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Co-phylogeographic study of the flatworm *Gyrodactylus gondae* and its goby host *Pomatoschistus minutus*

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ABSTRACT

We performed a comparative phylogeographic study on the monogenean flatworm *Gyrodactylus gondae* Huyse, Malmberg & Volckaert 2005 (Gyrodactylidae) and its sand goby host *Pomatoschistus minutus* (Pallas, 1770) (Gobiidae). *G. gondae* is a host-specific parasite with a direct life cycle and a very short generation time. These properties are expected to increase the chance to track the genealogical history of the host with genetic data of the parasite ('magnifying glass principle'). To investigate this hypothesis we screened nine sand goby populations ($n = 326$) along the Atlantic coasts of Europe for *Gyrodactylus* specimens. Low parasite prevalence resulted in partially overlapping host and parasite datasets. Ninety-two *G. gondae* collected on five sand goby populations were subsequently sequenced for a 460 bp cytochrome *c* oxidase subunit II (*coxII*) fragment, which, in combination with previously published haplotype data for the hosts, allowed for partially overlapping host and parasite datasets. Haplotype diversity was lowest in the Irish Sea while nucleotide diversity was highest in the Southern North Sea. The host population also showed the lowest diversity in the Irish Sea but the highest nucleotide diversity, based on cytochrome *b* sequences of 850 bp, was found in Skagerrak. Phylogeographic networks suggest postglacial expansion in both the host and the parasite. Pair-wise population differentiation was however not consistently higher in the parasite than in the host, rejecting the magnifying glass hypothesis for this host-parasite system. The parasite network offered limited resolution and was characterized by many extinctions and/or missing haplotypes, which could be attributed to 1) sampling bias, 2) size fluctuations in the parasite populations resulting in frequent extinctions and genetic drift and 3) the relatively young age of the host-parasite association. A more exhaustive study including a broader geographical and genomic coverage is needed to discriminate among these competing hypotheses.

1. Introduction

Although parasites and their vertebrate hosts are distantly related and dissimilar in many ways, they live in intimate relationships. The parasite has to adapt to the host that represents a specific and changing environment, and the host in turn has to adapt to the parasite, in order to minimize potential adverse effects. This may induce co-evolutionary arms races between host and parasite [1], which may lead to reciprocal evolutionary change in the interacting species, and ultimately to joint speciation. On an evolutionary timescale co-speciation may result in mirror-image phylogenies [2]. Congruent patterns of host and parasite phylogenies can also evolve from a one-way interaction, where speciation of the host induces speciation of the parasite, without parasite-induced speciation of the host.

So far, most studies on host-parasite co-speciation focused on the phylogenetic level, examining co-evolutionary relationships between species pairs [2]. However, speciation is a continuous process and processes acting at the population-level influence patterns of co-speciation. Phylogeography offers the tools to unravel co-evolutionary relationships in different host and parasite populations across their shared distribution range [3]. When host populations become separated, gene flow between their parasites may be severed and initiate allopatric co-speciation. The reduction in gene flow depends on the parasite dispersing capabilities, the transmission mode of the parasite and the degree of co-occurrence between the newly diverged host species. In the case of host-specific parasites with a vertical transmission mode or low dispersal abilities, this may lead to a strong concordance among the phylogeographic patterns of distant host and parasite taxa (co-evolution

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hypothesis). This is illustrated by the mirror-image phylogenetic trees of *Gyrodactylus teuchis* Lautraite, Blanc, Thiery, Daniel & Vigneulle, 1999 and its salmonid hosts *Salmo trutta* Linnaeus, 1758 and *S. salar* Linnaeus, 1758. Moreover, if the generation time of the parasite is shorter than that of the host, genetic diversity and differentiation may be higher in the parasite [4]. In that case parasite genetic analyses may offer increased resolution for understanding host evolution. This so-called 'magnifying glass' hypothesis looks conceptually very attractive. Nieberding & Olivieri [4] provide a list of cases where parasites were successfully used as proxies for their host history. Among those is the case where the phylogenetic tree of helminth parasites of wood mouse revealed previously unknown refugia during the Pleistocene ice ages because the resolution was much higher than in the host tree [5]. Another study nicely demonstrated the use of digenean parasites to discriminate among fish stocks of steelhead trout *Oncorhynchus mykiss* Walbaum, 1792. Microsatellite genotyping of the trematode parasite *Plagioporus shawi* (McIntosh, 1939) allowed discrimination of host populations from rivers separated by as little as ca. 50 km, whereas patterns of host microsatellite diversity failed to resolve such fine-scale population assignment [6].

