

**Research Article** 

## *Bratislavia dadayi* (Michaelsen, 1905) (Annelida: Clitellata: Naididae): a new non-indigenous species for Europe, and other non-native annelids in the Schelde estuary

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#### Abstract

For the first time, the freshwater oligochaete species *Bratislavia dadayi* (Michaelsen, 1905) is recorded in Europe. The species was found at three subtidal stations in the Schelde estuary in Belgium, where it was probably introduced from the Americas. We provide an overview of the species' nomenclature, diagnostics, distribution, and ecology. *Bratislavia dadayi* is one of 11 non-indigenous annelids currently known to occur in the Schelde estuary.

Key words: alien species; Annelida; Clitellata; Oligochaeta; Polychaeta; Belgium

#### Introduction

Over the last 150 years, the number of nonnative species turning up in areas far from their original range has increased significantly (Bax et al. 2003), including in the North Sea region and its estuaries (Kerckhof et al. 2007). The impact of these introductions is often not known, although several are known to have high ecological impact, e.g., the American jack-knife clam Ensis directus (Conrad, 1843), the Pacific oyster Crassostrea gigas (Thunberg, 1793), the New Zealand barnacle Elminius modestus Darwin, 1854, and the slipper limpet Crepidula fornicata (Linnaeus, 1758). Kerckhof et al. (2007) stated that 'these species are invasive, competing with native species, changing the original habitat and significantly altering the overall biodiversity and biomass'. Most introductions of non-native species, however, have no clear impact or fail to establish sustainable populations (Manchester and Bullock 2000).

Annelids, and oligochaetes in particular, are a less-studied group, often overlooked when considering alien species. Yet the best studied Annelid species, *Lumbricus terrestris* (L., 1758), is now considered a widespread invasive species that has invaded ecosystems previously devoid of earthworms or has replaced species with a comparable ecology (Hendrix et al. 2008).

This study documents the first European records of *Bratislavia dadayi* (Michaelsen, 1905). Nomenclature, ecology and diagnostic features are presented and the species is 'virtually' added herein to the most commonly used European oligochaete key (Timm 2009). We also include an annotated checklist of non-indigenous annelids recorded from the Schelde estuary.

#### Material and methods

#### Study area

*Bratislavia. dadayi* was first discovered in samples collected from the Schelde estuary near

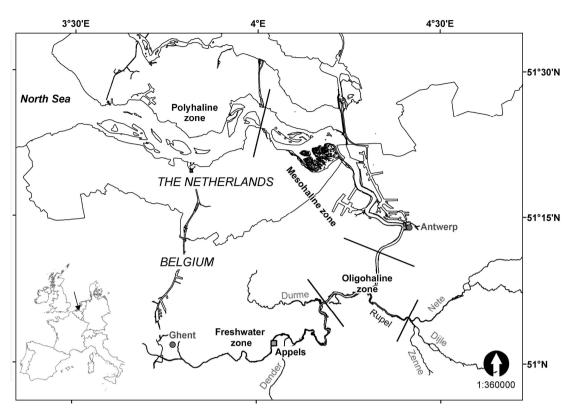


Figure 1. The Schelde estuary. Bratislava dadayi was found near the small town of Appels.

the town of Appels (Figure 1). The river Schelde originates in the north of France, flows through Belgium where it is called the 'Zeeschelde', then into the North Sea near Vlissingen, The Netherlands (Figure 1). It is a lowland river with a total length of 355 km and a fall of 100 m at most. About ten million people live in its catchment area of around 21,000 km<sup>2</sup>. The estuarine influence extends to Gent, 160 km from the mouth, where tidal influence is artificially interrupted by sluices. A number of tributaries are also under tidal influence and are considered to be part of the estuary (Meire et al. 2005). The presence of several large ports in the estuary, Antwerp Harbour is the most important, makes the Schelde vulnerable to introductions of non-native species (Soors et al. 2010).

