



New Data on the Distribution and Relation to Environmental Factors of the Alien *Branchiura sowerbyi* Beddard, 1892 (Oligochaeta: Naididae) in Bulgaria

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Abstract: New data on the distribution of the Asian alien species *Branchiura sowerbyi* (Oligochaeta: Naididae) were reported for Bulgaria. During the period 2012–2020, the species was found in 40 lotic and lentic water bodies (17 rivers and 23 reservoirs and lakes). Eight new inhabited water bodies of different types (4 reservoirs, 2 natural lakes and 2 rivers) were registered. The spread of the species in relation to environmental parameters was analysed. The ecological preferences of the *B. sowerbyi* showed a great adaptability and tolerance towards water temperature and oxygen concentration as well characteristics of the water bodies' type. The highest number of individuals was recorded in silt-sand, natural organic matter and coarse detritus substrates.

Key words: Aquatic oligochaetes, substrate type, water parameters

Introduction

The alien species *Branchiura sowerbyi* Beddard, 1892 (formerly considered a tubificid, now placed in the family Naididae, see ERSEUS et al. 2008) is a widespread branchiate oligochaete, ubiquitous in various types of freshwater ecosystems. The species originates from Southeast and South Asia (TITZLER 1996); it is native to the tropical and subtropical Asian regions (MILLS et al. 1993, TIMM 2009). It has been firstly registered in Europe in the Royal Botanical Society's Gardens, Regents Park in London (UK) (BEDDARD 1892), presumably introduced with decorative aquatic plants. MICHAELSEN (1908) found it in a warm water tank of the Botanical Gar-

dens in Hamburg. In natural habitats, it was registered for the first time in the Kennet and Avon Canal in Britain (MANN 1958). According to TIMM (2009), the introduction in the wild in Europe cannot be precisely dated but probably has taken place in the first half of the 20th century, only in warmer waters and never in the profundal of lakes. Since the first record, *B. sowerbyi* has been reported from 23 countries throughout Europe (GIANI 2004). As an alien species, it is included in the “watch list” (according to the online information system on the aquatic species – AquaNIS 1915).

The first record of *B. sowerbyi* on the Balkan Peninsula was in Croatia in 1956 (after Žganec et al. 2020). In Bulgaria, DIMITROV (1966) reported

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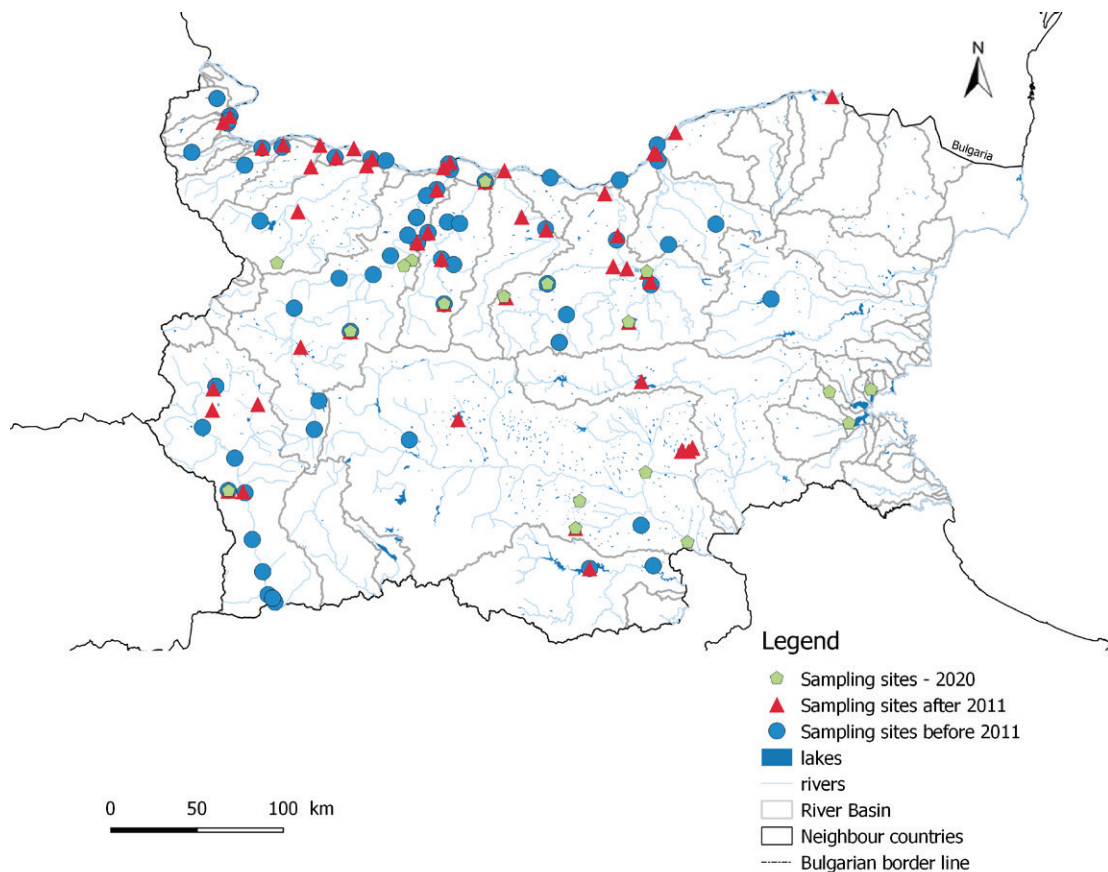


