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Host switching *via* rare sex: *Oncorhynchus mykiss* as a vector in fusion of three *Gyrodactylus salaris* genomes into a sexual-clonal population on a landlocked *Salmo salar* (Monogenea, Platyhelminthes)

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Abstract: The monogenean ectoparasite *Gyrodactylus salaris* Malmberg, 1957 was detected in 2001 on juveniles of landlocked *Salmo salar* Linnaeus in three rivers draining to Lake Kuitozero in the White Sea basin. The ancestry of the colony was studied by internal transcribed spacers of the nuclear ribosomal operon (ITS), mitochondrial COI gene and by 12 microsatellite markers. The maternal ancestor was a frozen heterozygotic and heteroplasmic diploid clone *RBT* marked by a ITS-1068^{R}, spread by rainbow trout and observed in Denmark, Norway, Finland and Russia. The clone combined genomes from two divergent matrilines, probably from grayling (*Thymallus* Linck) in the Southern Baltic basin (COI ^{alien}, 84 % bootstrap support) and in Balkans (COI ^{rbt}, 99 %). The genetic distance of the 1545 bp sequences was d_{MCL} = 0.025. The population in Kuitozero was a progeny of *RBT* females repeatedly inseminated by conspecific males. It was sexual but propagated as parthenogenetic clones. The derived population carried only COI ^{rbt}. The microsatellite alleles of the *RBT* clone segregated with the paternal alleles following Mendelian model, with an excess of heterozygotes: H_e = 0.608, H_o = 0.883. Among 244 genotyped worms, 27 multilocus clones were observed, as segregated among the parr (F_{ST} = 0.824). Parental genotypes were not detected. The largest clone (104 worms) was heterozygotic in all 12 loci and was identified on fifteen fish, in two rivers and four years. The balancing selection (excess of heterozygotes) equalised the allelic frequencies and maximised the genetic variance available for purifying and adaptive natural selection on a new host. The two sexual episodes associated with three host switch steps offers a model to understand the evolution of the diverse host spectrum of host-specific *Gyrodactylus* von Nordmann, 1832.

Keywords: mitochondrial heteroplasmy, hybrid clones, life cycle transition, host switch, anthropogenic translocation, Muller's ratchet

The fish ectoparasites of the genus *Gyrodactylus* von Nordmann, 1832 have a simple (monoxenous) and direct life cycle, utilising one host fish species in a stable environment. The anatomy and physiology of this lifestyle were described in great detail by Harris (1998), Cable and Harris (2002) and Bakke et al. (2002, 2007). The worms exist as adult females only, which give birth to next two generations as fully grown gravid daughters. The interval between births may be as short as two days, but the mean in annual temperatures was 6.3 days (Harris 1998). There are no freely dispersing eggs or larvae, but the adult worm has many characteristics and skills of trematode miracidia, such as the posterior attachment organ, which is assumed to be an apomorphic novelty for ectoparasitism shared with all monogeneans (Brooks and McLennan 1993).

The adult also has all the necessary instincts and photo- and chemoreceptors to orient, identify, attach to, and survive on a new host. It is noteworthy that the risky phase of host transition is not safeguarded by the release of multiplied propagules, unlike in the ancestral egg-laying monogeneans (Brooks and McLennan 1993). Multiplication occurs in an established colony prior to transition, which can happen at any time if a new resource appears, but not blindly. This strategy seems to be an adaptation to a stable host expected to support the colony for a long time, perhaps years. The nursery populations of *Gyrodactylus salaris* Malmberg, 1957 in the large salmon river Tornio, bordering Sweden and Finland, were observed to be genetically stable and locally segregated for years, despite the annual host migrations (Lumme et al. 2016).

The genus *Gyrodactylus* is highly speciose (Bakke et al. 2002). Swarms of new species are being recorded and identified globally in newly explored freshwater and marine environments However, the life cycle characteristics remain con-

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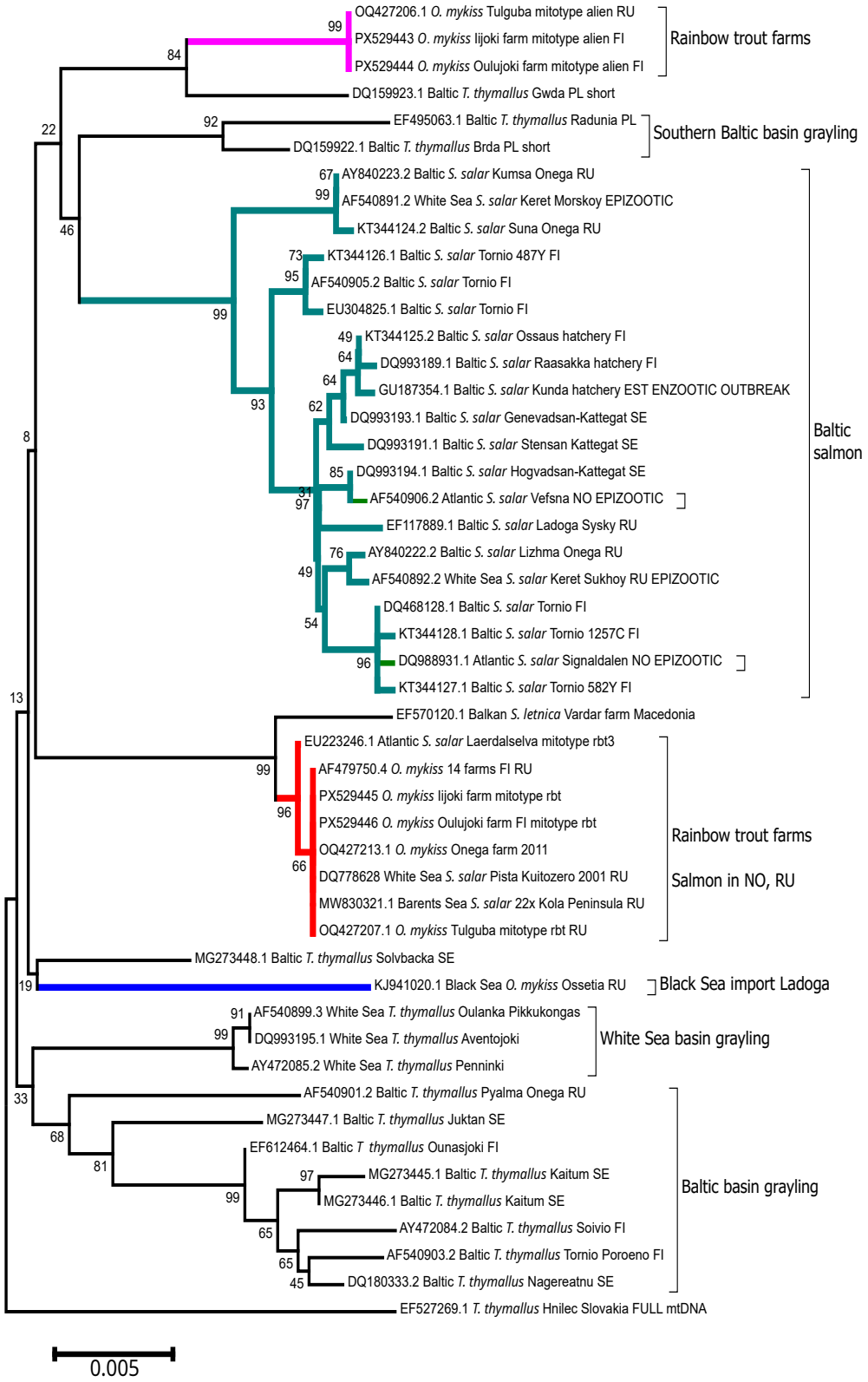

