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Research Article

The invasive round goby *Neogobius melanostomus* and tubenose goby *Proterorhinus semilunaris*: two introduction routes into Belgium

Merlijn Mombaerts[1], Hugo Verreycken[2,*], Filip A.M. Volckaert[1] and Tine Huyse[1,3]

[1]*University of Leuven, Laboratory of Biodiversity and Evolutionary Genomics, Charles Deberiotstraat 32, B-3000 Leuven, Belgium*
[2]*Research Institute for Nature and Forest (INBO), Dept. of Biodiversity and Natural Environment, Species Diversity group, Duboislaan 14, B-1560 Hoeilaart, Belgium*
[3]*Royal Museum for Central Africa, Department of Biology, Leuvensesteenweg 13, B-3080 Tervuren, Belgium*

E-mail: merlijn.mombaerts@hotmail.be (MM), hugo.verreycken@inbo.be (HV), filip.volckaert@bio.kuleuven.be (FV), tine.huyse@africamuseum.be (TH)

**Corresponding author*

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Abstract

The invasion of Ponto-Caspian taxa in Western Europe has increased steadily since the connection of the Danube basin with the Rhine basin in 1992, in combination with transfers through interbasin shipping. In 2010, the tubenose goby (*Proterorhinus semilunaris*) and round goby (*Neogobius melanostomus*) were observed in Belgium for the first time. To gain insight in the introduction pathways in Belgium and to identify potential source populations, a phylogeographical and parasitological study was initiated on both species. The mitochondrial cytochrome *b* gene was sequenced, its haplotype diversity calculated and a statistical parsimony haplotype network built. Both species exhibited low haplotype diversity compared to native and other non-native populations. The network revealed potential source locations in the Northern Black Sea for the round goby and in the Danube at the Serbian-Romanian border for the tubenose goby. Fins, gills and body were examined for the presence of ectoparasites. Prevalence, abundance and infection intensity was much higher in tubenose goby, which might be the consequence of a different introduction pathway. Our data provides evidence that tubenose goby entered Belgium through active dispersal. The round goby, however, was most likely introduced with ballast water.

Key words: ballast water, Ponto-Caspian gobies, *Gyrodactylus*, parasitology, population genetics

Introduction

The introduction of non-indigenous species (NIS) is regarded as the second leading cause of species extinction and endangerment worldwide (Mace et al. 2005) and they are listed first among the principal threats to freshwater fishes (Cowx 2002). They can destabilize local ecosystems, sometimes with major economic consequences (Grosholz et al. 2011). The negative impact of NIS on native fauna is due to competition for food and habitat, and the introduction of alien pathogens, sometimes with devastating consequences (Johnsen and Jensen 1991).

In recent years the rate of biological invasions has substantially increased, mostly due to the globalization of the economy (Lin et al. 2011) and infrastructure works like the construction of canals and harbours. The range expansion of invasive Ponto-Caspian aquatic species towards Central and Western Europe illustrates the impact of interconnections between river basins. Bij de Vaate et al. (2002) documented three corridors connecting Eastern, Central and Western European river basins. The northern corridor (connecting the Black and Baltic Sea through the Volga) and the central corridor (connecting the Black and Baltic Sea through the Dnieper) have been important introduction routes for invasive species to reach the Baltic region (e.g. the amphipod *Chelicorophium curvispinum* (Sars, 1895) and the bivalve *Dreissena polymorpha* (Pallas, 1771)). Since the opening of the Danube-Main-Rhine Canal in 1992, the southern

corridor has become the most important route for Ponto-Caspian species to invade Western Europe (e.g. the amphipod *Dikerogammarus villosus* (Sowinsky, 1894) and the isopod *Jaera istri* (Veuille, 1979)) (Bij de Vaate et al. 2002). Intercontinental invasions of aquatic species are mainly facilitated through the transport of ballast water (Carlton and Geller 1993). For example, in the Laurentian Great Lakes of North America, 70 % of the introduced aquatic species originate from the Ponto-Caspian region. These species have invaded North America directly via ballast water from an international harbour in the Ponto-Caspian region (e.g. the bivalve *Dreissena bugensis* (Andrusov, 1897)) or have dispersed first to the North Sea or Baltic Sea to be picked up there for intercontinental transfer (e.g. the amphipod *Echinogammarus ischnus* (Stebbing, 1899)) (Ricciardi and MacIsaac 2000). Also several freshwater fish species of Ponto-Caspian origin have been 'on the move' since these corridors were created (Copp et al. 2005).

Two of these Ponto-Caspian fish species rapidly expanding throughout Europe are the round goby (*Neogobius melanostomus*) (Pallas, 1814) and the tubenose goby (*Proterorhinus semilunaris*) (Heckel, 1937) (Copp et al. 2005). Established non-native populations of both gobies inhabit the Great Lakes of North America and Eastern and Central Europe. In Western Europe, first reports originate from the Netherlands, where round goby was caught for the first time in 2004 (van Beek 2006) and tubenose goby in 2002 (van Kessel et al. 2009). In Belgium, both species were discovered much later in 2010 (Verreycken et al. 2011; Cammaerts et al. 2012). Recently, Manné et al. (2013) documented the occurrence of *N. melanostomus* in France (first record in 2011) whereas *P. semilunairs* was discovered there in 2007 (Manné and Poulet 2008). Numbers of sites as well as population densities of both gobies have increased enormously since their arrival in Western Europe (Spikmans et al. 2010; Manné et al. 2013; Verreycken 2013).

Aggressive behaviour towards competitors, high environmental tolerance, fast reproduction and high growth rates facilitate the successful establishment of their populations in invaded areas (Charlebois et al. 2001; Van Kessel et al. 2011; Kornis et al. 2012). High dispersal rates are observed despite a benthic lifestyle; fast natural dispersal is mostly possible through a combination of short-distance diffusion and long-distance active dispersal events (Bronnenhüber et al. 2011) while chronic human mediated dispersal (propagule pressure) is mainly due to ballast water transfer and the opening of canals interconnecting distant river basins (Copp et al. 2005; Kocovsky et al. 2011).

