

## Rapid Communication

**Oriental or not: First record of an alien weatherfish (*Misgurnus*) species in Austria verified by molecular data**Lukas Zangl<sup>1,2,\*</sup>, Michael Jung<sup>3</sup>, Wolfgang Gessl<sup>1</sup>, Stephan Koblmüller<sup>1</sup> and Clemens Ratschan<sup>3</sup><sup>1</sup>University of Graz, Institute of Biology, Universitätsplatz 2, 8010 Graz, Austria<sup>2</sup>Universalmuseum Joanneum, Studienzentrum Naturkunde, Weinzöttlstraße 16, 8045 Graz, Austria<sup>3</sup>ezb-TB Zauner GmbH, Marktstraße 35, 4090 Engelhartzell, Austria

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**Abstract**

Weatherfishes of the genus *Misgurnus* are natively distributed across large parts of Eurasia. Since the end of the 20<sup>th</sup> century, two alien weatherfish species, the oriental weatherfish, *Misgurnus anguillicaudatus*, and the large-scaled loach, *Paramisgurnus dabryanus*, have been reported from Europe. Here, we provide a first record of alien *Misgurnus* for Austria (Inn river). Based on morphology and DNA barcoding in combination with sequences of the nuclear RAG1 gene we found that this alien Austrian weatherfish is neither *M. anguillicaudatus* nor *P. dabryanus*, but *Misgurnus bipartitus*, the northern weatherfish. Fish from further upstream the Inn in Germany, previously identified as *M. anguillicaudatus*, share their COI haplotype with the Austrian samples and other *M. bipartitus*, suggesting a misidentification of these German fishes and raising alarm that alien *Misgurnus* might be already present across large parts of the middle and lower Inn drainage.

**Key words:** DNA barcoding, invasive species, *COI*, *RAG1*, *Misgurnus anguillicaudatus*, *Misgurnus bipartitus*

**Introduction**

Throughout the world, there is an increasing number of alien species, sometimes with negative effects on indigenous taxa (Seebens et al. 2017). This is also true for weatherfishes of the family Cobitidae. Eschmeyer's catalogue of fishes lists seven species of *Misgurnus* and one species of *Paramisgurnus*, mostly known from East Asia (Table 1; Fricke et al. 2019).

*Misgurnus fossilis* (Linnaeus, 1758) is the only weatherfish native to Europe. It is a species of special national and European conservation concern (Council of the European Union 1992, listed in Annex II of the European Habitat Directive) and mainly threatened by the loss of suitable habitats (Belle et al. 2017). Negative impacts on European weatherfish populations may further arise by the spread of allochthonous weatherfish due to potential interspecific competition, but also by potential hybridization. Hybridization has at least been documented in natural populations of *P. dabryanus* and *M. anguillicaudatus* (Stoockle et al. 2019 and authors therein).

**Table 1.** List of species in the genera *Misgurnus* and *Paramisgurnus* and their distribution ranges.

Species	Distribution
<i>Misgurnus anguillicaudatus</i>	widely distributed in the middle and lower reaches of the Yangtze river Basin, East Asia
<i>M. mohoity</i>	Amur River Basin in northeast China, Mongolia and the Far East region of Russia
<i>M. bipartitus</i>	north of the Yellow River in China
<i>M. buphoensis</i>	Korea
<i>M. fossilis</i>	Europe and Eastern Asia
<i>M. multimaculatus</i>	Vietnam
<i>M. nikolskyi</i>	East Asia (Amur River drainage)
<i>M. tonkinensis</i>	Vietnam
<i>Paramisgurnus dabryanus</i>	China (Tijanjin)