Fig. 1. Map of the records of *Branchiura sowerbyi* in Bulgaria in 2012–2020 (present study) and before 2011 (after GEORGIEVA et al. 2012).

the species from the benthos samples of a fish farm on the Belene Island in the Danube R. since 1964. Later, UZUNOV (1976) reported it from the Struma River. Up to now the species has been reported from different water body types in Bulgaria as part of the macroinvertebrate community (TRICHKOVA et al. 2013, VARADINOVA et al. 2013, GEORGIEVA & UZUNOV 2015, GEORGIEVA et al. 2017, VIDINOVA et al. 2016, SUBEVA et al. 2019, VARADINOVA et al., 2022). The distribution pattern of this species within nearly five decades (1964–2011) was summarized by GEORGIEVA et al. (2012). In Serbia, *B. sowerbyi* was found in 1972 (PAUNOVIC et al. 2005) and much later, in 2008, in Greece (GRABOWSKI & JABLOŃSKA 2009). ZORIĆ et al. (2020) registered this species in the main course of the Danube River and its tributaries; it has even been found that the species dominated in the composition of the oligochaete group at some localities.

This article aims to summarise the data on the distribution of *B. sowerbyi* in Bulgaria in the time-frame 2012–2020 and to analyse the information on the species dispersal in relation to some environmental factors during 2020.

Materials and Methods

The study on the distribution of *B. sowerbyi* on the territory of Bulgaria was conducted in 2012–2020. The relation between distribution of the alien species and the environmental parameters was analysed in 2020. Samples from 14 rivers' catchments of the rivers Danube, Vidbol, Lom, Tsbritsa, Ogosta, Osam, Iskar, Vit, Yantra, Rusenski Lom, BlackSea Rivers, Maritsa, Arda and Struma were checked (Fig. 1). The macrozoobenthos samples were taken following the multi-habitat sampling methods (CHESHMEDJIEV et al. 2011) according to the standards BDS EN ISO 10870:2012 and EN 16150:2012. The identification of the species was made using species keys' of CHEKANOVSKAYA (1962) and VAN HAAREN & SOORS (2012).

In 2020, in parallel with benthic samples, basic physical and chemical water parameters were analysed with a portable Windaus Labortechnik Package. Water temperature ($^{\circ}\text{C}$) (TEMP), dissolved oxygen concentration (mg/L) (DIOX), electrical conductivity ($\mu\text{S}/\text{cm}^{-1}$) (COND) and hydrogen ion concentration (pH) were measured *in situ*. Total

phosphorus (mg/L) (TP) and total nitrogen (mg/L) (TN) were measured in the laboratory using HANNA multi-parameter instruments. In 2020, characterisation of the bottom substrates at the studied localities was done based on the percentage distribution of the different types of substrate, presented at the sampling sites.

