Selce	23.9	7.8	32.5	12.5	52.2	13.5	56.3	6.3	26.1	5.7	23.9	3.9	16.2	not clear		4.9	20.3
G. vittatus	3	Selce	17.3	6.4	36.6	9.3	53.7	9.8	56.7	3.4	19.4	4.2	24.3	3.6	20.6	4.2	24.3	3.0	17.4
G. vittatus	4	Selce	20.8	7.5	36.1	11.1	53.1	11.8	56.8	3.6	17.5	4.4	21.1	3.6	17.4	5.6	26.7	4.6	22.2
G. vittatus	5	Selce	39.3	14.2	36.1	22.1	56.3	23.5	59.7	6.3	16.1	8.0	20.4	8.4	21.4	10.0	25.5	7.4	18.9
G. vittatus	6	Selce	17.6	6.6	37.4	9.5	53.9	10.2	57.7	3.2	18.1	3.1	17.9	3.4	19.2	4.2	23.9	3.2	18.1
G. vittatus	7	Selce	38.2	13.5	35.4	20.6	54.0	22.1	57.9	6.1	15.9	8.3	21.6	7.7	20.2	10.3	27.0	7.6	19.9
G. vittatus	8	Selce	43.2	15.2	35.1	23.6	54.5	26.1	60.4	6.8	15.7	8.6	20.0	8.1	18.7	11.8	27.3	8.6	19.8
G. vittatus	9	Selce	41.9	14.5	34.5	22.0	52.5	25.1	59.8	6.8	16.1	9.1	21.6	7.4	17.5	10.6	25.2	8.1	19.3
G. vittatus	1	Krk	31.0	10.8	34.9	17.4	56.3	18.6	60.2	4.4	14.1	5.6	18.0	6.5	20.8	8.7	27.9	6.8	21.9
G. vittatus	2	Krk	38.1	13.0	34.2	21.1	55.5	22.5	59.2	6.4	16.7	7.8	20.4	7.3	19.2	10.2	26.8	7.7	20.1
P. kneri	1	Krk	21.6	8.1	37.6	11.9	55.2	12.4	57.3	5.2	23.9	5.1	23.4	2.9	13.5	4.1	18.8	4.4	20.3
P. kneri	2	Krk	23.3	8.8	37.7	13.3	57.0	13.5	58.0	5.0	21.3	6.0	25.5	3.4	14.5	4.5	19.5	3.7	16.0
P. kneri	3	Krk	26.3	9.6	36.3	14.9	56.8	15.0	57.1	5.8	22.2	6.8	25.6	4.4	16.5	4.8	18.1	4.2	16.1
P. kneri	4	Krk	27.0	10.4	38.6	15.3	56.7	15.6	57.7	5.7	21.1	7.5	27.6	4.0	15.0	4.9	18.2	4.1	15.3
P. kneri	5	Krk	25.2	9.5	37.8	14.1	56.1	14.7	58.2	5.6	22.4	6.5	25.8	3.4	13.3	4.6	18.4	4.0	15.7
P. kneri	6	Krk	26.2	10.1	38.5	15.1	57.7	15.3	58.4	5.6	21.3	7.1	27.0	3.7	14.3	4.7	17.8	3.9	15.0
P. kneri	7	Krk	23.1	8.6	37.1	13.1	56.8	13.1	56.7	5.5	23.9	6.2	26.8	3.3	14.2	3.8	16.3	3.8	16.5
P. kneri	8	Krk	26.0	9.3	35.7	14.4	55.3	14.7	56.4	6.1	23.4	6.9	26.6	3.7	14.2	4.8	18.6	4.3	16.7
P. kneri	9	Krk	25.7	9.4	36.5	14.4	55.8	14.6	56.6	6.2	24.0	6.9	26.8	3.8	14.9	4.1	15.7	4.1	15.9
P. kneri	10	Krk	23.3	8.3	35.6	12.8	54.9	13.0	55.7	5.6	24.1	6.3	27.1	3.4	14.5	4.4	18.8	3.8	16.1
P. marmoratus	1	Selce	25.0	9.6	38.6	14.8	59.1	15.5	62.1	4.7	18.8	5.9	23.7	3.8	15.1	4.3	17.3	3.7	14.9
P. marmoratus	2	Selce	24.8	9.9	39.9	14.7	59.2	15.5	62.4	4.9	19.8	5.6	22.8	4.1	16.6	4.1	16.4	3.8	15.3
P. marmoratus	3	Selce	30.6	11.2	36.7	17.3	56.6	17.3	56.6	6.5	21.1	8.4	27.5	4.6	15.1	5.5	17.9	4.4	14.3
P. marmoratus	4	Selce	32.8	12.3	37.5	19.1	58.3	19.0	57.9	6.5	19.7	8.7	26.6	5.5	16.8	5.9	18.0	5.1	15.4
P. marmoratus	5	Selce	26.7	10.7	39.9	15.9	59.6	16.1	60.4	5.8	21.5	6.7	24.9	4.9	18.5	4.3	16.2	3.8	14.3
P. marmoratus	6	Selce	26.7	9.7	36.3	15.4	57.7	15.5	58.2	5.4	20.0	6.6	24.7	4.3	16.1	4.9	18.2	4.3	15.9
P. marmoratus	7	Selce	37.1	13.4	36.2	20.9	56.4	21.1	56.9	7.1	19.2	9.4	25.3	5.5	14.8	7.4	19.9	6.8	18.3

P. marmoratus	2	Albania	28.6	9.5	33.2	15.9	55.5	16.2	56.7	6.6	22.9	7.9	27.7	4.1	14.2	4.8	16.9	4.4	15.5
P. marmoratus	3	Albania	26.2	8.8	33.6	14.5	55.5	14.6	55.9	5.9	?	7.1	27.3	3.8	14.6	4.6	17.6	4.3	16.3
P. microps	2	Stralsund	29.3	10.5	35.6	16.3	55.7	17.1	58.3	5.1	17.4	7.2	24.6	4.9	16.6	5.1	17.3	4.2	14.2
P. microps	3	Stralsund	28.9	11.0	38.1	16.7	57.8	17.1	59.1	4.6	16.0	7.8	26.9	5.0	17.3	4.4	15.3	3.9	13.4
P. microps	4	Stralsund	30.0	11.4	38.0	17.3	57.6	17.7	58.8	5.4	17.9	7.6	25.3	4.6	15.4	5.2	17.3	4.7	15.5
P. microps	5	Stralsund	27.8	10.5	37.6	15.8	56.7	16.5	59.4	4.8	17.2	7.4	26.6	4.8	17.4	4.5	16.1	3.8	13.6
P. microps	6	Stralsund	28.7	10.8	37.7	16.3	56.9	17.3	60.2	4.4	15.2	7.2	25.1	4.8	16.7	4.4	15.3	3.5	12.4
P. microps	7	Stralsund	28.6	10.3	35.9	15.6	54.5	16.3	57.2	4.4	15.4	7.8	27.3	5.2	18.3	4.0	14.1	3.9	13.5
P. microps	8	Stralsund	26.4	9.9	37.4	15.1	57.3	15.6	59.0	4.6	17.6	6.1	23.0	5.0	19.1	4.5	16.9	3.8	14.5
P. microps	9	Stralsund	27.4	11.2	41.0	16.7	61.1	17.1	62.5	4.7	17.3	6.1	22.2	5.1	18.6	4.3	15.6	4.2	15.5
P. microps	10	Stralsund	21.7	8.5	39.2	12.9	59.5	13.2	60.9	3.6	16.4	5.5	25.4	5.4	24.8	4.1	18.8	3.4	15.8
P. microps	11	Stralsund	29.4	10.6	35.9	16.3	55.6	17.3	58.9	5.2	17.5	7.6	25.9	3.6	12.1	5.2	17.6	4.5	15.4
P. microps	12	Stralsund	24.5	10.1	41.2	15.4	63.1	16.2	66.1	4.2	17.2	6.3	25.6	5.1	20.8	4.2	17.2	3.6	14.6
P. microps	13	Stralsund	27.5	10.6	38.3	16.1	58.3	16.5	60.0	4.6	16.6	6.3	22.8	4.2	15.3	4.2	15.4	3.7	13.5
P. microps	14	Stralsund	31.6	11.6	36.6	18.2	57.7	18.8	59.5	4.8	15.2	7.8	24.6	4.7	14.9	4.7	14.8	4.0	12.8
P. microps	15	Stralsund	27.6	10.6	38.5	15.8	57.0	16.6	60.2	4.3	15.7	6.5	23.4	4.9	17.6	4.9	17.6	3.9	14.2
P. microps	16	Stralsund	28.8	10.8	37.3	15.4	53.4	15.8	54.7	5.6	19.6	8.3	28.7	3.4	11.7	5.2	18.0	4.1	14.3
P. minutus	1	Stralsund	48.0	14.9	31.1	23.0	47.9	23.4	48.7	8.6	18.0	8.7	18.2	8.8	18.4	9.7	20.2	9.0	18.7
P. minutus	17	Stralsund	51.7	14.8	28.6	23.6	45.5	24.2	46.8	9.0	17.3	9.7	18.7	7.2	14.0	11.9	23.1	11.1	21.4
P. montenegrensis	1	Skadar Lake	21.5	8.5	39.3	12.6	58.5	12.9	59.9	5.1	23.5	6.1	28.2	3.6	16.9	3.1	14.2	2.5	11.8
P. montenegrensis	2	Skadar Lake	17.7	7.2	41.0	10.5	59.2	10.6	60.3	4.2	23.6	4.7	26.6	3.4	19.2	2.6	15.0	2.4	13.5
P. montenegrensis	3	Skadar Lake	21.3	8.2	38.6	12.1	57.0	12.6	59.2	5.3	24.8	6.1	28.8	3.5	16.6	2.7	12.6	2.7	12.6
P. montenegrensis	4	Skadar Lake	21.3	8.5	39.7	12.1	56.7	12.7	59.6	4.8	22.5	6.1	28.6	3.8	18.0	3.7	17.3	2.9	13.6
P. montenegrensis	5	Skadar Lake	19.2	7.4	38.7	10.8	56.2	11.3	58.7	5.2	26.9	5.9	30.6	3.5	18.1	2.6	13.3	1.9	10.0
P. montenegrensis	6	Skadar Lake	23.7	9.5	40.2	13.6	57.4	14.4	60.7	5.1	21.4	6.2	26.0	3.7	15.7	4.2	17.5	3.2	13.3
P. montenegrensis	7	Skadar Lake	10.3	8.1	78.9	11.6	112.8	12.3	119.9	5.2	50.6	5.6	54.7	3.3	32.4	2.7	26.7	2.5	24.1
P. montenegrensis	8	Skadar Lake	22.5	8.9	39.4	13.1	58.3	13.5	59.8	5.2	23.3	6.2	27.3	4.4	19.4	3.4	15.3	2.9	12.6
P. montenegrensis	9	Skadar Lake	22.9	9.1	40.0	13.3	58.3	14.0	61.4	5.1	22.4	5.9	25.9	4.1	17.9	3.7	16.2	2.9	12.9
P. montenegrensis	10	Skadar Lake	21.6	8.5	39.3	12.5	57.9	13.2	61.1	5.0	23.3	5.5	25.7	3.8	17.6	3.3	15.1	2.7	12.6
P. pictus	1	Norway	25.2	8.9	35.5	13.8	54.8	13.9	55.2	4.5	17.9	5.9	23.6	4.7	18.6	5.9	23.2	5.5	21.9
P. pictus	2	Norway	28.4	9.1	32.1	14.9	52.6	15.2	53.6	5.9	20.8	7.2	25.4	5.0	17.7	6.5	22.7	6.0	21.0
P. quagga	1	Krk	26.3	9.2	34.9	14.6	55.4	14.8	56.1	6.3	23.9	7.8	29.5	3.1	11.9	4.5	17.0	4.0	15.1
P. quagga	2	Krk	27.4	9.5	34.5	14.9	54.3	15.3	55.6	6.6	24.2	8.0	29.2	3.1	11.3	4.8	17.3	4.0	14.6
P. quagga	3	Krk	27.6	9.4	34.2	15.1	54.7	15.5	56.0	7.3	26.3	8.4	30.6	3.2	11.7	4.4	15.8	3.7	13.4
P. quagga	4	Krk	28.8	9.5	32.9	15.5	53.8	15.9	55.1	7.0	24.2	8.4	29.2	3.3	11.5	5.1	17.6	4.6	16.0
P. quagga	5	Krk	28.4	9.9	35.0	15.8	55.6	16.2	57.0	6.5	22.8	7.8	27.6	3.5	12.5	4.9	17.4	4.4	15.5
P. quagga	6	Krk	28.5	9.8	34.3	15.4	54.2	15.7	55.1	6.7	23.6	8.2	28.9	3.4	12.0	5.2	18.4	4.6	16.3
P. quagga	7	Krk	28.2	9.9	35.3	15.7	55.7	16.2	57.4	6.8	24.0	8.2	29.1	3.4	12.2	4.6	16.4	3.8	13.4
P. quagga	8	Krk	27.4	9.5	34.8	14.9	54.5	15.3	55.7	6.6	24.0	7.9	28.8	3.3	12.0	4.6	16.7	4.2	15.3
P. quagga	9	Krk	29.9	9.8	33.0	15.9	53.3	16.4	54.9	7.4	24.8	8.9	29.8	3.5	11.7	5.2	17.5	4.6	15.5
P. quagga	10	Krk	28.3	9.7	34.4	15.3	54.2	15.1	53.5	6.8	23.9	8.4	29.7	3.8	13.4	5.1	17.9	4.8	16.9