Fig. 1. Mitochondrial phylogenetic tree of *Gyrodactylus salaris* Malmberg, 1957 based on 1,545 bp of COI gene. The hosts are *Salmo salar* Linnaeus, 1758, *Thymallus thymallus* (Linnaeus, 1758), *Oncorhynchus mykiss* (Walbaum, 1792), and *Salmo letnica* (Karaman, 1924). The widespread clades on grayling are black. The blue, red and purple clades were found on rainbow trout, and the red and purple were sexually combined in the RBT clone. The phylogeny indicates that the three lineages on rainbow trout are European parasites adopted by the immigrant fish. . The embedded monophyletic clade on the Baltic salmon is marked as green and gave the name for the species, due to priority rules. All strains in the tree share the ITS within one or two substitutions. The tree was rooted by the most distant farm-living strain of *G. salaris* found in Danish, Macedonian and Polish farms, separated from the standard ITS by three substitutions (AJ515912, EF524573; Lindenstrøm et al. 2003), but carrying about 20% divergent COI (EF524576; Ziętara et al. 2010), as an example of mitochondrial introgression *via* interspecific hybridisation.

servative. Some next-generation researchers are tempted to split the genus into several genera utilising only ITS sequence, but this is unnecessary and even harmful when scalable molecular barcoding already handles systematic bookkeeping.

Malmberg (1970) divided the genus into several subgenera based on the osmoregulatory system, separating freshwater and saline segments. Molecular methods, first ITS (Cunningham 1997) and later mitochondrial DNA (Meinilä et al. 2002), have supported and partially replaced other systematic approaches. For example, researchers have easily added species to the subgenus *Limnonephrotus* Malmberg, 1964 without inspecting the secretory system. Malmberg's two freshwater subgenera remain valid, but the euryhaline and marine groups have proven to be more diverse than predicted from the Stockholm horizon, now that the scope is global from the Arctic to the Antarctic.

Malmberg's subgenus *Gyrodactylus* (*Limnonephrotus*) remains coherent after molecular barcoding and is focused on Eurasian and North African freshwaters. Species of this subgenus are hosted by at least nine fish families: Cyprinidae, Leuciscidae, Acheilognathidae, Salmonidae, Osmeridae, Esocidae, Percidae, Gasterosteidae, and Gobiidae, and the list will certainly grow (Ziętara and Lumme 2002, Leis et al. 2021, Lebedeva et al. 2023, Rahmouni et al. 2023, 2025). Most importantly for the present topic, this subgenus contains all known salmonid parasites of *Gyrodactylus*, reported on the genera *Salmo* Linnaeus, *Hucho* Günther, *Salvelinus* Richardson, *Oncorhynchus* Suckley, *Coregonus* Linnaeus, and *Thymallus* Linck. The salmonid parasites are not a monophyletic group coevolved with the host, but several independent derivatives from the common base of parasites infecting diverse families (Leis et al. 2021). This diversity clearly demonstrates that, while the species are considered host-specific, distant transitions to new hosts have occurred. What kinds of evolutionary changes are needed?

Our focal species, *G. salaris*, was described as a benign commensal in a Baltic salmon hatchery in the Swedish Vindelälven (Malmberg 1957). Only after the catastrophic epizootic in Norway stimulated research, and with the advent of molecular barcoding, the species was elevated to a major study object. First, it was demonstrated that the name *Gyrodactylus thymalli* Žitňan, 1960, was a junior synonym. According to taxonomic rules, the proper name for the species found most widely on European grayling *Thymallus thymallus* (Linnaeus) is *G. salaris*. The matrilineal (COI) phylogenetic tree in Fig. 1 illustrates the diversity of the species from the Balkans to the Black and White Seas. Grayling is indeed the basal host of the parasite, and the Baltic salmon-specific monophyletic clade is a younger branch from the base, suggested to be a consequence of hybridisation between two grayling lineages after the Eemian interglacial 130,000 years ago (Kuusela et al. 2007).

Within the species *G. salaris* numerous other host-switch events have been detected. A strain of *G. salaris* was found on Ohrid trout *Salmo letnica* (Karaman) in a Macedonian fish farm (Ziętara et al. 2010). Transitions to Arctic charr *Salvelinus alpinus* (Linnaeus), Robertsen et al. 2008, Winger et al. 2008, 2009, Mo et al. 2023) were possibly mediated by Atlantic salmon and associated with the large Norwegian epizootics. Imported American rainbow trout *Oncorhynchus mykiss* (Walbaum) and brook trout *Salvelinus fontinalis* (Mitchill); carry their own variants of *G. salaris* of European ancestry (Hansen et al. 2016). The tree in Fig. 1 is pruned, and the new branches from Arctic charr and Romanian brook trout farms are not included because only short COI sequences were published. Fish farms attract the parasites.

Contemporary host switches are amenable to analysis. The parental populations can be deduced, if not directly found, and Mendelian inheritance may perhaps be resolved. Population genetics requires nuclear markers, and the use of microsatellites for the first time truly demonstrated that sex (recombination) and clonality are real alternatives in *Gyrodactylus* (Faria et al. 2011, Schelkle et al. 2012). A new set of microsatellite markers specific for *G. salaris* was recently published by Aisala et al. (2026). This opens opportunities to study the genetics of contemporary host-switch cases of *G. salaris* in detail. The transitions from tolerant Baltic salmon to susceptible populations in the Atlantic and White Sea basins were perhaps direct events because the host was the same species, and the transitions may have occurred without major genomic reorganisations. The complex origin of the unexpected infection on a landlocked and isolated salmon in Lake Kuitozero involved three host-switch events and will be analysed at the population genetic level in this paper.

