

Rapid Communication

Molecular confirmation of the large-scale loach *Paramisgurnus dabryanus* Dabry de Thiersant, 1872 (Cypriniformes, Cobitidae) in Europe

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Abstract

Asian weather loach species of the genus *Paramisgurnus* and *Misgurnus* can have a great invasive potential. In Europe, several feral populations of Asian weather loach have been reported, many of them within the distribution range of the native European weather loach *Misgurnus fossilis*, a declining species of high conservation concern. Distinguishing the different exotic species of weather loach can be difficult for the non-expert, as their general morphology is very similar, the coloration patterns are highly variable and the morphological characteristics may become degraded after preservation. We present the first molecular genetic confirmation of the presence of the large-scale loach *Paramisgurnus dabryanus* in Europe, after analyzing two specimens from a small pond along the brook "Wörthlinger Bach" (Danube catchment) in southern Germany. Accurate species identification is crucial both in the context of monitoring and conserving endangered species, and in detection and prevention of biological invasions. Therefore, we suggest the standard use of genetic species assignment based on mitochondrial DNA barcoding in monitoring cryptic fish species complexes, especially during potentially cryptic biological invasions.

Key words: *Misgurnus fossilis*, Asian weather loach, cytochrome oxidase subunit I (COI) DNA barcoding, genetic species assignment, biological invasions

Introduction

Several species of the genus *Paramisgurnus* and *Misgurnus*, belonging to the family Cobitidae (true loaches), have a great invasive potential (Kirsch et al. 2018). Numerous records of the large-scale loach *Paramisgurnus dabryanus* Dabry de Thiersant, 1872 or the oriental weather loach *Misgurnus anguillicaudatus* (Cantor, 1842) were reported outside their native distribution range, e.g. from the USA (Kirsch et al. 2018), Europe (Riffel et al. 1994; Zięba et al. 2010; Freyhof 2013), and Australia (Kottelat and Freyhof 2007). Several feral populations of these invasive Asian loaches are within the

distribution range of the native European weather loach *Misgurnus fossilis* (Linnaeus, 1758), a declining species in Europe of high conservation concern (Freyhof and Korte 2005; Freyhof 2013; Belle et al. 2017), listed in Annex II of the European Habitat Directive (Council of the European Communities 1992). In central and northern Europe, Asian loach species like *P. dabryanus* and *M. anguillicaudatus* are regularly imported from southern China as ornamental species for aquaria and garden ponds (Freyhof and Korte 2005; Freyhof 2013). Currently, both loach species are globally expanding invaders (e.g. Mukai et al. 2011; Kirsch et al. 2018).

The large-scale loach *P. dabryanus* is native to the East-Asian temperate zones (China) and distributed in the middle and lower reaches of the Yangtze River, Pearl River, Nujiang River, and in inland waters of Taiwan (You et al. 2009; Chen et al. 2015). Populations of *P. dabryanus* have established outside its native range in Japan (Mukai et al. 2011) and reproduce in several regions of Honshu Island (Kanou et al. 2007). It was also found in the USA, where its occurrence was earlier reported as *M. anguillicaudatus* (Kirsch et al. 2018). It is assumed that this species occurs also in Great Britain (Zięba et al. 2010), in Switzerland (Freyhof 2013; Bundesamt für Umwelt BAFU 2014), and in northern Germany (Riffel et al. 1994). However, there is still no molecular genetic evidence of the large-scale loach within Europe in the wild.

Taxonomic literature and published illustrations show large morphological variation in different populations referred to as *P. dabryanus* (Kottelat 2012). Moreover, the diagnostic features in the Asian species of *Misgurnus* and *Paramisgurnus* are very difficult to classify for the non-expert (Chen 1981). However, the native loach (*M. fossilis*) can be morphologically identified by having a broad midlateral stripe and a narrower stripe between lateral midline and dorsum. This character is expressed in all specimens of *M. fossilis* since their early juvenile stage. This morphological characteristic can be used as a distinctive feature between the native and the invasive Asian species *P. dabryanus* and *M. anguillicaudatus*. Also, *M. fossilis* is the only species of weather loach where males do not develop a lamina circularis on the pectoral fins. Among the invasive Asian loach species, *P. dabryanus* can be identified by its elongated lamina circularis and the absence of a black dot on the base of the caudal fin. Moreover, most individuals display a high adipose crest (Kottelat and Freyhof 2007).

