Research Article

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A minimalist macroparasite diversity in the round goby of the Upper Rhine reduced to an exotic acanthocephalan lineage

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Abstract

The round goby, *Neogobius melanostomus*, is a Ponto-Caspian fish considered as an invasive species in a wide range of aquatic ecosystems. To understand the role that parasites may play in its successful invasion across Western Europe, we investigated the parasitic diversity of the round goby along its invasion corridor, from the Danube to the Upper Rhine rivers, using data from literature and a molecular barcoding approach, respectively. Among 1666 parasites extracted from 179 gobies of the Upper Rhine, all of the 248 parasites barcoded on the c oxidase subunit I gene were identified as *Pomphorhynchus laevis*. This lack of macroparasite diversity was interpreted as a loss of parasites along its invasion corridor without spillback compensation. The genetic diversity of *P. laevis* was represented by 33 haplotypes corresponding to a haplotype diversity of 0.65 ± 0.032, but a weak nucleotide diversity of 0.0018 ± 0.00015. Eight of these haplotypes were found in 88.4% of the 248 parasites. These haplotypes belong to a single lineage so far restricted to the Danube, Vistula and Volga rivers (Eastern Europe). This result underlines the exotic status of this Ponto-Caspian lineage in the Upper Rhine, putatively disseminated by the round goby along its invasion corridor.

Introduction

Freshwater ecosystems have been especially affected by the unintentional introduction of exotic species (Sala *et al.* 2000; Gherardi, 2007; Havel *et al.* 2015). The analysis of the mechanisms underlying successful invasions contributes to the protection of aquatic ecosystems against the impacts of future invaders (Nunes *et al.* 2015). Among these mechanisms, parasitic interactions could play a critical role in the invasion success of an exotic host, through their impact on both exotic and native species (Dunn and Hatcher, 2015). Since the review of Torchin *et al.* (2003) and subsequent studies (Torchin and Mitchell, 2004; Gendron *et al.* 2012; Paterson *et al.* 2012), it is suggested that invasive species are released from their enemies as they occupy new areas – a phenomenon named the 'enemy release hypothesis' (Williamson, 1996); but some exotic parasites may also be introduced with the exotic host. In this case, they can colonize native communities and decrease their performance, giving a competitive advantage to their initial exotic host (Gendron *et al.* 2012). On the other hand, introduced species can be then colonized by non-specific native parasites from their new environment (Poulin and Mouillot, 2003; Gendron *et al.* 2012). These spillover–spillback phenomena, i.e. the transfer of parasites from invasive hosts to native ones (spillover), and from native hosts to invasive ones (spillback), can lead to the partial substitution of the parasite community in an invasive host along its invasive pathway (Kelly *et al.* 2009).

The round goby, *Neogobius melanostomus* (Pallas, 1814), is a Ponto-Caspian species that spread only recently in Western Europe (Roche *et al.* 2013). Until the 1980s, its uppermost distribution range was limited to the northeast of Bulgaria along the Danube River (Francová *et al.* 2011). The Danube and the Rhine rivers have been connected after the construction of the Rhine–Main–Danube corridor in 1992 (Leuven *et al.* 2009). The round goby was recorded downstream of the Vienna hydropower dam (Austrian stretch of the Danube) in 2000 and soon after in the Dutch Rhine Delta in 2004 (Van Beek, 2006). First records in the Upper Rhine date back to 2011 and 2012 in the Gambsheim fishway and several places along the French–German border, i.e. the unchannelled part of the Upper Rhine (Manne *et al.* 2013). The round goby is now widespread along the Rhine River and its abundance during an electrofishing can reach 84% of the total catch (Manne *et al.* 2013), with an estimated density ranging from 2 to 8 individuals m^{-2}. The geographic expansion of *N. melanostomus* is still in progress, since it was observed for the first time in the Seine Basin in 2015, within the Rouen harbour (Agence Française de la Biodiversité, personal communication). Its flexible trophic diet, with a majority of crustaceans and insects (Brandner *et al.* 2013), and its aggressive behaviour are considered as the main reasons for its invasion success in introduced aquatic communities (Steinhart *et al.* 2004; Borza *et al.* 2009; Stevove and Kovac, 2013).