Table S5: Meristic counts of total, caudal, abdominal vertebrae (TotVert, CaudVert, AbVert), spines of first dorsal fin (D1), rays of second dorsal fin (D2), rays of anal fin (A), anal fin pterygiophores before first caudal vertebra (AP),and dorsal and ventral procurrent rays (DProCur, VProCur).

Species	specimen number	locality	TotVert	CaudVert	AbVert	D1 spine	D2rays	Arays	AP	DProCur	VProCur
Gobius auratus	1	Selce	29	18	11	6	14	14	3	10	9
Gobius auratus	2	Selce	28	17	11	6	14	12	3	10	8
Gobius auratus	3	Selce	28	17	11	6	14	13	3	8	8
Gobius auratus	4	Selce	28	17	11	6	14	13	3	9	8
Gobius auratus	5	Selce	28	17	11	6	14	13	3	9	9
Gobius auratus	6	Selce	28	17	11	6	14	13	3	8	8
Gobius auratus	7	Selce	28	17	11	6	13	12	2	9	9
Gobius auratus	8	Selce	28	17	11	6	14	13	3	9	8
Gobius auratus	9	Selce	28	17	11	6	13	12	3	8	6
Gobius auratus	1	Krk	28	17	11	6	14	13	3	10	11
Gobius auratus	2	Krk	28	17	11	6	14	14	3	9	9
Gobius bucchichi	1	Kraljevica	28	17	10	6	14	13	3	8	9
Gobius bucchichi	1	Selce	28	17	11	6	14	13	3	8	7
Gobius bucchichi	2	Selce	28	17	11	6	14	12	3	8	9
Gobius bucchichi	3	Selce	28	17	11	6	14	13	3	8	7
Gobius bucchichi	4	Selce	28	17	11	6	14	13	3	9	9
Gobius bucchichi	5	Selce	28	17	11	6	14	13	3	9	8
Gobius bucchichi	6	Selce	28	17	11	6	14	13	3	9	9
Gobius bucchichi	7	Selce	28	17	11	6	14	13	3	8	9
Gobius bucchichi	8	Selce	28	17	11	6	14	13	3	8	8
Gobius bucchichi	9	Selce	29	17	12	6	14	12	3	9	9
Gobius cobitis	1	Montenegro	28	17	11	6	14	12	2	8	10
Gobius cobitis	2	Montenegro	28	17	11	6	14	12	2	9	8
Gobius cobitis	3	Montenegro	28	17	11	6	14	11	2	?	8?
Gobius cobitis	4	Montenegro	28	17	11	6	14	13	2	9	10
Gobius cobitis	5	Montenegro	28	17	11	6	15	12	2	9	8
Gobius cobitis	6	Montenegro	28	17	11	6	14	12	2	8	8
Gobius cobitis	7	Montenegro	28	17	11	6	14	11	2	10	8
Gobius cobitis	8	Montenegro	28	17	11	6	14	12	2	10	10
Gobius cobitis	9	Montenegro	28	17	11	6	14	13	2	8	8
Gobius cobitis	10	Montenegro	28	17	11	6	14	12	2	8	10
Gobius couchi	1	Krk	28	17	11	6	13	12	3	9	9
Gobius couchi	2	Krk	28	17	11	6	13	12	3	8	8
Gobius couchi	3	Krk	28	17	11	6	13	12	3	9	10
Gobius couchi	4	Krk	28	18	10	6	13	11	3	8	9
Gobius cruentatus	1	Selce	28	17	11	6	14	13	3	11	11
Gobius cruentatus	2	Selce	28	17	11	6	14	12	3	12	12
Gobius cruentatus	3	Selce	28	17	11	6	14	13	3	11	11
Gobius cruentatus	4	Selce	28	17	11	6	14	13	3	9	10
Gobius cruentatus	5	Selce	28	17	11	6	14	13	3	11	12

Gobius cruentatus	6	Selce	28	17	11	6	14	13	3	11	11
Gobius cruentatus	7	Selce	28	17	11	6	14	13	3	11	11
Gobius cruentatus	8	Selce	28	17	11	6	14	13	3	11	11
Gobius cruentatus	9	Selce	28	17	11	6	14	13	3	12	12
Gobius cruentatus	1	Krk	28	17	11	6	14	13	3	12	11
Gobius cruentatus	2	Krk	28	17	11	6	14	13	3	11	10
Gobius fallax	1	Croatia	28	17	11	6	14	13	3	9	10
Gobius fallax	2	Croatia	28	17	11	6	14	14	3	9	12
Gobius gasteveni	1	Galicia	28	17	11	6	14	13	3	10	11
Gobius geniporus	1	Montenegro	28	17	11	6	13	12	3	?	?
Gobius geniporus	2	Montenegro	28	17	11	6	13	12	3	11	11
Gobius geniporus	3	Montenegro	28	17	11	6	13	12	3	9	12
Gobius geniporus	4	Montenegro	28	17	11	6	13	12	3	8?	10
Gobius geniporus	1	Selce	28	17	11	6	13	12	3	10	11
Gobius geniporus	2	Selce	28	17	11	6	13	12	3	?	?
Gobius geniporus	3	Selce	28	17	11	6	13	12	3	11	11
Gobius geniporus	small	Selce	28	17	11	5	12	12	?4	11	9
Gobius geniporus	medium	Selce	28	17	11	6	13	12	4	11	10
Gobius geniporus	large	Selce	28	17	11	6	13	13	3	11	10
Gobius incognitus	1904	Croatia	28	17	11	6	14	13	3	10	9
Gobius incognitus	1906	Croatia	28	17	11	6	14	13	3	8	10
Gobius incognitus	1907	Croatia	28	17	11	6	14	12	3	10	11
Gobius incognitus	1910	Croatia	28	17	11	6	14	13	3	9	8
Gobius incognitus	1911	Croatia	28	17	11	6	14	13	3	8	10
Gobius incognitus	1912	Croatia	28	17	11	6	13	12	3	?	10
Gobius incognitus	1913	Croatia	29	17	12	6	14	13	3	9	8
Gobius incognitus	1914	Croatia	28	17	11	6	14	13	3	9	10
Gobius incognitus	1915	Croatia	28	17	11	6	14	13	3	9	9
Gobius kolombatovici	1	Krk	28	17	11	6	14	13	2	12	11
Gobius kolombatovici	2	Krk	28	17	11	6	14	13	3	9	10
Gobius niger	1	Pilsey Island	28	17	11	6	12	11	3	9	8
Gobius niger	2	Pilsey Island	28	17	11	6	11	10	3	10	9
Gobius niger	3	Pilsey Island	28	17	11	6	12	11	3	9	9
Gobius niger	4	Pilsey Island	28	17	11	6	12	12	3	8	7
Gobius niger	5	Pilsey Island	27	17	10	5	12	10	3	9	10
Gobius niger	6	Pilsey Island	28	17	11	6	12	11	3	9	10
Gobius niger	7	Pilsey Island	28	17	11	6	11	11	3	?	10
Gobius paganellus	1	Faro	28	17	11	6	14	12	3	10	9
Gobius paganellus	2	Faro	28	17	11	6	13	12	3	10	10
Gobius paganellus	3	Faro	28	17	11	6	13	11	1	10	10
Gobius paganellus	4	Faro	28	17	11	6	14	12	3	10	10
Gobius paganellus	5	Faro	28	17	11	6	14	12	3	10	10
Gobius paganellus	6	Faro	28	17	11	6	13	11	3	9	9
Gobius paganellus	7	Faro	28	17	11	6	14	12	2	9	9

Gobius paganellus	8	Faro	28	17	11	6	14	12	2	10	9
Gobius paganellus	9	Faro	28	17	11	6	14	12	2	9	10
Gobius paganellus	10	Faro	28	17	11	6	14	12	2	10	10
Gobius roulei	1	Selce	28	17	11	6	12	12	3	8	8
Gobius roulei	2	Selce	28	17	11	6	13	12	3	9	9
Gobius roulei	3	Selce	28	17	11	6	13	12	3	10	9
Gobius roulei	4	Selce	28	17	11	6	12	12	3	9	10
Gobius roulei	Gr1	Selce	28	17	11	6	13	12	3	8	6
Gobius roulei	Gr2	Selce	28	17	11	6	13	12	3	8	8
Gobius roulei	Gr3	Selce	28	17	11	6	12	10	3	8	7
Gobius roulei	Gr4	Selce	28	17	11	6	13	12	3	9	9
Gobius roulei	Gr5	Selce	28	17	11	6	13	12	3	9	9
Gobius roulei	Gr6	Selce	28	17	11	6	13	12	3	8	8
Gobius vittatus	1	Selce	29	18	11	6	12	11	3	7?	7?
Gobius vittatus	2	Selce	28	17	11	6	9	11	3	7	7
Gobius vittatus	3	Selce	28	17	11	6	11	10	3	5?	6?
Gobius vittatus	4	Selce	28	17	11	6	11	10	3	8	8
Gobius vittatus	5	Selce	28	17	11	6	11	11	3	8	8
Gobius vittatus	6	Selce	28	17	11	6	11	9	3	7	7
Gobius vittatus	7	Selce	28	17	11	6	12	10	3	8	8
Gobius vittatus	8	Selce	28	18	10	6	12	11	3	7	8
Gobius vittatus	9	Selce	28	17	11	6	12	11	3	8	7
Gobius vittatus	1	Krk	28	17	11	6	12	11	3	7	5
Gobius vittatus	2	Krk	28	17	11	6	12	11	3	6	7
Pomatoschistus kneri	1	Krk	31	19	12	6	9	11	3	8	?
Pomatoschistus kneri	2	Krk	31	19	12	6	10	9	2	9	9
Pomatoschistus kneri	3	Krk	31	19	12	6	9	9	3	13	13
Pomatoschistus kneri	4	Krk	31	19	12	6	9	9	2	14	13
Pomatoschistus kneri	5	Krk	31	19	12	6	9	9	3	13	12
Pomatoschistus kneri	6	Krk	31	19	12	6	9	8	2	12	12
Pomatoschistus kneri	7	Krk	31	19	12	6	9	9	3	11	10
Pomatoschistus kneri	8	Krk	32	20	12	6	10	10	3	11	10
Pomatoschistus kneri	9	Krk	31	19	12	6	8	9	3	13	10
Pomatoschistus kneri	10	Krk	31	19	12	6	9	9	3	8	7
Pomatoschistus marmoratus	1	Selce	31	19	12	5	8	9	3	12	10
Pomatoschistus marmoratus	2	Selce	31	19	12	6	8	9	3	11	8
Pomatoschistus marmoratus	3	Selce	31	19	12	6	8	8	3	11	12
Pomatoschistus marmoratus	4	Selce	32	20	12	6	8	8	2	12	12
Pomatoschistus marmoratus	5	Selce	32	20	12	7	8	8	2	8	9
Pomatoschistus marmoratus	6	Selce	31	19	12	6	8	8	2	12	12
Pomatoschistus marmoratus	7	Selce	32	20	12	6	9	9	2	14	13
Pomatoschistus marmoratus	2	Albania	33	20	13	6	8	9	2	13	12
Pomatoschistus marmoratus	3	Albania	31	19	12	6	9	8	2	12	9
Pomatoschistus microps	2	Stralsund	31	20	11	6	9	8	1	11	11

