



# Distribution of the invasive polychaete *Hypania invalida* (Grube, 1860) against the background of the benthic fauna in the upper Oder River catchment (Poland)

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**Abstract** – Biological invasions are one of the greatest threats to biodiversity, economic development, and human health. Therefore an important challenge is to understand the mechanisms and factors that facilitate the spread of invasive species. The Ponto-Caspian polychaete *Hypania invalida* is one of the invaders that have been colonizing the river systems in Europe since the 1950s. The research aim was to map the distribution of *H. invalida* in the upper Oder and associated aquatic environments, to assess the contribution of *H. invalida* to the benthic fauna, to identify environmental factors linked to the occurrence of *H. invalida*, and to provide genetic markers that can be used to identify *H. invalida* and monitor its dispersal. *H. invalida* was found at two sites in the canalized section of the upper Oder and one site at the initial section of the Gliwice Canal. It was found at low abundance and coexisted with other alien macroinvertebrates. *H. invalida* was not recorded in any smaller river, whether natural, semi-natural, or anthropogenically modified. Apart from the watercourse width, no significant relationships between this polychaete and habitat drivers were found. Genetic analysis showed that the amplified cytochrome c oxidase subunit I and 18S gene fragments showed no sequence variation across all analyzed specimens. Further research is needed to follow the spread of *H. invalida* in the upper Oder River catchment, as at high densities it can adversely affect inhabited ecosystems.

**Keywords:** Alien species / Polychaeta / inland waters / DNA barcoding

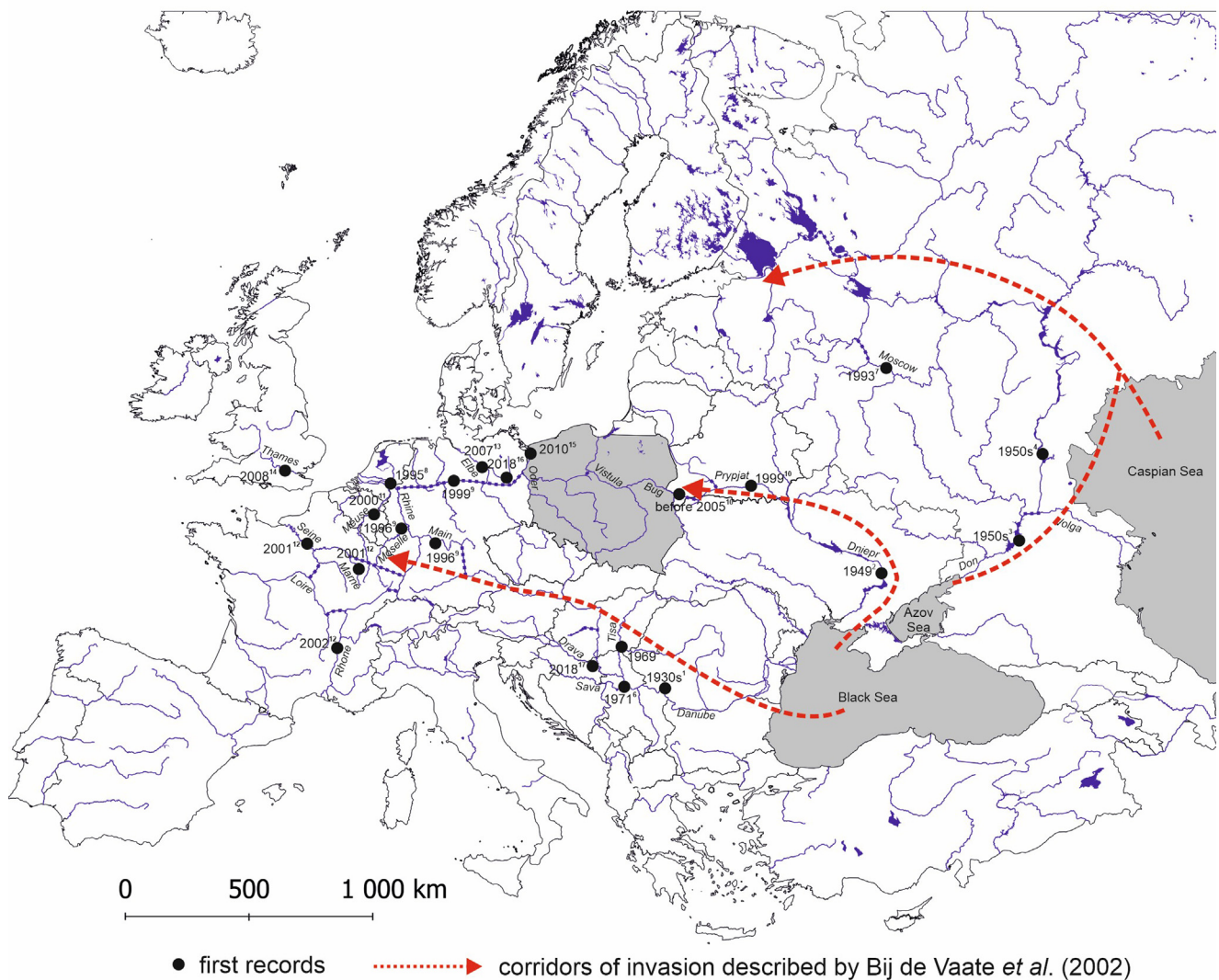
## 1 Introduction

Biological invasions are one of the greatest threats to global biodiversity, economic development, and human health (Pyšek *et al.*, 2020). Inland waters, especially river systems, are particularly vulnerable to invasions due to their location in the landscape, high levels of connectivity, and human usage (Olden *et al.*, 2021). The assessment and management of invasive species is difficult and costly (Cuthbert *et al.*, 2021), so it is crucial to understand the mechanisms and factors that facilitate their expansion in aquatic ecosystems.

