

## Rapid Communication

## Most northerly record of the bighead goby *Ponticola kessleri* (Günther, 1861) in the Elbe River, Germany

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### Abstract

We report the most northerly occurrence of the bighead goby *Ponticola kessleri* (Günther, 1861) in the Elbe River in Germany, where the fish was first observed in August 2015. Previously, the northernmost record of this Ponto-Caspian gobiid species originated from the lower Rhine River. Twelve specimens of *P. kessleri* were recorded in the Elbe River between Hamburg and Lauenburg between the 7<sup>th</sup> of August 2015 and the 13<sup>th</sup> of June 2016. Detailed morphometric and meristic data for nine specimens are provided. We further provide DNA barcode sequences for two of the specimens and compare these to published records. The newly sequenced specimens and previously published sequences from German and Austrian locations showed no genetic variation for the COI gene.

**Key words:** Gobiidae, Ponto-Caspian goby species, range expansion, morphology, DNA barcoding

### Introduction

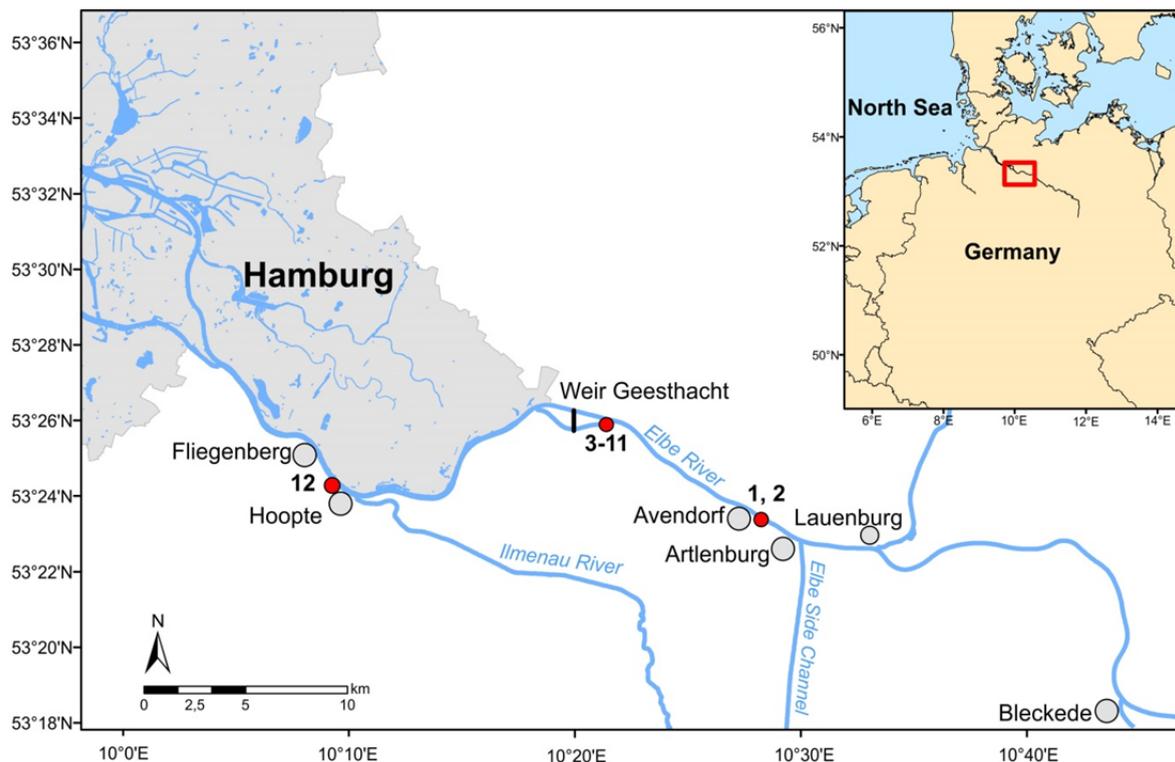
The natural distribution of the bighead goby *Ponticola kessleri* (Günther, 1861) is the Black Sea basin, especially the brackish zone of the northern and western shores of the Black Sea as well as the Danube, Dniester, South Bug and Dnieper drainages (Svetovidov 1964; Kottelat and Freyhof 2007). The species does not occur in the Azov Sea basin.