In this study we propose the sand goby *Pomatoschistus minutus* and monogenean parasites of the genus *Gyrodactylus* as a model system. The sand goby is a marine demersal fish, commonly distributed along the European coast, living up to maximally two years of age and showing a unique brooding behavior with paternal care [7]. Its phylogeographic pattern points to a monophyletic Atlantic clade with an Iberian Peninsula and a North Atlantic group [8,9]. Late Pleistocene population expansions characterize the sand goby populations of the Baltic Sea, Irish Sea, North Sea and Bay of Biscay. *Gyrodactylus* is a parasite genus with a direct life cycle and no free-living phase, which makes *Gyrodactylus* species highly dependent on their host for dispersal and gene flow [10]. Due to viviparous reproduction several generations can live on the same host individual, resulting in a close relationship with the host. The *Gyrodactylus* species described on *Pomatoschistus* gobiids are exclusively found on this genus, suggesting strong phylogenetic host specificity, even though host-switching among closely related *Pomatoschistus* hosts can occur [11,12]. All these aspects suggest that the respective host-parasite system would represent a good case to test the co-evolution and 'magnifying glass' hypotheses.

The aim of this study is threefold: (1) To collect information on the infection pattern of *P. minutus* gobies by *Gyrodactylus* parasites along its distribution range, (2) to elucidate the co-evolutionary relationships between *P. minutus* and its most prevalent *Gyrodactylus* parasite(s) at the population level and (3) to test the 'magnifying glass' hypothesis, which predicts stronger genetic differentiation and thus increased phylogeographic resolution based on parasite data, compared to the hosts' data.

2. Materials and methods

2.1. Sampling and DNA-extraction

Specimens of the sand goby *Pomatoschistus minutus* (Pallas, 1770) (Gobiidae) were caught across Europe between 2000 and 2008 by [8,13] and screened for gyrodactylids (Fig. 1, Table 1). Only populations of Llanfairfechan (Wales), Texel (the Netherlands), Fiskebäckskil (Sweden), Bergen and Trondheim (Norway) hosted gyrodactylids that were identified as *Gyrodactylus gondae* Huyse, Malmberg & Volckaert 2005 (Table 2). Additional gobies were collected at Oostende (Belgium) and Fiskebäckskil (Sweden) in 2012 in an attempt to complement host coverage. All fish were fixed in analytical-grade ethanol (80%). We screened fins, skin, gills, head and body for *Gyrodactylus* sp. together with parasites at the bottom of the collection jar in an embryo dish filled with ethanol, using a stereomicroscope (Olympus SZX 12). Abundance and prevalence of the total number of *Gyrodactylus* spp. infecting sand gobies were calculated according to Bush et al. [14]. We sequenced parasites from as many hosts as possible because parasites from the same individual might represent clones [10]. Each parasite specimen was individually stored in 10 μL of milli-Q water at −20 °C.

DNA extraction was performed following a modified version of [15]. We added 10 μL of a double concentrated lysis solution containing 1× PCR buffer (Eurogentec), 0.45% Tween 20 (Merck), 0.45% NP40

Fig. 1. Geographical distribution of the collection sites of sand goby *Pomatoschistus minutus* (grey bullets) and *Gyrodactylus gondae* (circled bullets).

Table 1
Summary of the sand goby samples screened for *Gyrodactylus* spp., including code, location, sea, date of sampling, number of screened sand gobies and isolate *Gyrodactylus* spp., and number of hosts infected with *Gyrodactylus* spp.