Pomatoschistus microps	3	Stralsund	31	19	12	6	8	8	3	10?	10?
Pomatoschistus microps	4	Stralsund	31	20	11	6	9	9	1	11	11
Pomatoschistus microps	5	Stralsund	31	20	11	6	9	9	2	11	10
Pomatoschistus microps	6	Stralsund	31	20	11	6	8	8	2	11	10
Pomatoschistus microps	7	Stralsund	30	19	11	6	8	7	1	10	12
Pomatoschistus microps	8	Stralsund	30	19	11	6	8	8	2	10	9
Pomatoschistus microps	9	Stralsund	31	19	12	6	9	8	1	?	?
Pomatoschistus microps	10	Stralsund	30	19	11	6	9	9	2	10	9
Pomatoschistus microps	11	Stralsund	31	20	11	6	9	9	3	12	12
Pomatoschistus microps	12	Stralsund	31	20	11	6	8	8	1	10	10
Pomatoschistus microps	13	Stralsund	30	19	11	6	8	8	2	11	11
Pomatoschistus microps	14	Stralsund	31	20	11	6	8	8	2	11	10
Pomatoschistus microps	15	Stralsund	30	19	11	6	9	8	3	?	?
Pomatoschistus microps	16	Stralsund	30	19	11	6	8	8	2	11	12
Pomatoschistus minutus	1	Stralsund	33	21	12	6	11	11	2	12	13
Pomatoschistus minutus	17	Stralsund	33	20	13	6	11	11	3	13	13
Pomatoschistus montenegrensis	1	Skadar Lake	30	18	12	6	7	7	2	10	10
Pomatoschistus montenegrensis	2	Skadar Lake	29	17	12	6	7	7	2	?	8?
Pomatoschistus montenegrensis	3	Skadar Lake	30	18	12	6	7	7	2	9?	8
Pomatoschistus montenegrensis	4	Skadar Lake	30	18	12	6	9	8	2	9	9
Pomatoschistus montenegrensis	5	Skadar Lake	30	18	12	6	7	6	2	?	?
Pomatoschistus montenegrensis	6	Skadar Lake	30	18	12	6	9	8	2	9	9
Pomatoschistus montenegrensis	7	Skadar Lake	30	18	12	6	7	7	2	8	9
Pomatoschistus montenegrensis	8	Skadar Lake	30	18	12	6	8	8	2	10	10
Pomatoschistus montenegrensis	9	Skadar Lake	30	18	12	6	9	8	2	9	10
Pomatoschistus montenegrensis	10	Skadar Lake	30	18	12	6	9	8	2	9	?
Pomatoschistus pictus	1	Norway	30	19	11	6	9?	8?	2	?	?
Pomatoschistus pictus	2	Norway	30	19	11	6	8	9	2	9?	9?
Pomatoschistus quagga	1	Krk	33	20	13	6	9	9	3	12	13
Pomatoschistus quagga	2	Krk	34	21	13	6	9	9	3	14	13
Pomatoschistus quagga	3	Krk	33	20	13	7	8	8	2	12	11
Pomatoschistus quagga	4	Krk	33	20	13	6	9	9	3	13	12
Pomatoschistus quagga	5	Krk	33	20	13	5	9	9	3	13	12
Pomatoschistus quagga	6	Krk	33	20	13	6	9	9	3	13	12
Pomatoschistus quagga	7	Krk	33	20	13	6	9	8	3	12	12
Pomatoschistus quagga	8	Krk	33	20	13	6	9	9	3	13	11
Pomatoschistus quagga	9	Krk	34	21	13	6	9	9	2	13	11
Pomatoschistus quagga	10	Krk	33	20	13	6	9	10	3	13	12

Appendix for chapter 3

Table S1: RAW measurements of otoliths and their calculated indices. Locality: K = Kienberg, V = Vöslau; Type: REM = electron raster scan; Digi = digital image

Locality	Species	Type	Sample	TED	OSH	SuTip	SuEnd	SuL	SuH	OL	OH	SuTip/SuEnd	SuL/SuH	OL/OH	TED/OL	OSH/OH	OSH/TED
K	*Paromiogobius johnsonae*	REM	3_32	1.049	0.440	0.698	0.976	1.012	0.540	2.134	2.129	0.715	1.874	1.002	0.492	0.207	0.419
K	*Paromiogobius johnsonae*	REM	R_23	0.930	0.343	0.507	0.862	0.869	0.465	1.784	1.833	0.588	1.869	0.973	0.521	0.187	0.369
K	*Paromiogobius johnsonae*	REM	R_9	0.831	0.327	0.490	0.708	0.798	0.379	1.445	1.565	0.692	2.106	0.923	0.575	0.209	0.394
K	*Lesueurigobius harzhauseri*	REM	1_13	0.673	0.236	0.414	0.649	0.638	0.328	1.229	1.400	0.638	1.945	0.878	0.548	0.169	0.351
K	*Lesueurigobius harzhauseri*	REM	1_27	0.808	0.325	0.615	0.892	0.774	0.394	1.654	1.903	0.689	1.964	0.869	0.489	0.171	0.402
K	*Lesueurigobius harzhauseri*	REM	11_7	0.737	0.323	0.394	0.697	0.688	0.360	1.375	1.490	0.565	1.911	0.923	0.536	0.217	0.438
K	*Lesueurigobius harzhauseri*	REM	R_35	0.797	0.346	0.494	0.769	0.711	0.465	1.501	1.657	0.642	1.529	0.906	0.531	0.209	0.434
K	*Lesueurigobius harzhauseri*	REM	1_2	0.931	0.373	0.520	0.849	0.886	0.459	1.655	1.778	0.612	1.930	0.931	0.563	0.210	0.401
K	*Lesueurigobius harzhauseri*	REM	1_4	0.852	0.331	0.614	0.840	0.842	0.369	1.859	1.897	0.731	2.282	0.980	0.458	0.174	0.388
K	*Lesueurigobius harzhauseri*	REM	1_82	0.859	0.461	0.598	0.862	0.833	0.471	1.616	1.768	0.694	1.769	0.914	0.532	0.261	0.537
K	*Lesueurigobius harzhauseri*	REM	1_87	0.763	0.276	0.480	0.829	0.689	0.457	1.549	1.663	0.579	1.508	0.931	0.493	0.166	0.362
K	*Lesueurigobius harzhauseri*	REM	1_56	0.956	0.386	0.638	0.974	0.911	0.542	1.793	1.938	0.655	1.681	0.925	0.533	0.199	0.404
K	*Lesueurigobius harzhauseri*	REM	10_4	0.644	0.312	0.496	0.635	0.631	0.352	1.318	1.413	0.781	1.793	0.933	0.489	0.221	0.484
K	*Lesueurigobius harzhauseri*	REM	10_7	0.743	0.219	0.455	0.717	0.666	0.271	1.407	1.480	0.635	2.458	0.951	0.528	0.148	0.295
K	*Lesueurigobius harzhauseri*	REM	6_25	0.746	0.366	0.595	0.821	0.708	0.441	1.746	1.829	0.725	1.605	0.955	0.427	0.200	0.491
K	*Lesueurigobius harzhauseri*	REM	8_6	0.798	0.252	0.498	0.761	0.743	0.433	1.464	1.617	0.654	1.716	0.905	0.545	0.156	0.316
K	*Lesueurigobius harzhauseri*	REM	8_8	0.850	0.318	0.565	0.836	0.820	0.371	1.815	1.855	0.676	2.210	0.978	0.468	0.171	0.374
K	*Lesueurigobius magniiugis*	REM	11_14	0.853	0.339	0.578	0.850	0.818	0.467	1.721	1.923	0.680	1.752	0.895	0.496	0.176	0.397
K	*Lesueurigobius magniiugis*	REM	11_22	0.859	0.351	0.527	0.799	0.810	0.440	1.637	1.776	0.660	1.841	0.922	0.525	0.198	0.409
K	*Lesueurigobius magniiugis*	REM	14_1	0.934	0.310	0.613	0.982	0.847	0.532	1.879	2.076	0.624	1.592	0.905	0.497	0.149	0.332
K	*Lesueurigobius magniiugis*	REM	14_2	0.884	0.318	0.505	0.839	0.801	0.475	1.779	1.858	0.602	1.686	0.957	0.497	0.171	0.360
K	*Lesueurigobius magniiugis*	REM	3_28	0.923	0.366	0.493	0.867	0.871	0.449	1.744	1.851	0.569	1.940	0.942	0.529	0.198	0.397
K	*Lesueurigobius brzobohatyi*	REM	1_25	0.841	0.307	0.511	0.742	0.816	0.326	1.632	1.768	0.689	2.503	0.923	0.515	0.174	0.365
K	*Lesueurigobius brzobohatyi*	REM	1_70	0.797	0.317	0.574	0.903	0.742	0.459	1.596	1.845	0.636	1.617	0.865	0.499	0.172	0.398
K	*Lesueurigobius brzobohatyi*	REM	3_24	0.867	0.290	0.543	0.897	0.805	0.425	1.706	1.901	0.605	1.894	0.897	0.508	0.153	0.334
K	*Thorogobius iucundus*	REM	12_1	0.786	0.421	0.571	0.677	0.777	0.414	2.020	1.661	0.843	1.877	1.216	0.389	0.253	0.536
K	*Thorogobius iucundus*	REM	3_7	0.844	0.448	0.673	0.832	0.827	0.456	2.128	1.799	0.809	1.814	1.183	0.397	0.249	0.531
K	*Thorogobius iucundus*	REM	4_31	0.694	0.341	0.445	0.622	0.666	0.334	1.696	1.388	0.715	1.994	1.222	0.409	0.246	0.491
K	*Thorogobius iucundus*	REM	4_56	0.823	0.396	0.568	0.633	0.813	0.391	1.958	1.563	0.897	2.079	1.253	0.420	0.253	0.481
K	*Thorogobius iucundus*	REM	4_6	0.771	0.356	0.485	0.585	0.762	0.337	1.624	1.315	0.829	2.261	1.235	0.475	0.271	0.462
K	*Thorogobius iucundus*	REM	4_8	1.242	0.617	0.669	0.922	1.233	0.610	2.510	2.132	0.726	2.021	1.177	0.495	0.289	0.497
K	*Thorogobius iucundus*	REM	4_33	0.758	0.374	0.536	0.650	0.747	0.376	1.667	1.489	0.825	1.987	1.120	0.455	0.251	0.493

