

Letter to BioInvasions Records

Taxonomic requirements for better documenting and understanding biological invasions – the example of genetic weatherfish *Misgurnus/Paramisgurnus* sp. identification

Christina C. Belle¹, Bernhard C. Stoeckle¹, Alexander F. Cerwenka², Ralph Kuehn^{3,4}, Joachim Pander¹ and Juergen P. Geist^{1,*}

¹Aquatic Systems Biology Unit, TUM School of Life Sciences, Technical University of Munich, D-85354 Freising, Bavaria, Germany

²Section Evertabrata varia, SNSB-Bavarian State Collection of Zoology, D-81247 Munich, Bavaria, Germany

³Unit of Molecular Zoology, TUM School of Life Sciences, D-85354 Freising, Bavaria, Germany

⁴Department of Fish, Wildlife and Conservation Ecology, New Mexico State University, Box 30003, MSC 4901, Las Cruces, NM 88003-8003, USA

Author e-mails: christina.belle@tum.de (CCB), bernhard.stoeckle@tum.de (BCS), cerwenka@snsb.de (AFC), kuehn@wzw.tum.de (RK), joachim.pander@tum.de (JP), geist@wzw.tum.de (JPG)

*Corresponding author

Citation: Belle CC, Stoeckle BC, Cerwenka AF, Kuehn R, Pander J, Geist JP (2021) Taxonomic requirements for better documenting and understanding biological invasions – the example of genetic weatherfish *Misgurnus/Paramisgurnus* sp. identification. *BioInvasions Records* 10(3): 506–520, <https://doi.org/10.3391/bir.2021.10.3.01>

Received: 12 September 2020

Accepted: 26 April 2021

Published: 23 July 2021

Handling editor: Cathryn Abbott

Thematic editor: Andrew David

Copyright: © Belle et al.

This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International - CC BY 4.0).

OPEN ACCESS

Abstract

Management of biological invasions strongly depends on early and accurate detection of non-native species, yet species identification is often complicated for various reasons. One prominent example relates to the controversy about the genetic specimen assignment of Asian and Oriental weatherfish species introduced into Europe. Weatherfishes, comprising the genera *Misgurnus* and *Paramisgurnus* (Cobitidae) are small benthic freshwater fishes with a wide range of habitats in the temperate to subtropical regions of Eurasia. Many of the eleven described species have been introduced outside their native ranges, mainly through ornamental trade and as food. Due to their poorly known life cycles, unclear morphology, overlapping meristic features and frequent hybridisation, the challenges associated with accurate species identification in this group comprise cryptic species and cryptic invasions, unresolved classical and molecular taxonomy, haplotype sharing and incomplete molecular genetic reference databases. Based on our newly generated molecular phylogeny comprising 289 published weatherfish COI barcodes, the existence of distinct phylogenetic clusters is evident. Except for the endangered Central European species, *Misgurnus fossilis*, and an unnamed cluster from Vietnam, all clusters were polyphyletic. Haplotype sharing was frequently observed, as well as specimens only labelled to genus or higher taxonomic levels. We conclude that genetic analysis of type specimens or type regions to resolve the underlying taxonomy and complete the reference databases would be necessary as prerequisite for accurate species identification in the weatherfish group. Such information is crucial in assessing their worldwide species distribution patterns, ecosystem impacts and invasive potential. As molecular genetic databases are constantly growing, new taxa are being proposed, and taxonomies are being changed in light of new data, it is obligatory to consider past publications in light of the dynamics of species names and taxonomic phylogenies. We still recommend early sharing of exotic species records since such knowledge is particularly crucial when it comes to management of invasive species.

Key words: Asian weatherfish, invasive species management, *Misgurnus fossilis*, mitochondrial DNA barcoding, molecular taxonomy, Oriental weatherfish

Introduction

Biological invasions are considered a main factor affecting biodiversity, with multiple societal and economic impacts (e.g., Keller et al. 2011). In addition to the necessity for unified frameworks to describe biological invasions (Robertson et al. 2020), management of biological invasions strongly depends on early and accurate recordings of invasive species. As illustrated by recent examples related to the controversy about Asian and Oriental weatherfish species introduced into Europe (Riffel et al. 1994; Razzetti et al. 2001; Freyhof and Korte 2005; Belle et al. 2017; Stoeckle et al. 2019; Zangl et al. 2020), there are many challenges associated with accurate species assignment in this group (Chen 1981; Vasil'eva 2001; Kottelat 2012). For instance, a recent rapid communication paper by Zangl et al. (2020) concludes that previous samples of weatherfish analysed in a paper from Belle et al. (2017) were misidentified using molecular taxonomy, potentially overlooking that several new mtDNA barcoding sequences that Zangl et al. (2020) were able to include in their paper were not available at the time of publication. Other challenges related to genetic weatherfish identification lie in (i) the accessibility of type specimens and their genetic reference sequences, (ii) classical taxonomy with many synonymous species names, (iii) hybridisation, as well as (iv) a not yet fully analysed suite of observed species and forms from different geographical regions and habitats. Many of these challenges are also relevant to other biological invasions. We thus use the example of weatherfishes to discuss current deficits and useful principles that should generally be considered when it comes to genetic species identification and documentation of invasive species.

Weatherfishes (Cobitidae) contain the two genera *Misgurnus* and *Paramisgurnus*. They are small benthic freshwater fishes with a wide range of habitats; still or slowly flowing rivers, lakes and ponds with muddy bottoms, and agricultural landscapes including rice fields and ditches (Meyer and Hinrich 2000; Kanou et al. 2007; Chen et al. 2015b). The native ranges of the eleven valid species cover the temperate to subtropical regions of Eurasia including Japan (see Table 1, Fricke et al. 2020). No native weatherfish species are known from the Americas, Africa and Australia. Depending on the regional context, weatherfishes are used as ornamental fish, food, and live bait and are frequently traded and sold in pet shops. Several weatherfish species have established globally outside their native distribution ranges. For instance, *M. anguillicaudatus* was recorded in Australia (Allen 1984), Europe (Razzetti et al. 2001; Franch et al. 2008; van Kessel et al. 2013), North America (Simon et al. 2006), South America (Abilhoa et al. 2013) and several Asian countries (e.g. Juliano 1989; Sal'nikov 1998). *Paramisgurnus dabryanus* was recorded in Europe (Riffel et al. 1994; Zięba et al. 2010; Freyhof 2013; Stoeckle et al. 2019), Japan (Mukai et al. 2011; Kanou et al. 2007), and the USA (Kirsch et al. 2018).

Table 1. List of valid taxonomic names and synonyms in the genera *Misgurnus* and *Paramisgurnus*, their native ranges, and the associated type localities (Kottelat 2012; Fricke et al. 2020). Species names in bold indicate that the species is currently recorded as introduced elsewhere outside its native range.