Code	Location	Sea	Longitude	Latitude	Sampling date	Number of gobies screened	Number of *Gyrodactylus* spp. found	Number of hosts infected %
TRO	Trondheim (NO)	Norwegian Sea	63°29′N	10°21′E	June 2000	3	14	67
					September 2000	NA	10	NA
BER	Bergen (NO)	Northern North Sea	60°24′N	05°14′E	June 2000	10	18	60
					June 2008	18	21	56
FIS	Fiskebäckskil (SE)	Skagerrak	58°15′N	11°27′E	October 2012	64	45	30
TEX	Texel (NL)	Southern North Sea	52°59′N	04°45′E	November 2000	23	80	61
BAL	Balgzand (NL)	Southern North Sea	52°56′N	04°51′E	August 2007	18	1	6
OOS	Oostende (BE)	Southern North Sea	51°15′N	02°58′E	June 2005	5	0	0
					October 2012	36	6	6
					August 2008	19	4	20
GUA	Coto Doñana, Guadalquivir estuary (ES)	Iberian Peninsula	36°55′N	06°22′W	November 2006	42	0	0
GIR	Royan, Gironde estuary (FR)	Bay of Biscay	45°37′N	01°01′W	August 2006	39	0	0
WIS	Llanfairfechan, Wales (UK)	Irish Sea	53°59′N	03°59′W	November 2006	49	88	46

NA: no data available.

(Calbiochem) and 60 $\mu g \cdot L^{-1}$ proteinase *K* (Sigma). Enzymatic digestion was carried out at 65 °C for 10 h instead of 25 min, followed by inactivation of the enzyme at 95 °C for 25 min. Additional unidentified *Gyrodactylus* specimens from Texel, Bergen, and Trondheim, collected in the year 2000, were already extracted and kept at −20 °C [11].

2.2. Molecular analyses and species identification

Polymerase Chain Reaction (PCR) reactions were performed with a TGradient Thermocycler (Biometra) using a primer pair amplifying a 460 bp fragment of the cytochrome oxidase c subunit II (*coxII*) [16,43]: Cox2F (5′-TACAYAYCGCCCGTCAAYYTCG-3′) and Cox2R (5′-AATAMWKATWGGCATRWAAGARTG-3′). The PCR cocktail (25 μL) consisted of 2.5 μL PCR buffer (10×; Invitrogen), 5 μL dNTPs (2 mM; Fermentas), 1.5 μL $MgCl_2$ (50 mM; Invitrogen), 0.2 μL (Platinum *Taq*, Invitrogen), 1 μL of both primers, topped up with mQ water. The following cycling conditions were applied: initial denaturation for 5 min at 95 °C followed by 35 cycles of 30 s at 94 °C, 45 s at 50 °C and 45 s at 72 °C, followed by a final elongation step for 7 min at 72 °C. Since the *Gyrodactylus* fauna of the sand gobies has only been characterized by ITS rDNA we used the ITS rDNA region for species identification. For each unique *coxII* haplotype the ITS rDNA was sequenced using ITS1A (5′-GTAACAAGGTTTCCGTAGGTG-3′) and ITS2 (5′-TCCTCCGCTTAGTGATA-3′ [17].

We used the NucleoSpin® 96 PCR Clean-up kit (Macherey-Nagel) following the manufacturer's instructions for purification of the samples from Texel and Llanfairfechan. Samples were eluted with 90 μL NE elution buffer. The remaining samples were purified with the Illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare) and eluted in 30 μL elution buffer. A 1/8 dilution of the Big Dye Terminator v3.1 sequencing protocol (Applied Biosystems) was applied for sequencing, using the initial PCR primers. Products and negative control samples were run on an ABI PRISM 3130 Avant Genetic Analyser automated sequencer (Applied Biosystems).

Sequences were visually inspected and manually edited using Sequencing Analysis v5.2 and SeqScape v2.5 software (Applied Biosystems). They were aligned with MUSCLE [18] as implemented in MEGA v5.05 [19]. Substitution models were evaluated using the Akaike information Criterion (AIC) via the FindModel web server (based on Posada and Crandall [20]).

2.3. Network analysis, genetic diversity and demographic analyses

A statistical parsimony network was constructed based on the parasite *coxII* sequences using the software TCS v1.21 [21]. For the host the network was constructed with previously obtained cytochrome *b* (cyt *b*) sequences [8]. The number of haplotypes (*R*), nucleotide (π) and haplotype (*h*) diversities were calculated with the software DnaSP v5.10.1 [22]. Pair-wise F_{ST}-values for the *coxII* rDNA sequences were calculated in Arlequin v3.11 [23] and Bonferroni corrected.

