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Rapid Communication**First record of an established population of *Misgurnus anguillicaudatus* (Cantor, 1842) (Teleostei, Cobitidae) in France**Gaël P.J. Denys^{1,2} and Sébastien Manné³¹Unité Patrimoine Naturel – Centre d'expertise et de données (2006 OFB – CNRS – MNHN – IRD), Muséum national d'Histoire naturelle, 36 rue Geoffroy-Saint-Hilaire CP 41, 75005 Paris, France²UMR Biologie des organismes et écosystèmes aquatiques (BOREA 8067), MNHN, CNRS, IRD, SU, UCN, UA, 57 rue Cuvier CP26, 75005 Paris, France³Office Français de la Biodiversité, Direction Régionale du Grand Est, Chemin du Longeau Rozérieulles, 57160 Moulins-les-Metz, FranceCorresponding author: Gaël P.J. Denys (gael.denys@mnhn.fr)

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OPEN ACCESS**Abstract**

A population of weatherloach has been detected in the Schadgraben stream at Geispolsheim, near Strasbourg (Rhine catchment, eastern France) in autumn 2021 and identified as the European weatherloach *Misgurnus fossilis*. Another sixty-three weatherloaches were caught in this location the 19 May 2022. The examination of morphological and molecular data on preserved specimens allows to identify this population as the dojo weatherloach *Misgurnus anguillicaudatus*, an invasive species. The length-weight relationship of this population highlights its good health and establishment. A review of the knowledges about this invasive species is also done. Monitoring must be put in place in order to follow its propagation, threatening native fishes like the endangered *M. fossilis*.

Key words: Dojo weatherloach, Rhine drainage, invasive species; DNA Barcoding; length-weight relationship**Introduction**

Weatherloach *Misgurnus* spp. *sensu lato* species (as a paraphyletic genus including *Paramisgurnus*) are the largest cobitids (with lengths up to 30 cm); they live buried in the mud of ponds and lentic Eurasian waterways but also happen in lotic systems (Kottelat and Freyhof 2007; Kottelat 2012). Among them, only the European weatherloach *Misgurnus fossilis* (Linnaeus, 1758) is native in Northern Europe with a septentrional distribution from the Meuse drainage (North Sea basin) to the Volga and Ural drainages (Caspian basin), including the whole Danube drainage (Kottelat and Freyhof 2007). The main character to easily identify this species is the presence of a broad midlateral stripe from the eye to the caudal base bracketed by two narrow stripes, from the opercula to at least the pelvic origin (Kottelat and Freyhof 2007). This species is threatened by stream regulations and agriculture activities (draining of wetlands and pesticides) causing populations to dwindle (see Hartvich et al. 2010; Freyhof 2011). The European weatherloach

is then a species of international conservation concern, listed in the Annex II of the European Habitat Directive (Council of the European Union 1992).

However, three Asian weatherloach species have been introduced in Europe mostly for their aquariophilic interest as ornamental fish, even if their use as food resources and live baits have been recorded elsewhere in the world (Froese and Pauly 2022). The dojo weatherloach *Misgurnus anguillicaudatus* (Cantor, 1842) was detected in Italy (Razzetti et al. 2001; Geiger et al. 2014), in Germany (Freyhof and Korte 2005; Belle et al. 2017), in Spain (Franch et al. 2008; Aparicio et al. 2016; Clavero et al. 2023), in England (Zięba et al. 2010) and in Netherlands (van Kessel et al. 2013). In France, dojo weatherloach was previously reported by only one specimen caught in the Orge river (Seine drainage) in the south suburb of Paris at Sainte-Geneviève-des-Bois in 2010. However, no more specimen was caught since then, despite some attempts (electrofishing and netting). This species was then considered as non-established (Chauvigné 2011), and was listed as introduced non-established in France in the French taxonomic register TAXREF (Gargominy et al. 2022) of the National Inventory of Natural Heritage (*Inventaire du Patrimoine Naturel* – INPN). The large-scale loach *Misgurnus dabryanus* (Dabry de Thiersant, 1872) was encountered in Germany (Riffel et al. 1994; Stoeckle et al. 2019), in Switzerland (Marchesi 2010), in Catalonia (Clavero et al. 2023) and also in France (Cuinet et al. 2024). Finally, *Misgurnus bipartitus* (Sauvage & Dabry de Thiersant, 1874) was detected in Austria (Zangl et al. 2020) and in Catalonia (Clavero et al. 2023).

