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Molecular faunistics of accidental infections of *Gyrodactylus* Nordmann, 1832 (Monogenea) parasitic on salmon *Salmo salar* L. and brown trout *Salmo trutta* L. in NW Russia

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Abstract Salmon *Salmo salar* L. and brown trout *S. trutta* L. juveniles were examined for the presence of accidental monogenean ectoparasitic species of *Gyrodactylus* Nordmann, 1832 in the Baltic and White Sea basins of Russian Karelia in order to estimate the frequency of host-switching attempts on an ecological timescale. To collect phylogeographical information and for exact species identification, the parasites were characterised by nuclear internal transcribed spacer sequences of rDNA (ITS) and, for some species, also by their mitochondrial DNA (CO1 gene) sequences. Four accidental *Gyrodactylus* species were observed on salmon and brown trout. A few specimens of *G. aphyae* Malmberg, 1957, the normal host of which is the Eurasian minnow *Phoxinus phoxinus* (L.), were observed on lake salmon from the Rivers Kurzhma (Lake Kuito, White Sea basin) and Vidlitsa (Lake Ladoga, Baltic basin). *G. lucii* Kulakovskaya, 1952, a parasite of the northern pike *Esox lucius* L., was observed on salmon in the Kurzhma. In the River Vidlitsa, two specimens of *G. papernai* Ergens & Bychowsky, 1967, normally on stone loach *Barbatula barbatula* (L.), were found on salmon. On anadromous White Sea salmon in the River Pulonga in Chupa Bay, a few salmon parr carried small colonies of *G. arcuatus* Bychowsky, 1933, which were shown to have originated from the local three-spined stickleback *Gasterosteus aculeatus* L. consumed as prey. No specimens of *Gyrodactylus salaris* Malmberg, 1957 were observed, although the Pulonga is the nearest salmon spawning river to the River Keret', which is heavily infected with introduced *G. salaris*. In the River Satulinoja, Lake Ladoga, three specimens of *G. lotae* Gusev, 1953, from burbot *Lota lota* (L.), were collected from a single brown trout *S. trutta*. All nonspecific gyrodactylid infections on salmonids were judged to be temporary, because only a few specimens were observed on each of the small number of infected fishes. The prevalence of endemic *G. salaris* was also low, only 1% (N_{fish} = 296) in Lake Onega and 0.7% (N_{fish} = 255) in Lake Ladoga, while brown trout specific *Gyrodactylus* species were not observed on any of the 429 trout examined from the Ladoga basin. The host-specific and unspecific burden of *Gyrodactylus* spp. on these 'glacial relict' populations of salmon and brown trout was very low, suggesting a generalised resistance against the co-evolved freshwater parasite community, or some kind of 'vaccination' effect. These hypotheses deserve further testing.

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Introduction

The salmon *Salmo salar* L. pathogen *Gyrodactylus salaris* Malmberg, 1957 is endemic in the Baltic Basin and pathogenic to salmon stocks outside of this area, especially along the Atlantic coast of Norway and on the White Sea. Reports of *G. salaris* in the literature extend to Portugal, but, so far, all observations of *G. salaris* in fish farms west from Denmark have proved to be misidentifications when tested by molecular means (Bakke et al., 2007). For example, *G. teuchis* Lautraite, Blanc, Thiery, Daniel & Vigneulle, 1999 was described as a new species only after molecular identification (Cunningham et al., 2001). In the important salmon spawning River Keret', on the White Sea in Russian Karelia, there was a serious epidemic among juvenile salmon caused by *G. salaris* (see Kudersky et al., 2003), the origin of which was confirmed by mitochondrial DNA to be in Lake Onega (Meinilä et al., 2004). *G. salaris* was also observed during 2001 in the landlocked salmon population in the River Pista, Lake Kuito, but a single sample collected in July was not enough to conclude whether the infection was pathogenic or benign (Meinilä et al., 2004). This infection was subsequently found to be permanent and possibly non-pathogenic, despite being introduced (Ziętara et al., 2006).

Therefore, it became obvious that more effort to monitor salmonid populations in Russian Karelia and the Kola Peninsula is needed. Not only was it necessary to test the hypothesis concerning the endemism of *G. salaris* in Lake Onega (Meinilä et al., 2004), but it is interesting to consider how frequently other freshwater species of *Gyrodactylus* Nordmann, 1832 may infect salmonids, and with what consequence. Host-switching was considered as the evolutionary explanation of the high species richness of *Gyrodactylus* (see Ziętara & Lumme, 2002).

The screening of *Gyrodactylus* species is challenging, knowing that this is a very species-rich genus. There are more than 409 formally described species (Harris et al., 2004), which is estimated to be only 2% of their true number (Bakke et al., 2002). Furthermore, the traditional species diagnostics based on the morphometrics of the haptor and, implicitly, on the host fish species, leads to many ambiguities and fails to reveal the real species richness. The recent results on gyrodactylids of North Sea gobies show that the number of species can be multiplied if molecular methods are utilised (Huyse & Volckaert, 2002; Huyse et al., 2004). Therefore, all parasite specimens collected from salmonids in the present study were checked using DNA sequencing.