Genetic species identification using mitochondrial DNA (mtDNA) barcoding (Hebert et al. 2003) is a reliable tool to identify co-occurring native and non-native loach species in Europe (Belle et al. 2017; Yi et al. 2016, 2017). Herein, we analysed weather loach individuals exhibiting morphological characteristics of Asian loaches from southern Germany for specimen assignment using mtDNA barcoding, providing the first confirmed record of feral large-scale loaches *P. dabryanus* in Europe based on molecular sequence data.



Figure 1. Lateral view of the specimens caught in the “Wörthlinger Bach” which were identified as *Paramisgurnus dabryanus* by molecular genetic analysis in this study. Numbers refer to cm scale. Photograph by Benedikt Beck.

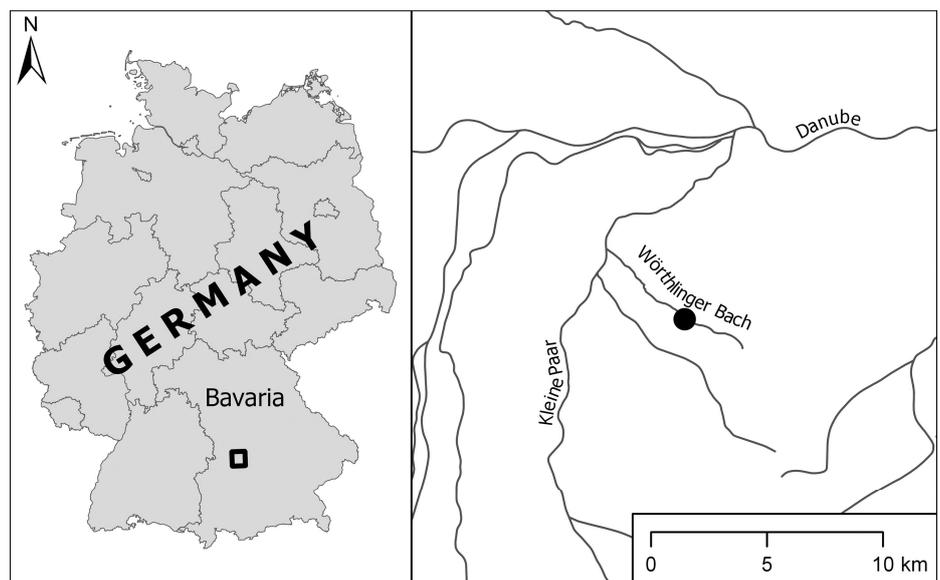


Figure 2. Location where two weather loach specimens were captured in the stream “Wörthlinger Bach” by electrofishing.

Materials and methods

Intense field samplings using electrofishing of several southern German streams, backwaters, and ponds in 2017 were conducted by the Bavarian Environment Agency (Augsburg, Germany). During this survey, two weather loach individuals (total length: fish A: 22.0 cm, fish B: 19.5 cm, Figure 1), missing the key characteristics of *M. fossilis* (typical stripes) and *M. anquilicaudatus* (dark spot at upper caudal base), were caught in a pond located in the former channel of the small stream “Wörthlinger Bach” (Lat: 48°39'45.318384”; Lon: 11°1' 54.120648”, Bavaria, Germany, Figure 2), on May 18th, 2017.

Morphological species identification followed the taxonomic key provided by Kottelat and Freyhof (2007), and the species description given in Kottelat (2012). For both fish, fin clips of the right pelvic fins were taken and subsequently stored in 80% pure ethanol until further processing in the laboratory.