Species name	Synonyms	Native distributional range	Type locality
<i>Misgurnus anguillicaudatus</i> (Cantor, 1842)	<i>Cobitis anguillicaudata</i> Cantor, 1842 <i>Cobitis bifurcata</i> McClelland, 1843 <i>Cobitis cirrhifurcata</i> Dabry de Thiersant, 1872 <i>Cobitis decemcirrosus</i> Basilewsky, 1855 <i>Cobitis haematopterus</i> Richardson, 1846 <i>Cobitis maculata</i> Temminck & Schlegel, 1846 <i>Cobitis micropus</i> Cuvier & Valenciennes, 1846 <i>Cobitichthys dichachrous</i> Bleeker, 1860 <i>Cobitichthys enalios</i> Bleeker, 1860 <i>Misgurnus anguillicaudatus formosanus</i> Rendahl, 1936 <i>Misgurnus crossochilus</i> Sauvage, 1878 <i>Misgurnus elongatus</i> Kimura, 1934 <i>Misgurnus fossilis</i> ssp. <i>Anguillicaudatus</i> Cantor, 1842 <i>Misgurnus mizolepis fukien</i> Nichols, 1925 <i>Misgurnus mizolepis grangeri</i> Nichols, 1925 <i>Misgurnus mizolepis hainan</i> Nichols & Pope, 1927 (also: synonym of <i>M. mizolepis</i> Günther, 1888) <i>Misgurnus mizolepis heungchow</i> Lin, 1932 <i>Misgurnus mizolepis unicolor</i> Lin, 1932 <i>Misgurnus mohoity leopardus</i> Nichols, 1925 <i>Misgurnus mohoity yunnan</i> Nichols, 1925 <i>Misgurnus punctatus</i> Oshima, 1926 <i>Nemacheilus lividus</i> Sauvage & Dabry de Thiersant, 1874	East Asia	China, Chusan Island
<i>Misgurnus bipartitus</i> (Sauvage & Dabry de Thiersant, 1874)	<i>Nemacheilus bipartitus</i> Sauvage & Dabry de Thiersant, 1874, same time valid and synonym of <i>Misgurnus mohoity</i> (Dybowski, 1869)	Yellow River, China	North China (North of Yellow River) and Central China (Sichuan)
<i>Misgurnus buphoensis</i> Kim & Park, 1995	no listed synonyms (July 2020)	Korea	North Korea, North Hamgyong province, Sonbong county, Bupori
<i>Misgurnus fossilis</i> (Linnaeus, 1758)	<i>Cobitis fossilis</i> Linnaeus, 1758	Europe and western Asia	“in Europa”
<i>Misgurnus mohoity</i> (Dybowski, 1869)	<i>Cobitis fossilis mohoity</i> var. Dybowski, 1869 <i>Misgurnus bipartitus</i> (Sauvage & Dabry de Thiersant, 1874) <i>Misgurnus cestoides</i> Kessler, 1876 <i>Misgurnus erikssoni</i> Rendahl, 1922 <i>Nemacheilus bipartitus</i> Sauvage & Dabry de Thiersant, 1874	Russia, Mongolia and China	Russia, Siberia, Zabaykalsky Krai, muddy lakes of Onon and Ingoda drainages, near Duldurga on Ila River
<i>Misgurnus mizolepis</i> Günther, 1888	Same time valid and synonym of <i>Paramisgurnus dabryanus</i> Dabry de Thiersant, 1872 (if not valid)	Russia, Mongolia and China	Yangtze River at Kiu-Kiang, China
<i>Misgurnus multimaculatus</i> Rendahl, 1944	<i>Misgurnus mizolepis multimaculatus</i> Rendahl, 1944	Vietnam, Hue province	Vietnam, Annam, Thua Luu, 50 km southeast of Hue
<i>Misgurnus nikolskyi</i> Vasil'eva, 2001	no listed synonyms (July 2020)	Amur River basin, Russia and China	Russia, Primorsky Krai, a lake near Lefu River (Ilistaya, a tributary of Lake Khanka), near Novokorovinskaya
<i>Misgurnus tonkinensis</i> Rendahl, 1937	<i>Misgurnus mizolepis tonkinensis</i> Rendahl, 1937	Northern Vietnam	Vietnam, Tonkin, Hanoi
<i>Paramisgurnus dabryanus</i> Dabry de Thiersant, 1872	<i>Misgurnus mizolepis</i> Günther, 1888 <i>Misgurnus oligolepos</i> Li, 1993	Tianjin, China (if valid); East Asia: China, Taiwan	China, Yangtze River
<i>Paramisgurnus nahangensis</i> Nguyen & Bui, 2009	no listed synonyms (July 2020)	Vietnam, Quang Province	Vietnam, Tuyen Quang Province, Na Hang district, Lo Gam River

Recently, *M. nikolskyi* was reported from Russian Siberia as presumably introduced (Interesova et al. 2010) and *M. bipartitus* from Austria and the Netherlands (Brys et al. 2020; Zangl et al. 2020). On the other hand, the Central European species *M. fossilis* is endangered (Council of the European Communities 1992) and a target species for conservation, in which local extinctions partly remained unnoticed because of the simultaneous introduction of morphologically similar exotic weatherfish species (Freyhof 2013). Other species like *M. anguillicaudatus* and *P. dabryanus* were introduced and established in regions where they are considered undesired alien species; yet they are threatened in their native ranges due to overexploitation (Chen et al. 2015a; Yi et al. 2017, 2018).

Morphologically distinguishing the different species of weatherfish can be challenging, as meristic and morphological features are not easily accessible by the non-expert (Chen 1981). For example, the number of vertebrae or the form of the lamina circularis, an enlarged pectoral fin ray feature displayed in males of some species either need to be characterized based on x-ray imaging or dissection. Also, field identification based on the specific position of dorsal and ventral fins typically requires direct comparison with other species (e.g., Vasil'eva 2001). Several meristic measurements for different species partially overlap (e.g., Kim and Park 1995; Vasil'eva 2001), and metric characteristics can be disguised in ethanol-preserved specimens (Kotusz 1995, own observation), further complicating correct identification. Additionally, the coloration of many species seems to be highly variable and variants with different colour are being cultured for ornamental trade (see Figure 1H) which all contributes to the morphological confusion of cryptic species under one name (Kottelat 2012). Hybridization between the East Asian species (*M. anguillicaudatus* × *P. dabryanus* ssp.) occurs in the wild and is artificially implemented to enhance food resources (You et al. 2009; Zhang et al. 2018). Hybrid vigour also can enhance the invasive potential of hybrids (Cucherousset and Olden 2011; Huang et al. 2017), and hybrids cannot be assigned to a species. Additionally, polyploidy occurs frequently, even within the populations of one species (Drozd et al. 2010; Zhao et al. 2012).