Several *Gyrodactylus* species have been reported on Atlantic salmon as non-pathogenic transient infections (Harris et al., 2004; see also the *Gyrodactylus* database GyroDB at http://www.gyrodb.net/index.php. Seven of the 10 parasite species reported as being temporary on salmon already have their ITS rDNA region sequences deposited in GenBank. The sequences were determined from *Gyrodactylus* specimens collected from their primary hosts. The same is also true for parasites from brown trout *Salmo trutta* L. ITS sequences are available for five of the eight reported species. So far, two new species of parasites specific to salmonids have been found and confirmed only after molecular methods were utilised: *G. teuchis* (see Cunningham et al., 2001) and *G. derjavinoides* Malmberg, Collins, Cunningham & Jalali, 2007 [previously misidentified as *G. derjavini* Mikailov, 1975] (see Malmberg et al., 2007). Consequently, every *Gyrodactylus* species infection on salmonids, whether in farmed or wild fishes, warrants a molecular identification.

The present paper summarises the 'bycatch' results of expeditions conducted in 1999–2006 in relation to landlocked salmon and brown trout populations in Karelia, the Lakes Onega and Ladoga on the Baltic Basin and Lake Kuito (White Sea basin), plus anadromous salmon from the White Sea coast of Russia.

Materials and methods

Fish were caught in spawning and nursery rapids by electrofishing. Salmonids were always collected in a separate bucket, with bycatch fish species in another. When possible, the *Gyrodactylus* parasites on salmonids were inspected at the fishing site with a binocular stereomicroscope after the fish had been anaesthetised in a 0.005% solution of benzocain (ethyl p-aminobenzoate) in local water, which was freshly prepared from a stock solution of 10% benzocain in absolute ethanol. Infected fish were then killed by destroying their brain with a needle and stored directly in 96% ethanol. However, during most

expeditions immediate inspection of the parasites was not possible, and so clipped fins, and later whole fishes, were stored directly in 96% ethanol and subsequently inspected in the laboratory. In this paper, only the results of those fish completely examined during 2004–2006 are presented. Previously, about 1,700 pairs of pectoral fins from White Sea and Barents Sea salmon had been examined along the coasts, without finding a single *Gyrodactylus* other than those in the River Keret'. However, the examination of pectoral and dorsal fins only is not adequate for a very low density of parasites.

For morphological identification, the haptors of the parasites were dissected, digested slightly by proteinase K and preserved in Malmberg's fixative on microscope slides (Malmberg, 1970). The rest of the body was used for molecular analysis.

Localities and accession numbers of the DNA sequences for all of the taxa presented in this paper are listed in Table 1.

Molecular species recognition and analysis of the ITS

For all PCR procedures, single ethanol-preserved worms without haptors were digested in 10 µl of solution containing 1× Dynazyme PCR-buffer, 0.5% Tween 20, 0.5% Igepal and 0.6 µg of Proteinase K. The worms were spun to the bottom of 200 µl Eppendorf vials and incubated at 65°C for 25 min, and then denatured at 94°C for 10 minutes. Enzymatic digestion was ended at 4°C.

The whole of ITS1 and ITS2 (internal transcribed spacer) regions of the nuclear ribosomal DNA (Ziętara et al., 2000) was amplified by primers *ITS1F* 5′-GTT TCC GTA GGT GAA CCT and *ITS2R* 5′-GGT AAT CAC GCT TGA ATC and sequenced as described previously (Ziętara & Lumme, 2002).

Mitochondrial cytochrome oxidase 1 (CO1)

The partial mitochondrial CO1 gene was processed as in Ziętara et al. (2006). FCox6 (5′-TTG GAT CAT AAG CGC ATY GGT AT-3′) and 16SR (5′-CAT TTA ATC ATG ATG CAA AAG G-3′) primers were used to amplify 1598 bp fragment of mitochondrial DNA of *G. lucii* Kulakovskaya, 1952. Three internal primers LA1 (5′-TAA TAG GGG GGT TTG GTA A-3′), FCox7 (5′-TTT TCA ATA GGT ATG GAC GT-3′) and RCox6 (5′-AAA TGC TGG AAT AAC ACT GG-3′) were used for sequencing.

For the *G. arcuatus* Bychowsky, 1933 mitochondrial CO1 marker, the PCR primers were: GarcL (5′-TAT TAT TAC CTT CAA TGG TGT TAG-3′) and GarcH (5′-CAT AAT GAA AAT GTG CTA CCA CAA-3′), producing a segment of 777 base pairs. Three internal primers were designed for sequencing: FCox1 (5′-TGT TAG TGA TAC CCC CAT AG-3′), RCox1 (5′-TTG CTT AAC TTT GAT CTT CT-3′) and RCox3 (5′-ATA AAC CTCA GGA TGT CCA A-3′).

GenBank comparisons

The DNA sequences were compared with sequences already in GenBank by a BLAST search, and with several new sequences, obtained from geographically distant localities for *G. arcuatus* and *G. lucii*. The new ITS sequences are given in Table 1. Sequences from GenBank are as follows: ITS – *G. alexgusevi* Ziętara & Lumme, 2003 (AY061979), *G. aphyae* Malmberg, 1957 (AF484527, AF484528, Ziętara & Lumme, 2002), *G. branchicus* Malmberg, 1964 (AY061977, Ziętara & Lumme, 2003), *G. lotae* Gusev, 1953 (AJ407884, Matějusová et al., 2001; AY061978, Ziętara & Lumme, 2003), *G. lucii* (AF484539, Ziętara & Lumme, 2002), *G. papernai* Ergens & Bychowsky, 1967 (AJ407877 + AJ407925, Matějusová et al., 2001, Přikrylová et al., 2007, *G. pseudonemachili* Ergens & Bychowsky, 1967 (AJ567674, Matějusová et al., 2003, Přikrylová et al., 2007), *G. papernai* (AF484533, published as *G. jiroveci* by Ziętara & Lumme, 2002; the name is changed here following Přikrylová et al., 2007), *G. jiroveci* (AM502860, Přikrylová et al., 2007), *G. gasterostei* Gläser, 1974 (AJ001841, Cable et al., 1999; AF328867, Ziętara et al., 2002), and CO1 – *G. salaris* (DQ517533, Ziętara et al., 2006).

