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Research Article

New evidence supporting upstream pathways of *Hypania invalida* (Grube, 1860) invasion

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Abstract

The freshwater polychaete *Hypania invalida* is indigenous to the Ponto-Caspian region. The transfer of the species from the Black Sea to most of Europe was mainly via the Danube River, and its distribution increased rapidly after the opening of the Rhine-Main-Danube Canal in 1992. The presence of this species may influence the relationships of other species within the benthic community and thus has significant implications for the food webs of aquatic ecosystems. Our aim was to provide detailed records of the distribution of this potentially invasive species in Croatia, with evidence of upstream invasion and a description of environmental conditions at the sites, together with species co-occurrence, as a basis for assessing the future risk of upstream invasion and for monitoring purposes. *Hypania invalida* individuals were recently found at five different sites in Croatia in the Drava and Sava River systems and at one additional site in the Danube. Most of the new records support the idea of upstream invasion predominantly via large rivers. We found that the species inhabits both semi-natural river sections, and smaller canals, contradicting the idea that it inhabits only hydromorphologically heavily impacted main river channels.

Key words: distribution, Ponto-Caspian, Danube River, Drava River, Sava River

Introduction

Most polychaete species live in marine habitats. However, a small number inhabit fresh and brackish waters. The polychaete *Hypania invalida* (Grube, 1860) lives mainly in freshwater, but still has a high tolerance to salinity (0–12 PSU) and temperature (2–25 °C) (Mordukhai-Boltovskoi 1964). *Hypania invalida* has separate sexes with no evidence of a distinct sexual mode and has several life stages, one of which is a planktonic larva (Norf et al. 2010). The species forms a tube around its body by viscous secretion, using fine sediments as building blocks (Manoleli 1975). It is usually found in microhabitats with fine sediments, but can also be found on a large substrate such as pebbles or cobbles and at sites with reduced flow velocity and turbulence (Devin et al. 2006; Filinova et al. 2008; Norf et al. 2010). *Hypania invalida* is a facultative suspension-feeder (Manoleli 1975) but it also feeds on benthic algae (Surugiu 2005), which means that it can find food resources in a variety of aquatic habitats. Its successful

distribution may be related to a combination of different ecological and biological traits, such as short generation time, early sexual maturity, rapid growth, and high fecundity (Ricciardi and Rasmussen 1998; Devin and Beisel 2007).

The native range of this species is the Ponto-Caspian region (Norf et al. 2010). The accelerated upstream dispersal of *H. invalida* was probably facilitated by the transport of early life-stage individuals in the ballast water of ships, whereas the downstream dispersal occurred by passive drift (Bij De Vaate 2002; Norf et al. 2010). Dispersal from the Caspian Sea and the Black Sea (Surugiu 2005) to the rest of Europe occurred via three corridors/directions: Northern corridor encompassing the Volga River catchment; Central corridor of the Dnieper and Vistula catchments; and Southern corridor of the Danube River catchment (Bij de Vaate et al. 2002). The introduction of the species into most of Europe was mainly via the Danube River (Panov et al. 2009), where the species was first observed in 1967 in the upper section of the Danube River (Germany) (Kothé 1968) and spread rapidly after the opening of the Rhine-Main-Danube Canal in 1992. The species spread from the Danube upstream through the Rhine and Mosel Rivers. Although colonization of the Main River was not reported until 1996, *H. invalida* was already observed in 1995 in the lower Rhine River, where a maximum of five individuals per m^2 were found (Klink and Bij de Vaate 1996), which may indicate a cryptic potential occurrence of *H. invalida* at the beginning of colonization of new river stretches. In 1998, more than 3000 individuals/m^2 were recorded in the Main River (Schmidt et al. 1998).

Hypania invalida has been detected at many sites in the Danube River, including three sites on the border between Croatia and Serbia (Zorić et al. 2011), but prior to this study, it was not found in any Danube tributary in Croatia.

Our aim was to provide the first records and distribution of this potentially invasive species in Croatia with evidence of upstream invasion and a description of environmental conditions at the sites as a basis for assessing future upstream invasion risk and for monitoring purposes.

