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Occurrence and Habitat Preferences of the Euryhaline Oligochaete *Paranais litoralis* (Müller, 1784) (Clitellata: Naididae) in a Tributary of the Danube River Middle Stretch

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Abstract: *Paranais litoralis*, a euryhaline aquatic oligochaete, was recently recorded in the middle stretch of the Danube River basin. This study provides more details about population abundance and habitat preferences of the species, inhabiting a lowland stretch of the Váh River in Slovakia. For the first time, few specimens were identified at one sampling site in September 2015, whereas in May 2016 the species was found at three sampling sites, with abundance ranging from 86 to 614 individuals per square meter. Regarding its habitat distribution, *P. litoralis* prefers sandy substrata but occurs also on muddy and stony substrata. The possible origin of this isolated population is discussed.

Key words: *Paranais litoralis,* freshwater record, Váh River, substratum requirements

Introduction

The distribution of the aquatic oligochaete *Paranais litoralis* (Müller, 1784) is rather well-known. The majority of studies reported *P. litoralis* from brackish waters and river estuaries of Europe, North America, Africa, Australia and Asia (Timm 1999, McEvoy & Goonan 2003, Baturina 2012, GBIF 2018). As the occurrence of the species is frequently associated with brackish and marine waters, it has been classified as euryhaline or euryhaline-marine species (Seys et al. 1999, Wolf et al. 2008). However, the presence of *P. litoralis* has been documented in freshwater habitats by several researchers (e.g. Uzunov 2008, Baturina 2012). Its occurrence in freshwaters is frequently associated with an increased salinity of the water (e.g. Sowa et al. 2018). In other cases, its presence is also related to an industrial and domestic pollution (e.g. Jabłońska 2014). Some studies have recorded the species in freshwater sections of rivers upstream of brackish estuaries (e.g. Seys et al. 1999). However, we still have enough information about the characteristics of populations and their habitat preferences in freshwaters.

Previously, *P. litoralis* has never been listed in the oligochaete fauna of the Danube River, except for the area of the Danube River Delta (Atanacković et al. 2013, Lyashenko et al. 2013). For the first time, the occurrence of *P. litoralis* in the middle stretch of the Danube River basin has been mentioned by Kokavec et al. (2018). The aim of the present study is to point out and discuss the occurrence of the species in the Váh River near its confluence with the Danube River in Slovakia. Due to a lack of information about the autecology of *P. litoralis* in freshwater habitats, we also provide detailed information about the ecology and habitat preferences of this new member of the aquatic oligochaete fauna of Slovakia.

† Deceased

Materials and Methods

Study area

The Váh River belongs to the Danube River basin. With its total length of 367 km and a catchment area of almost 17,000 km^2, it is the longest river in Slovakia. From source to mouth, the Váh River basin is characterised by a decreasing number of tributaries. On the other hand, the number of reservoirs increases as well as the organic enrichment of the water is growing (Kokavec et al. 2018).

Paranais litoralis was found at three sites in the lower stretch of the Váh River, near its confluence with the Danube River (Fig. 1). The stretch is influenced by a hydropower plant operation (Kráľová Reservoir), which causes daily water-level fluctuations accompanied by a dynamic change in the flow of the water. Moreover, the riverbed is regulated considerably, the riverbank is reinforced by stones and the river is segregated from its inundation zone by artificial banks to protect against floods.

Macroinvertebrate sampling

Sampling was performed at three sites in September 2015 and May 2016. Samples were taken quantitatively using a modified Surber sampler with a surface of 0.063 m^2 and a mesh size of 500 µm. In order to take a sufficient quantity of benthic material, eight to ten subsamples were taken from the dominant substratum at each site in September and May. Benthic material was stored in a 4 % formaldehyde solution in plastic bottles. In the laboratory, benthic invertebrates were manually picked out from detritus and preserved in 70 % ethanol. Aquatic worms were identified at the species level using the identification keys of Hrabě (1979) and Timm (2009).

Environmental variables

During the sampling, water temperature, oxygen saturation, oxygen concentration, pH and conductivity were measured using a Hach Lange HQ4D multimeter, WTW pH/cond 340i or Aquaread GPS Aquameter with an AP-700 Aquaprobe sensor. Concentrations of phosphates, nitrates and chemical oxygen demand were measured with a Merck Spectroquant ®Move portable colorimeter. At each site, transported and particulated organic matter were also sampled and then processed in a laboratory (for further details, see Kokavec et al. 2018). The relative percentage of microhabitats was determined visually.