3. Results

3.1. Prevalence

The 326 sand gobies collected before [8,11,13] and for the purpose of this study varied in prevalence of infection and abundance of *Gyrodactylus* flatworms (Table 1). Abundance was highest in the Irish Sea and prevalence of infection varied from zero up to 67% in the Southern North Sea.

Low parasite prevalence resulted in only partially overlapping host and parasite datasets (Table 1). Since the parasites were immediately collected in a 2 mL tube for subsequent DNA extraction, no morphological identification was made. Sequence analysis revealed that 107 out of the 278 parasite specimens belonged to the gill parasite *G. gondae*. The

Table 2
List with sampling sites, number of individuals, and genetic diversity at the partial mitochondrial *coxII* fragment (460 bp) of *Gyrodactylus gondae*. Values in brackets are standard deviations. See Table 1 for geographical position of sampling location.

Code	Location	Sea	Sampling date	n	Number of polymorphic sites, *S*	Number of haplotypes, *R*	Haplotype diversity, *h*	Nucleotide diversity, Π	Average number of nucleotide differences, *k*
TEX	Texel	Southern North Sea	2000	38	21	13	0.784 (0.040)	0.0102 (0.0008)	4.706
BER	Bergen	Northern North Sea	2000	11	4	4	0.675 (0.063)	0.0023 (0.0005)	1.039
TRO	Trondheim	Norwegian Sea	2000	2	6	2	0.667 (0.204)	0.0087 (0.0027)	4.000
FIS	Fiskebäckskil	Skagerrak	2012	17	18	6	0.777 (0.039)	0.0015 (0.0009)	4.520
WIS	Llanfairfechan	Irish Sea	2006	24	18	8	0.624 (0.074)	0.0082 (0.0010)	3.749
Total				92	45	30	0.906 (0.012)	0.0121 (0.0003)	5.573

Table 3
List with sampling sites, number of individuals (n), and genetic diversity at the partial mitochondrial cyt *b* fragment (850 bp) of *Pomatoschistus minutus* (as reported in [8]). Values in brackets are standard deviations.

Code	Location	Sea	Longitude	Latitude	Sampling date	n	Number of polymorphic sites, *S*	Number of haplotypes, *R*	Haplotype diversity, *h*	Nucleotide diversity, π	Average number of nucleotide differences, *k*
BNS	Oostduinkerke	Southern North Sea	51°08′N	02°40′E	2006	12	9	5	0.864 (0.064)	0.0049 (0.0006)	4.1364
RNS	Renesse	Southern North Sea	51°44′N	03°47′E	2006	22	21	14	0.866 (0.066)	0.0053 (0.0005)	4.4805
FIS	Fiskebäckskil	Skagerrak	58°14′N	11°26′E	2006	17	24	12	0.919 (0.057)	0.0056 (0.0008)	4.7206
WIS	Llanfairfechan	Irish Sea	53°59′N	03°59′W	2006	30	20	13	0.685 (0.097)	0.0029 (0.0008)	2.4276
Total						81	44	35	0.876 (0.030)	0.0049 (0.0003)	4.1867

remainder of the samples included predominantly *G. rugiensoides* Huyse & Volckaert, 2002, a common fin parasite of *P. minutus*, and in a few exceptions *G. rugiensis* Gläser, 1974 and *G. branchialis* Huyse, Malmberg & Volckaert, 2004, which normally infect the common goby *Pomatoschistus microps* [11]. *G. gondae* was the most frequently encountered species across the sampling sites/times and was therefore selected for the co-phylogeographic analysis. Still only five goby populations were infected with *G. gondae*, with parasite abundance varying from a low of two at Trondheim up to 38 in Texel (Table 2).

