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Rapid Communication

Invading Europe: the tropical aquatic worm *Branchiodrilus hortensis* (Stephenson, 1910) (Clitellata, Naididae) extends its range

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Abstract

We report the occurrence of non-indigenous *Branchiodrilus hortensis* (Stephenson, 1910) in Serbia and its rapid spread in Southeast Europe. These new findings were observed at locations about 1000 Rkm downstream from the nearest known record in the Danube (in July 2008) in the middle part of the river section in the Slovak-Hungarian part. *Branchiodrilus hortensis* was registered for the first time in Serbia in September 2018 during routine monitoring of the Danube River in the lower part of the river section. Since then, we have continuously found this species in the Serbian part of the Danube, and it has spread to the main tributaries and canals of the Danube basin. These new records are a valuable contribution to the knowledge of this species' distribution. Recent findings and the native distribution of this worm suggest that its introduction in the Danube was probably human-mediated, and it has successfully established its niche in the new environment. At this phase we cannot predict the possible effects of its presence on aquatic ecosystems and therefore further monitoring of its distribution and population dynamics is necessary.

Key words: Oligochaeta, new records, invasive species, Serbia, Danube, Southeast Europe

Introduction

Information on the distribution of non-indigenous *Branchiodrilus hortensis* (Stephenson, 1910) (Annelida: Oligochaeta) in Europe is scarce. This organism belongs to a species of the family Naididae, subfamily Naidinae, rare in Europe and with native distribution related to Asia, Australia and Africa. Within the genus *Branchiodrilus* Michaelsen, 1900 that includes 3 known species distributed in the tropics, only *Branchiodrilus hortensis* was registered in the Holarctic (van Haaren et al. 2005; Šporka 2009). According to van Haaren and Soors (2013) it was probably introduced into European freshwaters from aquaria. The first discovery of this aquatic oligochaete in Europe was together with a tubificid species, *Branchiura sowerbyi* Beddard, 1892 in 1890–1892 in the Botanical Garden in London. This specimen was reported by Beddard (1895) as *Chaetobranchus*, referring to *C. semperi* Bourne, 1890 since it was the only species known at that time. Since then, it

has been recorded in the Netherlands, Belgium, France, Slovakia and Germany as the species *Branchiodrilus hortensis* (van Haaren et al. 2005; Šporka 2009; van Haaren and Soors 2013; Martin et al. 2018; Baumgartner et al. 2020).

Recent molecular phylogeny suggests that species' complexes can occur within the genus *Branchiodrilus*, which potentially represents 10 different species (Martin et al. 2018). However, morphological examination of groups of all specimens from the Palaearctic region suggested that they all belong to the same species, probably *B. hortensis*, one of three nominal species of the genus (Martin et al. 2018). Furthermore, most recent molecular analysis by Martin et al. (2018) confirmed that the Oriental region might be the centre of origin from which *Branchiodrilus* species have dispersed and radiated.

Branchiodrilus hortensis was recorded in Serbia for the first time in September 2018, representing at the same time the first record for East and Southeast Europe. In this paper we present new records that are a valuable contribution to the knowledge of the distribution of the species. The new findings provided herein extend its distribution from west and central to southeast Europe. This worm is expected to expand its areal to neighbouring watercourses. In this phase we cannot predict the possible effects of the presence of this organism on aquatic ecosystems and therefore further monitoring of its distribution and population dynamics is necessary.

Materials and methods

The investigation was performed as a part of several independent projects focused on the monitoring of the watercourses in Serbia. Field survey included investigation of the Danube, a river section that flows through Serbia, its main tributaries, Tisa, Sava, Velika Morava and Pek, as well as canals of the Belgrade area and most of the watercourses in the Pannonian Plain and hilly mountainous parts of Serbia. Over a hundred sampling sites were investigated during the research. The specimens were collected during routine monitoring along the Danube River in Serbia from September 2018 until September 2020 (within the program for monitoring, measuring and analysing the impact of Danube flow slowdown caused by the construction and operation of the hydro power plants (HPP) "Đerdap 1" and HPP "Đerdap 2"). Additional findings were also recorded during the Joint Danube Survey (JDS) in 2019 (JDS 4, supported by the International Commission for the Protection of the Danube River (ICPDR)), and during the project entitled Conducting Research Monitoring of Phytobenthos and Aquatic Macroinvertebrates, at selected locations with the aim of drafting the Water Management Plan for the Republic of Serbia (Project No. 01-2570, 2019).

Samples were collected with a benthological hand net, mesh size 500 μm, using the kick and sweep technique (EN 16150:2012: Water quality –

Table 1. Findings of *Branchiodrilus hortensis* (Stephenson, 1910) specimens.