The polychaete *Hypania invalida* (Grube, 1860) is one of the invaders that have colonized many river systems in Europe since the 1950s (Bij de Vaate *et al.*, 2002; Gherardi *et al.*, 2009). This Ponto-Caspian species used three corridors of migration described by Bij de Vaate *et al.* (2002): the Northern (Volga basin), the Central (Dnieper and Vistula basins), and the Southern (Danube basin), to spread in European waters

(Fig. 1). The Danube River catchment has played a crucial role in the dispersion of *H. invalida* (Pavel *et al.*, 2021), allowing its colonization in numerous river systems across Western and Central Europe. The species has settled in the Rhine, the Main (Schmidt *et al.*, 1998; Tittizer *et al.*, 2000), the Elbe, the Havel, the Moselle (Tittizer *et al.*, 2000; Eggers and Anlauf, 2008; Straka *et al.*, 2015; Müller *et al.*, 2018), the Meuse (Vanden Bossche *et al.*, 2001; Vanden Bossche, 2002), the Seine, the Marne, the Rhone (Devin *et al.*, 2006; Bij de Vaate *et al.*, 2007; Besacier-Monbertrand *et al.*, 2014), the Sava (Zorić *et al.*, 2011; Vučković *et al.*, 2021), the Drava (Vučković *et al.*, 2021), the Tisa (Ferencz, 1969; Szító, 1996; Zorić *et al.*, 2011), and the Oder rivers (Wozniczka *et al.*, 2011; Pabis *et al.*, 2017). It has also been found in various canals linking river systems (Tittizer *et al.*, 2000; Müller *et al.*, 2006). Beyond continental Europe, it was found in the Thames in England in 2008 (Gallardo and Aldridge, 2013) (Fig. 1). The expansion of *H. invalida* in inland waters has been made easier by the rapid upstream movement of juveniles through ballast water of ships. Additionally, natural passive drift, especially during floods, has increased downstream spread (Bij de Vaate, 2003;

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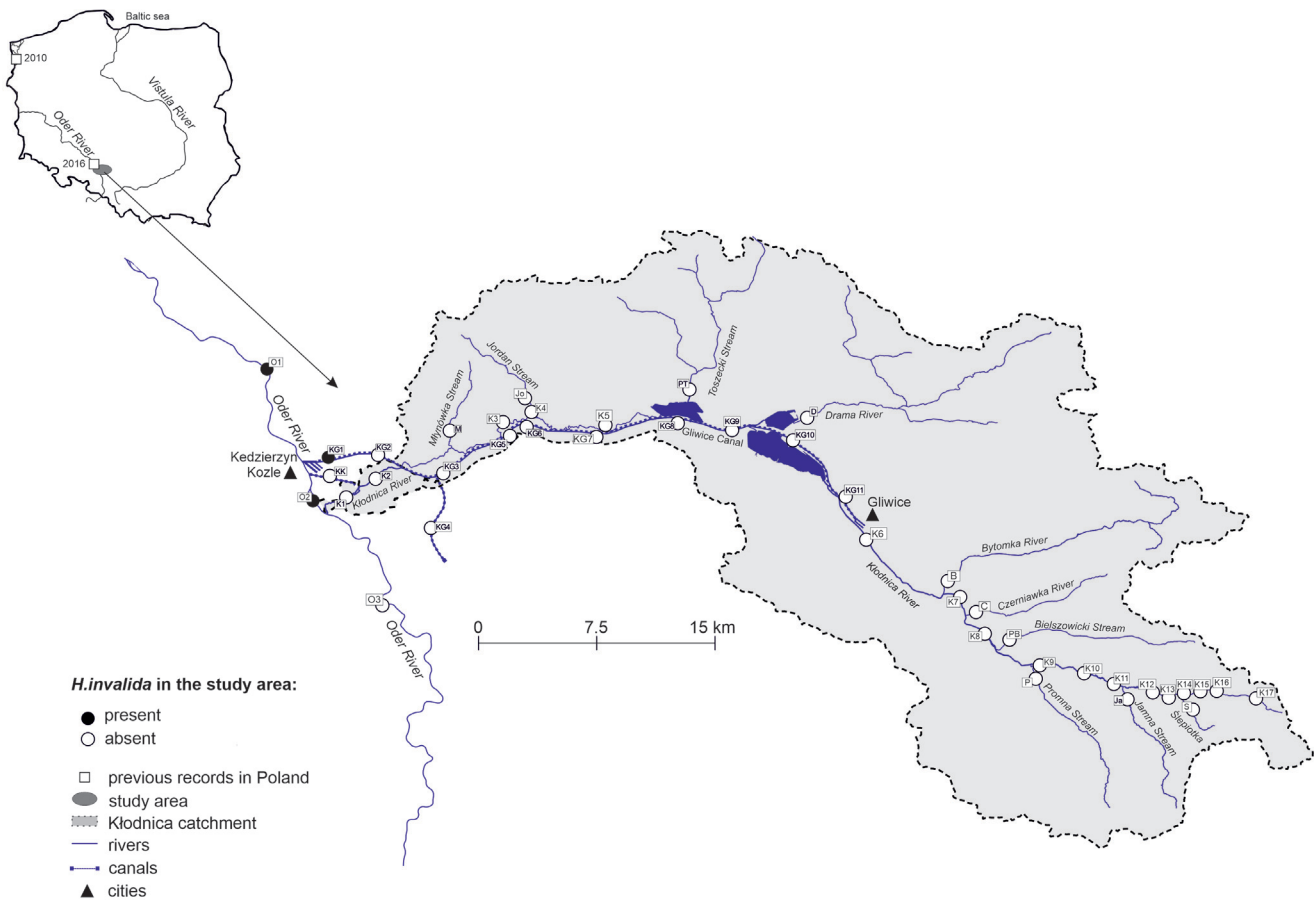


**Fig. 1.** The first records of *H. invalida* in European rivers outside the area of natural occurrence based on reference data. Abbreviation: sof references: <sup>1</sup>Popescu-Marinescu (1992), <sup>2</sup>Semenchenko *et al.* (2016), <sup>3</sup>Ketelaars (2004), <sup>4</sup>Dzyuban and Slobodchikov (1980), <sup>5</sup>Ferencz (1969), <sup>6</sup>Zorić *et al.* (2011), <sup>7</sup>Lvova *et al.*, (1996), <sup>8</sup>Klink and Bij de Vaate (1996), <sup>9</sup>Tittizer *et al.* (2000), <sup>10</sup>Tischikov and Tischikov (2005), <sup>11</sup>Vanden Bossche *et al.* (2001), <sup>12</sup>Devin *et al.* (2006), <sup>13</sup>Eggers and Anlauf (2008), <sup>14</sup>Gallardo and Aldridge (2013), <sup>15</sup>Wozniczka *et al.* (2011), <sup>16</sup>Müller *et al.* (2018), <sup>17</sup>Vučković *et al.* (2021).

Norf *et al.*, 2010; Wozniczka *et al.*, 2011). In the future, it cannot be ruled out that *H. invalida* will be introduced to waterways in North America through transport in ballast water, like many other macroinvertebrates of Ponto-Caspian origin. However, current ballast water regulations are likely to hinder this process (ballast water exchange, 30 parts per thousand (ppt) flushing) (Baker *et al.*, 2023).

*H. invalida* is a eurytopic species that can be found in a wide range of environmental conditions. It is one of the few polychaetes that inhabits freshwater (Glasby and Timm, 2008), but it can also tolerate waters with salinities up to 12 ppt (Mordukhai-Boltovskoi, 1964; Norf *et al.*, 2010). It can tolerate a wide range of temperatures (2–25°C), depths (from the nearshore to above 900 m) (Norf *et al.*, 2010), water with alkaline pH (Vanden Bossche *et al.*, 2001), and various substrates (Filinova *et al.*, 2008; Norf *et al.*, 2010).