Phylogeographic analysis may uncover the introduction pathways by identifying the possible sources of introduced populations and by discriminating among competing hypotheses of natural spreading *versus* (multiple) introductions (Stepien and Tumeo 2006). Comparing the genetic diversity of native and non-native populations also provides the means to test for founder events and to assess the importance of genetic variability for invasive success. It is believed that the genetic composition of an exotic population is a key factor in the survival and establishment in an invaded area (Williamson 1996).

Another useful method to gain insights in the colonization route is the examination of the parasite fauna of NIS. Presence or absence of parasites can inform about diet and migration of their host, and parasites have been successfully used as biological tags in fish stock discrimination (MacKenzie 2002; Barson et al. 2010). Comparing the parasite fauna of introduced and native populations might therefore hold information on the source or pathway of species introduction.

In this study, we test the hypothesis that Ponto-Caspian freshwater taxa have reached the Atlantic region through direct (ballast water) or indirect (the opening of canals) human mediated mechanisms. We reconstruct the invasion pathway of the round and tubenose goby in Belgium through a multidisciplinary approach combining molecular phylogeographic studies with parasitological investigations.

Materials and methods

Fish sampling

Belgian fish samples were collected by INBO (Research Institute for Nature and Forest) between September and November 2011 and in April and October 2012. Round gobies were captured at three different water courses in Belgium by electrofishing, fyke netting and angling (Figure 1). They were supplemented with specimens from the river Waal in the Netherlands for phylogeographical analysis (Table 1). For tubenose goby, three different sites were sampled using electrofishing only (Figure 1). In total, 193 specimens were collected including 77 tubenose gobies, 44 round gobies,

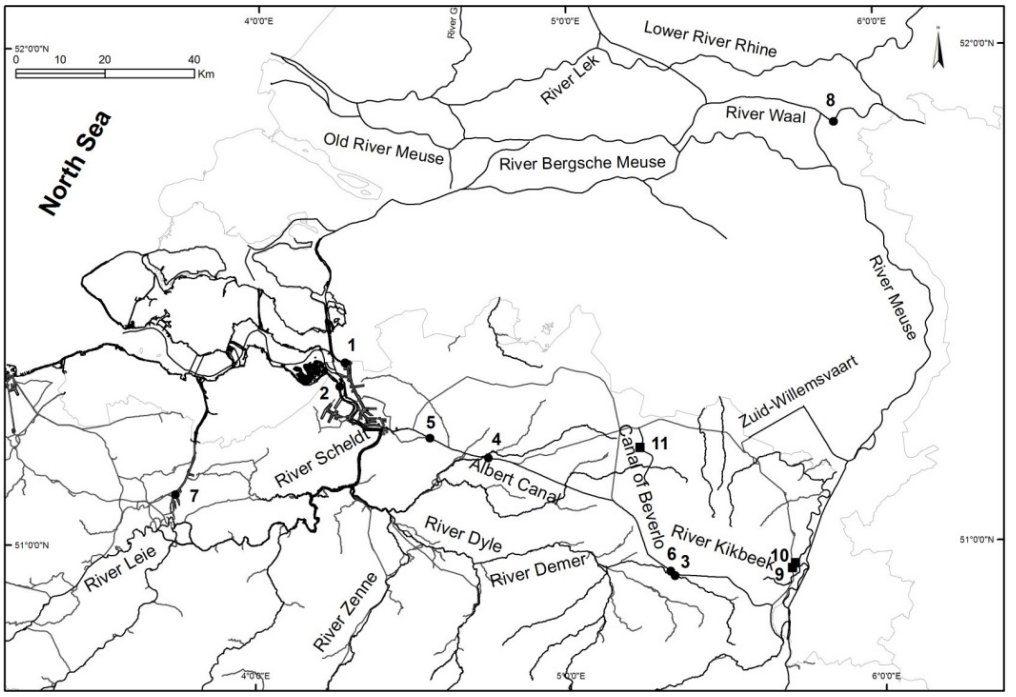

Figure 1. Sampling locations of the round and tubenose goby in Belgium and the Netherlands. Round goby sample locations (●): 1) River Zeescheldt near Zandvliet, 2) River Zeescheldt near Doel, 3) Albert Canal near Hasselt, 4) Albert Canal near Grobbendonk, 5) Albert Canal near Zandhoven, 6) Albert Canal near Kuringen, 7) Canal Gent-Terneuzen near Gent, 8) River Waal near Nijmegen (NL). Tubenose goby sample locations (■): 9) Rivers Kikbeek and Ziepbeek mouth near Border Meuse, 10) Canal of Zuid-Willems, 11) Canal of Beverlo.

Table 1. Sequence diversity (cytochrome *b* mtDNA) of round goby populations from this study and from Brown and Stepien (2008, 2009)*

		N_s	N_h	H_d	P_i ($x10^{-3}$)	P_i' ($x10^{3}$)	Native
Belgium	Albert Canal	22	2	0.173	0.27	0.89	-
Belgium	Canal Gent-Terneuzen	10	3	0.378	0.38	1.18	-
Belgium	Zeescheldt	2	1	0	/	/	-
Belgium	**Total**	**34**	**3**	**0.308**	**0.37**	**1.18**	**-**
The Netherlands	**River Waal**	**11**	**1**	**0**	**0**	**0**	**-**
U.S.A.	Lake St. Clair	39	4	0.461		2.08	-
U.S.A.	Lake Huron	117	4	0.447		2.08	-
U.S.A.	Lake Michigan	119	4	0.230		2.08	-
U.S.A.	Lake Superior	26	3	0.151		1.11	-
U.S.A.	Lake Erie	245	5	0.497		1.99	-
Canada	Lake Ontario	68	6	0.657		2.27	-
Canada	St. Lawrence River	96	4	0.166		2.08	-
Poland	Gulf of Gdansk	20	1	0		0	-
Russia	Volga River	82	10	0.425		9.41	partially
Russia	Moscow River	9	5	0.857		7.48	-
Serbia	Danube River	45	2	0.044		0.83	-
Slovakia	Danube River	39	2	0.051		0.83	-
Ukraine	Dnieper River	57	6	0.363		1.99	partially
Ukraine	Bug River	27	8	0.459		2.02	+
-	Sea of Azov	20	17	0.978		2.58	+
-	Black Sea	61	22	0.838		3.56	+
-	Caspian Sea	66	18	0.452		3.60	+

