Occurrence of the European weatherfish *Misgurnus fossilis* (Linnaeus, 1758) in the Danube floodplains of the Lobau in Vienna, Austria

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The European weatherfish (*Misgurnus fossilis*) is a rare and protected species known to occur in the Danube floodplains of the Lobau in Vienna, Austria. A single specimen was recently caught andsubsequently used for sequencing of a part of the mitochondrial cytochrome c oxidase subunit 1 gene (CO1). Thus, we established the first DNA barcode of this species from a Viennese water body, constituting an important addition to the *Austrian Barcode of Life* project – ABOL. Moreover, for the first time, DNA evidence of this species was obtained from a water sample via environmental DNA barcoding from the Lobau.

Kirchner S, Schindelar J, Sittenthaler M, Christ M, Zangl L, Sattmann H, Fischer I, Schubert H, Haring E (2023) Ein Vorkommen des Schlammpeitzgers *Misgurnus fossilis* (L.) in der Donauaue der Lobau in Wien, Österreich. Ein jüngster Fund des seltenen Europäischen Schlammpeitzgers *Misgurnus fossilis* aus dem Schwarzen Loch in der Wiener Lobau erlaubte die Sequenzierung eines Abschnitts des mitochondriellen Cytochrom-c-Oxidase-Untereinheit-1-Gens (CO1). Somit konnte der erste DNA-Barcode dieser seltenen Art aus einem Wiener Gewässer erstellt werden, eine wichtige Bereicherung für das Projekt *Austrian Barcode of Life* – ABOL. Erstmals gelang auch der DNA-Nachweis dieser Art aus einer Gewässerprobe aus der Lobau.

Keywords: DNA barcoding, eDNA detection, environmental DNA, rare species.

Introduction

Misgurnus fossilis (Linnaeus, 1758), the European weatherfish, is a stationary, crepuscular and nocturnal bottom-dwelling fish of slow-flowing or stagnant waters with muddy bottoms and lush vegetation. *Misgurnus fossilis* has an elongated, eel-like body, thick, slimy skin, and grows to a length of 30 cm. Its lateral line is only rudimentary, and it is unmistakable with its characteristic longitudinally striped yellow-brown colour, the orange-coloured belly and a sub-terminal mouth with 10 barbels (Fig. 1). The nocturnal fish feeds on insect larvae, small snails and mussels, and during the day it is usually buried in the bottom mud. In winter or during dry periods, it is usually dormant, due to its ability to breathe through the intestinal mucosa and the skin. This stage may even take up to one year when conditions are bad. The distribution of *M. fossilis* extends over central and western Europe (NW France to east of the Volga basin, also in Great Britain, Scandinavia, Italy, southern France and Greece) (Kottelat & Freyhof 2007; Hauer 2020).

In Austria, *M. fossilis* has been detected in Vorarlberg, Styria, Burgenland, Upper and Lower Austria and Vienna. It is mainly found in floodplain waters along the Danube and its large tributaries (Hein et al. 2016). In the east of the country, the focus of distribution is the oxbow system of the Danube floodplains that accompanies the river, especially in the area of the Tullner Feld and east of Vienna. In the "Lower Danube" study area, European weatherfish occur in waterbodies of the Danube wetlands with no or very little connec-

tion to the Danube River (Spindler 1997; Zauner et al. 2006). For Vienna, the evidence (all from Lobau) is quite sporadic and recorded in the literature since the 1980s, the last

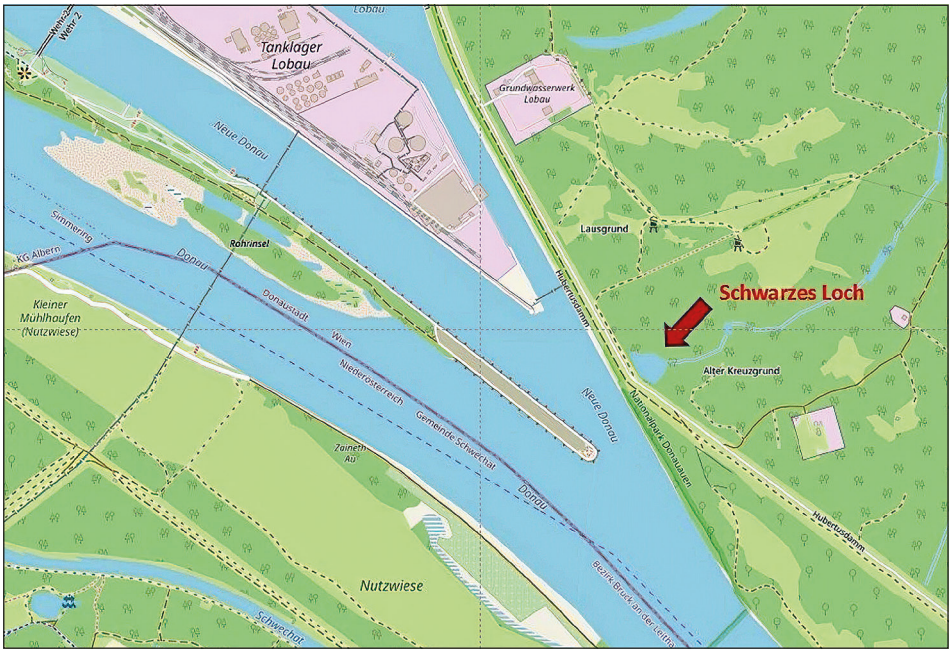
Fig. 1: *Misgurnus fossilis*. Photo Norbert Sendor. – Abb. 1: *Misgurnus fossilis*. Foto Norbert Sendor.