Sampling site	Coordinates	Number of specimens					
		September 2018	April 2019	July 2019	October 2019	May 2020	October 2020
Danube, Ram	N44.814333°; E21.330509°	8	–	–	–	–	5
Danube, Veliko Gradište	N44.768018°; E21.524497°	9	5	–	–	–	12
Danube, Donji Milanovac	N44.464573°; E22.137682°	2	–	–	–	–	–
Danube, Kladovo	N44.612264°; E22.639825°	56	–	–	–	–	–
Tisa, Martonoš	N46.133904°; E20.061427°	–	–	4	–	–	–
Karaš Canal	N45.096656°; E20.376144°	–	–	–	10	–	–
Begej, Ečka	N45.305356°; E20.449528°	–	–	–	–	5	–
Tamiš, Opovo	N45.006645°; E20.470348°	–	–	–	–	7	–

Guidance on pro-rata Multi-Habitat sampling of benthic macro-invertebrates from wadeable rivers. European Committee for Standardisation 2012) from an area of 0.0625 m^2 and from all available habitats, represented with more than 5% of the total habitat area on the sampling stretch according to the multi-habitat sampling procedure.

The worms are preserved in 70% ethanol and stored in the collection of biological materials of the Benthological Section of the Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, University of Belgrade.

Results

Since the first finding in September 2018, when a total of 75 specimens were recorded in the Danube at four localities downstream from the Belgrade area, several new records of this alien species have been recorded. To date, *Branchiodrilus hortensis* were observed at eight sampling sites, listed in Table 1 and presented on Figure 1. The largest number of findings was recorded in the mainstream of the Danube, with a total of 92 specimens at four sampling sites. But this tropical aquatic worm extended its distribution along the tributaries, Tisa and Tamiš Rivers, as well as the canal Karaš which links the Danube and Tamiš rivers. The only locality not connected directly with Danube main canal, where findings were recorded was in the Begej River, a tributary of the Tisa River.

All of the morphological characters of the examined individuals corresponded to the species *B. hortensis* according to the Nesemann et al. (2004), Timm (2009) and Van Haaren and Soors (2013) guides for species identification (Figure 2A–C). The genus *Branchiodrilus* can easily be recognized by the presence of pairs of dorso-lateral gills almost throughout its entire body. Simple pointed and fine needles are enclosed within these gills in the anterior segments (Figure 2B). In the posterior segments, the needles are free and are not enclosed within the gills (Figure 2C).

Oligochaete communities in samples with *Branchiodrilus hortensis* were typical for lowland rivers with a soft-bottom substrate. In general, in the samples from the Danube, beside *B. hortensis*, other naidines were present, such as *Nais bretscheri* Michaelsen, 1899, *Ophidonais serpentina* Müller, 1774