The sequence alignments were made by ClustalW, and the gaps in ITS alignments were hand-edited to be most parsimonious. The phylogenetic trees were constructed by the Neighbor Joining method based on Kimura's two-parameter distances, as implemented in MEGA3.1 program package (Kumar et al., 2004).

Results

Because of the exploratory nature of this study, we will present here the faunistic results and taxonomic observations based on molecular analysis of each parasite, combined with a comparison with other

Table 1 Localities and accession numbers of the DNA sequences of *Gyrodactylus* species presented in this paper

Gyrodactylus species	Host	Locality	Collection date	ITS ac. No.	No.	CO1 ac. No.	No.
G. aphyae Malmberg, 1957	*Phoxinus phoxinus* (L.)	R. Oulankajoki, Oulanka, Fi 66°21′N, 29°19′E	May 2000	EF446716	1	–	–
		R. Salnica, Kola Peninsula, Ru 66°21′N, 35°43′E	Jul 2000	EF446717	1	–	–
		Bothnian Bay, Oulu, Fi 65°02′N, 25°24′E	Jul 2000	EF446718	1	–	–
		R. Bolshie Kozli, Ru 65°34′N, 39°56′E	Jun 2003	EF446719	2	–	–
		Bothnian Bay, Lumijoki, Fi 64°55′N, 25°05′E	May 2005	Same as AF484527	1	–	–
		R. Vidlitsa, Ru	Jul 2006	EF446720	1	–	–
		60°76′N, 32°30′E		EF446721	1	–	–
	Salmo salar L.	R. Kurzhma, Ru 65°12′N, 30°09′E	Jul 2005	EF446722	2	–	–
		R. Vidlitsa, Ru 60°76′N, 32°30′E	Jul 2006	EF446723	1	–	–
G. arcuatus Bychowsky, 1933	*Gasterosteus aculeatus* L.	Bothnian Bay, Lumijoki, Fi 64°55′N, 25°05′E	Aug 2004	Same as AF328865	1	DQ078701-DQ078719	34
		R. Pulonga, Ru	Jul 2005	–	–	EF446742	6
		66°18′N, 33°15′E		EF446724	1	EF446743	1
				–	–	EF446744	3
				EF446724	1	EF446745	4
	Salmo salar L.	R. Tenojoki, Boratbokca 70°04′N, 27°45′E	Sep 2004	EF495225	1	–	–
		R. Pulonga, Ru	Jul 2005	EF446725	1	EF446746	2
		66°18′N, 33°15′E		EF446725	1	EF446747	6
	Spinachia spinachia (L.)	Lyngen, Skibotn, No	Aug 2006	EF446726	2	EF446748	2
		69°23′N, 20°16′E		EF446727	1	EF446748	1
G. gasterostei Gläser, 1974*	*Gasterosteus aculeatus* L.	R. Endrick, UK 56°55′N, 04°22′W	2003	EF446728	1	–	–

Table 1 continued

Gyrodactylus species	Host	Locality	Collection date	ITS ac. No.	No.	CO1 ac. No.	No.
G. lotae Gusev, 1953	*Lota lota* (L.)	L. Konchozero, Ru, 62°07′N, 34°00′E	Jul 2005	EF446730	4	–	–
	Salmo trutta L.	R. Satulinoja, Ru	Jul 2006	EF446731	1	–	–
		61°28′N, 31°39′E		EF446732	2	–	–
G. lucii Kulakovskaya, 1952	*Esox lucius* L.	R. Jaziewianka, Jaziewo, Pl 53°40′N, 22°54′E	Aug 2004	DQ993187	1	–	–
		R. Jaziewianka, Dębowo, Pl 53°36′N, 22°56′E	Jun 2005	EF446733	2	EF446749	2
		R. Chirko-Kem', Ru 64°12′N, 32°23′E	Jul 2005	EF446734	2	EF446750	2
		R. Merenoja, Oulanka, Fi 66°21′N, 29°21′E	Aug 2005	EF446735	2	EF446751	2
		R. Kiekeröjoki, Fi 66°24′N, 29°14′E	Aug 2005	EF446736	2	EF446752	2
		L. Kuorinki, Fi 66°01′N, 28°28′E	Oct 2005	EF446737	2	EF446753	2
		L. Kuohkimajärvi 69°03′N, 20°33′E	Sep 2004	EF113105	5	–	–
	Rutilus rutilus (L.)	R. Einojoki, Ru 61°20′N, 32°09′E	Jul 2006	EF446738	2	EF446754	2
		R. Hiitolanjoki, Ru	Jul 2006	EF446739	1	EF446755	1
		61°10′N, 29°51′		EF446740	2	EF446756	2
	Salmo salar L.	R. Kurzhma, Ru 65°12′N, 30°09′E	Jul 2005	EF446741	1	EF446757	1
G. papernai Ergens & Bychowsky, 1967	*Salmo salar* L.	R. Vidlitsa, Ru 60°76′N, 32°30′E	Jul 2006	EF446729	2	–	–

* Sample kindly provided by Hannu Mäkinen

relevant species, and a short commentary on the significance of the observations.