The map of the surveyed localities (Fig. 1) was visualised with the software product Quantum GIS Version 2.18 Las Palmas. The method of DE VRIES (1937) was applied to analyse and calculate the indicator “frequency of occurrence” (*pF*) of the *B. sowerbyi* in the studied water bodies during 2020. The method presents the number of samples in which the species was found, in relation to the total number of collected samples. Principal Component Ordination (PCA) analysis techniques supported by the package CANOCO 4 (TER BRAAK & Šmilauer 1998) was applied to present the distributional preferences of the oligochaete at the studied sites in relation to the measured environmental parameters – parametric (TEMP, DIOX, pH, COND, TP and TN) and non-parametric (substrate types).

Results

During the whole studied period (2012–2020), *B. sowerbyi* was registered in 80 benthic samples. They belonged to 11 types of standing water bodies and five river types, including the Danube River. Most of the sampling sites belonged to six water bodies

from the Yantra River basin in the Ecoregion (ER) Pontic Province (ER 12). Eight sites were in the Maritsa River basin and five sites were in the Struma River basin, both basins belonging to the ER Eastern Balkans (ER 7). In 2020, *B. sowerbyi* was found at 18 sites – 4 lotic and 14 lentic water bodies (Table 1). Additionally, *B. sowerbyi* was recorded in the α -mesohaline (AquaNIS) Mandra Lake – East (16.7 psu) with 18 specimens and in the euhaline Atanasovsko Lake – North (36 psu) with two specimens.

Of all 18 samples from 2020, the species was more abundant in water bodies with predominance of silt (7 samples), gravel (stones between 2 and 6 cm – 4 samples) and silt-sand (3 samples) on the bottom (Fig. 2).

The lowest value of pH was registered at Krpets Reservoir (6.9) and the highest – in the tail of Gorni Dubnik Reservoir (10.2) (Table 2). The range of water temperature varied between 22.3°C (Gorni Dubnik Reservoir tail) and 29.3°C (in Rozov Kladenets Reservoir). The average temperature in the studied water bodies was 25.3°C. The average amount of dissolved oxygen in the water was 8.76 mg/L and fluctuated between 5.02 mg/L (Vit River – Gulyantsi) and 13 mg/L (Rozov Kladenets Reservoir). In the latest reservoir, the highest amounts of nutrients (total phosphorus 5.66 mg/L and total nitrogen 0.818 mg/L) were registered.

According to the PCA diagram, the first two principal components (PCs) explained 66.16 % of the total variance in environmental data (Fig. 3). The

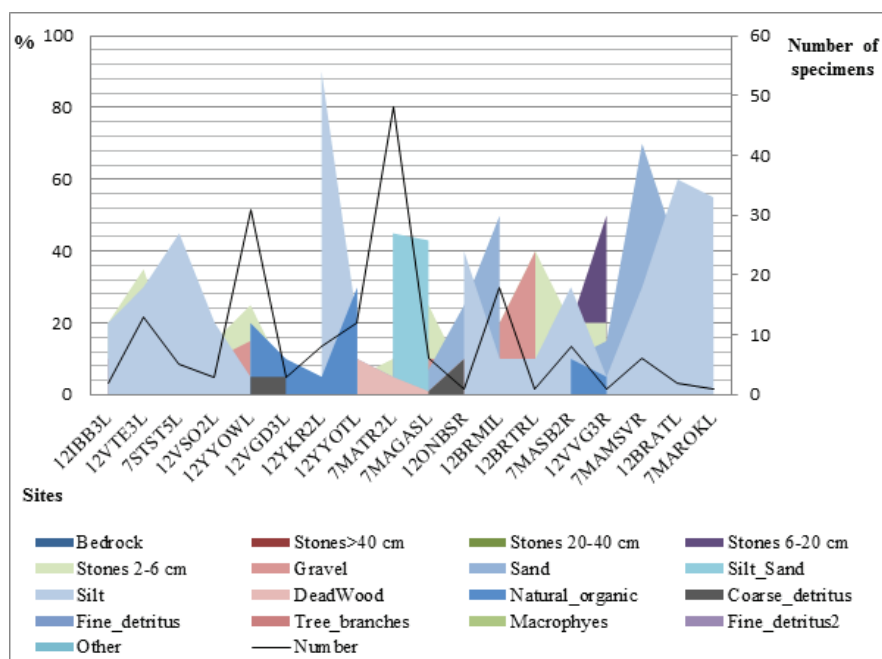


Fig. 2. Substrate types proportion (in %) and number of the specimens of *B. sowerbyi* at the sampled sites. The codes of sites are indicated in Table 1.