Molecular genetic tools included in integrative taxonomy are increasingly considered a reliable and unambiguous alternative to classical methods of species identification (e.g., Beggel et al. 2015; Pieri et al. 2018; Weiss et al. 2018). In recent years, various biochemical, mitochondrial, and nuclear molecular genetic markers have been used to investigate the phylogeny, phylogeography, distribution and species delimitation, and the intra-specific or population genetic structure in the genera *Misgurnus* and *Paramisgurnus* (e.g., Perdices et al. 2012; Thomsen et al. 2012; Jakovlić et al. 2013; Chen et al. 2015a; Yi et al. 2016a, b, 2017; Brys et al. 2021). As in other metazoan animal groups, an approximately 650 bp long segment of the mitochondrial cytochrome oxidase I (COI) gene is the predominantly used molecular marker



Figure 1. Pictures of different weatherfish species and specimens encountered in Central Europe. A–E = Different colour morphs of *Misgurnus fossilis* from Southern Germany (A to C = River Inn catchment; D, E = specimens from the Danube catchment). F = *Misgurnus* sp. (cf. *bipartitus*) from the River Inn catchment displaying the distinctive black dot at the posterior upper base of the caudal peduncle (white arrow). G = Male *Misgurnus fossilis* from the same location displaying the distinctive lateral black stripes and enlarged second fin ray (white arrow) of the pectoral fins. H = Female weatherfish individual obtained from the ornamental aquarium trade in Southern Germany in 2018. Initially labelled as “*Misgurnus anguillicaudatus* var. Gold”, morphological re-analysis classified the specimens as *Paramisgurnus* sp. or “Golden Dojo”. Scale bar denotes 1 cm. Photographs by Chair of Aquatic Systems Biology, Technical University of Munich, Freising, Germany.

for genetic species identification including weatherfishes (Yi et al. 2016b, 2017; Belle et al. 2017; Stoeckle et al. 2019; Zangl et al. 2020).

The issues outlined above, i.e. unresolved taxonomy and synonymous species names, challenging morphology, and possible hybridisation even in the wild, lead to frequently encountered problems in species identification, not only in weatherfishes (e.g., Meier et al. 2006; Steinke et al. 2009; Jones et al. 2013; Pyšek et al. 2013; Ryberg and Nilsson 2018). To exemplify the existing problems and difficulties that can be encountered in genetic species identification and management, we computed and assessed an updated phylogenetic tree of the genera *Misgurnus* and *Paramisgurnus* comprising 289 published weatherfish COI barcodes and two outgroups.

Materials and methods

A condensed phylogenetic tree of the genera *Misgurnus* and *Paramisgurnus* displaying the maximum likelihood estimates of phylogenetic relationship of COI-5-P mtDNA barcode sequences sourced on July 15, 2020, was computed. The databases GenBank/NCBI and BOLD version 4 (“Barcode of Life Data System”, Ratnasingham and Hebert 2007) were searched using the phrases “*Misgurnus* OR *Paramisgurnus*” AND “COI” or “cox1” or “mitochondrial genome” in GenBank/NCBI, and “*Misgurnus* OR *Paramisgurnus*” in a public database query in BOLD. Subsequently, all duplicates of the resulting 361 sequences, and other mtDNA region barcodes available in BOLD, e.g. cytb, COII, COIII, or ND, were removed in Excel. For the phylogenetic analysis, the remaining sequences were aligned using the muscle algorithm implemented in MEGA X (Kumar et al. 2018) including published *Pangia pangio* (Hamilton, 1822) and *Cobitis taenia* Linnaeus, 1758 COI-sequences from India (Rahman et al. 2016) and Germany (Knebelsberger et al. 2015) as outgroups. The resulting alignment was trimmed to an overall length of 607 bp, removing all shorter barcode sequences, resulting in a total of 291 sequences. Maximum Likelihood (ML) method implemented in MEGA X was used to determine the best substitution model. The phylogenetic clustering of all sequences using the resulting best-fit model (HKY+G), and the maximum intra- and minimum interspecific uncorrected p-distances between the phylogenetic clusters representing different species or sub-groups were computed with the same software (see Supplementary material Table S1). The phylogenetic tree was subsequently condensed in collapsing each branch with less than 70% bootstrap support values obtained after 1000 replications.

Results and discussion

Figure 2 shows the condensed phylogenetic Maximum Likelihood tree of 289 available COI barcode sequences named either *Misgurnus* or *Paramisgurnus* plus two outgroups (total 291) with a minimum length of 607 bp. Overall,