DNA-extraction followed the phenol-chloroform protocol according to Sambrook et al. (1989). Amplification of the mitochondrial COI-5P gene was performed using the fish barcoding protocol with the M13 tailed primer cocktail C_FishF1t1 and C_FishR1t1 (Ivanova et al. 2007). The approximately 730 bp PCR products were cleaned (NucleoSpin®Gel and PCR Clean-up Kit, Macherey & Nagel) and sequenced (Eurofins GmbH, Munich).

The obtained sequences were inspected manually, trimmed and submitted to GenBank (accession numbers MH107300 and MH107301). The sequences were included in a query search using GenBank's BLASTN (highly similar sequences [megablast], Zhang et al. 2000) and the species identification tool in BOLD (Barcode of Life Data System, Ratnasingham and Hebert 2007) version 4 in October 2017 for species identification.

For the phylogenetic analysis and species assignment, sequences were aligned with published *M. anguillicaudatus*, *M. fossilis*, *P. dabryanus*, *Cobitis taenia* and *Pangia pangio* (as outgroup) COI-sequences from Chen et al. (2015), Geiger et al. (2014) and Knebelsberger et al. (2015) sourced in BOLD and GenBank. Maximum Likelihood method implemented in MEGA7 (Kumar et al. 2016) was used to determine the best substitution model. A phylogenetic tree of these sequences using the best-fit model (HKY+I) and the maximum intra- and minimum interspecific uncorrected p-distances (Meier et al. 2008) to estimate the “barcoding gap” between the species were also computed with MEGA7. The nucleotide and haplotype diversity within species was estimated with ARLEQUIN 3.5 (Excoffier and Lischer 2010).

Results

Both individuals were suspected as female *P. dabryanus* based on the following diagnostic morphological characters: (1) no enlarged second rays of the pectoral fins, thus female; (2) the absence of a lamina circularis on the pectorals; (3) the absence of a dark dot on the upper caudal base, therefore excluding *M. anguillicaudatus sensu lato*; and (4) the absence of a broad midlateral stripe from eye to caudal base, and absence of the narrow stripe from opercle to the pelvic origin, thus excluding *M. fossilis* (Kottelat and Freyhof 2007) (Figure 1).

Genetically, both individuals displayed different haplotypes, and retrieved maximum similarity of the 630 bp COI-sequences with database entries of the species *P. dabryanus* clade 2. Best-match sequences were originating from either Hubei province (unpublished sequences in BOLD taxon tree results) or along the Nujiang River, China (99% GenBank BLAST; 99.85% BOLD species identification tool, Figure 3). Similarity with the other *Paramisgurnus* clade (clade 1) was slightly lower, with 97% to 96%. Similarities to other weather loach species were much lower, 86% for