#### Sampling

Within the Belgian part of the Schelde estuary, benthos has been monitored since 1996 (Seys et al. 1999; Soors et al. 2010, J. Speybroeck, unpublished data). Prior to 2008, sampling was conducted every third year at fixed intertidal and subtidal stations in an increasing quantity of sites: from 51 sampling sites in 1999 up to 75 in 2005. At each sampling site, 3 core samples (diameter: 3.5 cm; depth: 10 cm) were collected. In case of subtidal samples, these were obtained from a Reineck boxcorer (diameter: 15 cm; depth: 10 cm). Sampler size was chosen to reduce highly laborious sorting effort of samples with high densities of oligochaetes (occasionally more than  $1 \times 10^6$  individuals m<sup>-2</sup>). Sediment samples were stored in 4% formaldehyde. In the laboratory, samples were sieved in two steps over mesh sizes of 250 µm and 1000 µm. Benthic invertebrates were sorted, identified to species level, and counted.

Since 2008, samples have been collected each year, following a stratified random sampling design; 212 stations are stratified by salinity zone, subtidal depth, and intertidal elevation. Two core samples (diameter: 4.5 cm; depth: 15cm) were collected at each station, fixed in 4% formaldehyde, and taken to the laboratory. These were sieved over mesh sizes of 500  $\mu$ m and 1000  $\mu$ m and subsequently treated as described for the samples collected prior to 2008.

# Nomenclature, diagnostics, distribution and ecology

We provide an overview of the nomenclature, diagnostics, distribution and ecology of *B.dadayi* based on literature and observations of Belgian specimens. Adaptations of the identification key of Timm (2009) are proposed to facilitate the differentiation of *B. dadayi* from *Pristina* species.

Nomenclature (after Christoffersen 2009) and remarks on the classification of aquatic oligochaetes

## Bratislavia dadayi (Michaelsen, 1905)

Syn.: Naidium dadayi Michaelsen, 1905. Pristina unidentata Harman, 1973. Bratislavia unidentata: Harman and Loden 1978; Davis 1982; Gluzman de Pascar 1987; Wang and Liang 2001. Bratislavia dadayi: Righi and Hamoui 2002.

A recent study by Erséus et al. (2008) showed that the former Tubificidae Vejdovský, 1876 should be regarded as members of the Naididae Ehrenberg, 1828. This means that the long established name Tubificidae is no longer valid. To avoid any confusion, we will use the terms **naidids** and **tubificids**. As a consequence, we now recognize seven different subfamilies within the family Naididae: Naidinae, Pristininae, Tubificinae, Rhyacodrilinae, Phallodrilinae, Telmatodrilinae, and Limnodriloidinae - with the latter five being the former Tubificidae.

## Diagnostics

Bratislavia dadayi is a small, eyeless oligochaete with dorsal bundles containing both hair and needle chaetae, starting in II (Figure 2). Because of these characters (shared only with other naidids in the genus Pristina and in the nongenus European Stephensoniana), it had originally been assigned to the genus Pristina (syn. Naidium). The unique dorsal needles are simple-pointed, sigmoid and remarkably stout, with a distinct distal nodulus (Figure 3). Hairs (also one per bundle) are gene-rally very thin and were considered to be present in all dorsal bundles according to the description by Harman (1973). Harman and Loden (1978) mentioned, however, that hairs may be occasionally absent in the most anterior bundles, which is supported by Gluzman de Pascar (1987), Kathman and Brinkhurst (1998) and Wang and Liang (2001). The latter mention even the occasional occurrence of two hairs per bundle. In the Belgian specimens, the first segments (II-III) also lack hairs in the dorsal bundles (Figure 3). The anterior segments II-V usually have 3 ventral chaetae, with the upper tooth about two times longer and thinner than the lower tooth; the posterior segments (from VI onwards) mostly have 3 shorter ventral chaetae, with the distal tooth as long as the lower tooth, but thinner. The species is relatively short (4-9 mm with up to 80 segments (Wang and Liang 2001), but longer than Pristina spp. Note that in its only other European congener, B. palmeni (Munsterhjelm, 1905), the dorsal bundles are starting in III, while the needles are bifid. The marked difference between anterior and posterior ventral chaetae, as well as the unusually thick simple-pointed needles, separate B. dadayi from Pristina spp.