Gyrodactylus aphyae on salmon in basins of Lakes Kuito and Ladoga

In the River Kurzhma (Lake Kuito, Karelian Republic, Russia), 38 juveniles of salmon were collected in 2005. In this locality, the bycatch consisted of bullhead *Cottus gobio* L., grayling *Thymallus thymallus* (L.) (one small individual without *Gyrodactylus*), and Eurasian minnow *Phoxinus phoxinus* (L.). The River Kurzhma salmon were sampled by us for the first time in 1999, but at this time and during the next visit in 2001 the fish were examined for *Gyrodactylus* only superficially. There was no *a priori* expectation of a *Gyrodactylus* infection in Lake Kuito, but *G. salaris* was observed in the River Pista (also draining into Lake Kuito) in 2001 (Meinilä et al., 2004; Ziętara et al., 2006). In 2005, *Gyrodactylus* spp. were found on six juvenile salmon. Four fish harboured only a single *Gyrodactylus* specimen, one fish had two parasites and one had eight. The parasites on four fish were identified as *G. salaris* (accession number of ITS rDNA EF117880; mtDNA DQ517533), as reported by Ziętara et al. (2006). One salmon parr in the Kurzhma carried two specimens of *G. aphyae*, normally a parasite of the Eurasian minnow *P. phoxinus.*

In the River Vidlitsa (Lake Ladoga, Karelian Republic, Russia), 44 juvenile lake salmon were collected. The bycatch consisted of chub *Alburnoides bipunctatus* (Bloch), stone loach *Barbatula barbatula*, bullhead *Cottus gobio* L. and Eurasian minnow *P. phoxinus*. No *G. salaris* was observed, but two accidental parasite species were found, including *G. aphyae*. The *G. aphyae* specimens on salmon were identified both morphologically and by sequencing the ITS region. In Fig. 1, the *G. aphyae* parasites found on Kurzhma and Vidlitsa salmon are embedded in the ITS phylogeny of representative sequences of parasites on their normal host and, as an outgroup, the very closely related *G. gasterostei* Gläser, 1974. The salmon parasites were in both cases identical with the nearest sequenced neighbours on the normal host, *P. phoxinus*, and therefore judged to be temporary visitors.

Interestingly, the ITS sequences of *G. aphyae* from the River Vidlitsa in the Baltic Basin and from several localities in the White Sea basin differed by only two nucleotides, (0.08% estimated by Kimura's two parameter distance × 100, see Fig. 1). The two identical sequences from the Bothnian Bay population on *Phoxinus* differed from the Vidlitsa sequences

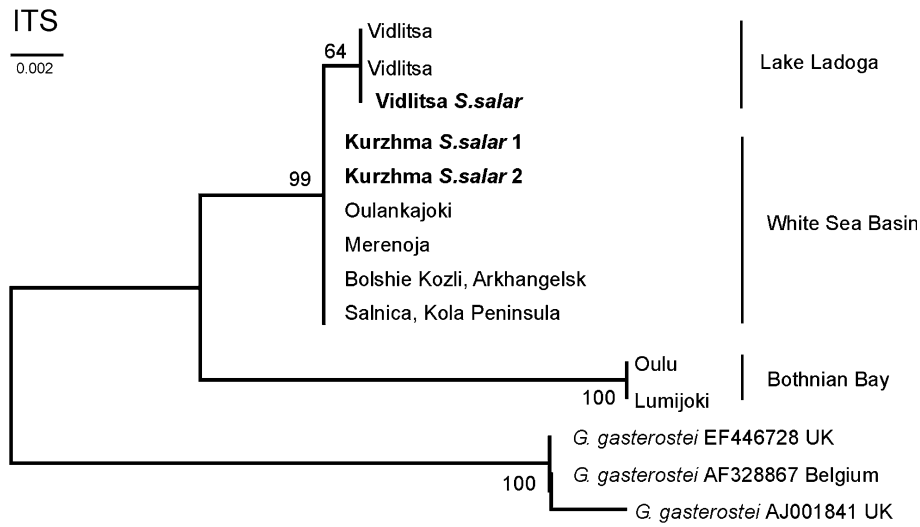

Fig. 1 Phylogenetic comparison of the ITS sequence of *Gyrodactylus aphya*e collected on salmon *Salmo salar* with *G. aphyae* from Eurasian minnow *Phoxinus phoxinus* in the White Sea and Baltic basins. The species name is not given in the tree, only locality and the unusual host. As an outgroup, we use the closely related *G. gasterostei* (from *Gasterosteus aculaetus*) in Belgium and the UK. Kimura's two parameter distance and Neighbor Joining tree, with bootstrap percentages on the nodes (MEGA3, Kumar et al., 2004)

by 1.3%, which was relatively large when compared to the distance of 2.6% between the Bothnian Bay *G. aphyae* and its nearest known relative, *G. gasterostei*, a parasite on the three-spined stickeback *Gasterosteus aculeatus* L., reported from the UK (Cable et al., 1999) and Belgium (Ziętara et al., 2002).

Gyrodactylus lucii on salmon from the River Kurzhma, Lake Kuito

In Fig. 2 the ITS of *G. lucii* from salmon in the River Kurzhma (bycatch reported above) was compared with pike parasites from the Rivers Oulanka,