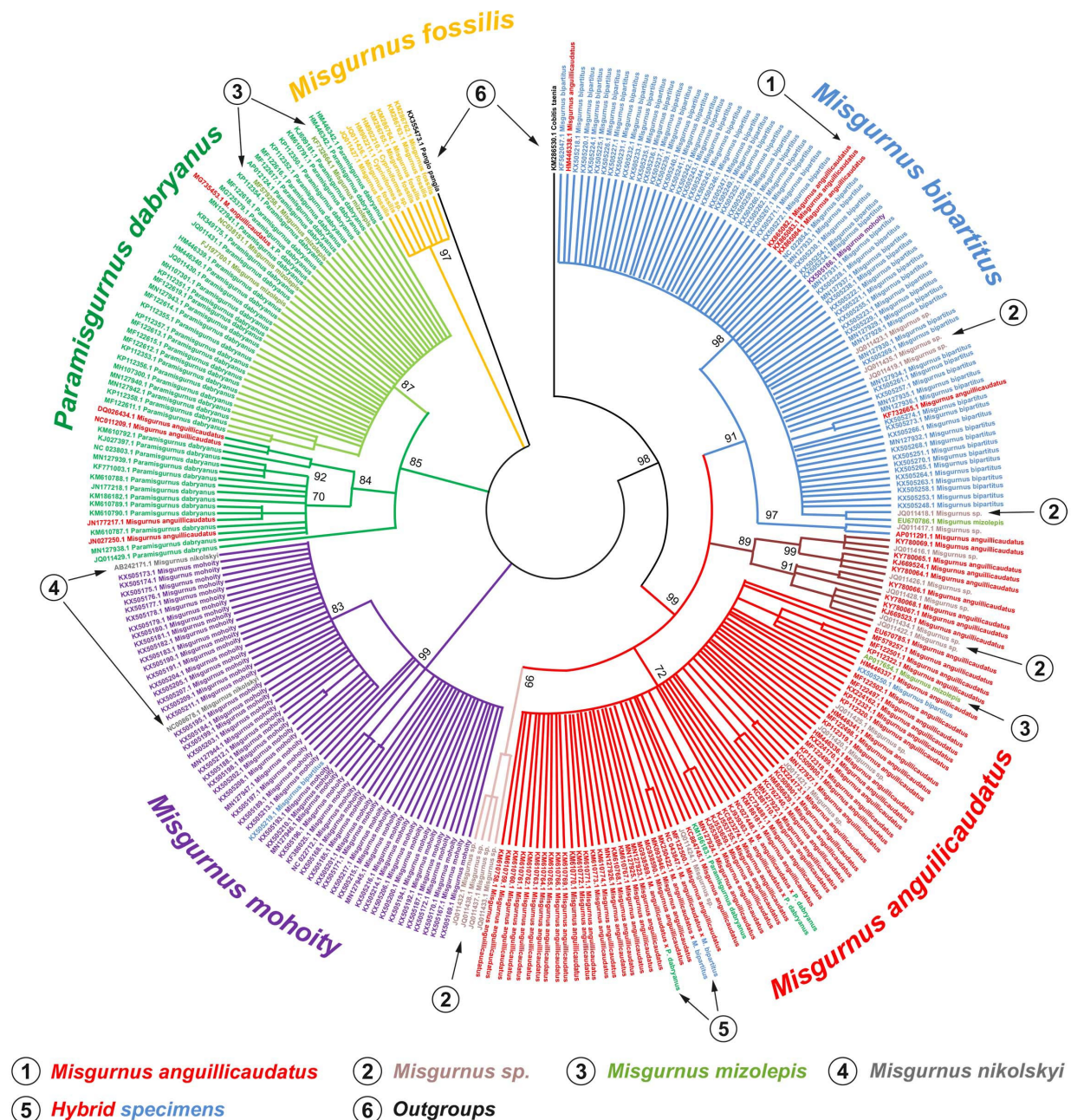


Figure 2. Condensed phylogenetic tree of the genera *Misgurnus* and *Paramisgurnus* displaying the Maximum Likelihood estimates of phylogenetic relationship of 291 COI-5-P mtDNA barcode sequences sourced in the public databases GenBank and BOLD on July 15, 2020. The numbers above branches display their respective bootstrap support values obtained after 1000 replications. Identical haplotype labels of the different species names, the associated tree branches and genetic clusters of the phylogenetic tree are indicated by different colours clockwise starting from the outgroups (given in black): blue = *M. bipartitus*; red = *M. anguillicaudatus*, split in “normal red” (group 1) and “dark red” (group 2), rose = *Misgurnus* sp.; violet = *M. mohoity*; green = *Paramisgurnus dabryanus*, split in “light green” (clade 1) and “dark green” (clade 2), yellow = *M. fossilis*. The encircled numbers 1 to 5 illustrate the discussed general examples of challenges and difficulties that can be encountered in invasive species assignment and molecular taxonomic identification, not just in the genera of weatherfishes. For instance, in cluster 1, the inclusion of *M. anguillicaudatus* is probably also due to morphological misidentification. Number 6 indicates the two sequences used as outgroups (left side *Pangio pangia*, right side *Cobitis taenia*).

the phylogenetic clustering revealed seven distinct groups (*M. bipartitus*, *M. anguillicaudatus* group 1, *M. anguillicaudatus* group 2, *Misgurnus* sp., *M. mohoity*, *P. dabryanus* and *M. fossilis*), separated by minimum interspecific uncorrected p-distances between 6.3% (*Misgurnus* sp. GenBank accession numbers #JQ011433 and *M. anguillicaudatus* #MF122497, #KP112320)

and 16.3% (all *Misgurnus* sp. and *P. dabryanus* #JQ011429, #KM610788 and #MN127938), proving the general validity of recognizing different clusters on species level within the group (Table S1; see also Yi et al. 2016b, 2017). However, the clusters do not always reflect the currently valid species names as listed in Table 1. For instance, the three Vietnamese and the one Korean species were excluded from the analysis because no barcodes have been published, and only two barcodes are available for *M. nikolskyi*. Six of the eleven valid species names occur throughout the phylogenetic tree (*M. anguillicaudatus*, *M. bipartitus*, *M. fossilis*, *M. mizolepis*, *M. mohoity*, and *M. nikolskyi*). All this indicates an incomplete reference database (as of July 2020) for the universal mtDNA barcode or COI-sequences, as well as potential morphological misidentification. This is not surprising, and 55% species or species names represented by barcodes is actually a good result, as public barcoding databases coverage even in taxonomic well-known groups, such as European freshwater fishes, is on average only 66 to 88% of the known taxa (Weigand et al. 2019).

Most surprising, each of the two distinct *M. anguillicaudatus* clusters separates from *M. bipartitus* with roughly the same minimum interspecific uncorrected p-distances (6.7% to 7.2%, as also reported by Yi et al. (2017)). We cannot confirm the proposed large interspecific distances between *M. bipartitus* and *M. anguillicaudatus* detected by Zangl et al. (2020). In contrast to our analyses, Zangl et al. (2020) included very few sequences named *M. anguillicaudatus* and excluded the ones from the native ranges published by Yi et al. (2017). This example illustrates the importance of a complete database sampling to avoid artificially increasing genetic differentiation between phylogenetic clusters due to incomplete coverage. The same problem also arises if there are only few divergent specimens available; this warrants more complete geographic sampling before drawing conclusions about species divergences.

In contrast to *M. anguillicaudatus*, only one monophyletic group is formed by *M. fossilis* (Figure 2). A potentially cryptic species identified to genus level, i.e. *Misgurnus* sp. from Hue province, Vietnam, also forms a distinct cluster (Figure 2, no. 2), but was only represented by four sequences. Other groups, e.g., *M. mohoity* and *P. dabryanus*, harbour large intraspecific genetic distances that may reflect true cryptic diversity due to the unresolved classical taxonomy, difficult morphological species identification, or simply a more exhaustive geographic sampling of genetically distinct sub-populations (Figure 2, Table S1). Nevertheless, extensive haplotype- or label-sharing occurred, for which we cannot exclude true haplotype sharing due to incomplete lineage sorting or introgression. Introgression and haplotype sharing were also detected by Perdices et al. (2012) using the cytochrome *b* gene (Cyt *b*) as the molecular mitochondrial marker. In our dataset, *M. nikolskyi* clusters completely in *M. mohoity*, rejecting a separate clade for this species, whereas the same

holds true for *M. mizolepis* sequences that are distributed in many phylogenetic groups all over the tree. Owing to the large interspecific genetic distances, and in considering only one single molecular marker, and in line with Jakovlić et al. (2013), we cannot confirm the hypothesis that *M. bipartitus* is a synonym of *M. mohoity* as suggested by Fricke et al. (2020).

Overall, 12% (35 out of 289) of the analysed sequences are labelled with names not belonging to their respective phylogenetic clusters, and roughly twice the number of these sequences originate from introduced ranges versus native origin (23 vs. 12 sequences, see Table S1). This clearly illustrates the difficulties with morphological identification of specimens, even within the native ranges. The problem is further complicated by changing taxonomy or species names over time. The large discrepancy between native and introduced ranges may be due to the lack of taxonomic expertise or taxonomic literature in the introduced ranges. Whereas old (i.e., before 1920) weatherfish species descriptions and taxonomic literature are readily available digitized through the “Biodiversity Heritage Library” (Gwinn and Rinaldo 2009, <https://www.biodiversitylibrary.org/>, accessed August 23, 2020), it is difficult for the scientific community to obtain some recent taxonomic keys and literature. Many of the respective journals are not yet digitized or open access (“dark texts” *sensu* Page 2016), and some species descriptions and keys are not yet fully available in English (e.g., Kim and Park 1995; Nguyen and Bui 2009).

