BioInvasions Records (2025) Volume 14, Issue 4: 929–943

Research Article

First records of the black bullhead *Ameiurus melas* (Rafinesque, 1820) and its parasites in Lithuania

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Citation: Rakauskas V, Kaupinis A, Binkienė R, Baker NJ, Virbickas T, Kudlai O (2025) First records of the black bullhead *Ameiurus melas* (Rafinesque, 1820) and its parasites in Lithuania. *BioInvasions Records* 14(4): 929–943, https://doi.org/10.3391/bir.2025.14.4.14

Received: 28 March 2025
Accepted: 5 September 2025
Published: 29 October 2025

Handling editor: Kęstutis Arbačiauskas
Thematic editor: Karolina Bącela-Spychalska

Abstract

This study documents a new introduction of *Ameiurus melas* in Lithuania, discovered within two lentic waterbodies in the summer of 2024. Specimens of *A. melas* were found at high densities, raising many questions about their potential impacts in the shallow, eutrophic waterbodies in which they were found. Using molecular and morphological identification, the identity of *A. melas* was confirmed from both waterbodies. The abundance and age structure of *A. melas* were evaluated and their potential introduction pathways are discussed. An initial parasitological survey revealed that the newcomer co-introduced a monogenean species of North American origin, *Ligictaluridus pricei*. The study also found that *A. melas* could facilitate the transmission of local trematodes of the genus *Diplostomum* in Lithuanian freshwater ecosystems.

Key words: invasive alien fish, Ictaluridae, nuclear and mitochondrial DNA, ecological impact, fish assemblage

Introduction

North America is a donor of many catfish species, which tend to be highly tolerant to abiotic stressors such as high water temperatures and low oxygen levels (Scott and Crossman 1973; Stuber 1982; Ribeiro et al. 2008), and are capable of establishing, spreading, and exerting impacts of freshwater ecosystems around the world (Wheeler 1978; Copp et al. 2005; Kottelat and Freyhof 2007). Some of these catfish species belong to the genus *Ameiurus*, which continue to spread across Europe. Catfishes of the genus *Ameiurus* have been established in Europe for over 120 years (Kottelat and Freyhof 2007; Nowak et al. 2010; Rutkayová et al. 2013) and their recent northward expansion is of great concern in view of their high fecundity, ecological plasticity, and adverse ecological impact on native fish populations (Commission Implementing Regulation (EU) 2022/1203). For many years, the brown bullhead *Ameiurus nebulosus* (Lesueur, 1819) and the black bullhead *Ameiurus melas* (Rafinesque, 1820) have been present in waters of countries neighbouring Lithuania, such as Poland (Nowak et al. 2010) and Belarus (Okhremenko and Gajduchenko 2024). Given that these countries share waterbodies such as the Neris and Nemunas rivers, and invasive species do not recognize political boundaries, it was inevitable that one or both species would eventually reach Lithuanian waters. However, no bullhead had been recorded in Lithuania until now. This study presents the first records of *A. melas* from the inland waters of Lithuania.

Ameiurus melas is a small catfish of the family Ictaluridae. The native range of *A. melas* is the eastern half of North America, from southern Canada to northern Mexico (Kottelat and Freyhof 2007). It has become successfully established in many European countries, including Germany, Switzerland, Czechia, Poland, Slovakia, Hungary, Serbia, Montenegro, Bulgaria, Romania, and Albania (Kottelat and Freyhof 2007; Copp et al. 2016; Rutkayová et al. 2013; Vancheva et al. 2020). *Ameiurus melas* is a benthic fish capable of tolerating low oxygen levels and high water temperatures (Moyle 1986; Ribeiro et al. 2008). The species is found in lakes, ponds, oxbows, and reservoir meliorative canals characterised by dense aquatic vegetation and muddy bottoms (Kottelat and Freyhof 2007).