*H. invalida* exhibits many characteristics of an invasive species, such as tolerance of various habitat conditions, ease of dispersal, early sexual maturity, rapid growth, high fecundity, high reproductive potential, and a broad diet. It is an active filter and deposit feeder with a non-specific food preference (Bij de Vaate *et al.*, 2002), feeding primarily upon diatoms (Gruia and Manoleli, 1974; Manoleli, 1975). These traits have led to its recognition as an invasive species that can cause changes in the food web of colonized aquatic ecosystems (Norf *et al.*, 2010). Thus far, however, no negative impact of *H. invalida* on the functioning of newly inhabited ecosystems has been demonstrated (Devin *et al.*, 2006; Vučković *et al.*, 2021). Nevertheless, if its populations reach very high densities, some effects may be expected (Straka *et al.*, 2015). It should be noted that this polychaete can improve water quality by reducing the amount of organic matter in the



**Fig. 2.** Area of the study. Abbreviations of study sites: the Oder River (O1-O3), the Gliwice Canal (KG1-KG11), the Kłodnica Canal (KK), the Kłodnica River (K1-K17), tributaries of the Kłodnica River (M-Młynówka Stream, Jo-Jordan Stream, PT-Toszecki Stream, D-Drama River, B-Bytomka River, C-Czerniawka River, PB-Bielszowicki Stream, P-Promna Stream, Ja-Jamna Stream, S-Ślepiotka Stream).

water and sediment. It can also promote the activity of aerobic bacteria by aerating the sediment through bioturbation (Surugi, 2005). *H. invalida* builds tubes made of sand and detritus and can change substrates through such a lifestyle (Pavel *et al.*, 2021). It can also serve as food for various fish species, e.g. bream, white bream, and round goby (Băcescu and Dumitrescu, 1958; Yakovlev and Yakovleva, 2010; Shcherbina, 2012).

To date in Poland, *H. invalida* has only been recorded in the Oder River. It has been found in the estuary area (Wozniczka *et al.*, 2011) and the upper reaches of the river (Pabis *et al.*, 2017). Taking the above findings into account, the aims of our research were: (1) to map the distribution of *H. invalida* in the upper Oder and associated aquatic environments, (2) to assess the contribution of *H. invalida* to the benthic fauna, (3) to identify environmental factors linked to the occurrence of *H. invalida*, (4) to provide genetic markers that can be used to identify *H. invalida* and monitor its dispersal.

## 2 Materials and methods

### 2.1 Study area

The study was carried out along a stretch of the upper Oder River between 108 and 85 km, in the Gliwice and Kłodnica

canals, which are related to the upper Oder River, and in the Kłodnica River and its tributaries (Fig. 2).

The Oder River is Poland's second-longest river and an important transborder river in Central Europe. It flows through the Czech Republic, Poland and Germany. The length of the Oder is 854 km (742 km in Poland), and it drains an area of 118 861 km<sup>2</sup> (106 056 km<sup>2</sup> in Poland). The river has been significantly altered by human activities over the decades, including regulatory and reservoir developments in its upper and middle reaches, and its connection to other European river systems via the Oder-Havel and Oder-Spree canals.

The Gliwice Canal connects the port on the Oder River in Kedzierzyn-Kozle to the inland port in Gliwice. It is 41.2 km long and has a difference in level of 43.6 m between the beginning and end of the canal. The bottom of the canal is covered with a layer of clay and the banks are reinforced with a bed of stones. The canal is mainly fed by the Kłodnica and Drama rivers. The Gliwice Canal plays an important role in the Oder River Waterway, which is the most significant waterway for shipping and cargo transport in Poland, connecting the port of Szczecin and Western Europe via the Oder River and Germany's inland waterways (Kozerska, 2016).

The Kłodnica Canal linked the industrial Upper Silesia with the Oder River but was closed in the 1930s after the opening of the Gliwice Canal. Nowadays, only 3.5 km of the

canal remains and it serves as both a technical monument and tourist attraction. The Kłodnica River feeds it.

The Kłodnica River is a right tributary of the upper Oder River, discharging into the Oder at 94 km. It has a length of 84 km and a catchment area of 1126 km<sup>2</sup>. The upper reaches of the river flow through heavily urbanized and industrialized regions, while the further course of the river drains mostly rural areas. The river is heavily polluted with industrial and municipal waste, including saline waste from coal mines and runoff from industrialized and urban areas (Olkowska *et al.*, 2014).

The selected tributaries of the Kłodnica River (Fig. 2) exhibit varying degrees of anthropogenic impact. The lower and middle course tributaries are predominantly fed surface runoff from agricultural areas. They are mostly streams with natural, unregulated channels. In contrast, the tributaries in the upper reach of the Kłodnica River flow through densely populated and industrial areas of the Upper Silesian Coal Basin and are anthropogenically transformed, their beds being strengthened and often concreted.

## 2.2 Methods

### 2.2.1 Field and laboratory study

The material for the study was collected at 42 locations, i.e. 3 sites in the Oder River, 11 sites in the Gliwice Canal, 1 site in the Kłodnica Canal, 17 sites in the Kłodnica River, and 10 sites in the Kłodnica River tributaries. Each locality was sampled in the summer of 2017 and 2018. Apart from the Oder River, at all sites samples were only taken from the riverbank using a cuboid frame (frame dimensions:  $W 25 \times D 25 \times H 50$  cm) out of which stones were manually removed, and invertebrates were brushed off into a plastic container. The residual material within the frame, approximately 5 cm in depth, was collected using a 500 µm mesh and transferred to a container. At all sites in the Oder River, two samples were taken, one from the riverbank using a cuboid frame and the other at a distance of approximately 2 m from the riverbank using an Ekman grab (surface area:  $16 \times 16$  cm). At the Oder sites, one sample was created by combining material from three Ekman grabs (collection area = 768 cm<sup>2</sup> of bottom) or three frame samples (collection area = 1875 cm<sup>2</sup> of bottom). The collected samples were sieved through a 500 µm mesh sieve in the laboratory and stored in 80% ethanol. The macroinvertebrates were separated from the sediments under a dissection microscope, identified to the lowest possible level, and enumerated.