Many available sequences are labelled to a higher taxonomic level such as the genus (e.g., “*Misgurnus*”, Figure 2). This may reflect difficulties in morphological species determination or the absence of a genetic species match at the time of analyses. It is important to keep in mind that molecular genetic databases like GenBank/NCBI and BOLD are constantly growing and changing, and that specimen assignments to taxonomic groups or phylogenetic clusters are dynamic. Thus, our analyses can also only provide a summary of the current picture on weatherfish phylogenetics and diversity, which may be outdated as soon as additional results become available. In our case, all data were downloaded on July 15, 2020, and all associated analyses can only reflect the knowledge of that time. This also illustrates that the indication of “misidentification” as made by Zangl et al. (2020) is due to the fact that Belle et al. (2017) conducted the analysis in September 2016 and correctly labelled the results according to the data available at that time. We agree that our COI sequences right now (July 15, 2020) cluster with COI sequences that are named *M. bipartitus* (Figure 2, no. 1). We also agree that there is published biogeographical, ecological, physiological and barcoding evidence that supports *M. bipartitus* at least as a distinct genetic sub-group or species (Yi et al. 2016b, 2017, 2018). The same issue applies to the sequences labelled *Misgurnus* sp. in our cladogram (Figure 2, no. 2). The specimens from which those COI sequences originate could neither be named nor assigned to any weatherfish species cluster

using DNA barcodes at the time of analysis in 2011, as those sequences were the first ones published in the genera *Misgurnus* and *Paramisgurnus* (Table S1). The assignment in our phylogenetic tree and especially the distinct cluster formed by four of the sequences originating from Hue province, Vietnam (Figure 2, branches in rose), the type locality of *M. multimaculatus* Rendahl, 1944, underpins the cryptic diversity in exotic weatherfish species introduced to Australia already hypothesised by Kearns et al. (2011).

This controversy is not new (Lis et al. 2016; Page 2016; Steinke 2016), but at the same time should not prevent early sharing of data, which is particularly crucial when it comes to management of invasive species (Pergl et al. 2020). In this context, we want to stress the importance of resolving the classical taxonomy and completing genetic reference databases of not only weatherfishes, as the same problems apply to many other exotic fish species (Gomes et al. 2015; Dahrudin et al. 2017; Kundu et al. 2019).

Nevertheless, it might be possible that, in retro-perspective, available publications in the context of genetic weatherfish identification (Belle et al. 2017; Stoeckle et al. 2019; Zangl et al. 2020) are all missing the target with their genetic specimen assignments because, in reality, there may also be non-native hybrid weatherfish specimens which are difficult to identify. As outlined above, hybridisation even in the wild occurs between Far Eastern weatherfish species. Under laboratory conditions, hybridisation between the European endangered *M. fossilis* and exotic specimens was successful (Josef Wanzenböck, Research Department for Limnology Mondsee, University of Innsbruck, *pers. comm.* December 05, 2019). As expected from mitochondrial DNA in vertebrates, sequences derived from hybrid specimens unambiguously cluster within the respective maternal lines in the phylogenetic tree (Figure 2, no. 5). Hybrid specimens thus cannot be identified by relying solely on a mitochondrial marker like COI. As Zangl et al. (2020) and others implemented in their studies, additional nuclear molecular markers, for example the “recombination activating gene 1” (RAG1), should be analysed in addition to mtDNA markers, foremost from specimens, populations and species from the native ranges.

Conclusions

Overall, our analysis of genetic weatherfish identification using mtDNA barcodes confirms their still incomplete and unresolved classical and molecular taxonomy, as well as an incomplete coverage of species in public databases. Nonetheless, given the distinct existing phylogenetic clusters facilitating a genetic specimen assignment for the genera *Misgurnus* and *Paramisgurnus*, we suggest DNA barcoding and genotyping of detected non-native or traded weatherfish specimens. In any case, such analyses appear mandatory for source populations of any re-stocking in the context of conservation programs. A genetic assessment is also especially crucial to

discover potential cryptic invasions of non-native genotypes or the presence of cryptic exotic species. For incomplete reference databases, the current situation also suggests clusters and differences that may change if taxon sampling is expanded. To tackle this problem, it might be sensible to expand “museomics” or barcoding projects to established non-native taxa to investigate their type specimens and type locations. This is also important as some introduced populations may become “extinct in the native ranges” and potentially could persist in the wild in introduced areas. However, an artificially driven distribution of individuals outside their native geographic range (e.g., “faunal enrichment”) is not recommended. Second, hybridisation impedes classical taxonomic species identification in weatherfish and is still challenging if using molecular genetic methods such as barcoding. Therefore, we suggest expanding the use and standardising of nuclear markers. Finally, educating taxonomists and practitioners in the introduced ranges is important, as any gap of knowledge may facilitate cryptic invasions during which superficially similarly looking species and their impact on the introduced area might be overlooked for a long time. Therefore, it is important to keep up-to-date freely available identification literature accessible and comparable between biogeographic regions. We would additionally encourage the use of standard genetic analyses during ecological monitoring of known morphologically variable and difficult species that can easily be confused with similar-looking native ones. Such a situation is not only existent for weatherfishes, but also for other fishes such as bitterling (*Rhodeus* sp.), and freshwater mussels (Pieri et al. 2018; Bartáková et al. 2019; Kondakov et al. 2020).

Acknowledgements

We would like to thank the Associate Editor of *BioInvasions Records*, Dr. Cathryn Abbott, as well as two anonymous reviewers for their helpful comments on an earlier version of this paper.

Author’s Contribution

CCB, JPG, and RK conceived the study, sample design and methodology; CCB did the investigation and data collection; CCB and BCS led the data analysis and interpretation; BCS, AFC, JP and CCB visualized the results; the original draft was written by CCB and JPG; all authors were responsible for reviewing and editing of the manuscript.

References

- Abilhoa V, Bornatowski H, Vitule JRS (2013) Occurrence of the alien invasive loach *Misgurnus anguillicaudatus* in the Iguaçú River basin in southern Brazil: a note of concern. *Journal of Applied Ichthyology* 29: 257–259, <https://doi.org/10.1111/jai.12007>
- Allen S (1984) Occurrence of juvenile weatherfish *Misgurnus anguillicaudatus* (Pisces: Cobitidae) in the Yarra River. *Victorian Naturalist* 101: 240–242, <https://www.biodiversitylibrary.org/page/39961466>
- Bartáková V, Bryja J, Šanda R, Bektas Y, Stefanov T, Choleva L, Smith C, Reichard M (2019) High cryptic diversity of bitterling fish in the southern West Palearctic. *Molecular Phylogenetics and Evolution* 133: 1–11, <https://doi.org/10.1016/j.ympev.2018.12.025>
- Beggel S, Cerwenka A, Brandner J, Geist J (2015) Shell morphological versus genetic identification of quagga mussel (*Dreissena bugensis*) and zebra mussel (*Dreissena polymorpha*). *Aquatic Invasions* 10: 93–99, <https://doi.org/10.3391/ai.2015.10.1.09>

