A fresh look on fossil gobioids and gobioid phylogeny

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

Vorgelegt von

Christoph Siegfried Gierl

München, 06.03.2019

Erstgutachter:	Prof. Dr. Bettina Reichenbacher
Zweitgutachter:	Prof. Dr. Alexander Nützel

Tag der Disputation:	09.07.2019

Statutory declaration and statement

I hereby confirm that my thesis entitled “**A fresh look on fossil gobioids and gobioid phylogeny**”, 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 knowledge and 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-Universität München.

München, 29.10.2019

Christoph Gierl

Abstract

Gobioidei are commonly small benthic fish forming one of the most species rich groups among Teleostei. There are about 270 genera with in total more than 2200 species, which can be found in all aquatic habitats forming a substantial part of the fish diversity in those ecosystems, especially coral reefs. Gobioid systematics was previously based on few morphological characters like the sensory papillae pattern or the pterygiophore formula. Recent molecular studies confirmed, by and large, those morphological classifications, but some taxa, like the Oxudercidae, are identified as a family only by molecular data. At the moment eight families are recognized: Rhyacichthyidae, Odontobutidae, Milyeringidae, Eleotridae, Butidae, Thalasseleotrididae, Gobiidae and Oxudercidae. The fossil record of Gobioidei consists of skeletons, which are rather rare discoveries, and isolated otoliths, which are much more abundant. Of more than 100 fossil species about 70% are only preserved by their otoliths. The rest are more or less well-preserved articulated skeletons. In unusual cases, otoliths are even preserved in the skeleton *in situ*. Previous descriptions of fossils often refrained from assigning them to a family and put them into the genus *Gobius sensu lato*.

So, the aims of this work are: to revise previously described fossil gobioids; to assess the potential of otoliths as additional holders of phylogenetic information; to create with the help of morphological and molecular data a phylogenetic framework to place the fossils in the gobioid family tree. With this framework it will be possible to get a better understanding of the evolutionary history of the Gobioidei.

This thesis comprises 6 chapters; chapters 2–4 are published, chapter 5 will be submitted soon, and chapter 6 contains a conclusion. In chapter 2, articulated skeletons, some with otoliths *in situ*, from the lower and middle Miocene of Southern Germany (Illerkirchberg and Öhningen) are re-examined to find out if the previously described species "*Cottus brevis* Ag." and "*Cottus brevis* Ag.?" belong to the same species. The revision showed that "*Cottus brevis* Ag.?" represents a new, previously unrecognized species which was given the species epithet *gaudanti*. Both species have a palatine that is T-shaped and no entopterygoid which is characteristic for Gobiidae + Oxudercidae (Gobioidei with five branchiostegals, 5brG). On the other hand, they have six branchiostegals, which is typical for all other Gobioid families. This character combination is unique and therefore it is justified to introduce the new genus †*Eleogobius* which includes both †*E. brevis* (as type species) and the new species †*E. gaudanti*. Furthermore, it was asserted that all otoliths that were previously described as †*Gobius multipinnatus* (von Meyer) belong to †*E. gaudanti*. Therefore, the otoliths of †*G. multipinnatus* remain unknown until a skeleton with otoliths *in situ* is discovered.

In the next chapter, "*Pomatoschistus bleicheri* (Sauvage)" from the lower Oligocene of Rouffach (France) and "*Pomatoschistus*(?) cf. *bleicheri* (Sauvage)" from the upper Eocene of the Isle of Wight (Great Britain) have been revised. If the current generic assignment was true these fossils would be the oldest representatives of the 5brG. The re-examination of the

type material of both species shows the presence of a postmaxillary process on the premaxilla which contradicts their assignment to *Pomatoschistus*. The fossils do not even belong to the 5brG, because they have six branchiostegals. Therefore, the previous established name †*Paralates* Sauvage has to be retained. Differences in the caudal fin ray counts and the shape of the frontals led to the assignment of the French material to †*Paralates bleicheri* Sauvage and the English material to †*Pa. chapelcorneri* n. sp. So, the oldest known representative of the 5brG is currently †*Gobius jarosi* Přikryl & Reichenbacher, 2018 from the lower Miocene of the Czech Republic.

An important part in the taxonomy of especially fossil fishes are the otoliths because they are often the only remains found. However, in ichthyology they are often ignored regarding taxonomy because their descriptions are of a qualitative nature. The case study presented in chapter 4 tries to find out if a quantification of otolith morphology of the family Oxudercidae can be used to distinguish between the five extant oxudercid lineages (*Mugilogobius*, *Acanthogobius*, *Pomatoschistus*, *Stenogobius*, *Periophthalmus*) and individual species of the *Pomatoschistus*-lineage from Europe. The data set consists of otoliths extracted from 84 specimens of 20 extant species representing all oxudercid lineages. Five fossil otoliths of †*Pomatoschistus* sp. (*sensu* Brzobohatý 1994) were added. On the basis of SEM images of the otoliths, ten measurements were partly newly defined and 23 otolith variables were computed and used for univariate and multivariate analysis. The results show that the used otolith morphometry can identify the *Pomatoschistus* and *Periophtalmus* lineages within the Oxudercidae; sand gobies (a distinct clade within the *Pomatoschistus* lineage) can be separated from other members of the *Pomatoschistus* lineage; the previous assignment of the fossil otoliths to †*Pomatoschistus* sp. is supported. This makes these fossils currently the oldest representatives of sand gobies. So, otolith morphology and morphometry can be a powerful taxonomic tool for certain taxa among Gobioidei.

Using these results, material of other fossils gobioids and a newly assembled data set of recent representatives of all gobioid families, a phylogenetic framework containing molecular and morphological data is presented based on parsimony and Bayesian inference. The molecular trees are in congruence with those of previous molecular studies. The morphological trees are partly poorly resolved but showed general patterns known from molecular phylogenies. The total evidence trees are better resolved with all gobioid families retained and the fossils are placed according to their previously assumed positions. This framework will be an essential starting point for further investigations of gobioid phylogeny.

These results indicate that fossil gobioids may have been much more diverse than the currently known record suggests, and that many fossil species of "*Gobius*" might not belong to this genus, or indeed even not to the Gobiidae. Besides it could be shown that otolith morphology can be quantified and used for discrimination between certain taxa. Furthermore, incorporating morphology into phylogenies leads to a confident placement of fossil gobioids which will help to further investigate and understand the evolutionary history of Gobioidei.

Zusammenfassung

Gobioidei sind meist benthisch lebende Fische und bilden eine der artenreichsten Gruppen unter den Teleostei. Es gibt ca. 270 Gattungen und insgesamt mehr als 2200 Arten, welche in allen auqatischen Habitaten zu finden sind und dort einen erheblichen Teil der Fischdiversität bilden, besonders in Korallenriffen. Die Systematik der Gobioidei basierte früher auf wenigen morphologischen Merkmalen wie z.B. das Muster der Sinnespapillen oder die Pterygiophorenformel. Molekulare Studien bestätigten im Großen und Ganzen diese auf Morphologie basierten Klassifikationen, aber einige Taxa, wie die Oxudercidae, konnten nur basierend auf molekularen Daten als eigen Familie identifiziert werden. Momentan gibt es acht akzeptierte Familien: Rhyacichthyidae, Odontobutidae, Milyeringidae, Eleotridae, Butidae, Thalasseleotrididae, Gobiidae und Oxudercidae. Der Fossilbericht der Gobioidei besteht aus eher selten zu findenden Skeletten und isolierten Otolithen, welche deutlich häufiger sind. Von mehr als 100 fossilen Arten sind ca. 70% nur durch Otolithen erhalten; der Rest durch mehr oder weniger gut erhaltene Skelette. In ungewöhnlichen Fällen kann es vorkommen, dass Otolithen *in situ* erhalten sind. Bisherige Fossilbeschreibungen sahen oft davon ab, diese einer Familie zuzuordnen und stellten sie stattdessen in die Gattung *Gobius sensu lato*.

Die Ziele dieser Arbeit sind: schon beschriebene fossile Gobioidei neu zu untersuchen; das Potential von Otolithen als zusätzliche Informationsquelle für phylogenetische Information zu bewerten; eine Phylogenie auf Basis morphologischer und molekularer Daten zu erstellen um die Fossilien in den Stammbaum der Gobioidei zu platzieren. Damit wird es möglich sein, ein besseres Verständnis der Evolutionsgeschichte der Gobioidei zu erhalten.

Diese Arbeit enthält sechs Kapitel; Kapitel zwei bis vier sind publiziert, Kapitel fünf wird bald eingereicht und Kapitel sechs fasst die Ergebnisse nochmals zusammen und gibt einen Ausblick. In Kapitel zwei werden Skelette aus dem unteren und mittleren Miozän von Süddeutschland (Illerkirchberg und Öhningen), manche mit Otolithen *in situ*, neu untersucht. Es soll herausgefunden werden, ob “*Cottus brevis* Ag.” und “*Cottus brevis* Ag.?” zur selben Art gehören. Die Untersuchung zeigte, dass “*Cottus brevis* Ag.?” eine neue, bisher unerkannte Art darstellt. Sie soll das Artepithet *gaudanti* erhalten. Beide Arten haben ein T-förmiges Palatinum und kein Entopterygoid, ein Charakteristikum der Gobiidae + Oxudercidae (Gobioidei mit fünf Kiemenhautstrahlen, 5brG). Andererseits haben sie sechs Kiemenhautstrahlen, was typisch für alle anderen Familien der Gobioidei ist. Diese einzigartige Merkmalskombination rechtfertigt die Einführung der neuen Gattung †*Eleogobius*, welche †*E. brevis* (Typusart) und die neue Art †*E. gaudanti* enthält. Außerdem wurde dadurch festgestellt, dass alle bisher als †*Gobius multipinnatus* (von Meyer) beschriebenen Otolithen zu †*E. gaudanti* gehören und somit die Otolithen von †*G. multipinnatus* noch auf ihre Entdeckung warten.

Im nächsten Kapitel werden “*Pomatoschistus bleicheri* (Sauvage)” (unteres Oligozän, Rouffach, Frankreich) und “*Pomatoschistus*(?) cf. *bleicheri* (Sauvage)” (oberes Eozän, Isle of Wight, Großbritannien) neu untersucht. Falls die bestehende Gattungszuordnung stimmte, wären diese Fossilien die ältesten Vertreter der 5brG. Die Untersuchung des Typusmaterials beider Arten zeigt das Vorhandensein eines postmaxillaren Prozesses auf dem Prämaxillare, was der Zuordnung zu *Pomatoschistus* widerspricht. Die Fossilien sind nicht einmal den 5brG zugehörig, denn sie haben sechs Kiemenhautstrahlen. Deshalb wird der ursprüngliche Name †*Paralates* wiederverwendet. Unterschiede in der Anzahl der Caudalstrahlen und der Form der Frontalia führten dazu, das französische Material †*Paralates bleicheri* Sauvage und das englische Material †*Paralates chapelcorneri* n. sp. zuzuordnen. Daher ist derzeit der älteste Vertreter der 5brG †*Gobius jarosi* Přikryl & Reichenbacher, 2018 aus dem unteren Miozän der Tschechischen Republik.

Ein wichtiger Teil der Taxonomie besonders fossiler Fische sind die Otolithen, denn sie sind oft ihre einzigen Überbleibsel. In der Ichthyologie werden sie trotzdem oft ignoriert, denn Otolithenbeschreibungen sind eher von qualitativer Natur. Die Fallstudie in Kapitel vier versucht herauszufinden, ob ein quantitativer Ansatz mit der Otolithenmorphologie der Familie Oxudercidae es möglich macht, deren fünf Linien voneinander zu unterscheiden (*Mugilogobius*, *Acanthogobius*, *Pomatoschistus*, *Stenogobius*, *Periophthalmus*). Ferner soll dieser Ansatz mit Arten innerhalb der europäischen *Pomatoschistus*-Linie abermals getestet werden. Der Datensatz besteht aus Otolithen aus 84 Individuen von 20 rezenten Arten aller Linien der Oxudercidae und fünf fossilen Otolithen beschrieben als †*Pomatoschistus* sp. (von Brzobohatý 1994). Auf Basis von REM-Bildern der Otolithen wurden zehn, zum Teil neu definierte, Messungen vorgenommen und 23 Variablen wurden analysiert mit uni- und multivariater Statistik. Die Ergebnisse zeigen, dass die verwendete Otolithenmorphometrie die *Pomatoschistus*- und *Periophtalmus*-Linien innerhalb der Oxudercidae identifizieren kann. Außerdem können die Sandgrundeln, eine Klade innerhalb der *Pomatoschistus*-Linie, von anderen Mitgliedern der Linie getrennt werden. Zusätzlich konnten die als †*Pomatoschistus* sp. beschriebenen Otolithen als zu dieser Gattung gehörig bestätigt werden, was diese Fossilien somit zu den ältesten Vertretern der Sandgrundeln macht. Dies zeigt, dass Otolithenmorphologie bzw. -morphometrie ein mächtiges taxonomisches Werkzeug für bestimmte Gruppen innerhalb der Gobioidei sein kann.

Mit all diesen Ergebnissen, Material von anderen fossilen Gobioidei und einem neu zusammengestellten Datensatz rezenter Vertreter aller Gobioidenfamilien, wird eine Phylogenie basierend auf morphologischen und molekularen Daten mit Hilfe von Parsimonie und bayesscher Methodik präsentiert. Die molekularen Bäume sind kongruent zu jenen aus früherern molekularen Studien. Die morphologischen Bäume sind teilweise schlecht aufgelöst, geben aber das generelle Muster der molekularen Bäume wieder. Die Bäume nach dem „total evidence“-Ansatz sind besser aufgelöst. Es sind alle Gobioidenfamilien erhalten und die Fossilien sind ungefähr dort platziert, wo es erwartet wurde. Diese Phylogenie wird ein wichtiger Anfangspunkt für weitere Studien zur Stammesgeschichte der Gobioidei.

All diese Ergebnisse deuten darauf hin, dass fossile Gobioidei viel diverser waren als bisher angenommen und dass viele fossile Arten der Gattung „*Gobius*“ gar nicht zu dieser gehören oder nicht einmal zu den Gobiidae. Außerdem konnte gezeigt werden, dass Otolithenmorphologie quantifizierbar und nützlich für die Unterscheidung bestimmter Taxa ist. Ferner führen Phylogenien, die auch morphologische Daten enthalten dazu, dass man fossile Gobioidei gut darin einordnen kann und so kann damit ein weiterer Beitrag zum besseren Verständnis der Evolutionsgeschichte der Gobioidei geleistet werden.

TABLE OF CONTENTS

1. Introduction

1.1 Pisces

Among the vertebrates fishes are the most species rich group with more than 35000 species currently recognized (Fricke et al. 2019). That is about half of all vertebrates (Helfman et al. 2009; Nelson et al. 2016). The fishes have their origin more than 500 million years ago and their high diversity in morphology, habitat occupation and behaviour is unparalleled (see Nelson et al. 2016). They can be found in all aquatic habitats from deep oceans, torrential streams, completely dark caves, to muddy swamps and even partially living on land (e.g. Helfman et al. 2009). These highly different habitats required a huge range of different adaptations in size, shape, behaviour and physiology. This makes fish systematics a difficult, some would say an impossible venture. The fossil record is even more challenging. That is mainly because soft tissue characters play an important role in fish systematics (e.g. muscular structures, see Datovo et al. 2014). And chances are very low that soft tissue is preserved in fossils. The other problem is convergent evolution that already gave ichthyologists dealing with recent fish headaches, because morphology and molecules do not always tell the same story and new classifications based on molecular phylogeny often lack a backing in morphological apomorphies (e.g. Mooi and Gill 2010).

Fishes today comprise two major groups: the jawless fishes (Myxinomorphi and Petromyzontomorphi) and the Gnathostomata (jawed fishes and the tetrapods, see Helfman et al. 2009). This group can be further subdivided into the Chondrichthyes (cartilaginous fishes) and the Osteichthyes (bony fishes) based on the nature of their endoskeleton (see Nelson et al. 2016). Among the Osteichthyes there are two subclasses: the Sarcopterygii containing the lobe-finned fishes and tetrapods, and the Actinopterygii (see Nelson et al. 2016). The latter contains the most species-rich fish group, the Teleostei or modern bony fish (Helfman et al. 2009). Within this group lies the Percomorphaceae or Percomorpha (Betancur-R et al. 2017) containing the group of interest in this thesis, the Gobioidei or gobies.

1.2 Otoliths

Before expanding on the Gobioidei it is necessary to draw the reader’s attention to a small feature (with a huge impact) in the skull of bony fishes: the otoliths. Otoliths are small calcareous structures developing independently from the skeleton (e.g. Popper 1981). In fact, they are present in all vertebrates, but only in fishes (except sharks, rays and lampreys, see Campana 2004) they are compact stones with characteristic features, opposed to tetrapods, where they are only “microcristalline dust” (Nolf, 2013, p. 1). Similar structures can also be found in other animal groups like the cephalopods (e.g. see Radtke 1983, using the more general term statolith). The bony fishes possess three pairs of otoliths. They are situated in the inner ear containing the labyrinth, which comprises three otolithic organs, the saccule (holding the saccular otolith or sagitta), the lagena (holding the lagenar otolith or asteriscus) and the utricle (holding the utricular otolith or lapillus) (e.g. see Popper and Lu 2000). So, they are part of the auditory system and the equilibrium sense of bony fishes. The otoliths are connected to a sensory epithelium containing hair cells. Since the otoliths are much denser than the rest of the fish they oscillate at a different phase and amplitude compared to the body (see Popper and Lu 2000). In this way the hair cells are stimulated resulting in hearing. In a similar way the equilibrium sense is working by stimulating the hair cells reacting to different directions of acceleration of the denser otolith; of course, the equilibrium sense consists additionally of the lateral line and the eyes, if present (see Kasumyan 2004).

But why the interest in otoliths? As already mentioned above they show characteristic features that are most prominent on the biggest otolith, the sagitta (in most teleosts; simple referred to as otolith in the following). These features can help to determine the taxonomic affinities of the whole specimen by only looking at the otolith (e.g. Nolf 1985). Important are the general outline of the otolith, if it is smooth or lobed and if projections are present. On the inner face of the otolith the most prominent feature is the sulcus and its shape is extremely variable. The sulcus shape is often characteristic on higher taxonomic levels and the here studied group, the Gobioidei, have a sulcus that shows the shape of a shoe sole (e.g. Gierl et al. 2013). Addtionally, otolith morphometrics can be used to discriminate fish taxa on different taxonomic levels (e.g. Gierl et al. 2018). Otoliths are therefore valuable taxonomic tools that have been used for species identification for more than a century in palaeontology (e.g. Koken 1884). The reason is that often the only remains of fossil fish are otoliths. They have a higher chance of fossilization because of their small size and their better surviving of digestion compared to bones (see Nolf 2013). There are currently about 1400 valid extinct species, and about 400 valid both fossil and recent fish species documented by their otoliths (Nolf 2013). In total there are 78 families that are only known by otoliths in the fossil record (Nolf 2013). In the taxonomy of recent fish, otoliths unfortunately play a minor role and biologists mainly use them for trace element and isotope analysis (e.g. Tabouret et al. 2014; Crook et al. 2015), age estimation via growth rings (e.g. Fablet et al. 2009) and stock discrimination (e.g Campana and Casselman 1993).

1.3 Gobioidei or gobies

1.3.1 General information

Gobioidei, commonly known as gobies are among the most species rich fish groups with more than 2200 species (Fricke et al. 2019). They can be found in fresh, brackish and marine habitats and form a big part of the fish community in coral reefs, especially within the cryptobenthic fishes (Brandl et al. 2018). They rarely become bigger than 30 centimeters and have in general a sub-cylindrical body and most species show a benthic lifestyle (see Patzner et al. 2011). Because of their huge species number, comprehensive studies on morphology are very challenging (Nelson et al. 2016). Gobies are also of a limited commercial use, e.g. in Bulgaria, Ukraine and Russia (Zarev et al. 2013).

1.3.2 Adaptations

Gobies show some very interesting specializations. In the following some examples are given.

Rhyacichcthys aspro is a species found in hill-streams of the Indo-Australian archipelago (Miller 1973). Its body shows adaptations to fast waters like the broadened pectoral and pelvic fins and the flattened head and snout for showing only a low profile to the current and adhering to rocks (Miller 1973). This makes *Rhyacichthys aspro* looking rather atypical for a goby explaining its trivial name “loach goby”.

Guardian gobies (like e.g. *Cryptocentrus cinctus*) live in association with burrowing alpheid shrimps (see Karplus 1987). The shrimp is building the burrow and the goby uses it as shelter and a place for breeding. In return the goby warns the shrimp if predators approach. For details see Karplus and Thompson (2011).

The waterfall-climbing gobies of the genus *Sicyopterus* from Hawaii show extraordinary adaptations to living in steep streams with high waterfalls (Cullen et al. 2013). Its amphidromous life cycle where the hatched larvae are swept into the ocean and later after fully developing return to the streams requires a special adaptation: in addition to the ventral sucker present in most Gobiidae they develop an oral sucker when returning to freshwater (Cullen et al. 2013). Their climbing style is characterized by an alternating attachment of the pelvic and oral sucking disc to the substrate and so they can climb vertical waterfalls with heights of ten meters and more (Cediel et al. 2008; Cullen et al. 2013).

The mudskippers (e.g. *Periophthalmus waltoni*) are maybe the most prominent example for specialization among fish in general because of their amphibious lifestyle. They developed certain traits that enabled them to move on land and even spend more time there than in water (see Jaafar and Murdy 2017). The respiratory system consists of the gills, the bucco-opercular cavity and the skin (see Jaafar and Murdy 2017). The pectoral and pelvic girdles are more massively built (see Harris 1960). With the pectoral fins the locomotion is achieved. The pelvic fins serve as support for the body when the pectoral fins are in movement (“crutching”, see Harris 1960, Fig. 12). Also, the caudal skeleton and fin is strengthened by expanded hemal and neural spines of preural vertebra 2 and the lower procurrent rays (see Harris 1960). Recent studies suggest that this mudskipper-like “crutching” was the style of locomotion in stem tetrapods like *Ichthyostega* (Pierce et al. 2013).

1.3.3 Phylogeny

Gobioid phylogeny and sister-group relationships of the taxon have been widely discussed in the last decades (e.g. Regan 1911; Gosline 1955; Miller 1973; Hoese 1984; Akihito 1986; Hoese and Gill 1993; Johnson and Brothers 1993; Winterbottom 1993; Thacker 2009; Gill and Mooi 2012; Agorreta et al. 2013; Near et al. 2013; Thacker et al. 2015). Before using molecular data, the classification of gobioids was based on osteology and external features like sensory papillae patterns (e.g. Miller 1973, 1986). The history of the classification of gobioids is well summarized in Van Tassell et al. (2011). The current phylogeny of gobioids is mainly based on Thacker et al. (2015) and Betancur-R et al. (2017). Kurtidae + Apogonidae are sister to the Trichonotidae and they are sister to the Gobioidei. The Gobioidei consist currently of eight families: Rhyacichthyidae, Odontobutidae, Milyeringidae, Eleotridae (= Eleotrididae), Butidae, Thalasseleotrididae, Gobiidae and Oxudercidae (= Gobionellidae).

1.3.4 Modern distribution and fossil record

Gobioidei are found worldwide in all aquatic habitats (see Patzner et al. 2011). Their current diversity hotspot is the Indo-Australian Archipelago (Thacker 2015).

The fossil record of Gobioidei comprises more than 100 species. Most species, about 70 % are only known from otoliths. Twelve species have skeletons with otoliths in situ. The oldest known gobioid fossils are isolated otoliths from the early Eocene of India (Bajpai and Kapur 2004). The oldest known skeletons of fossil gobioids is *Gobius*? *praecursor* from the middle Eocene of Spain (Gaudant 1996; Gaudant and Busquets 1996). During the Oligocene there appear only few species. In the early-middle Miocene there is a significant increase in diversity (see Reichenbacher et al. 2018, 2019).

Fossil gobioids have been found so far in Eurasia (e.g. Arambourg 1927; Vorstman 1927; Weiler 1963; Bajpai and Kapur 2004; Carnevale et al. 2006; Reichenbacher et al. 2018), Australia and New Zealand (e.g. McDowall et al. 2006; Schwarzhans et al. 2012), Japan (e.g. Aoki 1967) and North America (e.g. Nolf and Stringer 2003).

1.4 Study significance

The fossil record is a valuable source of information that can help to understand the phylogeny of Gobioidei. However, many previously described fossils have not been assigned on family level (e.g. see Gierl et al. 2013; Gierl and Reichenbacher 2015). Therefore, it is important to review those fossils. Many people are currently working on gobioid phylogeny or fish phylogeny in general and well determined fossils can help to resolve previously unresolved deep interrelationships or to get time-calibrated trees on a solid basis (e.g. see Davesne et al. 2016; Betancur-R et al. 2017).

1.5 Aim and outline of thesis

1.5.1 Aim

The aim of this work is to revise previously described fossil goboids, to assess the usefulness of otolith morphometry for taxonomy and to give a first total evidence phylogeny incorporating fossils to gain new insights into gobioid evolutionary history.

1.5.2 Overview of manuscripts

Chapter 2 is a taxonomic revision of fossil gobioids from Southern Germany. The outcome is that "*Cottus brevis* Ag." and "*Cottus brevis* Ag.?" are two different species. Based on a unique characer combination the new genus †*Eleogobius* containing †*E. brevis* and the new species †*E. gaudanti* is established. Furthermore, all otoliths previously described as †*Gobius multipinnatus* are assigned to †*E. gaudanti*. The familial affinity of †*Eleogobius* could not be clarified and otoliths of †*G. multipinnatus* remain to be explored.

Chapter 3 is a taxonomic revision of fossil gobioids from Britain and France. The results show that those fossils represent two species, †*Paralates bleicheri* and the new species †*Paralates chapelcorneri*. Because of the presence of six branchiostegals †*Paralates* cannot be assigned to the Gobiidae or Oxudercidae and thus is a member of one of the remaining gobioid families.

Chapter 4 is a case study on the basis of otoliths from recent species representing the five oxudercid lineages and some fossil otoliths previously assigned to the genus *Pomatoschistus*. The outcome of the study showed that otolith morphometry can be used to identify at least two lineages and that within the *Pomatoschistus* lineage the subgroup "sand gobies" can be distinguished from other members of the *Pomatoschistus* lineage. The data support also the assignment of the fossil otoliths to *Pomatoschistus*.

Chapter 5 (containing preliminary results) is the establishment of a phylogenetic framework for Gobioidei incorporating representatives of all families and some fossil species using the total evidence approach. The resulting phylogenies recreate the current gobioid phylogeny and place the fossils fairly well where they have been formerly placed with comparative taxonomy only.

Author contributions

Chapter 2: **Christoph Gierl**, Bettina Reichenbacher: A New Fossil Genus of Gobiiformes from the Miocene Characterized by a Mosaic Set of Characters.
CG designed and conducted the analyses, acquired, analysed, interpreted the data and drafted the manuscript. CG and BR contributed to discussions and the final manuscript.
Manuscript published in *Copeia* 103 (4): 792–805. doi: 10.1643/CI-14-146.

Chapter 3: **Christoph Gierl**, Bettina Reichenbacher: Revision of so-called *Pomatoschistus* (Gobiiformes, Teleostei) from the late Eocene and early Oligocene.

CG designed and conducted the analyses, acquired, analysed, interpreted the data and drafted the manuscript. CG and BR contributed to discussions and the final manuscript.
Manuscript published in *Palaeontologia Electronica* 20.2.33A: 1–7. doi: 10.26879/721.

Chapter 4: **Christoph Gierl**, Dorothea Liebl, Radek Šanda, Jasna Vukić, Hamid Reza Esmaeili, Bettina Reichenbacher: What can goby otolith morphology tell us?
CG and BR designed and conducted the analyses, acquired, analysed, interpreted the data and drafted the manuscript. DL also acquired data. RŠ, JV and HRE provided specimens. All authors contributed to discussions and the final manuscript.
Manuscript published in *Cybium* 42 (4): 349–363. doi: 10.26028/cybium/2018-424-006.

Chapter 5: **Christoph Gierl**, Bettina Reichenbacher, Martin Dohrmann et al.
CG designed and conducted the analyses, acquired, analysed, interpreted the data and drafted the manuscript. MD also analysed data. CG, BR and MD contributed to discussions and the manuscript.
Manuscript to be submitted as standalone publication.

1.6 References

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

Akihito. 1986. Some morphological characters considered to be important in gobiid phylogeny. In *Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fishes*, ed. K. Matsuura, 629–639. Tokyo: Ichthyological Society of Japan.

Aoki, N. 1967. 526. Some fossil *Gobius* from Japan. *Transactions and Proceedings of the Palaeontological Society of Japan. New Series* 67: 125–128.

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

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

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

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

Campana, S.E. 2004. *Photographic atlas of fish otoliths of the northwest Atlantic Ocean*. Ottawa, Ontario: NRC Research Press.

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

Carnevale, G., A.F. Bannikov, W. Landini, and C. Sorbini. 2006. Volhynian (early Sarmatian sensu lato) fishes from Tsurevsky, North Caucasus, Russia. *Journal of Paleontology* 80 (4): 684–699.

Cediel, R.A., R.W. Blob, G.D. Schrank, R.C. Plourde, and H.L. Schoenfuss. 2008. Muscle fiber type distribution in climbing Hawaiian gobioid fishes: ontogeny and correlations with locomotor performance. *Zoology* 111 (2): 114–122.

Crook, D.A., D. Wedd, and T.M. Berra. 2015. Analysis of otolith Sr-87/Sr-86 to elucidate salinity histories of Nurseryfish *Kurtus gulliveri* (Perciformes: Kurtidae) in a tropical lowland river in northern Australia. *Freshwater Science* 34 (2): 609–619.

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

Datovo, A., M.C.C. de Pinna, and G.D. Johnson. 2014. The infrabranchial musculature and its bearing on the phylogeny of percomorph fishes (Osteichthyes: Teleostei). *PLOS ONE* 9 (10): e110129.

Davesne, D., C. Gallut, V. Barriel, P. Janvier, G. Lecointre, and O. Otero. 2016. The phylogenetic intrarelationships of spiny-rayed fishes (Acanthomorpha, Teleostei, Actinopterygii): fossil taxa increase the congruence of morphology with molecular data. *Frontiers in Ecology and Evolution* 4: 129.

Fablet, R., A. Chessel, S. Carbini, A. Benzinou, and H. de Pontual. 2009. Reconstructing individual shape histories of fish otoliths: A new image-based tool for otolith growth analysis and modeling. *Fisheries Research* 96 (2–3): 148–159.

Fricke, R., W.N. Eschmeyer, and J.D. Fong. 2019. Species by Family/Subfamily. http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp Electronic version accessed February 2019.

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 II, Sciences de la terre et des planètes* 322 (1): 71–76.

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

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

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

Gierl, C., B. Reichenbacher, J. Gaudant, D. Erpenbeck, and A. Pharisat. 2013. An extraordinary gobioid fish fossil from southern France. *PLOS ONE* 8 (5): e64117.

Gill, A.C., and R.D. Mooi. 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: 41–52.

Gosline, W.A. 1955. The osteology and relationships of certain gobioid fishes, with particular reference to the genera *Kraemeria* and *Microdesmus*. *Pacific Science* IX: 158–170.

Harris, V.A. 1960. On the locomotion of the mud-skipper *Periophthalmus koelreuteri* (Pallas): (Gobiidae). *Proceedings of the Zoological Society of London* 134 (1): 107–135.

Helfman, G.S., B.B. Collette, D.E. Facey, and B.W. Bowen. 2009. *The diversity of fishes: biology, evolution, and ecology, 2nd edition*. Oxford: Wiley-Blackwell.

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

Hoese, D.F., and A.C. Gill. 1993. Phylogenetic relationships of eleotridid fishes (Perciformes, Gobioidei). *Bulletin of Marine Science* 52 (1): 415–440.

Jaafar, Z., and E.O. Murdy eds. 2017. *Fishes out of water: biology and ecology of mudskippers*. Boca Raton: Taylor & Francis.

Johnson, G.D., and E.B. Brothers. 1993. *Schindleria*: a paedomorphic goby (Teleostei, Gobioidei). *Bulletin of Marine Science* 52 (1): 441–471.

Karplus, I. 1987. The association between gobiid fishes and burrowing alpheid shrimps. *Oceanography and Marine Biology Annual Review* 25: 507–562.

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

Kasumyan, A.O. 2004. The vestibular system and sense of equilibrium in fish. *Journal of Ichthyology* 44 (Supplement 2): S224–S268.

Koken, E. 1884. Über Fisch-Otolithen, insbesondere über diejenigen der norddeutschen Oligocän-Ablagerungen. *Zeitschrift der Deutschen Geologischen Gesellschaft* 36: 500–565.

Leonard, G., T. Maie, K.N. Moody, G.D. Schrank, R.W. Blob, and H.L. Schoenfuss. 2012. Finding paradise: cues directing the migration of the waterfall climbing Hawaiian gobioid *Sicyopterus stimpsoni*. *Journal of Fish Biology* 81 (2): 903–920.

McDowall, R.M., E.M. Kennedy, J.K. Lindqvist, D.E. Lee, B.V. Alloway, and M.R. Gregory. 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.J. 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.J. 1986. Gobiidae. In *Fishes of the north-eastern Atlantic and the Mediterranean (FNAM)*, eds. P. J. P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen, and E. Tortonese, 1019–1085. Paris: UNESCO.

Mooi, R.D., and A.C. Gill. 2010. Phylogenies without synapomorphies - a crisis in fish systematics: time to show some character. *Zootaxa* 2450: 26–40.

Near, T.J., A. Dornburg, R.I. Eytan, B.P. Keck, W.L. Smith, K.L. Kuhn, J.A. Moore, S.A. Price, F.T. Burbrink, M. Friedman, and P.C. Wainwright. 2013. Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. *Proceedings of the National Academy of Sciences of the United States of America* 110 (31): 12738–12743.

Nelson, J.S., T.C. Grande, and M.V.H. Wilson. 2016. *Fishes of the World, Fifth Edition*. Hoboken, New Jersey: John Wiley & Sons, inc.

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

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

Nolf, D., and G.L. Stringer. 2003. Late Eocene (Priabonian) fish otoliths from the Yazoo Clay at Copenhagen, Louisiana. *Louisiana Geological Survey, Geological Pamphlet* 13: 1–23.

Patzner, R.A., J.L. Van Tassell, M. Kovačić, and B.G. Kapoor eds. 2011. *The biology of gobies*. Enfield, New Hampshire: Science Publishers Inc.

Pierce, S.E., J.R. Hutchinson, and J.A. Clack. 2013. Historical perspectives on the evolution of tetrapodomorph movement. *Integrative and Comparative Biology* 53 (2): 209–223.

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., and Z. Lu. 2000. Structure–function relationships in fish otolith organs. *Fisheries Research* 46 (1–3): 15–25.

Radtke, R.L. 1983. Chemical and structural characteristics of statoliths from the short-finned squid *Illex illecebrosus*. *Marine Biology* 76 (1): 47–54.

Regan, C.T. 1911. The osteology and classification of the gobioid fishes. *The Annals and Magazine of Natural History [Eighth Series]* 8 (48): 729–733.

Reichenbacher, B., S. Filipescu, and A. Miclea. 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., R. Gregorová, K. Holcová, R. Šanda, J. Vukić, and T. Přikryl. 2018.

Discovery of the oldest *Gobius* (Teleostei, Gobiiformes) from a marine ecosystem of Early Miocene age. *Journal of Systematic Palaeontology* 16 (6): 493–513.

Schwarzhans, W., R.P. Scofield, A.J.D. Tennyson, J.P. Worthy, and T.H. Worthy. 2012. Fish remains, mostly otoliths, from the non-marine early Miocene of Otago, New Zealand. *Acta Palaeontologica Polonica* 57 (2): 319–350.

Tabouret, H., M. Tomadin, L. Taillebois, M. Iida, C. Lord, C. Pécheyran, and P. Keith. 2014. Amphidromy and marine larval phase of ancestral gobioids *Rhyacichthys guilberti* and *Protogobius attiti* (Teleostei: Rhyacichthyidae). *Marine and Freshwater Research* 65 (9): 776–783.

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.

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

Thacker, C.E., T.P. Satoh, E. Katayama, R.C. Harrington, R.I. Eytan, and T.J. Near. 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.

Van Tassell, J.L., L. Tornabene, and M.S. Taylor. 2011. A history of gobioid morphological systematics. In *The biology of gobies*, eds. R. A. Patzner, J. L. Van Tassell, M. Kovačić, and B. G. Kapoor, 3–22. Enfield, NH: Science Publishers Inc.

Vorstman, A.G. 1927. Tertiaire vischotolieten van Java. *Wetenschappelijke Mededeelingen Dienst van den mijnbouw in Nederlandsch-Indie* 5: 1–24.

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.

Wiley, E.O., and G.D. Johnson. 2010. A teleost classification based on monophyletic groups. In *Origin and phylogenetic interrelationships of teleosts*, eds. J. S. Nelson, H.-P. Schultze, and M. V. H. Wilson, 123–182. München: Verlag Dr. Friedrich Pfeil.

Winterbottom, R. 1993. Search for the gobioid sister group (Actinopterygii: Percomorpha). *Bulletin of Marine Science* 52 (1): 395–414.

Zarev, V., A. Apostolou, B. Velkov, and M. Vassilev. 2013. Bulgarian black sea gobies as important object in the commercial fishing. *Bulgarian Journal of Agricultural Science* 19 (Supplement 2): 233–236.

Results

The following subchapters are presented in the form of scientific articles.

2. A new Fossil Genus of Gobiiformes From the Miocene Characterized by a Mosaic Set of Characters

Christoph Gierl[1], Bettina Reichenbacher[1]

[1]Department of Earth and Environmental Sciences, Ludwig-Maximilians University Munich, Richard-Wagner Straße 10, 80333 Munich, Bavaria, Germany; E-mail: c.gierl@lrz.uni-muenchen.de (send reprint requests to this address); b.reichenbacher@lrz.uni-muenchen.de.

ABSTRACT

Gobiiformes are one of the most versatile groups among teleosts and show countless adaptations. Their fossil record encompasses numerous otolith-based species, but only a few species that are based on skeletons, and fossil skeletons with otoliths preserved in situ are exceptionally rare. Here, articulated skeletons, some with otoliths in situ, from the lower and middle Miocene of Southern Germany (Illerkirchberg, Öhningen) are re-investigated to clarify whether "*Cottus brevis* Ag." and "*Cottus brevis* Ag.?" correspond to the same species. Our data show that the latter actually represents a previously unrecognized new species, for which we introduce the species epithet *gaudanti*. In both species, the palatine is T-shaped and the entopterygoid is absent, as is characteristic for the Gobiidae – but the number of branchiostegals is six, which is typical for the sleeper gobies (Eleotrididae and Butidae). This character combination is so far unique, and justifies the introduction of *Eleogobius* n. gen. including *E. brevis* (Ag.) (as type species) and the new species *E. gaudanti*. A further outcome is that all otoliths previously described as *Gobius multipinnatus* (H. v. Meyer) rather belong to *E. gaudanti* n. sp. and that the otoliths of *G. multipinnatus* remain unknown until a skeleton with otoliths in situ is found. Our results indicate that fossil Gobiiformes may have been much more diverse than the currently known record suggests, and that many fossil species of "*Gobius*" might not belong to this genus, or indeed even to the Gobiidae.

2.1 Introduction

The Gobiiformes (collectively referred to as gobies in the following) display astonishing species diversity in marine, brackish and freshwater habitats (Patzner et al. 2011) and exhibit a wealth of adaptations (e.g. Thacker 2011). Their fossil record extends back to the Eocene

(Gaudant 1996; Bajpai and Kapur 2004), but most fossil species have been defined on the basis of isolated otoliths. Complete skeletons are uncommon, and articulated skeletons with otoliths in situ are exceedingly rare. The present study focuses on such exceptional fossils from the lower and middle Miocene of the North Alpine Foreland Basin (the Molasse Basin) of Southern Germany. These were first described as *Cottus brevis* by Agassiz (1839) from freshwater sediments at Öhningen (lower middle Miocene, c. 14–16 Ma), and as *C. brevis* Ag.? by H. v. Meyer in 1852 from brackish sediments at Illerkirchberg (uppermost lower Miocene, c. 17 Ma). From the latter locality, and co-occurring with *C. brevis* Ag.?, H. v. Meyer (1852) also described the new species *C. multipinnatus*, based on a single articulated skeleton (without otoliths).

Cottus brevis has been considered to be a species of *Gobius* since Gaudant (1980). The two species of *Cottus* described by H. v. Meyer (1852) from Illerkirchberg were merged into a single species termed *Lepidocottus multipinnatus* (H. v. Meyer) by Woodward (1901). Weiler (1955) confirmed Woodward's view that only a single species is present and recognized this species as belonging to the genus *Gobius*. He also studied the otoliths preserved in situ within a skeleton that had been assigned to "*Cottus brevis* Ag.?" by H. v. Meyer (1852). As Weiler believed that only a single species of *Gobius* is represented at Illerkirchberg, he attributed these otoliths to *Gobius multipinnatus*. Since then, otoliths assigned to *G. multipinnatus* (H. v. Meyer) have been reported from the brackish Kirchberg Formation (Reichenbacher 1989, 1993; Reichenbacher et al. 2013), and also from marine sediments of the Mediterranean (Reichenbacher and Cappetta 1999; Carnevale et al. 2006) and the Central Paratethys (Vienna Basin) (Śmigielska 1966; Brzobohatý 1989; Brzobohatý et al. 2007; Nolf and Brzobohatý 2009).

Reichenbacher et al. (2007) indicated that H. v. Meyer (1852) was correct in assuming that "*Cottus brevis* Ag.?" from Illerkirchberg is not the same species as *Gobius brevis* from Öhningen, but did not review the issue in detail. The present study provides a thorough re-investigation of *Gobius brevis* (Ag.) and "*Cottus brevis* Ag.?". The phylogenetic relationships are discussed, and a new fossil genus for both *G. brevis* and "*Cottus brevis* Ag.?" and a new species name for the latter are introduced.

2.2 Geographical and geological setting

Both the Öhningen and Illerkirchberg sites are located in the Molasse Basin of Southwestern Germany (Baden-Württemberg). Öhningen is situated near Lake Constance close to the border with Switzerland, and encompasses lacustrine sediments of the Upper Freshwater Molasse of middle Miocene age (Stauber 1939; Gaudant 1980; Gregor 1982). Illerkirchberg lies to the south of Ulm and represents the type locality of the Kirchberg Formation, which is marked by a typical brackish faunal assemblage (Kranz 1904; Reichenbacher 1989). The Kirchberg Formation is part of the Upper Brackish Molasse and corresponds to the late early Miocene according to Reichenbacher et al. (2013).

2.3 Materials and methods

The material discussed here comprises nine articulated skeletons of *Eleogobius gaudanti* (formerly described as *Gobius multipinnatus* or *G. brevis*) from Illerkirchberg, four of which have otoliths in situ, and five articulated skeletons of "*Gobius brevis*" from Öhningen (see species description for further details). Furthermore, 24 isolated otoliths recovered from the Kirchberg Fm in cores obtained at the Dillingen, Druisheim and Holzheim drilling sites and assigned to *G. multipinnatus* (according to Weiler (1955) and Reichenbacher (1993)) are considered, together with 11 isolated otoliths of *G. brevis* from a previously studied exposure (St. Martin) near Edenkoben (see species description for further details).

Specimen numbers preceded by an asterisk have otoliths in situ. In the following the term "otolith" refers to the saccular otolith or sagitta.

The comparative material comprises seven articulated skeletons of *Gobius multipinnatus* from Illerkirchberg (HLMD V3260, V3262, *V3407 a-b (poorly preserved, indeterminable skeleton with strongly corroded otoliths), V3410; SMNS 3686/4, 50960, 87132), 15 articulated skeletons plus parts of skeletons (e.g. just the skull) of *Gobius brevis* from Edenkoben, 16 of which retain otoliths in situ (material from Reichenbacher et al. 2007) and 14 isolated otoliths from the NMW collection, labelled as "*Gobius pretiosus* Pr." from the Kienberg site (NHM 1860 I 50, Kienberg/Gp/1–14).

Meristic, morphometric and osteological characters of the skeletons and characters of otoliths were examined under a stereomicroscope equipped with a digital camera, and imaged using a SEM (LEO 1430VP at 15 kV). Measurements of otoliths (see Fig. 1) were taken with ImageJ v1.49e 64-bit (Schneider et al. 2012) based on the digital and SEM images. Counts of caudal fin rays follow Fricke (1983).

Pictures were taken by the first author if not otherwise noted, and figures were prepared using Adobe Photoshop CS4 (11.0).

Institutional abbreviations: HLMD (Hessisches Landesmuseum Darmstadt), MCZ (Museum of Comparative Zoology, Harvard University), NMB (Naturhistorisches Museum Basel), NMW (Naturhistorisches Museum, Wien), SMNS (Staatliches Museum für Naturkunde Stuttgart) (see Sabaj Pérez 2016).

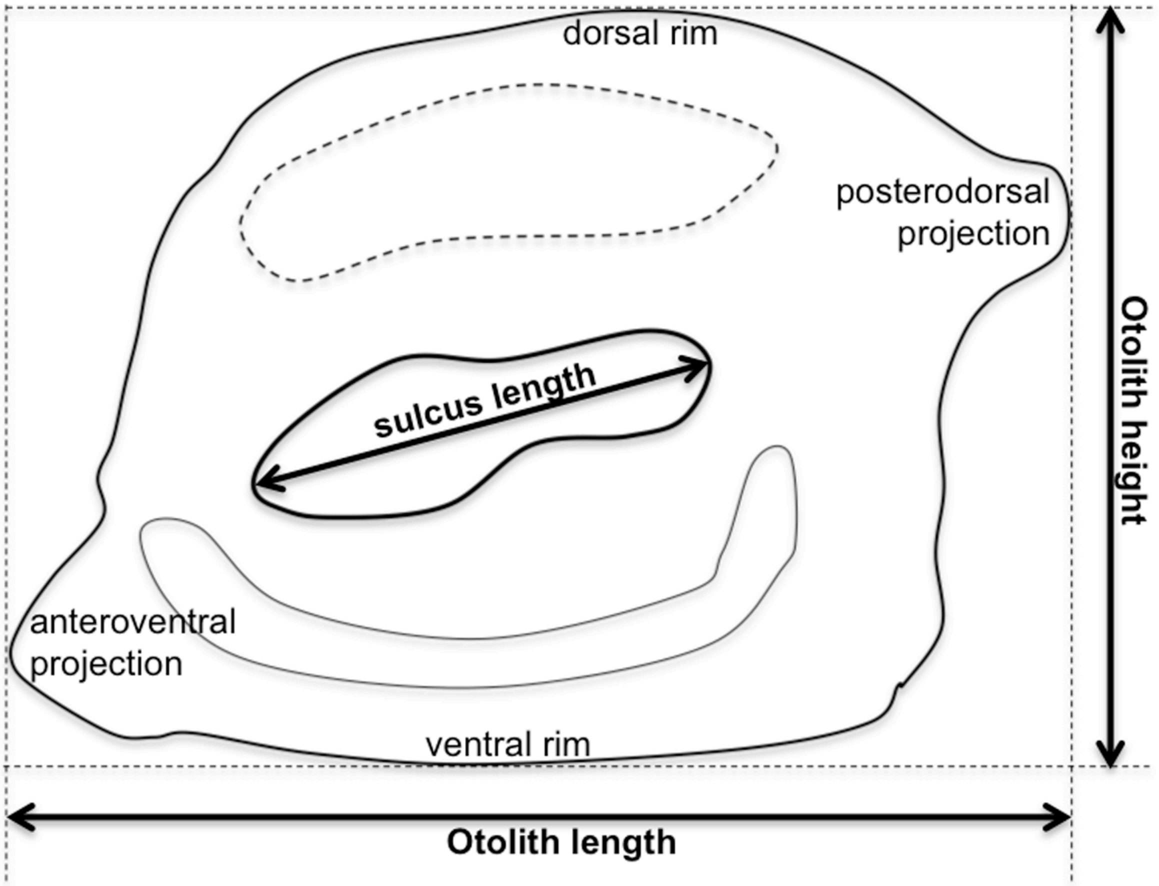

Fig. 1. Drawing of an otolith showing the parameters measured.

2.4 Results

Our re-examination confirms the view of H. v. Meyer (1852) that two species of "*Cottus*" (now Gobiiformes) are present in the Kirchberg Fm at Illerkirchberg. One is *Gobius multipinnatus* (H. v. Meyer, 1852), and the other corresponds to "*Cottus brevis* Ag.?" sensu H. v. Meyer, 1852. However, "*Cottus brevis* Ag.?" cannot be assigned to *Gobius brevis* (Agassiz, 1839) and is therefore introduced here as a new species with the epithet "*gaudanti*". Both the new species from Illerkirchberg and *G. brevis* from Öhningen show a combination of characters that is unprecedented in the known record of extant Gobiiformes: The palatine is T-shaped and the entopterygoid is absent, as is usually seen in the Gobiidae, but the number of branchiostegals is six, which is typical for the sleeper gobies (Eleotrididae and Butidae). This unique set of characters justifies the introduction of the new genus *Eleogobius* to accommodate the fossil species previously described as *G. brevis* and "*Cottus brevis* Ag.?". It should, however, be mentioned here that Gaudant (2000) has described a specimen of "*Gobius brevis* (Ag.)" from Eibiswald in which he found a T-shaped palatine and six branchiostegals (i.e. same unique combination of characters as described above), but he did not comment on this.

As *Eleogobius* cannot be assigned to any of the known families of the Gobiiformes, we consider it as incertae sedis at the family level.

2.4.1 Systematic Palaeontology

Division Percomorphacea (= Percomorphi Cope, 1871) sensu Wiley and Johnson (2010)
Order Gobiiformes Günther, 1880 (Suborder Gobioidei Agassiz, 1835)
Family incertae sedis
***Eleogobius* n. gen.**

Type species.—*Eleogobius brevis* (Agassiz, 1839) [*Cottus brevis* Agassiz, 1839]

Included species.—*Eleogobius gaudanti* sp. nov.

Diagnosis.— Unique combination of a palatine with a long ventral maxillary process and a long dorsal ethmoid process (= T-shaped palatine) and six branchiostegals. Entopterygoid absent. The otoliths are rectangular with a small anteroventral projection and a very prominent posterodorsal projection.

General description.—See description of type species.

Etymology.—Blend of "eleotridid" and "*Gobius*" to denote the fact that the new genus combines characters of eleotridids and gobiids.

***Eleogobius brevis* (Agassiz, 1839)**

Figs. 2C, 2C1, 2D, 3A, 5Q–Y

	1832	*Cottus brevis*. – Agassiz: 137 [name only].
*	1839	*Cottus brevis* Agass.: 185, pl. 32, figs. **2**–4.
v	1967	*Gobius* cf. *francofurtanus* Koken [pro parte]. – Salis: 45, figs. 13/2–4, 6.
v	1992	*Gobius latiformis* n. sp. Reichenbacher & Weidmann: 42, pl. 7, figs. 7–10, pl. 8, figs. 7–10.
v	1992	*Gobius altiformis* n. sp. Reichenbacher & Weidmann: 40, pl. 7, figs. 1–6, pl. 8, figs. 1–3.
v	1993	*Gobius latiformis* Reichenbacher 1992, in Reichenbacher & Weidmann. – Reichenbacher: 359, pl. 10, figs. 147–148.
v	1993	*Gobius altiformis* Reichenbacher 1992, in Reichenbacher & Weidmann. – Reichenbacher: 355, pl. 9, figs. 138–140.
	2000	*Gobius brevis* (Agassiz). – Gaudant: 28, pl. 2 & figs. 6–8
?	2003	*Gobius multipinnatus* (H. v. Meyer, 1852). – Brzobohatý et al.: pl. 5, fig. 2 (non pl. 3, fig. 10).
v	2003	*Gobius latiformis* Reichenbacher, in Reichenbacher & Weidmann 1992. – Sach et al.: 14, pl. 3, figs, 7–15.

v 2006 *Gobius latiformis* Reichenbacher, in Reichenbacher & Weidmann 1992.– Jost et al.: 320, fig.14.
v 2007 *Gobius brevis* (Agassiz, 1839). – Reichenbacher et al.: 367, figs. 2–4.
2009 *Gobius brevis* (Agassiz, 1839). – Brzobohatý & Gaudant: 248, figs. 2–4.

Remark.—Schultz (2013) gives additional synonymies that are difficult to verify without considering the original material.

Material.—Five articulated skeletons (*NMB On. 14, *On. 19, *On. 28, On. 29, On. 86) from the type locality. Comparative material includes articulated skeletons from Edenkoben and otoliths from St. Martin (StMartin_Z1_a–b, StMartin_Z2_a–f).

Provenance of type specimens.—Öhningen, SW Germany (Upper Freshwater Molasse, lower middle Miocene, c. 14–16 Ma).

General description.—Up to 37 mm SL (standard length). Cylindrical body with massive skull, T-shaped palatine, no entopterygoid and six branchiostegals. Number of vertebrae: 27 to 29. First dorsal fin with six spines, the last separated by a small gap from the first five. Second dorsal fin with one spine and ten or eleven soft rays. Anal fin with one spine and eight to nine soft rays. Otoliths rectangular with a broad posterodorsal projection; posteroventral projection weak or absent; if absent, posteroventral transition angled.