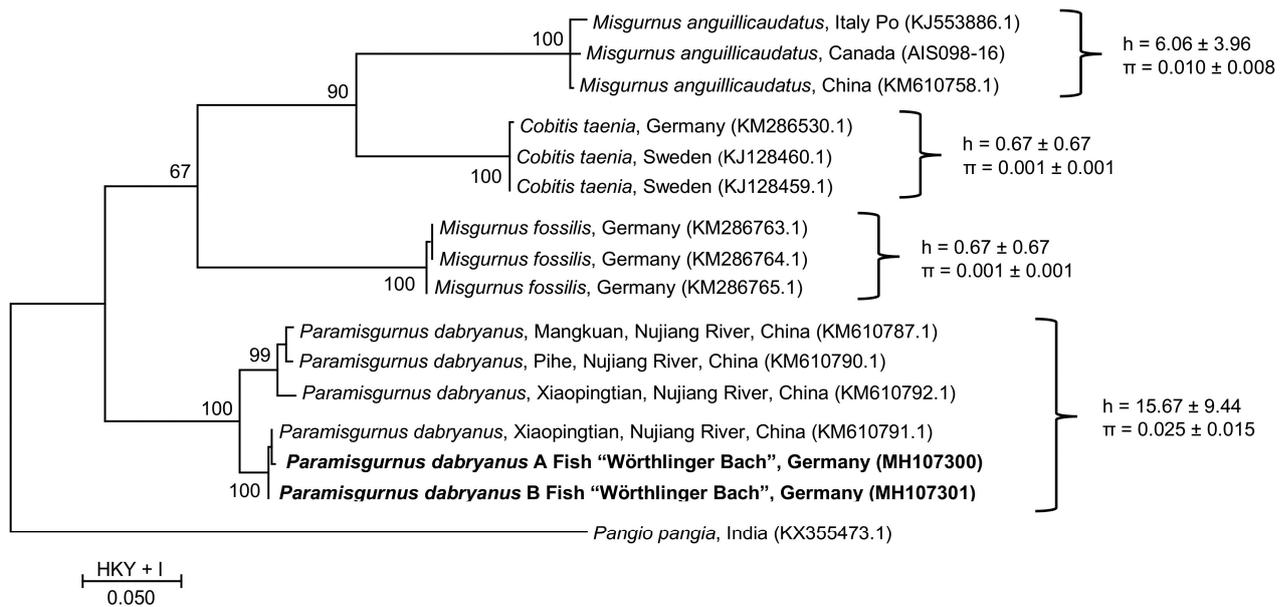


Figure 3. Maximum likelihood (ML) estimates of phylogenetic relationships of *Paramisgurnus dabryanus* (studied individuals in bold), *Misgurnus fossilis*, *M. anguillicaudatus*, and *Cobitis taenia* COI sequences (630 bp). Nodes are labeled with the highest bootstrap support by ML (1.000 replications). All branches had bootstrap support above 60%. The scale bar shows the best fit model chosen based on the Bayesian Information Criterion approach (Hasegawa-Kishino-Yano model with evolutionarily invariable sites, HKY+I, above), and substitutions/site (below). In brackets, the GenBank accession numbers of the published sequences used for this analysis are shown. Values beside curly brackets indicate the estimated nucleotide (π) and haplotype (h) diversity.

M. fossilis, and 85% for *M. anguillicaudatus*, respectively. Consequently, the phylogenetic clustering of the trimmed barcodes using the Maximum Likelihood method implemented in MEGA7 clearly suggests an assignment of the specimens to *P. dabryanus* clade 2 (Figure 3).

The minimum interspecific genetic distance between COI sequences of *P. dabryanus* and *M. fossilis* / *M. anguillicaudatus* was 14.5%, and 15.1%, respectively. The maximum intraspecific uncorrected p-distance in the species *P. dabryanus* was 3.8%. Intra-clade distances were much smaller, with a maximum of 1.6% for clade 1, and 0.3% for clade 2 to which both individuals could be assigned to. The minimum inter-clade distance was 3.3%.

Discussion

In our study, we report a new record of feral large-scale loach, *P. dabryanus*, in Europe, and verify it by morphological as well as genetic species identification.

So far, the invasion history of *P. dabryanus* in the studied pond remains unknown. A release of specimens in the context of ornamental trade is a likely option which was also suggested for Great Britain (Zięba et al. 2010) and the USA (Kirsch et al. 2018), with the same explanation being likely for populations of feral *M. anguillicaudatus* in southern Germany (Belle et al. 2017). The unintentional introduction of the Asian weather loaches in the context of *M. fossilis* conservation-related restocking programmes could also be possible, but up to now both ways of introduction cannot be proven. So far, it is not known whether the specimens belong to a self-sustaining

population. Furthermore, nothing is known about any possible impacts of *P. dabryanus* on other species and the ecosystem of the introduced area. The species has high reproductive potential (Chu et al. 2012) and is an omnivorous benthic feeder preying on zooplankton, macroinvertebrates, and algae (Wang and Li 2005; Kanou et al. 2007). Therefore, interference with other benthic feeders is possible, especially when population densities are high (Hazelton and Grossman 2009). It is likely, that the occurrence of *P. dabryanus* can influence populations of the congeneric native species, but further research is needed to clarify this issue. Hybridization between native and invasive fish species resulting in major consequences for the native fish fauna is also a widespread problem worldwide (Fukui et al. 2016). It has been shown that *P. dabryanus* and *M. anguillicaudatus* can hybridize in natural populations (You et al. 2009). Hybridization could also occur between native and invasive Asian loach species, resulting in a serious threat for *M. fossilis* populations as already described for many other fish species (e.g., Allendorf and Leary 1988; Leary et al. 1993).