Parasites, especially helminths, often co-invade ecosystems outside their native distribution range along with their non-native fish hosts (Lymbery et al. 2014). When a non-native fish species is introduced to an ecosystem outside its native distribution range, several scenarios can occur. For instance, non-native fish can lose their parasites and pathogens in their newly invaded ecosystems (enemy release), providing them a competitive advantage over native fish burdened by parasites, and parasite transmission may reoccur from native hosts back to non-native hosts (acquisition, spillback). Alternatively, non-native hosts can be introduced with their parasites, which may be transmitted to native hosts (spillover, co-invasion). The consequences of fish introductions with parasites and other pathogens are often neglected in wild fish populations (Lagrue 2017; Gendron and Marcogliese 2017; Hohenadler et al. 2019; Magalhães et al. 2024). It is therefore vital to examine invasive fish for parasites as soon as they enter new ecosystems to understand and prevent the potential ecological, economic, and health risks posed by these fish and their parasites.

The objective of this study was to provide proof of the occurrence of *A. melas* in Lithuanian freshwaters. We applied morphological and molecular analysis for species identification. Furthermore, we examined the first recorded specimens of *A. melas* caught within Lithuanian freshwaters for the presence of helminth parasites. We compared our results to previous studies reporting on parasites from *A. melas* in Europe. Finally, we discuss the most likely species introduction vectors and pathways into Lithuanian freshwaters.

Materials and methods

Assessment of fish communities

Local anglers reported the presence of an exotic catfish (Figure 1) in two waterbodies within the Lithuanian portion of the Nemunas River basin during

Figure 1. Non-native *Ameiurus melas* collected in the studied sites located in Lithuania. Photo made by V. Rakauskas.

Table 1. Water bodies sampled for the presence of *Ameiurus melas*, indicating the number of collected specimens (N), as well as the surface area (A) and mean depth (D) of the waterbodies in Lithuania.

Site	Date	N	Coordinates (WGS84)	A (ha)	D (m)
Gravel pit	15.06.2024	29	55.603364, 21.200009	0.15	~ 2.0
Lake Privalskis	21.07.2024	41	54.573491, 24.525181	2.32	~ 1.5

the summer of 2024 (Table 1). The two waterbodies were 240 km apart (Figure 2). These water bodies were monitored and found to be overgrown by aquatic plants and to have muddy bottoms. They are both closed waters with no inflow or outflow. Fish communities were assessed once at each sampling site using multi-mesh benthic gillnets, each 40 m long and 3 m high, with mesh size (bar length) varying every 5 m from 14 mm to 60 mm. Two (Gravel pit) to four (Lake Privalskis) benthic gillnets, clustered into one set, were used. Nets were positioned to cover different depths and multiple habitats, and left for 12 hours overnight, including sunset and sunrise. All trapped fish were identified to species level, measured to the nearest 1 mm (total body length – TL) and weighed to the nearest 0.1 g. The standard catch per unit of effort (CPUE) was estimated as fish biomass

Figure 2. Map showing the sampling sites of non-native *Ameiurus melas* in Lithuania.

or number trapped per 40 m long multi-mesh benthic gillnet per sampling occasion. The fish taxonomy used in this paper followed FishBase (http://www.fishbase.org, accessed 2022.01.06). The age of *A. melas* was determined by examining the growth rings on the sagittal otoliths.

Morphological identification

We used the following morphological characters to distinguish *A. melas* from *A. nebulosus*: 1. enlargement and pigmentation of the anal fin membrane; 2. number of gill rakers; and 3. serrations on the pectoral fin spine. According to literature, these characteristics are best for distinguishing between these two species of *Ameiurus* (Kutsokon et al. 2024). Fifteen individuals from each waterbody were assessed using these characteristics.

Molecular identification

Since the morphological characteristics used for identifying *A. melas* are highly variable and overlap considerably with the characteristics of other *Ameiurus* species present in Europe, e.g., *A. nebulosus*, molecular identification was further used to confirm the identity of this species.