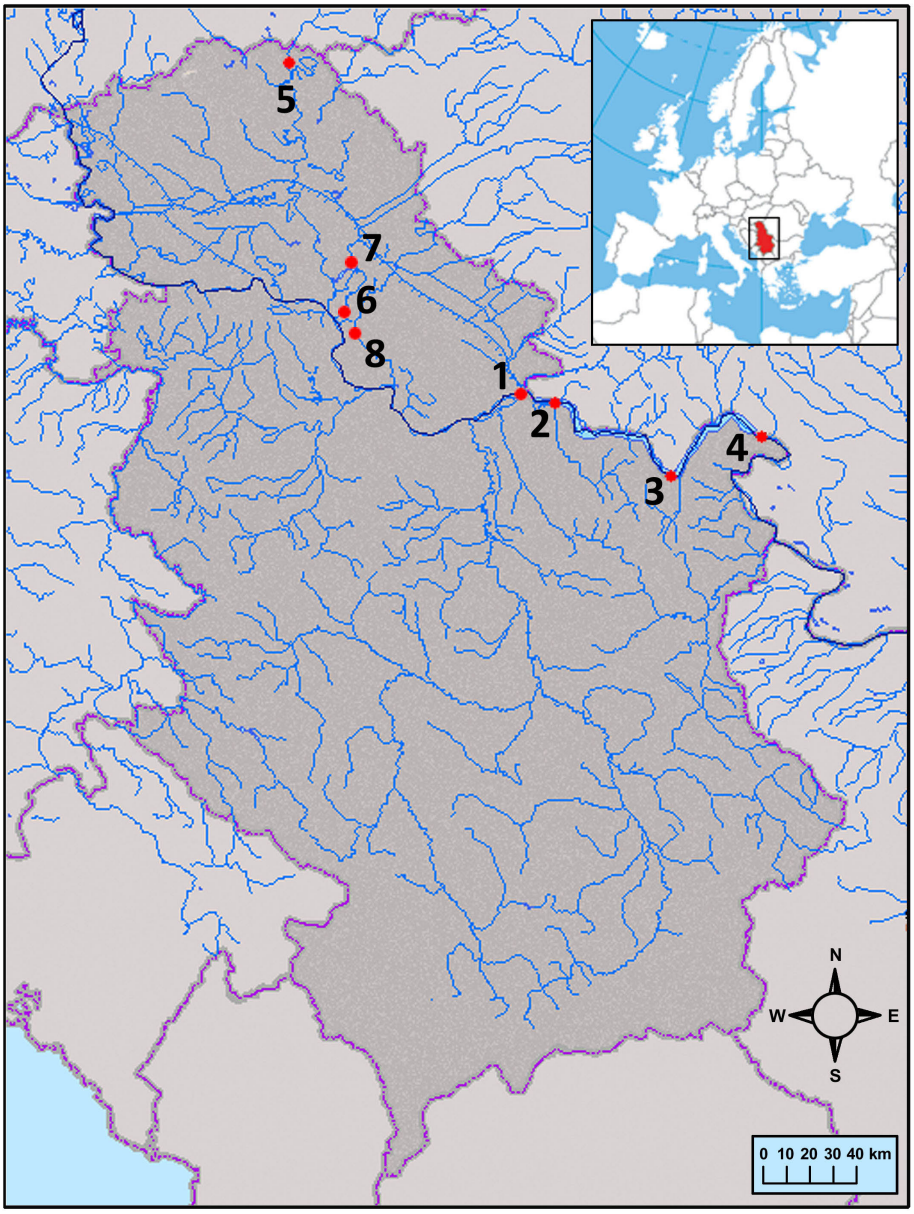

Figure 1. Map with sampling sites where *Branchiodrilus hortensis* (Stephenson, 1910) has been recorded. Sampling sites are numbered in order of the findings from the first to the last (according to Table 1).

and *Stylaria lacustris* (Linnaeus, 1758), as well as tubificines *Limnodrilus*, *Potamothrix*, *Psammoryctides*, *Branchiura sowerbyi*, *Isochaetides michaelseni* (Lastočkin, 1937). The most abundant species in the samples was *Limnodrilus hoffmeisteri* Claparède, 1862. A similar oligochaete assemblage with domination of *L. hoffmeisteri* was noted in a sample from the river Tamiš, however, in the Begej River only the following naidines were recorded: *Dero dorsalis* Ferronnière, 1899, *N. bretscheri*, *O. serpentina*, *S. lacustris* and *B. hortensis*.

Discussion

Our records are in agreement with previous records in Europe of species collected in lowland rivers and municipal canals in suburban areas on clay