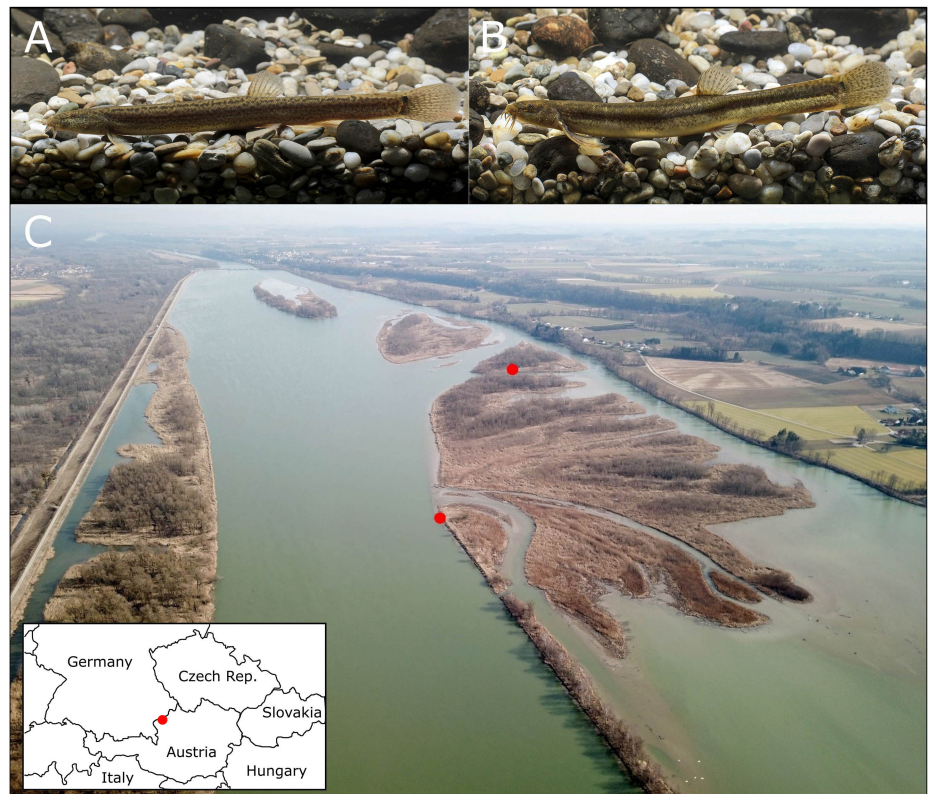
Hence, identifying potentially invasive alien weatherfishes and taking actions against their further spread might be crucial to the long-term survival of the indigenous species.

Initially farmed for the food industry in Asia (Belle et al. 2017; Yi et al. 2017b), the aquarium trade of ornamental pond fishes fueled the global spread and concordantly reports of alien weatherfish have been ever increasing (van Kessel et al. 2013). Today, populations of Asian weatherfishes are known from Australia, North America, South America and Asia outside of their natural range (Belle et al. 2017). From Europe, *Misgurnus anguillicaudatus*, the oriental weatherfish has been reported from Italy (Razzetti et al. 2001), Spain (Franch et al. 2008), Germany (Freyhof and Korte 2005; Belle et al. 2017) and the Netherlands (van Kessel et al. 2013). Furthermore, Marchesi (2010) and Stoeckle et al. (2019) have confirmed *Paramisgurnus dabryanus*, from Switzerland and Germany, respectively.

In this study we report the first record of an alien *Misgurnus* species in Austria. Based on morphology, DNA barcodes (part of the mitochondrial COI gene) and sequences of the nuclear RAG1 gene we aim to identify it to the species level and relate it to recent findings of alien weatherfish species in Germany. Furthermore, we discuss difficulties in the correct species identification of alien weatherfish species, even when DNA sequence data are available, as systematics and taxonomy, especially of the genera *Misgurnus* and *Paramisgurnus*, are still not fully resolved.

## Materials and methods

On October 18<sup>th</sup> 2018 two strange cobitid individuals (both with a total length of 125 mm, Figure 1A, B) were caught in the central area of the impoundment of the Egglfing-Obernberg power plant (48.301614°N; 13.282020°E, Upper Austria, Figure 1C) at the river Inn during an electrofishing survey. Specimens from the river Inn were determined morphologically following identification keys published by Kottelat and Freyhof (2007) and Vasil'eva (2001, and a short Chinese key therein), sampled (finclips put in 99% EtOH), fixed in formalin and deposited at the Biologiezentrum Linz (Museum IDs 2019/166-167). Extraction of genomic DNA employed a rapid



**Figure 1.** A + B) Pictures of the two *Misgurnus bipartitus* caught in the Inn impoundment lake close to the German border. C) Bird's eye view picture of the impoundment lake at the Egglfing-Obernberg power plant and a map of Central Europe showing the sampling site close to the German border.