In total, five specimens of *A. melas* (Lake Privalskis n = 1; gravel pit n = 4) were used for molecular identification. The caudal fin of collected fish was preserved in 96% ethanol. Total genomic DNA of each specimen of *A. melas* was extracted from the ethanol-fixed fin tissue using the KAPA Express Extract Kit (KAPA Biosystems, Cape Town, South Africa). Two mitochondrial gene fragments, cytochrome *b* (*cyt*b) and cytochrome *c* oxidase subunit 1 (*cox*1) were amplified using the primers CytB_L_14724F (5′-GACTTGAA

AACCCACCGTTG-3′) and Cytbasa_R (5′-GAAGGCGGTCATCATAAC TAG-3′) following the polymerase chain reaction (PCR) protocol provided in Ondračková et al. (2020), and Fish1F (5′-TCAACCAACCACAAA GACATTGGCAC-3′) and Fish1R (5′-TAGACTTCTGGGTGGCCAAAG AATCA-3′) following the PRC protocol provided in Ward et al. (2005), respectively. The PCR products were purified using the ExoSAP-IT PCR Cleanup enzymatic kit from Thermo Fisher Scientific, Inc. (Waltham, MA, USA) and thereafter sequenced from both strands using the PCR primers. Geneious Prime software ver. 2023.2.5 (Biomatters, Auckland, New Zealand) was used to assemble and revise sequences. The newly obtained sequences were deposited in GenBank (PX310526, PX310527). The species identity was confirmed via the comparison of novel sequences of *A. melas* to those available in the GenBank database.

Parasite assessment

For parasite assessment, a total of 23 specimens of *A. melas* from both study sites (Lake Privalskis n = 15; Gravel pit n = 8) were examined in June and July of 2024. Fish were individually labelled and examined for helminth parasites under dissecting microscopes according to a standardized necropsy protocol in which the fins, body surface, gills, muscles, and all internal organs were separately examined. The total length of examined fish varied from 9.9 to 11.8 cm (mean length = 10.6 ± 0.4 cm), and they were determined to be 1–2 years old.

All recorded helminths were collected, rinsed in saline, counted, heat killed in saline, and preserved in 80% ethanol for morphological or in 96% ethanol for molecular analyses. The morphology of helminths selected for molecular analyses was studied using live specimens and a series of photomicrographs were made for each isolate prior its fixation; detailed procedures are described in Kudlai et al. (2024). The parasitological indices, prevalence (P %) and intensity of infection presented as min–max, were calculated according to Bush et al. (1997). Helminthological material collected during the present study is stored in the helminthological collection of State Scientific Research Institute Nature Research Centre, Lithuania.

Total genomic DNA was extracted from a single ethanol-fixed helminth specimen using the KAPA Express Extract Kit. For trematodes, partial fragments of *cox*1 gene were amplified using the primers Dice1F (5'-ATTA ACCCTCACTAAATTWCNT TRGATCAT AAG-3') and Dice14R (5'-TAATACGACTCACTATACCHACMRTAAACATAT GATG-3') following the PCR protocol provided in Van Steenkiste et al. (2015). For monogeneans, partial 28S rDNA sequences were amplified using the primers C1 (5′-ACC CGCTGAATTTAAGCA-3′) and D2 (5′-TGGTCCGTGTTTCAAGAC-3′) following the PCR protocol described in Hassouna et al. (1984). The methods of purification of PCR products, sequencing, and assembling of novel sequences

Table 2. Morphological characteristics used for the identification of *A. melas* introduced in Lithuania: number of specimens investigated (N); anal fin base enlargement (BE); anal fin membrane pigmentation (MP); number of spinae branchiales (gill rakers) (Sp. br.); serrations on pectoral-fin spine (SE).

Site	N	BE	MP	Sp. br.	SE
Gravel pit	15	Present	Black	18 – 19	Small, absent near tip
Lake Privalskis	15	Present	Black	18 – 19	Small, absent near tip

of parasites were the same as for fish samples described above. The taxonomic identity of the parasites was achieved using the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST/ accessed on February 2025). The newly obtained sequences were deposited in GenBank (Monogenea: PX369156, PX369157; Trematoda: PX369151–PX369155).