A wider geographic distribution of *P. dabryanus* in Europe than currently assumed seems to be highly likely due to recent reports of this species based on morphological characteristics (Zięba et al. 2010; Freyhof 2013; Bundesamt für Umwelt BAFU 2014) and allozyme electrophoresis (Riffel et al. 1994). Furthermore, invasions might be disguised by the occurrence of morphologically highly similar Asian species of *Misgurnus* (Chen 1981; Shen et al. 2016; Yi et al. 2017). Luckily, from a conservation perspective, the native and threatened *M. fossilis* still can reliably be identified by its pigmentation pattern and the lack of a lamina circularis in males.

Further studies are needed to reveal the actual geographic distribution, the dynamics of dispersal, and potential connectivity between populations of *P. dabryanus* in Europe. This is especially important with respect to the difficult phenotypic species identification in the genera *Misgurnus* and *Paramisgurnus* (Chen 1981; Shen et al. 2016; Yi et al. 2017). Since the ability of interspecific hybridization (You et al. 2009) might also hinder, or even preclude, the identification of Asian species of *Misgurnus* and *Paramisgurnus* based solely on mitochondrial DNA sequences or morphology, we recommend further molecular studies using nuclear markers, e.g., microsatellites and SNPs, for such purposes.

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References

- Allendorf FW, Leary RF (1988) Conservation and distribution of genetic variation in a polytypic species: the cutthroat trout. *Conservation Biology* 2: 170–184, <https://doi.org/10.1111/j.1523-1739.1988.tb00168.x>
- 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 and Management of Aquatic Ecosystems* 418: 1–5, <https://doi.org/10.1051/kmae/2017025>
- Bundesamt für Umwelt BAFU (2014) BASISDATEN AUS DEM BIODIVERSITÄTS-MONITORING SCHWEIZ BDM; Artenvielfalt in der Schweiz und in den Regionen. http://www.biodiversitymonitoring.ch/fileadmin/user_upload/documents/daten/basisdaten_dt/1180_Z3_Basisdaten_2013_V1_dt.pdf
- Chen JX (1981) A study on the classification of the subfamily Cobitinae of China. *Transactions of the Chinese Ichthyological Society* 1: 21–31
- Chen W, Ma X, Shen Y, Mao Y, He S (2015) The fish diversity in the upper reaches of the Salween River, Nujiang River, revealed by DNA barcoding. *Scientific Reports* 5: 17437, <https://doi.org/10.1038/srep17437>
- Chu Z, Lu G, Hu T, Wang H, Dai L, Huang W (2012) Comparative analysis on fecundity of *Misgurnus anguillicaudatus* and *Paramisgurnus dabryanus*. *Hubei Agricultural Sciences* 13: 2794–2796
- Council of the European Communities (1992) Directive 92/43/EEC of the European Parliament and the Council of 21st May 1992 on the conservation of natural habitats and of wild fauna and flora. *Official Journal of the European Communities* L206: 7–50
- Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564–567, <https://doi.org/10.1111/j.1755-0998.2010.02847.x>
- Freyhof J (2013) *Misgurnus fossilis*. The IUCN Red List of Threatened Species 2013, e.T40698A10351495, <https://www.iucnredlist.org/species/pdf/10351495> (accessed 16 August 2017)
- 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>
- Fukui S, May-McNally SL, Katahira H, Kitano S, Koizumi I (2016) Temporal change in the distribution and composition of native, introduced, and hybrid charrs in northern Japan. *Hydrobiologia* 783: 309–316, <https://doi.org/10.1007/s10750-016-2688-8>