report from 2012 at the Lausgrund (Fusko 1987, 1990; Schabuss & Baranyi 2006; Schabuss & Zornig 2013a, b).

While related species of the genus *Misgurnus* (originally) all have purely Asiatic distributions (e.g., *Misgurnus bipartitus* Sauvage & Dabry de Thiersant, 1874 and *Misgurnus anguillicaudatus* (Cantor, 1842) in China), *M. fossilis* is the only representative of the genus which has its core distribution area spread across Europe and Western Asia (Kottelat & Freyhof 2007; Zangl et al. 2020; Fricke 2022). Because of its hidden lifestyle, data on distribution and frequency are patchy.

Misgurnus fossilis is listed in the Appendix III of the Bern Convention and in Annex II of the European Habitat Directive (Council of the European Union 1992). According to the Vienna Conservation Regulation, *M. fossilis* is listed as a species of high priority. This status would demand compulsory habitat protection measures according to the § 15, Vienna Nature Conservation Act. In addition to habitat loss, the introduction of related species poses a potential risk due to possible hybridization between native and alien species. At least three species have been introduced (probably as escapees via the ornamental fish trade; Belle et al. 2017; Zangl et al. 2020) into Europe so far: the Asian representatives *M. bipartitus* and *M. anguillicaudatus* mentioned above, and *Paramisgurnus dabryanus* Dabry de Thiersant, 1872 (which also originated in China). For Austria, *M. bipartitus* (Zangl et al. 2020) and most likely *P. dabryanus* (Jung et al. 2021) have been recorded in natural water bodies. During the ABOL project, which also aimed to provide a genetic reference

Fig. 2: A. Schwarzes Loch, Lobau, 1220 Vienna, Austria. Photo: NHMW. B. Location of Schwarzes Loch in the Lower Lobau. © OpenStreetMap. – Abb. 2: A. Schwarzes Loch, Lobau, Wien, Österreich. Foto: NHMW. B. Position des Schwarzen Loches in der Unteren Lobau. © OpenStreetMap.



database for all Austrian fish species, further CO1 barcode sequences of the native *M. fossilis* were generated and made publicly available for future identification and monitoring purposes (Zangl et al. 2022).

Fig. 3: *Misgurnus fossilis* (NHMW 100424), individual analysed. – Abb. 3: Untersuchtes Exemplar von *Misgurnus fossilis* (NHMW 100424).

Here we report the most recent finding of a *M. fossilis* individual in Vienna as well as the first detection of the species via environmental DNA (eDNA) from a water sample from "Schwarzes Loch", a small pond within the Lobau, Vienna, Austria (Fig. 2).

Material and Methods

The investigation was carried out in the Viennese part of the Lobau (Schwarzes Loch, 1220 Vienna, Austria; coordinates 48.156606, 16.530642). The DNA barcode sequence of the mitochondrial cytochrome c oxidase subunit 1 gene (CO1) was sequenced from a juvenile *M. fossilis* individual collected on 12.3.2021 (Fig. 3), a specimen unintentionally caught with a collected sample of macrophytes. At the same location, water samples had been collected for a different study (dealing with the detection of dragonfly larvae by environmental DNA analysis). Two of those samples have been used to test for the presence of *M. fossilis* DNA.

DNA extraction

For DNA extraction, a finclip was taken from the fish which was subsequently included in the Fish collection of the Natural History Museum Vienna (NHM). Collection number: NHMW 100424. DNA extraction was performed in the clean room of the NHM DNA laboratory following standard routines to avoid/detect contaminations. For DNA extraction of the finclip sample, the DNeasy Blood & Tissue kit (QIAGEN N.V., Venlo, Netherlands) was used according to the manufacturer's protocol. In the last step, the DNA was eluted with 30 µl AE buffer. Remaining DNA is stored in the DNA and Tissue Collection of the NHMW. All post-PCR work was performed in a separate laboratory. DNA extractions included control extractions without samples to screen for contaminated reagents. Likewise, all PCRs included negative control reactions without template DNA.

For eDNA analysis, two water samples (300 and 500 ml) were taken using sterile, DNA-free plastic syringes and manually pressed through Sterivex-HV filter units with pore size 0.45 µm (Merck Millipore, Germany). The filters were transported cooled to the lab where they were stored at minus 80° C until DNA extraction. For extraction of the DNA bound on the filters, the DNeasy PowerWater Sterivex Kit (Qiagen, Hilden, Germany) was used. The extraction was performed according to the manufacturer's protocol except omitting the incubation of the Sterivex filter at 90° C (Step 7 of the protocol). In the last step, the DNA was eluted with 50 µl of EB solution.