Materials and methods

Study area

The study was conducted in the Hungarian Lowlands ecoregion (ER 11; Illies 1978) in Croatia (Figure 1). This region of Croatia is mainly characterized by a temperate humid climate with an average annual temperature of about 10.5 °C and an average annual precipitation rate of 1.050 mm (Šegota and Filipčić 2003). Biological data were obtained as part of the monitoring and assessment system development programs in Croatia, within which 141 sites were sampled (Supplementary material Table S1). Benthic invertebrates were

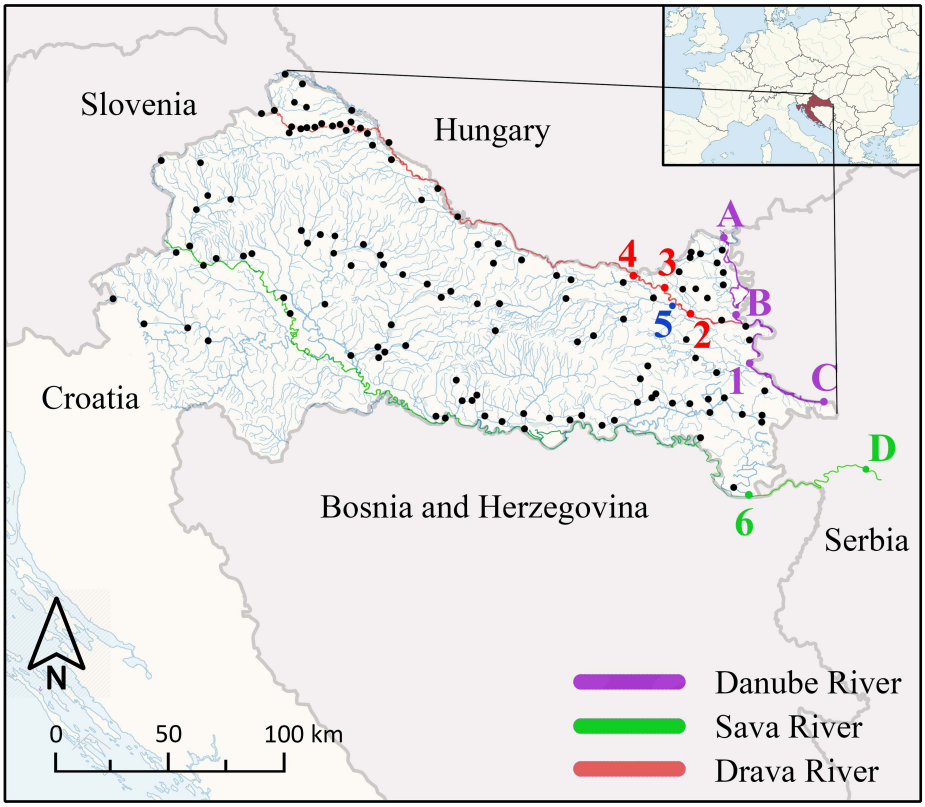

Figure 1. Map of the study area showing localities where *Hypania invalida* was found. The numbers indicate sites in Croatia and the letters sites in Serbia (near Croatia). 1 Danube River, Borovo; 2 Drava River, upstream from Osijek; 3 Drava River, Nard; 4 Drava River, Petrovo selo; 5 Canal Pongračevo-Kravnički, Josipovac; 6 Sava River, Račinovci; A Danube River, Bezdan; B Danube River, Upstream from mouth of the Drava River; C Danube River, Bačka Palanka, D Sava River, Sremska Mitrovica. Black dots represent sampling sites where *H. invalida* was not found.

collected in 2018–2020 during low discharge rates of the sampled water bodies. The Danube, Drava, Sava, Mura and other large rivers were included in the survey, as well as smaller rivers, streams and canals. Floodplains and lakes connected by canals to the Danube and Drava, which are part of the Kopački Rit Nature Park, were also included in the monitoring.

Sampling protocol

At each sampling site, the AQEM sampling protocol (AQEM Consortium 2002) was used, i.e., twenty samples were collected from a 25 × 25 cm area using a 500 µm mesh hand net. The hand net was used to proportionally sample different substrates to capture microhabitat heterogeneity in the wadeable parts of the rivers. Samples were collected from microhabitats that covered at least 5% of the sampling area, proportional to their coverage at the sampling sites. The channel substrate of each sampling site was classified according to the AQEM Consortium (2002), with the first 700 individuals identified within the sample, but the entire sample was checked for new or rare species. Macroinvertebrates were identified at the finest possible taxonomic resolution using appropriate taxonomic literature. Individuals of *H. invalida* (Figure 2) were identified using the identification