N_s, number of samples; N_h; number of haplotypes; H_d, haplotype diversity; P_i, nucleotide diversity.
* P_i was calculated in this study over all sequences;. P_i' was calculated over one sequence for each haplotype; P_i' of populations outside Belgium and the Netherlands were calculated accordingly and based on data presented in Brown and Stepien (2008; 2009). All other values are from Brown and Stepien (2008; 2009).

and 72 fin samples of round goby. All captured fish were measured to the nearest millimeter (total length, TL), weighed to the nearest milligram and stored in 70 % ethanol for further research. Dutch samples were stored in the freezer (-20°C).

Parasitological analysis

The body, fins and gills were inspected for parasites under an Olympus SZX12 stereomicroscope. Parasites were removed with a dissection needle and stored in 5 µL of milli-Q water at -20°C. Parasite prevalence (percentage of infected gobies in a population), abundance (mean number of parasites in a goby population) and infection intensity (mean number of parasites on infected gobies in a population) were calculated for each fish species and each site. These statistics were compared using a Kruskal-Wallis test in Statistica 7 (Statsoft 1995). Fin clips of each fish sample were stored in 70 % ethanol for DNA extraction.

Molecular analysis

Genomic DNA was extracted from fin tissue of a subsample of 47 round goby and 41 tubenose goby samples using a NucleoSpin® Tissue kit (Macherey-Nagel, Germany) following manufacturer's instructions. The mitochondrial cytochrome b (cyt *b*) and partial threonine tRNA gene (in total 1204 base pairs (bp)) were amplified using primers AJG15 (5'-CAAAAACCATCGTTGTAATT CAA CT-3') and H5 (5'-GAATTYTRGCTTTGGGAG-3') (Neilson and Stepien 2009). Polymerase Chain Reaction (PCR) was performed using a GeneAmp PCR system 2700 thermocycler (Applied Biosystems, Belgium) containing 1x PCR buffer (Eurogentec, Belgium), 200 µM of dNTPs (Amersham Pharmacia Biotech, the Netherlands), 2 mM of $MgCl_2$ (50 mM) (Eurogentec), 0.5 U of *Taq*-polymerase (Eurogentec), 0.5 µM of each primer (Eurogentec), 1 µL of template DNA, topped up with milli-Q water to 25 µl. Samples were initially denatured at 94 °C for 2 min followed by 35 cycles of 45 s denaturation at 94 °C, 30 s annealing at 52 °C and 60 s extension at 72 °C and followed by a final extension at 72 °C for 3 min. PCR products were purified using NucleoSpin® 96 PCR Clean-up (Macherey-Nagel) following the manufacturer's instructions and sequenced using a 1/8 dilution of the Big Dye Terminator 3.1 sequencing protocol (Applied Biosystems) using the initial PCR primers. Samples were run on an ABI PRISM 3130 Avant Genetic Analyser automated sequencer (Applied Biosystems). Sequences were manually adjusted using SeqScape® Software v2.7 (Applied Biosystems). Two samples of the round goby produced sequences of low quality and were excluded from further analysis.

Sequences were aligned in MEGA v5.10 (Kumar et al. 1994) according to the MUSCLE algorithm (Edgar 2004) using default distance measures and sequence weighting schemes. Haplotype and nucleotide diversity were calculated in DNAsp v5.10 (Librado and Rozas 2009) and compared with sequences on GenBank (2012) using the Basic Local Alignment Search Tool (BLAST). A statistical parsimony haplotype analysis was conducted using TCS 1.21 (Clement et al. 2000) with a 95 % connection limit.

Results

Phylogeography of round and tubenose goby

The 45 sequences of round goby belonged to three haplotypes, each differing in one mutation (Table 1; GenBank Accession Nos. KJ654330-KJ654332). All Dutch samples, 21 samples from the Albert Canal and nine samples from the Canal Gent-Terneuzen contained NSB1 (KJ654330), the most common haplotype (88%). Two samples from the Zeescheldt, one from the Albert Canal and another one from the Canal Gent-Terneuzen contained NSB2 (8 %; KJ654331). One single sample from the Canal Gent-Terneuzen contained NSB3 (2%; KJ654332). Haplotype diversity (H_d) and nucleotide diversity (P_i) was highest in the Canal Gent-Terneuzen, where three haplotypes were found. A single haplotype was found in the Zeescheldt and the Dutch samples. The network analysis was complemented with the known *Neogobius melanostomus melanostomus* haplotypes from GenBank (Figure 2, Table 1, Table S1). The resulting statistical parsimony network slightly differs from Brown and Stepien (2009), probably because shorter sequences were used in the present study (1130 bp *vs* 1204 bp in Brown and Stepien (2009)). NSB1 is identical to 'ame1' as defined by Brown and Stepien (2008) and clusters with other haplotypes from the Black Sea. NSB2 is identical to 'ame18' and clusters with haplotypes found in the Sea of Azov and the Volga River. NSB1 and NSB2 are linked directly, either via 'ame7' or via 'ame5' and 'ame42' of Brown and Stepien (2008). NSB3 (KJ654332) did not have an exact match with any other GenBank sequence, and differs in just one mutation from NSB1.