Table 1. General information on the distribution of *Branchiura sowerbyi* in Bulgaria in 2012–2020. Abbreviations: d/s – downstream and u/s – upstream

Site Code	Site	Coordinates		River/ lake type	Year of finding
		Y	X		
	Danube River				
12DD01R	Danube River–Orsoya	43.777380	23.094800	R6	2012
12DD02R	Danube River–Kozloduy	43.796230	23.755620	R6	2012
12DD03R	Danube River–Aydemir	44.114302	27.205182	R6	2013
12DD04R	Danube River–d/s Iskar River	43.739770	24.450830	R6	2013
12DD05R	Danube River–d/s Ogosta River	43.745350	23.889440	R6	2013
12DD06R	Danube River–Ardjef	44.054950	26.624833	R6	2013
12DD07R	Danube River–u/s Ruse	43.922530	26.073090	R6	2014
12DD08R	Danube River–Novo selo	44.163097	22.788319	R6	2019
12DD09R	Danube River–Vetren	44.142219	27.053184	R6	2019
12DD10R	Danube River–Cherkovitsa	43.705760	24.844590	R6	2014
12DD11R	Danube River–Baikal	43.713770	24.406720	R6	2014
	Vidbol River				
12VID2R	Vidbol River–Dunavtsi	43.903020	22.805330	R8	2014
12VIS3R	Vidbol River–Sinagovtsi	43.903020	22.805330	R8	2015
	Lom River				
12LLOMR	Lom River–u/s Lom	43.800430	23.244890	R8	2015
	Tsibritsa River				
12TSGTR	Tsibritsa River–Gorni Tsibar	43.806380	23.510530	R8	2012
12TSVDR	Tsibritsa River–Valchedram	43.691980	23.451980	R8	2015
	Ogosta River				
12OBOHR	Botunya River–Ohrid	43.454120	23.372730	R8	2014
12OSMIR	Skat River–d/s Mizia	43.708730	23.850940	R8	2015
12ONBSR	Nechinska bara River–u/s Lom	43.706690	23.129330	R8	2020
	Osam River				
12OOI1R	Osam River–u/s Izgrev	43.466260	24.976770	R7	2014
12OOL2R, 12OOL3R	Osam River–u/s Levski	43.401110	25.155100	R7	2014, 2015
	Iskar River				
12INNGL	Negovan Reservoir	42.747070	23.433610		2013
12IIO1R	Iskar River–Orehovitsa	43.379040	25.667740	R7	2014
12IBB1L, 12IBB2L, 12IBB3L	Bebresh Reservoir	42.838969	23.780936	L2	2015, 2016, 2020
	Vit River				
12VVB1R, 12VVB2R, 12VVB3R	Vit River–Bezhanovo	43.233203	24.411192	R7	2012
12VVG2R, 12VVG3R	Vit River–Gulyantsi	43.645236	24.708386	R7	2012, 2020
12VGD1L, 12VGD2L, 12VGD3L	Gorni Dabnik Reservoir–wall	43.369925	24.308892	L14	2012, 2016, 2020
12VSO1L, 12VSO2L	Sopot Reservoir	42.999475	24.440300	L12	2015, 2020
12VTE0L, 12VTE1L, 12VTE2L, 12VTE3L	Telish Reservoir	43.314750	24.237508	L16	2012, 2015, 2016, 2020
	Yantra River				
12YDJ1R, 12YDJ2R	Dzhulyunitsa River – Dzhulyunitsa village	43.139000	25.906520	R4	2014, 2015
12YKR1P, 12YKR2P	Krapets Reservoir–Malinovo	43.042670	24.880200	L12	2014, 2020
12YRP1R, 12YRP2R	Rositsa River–Polikraishte	43.216660	25.638650	R7	2014, 2015
12YST1R	Studena River–Novgrad	43.597220	25.569760	R8	2014
12YST2R	Studena River–u/s Yantra	43.597220	25.569760	R8	2015
12YYPTR	Yantra River–Polski Trambesh	43.206770	25.736940	R7	2014