K	*Thorogobius iucundus*	REM	4_38	0.539	0.185	0.450	0.639	0.505	0.267	1.439	1.343	0.704	1.891	1.071	0.375	0.138	0.343
K	*Gobius mustus*	REM	5_1	0.990	0.436	0.469	0.755	0.951	0.439	1.862	1.607	0.621	2.166	1.159	0.532	0.271	0.440
K	*Gobius mustus*	REM	5_3	0.792	0.305	0.440	0.581	0.782	0.316	1.450	1.198	0.757	2.475	1.210	0.546	0.255	0.385
K	*Gobius mustus*	REM	5_6	1.590	0.660	0.700	0.977	1.545	0.688	2.616	2.264	0.716	2.246	1.155	0.608	0.292	0.415
K	*Lesueurigobius annajanae*	REM	9_1	1.153	0.554	0.844	1.168	1.077	0.724	2.409	2.455	0.723	1.488	0.981	0.479	0.226	0.480
K	*Lesueurigobius annajanae*	REM	Gv_8	0.845	0.281	0.535	0.838	0.767	0.446	1.611	1.802	0.638	1.720	0.894	0.525	0.156	0.333
K	*Lesueurigobius annajanae*	REM	R_4	0.987	0.429	0.518	0.815	0.925	0.571	1.657	1.868	0.636	1.620	0.887	0.596	0.230	0.435
V	*Lesueurigobius harzhauseri*	REM	7_7	0.950	0.365	0.675	0.901	0.934	0.426	2.037	1.901	0.749	2.192	1.072	0.466	0.192	0.384
V	*Lesueurigobius harzhauseri*	REM	7_10	0.959	0.419	0.695	1.002	0.949	0.533	1.963	2.222	0.694	1.780	0.883	0.489	0.189	0.437
V	*Lesueurigobius harzhauseri*	REM	7_12	0.975	0.439	0.797	0.894	0.958	0.501	1.973	2.123	0.891	1.912	0.929	0.494	0.207	0.450
V	*Lesueurigobius harzhauseri*	REM	5_6	1.052	0.402	0.836	1.044	1.001	0.505	2.300	2.423	0.801	1.982	0.949	0.457	0.166	0.382
V	*Lesueurigobius harzhauseri*	REM	5_8	1.345	0.687	0.857	1.109	1.319	0.676	2.632	2.641	0.773	1.951	0.997	0.511	0.260	0.511
V	*Lesueurigobius magniiugis*	REM	1_20	0.844	0.373	0.614	0.915	0.780	0.438	1.709	1.979	0.671	1.781	0.864	0.494	0.188	0.442
V	*Lesueurigobius magniiugis*	REM	1_72	0.849	0.392	0.651	0.938	0.809	0.462	1.835	2.128	0.694	1.751	0.862	0.463	0.184	0.462
V	*Lesueurigobius magniiugis*	REM	3_4	0.998	0.408	0.620	0.886	0.951	0.504	1.975	2.035	0.700	1.887	0.971	0.505	0.200	0.409
V	*Lesueurigobius magniiugis*	REM	4_5	0.944	0.393	0.596	0.826	0.914	0.422	1.801	1.882	0.722	2.166	0.957	0.524	0.209	0.416
V	*Lesueurigobius magniiugis*	REM	1_81	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
V	*Lesueurigobius magniiugis*	REM	1_82	0.846	0.300	0.675	0.890	0.799	0.403	1.792	1.954	0.758	1.983	0.917	0.472	0.154	0.355
V	*Lesueurigobius magniiugis*	REM	8_6	0.833	0.405	0.745	0.974	0.791	0.522	1.929	2.123	0.765	1.515	0.909	0.432	0.191	0.486
V	*Lesueurigobius magniiugis*	REM	1_71	0.762	0.297	0.635	0.920	0.729	0.410	1.784	1.982	0.690	1.778	0.900	0.427	0.150	0.390
V	*Lesueurigobius magniiugis*	REM	1_2	0.811	0.374	0.650	0.900	0.757	0.441	1.789	1.976	0.722	1.717	0.905	0.453	0.189	0.461
V	*Lesueurigobius magniiugis*	REM	1_4	0.804	0.335	0.670	0.874	0.787	0.406	1.827	1.958	0.767	1.938	0.933	0.440	0.171	0.417
V	*Lesueurigobius magniiugis*	REM	7_6	0.956	0.471	0.741	1.038	0.913	0.520	2.045	2.262	0.714	1.756	0.904	0.467	0.208	0.493
V	*Lesueurigobius magniiugis*	REM	9_1	0.790	0.301	0.500	0.746	0.747	0.394	1.515	1.616	0.670	1.896	0.938	0.521	0.186	0.381
V	*Lesueurigobius magniiugis*	REM	9_13	0.680	0.290	0.514	0.763	0.639	0.344	1.404	1.532	0.674	1.858	0.916	0.484	0.189	0.426
V	*Lesueurigobius magniiugis*	REM	9_9	0.827	0.376	0.605	0.928	0.787	0.446	1.809	1.980	0.652	1.765	0.914	0.457	0.190	0.455
K	*Lesueurigobius harzhauseri*	Digi	1_40	1.084	0.413	0.613	0.930	1.017	0.546	1.795	2.053	0.659	1.863	0.874	0.604	0.201	0.381
K	*Lesueurigobius harzhauseri*	Digi	1_44	0.809	0.268	0.551	0.802	0.766	0.385	1.594	1.753	0.687	1.990	0.909	0.508	0.153	0.331
K	*Lesueurigobius harzhauseri*	Digi	10_20	0.948	0.474	0.569	0.845	0.900	0.465	1.621	1.817	0.673	1.935	0.892	0.585	0.261	0.500
K	*Lesueurigobius harzhauseri*	Digi	10_6	0.701	0.288	0.473	0.704	0.678	0.328	1.255	1.474	0.672	2.067	0.851	0.559	0.195	0.411
K	*Lesueurigobius harzhauseri*	Digi	10_9	0.784	0.288	0.483	0.781	0.686	0.496	1.498	1.716	0.618	1.383	0.873	0.523	0.168	0.367
K	*Lesueurigobius harzhauseri*	Digi	1_68	0.796	0.342	0.467	0.711	0.732	0.447	1.406	1.642	0.657	1.638	0.856	0.566	0.208	0.430
K	*Lesueurigobius harzhauseri*	Digi	6_13	0.805	0.358	0.453	0.693	0.779	0.395	1.366	1.484	0.654	1.972	0.920	0.589	0.241	0.445
K	*Lesueurigobius harzhauseri*	Digi	6_16	0.775	0.403	0.475	0.728	0.724	0.415	1.422	1.605	0.652	1.745	0.886	0.545	0.251	0.520
K	*Lesueurigobius harzhauseri*	Digi	R_41	0.752	0.384	0.649	0.868	0.721	0.426	1.687	1.846	0.748	1.692	0.914	0.446	0.208	0.511
K	*Lesueurigobius harzhauseri*	Digi	11_26	0.908	0.318	0.585	0.898	0.861	0.381	1.775	1.983	0.651	2.260	0.895	0.512	0.160	0.350
K	*Paromiogobius johnsonae*	Digi	11_20	0.722	0.311	0.478	0.724	0.698	0.385	1.585	1.615	0.660	1.813	0.981	0.456	0.193	0.431
K	*Paromiogobius johnsonae*	Digi	3_16	0.750	0.317	0.442	0.734	0.688	0.425	1.488	1.508	0.602	1.619	0.987	0.504	0.210	0.423
K	*Lesueurigobius harzhauseri*	Digi	10_5	0.996	0.442	0.523	0.929	0.890	0.551	1.714	1.887	0.563	1.615	0.908	0.581	0.234	0.444