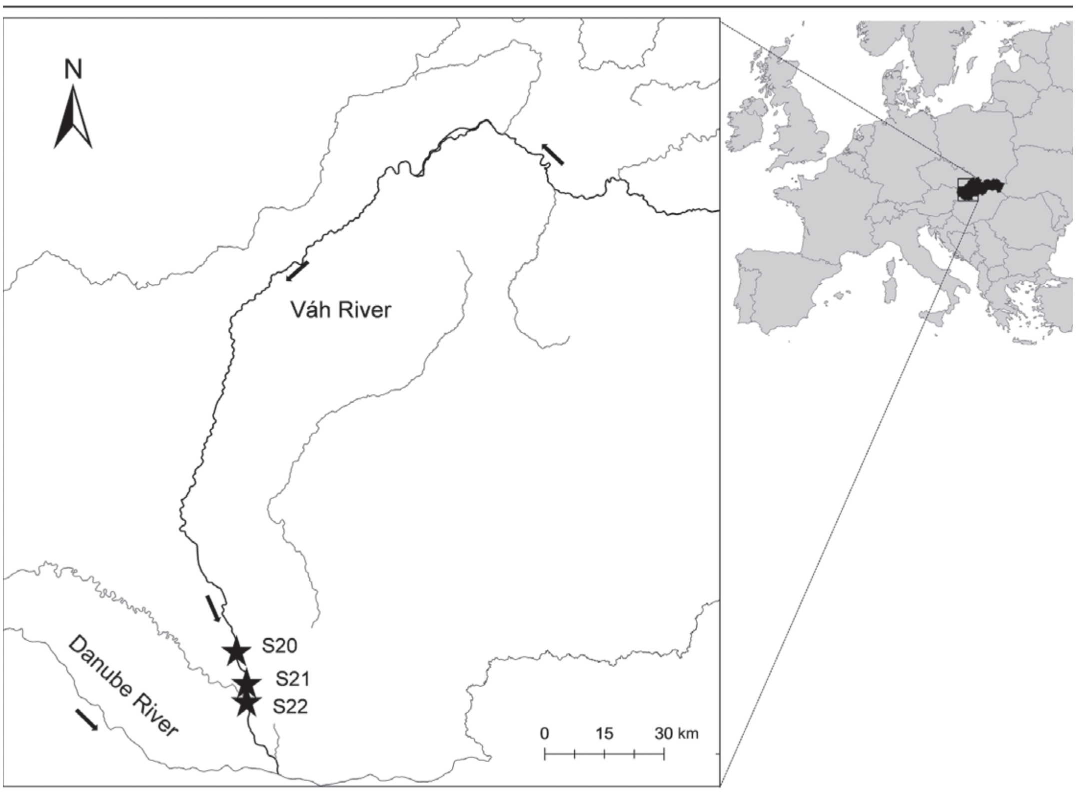

Fig. 1. Map of the study area with the sampling sites where *P. litoralis* was recorded: Site S20 (N 48°00.893, W 17°58.442, 113 m a.s.l.), Site S21 (N 47°56.747, W 18°00.744, 111 m a.s.l.), Site S22 (N 47°54.718, W 18°00.762, 111 m a.s.l.).

