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 Kuehn³, Joachim Pander¹ and Juergen P. Geist¹,*

¹Aquatic Systems Biology Unit, TUM School of Life Sciences, Technical University of Munich, D-85354 Freising, Bavaria, Germany
²Section Evertebrata 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

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

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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.

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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).

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