During the field work, water parameters, such as water conductivity, total dissolved solids, salinity, pH, temperature, oxygen and oxygenation, were measured at each sampling site using HI 9811-5 Hanna Instruments and Multi 3410 WTW portable meters. The laboratory meters from Hanna Instruments and Merck were used to test other water parameters such as chlorides, sulfates, potassium, nitrates, nitrites, ammonium and phosphate content, total hardness, and alkalinity. These were tested following standard methods (Hermanowicz *et al.*, 1999). Sediment samples from all sites were collected to assess the organic matter content and grain size composition of the bottom sediments. The total organic matter content (%) in the sediments was determined using the loss-on-ignition technique by combusting them at 550 °C for 4 h (Myslinska, 2001). The grain size composition of the sediments was examined using the sieve method according to the Polish

standard (PKN-CEN ISO/TS 17892-4, 2009). The water velocity was measured using the float method in the canals, the Kłodnica River, and its tributaries (Dobryjal *et al.*, 2017). Data on water velocity in the Oder River were obtained from the Institute of Meteorology and Water Management – National Research Institute (IMWM-NRI, 2020). Depth was measured at all sampling sites.

### 2.2.2 DNA barcoding

The material for molecular studies consisted of 17 individuals collected from two locations (eight individuals from the Odra River and nine individuals from the Gliwice Canal). Pieces of the anterior and posterior parts of the body were used for DNA isolation, avoiding fragments with remnants of gut contents. The remaining parts of the individuals were preserved in a 96% ethanol-filled tube as a backup and stored at –20°C. DNA isolation was performed using a column method with proteinase K and RNase A digestion step (GeneMATRIX Tissue DNA Purification Kit) (EURx, Gdansk, Poland) according to the manufacturer's protocol. Fragments of two commonly used marker genes, mitochondrial cytochrome c oxidase subunit I (COI) and nuclear 18S rRNA (18S), were amplified. COI amplification was amplified on all 17 individuals, while 18S was analyzed on seven individuals from the same pool. Polymerase chain reactions (PCRs) were carried out in 50 µL reactions containing 21 µL of double-distilled water, 25 µL of Color OptiTaQ PCR Master Mix (2x) (EURx, Gdansk, Poland), 1 µL of each primer at a 10 mM concentration, and 1 µL of total genomic DNA as a template. For COI amplification, the LCO1490 and HCO2198 primer set was used (Folmer *et al.*, 1994). Initially, we attempted to amplify 18S as two partially overlapping segments using primer A combined with Rev1044a18S for “segment A” and Frw921b18S combined with primer B for “segment B” (Apakupakul *et al.*, 1999; de Carle *et al.*, 2022). However, this approach, usually successful for other annelids, produced low-quality reads for *H. invalida*. Therefore, we designed and tested a series of new primer sets based on the consensus sequence assembled with those low-quality reads. Finally, the 18S was successfully amplified and sequenced using Hyp18s\_54F combined with Hyp18s\_1135R and Hyp18s\_833F combined with Hyp18s\_1951R, which allowed us to obtain high-quality reads for the gene. The amplification products were sent to GenoMed (Warsaw, Poland) and sequenced in both directions. Table 1 lists all effective primers and thermal cycling profiles.

### 2.2.3 Statistical analysis

To describe the structure of the benthic fauna, the abundance of taxa and their contribution to total zoobenthos were calculated. Ordination statistics were conducted using the program Canoco Version 5.0 (Šmilauer and Lepš, 2014). Analyses were carried out on a biological data matrix (density of alien species and density of other macroinvertebrates identified to the family level) and an environmental data matrix. The results of the preliminary analysis (detrended correspondence analysis) indicated that the variation in the biological data was best described by a unimodal model (the length of the gradient was 3.198). Therefore, a canonical

**Table 1.** Primers and thermal cycling profiles used for DNA barcoding of *H. invalida*.

Gene	Primer name	Primer sequence 5'-3'	PCR thermal condition	References
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	1 cycle of 95 °C for 180 s (initial denaturation); 35 cycles each of 95 °C for 30 s, 45 °C for 30 s and 72 °C for 45 s; with a final 72 °C extension for 120 s	Folmer <i>et al.</i> (1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA		
18S rRNA	Hyp18s_54F	AACGGCTCATTAGATCAGTTGATA	1 cycle of 95 °C for 260 s (initial denaturation); 35 cycles each of 95 °C for 40 s, 50 °C for 30 s, 72 °C for 55 s; with a final 72 °C extension for 120 s	This study
	Hyp18s_1135R	GCAGTAGTCGTAAAGACTGACG		
	Hyp18s_833F	AACGACCGCCTGAATAATGTTGC		
	Hyp18s_1951R	GGGTAAGATCCGTGGTTCTTGCT		

correspondence analysis (CCA) was used to estimate the relative importance of environmental variables for biological data. First, a CCA with a stepwise forward selection (with Monte Carlo permutations = 499 runs) including all environmental variables (water parameters, granulometric composition of sediments, content of organic matter in sediments, current velocity, width, depth, nature of the river section, i.e. natural section or hydromorphologically modified section) was conducted to select the variables that explained most of the variability in macroinvertebrate density. The Pearson product-moment correlations were then calculated among the selected environmental variables to check for redundancy. Rare taxa (those that occurred in less than 5% of samples) were removed from the analysis to reduce noise in the data set (Gauch, 1982). After removing the rare taxa and redundancy among the significant environmental variables (chlorides and sulfates were excluded from the analysis because they were correlated with conductivity), 10 environmental variables and 36 taxa were used in the final CCA. The explanatory variables included conductivity, current velocity, width, the content of organic matter in sediments, %stone, %gravel, % sand, %silt, and the character of the river stretch (natural versus hydromorphologically modified). Analyses were conducted on log ( $x + 1$ )-transformed invertebrate density and environmental data. The results were presented graphically in an ordination diagram.

Multiple regression analysis (backward variable elimination) was used to assess the relationship between environmental variables and the contribution of alien species to the zoobenthos community. With each step in a regression, the environmental variable with the lowest partial effect indicated by the highest  $p$ -value was removed until only environmental variables related ( $p \leq 0.05$ ) remained. This analysis was carried out using Statistica ver. 13.1.

## 3 Results

### 3.1 Habitat characteristics

The characteristics of the habitat parameters of the studied environments are presented in Table 2. The salinity and salinity-related parameters such as chlorides, sulfates, potassium, and total hardness were elevated in the Oder River and

both canals. In contrast, these parameters exhibited substantial variation in the Kłodnica River and its tributaries (Tab. 2). High values were recorded along the entire course of the Kłodnica River, except for the river's source stretch, and in the tributaries upstream. In contrast, salinity-related parameters were low in the tributaries in the middle and lower reaches of the Kłodnica River.

The pH of water at all sites was either neutral or slightly alkaline. The concentrations in the water of nitrite, nitrate, ammonium and phosphate varied considerably at the sampling sites in all watercourses. They were usually higher in the upper Kłodnica and its upper tributaries. The sediments in the upper reaches of the Kłodnica River and these tributaries were characterized by a higher content of the fine-grained fraction and content of organic matter than in other environments.