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The aim of our study is to document the parasite assemblage of the round goby *N. melanostomus* in populations recently established in the Upper Rhine (French–German border), and compare it to the parasite assemblages reported from populations closest from its native area. We hypothesized that the round goby has a poor macroparasite assemblage in the newly invaded area compared with the native one. Depending on the phenomenon driving the changes in its parasite community, the parasite assemblage of the round goby from the Old Rhine should be more similar to the Danube ones or to local ones (spillback phenomenon; Kelly *et al.* 2009). To test these hypotheses, we first documented the potential changes in the parasite assemblage reported from the round goby throughout its European invasive pathway along the Rhine–Main–Danube corridor through a literature review. We also collected a large number of *N. melanostomus* from the Upper Rhine (and some native fishes) to establish parasite assemblage in the newly invaded area. We expected the acanthocephalan *Pomphorhynchus laevis* to be one of the most abundant parasite of the round goby (Francová *et al.* 2011), and we therefore focused on this species to better address the changes in parasite diversity driven by invasion. With a wide geographical distribution across Europe, *P. laevis* is one of the most common acanthocephalan parasites of freshwater fishes (Perrot-Minnot *et al.* in press, and references therein). This intestinal parasite of freshwater fish uses several amphipod species as intermediate hosts. It uses a broad range of freshwater and brackish-water fish species as final hosts, mainly Cyprinidae, the largest family of freshwater fish, but also Salmonidae (Médoc *et al.* 2011; Perrot-Minnot *et al.* in press). *Pomphorhynchus laevis* has occasionally and locally integrated in its life cycle an additional fish species as paratenic host, i.e. a facultative host used for the completion of the life cycle but in which no development occurs (Médoc *et al.* 2011).

The biogeographic history of *P. laevis* has been recently reconstructed, and reveals the existence of two lineages genetically and geographically distinct in the Danubian system, one in the Danube, Volga and Vistula rivers, and the other in its tributaries (Perrot-Minnot *et al.* in press). We therefore analysed the genetic diversity of *P. laevis* in our samples based on the mitochondrial DNA c oxidase subunit I (COI) gene, and used this sequence information to identify the geographic origin of the Upper Rhine lineages of *P. laevis* and propose a scenario for its introduction.

Material and methods

Literature review

A literature review was performed on Web of Science (up to July 2017) to make a census of papers focusing on round goby parasites along the Rhine–Main–Danube corridor. The main keywords used were 'Neogobius melanostomus', 'parasite' and 'European freshwater'. The references in each paper have also been checked to decrease the chance of missed studies. A total of 13 papers were found, covering 20 locations along the invasion corridor (Fig. 1). The presence and the prevalence of each parasite species were recorded for each location (Table 1).

Host sampling

Fish were collected in three sites of the Upper Rhine River located near Ottmarsheim (Grand Est, France), 20 km downstream of Basel, along the left bank. Two samplings were collected, one in February and one in May 2016, e.g. before the spring flood of the Rhine River. The sites are located on a relic of the uppermost stretch of the Upper Rhine, called the Old Rhine, a 50 km long by-passed single-bed channel located downstream of the Kembs dam. Fish were sampled from the three sites (A, B, C hereafter) that are quite close from each other. These locations belong to a morphodynamic restoration programme of controlled bank erosion conducted by Electricité de France (EDF) (Garnier and Barillier,
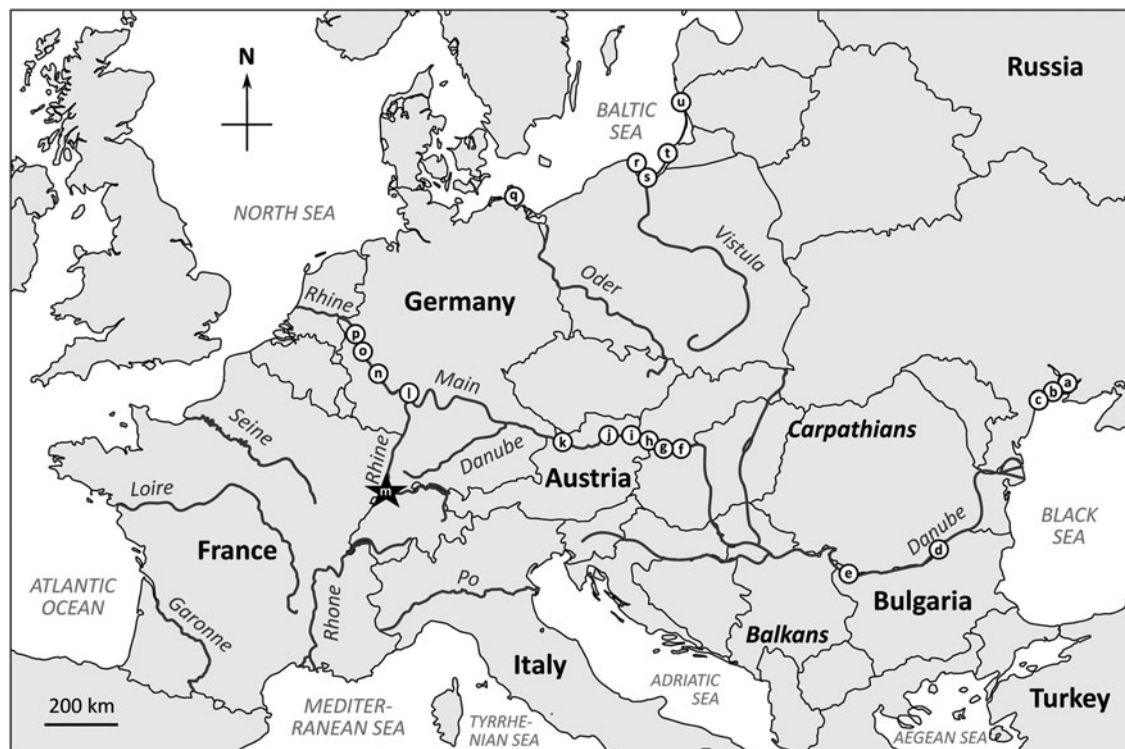