On the 11th of October 2021, a French consulting engineer inventoried the Schadgraben stream at Geispolsheim, near Strasbourg (Rhine catchment, eastern France). They caught several dozens of weatherloach identified as *M. fossilis*, but the coloration pattern presented black stains on the flanks instead of the three longitudinal stripes. So, their identification was erroneous and must be an Asian weatherloach species. So the French Biodiversity Agency (OFB), with the collaboration of the Muséum national d'Histoire naturelle (MNHN), collected specimens at this location on the 19th of May 2022. The aim of this study is to identify the species present in this location with morphological and molecular data in order to avoid the risk of misidentification (Belle et al. 2017, 2021), as well as to characterize the population establishment.

Materials and methods

Studied area

From its source at Geispolsheim, the Schadgraben stream flows about 7 km into the Ill river, an affluent of the River Rhine (Figure 1). At Geispolsheim, the entire stretch is represented by a very artificial channel, straight and deep (banks 1 to 1.8 m high). The wetted width changes from 0.5 m to 5–6 m, upstream to downstream. Its typology is an eutrophic system stream with

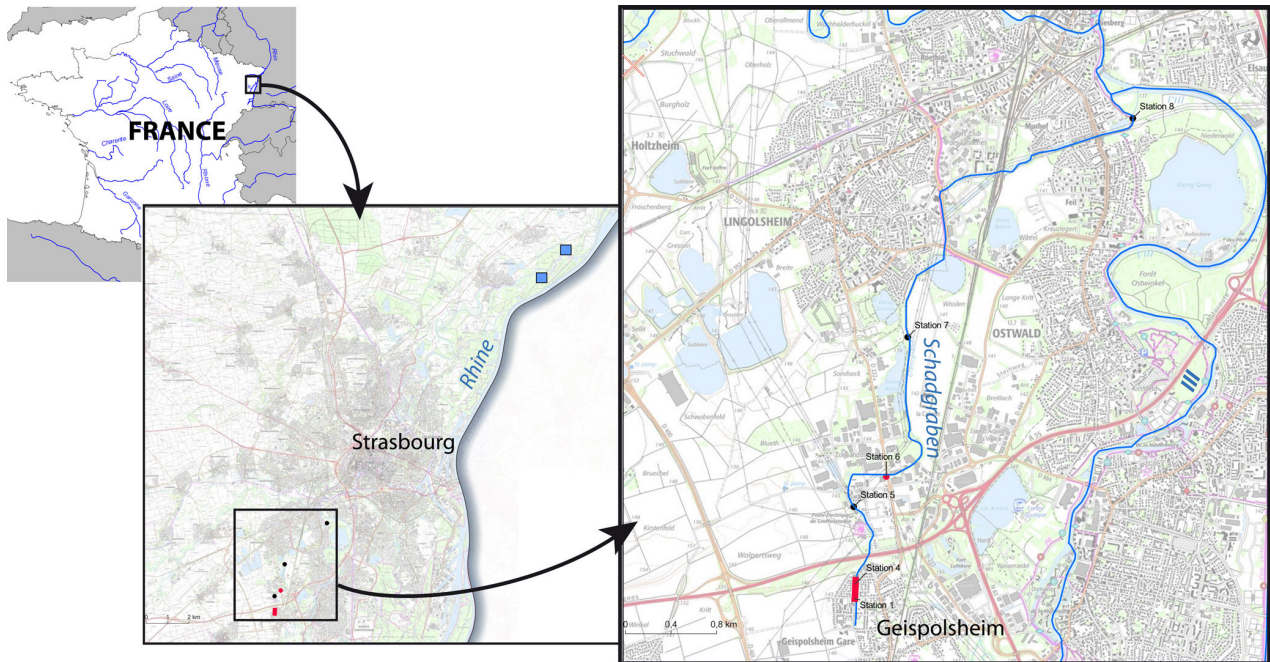


Figure 1. Sampling locations prospecting the presence of dojo weatherloach *M. anguillicaudatus* in the Schadgraben stream at Geispolsheim on 19th May 2022. Red dots indicate locations where dojo weatherloach were fished, black dots indicate absences of capture, and blue squares mean location of the native and endangered European weatherloach *M. fossilis*.

no flow at the time of the inventories, no current speed, a water height of about 20 to 40 cm with some deepest zones (> 1 m of depth), and sediments composed by decaying organic matter at the bottom (Figure 2).

Sampling

Fishes were caught by electrofishing from the shore, with a portable device (“Martin Pêcheur” from Dream Electronique), at seven stations along the stream where the consulting engineer detected Asian weatherloaches (Figure 1; Table 1). Individuals were measured in total lengths and weighted but not sexed.