Chelex protocol (Richlen and Barber 2005) and PCR and sequencing followed Koblmüller et al. (2011) and Duftner et al. (2005), respectively, using the primer cocktail C\_FishF1t1 and C\_FishR1t1 (Ivanova et al. 2007) for COI and RAG1-2533F (Lopez et al. 2004) and RAG1-3261 (Li and Ortí 2007) for RAG1. Sequences were visualized on a 3130xl capillary sequencer (Applied Biosystems). Sequences were edited and aligned by eye in MEGA 6.06 (Tamura et al. 2013). All newly generated sequences are available on GenBank under the accession numbers MT108218–MT108221. Additional COI sequences (the same set as compiled by Belle et al. 2017, as well as sequences of *M. mohoity*, the Amur weatherfish and *M. bipartitus* from Yi et al. 2016) and RAG1 sequences were downloaded from GenBank (Table 2). Minimum inter- as well as maximum intraspecific genetic distances were calculated using SPIDER (Brown et al. 2012). Maximum likelihood (ML) and Bayesian Inference (BI) analyses were performed for both COI and RAG1 data using PhyML 3.0 (Guindon et al. 2010) and MrBayes 3.2 (Ronquist and Huelsenbeck 2003), respectively, employing the best fitting models of evolution as suggested by the Smart Model Selection tool in PhyML (Lefort et al. 2017).