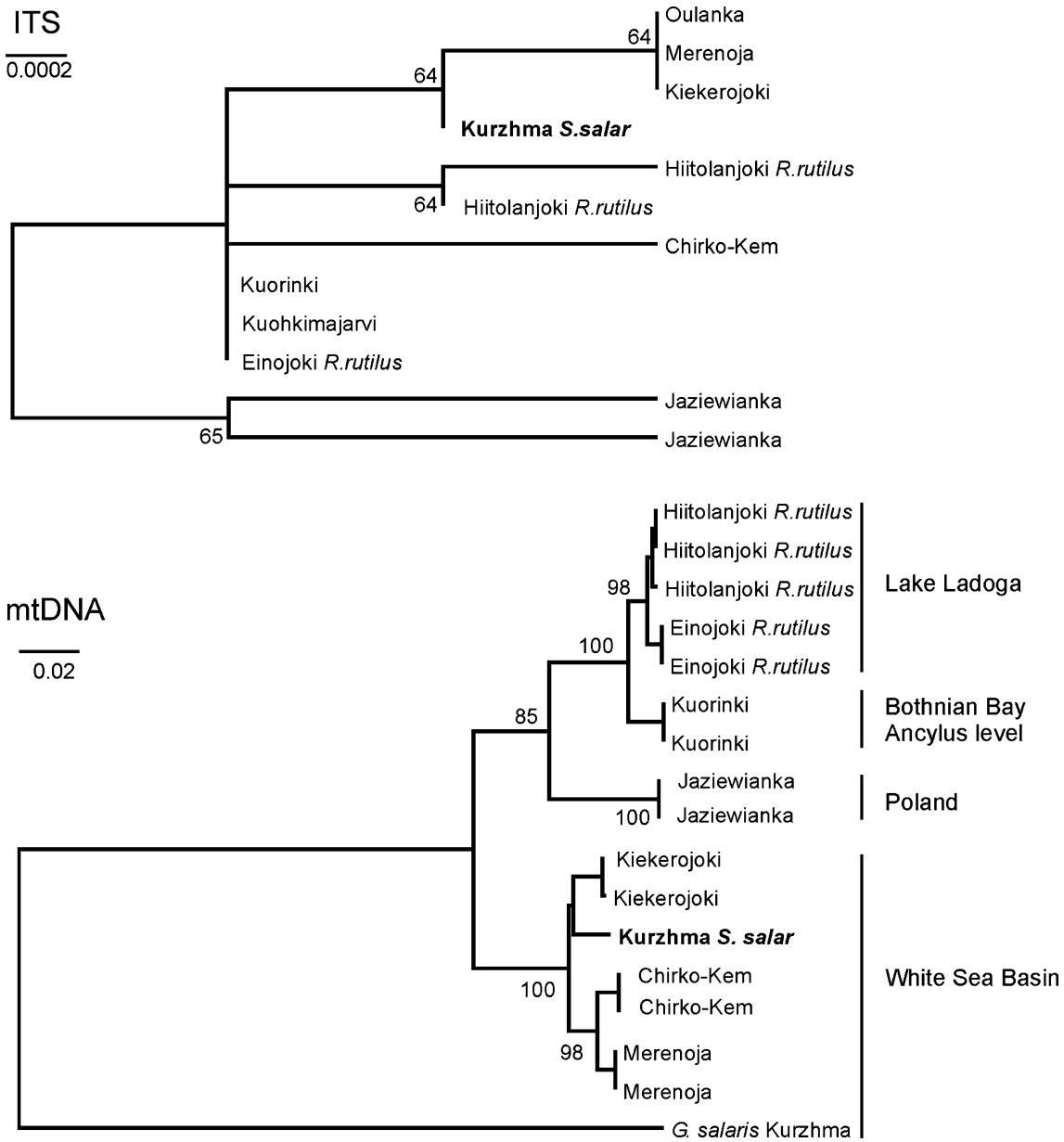

Fig. 2 The phylogenetic hypotheses for *Gyrodactylus lucii* based on its ITS and mtDNA, depicting the position of parasites found on *Salmo salar* in the River Kurzhma among other isolates of this species, some of them from roach *Rutilus rutilus* and most from the normal host *Esox lucius*. In the ITS tree, the salmon parasite was intermediate (heterozygous Y in site 132) between the Baltic (T) and White Sea basin (C) types. The mitochondrial tree is rooted with the sequence of *G. salaris* from the Kurzhma (Ziętara et al., 2006)

Merenoja, Kiekeröjoki and Chirko-Kem' from the White Sea basin plus Lakes Kuohkimajärvi and Kuorinki in Finland and the River Jaziewianka in Poland from the Baltic Basin. Sequences of *G. lucii* were also obtained from several parasites on roach *Rutilus rutilus* (L.) from Lake Ladoga (Baltic Basin) in the Rivers Einojoki and Hiitolanjoki. The differences between the different clones were very small, and the ITS contained only six variable sites. However, it is known that even a few nucleotide substitutions either homozygous or heterozygous can be a very definitive indication of the divergent phylogenetic history of *Gyrodactylus* populations (see e.g. Lindenstrøm et al., 2003, for the significance of three nucleotide difference in the ITS of *G. salaris*).

For *G. lucii*, we also sequenced a 1,598 bp segment of the mtDNA, which is presented in the lower panel in Fig. 2. The comparison of ITS data with the mitchondrial phylogeny leads to interesting conclusions. The mitochondrial phylogenetic hypothesis clearly separates two clades of *G. lucii*, one in the Baltic (including Poland) and the other in the White Sea basin. The mean genetic distance between the two mtDNA clades was 6.7% (K2P × 100), which is more than twice the distance between *G. salaris* phylogroups on grayling *Thymallus thymallus* (L.) and salmon in the two sea basins (Meinilä et al., 2004), and also twice the maximum distance of *G. arcuatus* phylogroups in the Baltic and White Sea basins presented in this paper (Fig. 3).