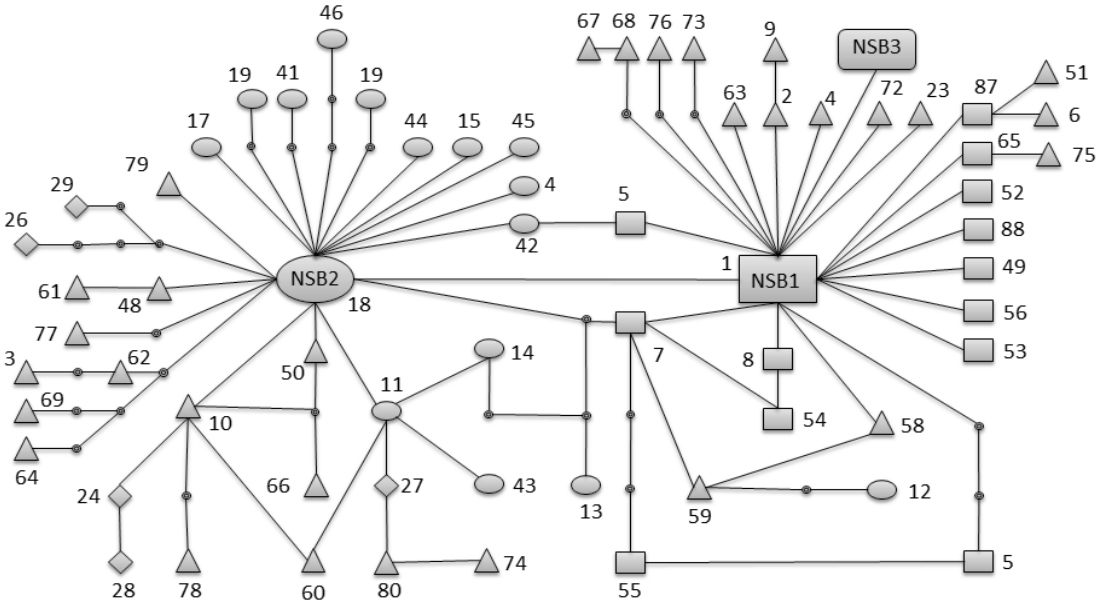

Figure 2. Statistical parsimony haplotype network of round goby cytochrome *b* haplotypes constructed with TCS 1.21 (Clement et al. 2000). Numbers next to the symbols correspond to the haplotypes in Brown and Stepien (2008, 2009) (e.g. 6 = ame6). Haplotype 1-3 in the larger sized symbols are the haplotypes discovered in the Netherlands and Belgium. Symbol size is not linked with haplotype frequency. Black dots represent a mutation. Symbols correspond with the sample sites where the haplotype is already discovered: (■) the Great Lakes and Black Sea drainages, (▲) Black Sea drainage, (⬬) Sea of Azov drainage, (♦) Volga. Haplotype frequency of the Belgian and Dutch samples: NSB1 (46/52), NSB2 (5/52) and NSB3 (1/52).

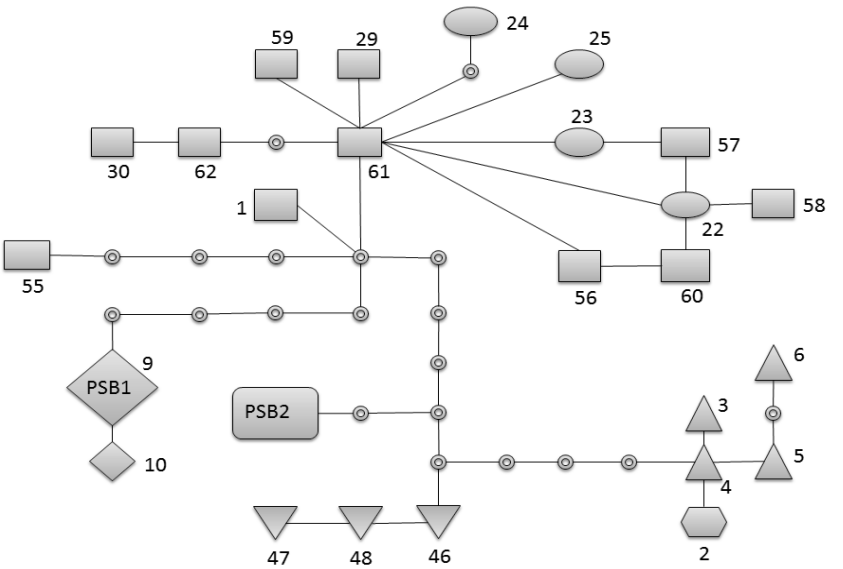

Figure 3. Statistical parsimony haplotype network of tubenose goby cytochrome *b* haplotypes assessed in TCS 1.21 (Clement et al. 2000). Numbers next to the symbols correspond to the haplotypes in Neilson and Stepien (2009) (e.g. 61 = Pro61). PSB1 and PSB2 in the larger sized symbols are the haplotypes discovered in the Netherlands and Belgium in this study. Size is not linked with haplotype frequency. Small double circles represent a mutation. Symbols correspond with the sample sites where the haplotype is already discovered: (■) Dniester and tributaries, Ukraine, (⬬) Odessa bay, Ukraine, (▲) Lake St. Clair and Superior, U.S.A., (▼) Simferopol, Ukraine, (⬢) Dnieper, Ukraine, (♦) Danube, Serbia, (▬) unknown outside Belgium. Haplotype frequency of the Belgian and Dutch samples: PSB1 (40/41) and PSB2 (1/41).

Table 2. Sequence diversity (cytochrome *b* mtDNA) of tubenose goby populations from this study and those from Neilson and Stepien (2009)*.