We detected the population of *G. salaris* in Lake Kuitozero in 2001. The mitochondrial COI suggested that the salmon had been infected by a parasite strain dispersed with rainbow trout. Using an *ad hoc* nuclear marker, it was demonstrated that the Kuitozero infection was not caused directly by the asexual farm parasite, but was instead sexually derived (Ziętara et al. 2006). Tempted by the known role of polyploids in parthenogenesis, and based on an extra unlinked copy of the marker in the rainbow trout strain, we erroneously suggested that the asexual rainbow trout farm strain of *G. salaris* was triploid (Ziętara et al. 2006). Kovalov and Trubenova (2026) cited this error 20 years later. Here, we revisit the pedigree of the Kuitozero population. Using conventional but new nuclear markers, we demonstrate that the asexual rainbow trout strain of *G. salaris* is a diploid frozen hybrid and that it was liberated into a sexual lifestyle after meeting a third partner, producing the Kuitozero population.

The analysis conducted in this paper gives answers to the problems presented in the Box 1. in a review 'Policy, phylogeny, and the parasite' by Stentiford et al. (2014). A single taxonomic name for a notifiable parasite may be insufficient, and a single genetic marker is as well unsatisfactory to identify the invasive pests and their roots and reservoirs.

MATERIALS AND METHODS

Wild salmon (*Salmo salar*) and the parasite *Gyrodacylus salaris* in Lake Kuitozero in Russian Karelia

The Upper Lake Kuito (Ylä-Kuittijärvi, Озеро Верхнее Куйто) is the western source of the large Karelian river Kem draining to the White Sea. The area of the lake is 168 km^2, it is 103 m above

sea level and it has four confirmed salmon spawning rivers: Pisto, Kurzhma, Vuonitsa and Vuokinjoki (Vokna). The longest distance between the river mouths along the lake is about 45 km. The River Pisto flows from Finland, town Kuusamo and Kuusamo Lake. Also, the uppermost waters of Kurzhma cross the border. It is important to note the salmon population is not descending to the White Sea via Kem river, but remains feeding in the lake.

Site No. 1. River Pisto, above Korpijärvi (65.2844N, 30.5111E, 115 m asl, about 18 km upstream from Kuitozero). First salmon sample was collected in 1999. Fifty nine juveniles of *Salmo salar* Linnaeus juveniles were caught, but *Gyrodactylus salaris* was not expected or inspected. Salmon data from this sample has been utilised in numerous publications (Tonteri et al. 2005, 2007, 2009, Makhrov and Bolotov 2006, Ozerov et al. 2010, 2012, Bourret et al. 2013, Zueva et al. 2014, 2018, 2020), demonstrating that the Kuitozero salmon population was postglacially derived and isolated from the Western White Sea colonisers, and is not related with the salmon stocks in the Baltic Basin which are known to be a tolerant reservoir of the most pathogenic strains of *G. salaris* (Lumme et al. 2015).

Site No. 2. River Pisto at the upper bridge (65.3525N, 30.5603E, 125 m asl, about 30 km upstream). The 2001 expedition aimed to the infected river Keret', but the Pisto site along the road was sampled and inspected by J. Kuusela. This was the first time *G. salaris* was found in Kuitozero. It was identified as *RBT* strain by mitochondrial sequence AF479750 (Meinilä et al. 2004, Kuusela et al. 2005). Further samples were obtained in 2005, 2008 and 2009. In 2018, electrofishing in this site produced only three specimens of *S. salar* and no parasites.

Site No. 3. River Kurzhma (65.2017N, 30.2181E, 113 m asl, about 11 km upstream).The river begins from Kalmojärvi (Cadaver Lake, 216 m asl), Suomussalmi, on the Finnish-Russian border. The sampling site was visited by J. Kuusela in 2001 (18 *S. salar*, parasites were checked but not found). In 2005, only five salmon parr were caught. Two parr carried five specimens (three genotypes) of *G. salaris* (*COI* DQ517533). Among the five salmon juveniles, few specimens of *Gyrodactylus lucii* Kulakovskaya, 1952 (main host *Esox lucius* Linnaeus) and *Gyrodactylus aphyae* Malmberg, 1957 (main host in this area *Phoxinus isetensis* [Georgi, 1775]) were observed as accidental visitors (Ziętara et al. 2008a). In 2018, electrofishing in the same site produced no salmon.

Site No. 4. River Voynitsa (Vuonitsa; Kumi-Porog, 65.2756N, 30.1978E, 135 m asl). Eight salmon parr were caught on 24 July 2005, but they were not infected by *Gyrodactylus.*

Site No. 5. River Vuokinjoki (Vokna, 64.9208N, 30.1325E, 122 m asl, about 25 km upstream) was sampled in 2012 for this study by E. Ieshko. Parasites on six infected fish were genotyped. In 2013, 21 salmon parr were caught, 19 of them were infected but not transferred to Oulu lab (E.I., personal communication). In October 2018 (N = 19) and October 2019 (N = 5), the salmon parr were not infected.

Gyrodactylus salaris in rainbow trout farms

The *Gyrodactylus* parasites in rainbow trout farms have been identified by different markers and reported earlier (Kuusela et al. 2005, Evseyeva 2009, Ieshko et al. 2015, 2016). The material analysed here by mitochondrial LA/HA primers and microsatellites consisted of 15 samples, three from Finland (Farms Hyrynsalmi, Oulujoki, Iijoki) and 12 from Russian Karelia (Farms A to K), along lakes Onega, Ladoga and Segozero, from 2003 to 2009. The exact localities and commercial details of the farms are not listed, because the rainbow trout industry was interconnected prior to 2022. Few Finnish breeders produced the juveniles to both Finnish and Karelian production ponds. The microsatellite genotype was identified from 189 parasite specimens. The mitochondrial COI sequences from Kuitozero and rainbow trout farms are displayed in the phylogenetic hypothesis in Fig. 1.