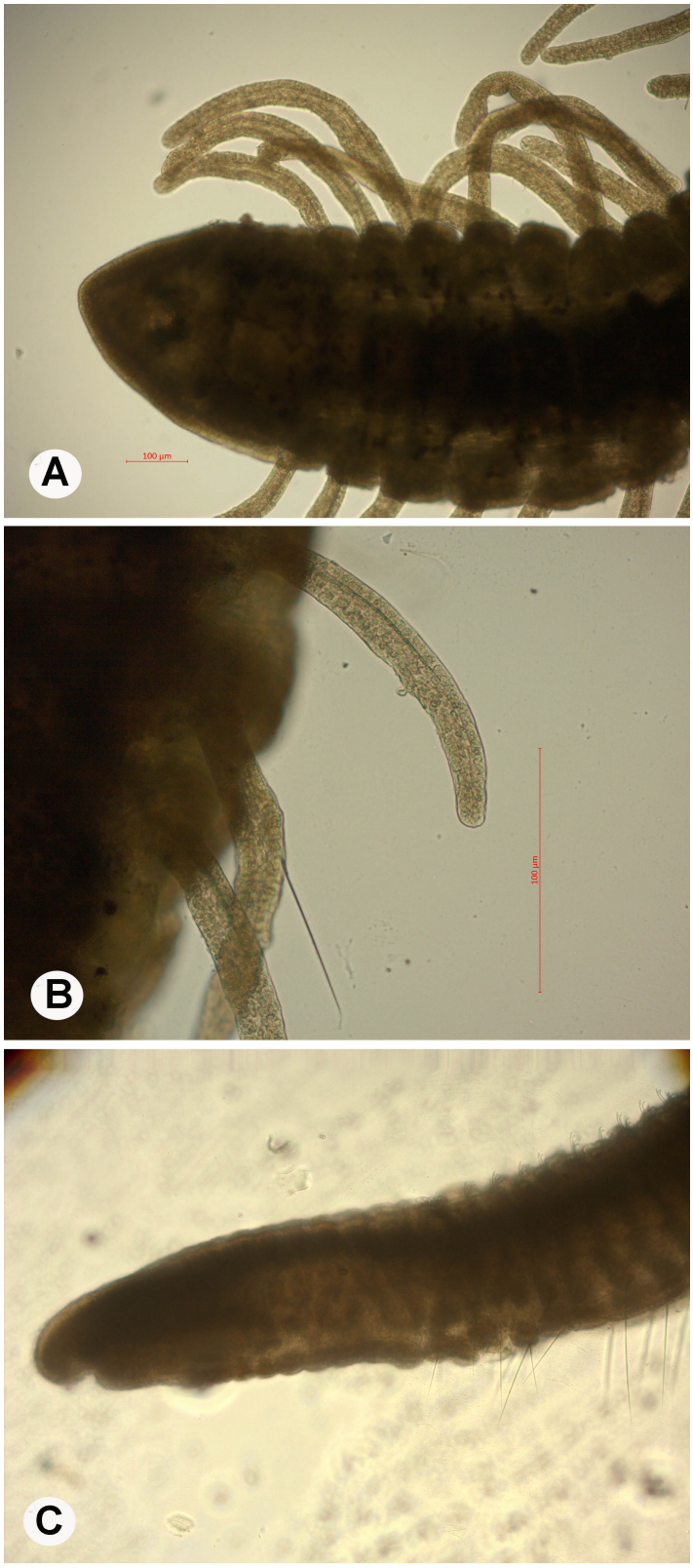

Figure 2. *Branchiodrilus hortensis* (Stephenson, 1910), specimen found in September 2018 from the Danube River; A) anterior part of the body; B) needles and gills; C) posterior part of the body. Photomicrographs by Atanacković et al.

soil. As van Haaren and Soors (2013) pointed out, the specific demands for this species, which resides on silty and silty-sand types of substrates, remain to be determined. All findings of *Branchiodrilus hortensis* in the

Danube were upstream from the Iron Gate dam in the zone of backwater effect that extends to Belgrade. Construction of the dam significantly slowed down the river current along this stretch of the river, which is under intensive sedimentation, impounding, heating, retention of organic matter and bed load suspension (Atanacković et al. 2013).

The vector of introduction for *Branchiodrilus hortensis* remains unknown. All reported populations consisted of asexual specimens. Sexually mature specimens are rarely found in Europe but have been observed in large numbers in native habitats in Asia (Nesemann et al. 2004). As a result, asexual reproduction by paratomy is typical for this species and its spread is probably supported by the water current. Although we found only small populations, *Branchiodrilus* is generally capable of forming dense populations and together with *Limnodrilus hoffmeisteri*, it can be dominant in oligochaete assemblages (Nesemann et al. 2004). As for its ecology, the species is adapted to a wide range of substrate type, from small wetland pools to large ponds (Nesemann et al. 2004), and it seems to be tolerant to organic load and pollution given the localities where it has been recorded in Europe in suburban areas. According to Nesemann et al. (2004), the species was only found among aquatic plants near the bank, floating or submerged macrophytes, and according to van Haaren and Soors (2013), the presence of aquatic weeds is not obligatory. In our investigations, the worms were observed from November to March in larger numbers. We still do not know enough which environmental factors could limit the distribution of this species (salinity maybe), but the species does not appear to be limited by the presence or absence of detritus.

Scattered findings and the native distribution of this worm suggest that its introduction in the Danube was primarily human-assisted. A natural spread from Slovak-Hungarian stretch of the Danube is a possibility, but we must consider that the extensive studies of the entire course of the Danube has been conducted since then – Joint Danube Surveys JDS 3 (Liška et al. 2015; Atanacković et al. 2020) and JDS 4 (Liška et al. 2021). From initial finding in the Danube reported by Šporka (2009) *B. hortensis* was not recorded anywhere in those 1000 kilometres of the river between Gabčikovo-side arm (Slovak-Hungarian part) and Ram (Serbia). The populations in the Danube could be the result of ballast water introduction (first introduction in Slovakian river stretch and second introduction in Serbian river stretch), but this vector is less likely in canals where there are no commercial shipping or barging routes that could connect these populations. The secondary introduction into other watercourses in Serbia is probably a result of natural dispersal of the species.

These new records represent a valuable contribution to the knowledge of the distribution of this species. Since the first introduction in Europe, *Branchiodrilus hortensis* has been recorded in 6 European countries. The present finding extends further the limits of its distribution to 7 countries, including Serbia.

At this stage of initial invasion, we cannot predict the possible effects of this organism on aquatic ecosystems and food webs, and therefore further monitoring of its distribution, population dynamics as well as the potential impact of *Branchiodrilus hortensis* is necessary. As our findings are rather scattered and single, additional studies are needed to investigate the presence of this species in other Serbian watercourses that provide favourable conditions, and to establish which environmental parameters influence its distribution and reproduction.

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Authors' Contribution

AA – research conceptualization, investigation and data collection, data analysis and interpretation, writing – original draft; KZ – research conceptualization, investigation and data collection, writing – review and editing; MP – research conceptualization, investigation and data collection, ethics approval, funding provision and writing – review and editing.

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