Results

Identification of A. melas

Anal-fin base enlargement was clearly visible in all examined individuals, especially in the older specimens (2–3+ years). The anal-fin membrane pigmentation on all investigated specimens was dark or black. The fin rays were always lighter in colour compared to the membrane pigmentation. The serrations on the pectoral-fin spine were consistently small or absent near the tip. The number of spinae branchiales (i.e., gill rakers) on the first gill arch varied between 18 and 19, thus always being greater than or equal to 18, conforming to the characteristics of *A. melas* (Table 2). Accordingly, the morphological analysis of the *Ameiurus* specimens collected from both waterbodies confirm their identity as *A. melas*.

The *cox*1 sequences (250 bp) obtained from the specimens sampled in both waterbodies were identical. They were also identical to sequences of both *A. melas* (OL806597) and *A. nebulosus* (NC068674) available in GenBank. However, the comparison of *cyt*b sequences (368 bp) to those of *A. melas* from North America (AY184263) and Europe (PP477076) and *A. nebulosus* from North America (AY184271) and Europe (PP496301) revealed that our sequences were identical to sequences of *A. melas* and differed from sequences of *A. nebulosus* by 1.4% (5 bp). Thus, molecular identification of the *Ameiurus* specimens collected from both sites confirmed morphological identification and demonstrated their taxonomic identity as *A. melas*.

Fish communities

A total of 243 specimens representing seven fish species were sampled during this study. These were *Alburnus alburnus* (Linnaeus, 1758), *A. melas*, *Carassius gibelio* (Bloch, 1782), *Gobio gobio* (Linnaeus, 1758), *Perca fluviatilis* (Linnaeus, 1758), *Rutilus rutilus* (Linnaeus, 1758), and *Scardinius erythrophthalmus* (Linnaeus, 1758). *Ameiurus melas* was recorded at both

Table 3. Number of individuals of each fish species caught (N) and the combined weight of these individuals (W) in the mean catch per unit of effort (CPUE, per 40-m-long multi-mesh benthic gillnet) and their percentage share in the total fish catch of different fish species in studied water bodies located in Lithuania.

Fish species	N, ind.	%	Weight, g	%
Gravel pit				
Ameiurus melas	14.5	50.0	151.5	23.1
Carassius gibelio	8.0	27.6	307.5	46.9
Perca fluviatilis	0.5	1.7	7.5	1.1
Rutilus rutilus	2.5	8.6	99.0	15.1
Scardinius erythrophthalmus	3.5	12.1	117.5	17.9
Lake Privalskis				
Alburnus alburnus	0.3	0.5	6.0	0.3
Ameiurus melas	10.3	22.2	193.5	8.6
Carassius gibelio	23.8	51.4	1610.0	71.7
Gobio gobio	0.3	0.5	2.5	0.1
Perca fluviatilis	6.0	13.0	306.5	13.7
Rutilus rutilus	0.5	1.1	18.3	0.8
Scardinius erythrophthalmus	5.0	10.8	107.5	4.8

study sites, with the other six fish species encountered considered as accompanying species (Table 3). The fish communities from both sampling localities primarily comprised fish species commonly found in lentic environments. The European perch *P. fluviatilis* was the only local piscivorous fish species in the fish communities at both sampling localities.

The fish community of the Lake Privalskis contained seven species, with an average catch per unit effort of 2.2 kg of fish per 40-m-long multi-mesh benthic gillnet. Standard catches showed *C. gibelio* to be the dominant fish species by the proportion of biomass (71.7%) and number of fish caught (51.4%), while *P. fluviatilis* and *S. erythrophthalmus* could be regarded as subdominant benthivores. *Ameiurus melas* also reached a subdominant proportion of biomass (8.6%) and number (22.2%) in the total fish catch, with a CPUE of 193.5 g and 10.3 individuals. The other three fish species, *Al. alburnus*, *G. gobio*, and *R. rutilus*, were present in low numbers, constituting less than 2% of the total biomass and number (see Table 3).

The diversity of the fish community in the gravel pit was lower than that of the Lake Privalskis, containing only five fish species. The average CPUE was also much lower, reaching only 0.7 kg. Standard catches showed *C. gibelio* to be the dominant fish species by biomass (46.9%), while *A. melas* dominated the fish community by number of fish caught (50.0%). *Rutilus rutilus* and *S. erythrophthalmus* could be regarded as subdominant benthivores, while the abundance of *P. fluviatilis* was very low (Table 3).