Site Code	Site	Coordinates		River/ lake type	Year of finding
		Y	X		
12YYORL, 12YYOW, 12YYOTL	Yovkovtsi Reservoir	42.926310	25.755030	L2	2012, 2020
	Rusenski Lom River				
12RLRLR	Rusenski Lom River–mouth	43.808410	25.937700	R7	2015
	BlackSea Rivers				
12BRMIL	Mandra Lake–east	42.402787	27.319357	L9	2020
12BRAVL	Asparuhov val Reservoir	43.744624	23.631389	L16	2012
12BRATL	Atanasovsko Lake–north	42.578458	27.475245	L10	2020
12BRTRL	Troyanovo Reservoir	42.566534	27.181035	L16	2020
	Maritsa River				
7MATR0L, 7MATR1L	Trakiets Reservoir	41.841640	25.405250	L15	2012, 2020
9997MAOS1L	Ovcharitsa Reservoir–cold	42.253900	26.140710	L15	2013
7MAOT5L	Ovcharitsa Reservoir–cooling	42.259520	26.144060	L15	2013
7MASB1R, 7MASB2R	Stara River–Bregovitsa	43.190720	25.880300	R7	2014, 2020
7MAPIAL	Pyasachnik Reservoir	42.397528	24.563000	L15	2018
7MAROKL	Rozov Kladenets Reservoir	42.139963	25.889729	L15	2020
7MAMSVR	Maritsa River	41.776623	26.189420	R12	2020
7MAGASL	Garvanovo Reservoir–wall	41.984918	25.430597	L15	2020
	Arda River				
7ARSTKL	Studen Kladenets Reservoir	41.633158	25.508156	L11	2012
	Struma River				
7STCHBL	Chokliovo Blato	42.397330	22.831190	L4	2012
7STPCH1, 7STPCH2	Pchelina Reservoir	42.508282	22.829263	L13	2018
7STBISL	Bistraka Reservoir	41.978368	23.073456	L6	2019
7STDDIL	Dolna Dikanya Reservoir	42.437551	23.148752	L13	2018
7STST1L, 7STST2L, 7STST3L, 7STST4L	Stoykovtsi Reservoir	41.980502	22.969856	L13	2012, 2018, 2019, 2020

Table 2. Values of the physical and chemical parameters measured at the sampling sites. For the codes of sites, see Table 1.

Code	Water temperature, °C	pH	Conductivity, µS/cm ⁻¹	Dissolved oxygen, mg/L	Total nitrogen, mg/L	Total phosphorus, mg/L
12IBB3L	23.5	7.8	204	9.48	0.1	0.01
12VTE3L	24.3	9.9	213	10.82	0.16	0.01
7STST5L	25.4	8.02	395	7.9	0.18	0.01
12VSO2L	24.8	7.7	249	10.69	0.29	0.01
12YYOWL	25.5	8	208	10.96	0.48	0.01
12VGD3L	22.3	10.2	245	9.23	2.37	0.01
12YKR2L	26.2	6.9	304	10.31	0.15	0.014
12YYOTL	26.8	8.1	228	10.4	0.5	0.033
7MATR2L	25.9	7.58	292	5.9	0.35	0.086
7MAGASL	25.2	7.63	285	5.5	0.9	0.092
12ONBSR	24.9	7.55	435	9.55	0.69	0.102
12BRMIL	23.4	8.7	581	7.84	1.6	0.12
12BRTRL	26.2	8.92	842	8.71	2.78	0.14
7MASB2R	25.8	7.63	494	8.3	1.61	0.175
12VVG3R	23.8	7.72	503	5.02	2.26	0.263
7MAMSVR	23.6	7.63	541	6.6	0.47	0.36
12BRATL	27.9	9.3	542	7.44	0.7	0.41
7MAROKL	29.3	8.31	730	13	5.66	0.818