Harman and Loden (1978) described the reproductive organs of *Pristina unidentata* and concluded that it belonged to the genus Bratislavia (Košel, 1976). Later, Righi and Hamoui (2002) synonymised B. unidentata with the long-neglected Naidium dadavi Michaelsen, 1905, originally described from specimens collected in Paraguay. Harman and Loden (1978) also noticed that the species reproduces asexually by fragmentation, not by budding, which is the case in Pristina. This observation was supported by Gluzman de Pascar (1987). According to the above authors, the clitellum of mature worms extends from the middle of segment V to the intersegmental furrow 7/8, spermathecae are in V, atria and male pores in VI, and a large sperm sac lies in VII-IX. Atria are surrounded with diffuse prostate glands.

## Key

In order to insert *B. dadayi* into Timm's key without applying further alterations to it, we have refrained from implementing the new classification of Erséus et al. (2008), mentioned above; following that classification would have involved considerable changes to the key, which is beyond the scope of the present paper.

*Bratislavia dadayi* will key out for 'Pristinidae' instead of 'Naididae' when using the key of Timm (2009). To avoid this, we suggest the following adaptations (indicated in bold):

## In the Family key (p. 26):

8 (7) Dorsal chaetal bundles lacking in a fixed number of anterior segments, most often beginning in VI (but also in II, III, IV, V, or

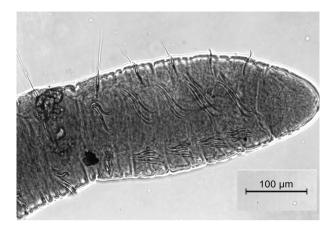


Figure 2. *Bratislavia dadayi* from the Schelde, anterior part (Photograph by Jan Soors).

#### In Naididae key (p. 44):

23 (18).....Haemonais waldvogeli - Dorsal bundles beginning in II–VI......24 24 (23) Dorsal bundles beginning from II, needles stout and simple-pointed...Bratislavia dadayi - Dorsal bundles beginning from III, needles bifid.....Bratislavia palmeni - Dorsal bundles beginning from VI......25

#### Distribution

After the original description from Paraguay by Michaelsen (1905), *Bratislavia dadayi* was recorded from Surinam (Harman 1974), the USA (Harman 1973; Harman and Loden 1978; Davis 1982; Wetzel 1992; Milligan 1997), Australia (first record in 1979; Adrian Pinder (Dept of Environ-ment and Conservation, Western Australia, pers. comm.), Argentina (Varela 1984;

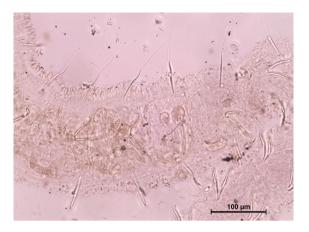


Figure 3. *Bratislavia dadayi* from the Schelde, posterior dorsal and ventral bundles (Photograph by Jan Soors).

Pujals 1985; Gluzman de Pascar 1987), southern Brazil (Takeda et al. 2000; Montanholi-Martins and Takeda 2001), the Bahamas and Lesser Antilles (Righi and Hamoui 2002), and Lake Poyang in the Yangtze River basin in southern China (Wang and Liang 2001).

#### Ecology

Little is known about the ecology of *B. dadayi*. Most records by Harman (1974) in Surinam were from a range of vegetation-rich lentic waters. Some records in Texas were from gravel riffles supporting dense filamentous algae and some macrophytes in moderately polluted streams, while the animals' gut content was dominated by detritus (Davis 1982). In Brazil and China, the species is known from floodplain lakes (Montanholi-Martins and Takeda 2001; Wang and Liang 2001). Spencer (1977) reported the species from a sandy substrate in the shallow littoral (3 m depth) of Lake Erie (Great Lakes, North America). Harman (1974) suggested that the species is primarily neotropical, due to its successful exploitation of a wide variety of habitats in Surinam, including a marsh, a swamp, a pond, a roadside ditch, and an acid stream. However, in Australia, the species is found wellaway from ports, mainly in seasonal tropical creeks with permanent pools. These records are often from remote areas of Australia, which suggests the species might be native (Adrian Pinder pers. comm.).