*Ponticola kessleri* reaches a maximum total length of 200 mm (Miller 1986) and spawns for the first time at 2 years of age. The spawning period ranges from March until May (Kottelat and Freyhof 2007). *Ponticola kessleri* has a wide diet range including different mollusks, insect larvae, crustaceans (e.g. mysids, corophiids, amphipods) and small fish (Miller 2004). The species prefers habitats with well vegetated bottom and different types of hard stony substrates, for instance pebbles, but also man-made stone packings at groynes and river banks (e.g. Kottelat and Freyhof 2007; Miller 2004; van Kessel

et al. 2016). According to Ahnelt et al. (1998) and Jurajda et al. (2005) the western most native distribution limit of *P. kessleri* in the Danube River corresponded to the mouths of the Rivers Velika Morava and Nera in Serbia. At the beginning of the 1990s a first range expansion of *P. kessleri* was reported into the middle Danube (e.g. Roche et al. 2013). About one decade later the species was found in the Rhine system (Borcherding et al. 2011; van Kessel et al. 2009; Kalchhauser et al. 2013). This study reports the first records of *P. kessleri* in the Elbe River, representing the most northerly expansion of this species.

### Material and methods

On August 7<sup>th</sup> 2015, the first two specimens of *P. kessleri* (Supplementary material Table S1, records No. 1 and 2) were caught with a trap in the Elbe River, Germany at 53.38306N and 10.47111E (Figure 1) by a commercial fisherman (Eckhard Panz, Hohnstorf). These individuals were first passed alive



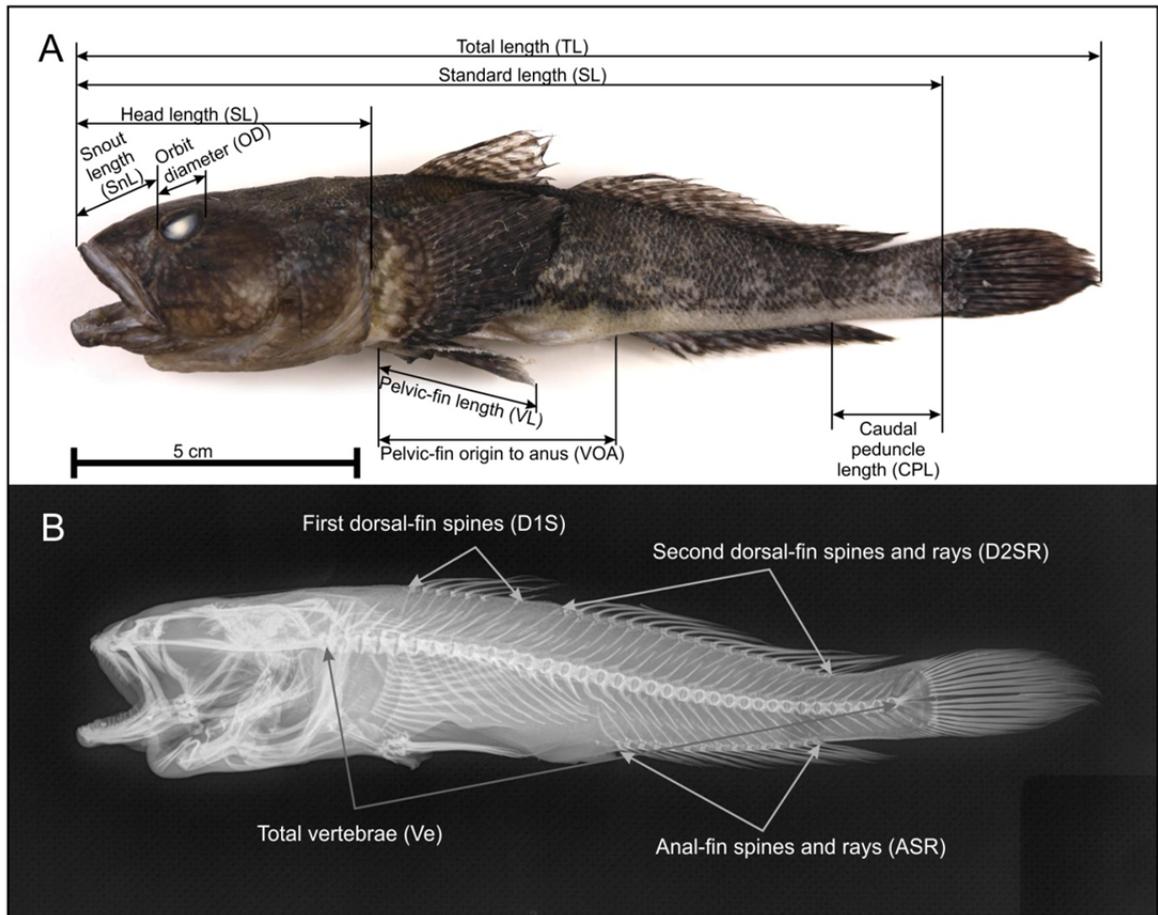
**Figure 1.** Map with the new records of *Ponticola kessleri* in the Elbe River. Red spots with numbers indicate the locations of the recorded specimens (for details see Supplementary material Table S1).