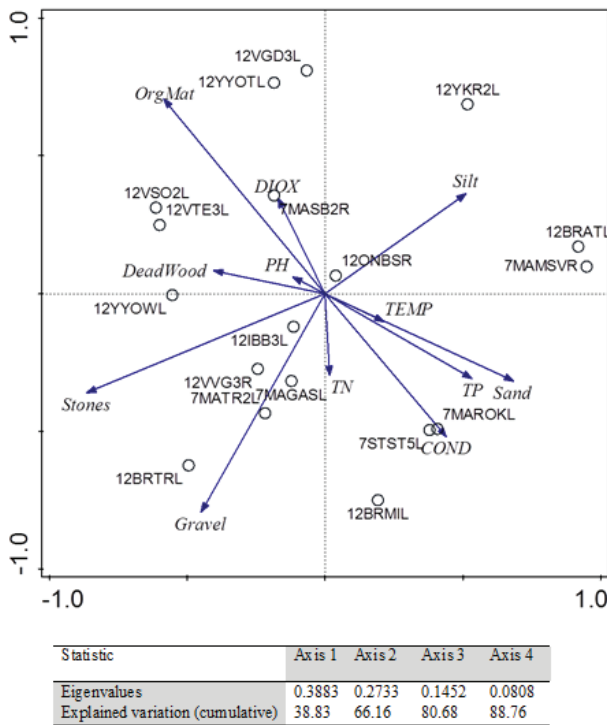


Fig. 3. Ordination diagram based on PCA of physical and chemical parameters as well as substrate types in the studied sites (for codes of sites and environmental parameters, see Table 1 and Materials and Methods).

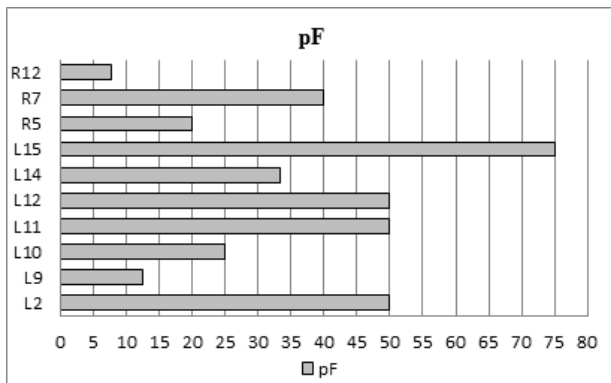


Fig. 4. Frequency of occurrence of *Branchiura sowerbyi* based on data obtained in 2020.

ordination illustrated that the type of the substrate, organic matter and conductivity played a main role in the propagation of the species. The ecoregion is also essential for the distribution of studied sites. There was no clear differentiation between the lotic and lentic sites, where *B. sowerbyi* has been found.

The data from 2020 showed that this species made up between 0.07% and 7.7% of the studied oligochaete assemblages. The frequency of occurrence (pF) according to DE VRIES (1937) for this year showed that the species was more frequent in lentic water bodies of the lake type L15 (big lowland, from

shallow to medium in depth reservoirs in ER7) (Fig. 4). In the rivers, the species was the most common in the larger Danube tributaries of the Ecotype R7.

Discussion

B. sowerbyi is an example of an alien subtropical species that not just successfully introduces itself but also develops stable colonies worldwide in surface water bodies with different conditions. In China, CUI & WANG (2008) reported that *B. sowerbyi*, together with *Limnodrilus hoffmeisteri* (Claparède, 1862), were the predominant taxa in the macrozoobenthic communities. Later studies, ALVES & STRIXINO (2000) and DORNFELD et al. (2006) for Brazil and VUČKOVIĆ et al. (2019) for Croatia, also demonstrated a prevailing abundance of *B. sowerbyi* in the benthic samples from different aquatic habitats.