Fig. 1. Location of the scientific studies published on *Neogobius melanostomus* and its parasites along the Rhine–Main–Danube corridor. (a, b) Kvach and Skóra (2007); (c, s) Kvach *et al.* (2014); (d, g, i) Francová *et al.* (2011); (e, i) Ondračková *et al.* (2010); (f) Košuthová *et al.* (2009); (h) Ondračková *et al.* (2005); (j, k) Mühlegger (2008); (l, n) Emde *et al.* (2014); (m) sampling location for the present study (vicinity of Ottmarsheim, Upper Rhine); (o) Emde *et al.* (2012); (p) Ondračková *et al.* (2015); (q) Kvach and Winkler (2011); (r) Kvach and Skóra (2007); (t) Rolbiecki (2006); (u) Herlevi *et al.* (2017).

Table 1. Data from a literature review of parasite assemblages in *Neogobius melanostomus* along the Rhine–Main–Danube corridor, restricted to the three mean macroparasites reported

Parasite taxa	Years	Black Sea[a, b, c]	Danube Native[e, d]	Danube Non-native[f, g, h, i, j, k]	Main[l]	Upper Rhine[m]	Rhine[n, o, p]	Baltic Sea[q, r, s, t, u]
		2005 2010–2012	2004–2005 2005–2006	2003 2004 2004–2005 2005–2006 2007	2011	2016	2009–2011 2011 2012	2006 2010 2010–2012 2015
All	Number of sites	3	3	8	1	3	4	5
	Number of fishes	848	112	381	175	179	316	914
	Richness	20	14	18	2	1	6	20
Pomphorhynchus laevis	Number of sites	0	3	5	1	3	4	5
	Prevalence	0	97·7	70·8	46·8	60·9	87·8	7·6
	Mean abundance	0	54·4	21·5	1·4	9·3	14·4	0·14
Raphidascaris acus	Number of sites	1	2	4	1	0	4	0
	Prevalence	0·3	16·2	83·3	84·6	0	39·1	0
	Mean abundance	0·003	0·56	13·2	4·3	0	2·1	0
Diplostomus spp.	Number of sites	1	2	5	0	0	0	3
	Prevalence	88·9	18·2	36·7	0	0	0	28
	Mean abundance	7·8	0·22	2·62	0	0	0	4·9

Richness: total number of parasite species found in the study; prevalence: the percentage of fishes parasitized; mean abundance: mean number of parasites found in all individual fishes sampled; native area: lower part of the Danube River (Francová et al. 2011); non-native area: area where the round goby is considered as an invasive fish.
(a, b) Kvach and Skóra (2007); (c, s) Kvach et al. (2014); (d, g, i) Francová et al. (2011); (e, i) Ondračková et al. (2010); (f) Košuthová et al. (2009); (h) Ondračková et al. (2005); (j, k) Mühlegger (2008); (l, n) Emde et al. (2014); (m) sampling location for the present study (vicinity of Ottmarsheim, Upper Rhine); (o) Emde et al. (2012); (p) Ondračková et al. (2015); (q) Kvach and Winkler (2011); (r) Kvach and Skóra (2007); (t) Rolbiecki (2006); (u) Herlevi et al. (2017).