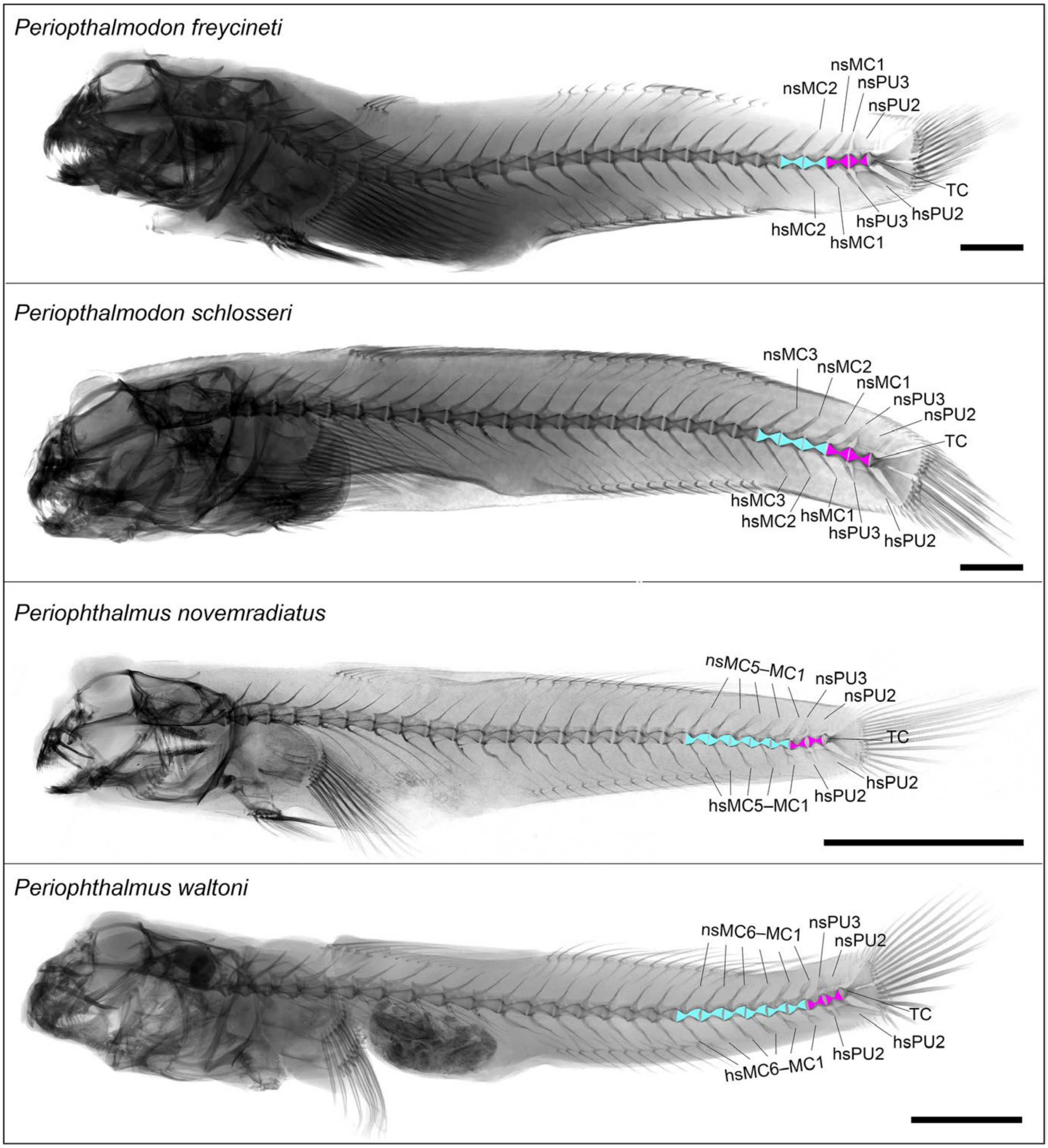
K	*Lesueurigobius harzhauseri*	Digi	1_10	0.770	0.370	0.607	0.813	0.734	0.435	1.608	1.814	0.747	1.687	0.886	0.479	0.204	0.481
K	*Lesueurigobius harzhauseri*	Digi	1_12	0.768	0.309	0.479	0.757	0.734	0.348	1.533	1.638	0.633	2.109	0.936	0.501	0.189	0.402
K	*Lesueurigobius harzhauseri*	Digi	1_16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Lesueurigobius harzhauseri*	Digi	1_18	0.859	0.394	0.568	0.839	0.821	0.428	1.582	1.763	0.677	1.918	0.897	0.543	0.223	0.459
K	*Lesueurigobius harzhauseri*	Digi	1_20	0.856	0.354	0.512	0.819	0.795	0.452	1.665	1.791	0.625	1.759	0.930	0.514	0.198	0.414
K	*Lesueurigobius harzhauseri*	Digi	1_3	0.932	0.403	0.520	0.882	0.866	0.482	1.715	1.837	0.590	1.797	0.934	0.543	0.219	0.432
K	*Lesueurigobius harzhauseri*	Digi	1_38	0.799	0.295	0.498	0.732	0.772	0.341	1.523	1.614	0.680	2.264	0.944	0.525	0.183	0.369
K	*Lesueurigobius harzhauseri*	Digi	1_42	1.089	0.440	0.553	0.832	1.033	0.555	1.785	1.941	0.665	1.861	0.920	0.610	0.227	0.404
K	*Lesueurigobius harzhauseri*	Digi	1_49	1.045	0.519	0.495	0.978	0.920	0.647	1.798	2.025	0.506	1.422	0.888	0.581	0.256	0.497
K	*Lesueurigobius harzhauseri*	Digi	1_5	0.934	0.307	0.670	0.982	0.843	0.534	1.805	2.108	0.682	1.579	0.856	0.517	0.146	0.329
K	*Lesueurigobius harzhauseri*	Digi	1_6	0.849	0.307	0.534	0.849	0.780	0.470	1.576	1.785	0.629	1.660	0.883	0.539	0.172	0.362
K	*Lesueurigobius harzhauseri*	Digi	1_7	0.738	0.319	0.582	0.767	0.702	0.421	1.613	1.732	0.759	1.667	0.931	0.458	0.184	0.432
K	*Lesueurigobius harzhauseri*	Digi	1_74	0.561	0.223	0.493	0.669	0.526	0.297	1.291	1.476	0.737	1.771	0.875	0.435	0.151	0.398
K	*Lesueurigobius harzhauseri*	Digi	1_76	0.669	0.338	0.544	0.814	0.610	0.431	1.590	1.679	0.668	1.415	0.947	0.421	0.201	0.505
K	*Lesueurigobius harzhauseri*	Digi	1_77	0.768	0.326	0.544	0.833	0.688	0.457	1.594	1.785	0.653	1.505	0.893	0.482	0.183	0.424
K	*Lesueurigobius harzhauseri*	Digi	1_8	0.945	0.342	0.645	0.772	0.929	0.370	1.722	1.838	0.835	2.511	0.937	0.549	0.186	0.362
K	*Lesueurigobius harzhauseri*	Digi	11_1	0.839	0.400	0.546	0.813	0.800	0.438	1.652	1.733	0.672	1.826	0.953	0.508	0.231	0.477
K	*Lesueurigobius harzhauseri*	Digi	11_13	0.847	0.352	0.611	0.897	0.806	0.399	1.757	1.935	0.681	2.020	0.908	0.482	0.182	0.416
K	*Lesueurigobius harzhauseri*	Digi	2_17	0.765	0.330	0.531	0.774	0.735	0.415	1.516	1.618	0.686	1.771	0.937	0.505	0.204	0.431
K	*Lesueurigobius harzhauseri*	Digi	9_11	0.748	0.323	0.543	0.826	0.723	0.387	1.610	1.748	0.657	1.868	0.921	0.465	0.185	0.432
K	*Lesueurigobius harzhauseri*	Digi	Gv_10	0.684	0.278	0.449	0.673	0.655	0.365	1.311	1.401	0.667	1.795	0.936	0.522	0.198	0.406
K	*Lesueurigobius harzhauseri*	Digi	Gv_11	0.918	0.384	0.661	0.939	0.862	0.500	1.853	1.990	0.704	1.724	0.931	0.495	0.193	0.418
K	*Lesueurigobius harzhauseri*	Digi	Gv_12	0.817	0.298	0.460	0.776	0.752	0.435	1.550	1.671	0.593	1.729	0.928	0.527	0.178	0.365
K	*Lesueurigobius harzhauseri*	Digi	Gv_6	0.741	0.253	0.536	0.799	0.700	0.346	1.606	1.677	0.671	2.023	0.958	0.461	0.151	0.341
K	*Lesueurigobius harzhauseri*	Digi	Gv_7	0.626	0.252	0.465	0.700	0.593	0.305	1.421	1.462	0.664	1.944	0.972	0.441	0.172	0.403
K	*Lesueurigobius harzhauseri*	Digi	R_32	0.755	0.269	0.519	0.750	0.708	0.379	1.468	1.570	0.692	1.868	0.935	0.514	0.171	0.356
K	*Lesueurigobius harzhauseri*	Digi	R_39	0.704	0.306	0.547	0.789	0.655	0.391	1.406	1.619	0.693	1.675	0.868	0.501	0.189	0.435
K	*Lesueurigobius harzhauseri*	Digi	9_7	0.766	0.250	0.526	0.773	0.733	0.355	1.662	1.680	0.680	2.065	0.989	0.461	0.149	0.326
K	*Lesueurigobius harzhauseri*	Digi	1_34	0.834	0.282	0.523	0.797	0.771	0.429	1.547	1.687	0.656	1.797	0.917	0.539	0.167	0.338
K	*Lesueurigobius harzhauseri*	Digi	1_71	0.859	0.330	0.557	0.795	0.817	0.414	1.635	1.790	0.701	1.973	0.913	0.525	0.184	0.384
K	*Lesueurigobius harzhauseri*	Digi	1_84	0.806	0.366	0.501	0.810	0.755	0.547	1.571	1.747	0.619	1.380	0.899	0.513	0.210	0.454
K	*Lesueurigobius harzhauseri*	Digi	1_9	0.950	0.371	0.625	0.920	0.892	0.495	1.971	2.012	0.679	1.802	0.980	0.482	0.184	0.391
K	*Lesueurigobius harzhauseri*	Digi	10_26	0.976	0.430	0.581	0.847	0.917	0.500	1.718	1.901	0.686	1.834	0.904	0.568	0.226	0.441
K	*Lesueurigobius harzhauseri*	Digi	1_1	1.002	0.426	0.546	0.859	0.967	0.453	1.824	1.888	0.636	2.135	0.966	0.549	0.226	0.425
K	*Lesueurigobius harzhauseri*	Digi	1_30	0.877	0.414	0.677	0.867	0.871	0.462	1.820	2.071	0.781	1.885	0.879	0.482	0.200	0.472
K	*Lesueurigobius harzhauseri*	Digi	1_33	0.810	0.382	0.587	0.856	0.756	0.427	1.612	1.722	0.686	1.770	0.936	0.502	0.222	0.472
K	*Lesueurigobius harzhauseri*	Digi	1_57	0.884	0.357	0.598	0.862	0.855	0.404	1.850	1.892	0.694	2.116	0.978	0.478	0.189	0.404
K	*Lesueurigobius harzhauseri*	Digi	1_93	0.698	0.219	0.503	0.715	0.648	0.354	1.422	1.470	0.703	1.831	0.967	0.491	0.149	0.314
K	*Lesueurigobius harzhauseri*	Digi	10_15	0.826	0.433	0.506	0.734	0.790	0.444	1.437	1.588	0.689	1.779	0.905	0.575	0.273	0.524

K	*Lesueurigobius harzhauseri*	Digi	10_23	0.761	0.318	0.582	0.780	0.736	0.371	1.568	1.729	0.746	1.984	0.907	0.485	0.184	0.418
K	*Lesueurigobius harzhauseri*	Digi	13_11	0.842	0.346	0.561	0.836	0.793	0.399	1.653	1.776	0.671	1.987	0.931	0.509	0.195	0.411
K	*Lesueurigobius harzhauseri*	Digi	13_4	0.753	0.283	0.503	0.606	0.745	0.311	1.340	1.298	0.830	2.395	1.032	0.562	0.218	0.376
K	*Lesueurigobius harzhauseri*	Digi	8_24	0.759	0.361	0.582	0.742	0.688	0.361	1.538	1.646	0.784	1.906	0.934	0.493	0.219	0.476
K	*Lesueurigobius harzhauseri*	Digi	Gv_1	0.648	0.301	0.454	0.637	0.623	0.317	1.265	1.324	0.713	1.965	0.955	0.512	0.227	0.465
K	*Lesueurigobius harzhauseri*	Digi	R_16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Lesueurigobius magniiugis*	Digi	11_16	0.970	0.371	0.926	0.690	0.926	0.491	1.919	1.949	1.342	1.886	0.985	0.505	0.190	0.382
K	*Lesueurigobius magniiugis*	Digi	2_15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Lesueurigobius magniiugis*	Digi	11_5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Lesueurigobius magniiugis*	Digi	14_5	0.811	0.311	0.546	0.853	0.741	0.452	1.786	1.843	0.640	1.639	0.969	0.454	0.169	0.383
K	*Lesueurigobius magniiugis*	Digi	3_17	0.811	0.292	0.555	0.784	0.754	0.408	1.656	1.724	0.708	1.848	0.961	0.490	0.169	0.360
K	*Lesueurigobius brzobohatyi*	Digi	1_14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Lesueurigobius brzobohatyi*	Digi	1_15	0.981	0.371	0.570	0.946	0.909	0.467	1.893	2.047	0.603	1.946	0.925	0.518	0.181	0.378
K	*Lesueurigobius brzobohatyi*	Digi	1_19	0.806	0.282	0.597	0.883	0.744	0.409	1.794	1.870	0.676	1.819	0.959	0.449	0.151	0.350
K	*Lesueurigobius brzobohatyi*	Digi	1_32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Lesueurigobius brzobohatyi*	Digi	1_58	0.782	0.310	0.571	0.867	0.731	0.422	1.728	1.860	0.659	1.732	0.929	0.453	0.167	0.396
K	*Lesueurigobius brzobohatyi*	Digi	1_69	0.880	0.461	0.609	0.871	0.839	0.438	1.709	1.894	0.699	1.916	0.902	0.515	0.243	0.524
K	*Lesueurigobius brzobohatyi*	Digi	1_89	0.662	0.272	0.560	0.703	0.600	0.371	1.482	1.556	0.797	1.617	0.952	0.447	0.175	0.411
K	*Lesueurigobius brzobohatyi*	Digi	11_6	0.839	0.326	0.537	0.868	0.775	0.445	1.767	1.867	0.619	1.742	0.946	0.475	0.175	0.389
K	*Thorogobius iucundus*	Digi	2_3	0.818	0.318	0.508	0.612	0.795	0.329	1.664	1.339	0.830	2.416	1.243	0.492	0.237	0.389
K	*Thorogobius iucundus*	Digi	4_10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Thorogobius iucundus*	Digi	4_11	0.730	0.351	0.531	0.656	0.714	0.341	1.749	1.469	0.809	2.094	1.191	0.417	0.239	0.481
K	*Thorogobius iucundus*	Digi	4_13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Thorogobius iucundus*	Digi	4_18	1.124	0.506	0.682	0.871	1.110	0.524	2.373	1.923	0.783	2.118	1.234	0.474	0.263	0.450
K	*Thorogobius iucundus*	Digi	4_21	0.597	0.308	0.452	0.591	0.578	0.344	1.433	1.233	0.765	1.680	1.162	0.417	0.250	0.516
K	*Thorogobius iucundus*	Digi	4_22	1.309	0.712	0.695	0.902	1.258	0.670	2.427	1.248	0.771	1.878	1.945	0.539	0.571	0.544
K	*Thorogobius iucundus*	Digi	4_24	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Thorogobius iucundus*	Digi	4_26	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Thorogobius iucundus*	Digi	4_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Thorogobius iucundus*	Digi	4_30	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Thorogobius iucundus*	Digi	4_34	0.829	0.407	0.583	0.697	0.817	0.398	1.876	1.557	0.836	2.053	1.205	0.442	0.261	0.491
K	*Thorogobius iucundus*	Digi	4_35	0.875	0.368	0.577	0.763	0.852	0.439	1.898	1.646	0.756	1.941	1.153	0.461	0.224	0.421
K	*Thorogobius iucundus*	Digi	4_37	0.877	0.430	0.589	0.666	0.867	0.426	1.969	1.552	0.884	2.035	1.269	0.445	0.277	0.490
K	*Thorogobius iucundus*	Digi	4_40	1.072	0.604	0.617	0.863	1.009	0.583	2.300	1.852	0.715	1.731	1.242	0.466	0.326	0.563
K	*Thorogobius iucundus*	Digi	4_41	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Thorogobius iucundus*	Digi	4_42	0.986	0.479	0.531	0.767	0.966	0.440	2.152	1.774	0.692	2.195	1.213	0.458	0.270	0.486
K	*Thorogobius iucundus*	Digi	4_43	0.968	0.424	0.579	0.772	0.941	0.440	1.959	1.699	0.750	2.139	1.153	0.494	0.250	0.438
K	*Thorogobius iucundus*	Digi	4_48	0.975	0.449	0.613	0.785	0.967	0.455	2.385	1.794	0.781	2.125	1.329	0.409	0.250	0.461
K	*Thorogobius iucundus*	Digi	4_49	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