Gyrodactylus salaris on grayling (*Thymallus thymallus*) in the White Sea basin

The grayling parasites have not been searched in Kuitozero, while they could be candidates for the paternity of the *G. salaris* population. In the 1999 expedition to site No. 1 Pisto, five grayling juveniles were caught by electrofishing (published by Koskinen et al. 2000), but they were not inspected for *G. salaris,* which was not yet an issue. In the White Sea basin, *G. salaris* on grayling was recorded in headwaters of Pisto (Peninki) and in the parallel Kovda river system Pikkuköngäs and Aventojoki (Fig. 1). The samples of *G. salaris* on grayling in Kovda upwaters had a unique substitution ITS 406^{C} (Kuusela et al. 2007), excluding them from Kuitozero paternity case. The ITS from Peninki is not known.

Molecular methods

Whole fish or fin clips were stored in 96% ethanol in field and maintained later in refrigerator temperatures, in darkness. The worms were picked from host tissue just before further processing in lab.

DNA extraction was from single ethanol preserved worms (triplets due to hyperviviparity), by standard methods, but our process had no purification after lysis, improving greatly the PCR gain (Ziętara et al. 2002, 2006). Prior to the enzymatic release of the DNA, the ethanol was evaporated. In the Oulu protocol, DNA was released using proteinase K lysis (Fermentas) with the following program: 65 °C for 25 min, 94 °C for 10 min to denature the proteinase, and cooling down to 4 °C. This simplified method produced a good harvest of DNA for polymerase chain reaction for multiplexed microsatellites.

The sequencing of the ITS1-5.8S-ITS2 was adequately described earlier (Cunningham 1997, Ziętara et al. 2002, 2006, Hansen et al. 2003, Kuusela et al. 2007, Lumme et al. 2015). ITS serves well as the barcode for species identification among *Gyrodactylus*. The flatbed DNA sequencer PE ABI 377 utilised in first identifications missed the G/A transition heterozygosity ITS-1068^{R} in the *RBT* clone, and some of the early GenBank sequences from rainbow trout farms display G instead of R.

The mitochondrial cytochrome c oxidase sub-unit I (COI) gene of *G. salaris* was sequenced as earlier (Meinilä et al. 2004, Huyse et al. 2006, Ziętara et al. 2006, Kuusela et al. 2007). Also in the COI, the heteroplasmy in *RBT* was overlooked or misunderstood in early recordings. The Thermofisher ABI 3730 capillary sequencer allowed the confirmation of heteroplasmy.

The microsatellite markers were published by Aisala et al. (2026). The markers were identified by Heidi Aisala from an early unpublished draft genome of *G. salaris*, derived from 50 unborn specimens collected from the Suna River, Lake Onega, in Russian Karelia by Julia Barskaya and Dariya Lebedeva. The variability was tested by utilising the *RBT* strain, but the data are published first time here. The final marker panel comprises

Table 1. Primers used in this study to amplify 12 variable microsatellite markers in *Gyrodactylus salaris* Malmberg, 1957 (see Aisala et al. 2026). The length distribution of the amplified sequences in rainbow trout farms and in Kuitozero population is displayed in Fig. 4.

Name of marker	Forward primer (5'-3')	Reverse primer (5'-3')
µs18122	GCAATTTTGCCGATTCTCTC	TCTCCAACATACCCTGCACA
µs19077	AGAATGCCCTGCATCAAAAG	ACGCCATGCAATTCCTCTAA
µs21946A	CCACTCTCTTATCCATCATTGTG	ACAGTCGGAGGAAGCTTGAG
µs21946AB	shared with µs21946 A	TGAATGGCAAGTGAATTGGA
µs22089	TTTCTTGCCCATTGTGATGA	AAACACCACGTATGCAACCA
µs2274	GTCCCAACGCATTCTACCC	GGACTTTGTGGCAGAGAGGA
µs25338	AGACCCCATTTTGACCCAAT	TTCCCAGGTTAGATGGCAT
µs27948	CCGTGAGCTAGATGGGAAAT	GCAAATTCTCTCTCCCCAAA
µs4137	TTGGGTACTTGAACGCAAAA	TCAGAATAACCAGCGCCATA
µs4315	GAGCGAGAGACTCCTGATGG	AATGACAAAAATCATTAAGTGAAG
µs5780	CATGCTGGATGTGCATTAGG	GCAAGGCAACTCTCAAGTCC
µs697A	AAGTGTTACTATAAATGGGACACG	CCTTTTCAAATGGGAGTTTG