G. lucii on the salmon in the Kurzhma was a hybrid between the widely distributed Baltic clade and White Sea clade haplotypes from northern pike. The samples from the Baltic basin extend from the extreme point of Lake Kuohkimajärvi at the bifurcation of the Atlantic and Baltic watersheds on the border junction of Norway, Sweden and Finland, to the River Einojoki which drains to Lake Ladoga in the Karelian Republic. These populations all had an identical ITS. The White Sea type of *G. lucii* ITS was found upstream in the Koutajoki River system (Oulanka, Merenoja and Kiekeröjoki). These Baltic and White Sea ITS types differed by only one nucleotide at site 132 of ITS1, which was either C or T, but, in the parasite on Kurzhma salmon, a heterozygous Y. This observation supports the hypothesis that the secondary contact zone of the Baltic and White Sea basin at the latitude of 65°N is 'leaky' due to postglacial upwater sluicing, which turned some of the originally Baltic waters to the east. Another, equally possible, explanation may be the fish and parasite trafficking on the Finnish side of the border, where the watershed is located.

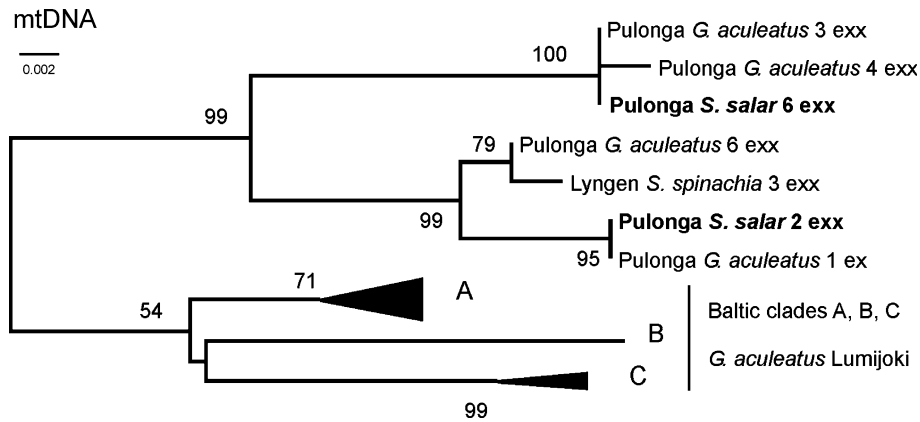

Fig. 3 Mitochondrial phylogenetic hypothesis of selected strains of *Gyrodactylus arcuatus*. The locality and host fish are indicated, as are the number of specimens of each haplotype. The three Baltic clades A, B and C represent 19 haplotypes found in 34 parasites collected from seven adult three-spined sticklebacks *Gasterosteus aculeatus* in one locality in the Bothnian Bay in Lumijoki Varjakka, near Oulu, Finland. The parasites sampled from the River Pulonga, either on salmon or three-spined stickleback, were divided into two well-supported mtDNA clades, which were randomly mixed among the two host species and also among stickleback individuals. The specimens obtained from the fifteen-spined stickleback *Spinachia spinachia* from Lyngen Fjord, Barents Sea, Norway showed that the mtDNA clades were not strictly local or host-specific. The tree is based on 777 bp long alignment, Kimura's two parameter distance, NJ and bootstrapping 500 times

Previously, it has been suggested that the mitochondrial haplotype of *G. salaris* clones in Lake Kuito had an origin in Finnish rainbow trout *Oncorhynchus mykiss* (Walbaum) farms (Ziętara et al., 2006).

Gyrodactylus arcuatus on anadromous salmon in the River Pulonga, White Sea

In the River Pulonga (Karelian Republic, Russia), 62 juveniles of anadromous White Sea salmon *Salmo salar* were collected. The bycatch consisted of three-spined stickleback *Gasterosteus aculeatus*, burbot *Lota lota*, nine-spined stickleback *Pungitius pungitius* (L.) and flounder *Platichthys flesus* (L.). Three salmon in the Pulonga were infected by *Gyrodactylus*, one fish by seven specimens and the other two by two specimens each. Because the Pulonga is in Chupa Bay, almost opposite to the River Keret', which is infected with introduced *G. salaris*, the first impression in the field was that the infection was *G. salaris*. However, the parasites were subsequently identified as *G. arcuatus*. In the Pulonga rapid, and in the stomachs of the juvenile salmon, there were many small first-summer sticklebacks from which the salmon infections were derived. We analysed 777 bp long fragments of the mitochondrial CO1 gene of the parasites and compared them to previously known sequences of *G. arcuatus* from the Baltic Basin. The mtDNA haplotypes found on salmon in the Pulonga were not identical to the Baltic reference sequences (Fig. 3). Therefore, we sequenced the CO1 of *G. arcuatus*, also from the small juvenile sticklebacks in the same rapid as the salmon juveniles, and thus confirmed that *G. arcuatus* was shared by the prey and the predator. It is noteworthy that the parasites had most probably multiplied on salmon, forming small colonies, because the parasites on each fish were of the same mtDNA clone, out of four types available on local sticklebacks (Fig. 3).

Comparison of the *G. arcuatus* sequence data obtained from seven adult three-spined sticklebacks in the Baltic Sea basin show that the two mtDNA clades in the Pulonga were clearly divergent from the three Baltic clades (99% bootstrap support). The ITS sequences of the Baltic and White Sea *G. arcuatus* were very similar, and they were also identical with more exotic specimens reported by Huyse et al. (2006), i.e. *G. arcuatus* on 'ghiozzetto di laguna' *Knipowitschia panizzae* (Verga) at Venice, Italy, on the nine-spined stickleback *Pungitius pungitius* at Bergen, Norway and Edesö, Sweden, on the two-spotted goby *Gobiusculus flavescens* (Fabricius) at Trondheim, Norway, and on the fifteen-spined stickleback *Spinachia spinachia* (L.) at Skibotn, Lyngen Fjord, Norway, which was also added to the mtDNA tree in Fig. 3.