to the public aquarium of the Biosphaerium Elbtaulae in the city of Bleckede by the Elbe River. While one of the two individuals (Table S1, record No. 2) died during the acclimatization process after 40 days in September 2015, the other specimen (Table S1, record No. 1) survived in the public aquarium until the 29<sup>th</sup> of May 2016. In June 2016 this individual was transferred deep frozen to the fish collection of the Zoological Museum Hamburg (ZMH) and was preserved in 70% ethanol after defrosting. The specimen was deposited in the ZMH fish collection under catalogue number ZMH 26193. A third individual of *P. kessleri*, caught in the tidal Elbe River at 53.39917N and 10.16222E (Figure 1) in August 2015 (Table S1, record No. 12), was reported by fisherman Per-Willem Grube via photographs immediately taken after its catch.

In May and June 2016 nine additional specimens of *P. kessleri* were caught with a trap in the Elbe River at 53.42750N and 10.36083E (Figure 1) by fisherman E. Panz. One of these individuals died after its catch (Table S1, record No. 6) and was preserved in 70% ethanol. After detailed morphological analysis the individual was stored in the ZMH

fish collection under catalogue number ZMH 26198. Another specimen (Table S1, record No. 10) was transferred to the aquarium of the Biosphaerium Elbtaulae in Bleckede for public exhibition. After morphological analysis, the remaining seven individuals (Table S1, records No. 3–5, 7–9, 11) were released into the Elbe River at the place of capture.

Morphometric measurements of all specimens were performed on the left body side following Hubbs and Lagler (1958). Meristic characters were counted and fish were identified according to Miller (1986) and Kottelat and Freyhof (2007). Vertebrae, and the spines and rays of dorsal and anal fins of preserved specimens (Table S2, records No. 1 and 6) were additionally counted from radiographs (Figure 2) using an X-ray imaging system (Faxitron LX-60). Sexes were identified following Kornis et al. (2012), based on the shape and length of the urogenital papilla. The estimated morphometric measurements and meristic counts are presented in Table S2. Furthermore, the following seven general morphological characters were estimated for specimens 1 and 3–11: (1) predorsal area and nape completely covered by ctenoid scales, (2) yellow pelvic fins, (3) first dorsal-



**Figure 2.** *Ponticola kessleri*, ZMH 26193, 187 mm TL, captured from the River Elbe, Germany, at Elbekilometer (Ekm) 575 on August 7<sup>th</sup> 2015. A) picture and B) X-ray taken after preservation in ethanol; morphometric measurements taken along the body are indicated in A); meristic characters of preserved specimens taken from X-rays are indicated in B).

fin without black spot on posterior part, (4) first branched ray of second dorsal about as long as penultimate ray, (5) snout length 1.5–2.0 times orbit diameter, (6) pelvic disc reaching 75–95% of distance between its origin and anus, and (7) pelvic-disc fraenum with angular lobes whose length is 1/6–1/2 of fraenum width at base. A reduced set of four general morphological characters was estimated for the specimens 2 (characters 1, 3, 4 and 5) and 12 (characters 2, 5, 6 and 7).

Prior to their preservation in 70% ethanol, tissue samples were taken from ZMH 26193 and ZMH 26198 and preserved in 96% ethanol for molecular analysis. For molecular identification we amplified the barcoding fragment of the mitochondrial Cytochrome Oxidase I (COI) gene. Whole genomic DNA was extracted from muscle tissue using a standard Chelex protocol (Walsh et al. 1991). PCR was performed with DreamTaq DNA Polymerase

(Thermo Fisher, Scientific, Bremen, Germany) in a total volume of 20  $\mu$ l using a standard PCR protocol with the primers HCO and LCO (Folmer et al. 1994) and an annealing temperature of 50 °C. PCR products were checked on a 1.5% agarose gel stained with GelRed (Biotium, Fremont, CA, USA) and purified using a mix of Exo I and SAP (Werle et al. 1994). The purified products were sequenced in both directions at the core facility of the Ludwig Maximilian University (LMU, Munich, Germany).