Water flow velocity in the Gliwice and Kłodnica canals was lower than in other environments (Tab. 2).

### 3.2 Distribution of *H. invalida* in the study area

In the study area, the polychaete *H. invalida* was exclusively found in three localities, i.e. two sites in the canalized section of the upper Oder River (site O1 at 108 km and site O2 at 95.3 km) and one site in the initial section of the Gliwice Canal (site KG1) (Fig. 2). At site O1, in the area up to a depth of 1 m, it reached densities of 16 individuals/m<sup>2</sup> to 171 individuals/m<sup>2</sup> on sandy substrate, and at depths above 1 m it achieved densities of 800 individuals/m<sup>2</sup> to 1133 individuals/m<sup>2</sup> on muddy sediments. At site O2, the density of *H. invalida* ranged from 15 individuals/m<sup>2</sup> to 2030 individuals/m<sup>2</sup> on a silty-sandy substrate. At the Gliwice Canal site (site KG1), its density was 176 individuals/m<sup>2</sup> on the bottom, which was covered by a thick layer of live zebra mussels and empty shells (Tab. 3).

*H. invalida* individuals accounted for up to 13.2% of the total benthic macroinvertebrate abundance in the benthic invertebrate communities (Tab. 4). Other non-native macroinvertebrates were co-occurring with *H. invalida* (Tab. 4). These included the polychaete *Laonome xeprovala* (Bick, Bastrop 2018), the amphipods *Dikerogammarus villosus* (Sowinsky, 1894) and *Gammarus tigrinus* (Sexton, 1939), the gastropod *Potamopyrgus antipodarum* (Gray, 1843), and the bivalves *Corbicula fluminea* (Müller, 1774), and *Dreissena*

**Table 2.** Characteristics of the sampling sites in the studied area.

Variables	Oder river	Gliwice and Kłodnica canals	Kłodnica river	Kłodnica tributaries
Water				
Temperature (°C)	13.1–18.3	12.8–25.4	11.4–22.7	13.0–22.4
Oxygen (mg L <sup>-1</sup> )	5.3–10.2	4.3–16.8	4.0–9.4	3.2–10.1
Oxygenation (%)	57.8–103.3	53.2–198.8	43–112.1	35.7–98.5
pH	7.4–7.8	7.4–8.3	7.4–7.9	6.9–8.0
Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	120–160	105–235	150–310	15–335
Salinity (‰)	1.1–1.5	1.3–3.7	0.1–5.8	0.2–12.1
Conductivity (µS cm <sup>-1</sup> )	2250–3000	2530–6780	420–10 340	520–20 300
TDS (mg L <sup>-1</sup> )	1125–1502	1268–2720	210–5170	260–10 150
Chlorides (mg L <sup>-1</sup> )	468–750	530–3220	125–2960	18–6400
Sulfates (mg L <sup>-1</sup> )	97–210	236–436	104–512	41–1020
Potassium (mg L <sup>-1</sup> )	7.4–15.6	10.4–36	5–32	2–62
Total hardness (mg CaCO <sub>3</sub> L <sup>-1</sup> )	240–325	315–540	320–940	195–1390
Nitrite (mg L <sup>-1</sup> )	0–0.32	0.05–0.92	0.09–2.43	0.01–1.03
Nitrate (mg L <sup>-1</sup> )	2.7–13.3	0.1–10.6	0–20.4	1.3–16.4
Ammonium (mg L <sup>-1</sup> )	0.25–0.73	0.24–1.2	0.19–6.74	0.01–1.58
Phosphates (mg L <sup>-1</sup> )	0.35–0.65	0.02–0.96	0.29–1.5	0.14–1.08
Iron (mg L <sup>-1</sup> )	0.34–0.78	0.16–0.97	0.22–1.46	0.25–1.5
Sediments				
Stone (%)	20–70	10–85	0–80	0–90
Gravel (%)	3–23.3	2.1–68.4	0–39.6	0–25
Sand (%)	20.3–56.1	1.9–20.8	7.2–86.3	4.7–68
Silt and clay (%)	0.3–36.1	0.8–11	0–82.8	0.1–72
Organic matter (%)	1–10	1.7–7.0	0.4–39.2	0.3–17.0
Width (m)	50–79	4–41	2–22	1–7
Depth (m)	0.5–2.5	0.5–1.5	0.2–0.7	0.1–0.8
Velocity (m s <sup>-1</sup> )	0.15–1.10	0.07–0.18	0.07–1.02	0.03–0.71

*polymorpha* (Pallas, 1771) at all sites. The contribution of non-native species to the total fauna was high, and especially very high at one site in the Oder River (site O1) and one in the Gliwice Canal (site KG1) (Tab. 4). Beside alien species, only the native brackish amphipod *Apocorophum lacustre* (Vanhöffen, 1911) was abundant at all these sites (Tab. 3).

In the CCA for all studied sites, which included only the environmental variables determined by the forward selection (conductivity, current velocity, width, the content of organic matter in sediments, %stone, %gravel, %sand, %silt, and nature of the river stretch), the first two axes explained 46.6% of the variability in the data on the composition of the benthic fauna. The relationship between the data of the macroinvertebrate fauna and the explanatory environmental variables accounted for 74.1%. The Monte Carlo permutation tests showed that the CCA's ordination axes were significant (Tab. 5). The most alien species, except for the two oligochaetes *P. bavaricus* and *P. albicola*, were associated with larger streams of altered hydromorphology than with smaller natural streams. Along the first axis, the main gradient of change in benthic invertebrate composition, including the abundance of *H. invalida*, was related to river width (Fig. 3). The second ordinate axis was related to the gradient of salinity parameters (conductivity, chloride and sulphate content), as salinity increased, the density of alien species as *P. antipodarum*, *P. acuta*, *D. polymorpha* and *G. tigrinus* increased (Fig. 3). Regression analysis showed that the share of alien species in the macroinvertebrate community was

positively related to salinity-related parameters, e.g. conductivity, chlorides and sulphates (adj.  $R^2 = 0.58597$ ,  $p < 0.0001$ ).

At sites in the Oder River, empty tubes and tubes containing *H. invalida* formed a thin layer of silt on coarse-grained sediments, which was often colonized by other benthic macroinvertebrates, e.g. mollusks and amphipods (Fig. 4).

### 3.3 DNA barcoding

As a result of molecular analysis, sequences for the COI and 18S genes of *H. invalida* were acquired. The study did not reveal any differences among specimens at the analyzed loci. The generated sequences were deposited in the GenBank database under accession numbers MK757472 and MT019843, respectively.