3.2. Genetic diversity and population differentiation

We obtained *coxII* fragments for 92 out of the 107 sequenced *Gyrodactylus gondae* specimens (GenBank Accession Numbers KX519729 to KX519757). Alignment of the *G. gondae coxII* sequences (460 bp) did not pose any particular problem as there were no gaps, nonsense mutations or stop codons. Aligned sequences revealed 30 unique haplotypes, 18 of them singletons. A total of 45 variable sites were detected, of which 24 were parsimony-informative and seven nonsynonymous mutations (Table 2). Haplotype diversity was lowest in Llanfairfechan (0.62) and highest in Texel and Fiskebäckskil (0.78). However, Llanfairfechan included six *coxII* sequences from parasites collected from the same host individual; since these might be clonal individuals they might artificially lower haplotype diversity. Nucleotide diversity was lowest in Skagerrak (0.0015) and highest in Southern North Sea (0.0102) while haplotype diversity was lowest in the Irish Sea (0.624) and highest in Southern North Sea (0.784). Comparisonwise, nucleotide diversity of the sand goby populations (cyt *b*) was lowest in the Irish Sea (0.0029) and highest in Skagerrak (0.0056) and Southern North Sea (0.0053); haplotype diversity was lowest in the Irish Sea (0.685) and highest in Skagerrak (0.92) [8]. Haplotype diversity is higher in the sand goby populations but nucleotide diversity (π value) is generally higher in the parasite populations (the maximum π value in the parasite is almost double the value in the host) (Table 3). For both host and parasite populations the lowest haplotype diversity was found in Llanfairfechan, and nucleotide diversity was highest in the Southern North Sea (but equally high in Skagerrak for the host). Pair-wise population differentiation of *G. gondae* is consistently high but not always higher than for the sand goby host (Table 4). Pair-wise genetic differences between the four sites evaluated are significant. For comparison pair-wise differences between host populations are also presented (Table 4).

3.3. Network analysis

The network pattern of the parasite *G. gondae* is heterogeneous with evidence of a centrally located rare haplotype and two peripheral very common haplotypes in Texel and Llanfairfechan (Fig. 2a). Haplotypes from Bergen and Trondheim are clustered, in contrast to the other populations that are spread across the network. Fiskebäckskil is characterized by distinct haplotypes. We included an - for this study - adapted version of the network of the sand goby host [8] by selectively highlighting the haplotypes involved in this study (Fig. 2b). The network analysis for all cyt *b* haplotypes (850 bp) of the North Atlantic sand gobies displays a pattern with several highly frequent haplotypes instead of a star-like pattern with one central haplotype. The network shows one highly frequent haplotype that occurs in each sampled North Atlantic location, and a few other frequent haplotypes that are common in the northern Baltic Sea or in the southern North Sea. Many haplotypes are connected with single mutation steps to those highly frequent haplotypes, providing evidence of a recent expansion across the North Atlantic marine ecosystem (Fig. 2b).

4. Discussion

4.1. Infection pattern of the sand goby

Parasite prevalence across the distribution range of the sand goby host was highly variable with some populations not infected with *Gyrodactylus* parasites, especially those living in the brackish waters of the Baltic Sea and the estuary of the Guadalquivir. Zander and Reimer [24] also observed a lower parasite load in the brackish Baltic Sea due to a decreasing salinity from west to east, while a previous study by Huyse et al. [11] only found *Gyrodactylus rugiensis* on the common goby collected in Edesö (near Stockholm, Sweden), unlike other associated *Gyrodactylus* parasites that are closely related to *G. gondae*. While sampling bias can't be ruled out, these patterns might reflect the differential salinity tolerance of *Gyrodactylus* taxa. Some *Gyrodactylus* species have a wide salinity tolerance, like *G. ostendicus* [25], which lives both in the Mediterranean Vaccarès (~10 ppm) and Venetian lagoon (33 ppm),

Table 4
Pair-wise F_{ST} values of *Gyrodactylus gondae* (below diagonal) and *Pomatoschistus minutus* (above diagonal); significant values ($P < 0.05$) are listed in bold, with * significant after Bonferroni correction ($P < 0.05$).