K	*Thorogobius iucundus*	Digi	4_55	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Thorogobius iucundus*	Digi	4_9	1.097	0.531	0.695	0.889	1.055	0.525	2.244	1.926	0.782	2.010	1.165	0.489	0.276	0.484
K	*Thorogobius iucundus*	Digi	R_17	0.972	0.511	0.695	0.889	0.947	0.585	2.434	2.065	0.782	1.619	1.179	0.399	0.247	0.526
K	*Gobius mustus*	Digi	5_2	1.590	0.615	0.716	1.047	1.546	0.660	3.047	2.343	0.684	2.342	1.300	0.522	0.262	0.387
K	*Gobius mustus*	Digi	5_4	0.827	0.348	0.480	0.722	0.782	0.344	1.702	1.451	0.665	2.273	1.173	0.486	0.240	0.421
K	*Gobius mustus*	Digi	5_5	1.245	0.471	0.573	1.063	1.179	0.557	2.135	1.766	0.539	2.117	1.209	0.583	0.267	0.378
K	*Lesueurigobius annajanae*	Digi	1_91	0.756	0.288	0.455	0.677	0.713	0.360	1.404	1.556	0.672	1.981	0.902	0.538	0.185	0.381
K	*Lesueurigobius annajanae*	Digi	9_2	0.721	0.355	0.564	0.807	0.664	0.423	1.589	1.640	0.699	1.570	0.969	0.454	0.216	0.492
K	*Lesueurigobius annajanae*	Digi	9_6	1.220	0.555	0.710	1.070	1.147	0.622	2.432	2.510	0.664	1.844	0.969	0.502	0.221	0.455
K	*Lesueurigobius annajanae*	Digi	Gv_3	0.620	0.251	0.699	0.494	0.571	0.367	1.386	1.576	1.415	1.556	0.879	0.447	0.159	0.405
K	*Lesueurigobius annajanae*	Digi	R_27	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
K	*Lesueurigobius annajanae*	Digi	R_31	1.538	0.698	0.816	1.203	1.466	0.793	2.774	2.838	0.678	1.849	0.977	0.554	0.246	0.454
V	*Lesueurigobius annajanae*	Digi	Fuchs_32_2	0.901	0.345	0.637	0.846	0.868	0.367	2.030	2.043	0.753	2.365	0.994	0.444	0.169	0.383
V	*Lesueurigobius annajanae*	Digi	Fuchs_32_3	0.991	0.420	0.656	0.935	0.951	0.509	2.070	2.062	0.702	1.868	1.004	0.479	0.204	0.424
V	*Lesueurigobius annajanae*	Digi	Fuchs_33_10	0.901	0.421	0.688	0.873	0.853	0.483	1.850	2.026	0.788	1.766	0.913	0.487	0.208	0.467
V	*Lesueurigobius annajanae*	Digi	Fuchs_32_1	0.848	0.364	0.838	1.021	0.824	0.405	2.111	2.201	0.821	2.035	0.959	0.402	0.165	0.429
V	*Lesueurigobius harzhauseri*	Digi	7_4	1.039	0.459	0.782	1.044	0.986	0.507	2.263	2.355	0.749	1.945	0.961	0.459	0.195	0.442
V	*Lesueurigobius harzhauseri*	Digi	Fuchs_32_5	0.766	0.359	0.628	0.873	0.722	0.384	1.772	1.788	0.719	1.880	0.991	0.432	0.201	0.469
V	*Lesueurigobius harzhauseri*	Digi	Fuchs_33_11	0.791	0.314	0.637	0.859	0.778	0.352	1.846	1.851	0.742	2.210	0.997	0.428	0.170	0.397
V	*Lesueurigobius harzhauseri*	Digi	Fuchs_33_3	0.847	0.411	0.647	0.898	0.801	0.454	1.817	1.897	0.720	1.764	0.958	0.466	0.217	0.485
V	*Lesueurigobius harzhauseri*	Digi	Fuchs_33_5	1.026	0.493	0.744	1.021	0.980	0.523	2.211	2.320	0.729	1.874	0.953	0.464	0.213	0.481
V	*Lesueurigobius harzhauseri*	Digi	Fuchs_33_7	0.993	0.383	0.700	0.970	0.939	0.493	2.137	2.240	0.722	1.905	0.954	0.465	0.171	0.386
V	*Lesueurigobius harzhauseri*	Digi	Fuchs_33_9	1.110	0.466	0.817	1.116	1.074	0.528	2.423	2.485	0.732	2.034	0.975	0.458	0.188	0.420
V	*Lesueurigobius harzhauseri*	Digi	7_14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
V	*Lesueurigobius harzhauseri*	Digi	5_11	0.871	0.336	0.652	0.867	0.833	0.422	1.946	2.053	0.752	1.974	0.948	0.448	0.164	0.386
V	*Lesueurigobius magniiugis*	Digi	Fuchs_33_6	0.889	0.344	0.587	0.836	0.844	0.382	1.784	1.904	0.702	2.209	0.937	0.498	0.181	0.387
V	*Lesueurigobius magniiugis*	Digi	1_16	0.717	0.317	0.574	0.751	0.672	0.347	1.474	1.634	0.764	1.937	0.902	0.486	0.194	0.442
V	*Lesueurigobius magniiugis*	Digi	1_32	1.046	0.398	0.635	0.985	0.959	0.552	2.073	2.199	0.645	1.737	0.943	0.505	0.181	0.380
V	*Lesueurigobius magniiugis*	Digi	1_33	1.154	0.543	0.871	1.117	1.122	0.615	2.603	2.572	0.780	1.824	1.012	0.443	0.211	0.471
V	*Lesueurigobius magniiugis*	Digi	Fuchs_33_1	0.823	0.389	0.546	0.871	0.750	0.443	1.600	1.723	0.627	1.693	0.929	0.514	0.226	0.473
V	*Lesueurigobius magniiugis*	Digi	1_49	0.917	0.407	0.643	0.948	0.822	0.523	1.751	1.945	0.678	1.572	0.900	0.524	0.209	0.444
V	*Lesueurigobius magniiugis*	Digi	1_7	0.856	0.331	0.703	0.916	0.805	0.419	1.842	2.002	0.767	1.921	0.920	0.465	0.165	0.387
V	*Lesueurigobius magniiugis*	Digi	Fuchs_33_2	0.800	0.321	0.545	0.808	0.747	0.397	1.692	1.845	0.675	1.882	0.917	0.473	0.174	0.401
V	*Lesueurigobius magniiugis*	Digi	1_25	0.711	0.307	0.570	0.819	0.673	0.337	1.709	1.791	0.696	1.997	0.954	0.416	0.171	0.432
V	*Lesueurigobius magniiugis*	Digi	1_18	0.678	0.264	0.698	0.833	0.630	0.349	1.663	1.849	0.838	1.805	0.899	0.408	0.143	0.389
V	*Lesueurigobius magniiugis*	Digi	1_60	0.867	0.390	0.667	0.859	0.829	0.457	1.823	2.058	0.776	1.814	0.886	0.476	0.190	0.450
V	*Lesueurigobius magniiugis*	Digi	1_24	0.846	0.339	0.863	0.632	0.797	0.421	1.666	1.938	1.366	1.893	0.860	0.508	0.175	0.401
V	*Lesueurigobius magniiugis*	Digi	1_46	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
V	*Lesueurigobius magniiugis*	Digi	3_2	0.788	0.297	0.614	0.836	0.746	0.372	1.741	1.853	0.734	2.005	0.940	0.453	0.160	0.377

V	*Lesueurigobius magniiugis*	Digi	8_5	0.715	0.313	0.555	0.740	0.700	0.319	1.602	1.599	0.750	2.194	1.002	0.446	0.196	0.438
V	*Lesueurigobius magniiugis*	Digi	Fuchs_33_8	0.997	0.405	0.665	0.898	0.968	0.475	1.908	1.999	0.741	2.038	0.954	0.523	0.203	0.406
V	*Lesueurigobius magniiugis*	Digi	1_12	0.941	0.355	0.645	0.942	0.886	0.446	1.830	2.066	0.685	1.987	0.886	0.514	0.172	0.377
V	*Lesueurigobius magniiugis*	Digi	5_2	0.881	0.362	0.616	0.891	0.847	0.447	1.543	1.742	0.691	1.895	0.886	0.571	0.208	0.411
V	*Lesueurigobius magniiugis*	Digi	7_1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
V	*Lesueurigobius magniiugis*	Digi	5_12	1.037	0.512	0.718	1.002	0.980	0.556	2.272	2.381	0.717	1.763	0.954	0.456	0.215	0.494
V	*Lesueurigobius magniiugis*	Digi	1_6	0.907	0.403	0.783	1.065	0.862	0.499	2.055	2.280	0.735	1.727	0.901	0.441	0.177	0.444
V	*Lesueurigobius magniiugis*	Digi	7_11	1.071	0.441	0.755	1.167	0.998	0.551	2.247	2.400	0.647	1.811	0.936	0.477	0.184	0.412
V	*Lesueurigobius magniiugis*	Digi	1_14	0.978	0.373	0.695	0.994	0.941	0.445	1.997	2.201	0.699	2.115	0.907	0.490	0.169	0.381
V	*Lesueurigobius magniiugis*	Digi	1_21	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
V	*Lesueurigobius magniiugis*	Digi	1_29	0.768	0.297	0.625	0.815	0.741	0.351	1.738	1.788	0.767	2.111	0.972	0.442	0.166	0.387
V	*Lesueurigobius magniiugis*	Digi	1_95	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
V	*Lesueurigobius magniiugis*	Digi	Fuchs_33_12	0.925	0.421	0.667	0.945	0.893	0.435	2.034	2.066	0.706	2.053	0.985	0.455	0.204	0.455
V	*Lesueurigobius magniiugis*	Digi	1_35	0.755	0.247	0.489	0.742	0.690	0.421	1.457	1.638	0.659	1.639	0.889	0.518	0.151	0.327
V	*Lesueurigobius magniiugis*	Digi	4_2	1.074	0.490	0.705	0.999	0.994	0.557	2.040	2.250	0.706	1.785	0.907	0.526	0.218	0.456
V	*Lesueurigobius magniiugis*	Digi	4_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
V	*Lesueurigobius magniiugis*	Digi	9_11	0.675	0.332	0.533	0.725	0.636	0.359	1.451	1.439	0.735	1.772	1.008	0.465	0.231	0.492

Appendix for chapter 4


Periopthalmodon freycineti

Periopthalmodon schlosseri

Periophthalmus novemradiatus

Periophthalmus waltoni

Figure A5: X-ray images showing the skeletons of *Pn. freycineti* (ZSMPIS- 019650), *Pn. schlosseri* (ZSMPIS-023806), *Ps. novemradiatus* (ZMB 5987–1) and *Ps. waltoni* (ZM-CBSU 96); see Table 1 for acronyms of collections. Light blue indicates a modified caudal vertebra, purple depicts a preural vertebra, their respective neural (ns) and haemal spines (hs) are labelled. The terminal centrum (TC) is also indicated. Scale bars =1 cm.