Within nearly 50 years period of observations (1964–2011), *B. sowerbyi* was reported from 74 water bodies in Bulgaria (GEORGIEVA et al. 2012) and in the last nine years (2012–2020) the species has been found at 40 sites. Compared to the first studied period, eight new inhabited water bodies of different types (4 reservoirs, 2 natural lakes and 2 rivers) were registered in 2020. In this year, the species was registered as the most abundant (48 specimens) in the Trakiets Reservoir (Maritsa River basin). Within the whole period 2012–2020, the species was recorded with highest number (206 specimens) in the the Studen Kladenets Res. (Arda R. basin), unlike most of the studied samples where it was presented with lower numbers (<10).

Since the first record at 20 m a. s. l. in the Belene Island (Danube River) (DIMITROV 1966), the species distribution spreads in running and stagnant waters at higher altitudes. The average altitude the species has been recorded at in Bulgaria was 205 m a. s. l. in 2012–2019 and 214 m a. s. l. in 2020; however, during the latest year, it was found between 0 m a. s. l. (Atanasovsko Lake – North) and 880 m a. s. l. (Chokliovo Blato).

Firstly found in the Danube River and Struma River, our results showed that nowadays the species could be recognised in the oligochaete complex of all Bulgarian surface waters bodies. Currently, *B. sowerbyi* is the most frequent within the Danube River Basin and lower stream river sections where the aquatic environment is characterised by alkaline values. In Poland, CEBULSKA & KRODKIEWSKA (2017) reported new localities 400 km away from the previous recordings in the Oder River and suggested that the species had spread upstream. COSTA et al. (2021) define the transport contamination and

release due to human activities as the most likely pathways of introduction and spread.

Our results demonstrated that the species prefer neutral (pH 6.9 in Krapets Reservoir) to alkaline (pH 10.2 in the tail of Gorni Dubnik Reservoir) waters (SUBEVA et al. 2019; unpublished data).

The species has got a great adaptability and it could be assumed that the changes in the environmental conditions, triggered by the likely climate changes, could open new niches in previously unapproached habitats for the species.

The species originated from wild freshwaters in the Asian climate and according to the Venice system of 1958, it inhabits the limnetic [<0.5 psu] salinity zone (AquaNIS). It was mentioned as an alien species invertebrate in the Odra River estuary, which is thought to be one of the gateways for alien species immigration to the Baltic Sea Basin (GRUZHKA 1999). Regardless the fact that the species prefers the limnetic [<0.5 psu] salinity zone, during the 2020 sampling period, it was recorded in two coastal lakes – the α -mesohaline (AquaNIS) Mandra Lake – East (16.7 psu) and the euhaline Atanasovsko Lake – North (36 psu) in the Black Sea Basin. These are the first records in brackish waters in Bulgaria, although the species was presented by only two specimens per site.

Our previous studies showed that *B. sowerbyi* is a typical inhabitant of slow-running waters with intensive sedimentation, so the hydrological conditions in the plain rivers contribute to the successful distribution and adaptation of this species (GEORGIEVA et al. 2012). As the environmental conditions in the indicated river sections are similar to those in the standing waters, we did not establish a clear distinction between the running and standing sites where the species has been registered (Fig. 2). In September 2014, the species was recorded with the number of 89 specimens in the semi-mountain Dzhuliunitsa River where the aquatic environment was not typical for *B. sowerbyi*. During this particular sampling, the water temperature was 19°C and the amount of oxygen was 8.6 mg/L. The flexibility and adaptability of this thermophile species show that in Bulgaria it can survive and thrive in different regions and diverse ecological conditions.

GEISSEN (1999) defined the sites where the species was registered with mostly fine sand to silty-clay, partly gravel or mixed sediments. *Branchiura sowerbyi* buries itself in the soft substrate and leaves the gilled posterior part of the body to perform undulatory breathing movements above the surface. This species plays an important role in the sediment particles' transportation and affects nitrification by

changing the oxidation-reduction conditions in the sediments (FUKUHARA et al. 1980).