Parasite/host	Renesse	Texel	Bergen	Trondheim	Fiskebäckskil	Llanfairfechan
Oostduinkerke	−0.049	No data	No data	No data	**0.133**	**0.330***
Renesse	–	No data	No data	No data	**0.093**	**0.225***
Texel	No data	–	No data	No data	No data	No data
Bergen	No data	**0.440***	–	No data	No data	No data
Trondheim	No data	**0.323**	**0.606**	–	No data	No data
Fiskebäckskil	No data	**0.324***	**0.556***	**0.451***	–	0.036
Llanfairfechan	No data	**0.203***	**0.590***	**0.480***	**0.251***	–

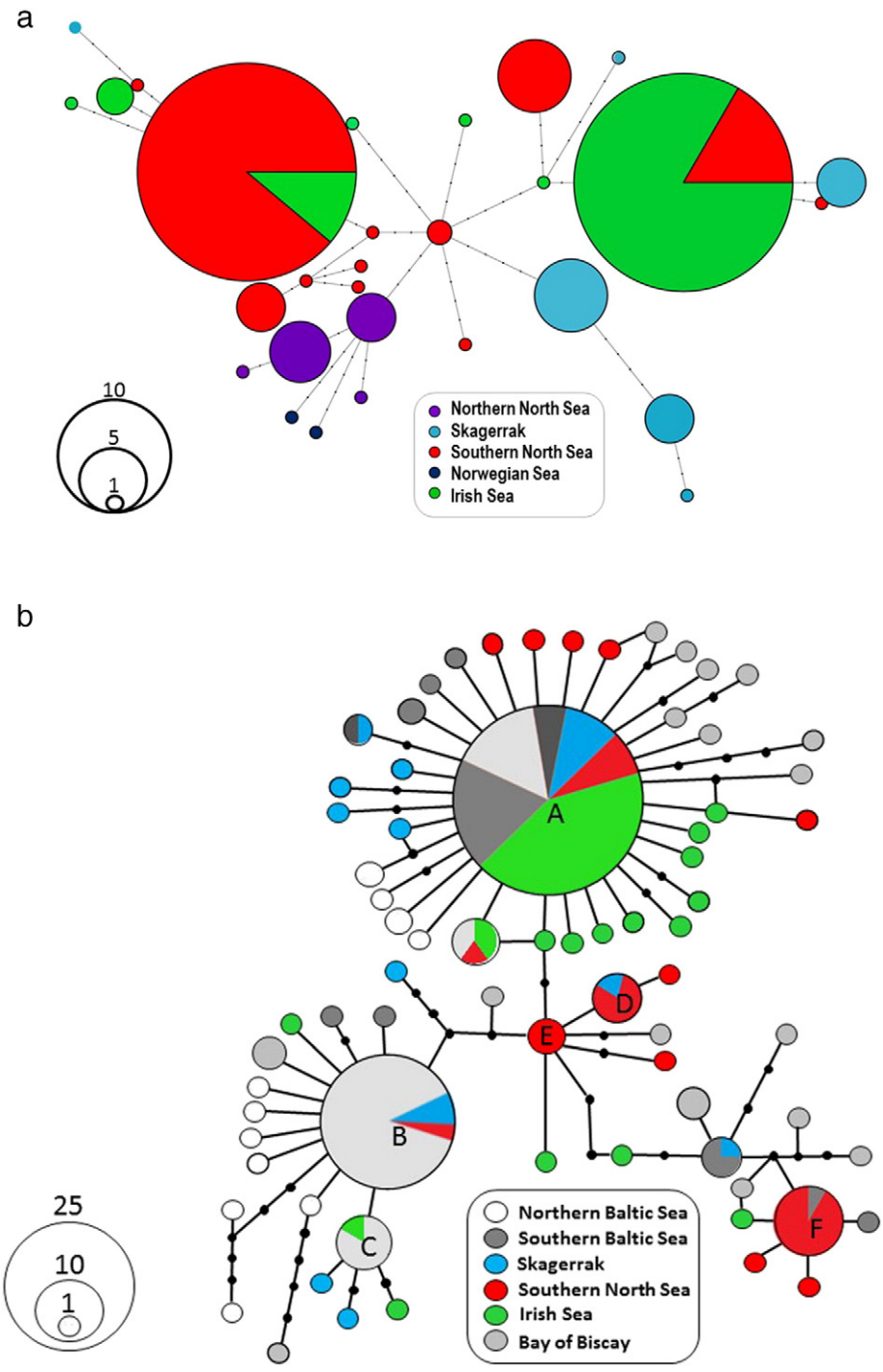

Fig. 2. Statistical parsimony network of (a) the *coxII* haplotypes of *Gyrodactylus gondae* and (b) the cyt *b* haplotypes on the North Atlantic group of *Pomatoschistus minutus* as modified from Larmuseau et al. [8].

but others are more restricted to either freshwater or marine ecosystems [11,12,26].