		N_s	N_h	H_d	P_i ($.10^{-3}$)	P_i' ($x10^3$)	Native
Belgium	Canal of Zuid-Willems	20	1	0	/	/	-
Belgium	Mouth Kik -en Ziepbeek	20	2	0.10	2.39	10.20	-
Belgium	Canal of Beverlo	1	1	0	/	/	-
Belgium	**Total**	**41**	**2**	**0.049**	**1.17**	**10.20**	**-**
U.S.A.	Lake St. Clair	10	4	0.53	0.84	1.90	-
U.S.A.	Lake Superior	9	1	0	/	/	-
Serbia	Danube River	3	2	0.67	0.59	0.88	-
Ukraine	Dnieper River	3	1	0	/	/	partially
Ukraine	Dniester River	6	5	0.93	2.05	2.46	partially
Ukraine	Simferopol	8	3	0.46	0.82	1.76	+

N_s, number of samples; N_h; number of haplotypes; H_d, haplotype diversity; P_i, nucleotide diversity. Samples from the Kurchurgan reservoir were grouped into the Dniester River.
* P_i was calculated in this study over all sequences; P_i' was calculated over one sequence for each haplotype; P_i and P_i' of populations outside Belgium and the Netherlands were calculated accordingly and together with Hd based on data presented in Neilson and Stepien (2009). All other values are from Neilson and Stepien (2009).

Table 3. Total number of fish screened (N_f), number of *Gyrodactylus* (N_p), prevalence (P in %), abundance (A) and infection intensity (I) of *Gyrodactylus* for each goby and waterway.

	N_f	N_p	P (%)	A	I
Round goby	**44**	**3**	**4.50**	**0.07**	**1.5**
Albert Canal Hasselt	11	0	0	0	/
Albert Canal Kuringen	14	0	0	0	/
Albert Canal Grobbendonk	4	0	0	0	/
Albert Canal Zandhoven	2	0	0	0	/
Zeescheldt	2	0	0	0	/
Canal Gent-Terneuzen	11	3	18.18	0.27	1.5
Tubenose goby	**73**	**163**	**82.19**	**2.23**	**2.72**
Mouth Kikbeek	37	92	83.78	2.49	2.97
Mouth Ziepbeek	7	13	85.71	1.86	2.17
Canal of Beverlo	1	0	0	0	x
Zuid-Willems Canal	28	58	82.14	2.07	2.52

In the 41 tubenose goby samples, two haplotypes were found differing by seven mutations (Table 2; GenBank Accession Nos. KJ654333-KJ654334). PSB1 was the most common haplotype (40 individuals); only one specimen from Ziepbeek contained PSB2. The BLAST search revealed that PSB2 is unique (KJ654334), while PSB1 is identical to Pro9 (KJ654333). Together with Pro10 these are the only haplotypes found in the Danube River in Serbia described by Neilson and Stepien (2009) (Table 2, Table S2). In the haplotype network (Figure 3), Pro9 and Pro10 differ by at least six bp from haplotypes found in the Dniester River and 14 bp from haplotypes found in North America. PSB2 does not group with any haplotype and differs with three bp from haplotypes found in Simferopol, Ukraine and seven bp from haplotypes found in North America and the Dniester River, Ukraine (Neilson and Stepien 2009).

Parasite infection characteristics

After screening 44 round gobies and 73 tubenose gobies, respectively 10 and 163 parasites were found. Most parasites (85 %) were discovered on the fins; only 2% were found on the body, 4 % on the head and another 4% on the gills. The remaining 5% were found detached from the fish on the bottom of the petri dish after screening. In total, seven were categorized as fungi while the others (n=166) were identified as the flatworm genus *Gyrodactylus* (Monogenea). Fungi and *Gyrodactylus* specimens were only found on round

goby specimens from the Canal Gent-Terneuzen. Tubenose gobies from all sites were infected with *Gyrodactylus* spp. except for the single specimen from the Canal of Beverlo (Table 3). *Gyrodactylus* prevalence reached 4.5% and 82.2% for the round and the tubenose goby respectively. Parasite abundance was significantly higher in tubenose gobies compared to round gobies ($p < 0.001$); also prevalence and intensity were much higher in tubenose gobies (Table 3).

A morphological and molecular study by Huyse et al. (in prep.) identified the *Gyrodactylus* species on the tubenose goby as *Gyrodactylus proterorhini* (Ergens, 1967), which was originally described on tubenose goby in Southern Slovakia and other gobiids from the Black and Azov Seas (http://bsmonogenea.ibss.org.ua). The *Gyrodactylus* specimens on the round goby could not be identified to species level since there was not sufficient material for morphological analyses.

Discussion

In Central Europe, the round goby has been recorded as far west as the upper Danube in Austria in 2005 (Copp et al. 2005; Jurajda et al. 2005). Kalchhauser et al. (2013) reported the presence of round gobies in the Danube-Main-Rhine Canal in 2006 and Roche et al. (2013) in the Main River in 2006–2007. In Western Europe, the round goby was reported for the first time in 2004 in the Lek River, 25 km upstream eastwards of the port of Rotterdam in the Netherlands, with a suspicion that ballast water represented the introduction means (van Beek 2006). Verreycken et al. (2011) suspected that the initial spread through Belgium started at the international port of Antwerp, which is heavily frequented by international vessels. Due to the remarkable rapid dispersal through some of the greater Central and Western European river basins, it has also been suggested that inland transport through ballast water led to the rapid range expansion of the round goby (Jurajda et al. 2005; Manné et al. 2013).