Population traits of A. melas

Ameiurus melas accounted for between 22 and 50% of the total numbers and between 9 and 23% of the total biomass of all the fish caught in the assessed communities. However, the CPUE of *A. melas* was similar in both the studied waterbodies, varying from 151.5 to 193.5 g and from 10.3 to

Table 4. The mean body total length (TL) and weight (W) at different fish age classes at two assessed *A. melas* populations in Lithuania.

Age (years)	Gravel pit			Lake Privalskis		
	n	TL (cm)	W (g)	n	TL (cm)	W (g)
1+	29	10.5 ± 0.5	10.4 ± 1.7	38	10.4 ± 1.0	13.3 ± 3.9
2+	–	–	–	1	13.3	28
3+	–	–	–	2	21.0 ± 1.4	117.0 ± 28.3
Mean	29	10.5 ± 0.5	10.4 ± 1.7	41	12.3 ± 4.1	59.5 ± 81.9

14.5 individuals (Table 3). The age structure of the *A. melas* populations differed in the two studied waterbodies. Three age classes of *A. melas* specimens were caught in the Lake Privalskis, meanwhile, only second-year individuals were caught in the Gravel pit (Table 4). However, both populations were dominated by small second-year individuals. The observed sex ratio was similar in both waterbodies. The ratio of males to females in the Gravel pit was 1.1, while it was 1.2 in the Lake Privalskis.

Parasites of A. melas

All 23 examined individuals of *A. melas* were infected with helminth parasites (P = 100%). Specimens of *A. melas* collected in the Lake Privalskis were infected with monogeneans found on gills (P = 80%, 1–17 specimens) and trematode metacercariae found in eye lenses (P = 100%, 20–114 specimens), whereas specimens from the Gravel pit were only infected with trematode metacercariae in eye lenses (P = 100%, 8 out of 8, 1–12 specimens).

Molecular identification of monogenean specimens revealed their taxonomic identity as *Ligictaluridus pricei* (Mueller, 1936) (Monogenea: Ancyrocephalidae) (Figure 3A, B). Our 28S rDNA sequences (825 bp) were identical to a sequence of *L. pricei* (PQ111545) recently published by Ondračková et al. (2025). The authors reported this species from both invasive bullhead catfish species, *A. melas* and *A. nebulosus,* in the Czech Republic.

Molecular identification of trematode metacercariae confirmed their morphological identification at the generic level as *Diplostomum* and furthered identification at the species level as *Diplostomum pseudospathaceum* and *D. spathaceum* (Trematoda: Diplostomidae) (Figure 3C, D). Our *cox*1

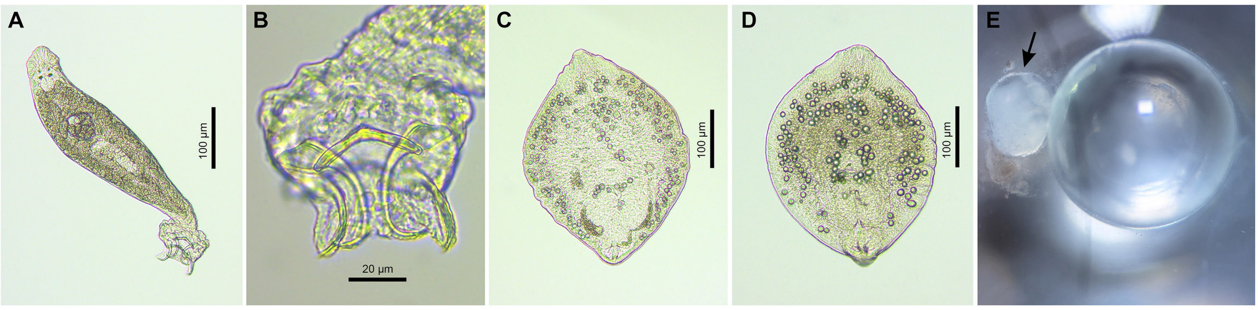

Figure 3. Photomicrographs of helminth parasites collected from the black bullhead catfish *Ameiurus melas* in Lithuania: A, B non-native *Ligictaluridus pricei* (voucher, PX369156); C, *Diplostomum pseudospathaceum* (voucher, PX369151); D, *Diplostomum spathaceum* (voucher, PX369155); E, infection with *D. spathaceum* in "lens poach" (arrow). Photos made by the two authors Olena Kudlai and Rasa Binkiene.