2015). Site A is the upstream site (47°44′51·87″N, 7°32′38·72″E) and can be considered as a positive control where geomorphic units and microhabitats are varied. Site B (47°44′43·95″N, 7°32′38·72″E) is 400 m downstream and has been the subject of an ecological restoration, with controlled bank erosion and artificial groynes implementation. The project was initiated by EDF. These actions are aimed at using the natural erosion capacity of floods to supply the Old Rhine River with aggregates, and to diversify the river mosaic thus allowing a potential gain in the biodiversity of alluvial environments. Site C (47°46′03·15″N, 7°31′54·84″E) is 2 km downstream of site B and can be considered as a negative control with a low diversity of habitats and a bank mostly composed of big rocks and a concrete area.

Electrofishing was used to collect the dominant fish species at each site. On completion of sampling, a total of 179 *N. melanostomus* were collected, 63 in site A, 57 in site B and 59 in site C. We also collected 18 barbels, *Barbus barbus* (L., 1758), and 11 chubs, *Leuciscus cephalus* (L., 1758) at the same time. To comply with animal welfare rules, fish were anaesthetized with precise doses of clove oil to cause death before transportation. The length (±1 mm) and the weight (±0·01 g) of each fish were determined before the dissection with aseptic precautions. During dissections, fish were sexed based on gonadal structure. For each individual, the eight gill arches were dissected off, and observed under a Leica ×40 binocular microscope. Macroparasites were also collected in the body cavity, and within the gut dissected under a binocular microscope. All parasites collected were stored separately in 99% ethanol.

DNA extraction, sequencing and phylogenetic analyses

Most of the parasites collected were surrounded by a membranous layer, which could represent a major host–parasite interface containing host haemocytes (Dezfuli et al. 2008). In order to limit contamination with host DNA during the extraction of parasite DNA, this membranous layer was systematically removed with sterile material. Each parasite was then placed in 99% ethanol. The DNA extraction was made on a selected number of *P. laevis* of each *N. melanostomus*, between one and three parasites in each sampled organ of each fish.

Individual parasite samples were incubated during 90 min at 55 °C in 700 µL of proteinase K 1 mg/mL (Euromedex, Souffelweyersheim, France) in 1% SDS, 500 mM NaCl, 10 mM TrisHCl, 50 mM EDTA. Then, the samples were mixed with 700 µL of phenol : chloroform : isoamyl alcohol (25 : 24 : 1; Euromedex). After centrifugation, the supernatant was collected and mixed with an equal volume of NaAc:ethanol and placed at −20 °C overnight for DNA precipitation. After centrifugation and washing with 70% EtOH, the dried pellets were suspended in 50 µL of Tris-EDTA with RNase solution (6 µL of RNAse, 594 µL of TE) at 55 °C for 60 min.

The first sequencing trials of cytochrome COI using universal primers (Folmer et al. 1994) systematically led to the amplification of COI from the host *N. melanostomus*. After having tested several alternatives including blocking primers, we chose to design new primers with limited hybridization to *N. melanostomus*: the forward primer 5′-TGTATGTTTTGGTTGGTGTGT GAGG and the reverse primer 5′-GGTGCTGATACAAAATA GGTGAACC (synthetized by Eurofins Scientific, Luxembourg). The condition of PCR amplification followed Perrot-Minnot (2004): 1× reaction buffer, 1·5 mM of MgCl, 0·1 µM of each primer, 0·1 mM of dNTP, 0·5 unit of GoTaqG2 polymerase (Promega, Madison, Wisconsin, USA) and 5 µL of DNA diluted at 50 ng/µL. The thermal cycling was programmed as 94 °C for 2 min, followed by 40 cycles with 20 s at 94 °C, 20 s at 50 °C and 50 s at 65 °C, with a final elongation of 5 min at 65 °C. PCR products were checked on 1·5% agarose gel and sequenced by Eurofins.