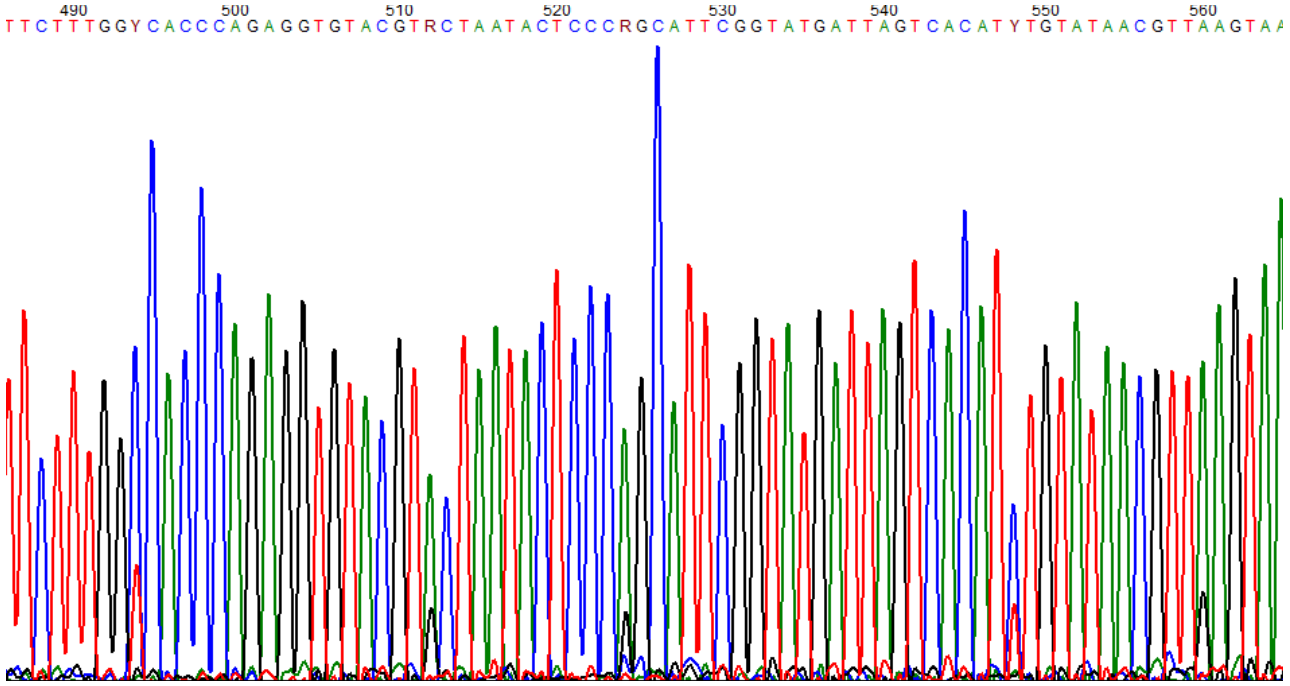

Fig. 2. A fragment of ABI phenogram (interval 710–785) of the heteroplasmic COI of *Gyrodactylus salaris* Malmberg, 1957 recorded in the farm Tulguba, Onega, Karelia (GenBank accessions OQ427206 and OQ427207). In this individual worm, the signal of COI ^{rbt} is stronger than the COI ^{alien}, which may be due to drift in the germ line, or somatic drift in the triplet of mother, daughter and grand-daughter. The last R (560 in this segment) was overlooked by the machine as A.

14 variable microsatellites representing 12 distinct genomic loci (Table 1). The forward primers were tagged with four different fluorescent labels, and the lengths of the PCR products ranged from 150 bp to 600 bp (Table 1). This allowed multiplexing all loci into the same reaction, using the Type-it Microsatellite PCR kit (Qiagen). The details of the laboratory protocol were as in Aisala et al. (2026). The sequences amplified with the primers in Suna (unpublished) and Lierelva (Hahn et al. 2014) references are as fasta file in Supplement A.

For population genetic analysis, we treated the Kuitozero data as a Mendelian crossing experiment, modified by the clonality option. For population genetic analysis, simple parameters were calculated (Hedrick 2000). The unbiased expected heterozygosity measures the nuclear allelic diversity: $He = (N/(N - 1)) \times (1 - \Sigma p^2)$. The clonal diversity on parr was measured by the standard $F_{ST} = (H_T - H_S)/ H_T$.

The mitochondrial phylogenetic tree (Fig.1) was based on Maximum Composite Likelihood distance (d_{MCL}), constructed by Neighbor Joining algorithm and tested by bootstrapping in MEGA 12 (Kumar et al. 2024). Only published full length (1,545 bp) COI sequences were included, but the DQ159923 (816 bp) from Polish grayling in the River Gwda was accepted as a nearest relative of the *cox*1^{alien} mitotype.

RESULTS

Mitochondrial heritage of the heteroplasmic *RBT* strain

The rainbow trout farm specific strain of *Gyrodactylus salaris* was in several samples heteroplasmic. Because the *RBT* strain is a permanently heterozygotic, asexual clone, we have to conclude that the heteroplasmy was derived from the original hybridisation. In the phylogenetic tree presented in Fig. 1, the two COI lineages found in farm

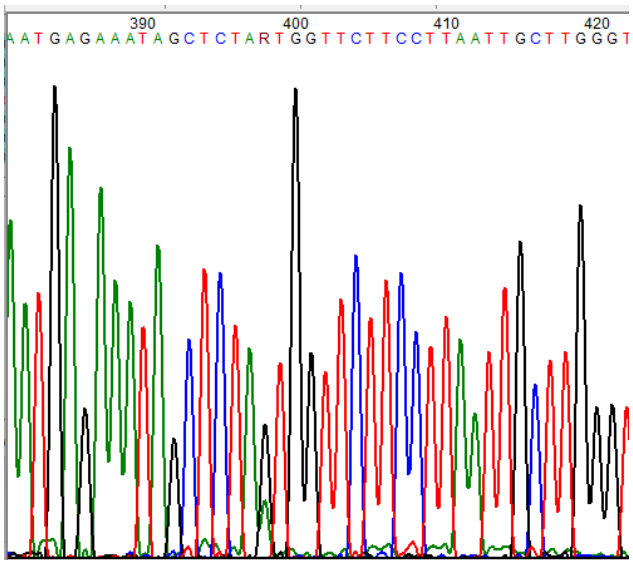

Fig. 3. The G/A transition heterozygosity ITS-1068^{R} in *Gyrodactylus salaris* Malmberg, 1957 specimen *10Pi1* from Pistojoki. This unique variant connects the rainbow trout farm parasites from Norway and Denmark (Kania et al. 2007) with the Finnish and Russian material, as evidence of the single hybrid origin of the frozen heterozygotic *RBT* clone.

specimens belong to two distant clades, purple and red. The mitochondrial COI ^{rbt} was first sequenced in few samples in Finland by Meinilä et al. (2002, 2004) and Ziętara et al. (2008a). This mitotype belongs to a clade containing also the Norwegian pathogenic *F* strain on salmon in Lærdalen and the less pathogenic *F* strain on *Salvelinus alpinus* (Hansen et al. 2003). A divergent type with 10/1545 substitutions was found on Ohrid trout *Salmo letnica* in a Macedonian farm (Ziętara et al. 2010). This may point the origin of this clade in Balkans, but this is uncertain. Farm parasites are not necessarily from nearest neighbourhood.

The heteroplasmic partner COI ^{alien} was labelled as alien because it was observed later, and was more often drifted to low intensity, and was neglected in early samples. However, it was almost fixed in some Finnish farms. It has a relative found on grayling in River Gwda on the Baltic South Coast, Poland (84% bootstrap support) (Hansen et al. 2007). This is the area (Pomerania) where the rainbow trout farming arrived to Europe (Crawford and Muir 2008, Stanković et al. 2015). The COI ^{alien} differs from COI ^{rbt} in 38 sites along the 1,545 bases: 34 transitions 25 Y (T/C), 9 R (A/G), and four transversions 2 M (C/A), 1 S (C/G), and 1 W (A/T). The distance estimate was d_{MCL} = 0.025. There were five non-synonymous changes, but no indels. Presumably, both mitochondrial DNAs were functional.

The degree of observed heteroplasmy of the *RBT* clone was variable (Fig. 2). The proportion of the two types COI ^{rbt} and COI ^{alien} drifted, both between farms, and even between worms (gravid females) in a farm. The drift may occur in either direction, but the COI ^{rbt} was fixed in ten farm samples. The original proportions of the two mitochondrial types in the primary hybrid, as well as the maternal *vs* and paternal phase are not known. Drifting is what we expect to the neutral mitochondrial heteroplasmy.