After being euthanised by eugenol overdose, a subsample of five specimens were fixed and preserved in EtOH, concentration progressively increasing from 40% to 95% in the span of a few hours in order to reduce shrinking effects. They were sent to the MNHN (catalogue number MNHN-IC-2022-0360) for morphological and molecular identification.

Identification

Specimens were identified morphologically following Kottelat and Freyhof (2007) and Zhang et al. (2021), as well as with molecular data. Measurements of the caudal peduncle length and depth were done with a digital calliper to the nearest 0.1 mm, point by point, never by projection.

A DNA Barcoding analysis *sensu* Hebert et al. (2003) with a mitochondrial marker (cytochrome *c* oxidase subunit 1: COI) was also performed. DNA extraction, PCR, sequencing and sequence treatments follow Denys et al. (2014). Obtained COI sequences (652 bp) were deposited in GenBank (accession number OR978333 to OR978337).



Figure 2. Stations features where dojo weatherloaches *M. anguillicaudatus* were caught in the Schadgraben stream at Geispolsheim on the 19th May 2022: upstream and downstream from the bridge rue des Suédois (stations 1 and 2), upstream the bridge of Lingolsheim road (station 6). Credit photos: S. Manné / OFB.

Table 1. Locations of electrofishing with the number of weatherloaches caught in the Schadgraben stream at Geispolsheim on 19th May 2022.

	Precise location	Latitude	Longitude	Location length	Number of weatherloaches caught
Station 1	Upstream the bridge (rue des Suédois)	48.52378 N	7.68001 E	6 m	40
Station 2	Downstream the bridge (rue des Suédois)	48.52389 N	7.68002 E	4 m	11
Station 3	60 m upstream the bridge (rue Georges Bizet)	48.52475 N	7.68016 E	3 m	2
Station 4	40 m upstream the bridge (rue Georges Bizet)	48.52523 N	7.68154 E	3 m	4
Station 5	Stable	48.53148 N	7.68011 E	10 m	0, only two <i>Carassius auratus</i>
Station 6	Upstream the bridge of Lingolsheim road	48.53325 N	7.68457 E	10 m	5 (not measured)
Station 7	North of Wiesel pond	48.54413 N	7.68813 E	10 m	0

The molecular identification was made with a data frame composed by sequences used by Belle et al. (2021), excluding the ones for which the identification did not match with the mitochondrial affiliation. Thus, our molecular data frame (Supplementary material Appendix 1) contains 235 sequences.