We identified three haplotypes in the Belgian and Dutch round goby samples. NSB1 is the most dominant haplotype in our samples, similar to North America and Eurasia (Brown and Stepien 2009). It occurs, together with the three other major Great Lakes haplotypes, in the southern Dnieper River near the port of Kherson, which is an important shipping port in Ukraine where ballast water is exchanged. This site is therefore suggested by Brown and Stepien (2009) as the primary source for the introduction into the Great Lakes. A similar scenario could be envisaged for the Belgian and Dutch population that share NSB1; however, based on the current data, an introduction from the Great Lakes back to Western Europe can't be excluded. Both scenarios would imply an introduction route via ballast water that is supported by our parasitological data. In contrast to the tubenose goby, the round goby was hardly infected with *Gyrodactylus* parasites, even though more than three different *Gyrodactylus* species have been recorded on round goby populations in their native range (Francova et al. 2011). The exchange of ballast water occurs just below the water surface, facilitating the selective uptake of pelagic juveniles unlike benthic adult gobies (Kocovsky et al. 2011). Because *Gyrodactylus* species mainly depend on physical contact between fish and contact with the riverbed for their transmission, pelagic juveniles are rarely infected (Bakke et al. 2007). Kvach et al. (2014) also suggested this scenario: they found far fewer parasite species on the invaded round goby populations in Poland than on goby populations from the native range. They also argued that the rapid translocation during ballast water transport, together with changes in water salinity, would result in a "disinfection" effect due to the loss of native parasite species (Kvach et al. 2014).

The two remaining Belgian haplotypes do not occur in the Great Lakes or in our samples from the Netherlands. One haplotype is unique whereas the other one has been described in the Dniester River draining into the Black Sea (Brown and Stepien 2008). Denser sampling is needed to conclude whether the presence of these haplotypes reflects independent introduction events in Belgium or introductions from different source populations.

The genetic diversity of the Belgian round goby population is rather low in comparison to the endemic populations and the introduced populations in North America (Brown and Stepien 2009; Table 1). Only Lake Michigan and Lake Superior display similar values. Since all haplotypes from the presumed founding source in Ukraine were present in the North American samples, a very large propagule pressure was assumed (Brown and Stepien 2009). This contrasts with the present study, and might indicate a rather low number of introductions in Belgium (i.e. low propagule pressure) or a limited number of source populations. However, many more North American samples have been genotyped compared to our study, precluding any firm conclusions.

Based on catchment data, it seems likely that the introduction of tubenose goby in Western Europe occurred through natural migration after the opening of the Danube-Main-Rhine Canal in 1992 (Von Landwüst 2006). Observations of tubenose goby in the river Roth, a River Main tributary, date back from 1997 (Von Landwüst 2006). It was recorded in the German River Rhine in 2000, in the Dutch River Waal in 2002 (van Kessel et al. 2009) and in the French canalized Rhine in 2007 (Manné and Poulet 2008). In 2002, it was recorded in the River Meuse and in 2008 for the first time in the Border Meuse on the Dutch and Belgian border (Cammaerts et al. 2011). The range expansion of tubenose goby was depicted well in Manné et al. (2013) and they assumed active migration was the primary driver for the expansion of the tubenose goby in Western Europe. Our genetic analysis supports this assumption of active migration from Eastern to Western Europe through the southern corridor (Bij de Vaate et al. 2002). All but one tubenose goby sequence belonged to PSB1, which was previously discovered in the Danube at the Serbian-Romanian border. PSB2 is most closely related to haplotypes discovered in Simferopol (Ukraine) and only recorded once in this study; it has not yet been described in other regions and is thus unique for Belgian waters. Again only extended sampling can provide insights on the distribution and rareness of this haplotype, and whether this represents a separate introduction or not.

Haplotype diversity of the Belgian tubenose goby population was about six times lower than the Belgian round goby population (Table 2). Compared to the tubenose goby populations from North America and the Ponto-Caspian region, fewer haplotypes have been found in relation to the number of samples. Nucleotide diversity on the other hand was high in comparison with the North American and East European population. This is due to the presence of two very divergent haplotypes in the Belgian samples (Figure 3). This high genetic variation in the tubenose goby has been previously reported (Neilson and Stepien 2009).

Parasite prevalence was very high in tubenose goby populations (82 %). This strongly contrasts with the near absence of parasites in round gobies and might be the result of different introduction pathways. In case of active migration (gradual dispersal), the parasite fauna experiences much less abrupt environmental gradients than during transport through ballast water. The identification of the *Gyrodactylus* species on the tubenose goby as *Gyrodactylus proterorhini* (Ergens, 1967) suggests that this parasite species has 'travelled' together with its host throughout Europe. This parasite has a Ponto-Caspian origin where it also infects round goby populations (Francova et al. 2011), but has never been recorded in Belgium before. It has been introduced in Poland together with the monkey goby *Neogobius fluviatilis* (Pallas, 1814), where it reached higher infection intensities compared to those in the native range (Kvach et al. 2014). Another possibility leading to the contrasting parasite characteristics between the two goby species is a different habitat preference. Differences in habitat preference have been reported several times (e.g. Jude and Deboe 1995; Spikmans et al. 2010) for round and tubenose gobies but all specimens used in this research were caught in a very similar rip-rap environment (H. Verreycken pers. obs.) and therefore differential habitat preference was possibly not the cause of the strong differences in parasite prevalence.

In conclusion, both the parasitological and the phylogeographical data point to introduction of the Belgian round goby population through ballast water, with the Black Sea as a potential introduction source. The tubenose goby, in contrast, seems to have spread naturally throughout Central and Western Europe. Our molecular and parasitological data supports previous observations that show a gradual expansion along the southern corridor (Bij de Vaate et al. 2002; Von Landwüst 2006; Manné et al. 2013). As previously stated, this corridor is the most important route for Ponto-Caspian species to invade Western Europe (Bij de Vaate et al. 2002).

With the steady pressure from Ponto-Caspian species, the already weakened food webs of Western European river basins increasingly destabilize. Newly established species may also facilitate the introduction of other species and their parasites, leading to an invasional meltdown (Simberloff and Von Holle 1999). Western Europe might be on a track comparable to the North American Great Lakes where, due to the chronic invasions, Ponto-Caspian food webs are being reassembled (Ricciardi and MacIsaac 2000). To prevent further introductions and a possible invasional meltdown, qualitative and quantitative models are needed. Genetic comparisons between native and non-native populations supported by parasitological observations may provide complementary information for heuristic and predictive models such as introduction routes and propagule pressure (Ricciardi and MacIsaac 2000).