4.2. Co-evolution between sand goby and Gyrodactylus populations?

The phylogeographic network of the sand goby reveals the impact of Pleistocene glaciations with the presence of reduced population sizes, the retreat to a limited number of refugia and the postglacial expansion of populations to higher latitudes [8,27]. At first sight this network is quite dissimilar from the parasite network, which can at least partly be explained by the non-overlapping sampling localities for host and parasites. For example, goby specimens from the northern North Sea and Norwegian Sea are lacking, while no parasites were included from the Baltic and the Bay of Biscay due to parasite absence. Nevertheless, of the three main lineages found in the host network, two of them are also found in the parasite network (the third lineage is from the Bay of Biscay, which was not sampled for the parasite). These two dominant haplotypes successfully established in the southern North Sea and Irish Sea. The characteristic star-like pattern of expanding populations following glaciation is however less pronounced in the parasite network. This might be due to parasite characteristics and our sampling design. *Gyrodactylus* species display a combination of sexual and asexual reproduction [10]. The latter can result in the presence of clones on a single fish individual. Since we included parasites originating from the same

host fish in our analysis, the presence of clones cannot be excluded [28]. Selecting one parasite per fish would overcome this problem but it might also underestimate true population diversity and it would decrease our total parasite population size considerably. We therefore opted to include all parasite specimens in the analysis but take this potential confounding factor into account during data interpretation. Another possibility is that the missing haplotypes reflect extinction events. Extinctions and population collapses are a common feature of *Gyrodactylus* populations because of seasonal variation in population size [29]. Moreover, during asexual population growth, inbreeding can create bottleneck effects and increased genetic drift. This can be counteracted by sexual episodes unless mating occurs within the same clone [28].

Rannala & Michalakis [30] studied the effect of population-level processes on patterns of cospeciation using the coalescent theory of population genetics. They showed that the chance for identical host and parasite gene trees is small because of the confounding effect of ancestral polymorphism and lineage sorting in recently diverging lineages. Indeed, this is illustrated by a case of co-phylogeographic congruence between Galapagos mocking birds and their louse and mite ectoparasites [31]. In this particular island system chances for multiple introductions are rare, and with only a few founders per island coalescence is fast, minimizing the confounding effects of gene flow and retention of ancient polymorphism. Congruent host and parasite trees can also be obtained if host and parasite have a similar effective population size and a similar generation time [30]. This is clearly not the case for the current host-parasite system. The sand goby can reach very high population densities and lives up to 32 months [32]. *Gyrodactylus* on *P. minutus* on the other hand, appear to have a much lower effective population size judging by the low prevalence and infection intensities (see above). Hyperviviparity combined with advanced progenesis in *Gyrodactylus* result in a very short generation time, which can be as short as 24 h, depending on the species [10]. This should result in a faster accumulation of mutations in comparison with the host. These pronounced differences could therefore explain why host and parasite networks are not perfectly matching. However, these features should also result in a parasite network with a higher resolution compared to the host network. Indeed, according to Nieberding and Oliveiri [30], the organism with the smallest effective population size and the shortest generation time will be more informative on the common genealogical history (i.e. the magnifying glass principle).

4.3. Does the magnifying glass principle hold for this host-parasite system?

Our initial hypothesis, predicting a higher diversity and genetic differentiation in the parasite compared to the host, could not be confirmed. Neither haplotype diversity nor nucleotide diversity is consistently higher in the parasite. Population differentiation between parasite populations is strong but again not consistently higher than found in host populations. Some similar trends do emerge however, with host and parasite diversity being lowest in the Irish Sea, and highest in Skagerrak and the Southern North Sea. Such spatially structured diversity might be attributed to the impact of glacial cycling with its spatially structured population dynamics [27].