Table 2. Segregation of the maternal and paternal alleles in the triple hybrid population of *Gyrodactylus salaris* Malmberg, 1957 in Kuitozero. Length distribution of the classes is in Fig. 4. Data were pooled over three rivers and all year classes. The variation parameters H_e and H_o are the proportions of expected and observed heterozygotes counted on real allele distribution, not on the three classes.

Locus	N genotypes	Maternal Yellow	Maternal Blue	Paternal Red	H_e	H_o
µs18122	230	188	192	82	0.632	0.983
µs19077	230	224	236	0*	0.501	0.974
µs21946A	230	232	123	106	0.623	0.991
µs21946B	224	225	118	103	0.624	0.991
µs22089	225	180	108	161	0.656	0.809
µs2274	229	272	140	46	0.545	0.790
µs25338	225	258	127	66	0.634	0.884
µs27948	231	181	235	67	0.620	0.797
µs4137	225	176	274	0*	0.477	0.764
µs4315	229	233	4*	219	0.612	0.974
µs5780	223	64	236	142	0.780	0.830
µs697A	230	114	78	268	0.586	0.804
Average		195.6	155.9	126.0	0.608	0.883

*Aberrant proportions, discussion in text

Nuclear genotype of the *RBT* clone

The twelve microsatellite loci were consistently heterozygotic in the rainbow trout farm *G. salaris*. Two loci µs52338 and µs5780 displayed some variation in the length estimates (perhaps process errors), but in no case any indication of sex, i.e., homozygotes. The genotype presented in the first row in Fig. 5 was confirmed in 189 specimens. Because most worms were pregnant mothers, the real N is even larger. The heterozygosity of the *RBT* clone is thus confirmed as 'frozen'. The sampling for microsatellite genotyping ranged from 2003 to 2009, and the strain was actually studied by other markers from 2001 until 2012, and over numerous farms. Sexual recombinants were either never born, or were immediately eliminated. The *RBT* clone is truly asexual ever since the original unique hybridisation.

It is important to note that there is a single heterozygous nucleotide also in the ITS (internal transcribed spacer) in the non-pathogenic COI ^{F} clone on *S. alpinus* in Norway and in *Oncorhynchus mykiss* farms in Denmark (Kania et al. 2007), and in the COI ^{rbt} clone in Kola Peninsula (Hansen et al. 2022). The ITS-1068^{R} was also found in three specimens after the hybridisation in the worms in Kuitozero (Fig. 3). This heterozygosity connects all the Norwegian, Danish, Finnish and Russian farm strains of *G. salaris* in northern Europe together, as descendants of a single hybridisation associated with the switch and lucky preadaptation to the novel North American host.

Maternal ancestry of the Kuitozero population

The mitochondrial gene COI was sequenced from 69 specimens from Kuitozero salmon parasites. Only the COI ^{rbt} was found in Kuitozero salmon parasites, meaning non-reciprocal hybridisation, the *RBT* strain as the maternal parent. No trace of the heteroplasmic COI ^{alien} was detected. The disappearance of the heteroplasmy may well predate this secondary hybridisation episode, which involved more than one *RBT* female. The paternal mitochondria had not leaked into the progeny in the Kuitozero fusion. The GenBank accession codes of mitochondrial COI in Kuitozero are

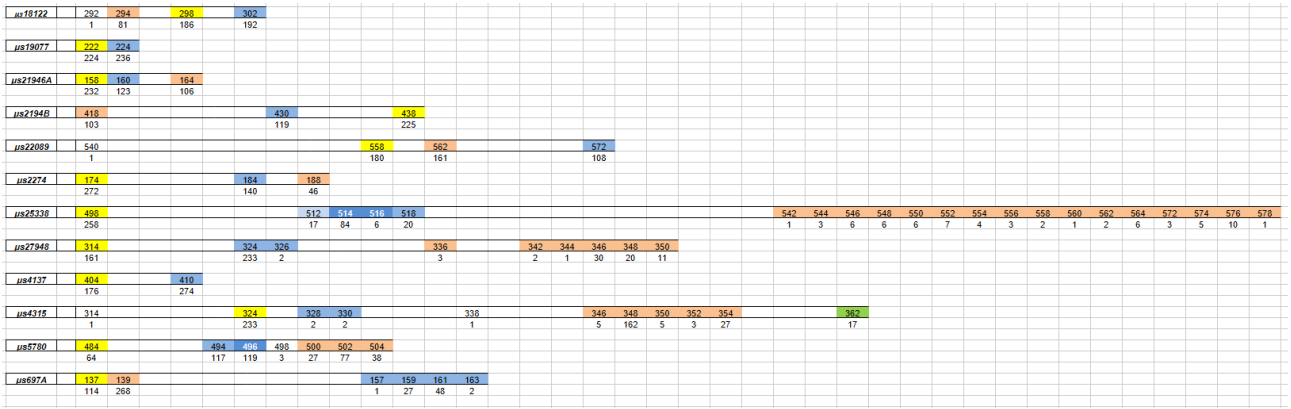

Fig. 4. Discontinuous size distributions of the alleles in the microsatellite loci of *Gyrodactylus salaris* Malmberg, 1957 in Kuitozero hybrid swarm. Allele counts are below the bars. The two alleles from the *RBT* strain are marked as *yellow* and *blue* (Fig. 5), and the alleles from the unlocalised paternal source as *red,* or *green*. We present the µs21946B as an independent allele by subtracting AB – A = B.

DQ517533 (Kurzhma) and DQ778628 (Pisto) (Kuusela et al. 2007). New sequences OQ427214–OQ427221 were produced for this report. They all are identical with the COI ^{rbt} in Finnish and Russian rainbow trout farms, as well as with the 22 recently published sequences from northwestern Russia, detected both on salmon and rainbow trout (MW830296–MW830326; table 3 in Hansen et al. 2022). The sequence EU223246 of the Norwegian pathogenic *F* strain differs by one synonymous nucleotide (Ziętara et al. 2008b).

ITS defined the paternal species of Kuitozero hybrid swarm as *G. salaris*

The ITS was determined for a small sample of Kuitozero parasites, which demonstrated that the sexual partner was not any alien species, but carried the standard ITS of *G. salaris,* originating either from grayling or salmon. The specific G/A heterozygosity (ITS 1068^{R}) of the *RBT* strain was observed in three specimens (Fig. 3), and the standard G in eight.