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References

Bakke TA, Cable J, Harris PD (2007) The biology of gyrodactylid monogeneans: the ''Russian-doll Killers''. *Advances in Parasitology* 64: 161–376, http://dx.doi.org/10.1016/S0065-308X(06)64003-7

Barson M, Přikrylová I, Vanhove MPM, Huyse T (2010) Parasite hybridization in African *Macrogyrodactylus* spp. (Monogenea, Platyhelminthes) signals historical host distribution. *Parasitology* 137: 1585–1595, http://dx.doi.org/10.1017/S0031182010000302

Bij de Vaate A, Jazdzewski K, Ketelaars HAM, Gollasch S, Van Der Velde G (2002) Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1159–1174, http://dx.doi.org/10.1139/f02-098

Bronnenhüber JE, Dufour BA, Higgs DM, Heath DD (2011) Dispersal strategies, secondary range expansion and invasion genetics of the nonindigenous round goby, *Neogobius melanostomus*, in Great Lakes tributaries. *Molecular Ecology* 20: 1845–1859, http://dx.doi.org/10.1111/j.1365-294X.2011.05030

Brown JE, Stepien CA (2008) Ancient divisions, recent expansions: phylogeography and population genetics of the round goby *Apollonia melanostoma*. *Molecular Ecology* 17: 2598–2615, http://dx.doi.org/10.1111/j.1365-294X.2008.03777.x

Brown JE, Stepien CA (2009) Invasion genetics of the Eurasian round goby in North America: tracing sources and spread patterns. *Molecular Ecology* 18: 64–79, http://dx.doi.org/10.1111/j.1365-294X.2008.04014.x

Cammaerts R, Spikmans F, van Kessel N, Verreycken H, Cherot F, Demol T, Richez S (2012) Colonization of the Border Meuse area (The Netherlands and Belgium) by the non-native western tubenose goby *Proterorhinus semilunaris* (Heckel, 1837) (Teleostei, Gobiidae). *Aquatic Invasions* 7: 251–258, http://dx.doi.org/10.3391/ai.2012.7.2.011

Carlton JT, Geller JB (1993) Ecological roulette: The global transport of non-indigenous marine organisms. *Science* 261: 78–82, http://dx.doi.org/10.1126/science.261.5117.78

Charlebois PM, Corkum LD, Jude DJ, Knight C (2001) The round goby (*Neogobius melanostomus*) invasion: current research and future needs. *Journal of Great Lakes Research* 27: 263–266, http://dx.doi.org/10.1016/S0380-1330(01)70641-7

Clement M, Posada D, Crandall KA (2000) TCS: computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657–1659, http://dx.doi.org/10.1046/j.1365-294x.2000.01020.x

Copp GH, Bianco PG, Bogutskaya N, Erős T, Falka I, Ferreira MT, Fox MG, Freyhof J, Gozlan RE, Grabowska J, Kováč V, Moreno-Amich R, Naseka AM, Peňáz M, Povž M, Przybylski M, Robillard M, Russell IC, Stakėnas S, Šumer S, Vila-Gispert A, Wiesner C (2005) To be, or not to be, a non-native freshwater fish? *Journal of Applied Ichthyology* 21: 242–262, http://dx.doi.org/10.1111/j.1439-0426.2005.00690

Cowx IG (2002) Recreational fisheries. In Hart PBJ, Reynolds JD (eds), Handbook of fish biology and fisheries, vol. II. Oxford (United Kingdom): Blackwell Science, pp 367–390, http://dx.doi.org/10.1093/nar/gkh340

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acid Research* 32: 1792–1797, http://dx.doi.org/10.1093/nar/gkh340

Francova K, Ondrackova M, Polacik M, Jurajda P (2011) Parasite fauna of native and non- native populations of *Neogobius melanostomus* (Pallas, 1814) (Gobiidae) in the longitudinal profile of the Danube River. *Joural of Applied Ichthyology* 27: 879–886, http://dx.doi.org/10.1111/j.1439-0426.2010.01582.x

Grosholz E, Lovell S, Besedin E, Katz M (2011) Modeling the impacts of the European green crab on commercial shellfisheries. *Ecological Applications* 21: 915–924, http://dx.doi.org/10.1890/09-1657.1

Johnsen BO, Jenser AJ (1991) The *Gyrodactylus* story in Norway. *Aquaculture* 98: 289–302, http://dx.doi.org/10.1016/0044-8486(91)90393-L

Jude DJ, DeBoe SF (1995) Possible impact of gobies and other introduced species on habitat restoration efforts. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 136–141, http://dx.doi.org/10.1139/f2011-139

Jurajda P, Černý J, Polačik M, Valová Z, Janáč M, Blažek R, Ondračková M (2005) The recent distribution and abundance of non-native *Neogobius* fishes in the Slovak section of the River Danube. *Journal of Applied Ichthyology* 21: 319–323, http://dx.doi.org/10.1111/j.1439-0426.2005.00688.x

Kalchhauser I, Mutzner P, Hirsch PE, Burkhardt-Holm P (2013) Arrival of round goby *Neogobius melanostomus* (Pallas, 1814) and bighead goby *Ponticola kessleri* (Günther, 1861) in the High Rhine (Switzerland). *BioInvasions Records* 2: 79–83, http://dx.doi.org/10.3391/bir.2013.2.1.14

Kocovsky PM, Tallman JA, Jude DJ, Murphy DM, Brown JE, Stepien CA (2011) Expansion of tubenose gobies *Proterorhinus semilunaris* into western Lake Erie and potential effects on native species. *Biological Invasions* 3: 2775–2784, http://dx.doi.org/10.1007/s10530-011-9962-51