- Belle CC, Stoeckle BC, Cerwenka AF, Kuehn R, Mueller M, Pander J, Geist J (2017) Genetic species identification in weatherfish and first molecular confirmation of Oriental Weatherfish *Misgurnus anguillicaudatus* (Cantor, 1842) in Central Europe. *Knowledge & Management of Aquatic Ecosystems* 418: 31, <https://doi.org/10.1051/kmae/2017025>
- Brys R, Neyrinck S, Halfmaerten D, Auwerx J, Van Wichelen J, Verreycken H (2020) De Noord-Aziatische modderkruiper - Nieuwe invasieve vissoort duikt dankzij eDNA niet langer ongezien de grens over [Oriental Weather Loach: a new invasive fish species in Flanders that remains no longer invisible via its eDNA]. *Natuur focus* 19: 70–74
- Brys R, Halfmaerten D, Neyrinck S, Mauvisseau Q, Auwerx J, Sweet M, Mergeay J (2021) Reliable eDNA detection and quantification of the European weather loach (*Misgurnus fossilis*). *Journal of Fish Biology* 98: 399–414, <https://doi.org/10.1111/jfb.14315>
- Chen J (1981) A study on the classification of the subfamily Cobitinae of China. *Transactions of the Chinese Ichthyological Society* 1: 21–32 [in Chinese with English summary], <https://doi.org/10.1016/B978-0-08-023861-6.50007-9>
- Chen G, Huang S, Gao J, Bai X, Wang W, Cao X (2015a) Development and characterization of microsatellite markers via cross-species amplification of *Paramisgurnus dabryanus*. *Genetics and Molecular Research* 14: 5694–5698, <https://doi.org/10.4238/2015.May.29.1>
- Chen W, Ma X, Shen Y, Mao Y, He S (2015b) The fish diversity in the upper reaches of the Salween River, Nujiang River, revealed by DNA barcoding. *Scientific Reports* 5: 1–12, <https://doi.org/10.1038/srep17437>
- Council of the European Communities (1992) Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. *Official Journal of the European Union* L206: 7–50
- Cucherousset J, Olden JD (2011) Ecological impacts of nonnative freshwater fishes. *Fisheries* 36: 215–230, <https://doi.org/10.1080/03632415.2011.574578>
- Dahrudin H, Hutama A, Busson F, Sauri S, Hanner R, Keith P, Hadiaty R, Hubert N (2017) Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. *Molecular Ecology Resources* 17: 288–299, <https://doi.org/10.1111/1755-0998.12528>
- Drozd B, Flajšhans M, Ráb P (2010) Sympatric occurrence of triploid, aneuploid and tetraploid weatherfish *Misgurnus fossilis* (Cypriniformes, Cobitidae). *Journal of Fish Biology* 77: 2163–2170, <https://doi.org/10.1111/j.1095-8649.2010.02794.x>
- Franch N, Clavero M, Garrido M, Gaya N, López V, Pou-Rovira Q, Queral J (2008) On the establishment and range expansion of oriental weatherfish (*Misgurnus anguillicaudatus*) in NE Iberian Peninsula. *Biological Invasions* 10: 1327–1331, <https://doi.org/10.1007/s10530-007-9207-9>
- Freyhof J (2013) *Misgurnus fossilis*. The IUCN Red List of Threatened Species 2013. e.T40698A10351495, <https://www.iucnredlist.org/species/40698/10351495> (accessed 16 August 2020)
- Freyhof J, Korte E (2005) The first record of *Misgurnus anguillicaudatus* in Germany. *Journal of Fish Biology* 66: 568–571, <https://doi.org/10.1111/j.0022-1112.2005.00606.x>
- Fricke R, Eschmeyer W, Van der Laan R (2020) Catalog of fishes: genera, species, references. California Academy of Sciences, San Francisco, CA, USA, <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp> (accessed 12 September 2020)
- Gomes LC, Pessali TC, Sales NG, Pompeu PS, Carvalho DC (2015) Integrative taxonomy detects cryptic and overlooked fish species in a neotropical river basin. *Genetica* 143: 581–588, <https://doi.org/10.1007/s10709-015-9856-z>
- Gwinn NE, Rinaldo CA (2009) The Biodiversity Heritage Library: Sharing biodiversity with the world. *IFLA Journal* 35: 25–34, <https://doi.org/10.1177/0340035208102032>
- Huang S, Cao X, Tian X, Wang W (2017) Ploidy and growth performance of hybrid progeny between tetraploid *Misgurnus anguillicaudatus* and *Paramisgurnus dabryanus*. *Aquaculture Research* 48: 2981–2988, <https://doi.org/10.1111/are.13131>
- Interesova E, Yadrenkina E, Vasil'eva E (2010) The first record of *Misgurnus nikolskyi* (Cobitidae) in the South of Western Siberia. *Journal of Ichthyology* 50: 281–284, <https://doi.org/10.1134/S0032945210030082>
- Jakovlić I, Wu Q-J, Treer T, Šprem N, Gui J-F (2013) Introgression evidence and phylogenetic relationships among three (*Para*)*Misgurnus* species as revealed by mitochondrial and nuclear DNA markers. *Archives of Biological Sciences* 65: 1463–1467, <https://doi.org/10.2298/ABS1304463J>
- Jones RW, Weyl OL, Swartz ER, Hill MP (2013) Using a unified invasion framework to characterize Africa's first loricariid catfish invasion. *Biological Invasions* 15: 2139–2145, <https://doi.org/10.1007/s10530-013-0438-7>
- Juliano R (1989) The introduction of exotic aquatic species in the Philippines. In: de Silva SS (ed), *Exotic Aquatic Organisms in Asia*, Proceedings of the Workshop on Introduction of Exotic Aquatic Organisms in Asia. Asian Fisheries Society, pp 83–90
- Kanou K, Saito S, Fuchigami S, Imamura A, Imai H, Taki Y (2007) Occurrence patterns and food habits of introduced alien loach *Paramisgurnus dabryanus* and native loach *Misgurnus anguillicaudatus* at irrigation drainages around rice fields in the Watarase River system, central Honshu, Japan. *Aquaculture Science* 55: 109–114, https://doi.org/10.11233/aquaculture_sci1953.55.109