As these wide-ranging collection sites demonstrate, the ecological niche and distribution of B. *dadayi* clearly deserve additional investigation. Also, a careful examination of specimens from the various locations around the world to confirm that they are in fact the same species should be undertaken.

## Results

## Belgian records of <u>Bratislava dadayi</u>

Bratislava dadayi was detected at three stations, in three different years, in the freshwater tidal part of the Schelde estuary, near the town of Appels (Figure 1), at about 130 km from the river mouth and about 75 km from the Dutch-Belgian border, following the thalweg. The 2002 station is situated at N 51°02'56" E 004°04'06". the 2008 station at N 51°02'11" E 004°02'35", and the 2011 station at N 51°02'36" E 004°05' 35" (WGS84). The stations are only 4.7 km apart along the thalweg. All are subtidal stations - both the 2002 and 2008 stations are located at a little more than 5 m below the mean low tide reference level, whereas the 2011 station is situated between 0 m and 2 m below that same reference level. The mean tidal range in the area is 4.2 m (spring tide 4.5 m; neap tide 3.75 m).

On 3 October 2002, nine specimens of B. dadayi were found at a subtidal station with a sediment of median grain size of 315 µm and an organic matter content of 1.4%. This represents the first European record of B. dadavi. On 8 October 2008, three additional specimens were found at a subtidal station with sediment of a median grain size of 191 µm and an organic matter content of 47%. An additional three specimens were collected in 2011 at a subtidal station with a sediment characterised by a median grain size of 18 µm and an organic matter content of 7.7%. No sexually mature worms were encountered in these samples. One specimen was atypical in having dorsal bundles from VI, thus closely resembling representatives of the genus Nais quite well.

Total abundance of benthic fauna in the subtidal zone of this part of the estuary is typically low, e.g., total density of all organisms per station was 10,134 individuals m<sup>-2</sup> in 2002 and 1,886 individuals m<sup>-2</sup> in both 2008 and 2011. In contrast, the local (mainly) high intertidal stations typically have supported much higher frequently exceeding 50,000 densities. individuals m<sup>-2</sup>. In the 2002 sample, Aulodrilus pluriseta (Piguet, 1908), Monopylephorus rubroniveus Levinsen, 1884, and juvenile tubificids with and without hair chaetae were

found together with *B. dadayi*, the latter representing 23% of benthic density. The 2008 sample contained only *B. dadayi*, whereas the *B. dadayi* present in the samples collected in 2011 were only accompanied by two *Hydra* polyps. Intertidal samples of this part of the estuary are dominated by pollution-tolerant species, such as: *Limnodrilus hoffmeisteri* Claparède, 1862; *L. claparedeianus* Ratzel, 1868; *Quistadrilus multisetosus* (Smith, 1900); *L. udekemianus* Claparède, 1862; an as yet undescribed *Limnodrilus* species (Van Haaren and Soors 2013); *Paranais frici* Hrabě, 1941; and the more typically estuarine species *Tubifex blanchardi* Vejdovský, 1891 and *P. litoralis* (Müller, 1784).