The DNA sequences and electropherograms were visualized, checked and aligned using MEGA6 (Tamura et al. 2013) and the haplotype reduction of the whole alignment was led on the

FaBox web interface (http://users-birc.au.dk/biopv/php/fabox/). The haplotype network was generated using *pegas* R-package (Paradis, 2010) in R v.3.2 (R Core Team, 2017). In order to identify the lineages occurring in the Upper Rhine, we compared these sequences to recently released sequences of *P. laevis* from the Western Europe (Perrot-Minnot *et al.* in press) (Genbank accessions MF563495–MF563527). For this purpose, the neighbour-joining phonetic tree was built in MEGA.

Results

Variation of the parasite assemblage of N. melanostomus along its invasion corridor

The bibliographical study along the corridor Rhine–Main–Danube revealed the presence of 37 different macroparasite species. Their presence and their prevalence vary depending on the site and on the river considered (Fig. 2). Three locations have been investigated in the Black Sea (Kvach and Skóra, 2007; Kvach *et al.* 2014) revealing a total of 20 different macroparasites, five of them being found in the three locations. In the Danube, studies showed a decrease of parasite diversity compared with the Black Sea (Ondračková *et al.* 2005, 2010; Mühlegger, 2008; Košuthová *et al.* 2009; Francová *et al.* 2011). Eighteen parasite species were reported in eight locations, with a mean diversity of 4·8 species per location. Eight parasite species found in the Danube samples have already been reported from the Black Sea. Only one study focuses on the parasites of *N. melanostomus* in the Main River (Emde *et al.* 2014). The two main parasites observed in the round goby were the nematode *Raphidascaris acus* (Bloch, 1779), already reported in the Black Sea, and *P. laevis*, already reported in the downstream stretch of the Danube but not in the Black Sea. In the Baltic Sea, five studies conducted on the parasite community of the round goby reported a total of 20 species, including five species that had never been reported in the other hydrosystems (Rolbiecki, 2006; Kvach and Skóra, 2007; Kvach and Winkler, 2011; Kvach *et al.* 2014; Herlevi *et al.* 2017). In the Rhine, three studies focus on three locations, but only in the lower (and middle) part of the river, downstream of the confluence of the Main–Danube canal (Emde *et al.* 2012, 2014; Ondračková *et al.* 2015). One location has been surveyed in two periods of the year, autumn and spring (Ondračková *et al.* 2015). Only six species have been reported, including one which had never been observed before in the Main, the Danube or the Black Sea, the nematode *Paracuaria adunca* (Emde *et al.* 2012).

The present overview of parasite diversity along the Rhine–Main–Danube corridor therefore revealed three dominant parasite species in most sites along the corridor, with a high prevalence (Table 1): the acanthocephalan *P. laevis*, the nematode *R. acus* and the digenean trematode *Diplostomum* spp. In this study, we only observed *P. laevis* parasites in the 179 round goby collected in the Upper Rhine River.