- Geiger MF, Herder F, Monaghan MT, Almada V, Barbieri R, Bariche M, Berrebi P, Bohlen J, Casal-Lopez M, Delmastro GB, Denys GPJ, Dettai A, Doadrio I, Kalogianni E, Käst H, Kottelat M, Kovačić M, Laporte M, Lorenzoni M, Marčić Z, Özuluğ M, Perdices A, Perea S, Persat H, Porcelotti S, Puzzi C, Robalo J, Šanda R, Schneider M, Šlechtová V, Stoumboudi M, Walter S, Freyhof J (2014) Spatial heterogeneity in the Mediterranean Biodiversity Hotspot affects barcoding accuracy of its freshwater fishes. *Molecular Ecology Resources* 14: 1210–1221, <https://doi.org/10.1111/1755-0998.12257>
- Hazelton PD, Grossman GD (2009) Turbidity, velocity and interspecific interactions affect foraging behavior of rosyside dace (*Clinostomus funduloides*) and yellowfin shiners (*Notropis lutipinnis*). *Ecology of Freshwater Fish* 18: 427–436, <https://doi.org/10.1111/j.1600-0633.2009.00359.x>
- Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B - Biological Sciences* 270: 313–321, <https://doi.org/10.1098/rspb.2002.2218>
- Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes* 7: 544–548, <https://doi.org/10.1111/j.1471-8286.2007.01748.x>
- 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
- Kirsch JE, Feeney RF, Goodbla A, Hart C, Jackson ZJ, Schreier A, Smith R (2018) The 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>
- Kottelat M (2012) Conspectus cobitidum: an inventory of the loaches of the world (Teleostei: Cypriniformes: Cobitoidei). *Raffles Bulletin of Zoology* (Suppl. 26): 1–199
- Kottelat M, Freyhof J (2007) Handbook of European Freshwater Fishes, Publications Kottelat, Cornol, 646 pp
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger data sets. *Molecular Biology and Evolution* 33: 1870–1874, <https://doi.org/10.1093/molbev/msw054>

- Leary RF, Allendorf FW, Forbes SH (1993) Conservation genetics of bull trout in the Columbia and Klamath River drainages. *Conservation Biology* 7: 856–865, <https://doi.org/10.1046/j.1523-1739.1993.740856.x>
- Meier R, Zhang G, Ali F (2008) The use of mean instead of smallest interspecific distances exaggerates the size of the “barcoding gap” and leads to misidentification. *Systematic Biology* 57: 809–813, <https://doi.org/10.1080/10635150802406343>
- 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
- Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* 7: 355–364, <https://doi.org/10.1111/j.1471-8286.2007.01678.x>
- Riffel M, Schenk M, Schreiber A (1994) Electrophoretic differentiation between European loach (*Misgurnus fossilis* L.) and Oriental weatherfish (*Misgurnus mizolepis* Günther), an autochthonous and a feral species of central European freshwater fish. *Zeitschrift für angewandte Zoologie* 80: 473–483
- Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbour, 1626 pp
- Shen Y, Kang J, Chen W, He S (2016) DNA barcoding for the identification of common economic aquatic products in Central China and its application for the supervision of the market trade. *Food Control* 61: 79–91, <https://doi.org/10.1016/j.foodcont.2015.08.038>
- Wang YJ, Li D (2005) Research on biological characters and exploitations of *Paramisgurnus dabryanus* Sauvage [J]. *Special Wild Economic Animal and Plant Research* 1: 60–62
- Yi S, Zhong J, Wang S, Huang S, Wang W (2016) 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>
- 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>
- Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7: 203–214, <https://doi.org/10.1089/10665270050081478>
- 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 *Leucaspius delineatus* (Heckel, 1843). *Aquatic Invasions* 5: 155–161, <https://doi.org/10.3391/ai.2010.5.2.04>