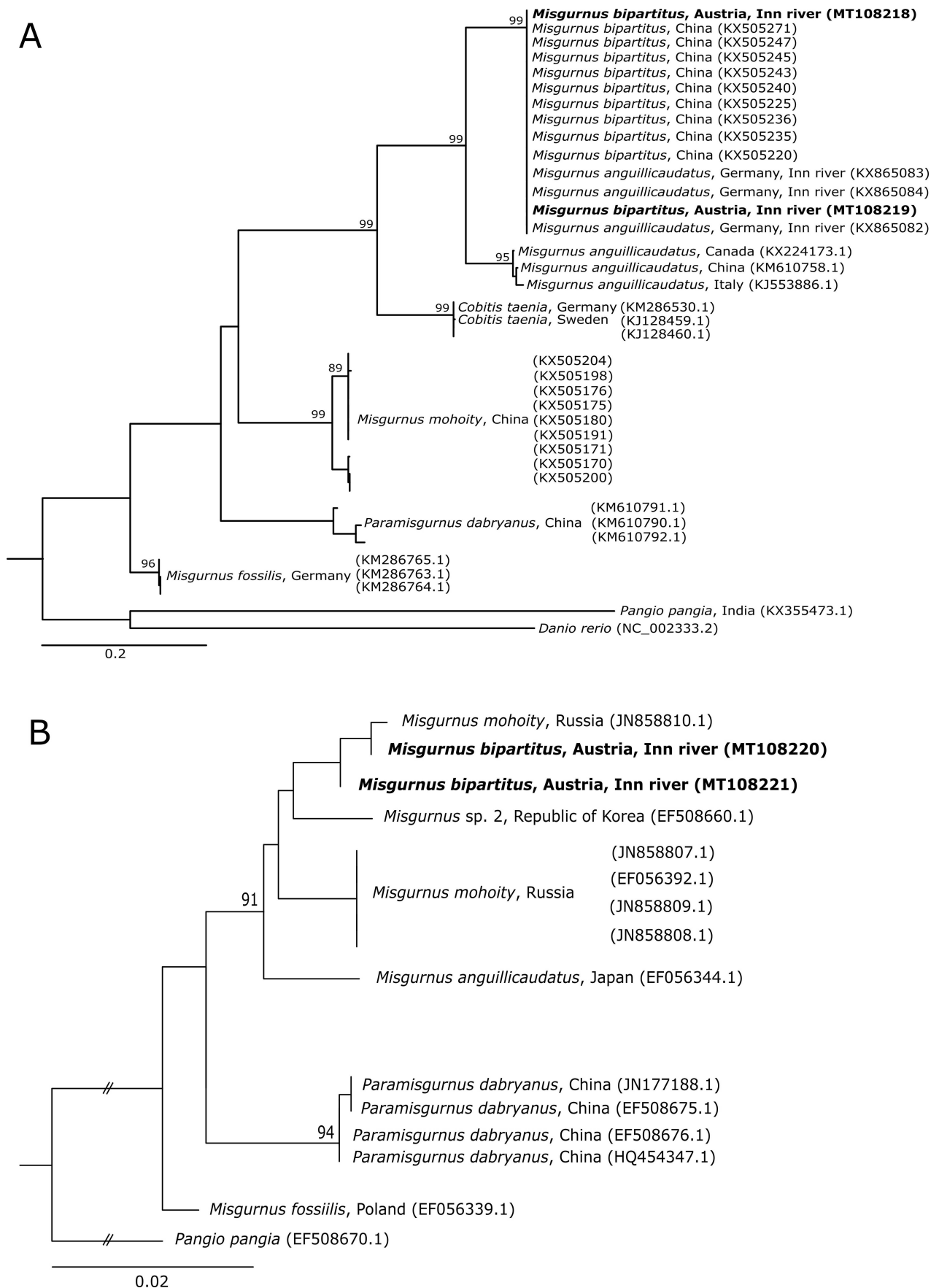
## Results

Based on characters given in the key by Vasil'eva (2001 and the translated Chinese key therein) (caudal peduncle depth 2.4–2.5 times in caudal peduncle

**Table 2.** Accession numbers of previously published sequences downloaded from GenBank and included in this study. \* denote *M. bipartitus* sequences labelled as *M. anguillicaudatus*.

Species	Sequence ID, Reference	
	COI	RAG1
<i>M. anguillicaudatus</i>	KX865083*, KX865084*, KX865082* (Belle et al. 2017); KX224173.1; KM610758.1 (Chen et al. 2015); KJ553886.1 (Geiger et al. 2014)	EF056344.1 (Šlechtová et al. 2007)
<i>M. bipartitus</i>	KX505271, KX505247, KX505245, KX505243, KX505240, KX505225, KX505236, KX505235, KX505220 (Yi et al. 2016)	–
<i>M. fossilis</i>	KM286765.1, KM286763.1, KM286764.1 (Knebelsberger et al. 2015)	EF056339.1 (Šlechtová et al. 2007)
<i>M. mohoity</i>	KX505204, KX505198, KX505176, KX505175, KX505180, KX505191, KX505171, KX505170, KX505200 (Yi et al. 2016)	JN858807.1, JN858809.1, JN858808.1, JN858810.1 (Perdices et al. 2012); EF056392.1 (Šlechtová et al. 2007)
<i>Misgurnus</i> sp. 2	–	EF508660.1 (Šlechtová et al. 2008)
<i>C. taenia</i>	KM286530.1 (Knebelsberger et al. 2015); KJ128459.1, KJ128460.1	–
<i>Danio rerio</i>	NC_002333.2 (Broughton et al. 2001)	–
<i>Pangio pangia</i>	KX355473.1	EF508670.1 (Šlechtová et al. 2008)
<i>P. dabryanus</i>	KM610791.1, KM610790.1, KM610792.1 (Chen et al. 2015)	JN177188.1 (Liu et al. 2012); EF508675.1, EF508676.1 (Šlechtová et al. 2008); HQ454347.1

length vs. 1.3–1.8 times in *M. anguillicaudatus*, maximum body depth 8.2–8.6 times in SL vs. < 7.5 times in *M. anguillicaudatus*) our specimens from the Inn were identified as *M. bipartitus*. Phylogenetic analysis of 612 bp of the COI gene (Figure 2A; Supplementary material Figure S1) also grouped the alien Austrian *Misgurnus* with *M. bipartitus*. Interestingly, the Austrian fish and *M. bipartitus* share their haplotype with German fish previously identified as *M. anguillicaudatus* (KX865082, KX865083, KX865084; Belle et al. 2017) collected 145 km further upstream in an oxbow of the Inn in Germany. Consistent with previous studies (Perdices et al. 2012; Yi et al. 2016), the genus *Misgurnus* resulted as paraphyletic. Phylogenetic relationships based on 658 bp of the nuclear RAG1 gene (Figure 2B, Figure S2) mirrored the results of the mitochondrial COI data, although node support was generally lower in the nuclear trees. In the RAG1 tree, the Austrian fishes grouped with a *Misgurnus* sp. 2 from Korea and a single *M. mohoity* from Russia. All other *M. mohoity* from their native range form a distinct clade, sister to that including the Austrian samples, albeit with limited bootstrap support. It is noteworthy though, that no RAG1 sequences of alleged *M. bipartitus* (see below) were available to be included in our analysis. The maximum intraspecific genetic distance, based on uncorrected p-distances of the COI gene, varied from 0% within *M. bipartitus* to 3.6% within *M. mohoity*. The minimum interspecific distance was 9% between *M. anguillicaudatus* and *M. bipartitus*.