- Kearns JE, Pettigrove PJ, Hoffmann AA, Carew ME (2011) DNA evidence for multiple species of invasive oriental weatherloach (*Misgurnus* sp.) in Australia. https://www.ncbi.nlm.nih.gov/popset/DbFrom=nuccore&Cmd=Link&LinkName=nuccore_popset&IdsFromResult=363720397 (accessed on 15 July 2020)
- Keller RP, Geist J, Jeschke JM, Kühn I (2011) Invasive species in Europe: ecology, status, and policy. *Environmental Sciences Europe* 23: 23, <https://doi.org/10.1186/2190-4715-23-23>
- Kim R, Park S (1995) A new species of a loach, *Misgurnus* from DPR of Korea. *Bulletin of Academy of Sciences of the Democratic Peoples Republic of Korea* 1: 54–56 [in Korean and English]
- Kirsch JE, Feeney RF, Goodbla A, Hart C, Jackson ZJ, Schreier A, Smith R (2018) First record of the Large-Scale Loach *Paramisgurnus dabryanus* (Cobitidae) in the United States. *Journal of Fish and Wildlife Management* 9: 246–254, <https://doi.org/10.3996/012017-JFWM-008>
- Kneibelsberger T, Dunz AR, Neumann D, Geiger MF (2015) Molecular diversity of Germany's freshwater fishes and lampreys assessed by DNA barcoding. *Molecular Ecology Resources* 15: 562–572, <https://doi.org/10.1111/1755-0998.12322>
- Kondakov AV, Bepalaya YV, Vikhrev IV, Konopleva ES, Gofarov MY, Tomilova AA, Vinarski MV, Bolotov IN (2020) The Asian pond mussels rapidly colonize Russia: successful invasions of two cryptic species to the Volga and Ob rivers. *BioInvasions Records* 9: 504–518, <https://doi.org/10.3391/bir.2020.9.3.07>
- Kottelat M (2012) Conspectus cobitidum: an inventory of the loaches of the world (Teleostei: Cypriniformes: Cobitoidei). *Raffles Bulletin of Zoology Suppl.* 26: 1–199
- Kotusz J (1995) Morphological characteristics of the mud loach *Misgurnus fossilis* [L.] [Pisces: Cobitidae] from the mid Odra and Vistula River basins. *Acta Ichthyologica et Piscatoria* 2: 3–14, <https://doi.org/10.3750/AIP1995.25.2.01>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549, <https://doi.org/10.1093/molbev/msy096>
- Kundu S, Tyagi K, Pakrashi A, Kumar V, Kosygin L, Rath S, Das U, Chandra K (2019) DNA barcoding of freshwater fishes from the transboundary river of Indo-Bhutan: multiple clades and cryptic diversity. *Mitochondrial DNA Part B* 4: 2527–2532, <https://doi.org/10.1080/23802359.2019.1640079>
- Lis JA, Lis B, Ziaja DJ (2016) In BOLD we trust? A commentary on the reliability of specimen identification for DNA barcoding: a case study on burrower bugs (Hemiptera: Heteroptera: Cydnidae). *Zootaxa* 4114: 83–86, <https://doi.org/10.11646/zootaxa.4114.1.6>
- Meier R, Shiyang K, Vaidya G, Ng PK (2006) DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology* 55: 715–728, <https://doi.org/10.1080/10635150600969864>
- Meyer L, Hinrichs D (2000) Microhabitat preferences and movements of the weatherfish, *Misgurnus fossilis*, in a drainage channel. *Environmental Biology of Fishes* 58: 297–306, <https://doi.org/10.1023/A:1007681313916>
- Mukai T, Umemura K, Takagi M (2011) First record of *Paramisgurnus dabryanus* accompanied with the invasion of Chinese lineage of *Misgurnus anguillicaudatus* in Gifu Prefecture, Japan. *Bulletin of the Biogeographical Society of Japan* 66: 85–92
- Nguyen VH, Bui TA (2009) Mô tả một loài Cá Chạch mới trong giống *Paramisgurnus* (họ Cobitidae) tại tỉnh Tuyên Quang [A finding of new species in *Paramisgurnus* (Cobitidae) in Tuyen Quang province]. *Science and Technology Journal of Agriculture & Rural Development Nông Nghiệp & Phát Triển Nông Thôn* 8: 1–11
- Page RD (2016) DNA barcoding and taxonomy: dark taxa and dark texts. *Philosophical Transactions of the Royal Society B: Biological Sciences* 371: 20150334, <https://doi.org/10.1098/rstb.2015.0334>
- Perdices A, Vasil'ev V, Vasil'eva E (2012) Molecular phylogeny and intraspecific structure of loaches (genera *Cobitis* and *Misgurnus*) from the Far East region of Russia and some conclusions on their systematics. *Ichthyological Research* 59: 113–123, <https://doi.org/10.1007/s10228-011-0259-6>
- Pergl J, Pyšek P, Essl F, Jeschke JM, Courchamp F, Geist J, Hejda M, Kowarik I, Mill A, Musseau C, Pipek P, Saul W-C, von Schmalensee M, Strayer D (2020) Need for routine tracking of biological invasions. *Conservation Biology* 34: 1311–1314, <https://doi.org/10.1111/cobi.13445>
- Pieri AM, Inoue K, Johnson NA, Smith CH, Harris JL, Robertson C, Randklev CR (2018) Molecular and morphometric analyses reveal cryptic diversity within freshwater mussels (Bivalvia: Unionidae) of the western Gulf coastal drainages of the USA. *Biological Journal of the Linnean Society* 124: 261–277, <https://doi.org/10.1093/biolinnean/bly046>
- Pyšek P, Hulme PE, Meyerson LA, Smith GF, Boatwright JS, Crouch NR, Figueiredo E, Foxcroft LC, Jarošík V, Richardson DM, Suda J, Wilson JR (2013) Hitting the right target: taxonomic challenges for, and of, plant invasions. *AoB Plants* 5: plt042, <https://doi.org/10.1093/aobpla/plt042>