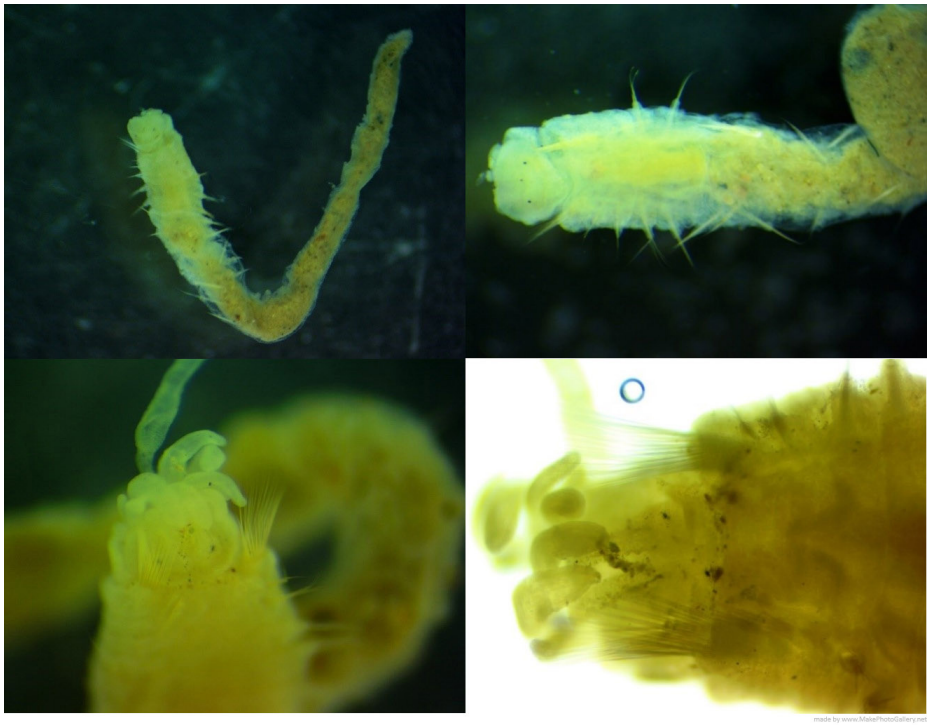

Figure 2. *Hypania invalida* from the Sava River in Račinovci.

key of Hartmann-Schröder (1996). All collected polychaete specimens are deposited in the Department of Biology, Faculty of Science, University of Zagreb, Croatia.

Environmental parameters

Data on twelve environmental parameters (measured monthly from 2017–2020) were obtained from Hrvatske vode (Croatian Waters), the legal entity for water management in Croatia. The parameters used as descriptors of environmental conditions during an annual cycle at each sampling site were: water temperature (°C); alkalinity (mg $CaCO_3$/l); conductivity (µS/cm); pH; nitrates (mg N/l); nitrites (mg N/l); organic nitrogen (mg N/l); orthophosphates (mg P/l); BOD_5 (mg O_2/l); COD (mg O_2/l); dissolved oxygen (mg O_2/l) and oxygen saturation (%).

The general physico-chemical water parameters at the sites where *H. invalida* was found were averaged and the standard deviation was calculated for tabular presentation.

Results

Of the 141 sampling sites, *H. invalida* was found at six sites, mostly in semi-natural sections of the large rivers Drava and Sava (Figure 1). It was found on heterogeneous substrates, ranging from stony substrates (megalithal, macrolithal) to fine sediments (argyllal, psammal) with macrophytes (phytal) present, as at Site 4 in the Drava River (Table 1). Annual average values of general physico-chemical parameters indicate good water quality, with occasionally recorded higher values (e.g. for conductivity) at the Pongračevo-

Table 1. Types of microhabitats found at sites and percentage of *Hypania invalida* in the benthic macroinvertebrate community.

River and Site name	1. Danube River, Borovo	2. Drava River, upstream from Osijek	3. Drava River, Nard	4. Drava River, Petrovo selo	5. Canal Pongračevo-Kravnički, Josipovac	6. Sava River, Račinovci
Latitude Longitude	45.38091N; 18.96729E	45.57209N; 18.64860E	45.66696N; 18.48880E	45.72773N; 18.32041E	45.57902N; 18.58732E	44.85250N; 18.90850E
Time of sampling	25.09.2018	27.09.2018	27.06.2018	27.06.2018	09.05.2019	16.07.2019
% of *H. invalida* in total benthic community	1.9	0.3	0.4	3.5	0.1	0.6
Number of individuals /m^2	15	2	5	49	1	2
Substrate composition %:						
Psammal		10	90	85		55
Phytal			10	15	20	
Microlithal		30				
Mesolithal		30				
Macrolithal	50	30				
Megalithal	50					
Argyllal					80	45

Table 2. Annual average values of basic physico-chemical water parameters at sites where *Hypania invalida* was found, presented as average ± standard deviation.