Forward and reverse sequences were assembled using Geneious v.6.1.8 (Kearse et al. 2012). The newly generated sequences were submitted to NCBI GenBank (Accession numbers KX857485, KX857486). We compared the sequences to database records in NCBI GenBank using Blast and to the Barcode of Life Database (BOLD). We then downloaded all records for the species from previous studies (Keskin and Atar 2013; Kneblsberger et al. 2014)

from BOLD and GenBank (Table S3) and aligned all sequences using Mafft (Kato and Standley 2013) as implemented in Geneious. The alignment served as input for PopArt (<http://popart.otago.ac.nz>) to construct a haplotype network using the TCS algorithm (Clement et al. 2002).

## Results and discussion

### *Brief description and identification of the recorded specimens*

The ten specimens of *P. kessleri* for which sex could be analysed were represented by three males and seven females with total weights between 20.7–73.3 g, total lengths between 125–187 mm, and standard lengths between 110–158 mm (Table S1).

All reported specimens matched the estimated general morphological characters of the species. The specimens analysed in detail also matched with the detailed morphometric measurements and meristic counts presented by Miller (1986) and Kottelat and Freyhof (2007). In addition to the seven morphological characters which are characteristic for the species mentioned above, we also estimated caudal peduncle depth of 0.6–0.8 caudal peduncle length; D1S V–VII; D2SR I,16–19; ASR I,14–16; PR 19–20; VSR I,5; CF 15; LL 69–71; Ve 34–35. The specimen specific results of the morphometric measurements and meristic counts are given in Table S2.

Sequencing resulted in a sequence length of 682 bp for ZMH 26193 and 636 bp for ZMH 26198. No polymorphisms were detected between the two sequences. Database searches resulted in 100% matches (no polymorphisms) in both databases for *Ponticola kessleri* specimens collected from other drainage systems in Germany and Austria (Table S3), therefore confirming the morphological identification.

### *Distribution and establishment status*

During the 1990s an upstream range expansion of *P. kessleri* was observed in the Danube (Roche et al. 2013). In 1999 the species was first recorded in the Danube in Germany (Seifert and Hartmann 2000). In 2005 *P. kessleri* was found in the upper reaches of the Rhine drainage close to a canal connecting it with the Danube (Kottelat and Freyhof 2007). In 2006 the species was observed in lower reaches of the Rhine in Germany (Borcherding et al. 2011), in 2007 in the Dutch Rhine delta (van Kessel et al. 2009), and in 2011 in the upper reaches of the Rhine in Switzerland (Kalchhauser et al. 2013). These records demonstrate that *P. kessleri* is currently spreading across European rivers as well as within the German

waterway network (Kalchhauser et al. 2013, Brandner 2014). Therefore, new occurrences of this species in other rivers or channel systems may be expected after a relatively short time. Hence, the new record of *P. kessleri* in the Elbe River is not so surprising, especially since *Neogobius melanostomus*, another Ponto-Caspian goby species, has spread in the Elbe River since 2008 (Hempel and Thiel 2013). Brandner (2014) distinguished a northern, central and southern migration corridor of Ponto-Caspian species in Europe. While the route of *P. kessleri* seems to follow the southern corridor (Danube–Main–Rhine), extending it to the Elbe River, we cannot dismiss other putative sources, including direct transport from the native area. Therefore, the route of the invasion of *P. kessleri* into the Elbe River is somewhat unclear.

Generally, Ponto-Caspian goby invasions have mainly been attributed to transport via ballast water of ships (Roche et al. 2013), although the importance of this human mediated transport vs. natural dispersal processes is still under discussion (e.g. Harka and Biró 2007, Kornis et al. 2012). Recently Adrian-Kalchhauser et al. (2016) analysed samples of *P. kessleri* along the river Rhine using molecular methods (microsatellites, mitochondrial D-loop sequences) and morphological methods (geometric morphometrics). The data showed weak temporal and strong geographic patterns. To explain the results of their molecular and morphological studies, Adrian-Kalchhauser et al. (2016) analysed the vector properties and travel patterns of commercial vessels on the Rhine. They concluded that freshwater cargo ships and tankers are plausible vectors for larvae of invasive goby species. The new records of *P. kessleri* in the Elbe River could likely also be explained by its transport via ballast water of ships. This invasion mechanism could explain why this species was found first in the Elbe River between Avendorf and Artlenburg at Elbekilometer (Ekm) 575 (Table S1), in the vicinity of the connection of the Elbe Side Channel with the Elbe River at Ekm 573 (Figure 1). The Rhine, where *P. kessleri* has already established about 10 years ago, is connected with the Elbe River via the Elbe Side Channel and the Mittelland Canal. Natural dispersal through these canals can also not be completely excluded as an explanation. An indication for this possibility is that *N. melanostomus*, found in the river Rhine (Borcherding et al. 2011) and recently in the Elbe River (Hempel and Thiel 2013), was also recorded in the Mittelland Canal (Matteikat et al. 2016) as well as in the Elbe Side Channel (Emmrich 2014). However, *P. kessleri* has not been recorded from these canals yet.