As stated above, this is rather unexpected, given the biology of *Gyrodactylus*. Gene flow is expected to be more restricted in the parasite than the host because *Gyrodactylus* spp., lacking a free-living (larval) stage, mainly depends on the adult host as vector for dispersal. They infect the host upon physical contact between fish and contact with the substrate [10]. Therefore pelagic juveniles are rarely infected with *Gyrodactylus* parasites [33]. However, pelagic fish larvae have a higher potential for dispersal than adults because of passive drift with coastal currents [34]. Gene flow was indeed much higher in the case of an egg laying monogenean parasite that was studied along the coast of the South and East China Sea [35]. Dispersal of the free-living larvae with ocean currents was put forward as an explanation for their homogeneous population structure. Also, given the relatively high mutation rate compared to the host, a similarly high genetic differentiation would be expected. Meinila et al. [36] estimated a nucleotide substitution rate of 13.7 to 20.3% per million years for *Gyrodactylus salaris* Malmberg, 1957 based on partial *coxI* gene sequences.

The relatively low diversity and population diversity found in *G. gondae* could be explained by the age of the host-parasite relationship. A previous study that tested for co-speciation between *Gyrodactylus* species and their goby hosts [12] suggested that *G. gondae* originated from an initial host transfer from *Gobiusculus flavescens* (Fabricius, 1779) onto *P. minutus* in the Late Pleistocene (refugia-mediated mixing). This suggests a relatively young association between *P. minutus* and *G. gondae* and might explain why the magnifying class hypothesis does not yet hold in this particular case. A similar scenario was found for the association between *Gyrodactylus truttae* Gläser, 1974 and its salmonid host, which also appeared to have arisen through a relative recent host-switching event (<60 ky BP). This might explain the low intraspecific diversity and hence lack of co-phylogeography, in contrast to the co-speciation between *Gyrodactylus teuchis* Lautraite, Blanc, Thiery, Daniel & Vigneulle, 1999 and its salmonid hosts [3]. *G. teuchis* has a much older association with its salmonid hosts and hence a higher sequence diversity and more time for co-evolution compared to *G. truttae*.

On the one hand, the direct life-cycle and the high host specificity enforce a tight relationship of *Gyrodactylus* and its host, promoting co-evolution [37,38]. On the other hand, *Gyrodactylus* species are very mobile and the ability to produce a viable deme from a single individual increases the chance for successful host switching. Speciation by host switching seems to have played an enhanced role in gyrodactylid speciation as many cases of ecological radiation onto distant-related hosts have been described [28,39]. Therefore host switching between closely related hosts that share the same habitat should be taken into account. *Pomatoschistus minutus* is closely related to lozano's goby *P. lozanoi* (de Buen, 1923) and both species may hybridise [40,41]. They occupy slightly different ecological niches, but since their breeding distributions overlap they must be regarded as truly sympatric. Both species share several *Gyrodactylus* spp. like *G. gondae*, *G. rugiensoides* and *G.* cf. *micropsi* [11]. The likelihood of strict co-evolution between host and parasite is expected to be smaller if the parasite infects closely related host species [37]. Of additional relevance is the recent host switch of *G. gondae* to the sand goby in the late Pleistocene [12], which is reflected in a lower haplotype diversity, similar to the switch of *G. trutta* to brown trout [3].

Finally, our study was handicapped by some technical constraints. The initial plan to cover all sites of sand goby sampled by Larmuseau et al. [8,9] had to be modified because some goby populations were not infected with *Gyrodactylus*. Therefore the sampling localities of the parasite and host do not completely overlap, which precludes a robust comparison. The overdispersed distribution of parasites has led to the possible inclusion of clonal haplotypes, which might influence diversity estimates. The low prevalence also leads to the inclusion of parasite specimens collected at other time periods, sometimes 12 years apart. We don't have any knowledge on the extent of temporal variation in *Gyrodactylus* but 12 years encompasses many parasite generations that might have experienced different demographic changes (and hence changes in haplotype frequencies), impacting the population genetic structure. In addition, the *coxII* mitochondrial fragment of 460 bp is rather short for a detailed evaluation of the dynamics of the haplotypes, including the network pattern; it is about half the length of the host sequences. Moreover, the cyt *b* and D-loop marker used for the host [8] are probably less conserved than *coxII* used in *Gyrodactylus* displaying higher levels of variation [42]. Therefore a larger database covering samples collected in time and space, and optimized genomic sampling of host and parasite would help to conclude whether or not *G. gondae* can be used as a magnifying glass for the host's history.

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