sequences of *D. pseudospathaceum* (802–836 bp) differed by 0.4% (3 bp) and showed low intraspecific divergence to most of sequences identified as *D. pseudospathaceum* in GenBank. Similar to sequences of *D. pseudospathaceum*, our *cox*1 sequences of *D. spathaceum* (836 bp) were either identical or differed by 0.4% (3 bp) and showed low intraspecific divergence to most of the sequences identified as *D. spathaceum* in GenBank.

Specimens of *A. melas* from the Lake Privalskis were infected with metacercariae of *D. pseudospathaceum*, whereas specimens from the gravel pit near Klaipėda were infected with metacercariae of *D. spathaceum*. Interestingly, the specimens of *D. spathaceum* were found either inside of the eye lens of fish or in a pouch connected to the lens (Figure 2E).

Discussion

Introduction pathways

In Europe, two North American catfishes (*A. melas* and *A. nebulosus*) were intentionally introduced to freshwaters at the end of the 19th century for aquaculture, laboratory studies, and aquaria (Wheeler 1978; Welcomme 1988). These species have been reported from most of European countries (review in Rutkayová et al. 2013), including countries neighbouring Lithuania, such as Poland (Nowak et al. 2010) and Belarus (Okhremenko and Gajduchenko 2024).

This study reports on two populations of *A. melas* from the inland waters of Lithuania. As both inhabited waters are closed and situated far from the state border, the appearance of *A. melas* in Lithuania was not the result of natural dispersion from adjacent countries (i.e., Poland or Belarus). Due to the well-expressed morphological differences of *A. melas* to local fish, the accidental co-introduction of this species with imported fish during stocking is also likely not possible. Thus, the most likely introduction pathway of *A. melas* into the inland waters of Lithuania was through illegal and intentional introduction by anglers. This introduction pathway is also supported by several observed cases of online advertisements in 2024 offering *Ameiurus* sp. fry for stocking in Lithuania (V. Rakauskas *personal observation*).

Population traits

The populations of *A. melas* assessed in this study were dominated by very young (1+) specimens. However, a few specimens older than two years were caught in the Lake Privalskis, indicating a multigenerational population structure and successful recruitment of juveniles in the *A. melas* population of this lake. On the contrary, the Gravel pit fish were all 1+ years, indicating a very recent introduction. Similarly, it was noted that *A. melas* in Europe tends to create concentrated and dense populations consisting of small individuals (Rutkayová et al. 2013). This phenomenon is called stunted growth and occurs when fish populations become too large for the available food and space resources, leading to slow growth and small size. This is common in small ponds and lakes. Overpopulation, lack of predators, and inadequate food sources are key factors contributing to stunted growth (Ylikarjula et al. 1999). It was even concluded that *A. melas* is mostly unpopular in Europe because of this tendency (Rutkayová et al. 2013). The observed sex ratio of 1:1 (males/females) is similar to the mean sex ratio of 0.93 reported for other known non-native *A. melas* populations in Europe (Copp et al. 2016). Equal sex ratio should be very advantageous for a non-native fish species especially during their first years of invasion because there will always be at least one female available to mate with a male, although there is very low number of newcomers. Such phenomena could ensure successful reproduction in newly invaded sites. Our study also showed that *A. melas* was rather abundant and accounted for up to 50% of the total numbers and 23% of the total biomass of all caught fish in one of the studied waterbodies. This implies a very worrying scenario for the future of Lithuania's native aquatic biota.