The genetic data provide little information on the geographic origin of the invaders. Our final alignment included 32 sequences (including our two sequences, Table S3) with a post-trim length of 647 bp. Not all sequences were of the same length and the alignment was constructed to maximize overlap across sequences and maintain the maximum number of polymorphic sites. If the alignment was trimmed to complete overlap it would contain 603 bp and only two haplotypes separating six Turkish haplotypes from all other samples. In our longer alignment (647 bp), due to shorter sequences, two specimens from Austria (Thalinger et al. 2016) could not be assigned to a specific haplotype with certainty. We detected a total of 3 variable sites (position 4 A/C, position 75 A/G, position 634 C/T). Accordingly, the haplotype network resulted in three closely related haplotypes differentiated by a single or two mutations. The central most common haplotype A (4-C, 75-G 634-C), as well as the more distantly related haplotype B (4-A, 75-A, 634-C, 2 mutations apart from central haplotype) only occurred in Turkey, whereas the third haplotype C (1 mutation apart from central haplotype, 4-C, 75-G, 634-T), to which our samples also belonged, was sampled in Germany and Austria. This network configuration, mainly because of large geographic sampling gaps, does not allow for an assignment of an origin. However, the low diversity and close relationships between haplotypes represent signatures of a rapid range expansion. To infer the origin of the invaders, more complete sampling and more fine scale genetic markers will be necessary.

The present establishment status of *P. kessleri* in the Elbe River cannot clearly be assessed yet. However, there are the following indications that the species is already established in the Elbe River: (i) the species was recorded at three different locations along a river section of 25 km between the cities of Hamburg and Lauenburg (Figure 1), (ii) records at close locations upstream Geesthacht Weir at Ekm 584, were obtained on three consecutive dates between May and June 2016 (Table S1), and (iii) two of the three males caught showed a distinct black body color, which is a typical sign that these specimens were caught during their spawning period. Additionally, the values of available physicochemical parameters in 2015 (Table S4), which were calculated based on daily average values for 2015 according to FGG Elbe (2015), indicate that suitable habitat conditions for *P. kessleri* exist in the section of the Elbe River where this species was recorded. The range of water temperature (1.9–26.5 °C) corresponds with the range of water temperature in the lower Rhine (1.8–26.2 °C; Lelek and Buhse 1992), where *P. kessleri* has occurred for at least 10 years

(Borcherding et al. 2011). Furthermore, the mean values of dissolved oxygen (11.04 and 11.98 mgL<sup>-1</sup>) and pH (8.30 and 8.34) in the Elbe River in 2015 correspond with the mean values of the same parameters presented by Brandner (2014) for the Danube River sections where *P. kessleri* occurs. The range of conductivity (61.6–168.0 mSm<sup>-1</sup>) in the relevant Elbe River section is also well tolerable for *P. kessleri*, which lives mostly in freshwater and brackish water with very low salinity (Kottelat and Freyhof 2007). Since similar ranges and mean values of the above discussed physicochemical parameters exist in a number of European rivers (e.g. Tockner et al. 2009), where *P. kessleri* has not yet been recorded, a further spread of *P. kessleri* in such European waters is expected.

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## Supplementary material

The following supplementary material is available for this article:

**Table S1.** Sampling data for the twelve new records of *Ponticola kessleri* in the Elbe River.

**Table S2.** Morphological characters for the collected individuals of *Ponticola kessleri* in the Elbe River.

**Table S3.** Sampling information for the molecular data used in this study.

**Table S4.** Means (Mean), minimum (Min) and maximum (Max) values of selected physicochemical water parameters at measuring stations Schnackenburg (Ekm 474.5) and Bunthaus (Ekm 609.8) in 2015.

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