The analysis of the bottom substrate in the samples from 2020 showed that the highest number of specimens of the species was registered at sites where silt-sand, natural organic matter and coarse detritus prevailed over the other substrate types (Figs. 2 and 3). Earlier studies showed that the species prefers shallow, stagnant or slowly flowing waters, associated with sediments with lower levels of oxygen (BRINKHURST & JAMIESON, 1971, NAQVI, 1973, RAPOSEIRO et al. 2009). During the studied period (2012–2020), the species was found at both relatively low values and in oxygen-rich environments (Table 2). In 2020, in the Rozov Kladenets Reservoir, the oxygen levels reached the highest registered values of 13 mg/L and might operate as a restrictive factor for *B. sowerbyi* since only one specimen was registered.

Branchiura sowerbyi shows a selective feeding on organic-rich, fine-grained particles and an increase in particle selectivity with an increase in population density (MATISOFF et al. 1999). According to the functional feeding classification (CHESHMEDJIEV & VARADINOVA 2013), *B. sowerbyi* belongs to deposit feeders' group, which is characterised as the highest abundant group in the trophic structure of the studied oligochaete samples. As a burrowing organism capable of digging in sediments (detritivore), the species feeds on fragmented particulate organic matter in the substrate. In 2020, the silt-sand and natural organic matter predominated in bottom substrates in the Trakiets Reservoir and the Yovkovtsi Reservoir and these favourable conditions were pre-condition for the highest abundance of the species.

LOBO & ESPINDOLA (2014) pointed out that the introduction, distribution, growth, reproduction and formation of a stabile population of *B. sowerbyi* depends not only on temperature and the levels of dissolved oxygen but also on the concentration of organic matter in the sediments, especially the amount of total phosphorous and total nitrogen. Our data do not show correspondence between the level of nutrients and the species abundance. In the current study, the maximum level of TP (5.66 mg/L) and TN (0.818 mg/L) were registered in the Rozov Kladenets Reservoir, where a single specimen of the species was found. On the opposite side, the lowest level of TP (0.086 mg/L) and TN (0.03 mg/L) were registered at the Trakiets Reservoir, where the species was the most abundant (48 specimens).

During the last nine years in Bulgaria, the species was found in water bodies within a wide range of water temperatures, from 7°C (Danube R. – Or-

soya, December 2012) to 32°C (Ovcharitsa Reservoir – cold, May 2013). The samplings during 2020 were performed in the period of lower waters from July to September. The species is common to the tropical and subtropical climate with higher water temperatures and has two annual reproductive cycles (LOBO & ALVES 2011). It can reproduce both sexually and asexually with great reproductive potential at temperatures between 21 and 29°C. ASTON et al. (1968) reported that the temperature of more than 25°C promotes its sexual reproduction and the maturation continues during the coolest season (CASELLATO 1984). In a controlled environment, a larger number of juveniles per cocoon at 25°C were produced compared to those raised at 15–20°C (LOBO & ALVES 2009). In Europe, the species abundance is higher at warmer artificial canals and warm water effluents of power stations (MANN 1958, RODRIGUEZ & REYNOLDS 2011). ASTON (1970) also reported that the warm condensers effluent waters in some cases were likely to provide proper conditions for colonisation by the alien oligochaete *B. sowerbyi*. Even though it is thought to be a tropical-climate species, it can be found in surface water bodies in countries in the temperate climate zone where it is usually used as an indicator of thermal pollution (BONACINA et al. 1994). In our study (2012–2020), the species was found in water bodies with temperature range between 7 and 32°C, with an average value of 21.9°C. The range of surface water temperature during the last studied year (2020) varied between 22.3°C (Gorni Dubnik Reservoir tail) to 29.3°C (in Rozov Kladenets Reservoir).

Conclusion

The present study reported the distribution of *B. sowerbyi* on the whole Bulgarian aquatory, in different types of water bodies (lotic and lentic, natural and modified), with a variety of conditions registered in the aquatic environment. Ecological preferences characterised this aquatic oligochaete as a widely plastic and flexible species. Although *B. sowerbyi* was not dominant in the oligochaete complex, our results demonstrated that it is one of the most adaptive and fast-spreading alien oligochaete species in Bulgaria. An important approach for control is the monitoring of the pathways of the invasion and early detection of the *B. sowerbyi* colonisation.

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