Parasites

During the first parasitological survey of *A. melas,* three species of helminth parasites were recorded. All examined individuals of *A. melas* were infected with local trematodes. Trematode metacercariae, *D. speudospathaceum* and *D. spathaceum,* represent species native to Europe and are common in freshwater fish within Lithuania, and thus they were most probably acquired by *A. melas* after their introduction. Both species of *Diplostomum* exhibit low host specificity at their larval stages and infect a variety of local fish hosts. Thus, *A. melas* is capable of facilitating the transmission of *Diplostomum* spp. in Lithuanian freshwater ecosystems. The third recorded species, *L. pricei,* is a monogenean parasite native to North America and it was most likely co-introduced to Lithuania with its recently introduced fish host, *A. melas*. Thus, the introduction of *A. melas* into Lithuanian inland waters is very threatening, as it serves both parasite transmitting scenarios, via spillback (*Diplostomum* spp. case) and spillover (*L. pricei* case).

A recent study of parasites of *A. melas* conducted in the Czech Republic reported on 10 helminth species parasitising *A. melas,* of which seven are of European origin, two are of North American origin, and one is of Asian origin (Ondračková et al. 2025). Scholz and Cappellaro (1993) and Oros et al. (2024) reported on cestodes of American origin from *A. melas* in Italy and Slovakia, respectively, suggesting the potential introduction of North American parasites of *A. melas* to Europe and the ability of these parasites to complete their life cycle on the continent (Oros et al. 2024). Two monogenean species, *L. pricei* and *Gyrodactylus nebulosus* Kritsky and Mizelle, 1968, were found in *A. melas* from Lake Srebarna, Bulgaria by Vancheva et al. (2020).

In the present study, we only found three out of the 10 helminth species reported in *A. melas* in the Czech Republic (Ondračková et al. 2025). In our opinion, this is likely due to the recent introduction of this fish species to Lithuanian inland waters and the relatively small sample size of specimens examined in our study. Future studies are therefore needed to monitor how invasive fish interact with local species and potentially impact and contribute to native parasite community dynamics.

The present study adds to previous research on parasites of invasive fish in Lithuania, indicating that there are now three known monogenean species that have been co-introduced with their non-native fish hosts: *Dactylogyrus squameus* Gusev, 1955 (Dactylogyridae) with *Pseudorasbora parva* (Temminck and Schlegel, 1846), *Gyrodactylus proterorhini* Ergens, 1967 (Gyrodactylidae) with *Neogobius fluviatilis* (Pallas, 1814), and *L. pricei* with *A. melas* (Kudlai et al. 2024).

Accompanying species

Ameiurus melas was found in small, degraded, and hyper-eutrophic ecosystems with atypical fish assemblages dominated by *C. gibelio*. Although found in very low abundance, *P. fluviatilis* was the only piscivorous fish species recorded in our study which spatially overlapped with *A. melas*. The revealed absence of the native piscivorous fish species is certain to favour the expansion of *A. melas* in freshwater environments within the country. Previous studies have shown that local predators such as *Esox lucius* (Linnaeus, 1758) or *P. fluviatilis* can effectively regulate populations of some invasive species, reducing their abundance and biomass, without affecting native species (Davies and Britton 2015; Lemmens et al. 2015; Rakauskas et al. 2019). However, it is unlikely that *P. fluviatilis* can suppress populations of *A. melas*, as this species has sharp spines in their dorsal and pectoral fins, leaving it with no natural enemies within its introduced ranges. Furthermore, it is known that *A. melas* may even decrease the predation efficiency of local predatory fishes (Kreutzenberger et al. 2008).

Impact

Ameiurus melas is a typical limnophilic and highly tolerant fish species, capable of tolerating water pollution (Ribeiro et al. 2008). In its native range, it grows to a maximum length of 66 cm and a weight of 3.6 kg (Rutkayová et al. 2013). However, in Europe it grows much smaller and reaches a maximum length of 31 cm and a weight of 383 g (Rutkayová et al. 2013; Copp et al. 2016). It has been found that *A. melas* can rapidly adapt to new environments, increase its population size temporarily, and thereafter compete with native fish species (Copp et al. 2016). Moreover, most *Ameiurus* species are nocturnal generalists, foraging on the most abundant and available prey in the water column whose size allows for consumption. In general, *Ameiurus* species are predators of small fishes and other organisms that show similar microhabitat requirements. They feed on a wide variety of prey, including insects, molluscs, fish, algae, plants, and terrestrial invertebrates (Scott and Crossman 1973; Kottelat and Freyhof 2007; Leunda et al. 2008). As *A. melas* is considered a benthic feeder it could be expected that its highest diet overlap and competition for food resources is with other local benthic feeders (in this case *G. gobio* and *C. gibelio*), however, a further diet or stable isotope analyses is needed to clarify such theory.