River and Site name	1. Danube River, Borovo	2. Drava River, upstream from Osijek	3. Drava River, Nard	4. Drava River, Petrovo selo	5. Canal Pongračevo-Kravnički Josipovac	6. Sava River, Račinovci
Water temperature (°C)	15.5 ± 7.3	14.7 ± 7.2	17.6 ± 6.9	17.2 ± 6.8	14.3 ± 7.9	17.5 ± 5.7
Alkalinity ($mgCaCO_3/l$)	148.2 ± 99.7	134.6 ± 26.4	145.6 ± 39.6	150.6 ± 38.4	340.5 ± 99.7	184.9 ± 12.1
Conductivity (µS/cm)	398 ± 77	325 ± 110	316 ± 33	326 ± 38	1733 ± 1276	467 ± 28
pH	8.2 ± 0.1	8.1 ± 0.2	8.15 ± 0.13	8.08 ± 0.17	8.10 ± 0.35	8.00 ± 0.03
Nitrates (mgN/l)	1.53 ± 1.63	1.20 ± 0.66	0.88 ± 0.25	0.90 ± 0.18	1.09 ± 1.63	1.08 ± 0.14
Nitrites (mgN/l)	0.01 ± 0.02	0.01 ± 0.66	0.01 ± 0.25	0.01 ± 0.18	0.02 ± 1.63	0.01 ± 0.00
Organic nitrogen (mgN/l)	0.32 ± 0.16	0.28 ± 0.11	0.67 ± 0.19	0.69 ± 0.15	0.70 ± 0.65	0.27 ± 0.12
Orthophosphates (mgP/l)	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0	0.02 ± 0	0.06 ± 0.08	0.04 ± 0.01
BOD_5 (mgO_2/l)	1.8 ± 3.2	1.68 ± 0.43	1.72 ± 0.55	1.60 ± 0.66	5.35 ± 3.23	1.25 ± 0.44
COD (mgO_2/l)	3.6 ± 0.6	3.65 ± 1.04	3.93 ± 0.81	3.60 ± 0.52	6.82 ± 3.13	3.02 ± 0.66
Dissolved oxygen (mgO_2/l)	10.6 ± 1.2	10.1 ± 1.25	9.20 ± 0.78	9.12 ± 1.01	8.64 ± 4.22	9.03 ± 0.72
Oxygen saturation (%)	105 ± 11	98 ± 8.7	96.0 ± 10.4	93.9 ± 5.1	79.3 ± 31.3	92.8 ± 3.90

Kravnički Canal (Table 2). In the benthic invertebrate assemblages, individuals of *H. invalida* were relatively rare, contributing up to 3.5% of the total abundance of benthic macroinvertebrates. Other invasive/alien macroinvertebrate taxa co-occurring with *H. invalida* were amphipods *Dikerogammarus villosus* (Sowinsky, 1894), *D. haemobaphes* (Eichwald 1841), *Chelicorophium curvispinum* Sars, 1895, *Echinogammarus ischnus* (Stebbing, 1899), isopod *Jaera istri* Veuille, 1979, bivalves *Corbicula fluminea* (Müller, 1774), *Dreissena polymorpha* (Pallas, 1771) and oligochaete *Branchiura sowerbyi* Beddard, 1892 (Table 3). Invasive/alien species were dominant, but only at some sampling sites, while at others some native taxa (co-)dominated, e.g. snail *Lithoglyphus naticoides* (C. Pfeiffer, 1828), oligochaete *Limnodrilus hoffmeisteri* Claparède, 1862 and Chironomidae.

Discussion

The Danube River is the link between the Ponto-Caspian Region and the western part of Europe through the Black Sea. Prior to this study, *H. invalida*

Table 3. Dominant and alien/invasive (*) macroinvertebrate taxa and number of individuals per m^2 found at the same sampling site as *Hypania invalida*.