Aligning, p-distances and NJ-tree reconstruction based on the DNA barcodes were performed under MEGA X (Kumar et al. 2018) with the Kimura 2 parameter model (K2P; Kimura 1980). Bootstrap values (Felsenstein 1985) with 1000 replicates, were also calculated for evaluating the robustness of clusters.

Length-Weight relationship

Studying the length–weight relationship (LWR) allows the estimation of fish growth as well as ecological modelling (Froese 2006). An ANOVA was first computed to confirm the statistical significance of the regression model ($P < 0.01$). LWR was calculated following the formula $\log_{10}W = \log_{10} a + b \log_{10}L$, where W is the weight (g), a is the intercept on the Y -axis of the regression curve, b is the regression coefficient and L is the total length (cm) (Ricker 1975). A value of $b = 3.0$ represents an isometric growth due to some environmental circumstances or the intrinsic condition of the fish (Ricker and Carter 1958). A value of $b < 3.0$ means a “negative” growth, *ie* the fish become slimmer with increasing length. Whereas a value of $b > 3$ means a positive allometric growth, so the fish become heavier, reflecting optimum conditions for growth. LWR was calculated for unsexed individuals. The 95% confidence limits of a and b (CL 95%) were also computed following Froese (2006). Finally, the coefficient of determination (R^2) was calculated in order to measure the quality of a linear regression’s prediction.

To evaluate the suitability of the water body for fish growth, the relative condition factor K_n was computed following the formula $K_n = \frac{W_o}{W_c}$, where W_o is the observed weight and W_c is the calculated weight from the previously obtained regression equation (Le Cren 1951). However, there may be a bias because the stomachs of the fish were not emptied before weighting. Fish are then considered in good growth condition when $K_n \geq 1$, whereas an organism in poor growth condition would have a $K_n < 1$ compared to an average individual with the same length.

Statistics tests were performed using R package (R Core Team 2022).

Results

Sixty-four were totally caught during the operation, including 62 weatherloaches and two goldfish *Carassius auratus* (Linnaeus, 1758). Weatherloaches were collected mainly in the first four sites, corresponding to a linear length of about 170 m, but were not recorded in and tend to be absent from downstream ones (except in the station 6 located 1.3 km downstream from the station 4) (Figures 1, 2; Table 1).

Determination

Specimens differ from *M. fossilis* by a dark spotted body (*vs.* body with three dark bands) (Figure 3). They are also different from *M. dabryanus* by a caudal peduncle with low adipose crests (*vs.* high adipose crests) and the presence of a black spot at the upper caudal base (*vs.* no black spot at the upper caudal base). Finally, they are different from *M. bipartitus* by long barbels reaching or exceeding the eyes (*vs.* short barbels not reaching the eyes) and by a short caudal peduncle with a ratio length/depth 1.5 to 1.9 (*vs.* long caudal peduncle with a 2.2 to 3.5 ratio). So morphological data allow the identification as *M. anguillicaudatus*.



Figure 3. Two specimens of dojo weatherloach *M. anguillicaudatus* caught in the Schadgraben stream at Geispolsheim on the 19th May 2022. The upper picture shows the pigmentation pattern of the body, low adipose crests on the caudal peduncle and the presence of a block spot at upper caudal base. The bottom picture highlights the long barbels. Credit photos: S. Manné / OFB.

The Neighbour-Joining analysis with the 235 COI sequences (607 bp) of the reference dataframe puts the five weatherloaches from the Schadgraben stream within the *Misgurnus anguillicaudatus* cluster (Figure 4). Our specimens share the same haplotype with four sequences from the Yangtze drainage (Wuhan, China: KP112321, KP112320, MF122497, MF122502) and one from a Canadian aquarium (KX224162).

Length-Weight relationship

Of the 62 weatherloaches caught, 57 were measured from 8.7 to 15.4 cm in total length ($\bar{L} = 11.9$ cm) and weighted from 3 to 21 g ($\bar{w} = 9.4$ g). The regression (Appendix 2) is supported with an R^2 equal to 0.9455. The a parameter is equal to 0.0023 (Range a [95% CL] = 0.0014–0.0039), and the b parameter is equal to 3.3258 (Range b [95% CL] = 3.1101–3.5416).