The following description is mainly based on specimen NMB On. 19.

Neurocranium. The frontal bones have a very narrow anterior and a broad posterior part (bordering the eye). The parietals are absent. Sphenotic with anteriorly inclined tip. Further details of the skull roof are not discernible.

Jaws. The maxilla is bent with anterior processes inserting in the palatine and premaxilla. The premaxilla shows the three processes usually seen in gobies and bears conical teeth. The dentary bears conical teeth and has a posterior indentation for the anguloarticular. The teeth are up to 0.4 mm in length.

Suspensorium and hyoid arches. The ectopterygoid is wedge-shaped. An entopterygoid is absent. The palatine is T-shaped and ends about halfway to the quadrate (Fig. 2C1). The quadrate is triangular with a long and slender posterior process which, together with the preopercle and symplectic, forms the ventral rim for the non-ossified area below the hyomandibular. The opercle is triangular and rather small compared to the crescent-shaped subopercle.

The posterior ceratohyal (epihyal) is triangular with rounded edges and apparently carries one branchiostegal, which is the broadest of the set (of six). In its caudal section the anterior ceratohyal is as broad as the posterior ceratohyal, but then narrows abruptly less than one-third of the way along the bone. The broad portion carries three branchiostegals that decrease slightly in width from posterior to anterior. The slender part bears two thin branchiostegals, which are separated by a short gap from the other four (Fig. 2D).

Branchial arches. The basihyal (glossohyal) is shaped like an enclosed V. The urohyal is ventrally rounded and shows two posterior processes (see Fig. 2C1). Other elements of the branchial apparatus are not preserved.

Vertebral column. Vertebrae 27 to 29, of which 15 to 17, ?18 are postabdominal. There are eight or nine pairs of slender ribs. In the region of the ribs thin epineural bones (sensu Patterson & Johnson 1995: fig. 6; note that the same bones have also been termed epipleurals in previous works) are visible. Supraneurals are absent.

Pectoral girdle and fins. The cleithrum is a splint-like and almost straight bone, with the lower third curving slightly in the anterior direction, and it has a forked dorsal tip. The four proximal radials have a rounded hourglass shape, though not as pronounced as that of the vertebrae (Fig 2C). The articulation of the dorsalmost ray is not preserved.

There are 15 to 17 pectoral fin rays and each fin is almost as big as the caudal fin.

Pelvic girdle and fins. The pelvic girdle shows only some fragments. The pelvic fins consist of at least three rays.

Dorsal fins. The first dorsal fin is located at the level of the third/fourth abdominal vertebra. It has six spines. The first five are equidistant from one another, while the last is separated from the preceding by twice that distance. They are supported by one pterygiophore each.

The second dorsal fin inserts above vertebra 10. It has one spine and ten or eleven soft rays, which seem to be equal in length to those in the first dorsal.

Anal fin. The anal fin inserts below vertebra 13/14 and has a short spine followed by eight or nine soft rays.

Caudal endoskeleton and fin. The caudal fin has 13 or 14 branched segmented soft rays and one or two upper and lower unbranched and segmented soft rays. In addition, six to eight short unbranched and unsegmented rays are present dorsally and ventrally. The caudal skeleton has two large hypural plates (HY1+2 and HY3+4) of which the upper is fused with the terminal centrum (Fig. 3A). Hypural 5 is a short and slender bone. The parhypural is a bigger structure three quarters of the length of the lower hypural plate. A single epural is present, of which only the proximal part is preserved. The neural spine of preural centrum 2 is very small.

Scales. The scales are ctenoid with tiny spines in a single row at the posterior margin. The focus is positioned posteriorly, the number of radii could not be determined. The scales correspond to the peripheral ctenoid scales (Pe-type) sensu Roberts (1993).

Otoliths. The otoliths are rectangular in shape and sometimes almost quadratic (otolith length: 1.20–2.14 mm; otolith height: 1.04–1.93 mm; sulcus length: 0.57–1.01 mm; l/h: 1.07–1.25; Figs. 5Q–Y). The inner face is flat, the outer face slightly convex. The posterodorsal projection is rounded, sometimes slightly pointed. The dorsal rim is convex and shows in some otoliths a small tip in the middle. The anterodorsal angle is weakly defined and the anterior rim is more or less straight, and may be slightly concave. A rounded anteroventral projection is usually present. The ventral rim is straight but often also somewhat convex. The posteroventral projection is sometimes as pronounced as the anteroventral one but then more rounded and wider. The posterior rim is concave because of the two projections. The dorsal area is clearly defined and sometimes longer than the sulcus. The sulcus resembles the sole of a shoe in shape, and cauda and ostium are not clearly separated. The tip of the ostium is more often rounded than pointed. The crista superior is visible in about the middle one-third of the otolith. A crista inferior is not well developed because the subsulcal area above the broad ventral line is elevated.

Further distribution.—Edenkoben (Burdigalian), Upper Rhine Graben; Eibiswald, Styrian Basin (Karpatian); Závod, Vienna Basin (Karpatian); Molasse Basin (Karpatian–Badenian).

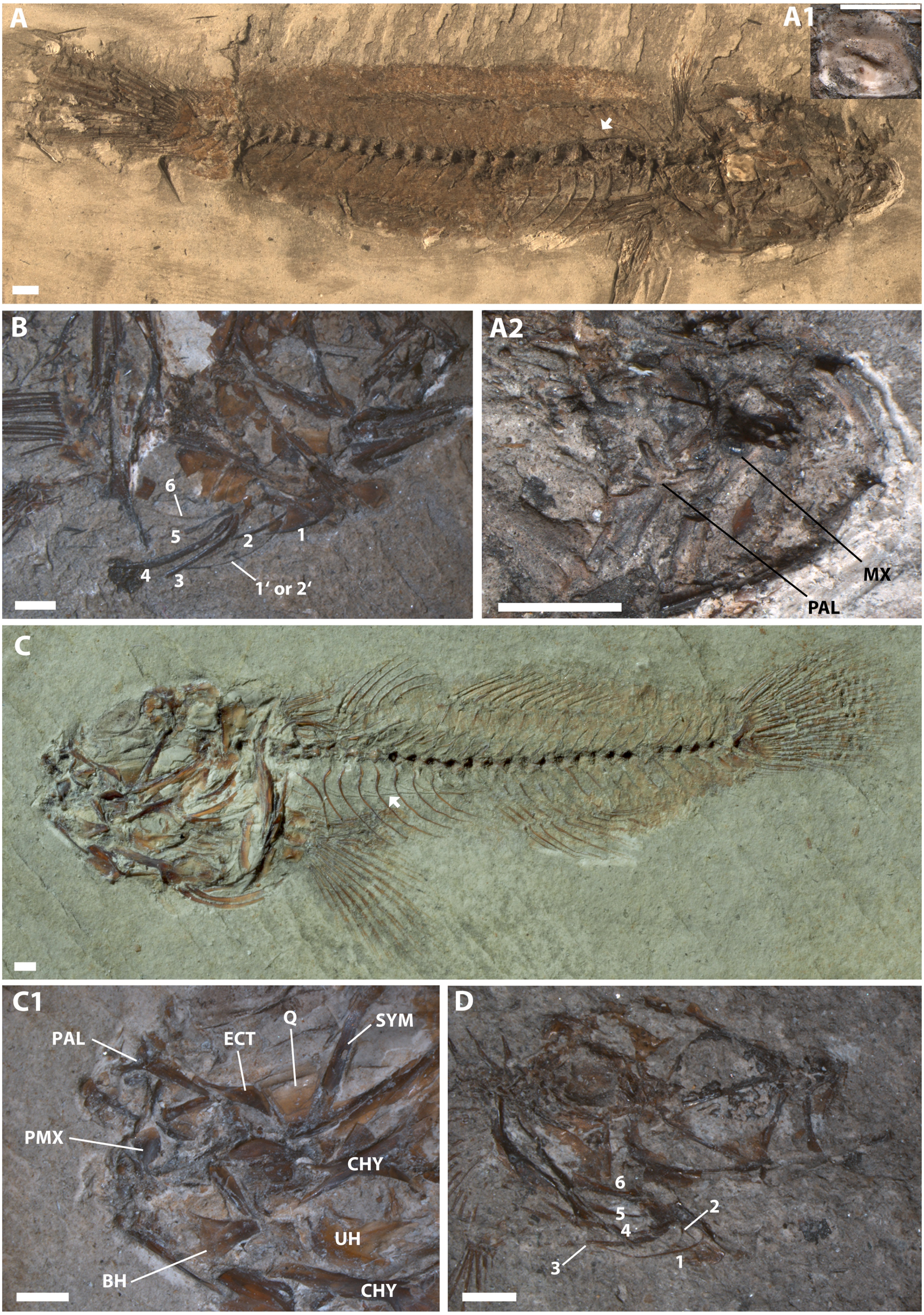

Fig. 2A–B. *Eleogobius gaudanti*. **A:** NMW 1857 XXVII 7. Skeleton with both sagittae preserved in situ. Arrow indicates epineurals. **A1:** Close-up of left sagitta. **A2:** Close-up of skull showing T-shaped palatine (PAL) and maxilla (MX). **B:** MCZ 5211-A. Part of skeleton showing branchiostegals 1 to 6 and one (1' or 2') ray from the other side. **C–D.** *Eleogobius* *brevis*. **C:** NMB On. 19. Skeleton with right sagitta preserved in situ (photo: M. Schellenberger). Arrow indicates epineurals **C1:** Close-up of skull showing T-shaped palatine, basihyal (BH), ceratohyal (CHY), ectopterygoid (ECT), premaxilla (PMX), quadrate (Q), symplectic (SYM), urohyal (UH). **D:** NMB On. 86. Part of skeleton showing branchiostegals 1 to 6. Scale bars: 1 mm.

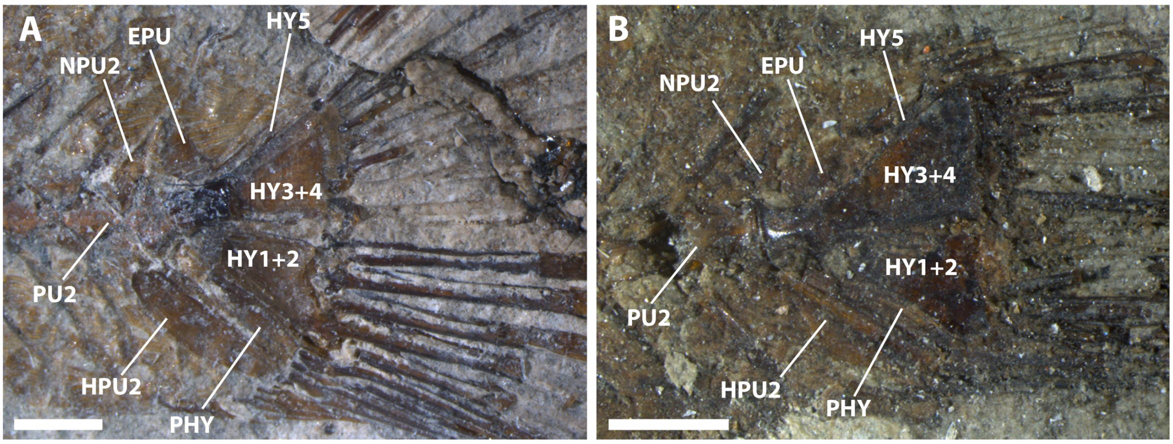

Fig. 3. Caudal fin skeletons of the two *Eleogobius* species. **A:** *Eleogobius brevis*. **B:** *Eleogobius gaudanti*. Epural (EPU), preural hemal spine (HPU), hypural (HY), preural neural spine (NPU), parhypural (PHY), preural centrum (PU). Scale bar: 1 mm.

Eleogobius gaudanti **n. sp.**

Figs. 2A, 2A2, 2B, 3B, 4, 5A–P

	1851	*Gobius* (?) *conicus*. – H. v. Meyer: 80 [name only].
* v	1852	*Cottus brevis*. Ag. ?. – H. v. Meyer: 107, pl. XVI, figs. 7–10.
v	1856	*Gobius*?. – H. v. Meyer: 27, pl. I, fig. 6.
v	1955	*Gobius multipinnatus* H. von Meyer. – Weiler: 93, figs. 5, 6, 8.
v	1988	*Gobius multipinnatus* (H. v. Meyer). – Reichenbacher: 22, pl. 4, figs. 17–18.
v	1993	*Gobius multipinnatus* (H. v. Meyer 1852). – Reichenbacher: 358, pl. 10, figs. 144–146.

The synonymy list contains only publications dealing with specimens from the type locality. Schultz (2013) gives an extensive synonymy list for *G. multipinnatus* (which does not include *Cottus brevis*. Ag. ?), but the status of the synonyms has to be checked in this new context.

Remark.—The year for H. v. Meyer's original description of the material we now assign to *Eleogobius gaudanti* is 1851. The complete volume of the Journal (Palaeontographica 2) containing his paper was published in 1852. However, six issues ("Lieferungen") are listed in the table of contents. The "Dritte Lieferung", which appeared in October 1851, contains H. v. Meyer's paper.

Holotype.—*NMW 1857 XXVII 7.

Paratypes.—*HLMD V3314a-b (a: skeleton, figured as "*Cottus brevis* Ag.?" in H. v. Meyer (1852), b: otoliths found in situ, now stored separately, depicted as "*Gobius multipinnatus* H. v. Meyer" in Weiler (1955)); MCZ 1586 (depicted as "*Cottus brevis* Ag.?" in H. v. Meyer (1852)), 5211A/B (part/counterpart; depicted as "*Gobius*?" in H. v. Meyer (1856: pl. 1, fig. 6)), 6791 (?), 6793 (?), 6794 (?);*SMNS 3466 (otolith not in situ, but found close to the skeleton), *51854.

Further material.—24 otoliths from the drill cores Dillingen, Druisheim and Holzheim: Dillingen/58.13–58.26m/a, d; Druisheim/67.23–67.27m; Druisheim/67.26–67.31m/2a–b; Druisheim/67.30–67.45; Druisheim/67.30–67.45/3a–c; Druisheim/67.30–67.45/5a, c–e; Druisheim/67.37–67.43; Druisheim/67.37–67.43/b; Druisheim/67.43–67.50/2a–b; Druisheim/67.43–67.50/a–c; Holzheim/40.55–40.60; Holzheim/40.93–41.00; Holzheim/41.00–41.11/a–b (see Pippèrr and Reichenbacher, unpubl.; deposited in the archive of the Landesamt für Umwelt, Munich, Germany)

Provenance of type specimens.—Illerkirchberg, Germany (Kirchberg Formation, uppermost lower Miocene, c. 17 Ma).

Diagnosis.—Maximum size 54 mm standard length. T-shaped palatine and six branchiostegals; entopterygoid absent. Vertebrae 26 to 27; 16 to 17 postabdominal. The first dorsal fin comprises six spines, with a gap present between spines V and VI. The second dorsal fin has one spine and nine or ten soft rays. Otoliths have a distinct posterodorsal projection, a small anteroventral projection and a strongly curved posteroventral corner.

General description.—The following description refers to the holotype if not indicated otherwise.

Neurocranium. The frontal bones are narrow anteriorly and wide in the posterior part behind the eye (SMNS 51854). The other bones of the skull roof are crushed and not differentiable. The vomer is the only bone of the ethmoid region that is partially recognizable (SMNS 3466).

Jaws. The premaxilla is slightly bent, the three processes that are usually seen in gobies appear to be present, but in none of the specimens is the premaxilla completely preserved. The toothless maxilla is slender with a pointed posterior tip. The head of the maxilla bears two massive spherical processes. The dentary and the premaxilla bear conical teeth (~0.5 mm long).

Suspensorium and hyoid arches. The T-shaped palatine, with its two processes, is rather gracile (Fig. 2A2), and inserts in the wedge-shaped ectopterygoid about halfway to the quadrate. An entopterygoid is not present. The quadrate is triangular and has a thin posteroventral process (HLMD V 3314). The symplectic is slender and widens dorsally, the metapterygoid is also slender, while the hyomandibular is massive but not well preserved

(MCZ 5211-A). The opercle is triangular with an anterior process and a rounded posterodorsal corner. The anterior rim is thickened. The preopercle is a curved bone with two arms of similar length. The subopercle has a half-moon shape, with an anteroventral hook for insertion of the ventrally pointing tip of the opercle. The interopercle is not recognisable. The ceratohyal is partly visible; other bones of the hyoid apparatus are crushed. There are six branchiostegals (MCZ 5211-B, Fig. 2B). Their articulation cannot be seen clearly, but seems to be the same as in *E. brevis*.

Branchial arches. The basihyal is triangular (HLMD V 3314) and neither the urohyal nor any of the branchial arches is preserved.

Vertebral column. Vertebrae 26 to 27; 16 to 17 are postabdominal. The first vertebrae are slightly shorter than the succeeding ones. At least seven pairs of slender ribs and slender epineurals (sensu Patterson & Johnson 1995: fig. 6; note that the same bones have also been termed epipleurals in previous works) are present. Supraneurals are absent.

Pectoral girdle and fins. The cleithrum is a splint-like and almost straight bone, with its lower third curving slightly anteriorly. Its dorsal tip is forked. The remaining bones in the pectoral girdle are not preserved. There are at least 15 or 16 pectoral fin rays.

Pelvic girdle and fins. Only the tips of the basipterygia are visible. The pelvic fins, each consisting of five soft rays and one spine (HLMD V 3314), are inserted beneath the pectoral fins.

Dorsal fins. The first dorsal fin is located at the level of the fourth abdominal vertebra. It has six spines. The first five are equidistant from one another, while the last is separated from the preceding by twice that distance. They are supported by one pterygiophore each.

The second dorsal fin inserts above vertebra 10/11 and has one spine and nine to ten rays (MCZ 5211A/B).

Anal fin. The anal fin inserts beneath vertebra 12. It seems to have ten or eleven rays (MCZ 5211A/B). The first ray should be a spine, but this is not unambiguously clear in the fossils.

Caudal endoskeleton and fin. The caudal fin has 13 branched segmented soft rays and one or two upper and lower unbranched and segmented soft rays. It has about seven short, unbranched and unsegmented rays dorsally and ventrally. The caudal skeleton appears to be similar to that of *E. brevis*, but the number of epurals (one or two) cannot be decided due to the poor preservation (Fig. 3B).

Scales. The body scales are ctenoid and have about 9–11 radii. They correspond to the Pe-type of Roberts (1993) as described above.

Otoliths. The saccular otoliths are rectangular to rhomboid in shape (otolith length 1.49–2.51 mm, otolith height: 1.03–1.58 mm, sulcus length: 0.68–1.09 mm; l/h-index: 1.15–1.59). The dorsal rim is convex. The anterodorsal angle (> 90°) is rounded and the steep anterior rim ends in a small anteroventral projection. The ventral rim is more or less straight; the posteroventral transition is rounded, not pronounced and is followed by a short posterior rim, which is dominated by the large pointed projection in the upper third. The dorsal area is about two-thirds the length of the sulcus. The sulcus is shaped like the sole of a shoe; the rounded cauda is shorter than the anteriorly pointed (sometimes rounded) ostium. The crista superior is present and shows a constant width. The crista inferior is not recognisable. The ventral line is prominent and extends more than ¾ of the otolith length. The two smaller otolith types (utricular and lagenar otoliths) are not preserved.

The degree of otolith variability in this species is very high (see Fig 5A–P). Even in a single specimen (holotype, NMW 1857 XXVII 7) variability between the two otoliths can be high, e.g. one otolith can have a straight anterior rim (and look smoother overall), the other can have a waved one; projections can also be developed to different degrees.

Further distribution.—The species appears to be restricted to the Kirchberg Fm and equivalent strata in the South German Molasse Basin (see Discussion).

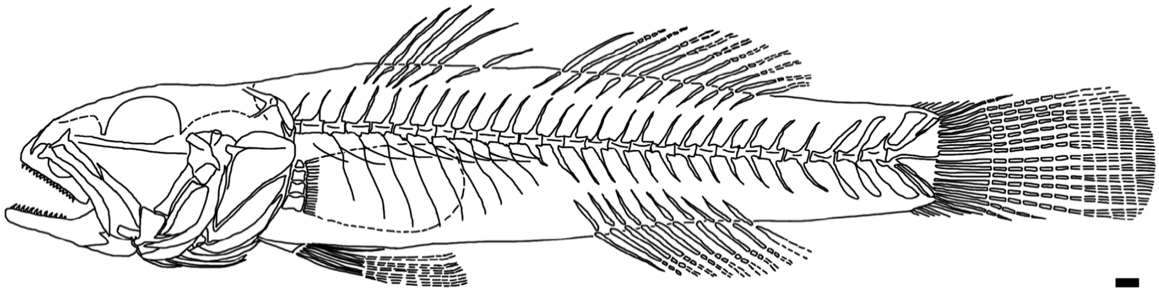

Fig. 4. Generalized schematic reconstruction of *Eleogobius gaudanti* n.sp. Note that this image should not serve as basis for pterygophore formula calculation or other quantitative estimations (all countable characters of the fossil specimens are provided in Table 1). Scale bar: 1 mm.

Comparisons of skeletons.—*Eleogobius brevis* and *E. gaudanti* can both be separated from *Gobius multipinnatus* by their more compact shape, smaller second dorsal fin, smaller anal fins, and lower number of vertebrae (Table 1). The first dorsal fin shows a gap between the D1 spines V and VI – a feature that is not found in *G. multipinnatus* (see also Table 2 in Reichenbacher et al. 2007). This gap can also be seen in recent gobies like *G. niger*, *Pomatoschistus minutus* and species of *Bathygobius* (see Lebour 1919; see also Birdsong et al. 1988; Allen and Robertson 1994). Unfortunately, this feature is not mentioned in most studies on fossil Gobiiformes.

Gobius multipinnatus has a T-shaped palatine, but none of the known specimens reveal how many branchiostegals this fossil species possesses. The gap between spines V and VI in the first dorsal fin is absent (as mentioned above) and the otoliths are unknown. It therefore remains within the genus "*Gobius*".

At the level of gross morphology, the two species of *Eleogobius* can perhaps be distinguished from each other by the shorter anal fin of *E. brevis,* but the two differ by only a single ray, and some specimens show overlap in the ray counts (Table 1). In addition, the two species differ slightly in the positions of their first and second dorsal fins: above vertebrae three/four and 10 in *E. brevis*, and above vertebrae four and 10/11 in *E. gaudanti*. Furthermore, the second dorsal fin of *E. brevis* appears to be slightly longer (I+10 to 11 vs. I+9 to 10 rays) than that of *E. gaudanti* (Table 1). With respect to their morphometric profiles, the two species are very similar (Table 2), but the head of *E. brevis* appears to be somewhat larger (33–35% of standard length vs. 27–32%) and more robust than that of *E. gaudanti*. The lack of other clear differences between the two fossil species in meristic or morphometric characters is a feature that also complicates the recognition of many of the recent goby species, which can often be discriminated only on the basis of sensory papillae patterns or scale counts (e.g. Miller 1998). Scale counts were not possible in the available fossil specimens.

Table 1. Meristic counts compiled from the original descriptions and counts of this study. D1 and D2 = first and second dorsal fin, A = anal fin, C = caudal fin, V = ventral fins, P = pectoral fins.

Ref./Taxon	**locality**	**D1**	**D2**	**A**	**C**	**V**	**P**	**Vertebrae[postabd]**
Agassiz (1839)/ *Gobius brevis*	Öhningen	6	2,I,10	2,I,8	5,I,8,7,I,5	5	many	12+15+1
Reichenbacher et al. (2007)/ *G. brevis*	Edenkoben	VI*	I+10–11	I+8	6/6	–	~15–16	28–29[(16)17]
Reichenbacher et al. (2007)/ *G. brevis*	Öhningen	VI*	I+9–10	I+7–8	–	–	–	27–28[15–17]
Reichenbacher et al. (2007)/ *G. brevis*	Eibiswald	VI*	I+9–11	I+8–9	–	–	–	26–27[16]
Brzobohatý and Gaudant (2009)/*G.* brevis	Závod-72	VI*	I+(8)9(10)	I+7–8	10,12–13,10	I+5	~15	27–28[15–16]
Brzobohatý and Gaudant (2009)/*G. brevis*	Illerkirchberg	VI	–	I+8	–	–	–	27–28[16]
this study/ *Eleogobius brevis*	Öhningen	VI*	I+10–?11	I+?8–9	13–?14	I+5	15–?17	27–?29[?16–?18]
this study/ *E. brevis*	Edenkoben	VI*	I+10–11	?9–?11	13–?14	6	16–?18	27–?29
v. Meyer (1852)/ *Cottus brevis* Ag.?	Illerkirchberg	–	–	–	–	6	10	28

this study/ *E. gaudanti*	Illerkirchberg	VI*	I+9–?10	?I+9–?10	?13	I+5	15–16	26–27[15–?17]
v. Meyer (1856)/ *Gobius*?	Illerkirchberg	6	I+9	10	–	5–?7	11–12	26[15]
v. Meyer (1852)/ *G. multipinnatus*	Illerkirchberg	5 or 6	13 or 14	12	dorsal part 9 or 10	–	> 8	10+18
Reichenbacher et al. (2007)/ *G. multipinnatus*	Illerkirchberg	VI	I+12–13	I+10(11)	–	–	–	29–30[18]
this study/ *G. multipinnatus*	Illerkirchberg	VI	I+13	I+12	12–13	> 4	18?	29[18]
Reichenbacher et al. (2007)/ *Gobius* sp.	Rieskrater lake.	VI–VII*	I+11	I+9	–	–	–	27[15]

Table 2. Morphometrics of four specimens with percentages relating to standard length in brackets. For abbreviations see Table 1.

	NMB On. 19 (*E. brevis*)	MCZ5211-A (*E. gaudanti*)	NMW 1857 XXVII 7 (*E. gaudanti*)	SMNS 3686/4 (*G. multipinnatus*)
Total length	40.5	38.1	33.3	95.7
Standard length	33	32.4	27.9	80.1
Body height	6.5 (20%)	9.2 (28%)	5.8 (20%)	15.0 (19%)
Head length	11.4 (35%)	9.1 (28%)	8.7 (31%)	22.3 (28%)
Head height	8.3 (20%)	8.1 (25%)	> 4.0	17.6 (22%)
Horizont. eye dia.	2.4 (7%)	1.8 (6%)	1.8 (6%)	-
Distance to D1	13.4 (41%)	10.1 (31%)	10.6 (38%)	27.9 (35%)
Distance to D2	18.7 (57%)	17.5 (54 %)	15.6 (56%)	45.4 (57%)
Distance to A	22.2 (67%)	18.9 (58%)	17.8 (64%)	52.1 (65%)
Distance to P	12.4 (38%)	9.8 (30%)	8.4 (30%)	25.7 (32%)
Distance to V	-	11.1 (34%)	9.4 (34%)	-
Length of D1	3.9 (12%)	> 2.7	-	8.5 (11%)
Length of D2	4.0 (12%)	> 3.2	> 1.2	9.8 (12%)

Length of A	4.3 (13%)	> 3.3	> 1.2	> 5.5
Length of P	7.9 (24%)	> 2.6	2.9 (10%)	-
Length of V	-	> 6.1	4.0 (14%)	-
Length of C	7.8 (24%)	> 5.1	5.7 (20%)	16.4 (20%)
D1 base length	2.8 (8%)	4.2 (13%)	3.0 (11%)	7.4 (9%)
D2 base length	7.1 (22%)	6.1 (19%)	4.9 (18%)	21.3 (27%)
A base length	4.3 (13%)	4.6 (14%)	3.7 (13%)	14.0 (17%)
Length caudal peduncle	6.8 (21%)	8.7 (27%)	6.4 (23%)	15.9 (20%)
D2C (see text)	5.6 (17%)	7.5 (23%)	5.2 (19%)	9.6 (12%)
Min. hight caud. Peduncle	3.2 (10%)	2.7 (8%)	2.4 (9%)	8.5 (12%)
Length of vertebrae	0.74–0.9 (2.2–2.7%)	0.77–1.05 (2.3–3.2%)	0.69–0.91 (2.5–3.3%)	1.78–2.06 (2.2–2.6%)

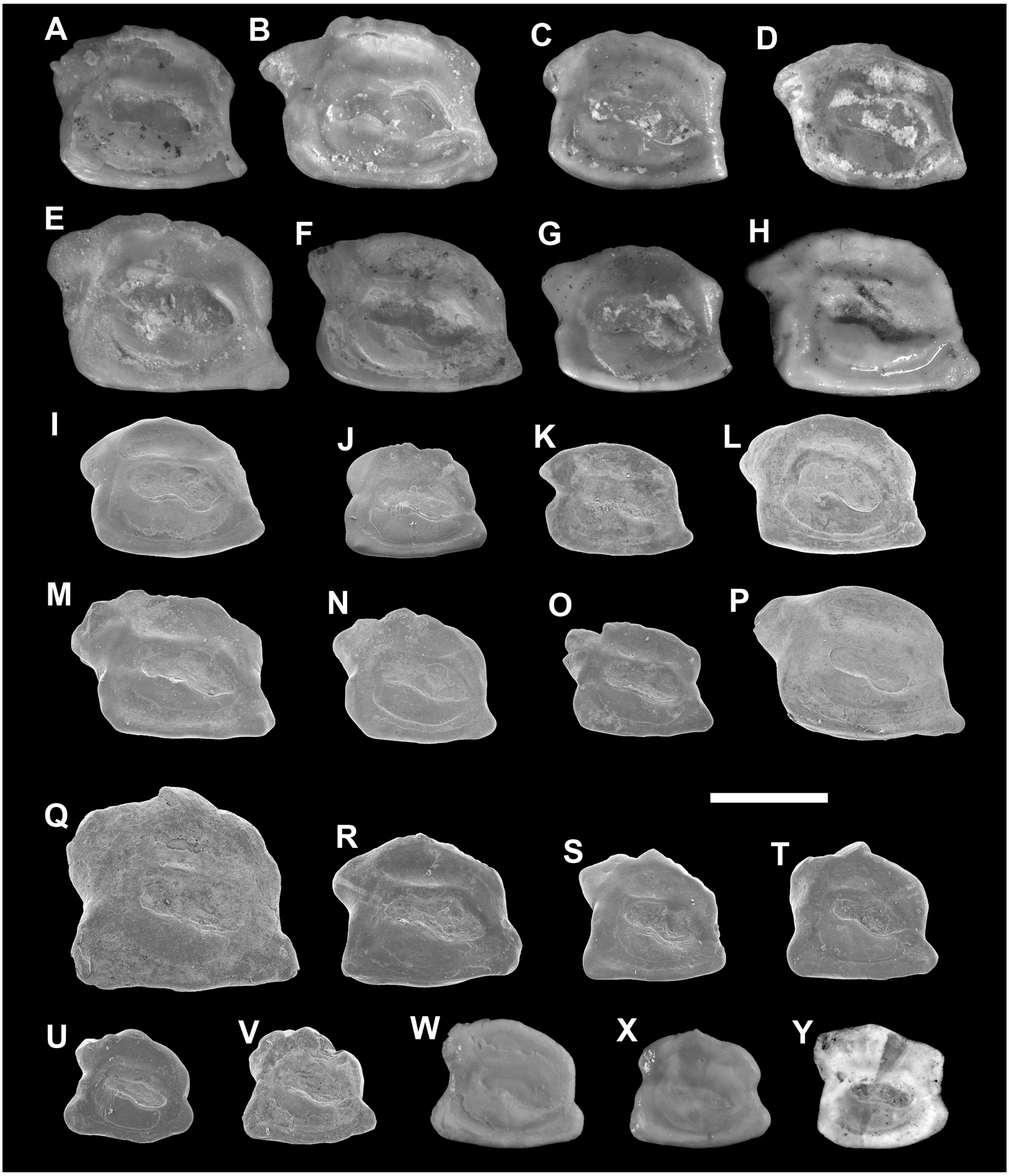

Fig. 5. A–P. Otoliths of *Eleogobius gaudanti*. **A:** Druisheim/67.43–67.50/a. **B:** Druisheim/67.30–67.45/5d. **C:** Druisheim/67.30–6745/5a. **D:** Druisheim/67.30–67.45/5c (mirrored). **E:** Druisheim/67.30–67.45/3c. **F:** Druisheim/67.43–67.50/c. **G:** Druisheim/67.43–67.50/2a. **H:** HLMD V 3314b (mirrored). **I:** Druisheim/67.26–67.31/2a (SEM image). **J:** Druisheim/67.37–67.43/b (SEM image). **K:** Druisheim/67.37–67.43 (mirrored, SEM image). **L:** Holzheim/41.00–41.11/a (SEM image). **M:** Druisheim/67.26–67.31/2b (mirrored, SEM image). **N:** Holzheim/40.55–40.60 (mirrored, SEM image). **O:** Druisheim/67.43–67.50/b (mirrored, SEM image). **P:** Druisheim/67.30–67.45 (SEM image). **Q–Y.** Otoliths of *Eleogobius brevis*. **Q:** StMartin_Z1_a (SEM image). **R:** StMartin_Z2_a (SEM image). **S:** StMartin_Z2_d (mirrored, SEM image). **T:** StMartin_Z1_d (mirrored, SEM image); **U:** StMartin_Z1_e (mirrored, SEM image). **V:** StMartin_Z2_f (mirrored, SEM image). **W:**

StMartin_Z2_c. **X:** StMartin_Z2_b. **Y:** In situ from specimen BSPG 2007 III 4; also depicted in Reichenbacher et al. (2007). Scale bar: 1 mm.

Comparisons of otoliths.—The otoliths attributed to "*Gobius multipinnatus*" by Weiler (1955) clearly belong to the new species *Eleogobius gaudanti*. The otoliths of the true *Gobius multipinnatus* remain unknown.

The otoliths of *Eleogobius brevis* and *E. gaudanti* can be distinguished by their overall shape, which is rectangular (almost quadratic) in *E. brevis* and rhomboid in *E. gaudanti*. Furthermore, otoliths of *E. brevis* display a small posteroventral projection that is usually lacking in *E. gaudanti*. The sulcus of *E. brevis* has a rounded tip, whereas the sulcus in the otoliths of *E. gaudanti* generally shows a rather pointed tip. Additionally, *E. gaudanti* normally lacks a posteroventral projection and displays a well-rounded posteroventral transition. The otoliths are well defined and clearly distinguishable from virtually all other otoliths of gobies (see Reichenbacher 1993; Reichenbacher et al. 2007); the exception is dealt with in the Discussion. In addition, the recently described otolith-based species *Gobius reichenbacherae* Schwarzhans, 2014 and *Gobius mustus* Schwarzhans, 2014 from the middle Miocene of Turkey show some similarities to *E. gaudanti* in their overall form (see Schwarzhans 2014, pls. 8–9). However, both species display a subcaudal iugum (not present in *E. gaudanti*), *G. reichenbacherae* usually bears a broader posterodorsal projection and the otoliths of *G. mustus* are relatively higher and thicker than those of *E. gaudanti*. It should be mentioned here that Schwarzhans (2014) illustrates an otolith from the middle Miocene of Slovakia (Central Paratethys) as *Gobius multipinnatus*, but this otolith does not correspond to *G. multipinnatus* as identified by Weiler (1955, 1966) and thus not to *E. gaudanti*.

Etymology.—In honour of Jean Gaudant (Paris, France), in recognition of his outstanding contribution to our knowledge of fossil fishes from the European Paleogene and Neogene.

2.5 Discussion

The two gobiiform species initially described by H. v. Meyer (1852) as "*Cottus brevis* Ag.?" and "*Cottus* (?) *multipinnatus* Myr." were synonymized as *Gobius multipinnatus* by Woodward (1901) and Weiler (1955), because these authors were unable to discern any difference between them. Our study indicates, however, that the two forms do not belong to a single species and that "*Cottus brevis* Ag.?" represents a new species. Moreover, the otoliths ascribed to "*G. multipinnatus*" by Weiler derive from the new species, while those of the true *Gobius multipinnatus* remain to be explored.

***The problem of* "Gobius pretiosus *Prochazka, 1893*".**—The otolith-based species *Gobius pretiosus* Procházka, 1893 is similar in appearance to *E. gaudanti*. This species was originally described from the middle Miocene marine deposits of Nussdorf and Neudorf (Vienna Basin, Austria) and of Seelowitz (Czech Republic). Procházka (1893) depicted a single specimen from Seelowitz, but this type specimen is unfortunately lost. Moreover, in his description of this species, Procházka stated that the anterior rim of the otolith is more strongly incised than the posterior, and that the posteroventral and anterodorsal parts are extended and bluntly pointed. In fact, Procházka's figure shows that the latter applies to the anteroventral and posterodorsal parts, and this part of the description is obviously erroneous.

Since then, several descriptions of *Gobius pretiosus* have been provided, but none has referred to specimens from the type locality Seelowitz. Schubert (1906) described *Gobius pretiosus* as having anteroventral and posterodorsal projections, and in some specimens, he observed a dorsal broadening of the ostial part of the sulcus. He mentioned the presence of this species at several sites, including Kienberg (Czech Republic) and Vöslau (Vienna Basin), but the specimens he illustrated all originate from Vöslau. Later, Nolf (1981) remarked that the otoliths published by Schubert as *G. pretiosus* from Vöslau do not belong to the species depicted by Prochazka, and can be tentatively assigned to the Recent species *G.* aff. *geniporus* Valenciennes, 1837. Further reports of *G. pretiosus* refer to the brackish deposits of the late Early Miocene of Illerkirchberg and Eibenschitz (Czech Republic) (Weiler 1955, 1966). Weiler (1955) considered *G. pretiosus* as a junior synonym of his *G. multipinnatus* (now *Eleogobius gaudanti*) because he could detect no diagnostic differences between the otoliths. In 1966, he changed his mind on this point, stating that the otoliths of *G. pretiosus* have a greater length/height index (l/h) compared to *E. gaudanti*. Other authors (e.g. Brzobohatý 1969; Śmigielska 1973; Martini 1983; Reichenbacher 1988) have followed Weiler in recognising *G. pretiosus* as a separate species on the basis of this difference. However, the actual l/h values given in Weiler (1955) and Weiler (1966) do not support the contention that this parameter differs between so-called *G. pretiosus* (l/h 1.33–1.56) and *E. gaudanti* (1.35–1.53). Moreover, the otoliths described as *G. pretiosus* by Weiler (1966) and also by Brzobohatý (1969) are not from the type locality Seelowitz, but from Eibenschitz and may actually derive from *E. gaudanti* (termed *G. multipinnatus* by Weiler) – which would explain why they are morphometrically indistinguishable from those of the latter.

As mentioned above, Schubert (1906) remarked on the presence of *G. pretiosus* at several localities, including Kienberg. Otoliths from Kienberg have subsequently been published and assigned to *Gobius multipinnatus* by Brzobohatý et al. (2007), but these authors did not comment on whether these specimens might be attributable to *G. pretiosus*. Our examination of 14 otoliths from the Kienberg location clearly indicates that two types of otoliths are present among specimens of "*G. multipinnatus*" sensu Brzobohatý et al. (2007) (Fig. 6).

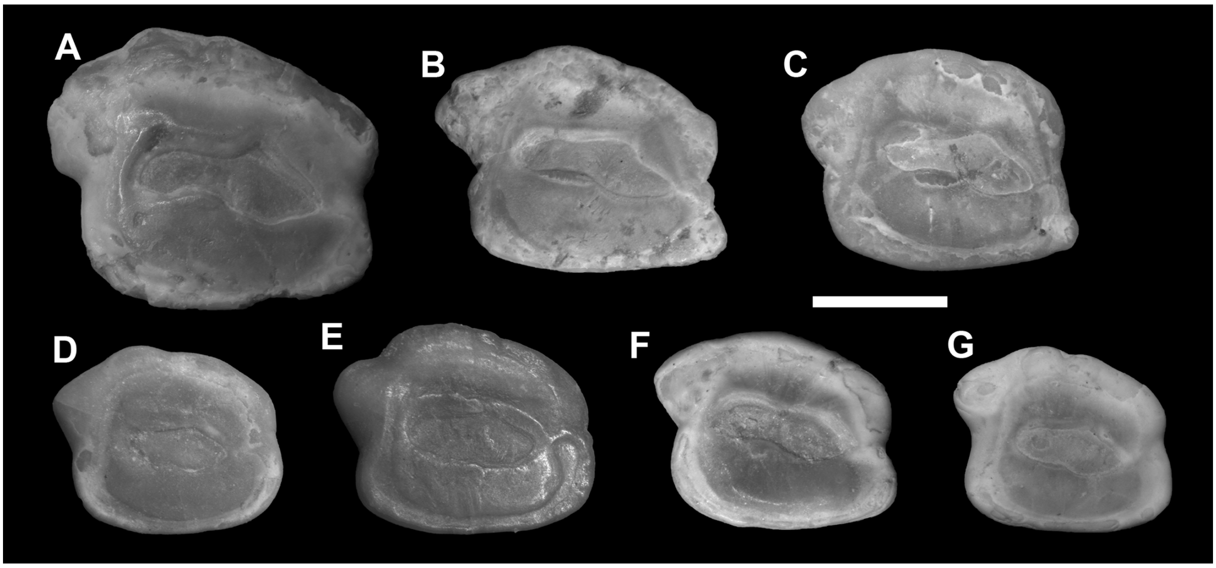

Fig. 6. Otoliths from Kienberg. **A:** Kienberg_Gp_1. **B:** Kienberg_Gp_7. **C:** Kienberg_Gp_11 (mirrored). **D:** Kienberg_Gp_14 (mirrored). **E:** Kienberg_Gp_13 (mirrored). **F:** Kienberg_Gp_5. **G:** Kienberg_Gp_9 (mirrored). Scale bar: 1 mm.

They are differentiated mainly by their sulcus, which is rather long and slender in one type (see here Figs. 6A–C, see also Brzobohatý et al. 2007, pl. 8, fig. 7) and compressed in the other (see here Figs. 6D–G, see also Brzobohatý et al. 2007, pl. 8, fig. 6, 8). The otolith length/sulcus length ratios support this observation, ranging from 1.81 to 1.92 (n = 5) for the elongate type, and from 2.03 to 2.35 (n = 9) for the stubbier form. The two types are similar in outline, although the type with the compressed sulcus has a slightly more rounded overall contour. These two types probably correspond to different genera, because sulcus differences are usually significant on a higher taxonomic level. The type with the compressed sulcus is clearly differentiated from *Eleogobius gaudanti* because of its more rounded overall outline and the aforementioned compression of the sulcus. The second type shows a more prominent posterodorsal projection compared to *E. gaudanti*, a clear subcaudal iugum (essentially a short crista inferior, see Schwarzhans 2014, fig. 2 for definition), which is absent in *E. gaudanti*, and the upper rim of the ostium displays a ventrally directed indentation that is not present in *E. gaudanti*. Based on these differences, we cannot follow Brzobohatý et al. (2007) in recognising *G. multipinnatus* (now *E. gaudanti*) at Kienberg. Moreover, if the true *G. pretiosus* is indeed present in the Kienberg material (as indicated by Schubert 1906), it should be one of the two types described above, and therefore is not conspecific with *E. gaudanti*. We refrain from describing the two taxa from Kienberg in the present paper until our re-examination of these types of otolith from other localities is complete.

In summary, due to the uncertainties in the description by Procházka (1893), and because no further specimens of *G. pretiosus* from the type locality are known, the status of *Gobius pretiosus* is unclear. This species is therefore considered here as a nomen dubium pending the (admittedly unlikely) rediscovery of the type material.

Gobiiform affiliation.—The species of *Eleogobius* can be assigned to the Gobiiformes based on several characters (as compiled by Wiley and Johnson 2010). Hypurals one and two are fused. Hypurals three and four are fused to each other and to the terminal centrum (Johnson and Brothers 1993). The parietals are absent (Regan 1911 and others) as are the supraneurals (Springer 1983; Johnson and Brothers 1993).

Whether the dorsalmost pectoral ray articulates with the dorsalmost radial cartilage rather than with the scapula (Johnson and Brothers 1993) could not be decided without a doubt due to the state of preservation of the fossils.

Additionally, together with the preopercle and symplectic, the quadrate forms the ventral rim for the non-ossified area below the hyomandibular, a feature that is a synapomorphy for Gobiiformes ("gap between symplectic and preopercle"; Johnson and Brothers 1993).

Family affinities.— The combination of six branchiostegals, a T-shaped palatine and no entopterygoid seen in the new genus *Eleogobius* is, to the best of our knowledge, unique among the Gobiiformes.

The Gobiidae as defined by Gill and Mooi (2012) are characterised by (among other features) the possession of five branchiostegals. All other Gobiiformes have either six (Rhyacichthyidae, Odontobutidae, Butidae, Eleotrididae, Milyeringidae, Thalasseleotrididae) or seven (†Pirskeniidae). Přikryl (2014) stated that the rare occurrence of seven branchiostegals in recent gobioid specimens, as reported by Akihito (1969) for a single specimen of *Odontobutis obscura*, might be a throwback.

The shape of the palatine was recognized long ago as a convenient character that distinguishes gobiids from eleotridids (Regan 1911). Gobiids have a T-shaped and eleotridids an L-shaped palatine. However, Gosline (1955) and later Hoese (1984) and Harrison (1989) cast doubt on this view, asserting that some gobiids have an L-shaped palatine. Furthermore, Harrison (1989) emphasised the presence of the L-shaped palatine in the eleotridids/butids. However, the latter assumption might also be erroneous, because Akihito (1969) illustrated some members of the eleotridids/butids with a palatine that more closely resembles a T than an L. Moreover, at least one of the representatives of the Thalasseleotrididae, *Grahamichthys radiata*, has an L-shaped palatine (unpublished data).

An additional apomorphy for *Eleogobius*, and also for the Gobiidae, is the absence of the entopterygoid. This bone is present in almost all basal Gobiiformes, and was used by Regan (1911) to differentiate between his Eleotrididae and Gobiidae. However, some exceptions are known. The Xenisthminae (which belong to the Eleotrididae; see Thacker 2009; Agorreta et al. 2013) also lack the entopterygoid (Akihito 1986), but they differ from the new genus in the specialized shape of their palatine (broadened and with forked tip) (Springer 1983). The family Thalasseleotrididae likewise lacks the entopterygoid, but apparently has an L-shaped palatine (see above). This family cannot be recognised in the fossil record to date, because it is defined by a single synapomorphy that concerns the soft tissue (see Gill and Mooi 2012).

The pterygiophore formula of the first dorsal fin can also be of taxonomic value within the Gobiiformes (see Birdsong et al. 1988), but the poor state of preservation of the fossils studied here does not allow it to be determined accurately.

The ratio between the length of the base of the second dorsal fin and the distance from the end of D2 to the base of the caudal fin ($\overline{\mathrm{D2C}}$) has been used as additional character to distinguish eleotridids/butids from gobiids in the key published by Larson et al. (2001). In gobiids the base of the second dorsal fin is usually longer than $\overline{\mathrm{D2C}}$; note that $\overline{\mathrm{D2C}}$ is not the same as the caudal peduncle length because it extends to the first procurrent ray, and not to the end of the caudal plate (see Larson et al. 2001, p. 3574). However, for sand gobies, this character may not apply because they have a very long caudal peduncle. In eleotridids/butids the D2 base is usually equal to or shorter than $\overline{\mathrm{D2C}}$ according to Larson et al. (2001). This is usually true for *E. brevis* and *E. gaudanti* also, which may perhaps indicate a relatively close relationship of *Eleogobius* to the Eleotrididae/Butidae. However, some specimens of *E. brevis* from Öhningen show the gobiid state, suggesting that this character may not be so definitive after all. Among the fossil skeletons from Illerkirchberg in which this character could be measured, only the specimen SMNS 3686/4, which represents a "true" *Gobius multipinnatus*, shows the "gobiid" state.

The caudal skeleton conforms to previous descriptions of gobiiform fishes (e.g. Hoese and Gill 1993). *Eleogobius brevis* has a single epural, and *E. gaudanti* probably has a single epural as well. The number of epurals may hint at family affiliation, frequently being one in Gobiidae and two in Eleotrididae and Butidae, although many exceptions are known (Birdsong et al. 1988; Hoese and Gill 1993).

Furthermore, the shape of the basihyal may be linked to the phylogenetic position of a gobiiform species. According to Takagi (1950), a staff-like shape is primitive, a closed triangle intermediate and a very broad fan (more than 90°) or a Y/V shape is more derived. The basihyal in the two species of *Eleogobius* (closed triangle or V) conforms to the intermediate state sensu Takagi (1950).

The problem with all these characters is their weighting. Which character is the most informative? Bearing in mind the most recent definition of Gill and Mooi (2012), recognition of the Gobiidae and the basal Gobiiformes (Eleotrididae, etc.) is based on, among other features, the numbers of branchiostegals (5 vs. 6). Accordingly, this character allows one to separate the Gobiidae from the remaining gobiiform families, whereas exceptions have been reported for the other characters (palatine, entopterygoid, number of epurals). Until phylogenetic analyses based on fossil Gobiiformes are available it seems reasonable to conclude that *Eleogobius* should be placed nearer the non-gobiid Gobiiformes than the gobiids.

***Other fossil species that may belong to* Eleogobius.**—Unlike *Lepidocottus aries* and *L. gracilis, L. papyraceus* (Agassiz, 1839) from the type locality Monteviale (Italy) shows a T-shaped palatine (Gaudant 1978). In the original description Agassiz mentioned six or seven branchiostegals, whereas Gaudant (1978) could not identify the precise number due to poor preservation. The first character (the T-shaped palatine) contradicts its assignment to the genus *Lepidocottus* by Gierl et al. (2013), although the otolith shape is similar to that of *L. aries*. This species may instead represent *Eleogobius*, but requires further investigation. *Gobius* sp. (Gaudant, 2010) from the Fohnsdorf Basin (middle Miocene) shares with *Eleogobius* spp. the gap before the last spine in the first dorsal fin, but the palatine shape and the number of branchiostegals could not be recognized in this fossil.

Gobius sp. from the Pleistocene of Italy (Barrier et al. 1993) has a T-shaped palatine, but the number of branchiostegals is not mentioned. The otoliths in situ have a fissured outline unlike those of the two *Eleogobius* species.

In other descriptions of fossil Gobiiformes the shape of the palatine or the number of branchiostegals is not mentioned, probably because these features could not be observed. In some species such as *Gobius francofurtanus* Koken, 1891 the “gobiid-state” of a T-shaped palatine and five branchiostegals is present (unpublished data).

The diversity of fossil gobies is definitely greater than the present state of our knowledge suggests. This study once more demonstrates that gobies are very difficult to discriminate and confidently assign. Future work will review further fossil species and may give us a clearer picture of the diversity of this group in the past, which can also clarify our understanding of recent relationships.

ACKNOWLEDGEMENTS

We would like to thank L. Costeur (NMB), R. Böttcher (SMNS), E. Maxwell (SMNS), N. Micklich (HLMD), U. Göhlich (NMW) and J. Cundiff (MCZ) for the loan of specimens required for this work, and I. Zorn (Geol. Bundesanstalt Wien) for informing us that the type specimen of *Gobius pretiosus* Prochazka, 1893 must be considered as lost.

2.6 References

Agassiz, L. 1832. Untersuchungen über die fossilen Süßwasser-Fische der tertiären Formationen. *Jahrbuch für Mineralogie, Geognosie, Geologie und Petrefaktenkunde* 3: 129–138.

Agassiz, L. 1833–1843. *Recherches sur les Poissons fossiles. Tome IV*. Neuchâtel: Petitpierre.

Agorreta, A., D. San Mauro, U. Schliewen, J.L. Van Tassell, M. Kovačić, R. Zardoya, and L.

Rüber. 2013. Molecular phylogenetics of Gobioidei and phylogenetic placement of European gobies. *Molecular Phylogenetics and Evolution* 69 (3): 619–633.

Akihito. 1969. A systematic examination of the gobiid fishes based on the Mesopterygoid, Postcleithra, Branchiostegals, pelvic fins, Scapula, and Suborbital. *Japanese Journal of Ichthyology* 16 (3): 93–104.

Akihito. 1986. Some morphological characters considered to be important in gobiid phylogeny. In *Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fishes*, ed. K. Matsuura, 629–639. Tokyo: Ichthyological Society of Japan.

Allen, G.R., and D.R. Robertson. 1994. *Fishes of the tropical eastern Pacific*. Honolulu: University of Hawaii Press.

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

Barrier, P., J. Gaudant, F. Raisson, D. Merle, and M. Toumarkine. 1993. La lagune Pléistocène à *Gobius* sp. du Monte Torre (Calabre méridionale, Italie): signification Paléogéographique. *Rivista Italiana di Paleontologia e Stratigrafia* 99 (1): 127–140.

Birdsong, R.S., E.O. Murdy, and F.L. Pezold. 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.

Brzobohatý, R. 1969. Die Fischfauna des südmährischen Untermiozäns. *Folia Facultatis Scientiarum Naturalium Universitatis Purkynianae Brunensis Geologia* 10 (1): 1–49.