Taxon \ River and site name	1. Danube River, Borovo	2. Drava River, upstream from Osijek	3. Drava River, Nard	4. Drava River, Petrovo selo	5. Canal Pongračevo-Kravnički, Josipovac	6. Sava River, Račinovci
CHIRONOMIDAE						
Chironomini	8	2	488	192	95	7
Tanypodinae	–	–	28	16	13	–
Tanytarsini	2	10	–	–	10	–
Ortholadiinae	4	2	260	32	40	–
AMPHIPODA						
**Chelicorophium curvispinum*	26	68	756	4944	–	38
**Dikerogammarus haemobaphes*						153
**Dikerogammarus villosus*	310	373	376	176	–	
**Echinogammarus ischnus*	–	–	–	8	–	–
ISOPODA						
**Jaera istri*	8	54	480	464	–	–
OLIGOCHAETA						
Enchytraeidae gen. sp.	–	10	104	56	47	–
**Branchiura sowerbyi*	–	10	–	544	–	13
Limnodrilus hoffmeisteri	205	67	168	1240	66	–
Limnodrilus sp.	–	–	580	–	47	–
Psammoryctides barbatus	92	–	272	–	–	–
GASTROPODA						
Lithoglyphus naticoides	–	–	8	1016	–	6
BIVALVIA						
**Corbicula fluminea*	–	37	284	240	–	3
**Dreissena polymorpha*	5	1	–	–	–	–
ODONATA						
Gomphus vulgatissimus	–	–	27	118	–	6

was found at three sites in the Danube River near Croatia and at six sites in the Sava River (Zorić et al. 2011), but not in the right tributaries of the Danube in Croatia. The distance between Danube Site 1 in Croatia and the nearest site (Site C) in Serbia was more than 35 km (SE). Site 2 at Drava River and the nearest site (Site B) in Serbia were 21 km (east) apart, and Site 6 at Sava River and Site D in Serbia were 80 km apart. From 2009, regular monitoring was conducted at Sava River in Račinovci (Site 6), and *H. invalida* was found for the first time in 2019. Since the closest finding of this species in Serbia was at Site D, 80 km downstream in 2006 (Zorić et al. 2011), and it took less than 13 years for it to colonize Sava Site 6 in Croatia upstream, it is evident that *H. invalida* is spreading upstream. Upstream migrations from the Danube to the Main, Rhine and Moselle Rivers have been described by several authors (Russev and Marinov 1964; Kothé 1968; Manoleli 1975; Schmidt et al. 1998; Devin et al. 2006).

We assume that *H. invalida* has spread from the Danube to the Drava River. A plausible explanation could be the transport of *H. invalida* individuals by flow and fluvial navigation. Transport is only possible from the mouth of Drava River to Donji Miholjac (waterway class II; ECMT, 1993) and the furthest upstream site where *H. invalida* was found is 10 km downstream from Donji Miholjac.

The transfer of these polychaetes from the Serbian part of the Sava River to the Croatian part is also possible by river transport. Zorić et al. (2011)

concluded that *H. invalida* is now widespread in the potamon riches of large rivers which are under the influence of hydromorphological alterations and exposed to intensive shipping traffic. However, according to our results, shipping traffic might be more important than hydromorphological alterations, as most of our sites were located at semi-natural river sections (Urbanič et al. 2020).

The species is supposedly typical of the Eupotamon of large rivers (Šporka 1998), but we found a single specimen inhabiting a small canal with well-developed macrophyte cover connected to the Drava River, about 6 km away (Site 5). Recreational anglers fish in this area, so we suspect that upstream migration of *H. invalida* is also possible through the use of fishing nets by recreational anglers (Smith et al. 2020). If nets are not properly disinfected, macroinvertebrate transmission is possible.

Hypania invalida is also known to tolerate a wide gradient of some environmental characteristics such as salinity and temperature (Norf et al. 2010; Parr et al. 2007), which was also confirmed in our study where a relatively wide range of these environmental gradients was present. Furthermore, our results suggest that this species may prefer a low-phosphorus aquatic environment. However, the relatively low observed abundances (1–49 individuals/m^2) compared to some other studies (e.g., more than 3000 individuals/m^2 in the Main River (Schmidt et al. 1998)) might be related to semi-natural conditions and the relatively low observed nutrient concentrations (phosphorus and nitrogen).

There are some reports of co-occurrence of species from the Ponto-Caspian region such as *Chelicorophium curvispinum*, *Dikerogammarus villosus* and *H. invalida* along a river section or lake without further information on their relationship, therefore the high number of interactions is considered neutral. To date, no cases of mutualism have been reported, suggesting that they may have mechanisms that facilitate their coexistence through processes in addition to habitat partitioning, such as community-level interactions (Gallardo and Aldrige 2015).

Further studies should address the issue of potential impacts of *H. invalida* on native biodiversity and ecosystem functioning. However, at present we cannot speculate on the possible impact of polychaetes on benthic communities in Croatian rivers due to low population density.

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Supplementary material

The following supplementary material is available for this article:

Table S1. List of sampling sites with coordinates and presence/absence indication of *Hypania invalida*.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2021/Supplements/BIR_2021_Vuckovic_etal_SupplementaryMaterial.xlsx