## 4 Discussion

### 4.1 Distribution of *H. invalida* and environmental conditions

Our research has revealed the upstream dispersion of *H. invalida* along the Oder River and to the initial section of the Gliwice Canal. Previous research identified its presence at 108 km in the Oder (Pabis *et al.*, 2017). Two years later, we detected this polychaete at 95.3 km, confirming its potential to move upstream. The rate of *H. invalida* spread up the Oder may have been aided by the river's use for shipping and cargo

**Table 3.** Abundance of macroinvertebrates (per m<sup>2</sup>) at the sites where *H. invalida* was found.

Taxa	O1		O1		O2		KG1	
	2017	2018	2017	2018	2017	2018	2017	
	depth <1 m		depth >1 m		depth >1 m		depth <1 m	
Polychaeta	<i>Hypania invalida</i> *	171	16	800	1133	15	2030	176
	<i>Laonome xeprovala</i> *	448	90	444	2182		607	21
Oligochaeta	<i>Psammoryctides barbatus</i> *	21	43	7	68			
	<i>Branchiura sowerbyi</i> *	16	26					
	<i>Spirosperma ferox</i>				10			
	<i>Potamotheix moldaviensis</i> *					26	13	
	<i>Limnodrilus claparedeanus</i>	59	27		29	130	508	
	<i>Limnodrilus hoffmeisteri</i>	59	587		68	208	1823	
	Tubicininae gen. spp.juv.		1103	13	78	234	1471	
	<i>Ophidonais serpentina</i>					39	52	
	<i>Dero digitata</i>				49			
Hirudinea	<i>Erpobdella testacea</i>							11
	<i>Helobdella stagnalis</i>							277
	<i>Piscicola</i> sp.	5					15	
Isopoda	<i>Asellus aquaticus</i>							208
Amphipoda	<i>Apocorophium lacustre</i>	1723	512	1504	63 600	4785	16 252	1264
	<i>Chelicorophium curvispinum</i> *	624	37	74	2000	59		
	<i>Dikerogammarus haemobaphes</i> *		11		33			5
	<i>Dikerogammarus villosus</i> *	800	80	67	100	59	15	277
	<i>Gammarus tigrinus</i> *	160	4651	30	8811	89	1733	9451
Odonata	Calopterygidae	5						
	Coenagrionidae		43					
	Libellulidae		5					
	Platycnemidae					59		
Ephemeroptera	Caenidae					30	15	
Neuroptera	Sisyridae							27
Diptera	Ceratopogonidae					15		
	Chironomidae	245	144	37	11	1200	1081	1552
Gastropoda	<i>Bithynia tentaculata</i>	261	16				15	677
	<i>Viviparus viviparus</i>	5	32	22		44		
	<i>Valvata naticina</i>		11			30		
	<i>Potamopyrgus antipodarum</i> *	1605	496	2289		1333	1393	64
	<i>Physa acuta</i> *	69	160					
	<i>Radix balthica</i>	16						112
	<i>Ancylus fluviatilis</i>	5				370	430	
	<i>Corbicula fluminea</i> *	16	16	311	311	74		16
Bivalvia	<i>Dreissena polymorpha</i> *	96	133	467	22	15		7803
	<i>Pisidium</i> sp.	11	53			30	148	
	<i>Sphaerium rivicola</i>		37		11		30	
	<i>Sphaerium</i> sp.	16		7				
	<i>Anodonta anatina</i>							5
	<i>Unio pictorum</i>					15	30	
Macroinvertebrate density (m <sup>2</sup> )	6437	8328	6071	78 518	8860	27 659	21 947	
Alien species density (individuals m <sup>-2</sup> )	4026	5759	4489	14 660	1670	5791	17813	

\*alien species.

transport (Maruszczak, 2019), particularly since juveniles can easily be transported in ballast water. Another factor that may have facilitated the spread of this euryhaline polychaete species (Norf *et al.*, 2010) is the increasing water salinity of the Oder River. The elevated salinity of the upper reaches of the river is caused by several factors, including the discharge

of underground saline water from the Upper Silesian Coal Basin's coal mines into its tributaries such as the Kłodnica River (Matysik, 2019). Salt-tolerant alien species often colonize environments with higher salinity, where native freshwater species cannot thrive (Grabowski *et al.*, 2007).

**Table 4.** Relative abundance (%) of alien versus native macroinvertebrates in benthic fauna at the sites where *H. invalida* occurred.

Higher taxa	Species	O1		O1		O2		KG1
		2017	2018	2017	2018	2017	2018	2017
		depth <1 m		depth >1 m		depth >1 m		depth <1 m
Polychaeta	<i>Hypania invalida</i>	2.7	0.2	13.2	1.4	0.2	7.3	0.8
	<i>Laonome xeprovala</i>	7.0	1.1	7.3	2.8		2.2	0.1
Oligochaeta	<i>Psammoryctides barbatus</i>	0.3	0.5	0.1	0.1			
	<i>Branchiura sowerbyi</i>	0.2	0.3					
	<i>Potamothenix moldaviensis</i>					0.3	0.05	
Amphipoda	<i>Chelicorophium curvispinum</i>	9.7	0.4	1.2	2.5	0.7		
	<i>Dikerogammarus haemobaphes</i>		0.1		0.04			0.02
	<i>Dikerogammarus villosus</i>	12.4	1.0	1.1	0.1	0.7	0.1	1.3
	<i>Gammarus tigrinus</i>	2.5	55.8	0.5	11.2	1.0	6.3	43.1
Gastropoda	<i>Potamopyrgus antipodarum</i>	24.9	6.0	37.7		15.0	5.0	0.3
	<i>Physa acuta</i>	1.1	1.9					
Bivalvia	<i>Corbicula fluminea</i>	0.2	0.2	5.1	0.4	0.8		0.1
	<i>Dreissena polymorpha</i>	1.5	1.6	7.7		0.2		35.6
Alien species		62.5	69.2	73.9	18.7	18.8	20.9	81.2
Native species		37.5	30.8	26.1	81.3	81.2	79.1	18.8

**Table 5.** Summary of canonical correspondence analysis (CCA) performed on macroinvertebrate data and environmental variables determined by forward selection.