- Rahman S, Dutta A, Kalita MC (2016) Development of DNA barcodes in fish species of Assam. https://www.ncbi.nlm.nih.gov/popset?LinkName=nuccore_popset&from_uid=1040784496 (accessed on 15 July 2020)
- Ratnasingham S, Hebert PD (2007) BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology Notes* 7: 355–364, <https://doi.org/10.1111/j.1471-8286.2007.01678.x>
- Razzetti E, Nardi P, Strosselli S, Bernini F (2001) Prima segnalazione di *Misgurnus anguillicaudatus* (Cantor, 1842) in acque interne italiane. *Annali del Museo Civico di Storia Naturale di Genova* 93: 559–563 [in Italian]
- Riffel M, Schenk M, Schreiber A (1994) Electrophoretic differentiation between European Loach (*Misgurnus fossilis* L.) and Oriental Weatherfish (*Misgurnus mizolepis* Guenther), an autochthonous and a feral species of Central European freshwater fish. *Zeitschrift für Angewandte Zoologie* 80: 473–484
- Robertson PA, Mill A, Novoa A, Jeschke JM, Essl F, Gallardo B, Geist J, Jarić I, Lambin X, Musseau C (2020) A proposed unified framework to describe the management of biological invasions. *Biological Invasions* 22: 2633–2645, <https://doi.org/10.1007/s10530-020-02298-2>
- Ryberg M, Nilsson RH (2018) New light on names and naming of dark taxa. *MycoKeys* 30: 31–39, <https://doi.org/10.3897/mycokeys.30.24376>
- Sal'nikov V (1998) Anthropogenic migration of fish in Turkmenistan. *Journal of Ichthyology* 38(8): 591–602
- Simon TP, Bright G, Veraldi F, Smith JR, Stahl JR (2006) New records for the alien Oriental Weatherfish, *Misgurnus anguillicaudatus*, in the Lake Michigan Basin, Indiana (Cypriniformes, Cobitidae). *Proceedings of the Indiana Academy of Science* 115(1): 32–36
- Steinke D (2016) DNA Barcoding. - Yes, I trust BOLD! <http://dna-barcoding.blogspot.com/2016/05/yes-i-trust-bold.html> (accessed 10 July 2020)
- Steinke D, Zemlak TS, Hebert PD (2009) Barcoding Nemo: DNA-based identifications for the ornamental fish trade. *PLoS ONE* 4: e6300, <https://doi.org/10.1371/journal.pone.0006300>
- Stoeckle BC, Belle CC, Geist J, Oehm J, Effenberger M, Heiss M, Seifert K, Kuehn R (2019) Molecular confirmation of the large-scale loach *Paramisgurnus dabryanus* Dabry de Thiersant, 1872 (Cypriniformes, Cobitidae) in Europe. *BioInvasions Records* 8: 419–426, <https://doi.org/10.3391/bir.2019.8.2.24>
- Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L, Willerslev E (2012) Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology* 21: 2565–2573, <https://doi.org/10.1111/j.1365-294X.2011.05418.x>
- van Kessel N, Dorenbosch M, Crombaghs B, Niemeijer B, Binnendijk E (2013) First record of Asian weather loach *Misgurnus anguillicaudatus* (Cantor, 1842) in the River Meuse basin. *BioInvasions Records* 2: 167–171, <https://doi.org/10.3391/bir.2013.2.2.14>
- Vasil'eva E (2001) Loaches (genus *Misgurnus*, Cobitidae) of Russian Asia. I. The species composition in waters of Russia (with a description of a new species) & some nomenclature and taxonomic problems of related forms from adjacent countries. *Journal of Ichthyology* 41: 553–563
- Weigand H, Beermann AJ, Čiampor F, Costa FO, Csabai Z, Duarte S, Geiger MF, Grabowski M, Rimet F, Rulík B, Strand M, Szucsich N, Weigand AM, Willassen E, Wyler SA, Bouchez A, Borja A, Čiamporová-Zaťovičová Z, Ferreira S, Dijkstra K-DB, Eisendle U, Freyhof J, Gadawski P, Graf W, Haegerbaeumer A, van der Hoorn BB, Japoshvili B, Keresztes L, Keskin E, Leese F, Macher JN, Mamos T, Paz G, Pešić V, Pfannkuchen DM, Pfannkuchen MA, Price BW, Rinkevich B, Teixeira MAL, Várbíró G, Ekrem T (2019) DNA barcode reference libraries for the monitoring of aquatic biota in Europe: Gap-analysis and recommendations for future work. *Science of the Total Environment* 678: 499–524, <https://doi.org/10.1016/j.scitotenv.2019.04.247>
- Weiss M, Weigand H, Weigand AM, Leese F (2018) Genome-wide single-nucleotide polymorphism data reveal cryptic species within cryptic freshwater snail species - The case of the *Ancylus fluviatilis* species complex. *Ecology and Evolution* 8: 1063–1072, <https://doi.org/10.1002/ece3.3706>
- Yi S, Wang S, Zhong J, Wang W (2016a) Comprehensive transcriptome analysis provides evidence of local thermal adaptation in three loaches (genus: *Misgurnus*). *International Journal of Molecular Sciences* 17: 1943, <https://doi.org/10.3390/ijms17121943>
- Yi S, Zhong J, Wang S, Huang S, Wang W (2016b) Mitochondrial DNA reveals evolutionary status and population genetics of two closely related fish (*Misgurnus bipartitus* and *Misgurnus mohoity*) in northeast China. *Biochemical Systematics and Ecology* 68: 192–199, <https://doi.org/10.1016/j.bse.2016.07.018>
- Yi S, Zhong J, Huang S, Wang S, Wang W (2017) Morphological comparison and DNA barcoding of four closely related species in the genera *Misgurnus* and *Paramisgurnus* (Cypriniformes: Cobitidae). *Biochemical Systematics and Ecology* 70: 50–59, <https://doi.org/10.1016/j.bse.2016.10.019>
- Yi S, Li Y, Wang W (2018) Selection shapes the patterns of codon usage in three closely related species of genus *Misgurnus*. *Genomics* 110: 134–142, <https://doi.org/10.1016/j.ygeno.2017.09.004>

- You C, Yu X, Tong J (2009) Detection of hybridization between two loach species (*Paramisgurnus dabryanus* and *Misgurnus anguillicaudatus*) in wild populations. *Environmental Biology of Fishes* 86: 65, <https://doi.org/10.1007/s10641-007-9282-x>
- Zangl L, Jung M, Gessl W, Koblmueller S, Ratschan C (2020) Oriental or not: First record of an alien weatherfish (*Misgurnus*) species in Austria verified by molecular data. *BioInvasions Records* 9: 375–383, <https://doi.org/10.3391/bir.2020.9.2.23>
- Zhang G, Sun X, Zhang G, Liang X, Cai K, Zhang H, Zhu D (2018) Molecular characteristics of mitochondrial DNA and phylogenetic analysis of the hybrid loach of *Misgurnus anguillicaudatus* (female) and *Paramisgurnus dabryanus* ssp. (male). *Mitochondrial DNA Part B* 3: 545–546, <https://doi.org/10.1080/23802359.2018.1467226>
- Zhao Y, Toda M, Hou J, Aso M, Arai K (2012) The occurrence of hypertetraploid and other unusual polyploid loaches *Misgurnus anguillicaudatus* among market specimens in Japan. *Fisheries Science* 78: 1219–1227, <https://doi.org/10.1007/s12562-012-0556-7>
- Zięba G, Copp GH, Davies GD, Stebbing P, Wesley KJ, Britton JR (2010) Recent releases and dispersal of non-native fishes in England and Wales, with emphasis on sunbleak *Leucaspis delineatus* (Heckel, 1843). *Aquatic Invasions* 5: 155–161, <https://doi.org/10.3391/ai.2010.5.2.04>

Supplementary material

The following supplementary material is available for this article:

Appendix 1. FASTA-file of sequences downloaded from GenBank/NCBI and BOLD (July 15, 2020) that are included in our analysis.

Figure S1. Uncondensed original phylogenetic tree including the bootstrap values and genetic distances of all 291 analysed sequences.

Table S1. Pairwise uncorrected genetic p-distances of all 291 analysed COI sequences and the associated standard error (Sheet 1); maximum intra- and minimum interspecific uncorrected p-distances of the weatherfish phylogenetic clusters representing the known species and of the paraphyletic *M. anguillicaudatus* sub-groups indicating cryptic diversity (Sheet 2).

This material is available as part of online article from:

http://www.reabic.net/journals/bir/2021/Supplements/BIR_2021_Belle_etal_Appendix_1.txt

http://www.reabic.net/journals/bir/2021/Supplements/BIR_2021_Belle_etal_Figure_S1.pdf

http://www.reabic.net/journals/bir/2021/Supplements/BIR_2021_Belle_etal_Table_S1.xlsx