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

Brzobohatý, R., and J. Gaudant. 2009. *Gobius brevis* (Agassiz, 1839), a gobiid fish with otoliths in situ (Pisces, Teleostei) in the Karpatian (Lower Miocene) of the Vienna Basin. *Annalen des Naturhistorischen Museums in Wien - Serie A (Mineralogie und Petrographie, Geologie und Paläontologie, Archäozoologie, Anthropologie und Prähistorie)* 111: 245–255.

Brzobohatý, R., D. Nolf, and O. Kroupa. 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., B. Reichenbacher, and R. Gregorová. 2003. Teleostei (Otoliths, Skeletons with Otoliths in situ) from the Karpatian of the Central Paratethys. In *The Karpatian - a Lower Miocene Stage of the Central Paratethys*, eds. R. Brzobohatý, I. Cicha, M. Kováč, and F. Rögl, 265–280. Brno: Masaryk University.

Carnevale, G., W. Landini, and G. Sarti. 2006. Mare versus Lago-mare: marine fishes and the

Mediterranean environment at the end of the Messinian Salinity Crisis. *Journal of the Geological Society* 163 (1): 75–80.

Fricke, R. 1983. A method for counting caudal fin rays of actinopterygian fishes. *Braunschweiger Naturkundliche Schriften* 1 (4): 729–733.

Gaudant, J. 1978. Nouvelles observations sur les poissons oligocènes de Monteviale (Vicenza - Italie). *Memorie di Scienze Geologiche* 32: 1–9.

Gaudant, J. 1980. Mise au point sur l'ichthyofaune miocène d'Öhningen (Baden, Allemagne). *Comptes Rendus des Séances de l'Académie des Sciences - Série D (Sciences naturelles)* 291: 1033–1036.

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 II, Sciences de la terre et des planètes* 322 (1): 71–76.

Gaudant, J. 2000. Nouvelles recherches sur l'ichthyofaune lacustre du Karpatien inférieur d'Eibiswald et observations sur quelques os isolés de poissons découverts aux environs de Wies (Styrie). *Sitzungsberichte Mathematisch-naturwissenschaftliche Klasse Abt. I Biologische Wissenschaften und Erdwissenschaften* 207: 15–43.

Gaudant, J. 2010. A short account on the Middle Miocene fish fauna from the Fohnsdorf Basin (Styria, Austria). *Annalen des Naturhistorischen Museums in Wien - Serie A (Mineralogie und Petrographie, Geologie und Paläontologie, Archäozoologie, Anthropologie und Prähistorie)* 112: 471–488.

Gierl, C., B. Reichenbacher, J. Gaudant, D. Erpenbeck, and A. Pharisat. 2013. An extraordinary gobioid fish fossil from southern France. *PLOS ONE* 8 (5): e64117.

Gill, A.C., and R.D. Mooi. 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: 41–52.

Gosline, W.A. 1955. The osteology and relationships of certain gobioid fishes, with particular reference to the genera *Kraemeria* and *Microdesmus*. *Pacific Science* IX: 158–170.

Gregor, H.-J. 1982. *Die jungtertiären Floren Süddeutschlands: Paläokarpologie, Phytostratographie, Paläoökologie, Paläoklimatologie*. Stuttgart: Enke.

Harrison, I.J. 1989. Specialization of the gobioid palatopterygoquadrate complex and its relevance to gobioid systematics. *Journal of Natural History* 23 (2): 325–353.

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

Hoese, D.F., and A.C. Gill. 1993. Phylogenetic relationships of eleotridid fishes (Perciformes, Gobioidei). *Bulletin of Marine Science* 52 (1): 415–440.

Johnson, G.D., and E.B. Brothers. 1993. *Schindleria*: a paedomorphic goby (Teleostei, Gobioidei). *Bulletin of Marine Science* 52 (1): 441–471.

Jost, J., D. Kälin, T. Schulz-Mirbach, and B. Reichenbacher. 2006. Late Early Miocene lake deposits near Mauensee, central Switzerland: fish fauna (otoliths, teeth), accompanying biota and palaeoecology. *Eclogae Geologicae Helvetiae* 99 (3): 309–326.

Kranz, W. 1904. Stratigraphie und Alter der Ablagerungen bei Unter- und Oberkirchberg, südlich Ulm a. D. *Centralblatt für Mineralogie, Geologie, Paläontologie* 1904: 481–502, 528–540, 545–566.

Larson, H.K., E.O. Murdy, and A.C. Gill. 2001. Suborder Gobioidei. In *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*, eds. K. E. Carpenter, and V. H. Niem, 3574–3609. Rome: FAO.

Lebour, M.V. 1919. The young of the Gobiidae from the neighbourhood of Plymouth. *Journal of the Marine Biological Association of the United Kingdom* 12 (1): 48–80.

Martini, E. 1983. Die Fischfauna von Langenau bei Ulm (Unter-Miozän, Ottnang-Stufe). *Stuttgarter Beiträge zur Naturkunde Serie B (Geologie und Paläontologie)* 91: 1–25.

Meyer, H.v. 1851. *Polyptychodon interruptus* im Flammen Mergel bei Goslar; Säugethier-Knochen in Braunkohle der Molasse der Schweitz; mittel-tertiäre Säugethiere und Reptilien-Knochen zu Heslach bei Ulm; über fossile *Emya*- und *Platemys*-Arten; Fische aus dem Tertiär-Thon von Unterkirchberg bei Ulm; *Dadocrinus*, *Nothosaurus* und Fische im Muschelkalke Oberschlesiens. *Neues Jahrbuch für Mineralogie, Geognosie, Geologie und Petrefakten-Kunde* 1851: 75–81.

Meyer, H.v. 1852. Fossile Fische aus dem Tertiärthon von Unter-Kirchberg an der Iller. *Palaeontographica. Beiträge zur Naturgeschichte der Vorwelt* 2 (Dritte Lieferung): 85–113.

Meyer, H.v. 1856. Neuer Beitrag zur Kenntnis der fossilen Fische aus dem Tertiärthon von Unter-Kirchberg. *Palaeontographica. Beiträge zur Naturgeschichte der Vorwelt* 6 (Erste Lieferung): 22–30.

Miller, P.J. 1998. The West African species of *Eleotris* and their systematic affinities (Teleostei: Gobioidei). *Journal of Natural History* 32 (2): 273–296.

Nolf, D. 1981. Révision des Types d'Otolithes de Poissons Fossiles décrits par R. Schubert. *Verhandlungen der Geologischen Bundesanstalt* 1981 (2): 133–183.

Nolf, D., and R. Brzobohatý. 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 (Mineralogie und Petrographie, Geologie und Paläontologie, Archäozoologie, Anthropologie und Prähistorie)* 111: 323–356.

Patterson, C., and G.D. Johnson. 1995. The intermuscular bones and ligaments of teleostean fishes. *Smithsonian Contributions to Zoology* 559: 1–83.

Patzner, R.A., J.L. Van Tassell, M. Kovačić, and B.G. Kapoor eds. 2011. *The biology of gobies*. Enfield, New Hampshire: Science Publishers Inc.

Přikryl, T. 2014. A new species of the sleeper goby (Gobioidei, Eleotridae) from the České Středohoří Mountains (Czech Republic, Oligocene) and analysis of the validity of the family Pirskeniidae. *Paläontologische Zeitschrift* 88 (2): 187–196.

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.

Regan, C.T. 1911. The osteology and classification of the gobioid fishes. *The Annals and Magazine of Natural History [Eighth Series]* 8 (48): 729–733.

Reichenbacher, B. 1988. Die Fischfauna der Kirchberger Schichten (Unter-Miozän) an der Typuslokalität Illerkirchberg bei Ulm. *Stuttgarter Beiträge zur Naturkunde Serie B (Geologie und Paläontologie)* 139: 1–53.

Reichenbacher, B. 1989. Feinstratigraphische Gliederung der Kirchberger Schichten (Unter-Miozän) an der Typuslokalität Illerkirchberg bei Ulm. *Geologica Bavarica* 94: 135–177.

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

Reichenbacher, B., J. Gaudant, and T.W. Griessemer. 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.

Reichenbacher, B., W. Krijgsman, Y. Lataster, M. Pippèrr, C.G.C. Van Baak, L. Chang, D. Kälin, J. Jost, G. Doppler, D. Jung, J. Prieto, H. Abdul Aziz, M. Böhme, J. Garnish, U. Kirscher, and V. Bachtadse. 2013. A new magnetostratigraphic framework for the Lower Miocene (Burdigalian/Ottnangian, Karpatian) in the North Alpine Foreland Basin. *Swiss Journal of Geosciences* 106 (2): 309–334.

Reichenbacher, B., and M. Weidmann. 1992. Fisch-Otolithen aus der oligo-/miozänen Molasse der West-Schweiz und der Haute-Savoie (Frankreich). *Stuttgarter Beiträge zur Naturkunde Serie B (Geologie und Paläontologie)* 184: 1–83.

Roberts, C.D. 1993. Comparative morphology of spined scales and their phylogenetic significance in the Teleostei. *Bulletin of Marine Science* 52 (1): 60–113.

Sabaj Pérez, M.H. ed 2016. *Standard symbolic codes for institutional resource collections in* *herpetology and ichthyology: an Online Reference. Version 6.5 (16 August 2016). Electronically accessible at http://www.asih.org/.* Washington, DC.: American Society of Ichthyologists and Herpetologists.

Sach, V.J., J. Gaudant, B. Reichenbacher, and M. Böhme. 2003. Die Fischfaunen der Fundstellen Edelbeuren-Maurerkopf und Wannenwaldtobel 2 (Miozän, Obere Süßwassermolasse, SW-Deutschland). *Stuttgarter Beiträge zur Naturkunde Serie B (Geologie und Paläontologie)* 334: 1–25.

Salis, K.v. 1967. Geologische und sedimentologische Untersuchungen in Molasse und Quartär südöstlich Wolhusen (Entlebuch, Kt. Luzern). *Mitteilungen der Naturforschenden Gesellschaft Luzern* XXI: 1–106.

Schneider, C.A., W.S. Rasband, and K.W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9 (7): 671–675.

Schubert, R.J. 1906. Die Fischotolithen des österr.-ungar. Tertiärs. III. *Jahrbuch der kaiserlich-königlichen geologischen Reichsanstalt* 56: 623–706.

Schultz, O. 2013. *Catalogus Fossilium Austriae. Band 3 Pisces*. Wien: Verlag der Österreichischen Akademie der Wissenschaften.

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

Śmigielska, T. 1966. Otolity Ryb z Tortonu Południowej Polski. *Roczink Polskiego Towarzystwa Geologicznego - Annales de la Société géologique de Pologne* 36 (3): 205–275.

Śmigielska, T. 1973. Fish otoliths from the Lower Tortonian deposits at Niskowa near Novy Sącz. *Annales de la Société géologique de Pologne* 43 (1): 3–40.

Springer, V.G. 1983. *Tyson belos, new genus and species of Western Pacific fish (Gobiidae, Xenisthminae), with discussions of gobioid osteology and classification*. Washington, D. C.: Smithsonian Institution Press.

Stauber, H. 1939. Erforschungsgeschichte der Öhninger Fundstätten und ihrer Versteinerungen. *Zentralblatt für Mineralogie, Geologie und Paläontologie B* 8: 314–332.

Takagi, K. 1950. On the glossohyal bone of the gobioid fishes of Japan, with some phylogenetic considerations. *Japanese Journal of Ichthyology* 1 (1): 37–52.

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.

Thacker, C.E. 2011. Systematics of Gobiidae. In *The biology of gobies*, eds. R. A. Patzner, J. L. Van Tassell, M. Kovačić, and B. G. Kapoor, 129–136. Enfield, NH: Science Publishers Inc.

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. 1966. Die Fischfauna des Helvets von Ivančice (Eibenschitz) in Mähren. *Paläontologische Zeitschrift* 40 (1/2): 118–143.

Wiley, E.O., and G.D. Johnson. 2010. A teleost classification based on monophyletic groups. In *Origin and phylogenetic interrelationships of teleosts*, eds. J. S. Nelson, H.-P. Schultze, and M. V. H. Wilson, 123–182. München: Verlag Dr. Friedrich Pfeil.

Woodward, A.S. 1901. *Catalogue of the fossil fishes in the British Museum (Natural History), Part 4*. London: Printed by order of the Trustees.

3. Revision of so-called *Pomatoschistus* (Gobiiformes, Teleostei) from the late Eocene and early Oligocene

Christoph Gierl[1], Bettina Reichenbacher[1]

[1]Department of Earth and Environmental Sciences, Ludwig-Maximilians University Munich, Richard-Wagner Straße 10, 80333 Munich, Bavaria, Germany; E-mail: c.gierl@lrz.uni-muenchen.de (send reprint requests to this address); b.reichenbacher@lrz.uni-muenchen.de.

ABSTRACT

The Gobiiformes (Teleostei) are among the most speciose groups of vertebrates, and are found in all aquatic habitats. Identification of extant gobiiform species is primarily based on soft-tissue characters, and their diversity and phenotypic variability often makes species determination difficult. Fossils normally lack soft-tissue features, and secure assignment of fossil Gobiiformes at family and genus levels can be extremely problematical. "*Pomatoschistus bleicheri* (Sauvage)" from the lower Oligocene of Rouffach (France) and "*Pomatoschistus*(?) cf. *bleicheri* (Sauvage)" from the upper Eocene of the Isle of Wight (England) exemplify these difficulties. These finds are of special interest, because they may represent the oldest fossil skeleton-based members of the Gobiidae + Oxudercidae (gobiiforms with five branchiostegals; hereafter 5brG). Re-examination of the type material of those two species now reveals the presence of a premaxilla with a postmaxillary process, which precludes assignment of these fossils to the genus *Pomatoschistus*. Indeed, they do not even belong to the 5brG because they display six branchiostegals. We conclude that the fossils from France and England both belong to †*Paralates* Sauvage. Differences in the shape of the frontal bones and the numbers of caudal fin rays allow us to assign the French material to †*Pa. bleicheri* Sauvage and the English specimens to †*Pa. chapelcorneri* n. sp. Thus the oldest currently known 5brG species based on articulated skeletons is †*Gobius jarosi* Přikryl and Reichenbacher, 2018 from the lower Miocene.

Keywords: fossil gobiiform; new species; Gobiidae; Oxudercidae; branchiostegals.

PLAIN LANGUAGE SUMMARY

Gobiiformes (or 'gobies') belong to the most diverse groups among vertebrates, and are found in both freshwater and marine habitats. However, it is often difficult to distinguish between morphologically similar forms. Moreover, species identification is based mostly on soft-tissue features, such as the pattern of sensory pores on the head, which makes fossil classification extremely challenging. *Pomatoschistus bleicheri* (Sauvage) from the lower Oligocene (~30

Ma old) of Rouffach (France) and *Pomatoschistus*(?) cf. *bleicheri* (Sauvage) from the upper Eocene (~35 Ma old) of the Isle of Wight (England) epitomize this problem. They are of special interest because they are regarded as the oldest fossil representatives of the more advanced gobies (Gobiidae + Oxudercidae). Here, we show that these fossils do not belong to the genus *Pomatoschistus* or indeed to the Gobiidae + Oxudercidae, nor can they be accommodated in any existing family of the "basal gobiiforms". Instead, the fossils from Rouffach and the Isle of Wight can both be placed in the same extinct genus, †*Paralates* Sauvage. Furthermore, differences in the shape of the frontal bones (on the forehead) and numbers of caudal fin rays lead to the conclusion that the material from Rouffach corresponds to †*Paralates bleicheri* Sauvage, while that from the Isle of Wight is assigned to a new species, which we name †*Paralates chapelcorneri* n. sp. Therefore, the oldest known fossil representative of the modern gobies remains †*Gobius jarosi* Přikryl and Reichenbacher, 2018 from the lower Miocene (20.4 Ma–19.1 Ma old) of Moravia (Czech Republic).

3.1 Introduction

Gobiiformes are a group that shows high species diversity in marine, brackish and freshwater habitats (Patzner et al. 2011) with numerous adaptations to varied niches in these environments (e.g. Thacker 2011). Their systematics at the family level was initially based on characteristic suites of morphological characters and has, more recently, been largely confirmed by studies using molecular data (e.g. Miller 1973; Hoese and Gill 1993; Johnson and Brothers 1993; Thacker 2009; Thacker and Roje 2009; Agorreta et al. 2013; Thacker et al. 2015). A simplified phylogeny is given in Figure 1. Note that we use Oxudercidae instead of Gobionellidae because the latter is a junior synonym of the former (Nelson et al. 2016).

The fossil record of the Gobiiformes dates back to the lower Eocene (Gaudant 1996; Bajpai and Kapur 2004; Bannikov and Carnevale 2016). Notably the Eocene skeleton-based fossils have not been assigned to any of the extant families, either owing to incomplete preservation (Gaudant 1996) or because of an unusual character set (Bannikov and Carnevale 2016). Also, the Eocene "gobiid" otoliths described by Bajpai and Kapur (2004) probably do not belong to the Gobiidae but rather represent basal Gobiiformes such as Odontobutidae and Eleotridae (see Gierl et al. 2013). The oldest skeleton-based records currently assigned to Gobiidae are those of "*Pomatoschistus*(?) cf. *bleicheri* (Sauvage, 1883)" from the upper Eocene of the Isle of Wight in England (Gaudant and Quayle 1988) and "*Pomatoschistus bleicheri* (Sauvage, 1883)" from the lower Oligocene of the southern sector of the Upper Rhine Graben in France (Sauvage 1883; Gaudant 1979).

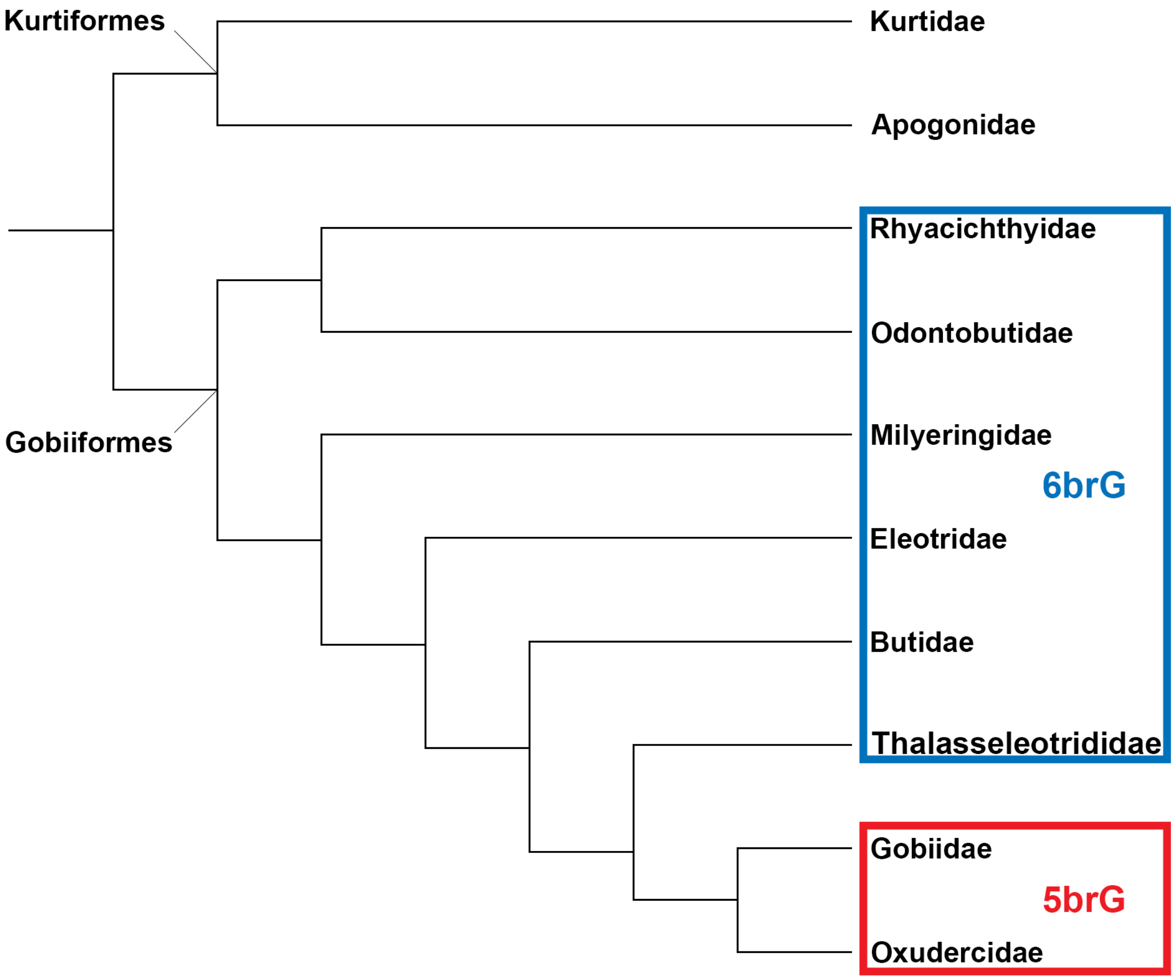

FIGURE 1. Phylogeny of the Gobiiformes according to Thacker et al. (2015) and Nelson et al. (2016). 6brG = gobiiforms with six branchiostegal rays, 5brG = gobiiforms with five branchiostegal rays.

Both have been provisionally assigned to *Pomatoschistus*, as these authors stated. Gaudant (1979) and Gaudant and Quayle (1988) observed certain features of the skeletons that were later recognized as important for distinguishing gobiids and oxudercids from all other gobiiform families (Gill and Mooi 2012) and for discrimination within gobiid and oxudercid groups (e.g. McKay and Miller 1997). The objective of this work is to re-investigate the previously described fossil material in the light of the newer literature and determine whether or not these specimens actually represent species of *Pomatoschistus* and thus a member of the Oxudercidae.

3.2 Materials and methods

The re-investigated specimens of "*Pomatoschistus bleicheri*" comprise seven articulated skeletons and one skull from the quarry at Rouffach (France). This material is kept in the Naturhistorisches Museum Basel (NMB) under the collection numbers NMB Ruf 13_1 (skull), NMB Ruf. 6a/b, NMB Ruf. 9_1, NMB Ruf. 9_2, NMB Ruf. 11, NMB Ruf. 13_2 (counterpart of NMB Ruf. 11), NMB Ruf. 15a/b, NMB Ruf. 18. Additional specimens from Rouffach are curated in the NMB, but their preservation is very poor.

The re-investigated "*Pomatoschistus* cf. *bleicheri*" fossils (n = 5) from the Isle of Wight include all available well-preserved specimens but one, which is in a private collection (see Gaudant and Quayle 1988, p. 31). Three of these specimens are deposited in the Natural History Museum, London (NHMUK, previously BMNH) under the numbers NHMUK PV P 59784, NHMUK PV P 59785 (plus 59785_counterpart) and NHMUK PV P 59786, further poorly preserved specimens are NHMUK PV P 59774, NHMUK PV P 59775, NHMUK PV P 59776, NHMUK PV P 59777, NHMUK PV P 59787, NHMUK PV P 59788, NHMUK PV P 59797. The other two well-preserved specimens are kept in the Sedgwick Museum of Earth Sciences, Cambridge (SM) as SM C.23632 and SM C.23633.

Meristic and osteological characters of the skeletons were studied under a stereomicroscope equipped with a digital camera. Counts of rays in the caudal fin follow Fricke (1983). Counts of vertebrae include the uroterminal centrum. Measurements were taken with ImageJ v1.51a 64-bit (Schneider et al. 2012) based on the digital images. Unless otherwise indicated, pictures were taken by the first author and the figures were prepared using Adobe Photoshop CS6 (13.0.6) 64-bit.

Institutional abbreviations: NHMUK (Natural History Museum, London), NMB (Naturhistorisches Museum Basel), SM (Sedgwick Museum of Earth Sciences, Cambridge). Other abbreviations: 6brG for gobiiforms with six branchiostegal rays; 5brG for gobiiforms with five branchiostegal rays; D2C for the distance between the end of second dorsal fin and the first dorsal (procurrent) ray of the caudal fin.

3.3 Systematic palaeontology

Taxonomic classification follows Nelson et al. (2016).
Series PERCOMORPHA sensu Johnson and Patterson, 1993
Subseries GOBIIDA Nelson et al., 2016
Order GOBIIFORMES Günther, 1880
Family incertae sedis

Genus †*Paralates* Sauvage, 1883

Emended Diagnosis. Six branchiostegals; first dorsal fin with seven spines; number of vertebrae 29 to 31; base of second dorsal fin shorter than D2C.

Type species. †*Paralates bleicheri* Sauvage, 1883

†*Paralates bleicheri* Sauvage, 1883

Figures 2, 3.1, 4

*	1883	*Paralates bleicheri* Sauvage: 483–485, pl. XI, figs. 1–2.
	1913	*Paralates* cf. *bleicheri* Sauvage. – Förster: 45, pl. III, fig. 37.
	1934	*Prolebias praecursor* Weiler. – Théobald: 122 (pro parte).
	1941	*Paralates bleicheri* Sauvage. – Maïkovsky: 55, pl. XII, fig. 86.
v	1979	*Pomatoschistus bleicheri* (Sauvage). – Gaudant: 131–137, pl. I, figs. 1–4.
?	1981	*Pomatoschistus bleicheri* (Sauvage). – Gaudant: 214, pl. I, fig. 4.

Material. A total of 29 specimens from Rouffach are curated in the NMB (NMB Ruf. 1 to NMB Ruf. 29); these specimens represent the entire material of the species available. Only seven articulated skeletons and one skull were well preserved and served as the basis for this study: NMB Ruf. 13_1 (skull), NMB Ruf. 6a/b, NMB Ruf. 9_1, NMB Ruf. 9_2, NMB Ruf. 11, NMB Ruf. 13_2 (counterpart of 11), NMB Ruf. 15a/b, NMB Ruf. 18. The type material described by Sauvage (1883) is lost. Therefore Gaudant (1979) designated NMB Ruf. 15b as neotype.

Emended Diagnosis. Small gobiiform fish ranging from 29 to about 55 mm in length. Body cylindrical. Head length about three times included in the standard length. Frontal bones anteriorly less than one third of the width of the posterior part. First dorsal with seven spines. Total number of vertebrae: 29–30. Four to six (?seven) simple rays (= unbranched, unsegmented) in caudal fin dorsally and ventrally.

Description. For meristic characters and morphometric distances see Tables 1 and 2. The description of the skull is mainly based on NMB Ruf. 13_1.

The orbital region of the frontals (Figures 2.1, 3.1) is very narrow, less than one-third as broad as their posterior parts (also in NMB Ruf. 11). Other elements of the skull roof are certainly present, but are unidentifiable within the brownish mass. Of the ethmoid region only parts of the mesethmoid and the vomer are also recognizable.

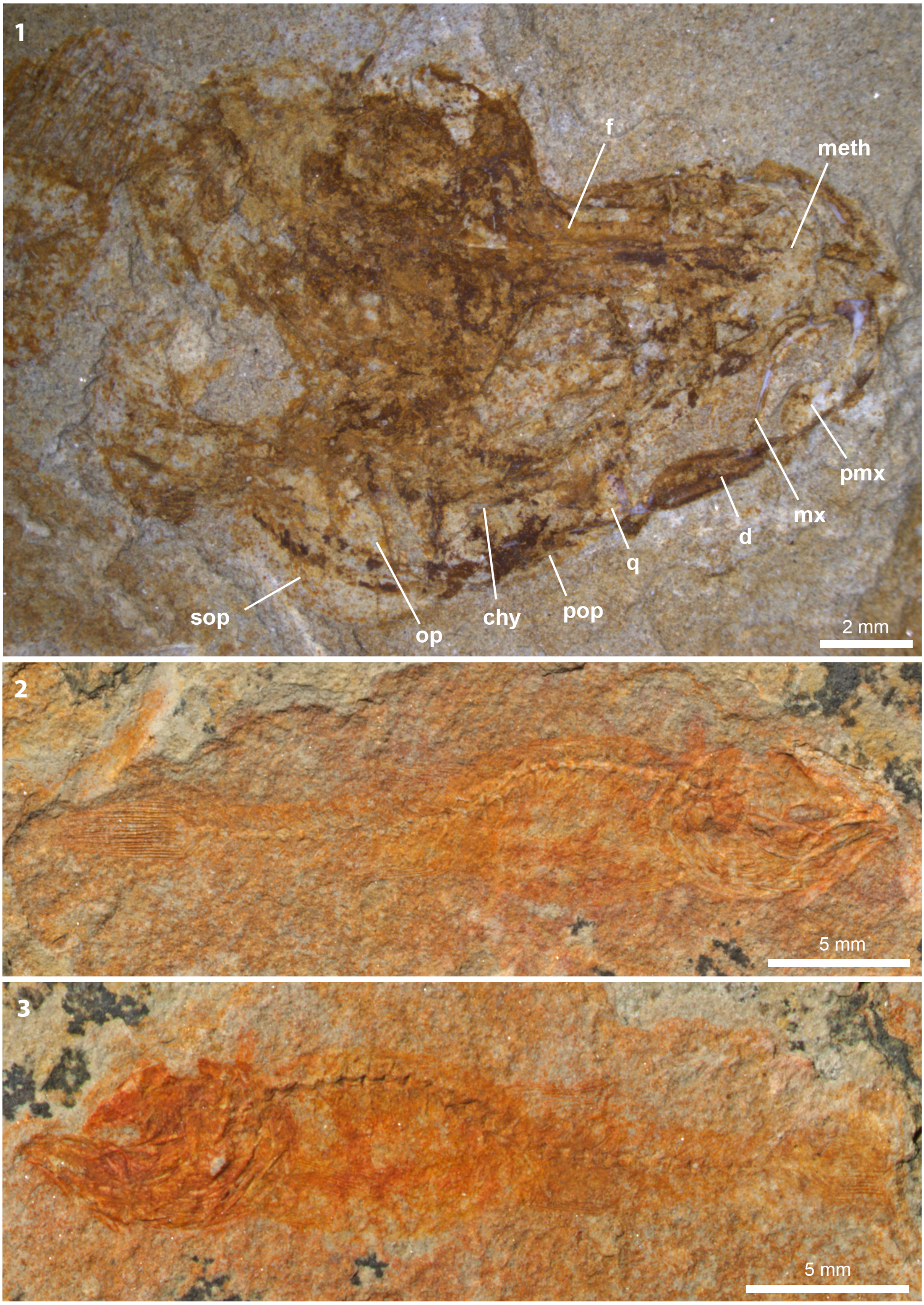

FIGURE 2. †*Paralates bleicheri* Sauvage, 1883. **1.** NMB Ruf. 13_1 skull. **2.** NMB Ruf. 15b (Neotype, designated by Gaudant 1979). **3.** NMB Ruf. 15a. Abbreviations: chy = ceratohyal, d = dentary, f = frontal, mx = maxilla, meth = mesethmoid, op = opercle, pmx = premaxilla, pop = preopercle, q = quadrate, sop = subopercle.

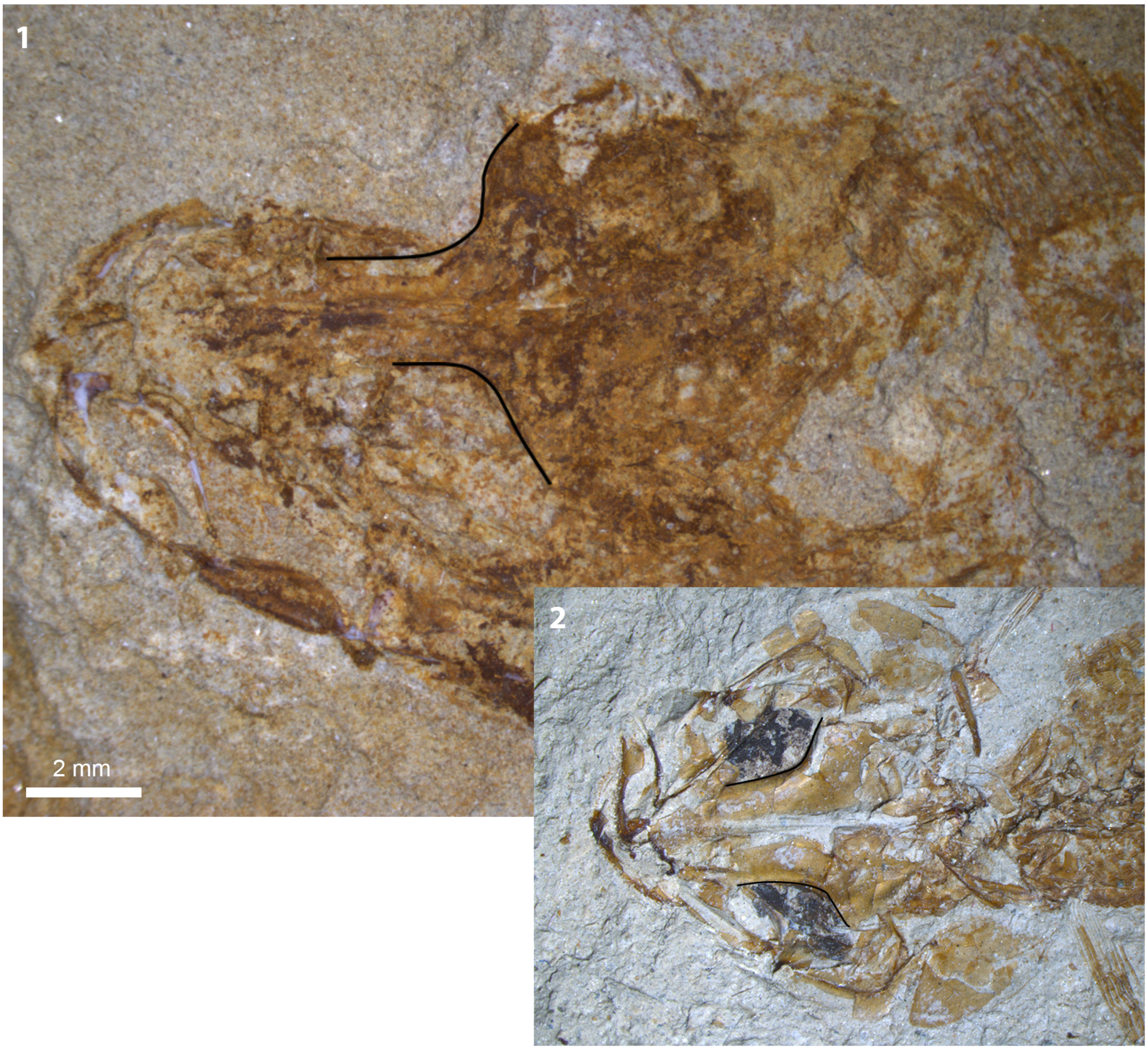

FIGURE 3. Comparison of frontals (highlighted by the black lines). **1.** †*Paralates bleicheri* Sauvage, 1883, NMB Ruf. 13_1 skull. **2.** †*Paralates chapelcorneri* n. sp., NHMUK PV P 59786. Same scale for both skulls.

In the jaws the premaxilla shows a broad postmaxillary process and a smaller articular process. A spine-like ascending process is not preserved (Figure 2.1). The maxilla is slender and bent, with a thick and forked articular process. The dentary is broad posteriorly and narrows anteriorly. Teeth are not preserved.

Most parts of the suspensorium and the hyoid arch are not identifiable. The quadrate is triangular and has a slender posteroventral process. The ceratohyal is broad in the short posterior part, while its long anterior part is quite slender. The epihyal (= posterior ceratohyal) is not preserved. Branchiostegals are not preserved.

The opercle is triangular and the subopercle is crescent-shaped. The preopercle is partially visible.

The vertebral column consists of 29 to 30 vertebrae, of which 17 or 18 are post-abdominal. The number of rib pairs seems to be at least eight.

The cleithrum is thick and slightly curved. The dorsally placed post-temporal is a rather robust V-shaped bone. The rest of the pectoral girdle is not recognizable.

The pelvic girdle is triangle-shaped in its posterior part and forms with its counterpart a rhombus-like structure. The fins consist of one spine and five rays each.

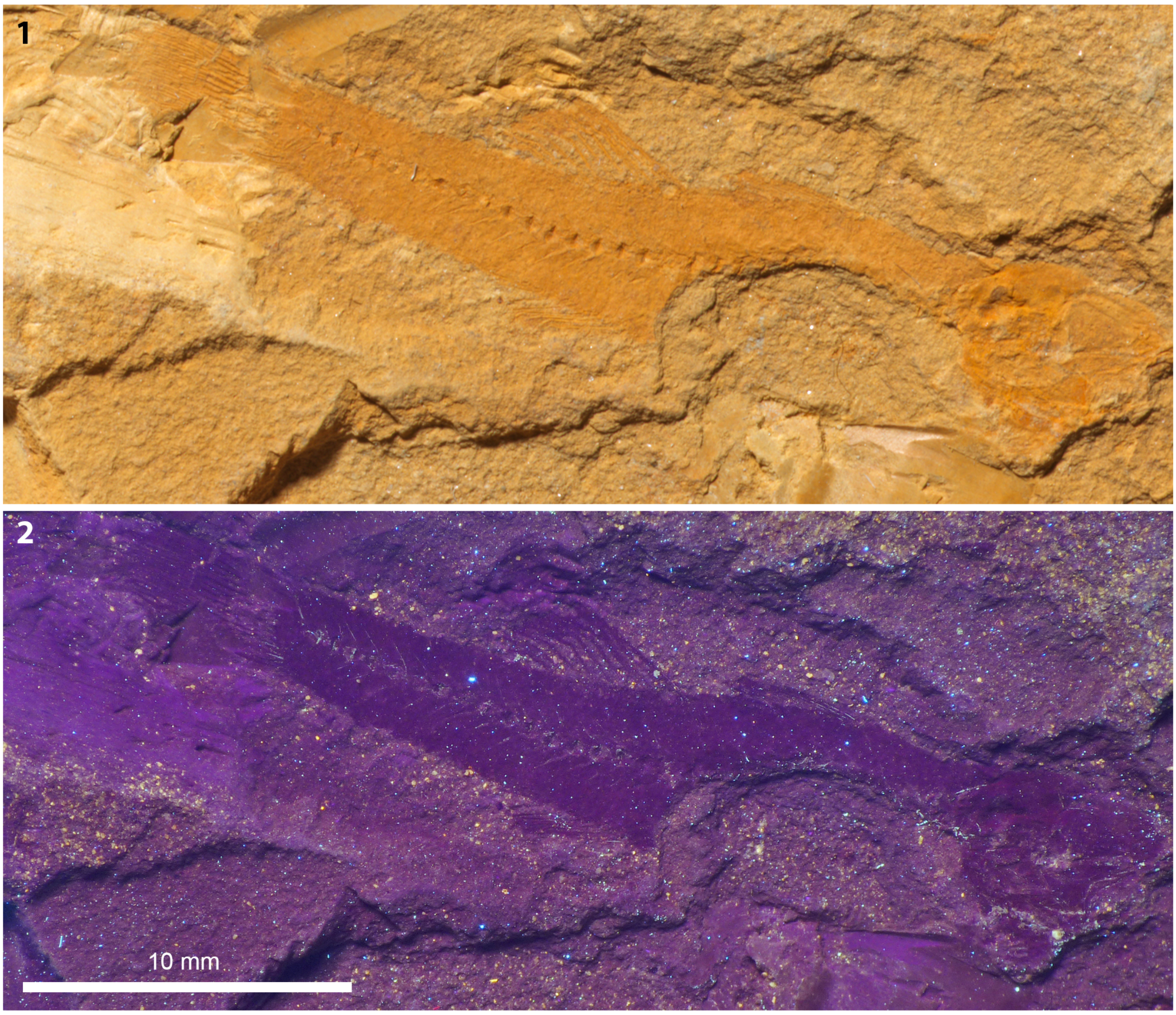

FIGURE 4. †*Paralates bleicheri* Sauvage, 1883, NMB Ruf. 18. **1.** Viewed under normal light. **2.** Imaged with UV light (picture by H. Tischlinger).

The first dorsal fin has seven spines, while the second has one spine and 10 rays. The base of the second dorsal is clearly shorter than the caudal peduncle. This is also true for the anal fin, which has one spine and eight or nine rays, and lies slightly behind the second dorsal fin. The caudal endoskeleton consists of two hypural plates (hy1+2, hy 3+4). Hypural 3+4 is fused to the terminal centrum (urostyle). Hypural 5 is long and slender, as is the parhypural.

Epurals are not preserved. The caudal fin has 12 segmented and branched rays, two specimens may have up to 13 segmented and branched rays (see Table 1). One or two segmented and unbranched rays occur dorsally and ventrally, and four to possibly seven simple rays (= unsegmented and unbranched) are inserted dorsally and ventrally.

Scales and otoliths are not preserved.

Remarks. The species †*Paralates bleicheri* was first described by Sauvage (1883) from the site Rouffach in the Upper Rhine Graben. As the genus name suggests, Sauvage thought this represented a perch-like fossil. Furthermore, he mentioned the presence of seven branchiostegals and two anal fin spines, but these characters are not mentioned in the later descriptions by Gaudant (1979, 1981) and are also absent in the specimens examined here. Nevertheless, judging by the rest of the original description (Sauvage 1883), e.g. the presence of a long caudal peduncle (longer than the second dorsal fin base), and the original figures, it is clear that the species described by Sauvage is the same as that reported by Gaudant (1979) from the same locality. Gaudant (1979) interpreted †*Paralates bleicheri* as a gobiid and assigned it to the genus *Pomatoschistus* based on meristics.

Moreover, in 1981 Gaudant described another specimen of †*Paralates bleicheri* from the sondage Niederhergheim, not far from Rouffach. This specimen has a slightly smaller anal (8 vs. 9 rays) and second dorsal fin (9 vs. 10–11 rays) and its frontals also differ in shape from those seen in the specimens from Rouffach. So, this fish might not represent †*Pa. bleicheri* and may need revision.

Range. Early Oligocene (Rupelian).

Occurrence. Southern Upper Rhine Graben (Rouffach, ?Niederhergheim; France).

TABLE 1. Meristic counts of all studied specimens of †*Paralates*. Numbers not in bold are subject to some uncertainty.

	Oligocene of Rouffach, no. Ruf						**Upper Eocene of Isle of Wight (Chapelcorner Fish Bed)**				
Specimen #	**6a/b**	**9_1**	**9_2**	**11/13_2**	**15a/b**	**18**	**P 59784**	**P 59785**	**P 59786**	**C 23632**	**C 23633**
First dorsal fin (D1)	–	–	> V	–	VII	**VII**	VII	**VII**	VII	–	–
Second dorsal fin (D2)	I,10	I,9	I,9	I,9	I,8	**I,10**	**I,9**	**I,10**	> 7	–	–
Anal fin (A)	10	> 8	10	> 7	I,8	I,8	I,8	I,9	–	–	–
C (branched, segmented)	12	13	?	13	12	**12**	**12**	**13**	–	–	–
C (unbranched, segmented)							2 + 1 or 2	**2 + 2**			
C (unbranched, unsegmented)						7	10 + ?	**10 + 12**			
Ventral fin (V)	–	–	–	–	I,5	–	I,5	**I,5**	–	–	–
Pectoral fin (P)	–	> 11	14	> 9	12	–	> 14	16	> 11	> 13	> 9
Vertebrae [postabdominal vert.]	> 23 [17]	> 21 [16]	> 27	> 23 [17]	**29** [17]	**30** [18]	30 [18]	**31 [19]**	> 23	> 11	29 [17]
Pterygiophore formula D1	–	–	–	–	–	–	–	3-2121100	–	–	–

†*Paralates chapelcorneri* n. sp.

Figures 3.2, 5, 6, 7, 8

1977 *Gobius*. – Ford: 109.
v 1988 *Pomatoschistus*(?) cf. *bleicheri* (Sauvage, 1883). – Gaudant & Quayle: 31–35, figs. 13–16.

Material. Five articulated skeletons: NHMUK PV P 59784, NHMUK PV P 59785 (plus 59785_counterpart), NHMUK PV P 59786, SM C.23632, SM C.23633. These specimens served as the basis for this study. Further poorly preserved specimens are NHMUK PV P 59774, NHMUK PV P 59775, NHMUK PV P 59776, NHMUK PV P 59777, NHMUK PV P 59787, NHMUK PV P 59788, NHMUK PV P 59797. The specimens listed above represent the entire material of the species that is accessible.

Holotype. NHMUK PV P 59785 (part and counter part); specimen in lateral view; complete skeleton.

Type locality and horizon. Chapelcorner Fish Bed (Colenutt's bed 3), collected at King's Quay, southeast of East Cowes; Isle of Wight (UK); Upper Eocene (Priabonian).

Etymology. Named after the unit in which the fossils were found: Chapelcorner Fish Bed.

Diagnosis. Small fish up to about 35 mm in total length; body cylindrical; frontals relatively broad anteriorly in comparison to their posterior portions (width greater than 40 % that of the posterior section; see Figures 3.2, 5); seven spines in first dorsal fin; 30 or 31 vertebrae; 10 to 12 simple rays (= unbranched and unsegmented) in the caudal fin dorsally and ventrally; pterygiophore formula 3-2121100.

Description. For meristic characters and morphometric distances see Tables 1 and 2.
The orbital region of the frontal bones is relatively broad (see Diagnosis and Figure 3.2). Other bones in the skull roof are not determinable.

The parasphenoid and basioccipital show a shape typical for gobiiform fishes. The parasphenoid is long and has a slender anterior and a broader posterior part. The basioccipital has a broad, roughly triangular shape and narrows posteriorly.

The premaxilla has a broad postmaxillary process, a smaller articular process and probably the spine-like ascending process, which seems premorse (Figure 5). Some conical teeth are visible. The maxilla is slender, with a thick articular head. The dentary is long and slender and bears conical teeth.

The shape of the palatine is not discernible. The ectopterygoid is slender and wedge-shaped. In one specimen (SM C.23632) bones bearing pharyngeal teeth of conical shape can be seen, which could correspond to ceratobranchial 5 and pharyngobranchial 3. The ceratohyal shows a slender anterior and a broader posterior part, the epihyal (= posterior ceratohyal) is not preserved. The number of branchiostegals is six, of which the two slender anterior ones attach to the narrow anterior part of the ceratohyal.

The triangular opercle has cycloid scales on its upper part (Figure 5).

The vertebral column consists of (29?) 30–31 vertebrae with 18?–19 post-abdominal vertebrae. There are at least seven rib pairs.

The pectoral fins have about 16 rays. The cleithrum is slightly curved, the supracleithrum is club-shaped (Figure 5). The post-temporal is V-shaped and has a process at the apex. Radials are present but not well preserved.

The pelvic girdle is recognizable but badly preserved. The pelvic fins have one spine and five rays.

The first dorsal fin has seven spines, the second dorsal fin one spine and 10 rays. The pterygiophore formula of the first dorsal fin is 3-2121100.

The anal fin inserts slightly behind the second dorsal, and has one spine and eight rays.

The caudal endoskeleton consists of two triangular hypural plates (hypural plate 1+2 and 3+4, see Figure 7). The upper one is fused to the terminal centrum. Hypural 5 is small and splint-like. The parhypural is slender and as long as the lower margin of hypural 1+2. At least one epural is present. The caudal fin has 12 or 13 segmented and branched rays, and one to two segmented und unbranched rays, dorsally and ventrally. There are 10 to 12 simple rays (= unbranched and unsegmented) dorsally and 12 ventrally (caudal fin formula according to the scheme of Fricke (1983) in specimen NHMUK PV P 59785_counterpart: (x), ii, 13, ii, (xii), see Figure 7).

The squamation consists of cycloid scales on the opercle and ctenoid scales on the rest of the body.

TABLE 2. Morphometrics of the genus †*Paralates* in mm. Values standardized to standard length are given in brackets. Abbreviations: A = anal fin, C = caudal fin, D1 = first dorsal fin, D2 = second dorsal fin, D2C = distance between end of D2 and first procurrent ray of C, P = pectoral fin, V = pelvic fin.

#	**Ruf. 11/13_2**	**Ruf. 15a/b**	**Ruf. 18**	**P 59784**	**P 59785**	**P 59786**
Total length	29.1 (115%)	29.5 (118%)	34.5 (119%)	35.3 (116%)	22.5 (116%)	> 22.3
Standard length	25.2	25.1	29.1	30.4	19.4	> 22.3
Max body height	5.4 (21.4%)	5.4 (21.5%)	5.5 (18.9%)	5.9 (19.4%)	4.4 (22.7%)	–
Head length	9.4 (37.3%)	6.9 (27.5%)	7.1 (24.4%)	9.5 (31.3%)	5.6 (28.9%)	6.6
Head height	6.6 (26.1%)	5.4 (21.5%)	5.2 (17.9%)	6.9 (22.7%)	4.9 (25.3%)	–
Horizontal eye diameter	1.5 (5.9%)	1.2 (4.8%)	1.3 (4.5%)	–	1.7 (8.8%)	1.4
Dist snout to D1	11.7 (46.4%)	9.2 (36.7%)	10.8 (37.1%)	11.7 (38.5%)	7.8 (40.2%)	7.9
Dist snout to D2	14.6 (57.9%)	14.5 (57.8%)	16.2 (55.7%)	17.4 (57.2%)	11.2 (57.7%)	12.8
Dist snout to A	15.8 (62.7%)	15.7 (62.5%)	16.5 (56.7%)	18.1 (59.5%)	11.5 (59,3%)	13.3
Dist snout to P	9.4 (37,3%)	8.7 (34.7%)	–	10.7 (35.2%)	6.8 (35.1%)	7.4
Dist snout to V	9.2 (36.5%)	8.2 (32.7%)	–	10.6 (34.9%)	6.6 (34.0%)	–
Length D1	> 2.1	> 2.4	> 2.4	> 3.4	> 1.7	–
Length D2	2.8 (11.1%)	> 2.4	4.4 (15.1%)	> 4.3	> 3.1	–
Length A	2.6 (10.3%)	> 2.4	> 2.3	> 3.5	> 2.9	–
Length P	> 0.8	> 1.3	–	> 1.9	> 0.8	> 2.8
Length V	> 1.2	> 1.5	–	> 2.7	2.7 (13.9%)	–
Base length D1	> 0.6	2.3 (9.2%)	2.3 (7.9%)	2.6 (8.6%)	1.6 (8.2%)	–
Base length D2	3.1 (12.3%)	3.0 (12.0%)	4.0 (13.7%)	> 3.5	2.4 (12.4%)	–
Base length A	3.0 (11.9%)	2.5 (19.0%)	3.7 (12.7%)	3.6 (11.8%)	1.9 (9.8%)	–
Length caudal peduncle	6.3 (25.0%)	6.8 (27.1%)	8.9 (30.6%)	8.3 (27.3%)	5.7 (29.4%)	–
D2C	6.7 (26.6%)	7.3 (29.1%)	8.4 (28.6%)	7.5 (24.7%)	5.0 (25.8%)	–
Min height of caud ped	2.0 (7.9%)	1.7 (6.8%)	2.6 (8.9%)	3.0(9.7%)	2.2 (11.3%)	–
Length of caudal fin	3.8 (15.1%)	> 3.8	> 5.0	5.4 (17.8%)	3.6 (18.6%)	–
Length of vertebrae	0.5–0.6 (1.9–2.3%)	0.6–0.7 (2.4–2.8%)	0.6–0.8 (2.1–2.7%)	0.5–0.7 (1.6–2.3%)	0.4–0.5 (2.1–2.6%)	0.4–0.5

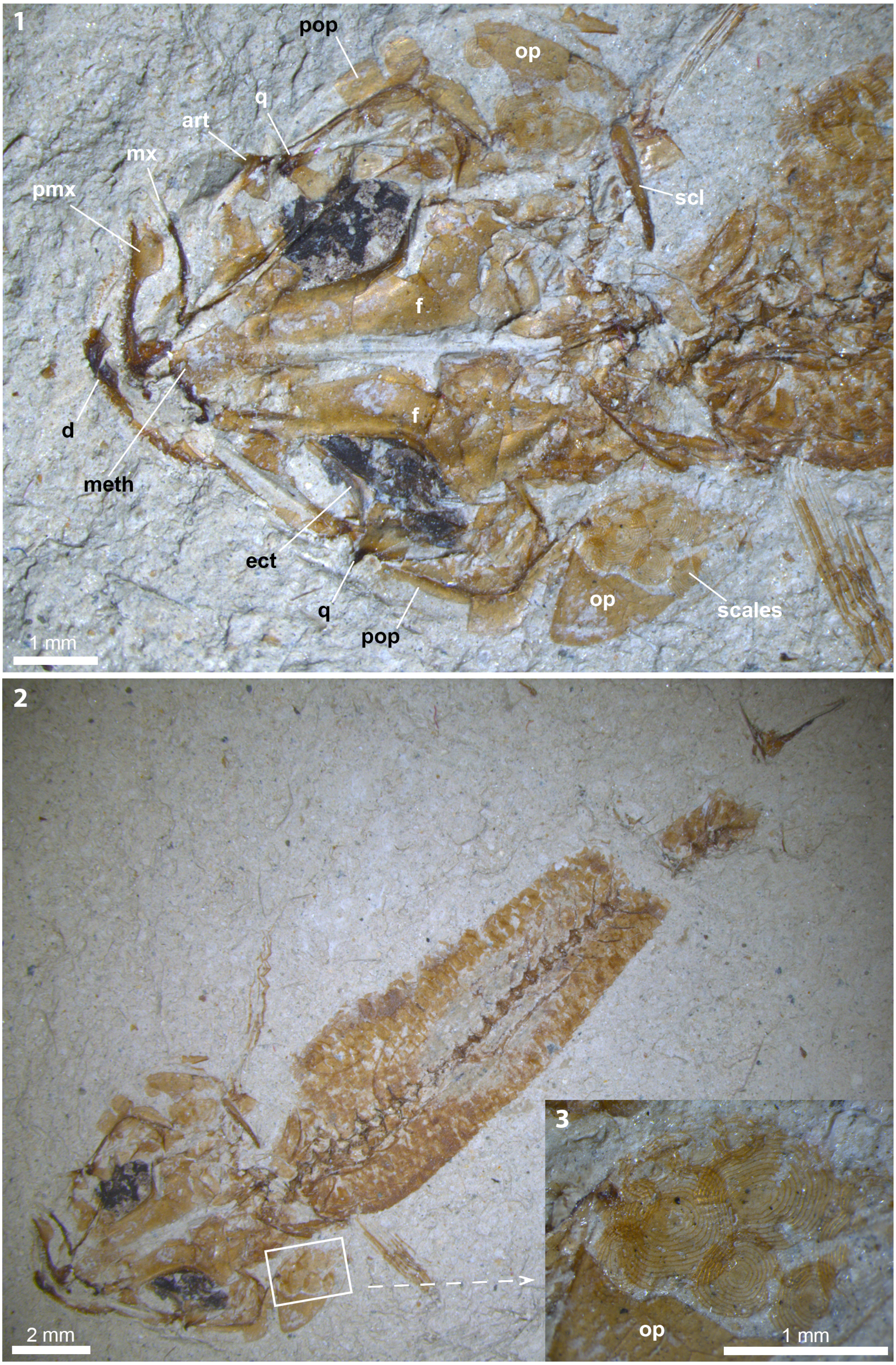
1
pop
op
q
art
mx
pmx
scl
f
f
d
meth
ect
q
pop
op
scales
1 mm
2
2 mm
3
op
1 mm

FIGURE 5. †*Paralates chapelcorneri* n. sp., NHMUK PV P 59786. **1.** Skull. **2.** Complete specimen. **3.** Detail of opercle with cycloid scales. Abbreviations: art = anguloarticular, d = dentary, ect = ectopterygoid, f = frontal, meth = mesethmoid, mx = maxilla, op = opercle, pmx = premaxilla, pop = preopercle, q = quadrate, scl = supracleithrum.