## Discussion

At present, B. dadayi seems to be rare in the Schelde estuary. It was found in only three samples, despite intensive sampling efforts since 1996. The species usually occurs in small streams and in the littoral zone of lakes and floodplains, often far away from coastal areas (Harman 1974; Montanholi-Martins and Takeda 2001; Wang and Liang 2001; Behrend et al. 2009). Therefore, estuaries may not provide ideal habitat for this species. However, in Belgium, B. dadavi was found in subtidal estuarine samples with a wide variation in sediment grain size and organic matter content. Another factor might be sampling season. Our samples were collected in autumn, which might not be optimal to detect maximal abundance of this species. Yet, B. dadayi had been found frequently in samples collected in autumn in most other worldwide localities (Harman 1973; Harman and Loden 1978; Wang and Liang 2001; Righi and Hamoui 2002). Thus, we tentatively consider the sampling season not to be a limiting factor in this case. Despite the low annelid density in the samples, it is striking that B. dadayi was accompanied in samples collected in 2002 by two species, A. pluriseta and M. rubroniveus, previously considered rare in this estuary, and then the *only* oligochaete species present in the 2008 samples. The combined local conditions (high hydrodynamics, sediment characteristics) might preclude rich oligochaete communities. Yet, these conditions might shape a habitat, which is rare or only locally found within the estuary. It is also possible that the species that are able to exist and thrive in these conditions characterised by values ranging from 0.6 to

Table 1. Annelid invaders in the Schelde.

Species	Salinity zone	Distribution in estuary	First record	n° records	Natural distribution
Branchiodrilus hortensis	Freshwater	upper most parts of estuary: Zenne tributary	2010 <sup>(1)</sup>	1	Asia, Africa & Australia
Limnodrilus cervix	Freshwater	upper most parts of estuary: Zeeschelde near Gent (= end of tidal influence) & Nete tributary	2009 <sup>(1)</sup>	2	N America
Potamothrix vejdovskyi	Freshwater	Zeeschelde subtidal near Oude Briel	$2009^{(1)}$	1	Ponto-Caspian
Marenzelleria neglecta	Freshwater	Probably a recent arrival but not uncommun in oligohaline and freshwater zone	2008 <sup>(1)</sup>	15	N America
Branchiura sowerbyi	Freshwater	two subtidal sites near Gent (Melle & Kwatrecht)	2008 <sup>(1)</sup>	2	Asia
Psammoryctides moravicus	Freshwater	almost exclusive subtidal, uppermost parts of estuary	2008(1)	6	Ponto-Caspian
Tasserkidrilus americanus	Freshwater	locally on a freshwater floodplain	2006 <sup>(1)</sup>	3	N America
Monopylephorus limosus	Freshwater	Zenne tributary	2002(1)	2	S and E Asia
Bratislavia dadayi	Freshwater	locally in the freshwater subtidal zone	2002 <sup>(1)</sup>	3	Southern hemisphere, China & USA
Quistadrilus multisetosus	Freshwater	common, mainly in the upper most part of the Zeeschelde, but also locally in tributaries	1996 <sup>(3)</sup>	68	N America
Marenzelleria viridis	Brackish	not uncommon, but low densities	1995 <sup>(2)</sup>	28	N America

<sup>1</sup>This study, <sup>2</sup>Y sebaert et al. 1997, <sup>3</sup>Seys et al. 1999

9.6%, the 2008 *B. dadayi* station had an exceptionally high organic matter content of 47%. The 2002 sample was characterised by the presence of an unusually coarse, cement-like substrate. It is possible that *B. dadayi* is outcompeted by indigenous species in more densely populated places.

In theory, natural presence of the species within Europe cannot be ruled out, as it is already known from at least four other continents, where it may or may not be native. However, this is not very plausible, because the species is relatively easy to recognise and Northwestern European oligochaetes have been surveyed relatively well and for several decades.

#### Other non-indigenous annelids

Other non-native annelids in the Schelde estuary include: the polychaetes *Marenzelleria viridis* (Verrill, 1873) (Ysebaert et al. 1997) and *M. neglecta* Sikorski et Bick, 2004; and the oligochaetes *Quistadrilus multisetosus* (Smith, 1900), *Monopylephorus limosus* (Hatai, 1898), *Psammoryctides moravicus* (Hrabě, 1934), *Potamothrix vejdovskyi* (Hrabě, 1941), *Tasserkidrilus americanus* (Brinkhurst et Cook, 1966), *Limnodrilus cervix* Brinkhurst, 1984, Branchiodrilus hortensis (Stephenson, 1910) and Branchiura sowerbyi Beddard, 1892 (Table 1).