Axes	1	2	3	4	Total variance
Eigenvalues	0.378 00.378	0.205 0.205	0.137 0.137	0.097 0.097	2.110
Taxa-environment correlations	0.919	0.923	0.895	0.804	
Cumulative percentage variance of taxa data	37.9	46.6	54.1	58.7	
of taxa-environment relationship	65.1	74.1	82.8	85.8	
The sum of all canonical eigenvalues					1.079
Summary of Monte Carlo test					
Test of significance of first canonical axis	$F = 7.645, p = 0.002$				
Test of significance of all canonical axes	$F = 3.327, p = 0.002$				

We observed a higher population density of *H. invalida* (up to 2030 individuals/m<sup>2</sup>) compared to the study by Pabis *et al.* (2017), which only found singular individuals in the upper Oder River. However, these densities were not as high as those found in other localities such as the Szczecin Lagoon (up to 11 466 individuals/m<sup>2</sup>) (Wozniczka *et al.*, 2011), the lower section of the Danube River (46 875 individuals/m<sup>2</sup> in 1072 km at Bazias, Romania) (Pavel *et al.*, 2021), or the lower part of the Moselle River (up to 11 431 individuals/m<sup>2</sup> at Sierck-les-Bains, France) (Devin *et al.*, 2006).

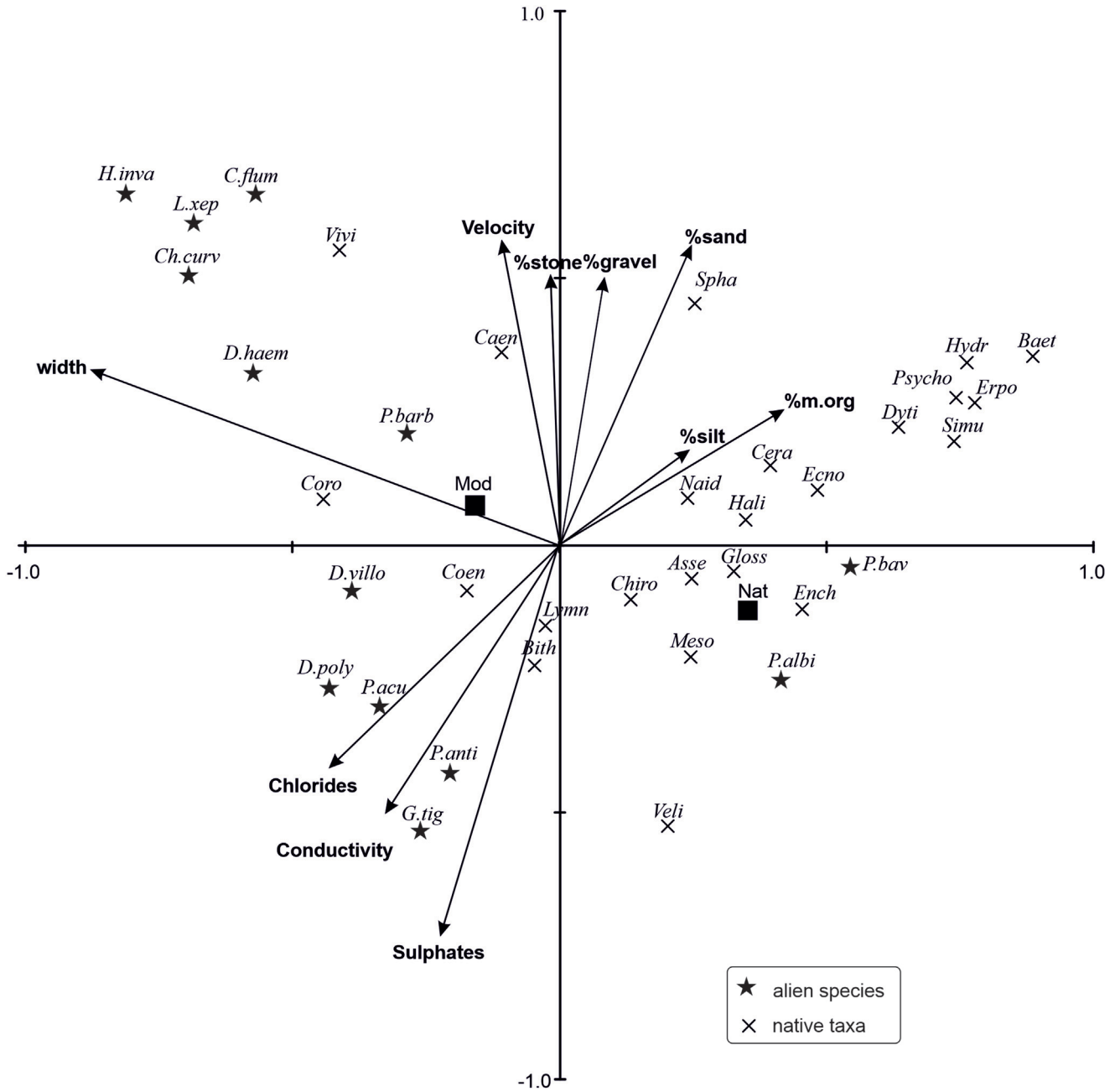
Our findings indicate that *H. invalida* is mainly associated with the stretch of the Oder River that has been hydromorphologically modified for shipping and cargo transport. Outside the Oder, it was only found in the initial section of the Gliwice Canal. Thus, our study aligns with previous research (Zorić *et al.*, 2011), suggesting that *H. invalida* is widely distributed in rivers undergoing hydromorphological changes and affected by heavy shipping traffic. However, Vučković *et al.* (2021) pointed out that this species also inhabits natural river sections and smaller channels in Croatia and suggested

that the migration of *H. invalida* into such environments could occur through the use of fishing nets by anglers.

Our research found no significant correlations between the occurrence of *H. invalida* and the studied factors except for the watercourse width, the parameter that is determined by Strahler stream order, slope and land use, among others (Downing *et al.*, 2012). Previous research indicates that *H. invalida* is more commonly found in high densities in environments with low-flow conditions where sedimentation rates are high, and rarely in environments with high-flow velocities, where coarse-grained sediments prevail (Băcescu, 1949; Devin *et al.*, 2006; Filinova *et al.*, 2008; Wozniczka *et al.*, 2011).

The finding of *H. invalida* in the Gliwice Canal on a bottom covered with a thick layer of live *D. polymorpha* and empty shells confirms the results of Shcherbina (2001), Norf *et al.* (2010) and Yakovlev and Yakovleva (2010), who reported that this alien polychaete often inhabits dreissenid druses (*D. polymorpha* and *D. bugensis*), which provide it with shelter, tube-building material and food (Straka *et al.*, 2015).