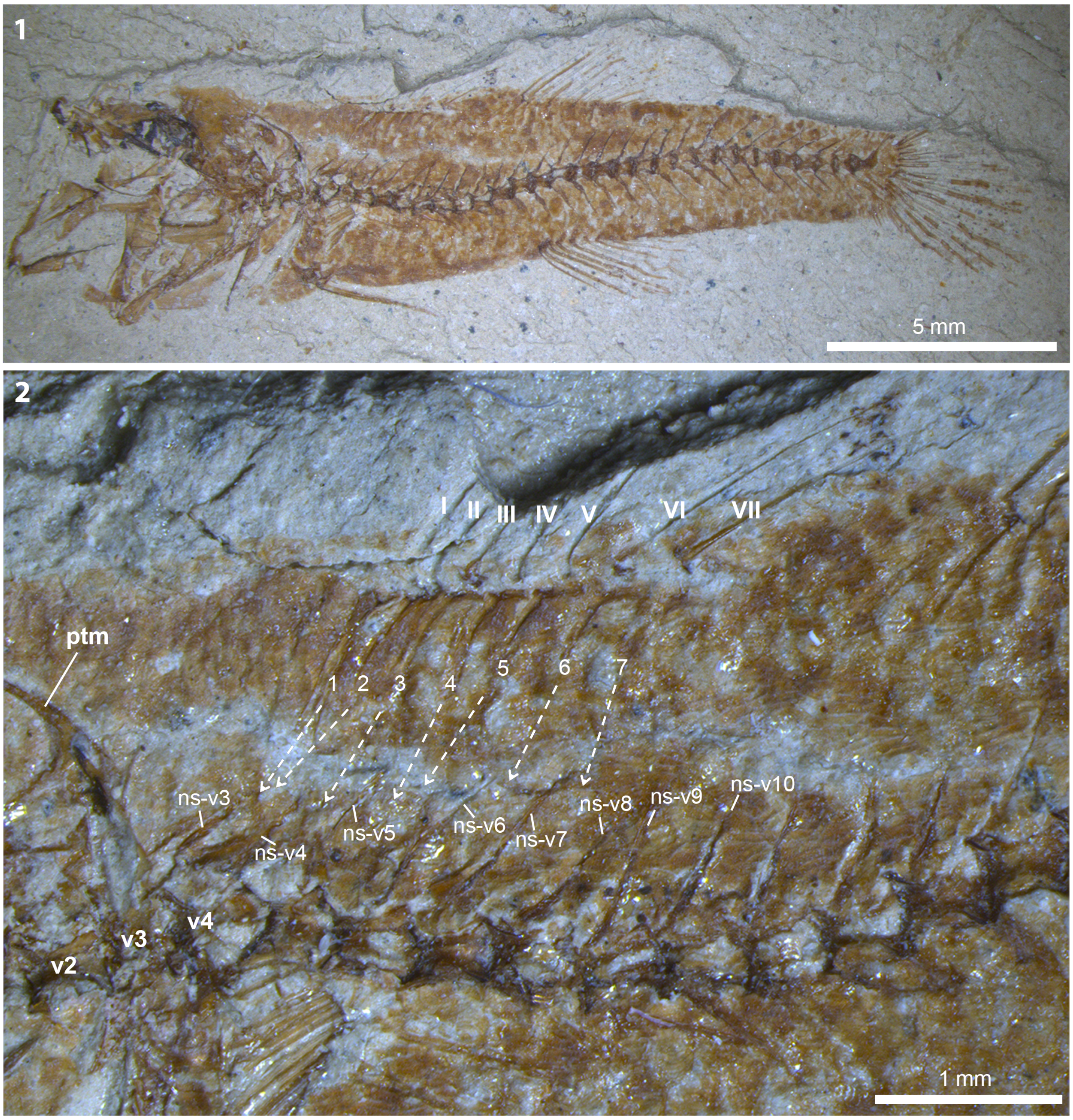

FIGURE 6. †*Paralates chapelcorneri* n. sp., NHMUK PV P 59785. **1.** Complete specimen. **2.** Region of the first dorsal fin. Arrows point from the pterygiophores to the interneural spaces into which each inserts. Pterygiophore formula = 3-2121100 The spinous rays of the first dorsal fin are marked with roman numerals. Abbreviations: 1–7 = pterygiophores, ptm = post-temporal, ns-v3–10 = neural spines of vertebrae 3–10, v2–4 = vertebral centra 2–4.

Remark. The meristic data for the specimens from Rouffach are rather similar to that for the fishes from the Isle of Wight (see Table 1), as is the long caudal peduncle, and we therefore place the species †*Paralates chapelcorneri* n. sp. in the same genus as †*P. bleicheri*. †*Paralates chapelcorneri* n. sp. from the Isle of Wight is different from †*Pa. bleicheri* from Rouffach for two reasons:

(1) In † *Pa. chapelcorneri* n. sp. the supraorbital part of the frontals is clearly broader than in the Rouffach fishes (see Figure 3.2 vs. 3.1). In NMB Ruf. 13 the width in the supraorbital part (0.63 mm) is 22 % of the width in the posterior part (2.86 mm); in NHMUK PV P 59786 from England it is 40 % (0.71 mm vs. 1.77 mm). Also Gaudant & Quayle (1988: 32) mentioned that the supraorbital part of the frontal is relatively broad in NHMUK PV P 59786 (now †*Pa. chapelcorneri* n. sp.). Although the specimen of †*Pa. chapelcorneri* n. sp. shown in Figure 3.2 is more dorso-ventrally compressed than the specimen of *Pa. bleicheri* shown in Figure 3.1, the shapes of their frontals are different and this cannot be explained by differences in preservation alone.

(2) †*Paralates chapelcorneri* n. sp. has more unbranched and unsegmented rays in the caudal fin (at least 10 vs. maximal seven).

3.4 Discussion

The specimens re-examined here display certain features, which clearly show that they belong neither to *Pomatoschistus* nor even to the Oxudercidae (see below).

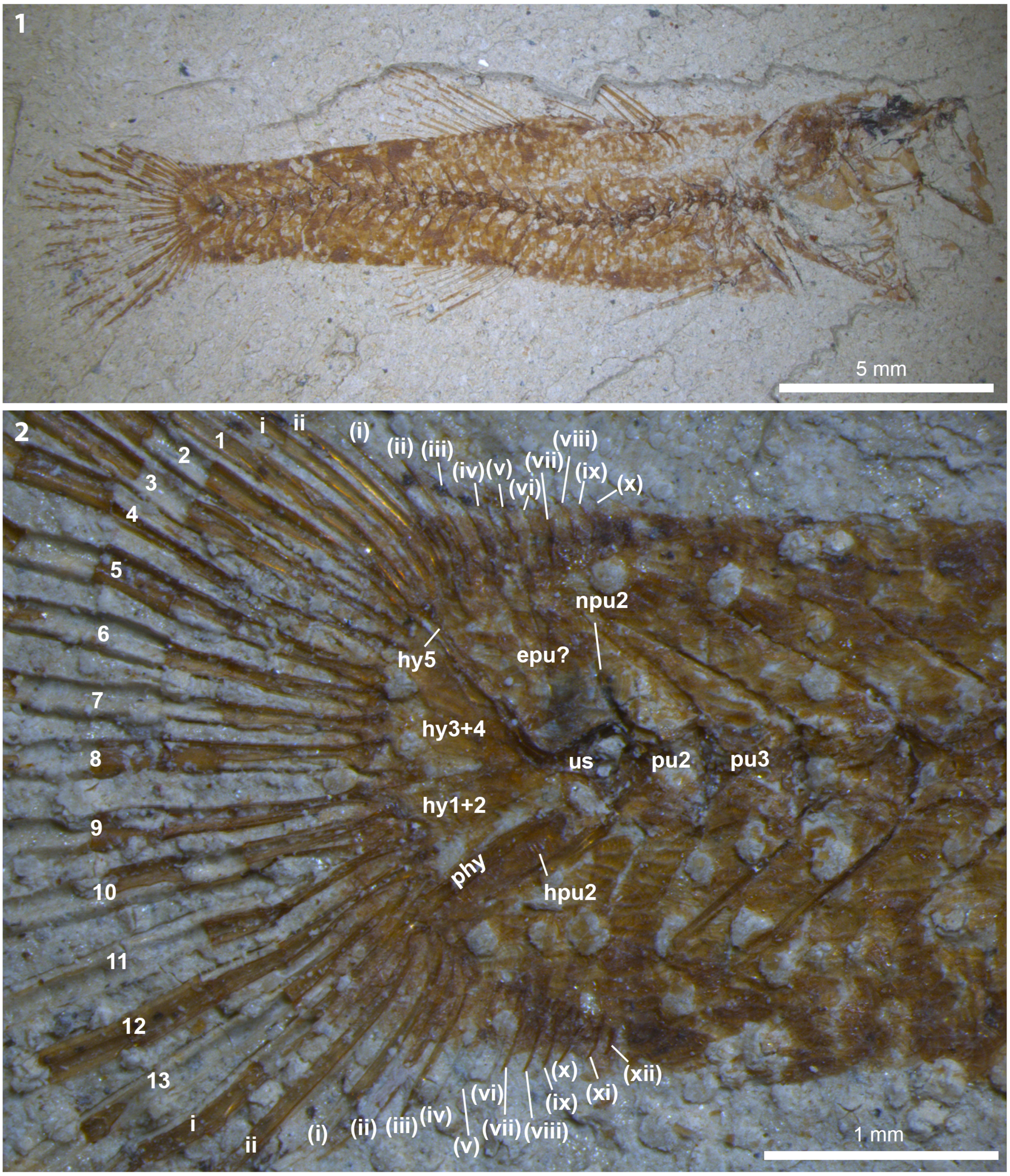

FIGURE 7. †*Paralates chapelcorneri* n. sp., NHMUK PV P59785_counterpart. **1.** Complete specimen. **2.** Caudal skeleton and fin. Small Roman numerals in *parentheses* label unbranched, unsegmented rays, small Roman numerals indicate segmented unbranched rays, and Arabic numerals designate segmented, branched rays (after Fricke 1983). Abbreviations: epu = epural, hpu = hemal spine of preural centrum, hy = hypural, npu = neural spine of preural centrum, phy = parhypural, pu = preural centrum, us = urostyle.

3.4.1 Assignment to the Gobiiformes

The fishes from both localities are members of the Gobiiformes because they exhibit the following features (see Springer 1983; Johnson and Brothers 1993; Winterbottom 1993; Wiley and Johnson 2010): suspensorium fenestrae are large (Figure 8) (Gosline 1955; see for illustration Parmentier et al. 2013, figure 4 A); hypurals 1 and 2 are fused; hypurals 3 and 4 are fused to each other and to the urostyle (Figure 7); parietals are absent.

FIGURE 8. †*Paralates chapelcorneri* n. sp., NHMUK PV P 59784. **1.** Complete specimen. **2.** Skull showing possible symplectic and six branchiostegals. Abbreviations: 1–6 = branchiostegals, ect = ectopterygoid, mpt = metapterygoid, sf = suspensorium fenestra, sym = symplectic.

3.4.2 Relationship to *Pomatoschistus*?

Pomatoschistus is a member of the 'sand-goby' group among the Oxudercidae (Agorreta et al. 2013). The 'sand-gobies' have been defined as a distinct clade based on morphological characters by McKay and Miller (1997). This clade has additionally been confirmed based on molecular work (see McKay and Miller 1997; Agorreta et al. 2013; Thacker 2013).

The informal name 'sand goby' refers to the preferred occurrence of these fishes on sandy substrates; no formal name exists for this clade. The group comprises the genus *Pomatoschistus*, together with *Gobiusculus*, *Knipowitschia*, *Economidichthys* and *Hyrcanogobius* (see McKay and Miller 1997; Agorreta et al. 2013). The 'sand gobies' show a distinctive feature in the jaws: the absence of a postmaxillary process on the premaxilla, except in some females of *Gobiusculus flavescens* (McKay and Miller 1997). Other oxudercids, most gobiids, as well as the members of the families with six branchiostegals (see Figure 1), possess such a postmaxillary process (McKay and Miller 1997). Moreover, McKay and Miller (1997) found that the pterygiophore formula in the 'sand gobies' starts with (12…) and that there is more than one interneural space.

A postmaxillary process on the premaxilla is present in both species of †*Paralates* (see Figures 2.1, 5.1). Additionally, the pterygiophore formula is discernible in the specimen NHMUK PV P 59785 of †*Paralates chapelcorneri* n. sp. (Figure 6.2) and starts with (21…). We therefore conclude that †*Paralates* is clearly different from the genus *Pomatoschistus* and not a member of the 'sand gobies'.

Moreover, †*Paralates* is not a member of the Gobiidae or Oxudercidae, because it has six branchiostegals. Gaudant and Quayle (1988) mentioned the presence of an entopterygoid, which would provide an additional criterion for exclusion of the fossil from the Gobiidae and Oxudercidae. However, the bone depicted as an entopterygoid by Gaudant and Quayle (1988) is not positioned near the ectopterygoid (as would be expected) and its anatomical assignment is therefore difficult to verify; it may be the distorted metapterygoid. The long caudal peduncle, which is longer than the base of the second dorsal fin, may also argue for an assignment to the "non-Gobiidae" (see Murdy et al. 2002).

TABLE 3. Meristic characters of recent gobioid taxa for which the pterygiophore formula 3-212110(0) has been observed in at least one species of the genus. The number of caudal fin rays refers to all segmented rays (branched and unbranched). Abbreviations: A = anal fin, C = caudal fin, D1 = first dorsal fin, D2 = second dorsal fin, P = pectoral fin, V = pelvic fin.

Taxon	**D1**	**D2**	**A**	**C**	**V**	**P**	**Vertebrae [postabd]**	**Source**	**Family**
†*Paralates bleicheri* Sauvage, 1883	VII	I,10	I,9	> 12–13	I,5	14	**29 [17–18?]**	this study	inc. sed.
†*Paralates chapelcorneri* n. sp.	VII	I,10	I,8–9	14–17	I,5	14–16	**30?–31 [18?–19]**	this study	inc. sed.
Gobiodon okinawae Sawada, Arai & Abe, 1972	VI	I,10	I,9	15	–	–	**27 [17]**	unpublished data	Gobiidae
Gobiosoma hemigymnum (Eigenmann & Eigenmann, 1888)	VII	I,11	I,9	17	I,5	17–19	**27 [16]**	van Tassell et al. 2015	Gobiidae
Gobiosoma spp.	–	–	–	–	–	–	**27–28 [15–17]**	Birdsong et al. 1988	Gobiidae
Gobulus spp.	–	–	–	–	–	–	**27–28 [16–17]**	Birdsong et al. 1988	Gobiidae
Gobulus spp.	VII–VIII	I,9–13	I,7–13	15–17	I,5	15–18	**27–28 [16–17]**	Hoese and Reader 2001	Gobiidae
Gobiomorphus alpinus Stokell, 1962	III–VII	I,7–9	I,8–10	–	–	16–19	**29–31**	McDowall 1994	Eleotridae
Gobiomorphus spp.	III–VIII	I,7–10	I,7–10	–	–	13–21	**27–31**	McDowall 1975	Eleotridae
Gobiomorphus spp.	–	–	–	–	–	–	**28–31 [16–19]**	Birdsong et al. 1988	Eleotridae
Thalasseleotris				16			**27 [17]**	Hoese and Larson 1987	Thalasseleotrididae

3.4.3 Affinity of †*Paralates* to previously described gobiiform fossils

One feature that clearly differentiates †*Paralates* from other fossil Gobiiformes is the number of spinous rays in the first dorsal fin. The only previously described species that has a first dorsal fin with seven spines is the Oligocene species †*Pirskenius radoni* Přikryl, 2014. However, the genus †*Pirskenius* is also characterized by seven branchiostegal rays (Obrhelová 1961; Přikryl 2014) whereas only six branchiostegal rays are present in †*Paralates*.

A few fossil gobiiforms with six branchiostegal rays have been described, i.e. †*Lepidocottus aries* (Agassiz, 1839) (see Gierl et al. 2013), †*L. papyraceus* (Agassiz, 1839) (see Pandolfi et al. 2016), †*Eleogobius brevis* (Agassiz, 1839) and †*E. gaudanti* Reichenbacher & Gierl, 2015. Whereas the species of †*Lepidocottus* have only 25-26 vertebrae, the two known species of †*Eleogobius* Gierl & Reichenbacher, 2015 from the lower Miocene of S. Germany, like †*Paralates*, have 29-31. However, in †*Eleogobius* the D2C can sometimes be slightly longer than the base of D2 (Gierl and Reichenbacher 2015), while †*Paralates* has a very long D2C that is about twice the length of the base of D2 (see Table 3).

Further species with large numbers of vertebrae compared to †*Paralates* include †*Gobius multipinnatus* (Meyer, 1852) from the lower Miocene of Germany and *Pomatoschistus* sp. from the upper Miocene of the North Caucasus, Russia. Also, these species, however, clearly differ from †*Paralates*: †*G. multipinnatus* has a bigger second dorsal fin (D2) (I+12–13 vs. I+10 in †*Paralates*) and the length of the base of D2 exceeds D2C (see Gierl and Reichenbacher 2015). *Pomatoschistus* sp., besides being younger (middle Miocene), has bigger anal and dorsal fins (see Carnevale et al. 2006).

3.4.4 Phenotypic affinity of †*Paralates* to the extant eleotrid *Gobiomorphus*

The pterygiophore formula 3-2121100 seen in †*Paralates* has been described (albeit with only one interneural space) in a few members of the Gobiidae, Thalasseleotrididae, and Eleotridae (see Birdsong et al. 1988). This specific formula, however, occurs only in genera that show some polymorphism with regard to the pterygiophore formula, e.g. in *Gobiomorphus*, *Gobiosoma*, *Gobulus*, *Gobiodon*, and *Thalasseleotris* (see Table 3). In addition, it is never the 'normal case' but always exceptional (see Hoese and Larson 1987; Birdsong et al. 1988; Lee 1993; our unpublished data). However, those genera of the Gobiidae and Thalasseleotrididae for which a pterygiophore formula 3-212110 has been reported always have more or fewer vertebrae than are seen in †*Paralates* (Hoese and Larson 1987; Birdsong et al. 1988; Lee 1993; our unpublished data) (see Table 3). Only the eleotrid genus *Gobiomorphus* reveals six branchiostegals (like in †*Paralates*) and a similar combination of the pterygiophore formula and vertebrae count as seen in †*Paralates*: The pterygiophore formula is 3-212110 (vs. 3-2121100) and the number of vertebrae is 12+19 (vs. 12+17–19).

Species of *Gobiomorphus* are now restricted to coastal rivers of Eastern Australia and rivers and lakes in New Zealand (Hoese and Larson 1987). Among the Gobiiformes, *Gobiomorphus* is difficult to define based on meristic and osteological characters because it belongs to the genera with the highest levels of variation in skeletal features (see McDowall 1975; Hoese and Larson 1987; Birdsong et al. 1988). For instance, the entopterygoid is present in some species, and lacking in others (Hoese 1984; Hoese and Larson 1987), the epural number varies between 1 and 2 (see Birdsong et al. 1988) and the number of vertebrae (excluding the terminal centrum) ranges from 26 to 31 (McDowall 1975). Nonetheless, the genus *Gobiomorphus* have been recognized as monophyletic based on molecular and morphological data (see McDowall 1975; Stevens and Hicks 2009).

Fossil skeletons of *Gobiomorphus*-like fossils have been described from the Miocene of New Zealand: †*Mataichthys bictenatus* Schwarzhans et al., 2012 has a lower vertebral count (28) and a smaller anal fin (I+7) (see Schwarzhans et al. 2012) than seen in †*Paralates*. *Gobiomorphus* sp. (described in McDowall et al. 2006) also has fewer vertebrae (26–28) and the count of its rays in the caudal fin falls into the range of †*Paralates*. Most other counts are uncertain, due to the moderate preservation of the fossils. So it is hard to say if there is a relationship between the fossil *Gobiomorphus* sp. from New Zealand and †*Paralates*, in spite of the similarities between the latter and extant *Gobiomorphus* species.

3.5 Conclusions

The question remains whether 'sand gobies' are represented in the fossil record. A specimen determined as "*Pomatoschistus* cf. *bleicheri*" from the lower Miocene of Bergama (Turkey) was described (Rückert-Ülkümen 2000), but the description given makes it hard to accept the proposed assignment (7 branchiostegals; only 27 vertebrae). The only reliable records of 'sand goby' skeletons seem to be *Pomatoschistus* sp. (with otoliths in situ) from the upper Miocene of the North Caucasus (Russia) described in Carnevale et al. (2006) and the species described in Schwarzhans et al. (2017) from the middle Miocene (Sarmatian) of the Central Paratethys (Croatia). Besides, there are some otolith-based species of the 'sand gobies' from the middle Miocene (see Weiler 1943; Wienrich et al. 2009; Schwarzhans 2010, 2014; Bratishko et al. 2015; Schwarzhans et al. 2015; Schwarzhans et al. 2017).

For now, the oldest member of modern Gobiiformes with five branchiostegals in the fossil record is the gobiid †*Gobius jarosi* Přikryl and Reichenbacher, 2018 in Reichenbacher et al. (2018) from the lower Miocene of Central Europe (Czech Republic). Phylogenetic analyses based on a combination of morphology and molecular data will be necessary to more accurately understand the phylogenetic relationships of the many different extant and extinct gobiiform fishes from Europe.

ACKNOWLEDGEMENTS

We would like to thank Emma Bernard (NHMUK, London, UK), Matt Riley (Sedgwick Museum, Cambridge, UK), and Loïc Costeur (NMB, Basel, Switzerland) for their kind support with the loan of the specimens described here. We also acknowledge the constructive comments of Dirk Erpenbeck (LMU Munich, Germany), two anonymous reviewers and the editorial crew of PE. This work was funded by a grant from the Deutsche Forschungsgemeinschaft (RE 1113/20-1).

3.6 References

Agassiz, L. 1833–1843. *Recherches sur les Poissons fossiles. Tome IV*. Neuchâtel: Petitpierre.

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

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

Bannikov, A.F., and G. Carnevale. 2016. †*Carlomonnius quasigobius* gen. et sp nov.: the first gobioid fish from the Eocene of Monte Bolca, Italy. *Bulletin of Geosciences* 91 (1): 13–22.

Birdsong, R.S., E.O. Murdy, and F.L. Pezold. 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.

Bratishko, A., W. Schwarzhans, B. Reichenbacher, Y. Vernyhorova, and S. Ćorić. 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.

Carnevale, G., A.F. Bannikov, W. Landini, and C. Sorbini. 2006. Volhynian (early Sarmatian sensu lato) fishes from Tsurevsky, North Caucasus, Russia. *Journal of Paleontology* 80 (4): 684–699.

Eigenmann, C.H., and R.S. Eigenmann. 1888. A list of the American species of Gobiidae and Callionymidae, with notes on the specimens contained in the Museum of Comparative Zoölogy, at Cambridge, Massachusetts. *Proceedings of the California Academy of Sciences. Second Series* 1: 51–78.

Ford, R.L.E. 1977. A genus of fossil fish (*Gobius*) new to the Isle of Wight. *Proceedings of the Isle of Wight natural History and archeological Society* 7 (2): 109–110.

Förster, B. 1913. Die Versteinerungen aus den Tiefbohrungen auf Kali im Oligocän des Oberelsass. *Mitteilungen der Geologischen Landesanstalt von Elsaß-Lothringen* 8 (1): 1–49.

Fricke, R. 1983. A method for counting caudal fin rays of actinopterygian fishes. *Braunschweiger Naturkundliche Schriften* 1 (4): 729–733.

Gaudant, J. 1979. Sur la présence de Gobiidae (Poissons téléostéens) dans l'Oligocène inférieur de Rouffach (Haut-Rhin). *Sciences Géologiques Bulletin* 32 (3): 131–137.

Gaudant, J. 1981. Nouvelles recherches sur l'ichthyofaune des Zones salifères moyenne et supérieure (Oligocène inférieur) du bassin potassique alsacien. *Sciences Géologiques Bulletin* 34 (4): 209–218.

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 II, Sciences de la terre et des planètes* 322 (1): 71–76.

Gaudant, J., and W.J. Quayle. 1988. New palaeontological studies on the Chapelcorner fish bed (Upper Eocene, Isle of Wight). *Bulletin of the British Museum of Natural History (Geology)* 44 (1): 15–39.

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

Gierl, C., B. Reichenbacher, J. Gaudant, D. Erpenbeck, and A. Pharisat. 2013. An extraordinary gobioid fish fossil from southern France. *PLOS ONE* 8 (5): e64117.

Gill, A.C., and R.D. Mooi. 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: 41–52.

Gosline, W.A. 1955. The osteology and relationships of certain gobioid fishes, with particular reference to the genera *Kraemeria* and *Microdesmus*. *Pacific Science* IX: 158–170.

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

Hoese, D.F., and A.C. Gill. 1993. Phylogenetic relationships of eleotridid fishes (Perciformes, Gobioidei). *Bulletin of Marine Science* 52 (1): 415–440.

Hoese, D.F., and H.K. Larson. 1987. New Australian fishes. Part 11. A new genus and species of eleotridid (Gobioidei) from Southern Australia with a discussion of relationships. *Memoirs of the Museum of Victoria* 48 (1): 43–50.

Hoese, D.F., and S. Reader. 2001. Revision of the eastern Pacific species of *Gobulus* (Perciformes: Gobiidae), with description of a new species. *Revista de Biologia Tropical* 49 (Supplement 1): 169–176.

Johnson, G.D., and E.B. Brothers. 1993. *Schindleria*: a paedomorphic goby (Teleostei, Gobioidei). *Bulletin of Marine Science* 52 (1): 441–471.

Lee, Y.-J. 1993. Study of vertebral column and pterygiophores in Gobiidae (Pisces, Perciformes) from Korea. *The Korean Journal of Systematic Zoology* 9 (1): 25–34.

Maïkovsky, V. 1941. Contribution à l'étude paléontologique et startigraphique du bassin potassique d'Alsace. *Mémoires du Service de la carte géologique d'Alsace et de Lorraine* 6: 1–192.

McDowall, R.M. 1975. A revision of the New Zealand species of *Gobiomorphus* (Pisces: Eleotridae). *National Museum of New Zealand Records* 1 (1): 1–32.

McDowall, R.M. 1994. The Tarndale bully, *Gobiomorphus alpinus* Stokell (Pisces: Eleotridae) revisited and redescribed. *Journal of the Royal Society of New Zealand* 24 (1): 117–124.

McDowall, R.M., E.M. Kennedy, J.K. Lindqvist, D.E. Lee, B.V. Alloway, and M.R. Gregory. 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.

McKay, S.I., and P.J. Miller. 1997. The affinities of European sand gobies (Teleostei: Gobiidae). *Journal of Natural History* 31 (10): 1457–1482.

Meyer, H.v. 1852. Fossile Fische aus dem Tertiärthon von Unter-Kirchberg an der Iller. *Palaeontographica. Beiträge zur Naturgeschichte der Vorwelt* 2 (Dritte Lieferung): 85–113.

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

Murdy, E.O., D.F. Hoese, and C.E. Thacker. 2002. Suborder Gobioidei. In *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Atlantic. Volume 3: Bony fishes part 2 (Opistognathidae to Molidae), sea turtles and marine mammals*, ed. K. E. Carpenter, 1778–1798. FAO Species Identification Guide for Fishery Purposes and American Society of Ichthyologists and Herpetologists Special Publication No. 5. Rome: Food and agriculture organization of the United Nations.

Nelson, J.S., T.C. Grande, and M.V.H. Wilson. 2016. *Fishes of the World, Fifth Edition*. Hoboken, New Jersey: John Wiley & Sons, inc.

Obrhelová, N. 1961. Vergleichende Osteologie der tertiären Süsswasserfische Böhmens (Gobioidei). *Sborník Ústředního ústavu geologického: oddíl paleontologiyký* 26: 103–192.

Pandolfi, L., G. Carnevale, L. Costeur, L. Del Favero, M. Fornasiero, E. Ghezzo, L. Maiorino, P. Mietto, P. Piras, L. Rook, G. Sansalone, and T. Kotsakis. 2017. Reassessing the earliest Oligocene vertebrate assemblage of Monteviale (Vicenza, Italy). *Journal of Systematic Palaeontology* 15 (2): 83–127.

Parmentier, E., L. Kéver, K. Boyle, Y.E. Corbisier, L. Sawelew, and S. Malavasi. 2013. Sound production mechanism in *Gobius paganellus* (Gobiidae). *The Journal of Experimental Biology* 216 (17): 3189–3199.

Patzner, R.A., J.L. Van Tassell, M. Kovačić, and B.G. Kapoor eds. 2011. *The biology of gobies*. Enfield, New Hampshire: Science Publishers Inc.

Přikryl, T. 2014. A new species of the sleeper goby (Gobioidei, Eleotridae) from the České Středohoří Mountains (Czech Republic, Oligocene) and analysis of the validity of the family Pirskeniidae. *Paläontologische Zeitschrift* 88 (2): 187–196.

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

Rückert-Ülkümen, N. 2000. Eine neue Fisch-Fauna aus dem Tertiär von Bergama (West-Türkei). *Mitteilungen der Bayerischen Staatssammlung für Paläontologie und historische Geologie* 40: 153–163.

Sauvage, M.H.E. 1883. Notes sur les poissons fossiles. *Bulletin de la Societe Geologique de France* 3 (11): 475–503.

Sawada, Y., R. Arai, and T. Abe. 1972. *Gobiodon okinawae*, a new Coral-Goby from the Ryukyu Islands, Japan. *Japanese Journal of Ichthyology* 19 (2): 57–62.

Schneider, C.A., W.S. Rasband, and K.W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9 (7): 671–675.

Schwarzhans, W. 2010. *The otoliths from the Miocene of the North Sea Basin*. Weikersheim: Backhuys Publishers, Leiden & Margraf Publishers.

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

Schwarzhans, W., H. Ahnelt, G. Carnevale, S. Japundžić, K. Bradić, and A. Bratishko. 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., K. Bradić, and L. Rundić. 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., R.P. Scofield, A.J.D. Tennyson, J.P. Worthy, and T.H. Worthy. 2012. Fish remains, mostly otoliths, from the non-marine early Miocene of Otago, New Zealand. *Acta Palaeontologica Polonica* 57 (2): 319–350.

Springer, V.G. 1983. *Tyson belos, new genus and species of Western Pacific fish (Gobiidae, Xenisthminae), with discussions of gobioid osteology and classification*. Washington, D. C.: Smithsonian Institution Press.

Stevens, M.I., and B.J. Hicks. 2009. Mitochondrial DNA reveals monophyly of New Zealand's *Gobiomorphus* (Teleostei: Eleotridae) amongst a morphological complex. *Evolutionary Ecology Research* 11 (1): 109–123.

Stokell, G. 1962. A new species of *Gobiomorphus*. *Transactions of the Royal Society of New Zealand. Zoology* 2 (3): 31–34.

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.

Thacker, C.E. 2011. Systematics of Gobiidae. In *The biology of gobies*, eds. R. A. Patzner, J. L. Van Tassell, M. Kovačić, and B. G. Kapoor, 129–136. Enfield, NH: Science Publishers Inc.

Thacker, C.E. 2013. Phylogenetic placement of the European sand gobies in Gobionellidae and characterization of gobionellid lineages (Gobiiformes: Gobioidei). *Zootaxa* 3619 (3): 369–382.

Thacker, C.E., and D.M. Roje. 2009. Phylogeny of cardinalfishes (Teleostei: Gobiiformes: Apogonidae) and the evolution of visceral bioluminescence. *Molecular Phylogenetics and Evolution* 52 (3): 735–745.

Thacker, C.E., T.P. Satoh, E. Katayama, R.C. Harrington, R.I. Eytan, and T.J. Near. 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.

Théobald, N. 1934. Contribution à la paléontologie du bassin oligocène du Haut-Rhin et du territoire de Belfort - Les poissons oligocènes. *Bulletin du Service de la Carte Géologique d'Alsace et de Lorraine* 2 (2): 117–162.

Van Tassell, J.L., J.-C. Joyeux, R.M. Macieira, and L. Tornabene. 2015. Status of *Gobiosoma* (Teleostei: Gobiidae) from Brazil: description of a new species, redescription of *G. hemigymnum*, molecular phylogeny of the genus, and key to Atlantic species. *Zootaxa* 4007 (4): 451–480.

Weiler, W. 1943. Die Otolithen aus dem Jungtertiär Süd-Rumäniens. 1. Buglow und Sarmat. *Senckenbergiana* 26 (1/3): 87–115.

Wienrich, G., T. Reinecke, and W. Schwarzhans eds. 2009. *Die Fauna des marinen Miozäns von Kevelaer (Niederrhein). Band 5: Vertebraten, Elasmobranchii, Otolithen*. Leiden, Weikersheim: Backhuys Publishers, Margraf Publishers.

Wiley, E.O., and G.D. Johnson. 2010. A teleost classification based on monophyletic groups. In *Origin and phylogenetic interrelationships of teleosts*, eds. J. S. Nelson, H.-P. Schultze, and M. V. H. Wilson, 123–182. München: Verlag Dr. Friedrich Pfeil.

Winterbottom, R. 1993. Search for the gobioid sister group (Actinopterygii: Percomorpha). *Bulletin of Marine Science* 52 (1): 395–414.

4. What can goby otolith morphology tell us?

Christoph Gierl[1], Dorothea Liebl[1], Radek Šanda[2], Jasna Vukić[3], Hamid Reza Esmaeili[1,4], Bettina Reichenbacher[1]

[1]Department of Earth and Environmental Sciences, Palaeontology & Geobiology and GeoBio-Center^{LMU}, Ludwig-Maximilians-University, Richard-Wagner-Straße 10, Munich, Bavaria, D-80333, Germany, c.gierl@lrz.uni-muenchen.de, b.reichenbacher@lrz.uni-muenchen.de (send reprint requests to this address);
[2]Department of Zoology, National Museum, Václavské nám. 68, CZ-115 79 Praha, Czech Republic, rsanda@seznam.cz
[3]Department of Ecology, Faculty of Science, Charles University, Viničná 7, CZ-128 44 Praha, Czech Republic, JVukic@seznam.cz
[4]Developmental Biosystematics Research Lab., Zoology Section, Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran, hresmaeili@shirazu.ac.ir

ABSTRACT

The taxonomic information inscribed in otoliths has been widely ignored in ichthyological research, especially in descriptions of new fish species. One reason for this is that otolith descriptions are *per se* qualitative, and only a few studies have presented quantitative data that can support assignments of otoliths to individual species or permit differentiation between higher taxonomic levels. On the other hand, in palaeontology, otoliths have been employed for the identification and taxonomic placement of fossil fish species for over 100 years. However, palaeontological otolith data is generally regarded with suspicion by ichthyologists. This is unfortunate because, in the Cenozoic, the fossil otolith record is much richer than that based on skeletons. Thus, fossil otoliths are a unique source of information to advance our understanding of the origin, biogeographical history and diversification of the Teleostei. This case study deals with otoliths of the Oxudercidae which, together with the Gobiidae, encompasses the 5-branchiostegal-rayed gobiiforms. The objective was to determine whether the five lineages of the Oxudercidae, and individual species of the European *Pomatoschistus* lineage, could be distinguished based on the quantification of otolith variations. The data set comprises otoliths from a total of 84 specimens belonging to 20 recent species, which represent all five lineages of the Oxudercidae (*Mugilogobius*, *Acanthogobius*, *Pomatoschistus*, *Stenogobius*, *Periophthalmus*), and five fossil otoliths of †*Pomatoschistus* sp. (sensu Brzobohatý 1994). Ten measurements were taken on each otolith and 23 otolith variables were computed and used for univariate and multivariate analyses. The results indicate that otolith morphometry (i) is capable of identifying the *Pomatoschistus* and

Periophthalmus lineages among the Oxudercidae, but is of limited use in the separation of the other three lineages; (ii) can reliably distinguish the sand gobies (a distinct clade within the *Pomatoschistus* lineage) from other members of the *Pomatoschistus* lineage; and (iii) supports a previous assignment of fossil otoliths to †*Pomatoschistus* sp. as an ancient genus of the sand gobies. With its middle Miocene age (15 m.y. ago), †*Pomatoschistus* sp. represents the oldest record of a sand goby species to date. We discuss possible relationships between distinct otolith morphologies, biogeographic distribution and lifestyles for *Pomatoschistus* and the *Periophthalmus* lineage, and also for the sand gobies. We conclude that otolith morphology, combined with morphometry, can be considered as an autapomorphy for an individual species from the studied groups. In addition, it also appears to contain a phylogenetic signal, but more work is needed to evaluate this fully.

RÉSUMÉ—Ce que peut révéler la morphologie des otolithes de gobies?
L'information taxonomique inscrite dans les otolithes a longtemps été ignorée en recherche en ichtyologie, surtout dans la description de nouvelles espèces de poissons. Une des raisons à cela, est que la description des otolithes est souvent qualitative, et il n'existe que peu d'études qui aient présentés des données quantitatives qui permettent d'assigner un otolithe à une espèce ou bien qui permettent une discrimination à des niveaux taxonomiques plus élevés. A l'inverse, en paléontologie, les otolithes sont utilisés depuis plus de 100 ans pour l'identification et le replacement taxonomique de poissons fossiles. Cependant, les données d'otolithes paléontologiques sont généralement vues de manière suspicieuse par les ichtyologues. Cela est bien dommage car, pour le Cénozoïque, il existe beaucoup plus de fossiles d'otolithes que de fossiles de squelettes. Ainsi, les otolithes fossiles représentent une source unique d'information qui peut nous aider à comprendre l'origine, l'histoire biogéographique et la diversification des téléostéens. Cette étude porte sur les otolithes d'Oxudercidae, qui, avec les Gobiidae, englobent les gobiiformes à 5 rayons branchiostégaux. L'objectif de ce travail était de déterminer si les 5 lignées d'Oxudercidae, et des espèces Européennes de la lignée *Pomatoschistus*, peuvent être différenciées grâce à des variables quantitatives des otolithes. Le jeu de données comprends des otolithes de 84 spécimens appartenant à 20 espèces récentes représentant les 5 lignées d'Oxudercidae (*Mugilogobius*, *Acanthogobius*, *Pomatoschistus*, *Stenogobius*, *Periophthalmus*), et 5 otolithes fossiles de †*Pomatoschistus* sp. (sensu Brzobohaty 1994). 10 mesures ont été prises sur chaque otolithe et 23 variables ont été mises au point pour des analyses statistiques uni et multivariées. Les résultats montrent que l'analyse morphométrique des otolithes permet (i) d'identifier les lignées de *Pomatoschistus* et *Periophthalmus* parmi les Oxudercidae, mais qu'elle est d'intérêt limité pour la discrimination des 3 autres lignées ; (ii) de distinguer de manière sûre les « sand gobies » (gobies vivant sur fonds sableux) des autres membres de la lignées *Pomatoschistus*; (iii) de valider l'assignation des otolithes fossiles de †*Pomatoschistus* à un genre ancien de « sand gobies». Datant du Miocène moyen (-15 Ma), †*Pomatoschistus* sp.

représente l'enregistrement le plus ancien de « sand gobies » connu à ce jour. Notre étude permet de discuter les relations possibles entre des morphologies distinctes d'otolithes, la distribution géographique des espèces ainsi que leur mode de vie pour les lignées de *Pomatoschistus* et *Periophthalmus*, ainsi qu'au sein des « sand gobies ». Nous concluons que la morphologie des otolithes, combinée à leur analyse morphométrique, peut constituer une autapomorphie spécifique au sein des groupes étudiés. De plus, il semblerait qu'il puisse y avoir un signal phylogénétique, mais des études approfondies restent à faire afin de clarifier ce point.

Keywords: otolith morphometry, taxonomy, Gobiiformes, Oxudercidae, sand gobies, fossils.

4.1 Introduction

Fish otoliths are aragonitic structures that develop independently from the skeleton as integral components of the hearing and balance organs of bony fishes (see Nolf 1985; Popper and Lu 2000). In the inner ear of teleost fishes, they are arranged in three pairs referred to as the saccular (sagittae), utricular (lapilli) and lagenar otoliths (asterisci), respectively (see Schulz-Mirbach et al. 2011). Examples for the usage of otoliths in biology include otolith shape analysis for fish stock discrimination (e.g. Campana and Casselman 1993; Lord et al. 2012), the study of otolith chemistry to gain information about life history and habitats (e.g. Tzeng and Tsai 1994; Avigliano et al. 2017), and use of otolith growth rings for estimation of age and duration of larval phases (e.g. Campana 2005; Lord et al. 2010).

The overall shape and detailed morphology of the sagitta ('otolith' in the following) provide taxonomically useful characters for species identification in most teleosts. However, although several atlases are available for the otoliths of recent species (e.g. Volpedo and Echeverría 2000; Campana 2004; Smale et al. 2005; Tuset et al. 2008), the taxonomic information inscribed in otoliths has been widely ignored in ichthyological research, especially in descriptions of new fish species (for exceptions e.g., see Gholami et al. 2014; Esmaeili et al. 2014; Teimori et al. 2012, 2014). On the other hand, in palaeontology, otoliths have been employed precisely for this purpose for over 100 years. Koken (1884) was one of the first to publish a comprehensive study of fossil otoliths, and some 1800 fossil fish species have now been documented solely on the basis of otoliths (Nolf 2013). Their identification mostly relies on comparative studies of the inner face of the sagitta, since this is the biggest otolith and exhibits the largest number of distinguishable features (except in Cyprinidae and Siluridae, see Assis 2003, 2005; Schulz-Mirbach and Reichenbacher 2006). As yet, however, only a few studies have attempted to collect quantitative data that could provide further support for otolith-based taxonomic assignments (Volpedo and Echeverría 2000; Reichenbacher et al. 2007; Schwarzhans 2014).

The Gobiiformes, as defined in the latest edition of "Fishes of the World" by Nelson et al. (2016), constitute one of the largest orders of teleost fishes, with approximately 2,200 species and a worldwide distribution. In this case study, we deal with the Oxudercidae, which together with the Gobiidae forms the group of the more derived 5-branchiostegal-rayed gobiiforms (see Gierl and Reichenbacher 2017: figure 1). Molecular work has recognised five lineages among the Oxudercidae, which are named for the genera *Acanthogobius*, *Mugilogobius*, *Periophthalmus*, *Pomatoschistus* and *Stenogobius*, respectively (Agorreta et al. 2013; Thacker 2015). The *Acanthogobius* lineage is a non-tropical taxon, distributed in the temperate Northern Pacific, the *Mugilogobius* lineage is distributed in the Indo-Pacific realm, and the *Periophthalmus* and *Stenogobius* lineages share a global tropical distribution (Thacker 2015); none of these lineages is present in Europe (Agorreta et al. 2013). In contrast, the *Pomatoschistus* lineage is represented in Europe by several species and additionally present in Asia (Agorreta et al. 2013; Thacker 2015).

Within the *Pomatoschistus* lineage the 'sand gobies' (for which no formal name exists) form a distinct clade, based on both morphological characters (McKay and Miller 1997) and molecular data (Agorreta et al. 2013). The sand gobies include the species of *Pomatoschistus*, *Gobiusculus*, *Knipowitschia*, *Economidichthys* and *Hyrcanogobius*; with the exception of some females of *Gobiusculus flavescens*, they all share the apomorphy of a premaxilla without a postmaxillary process (McKay and Miller 1997).

Whether or not fossil otoliths of the Oxudercidae can be assigned to one of the five newly recognized lineages or used to reliably distinguish sand gobies from non-sand gobies in the *Pomatoschistus* lineage has not yet been rigorously examined. The ability to do so would significantly advance our understanding of the origin, biogeographic history and diversification of the Oxudercidae clade (see Thacker 2015). The objectives of this study are to test whether otolith morphometry might be suitable (i) for identifying lineages among the Oxudercidae, (ii) distinguishing the sand gobies from other members of the *Pomatoschistus* lineage, and (iii) for supporting previous assignments of fossil otoliths as †*Pomatoschistus* sp.

Institutional Abbreviations: NHM, Natural History Museum, Vienna, Austria; NMP, National Museum Prague, Czech Republic; ZM-CBSU, Zoological Museum of Shiraz University, Collection of Biology Department, Iran; ZSM, Zoological State Collection, Munich, Germany.

4.2 Materials and methods

4.2.1 Material

The material discussed here comprises saccular otoliths isolated from a total of 84 specimens belonging to 20 recent species representing all five lineages of the Oxudercidae, and five fossil otoliths of †*Pomatoschistus* sp. (sensu Brzobohatý 1994). The *Stenogobius* lineage is represented by three species, the *Acanthogobius* and *Mugilogobius* lineages by four species each, the *Pomatoschistus* lineage by six and the *Periophthalmus* lineage by three species (see Table I). Only the saccular otoliths (referred to as otoliths in the following) of the left head side were used for this study (totally 89 left otoliths).

4.2.2 Methods

Otolith dissection. – For otolith dissection, we followed standard protocols (e.g. Gholami et al. 2014), with some slight modifications concerning cleaning and washing of the otoliths. The skull was opened dorsally with a scalpel and the otoliths were carefully removed using tweezers. Then the otoliths were treated with 5% KOH solution for at least 30 minutes to clean them from organic residuals. Subsequently, the otoliths were rinsed about five times with distilled water in a sample chamber under the microscope, and then air dried.

SEM imaging. – All otoliths from the recent species were coated with gold, and SEM images were taken using a LEO 1430 VP (15 kV) at the Zoologische Staatssammlung München or a HITACHI SU 5000 Schottky FE-SEM at the Department of Earth and Environmental Sciences (LMU Munich). The fossil otoliths of †*Pomatoschistus* sensu Brzobohatý (1994) were not gold-coated (because they represent the type material of Brzobohatý 1994) and images were taken using the low vacuum SEM facility (JSM-6490LV, 20 kV) at the Senckenberg Museum and Research Institute, Frankfurt am Main. Based on the SEM images of the studied otoliths we prepared morphological descriptions and qualitative comparisons.

Otolith measurements. – The SEM images of the otoliths were oriented so that the ventral margin was essentially horizontal (Fig. 1a). Only in the case of *Gobiusculus flavescens* was the posterior margin used as the base. Ten measurements were taken on each otolith (Fig. 1b) using imageJ 1.51r (Schneider et al. 2012). To measure the otolith length (OL), otolith height (OH), sulcus length (SuL) and sulcus height (SuH), a rectangle was drawn that enclosed the most dorsal, most ventral, most anterior and most posterior points of the otolith and the sulcus, respectively (yellow lines in Fig. 1b). The lengths of this rectangle's edges represent the maximum length and height of the otolith and sulcus, respectively. Measurements were also made of the otolith perimeter (OP), otolith area (OA), sulcus perimeter (SuP), sulcus area (SuA), distance from sulcus tip to the ventral margin (SuTipV) and distance from sulcus end to the ventral margin (SuEndV). The outline of the sulcus is usually recognisable because it is encompassed by a faintly etched line (Fig. 1a). Measurements of the SuP were taken along this line, or, if multiple lines were present, at the outermost margin (see Fig. 1b). In cases where parts of the sulcus were not clearly defined by a thin line, the sulcus perimeter was estimated by comparison with other otoliths of the same species. All measurements are presented in the appendix.

Table I. Material used to analyze otolith characters in different lineages; n, number of fish individuals per species and number of otoliths in the case of †*Pomatoschistus*.

Lineage	**Species**	**n**	**Designation**
Stenogobius	*Awaous flavus* (Valenciennes, 1837)	2	ZSM-PIS-43853 (P-GO-1050 L; 1051 L)
	Gobioides broussonnetii Lacepède, 1800	2	ZSM-PIS-43852 (P-GO-1048 L; 1049 L)
	Stiphodon atropurpureus (Herre, 1927)	2	ZSM_PIS-43862 (P-GO-1070 L, 1071 L)
Acanthogobius	*Rhinogobius candidianus* (Regan, 1908)	4	ZSM-PIS-43858 (P-GO-1060 L; 1060,61-1 L; 1060,61-2 L; 1061 L)
	Rhinogobius formosanus Oshima, 1919	4	ZSM-PIS-43864 (P-GO-1072 L; 1072,73-1 L; 1072,73-3 L; 1073 L)
	Rhinogobius rubromaculatus Lee & Chang, 1996	4	ZSM-PIS-43860 (P-GO-1064 L; 1064,65-2 L; 1064,65-3 L; 1065 L)
	Rhinogobius zhoui Li & Zhong, 2009	4	ZSM-PIS-43859 (P-GO-1062 L; 1062,63-2 L; 1062,63-6 L; 1063 L)
Mugilogobius	*Brachygobius xanthozonus* (Bleeker, 1849)	2	ZSM-PIS-43865 (P-GO-1074 L; 1075 L)
	Chlamydogobius eremius (Zietz, 1896)	2	ZSM-PIS-43854 (P-GO-1052 L; 1053 L)
	Schismatogobius roxasi Herre, 1936	2	ZSM-PIS-43866 (P-GO-1076 L; 1077 L)
	Stigmatogobius sadanundio (Hamilton, 1822)	2	ZSM-PIS-43856 (P-GO-1056 L; 1057 L)
Pomatoschistus	*Buenia affinis* Iljin, 1930	4	NMP P6d 30/2017-3, -6, -7, -9
	Deltentosteus quadrimaculatus (Valenciennes, 1837)	9	NMP P6d 34/2017-1–3, -5–10
	Gobiusculus flavescens (Fabricius, 1779)	4	NMP P6V 142775–142778
	Knipowitschia croatica Mrakovčić *et al.*, 1996	6	NMP P6d 31/2017-1–4, -6, -7
	Pomatoschistus marmoratus (Risso, 1810)	7	NMP P6d 32/2017-1–7
	Pomatoschistus quagga (Heckel, 1839)	7	NMP P6d 33/2017-4–10
Periophthalmus	*Bolephthalmus dussumieri* Valenciennes, 1837	4	ZM–CBSU Khamir 38, 40, 41, 54
	Periophthalmus waltoni Koumans, 1941	8	ZM–CBSU Gowater 1734, 1736, 1739, 1741, 1743, 1745, 1748, 1751
	Scartelaos tenuis (Day, 1876)	5	ZM–CBSU Helleh 82, 84–86, 89
fossil	†*Pomatoschistus* sp. (Brzobohatý, 1994)	5	NHM 1993/140/4–8

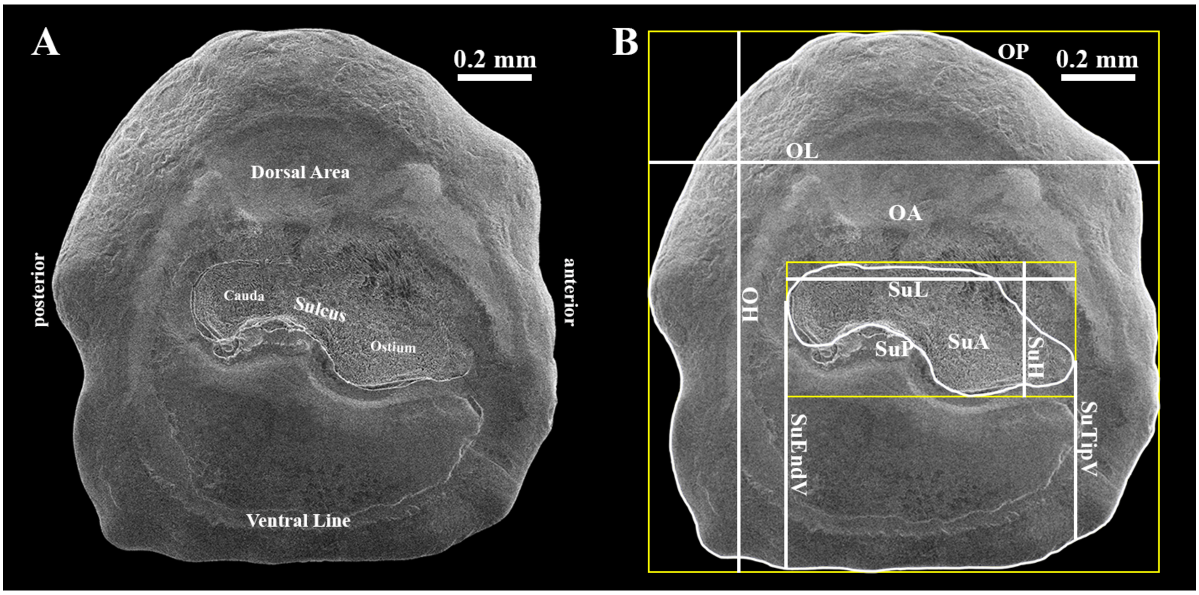

Figure 1. – Left otolith of *Rhinogobius candidianus* (ZSM-PIS-43858 (P-GO-1060 L)): nomenclature (**A**) and measurements (**B**). Abbreviations: OA – otolith area; OH – otolith height; OL – otolith length; OP – otolith perimeter; SuA – sulcus area; SuH – sulcus height; SuL – sulcus length; SuP – sulcus perimeter; SuEndV – distance from sulcus end to the ventral margin; SuTipV – distance from sulcus tip to the ventral margin.