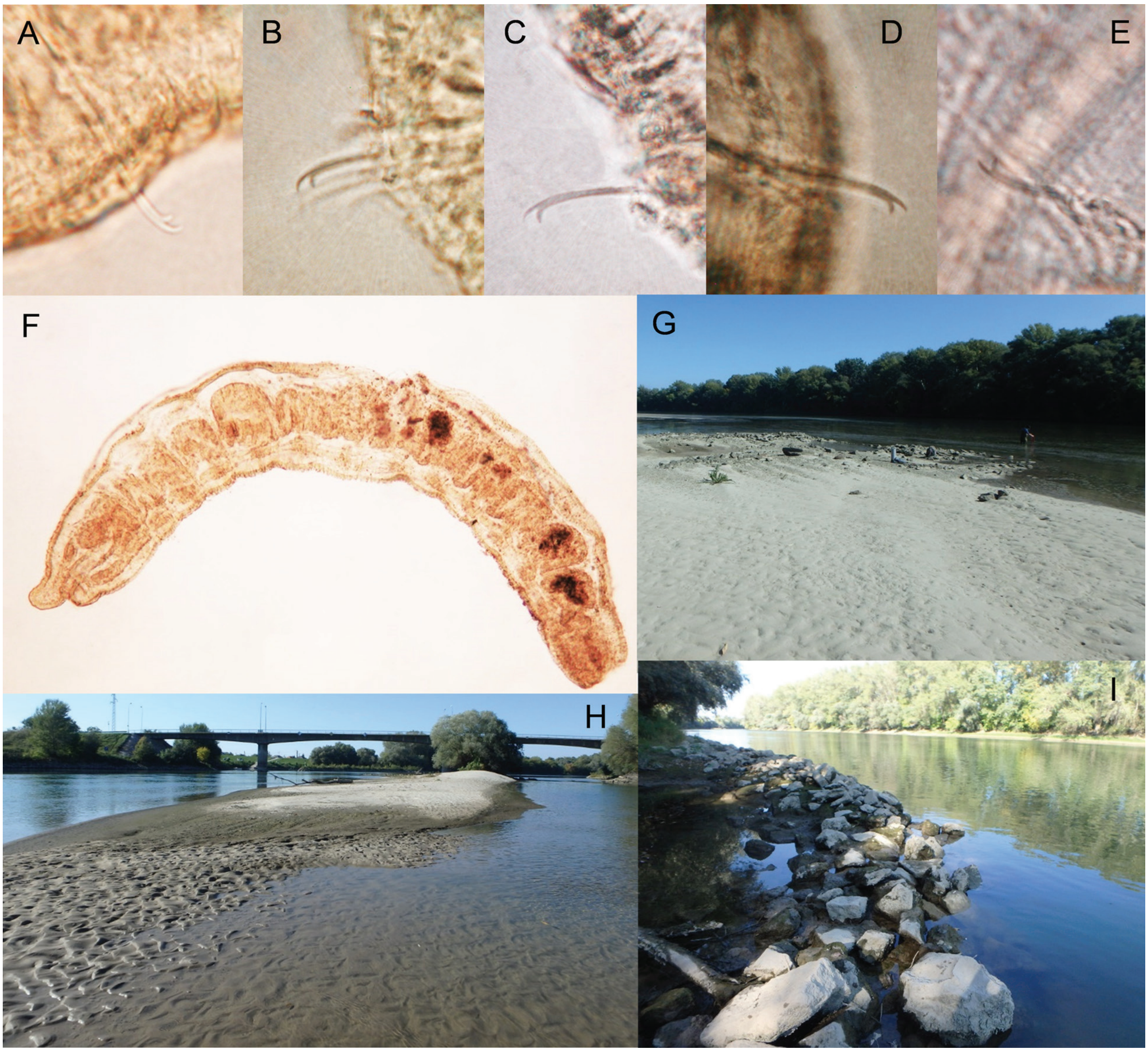

Fig. 2. Photos of body structures important for identification: A, B – distal end of ventral chaetae of 2nd segment, C, D – distal end of ventral chaetae of posterior segments, E – dorsal chaeta, F – body of preserved individual, G – site S20, H – site S21, I – site S22.

Results

Occurrence and abundance of *Paranais litoralis*

Ten individuals (ind.) were identified in macroinvertebrate samples at site S20 in September 2015 for the first time. A more accurate distribution of *P. litoralis* population in the Váh was documented in May 2016. With 282 ind. (614 ind.m^{-2}), its population was the most abundant at site S22. At site S21, 54 ind. (86 ind.m^{-2}) were found, while 384 ind. (451 ind.m^{-2}) were recorded in a sample taken from site S20. Photos of body structures important for identification are presented in Fig. 2. The percentage of *P. litoralis* in the benthic non-insect community (Tricladida, Gastropoda, Bivalvia, Polychaeta, Oligochaeta, Hirudinea, Mysida, Isopoda and Amphipoda) reached 13 % (S20), 14 % (S21) and 17 % (S22) in May, while it was just 2 % at S20 in September. At all sites, the species occurred along with other naidid species, such as *Paranais frici* Hrabě, 1941, *Nais bretscheri* Michaelsen, 1899, *N. christinae* Kasprzak, 1973, *N. elinguis* Müller, 1774 and *N. stolci* Hrabě, 1981.

Habitat characteristics

In general, all sampling sites had similar physico-chemical conditions in spring (Table 1). During autumn sampling, the difference between sites was more expressed. However, the values of the variables matched the lowland character of the river. With regard to the bottom substratum, sand dominated at sites S20 and S22. However,

Table 1. Physico-chemical water parameters and relative percentage of bottom substrata at each site in May and September (the latter in parentheses).