Ameiurus melas is an invasive species of European concern (Commission Implementing Regulation (EU) 2022/1203) due to its high fecundity, ecological plasticity, and adverse ecological impact on native fish populations. It is well-documented that *A. melas* increases water turbidity (Braig and Johnson 2003) and decreases the predation efficiency of local predatory fish such as *E. lucius* (Kreutzenberger et al. 2008), thereby negatively affecting local fish species through direct predation (Leunda et al. 2008). Due to its very recent introduction, the ecological effects of *A. melas* within Lithuanian inland waters remains unclear, with sustained monitoring of its distribution and further studies on its impact being required.

Future spread

The recent distribution of *A. melas* has been restricted to only a few water bodies within the country. Despite its recent introduction, *A. melas* is considered a pest in Lithuania, and European Union legislation forbids the release of this species into the wild (Commission Implementing Regulation (EU) 2022/1203). However, human-mediated transfer is facilitating its expansion. Intentional illegal stocking of *A. melas* is the only currently recognised pathway of introduction in Lithuania and the further spread of *A. melas* within the country is expected. Thus, increased public awareness and education is of crucial importance to reduce the further spread of *A. melas* within the country. However, it is highly probable that *A. melas* will invade multiple waterbodies that are currently devoid of this species. Previous research on *A. melas* suggests that it is highly tolerant to environmental degradation and can live in poor water quality conditions (Ribeiro et al. 2008). Furthermore, it was suggested that *A. melas* is generally associated with degraded or impacted ecosystems, which are more predisposed and susceptible to invasion (Moyle 1986). Thus, with the recent trend in warmer winter temperatures in Europe and less than par water quality in Lithuania (63% of freshwaters are below "good" ecological status, LEPA 2023), the species may soon become fully established in Lithuania, potentially overwintering and breeding. Despite its strong preference for lentic conditions, small rivers or canalized parts of main rivers may serve as dispersal corridors for *A. melas*. If the species reaches open-water systems (i.e., rivers or canals), it will disperse widely within the country; more than 70% of

Lithuania's landmass falls within the River Nemunas basin. There is thus a high risk of this species spreading within Lithuania and further north to the Latvian waters.

The spread of this species is not desirable, since it increases rapidly in new waterbodies and is difficult to track. Other Baltic countries should therefore be on the lookout of this species, both during state monitoring and via online advertisements. The pathway of introduction of *A. melas* in Lithuania via intentional stocking highlights the importance of controlling and screening human activities related to aquaculture, recreational angling, and the ornamental fish trade to avoid further expansion of *A. melas* and other invasive species of concern in north-eastern Europe. Thus, a joint effort between the Baltic countries is needed to prevent further *A. melas* spread within the region. However, such attempts require awareness, cooperation, and support of concerned citizens and the government of individual countries.

Authors' contribution

Conceptualisation: VR; conducting field research and sample processing: VR, AK, RB, NJB, TV, OK; molecular data generation and analysis: OK; preparation of figures: VR, OK, NJB; preparation of tables: VR; data interpretation and writing: VR, NJB, OK; critical editing of the manuscript: VR, AK, RB, NJB, TV, OK. All authors have read and agreed to the published version of the manuscript.

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Acknowledgements

We thank the two anonymous reviewers who provided helpful suggestions.

Funding declaration

This research was partly funded by the Environmental Protection Agency of Lithuania, grant number (34)-A4E-6574. Parasitological research of this study received funding from the Research Council of Lithuania (LMTLT), agreement No. S-MIP-22-53.

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