Morphometrics. – For the statistical analysis, 23 otolith variables in all were computed, based on the ratios between the individual otolith measurements (Table II).

Statistics. – All otolith variables were analysed using SPSS 24.0 (IBM Corp.) and PAST 3.17 (Hammer et al. 2001). The Shapiro-Wilk test ($p < 0.05$) indicated that several otolith variables are not normally distributed across the lineages and species, respectively. We suspect that the non-normally distributed variables represent artefacts resulting from the relatively small sample sizes; consequently, these data were not normalized. We used Mann-Whitney ($p < 0.05$) to examine the variation of non-normally distributed otolith variables and ANOVA with Dunnett T3 post-hoc test ($p < 0.05$) in case of normally distributed otolith variables. In addition, multivariate analysis based on Principal Components Analysis was done to find out whether the identification of the fossil otoliths as †*Pomatoschistus* sp. (sensu Brzobohatý 1994) is supported by our morphometric approach.

Table II. – Definitions of the 23 otolith variables used in this study. For measurements see Figure 1B.

Otolith variable	**Abbreviation**
Ratio of otolith length and otolith height	OL/OH
Ratio of otolith perimeter and otolith length	OP/OL
Ratio of otolith perimeter and otolith height	OP/OH
Ratio of sulcus area and otolith area	SuA/OA
Ratio of sulcus perimeter and otolith perimeter	SuP/OP
Ratio of sulcus perimeter and distance from sulcus tip to the ventral margin	SuP/SuTipV
Ratio of sulcus perimeter and distance from sulcus end to the ventral margin	SuP/SuEndV
Ratio of sulcus length and otolith length	SuL/OL
Ratio of sulcus length and otolith height	SuL/OH
Ratio of sulcus length and sulcus height	SuL/SuH
Ratio of sulcus length and distance from sulcus tip to the ventral margin	SuL/SuTipV
Ratio of sulcus length and distance from sulcus end to the ventral margin	SuL/SuEndV
Ratio of sulcus length and otolith perimeter	SuL/OP
Ratio of sulcus length and sulcus perimeter	SuL/SuP
Ratio of sulcus height and otolith length	SuH/OL
Ratio of sulcus height and otolith height	SuH/OH
Ratio of sulcus height and distance from sulcus tip to the ventral margin	SuH/SuTipV
Ratio of sulcus height and distance from sulcus end to the ventral margin	SuH/SuEndV
Ratio of sulcus height and otolith perimeter	SuH/OP
Ratio of sulcus height and sulcus perimeter	SuH/SuP
Ratio of distance from sulcus tip to the ventral margin and otolith perimeter	SuTipV/OP
Ratio of distance from sulcus tip to the ventral margin and distance from sulcus end to the ventral margin	SuTipV/SuEndV
Ratio of distance from sulcus end to the ventral margin and otolith perimeter	SuEndV/OP

4.3 Results

4.3.1 Otolith morphology among Oxudercidae lineages

Apart from those of the *Periophthalmus* lineage, the otoliths of the species studied here share a rectangular to slightly rounded overall shape and have mostly smooth rims. Accordingly, they show no posterodorsal or preventral projection. In contrast, species of the *Periophthalmus* lineage have otoliths with crenulated rims and a posterodorsal projection is present (Fig. 2).

In most otoliths of all lineages, the sulcus has the typical gobioid shape ('shoe sole-like'). Only two of the species examined, *Rhinogobius rubromaculatus* (Fig. 2I) and *Buenia affinis* (Fig. 2N), show a short thickening of the crista inferior along the cauda ('subcaudal iugum' sensu Schwarzhans 2014). Furthermore, some species display modifications of the general sulcus shape. Within the *Mugilogobius* lineage, this holds for *Chlamydogobius eremius* with its triangular ostium and thin, ventrally bent, appendix-like prolongation at the end of the cauda (Fig. 2A), as well as for *Stigmatogobius sadanundio* which has a relatively long cauda that is slender in the middle and widened at the end (Fig. 2B). Examples from the *Stenogobius* lineage are *Awaous flavus* (Fig. 2F, G) and *Stiphodon atropurpureus* (Fig. 2H), both of which display a very shallow sulcus; indeed, the sulcus is barely recognisable in the latter. Within the *Pomatoschistus* lineage, the cauda in both *Pomatoschistus marmoratus* (Fig. 2M) and *Gobiusculus flavescens* (Fig. 2O) is reduced in size, whereas in *Deltentosteus quadrimaculatus* there is a notable triangular extension of the upper ostial rim (Fig. 2P). Furthermore, one species of the *Mugilogobius* lineage, i.e. *Schismatogobius roxasi* (Fig. 2D) and one of the *Acanthogobius* lineage, i.e. *Rhinogobius zhoui* (Fig. 2J) lack a clear subdivision of the sulcus into ostium and cauda. Moreover, the four species of *Rhinogobius* differ among themselves in their sulcus morphology. Most distinct is the sagitta of *R. zhoui*, which has a slender and slightly pointed but relatively small sulcus lacking the constriction between ostium and cauda (Fig. 2J). In the remaining three species of *Rhinogobius*, the sulcus is clearly divided, but differences are recognisable in the contour of the ostium. In *R. formosanus* (Fig. 2L), the lower rim of the ostium has a distinctive, U-shaped indentation, in *R. candidianus* (Fig. 2K) a similar but less pronounced indentation is present, whereas no such structure is developed in *R. rubromaculatus* (Fig. 2I).

4.3.2 Otolith morphology of recent sand-gobies (*Pomatoschistus* lineage) and of †*Pomatoschistus* sp. (*sensu* Brzobohatý 1994)

The otoliths of four recent species of sand gobies were used for this study, i.e. *Gobiusculus flavescens* (Fig. 2O, 3J–K), *Knipowitschia croatica* (Fig. 3M–O), *Pomatoschistus marmoratus* (Fig. 2M, 3D–F), and *P. quagga* (Fig. 3G–I). In all species, the sulcus has the typical ‘shoe sole’ shape, but it is not centered (as is usual in the lineages of the Oxudercidae) and lies slightly closer to the anterior than to the posterior otolith margin. A further difference relative to the general ‘shoe sole’ sulcus condition concerns the cauda, which is distinctively smaller in width and length than the ostium.

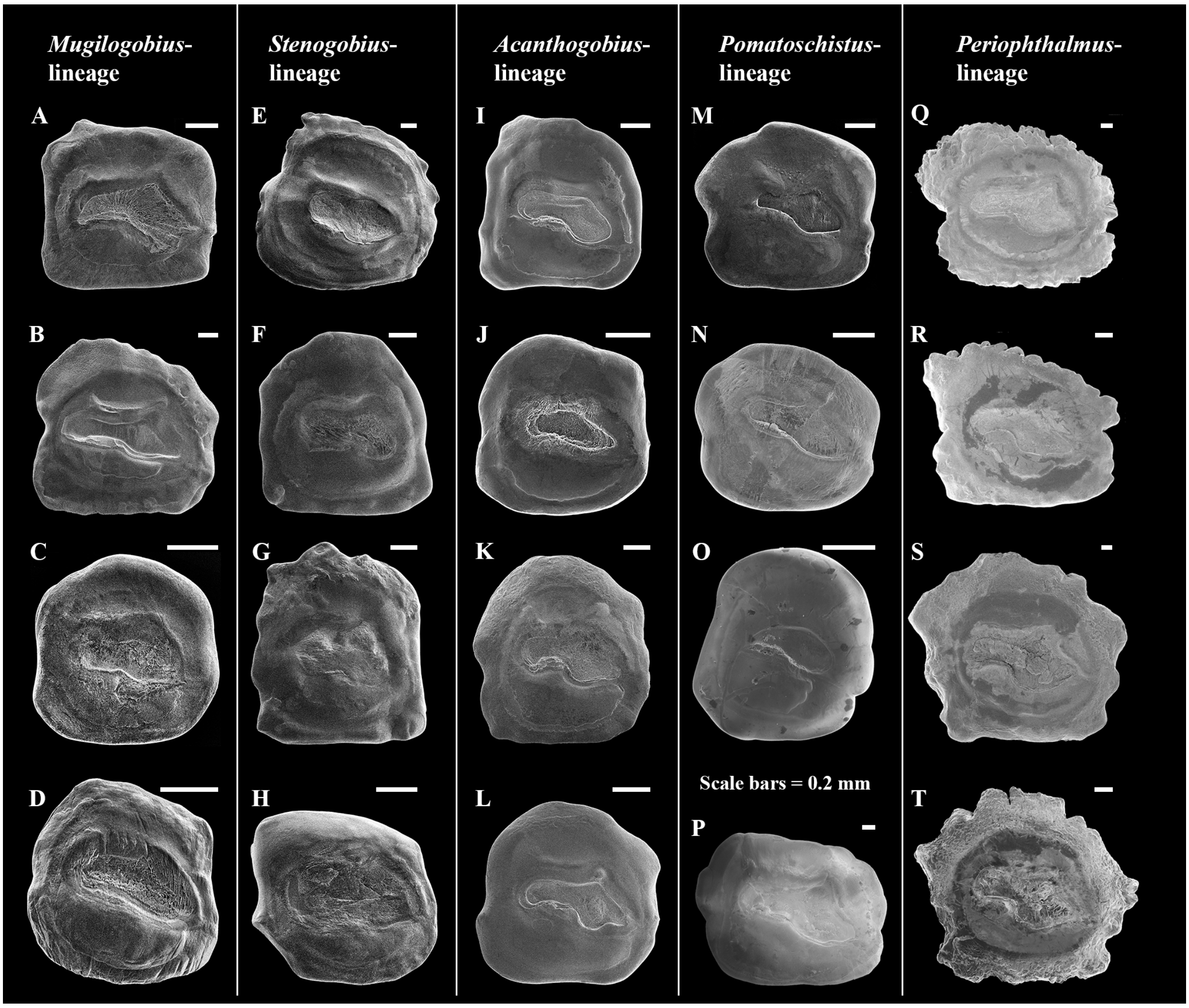

Figure 2. – Otolith morphology of the studied oxudercid species (left sagittae, inner face). ***Mugilogobius* lineage**: **A**: *Chlamydogobius eremius* ZSM-PIS-43854 (P-GO-1052). **B**: *Stigmatogobius sadanundio* ZSM-PIS-43856 (P-GO-1056). **C**: *Brachygobius xanthozonus* ZSM-PIS-43865 (P-GO-1075). **D**: *Schismatogobius roxasi* ZSM-PIS-43866 (P-GO-1077). ***Stenogobius* lineage**: **E**: *Gobioides broussonnetii* ZSM-PIS-43852 (P-GO-1048). **F, G**: *Awaous flavus* ZSM-PIS-43853 (P-GO-1051, -1050). **H**: *Stiphodon atropurpureus* ZSM-PIS-43862 (P-GO-1070). ***Acanthogobius* lineage**: **I**: *Rhinogobius rubromaculatus* ZSM-PIS-43860 (P-GO-1064). **J**: *R. zhoui* ZSM-PIS-43859 (P-GO-1062/63-2). **K**: *R. candidianus* ZSM-PIS-43858 (P-GO-1060). **L**: *R. formosanus* ZSM-PIS-43864 (P-GO-1073). ***Pomatoschistus* lineage: M**: *Pomatoschistus marmoratus* NMP P6d 32/2017-7. **N**: *Buenia affinis* NMP P6d 30/2017-6. **O**: *Gobiusculus flavescens* NMP P6V 142775. **P**: *Deltentosteus quadrimaculatus* NMP P6d 34/2017-9. ***Periophthalmus* lineage**: **Q**: *Boleophthalmus dussumieri* ZM–CBSU Khamirr 38. **R**: *Scartelaos tenuis* ZM–CBSU Helleh 86. **S, T**: *Periophthalmus waltoni* ZM–CBSU Gowater 1745 (male), ZM–CBSU Gowater 1736 (female).

The otoliths of *G. flavescens* are rounded-rectangular, with the height exceeding the length (Fig. 2O, 3J–K). The dorsal margin is rounded and smooth, the anterior margin straight but with a distinct convexity in the middle. The ventral margin is smooth or presents a few slight incisions; its deepest part is at the posterior end (and not in the middle), which gives the otolith a slightly irregular shape. The posterior margin is straight or has a small concavity approximately in the middle. The sulcus is as described above, but the cauda is straight or slightly inclined towards the ventral rim (instead of slightly inclined to the dorsal rim as in the other sand goby species).

The otoliths of *K. croatica* are almost rectangular in shape, but have a slightly rounded dorsal margin, and are higher than long (Fig. 3M–O). The dorsal margin sometimes displays a slight posterodorsal projection. The anterior margin is straight or shows a slightly undulating contour. The smooth ventral margin is straight in the middle and anteriorly and posteriorly curved. The posterior margin presents a slight posteroventral kink in some otoliths; otherwise it is straight or slightly concave.

The otoliths of *P. marmoratus* and *P. quagga* are similar to each other (Fig. 3D–F vs. 3G–I). The main difference is that *P. quagga* is higher than long, while *P. marmoratus* is approximately square-shaped. Both species display a dorsal margin that can be either rounded or presents a small ridge in the middle or slightly behind the middle; a short, broad posterodorsal projection is present in most specimens. Again in both species the anterior margin is slightly undulating, while the ventral margin is straight or slightly indented in the middle, posteriorly curved, but anteriorly angled or rounded. *Pomatoschistus marmoratus* has a cauda whose contour is mostly straight or slightly inclined towards the ventral rim (as seen in *G. flavescens*), while most otoliths of *P. quagga* have a cauda that is slightly inclined towards the dorsal rim.

The otoliths of †*Pomatoschistus* sp. are pictured in Brzobohatý (1994: pl. 6, figs. 9–119), but not been described. The sulcus has the same shape and position as described above for the recent sand goby species (Fig. 3A–C), but the cauda is straight or slightly inclined towards the ventral rim, as seen in *G. flavescens* and *P. marmoratus*. The otoliths are approximately square in overall shape. The rounded dorsal margin is smooth or mildly crenulated; a slight predorsal kink and a short posterodorsal projection are present in some otoliths. The anterior margin is straight or slightly inclined towards the ventral margin. The smooth ventral margin is straight or slightly notched in the middle and anteriorly and posteriorly curved. The posterior margin is smooth and can show a slight concavity.

4.3.3 Variation of otolith variables between the oxudercid lineages and †*Pomatoschistus* sp. (*sensu* Brzobohatý 1994)

We used non-parametric tests (Mann-Whitney, $p < 0.05$) to examine the significance of differences in otolith variables between the five recent lineages and †*Pomatoschistus* sp., because some variables were not normally distributed (Shapiro-Wilk, $p < 0.05$). The results indicate that eight out of the 23 otolith variables revealed differences between at least two of the studied groups (Fig. 4, Table III). Three of these eight variables incorporate the sulcus length (SuL/OL, SuL/SuEndV, SuL/OP), two the sulcus perimeter (SuP/OP, SuP/SuEndV), and the remainder are SuA/OA, SuTipV/SuEndV, and SuEndV/OP.

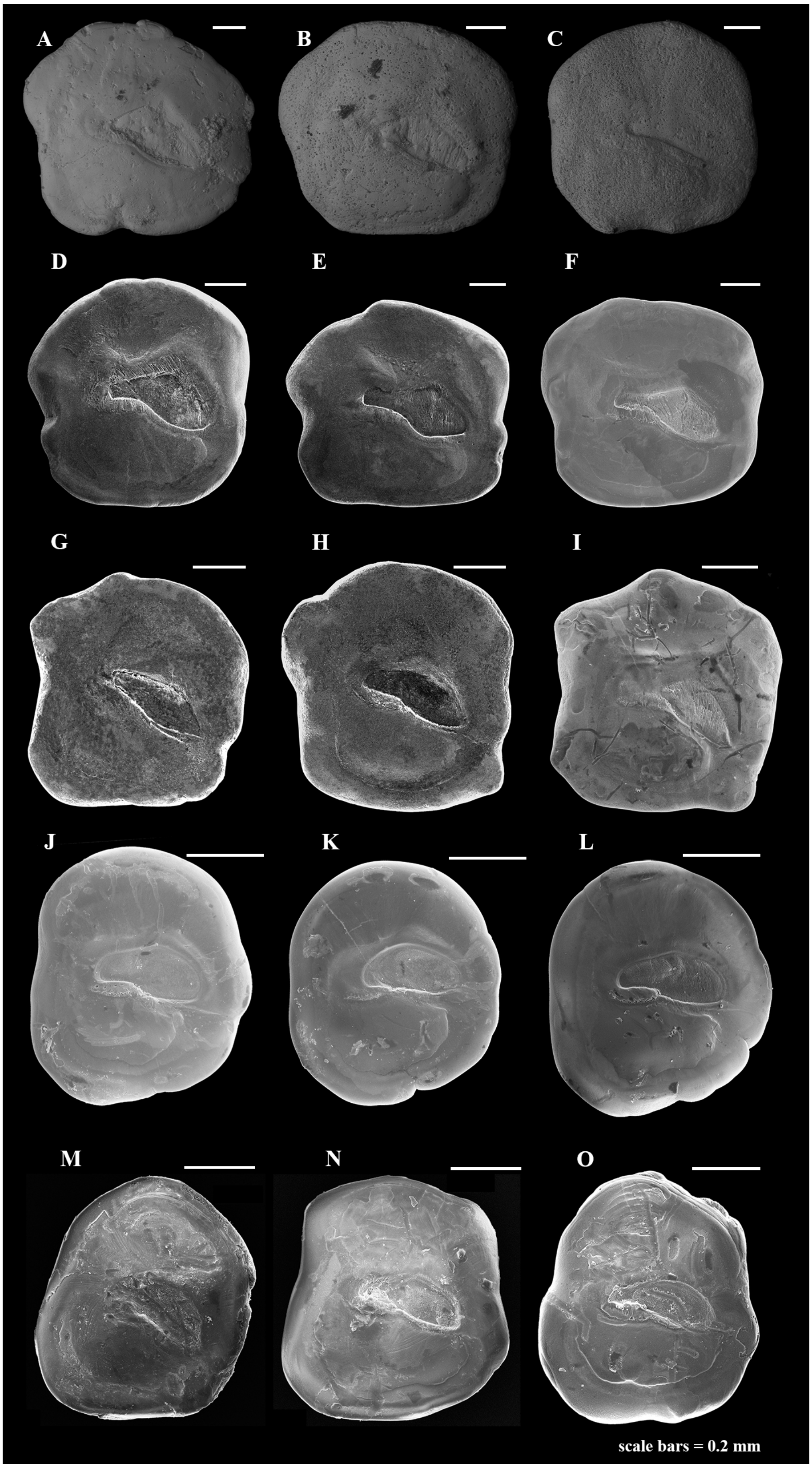

Figure 3. – Otolith morphology of †*Pomatoschistus* sp. **(A–C,** NHM 1993/140/4, NHM 1993/140/6, NHM 1993/140/8, all three mirrored**)** and the studied recent species of the sand-gobies, i.e. *Pomatoschistus marmoratus* **(D–F**, NMP P6d 32/2017-4, NMP P6d 32/2017-7, NMP P6d 32/2017-1**)**, *P. quagga* **(G–I,** NMP P6d 33/2017-4, NMP P6d 33/2017-5, NMP P6d 33/2017-7**),** *Gobiusculus flavescens* **(J–L,** NMP P6V 142777, NMP P6V 142778, NMP P6V

142776**)** and *Knipowitschia croatica* **(M–O,** NMP P6d 31/2017-7, NMP P6d 31/2017-2, NMP P6d 31/2017-3**)**. All images show the left sagitta from the inner face.

The *Pomatoschistus* lineage and †*Pomatoschistus* sp. were clearly separated from the *Mugilogobius*, *Acanthogobius* and *Periophthalmus* lineages, but not from the *Stenogobius* lineage (Table III). The *Acanthogobius* and *Periophthalmus* lineages could be separated from each other, and from the *Pomatoschistus* lineage and †*Pomatoschistus* sp., but not from the *Mugilogobius* and *Stenogobius* lineages. While the *Mugilogobius* lineage could be separated at least from the *Pomatoschistus* lineage and †*Pomatoschistus* sp., no separation was possible between the *Stenogobius* lineage and the others, and only a single otolith variable separated this lineage from †*Pomatoschistus* sp. (see Table III).

Table III lists the otolith variables that were most useful in separating groups from one another. The most powerful parameter in this respect is SuL/OL, as it contributes to the separation of all groups that could actually be distinguished, and is the only variable that could discriminate †*Pomatoschistus* sp. from the *Stenogobius* lineage. The variables SuL/SuEndV and SuTipV/SuEndV could separate six groups, SuA/OA and SuEndV/OP were useful in the separation of five groups, and SuL/OP could still separate four groups. The two variables related to the sulcus perimeter (SuP/OP, SuP/SuEndV) showed only little discriminatory power by separating only one and two groups, respectively, from the rest (Table III).

Considering which groups cluster together, one finds that the SuEndV/OP discriminates between three groups, one comprising the *Stenogobius* and *Mugilogobius* lineages, the second one the *Acanthogobius* and *Pomatoschistus* lineages, and the third one the *Periophthalmus* lineage alone (Fig. 4H). Furthermore, the variables SuP/OP, SuL/OL and SuL/OP can distinguish *Stenogobius*+*Acanthogobius*+*Mugilogobius* from *Pomatoschistus*+*Periophthalmus* (Figs. 4B, D, F). With respect to all of these variables, fossil otoliths of †*Pomatoschistus* fall either within the limits of the *Pomatoschistus* lineage or nearest to it.

A multivariate analyses based on a PCA using all 23 otolith variables shows that all lineages overlap, but that the fossil otoliths of †*Pomatoschistus* sp. only overlap with the *Pomatoschistus* lineage (Fig. 5). Very similar results were obtained when only the eight otolith variables shown to be informative in the univariate analyses were employed in the PCA (plot not shown).

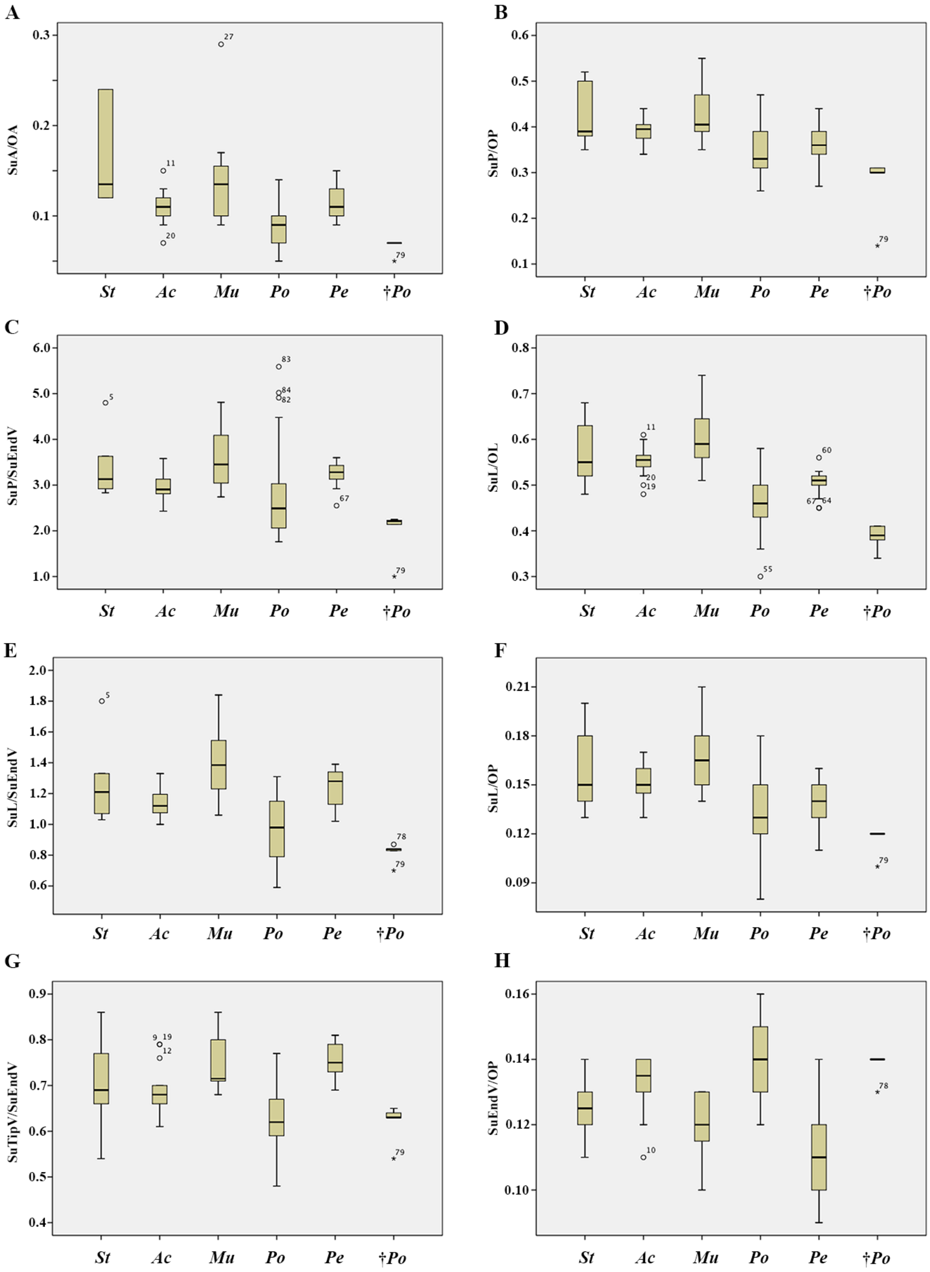

Figure 4. – Box plots of eight otolith variables that were useful in the separation of the five oxudercid lineages. **A**: ratio of sulcus area and otolith area. **B**: ratio of sulcus perimeter and otolith perimeter. **C**: ratio of sulcus perimeter and distance from sulcus end to the ventral margin. **D**: ratio of sulcus length and otolith length. **E**: ratio of sulcus length and distance from sulcus end to the ventral margin. **F**: ratio of sulcus length to otolith perimeter. **G**: ratio of distance from sulcus tip to the ventral margin and distance from sulcus end to the ventral margin. **H**: ratio of distance from sulcus end to the ventral margin and otolith perimeter. Abbreviations: *Ac* – *Acanthogobius* lineage; *Mu* – *Mugilogobius* lineage; *Pe* – *Periophthalmus* lineage; *Po* – *Pomatoschistus* lineage; *St* – *Stenogobius* lineage; †*Po* – †*Pomatoschistus* sp. from Brzobohatý (1994).

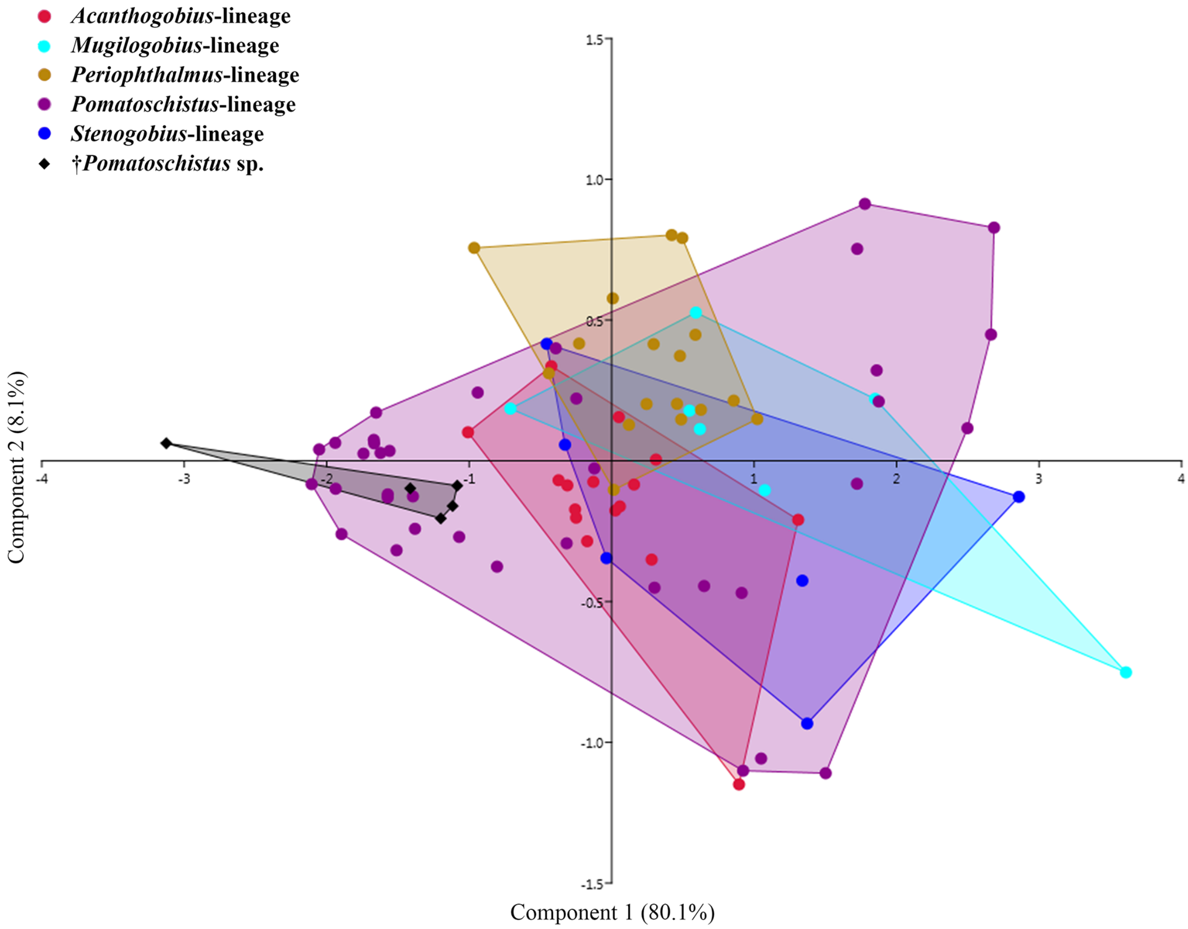

Figure 5. – PCA of the oxudercid lineages and †*Pomatoschistus* sp. based on all 23 otolith variables.

4.3.4 Variation of otolith variables between species of the *Pomatoschistus* lineage and †*Pomatoschistus* sp. (*sensu* Brzobohatý 1994)

Most of the otolith variables obtained for the recent species of the *Pomatoschistus* lineage were normally distributed, but in case of †*Pomatoschistus* sp. only OL/OH and SuL/OH revealed normal distribution (Shapiro-Wilk, $p < 0.05$). Accordingly, we used either parametric tests (ANOVA with Dunnett T3 post-hoc test, $p < 0.05$) or Mann-Whitney ($p < 0.05$) to examine the significance of differences in the individual otolith variables between the six recent species and †*Pomatoschistus* sp.

Table III. Otolith variables that differed significantly between the studied groups (Mann-Whitney test, $p < 0.05$). To facilitate comparison, the otolith variables are arranged in each cell in the same order. The symbol -- indicates that the respective variable was not significantly different; the symbol --- indicates that none of the otolith variables was different. For abbreviations of otolith variables see Table II. Since the content of the Table shows a symmetric matrix, we shaded the upper part.

	***Mugilogobius* lineage**	***Stenogobius* lineage**	***Acanthogobius* lineage**	***Pomatoschistus* lineage**	***Periophthalmus* lineage**	**†*Pomatoschistus* sp.**
***Mugilogobius* lineage**	X	---	---	-- -- -- SuL/OL SuL/SuEndV -- SuTipV/SuEndV SuEndV/OP	---	-- -- SuP/SuEndV SuL/OL SuL/SuEndV SuL/OP SuTipV/SuEndV SuEndV/OP
***Stenogobius* lineage**	---	X	---	---	---	-- -- -- SuL/OL -- -- -- --
***Acanthogobius* lineage**	---	---	X	SuA/OA SuP/OP - SuL/OL SuL/SuEndV SuL/OP SuTipV/SuEndV -	-- -- -- SuL/OL -- -- SuTipV/SuEndV SuEndV/OP	SuA/OA -- -- SuL/OL SuL/SuEndV SuL/OP -- --
***Pomatoschistus* lineage**	-- -- -- SuL/OL SuL/SuEndV -- SuTipV/SuEndV SuEndV/OP	---	SuA/OA SuP/OP -- SuL/OL SuL/SuEndV SuL/OP SuTipV/SuEndV --	X	SuA/OA -- -- SuL/OL SuL/SuEndV -- SuTipV/SuEndV SuEndV/OP	SuA/OA -- -- SuL/OL -- -- -- --
***Periophthalmus* lineage**	---	---	-- -- -- SuL/OL -- -- SuTipV/SuEndV SuEndV/OP	SuA/OA -- -- SuL/OL SuL/SuEndV -- SuTipV/SuEndV SuEndV/OP	X	SuA/OA -- SuP/SuEndV SuL/OL SuL/SuEndV SuL/OP SuTipV/SuEndV SuEndV/OP
†*Pomatoschistus* sp.	-- -- SuP/SuEndV SuL/OL SuL/SuEndV SuL/OP SuTipV/SuEndV SuEndV/OP	-- -- -- SuL/OL -- -- -- --	SuA/OA -- -- SuL/OL SuL/SuEndV SuL/OP -- --	SuA/OA -- -- SuL/OL -- -- -- --	SuA/OA -- SuP/SuEndV SuL/OL SuL/SuEndV SuL/OP SuTipV/SuEndV SuEndV/OP	X

We selected the eight otolith variables that revealed clear differences between at least two of the studied groups (Fig. 6, Table IV). As in the analysis of the lineage groups, three of these parameters incorporate the sulcus length (SuL/OH, SuL/OP, SuL/SuEndV), two the sulcus perimeter (SuP/SuTipV, SuP/SuEndV) and the remainder are SuA/OA, OL/OH and SuH/SuP. Four otolith variables (OL/OH, SuL/OH, SuH/SuP, SuP/SuTipV) showed discriminatory power in this analysis, although they had not done so with respect to the lineage groups (see above).

These results showed that the sand gobies plus †*Pomatoschistus* sp. can be clearly discriminated from the non-sand gobies (*B. affinis*, *D. quadrimaculatus*), but that the separation of *P. marmoratus* between either *B. affinis* or *D. quadrimaculatus* is not as clearcut as that obtained for the other sand gobies (Fig. 6, Table IV). On the other hand, only three otolith variables, two of them related to the sulcus perimeter, separated *B. affinis* from *D. quadrimaculatus* (Table IV). In the case of the recent sand gobies, *P. marmoratus* could be reliably separated from all studied sand gobies, and even from *P. quagga,* based on 5–7 otolith variables (see Table IV). In contrast, only two otolith variables discriminate between *G. flavescens* and *P. quagga*, and solely one otolith variable differs between *K. croatica* and *P. quagga* (SuH/SuP), and also between *G. flavescens* and *K. croatica* (SuA/OA) (Table IV). The fossil †*Pomatoschistus* sp. could be separated from *P. quagga* based on two variables ((OL/OH, SuH/SuP), and only a single otolith variable separates it from *G. flavescens* (SuA/OA) and *K. croatica* (OL/OH).

Table IV lists the otolith variables that were most useful in the separation of the studied species of the *Pomatoschistus* lineage. It appears that the most powerful parameters are OL/OH, SuL/OP, SuL/OH, SuL/SuEndV and SuP/SuEndV, as they contribute to almost all the discriminations achieved for the recent species. SuH/SuP is involved in fewer cases of successful separation between groups, but is nevertheless meaningful because it is the sole variable that could separate *K. croatica* from *P. quagga* and, moreover, one of only two variables that discriminate between *B. affinis* and *P. marmoratus* and between *G. flavescens* and *P. quagga*. Of the remaining two variables, SuP/SuTipV appears to be less informative, whereas SuA/OA, although involved only in the separation between few groups, is the single variable that could discriminate between *G. flavescens* and *K. croatica* as well as between *G. flavescens* and †*Pomatoschistus* sp., and one of only two variables that separate *G. flavescens* from *P. quagga.*

The PCA based on all 23 otolith variables for the recent species of the *Pomatoschistus* lineage and †*Pomatoschistus* sp. resolves three groups (Fig. 7). One group consists only of *D. quadrimaculatus*. Within the second group, *B. affinis* overlaps to some extent with *P. marmoratus*. The third group comprises the sand gobies *G. flavescens*, *K. croatica* and *P. quagga* as well as †*Pomatoschistus* sp.

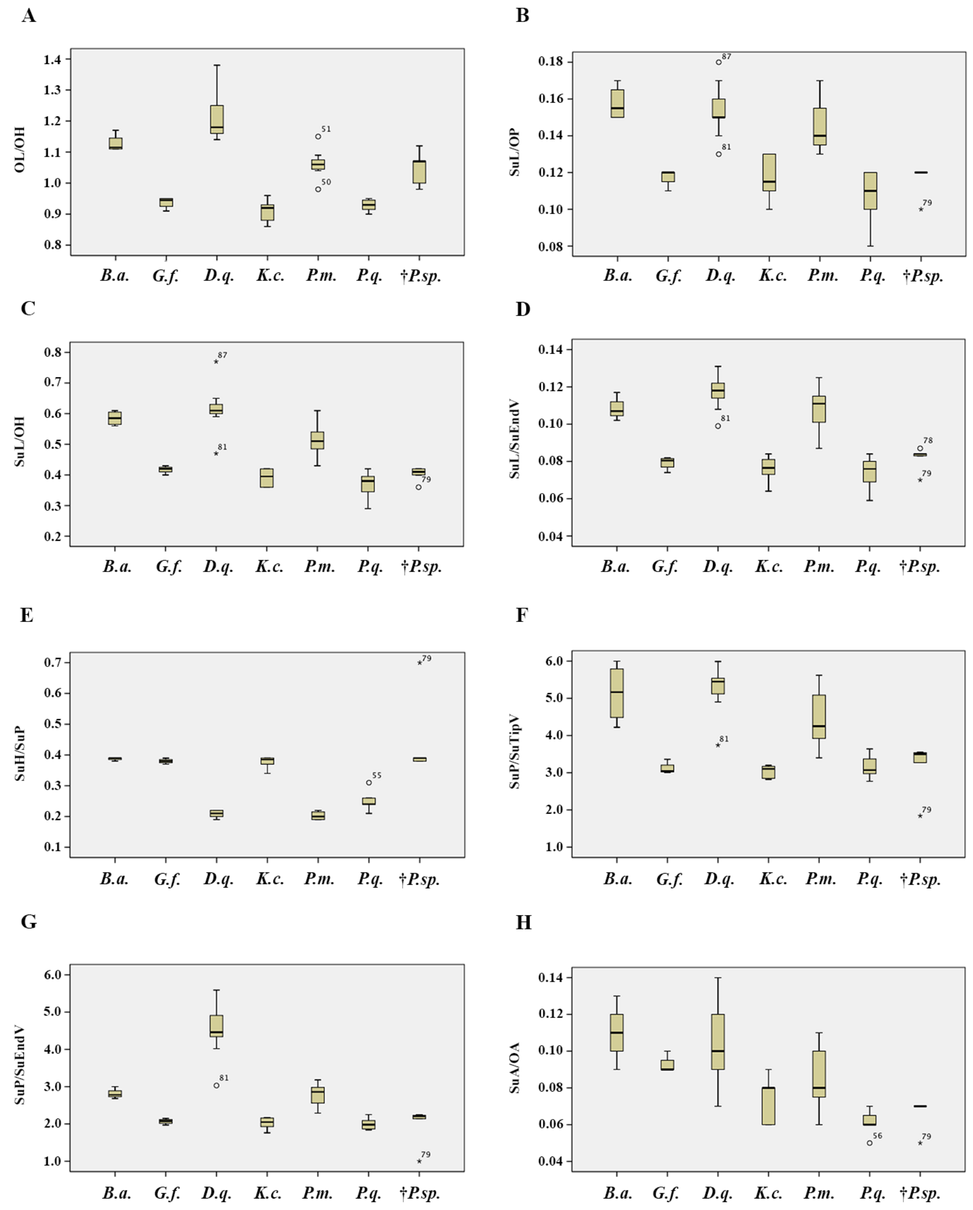

Figure 6. – Box plots of eight otolith variables that were useful in the separation of the recent species of the *Pomatoschistus* lineage and †*Pomatoschistus* sp. **A**: ratio of otolith length and otolith height. **B**: ratio of sulcus length and otolith perimeter. **C**: ratio of sulcus length and otolith length. **D**: ratio of sulcus length and distance from sulcus end to the ventral margin. **E**: ratio of sulcus height and sulcus perimeter. **F**: ratio of sulcus perimeter and distance from sulcus tip to the ventral margin. **G**: ratio of sulcus perimeter and distance from sulcus end to the ventral margin. **H**: ratio of sulcus area and otolith area. Abbreviations: *B.a.*: *Buenia affinis*; *G.f.*: *Gobiusculus flavescens*; *D.q.*: *Deltentosteus quadrimaculatus*; *K.c.*: *Knipowitschia croatica*; *P.m.*: *Pomatoschistus marmoratus*; *P.q.*: *Pomatoschistus quagga*; †*P.*sp.: †*Pomatoschistus* sp.

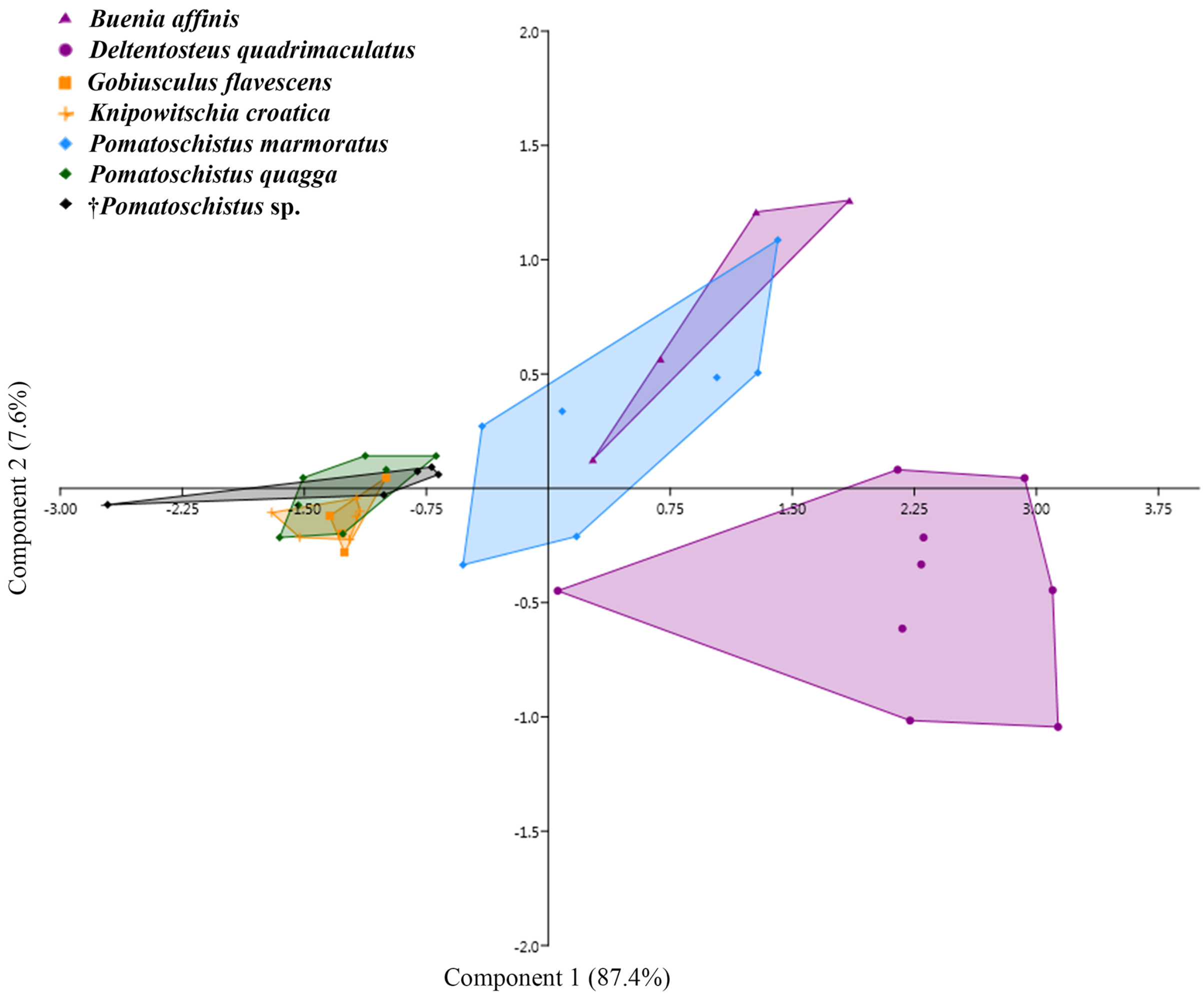

Figure 7. – PCA of the studied *Pomatoschistus* species and genera and †*Pomatoschistus* sp. based on all 23 otolith variables.

Table IV. Otolith variables that differed significantly between the studied groups ($p < 0.05$, ANOVA with Dunnett T3 post-hoc test for normally distributed variables, Mann-Whitney for non-normally ones). To facilitate comparison, the otolith variables are arranged in each cell in the same order. The symbol -- indicates that the respective variable was not significantly different. For complete species names see Table I, for abbreviations of otolith variables see Table II. Since the content of the Table shows a symmetric matrix, we shaded the upper part.

	Non sand-goby		**Recent species of sand-gobies**				
	Buenia affinis	***Deltentosteus quadrimac.***	***Gobiusculus flavescens***	***Knipow. croatica***	***Pomatosch. marmoratus***	***Pomatosch. quagga***	**†*Pomatosch.* sp.**
Buenia affinis	X	OL/OH -- -- -- SuH/SuP -- SuP/SuEndV --	OL/OH SuL/OP SuL/OH SuL/SuEndV -- SuP/SuTipV SuP/SuEndV --	OL/OH SuL/OP SuL/OH SuL/SuEndV -- -- SuP/SuEndV --	OL/OH -- -- -- SuH/SuP -- -- --	OL/OH SuL/OP SuL/OH SuL/SuEndV SuH/SuP -- SuP/SuEndV --	-- SuL/OP SuL/OH SuL/SuEndV -- SuP/SuTipV SuP/SuEndV SuA/OA
Deltentosteus quadrimac.	OL/OH -- -- -- SuH/SuP -- SuP/SuEndV --	X	OL/OH SuL/OP SuL/OH SuL/SuEndV SuH/SuP SuP/SuTipV SuP/SuEndV --	OL/OH SuL/OP SuL/OH SuL/SuEndV SuH/SuP SuP/SuTipV SuP/SuEndV --	OL/OH -- -- -- -- -- SuP/SuEndV --	OL/OH SuL/OP SuL/OH SuL/SuEndV -- SuP/SuTipV SuP/SuEndV SuA/OA	OL/OH SuL/OP SuL/OH SuL/SuEndV SuH/SuP SuP/SuTipV SuP/SuEndV SuA/OA
Gobiusculus flavescens	OL/OH SuL/OP SuL/OH SuL/SuEndV -- SuP/SuTipV SuP/SuEndV --	OL/OH SuL/OP SuL/OH SuL/SuEndV SuH/SuP SuP/SuTipV SuP/SuEndV --	X	-- -- -- -- -- -- -- SuA/OA	OL/OH SuL/OP -- SuL/SuEndV SuH/SuP SuP/SuTipV SuP/SuEndV --	-- -- -- -- SuH/SuP -- -- SuA/OA	-- -- -- -- -- -- -- SuA/OA
Knipow. croatica	OL/OH SuL/OP SuL/OH SuL/SuEndV -- -- SuP/SuEndV --	OL/OH SuL/OP SuL/OH SuL/SuEndV SuH/SuP SuP/SuTipV SuP/SuEndV --	-- -- -- -- -- -- -- SuA/OA	X	OL/OH SuL/OP SuL/OH SuL/SuEndV SuH/SuP SuP/SuTipV SuP/SuEndV --	-- -- -- -- SuH/SuP -- -- --	OL/OH -- -- -- -- -- -- --
Pomatosch. marmoratus	OL/OH -- -- -- SuH/SuP -- -- --	OL/OH -- -- -- -- -- SuP/SuEndV --	OL/OH SuL/OP -- SuL/SuEndV SuH/SuP SuP/SuTipV SuP/SuEndV --	OL/OH SuL/OP SuL/OH SuL/SuEndV SuH/SuP SuP/SuTipV SuP/SuEndV --	X	OL/OH SuL/OP SuL/OH SuL/SuEndV -- -- SuP/SuEndV --	-- SuL/OP SuL/OH SuL/SuEndV SuH/SuP SuP/SuTipV SuP/SuEndV --
Pomatosch. quagga	OL/OH SuL/OP SuL/OH SuL/SuEndV SuH/SuP -- SuP/SuEndV --	OL/OH SuL/OP SuL/OH SuL/SuEndV -- SuP/SuTipV SuP/SuEndV SuA/OA	-- -- -- -- SuH/SuP -- -- SuA/OA	-- -- -- -- SuH/SuP -- -- --	OL/OH SuL/OP SuL/OH SuL/SuEndV -- -- SuP/SuEndV --	X	OL/OH -- -- -- SuH/SuP -- -- --
†*Pomatosch.* sp.	-- SuL/OP SuL/OH SuL/SuEndV -- SuP/SuTipV SuP/SuEndV SuA/OA	OL/OH SuL/OP SuL/OH SuL/SuEndV SuH/SuP SuP/SuTipV SuP/SuEndV SuA/OA	-- -- -- -- -- -- -- SuA/OA	OL/OH -- -- -- -- -- -- --	-- SuL/OP SuL/OH SuL/SuEndV -- -- -- --	OL/OH -- -- -- SuH/SuP -- -- --	X

4.4 Discussion

The intention of this study was to test whether the five lineages of the Oxudercidae, and individual species of the European *Pomatoschistus* lineage, could be distinguished based on the quantification of otolith variations. The approach was applied to otoliths from a total of 20 extant oxudercid species representing the five extant lineages, and fossil otoliths of †*Pomatoschistus* sp. (sensu Brzobohatý 1994). Both otolith morphology (qualitative approach) and otolith morphometry (quantitative approach) were considered and a total of 23 otolith variables was assessed. The results showed that the otolith morphology of most Oxudercidae examined is clearly different from that found in their sister group, the Gobiidae (e.g. Agorreta et al. 2013), because no obvious posterodorsal projection is present (see Gierl et al. 2013: fig. 6D). However, the otolith samples obtained from the *Periophthalmus* lineage do have a prominent posterodorsal projection (see Fig. 2) and thus are similar to the otoliths of Gobiidae.

4.4.1 Separation at the lineage level using otoliths

Visual inspection of otolith morphology alone was insufficient to enable specimens to be assigned to an individual lineage, but the comparative analysis of otolith variables was partially successful. The overall rate of success is low (40%, see Table III), but appears to reflect to some extent the biogeographic distribution and, in the case of the *Periophthalmus* lineage, also a specific lifestyle (see below).