In the Baltic Sea, the three clades, A, B and C in the mtDNA tree in Fig. 3, differed by a maximum 2.1%, and the two clades in White Sea by 1.7%. The distance between Baltic Clade B and the White Sea clades was 2.9%, i.e. less than between the corresponding clades of the freshwater parasite *G. lucii* presented above.

G. arcuatus has previously been confirmed once on salmon in the River Tenojoki (Tana) by sequencing the ITS (EF495225).

Gyrodactylus papernai on salmon in the Ladoga Basin

One salmon parr in the River Vidlitsa, Lake Ladoga (bycatch reported above) harboured two parasites belonging to a species group normally found on stone loach *Barbatula barbatula*. The ITS tree including them (Fig. 4) also contains four different sequences, all from *B. barbatula*, deposited in the GenBank with species names *G. jiroveci* Ergens & Bychowsky, 1967, *G. papernai* Ergens & Bychowsky, 1967 and *G. pseudonemachili* Ergens & Bychowsky, 1967. One of them (River Kiiminkijoki, Finland, Baltic basin) was nearly identical with the Ladoga sequences. Three other sequences were from the River Vlara, Danube Basin, Czech Republic (AJ567674, Matějusová et al., 2003; AJ40877 + AJ407925, Matějusová et al., 2001; and AM502860, Přikrylová et al., 2007).

The Kimura two parameter distances (×100) between the three Czech species were 4.7–7.7%, calculated on the basis of 997 bp of the fully alignable segments of the ITS1 and ITS2. The genetic distance between the Baltic parasites on stone loach in the Kiiminkijoki and on salmon in the Vidlitsa was 0.1%, and they differed from *G. papernai* from the Czech Republic by only 0.6–0.7%. Therefore, we decided that the parasites in the Vidlitsa and

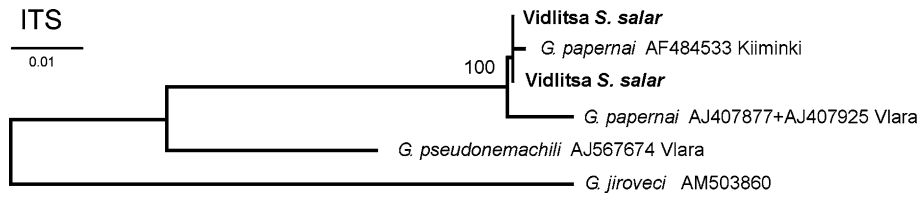

Fig. 4 An ITS phylogenetic tree of *Gyrodactylus papernai* found on salmon (*Salmo salar*) fron the River Vidlitsa compared with other parasites on stone loach *Barbatula barbatula*, which are deposited in GenBank. Neighbor joining tree, K2P distance, and the complete deletion option was chosen because the 5.8S rDNA is missing in *G. papernai* (AJ407877 + AJ407925)

Kiiminki belong to the species *G. papernai*. The sequence AF484533 was originally deposited in GenBank as *G. jiroveci*, but the identification was considered as problematical in a taxonomic note (Ziętara & Lumme, 2002).

The taxonomy of three of the parasites on stone loach was solved by this decision, but the ITS of the species *G. pavlovskyi* Ergens & Bychowsky, 1967 is still missing (Přikrylová et al., 2007). Until the latter species is redescribed with its ITS sequence from the North Sea basin in Czech Republic, where it was originally described, the question whether the specimens from Kiiminkijoki and Vidlitsa really are *G. papernai* or *G. pavlovskyi* remains open.

Gyrodactylus lotae on brown trout in the Ladoga Basin

In the River Satulinoja (Lake Ladoga, Karelian Republic, Russia), 24 juvenile brown trout *Salmo trutta* were collected together with a bycatch which consisted of northern pike *Esox lucius* L., ruffe *Gymnocephalus cernuus* (L.), burbot *Lota lota* and lampreys *Lampetra* sp. Three *Gyrodactylus lotae* specimens were found on a single brown trout. The ITS sequences were compared with samples collected from burbot from Lake Konchozero (Onega, i.e. the Baltic basin) and the River Oulanka (the White Sea basin) (Fig. 5). No genetic difference was observed in *G. lotae* between the White Sea and Baltic Sea basins. From *G. lotae*, another species *G. alexgusevi* Ziętara & Lumme, 2003 was separated recently. In the original species description, samples of *G. alexgusevi* were collected from the Baltic Sea basin, and the sequenced specimens of *G. lotae* originated from the White Sea basin (and from the River Morava, Czech Republic; Matějusová et al., 2001). Morphological inspection of the specimen in Malmberg's collection in Stockholm suggested that both species were present in the Baltic Basin. The three parasites on brown trout in the Satulinoja thus confirm that *G. lotae* occurs in the Baltic basin, although they were found on a transient host.