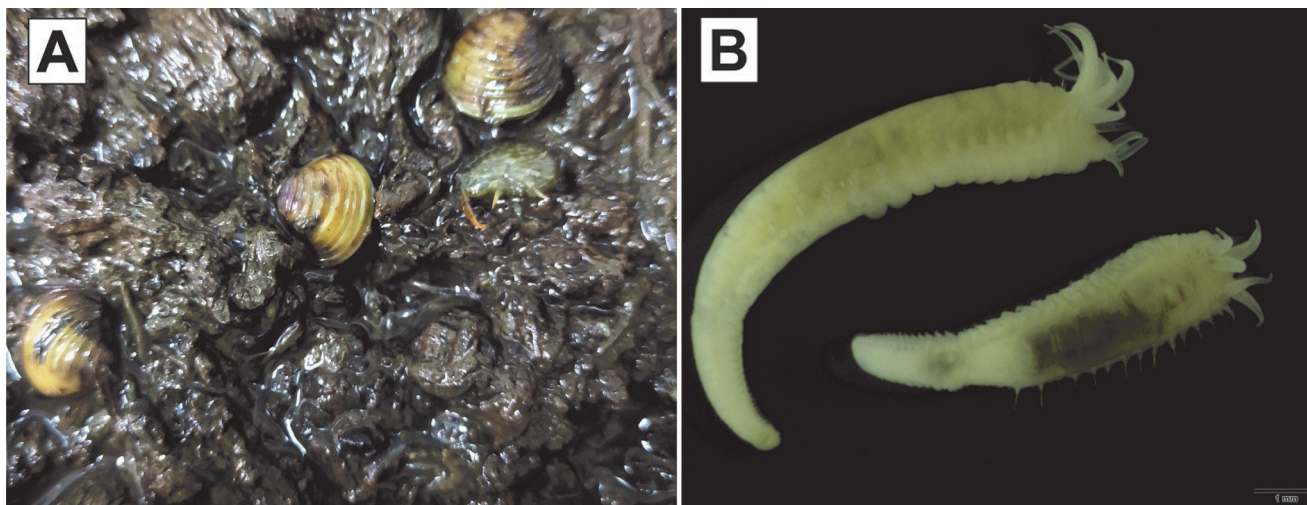




**Fig. 3.** Ordination diagram based on the canonical correspondence analysis of the taxa abundance data and the best explanatory variables. Abbreviations: alien species Ch.cur-Ch.curvispinum, C.flu-C.fluminea, D.hae-D.haemobaphes, D.pol-D.polymorpha, D.vill-D.villosus, G.tig-G.tigrinus, H.inv-H.invalida, L.xep-L.xeprovala, P.acu-P.acuta, P.bar-P.barbatus, P.bav-P.bavaricus, P.alb-P.albicola, P.ant-P.antipodarum, other macroinvertebrate taxa Asel-Asellidae, Baet-Baetidae, Bith-Bithyniidae, Caen-Caenidae, Cera-Ceratopogonidae, Chiro-Chironimidae, Coen-Coenagrionidae, Coro-Corophidae, Dyti-Dytiscidae, Ecno-Ecnomidae, Ench-Enchytraeidae, Erpo-Erpbodellidae, Gloss-Glossiphoniidae, Hali-Haliplidae, Hydr-Hydropsychidae, Lymn-Lymnaeidae, Meso-Mesoveliidae, Naid-Naididae, Psycho-Psychodidae, Simu-Simuliidae, Spha-Sphaeriidae, Veli-Veliidae, Vivi-Viviparidae, environmental variables Mod-hydromorphologically modified river stretch, Nat-natural river stretch

Our study, like the earlier research by [Klink and Bij de Vaate \(1996\)](#), [Vanden Bossche \*et al.\* \(2001\)](#), and [Pavel \*et al.\* \(2021\)](#), showed that *H. invalida* coexists with other alien macroinvertebrates from the Ponto-Caspian region, such as the oligochaetes *Potamothenis bavaricus* (Oschmann, 1913), *Potamothenis moldaviensis* Vejdovský and Mrázek, 1903,

*Psammoryctides barbatus* (Grube, 1860), and *Psammoryctides albicola* (Michaelson, 1901) ([Dumnicka, 2016](#)), the amphipods *Chelicorophium curvispinum* (G.O. Sars, 1895), *Dikerogammarus haemobaphes* (Eichwald, 1841), and *Dikerogammarus villosus* (Sovinsky, 1894), and the bivalve *Dreissena polymorpha*.



**Fig. 4.** (A) Sediment with tubes of *H. invalida* from the upper Oder River; (B) *Hypania invalida*.

Taking into account the low population density of *H. invalida* in the study area, it can be supposed that it has currently no impact on the benthic fauna. It is worth noting that the effect of *H. invalida* on colonized ecosystems outside its natural range has not yet been fully recognized. Zorić *et al.* (2011) suggested that *H. invalida* had limited influence on benthic assemblages due to its preference for soft sediment, and Devin *et al.* (2006) found that it was a weak competitor for environmental resources. Nevertheless, at high densities, the impact of this polychaete on the ecosystem cannot be ruled out (Straka *et al.*, 2015). With global climate change, shorter periods of ice cover and higher water temperatures may extend the breeding season of *H. invalida* (Norf *et al.*, 2010), potentially contributing to an increase in its population size. Thus, further research is necessary to evaluate the population changes of this euryhaline polychaete and its future spread in the upper Oder River.

#### 4.2 Molecular data

Although amplifying and sequencing COI for *H. invalida* were straightforward, obtaining the 18S rDNA barcode required additional effort. However, our persistence paid off, and we obtained reliable sequences for both genes. We believe that the newly designed primer sets used to amplify the 18S rRNA gene could be useful for other researchers monitoring the spread of *H. invalida*. We deposited the generated sequences in the GenBank database in 2020. However, it is surprising that, to date, no additional marker genes, mitochondrial haplotypes, or nuclear haplotypes for this invasive species have been publicly deposited in databases. Other marker gene sequences for *H. invalida*, obtained from Sanger sequencing and RNA-Seq data, can be found in the supplementary data provided in the paper by Stiller *et al.* (2020), who analyzed specimens collected in Magdeburg, Germany. Although the shorter partial COI sequence retrieved from that data was identical to our Polish haplotype (MK757472), the 18S sequence was missing, perhaps due to Sanger sequencing issues similar to the ones we encountered. However, mitochondrial 16S rDNA, nuclear

28S rDNA, and histone H3 sequences were provided based on transcriptomic data. *H. invalida* is currently defined by five commonly used marker genes, although this information may not be widely known. We encourage researchers studying *H. invalida* to deposit their data in publicly accessible databases to facilitate knowledge sharing and advance research on this important invasive species. The identical COI sequence of specimens from the Oder and Elbe in Magdeburg supports our suggestion that the Oder population may originate from the Elbe population, which was discovered early (Eggers and Anlauf, 2008), or from riverine ecosystems linked to the Elbe, given its connection to the Oder via the Elbe-Havel and Havel-Oder Canals. However, this hypothesis is based on limited evidence. Before any definitive conclusions can be drawn, more molecular studies are necessary to analyze the genetic diversity of *H. invalida* individuals from various locations in Europe and to determine whether there are any significant genetic differences between populations.

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