Kornis MS, Mercado-Silva N, Vander Zanden MJ (2012) Twenty years of invasion: a review of round goby *Neogobius melanostomus* biology, spread and ecological implications. *Journal of Fish Biology* 80: 235–285, http://dx.doi.org/10.1111/j.1095-8649.2011.03157.x

Kumar S, Tamura K, Nei M (1994) MEGA: Molecular evolutionary genetics analysis software for microcomputers. *Computational Applied Bioscience* 10: 189–191, http://dx.doi.org/10.1093/bioinformatics/10.2.189

Kvach Y, Kornyychuk Y, Mierzejewska K, Rubtsova N, Yurakhno V, Grabowska J, Ovcharenko M (2014) Parasitization of invasive gobiids in the eastern part of the Central trans-European corridor of invasion of Ponto-Caspian hydrobionts. *Parasitological Research* 113: 1605–1624, http://dx.doi.org/10.1007/s00436-014-3791-2

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451–1452, http://dx.doi.org/10.1093/bio informatics/btp18

Lin W, Cheng X, Xu R (2011) Impact of different economic factors on biological invasions on the global scale. *PLoS ONE* 6: e18797, http://dx.doi.org/10.1371/journal.pone.0018797

Mace G, Masundire H, Baillie J (2005) Millenium Ecosystem Assessment. Current State and Trends Assessment, Chapter 4: Biodiversity. Island Press, 36 pp

MacKenzie K (2002) Parasites as biological tags in population studies of marine organisms: an update. *Parasitology* 124: 153–163, http://dx.doi.org/10.1017/S0031182002001518

Manné S, Poulet N (2008) First record of the western tubenose goby *Proterorhinus semilunaris* (Heckel, 1837) in France. *Knowledge and Management of Aquatic Ecosystems* 389: 03 http://dx.doi.org/10.1051/kmae:2008009

Manné S, Poulet N, Dembski S (2013) Colonisation of the Rhine basin by non-native gobiids: an update of the situation in France. *Knowledge and Management of Aquatic Ecosystems* 411, http://dx.doi.org/10.1051/kmae/2013069

Neilson ME, Stepien CA (2009) Evolution and phylogeography of the tubenose goby genus *Proterorhinus* (Gobiidae: Teleostei): evidence for new cryptic species. *Biological Journal of the Linnean Society* 96: 664–684, http://dx.doi.org/10.1111/j.1095-8312.2008.01135.x

Ricciardi A, MacIsaac HJ (2000) Recent mass invasion of the North American Great Lakes by Ponto–Caspian species. *Trends in Ecology and Evolution* 15: 62–65, http://dx.doi.org/10.1016/S0169-5347(99)01745-0

Roche KF, Janac M, Jurajda P (2013) A review of Gobiid expansion along the Danube-Rhine corridor – geopolitical change as a driver for invasion. *Knowledge and Management of Aquatic Ecosystems* 411: 01–24, http://dx.doi.org/10.1051/kmae/2013066

Simberloff D, Von Holle B (1999) Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions* 1: 21–32, http://dx.doi.org/10.1023/A:1010086329619

Spikmans F, van Kessel N, Dorenbosch M, Kranenbarg J, Bosveld J, Leuven R (2010). Plaag risico analyses van tien exotische vissoorten in Nederland. Nederlands Centrum voor Natuuronderzoek: Stichting RAVON, Radboud Universiteit Nijmegen, Stichting Bargerveen and Natuurbalans – Limes Divergens, Nijmegen, 84 pp

Statsoft I (1995) STATISTICA for Windows (Computer Program Manual). Statsoft, Inc., Tulsa, OK, http://www.statsoft.com

Stepien CA, Tumeo MA (2006) Invasion genetics of Ponto-Caspian gobies in the Great Lakes: a 'cryptic' species, absence of founder effects, and comparative risk analysis. *Biological Invasions* 8: 61–78, http://dx.doi.org/10.1007/s10530-005-0237-x

van Beek GCW (2006) The round goby *Neogobius melanostomus* first recorded in the Netherlands. *Aquatic Invasions* 1: 42–43, http://dx.doi.org/10.3391/ai.2006.1.1.10

van Kessel N, Dorenbosch M, Spikmans F (2009) First record of Pontian monkey goby, *Neogobius fluviatilis* (Pallas, 1814), in the Dutch Rhine. *Aquatic Invasions* 4: 421–424, http://dx.doi.org/10.3391/ai.2009.4.2.24

van Kessel N, Dorenbosch M, De Boer M, Leuven R, Van der Velde G (2011) Competition for shelter between four invasive gobiids and two benthic fish species. *Current Zoology* 57: 844–851

Verreycken H, Breine JJ, Snoeks J, Belpaire C (2011) First record of the round goby, *Neogobius melanostomus* (Actinopterygii: Perciformes: Gobiidae) in Belgium. *Acta Ichthyologica Et Picatoria* 41: 137–140, http://dx.doi.org/10.3750/AIP2011.41.2.11

Verreycken H (2013) Risk analysis report of non-native organisms in Belgium: Risk analysis of the round goby, *Neogobius melanostomus*. Research Institute for Nature and Forest, 37 pp

Von Landwüst C (2006) Expansion of *Proterorhinus marmoratus* (Teleostei, Gobiidae) into the River Moselle (Germany). *Folia Zoologica* 55: 107–111

Williamson M (1996) Biological Invasions. Chapman and Hall, London, UK, 150 pp

Supplementary material

The following supplementary material is available for this article:

Table S1. Round goby haplotypes extracted from GenBank.

Table S2. Tubenose goby haplotypes extracted from GenBank.

This material is available as part of online article from:
http://www.aquaticinvasions.net/2014/Supplements/AI_2014_Mombaerts_etal_Supplement.xls