The *Pomatoschistus* lineage is the only lineage of the Oxudercidae that occurs in European waters (Agorreta et al. 2013). This biogeographic uniqueness may be reflected in its otoliths, as otolith variables separated the *Pomatoschistus* lineage from all others except the *Stenogobius* group (see Table III). Of the two tropical lineages, only the *Periophthalmus* lineage could be separated from the temperate ones (see Table III). This lineage includes the mudskippers, which are characterized by an amphibious lifestyle (Murdy 1989). Among the ten mudskipper genera that are recognised, the members of *Boleophthalmus* Valenciennes, 1937, *Scartelaos* Swainson, 1839, *Periophthalmodon* Bleeker, 1874 and *Periophthalmus* Bloch & Schneider, 1801 (Murdy 1989; Ishimatsu and Gonzales 2011) are especially derived, because they can move on muddy substrates, climb rocks or mangrove roots, and display, forage and defend territories during low tides (Murdy 2011; Jafaar and Parenti 2017). The otoliths of the *Periophthalmus* lineage used in this study were obtained from three of these species (Table I). It can therefore be assumed that the highly distinctive nature of their otoliths, relative to those of the other oxudercid lineages, reflects a functional change in the sensory system of the inner ear that is related to their unique lifestyle.

4.4.2 Separation of species and †*Pomatoschistus* sp. (*sensu* Brzobohatý 1994) within the *Pomatoschistus* lineage

The otoliths of the four recent species of the sand gobies used in our study (*Gobiusculus flavescens*, *Knipowitschia croatica*, *Pomatoschistus marmoratus*, *P. quagga*) all show a marked reduction in the size of the cauda (Figs. 2 and 3). Based on this character, they could be readily separated from the non-sand gobies studied here, i.e. *Deltentosteus quadrimaculatus* (Fig. 2P) and *Buenia affinis* (Fig. 2N). The name sand gobies refers to their preference for sandy substrates (see Gierl and Reichenbacher 2017) and it appears that this particular lifestyle has had an impact on their sensory system and otoliths, as discussed above for the mudskippers. Moreover, otolith variables proved useful for species classification within the *Pomatoschistus* lineage, as all cases were separated (Table IV).

Another interesting outcome of our study is the similarity observed between the otoliths of the sand goby *P. marmoratus* and those of the two non-sand goby species (*D. quadrimaculatus*, *B. affinis*). It could be speculated that this might reflect a more basal phylogenetic position of *P. marmoratus* among the sand-gobies, but such a placement is not supported by molecular studies (Vanhove et al. 2012; Agorreta et al. 2013). *P. marmoratus* is one of the most abundant benthic oxudercid species in the estuaries and sandy coastal lagoons of the Mediterranean Sea (see Mejri et al. 2011). It is also widespread throughout the eastern Atlantic, the Black Sea and the Sea of Azov (Miller 1986). Accordingly, *P. marmoratus* appears to be a generalist rather than a specialist, and this might explain its 'basal' otolith morphology and similarity to the two non-sand goby species, respectively.

Moreover, the otolith variables permit a surprisingly clear degree of distinction between *P. marmoratus* and *P. quagga* (five differing variables, see Table IV). In this case, the otolith data are fully confirmed by the molecular study of the sand gobies by Vanhove et al. (2012). These authors showed that *Pomatoschistus* splits into two main clades (one so far containing mainly *P. marmoratus*) and is, in addition, paraphyletic, because *P. quagga* is not a member of the two main clades, but falls within a *Knipowitschia* clade. The presence of two main clades within *Pomatoschistus*, and a basal position for *P. marmoratus* in one of those, has now been further confirmed by Engin and Seyhan (2017).

In addition, we found few differences between *P. quagga* and *G. flavescens* (two differing variables). According to Vanhove et al. (2012), *G. flavescens* belongs to the second of the two *Pomatoschistus* clades (which does not contain *P. marmoratus*), a conclusion that is consistent with its '*Pomatoschistus*-like' otoliths. This is further supported by Thacker et al. (2018) who place "*flavescens*" within the genus *Pomatoschistus*. Furthermore, both *G. flavescens* and *P. quagga* share a 'hyperbenthic' lifestyle, which means that they remain within 1 m above the sea bottom (Kovačić, 2003). So, the two species could also be a further example of otolith similarity because of a shared specialisation in lifestyle.

Another interesting point is that we found only one otolith variable that differed between *P. quagga* and *K. croatica*. In this case, related lifestyles cannot be responsible for this close similarity in otolith morphology, because *P. quagga* is marine, whereas *K. croatica* is a freshwater species. In the molecular tree of Vanhove et al. (2012), *P. quagga* is a member of a *Knipowitschia* clade, but *K. croatica* was not included in this tree. In the DNA sequence-based study by Geiger et al. (2014), a comprehensive molecular data set from *Knipowitschia* was used, and *K. croatica* did not fall in the *Knipowitschia* clade in that study. Also Vanhove et al. (2016) found two main clades within the *Knipowitschia* species. One clade comprised *K. croatica* + *K. punctatissima* and these two were sister to *Pomatoschistus canestrinii*. This group in turn was sister to the second clade of *Knipowitschia*. So more work is necessary to resolve the relationships between *P. quagga* and *K. croatica* and to evaluate whether their otolith similarity is a phylogenetic signal.

The fossil otoliths of †*Pomatoschistus* sp. were clearly similar to those of the sand gobies, but not to *P. marmoratus* (Fig. 7, Table IV). This, however, concurs precisely with the aforementioned results of the molecular studies. It cannot be definitively decided whether the fossil otoliths of †*Pomatoschistus* sp. represent a 'true' *Pomatoschistus* species. But this genus is certainly a member of the sand gobies and, with its middle Miocene age, represents the oldest record of a sand goby species to date. Since fossil otoliths have a much higher chance of preservation, they usually precede the fossil skeletal record. The oldest skeleton-based *Pomatoschistus* is †*Pomatoschistus* sp. from the Middle Miocene of Russia (Carnevale et al. 2006).

4.4.3 Concluding remarks

The ratio of otolith length to otolith height (OL/OH) has long been considered as a useful parameter for species separation in fossil otoliths (e.g., Weiler, 1963). The ratio of the sulcus area to overall otolith area (SuA/OA) has previously been used mainly to study the eco-morphological patterns of otoliths of recent species (Volpedo et al. 2008). Other already established otolith variables mainly relate to the rostrum and antirostrum (Reichenbacher et al. 2007; Volpedo et al. 2008; Vignon and Morat 2010). They cannot be used for the otoliths of the Gobiidae and Oxudercidae because these do not possess such structures.

Separation of otoliths at different taxonomic levels may require different otolith variables (see Reichenbacher et al. 2007). This was also the case in our study. While the SuL/OL was involved in all cases of successful separation at the level of lineages (see Table III), it was not meaningful at the level of species (see Table IV). This implies that the size of the sulcus may bear a phylogenetic signal at higher taxonomic levels. On the other hand, OL/OH was among the most useful variables at the species level, but was of little import in the separation of the lineages. So OL/OH can be considered as an autapomorphy for an individual species. However, further work based on additional species is needed to verify the possible phylogenetic value of the individual otolith variables.

ACKNOWLEDGEMENTS

First of all, we would like to thank David Lecchini (Paris) and the CRIOBE team for the organisation of the impressive Indo-Pacific Fish Conference in Tahiti in 2017 and for giving us the opportunity to contribute to a special Cybium volume dedicated to this congress. We are grateful to all participants of the IPFC symposium 'Ecology & Evolution of Gobies' for their interest in our work which encouraged us to prepare this manuscript. We thank Dr. Ulrich Schliewen (Zoological State Collection, Munich) for his valuable help in aquiring some of the recent species and fruitful discussions. We are grateful to Dr. Ursula Göhlich (Natural History Museum Vienna, Austria) for providing the fossil otoliths described in Brzobohatý (1994). We thank Prof. Dr. Roland Melzer (Zoological State Collection, Munich) and Claudia Franz (Senckenberg, Frankfurt am Main) for their help with the respective SEM facilities, and Dr. Zeinab Gholami (LMU Munich, Germany) for taking the SEM images of the species from the *Periophthalmus* lineage. We gratefully acknowledge the constructive and detailed comments of two anonymous reviewers. CG and BR acknowledge funding from the German Research Foundation (grant number RE 1113/20-1). RS received support from the Ministry of Culture of the Czech Republic (DKRVO 2017/15, National Museum, 00023272).

4.5 References

Agorreta, A., D. San Mauro, U. Schliewen, J.L. Van Tassell, M. Kovačić, R. Zardoya, and L. Rüber. 2013. Molecular phylogenetics of Gobioidei and phylogenetic placement of European gobies. *Molecular Phylogenetics and Evolution* 69 (3): 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 (6): 1268–1295.

Assis, C.A. 2005. The utricular otoliths, lapilli, of teleosts: their morphology and relevance for species identification and systematics studies. *Scientia Marina* 69 (2): 259–273.

Avigliano, E., A. Domanico, S. Sánchez, and A.V. Volpedo. 2017. Otolith elemental fingerprint and scale and otolith morphometry in *Prochilodus lineatus* provide identification of natal nurseries. *Fisheries Research* 186 (Part 1): 1–10.

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

Campana, S.E. 2004. *Photographic atlas of fish otoliths of the northwest Atlantic Ocean*. Ottawa, Ontario: NRC Research Press.

Campana, S.E. 2005. Otolith science entering the 21st century. *Marine and Freshwater Research* 56 (5): 485–495.

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

Carnevale, G., A.F. Bannikov, W. Landini, and C. Sorbini. 2006. Volhynian (early Sarmatian sensu lato) fishes from Tsurevsky, North Caucasus, Russia. *Journal of Paleontology* 80 (4): 684–699.

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

Esmaeili, H.R., A. Teimori, Z. Gholami, and B. Reichenbacher. 2014. Two new species of the tooth-carp *Aphanius* (Teleostei: Cyprinodontidae) and the evolutionary history of the Iranian inland and inland-related *Aphanius* species. *Zootaxa* 3786 (3): 246–268.

Geiger, M.F., F. Herder, M.T. Monaghan, V. Almada, R. Barbieri, M. Bariche, P. Berrebi, J. Bohlen, M. Casal-Lopez, G.B. Delmastro, G.P.J. Denys, A. Dettai, I. Doadrio, E. Kalogianni, H. Kärst, M. Kottelat, M. Kovačić, M. Laporte, M. Lorenzoni, Z. Marčić, M. Özuluğ, A. Perdices, S. Perea, H. Persat, S. Porcelotti, C. Puzzi, J. Robalo, R. Šanda, M. Schneider, V. Šlechtová, M. Stoumboudi, S. Walter, and J. Freyhof. 2014. Spatial heterogeneity in the Mediterranean Biodiversity Hotspot affects barcoding accuracy of its freshwater fishes. *Molecular Ecology Resources* 14 (6): 1210–1221.

Gholami, Z., H.R. Esmaeili, D. Erpenbeck, and B. Reichenbacher. 2014. Phylogenetic analysis of *Aphanius* from the endorheic Kor River Basin in the Zagros Mountains, South-western Iran (Teleostei: Cyprinodontiformes: Cyprinodontidae). *Journal of Zoological Systematics and Evolutionary Research* 52 (2): 130–141.

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

Gierl, C., B. Reichenbacher, J. Gaudant, D. Erpenbeck, and A. Pharisat. 2013. An extraordinary gobioid fish fossil from southern France. *PLOS ONE* 8 (5): e64117.

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

IBM Corp. 2016. IBM SPSS Statistics for Macintosh, Version 24.0. IBM Corp., Armonk, NY.

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

Jaafar, Z., and L.R. Parenti. 2017. Systematics of the mudskipper genus *Oxuderces* Eydoux & Souleyet 1848 (Teleostei: Gobiidae: Oxudercinae) with resurrection from synonymy of *O. nexipinnis* (Cantor 1849). *Zoological Journal of the Linnean Society* 180: 195–215.

Koken, E. 1884. Über Fisch-Otolithen, insbesondere über diejenigen der norddeutschen

Oligocän-Ablagerungen. *Zeitschrift der Deutschen Geologischen Gesellschaft* 36: 500–565.

Kovačić, M. 2003. Hyperbenthic gobies in the Kvarner area, Adriatic Sea. *Journal of Fish Biology* 63 (4): 1051–1055.

Lord, C., C. Brun, M. Hautecœur, and P. Keith. 2010. Insights on endemism: comparison of the duration of the marine larval phase estimated by otolith microstructural analysis of three amphidromous *Sicyopterus* species (Gobioidei: Sicydiinae) from Vanuatu and New Caledonia. *Ecology of Freshwater Fish* 19 (1): 26–38.

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

McKay, S.I., and P.J. Miller. 1997. The affinities of European sand gobies (Teleostei: Gobiidae). *Journal of Natural History* 31 (10): 1457–1482.

Mejri, R., M. Arculeo, O.K. Ben Hassine, and S. Lo Brutto. 2011. Genetic architecture of the marbled goby *Pomatoschistus marmoratus* (Perciformes, Gobiidae) in the Mediterranean Sea. *Molecular Phylogenetics and Evolution* 58 (2): 395–403.

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

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

Murdy, E.O. 2011. Systematics of Oxudercinae. In *The biology of gobies*, eds. R. A. Patzner, J. L. Van Tassell, M. Kovačić, and B. G. Kapoor, 99–106. Enfield, NH: Science Publishers Inc.

Nelson, J.S., T.C. Grande, and M.V.H. Wilson. 2016. *Fishes of the World, Fifth Edition*. Hoboken, New Jersey: John Wiley & Sons, inc.

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

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

Popper, A.N., and Z. Lu. 2000. Structure–function relationships in fish otolith organs. *Fisheries Research* 46 (1–3): 15–25.

Reichenbacher, B., U. Sienknecht, H. Küchenhoff, and N. Fenske. 2007. Combined otolith morphology and morphometry for assessing taxonomy and diversity in fossil and extant killifish (*Aphanius*, †*Prolebias*). *Journal of Morphology* 268 (10): 898–915.

Schneider, C.A., W.S. Rasband, and K.W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9 (7): 671–675.

Schulz-Mirbach, T., M. Heß, and M. Plath. 2011. Inner ear morphology in the Atlantic molly

Poecilia mexicana—first detailed microanatomical study of the inner ear of a cyprinodontiform species. *PLOS ONE* 6 (11): e27734.

Schulz-Mirbach, T., and B. Reichenbacher. 2006. Reconstruction of Oligocene and Neogene freshwater fish faunas – an actualistic study on cypriniform otoliths. *Acta Palaeontologica Polonica* 51 (2): 283–304.

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

Smale, M.J., G. Watson, and T. Hecht eds. 1995. *Otolith atlas of southern African marine fishes*. Grahamstown: J. L. B. Smith Institute of Ichthyology.

Teimori, A., H.R. Esmaeili, D. Erpenbeck, and B. Reichenbacher. 2014. A new and unique species of the genus *Aphanius* Nardo, 1827 (Teleostei: Cyprinodontidae) from Southern Iran: A case of regressive evolution. *Zoologischer Anzeiger - A Journal of Comparative Zoology* 253 (4): 327–337.

Teimori, A., H.R. Esmaeili, Z. Gholami, N. Zarei, and B. Reichenbacher. 2012. *Aphanius arakensis*, a new species of tooth-carp (Actinopterygii, Cyprinodontidae) from the endorheic Namak Lake basin in Iran. *Zookeys* 215: 55–76.

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

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

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

Tzeng, W.N., and Y.C. Tsai. 1994. Changes in otolith microchemistry of the Japanese eel, *Anguilla japonica*, during its migration from the ocean to the rivers of Taiwan. *Journal of Fish Biology* 45 (4): 671–683.

Vanhove, M.P.M., A.N. Economou, S. Zogaris, M.H.D. Larmuseau, S. Giakoumi, E. Kalogianni, F.A.M. Volckaert, and T. Huyse. 2012. Phylogenetics and biogeography of the Balkan 'sand gobies' (Teleostei: Gobiidae): vulnerable species in need of taxonomic revision. *Biological Journal of the Linnean Society* 105 (1): 73–91.

Vanhove, M.P.M., M. Kovačić, and S. Zogaris. 2016. A distinct island population of threatened freshwater fish: to split or lump? *Hydrobiologia* 777 (1): 79–93.

Vignon, M., and F. Morat. 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., and D.D. Echeverría. 2000. *Catálogo y claves de otolitos para la identificación de peces del Mar Argentino 1. Peces de importanica comercial*. Buenos Aires: Editorial Dunken.

Volpedo, A.V., A.D. Tombari, and D.D. Echeverría. 2008. Eco-morphological patterns of the sagitta of Antarctic fish. *Polar Biology* 31 (5): 635–640.

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.

5. First total evidence approach for Gobioidei

Christoph Gierl[1], Bettina Reichenbacher[1], Martin Dohrmann[1] et al.

[1]Department of Earth and Environmental Sciences, Ludwig-Maximilians University Munich, Richard-Wagner Straße 10, 80333 Munich, Bavaria, Germany; E-mail: c.gierl@lrz.uni-muenchen.de (send reprint requests to this address); b.reichenbacher@lrz.uni-muenchen.de; m.dohrmann@lrz.uni-muenchen.de.

This chapter is preliminary work and will not be published in this version.
Please do not cite or distribute without permission of the authors.

ABSTRACT

The Gobioidei or gobies is one of the most diverse groups among vertebrates with more than 2200 species in 320 genera. They can be found in fresh, brackish and marine habitats. Their high adaptiveness is shown in peculiar ways, e.g. the guardian gobies sharing shelter with a shrimp or the mudskippers showing a pronounced amphibious lifestyle. The fossil record is compared to the gobies' modern diversity with about 100 known species – 70 of which only based on otoliths – rather scarce. Earlier descriptions of fossil species have been mostly described as *Gobius* (*sensu lato*), because there are only few characters for defining families. So, in order to place fossils properly and understand the evolutionary history of the gobioids a phylogenetic framework that incorporates morphological data is needed. Such a phylogeny is currently not available. Here we present a morphology-based phylogeny comprising all currently recognized gobioid families represented by at least two species each and eight fossil species. Furthermore, we incorporate molecular data for a total evidence analysis. We used maximum parsimony and a Bayesian approach for tree inference. The resulting molecular trees were in congruence with previous published molecular phylogenies. The trees based on morphology were in part poorly resolved but still showed general patterns known from the molecular data. The total evidence trees were resolved better, all families were retained and the fossils were placed in accordance to the putative assignments of previous works based on comparative taxonomy. This total evidence framework can be used and expanded for future placement of gobioid fossils.

5.1 Introduction

Gobioidei or gobies are among the most diverse groups of vertebrates with about 320 genera and over 2200 species (Nelson et al. 2016). They inhabit marine, brackish and freshwater habitats and form an important and huge part in coral reefs (see Patzner et al. 2011; Tornabene et al. 2016, Brandl et al. 2018). They show a high range of very peculiar specializations like being able to live on land for a longer period of time (mudskippers, e.g. see Jaafar and Murdy 2017) or living in symbiosis with certain shrimp species (see Karplus 1987). The Gobioidei are a part of the Gobiiformes (sensu Thacker et al. 2015) which additionally contains the Kurtidae, Apogonidae and Trichonotidae (Fig. 1). The Gobioidei consists of eight families, the Rhyacichthyidae, the Odontobutidae; the Milyeringidae; the Eleotridae; the Butidae; the Thalasseleotrididae; the Gobiidae and the Oxudercidae (= former Gobionellidae, see Nelson et al. 2016) (Fig. 1). The relationships within the Gobioidei seem well resolved based on molecular data (e.g. see Agorreta et al. 2013; Thacker et al. 2015), however the relationships between the Odontobutidae and those species considered as being the most basal members of the Gobioidei (*Rhyacichthys*, *Protogobius* and *Terateleotris*) are not completely resolved (Shibukawa et al. 2001). Here we depict the basal groups that share the number of six branchiostegal rays as 6brG and the Gobiidae + Oxudercidae that are characterized by the number of five branchiostegals as 5brG.

In recent years the focus in gobioid phylogeny lay on molecular data. However, there are works that also used morphological data (e.g. Thacker 2009), but most of the recent works focused on smaller groups within the Gobioidei (e.g. Pezold 2004; Harold et al. 2008; Tornabene et al. 2018). A comprehensive compilation of different gobioid classifications using morphological characters is given in Van Tassell et al. (2011). Among the cited works, Hoese and Gill (1993) proposed interrelationships of three of the six recognized gobioid families in their work (Rhyacichthyidae, Odontobutidae, Gobiidae [including Butinae, Eleotridinae, Gobiinae]; the other three families [Xenisthmidae, Microdesmidae, Kraemeriidae] were not investigated) based on 16 characters. Harrison (1989) tried to resolve gobioid interrelationships with the help of features of the palatopterygoquadrate complex. Other authors used morphological data for phylogenies among subgroups of the families, like Larson (2001) who made an extensive study of the *Mugilogobius*-group (family Gobiidae).

One could ask why morphology is still important in the age of phylogenetics and phylogenomics, but evolution can only be comprehensively understood taking into account (functional) morphology, and of course it is the only source of information from extinct taxa. The value of fossils for phylogenetics consists not only of being a calibration point in a time-calibrated phylogeny but recent studies also showed that characters or character combinations of fossils can provide synapomorphies for clades otherwise only recognizable based on molecular data (Davesne et al. 2016). In the case of the gobioids such a clade would be the Butidae (Hoese and Gill 1993). Generally, the fossil record of gobioids with about 100 species is scarce compared to their recent species richness. Of these, about 70 fossil species are preserved by their (sagittal) otoliths only (e.g. see Nolf 2013). However, many of these species cannot be placed with confidence at family level; examples are †*Pirskenius* (see Přikryl 2014), †*Eleogobius* and †*Paralates* (see Gierl & Reichenbacher 2015, 2017), amongst others.

Phylogenetic frameworks that can place fossil gobioids would provide the foundation we need to understand the evolutionary history of the gobioids. The aim of this study is to provide for the first time such a phylogenetic context based on a total evidence approach.

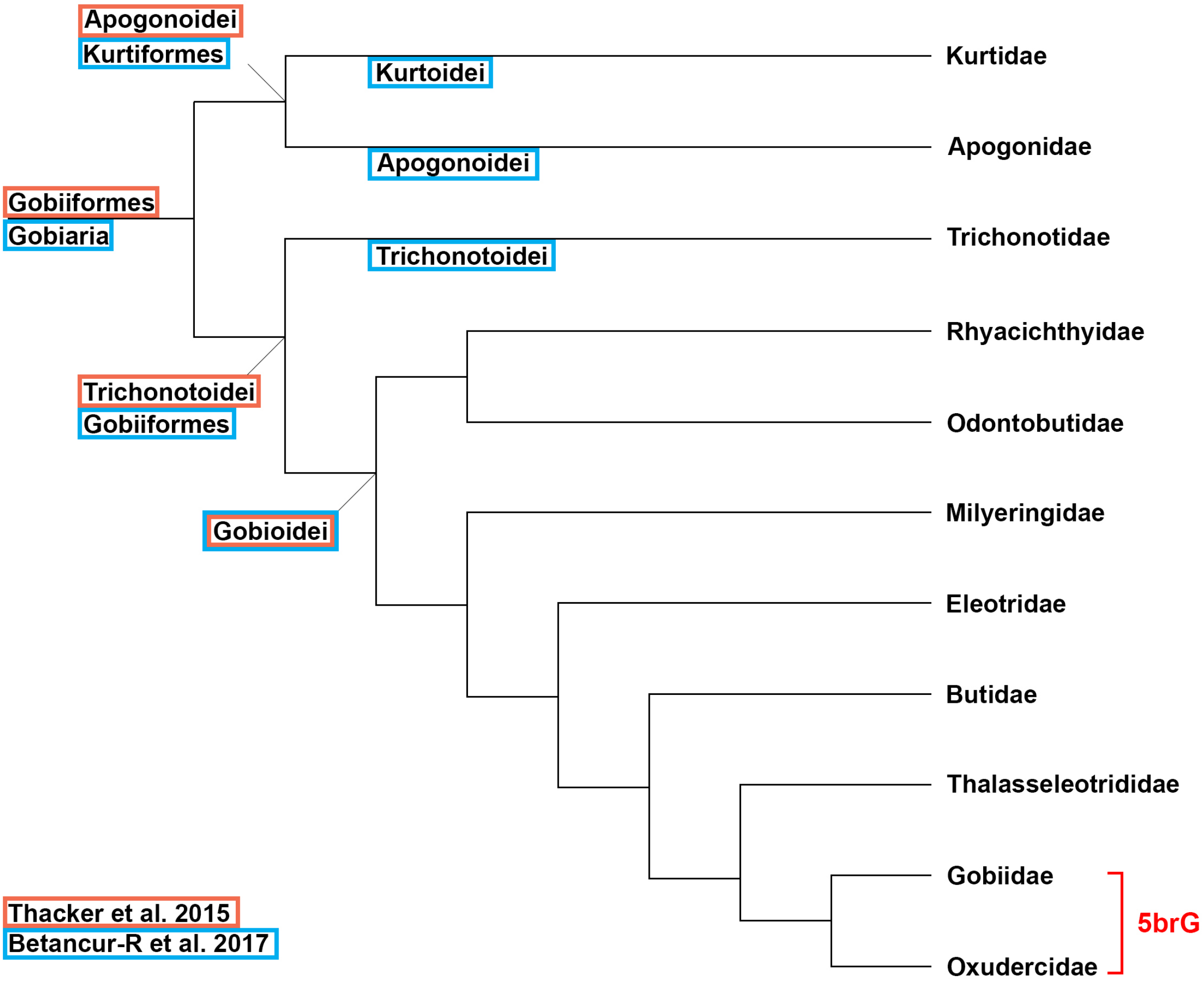

Figure 1. Goby relationships showing different nomenclatural uses. Note that Gobiiformes/Gobiaria is termed Gobiida in Nelson et al. (2016), but that the Trichonotidae are not included in their Gobiida. Therefore, the Gobiiformes sensu Nelson et al. (2016) corresponds to the Gobioidei of Thacker et al. (2015) and Betancur-R et al. (2017).

5.2 Materials and methods

The material for this study comprises 32 recent and 8 fossil species of the Gobiiformes (see Table 1). Every recent family of the Gobioidei is represented by at least two species. For the molecular analysis we used data (five genes: rRNA, *cytb*, *rag1*, *zic1*, *sreb2*) that has been published before. These are mainly Agorreta et al. (2013), Near et al. (2013), Thacker et al. (2015) and individual genes from Genbank (Tab. 1). E.g. we included molecular data of

Protogobius provided by Akihito et al. (2000), which was the only previous work considering this taxon.

Morphological studies of the fossils were carried out with the help of a Leica binocular microscope equipped with a digital camera. Of the recent species X-ray photographs have been made using the Faxitron Ultrafocus facility of the Zoological State Collection Munich (ZSM) to determine their morphological characters.

Meristic counts comprise the number of elements (spines and rays) in each fin, of vertebrae, of branchiostegals, of epurals, the pterygiophore formula of the first dorsal fin and the number of pterygiophores of the anal fin that precede the first haemal spine (see Birdsong et al. 1988).

Table 1. Species used here with Genbank (Clark et al. 2016) accession numbers, collection numbers and additional literature sources used for morphology. Green: Agorreta et al. (2013); blue: Near et al. (2013); red: Thacker et al. (2015); no color: from Genbank, but see entry for authors.

Family	**Species**	**Morphology**	**rRNA**	***cytb***	***rag1***	***zic1***	***sreb2***
Fossil	*Eleogobius brevis* (Agassiz 1839)	Gierl & Reichenbacher 2015	n/a	n/a	n/a	n/a	n/a
Fossil	*Eleogobius gaudanti* Gierl & Reichenbacher 2015	Gierl & Reichenbacher 2015	n/a	n/a	n/a	n/a	n/a
Fossil	*Gobius francofurtanus* Koken 1891	Weiler 1963; Gierl 2012	n/a	n/a	n/a	n/a	n/a
Fossil	*Gobius jarosi* Prikryl & Reichenbacher 2018	Reichenbacher et al. 2018	n/a	n/a	n/a	n/a	n/a
Fossil	*Lepidocottus aries* (Agassiz 1839)	Gierl et al 2013	n/a	n/a	n/a	n/a	n/a
Fossil	*Paralates bleicheri* Sauvage 1883	Gierl & Reichenbacher 2017	n/a	n/a	n/a	n/a	n/a
Fossil	*Pirskenius diatomaceus* Obrhelová 1961	Obrhelová 1961; Přikryl 2014	n/a	n/a	n/a	n/a	n/a
Fossil	*Pirskenius radoni* Přikryl 2014	Přikryl 2014	n/a	n/a	n/a	n/a	n/a
Apogonidae	*Sphaeramia nematoptera* (Bleeker 1856)	ZSM-PIS-P-GO-0621, -0634; Fraser 1972	AB889673		KT266401	KT266508	KT266456
Butidae	*Kribia nana* (Boulenger 1901)	#1 - #5; Wongrat 1977		AY722211	KF235468		
Butidae	*Oxyeleotris marmorata* (Bleeker 1852)	ZSM-PIS-43857 (P-GO-1058, -1059); Harrison 1989	KF415429	KF415623	KF415829	KF416035	KF416250
Eleotridae	*Dormitator maculatus* (Bloch 1792)	ZSM-PIS-009383 – 5; Harrison 1989	KF415347	KF415542	KF415739	KF415947	KF416157
Eleotridae	*Hypseleotris compressa* (Krefft 1864)	ZSM-PIS-43863 (P-GO-1068, -1069); Harrison 1989	KF415398	KF415596	KF415800	KF416004	KF416217
Eleotridae	*Tateurndina ocellicauda* Nichols 1955	ZSM-PIS-43855 (P-GO-1054, -1055)	KF415480	KF415672	KF415875	KF416085	KF416299
Gobiidae	*Amblygobius phalaena* (Valenciennes 1837)	ZSM-PIS-P-GO-0594, -0600	KF415301	KF415498	KF415693	KF415897	KF416109
Gobiidae	*Aphia minuta* (Risso 1810)	1 & 2 Selce, May 2014; Rojo 1985; Birdsong et al. 1988; Harrison 1989	KF415305	FR851417	KF415697	KF415901	KF416113
Gobiidae	*Asterropteryx semipunctata* Rüppell 1830	Birdsong et al. 1988; Van Tassell et al. 1988; Harrison 1989	KF415309	KF415506	KF415701	KF415906	KF416117
Gobiidae	*Austrolethops wardi* Whitley 1935	#1 - #4					
Gobiidae	*Cryptocentrus cinctus* (Herre 1936)	ZSM-PIS-P-GO-0619, -0639	KF415340	KF415536	KF415732	KF415940	KF416150
Gobiidae	*Discordipinna griessingeri* Hoese & Fourmanoir 1978	ZSM-PIS-P-GO-0632, -0633; Birdsong et al. 1988; Hoese & Fourmanoir 1978	KF415345	KF415540	KF415737	KF415945	KF416155
Gobiidae	*Glossogobius giuris* (Hamilton 1822)	1 Sarbaz; Hamilton 1822, Harrison 1989	KF415369	KF415566	KF415767	KF415972	KF416185
Gobiidae	*Gobius niger* Linnaeus 1758	1-Pilsey Island, Sanda, Prag; 10 (3), Pilsey Island, Sanda; Harrison 1989	KF415385	KF415583	KF415786	KF415990	KF416203
Gobiidae	*Lesueurigobius sanzi* (de Buen 1918)	Miller 1986; Birdsong et al. 1988; Harrison 1989; McKay & Miller 1997	KF415406	KF415603	KF415808	KF416012	
Gobiidae	*Ptereleotris evides* (Jordan & Hubbs 1925)	ZSM-PIS-P-GO-0587, -0588			KF141341	KF140623	KF140263
Gobiidae	*Tigrigobius multifasciatus* (Steindachner 1876)	ZSM-PIS-P-GO-0622, -0642	AF491102	AY846402	KF415878	KF416088	KF416302
Milyeringidae	*Milyeringa veritas* Whitley 1945	BES1151(B), BES18735; Larson et al. 2013		HM590598	KT266404	KT266511	KT266459
Milyeringidae	*Typhleotris madagascariensis* Petit 1933	Sparks & Chakrabarty 2012		JQ619661	KY981273		
Odontobutidae	*Odontobutis obscurus* (Temminck & Schlegel 1845)	Iwata et al. 1985	KF415424	KF415618	KF415825	KF416030	KF416245
Odontobutidae	*Perccottus glenii* Dybowski 1877	ZSM-PIS-43867 (P-GO-1078, -1079); Birdsong et al. 1988	KF415440	KF415632	KF415837	KF416044	JX190055
?Odontobutidae	*Terateleotris aspro* (Kottelat 1998)	Shibukawa et al. 2001					
Oxudercidae	*Awaous flavus* (Valenciennes 1837)	ZSM-PIS-43853 (P-GO-1050, -1051)	KF415311	KF415508	KF415703	KF415908	KF416119
Oxudercidae	*Chlamydogobius eremius* (Zietz 1896)	ZSM-PIS-43854 (P-GO-1052, -1053); Miller 1987	KF415329	KF415526	KF415720	KF415928	KF416138
Oxudercidae	*Eucyclogobius newberryi* (Girard 1856)	Birdsong et al. 1988; Kindermann et al. 2007	KF415355	EU380942	KF415751	KF415958	KF416169
Oxudercidae	*Gobioides broussonnetii* Lacepède 1800	ZSM-PIS-43852 (P-GO-1048, -1049)	KF415374		KF415772	KF415977	KF416189
Oxudercidae	*Pomatoschistus flavescens* (Fabricius 1779)	ZSM-PIS-043982, 2 Vigo, Spain, Sanda, Prag; Harrison 1989	KF415386	KF415584	KF415787	KF415991	KF416204
Rhyacichthyidae	*Protogobius attiti* Watson & Pöllabauer 1998	Philipe Keith; Akihito et al. 2000		AB021257			
Rhyacichthyidae	*Rhyacichthys aspro* (Valenciennes 1837)	ZSM-PIS-044262 (BAyFi 13481); Miller 1973; Birdsong et al. 1988	KF415462	KF415654	KF415858	KF416066	KF416282
Rhyacichthyidae	*Rhyacichthys guilberti* Dingerkus & Séret 1992	Philipe Keith		KF669052			
Thalasseleotrididae	*Grahamichthys radiatus* (Valenciennes 1837)	P-GO-0783; Gierl & Reichenbacher 2015			KT266412	KT266520	KT266467
Thalasseleotrididae	*Thalasseleotris iota* Hoese & Roberts 2005	P-GO-766; Hoese & Larson 1987			KT266413	KT266521	

The pterygiophore formula of the first dorsal fin indicates the insertion pattern of the pterygiophores in the spaces between the neural spines, e.g. 3-2211 means that after the third neural spine there insert two pterygiophores, after the fourth neural spine there insert two pterygiophores, after the fifth there inserts one and in the next space also one (see Birdsong et al. 1988). Osteological features are amongst others the shape of the palatine, the configuration of the palatopterygoquadrate complex (see Harrison 1989) or the presence or absence of the entopterygoid. In addition, the lengths of the base of the second dorsal fin (D2base) and the distance from the end of the second dorsal fin to the base of the caudal fin (D2C) have been compared, either by visual inspection (if the difference was obvious) or based on measurements (see Larson et al. 2001; Gierl and Reichenbacher 2015). Additional morphological information on the taxa, if necessary, has been taken from the literature (see Tab. 1). Otoliths have been extracted from each X-rayed specimen following the protocol of Gholami et al. (2014). Otolith imaging was based on digital microscopy and SEM. Otolith terminology (Fig. 2) follows previous work (e.g. Nolf 1985; Schwarzhans 2014; Gierl et al. 2018).

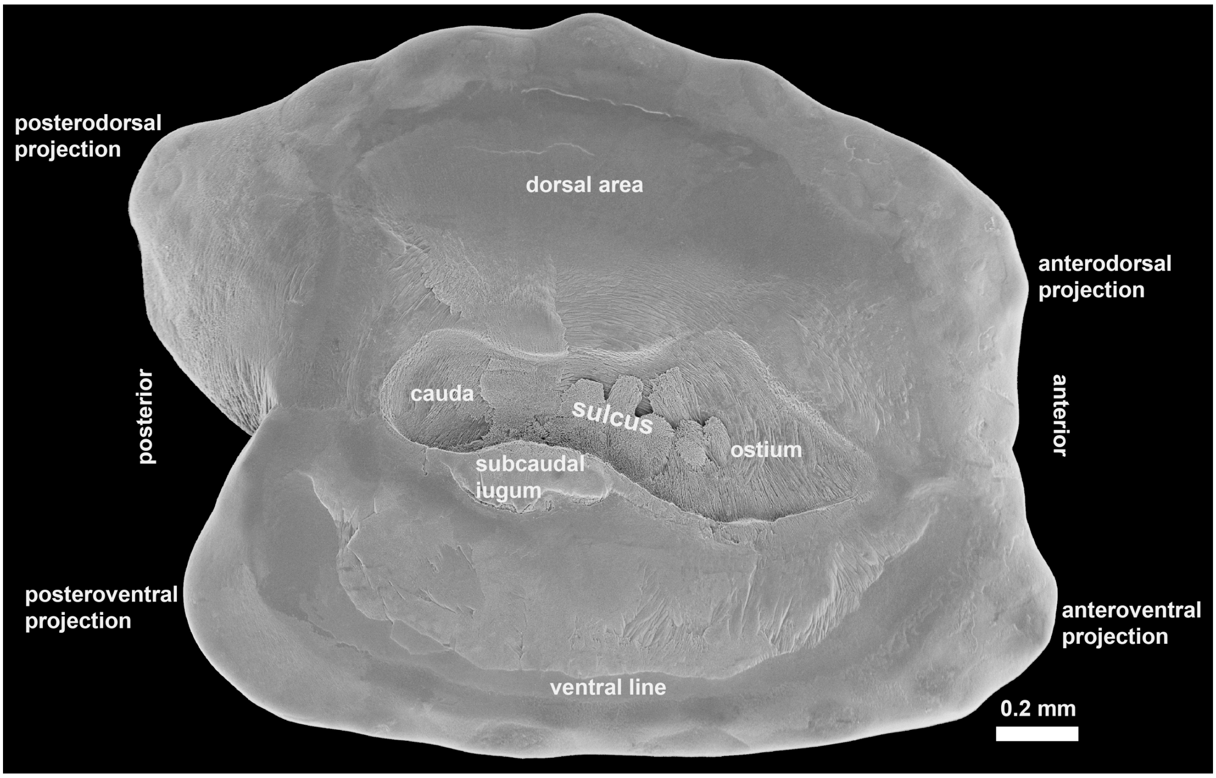

Figure 2. Nomenclature of a sagittal otolith. Left sagitta of *Gobius niger* (1 L, Pilsey Island).

Character matrix preparation. We built a morphology-based phylogeny (skeleton and otolith data) containing representatives of all eight gobioid families with an apogonid as outgroup. 341 (147 informative, see Appendix) morphological characters (263 meristic; 49 osteological; 2 morphometric; 6 soft tissue; 5 scale characters; 16 otolith characters) were assembled using Mesquite 3.51/3.6 (Maddison and Maddison 2018). Our coding strategy was to code every character as absent (coded with 0) and present (coded with 1) (see Pleijel 1995). However, in characters taken from the literature "0" can mean present and "1" absent (e.g. the absence of the transforming ctenii in ctenoid scales is coded as "1", because it is the derived state in gobioids, see Hoese and Gill 1993). This coding strategy means that, for example, meristic characters that would result in a high number of character states, have been split into single characters. So, every number of rays of a given fin counts as a separate character and is coded as present or absent. With the help of PAUP* (4.0a build 164; Swofford 2003) we generated a list of all characters (command: Cst full = y) that shows which are informative or uninformative; of 341 characters 147 are parsimony-informative; the other 194 were excluded from the phylogenetic analyses.

Furthermore, we use available molecular data of 30 species (including one apogonid as outgroup) for molecular phylogenetics and as a backbone for a total evidence approach (see Table 1).

Phylogenetic analysis. We used the parsimony criterion with TNT 1.5 (Goloboff and Catalano 2016) with the following settings: New Technology Search; Sect. search, ratchet, drift & tree fusing; init. addseqs = 100; find min. length = 10; implied weighting, K = 3.0. Resampling: bootstrap, standard; absolute frequencies; 1000 replicates; new tech search, Sect. search, ratchet, drift & tree fusing; init. addseqs = 10; find min. length = 5. Additionally, we did Bayesian inference with MrBayes v3.2.6 (Ronquist et al. 2012) We used for the morphological data the Mk + G model (Yang 1994; Lewis 2001), for molecular data the GTR+G model (Lanave et al. 1984; Yang 1994) and "coding = informative" because we included only parsimony-informative characters. We ran two MCMC chains in parallel for 5 x 10^6 generations. We used Tracer 1.7.1 (Rambaut et al. 2018) to check for convergences and discarded the first 10% of samples as burn-in and calculated then the 50% majority-rule consensus tree. For each method we performed three separate analyses, i.e. morphological, molecular and a combined (total evidence) analysis. For the latter two the molecular and morphological partitions were concatenated in SeaView 4.7 (Gouy et al. 2010). Alignment of molecular data was done in AliView 1.25 (Larsson 2014).

Trees were prepared using FigTree 1.4.4 (Rambaut 2018) and Adobe Photoshop CC 2017. To reconstruct ancestral character states, we used the "Trace character history" feature of Mesquite 3.6 with the option "parsimony ancestral states".

5.3 Results and Discussion

Phylogenies inferred from molecular data. The molecular trees of the 30 taxa created with MrBayes and TNT (see Fig. 3) are congruent with the trees published by Agorreta et al. (2013) and Thacker et al. (2015), i.e. all families are monophyletic. In our tree *Protogobius* is revealed as sister to *Rhyacichthys guilberti* + *R. aspro*, which is consistent with the results of Akihito et al. (2000), although only *Rhyacichthys aspro* was used by these authors.

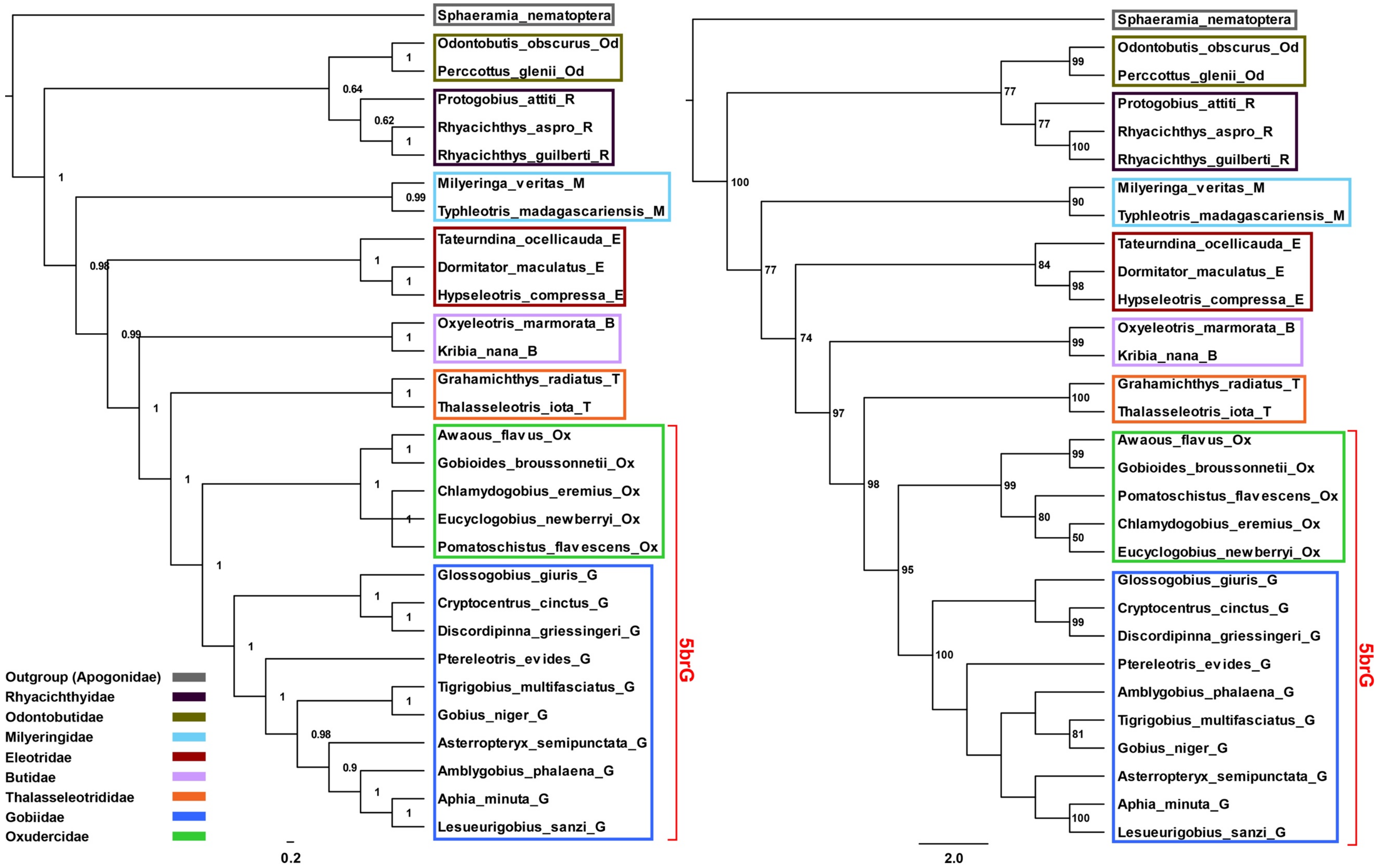

Figure 3. Phylogenetic trees based on molecular data. Left: 50% majority rule consensus tree inferred with MrBayes with posterior probabilities (4500000 post-burnin samples, average standard deviation of split frequencies between two independent runs was 0.003776). Right: most parsimonious tree (MPT, only one retained) inferred with TNT with bootstrap support values (< 50% not shown; based on 1000 pseudoreplicates); tree length (TL) = 10114, consistency index (CI) = 0.41, retention index (RI) = 0.38, rescaled CI (RC) = 0.15.

Phylogenies inferred from morphological data. In the MrBayes tree and the single most parsimonious (MPT) tree generated with TNT, the 5brG are separated from the 6brG and †*Pirskenius* (seven branchiostegals) with a posterior probability (PP) of 0.98 and a bootstrap support (BS) < 50%, respectively (Fig. 4). In both trees the families Rhyacichthyidae (PP = 0.7; BS = 52% including *Protogobius*) and Odontobutidae (PP = 0.96; BS = 65%) are retained as monophyletic each, but the difference is that the Bayesian tree yielded the two *Rhyacichthys* species as sister (PP = 0.7) and the relationship to *Protogobius* is unresolved, while the TNT tree depicts *Protogobius* as sister to R. aspro (BS < 50%), which would make *Rhyacichthys* paraphyletic.

The only other recent family retained in the MrBayes tree as monophyletic are the Thalasseleotrididae (PP = 0.89). In the TNT tree the †Pirskeniidae (BS = 70%), Thalasseleotrididae (BS < 50%) and the included members of the Eleotridae (BS < 50%) appear as monophyletic clades, whereas the included Butidae do not appear as monophyletic. The two families within the 5brG are not resolved: Gobiidae and Oxudercidae are mixed and the position of the two fossil *Gobius* species is not resolved either.

The Indo-Pacific *Austrolethops wardi* has been placed as *incertae sedis* within the Gobiidae by Thacker & Roje (2011) and was never included in a molecular phylogenetic study and is therefore the only member of the Gobiidae not assigned to a lineage (see Agorreta et al. 2013; Thacker 2015). It also shows some uncommon features for gobies, e.g. being scaleless (see Kneer et al. 2008). In our TNT tree, *A. wardi* is sister to the European genus *Aphia* (BS = 54%), which is interesting because the closest habitat of *A. wardi* with respect to the Mediterranean Sea are East African reefs (Kneer et al. 2008).

In both trees †*Pirskenius* is placed within the 6brG where it occurs in a clade (PP = 0.55, BS < 50%) which also contains the Eleotridae and the Thalasseleotrididae, and in the case of the TNT tree additionally *Kribia*. In the MrBayes tree the monophyly of †*Pirskenius* is particularly well supported (PP = 0.98).

In the MrBayes tree the fossil genus †*Paralates* is placed within the 6brG where it occurs as sister to a clade containg all gobioids except Odontobutidae, Rhyacichtyidae and *Terateleotris* (PP = 0.61). In the TNT tree †*Paralates* appears in a sister-relationship to *Terateleotris* (BS < 50%), and these two are sister to the clade of the Rhyacichtyidae (BS < 50%).

In the MrBayes tree the position of †*Lepidocottus* is unresolved; wheras in the TNT tree it is sister to a clade containing *Kribia* (Butidae), all studied members of the eleotrids, the thalasseleotridids and †*Pirskenius* (BS < 50%).

Again, in both trees †*Eleogobius* forms a monophyletic clade (PP = 0.69, BS = 71%), which is sister to the 5brG (PP = 0.94, BS < 50%).

The positions of the two *Gobius* fossils †*Gobius jarosi* and †*Gobius francofurtanus* is unresolved in the MrBayes tree and in the TNT tree they are placed in a clade with *Gobius niger* and *Aphia minuta* (BS < 50%), which represent two European lineages of the Gobiidae.

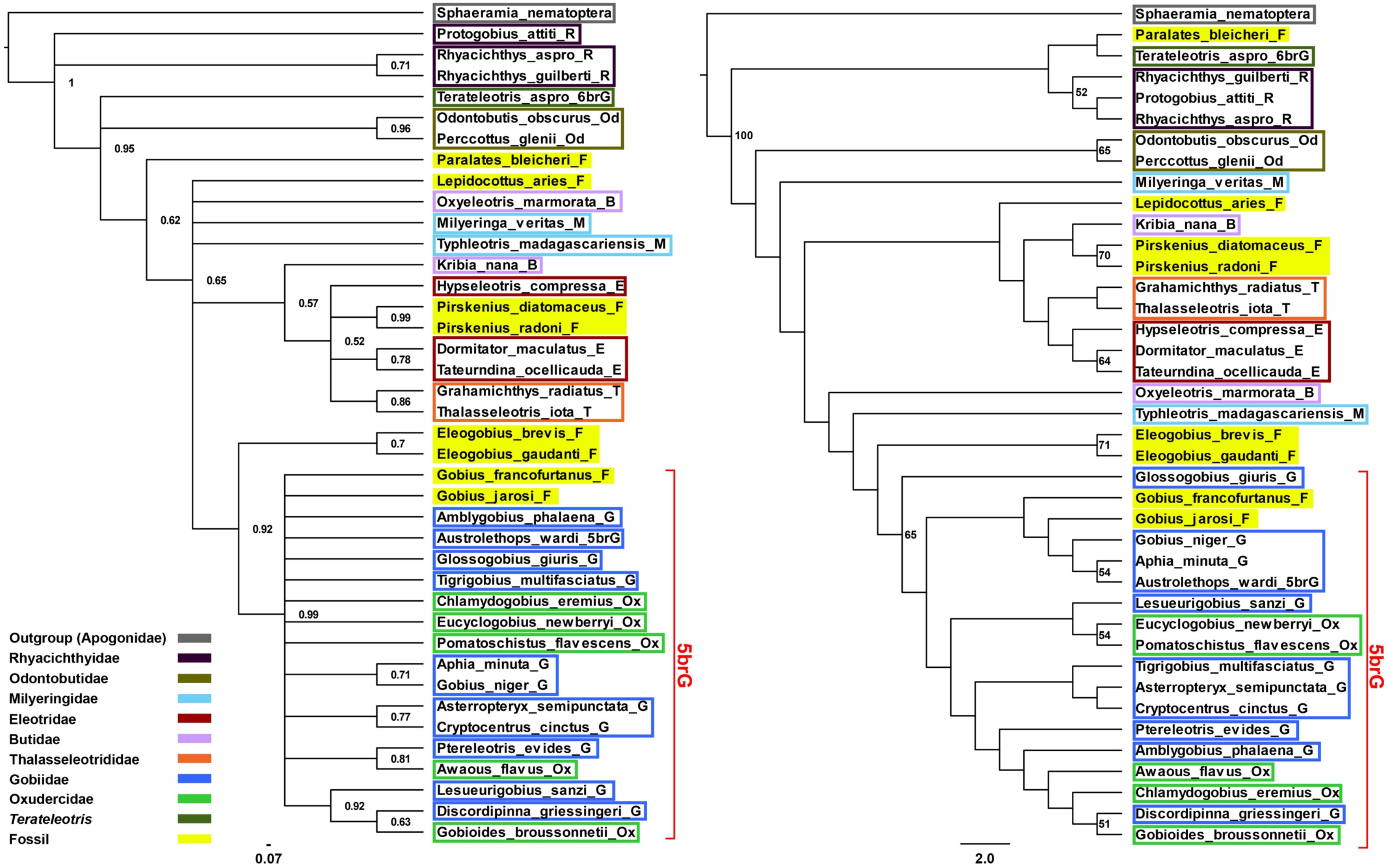

Figure 4. Phylogenetic trees based on morphological data. Left: 50% majority rule consensus tree inferred with MrBayes with posterior probabilities (4500000 post-burnin samples, average standard deviation of split frequencies between two independent runs was 0.006593). Right: MPT (only one retained) inferred with TNT with bootstrap support values (< 50% not shown; based on 1000 pseudoreplicates); TL = 576, CI = 0.26, RI = 0.52, RC = 0.13.