Table 3. Number of *Gyrodactylus salaris* Malmberg, 1957 assigned to each clone (code in Fig. 5) on 16 fish in Pisto 2005 and Vuokinjoki 2012. The most numerous clone *1Pi1* comprised 42.6 % of the total. The clonal propagation on fish overweights the transition between fish. Total H_e = 0.564, mean in fish H_e = 0.111, F_{ST} = 0.803.

Site	Fish	Parasite clone								
		1Pi1	*2Pi3*	*1K10*	*6K1*	*6K6*	*7K3*	*6K14*	0ther	Σ
Pisto	No. 1	7								7
2005	No. 2		8							8
	No. 3	8								8
	No. 4	5								5
	No. 5	13	1							14
	No. 6	1	10							11
	No. 7								1	1
	No. 8	38	1							39
	No. 9	3							2	5
Vuokinjoki	No. 1			16						16
2012	No. 2	9								9
	No. 3	1								1
	No. 5			1	13					14
	No. 6				1	2		1		4
	No. 7						1			1
	No. 8	16								16
	Σ	101	20	17	14	2	1	1	3	159

The early GenBank accession codes of the ITS segments in Kuitozero were EF117880 (Kurzhma) and EF117884 (Pisto) (Kuusela et al. 2007). They are identical with AF328871 from Finnish rainbow trout farms. Thirty-one recent sequences from salmon and rainbow trout in Barents area in northwestern Russia were published by Hansen et al. (2022): MW816676–MW816697 included the heterozygotic 1068^{R}, MW819698–MW819706 were standard 1068^{G}.

In Fig. 3 the peak of G is higher than the A, but the method is not quantitative, as obvious in the wide height variation among homozygous peaks. The ribosomal DNA consists of numerous tandem repeats, which tend to be homogenised via unequal crossing over in a process called concerted evolution, and the heterozygosity is not on/off like in single copy nuclear genes, but may fade away (or fix up) gradually.

Microsatellite variation in the Kuitozero population

The size variation of the microsatellites was wide and discontinuous (Fig. 4). We labeled the microsatellite alleles derived from the *RBT* strain arbitrarily as *Yellow* and *Blue.* The alleles derived from the unknown paternal strain were marked as *Red.* The *Red* alleles were in some loci longer and more variable, but the distribution was unimodal. It may well reflect real variation in the paternal population. The extreme outlier in *µs4315* marked as *Green* was found in one separate clone (Figs. 4 and 6). The paternal alleles represented about one third of the gene pool.

In all microsatellite loci, both alleles of the heterozygous *RBT* strain were present in the Kuitozero population (Figs. 4 and 5). This indicates that the mating of the females of the heterozygotic *RBT* strain with wild *G. salaris (Y/B × R/R)* had occurred repeatedly. In one fertilisation of a heterozygote, only one allele from a heterozygotic locus is transferred to the single daughter clone. The single daughter instead of segregating swarm is the main modification when adopting the *Drosophila* school model to *Gyrodactylus*.

The probability that one of the two alleles in *n* linkage groups (chromosomes) is missing after *c* fertilised eggs is $P = n \times (½)^c$. By six chromosomes, after three insemina-

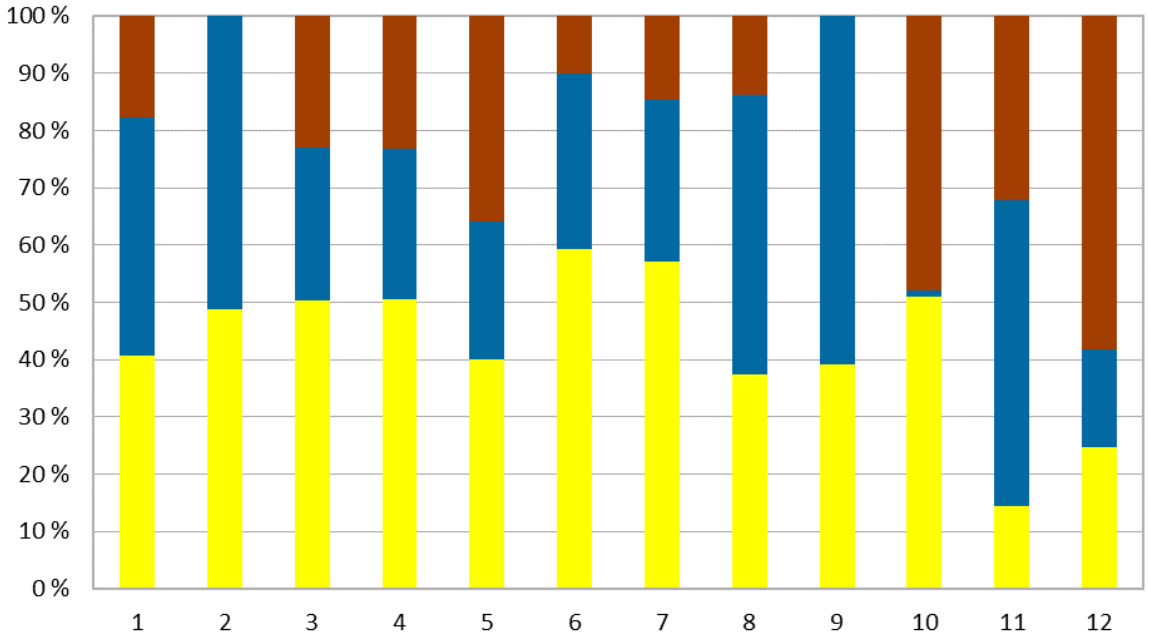

Fig. 5. Proportion of the maternal alleles *Yellow* and *Blue* from *RBT* clone of *Gyrodactylus salaris* Malmberg, 1957, and *Red* from the paternal partner. The aberrant proportions are missing *Red* in µs19077 (2) and nearly missing *Blue* in µs4315 (10). The *Red* may be mimicking Blue in µs4137 (9), but the other two aberrations suggest non-neutrality of the genomic area linked with the marker.