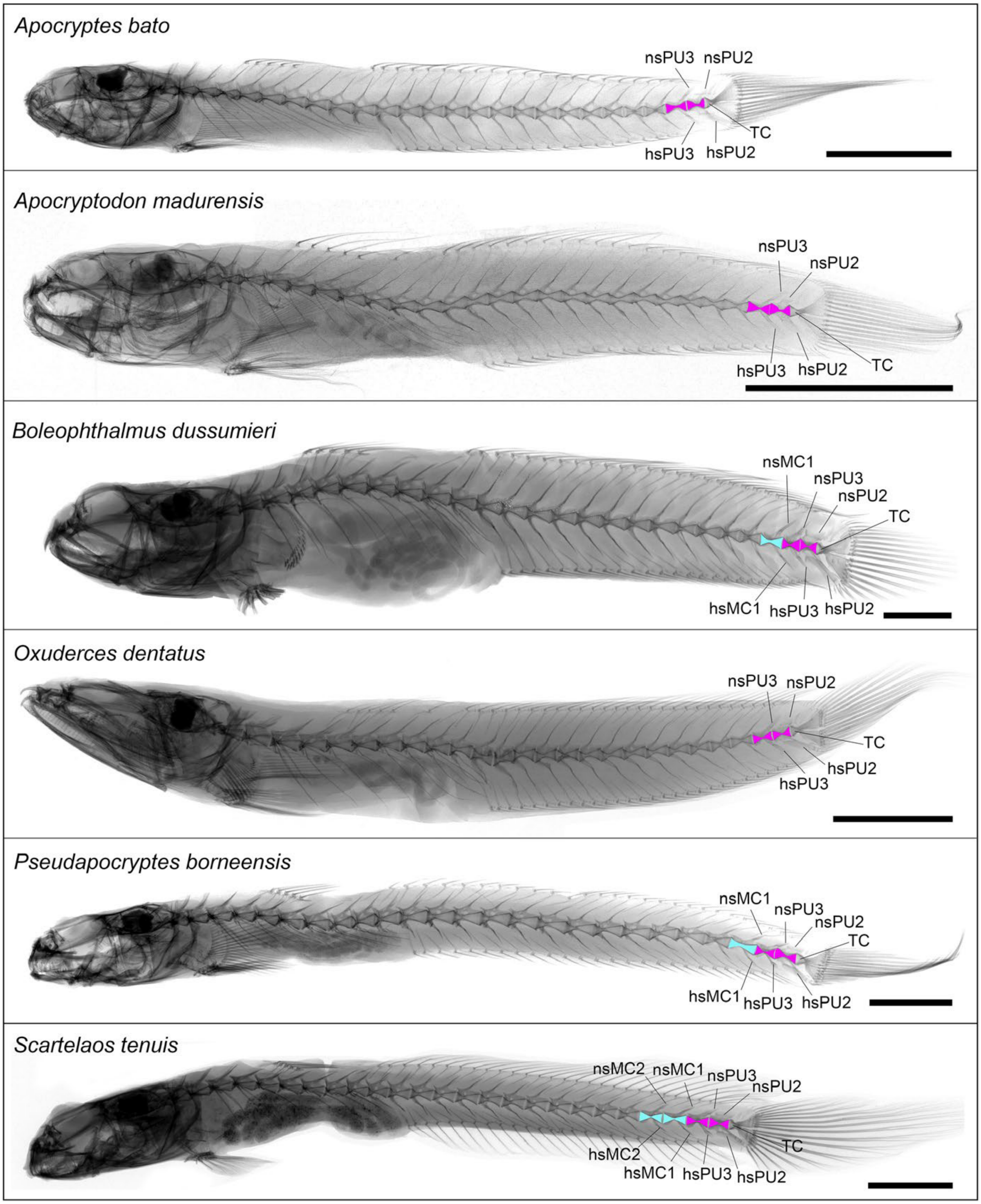


Figure A1: X-ray images showing the skeletons of *As. bato* (SMF 23715–2), *An. madurensis* (ZMB 6510), *B. dussumieri*(ZM-CBSU 8), *O. dentatus* (SMNS 3410–1), *P. borneensis* (ZMB 9931) and *S. tenuis* (ZM-CBSU 89); see Table 1 for acronyms of collections. Light blue indicates a modified caudal vertebra, purple depicts a preural vertebra, their respective neural (ns) and haemal spines (hs) are labelled. The terminal centrum (TC) is also indicated. Scale bars = 1 cm.

Appendix for chapter 5

Table S1: Raw data of otolith measurements, calculated variables, mean values and standard deviations.

Sp	Sp #	Locality	OL	OH	OL/OH	OP	OP/OL	OP/OH	SuP/OP	SuL/OP	SuH/OP
B. dussumieri	Bd 4	Khorramshahr	4.5420	4.0790	1.1135	16.4970	3.6321	4.0444	0.4204	0.1507	0.0555
	Bd 6	Khorramshahr	3.5990	3.4110	1.0551	15.2330	4.2326	4.4658	0.3021	0.1152	0.0540
	Bd 7	Khorramshahr	4.8890	4.1120	1.1890	17.7550	3.6316	4.3179	0.3299	0.1315	0.0554
	Bd 8	Khorramshahr	4.4620	4.1910	1.0647	16.6930	3.7411	3.9831	0.3778	0.1508	0.0704
	Bd 9	Khorramshahr	4.6300	4.3330	1.0685	16.1980	3.4985	3.7383	0.3755	0.1396	0.0697
	Bd 11	Khorramshahr	2.1857	2.0314	1.0759	8.3013	3.7980	4.0864	0.3469	0.1356	0.0659
	mean values		**4.0513**	**3.6929**	**1.0945**	**15.1129**	**3.7557**	**4.1060**	**0.3588**	**0.1372**	**0.0618**
	STDEV		1.0130	0.8744	0.0505	3.4346	0.2554	0.2565	0.0415	0.0134	0.0077
S. tenuis	St 82	Heleh	2.7890	2.4581	1.1346	9.2420	3.3137	3.7599	0.3324	0.1197	0.0773
	St 84	Heleh	2.3799	2.0285	1.1733	8.1323	3.4171	4.0091	0.3506	0.1412	0.0630
	St 85	Heleh	2.8980	2.3374	1.2398	9.5393	3.2917	4.0811	0.4235	0.1502	0.0937
	St 86	Heleh	2.4102	2.0460	1.1780	8.6162	3.5749	4.2113	0.3478	0.1381	0.0613
	St 89	Heleh	2.9020	2.3347	1.2430	9.8561	3.3963	4.2216	0.3683	0.1376	0.0820
	mean values		**2.6758**	**2.2409**	**1.1938**	**9.0772**	**3.3987**	**4.0566**	**0.3645**	**0.1373**	**0.0755**
	STDEV		0.2605	0.1926	0.0467	0.6987	0.1119	0.1885	0.0353	0.0111	0.0135
P. waltoni	Pw 92	Heleh	3.8710	3.5410	1.0932	13.1920	3.4079	3.7255	0.4015	0.1399	0.0912
	Pw 93	Heleh	3.9720	3.6200	1.0972	13.1360	3.3072	3.6287	0.4301	0.1611	0.0924
	Pw 95	Heleh	3.4000	2.8450	1.1951	11.3110	3.3268	3.9757	0.3626	0.1410	0.0707
	Pw 96	Heleh	3.2370	3.0340	1.0669	11.0350	3.4090	3.6371	0.4043	0.1446	0.0878
	Pw 101	Heleh	3.8090	3.3480	1.1377	13.4630	3.5345	4.0212	0.3764	0.1305	0.0832
	Pw 1716	Heleh	3.2630	2.8560	1.1425	11.3770	3.4867	3.9835	0.3262	0.1216	0.0715
	Pw 1727	Heleh	2.4860	2.2240	1.1178	10.2930	4.1404	4.6281	0.2765	0.1049	0.0583
	mean values		**3.4340**	**3.0669**	**1.1215**	**11.9724**	**3.5161**	**3.9429**	**0.3682**	**0.1348**	**0.0793**
	STDEV		0.5146	0.4856	0.0418	1.2619	0.2868	0.3459	0.0525	0.0180	0.0128

Species	Sp #	Locality	SuEndV/OP	OA	SuA/OA	SuL	SuL/OL	SuL/OH	SuL/SuH	SuL/SuP	SuL/SuTipV
B. dussumieri	Bd 4	Khorramshahr	0.1075	14.0710	0.0765	2.4860	0.5473	0.6095	2.7169	0.3585	1.3966
	Bd 6	Khorramshahr	0.1011	8.8200	0.0981	1.7550	0.4876	0.5145	2.1350	0.3814	1.4176
	Bd 7	Khorramshahr	0.1063	15.1670	0.0862	2.3340	0.4774	0.5676	2.3744	0.3984	1.4828
	Bd 8	Khorramshahr	0.1283	14.3630	0.1123	2.5170	0.5641	0.6006	2.1421	0.3991	1.7111
	Bd 9	Khorramshahr	0.1287	14.4330	0.1149	2.2620	0.4886	0.5220	2.0035	0.3719	1.4138
	Bd 11	Khorramshahr	0.1139	3.2935	0.0976	1.1257	0.5150	0.5541	2.0592	0.3909	1.4868
	mean values		**0.1143**	**11.6912**	**0.0976**	**2.0800**	**0.5133**	**0.5614**	**2.2385**	**0.3834**	**1.4848**
	STDEV		0.0117	4.7152	0.0148	0.5422	0.0355	0.0392	0.2663	0.0160	0.1171
S. tenuis	St 82	Heleh	0.1185	5.3055	0.0816	1.1061	0.3966	0.4500	1.5488	0.3600	1.4579
	St 84	Heleh	0.1050	3.6615	0.0900	1.1481	0.4824	0.5660	2.2418	0.4027	1.8641
	St 85	Heleh	0.1181	5.1912	0.1190	1.4327	0.4944	0.6129	1.6027	0.3546	1.6027
	St 86	Heleh	0.1114	3.8100	0.0954	1.1898	0.4936	0.5815	2.2512	0.3970	1.7651
	St 89	Heleh	0.1093	5.1977	0.1055	1.3561	0.4673	0.5809	1.6780	0.3736	1.7038
	mean values		**0.1125**	**4.6332**	**0.0983**	**1.2466**	**0.4669**	**0.5583**	**1.8645**	**0.3776**	**1.6788**
	STDEV		0.0058	0.8222	0.0145	0.1408	0.0408	0.0629	0.3517	0.0215	0.1557

P. waltoni	Pw 92	Heleh	0.1187	10.0510	0.1223	1.8460	0.4769	0.5213	1.5345	0.3486	1.3807
	Pw 93	Heleh	0.1175	10.4000	0.1447	2.1160	0.5327	0.5845	1.7430	0.3745	1.8497
	Pw 95	Heleh	0.1189	6.9850	0.1041	1.5950	0.4691	0.5606	1.9938	0.3889	1.5337
	Pw 96	Heleh	0.1343	7.4480	0.1141	1.5960	0.4930	0.5260	1.6471	0.3577	1.3423
	Pw 101	Heleh	0.1273	9.5740	0.1223	1.7570	0.4613	0.5248	1.5688	0.3468	1.5399
	Pw 1716	Heleh	0.1229	6.8120	0.0959	1.3830	0.4238	0.4842	1.7011	0.3727	1.3546
	Pw 1727	Heleh	0.1093	4.0070	0.0926	1.0800	0.4344	0.4856	1.8000	0.3795	1.2796
	mean values		**0.1213**	**7.8967**	**0.1137**	**1.6247**	**0.4702**	**0.5267**	**1.7126**	**0.3669**	**1.4686**
	STDEV		0.0079	2.2752	0.0181	0.3328	0.0365	0.0366	0.1552	0.0161	0.1945