Comparison of the placement of the fossils in the morphological MPT and Bayesian tree with previous results. The position of the fossils is somewhat in congruence with the conclusions drawn by previous work. †*Paralates bleicheri* was described as a member of the "basal gobioids" with 6 branchiostegals (Gierl and Reichenbacher 2017). But its placement in a clade with the rhyacichthyids and *Terateleotris* is interesting, although weakly supported (BS < 50%). In the Bayesian tree it is sister (PP = 0.61) to the remaining gobioids with six branchiostegals and the 5brG.

†*Lepidocottus* was described as a probable member of the Butidae based on its morphological and otolith characters (Gierl et al. 2013). Its position in both trees, although not well supported, is not in conflict with Gierl et al.'s conclusions.

The placement of †*Pirskenius* in one clade (PP = 0.98, BS = 70%) favours more its original assignment of Obrhelová (1969), who put it into the new family †Pirskeniidae. In the MrBayes tree the Thalasseleotrididae have the same rank as †*Pirskenius*, so one could argue that the Pirskeniidae is valid because the Thalasseleotrididae are. But it is not possible to exclude the conclusion of Přikryl (2014) who considered †*Pirskenius* as being an eleotrid, either. †*Pirskenius* still seems to be closely related to the Eleotridae, but the relationship is unresolved. However, in the MPT †*Pirskenius* is sister to the butid *Kribia nana* (BS < 50%).

The genus †*Eleogobius* as sister to the 5brG in both trees (PP = 0.94, BS < 50%) fits well to its character mix of basal and derived characters (6 branchiostegals; no entopterygoid; palatine with ethmoidal process, T-shaped) and is therefore in accordance with the assumptions of Gierl and Reichenbacher (2015).

In the MrBayes tree the position of the two fossil *Gobius* is in the 5brG but not further resolved. In the MPT the two *Gobius* fossils †*G. jarosi* and †*G. francofurtanus* are placed in a clade with the extant species *G. niger* and *Aphia minuta*, which represent the two European gobiid lineages (*Gobius*- and *Aphia*-lineage sensu Agorreta et al., 2013). This placement of the two fossils is not conflicting with previous assignments, because †*G. jarosi* was explicitely assigned to the genus *Gobius* (Reichenbacher et al. 2018) and †*G. francofurtanus* was also described as a gobiid (Weiler, 1963; Gierl 2012).

Phylogenies inferred from molecular and morphological data combined. The total evidence Bayesian and TNT analyses each (Fig. 5) produced both a (consensus) tree that is in congruence with the "molecular only" analyses regarding the positions of the included recent species, i.e. all recent families are retained. The positions of the recent species having molecular and morphological data will therefore not be discussed.

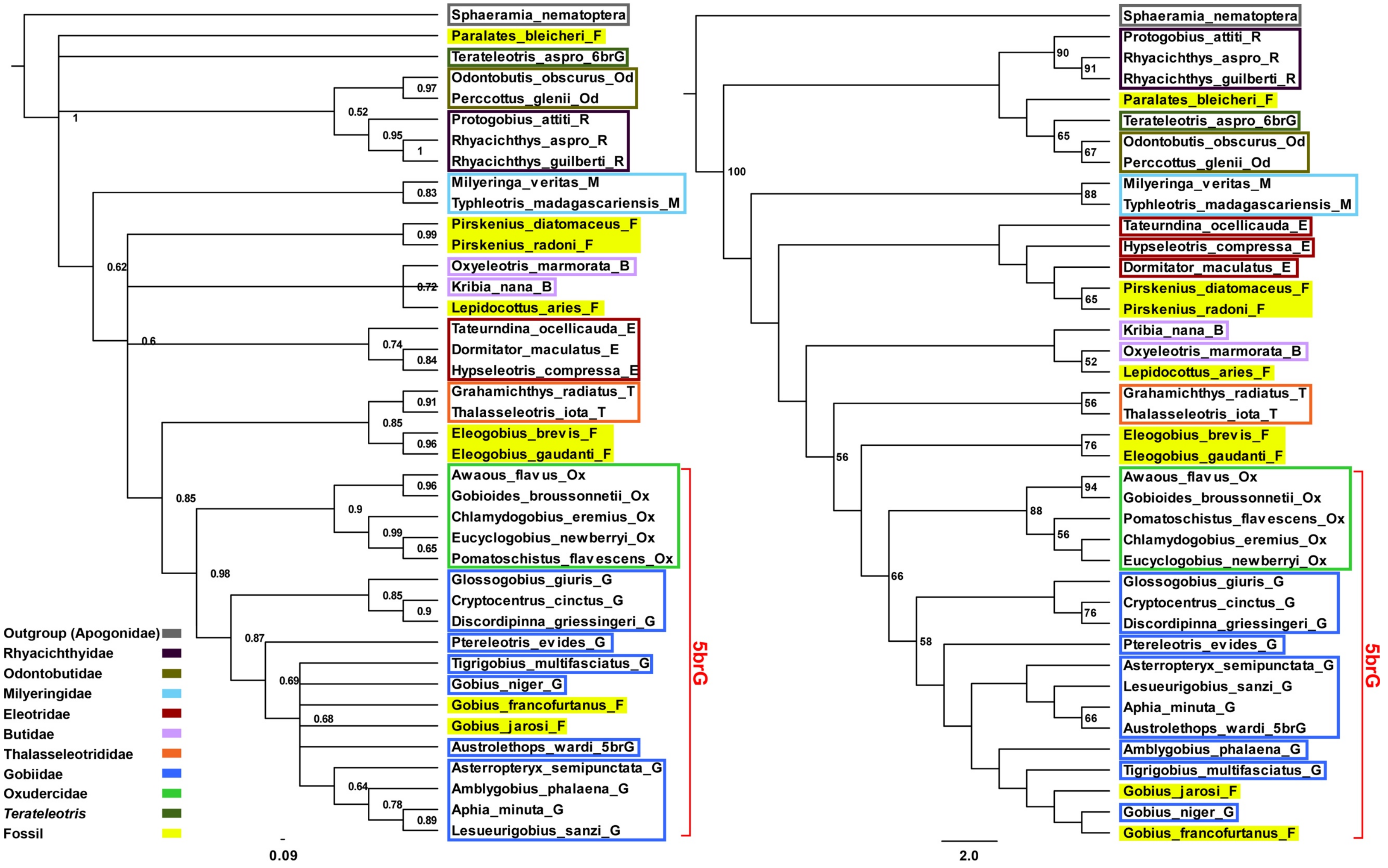

Figure 5. Phylogenetic trees based on combined (total evidence) data. Left: 50% majority rule consensus tree inferred with MrBayes with posterior probabilities (4500000 post-burnin samples, average standard deviation of split frequencies between two independent runs was 0.012711). Right: MPT (only one retained) inferred with TNT with bootstrap support values (< 50% not shown; based on 1000 pseudoreplicates); TL = 10714, CI = 0.4, RI = 0.39, RC = 0.1.

Comparison of the placement of the fossils in the combined (total evidence) TNT tree and MrBayes tree with previous results. †*Paralates* stands in the MrBayes tree in an unresolved position to *Terateleotris*, rhyacichtyids/odontobutids and the other gobioids. In the TNT tree it is placed (BS < 50%) as sister to *Terateleotris* and the two Odontobutidae. As †*Paralates* has six branchiostegals it is not unexpected that it is placed in the "basal gobioids" (6brG). However, its placement within or close to the Rhyacichthyidae and Odontobutidae/*Terateleotris* is somewhat surprising, because we see no hint in the fossils for that placement. One reason could be the character D2C (length between the end of the second dorsal fin and the first unsegmented and unbranched caudal fin ray). The D2C is in the basal gobioids always longer than the base of the second dorsal fin. But such a relatively long D2C can also be found in the Eleotridae and Butidae. Another feature responsible for this placement might be that †*Paralates* can have 18 caudal vertebrae, which he has in common with *Terateleotris* and *Odontobutis*.

†*Pirskenius* is in the MrBayes tree in a clade (PP = 0.6) with unresolved relationships to the butids and eleotrids, and this clade is sister to thalasseleotrids and †*Eleogobius* and the 5brG. Thus, one could argue, that the family Pirskeniidae can be sustained, since the two species of †*Pirskenius* form a distinct clade. However, the synapomorphy of this clade is the presence of seven branchiostegals (rather than six or five; see Obrhelová 1969; Přikryl 2014). In other gobioids a number of seven branchiostegals occurs only as exception. One (out of 12) *Odontobutis obscurus* specimen examined by Akihito (1969) had seven branchiostegals (three on the slender anterior part of the anterior ceratohyal, just like †*Pirskenius*). Besides, Akihito (1969, Table 3) reported the "wrong" number of branchiostegals in one specimen each of five additional gobioid species (Akihito 1969, Table 3). In the TNT (total evidence) analysis the position of †*Pirskenius* suits the conclusion by Přikryl (2014) that †*Pirskenius* is an eleotrid. However, the whole clade is not well supported (BS < 50%).

†*Lepidocottus* is in both the MrBayes and TNT total evidence analysis placed in a clade with the two butids. This is in accordance with Gierl et al. (2013) who considered †*Lepidocottus* as being a butid based on the number of branchiostegals (six), presence of an entopterygoid, a palatine without ethmoid process and an anteriorly shifted sulcus on its sagittal otolith.

†*Eleogobius* is placed in the TNT tree as sister to the 5brG and in the MrBayes tree forming a clade together with the Thalasseleotrididae which is sister to the 5brG. This fits well to the comments of Gierl and Reichenbacher (2015) who state that because of its mosaic-like characters (6 branchiostegals; T-shaped palatine; no entopterygoid) †*Eleogobius* might be a form that lies "in between". The sister relationship with the Thalasseleotrididae in the MrBayes tree (PP = 0.85) is interesting because Gierl and Reichenbacher (2015) do not mention the presence of one of the five synapomorphies that have been used by Gill and Mooi 2012 to justify the sister-relationship of Gobiidae and Thalasseleotridae (i.e. interhyal with cup-shaped lateral structure for articulation with preopercle; laterally directed posterior

process on the posterior ceratohyal supporting the interhyal; pharyngobranchial 4 absent; dorsal postcleithrum absent; urohyal without ventral shelf). If the position of †*Eleogobius* is retained in a bigger taxon sampling it could be justified to put it into its own family. †*Gobius francofurtanus* is placed in the MrBayes tree unresolved within a clade of the Gobiidae where †*Gobius jarosi* and *G. niger* are also placed unresolved. In the MPT of TNT it is sister to *Gobius niger*, but weakly supported.

†*Gobius jarosi* was explicitely described as being a member of the genus *Gobius* and this is not refuted by these phylogenies. The most important feature seems to be the high number of rays in the second dorsal fin (12). Additionally, the placement is supported by the pterygiophore formula typical for Gobiidae (3-22110) and the number of anal fin rays (11) (see Reichenbacher et al. 2018).

Character Analysis. The ancestral state reconstruction showed several characters that can be used to separate groups or families within the Gobioidei (trees not shown). Almost all of these characters have already been proposed as important for gobioid phylogeny based on comparative morphology. Based on the ancestral character tracing the following characters can be regarded as most relevant (numbers in brackets refer to complete character list, see appendix):

the number of branchiostegals defining the two big groups within the Gobioidei (5brG and the rest) as mentioned in e.g. Gill and Mooi (2012) (277–279);

the number of two anal pterygiophores preceding the first neural spine which is found in the 5brG and Rhyacichthyidae, Odontobutidae, *Terateleotris* and Milyeringidae (see Birdsong et al. 1988) (3);

the presence or absence of the first middle radial of the second dorsal fin and it is present in Rhyacichthyidae, Odontobutidae, *Terateleotris* and the Thalasseleotrididae (see Hoese and Gill 1993) (11);

of the pterygiophore formulas only 3-22110 (55) has proven to be somewhat indicative for Gobiidae, although it can also be found in the basal gobioids (e.g. see Birdsong et al. 1988);

the D2C (121–122) is also here in the analysis a feature most typically found within the 5brG (see Larson et al. 2001), but exceptions (e.g. *Pomatoschistus*) exist;

the epural number of one and two is also inconclusive, although one is most typical for 5brG and two for most basal gobioids except Rhyacichthyidae and *Terateleotris*, which have three (Miller 1973; Shibukawa et al. 2001) (126–128);

a high number of rays (12 or 13) in the second dorsal fin (239) is only found within 5brG and in particular in the genus *Gobius*, but also in the monotypic *Aphia* and *Austrolethops*, which are two very specialized genera (see Reichenbacher et al. 2018 and Results and Discusiion above);

the five characters used by Gill and Mooi (2012) as synapomorphies of Thalasseleotrididae+Gobiidae: dorsal postcleithrum absent (270); laterally directed posterior process on the posterior ceratohyal supporting the interhyal (288); interhyal with cup-shaped lateral structure for articulation with preopercle (295); pharyngobranchial 4 absent (305); urohyal without ventral shelf (315);

there is no contact of the upper proximal radial with the cleithrum (273) in Rhyacichthyidae, Odontobutidae and Terateleotris in contrast to all other gobioids (see Hoese and Gill 1993);

the palatine having a process directed dorsally for connection to the ethmoid (T-shape, 300) is a character to separate the 5brG from the rest, although exceptions exist in both groups (see Regan 1911; Hoese 1984; Harrison 1989);

The position of the penultimate branchiostegal is only in the Rhyacichthyidae and Odontobutidae on the posterior ceratohyal (Hoese and Gill 1993) (302–304);

the anteriorly shifted sulcus on the otolith is a hint at being within the Butidae (see Gierl et al. 2013) (330);

transforming cteni on the scales are only present in Rhyacichthyidae, Oodntobutidae and *Terateleotris* (Hoese and Gill 1993; Shibukawa et al. 2001) (335).

A character that we were interested to test because it would have a good chance for fossil preservation unfortunately is of no use: the presence or absence of a connection between symplectic and preopercle that is according to Miller (1973) typical for Oxudercidae (Gobionellinae then) and some other non-related genera. But Harrison (1989) already mentioned that this character might not be good for defining taxa. Other characters like the pelvic fins being separated or not can be of limited help at least for phylogenies but for the assignment of a goby caught in the field it can be one of the first steps to approach its proper assignment.

5.4 Conclusion

This study is the first try of a total evidence analysis for the Gobioidei. And although limited compared to the gobioid species richness it shows that many morphological characters or combinations of characters previously used to define gobioid taxa do not conflict molecular studies. Furthermore, with such a framework it will be possible to place fossil gobies with higher confidence in the gobioid phylogeny in order to get further insights into the evolutionary history of the group.

5.5 Future work

More osteological studies on gobioids are needed, because there are only a handful species that are well described with all of their osteological features (e.g. Miller 1973; Birdsong 1975; Kindermann et al. 2007) opposite to more than 2200 species overall. But that is a huge task and unfortunately morphological studies describing one species in detail seem to have lost their attractiveness.

So, future plans are to expand our dataset especially with a focus on the 5brG incorporating all representatives of every lineage within the Gobiidae and Oxudercidae to assess if morphology can be of any use when it comes to the assignment of taxa to those lineages. Furthermore, there are some species of high interest with no or only very limited molecular data available. For instance, there is no molecular data available for *Terateleotris aspro* (see Genbank, last access: 2/2019). Unfortunately, the latest molecular study which focused on Odontobutidae did not include *Terateleotris* (which is assigned to that group by some) because specimens were not available (Li et al. 2018). Therefore, it would be really important to get molecular data for this taxon. The same is true for *Austrolethops wardi*.

ACKNOWLEDGEMENTS
We thank Dr. Ulrich Schliewen (Zoological State Collection, Munich) for his valuable help in aquiring some of the recent species and fruitful discussions. We also thank Dr. Z. Gholami (LMU) who helped preparing X-ray photographs and we want to thank Inda Brinkmann (LMU) for being the second pair of eyes while preparing the morphological matrix.
CG and BR acknowledge funding from the German Research Foundation (grant number RE 1113/20-1).

5.6 References

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

Akihito. 1986. Some morphological characters considered to be important in gobiid phylogeny. In *Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fishes*, ed. K. Matsuura, 629–639. Tokyo: Ichthyological Society of Japan.

Akihito, M. Hayashi, T. Yoshino, K. Shimada, T. Yamamoto, and H. Senou. 1984. Suborder Gobioidei. In *The fishes of the Japanese archipelago*, eds. H. Masuda, K. Amaoka, C. Araga, T. Uyeno, and T. Yoshino, 236–289, pls. 235–258, 353–355. Tokyo: Tokai University Press.

Akihito, A. Iwata, T. Kobayashi, K. Ikeo, T. Imanishi, H. Ono, Y. Umehara, C. Hamamatsu, K. Sugiyama, Y. Ikeda, K. Sakamoto, A. Fumihito, S. Ohno, and T. Gojobori. 2000. Evolutionary aspects of gobioid fishes based upon a phylogenetic analysis of mitochondrial cytochrome *b* genes. *Gene* 259 (1–2): 5–15.

Betancur-R., R., E.O. Wiley, G. Arratia, A. Acero, N. Bailly, M. Miya, G. Lecointre, and G.

Ortí. 2017. Phylogenetic classification of bony fishes. *BMC Evolutionary Biology* 17 (1): 162.

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.

Birdsong, R.S., E.O. Murdy, and F.L. Pezold. 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.

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

Carpenter, K.E., and V.H. Niem. 2001. *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 5. Bony fishes part 3 (Menidae to Pomacentridae)*. Rome: FAO.

Clark, K., I. Karsch-Mizrachi, D.J. Lipman, J. Ostell, and E.W. Sayers. 2016. GenBank. *Nucleic Acids Research* 44 (Database issue): D67–D72.

Davesne, D., C. Gallut, V. Barriel, P. Janvier, G. Lecointre, and O. Otero. 2016. The phylogenetic intrarelationships of spiny-rayed fishes (Acanthomorpha, Teleostei, Actinopterygii): fossil taxa increase the congruence of morphology with molecular data. *Frontiers in Ecology and Evolution* 4: 129.

Fraser, T.H. 1972. Comparative osteology of the shallow water cardinal fishes (Perciformes: Apogonidae) with reference to the systematics and evolution of the family. *Ichthyological Bulletin of the J. L. B. Smith Institute of Ichthyology* 34: 1–105.

Fricke, R. 1983. A method for counting caudal fin rays of actinopterygian fishes. *Braunschweiger Naturkundliche Schriften* 1 (4): 729–733.

Gholami, Z., H.R. Esmaeili, D. Erpenbeck, and B. Reichenbacher. 2014. Phylogenetic analysis of *Aphanius* from the endorheic Kor River Basin in the Zagros Mountains, South-western Iran (Teleostei: Cyprinodontiformes: Cyprinodontidae). *Journal of Zoological Systematics and Evolutionary Research* 52 (2): 130–141.

Gierl, C. 2012. Articulated gobioid skeletons from the Frankfurt-Formation (Lower Miocene). Thesis. 50 pp., München (Ludwig-Maximilians-Universität München).

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

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

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

Gierl, C., B. Reichenbacher, J. Gaudant, D. Erpenbeck, and A. Pharisat. 2013. An extraordinary gobioid fish fossil from southern France. *PLOS ONE* 8 (5): e64117.

Gill, A.C., and R.D. Mooi. 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: 41–52.

Goloboff, P.A., and S.A. Catalano. 2016. TNT version 1.5, including a full implementation of phylogenetic morphometrics. *Cladistics* 32 (3): 221–238.

Gosline, W.A. 1955. The osteology and relationships of certain gobioid fishes, with particular reference to the genera *Kraemeria* and *Microdesmus*. *Pacific Science* IX: 158–170.

Gouy, M., S. Guindon, and O. Gascuel. 2010. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* 27 (2): 221–224.

Hamilton, F. 1822. *An account of the fishes found in the river Ganges and its branches*. Edinburgh, London: Archibald Constable and Company, Hurst, Robinson, and Co.

Harold, A.S., R. Winterbottom, P.L. Munday, and R.W. Chapman. 2008. Phylogenetic relationships of Indo-Pacific coral gobies of the genus *Gobiodon* (Teleostei: Gobiidae), based on morphological and molecular data. *Bulletin of Marine Science* 82 (1): 119–136.

Harrison, I.J. 1989. Specialization of the gobioid palatopterygoquadrate complex and its relevance to gobioid systematics. *Journal of Natural History* 23 (2): 325–353.

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

Hoese, D.F., and P. Fourmanoir. 1978. *Discordipinna griessingeri*, a new genus and species of gobiid fish from tropical Indo-West Pacific. *Japanese Journal of Ichthyology* 25 (1): 19–24.

Hoese, D.F., and A.C. Gill. 1993. Phylogenetic relationships of eleotridid fishes (Perciformes, Gobioidei). *Bulletin of Marine Science* 52 (1): 415–440.

Hoese, D.F., and H.K. Larson. 1987. New Australian fishes. Part 11. A new genus and species of eleotridid (Gobioidei) from Southern Australia with a discussion of relationships. *Memoirs of the Museum of Victoria* 48 (1): 43–50.

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

Iwata, A., S.-R. Jeon, N. Mizuno, and K.-C. Choi. 1985. A revision of the Eleotrid goby genus *Odontobutis* in Japan, Korea and China. *Japanese Journal of Ichthyology* 31 (4): 373–388.

Jaafar, Z., and E.O. Murdy eds. 2017. *Fishes out of water: biology and ecology of* *mudskippers*. Boca Raton: Taylor & Francis.

Karplus, I. 1987. The association between gobiid fishes and burrowing alpheid shrimps. *Oceanography and Marine Biology Annual Review* 25: 507–562.

Kindermann, G., N. Miljković, H. Ahnelt, and D.E. Stevenson. 2007. The osteology of *Eucyclogobius newberryi* and *Quietula guaymasiae* (Teleostei: Gobiidae), two closely related gobionellines from the East Pacific. *Annalen des Naturhistorischen Museums in Wien - Serie B (Botanik und Zoologie)* 108: 13–56.

Kneer, D., H. Asmus, H. Ahnelt, and J.A. Vonk. 2008. Records of *Austrolethops wardi* Whitley (Teleostei: Gobiidae) as an inhabitant of burrows of the thalassinid shrimp *Neaxius acanthus* in tropical seagrass beds of the Spermonde Archipelago, Sulawesi, Indonesia. *Journal of Fish Biology* 72 (4): 1095–1099.

Lanave, C., G. Preparata, C. Saccone, and G. Serio. 1984. A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution* 20 (1): 86–93.

Larson, H.K. 2001. A revision of the gobiid fish genus *Mugilogobius* (Teleostei: Gobioidei), and its systematic placement. *Records of the Western Australian Museum* Supplement No. 62: 1–233.

Larson, H.K., R. Foster, W.F. Humphreys, and M.I. Stevens. 2013. A new species of the blind cave gudgeon *Milyeringa* (Pisces: Gobioidei, Eleotridae) from Barrow Island, Western Australia, with a redescription of *M. veritas* Whitley. *Zootaxa* 3616 (2): 135–150.

Larson, H.K., E.O. Murdy, and A.C. Gill. 2001. Suborder Gobioidei. In *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*, eds. K. E. Carpenter, and V. H. Niem, 3574–3609. Rome: FAO.

Larsson, A. 2014. AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30 (22): 3276–3278.

Lee, Y.-J. 1993. Study of vertebral column and pterygiophores in Gobiidae (Pisces, Perciformes) from Korea. *The Korean Journal of Systematic Zoology* 9 (1): 25–34.

Lewis, P.O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* 50 (6): 913–925.

Maddison, W.P., and D.R. Maddison. 2018. Mesquite: a modular system for evolutionary analysis. Version 3.6 http://mesquiteproject.org.

McKay, S.I., and P.J. Miller. 1997. The affinities of European sand gobies (Teleostei: Gobiidae). *Journal of Natural History* 31 (10): 1457–1482.

Miller, P.J. 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.J. 1986. Gobiidae. In *Fishes of the north-eastern Atlantic and the Mediterranean (FNAM)*, eds. P. J. P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen, and E.

Tortonese, 1019–1085. Paris: UNESCO.

Miller, P.J. 1987. Affinities, origin and adaptive features of the Australian Desert Goby *Chlamydogobius eremius* (Zietz, 1896) (Teleostei: Gobiidae). *Journal of Natural History* 21 (3): 687–705.

Near, T.J., A. Dornburg, R.I. Eytan, B.P. Keck, W.L. Smith, K.L. Kuhn, J.A. Moore, S.A. Price, F.T. Burbrink, M. Friedman, and P.C. Wainwright. 2013. Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. *Proceedings of the National Academy of Sciences of the United States of America* 110 (31): 12738–12743.

Nelson, J.S., T.C. Grande, and M.V.H. Wilson. 2016. *Fishes of the World, Fifth Edition*. Hoboken, New Jersey: John Wiley & Sons, inc.

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

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

Obrhelová, N. 1961. Vergleichende Osteologie der tertiären Süsswasserfische Böhmens (Gobioidei). *Sborník Ústředního ústavu geologického: oddíl paleontologiyký* 26: 103–192.

Patzner, R.A., J.L. Van Tassell, M. Kovačić, and B.G. Kapoor eds. 2011. *The biology of gobies*. Enfield, New Hampshire: Science Publishers Inc.

Pezold, F. 2004. Phylogenctic analysis of the genus *Gobionellus* (Teleostei: Gobiidae). *Copeia* 2004 (2): 260–280.

Pleijel, F. 1995. On character coding for phylogeny reconstruction. *Cladistics* 11 (3): 309–315.

Přikryl, T. 2014. A new species of the sleeper goby (Gobioidei, Eleotridae) from the České Středohoří Mountains (Czech Republic, Oligocene) and analysis of the validity of the family Pirskeniidae. *Paläontologische Zeitschrift* 88 (2): 187–196.

Rambaut, A. 2018. FigTree. Tree Figure Drawing Tool version 1.4.4. http://tree.bio.ed.ac.uk/software/figtree/. University of Edinburgh, Edinburgh.

Rambaut, A., A.J. Drummond, D. Xie, G. Baele, and M.A. Suchard. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67 (5): 901-904.

Regan, C.T. 1911. The osteology and classification of the gobioid fishes. *The Annals and Magazine of Natural History [Eighth Series]* 8 (48): 729–733.

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

Rojo, A.L. 1985. Osteología del chanquete, *Aphya minuta* (Risso, 1810) (Pisces: Gobiidae). *Boletín del Instituto Español de Oceanografía* 2 (1): 165–179.

Ronquist, F., M. Teslenko, P. van der Mark, D.L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M.A. Suchard, and J.P. Huelsenbeck. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61 (3): 539–542.

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

Shibukawa, K., A. Iwata, and S. Viravong. 2001. *Terateleotris*, a new gobioid fish genus from the Laos (Teleostei, Perciformes), with comments on its relationships. *Bulletin of the National Science Museum Series A (Zoology)* 27 (4): 229–257.

Sparks, J.S., and P. Chakrabarty. 2012. Revision of the endemic Malagasy cavefish genus *Typhleotris* (Teleostei: Gobiiformes: Milyeringidae) with discussion of its phylogenetic placement and description of a new species. *American Museum Novitates* 3764: 1–28.

Swofford, D.L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

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.

Thacker, C.E., T.P. Satoh, E. Katayama, R.C. Harrington, R.I. Eytan, and T.J. Near. 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.

Tornabene, L., B. Deis, and M.V. Erdmann. 2018. Evaluating the phylogenetic position of the goby genus *Kelloggella* (Teleostei: Gobiidae), with notes on osteology of the genus and description of a new species from Niue in the South Central Pacific Ocean. *Zoological Journal of the Linnean Society* 183 (1): 143–162.

Tornabene, L., J.L. Van Tassell, D.R. Robertson, and C.C. Baldwin. 2016. Repeated invasions into the twilight zone: evolutionary origins of a novel assemblage of fishes from deep Caribbean reefs. *Molecular Ecology* 25 (15): 3662–3682.

Van Tassell, J.L., P.J. Miller, and A. Brito. 1988. A revision of *Vanneaugobius* (Teleostei: Gobiidae), with description of a new species. *Journal of Natural History* 22 (2): 545–567.

Van Tassell, J.L., L. Tornabene, and M.S. Taylor. 2011. A history of gobioid morphological systematics. In *The biology of gobies*, eds. R. A. Patzner, J. L. Van Tassell, M. Kovačić, and B. G. Kapoor, 3–22. Enfield, NH: Science Publishers Inc.

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.

Wiley, E.O., and G.D. Johnson. 2010. A teleost classification based on monophyletic groups. In *Origin and phylogenetic interrelationships of teleosts*, eds. J. S. Nelson, H.-P. Schultze, and M. V. H. Wilson, 123–182. München: Verlag Dr. Friedrich Pfeil.

Wongrat, P. 1977. Systematics, comparative anatomy, and phylogeny of eleotrine gobies (Teleostei: Gobioidei). Thesis. 460 pp., (University of Bristol).

Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. *Journal of Molecular Evolution* 39 (3): 306–314.

6. Conclusion and outlook

Several fossil gobioids have been revised in this work. Gierl and Reichenbacher (2015) focused on "*Cottus brevis* Ag." from Öhningen (Germany) and "*Cottus brevis* Ag.? from Illerkirchberg (Germany) which, after their initial description, had been assigned to the genus *Gobius*. It could be confirmed that they are indeed two different species. Moreover, their unique combination of characters, i.e. six branchiostegals, no entopterygoid and a T-shaped palatine indicate clearly that they do not belong to *Gobius* and not even to the Gobiidae. Therefore, the new genus †*Eleogobius* was erected and "*Cottus brevis* Ag.? was described as the new species †*Eleogobius gaudanti*. Thanks to the fact that both species are preserved with specimens bearing otoliths *in situ E. brevis* and *E. gaudanti* can be well separated from each other based on their different otolith morphology.

The revision of "*Pomatoschistus bleicheri* (Sauvage)" and "*Pomatoschistus*(?) *bleicheri* (Sauvage)" presented in Gierl and Reichenbacher (2017) yielded similar results. They both represent the extinct genus †*Paralates*, which was the originally established genus name for the type species. "*Pomatoschistus*(?) *bleicheri* (Sauvage)" was described as the new species †*Paralates chapelcorneri*. †*Pa. bleicheri* and †*Pa. chapelcorneri* are characterized by six branchiostegals and a high vertebrae number (29–31) and the two species can be distinguished from each other by their differences in fin ray counts and shape of frontal bones.

Gierl et al. (2018) focus on the importance and usefulness of otoliths as taxonomic tools. Background of this study is that otoliths are widely ignored by ichthyologists working on recent fish species. But who could actually blame them for that? Having a recent fish specimen with everything intact does often not require using every feature that is present to clearly identify a species. This is especially true for "hidden" features like osteology and otoliths. In palaeontology however it is a bit different, for obvious reasons. In almost every case soft tissue is not preserved, skeletons are incomplete and often disarticulated and, in many cases, only isolated bones, teeth or just the otoliths are what remains from a specimen. So palaeontologists have to use every source of information that is available. In palaeontology there is a long tradition of describing species only based on their otoliths (e.g. Koken 1884, 1891; Weiler 1942, 1963; Reichenbacher 2000). But these descriptions are mainly based on rather subjective criteria. A description of the shape and the surface of an otolith and the comparison to other partly very similar species can be pretty difficult to follow for non-specialists. This is definitely another reason why otoliths often are ignored in a taxonomical context. So, in the work of Gierl et al. (2018) a case study was undertaken to assess some objective criteria in otolith morphology. In a dataset of extant representatives of all oxudercid lineages it was possible to identify two of the five lineages with confidence, the *Periophthalmus* and the *Pomatoschistus* lineage. Within the latter it was possible to distinguish the sand goby subgroup from other members of this lineage and the assignment of fossil otoliths to *Pomatoschistus* was supported by this study. So, it is possible to find measurable criteria for otoliths.

The lack of a comprehensive gobioid phylogeny including molecular, morphological data and fossil species led to the first try to change this situation presented in chapter 5 (Gierl et al. manuscript). The total evidence approach shows promising results. All current families were retained and the fossils are more or less placed where it was expected by their previous descriptions. So, this will be the starting point for further studies incorporating more taxa and other methods like dating methods or methods to estimate palaeogeography.

The biggest problem of gobioid taxonomy and phylogeny is simply the fact that there are too many gobioids. More than 2200 species and only few have been studied extensively regarding their osteology (e.g. Birdsong 1975). As an ichthyologist working with gobies one can look envious at groups like the Esociformes, which consists only of 14 (!) species (Fricke et al. 2019). So, a whole army of ichthyologists would be required to get a broad overview of gobioid ichthyology. But this is only wishful thinking. Still this is what needs to be done in the future, using clearing and staining, X-ray and microCT techniques to gather more and more information to further expand our knowledge in gobioid osteology.

Another problem does not only affect gobioid phylogeny but phylogeny based on morphology in general. There is still no adequate method to incorporate meristic data into a matrix. There are attempts to solve this, like the approach with step matrices (see Lawing et al. 2008). But if it would be possible to just input meristic values in a matrix (that contains also presence-absence coded characters) as they are, it would make the whole coding process easier and more accessible. Additionally, there is still an issue how phylogeny programs deal with missing data and not applicable data. According to Vogt (2018), no current phylogeny software makes a difference between those two data types. But of course, it makes a difference if, for example, the scale type (ctenoid or cycloid) for two different species is coded in both cases with a question mark although one species does not even have scales (see also Lee and Bryant 1999). But solving these problems lies rather in the hands of mathematicians, computer scientists and bioinformaticions. Palaeontologists or biologists usually have only a very limited programming background.

This work has shown that many fossils are worth a second glance; especially in the context of new knowledge regarding morphology and its phylogenetic implications of the respective taxon. So gobioid taxonomy and phylogeny based on morphology are not a hopeless effort.

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.

Fricke, R., W.N. Eschmeyer, and J.D. Fong. 2019. Species by Family/Subfamily. http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp Electronic version accessed February 2019.

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

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

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

Gierl, C., B. Reichenabcher, M. Dohrmann, et al. In preparation. First total evidence approach for Gobioidei.

Koken, E. 1884. Über Fisch-Otolithen, insbesondere über diejenigen der norddeutschen Oligocän-Ablagerungen. *Zeitschrift der Deutschen Geologischen Gesellschaft* 36: 500–565.

Lawing, A.M., J.M. Meik, and W.E. Schargel. 2008. Coding meristic characters for phylogenetic analysis: a comparison of step-matrix gap-weighting and generalized frequency coding. *Systematic Biology* 57 (1): 167–173.

Lee, D.-C., and H.N. Bryant. 1999. A reconsideration of the coding of inapplicable characters: assumptions and problems. *Cladistics* 15 (4): 373–378.

Reichenbacher, B. 2000. Das brackisch-lakustrine Oligozän und Unter-Miozän im Mainzer Becken und Hanauer Becken: Fischfaunen, Paläoökologie, Biostratigraphie, Paläogeographie. *Courier Forschungsinstitut Senckenberg* 222: 1–143.

Vogt, L. 2018. The logical basis for coding ontologically dependent characters. *Cladistics* 34 (4): 438–458.

Weiler, W. 1942. Die Otolithen des rheinischen und nordwestdeutschen Tertiärs. *Abhandlungen des Reichsamts für Bodenforschung, Neue Folge* 206: 5–140.

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.

Appendix for chapter 4

Otolith measurements

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 the ventral margin; SuTipV – distance from sulcus tip to the ventral margin. OA and SuA are given in mm^2, the other measurements are given in mm.

Lineage	**Name**	**Specimen No.**	**OL**	**OH**	**OP**	**OA**	**SuL**	**SuH**	**SuP**	**SuA**	**SuTipV**	**SuEndV**
Stenogobius	*Awaous flavus*	ZSM-PIS-43853_(P-GO-1050 L)	1,37	1,60	5,51	1,83	0,76	0,53	2,16	0,26	0,49	0,74
Stenogobius	*Awaous flavus*	ZSM-PIS-43853_(P-GO-1051 L)	1,27	1,33	4,60	1,41	0,70	0,34	1,81	0,17	0,50	0,58
Stenogobius	*Gobioides broussonnetii*	ZSM-PIS-43852_(P-GO-1048 L)	2,43	2,35	8,84	4,38	1,17	0,71	3,08	0,55	0,77	1,09
Stenogobius	*Gobioides broussonnetii*	ZSM-PIS-43852_(P-GO-1049 L)	2,67	2,56	9,42	5,14	1,38	0,84	3,58	0,63	0,62	1,14
Stenogobius	*Stiphodon atropurpureus*	ZSM_PIS-43862_(P-GO-1070 L)	0,93	0,87	3,22	0,66	0,63	0,35	1,68	0,16	0,27	0,35
Stenogobius	*Stiphodon atropurpureus*	ZSM_PIS-43862_(P-GO-1071 L)	0,90	0,88	3,12	0,62	0,57	0,34	1,56	0,15	0,29	0,43
Acanthogobius	*Rhinogobius canidianus*	ZSM-PIS-43858_(P-GO-1060 L)	1,40	1,49	5,18	1,70	0,78	0,37	2,02	0,17	0,50	0,74
Acanthogobius	*Rhinogobius canidianus*	ZSM-PIS-43858_(P-GO-1060,61-1 L)	1,24	1,37	4,54	1,38	0,69	0,39	1,81	0,17	0,44	0,63
Acanthogobius	*Rhinogobius canidianus*	ZSM-PIS-43858_(P-GO-1060,61-2 L)	1,39	1,46	5,04	1,65	0,79	0,41	2,06	0,19	0,50	0,63
Acanthogobius	*Rhinogobius canidianus*	ZSM-PIS-43858_(P-GO-1061 L)	1,40	1,33	5,04	1,53	0,76	0,37	2,04	0,18	0,38	0,57
Acanthogobius	*Rhinogobius formosanus*	ZSM-PIS-43864_(P-GO-1072 L)	1,10	1,60	3,95	1,02	0,67	0,35	1,75	0,15	0,33	0,54
Acanthogobius	*Rhinogobius formosanus*	ZSM-PIS-43864_(P-GO-1072,73-1 L)	1,02	1,09	3,74	0,93	0,61	0,32	1,63	0,12	0,37	0,49
Acanthogobius	*Rhinogobius formosanus*	ZSM-PIS-43864_(P-GO-1072,73-3 L)	1,03	1,12	3,81	0,95	0,57	0,30	1,49	0,10	0,36	0,53
Acanthogobius	*Rhinogobius formosanus*	ZSM-PIS-43864_(P-GO-1073 L)	1,02	1,01	3,54	0,84	0,55	0,28	1,44	0,08	0,31	0,49
Acanthogobius	*Rhinogobius rubromaculatus*	ZSM-PIS-43860_(P-GO-1064 L)	1,16	1,23	4,31	1,21	0,66	0,38	1,73	0,14	0,40	0,58
Acanthogobius	*Rhinogobius rubromaculatus*	ZSM-PIS-43860_(P-GO-1064,65-2 L)	1,07	1,08	3,87	0,99	0,60	0,30	1,51	0,10	0,36	0,53
Acanthogobius	*Rhinogobius rubromaculatus*	ZSM-PIS-43860_(P-GO-1064,65-3 L)	1,00	1,10	3,80	0,93	0,56	0,32	1,50	0,09	0,35	0,54
Acanthogobius	*Rhinogobius rubromaculatus*	ZSM-PIS-43860_(P-GO-1065 L)	1,11	1,18	4,50	1,08	0,60	0,32	1,52	0,10	0,34	0,54

Acanthogobius	*Rhinogobius zhoui*	ZSM-PIS-43859_(P-GO-1062 L)	1,00	0,94	3,58	0,80	0,48	0,29	1,28	0,10	0,34	0,43
Acanthogobius	*Rhinogobius zhoui*	ZSM-PIS-43859_(P-GO-1062,63-2 L)	0,80	0,82	2,84	0,54	0,40	0,18	0,97	0,04	0,28	0,40
Acanthogobius	*Rhinogobius zhoui*	ZSM-PIS-43859_(P-GO-1062,63-6 L)	0,86	0,85	3,12	0,62	0,47	0,25	1,24	0,07	0,28	0,41
Acanthogobius	*Rhinogobius zhoui*	ZSM-PIS-43859_(P-GO-1063 L)	0,87	0,92	3,24	0,67	0,45	0,25	1,18	0,07	0,29	0,42
Mugilogobius	*Brachygobius xanthozonus*	ZSM-PIS-43865_(P-GO-1074 L)	0,99	0,95	3,43	0,78	0,65	0,30	1,60	0,13	0,32	0,45
Mugilogobius	*Brachygobius xanthozonus*	ZSM-PIS-43865_(P-GO-1075 L)	0,75	0,77	2,65	0,48	0,38	0,18	0,93	0,05	0,26	0,34
Mugilogobius	*Chlamydogobius eremius*	ZSM-PIS-43854_(P-GO-1052 L)	1,13	1,08	4,10	1,05	0,71	0,45	1,91	0,15	0,36	0,43
Mugilogobius	*Chlamydogobius eremius*	ZSM-PIS-43854_(P-GO-1053 L)	1,41	1,33	5,25	1,62	0,82	0,48	2,17	0,23	0,50	0,58
Mugilogobius	*Schismatogobius roxasi*	ZSM-PIS-43866_(P-GO-1076 L)	0,77	0,75	2,69	0,45	0,57	0,32	1,49	0,13	0,21	0,31
Mugilogobius	*Schismatogobius roxasi*	ZSM-PIS-43866_(P-GO-1077 L)	0,67	0,73	2,53	0,40	0,36	0,73	0,97	0,05	0,24	0,34
Mugilogobius	*Stigmatogobius sadanundio*	ZSM-PIS-43856_(P-GO-1056 L)	1,94	1,83	6,89	2,91	1,17	0,43	2,79	0,29	0,61	0,86
Mugilogobius	*Stigmatogobius sadanundio*	ZSM-PIS-43856_(P-GO-1057 L)	2,38	2,30	8,52	4,26	1,38	0,56	3,44	0,40	0,74	1,03
Pomatoschistus	*Buenia affinis*	NMP P6d 30/2017-3	0,90	0,77	2,97	0,57	0,44	0,24	1,14	0,06	0,27	0,41
Pomatoschistus	*Buenia affinis*	NMP P6d 30/2017-6	0,93	0,84	3,08	0,64	0,51	0,31	1,34	0,08	0,24	0,50
Pomatoschistus	*Buenia affinis*	NMP P6d 30/2017-7	1,00	0,90	3,31	0,74	0,54	0,29	1,38	0,07	0,23	0,46
Pomatoschistus	*Buenia affinis*	NMP P6d 30/2017-9	0,87	0,78	2,91	0,55	0,44	0,25	1,14	0,06	0,24	0,41
Pomatoschistus	*Deltentosteus quadrimaculatus*	NMP P6d 34/2017-1	3,04	2,58	9,76	6,26	1,22	0,67	3,09	0,43	0,83	1,24
Pomatoschistus	*Deltentosteus quadrimaculatus*	NMP P6d 34/2017-2	2,63	2,26	9,19	4,86	1,39	0,68	3,52	0,50	0,60	1,17
Pomatoschistus	*Deltentosteus quadrimaculatus*	NMP P6d 34/2017-3	2,21	1,93	7,47	3,48	1,15	0,64	2,99	0,35	0,54	0,99
Pomatoschistus	*Deltentosteus quadrimaculatus*	NMP P6d 34/2017-5	2,34	2,05	8,16	3,95	1,21	0,65	3,03	0,37	0,62	1,00
Pomatoschistus	*Deltentosteus quadrimaculatus*	NMP P6d 34/2017-6	2,95	2,44	9,39	5,92	1,58	0,79	3,94	0,70	0,74	1,21
Pomatoschistus	*Deltentosteus quadrimaculatus*	NMP P6d 34/2017-7	2,67	2,13	9,31	4,66	1,31	0,64	3,24	0,43	0,63	1,15
Pomatoschistus	*Deltentosteus quadrimaculatus*	NMP P6d 34/2017-8	3,13	2,27	9,55	5,77	1,75	0,93	4,50	0,84	0,75	1,34
Pomatoschistus	*Deltentosteus quadrimaculatus*	NMP P6d 34/2017-9	3,02	2,42	9,62	5,97	1,51	0,89	4,01	0,72	0,74	1,26
Pomatoschistus	*Deltentosteus quadrimaculatus*	NMP P6d 34/2017-10	2,96	2,50	9,74	6,03	1,49	0,85	3,86	0,58	0,70	1,38

Pomatoschistus	Gobiusculus flavescens	NMP P6V 142775	0,72	0,76	2,72	0,44	0,32	0,20	0,84	0,04	0,28	0,39
Pomatoschistus	Gobiusculus flavescens	NMP P6V 142776	0,63	0,67	2,34	0,33	0,29	0,16	0,74	0,03	0,22	0,36
Pomatoschistus	Gobiusculus flavescens	NMP P6V 142777	0,61	0,67	2,33	0,33	0,28	0,17	0,73	0,03	0,24	0,35
Pomatoschistus	Gobiusculus flavescens	NMP P6V 142778	0,60	0,63	2,29	0,30	0,25	0,17	0,67	0,03	0,22	0,34
Pomatoschistus	*Knipowitschia croatica*	NMP P6d 31/2017-1	0,63	0,73	2,40	0,37	0,31	0,17	0,80	0,03	0,25	0,39
Pomatoschistus	*Knipowitschia croatica*	NMP P6d 31/2017-2	0,66	0,71	2,51	0,38	0,30	0,16	0,76	0,03	0,24	0,37
Pomatoschistus	*Knipowitschia croatica*	NMP P6d 31/2017-3	0,67	0,76	2,47	0,40	0,32	0,17	0,82	0,03	0,27	0,38
Pomatoschistus	*Knipowitschia croatica*	NMP P6d 31/2017-4	0,74	0,81	2,76	0,48	0,29	0,20	0,79	0,03	0,28	0,45
Pomatoschistus	*Knipowitschia croatica*	NMP P6d 31/2017-6	0,77	0,80	2,77	0,50	0,29	0,18	0,77	0,03	0,27	0,40
Pomatoschistus	*Knipowitschia croatica*	NMP P6d 31/2017-7	0,65	0,70	2,36	0,35	0,26	0,21	0,76	0,03	0,24	0,35
Pomatoschistus	*Pomatoschistus marmoratus*	NMP P6d 32/2017-1	1,13	1,06	3,86	1,00	0,52	0,26	1,32	0,06	0,31	0,53
Pomatoschistus	*Pomatoschistus marmoratus*	NMP P6d 32/2017-2	1,17	1,13	3,94	1,04	0,58	0,32	1,50	0,10	0,27	0,52
Pomatoschistus	*Pomatoschistus marmoratus*	NMP P6d 32/2017-3	1,23	1,17	4,18	1,18	0,72	0,35	1,83	0,13	0,35	0,57
Pomatoschistus	*Pomatoschistus marmoratus*	NMP P6d 32/2017-4	1,37	1,29	4,59	1,46	0,62	0,35	1,58	0,12	0,46	0,60
Pomatoschistus	*Pomatoschistus marmoratus*	NMP P6d 32/2017-5	1,35	1,24	4,57	1,37	0,64	0,34	1,68	0,10	0,34	0,56
Pomatoschistus	*Pomatoschistus marmoratus*	NMP P6d 32/2017-6	1,12	1,14	3,90	1,04	0,49	0,28	1,30	0,08	0,33	0,57
Pomatoschistus	*Pomatoschistus marmoratus*	NMP P6d 32/2017-7	1,45	1,25	4,59	1,44	0,72	0,34	1,85	0,15	0,47	0,63
Pomatoschistus	*Pomatoschistus quagga*	NMP P6d 33/2017-4	0,85	0,90	3,05	0,63	0,37	0,20	0,93	0,04	0,31	0,45
Pomatoschistus	*Pomatoschistus quagga*	NMP P6d 33/2017-5	0,81	0,90	3,06	0,62	0,34	0,22	0,92	0,04	0,27	0,44
Pomatoschistus	*Pomatoschistus quagga*	NMP P6d 33/2017-6	0,85	0,91	3,12	0,64	0,31	0,21	0,83	0,04	0,28	0,45
Pomatoschistus	*Pomatoschistus quagga*	NMP P6d 33/2017-7	0,82	0,86	2,99	0,58	0,25	0,24	0,77	0,03	0,25	0,42
Pomatoschistus	*Pomatoschistus quagga*	NMP P6d 33/2017-8	0,78	0,86	2,87	0,56	0,33	0,21	0,86	0,03	0,26	0,44
Pomatoschistus	*Pomatoschistus quagga*	NMP P6d 33/2017-9	0,84	0,90	3,12	0,63	0,32	0,22	0,85	0,04	0,31	0,45
Pomatoschistus	*Pomatoschistus quagga*	NMP P6d 33/2017-10	0,82	0,88	3,03	0,60	0,37	0,23	0,98	0,04	0,27	0,44
Periophthalmus	*Bolephthalmus dussumieri*	ZM–CBSU Khamirr 38	3,69	3,02	13,73	8,51	1,91	0,93	4,75	0,95	1,12	1,38

Periophthalmus	*Bolephthalmus dussumieri*	ZM–CBSU Khamirr 40	3,62	3,25	12,99	8,75	2,02	0,95	5,07	1,06	1,06	1,45
Periophthalmus	*Bolephthalmus dussumieri*	ZM–CBSU Khamirr 41	3,29	2,86	11,90	7,13	1,72	0,95	4,53	0,93	0,98	1,42
Periophthalmus	*Bolephthalmus dussumieri*	ZM–CBSU Khamirr 54	2,90	2,52	11,90	5,27	1,53	0,80	3,84	0,70	0,88	1,12
Periophthalmus	*Periophthalmus waltoni*	ZM–CBSU Gowater 1734 (male)	4,43	4,09	15,61	13,63	1,99	1,14	5,35	1,32	1,41	1,83
Periophthalmus	*Periophthalmus waltoni*	ZM–CBSU Gowater 1736 (female)	2,63	2,32	9,56	4,30	1,18	0,74	3,29	0,47	0,75	1,09
Periophthalmus	*Periophthalmus waltoni*	ZM–CBSU Gowater 1739 (female)	4,41	4,44	16,62	14,41	2,30	1,51	6,43	1,88	1,50	1,89
Periophthalmus	*Periophthalmus waltoni*	ZM–CBSU Gowater 1741 (female)	2,66	2,51	8,56	4,67	1,37	0,87	3,79	0,72	0,90	1,21
Periophthalmus	*Periophthalmus waltoni*	ZM–CBSU Gowater 1743 (male)	4,30	3,90	16,00	12,93	2,14	1,17	5,70	1,43	1,44	1,91
Periophthalmus	*Periophthalmus waltoni*	ZM–CBSU Gowater 1745 (male)	4,23	3,68	14,52	11,68	2,15	1,23	5,79	1,54	1,37	1,69
Periophthalmus	*Periophthalmus waltoni*	ZM–CBSU Gowater 1748 (male)	3,96	3,76	16,79	11,02	1,78	0,90	4,46	0,94	1,32	1,75
Periophthalmus	*Periophthalmus waltoni*	ZM–CBSU Gowater 1751 (male)	4,64	4,38	16,80	14,74	2,44	1,37	6,52	1,96	1,46	1,91
Periophthalmus	*Scartelaos tenuis*	ZM–CBSU Helleh 82	2,76	2,40	9,55	5,14	1,37	0,68	3,56	0,53	0,72	0,99
Periophthalmus	*Scartelaos tenuis*	ZM–CBSU Helleh 84	2,40	2,05	8,20	3,72	1,19	0,50	2,89	0,34	0,62	0,89
Periophthalmus	*Scartelaos tenuis*	ZM–CBSU Helleh 85	2,86	2,31	9,41	5,10	1,48	0,66	3,65	0,56	0,92	1,15
Periophthalmus	*Scartelaos tenuis*	ZM–CBSU Helleh 86	2,39	2,03	8,99	3,75	1,19	0,54	3,05	0,35	0,66	0,93
Periophthalmus	*Scartelaos tenuis*	ZM–CBSU Helleh 89	2,86	2,31	9,68	5,10	1,34	0,72	3,52	0,51	0,79	1,05
fossil	*Pomatoschistus* sp.	NHM 1993/140/4	1,40	1,30	4,71	1,44	0,54	0,37	1,44	0,11	0,41	0,65
fossil	*Pomatoschistus* sp.	NHM 1993/140/5	1,21	1,20	4,13	1,20	0,49	0,30	1,25	0,09	0,38	0,59
fossil	*Pomatoschistus* sp.	NHM 1993/140/6	1,22	1,09	3,97	1,08	0,46	0,28	1,19	0,08	0,34	0,53
fossil	*Pomatoschistus* sp.	NHM 1993/140/7	1,15	1,08	3,93	1,02	0,39	0,25	0,56	0,05	0,31	0,56
fossil	*Pomatoschistus* sp.	NHM 1993/140/8	1,18	1,20	4,11	1,16	0,48	0,31	1,28	0,08	0,36	0,58

Appendix for chapter 5

Character list

Note that many characters were used in the spirit of an explorative study.