Lack of parasite diversity in the Upper Rhine River

Out of the 179 round gobies sampled, 109 were parasitized, which represents a prevalence of 60·9%. We collected 1666 parasites in the body cavity, the gut, the liver or the gonads, all of which belonging to *Pomphorhynchus* species based on visual inspection. The parasites were enveloped in a membranous layer, at the larval stage called cystacanth. Once this layer was removed, the parasites presented diverse morphologies: invaginated proboscis or not, large and short body or small and thin body, proboscis with spines or not. Their identification according to their morphology was therefore difficult. The intensity, i.e. the mean number of parasite per infected fish was 15·3, with a minimum of one parasite (mean length of the fishes: 7·1 cm) and a maximum of 120 parasites (length of the fish: 15 cm). Fifty per cent of the goby harboured between one and five parasites, and only 8·3% carried more than 40 parasites. Out of the 18 barbels sampled, four were parasitized. The 21 parasites from barbels were all collected inside the gut. They were all at the adult stage, with their proboscis attached to the inner side of the intestine wall. Out of the 11 chub collected, only one was parasitized, with one parasite. This parasite was also at the adult stage, inside the gut of the fish. A total of 242 *Pomphorhynchus* parasite samples from 109 round gobies were identified using molecular method, plus 10 parasites from four barbels and one parasite from one chub. The molecular barcoding approach on the mDNA COI assigned all the 253 partial sequences to *P. laevis*.
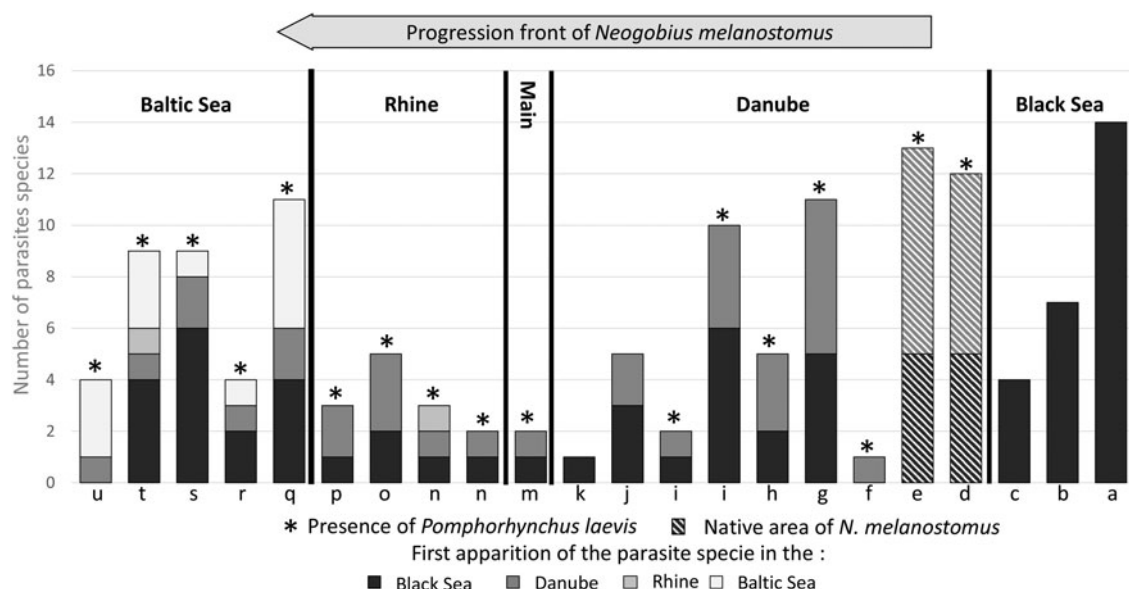

Fig. 2. Parasitic diversity along the Rhine–Main–Danube corridor. Each column represents one site except for the Rhine River, where the first two columns correspond to the same site surveyed at two different periods of the year (references in Fig. 1).

Table 2. Distribution of the eight most common haplotypes of *Pomphorhynchus laevis* recorded in the Upper Rhine River

Haplotype	Number of parasites	Number of parasites in hosts			Number of host fishes	Parasite location in hosts				Presence in study sites		
		Barbel	Chub	Goby		Cavity	Liver	Gut	Gonads	A	B	C
A	145	6	0	139	76	87	49	6	3	Yes	Yes	Yes
B	35	1	0	34	31	27	7	1	0	Yes	Yes	Yes
C	12	0	0	12	12	6	6	0	0	Yes	Yes	Yes
D	4	0	0	4	4	4	0	0	0	Yes	No	No
E	9	0	0	9	8	9	0	0	0	Yes	Yes	Yes
F	6	1	0	5	6	3	2	1	0	Yes	Yes	Yes
G	6	0	0	6	5	5	0	0	1	Yes	Yes	Yes
H	4	0	0	4	3	3	1	0	0	No	Yes	No

Genetic diversity of the P. laevis *in the Upper Rhine River*

Using the specific primers designed for this study, we obtained partial COI sequences of 557 bp length from the 253 parasites. This dataset included 33 haplotypes (Genbank accessions: MF563495–MF563527) representing a haplotype diversity of 0·65 ± 0·032, despite a weak nucleotide diversity of 0·0018 ± 0·00 015. Eight of the 33 haplotypes represented the majority (87·4%) of the samples (Table 2), the remaining haplotypes being represented by only one or two samples. The haplotypes A and B represented 145 and 35 samples, respectively. The median-joining network showed a radial structure centred on the haplotype A (Fig. 3). This radial unimodal shape is accentuated by the poor representation of the other haplotypes. The highest divergence between two haplotypes is brought by seven mutations, and the distance between two neighbour haplotypes does not exceed two mutations. There was no evidence for structuring in the haplotype network driven by spatial location, nor by fish hosts, nor by location within the host (viscera or body cavity).