Polymerase chain reaction and DNA sequencing

PCR was performed with the Multiplex PCR Kit (Qiagen, Germany) in a volume of 25 µl, containing 12.5 µl Multiplex PCR Master Mix, 0.5 µM of each primer and 1 µl of template DNA. The DNA extract of the finclip sample was used to amplify a 707-bp fragment of the *CO1* gene using the primers FishF1-50 (5´- TCAACCAACCACAAAGACATTGGCAC -3´) / FishR1-50 (5´-TAGACTTCTGGGTGGCCAAAGAATCA-3´) (Ward et al. 2010). The PCR protocol consisted of the following steps: initial denaturation 95° C (15 min); 35 cycles of 94° C (30 s) denaturation, 54° C (90 s) annealing, 72° C (90 s) polymerisation; final extension at 72° C (7 min). PCR with the eDNA samples was performed using the primers Mf-COI-F (5´- CCCCCGACATAGCATTTCCG -3´) / Mf-COI-R (5´-AACTGTTCAGCCTGTCCCAG -3´) which amplify a PCR product of 119 bp (Brys et al. 2021). PCR reactions were done in a step down PCR protocol comprising the following steps: initial denaturation 95° C (15 min); 5 cycles: 94° C (30 s) / 60° C (90 s) / 72° C (60 s); 40 cycles: 94° C (30 s) / 58° C (90 s) / 72° C (60 s); final extension at 72° C (10 min).

As for eDNA samples, PCR resulted in multiple PCR products. PCR products in relevant size were cut and extracted from agarose gels and purified with the QIAquick Gel Extraction Kit (Qiagen, Germany). All PCR products were sent for bidirectional Sanger sequencing to Microsynth Austria (Vienna, Austria) employing the amplification primers used in the PCRs. Sequences were edited with BioEdit 7.0.5.3 (Hall 1999) and blasted against the GenBank database (https://blast.ncbi.nlm.nih.gov) as well as BOLD (https://boldsystems.org). The barcode reference sequence generated in the course of the present study was deposited in BOLD (BCAFL791-22).

Results and Discussion

The 655-bp sequence obtained from the *M. fossilis* individual NHMW 100424 is the first DNA barcode of this species from a Viennese water body, which is a valuable addition to the Austrian Barcode of Life project (ABOL). The DNA barcode sequence was blasted against GenBank and delivered hits between 99.7-100 % similarity for *M. fossilis*, thus confirming the morphological species identification. For comparison, similarity scores with *M. anguillicaudatus* were only 88.89 %. Comparisons with the BOLD database delivered congruent results: 99.5-100 % similarity with *M. fossilis* as compared to only 88.0 % for the next closest related species *Misgurnus nikolskyi* Vasil'eva, 2001.

Moreover, DNA evidence of this species from a water sample via environmental DNA barcoding from the Lobau was obtained. The 119-bp *CO1* fragment was obtained from

both eDNA samples. For both samples, the sequences confirmed the species identification (100 % identity with *M. fossilis*).

Due to its biology and hidden way of life, detection of *M. fossilis* based on conventional sampling and monitoring techniques can be difficult and often unsuccessful (Sigsgaard et al. 2015). Yet, in the recent past molecular genetic approaches i.e., detection from water and/or sediment samples have been proposed (Kusanke et al. 2020) and since then were refined to even allow for quantification (as tested in an aquarium experiment by Brys et al. (2021)). Environmental DNA approaches have since been applied successfully to monitor the presence or absence of *Misgurnus* weatherfishes (Sigsgaard et al. 2015; Hinlo et al. 2018; Brys et al. 2021). Sigsgaard et al. (2015) reported better recovery rates of the European weatherfish and lower overall costs by eDNA than by conventional fishing techniques. Moreover, Jo et al. (2020) successfully detected *M. anguillicaudatus* among other threatened native as well as alien invasive species by targeted qPCR and hence increased distributional resolution of those species.

The success of eDNA approaches provides confidence that this technique may be useful to further non-invasively explore the distribution of the European weatherfish in Austria (as is currently being done in the Morava river floodplains (https://unece.org/sites/default/files/2021-05/3.7_Austria%20Florian%20Ott_eDNA.pdf; https://www.abol.ac.at/project/schlammpeitzger-nachweis-mittels-edna/) and also to monitor the potential spread of alien (invasive) species. This holds especially true since certain well-known pre-existing hurdles and limitations of eDNA/metabarcoding approaches (Schenekar et al. 2020) have been overcome for *Misgurnus* weather loaches specifically (e.g., Brys et al. 2021) and for freshwater fishes in general (e.g., Pont et al. 2021; Thalinger et al. 2021a,b). Based on all these results, perspectives for future monitoring efforts in similar water bodies open up.

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