Discussion

The host-specificity of *Gyrodactylus* spp. is frequently tested in laboratory experiments (Bakke et al., 2002, 2007; Lindenstrøm et al., 2003; King & Cable, 2007). Among the faunistic field reports,

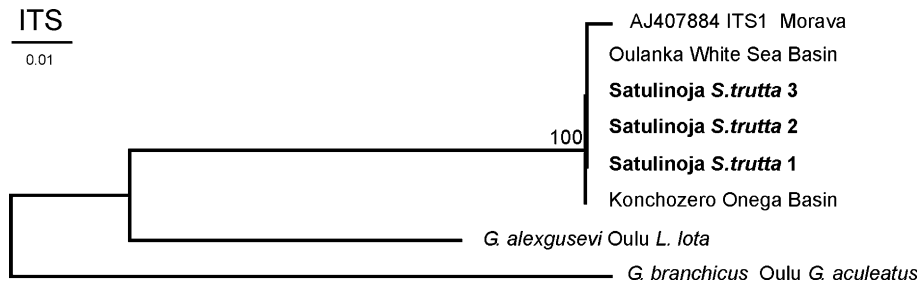

Fig. 5 The *Gyrodactylus lotae* ITS tree, confirming that the three parasites found on brown trout *Salmo trutta* were *G. lotae*, and not *G. alexgusevi*, which is morphologically very similar. The tree is based on the ITS1 and 5.8S rDNA, because the ITS2 was not available for the Moravian isolate. The outgroup *G. branchicus* is a parasite of *Gasterosteus aculeatus* from the Bothnian Bay, Finland

there are hundreds of records of *Gyrodactylus* on unusual hosts, and some *Gyrodactylus* species have been historically characterised as generalists. However, many of these reports should probably be rejected by modern standards. Occasional host-switching in aquaria or in a bucket containing several host species is not ecologically relevant. An inadequate species identification may also lead to reports of liberal generalists (such as the first species described, *G. elegans* Nordman, 1832, as explained by Harris et al., 2004). In this study, we tried to maintain the highest possible standards, by isolating the host fish species immediately from the bycatch and by identifying the specimens by molecular sequencing. The results show that the *Gyrodactylus* species in waters suitable for salmonids are indeed strict specialists, that transfers to atypical hosts are extremely rare and that, most probably, the latter do not lead to any successful colonisations. Thus, conditions for host-switching in the evolutionary sense need to be something very specific.

In this connection, the rich *Gyrodactylus* fauna observed on the introduced salmonid *Oncorhynchus mykiss*, the rainbow trout, deserves specific attention. GyroDb listed (June 2007) 12 species on rainbow trout, and recent reports under the names *G. derjavini* – *G. derjavinoides* (see Malmberg et al., 2007) and *G. brachymystacis* Ergens, 1978 (see You et al., 2006) were not yet registered. In a PCR-RFLP study on Polish fish farms, six different *Gyrodactylus* strains were observed (Rokicka et al., 2007). It seems obvious that this non-native salmonid offers a non-resistant 'training ground' for the local parasites, which have no success among the native fish fauna.

The *Gyrodactylus* species reported to specifically infect salmon or trout in Europe all belong to the 'wageneri species group' of the subgenus *Limnonephrotus* Malmberg, 1970. This species group is characterised by frequent (in evolutionary time) host-switches, apparently leading to speciation (Ziętara & Lumme, 2002). It was demonstrated recently that switching of *G. salaris* from rainbow trout to salmon in Lake Kuito coincided with a genetic reorganisation (Ziętara et al., 2006). Thus, switching may be rare, and it is not possible to predict when it happens.

Two of the accidental parasites observed here on salmonids are members of the 'wageneri group'. *G. lucii* has not previously been reported on salmon. The nearest relative of *G. lucii* is the brown trout parasite *G. derjavinoides* (previously misidentified as *G. derjavini*) (see Malmberg et al., 2007). A close relative of the minnow parasite *G. aphyae* is *G. gasterostei*, a parasite of three-spine sticklebacks (Fig. 1). Thus, the 'capacity' for switching exists in these evolutionary lineages. However, these species have always lived side-by-side with native salmonids without switching.

The species of the 'nemachili group' on stone loach *Barbatula barbatula* also belong to subgenus *Limnonephrotus*, but although this clade has speciated (Fig. 4) on its normal host, *B. barbatula*, no close relatives are found on other fish species. The species of the 'nemachili group' have never previously been reported on salmon or trout.

G. lotae is a freshwater member of subgenus *Paranephrotus* Malmberg, 1970, which contains mainly marine forms, and it is an example of a conservative host-specific parasite. The clade has followed the gadid *Lota lota* to freshwaters tens of millions of years ago and has speciated, at least to the extent of *G. lotae* and *G. alexgusevi*, on the same host.

The list of *Gyrodactylus* species reported as 'visitors' on salmon is already long: *G. arcuatus, G. phoxini* Malmberg, 1957, *G. lenoki* Gusev, 1953, *G. aphyae, G. truttae* Gläser, 1974, *G. salmonis* Yin & Sproston, 1948, *G. derjavinoides, G. caledoniensis* Shinn, Sommerville & Gibson, 1995 and *G. teuchis* (*Gyrodactylus* database, GyroDb, at http://www.gyrodb.net/). Here we have confirmed using molecules two of the listed species (*G. arcuatus* and *G. aphyae*) and added two others (*G. lucii* and *G. papernai*).

The GyroDb list of *Gyrodactylus* parasites on brown trout consists of *G. salaris*, *G. macronychus* Malmberg, 1957, *G. truttae*, *G. salmonis*, *G. colemanensis* Mitzelle & Kritsky, 1967, *G. derjavini* Mikailov, 1975, *G. derjavinoides*, *G. caledoniensis* and *G. teuchis*. An undescribed species from Arctic bullhead *Cottus poecilopus* Heckel (*G.* cf. *hrabei* of Malmberg (1973); see Hansen et al., 2003) has also been reported. In this study, we did not find any of the above parasite species, not even those specific to brown trout. In the summer of 2006, we examined 426 trout in the Lake Ladoga basin, and *G. lotae* was the only *Gyrodactylus* species found, thus adding one name to the list of temporary trout parasites.

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