Marenzelleria neglecta, Branchiodrilus hortensis, M. limosus, P. moravicus, P. vejdovskyi, L. cervix and T. americanus were found for the first time in Belgium. The latter species is in Europe only known from The Netherlands and Belgium (Van Haaren and Soors 2013). Because of the recent revision of *Marenzelleria* with the description of M. neglecta Sikorski and Bick, 2004 and the absence of recent records of M. viridis in the Schelde estuary, it cannot be excluded that the old records of *M. viridis* in fact relate to M. neglecta specimens. Future research will possibly reveal the co-occurrence of both species, as observed in the Elbe estuary (Blank et al. 2004). For the time being, we consider both species to be present.

All of the non-indigenous annelid species, except *Marenzelleria viridis*, are found exclusively in the freshwater part of the Schelde estuary. However, the brackish part of the estuary in Belgium is limited in size. This area is known to be poor in oligochaete diversity (Seys et al. 1999, Van Haaren and Soors 2013). The polyhaline part is situated exclusively in The Netherlands and is, to the best of our knowledge, not well surveyed for oligochaetes. In this lower river section, more coastal and marine alien species might be present. It is not clear how the observed exotic species have reached the estuary, but ballast water and inland navigable canals may have acted as routes of introduction (Ysebaert et al. 1997; Hulme 2009).

The presence of several large ports in the estuary makes the Schelde vulnerable to introduction of non-native species (Stevens et al. 2004). In addition, recent improvement in water quality (Cox et al. 2009), especially in the freshwater part of the estuary, may facilitate the establishment of non-indigenous species once they arrive (Soors et al. 2010). Both Marenzelleria species and Quistadrilus multisetosus are common, but all of the other non-indigenous annelids are rare and localised (Table 1). Remarkably, four of these species (B. dadavi, B. sowerbyi, P. moravicus, P. vejdovskyi) are almost exclusively found in subtidal samples despite the subtidal zone being characterised by a harsher environment (e.g., stronger currents, lower organic matter content) and generally lower densities of benthic invertebrates. This is also the case for indigenous species such as Potamothrix moldaviensis Vejdovský et Mrázek, 1903 and Psammorvctides barbatus (Grube, 1861), which are uncommon in the Belgian part of the Schelde estuary, but common elsewhere in Belgium and widespread in The Netherlands (Van Haaren and Soors 2013). A lot of limne-tic species do not develop important populations in the dynamic estuarine environment of the estuary. They are almost exclusively found at the edge of the estuarine influence and mostly avoid intertidal conditions. This may also explain why invaders such as the polychaete Hypania invalida (Grube, 1860) or the isopod Jaera sarsi Valkanov, 1936 (syn. J. istri Veuille, 1979) have not (yet) been found in the estuary.

Most of the annelid invaders in the estuary dwell on soft-bottom substrates and are restricted to the freshwater reaches of the estuary. In contrast, it is remarkable that most alien crustacean species such as the tanaid *Sinelobus stanfordi* (Richardson, 1901) and the isopod *Synidotea laticauda* (Benedict, 1897) are typically species from brackish waters and take advantage of the human introduction of hard substrates in estuaries, where soft sediments naturally prevail (van Haaren and Soors 2009; Soors et al. 2010).

Most annelid invaders are rare or present in low densities. For the moment, we assume that any ecological impact of their occurrence is limited. Marenzelleria neglecta may be an exception because of its presence in high subtidal and low intertidal samples in the oligohaline reaches of the Zeeschelde where other benthic organisms are present in low densities. As such, this species might represent an important food source for benthivorous organisms in this area. Marenzelleria neglecta is known to reach high densities and biomass in the southern Baltic, competing with indigenous the polychaete Hediste species such as diversicolor (Kotta and Kotta 1998), and the amphipods Corophium volutator (Zettler et al. 2007) and Monoporeia affinis (Kotta and Olafson 2003). In German Baltic coastal waters, however, an increase in most indigenous species was observed after Marenzelleria colonised the substrate (Zettler et al. 2002). Further research will be necessary to learn in which degree this invasive species affects indigenous species in the Schelde estuary.

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