**Figure 2.** Maximum Likelihood (ML) phylograms based on (A) 612 bp of the mitochondrial COI gene and (B) 658 bp of the nuclear RAG1 gene. Node labels indicate bootstrap support values (1000 bootstrap replicates; only values > 85 are shown). Samples in bold were obtained and sequenced in this study. Numbers in parentheses represent GenBank accession numbers.

## Discussion

In this study we present the first record of an alien *Misgurnus* species for Austria. Phylogenetic analysis of the COI gene clearly clustered the two specimens caught in the Inn impoundment with other previously published COI sequences of *M. bipartitus* (Yi et al. 2016, 2017a). This particular clade also contains three sequences of specimens likely misidentified as *M. anguillicaudatus* from further upstream the Inn in Germany (Belle et al. 2017), which share the exact same haplotype with the Austrian samples and all *M. bipartitus* included in our dataset. In theory, ancient hybridization could explain this finding, as hybridization is known to occur between *Misgurnus* species as well as between *Misgurnus* and *Paramisgurnus* (van Kessel et al. 2013). On the other hand, though, Belle et al. (2017) did not include any sequence of *M. mohoity* and *M. bipartitus*. Unfortunately, the systematic background of the genera *Misgurnus* and *Paramisgurnus* is still not fully resolved. Perdices et al. (2012), for example, suggested synonymy of *M. bipartitus* and *M. mohoity* based on DNA sequence data of the mitochondrial cytochrome *b* (cyt *b*) and the nuclear recombination activating gene 1 (RAG1) genes, a view concordant with Vasil'eva's (2001) morphological study. Yi et al. (2016, 2017a, b) and Li et al. (2010), on the other hand, granted species level to *M. bipartitus* as well, based on clear divergence in DNA sequence data of the mitochondrial cytochrome oxidase subunit 1 (COI) gene and morphology. Here, we follow Yi et al. (2016) and treat *M. bipartitus* as a valid and distinct species. Thus, based on our data we strongly argue that the three specimens found in the German stretches of the Inn river (Belle et al. 2017) and the two specimens found on the Austrian side do belong to *Misgurnus bipartitus*, the northern weatherfish and so might several other findings of alien loaches in Germany determined as *M. anguillicaudatus* down to 46 km upstream of the recent finding (*unpublished reports*) be as well. These findings are also corroborated by nuclear RAG1 data, which group the Austrian samples not with *M. anguillicaudatus*, but with a distinct lineage within *M. mohoity* (but note, no RAG1 data are available for morphologically identified *M. bipartitus*).

The close genetic relationship of German and Austrian alien *Misgurnus* from the Inn, however, further indicates a downstream range expansion of at least 145 km, which is also supported by several more recent catches of alien *Misgurnus* along the German stretch of the Inn downstream the site of first record by Belle et al. (2017). Thus, alien *Misgurnus* seem to use the main stem of the Alpine river Inn with low water temperatures and high turbidity at least as a dispersal corridor. Systematic uncertainties as well as scarce available ecological data on these species further complicate the prediction of the invasive potential, prime habitats and possible conflicts with native biota.

Despite the above-mentioned challenges, we agree with Belle et al. (2017) that DNA barcoding, and even more so eDNA metabarcoding or the application of diagnostic primers for detecting alien species in eDNA samples (e.g., Rees et al. 2014; Thalinger et al. 2019) helps to detect and identify non-native *Misgurnus/Paramisgurnus* species, which might be a threat to the autochthonous *Misgurnus* species (Franch et al. 2008). Confronted with a new alien species, facing potential interspecific competition and/or genetic dilution through hybridization (Stoeckle et al. 2019), protection and conservation of *M. fossilis* should be enforced. Furthermore, appropriate regulations on the trade of ornamental fish should be considered in order to reduce the risk of unintentional spread of alien species that could become invasive and threaten the native biodiversity (Franch et al. 2008).

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#### Supplementary material

The following supplementary material is available for this article:

**Figure S1.** Bayesian Inference (BI) phylogram of 612 bp COI sequences.

**Figure S2.** Bayesian Inference (BI) phylogram of 658 bp RAG1 sequences.

This material is available as part of online article from:

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