The relative condition factor was also calculated with $\bar{k}_n = 1.055$ (Range = 0.7165–1.2258; $sd = 0.1041$).

Discussion

Morphological and molecular data corroborate the identification of the Asian weatherloach population found in the Schadgraben stream in France as the dojo weatherloach *Misgurnus anguillicaudatus*.

(Logan et al. 1996), as well as a great tolerance to thermal amplitudes from 0 °C to 38 °C (Strecker et al. 2011) and can also survive fully enclosed in ice (Urquhart and Koetsier 2014a). Moreover, considering its high fecundity, with multiple spawning events during the breeding season, allowing a high reproductive potential (Suzuki 1983), and its large dietary range, from detritus to macroinvertebrates (Tabor et al. 2001; Urquhart and Koetsier 2014b), *M. anguillicaudatus* fulfil all criteria to be considered an invasive species (e.g., Keller et al. 2011).

Furthermore, the dojo weatherloach may induce several dire changes in the environment and the native aquatic fauna. It causes significant reductions in macroinvertebrate numbers and biomass and changes its environment, elevating ammonia, nitrate/nitrite and turbidity levels (Keller and Lake 2007). It is also a vector of Asian pathogens such as the Birnavirus LV1, larval nematodes *Contracaecum bancrofti* Johnston & Mawson, 1941 and several monogenea *Gyrodactylus* spp. infecting native fish, amphibians, piscivorous birds and humans as it was recorded in Australia and USA (Wolf 1988; Dove and Ernst 1998; Shamsi et al. 2019; Reyda et al. 2020). Finally, there is also a threat for the two populations of *M. fossilis*, a species evaluated as Endangered in the French Red List IUCN (UICN comité français et al. 2019), present about 20 km downstream from the dojo weatherloach population (Figure 1), with the risk of hybridization (Wanzenböck et al. 2021).

This dojo weatherloach population of the Schadgraben stream must be monitored realizing eradication operations before its spread in the Ill river; see Britton et al. (2011) and Donaldson and Cooke (2016) for the different methods. Adjacent waterbodies must also be surveyed with regular electrofishing operations and with molecular methods such as eDNA in order to maximize early detections and thus the eradication efficiencies due to rapid responses. The latter appears to be more efficient to detect this species (Hinlo et al. 2018; Roy et al. 2017) but see Jerde (2020) about the risk of results misinterpretation. Several studies were published optimizing the efficiency of the detection such as sampling a minimum of 10 L of water during the spring or early summer (Furlan et al. 2016; Jo et al. 2020).

Determination mistakes can have consequences for management (Bortolus 2008), since we went from a new report of an endangered species *M. fossilis* to the first occurrence of an invasive species *M. anguillicaudatus*. The taxonomic skills tend to disappear, it is however still important to train riverine actors, such as managers and consulting engineers, to identify the native but also the alien fish species in order to undertake as adapted monitoring and managing measures as possible.

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Ethics and permits

Electrofishing permit was provided according to the prefectural decree of the Bas-Rhin department dated 12 March 2018 allowing to the French Agency of Biodiversity all investigations in the department from 2018 to 2022. No ethics was required.

Authors' contribution

GPJD and SM conceptualized research. SM designed sampling and methodology, investigated and collected data, and provided funding. GPJD analysed and interpreted data. Both authors wrote and edited the manuscript.

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Supplementary material

The following supplementary material is available for this article:

Appendix 1. List of GenBank accession numbers used as molecular dataframe for the Barcoding analysis.

Appendix 2. Length-weight relationship of the population of dojo weatherloach *M. anguillicaudatus* caught in the Schadgraben stream at Geispolsheim.

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