Phylogenetic position of the Upper Rhine populations in the phylogeography of P. laevis

In order to establish the native or exotic status of the lineage of *P. laevis* found in the Upper Rhine, we placed these haplotypes in a phylogenetic tree comprising published sequences of *P. laevis* from Europe (Fig. 4). All samples from the Upper Rhine are clustering with haplotypes from the Danube and the Vistula rivers.
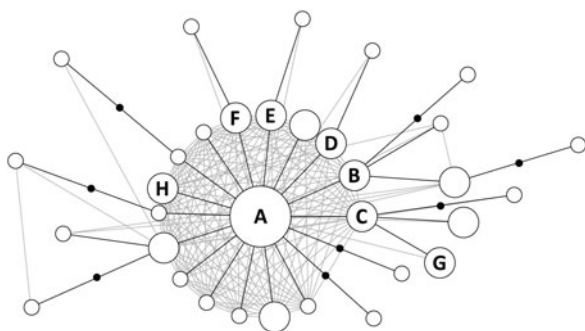
They are therefore gathered in a lineage distinct from the Western Europe lineage comprising samples from France (Rhone and Loire drainages, and Meuse River; Fig. 4).

Discussion

In the Upper Rhine, the invasive *N. melanostomus* is parasitized only by the acanthocephalan *P. laevis*. However, *N. melanostomus* is infected by a diversity of macroparasites along the Rhine–Main–Danube corridor (Molnar, 2006; Emde *et al.* 2012). Some macroparasites are specific to an area [e.g. the nematode *Cosmocephalus obvelatus* (Creplin, 1825), in the Baltic Sea (Kvach and Winkler, 2011; Kvach *et al.* 2014), while others are present at several locations along the corridor (e.g. the nematode *R. acus*)]. Torchin *et al.* (2003) proposed that at the beginning of invasion process, the newly introduced host loses a part of its parasite community. The differences in the parasite community between the native and the invasive populations would vanish after several
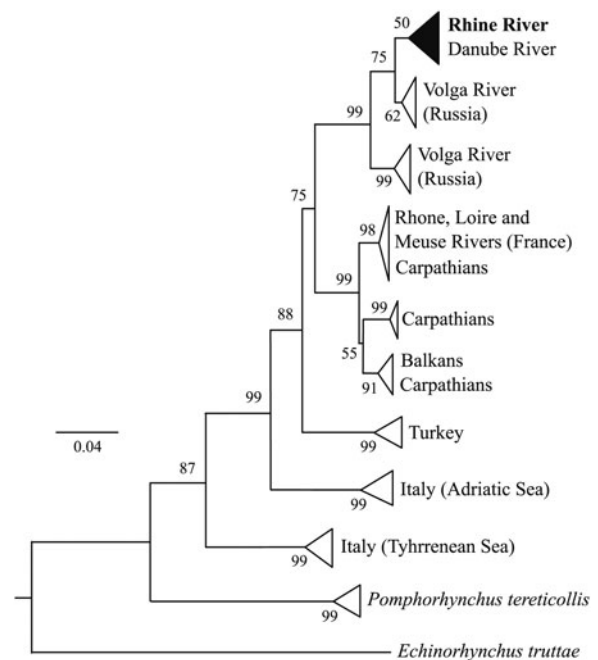

Fig. 3. Median-joining network comprising the 33 COI haplotypes of *Pomphorhynchus laevis* from the Upper Rhine River. Each circle represents a haplotype and its size is proportional to the haplotype frequency. Haplotype A gathers 145 individuals, haplotype B gathers 35 individuals, other median circle dots gather from 12 to 2 individuals. Small white circles were found in only one individuals. Line lengths in the network corresponds to the number of mutational changes between haplotypes, and grey lines represent other equivalent lops between close haplotypes. Black dots represent haplotypes missing in the study sampling.