Anal pterygiophores [10 characters]. Number of anal fin pterygiophores preceding the first hemal spine. This number has been examined by Birdsong et al. (1988) in order to subdivide the Gobioidei into different groups.

AP (no. 1–10, 4 informative: 2–5); 0 = absent; 1 = present.

Second dorsal fin first middle radial [1 character]. “Middle radial of first pterygiophore of second dorsal fin” (Hoese and Gill 1993: p. 423). This has been used in their morphological matrix as character 5.

D2 first middle radial (no. 11, informative); 0 = present; 1 = absent.

Pterygiophore D1 Formula [82 characters]. Pterygiophore formula of the first dorsal fin (Birdsong et al. 1988; see also Birdsong 1975; Akihito et al. 1984; Lee 1993). Birdsong et al. (1988) used this character to subdivide the Gobioidei into different groups.

PTG D1 Formula (no. 12–93, 6 informative: 22, 28, 32, 33, 35, 55); 0 = absent; 1 = present.

Vertebrae abdominal [12 characters]. Number of abdominal vertebrae.

Vertebrae abdominal 8–18, 26 (no. 94–105, 8 informative: 95–102): 0 = absent; 1 = present.

Vertebrae caudal [15 characters]. Number of caudal vertebrae including urostyle.

Vertebrae caudal 11–22, 31–33 (no. 106–120, 7 informative: 109–115). 0 = absent; 1 = present.

Length of second dorsal fin base (D2b) compared to length from end of D2 to beginning of first caudal (procurrent) ray (D2C) [2 characters]. One character is D2b ≤ D2C, the other D2b > D2C. This character was used as by Larson et al. (2001).

D2 base ≤ D2C (no. 121, informative): 0 = absent; 1 = present.

D2 base > D2C (no. 122, informative): 0 = absent; 1 = present.

Caudal fin shape [3 characters]. The shape of the caudal fin is rounded in most gobioids. Apogonids and kurtids have forked caudal fins as have the rhyacichthyids. In some specialized gobiids like *Lesueurigobius* a lanceolate caudal fin is present.

Caudal fin forked/straight (no. 123, informative): 0 = absent; 1 = present.

Caudal fin rounded (no. 124, informative): 0 = absent; 1 = present.

Caudal fin elongate/lanceolate (no. 125, informative): 0 = absent; 1 = present.

Epurals [3 characters]. This was discussed by Hoese and Gill (1993) but not used in their matrix because they found it to be too variable.

Epurals 1–3 (no. 126–128, informative); 0 = absent; 1 = present.

Hypural plates [3 characters]. Configuration of hypural plates; Hypurals 1+2 & 3+4+urostyle fused is typical for gobioidei (see Wiley and Johnson 2010).

Hypurals not fused (no. 129, uninformative): 0 = absent; 1 = present.

Hypurals 1+2 & 3+4+urostyle fused (no. 130, uninformative): 0 = absent; 1 = present.

Hypurals all fused (no. 131, uninformative): 0 = absent; 1 = present.

Caudal fin dorsal unbranched unsegmented rays [13 characters]. System follows Fricke (1983).

Caudal fin dorsal unbranched unsegmented rays 2–14 (no. 132–144, 9 informative: 135–140, 142–144); 0 = absent; 1 = present.

Caudal fin dorsal unbranched segmented rays [5 characters].

Caudal fin dorsal unbranched segmented rays 0–4 (no. 145–149, 2 informative: 146–147): 0 = absent; 1 = present.

Caudal fin branched segmented rays [7 characters].

Caudal fin branched segmented rays 11–17 (no. 150–156, 5 informative: 150–154): 0 = absent; 1 = present.

Caudal fin ventral unbranched segmented rays [4 characters].

Caudal fin ventral unbranched segmented rays 0–3 (no. 157–160, 3 informative: 157-159): 0 = absent; 1 = present.

Caudal fin ventral unbranched unsegmented rays [13 characters]. Number of caudal fin rays;

Caudal fin ventral unbranched unsegmented rays 1–13 (no. 161–173, 9 informative: 164–169, 171–173): 0 = absent; 1 = present.

Body lateral line [1 character]. Most gobioids lack the body lateral line. Only species placed at the base of the tree like *Rhyacichthys*, *Protogobius* and *Terateleotris* have it (Shibukawa et al. 2001)

Body lateral line (no. 174, uninformative); 0 = present; 1 = absent.

Anal fin rays [40 characters]. Number of anal fin rays.

Anal fin rays (no. 175–214, 11 informative: 175–185): 0 = absent; 1 = present.

Anal fin spines [3 characters]. Number of anal fin spines.

Anal fin spines (no. 215–217, 1 informative: 216): 0 = absent; 1 = present.

D1 spines [22 characters]. Number of first dorsal fin spines.

D1 spines (no. 218–230, 5 informative: 221, 223–226): 0 = absent; 1 = present.

D2 rays [34 characters]. Number of second dorsal fin rays.

D2 Rays (no. 231–264, 8 informative: 235–242): 0 = absent; 1 = present.

D2 spines [2 characters]. Number of second dorsal fin spines.

D2 spines 0 or 1 (no. 265–266, uninformative): 0 = absent; 1 = present.

Dorsal fin [2 characters]. Appearance of dorsal fin which can be a single fin or divided as in most gobioids.

Dorsal fin single/connected (no. 267, uninformative): 0 = absent; 1 = present.

Dorsal fin divided (no. 268, uninformative): 0 = absent; 1 = present.

Dorsal hemitrich of pelvic-fin rays complex [1 character]. “Complex base on dorsal hemitrich of pelvic fin rays” (Gill and Mooi 2012: p. 43). This is one of the three characters proposed by Gill and Mooi for defining their “Gobiidae”.

Dorsal hemitrich of pelvic-fin rays complex (no. 269, informative): 0 = absent; 1 = present.

Dorsal postcleithrum [1 character]. Absent in Thalasseleotrididae and 5brG; also absent in the eleotrid genus *Xenisthmus* (Gill and Mooi 2012).

Dorsal postcleithrum (no. 270, informative): 0 = absent; 1 = present.

Pelvic fins [2 characters]. Generally, the 5brG have united pelvic fins, but exceptions exist, e.g. *Vanneaugobius* (see Van Tassell et al. 1988). Character 16 in the matrix of Hoese and Gill (1993) who took it from Hoese (1984).

Pelvic fins separate (no. 271, informative): 0 = absent; 1 = present.

Pelvic fins united (no. 272, informative): 0 = absent; 1 = present.

Radial contact [2 characters]. Linked to Scapula development, character 4 in the matrix of Hoese and Gill (1993)

Radial not in contact with cleithrum (no. 273, informative): 0 = absent; 1 = present.

Radial in contact with cleithrum (no. 274, informative): 0 = absent; 1 = present.

Bony opercular canal support [2 characters]. These form character 6 in the matrix of Hoese and Gill (1993).

Bony opercular canal support on complete preopercle (no. 275, informative): 0 = absent; 1 = present.

Bony opercular canal support on vertical part of preopercle (no. 276, informative): 0 = absent; 1 = present.

Branchiostegal rays [3 characters]. The Oxudercidae and Gobiidae together form a distinct clade and one of their synapomorphies is five branchiostegals. All other recent gobioids have six. †*Pirskenius* and the Apogonidae have seven (see Přikryl 2014; Carpenter and Niem 2001).

Branchiostegals 7, 6 or 5(no. 277–278, informative): 0 = absent; 1 = present.

Connection between symplectic and preopercle [1 character]. The absence of this feature was mentioned by Miller (1973) as being indicative for Oxudercidae (Gobionellinae then).

Connection between symplectic and preopercle (no. 280, informative): 0 = absent; 1 = present.

Ectopterygoid depth [2 characters]. This character was used by Harrison (1989) as character F in his matrix.

Ectopterygoid deep (no. 281, informative): 0 = present; 1 = absent.

Ectopterygoid shallow (no. 282, informative): 0 = present; 1 = absent.

Ectopterygoid quadrate relationship [4 characters]. These charaters originate from Harrison (1989) as characters C, D and E.

Ectopterygoid expanded along anterior edge of quadrate (no. 283, informative): 0 = absent; 1 = present.

Ectopterygoid applied to dorsal anterior corner of the quadrate and not expanding along the anterior edge of the quadrate (no. 284, informative): 0 = absent; 1 = present.

Ectopterygoid applied to the dorsal edge of the quadrate (no. 285, uninformative): 0 = absent; 1 = present.

Ectopterygoid applied to the anteroventral corner of the quadrate (no. 286, uninformative): 0 = absent; 1 = present.

Entopterygoid (Endopterygoid) [1 character] The first to use this character to discriminate between Gobiidae and Eleotridae was Regan (1911). Since the absence is viewed as the derived state therefore the coding is reversed.

Entopterygoid (Endopterygoid) (no. 287, informative); 0 = present; 1 = absent.

Epihyal posterior process [1 character]. This is one of the characters supporting the sister relationship of Thalasseletrididae and Gobiidae (Gill and Mooi 2012).

Epihyal posterior process supporting interhyal (no. 288, informative): 0 = absent; 1 = present.

Infraorbital canal [1 character]. This is character 11 in the matrix of Hoese and Gill (1993).

Infraorbital canal extending below eye (no. 289, informative): 0 = present; 1 = absent.

Infraorbitals [4 characters]. The infraorbitals are reduced in most gobioids to only the lacrimal (e.g. see Akihito 1986)

Infraorbital number (no. 290 – 293, 1 informative: 292): 0 = absent; 1 = present.

Interhyal and symplectic separated [1 character]. This is character 14 of the matrix from Hoese and Gill (1993).

Interhyal and symplectic separated (no. 294, informative): 0 = absent; 1 = present.

Interhyal with cup-shaped structure [1 character]. "Interhyal with cup-shaped structure for articulation with preopercle" (Gill and Mooi 2012, p. 47). Synapomorphy of Thalasseleotrididae and their Gobiidae (Gobiidae + Oxudercidae).

Interhyal with cup-shaped structure (no. 295, informative): 0 = absent; 1 = present.

Membrane connecting hyoid arch to ceratobranchial 1 [1 character]. "Membrane connecting the hyoid arch to ceratobranchial 1 broad, extending most of the length of ceratobranchial 1 (= first gill slit restricted or closed)" (Gill and Mooi 2012, p. 46). This single synapomorphy supports the monophyly of the Thalasseleotridae according to Gill and Mooi (2012).

Membrane connecting hyoid arch to ceratobranchial 1 (no. 296, informative): 0 = absent; 1 = present.

Palatine shaft [3 characters]. This constitutes characters A and B used by Harrison (1989).

Pal extending about half the length of the ectopterygoid (no. 297, informative): 0 = absent; 1 = present.

Pal extending towards or meeting quadrate (no. 298, informative): 0 = absent; 1 = present.

Pal not extending much beyond the dorsal end of the ectopterygoid (no. 299, informative): 0 = absent; 1 = present.

Palatine ethmoid process [1 character]. Regan (1911) used this character to distinguish between Gobiidae (T-shaped palatine) and Eleotridae (L-shaped).

Palatine ethmoid process well developed (no. 300, informative): 0 = absent; 1 = present.

Parietals [1 character]. The lack of the parietals is characteristic for the Gobioidei (e.g. Wiley and Johnson 2010).

Parietals (no. 301, uninformative): 0 = present; 1 = absent.

Penultimate branchiostegal position [3 characters]. This character was used by Hoese and Gill (1993) as character 8, but without the state “in between”.

Penultimate branchiostegal on epihyal (no. 302, informative): 0 = absent; 1 = present.

Penultimate branchiostegal in between (no. 303, informative): 0 = absent; 1 = present.

Penultimate branchiostegal on ceratohyal (no. 304, informative): 0 = absent; 1 = present.

Pharyngobranchial 4 [1 character]. Gill and Mooi (2012) gives this as a synapomorphy of the Thalasseleotridae and their Gobiidae.

Pharyngobranchial 4 (no. 305, informative): 0 = absent; 1 = present.

Postmaxillary process of premaxilla [2 characters]. The postmaxillary process is a feature known from the sand goby group (see McKay and Miller 1997).

Postmaxillary process (no. 306, informative): 0 = absent; 1 = present.

Postmaxillary process well developed (no. 307, informative): 0 = absent; 1 = present.

Preopercular mandibular canal [1 character]. This is character 10 in the matrix of Hoese and Gill (1993).

Preopercular mandibular canal connected to the oculoscapular canal and extending onto the mandibular region (no. 308, informative): 0 = present; 1 = absent.

Process on ceratobranchial 5 [1 character]. This character was used by Gill and Mooi (2012) to define their Gobiidae.

Process on ceratobranchial 5 (no. 309, informative): 0 = absent; 1 = present.

Quadrate shape [4 characters]. These form characters G and H of the matrix of Harrison (1989).

Quadrate dorsal lamina triangular (no. 310, uninformative): 0 = absent; 1 = present.

Quadrate dorsal lamina deeply forked with anteroventral sloping edge (no. 311, uninformative): 0 = absent; 1 = present.

Quadrate dorsal lamina anteroposteriorly long (no. 312, uninformative): 0 = absent; 1 = present.

Quadrate dorsal lamina anteroposteriorly short (no. 313, uninformative): 0 = absent; 1 = present.

<u>Suspensorium fenestra</u> [1 character]. This character was mentioned as being characteristic for Gobioidei by Gosline (1955).

Suspensorium fenestra (no. 314, uninformative): 0 = absent; 1 = present.

<u>Urohyal ventral shelf</u> [1 character]. The absence of this character is established as a synapomorphy of the Thalasseleotrididae and Gobiidae (Gobiidae+Oxudercidae) by Gill and Mooi (2012).

Urohyal ventral shelf (no. 315, informative): 0 = absent; 1 = present.

<u>Otolith (Sagitta) [16 characters].</u>

Otolith anterodorsal projection (no. 316, informative): 0 = present; 1 = absent.

Otolith anteroventral projection (no. 317, informative): 0 = present; 1 = absent.

Otolith shape perch-like (no. 318, informative): 0 = absent; 1 = present.

Otolith posterodorsal projection (no. 319, informative): 0 = present; 1 = absent.

Otolith posteroventral projection (no. 320, informative): 0 = present; 1 = absent.

Otolith shape quadratic (no. 321, informative): 0 = absent; 1 = present.

Otolith shape rectangular (no. 322, informative): 0 = absent; 1 = present.

Otolith shape rectangular/higher than long (no. 323, informative): 0 = absent; 1 = present.

Otolith shape rounded (no. 324, informative): 0 = absent; 1 = present.

Otolith shape trapezoid/triangular (no. 325, informative): 0 = absent; 1 = present.

Otolith subcaudal iugum (no. 326, informative): 0 = absent; 1 = present. See Schwarzhans (2014).

Otolith subcaudal iugum short (no. 327, uninformative): 0 = absent; 1 = present.

Otolith subcaudal iugum long (no. 328, informative): 0 = absent; 1 = present.

Otolith sulcus centered (no. 329, informative): 0 = absent; 1 = present.

Otolith sulcus nearer to anterior rim (no. 330, informative): 0 = absent; 1 = present.

Otolith sulcus shoe-sole-like (no. 331, uninformative): 0 = absent; 1 = present.

<u>Scales</u> [4 characters].

Scales (no. 332, uninformative): 0 = absent; 1 = present.

Scales ctenoid (no. 333, informative): 0 = absent; 1 = present.

Scales cycloid (no. 334, informative): 0 = absent; 1 = present.

Scale transforming cteni (no. 335, informative): 0 = present; 1 = absent. This one is character 7 in the matrix of Hoese and Gill (1993).

Adductor mandibulae tendon [2 characters]. This is character 1 in the matrix of Hoese and Gill (1993).

Adductor mandibulae tendon attaches posterior to or on head of maxilla (no. 336, informative): 0 = absent; 1 = present.

Adductor mandibulae tendon inserts on posterodorsolateral margin of the maxilla (no. 337, informative): 0 = absent; 1 = present.

Anterior procurrent cartilage [2 characters]. This is character 2 in the matrix of Hoese and Gill (1993).

Anterior procurrent cartilage small (no. 338, informative): 0 = absent; 1 = present;

Anterior procurrent cartilage anteriorly expanded (339, informative): 0 = absent; 1 = present.

Posterior procurrent caudal cartilage [2 characters]. This is character 3 in the matrix of Hoese and Gill (1993).

Posterior procurrent caudal cartilage relatively short (no. 340, informative): 0 = absent; 1 = present;

Posterior procurrent caudal cartilage posteriorly and anteriorly elongate (no. 341, informative): 0 = absent; 1 = present.

Table of all characters

Character	**#**	**Group**
AP 00	1	Anal pterygiophore
AP 01	2	Anal pterygiophore
AP 02	3	Anal pterygiophore
AP 03	4	Anal pterygiophore
AP 04	5	Anal pterygiophore
AP 05	6	Anal pterygiophore
AP 06	7	Anal pterygiophore
AP 07	8	Anal pterygiophore
AP 08	9	Anal pterygiophore
AP 09	10	Anal pterygiophore
D2 first middle radial	11	Axial sk
PTG D1 F 0-1*1*1*+1211111	12	Pterygiophore formula
PTG D1 F 0-1*1*1*+1211122241	13	Pterygiophore formula
PTG D1 F 1-1*0022121(40)	14	Pterygiophore formula
PTG D1 F 2-121(20)	15	Pterygiophore formula
PTG D1 F 2-21(21)	16	Pterygiophore formula
PTG D1 F 3-11111	17	Pterygiophore formula
PTG D1 F 3-1121100	18	Pterygiophore formula

PTG D1 F 3-112111	19	Pterygiophore formula
PTG D1 F 3-11310	20	Pterygiophore formula
PTG D1 F 3-12110	21	Pterygiophore formula
PTG D1 F 3-121111	22	Pterygiophore formula
PTG D1 F 3-121201	23	Pterygiophore formula
PTG D1 F 3-12200	24	Pterygiophore formula
PTG D1 F 3-12201	25	Pterygiophore formula
PTG D1 F 3-1221	26	Pterygiophore formula
PTG D1 F 3-1221*	27	Pterygiophore formula
PTG D1 F 3-12210	28	Pterygiophore formula
PTG D1 F 3-122100	29	Pterygiophore formula
PTG D1 F 3-1221000	30	Pterygiophore formula
PTG D1 F 3-122102	31	Pterygiophore formula
PTG D1 F 3-12211	32	Pterygiophore formula
PTG D1 F 3-122110	33	Pterygiophore formula
PTG D1 F 3-1221100	34	Pterygiophore formula
PTG D1 F 3-122111	35	Pterygiophore formula
PTG D1 F 3-12220	36	Pterygiophore formula
PTG D1 F 3-1222111	37	Pterygiophore formula
PTG D1 F 3-13100	38	Pterygiophore formula
PTG D1 F 3-1311*	39	Pterygiophore formula
PTG D1 F 3-131111	40	Pterygiophore formula
PTG D1 F 3-2112111	41	Pterygiophore formula
PTG D1 F 3-21211	42	Pterygiophore formula
PTG D1 F 3-212110	43	Pterygiophore formula
PTG D1 F 3-2121100	44	Pterygiophore formula
PTG D1 F 3-212111	45	Pterygiophore formula
PTG D1 F 3-2121111	46	Pterygiophore formula
PTG D1 F 3-2121111*	47	Pterygiophore formula
PTG D1 F 3-2121210	48	Pterygiophore formula
PTG D1 F 3-2122110	49	Pterygiophore formula
PTG D1 F 3-2201*	50	Pterygiophore formula
PTG D1 F 3-221*001*1*	51	Pterygiophore formula
PTG D1 F 3-221*1*01*1*	52	Pterygiophore formula
PTG D1 F 3-2211	53	Pterygiophore formula
PTG D1 F 3-2211*	54	Pterygiophore formula
PTG D1 F 3-22110	55	Pterygiophore formula
PTG D1 F 3-221100	56	Pterygiophore formula
PTG D1 F 3-221110	57	Pterygiophore formula

PTG D1 F 3-221111*	58	Pterygiophore formula
PTG D1 F 3-22120	59	Pterygiophore formula
PTG D1 F 3-221220	60	Pterygiophore formula
PTG D1 F 3-22210	61	Pterygiophore formula
PTG D1 F 3-222100	62	Pterygiophore formula
PTG D1 F 3-2301000	63	Pterygiophore formula
PTG D1 F 3-32010	64	Pterygiophore formula
PTG D1 F 3-4100	65	Pterygiophore formula
PTG D1 F 3-4101*0	66	Pterygiophore formula
PTG D1 F 3-4101*00	67	Pterygiophore formula
PTG D1 F 4-11120110	68	Pterygiophore formula
PTG D1 F 4-1210100	69	Pterygiophore formula
PTG D1 F 4-132100	70	Pterygiophore formula
PTG D1 F 4-2110100	71	Pterygiophore formula
PTG D1 F 4-212111	72	Pterygiophore formula
PTG D1 F 4-212111*	73	Pterygiophore formula
PTG D1 F 4-22110	74	Pterygiophore formula
PTG D1 F 4-221110	75	Pterygiophore formula
PTG D1 F 4-221111*	76	Pterygiophore formula
PTG D1 F 4-222110	77	Pterygiophore formula
PTG D1 F 4-231	78	Pterygiophore formula
PTG D1 F 4-2310	79	Pterygiophore formula
PTG D1 F 4-312110	80	Pterygiophore formula
PTG D1 F 5-1111101*0	81	Pterygiophore formula
PTG D1 F 5-1211100	82	Pterygiophore formula
PTG D1 F 5-1220100	83	Pterygiophore formula
PTG D1 F 5-1221010	84	Pterygiophore formula
PTG D1 F 5-2111010	85	Pterygiophore formula
PTG D1 F 5-2111100	86	Pterygiophore formula
PTG D1 F 5-21200100	87	Pterygiophore formula
PTG D1 F 5-21200100	88	Pterygiophore formula
PTG D1 F 5-212010	89	Pterygiophore formula
PTG D1 F 5-2120100	90	Pterygiophore formula
PTG D1 F 5-2121010	91	Pterygiophore formula
PTG D1 F 5-221101*0	92	Pterygiophore formula
PTG D1 F 6-212010	93	Pterygiophore formula
Vertebrae abdominal 08	94	Axial sk
Vertebrae abdominal 09	95	Axial sk
Vertebrae abdominal 10	96	Axial sk

Vertebrae abdominal 11	97	Axial sk
Vertebrae abdominal 12	98	Axial sk
Vertebrae abdominal 13	99	Axial sk
Vertebrae abdominal 14	100	Axial sk
Vertebrae abdominal 15	101	Axial sk
Vertebrae abdominal 16	102	Axial sk
Vertebrae abdominal 17	103	Axial sk
Vertebrae abdominal 18	104	Axial sk
Vertebrae abdominal 26	105	Axial sk
Vertebrae caudal 11	106	Axial sk
Vertebrae caudal 12	107	Axial sk
Vertebrae caudal 13	108	Axial sk
Vertebrae caudal 14	109	Axial sk
Vertebrae caudal 15	110	Axial sk
Vertebrae caudal 16	111	Axial sk
Vertebrae caudal 17	112	Axial sk
Vertebrae caudal 18	113	Axial sk
Vertebrae caudal 19	114	Axial sk
Vertebrae caudal 20	115	Axial sk
Vertebrae caudal 21	116	Axial sk
Vertebrae caudal 22	117	Axial sk
Vertebrae caudal 31	118	Axial sk
Vertebrae caudal 32	119	Axial sk
Vertebrae caudal 33	120	Axial sk
D2 base ≤ D2C	121	Axial sk
D2 base > D2C	122	Axial sk
Caudal fin forked/straight	123	Caudal shape
Caudal fin rounded	124	Caudal shape
Caudal fin elongate/lanceolate	125	Caudal shape
Epurals 01	126	Caudal
Epurals 02	127	Caudal
Epurals 03	128	Caudal
Hypurals not fused	129	Hypural pattern
Hypurals 1+2&3+4+us fused	130	Hypural pattern
Hypurals all fused	131	Hypural pattern
I C dorsal unbranched unsegmented rays 02	132	Caudal
I C dorsal unbranched unsegmented rays 03	133	Caudal
I C dorsal unbranched unsegmented rays 04	134	Caudal
I C dorsal unbranched unsegmented rays 05	135	Caudal

I C dorsal unbranched unsegmented rays 06	136	Caudal
I C dorsal unbranched unsegmented rays 07	137	Caudal
I C dorsal unbranched unsegmented rays 08	138	Caudal
I C dorsal unbranched unsegmented rays 09	139	Caudal
I C dorsal unbranched unsegmented rays 10	140	Caudal
I C dorsal unbranched unsegmented rays 11	141	Caudal
I C dorsal unbranched unsegmented rays 12	142	Caudal
I C dorsal unbranched unsegmented rays 13	143	Caudal
I C dorsal unbranched unsegmented rays 14	144	Caudal
II C dorsal unbranched segmented rays 00	145	Caudal
II C dorsal unbranched segmented rays 01	146	Caudal
II C dorsal unbranched segmented rays 02	147	Caudal
II C dorsal unbranched segmented rays 03	148	Caudal
II C dorsal unbranched segmented rays 04	149	Caudal
III C branched segmented rays 11	150	Caudal
III C branched segmented rays 12	151	Caudal
III C branched segmented rays 13	152	Caudal
III C branched segmented rays 14	153	Caudal
III C branched segmented rays 15	154	Caudal
III C branched segmented rays 16	155	Caudal
III C branched segmented rays 17	156	Caudal
IIII C ventral unbranched segmented rays 00	157	Caudal
IIII C ventral unbranched segmented rays 01	158	Caudal
IIII C ventral unbranched segmented rays 02	159	Caudal
IIII C ventral unbranched segmented rays 03	160	Caudal
IIIII C ventral unbranched unsegmented rays 01	161	Caudal
IIIII C ventral unbranched unsegmented rays 02	162	Caudal
IIIII C ventral unbranched unsegmented rays 03	163	Caudal
IIIII C ventral unbranched unsegmented rays 04	164	Caudal
IIIII C ventral unbranched unsegmented rays 05	165	Caudal
IIIII C ventral unbranched unsegmented rays 06	166	Caudal
IIIII C ventral unbranched unsegmented rays 07	167	Caudal
IIIII C ventral unbranched unsegmented rays 08	168	Caudal
IIIII C ventral unbranched unsegmented rays 09	169	Caudal
IIIII C ventral unbranched unsegmented rays 10	170	Caudal
IIIII C ventral unbranched unsegmented rays 11	171	Caudal
IIIII C ventral unbranched unsegmented rays 12	172	Caudal
IIIII C ventral unbranched unsegmented rays 13	173	Caudal
Body lateral line	174	Ext. features

Anal fin rays 06	175	Fins+sk
Anal fin rays 07	176	Fins+sk
Anal fin rays 08	177	Fins+sk
Anal fin rays 09	178	Fins+sk
Anal fin rays 10	179	Fins+sk
Anal fin rays 11	180	Fins+sk
Anal fin rays 12	181	Fins+sk
Anal fin rays 13	182	Fins+sk
Anal fin rays 14	183	Fins+sk
Anal fin rays 15	184	Fins+sk
Anal fin rays 16	185	Fins+sk
Anal fin rays 20	186	Fins+sk
Anal fin rays 21	187	Fins+sk
Anal fin rays 22	188	Fins+sk
Anal fin rays 24	189	Fins+sk
Anal fin rays 25	190	Fins+sk
Anal fin rays 26	191	Fins+sk
Anal fin rays 27	192	Fins+sk
Anal fin rays 28	193	Fins+sk
Anal fin rays 29	194	Fins+sk
Anal fin rays 30	195	Fins+sk
Anal fin rays 31	196	Fins+sk
Anal fin rays 32	197	Fins+sk
Anal fin rays 33	198	Fins+sk
Anal fin rays 34	199	Fins+sk
Anal fin rays 35	200	Fins+sk
Anal fin rays 36	201	Fins+sk
Anal fin rays 37	202	Fins+sk
Anal fin rays 38	203	Fins+sk
Anal fin rays 39	204	Fins+sk
Anal fin rays 40	205	Fins+sk
Anal fin rays 41	206	Fins+sk
Anal fin rays 42	207	Fins+sk
Anal fin rays 43	208	Fins+sk
Anal fin rays 44	209	Fins+sk
Anal fin rays 45	210	Fins+sk
Anal fin rays 46	211	Fins+sk
Anal fin rays 47	212	Fins+sk
Anal fin rays 48	213	Fins+sk

Anal fin rays 49	214	Fins+sk
Anal fin spines 00	215	Fins+sk
Anal fin spines 01	216	Fins+sk
Anal fin spines 02	217	Fins+sk
D1 spines 00	218	Fins+sk
D1 spines 01	219	Fins+sk
D1 spines 02	220	Fins+sk
D1 spines 03	221	Fins+sk
D1 spines 04	222	Fins+sk
D1 spines 05	223	Fins+sk
D1 spines 06	224	Fins+sk
D1 spines 07	225	Fins+sk
D1 spines 08	226	Fins+sk
D1 spines 14	227	Fins+sk
D1 spines 15	228	Fins+sk
D1 spines 20	229	Fins+sk
D1 spines 21	230	Fins+sk
D2 rays 04	231	Fins+sk
D2 rays 05	232	Fins+sk
D2 rays 06	233	Fins+sk
D2 rays 07	234	Fins+sk
D2 rays 08	235	Fins+sk
D2 rays 09	236	Fins+sk
D2 rays 10	237	Fins+sk
D2 rays 11	238	Fins+sk
D2 rays 12	239	Fins+sk
D2 rays 13	240	Fins+sk
D2 rays 14	241	Fins+sk
D2 rays 15	242	Fins+sk
D2 rays 19	243	Fins+sk
D2 rays 20	244	Fins+sk
D2 rays 21	245	Fins+sk
D2 rays 25	246	Fins+sk
D2 rays 27	247	Fins+sk
D2 rays 28	248	Fins+sk
D2 rays 29	249	Fins+sk
D2 rays 30	250	Fins+sk
D2 rays 31	251	Fins+sk
D2 rays 32	252	Fins+sk

D2 rays 33	253	Fins+sk
D2 rays 34	254	Fins+sk
D2 rays 35	255	Fins+sk
D2 rays 39	256	Fins+sk
D2 rays 40	257	Fins+sk
D2 rays 41	258	Fins+sk
D2 rays 42	259	Fins+sk
D2 rays 43	260	Fins+sk
D2 rays 44	261	Fins+sk
D2 rays 45	262	Fins+sk
D2 rays 46	263	Fins+sk
D2 rays 47	264	Fins+sk
D2 spine 00	265	Fins+sk
D2 spine 01	266	Fins+sk
Dorsal fin single/connected	267	Dorsal fin
Dorsal fin divided	268	Dorsal fin
Dorsal hemitrich of pelvic-fin rays complex	269	Fins+sk
Dorsal postcleithrum	270	Fins+sk
Pelvic fins separate	271	Pelvic fins
Pelvic fins united	272	Pelvic fins
Radial not in contact with cleithrum	273	Scapula/radial position
Radial in contact with cleithrum	274	Scapula/radial position
Bony opercular canal support on complete pop	275	Bony opercular canal support
Bony opercular canal support on vertical part of pop	276	Bony opercular canal support
Branchiostegals 05	277	Head
Branchiostegals 06	278	Head
Branchiostegals 07	279	Head
Connection between symplectic & preopercle	280	Head
Ectopterygoid deep	281	Ectopterygoid depth
Ectopterygoid shallow	282	Ectopterygoid depth
Ect expanded along ant edge of q	283	Ectopterygoid quadrate relationship
Ect applied to dors. ant. corner of q, & not exp. along ant. edge of q	284	Ectopterygoid quadrate relationship
Ect appl. to dors. edge of q	285	Ectopterygoid quadrate relationship
Ect appl. to anterovent. corner of q	286	Ectopterygoid quadrate relationship
Entopterygoid	287	Head
Epihyal post. process supp. interhyal	288	Head
Infraorbital canal extending below eye	289	Head
Infraorbitals 00	290	Head
Infraorbitals 01	291	Head

Infraorbitals 02	292	Head
Infraorbitals 03 +	293	Head
Interhyal & symplectic separated	294	Head
Interhyal with cup-shaped structure	295	Head
Membrane connecting hyoid arch to cb1	296	Head
Pal extend. about 0.5xlength of ect	297	Palatine shaft
Pal extend. towards or meeting q	298	Palatine shaft
Pal not extend. much beyond dors end of ect	299	Palatine shaft
Palatine ethmoid process well developed	300	Head
Parietals	301	Head
Penultimate branchiostegal on epihyal	302	Penultimate branchiostegal position
Penultimate branchiostegal in between	303	Penultimate branchiostegal position
Penultimate branchiostegal on ch	304	Penultimate branchiostegal position
Pharyngobranchial 4	305	Head
Postmaxillary process of premaxilla	306	PMX postmaxillary process
Postmaxillary process well developed	307	PMX postmaxillary process
Preop mandibular canal con. to oc.scap. canal	308	Head
Process on cb 5	309	Head
Quadrate dorsal lamina triangular	310	Quadrate shape (G)
Quadrate dors lam. deeply forked w. anterovent. slop. edge	311	Quadrate shape (G)
Quadrate dorsal lamina anteropost. long	312	Quadrate shape (H)
Quadrate dorsal lamina anteropost. short	313	Quadrate shape (H)
Suspensorium fenestra	314	Head
Urohyal ventral shelf	315	Head
Otolith anterodorsal projection	316	Otolith
Otolith anteroventral projection	317	Otolith
Otolith perch-like	318	Otolith
Otolith posterodorsal projection	319	Otolith
Otolith posteroventral projection	320	Otolith
Otolith shape quadratic	321	Otolith
Otolith shape rectangular	322	Otolith
Otolith shape rectangular/higher than long	323	Otolith
Otolith shape rounded	324	Otolith
Otolith shape trapezoid/triangular	325	Otolith
Otolith subcaudal iugum	326	Otolith
Otolith subcaudal iugum long	327	Otolith
Otolith subcaudal iugum short	328	Otolith
Otolith sulcus centered	329	Otolith
Otolith sulcus nearer to anterior rim	330	Otolith

Otolith sulcus shoe-sole-like	331	Otolith
Scales	332	Scales
Scales ctenoid	333	Scales
Scales cycloid	334	Scales
Scale transforming cteni	335	Scales
Adductor mandibulae tendon attaches post. or on mx head	336	Adductor mandibulae tendon
Adductor mandibulae tendon inserts on podolat. margin of mx	337	Adductor mandibulae tendon
Anterior procurrent cartilage small	338	Anterior procurrent cartiladge
Anterior procurrent cartilage anter. expanded	339	Anterior procurrent cartiladge
Posterior procurrent caudal cartilage rel. short post.	340	Posterior procurrent caudal cartilage
Posterior procurrent caudal cartilage elongate	341	Posterior procurrent caudal cartilage

NEXUS-file created with Mesquite

```
#NEXUS
BEGIN TAXA;
	TITLE Taxa;
	DIMENSIONS NTAX = 40;
	TAXLABELS
		Sphaeramia_nematoptera Eleogobius_brevis_F Eleogobius_gaudanti_F
Gobius_francofurtanus_F Gobius_jarosi_F Lepidocottus_aries_F Paralates_bleicheri_F
Pirskenius_diatomaceus_F Pirskenius_radoni_F Kribia_nana_B Oxyeleotris_marmorata_B
Dormitator_maculatus_E Hypseleotris_compressa_E Tateurndina_ocellicauda_E
Amblygobius_phalaena_G Aphia_minuta_G Asterropteryx_semipunctata_G
Austrolethops_wardi_5brG Cryptocentrus_cinctus_G Discordipinna_griessingeri_G
Glossogobius_giuris_G Gobius_niger_G Lesueurigobius_sanzi_G Ptereleotris_evides_G
Tigrigobius_multifasciatus_G Milyeringa_veritas_M Typhleotris_madagascariensis_M
Odontobutis_obscurus_Od Perccottus_glenii_Od Terateleotris_aspro_6brG
Awaous_flavus_Ox Chlamydogobius_eremius_Ox Eucyclogobius_newberryi_Ox
Gobioides_broussonnetii_Ox Pomatoschistus_flavescens_Ox Protogobius_attiti_R
Rhyacichthys_aspro_R Rhyacichthys_guilberti_R Grahamichthys_radiatus_T
Thalasseleotris_iota_T
	;

END;

BEGIN CHARACTERS;
	TITLE  Character_Matrix;
	DIMENSIONS  NCHAR = 147;
```

```
FORMAT DATATYPE = STANDARD GAP = - MISSING = ? SYMBOLS = "  0 1";
CHARSTATELABELS
	1 AP_01 /  absent present,
	2 AP_02 /  absent present,
	3 AP_03 /  absent present,
	4 AP_04 /  absent present,
	5 D2_first_middle_radial /  present absent,
	6 'PTG D1 F 3-121111' /  absent present,
	7 'PTG D1 F 3-12210' /  absent present,
	8 'PTG D1 F 3-12211' /  absent present,
	9 'PTG D1 F 3-122110' /  absent present,
	10 'PTG D1 F 3-122111' /  absent present,
	11 'PTG D1 F 3-22110' /  absent present,
	12 Vertebrae_abdominal_09 /  absent present,
	13 Vertebrae_abdominal_10 /  absent present,
	14 Vertebrae_abdominal_11 /  absent present,
	15 Vertebrae_abdominal_12 /  absent present,
	16 Vertebrae_abdominal_13 /  absent present,
	17 Vertebrae_abdominal_14 /  absent present,
	18 Vertebrae_abdominal_15 /  absent present,
	19 Vertebrae_abdominal_16 /  absent present,
	20 Vertebrae_caudal_14 /  absent present,
	21 Vertebrae_caudal_15 /  absent present,
	22 Vertebrae_caudal_16 /  absent present,
	23 Vertebrae_caudal_17 /  absent present,
	24 Vertebrae_caudal_18 /  absent present,
	25 Vertebrae_caudal_19 /  absent present,
	26 Vertebrae_caudal_20 /  absent present,
	27 'D2 base ≤ D2C' /  absent present,
	28 'D2 base > D2C' /  absent present,
	29 'Caudal fin forked/straight' /  absent present,
	30 Caudal_fin_rounded /  absent present,
	31 'Caudal fin elongate/lanceolate' /  absent present,
	32 Epurals_01 /  absent present,
	33 Epurals_02 /  absent present,
	34 Epurals_03 /  absent present,
	35 I_C_dorsal_unbranched_unsegmented_rays_05 /  absent present,
	36 I_C_dorsal_unbranched_unsegmented_rays_06 /  absent present,
	37 I_C_dorsal_unbranched_unsegmented_rays_07 /  absent present,
```

38 I_C_dorsal_unbranched_unsegmented_rays_08 / absent present,
39 I_C_dorsal_unbranched_unsegmented_rays_09 / absent present,
40 I_C_dorsal_unbranched_unsegmented_rays_10 / absent present,
41 I_C_dorsal_unbranched_unsegmented_rays_12 / absent present,
42 I_C_dorsal_unbranched_unsegmented_rays_13 / absent present,
43 I_C_dorsal_unbranched_unsegmented_rays_14 / absent present,
44 II_C_dorsal_unbranched_segmented_rays_01 / absent present,
45 II_C_dorsal_unbranched_segmented_rays_02 / absent present,
46 III_C_branched_segmented_rays_11 / absent present,
47 III_C_branched_segmented_rays_12 / absent present,
48 III_C_branched_segmented_rays_13 / absent present,
49 III_C_branched_segmented_rays_14 / absent present,
50 III_C_branched_segmented_rays_15 / absent present,
51 IIII_C_ventral_unbranched_segmented_rays_00 / absent present,
52 IIII_C_ventral_unbranched_segmented_rays_01 / absent present,
53 IIII_C_ventral_unbranched_segmented_rays_02 / absent present,
54 IIIII_C_ventral_unbranched_unsegmented_rays_04 / absent present,
55 IIIII_C_ventral_unbranched_unsegmented_rays_05 / absent present,
56 IIIII_C_ventral_unbranched_unsegmented_rays_06 / absent present,
57 IIIII_C_ventral_unbranched_unsegmented_rays_07 / absent present,
58 IIIII_C_ventral_unbranched_unsegmented_rays_08 / absent present,
59 IIIII_C_ventral_unbranched_unsegmented_rays_09 / absent present,
60 IIIII_C_ventral_unbranched_unsegmented_rays_11 / absent present,
61 IIIII_C_ventral_unbranched_unsegmented_rays_12 / absent present,
62 IIIII_C_ventral_unbranched_unsegmented_rays_13 / absent present,
63 Anal_fin_rays_06 / absent present,
64 Anal_fin_rays_07 / absent present,
65 Anal_fin_rays_08 / absent present,
66 Anal_fin_rays_09 / absent present,
67 Anal_fin_rays_10 / absent present,
68 Anal_fin_rays_11 / absent present,
69 Anal_fin_rays_12 / absent present,
70 Anal_fin_rays_13 / absent present,
71 Anal_fin_rays_14 / absent present,
72 Anal_fin_rays_15 / absent present,
73 Anal_fin_rays_16 / absent present,
74 Anal_fin_spines_01 / absent present,
75 D1_spines_03 / absent present,
76 D1_spines_05 / absent present,

77 D1_spines_06 / absent present,

78 D1_spines_07 / absent present,

79 D1_spines_08 / absent present,

80 D2_rays_08 / absent present,

81 D2_rays_09 / absent present,

82 D2_rays_10 / absent present,

83 D2_rays_11 / absent present,

84 D2_rays_12 / absent present,

85 D2_rays_13 / absent present,

86 D2_rays_14 / absent present,

87 D2_rays_15 / absent present,

88 'Dorsal hemitrich of pelvic-fin rays complex' / absent present,

89 Dorsal_postcleithrum / absent present,

90 Pelvic_fins_separate / absent present,

91 Pelvic_fins_united / absent present,

92 Radial_not_in_contact_with_cleithrum / absent present,

93 Radial_in_contact_with_cleithrum / absent present,

94 Bony_opercular_canal_support_on__complete_pop / absent present,

95 Bony_opercular_canal_support_on_vertical_part_of_pop / absent present,

96 Branchiostegals_05 / absent present,

97 Branchiostegals_06 / absent present,

98 Branchiostegals_07 / absent present,

99 Connection_between_symplectic_&_preopercle / absent present,

100 Ectopterygoid_deep / absent present,

101 Ectopterygoid_shallow / absent present,

102 Ect_expanded_along_ant_edge_of_q / absent present,

103 'Ect applied to dors. ant. corner of q, & not exp. along ant. edge of q' / absent present,

104 Entopterygoid / present absent,

105 Epihyal_post._process_supp._interhyal_ / absent present,

106 Infraorbital_canal_extending_below_eye / present absent,

107 Infraorbitals_02 / absent present,

108 Interhyal_&_symplectic_separated / absent present,

109 'Interhyal with cup-shaped structure' / absent present,

110 Membrane_connecting_hyoid_arch_to_cb1 / absent present,

111 Pal_extend._about_0.5xlength_of_ect / absent present,

112 Pal_extend._towards_or_meeting_q / absent present,

113 Pal_not_extend._much_beyond_dors_end_of_ect / absent present,

114 Palatine_ethmoid_process_well_developed / absent present,

115 Penultimate_branchiostegal_on_epihyal / absent present,

116 Penultimate_branchiostegal_in_between / absent present,

117 Penultimate_branchiostegal_on_ch / absent present,

118 Pharyngobranchial_4 / absent present,

119 Postmaxillary_process_of_premaxilla / absent present,

120 Postmaxillary_process_well_developed / absent present,

121 Preop_mandibular_canal_con._to_oc.scap._canal / present absent,

122 Process_on_cb_5 / absent present,

123 Urohyal_ventral_shelf / absent present,

124 Otolith_anterodorsal_projection / absent present,

125 Otolith_anteroventral_projection / absent present,

126 'Otolith perch-like' / absent present,

127 Otolith_posterodorsal_projection / absent present,

128 Otolith_posteroventral_projection / absent present,

129 Otolith_shape_quadratic / absent present,

130 Otolith_shape_rectangular / absent present,

131 'Otolith shape rectangular/higher than long' / absent present,

132 Otolith_shape_rounded / absent present,

133 'Otolith shape trapezoid/triangular' / absent present,

134 Otolith_subcaudal_iugum / absent present,

135 Otolith_subcaudal_iugum_short / absent present,

136 Otolith_sulcus_centered / absent present,

137 Otolith_sulcus_nearer_to_anterior_rim / absent present,

138 Scales_ctenoid / absent present,

139 Scales_cycloid / absent present,

140 Scale_transforming_cteni / present absent,

141 Body_lateral_line / present absent,

142 Adductor_mandibulae_tendon_attaches_post._or_on_mx_head / absent present,

143 Adductor_mandibulae_tendon_inserts_on_podolat._margin_of_mx / absent present,

144 Anterior_procurrent_cartilage_small / absent present,

145 Anterior_procurrent_cartilage_anter._expanded / absent present,

146 Posterior_procurrent_caudal_cartilage_rel._short_post. / absent present,

147 Posterior_procurrent_caudal_cartilage_elongate / absent present ;

MATRIX

Sphaeramia_nematoptera

0100?00000001000000010000000101000001001000000010000110100100000000001000000000
001001100000??1010??0011????0??0???????100?11???10110000100-0110?0??????

Eleogobius_brevis_F

1000?010000001000000001000110101000111000001100110011001110000001100000001 0

010000110000????????010?10101??????1001????11??00101101000??101?00??????

Eleogobius_gaudanti_F

??????????01100000001100010010??00010000000110010001100010000000011000000010 01

0001100000????????010???101?????????1????11???0101001000??101?0???????

Gobius_francofurtanus_F

00100000001011000000011000??010100???????????00111????????????00011000000?0010

000111?001???????100???101??????1001????11???1101101000??10111???????

Gobius_jarosi_F

0100?0000010010000000011000001?????0???????????????????????????00000100000100100

00001000????????100?????1??????1001????11??????????????????1?1???????

Lepidocottus_aries_F

?????00000001000000011000010010010???????????00100????????????0011000000010010

000100000??10????010???100??????1000????11???0001101000??01111???????

Paralates_bleicheri_F

0100?0000000001000000011001001 0???00000100001011000110000000100011000000010

001001100000????????010?????????????????????11??????????????????????????

Pirskenius_diatomaceus_F

?????0001000011000001100001001010 0???????????00110????????????0001000000010010

001000000????01??001110100??0???1000????11??????????????????101???????

Pirskenius_radoni_F

0001???????00010000001000001001010 0???????????00010????????????0001000000010001

010000000????01??001010100??????1000????11??????????????????????????

Kribia_nana_B

00011010000000110000001100010010010????????????????????????????1100000000010010

0100000000110010101 0?????001?100???00011??1010000001001??01??11100110

Oxyeleotris_marmorata_B

001010000010100000000100001101001000000100011001010110000010000010001000010

01000100000000110011001011010001?10010000011??10111011010000-01??11100110

Dormitator_maculatus_E

00011001000000100000100000110100100001000000100001000000001000000000100000010

0010110000000110010101 0?0110001?10010000011??101?????????????????11010101

Hypseleotris_compressa_E

000010000010000010000000000100100100000100000111000001000001000000000100000 10

0100010000000110010101 0?1010001?10010000101101010000000100??10??11010101

Tateurndina_ocellicauda_E

0000100000000000110010100001010010000100000100001111000011000000000000001110

001000000011011001010 10?????001?1001000001111101000000001000-10??110101??

Amblygobius_phalaena_G
0110100000101100000011000001010100001100000010010001000010000000000000010010
01000000001010010110100?1001111?11000110010??11001010001000-10??11100110
Aphia_minuta_G
0100100000001000000000100001010100???????????????????????????0000011111001100
00001110010010110100?1111111?110101100100-11000000000101001??11100110
Asterropteryx_semipunctata_G
01001000001010000000001000001010100???????????????????????????0011000000010010
0011100001001011010001010111?11010010010??110??????????????1011100110
Austrolethops_wardi_5brG
110010000010110000000100000101010000001000001000010010000010000000000001100010
01000000110011100110100??????11?110???1???0??11000000001010-10---1100110
Cryptocentrus_cinctus_G
01001000001010000000001000001010100001100000100010000100110000000001000000010
01000010000010010110100?1010111?110100100100-11000000001100-10??11100110
Discordipinna_griessingeri_G
10001000000110000000001000001001100100000000100001001011000000000010000000010
100010000000010010110100????111?110???10010??11011011010000-10??11100110
Glossogobius_giuris_G
00101000000001000000000100001010100???????????00010????????????0010000000010010
001000000010010110100?1010111?11010010010??11000011010100-10??11100110
Gobius_niger_G
001010000010010000000001000010101000000001000100001001000001000000000011000010
1110000111001001011010011010111?1101001001011110010110100011101?11100110
Lesueurigobius_sanzi_G
??0010000001010000000000100001001100???????????????????????????000000000110010
00000000110010110100?1010111?110100100101111011011110001010??11100110
Ptereleotris_evides_G
100010000000100000000010000011001000000000101010010000100000011000000000000010
0100000000001001011010001010111?110100100101111011001001000-1001-1100110
Tigrigobius_multifasciatus_G
010010000000001000000000100001010100001000000010000110100011000000000100000000010
001000100000010010110100????111?110100100100-11001011001000-01??11100110
Milyeringa_veritas_M 011010000001100000011000001001010000000000000---
-------1000000001010000000011000000000000001100110010??????01?100100???10-
10111011000010-1001-1100110
Typhleotris_madagascariensis_M
????1?????????????????????10010?????????????????????????????????0010000000000100011
00000001010110010???????1?1?????????1??101???????????????1111100110

Odontobutis_obscurus_Od

011101000100000111111111001001001 0???????????????????????????1111000000010011

1111000000110101001 0??????01?100???0100111101010110000110101101101010

Perccottus_glenii_Od

0000010010000000110111000010010010000011000100010000100001000000000100000010

00110011000000110101001 0??????01?100???0100111101??1?1000011101??01101010

Terateleotris_aspro_6brG

0110000001000001000000001001001000100001000010000000100000000000011000000000 10

0001011000000011010??010010100??11?010000101111 01???????????????1100??????

Awaous_flavus_Ox

0100101000000100000000010000010101000000000110100011001000000001100000100000010

0100001000000100101101 00????111?110010100100-1100000000 1000-10??11100110

Chlamydogobius_eremius_Ox

1100100000000010000000011001001010000001000001000010100000001000010000000000010

010010000000100101101000????111?1100101001010110000001000 00-01??11100110

Eucyclogobius_newberryi_Ox

0111100000000000001100001110101011000001000000010000100000100000000010000010

0100000100001001011010001010111011010010 0100-110???????????????01-1100110

Gobioides_broussonnetii_Ox

0100100000011000000000100001001010110000000000000001000110000000000000001010

010000000000110010110100001001 11?11001010010??1100000000 0110-1001-1100110

Pomatoschistus_flavescens_Ox

0110100000000000100000000111010010000000001001000100010000000000000000100000010

0010001000001001011010 0?1010111?110100100101011000000110101010 1?11100110

Protogobius_attiti_R

0100000000000010000000001000101000110000000101000100001000000000100100000000010

00101000000001101010010?????00???00???????1???01???????????????1100??????

Rhyacichthys_aspro_R

0001000000000001000000010000101000010000000001100100001000000000001001000000000010

0010100000000110101001 01????00010001000100111001100010000 10-011000011010

Rhyacichthys_guilberti_R

00010??????000100000001000010100001000001000010010000100000001000010000000000100

0101000000001101010010?????000?000????1001??00101000000110-011?00011010

Grahamichthys_radiatus_T

100000000010100000000010000100101000000000010011000000100000000010000100000010

0100001000000010010101 0?101011??1111000001011100000000000101010??11010101

Thalasseleotris_iota_T

00010001000000010000001000001001001000100000000100100010000100000001000000000010

0100000100000001001010 10????11??111???000101010001010001001010111 1010101

Acknowledgements

I am deeply indebted to Professor Dr. Bettina Reichenbacher for her great support and her patient supervision. I am grateful for fruitful discussions with Dr. Ulrich Schliewen, Dirk Neumann (SNSB-ZSM, Munich), Dr. Gloria Arratia (University of Kansas, Lawrence, USA), PD Dr. Dirk Erpenbeck and Dr. Martin Dohrmann (LMU). I thank Professor Dr. Gert Wörheide, director of the Bavarian State Collection for Palaeontology and Geology, for his support. I want to thank René Neumaier, Domink Bastl, Elisabeth Lange, Dr. Nora Dotzler and Manuela Schellenberger for technical support. And I also want to thank Dr. Melanie Altner, Stefanie Penk, Carolin Gut, Charalampos Kevrekidis, Dr. Martina Pippèrr, Dr. Zeinab Gholami, Dr. Azad Teimori, Dr. Tanja Schulz-Mirbach, Dr. Nicola Heckeberg, Prof. Dr. Michael Krings, Prof. Dr. Oliver Rauhut, Dr. Adriana Lopez-Arbarello (all LMU) and Professer Dr. Alexander Nützel and PD Dr. Gertrud Rößner for their kind support (both SNSB-BSPG). I acknowledge the German Research Foundation (so basically the tax payer) for funding (grant number RE 1113/20-1). Special thanks go to my friends and first and foremost to my family. Without them none of this would have been possible.