Species	**Sp #**	**Locality**	**SuH/OH**	**SuH/SuP**	**SuH/SuTipV**	**SuH/SuEndV**	**SuP**	**SuP/SuTipV**	**SuP/SuEndV**	**SuA**	**SuTipV/SuEndV**
B. dussumieri	Bd 4	Khorramshahr	0.2243	0.1319	0.5140	0.5158	6.9350	3.8961	3.9092	1.0760	1.0034
	Bd 6	Khorramshahr	0.2410	0.1786	0.6640	0.5338	4.6020	3.7173	2.9883	0.8650	0.8039
	Bd 7	Khorramshahr	0.2391	0.1678	0.6245	0.5207	5.8580	3.7217	3.1028	1.3070	0.8337
	Bd 8	Khorramshahr	0.2804	0.1863	0.7988	0.5486	6.3060	4.2869	2.9440	1.6130	0.6867
	Bd 9	Khorramshahr	0.2606	0.1856	0.7056	0.5415	6.0820	3.8013	2.9170	1.6590	0.7674
	Bd 11	Khorramshahr	0.2691	0.1898	0.7220	0.5780	2.8799	3.8036	3.0452	0.3214	0.8006
	mean values		**0.2524**	**0.1734**	**0.6715**	**0.5397**	**5.4438**	**3.8711**	**3.1511**	**1.1402**	**0.8159**
	STDEV		0.0211	0.0217	0.0969	0.0225	1.4716	0.2140	0.3775	0.5042	0.1048
S. tenuis	St 82	Heleh	0.2906	0.2325	0.9413	0.6519	3.0723	4.0493	2.8045	0.4327	0.6926
	St 84	Heleh	0.2525	0.1796	0.8315	0.6000	2.8511	4.6291	3.3402	0.3297	0.7216
	St 85	Heleh	0.3824	0.2213	1.0000	0.7935	4.0396	4.5192	3.5859	0.6176	0.7935
	St 86	Heleh	0.2583	0.1763	0.7841	0.5505	2.9970	4.4463	3.1219	0.3636	0.7021
	St 89	Heleh	0.3462	0.2226	1.0154	0.7502	3.6300	4.5608	3.3698	0.5484	0.7389
	mean values		**0.3060**	**0.2065**	**0.9145**	**0.6692**	**3.3180**	**4.4409**	**3.2445**	**0.4584**	**0.7297**
	STDEV		0.0566	0.0264	0.1026	0.1015	0.4999	0.2288	0.2958	0.1221	0.0399
P. waltoni	Pw 92	Heleh	0.3397	0.2272	0.8998	0.7682	5.2960	3.9611	3.3819	1.2290	0.8538
	Pw 93	Heleh	0.3354	0.2149	1.0612	0.7868	5.6500	4.9388	3.6617	1.5050	0.7414
	Pw 95	Heleh	0.2812	0.1951	0.7692	0.5948	4.1010	3.9433	3.0491	0.7270	0.7732
	Pw 96	Heleh	0.3194	0.2172	0.8150	0.6538	4.4620	3.7527	3.0108	0.8500	0.8023
	Pw 101	Heleh	0.3345	0.2210	0.9816	0.6534	5.0670	4.4408	2.9562	1.1710	0.6657
	Pw 1716	Heleh	0.2847	0.2191	0.7963	0.5815	3.7110	3.6347	2.6545	0.6530	0.7303
	Pw 1727	Heleh	0.2698	0.2108	0.7109	0.5333	2.8460	3.3720	2.5298	0.3710	0.7502
	mean values		**0.3092**	**0.2150**	**0.8620**	**0.6531**	**4.4476**	**4.0062**	**3.0349**	**0.9294**	**0.7596**
	STDEV		0.0297	0.0102	0.1247	0.0949	0.9813	0.5274	0.3916	0.3905	0.0591

Species	**Sp #**	**Locality**	**SuTipV/OP**	**SuH**	**SuH/OL**	**SuTipV**	**SuL/SuEndV**	**SuEndV**
B. dussumieri	Bd 4	Khorramshahr	0.1079	0.9150	0.2015	1.7800	1.4014	1.7740
	Bd 6	Khorramshahr	0.0813	0.8220	0.2284	1.2380	1.1396	1.5400
	Bd 7	Khorramshahr	0.0887	0.9830	0.2011	1.5740	1.2362	1.8880
	Bd 8	Khorramshahr	0.0881	1.1750	0.2633	1.4710	1.1751	2.1420
	Bd 9	Khorramshahr	0.0988	1.1290	0.2438	1.6000	1.0849	2.0850

	Bd 11	Khorramshahr	0.0912	0.5467	0.2501	0.7571	1.1903	0.9457
	mean values		**0.0927**	**0.9284**	**0.2314**	**1.4034**	**1.2046**	**1.7291**
	STDEV		0.0094	0.2286	0.0259	0.3631	0.1089	0.4413
S. tenuis	St 82	Heleh	0.0821	0.7142	0.2561	0.7587	1.0097	1.0955
	St 84	Heleh	0.0757	0.5121	0.2152	0.6159	1.3451	0.8536
	St 85	Heleh	0.0937	0.8939	0.3085	0.8939	1.2717	1.1265
	St 86	Heleh	0.0782	0.5285	0.2193	0.6740	1.2394	0.9600
	St 89	Heleh	0.0808	0.8082	0.2785	0.7959	1.2589	1.0772
	mean values		**0.0821**	**0.6914**	**0.2555**	**0.7477**	**1.2250**	**1.0226**
	STDEV		0.0069	0.1687	0.0396	0.1079	0.1268	0.1136
P. waltoni	Pw 92	Heleh	0.1013	1.2030	0.3108	1.3370	1.1788	1.5660
	Pw 93	Heleh	0.0871	1.2140	0.3056	1.1440	1.3714	1.5430
	Pw 95	Heleh	0.0919	0.8000	0.2353	1.0400	1.1859	1.3450
	Pw 96	Heleh	0.1077	0.9690	0.2994	1.1890	1.0769	1.4820
	Pw 101	Heleh	0.0848	1.1200	0.2940	1.1410	1.0251	1.7140
	Pw 1716	Heleh	0.0897	0.8130	0.2492	1.0210	0.9893	1.3980
	Pw 1727	Heleh	0.0820	0.6000	0.2414	0.8440	0.9600	1.1250
	mean values		**0.0921**	**0.9599**	**0.2765**	**1.1023**	**1.1125**	**1.4533**
	STDEV		0.0093	0.2331	0.0330	0.1544	0.1438	0.1880

Table S2: Calculated otolith variables x 1000 used for the statistical analysis.

Sp	**Sp#**	**OL/OH**	**OP/OL**	**OP/OH**	**SuP/OP**	**SuL/OP**	**SuH/OP**	**SuTipV/OP**	**SuEndV/OP**	**SuA/OA**
B. dussumieri	Bd 4	1114	3632	4044	420	151	55	108	108	76
	Bd 6	1055	4233	4466	302	115	54	81	101	98
	Bd 7	1189	3632	4318	330	131	55	89	106	86
	Bd 8	1065	3741	3983	378	151	70	88	128	112
	Bd 9	1069	3498	3738	375	140	70	99	129	115
	Bd 11	1076	3798	4086	347	136	66	91	114	98
S. tenuis	St 82	1135	3314	3760	332	120	77	82	119	82
	St 84	1173	3417	4009	351	141	63	76	105	90
	St 85	1240	3292	4081	423	150	94	94	118	119
	St 86	1178	3575	4211	348	138	61	78	111	95
	St 89	1243	3396	4222	368	138	82	81	109	106
P. waltoni	Pw 92	1093	3408	3726	401	140	91	101	119	122
	Pw 93	1097	3307	3629	430	161	92	87	117	145
	Pw 95	1195	3327	3976	363	141	71	92	119	104
	Pw 96	1067	3409	3637	404	145	88	108	134	114
	Pw 101	1138	3535	4021	376	131	83	85	127	122
	Pw 1716	1143	3487	3984	326	122	71	90	123	96
	Pw 1727	1118	4140	4628	276	105	58	82	109	93

Sp	**Sp#**	**SuL/OL**	**SuL/OH**	**SuL/SuH**	**SuL/SuP**	**SuL/SuTipV**	**SuL/SuEndV**	**SuH/OL**	**SuH/OH**	**SuH/SuP**
B. dussumieri	Bd 4	547	609	2717	358	1397	1401	201	224	132
	Bd 6	488	515	2135	381	1418	1140	228	241	179
	Bd 7	477	568	2374	398	1483	1236	201	239	168
	Bd 8	564	601	2142	399	1711	1175	263	280	186
	Bd 9	489	522	2004	372	1414	1085	244	261	186
	Bd 11	515	554	2059	391	1487	1190	250	269	190
S. tenuis	St 82	397	450	1549	360	1458	1010	256	291	232
	St 84	482	566	2242	403	1864	1345	215	252	180
	St 85	494	613	1603	355	1603	1272	308	382	221
	St 86	494	582	2251	397	1765	1239	219	258	176
	St 89	467	581	1678	374	1704	1259	278	346	223
P. waltoni	Pw 92	477	521	1534	349	1381	1179	311	340	227
	Pw 93	533	585	1743	375	1850	1371	306	335	215
	Pw 95	469	561	1994	389	1534	1186	235	281	195
	Pw 96	493	526	1647	358	1342	1077	299	319	217
	Pw 101	461	525	1569	347	1540	1025	294	335	221
	Pw 1716	424	484	1701	373	1355	989	249	285	219
	Pw 1727	434	486	1800	379	1280	960	241	270	211

Sp	**Sp#**	**SuH/SuTipV**	**SuH/SuEndV**	**SuP/SuTipV**	**SuP/SuEndV**	**SuTipV/SuEndV**
B. dussumieri	Bd 4	514	516	3896	3909	1003
	Bd 6	664	534	3717	2988	804
	Bd 7	625	521	3722	3103	834

	Bd 8	799	549	4287	2944	687
	Bd 9	706	541	3801	2917	767
	Bd 11	722	578	3804	3045	801
S. tenuis	St 82	941	652	4049	2804	693
	St 84	832	600	4629	3340	722
	St 85	1000	793	4519	3586	793
	St 86	784	551	4446	3122	702
	St 89	1015	750	4561	3370	739
P. waltoni	Pw 92	900	768	3961	3382	854
	Pw 93	1061	787	4939	3662	741
	Pw 95	769	595	3943	3049	773
	Pw 96	815	654	3753	3011	802
	Pw 101	982	653	4441	2956	666
	Pw 1716	796	582	3635	2655	730
	Pw 1727	711	533	3372	2530	750

Acknowledgements

I am very grateful to Prof. Dr. Bettina Reichenbacher for her supervision, support, quick responses, and detailed comments throughout this study. I am grateful for fruitful discussions with Dr. Ulrich Schliewen, Dirk Neumann, Dr. Alexander Cerwenka (SNSB-ZSM, Munich), Prof. Dr. William Orsi, Dr. Sergio Vargas (LMU), PD. Dr. Mike Reich (SNSB-BSPG), Dr. Nora Dotzler, Prof. Dr. Michael Krings, PD. Dr. Ursula Göhlich, Elisabeth Lange, Gabriele Büttner, Simone Schätzler, Baran Karapunar (LMU) and Bork Ilsemann. I am grateful to Prof. Dr. Gert Wörheide, chair of Palaeontology & Geobiology, Department of Earth and Environmental Sciences, Director of the Bavarian State Collections of Palaeontology and Geology, Vice-Dean of the Faculty of Geosciences and Speaker of the Geobio-Center. Many thanks go to Neumaier René and Dominik Bastl (LMU) for their quick and continuous IT support. I also owe many thanks to Monika Brinkrolf and Britta Nüske-Illenberger for their patience and their willingness to help. I am thankful to my office mates Dr. Martin Dohrmann, Luis Porras and Imelda Hausmann (SNSB-BSPG) for their support and great advice. I would also like to warmly thank all the members of my working group, especially Dr. Christoph Gierl, Charalampos Kevrekidis, Stefanie Penk and Eleni Charmpila. Special thanks go out to my friends, my partner and especially to my family for their patience and kind-hearted support. Without these people this work wouldn't have been possible.