Fig. 4. Neighbour-joining phenetic tree of the lineage of *Pomphorhynchus laevis* in Europe, based on 45 European haplotypes from a previous study, and the 33 haplotypes identified in the present study. The numbers correspond to bootstrap values supported by each node. The phylogenetic tree has been built using two acanthocephalans species close to *P. laevis* as external groups, *Pomphorhynchus tereticollis* (*n* = 5) and *Echinorhynchus truttae*.

years, once the invasive population is well settled (Gendron *et al.* 2012). If the parasite community of *N. melanostomus* shows a large diversity along its invasive pathway, it is yet reduced to one species in the Upper Rhine River. Indeed, we did not find in the Upper Rhine the parasites associated to *N. melanostomus* in its native range (Kvach and Skóra, 2007; Kvach *et al.* 2014). This loss of parasites is in agreement with the 'enemy release hypothesis' (Williamson, 1996), which states that exotic species arrive almost without any parasite. In addition, the mDNA COI sequences of *P. laevis* from the Upper Rhine clearly fit within a phylogenetic lineage described in the Danube and the Vistula rivers (Perrot-Minnot *et al.* in press). This lineage could have been transported by *N. melanostomus* from the Danube to the Rhine, as a 'hitchhiker' parasite. Associated with the 'enemy release' phenomenon (i.e. the loss of initial parasites), the Danubian *P. laevis* found in *N. melonostomus* could testify a previous spillback event during the passing of the round goby along the Danube River. Our additional fishing of two well-known definitive hosts of *P. laevis*, the barbel, *B. barbus* and the chub, *L. cephalus* (Sures and Siddall, 1999; Thielen *et al.* 2004) demonstrate that *P. laevis* from the Danube uses these native fish to complete its life cycle in the Upper Rhine. This is in agreement with the fish hosts from which this lineage was recorded in the Danube (barbels and gobies, Perrot-Minnot *et al.* in press). This result could testify to a spillover event with the transfer of *P. laevis* from an exotic host (*N. melanostomus*) to a native one. Concomitantly with our study, the introduction of *P. laevis* in the Rhine River (Germany, Switzerland) from the Ponto-Caspian region has been recently reported, although the precise genetic lineage of *P. laevis* was not identified (Hohenadler *et al.* 2017). Interestingly, the introduction of Ponto-Caspian *P. laevis* was accompanied by the displacement of *Pomphorhynchus tereticollis* within about a decade, as evidenced based on historical sampling of *Pomphorhynchus* from eels (Hohenadler *et al.* 2017). From our data, we cannot conclude that *N. melanostomus* is the only dispersal vector of *P. laevis*, and other intermediate hosts could have played a role in this dispersal. For instance, a Ponto-Caspian amphipod, *Dikerogammarus villosus* (Sowinsky, 1894), is an intermediate host for *P. laevis* (Rewicz *et al.* 2014), and it has become an invasive species westward, including the Upper Rhine (Bollache *et al.* 2004). It is therefore a likely candidate for *P. laevis* introduction (Hohenadler *et al.* 2017), together with other species from the Ponto-Caspian region such as the gobies. In addition, other transfers of parasites, led by spillover and spillback phenomena, have probably occurred all along the corridor, resulting in a large diversity of parasite communities of the round goby.

With this study, we revealed some gaps in the knowledge of the life cycle of *P. laevis* in the Upper Rhine River. More specifically, most parasites found in the round goby were non-mature and found outside the intestinal tract (where sexual reproduction takes place), which indicates that the round goby is probably not a definitive host for *P. laevis*. The significance of this host as a paratenic host or as a dead-end for *P. laevis* still needs to be established. In the former case, predatory fish of the goby could act as definitive hosts and allow the parasite to resume its cycle. For instance, some large barbels could feed on *N. melanostomus* (Emde *et al.* 2012). This hypothesis awaits further investigations, for instance by testing the viability of the cystacanths found in the body cavity of the round goby (Médoc *et al.* 2011). According to Kennedy (2006), *N. melanostomus* could be a paratenic host for *P. laevis* only if the cystacanths can resume their development once they are transferred to a definitive host. In this case, parateny could have a positive effect on the life cycle of *P. laevis*: the concentration of parasites in the paratenic host allows a delayed and massive contamination of the definitive host (Kennedy, 2006). If the round goby is a dead-end host, the *P. laevis* in the round goby cannot resume their cycle at all,

which leads to a decrease of the parasite population. To test this hypothesis, the prevalence and intensity of the parasite in several hosts over time should be studied, in order to detect a dilution effect in the *P. laevis* community (Emde *et al.* 2012). The ecological consequences in the Upper Rhine River could be multiple, for instance a disappearance of the parasite and a lack of regulation of the round goby population, this latter already constituting a worrying percentage of fish abundance in the Upper Rhine.

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