	S20	S21	S22
O_2 (mg.l^{-1})	8.94 (10.4)	8.94 (9.69)	8.77 (10.17)
%_O_2	90.4 (106.8)	90.6 (97.8)	88.7 (100)
Water temperature (°C)	15.6 (16.9)	15.7 (16.2)	15.6 (14.9)
pH	8.15 (8.23)	8.2 (8.09)	8.14 (8.15)
Conductivity (μS.cm^{-1})	393 (443)	397 (451)	447 (472)
NO_3^- (mg.l^{-1})	3.54 (5.31)	15.05 (4.87)	5.75 (8.85)
PO_4^{3-} (mg.l^{-1})	0.28 (0.09)	0.31 (0.12)	0.52 (0.12)
COD (mg.l^{-1})	35 (4)	62 (6)	30 (4)
TOM (mg.l^{-1})	1.7 (1.36)	2.5 (2.7)	4.3 (3.6)
TIM (mg.l^{-1})	1.2 (0)	0 (0)	4 (0)
CPOM (g.m^{-1})	8.3 (3.4)	NA (3.6)	10.2 (0.8)
FPOM (g.m^{-1})	77.4 (13.8)	NA (55.8)	122.9 (47.7)
UFPOM (g.m^{-1})	67.9 (19.7)	NA (20.4)	52.9 (10)
Substrata			
Mud	5	50	20
Clay	2		
Sand	85		75
Gravel			
Cobbles			
Stones	5	50	
Macrophytes	2		3
Algae		+	
POM			2
Other	+		

Abbreviations: oxygen concentration (O_2), oxygen saturation (%_O_2), chemical oxygen demand (COD), transported organic matter (TOM), transported inorganic matter (TIM), coarse particulate organic matter (CPOM), fine POM (FPOM), ultra-fine POM (UFPOM).

the substratum at S20 was covered intermittently by numerous shells of the mussel *Corbicula fluminea* (O.F. Müller, 1774). At site S21, the substratum was formed by stones covered by green algae and periphyton on the banks and mud on the bottom (Fig. 2). The hydrualical conditions at all sites were close to a moderate flow of the water. However, the flow velocity changed depending on the electricity production by the hydropower plant located upstream.

Discussion

Apart from the preference of *P. litoralis* for habitats with increased salinity, records of the abundant, stable populations inhabiting pure freshwaters, such as those presented in this study, are very rare. The present records point out at least three important facts. Firstly, the population in the Váh River is the only known population of *P. litoralis* inhabiting the middle stretch of the Danube River basin. Secondly, this riverine population is not directly connected with estuarine population or other populations considering migration to play a role. Thirdly, the Váh River does not currently suffer from a severe saline or domestic pollution that may facilitate the distribution of this euryhaline species. In spite of the several records of the species in freshwaters, previous studies have not discussed factors allowing the existence of stable freshwater populations.

The absence of historical data does not allow us to reveal whether the populations of *P. litoralis* have inhabited the Váh River in the past or it is a recently established population. There are many reports of various non-native invertebrate species accidentally introduced into new aquatic environments by an anthropogenic activity (e.g. Pabis et al. 2017, Borza et al. 2019). Introduction of *P. litoralis* by ships is unlikely, since the river is not suitable for navigation. However, we cannot exclude the release of this species by aquarists.

According to our findings, the microhabitat preference of *P. litoralis* in the Váh River is similar to that in saltwaters. In brackish environments, the species inhabits a sand and gravel bottom (Laakso 1969) but one of the preferred habitats are rocks covered by algae (Grzelak & Kuklinski 2010). Taking into account the highest abundance of *P. litoralis* on sandy substrata in our study, sand seems to be the most preferred substratum of this species in the river but we have also observed it on stony substrata. Both substrata are frequently presented in the middle Danube River basin; however, the species has not been found anywhere else in this region. Thus, stony banks that lack stretches with a sandy bottom do not tend to be suitable for *P. litoralis*, although Wachs (1964) found *P. litoralis* on stones and gravel. Moreover, the absence of the species at particular places could be linked rather to flow velocity or physico-chemical conditions (Prenda & Gallardo 1992, Jabłońska 2014) than to the substratum characteristics.

The classification of *P. litoralis* as euryhaline–marine species by Wolf et al. (2008) is not appropriate. Regarding its records from various habitats, Seys et al. (1999) consider that *P. litoralis* is euryhaline, which corresponds to the occurrence of the species better. Its high tolerance for salinity and pollution, in combination with the species worldwide distribution, opens the possibility that we are dealing with a cryptic species. Earlier molecular studies reveal possible molecular diversity within *P. litoralis* (Bely & Wray 2004). However, the morphological differentiation of the species has never been evaluated. There is a need for detailed molecular studies to understand better the species diversification and distribution in aquatic environments.

Conclusion

Our record of *P. litoralis* in the Váh River is likely an accidental discovery of the species in a freshwater biotope. Regarding its environmental preferences, the population occurring in the river has similar habitat requirements with those documented in saltwaters. Due to the lack of faunistic data, it is difficult to clarify the history of the species presence in the Váh River. Further studies and molecular analyses could be useful to determine the possible interspecific differences within *P. litoralis* as well as the ecological exceptionality of a rare isolated populations in freshwaters.

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