Anchor exx	River	18122	19077	21946A	21946B	22089	2274	25338	27948	4137	4315	5780	697A	Nfish	Nworms	Years
	Farm RBT	298 302	222 224	158 160	438 430	558 572	174 184	498 514	314 324	404 410	324 328	484 494	137 161		189	2003 - 2009 14 farms
1Pi1	Pisto, Vuokinjoki	298 302	222 224	158 160	438 430	558 572	174 184	498 514	314 324	404 410	324 348	494 500	137 139	15	104	2005, 2008, 2009, 2012
4P2	Pisto	294 298	222 224	158 164	438 418	558 562	174 184	498 550	324 346	410 410	324 348	484 494	139 161	7	32	2008
2Pi3	Pisto	294 302	222 224	158 164	438 418	558 562	174 188	498 574	314 348	404 410	324 348	484 504	139 161	4	28	2001, 2005
8K10	Vuokinjoki	298 302	222 224	158 164	438 418	562 562	174 174	498 498	324 324	410 410	324 354	494 494	139 139	3	26	2012
1K10	Vuokinjoki	298 302	222 224	158 160	438 430	562 562	174 174	498 512	324 324	404 410	324 362	494 496	139 139	2	18	2012
P01-14	Pisto	294 302	222 224	158 164	438 418	558 562	174 188	498 564	314 314	404 410	324 348	484 504	139 161	1	6	2001
1Kr2	Kurzhma	294 298	224 224	158 164	438 418	558 562	174 188	498 546	324 324	404 410	324 324	496 496	137 161	1	4	2005
9Pi4	Pisto	298 302	222 224	158 160	438 430	558 572	174 184	498 514	314 350	404 410	324 348	496 502	137 139	1	2	2005
2PT2	Pisto	298 302	222 224	158 160	438 430	558 572	174 184	498 518	314 324	404 410	324 352	500 502	137 139	2	2	2008
6K6	Vuokinjoki	298 302	222 224	158 158	438 438	558 572	184 184	498 518	314 324	404 410	324 324	496 496	137 139	1	2	2012
3P4	Pisto	294 298	222 224	158 164	438 418	558 562	174 184	516 554	314 324	404 404	324 348	494 496	137 159	1	2	2008
2Pis4	Pisto	294 302	222 224	158 164	438 418	558 572	174 174	498 498	324 336	404 410	324 328	484 496	139 157	2	2	2008, 2009
PO91	Pisto	294 298	222 224	158 164	438 418	558 562	174 184	498 498	324 346	410 410	324 346	496 496	139 139	1	2	2009
Pisto08_3	Pisto	298 298	222 224	158 164	438 418	558 558	174 174	498 550	324 346	410 410	324 348	484 494	139 161	1	1	2008
6K14	Vuokinjoki	298 298	222 224	158 160	438 430	572 572	174 184	498 518	314 324	404 410	348 348	496 502	137 139	1	1	2012
14P2	Pisto	298 302	222 224	158 160	438 430	558 572	174 184	498 516	314 324	404 410	324 348	496 500	139 139	1	1	2008
P01-1	Pisto	294 294	222 224	158 164	438 418	558 562	174 188	498 568	314 346	404 410	324 348	502 504	139 161	1	1	2001
7Pi1	Pisto	294 294	222 224	158 164	438 418	558 562	174 188	498 498	314 342	404 410	324 348	502 502	139 161	1	1	2005
Pisto08_6	Pisto	294 294	222 222	158 164	438 418	562 562	174 174	498 558	324 346	410 410	324 324	484 494	139 159	1	1	2008
Pisto08_18	Pisto	294 294	222 222	158 164	438 418	558 562	174 184	498 546	324 346	410 410	348 348	484 494	139 159	1	1	2008
7K3	Vuokinjoki	294 294	222 224	158 164	438 418	558 562	174 188	498 518	314 324	404 410	324 348	502 502	137 139	1	1	2012
14P1	Pisto	294 298	222 224	158 164	438 418	558 562	174 184	498 552	324 346	410 410	324 348	484 494	139 139	1	1	2008
Pisto08_16	Pisto	294 298	222 224	158 164	438 418	558 562	174 184	498 514	324 346	410 410	324 348	484 494	139 159	1	1	2008
Pisto08_9	Pisto	294 298	222 224	158 164	438 418	558 562	174 184	498 498	324 346	410 410	324 348	484 492	139 159	1	1	2008
Pisto08_4	Pisto	294 298	222 224	158 164	438 418	558 562	174 184	498 546	324 346	410 410	324 348	484 494	139 159	1	1	2008
2PT3	Pisto	294 298	222 224	158 164	438 418	562 562	174 184	0 0	324 346	410 410	324 348	484 494	139 161	1	1	2008
14P9	Pisto	294 298	222 224	158 164	438 418	558 562	174 184	498 544	314 324	404 410	324 348	484 494	139 159	1	1	2008
Genotype	*Y/B*	7	25	6	6	6	16	9	9	15	1	12	2	114	*Y*	249
	Y/R	11		20	20	16	6	12	3		22	2	6	118	*B*	191
	B/R	3						1	12			4	15	35	*R*	174
	B/B		1			1	1		3	12		6		24		
	Y/Y	2	2	1	1	1	5	5	1	1	3			22		
	R/R	5				4					2	4	5	20		
														333		

Fig. 6. The separate multilocus clones of *Gyrodactylus salaris* Malmberg, 1957 found in Kuitozero parasite population (244 specimens, 27 clones). *Yellow* and *Blue* cells mark the alleles originating from the rainbow trout farm RBT clone. *Red* (or *green*) represents the alleles contributed by the wild male partner. The only *Green* cell represents an outlier allele found in one locality. The clones are named by the original notebook label of an anchor specimen.

tions, $P = 0.75$, and after six, still $P = 0.094$. It may be possible that a single mating leads to fertilisation of several sequential eggs: living sperm has been observed in tissues of *G. salaris* (Harris et al. 1997). The number of primary eggs in *Gyrodactylus* may be only two or three (Harris 1998). Thus, the exact number of matings and fertilisations remains open. The high number of *Red* alleles in highly variable loci supports the idea that numerous *RBT* females were sexually active with the new hermaphroditic partners. The hybridisation was not reciprocal and no trace of the paternal COI was left.

Evenness of the frequencies of the alleles from three ancestors was apparent after lumping the alleles in three groups, *Yellow*, *Blue* and *Red*. In ten microsatellite loci with all three allele groups, the two alleles (*Yellow* and *Blue*) from the farm strain were present with a mean sum frequency of 66.4 %, and the third group of alleles (*Red*) from the sexual partner by 33.6 %, indicating roughly the allelic frequency

ratio 1 : 1 : 1 (Fig. 5). Clonewise genotype frequencies are presented in bottom rows in Fig. 6.

The *Red* allele class was missing in two loci. In the µs19077, the *Yellow* and *Blue* alleles were present in about equal frequency (Fig. 4, 202/214). The *Y/B* heterozygote was almost the exclusive genotype (Fig. 5). If the µs19077 *Red* were a null allele, not amplified with the utilised primers, it should be visible as numerous apparent *Yellow* or *Blue* homozygotes, at least in *2Pi3* which looks like F_1. The *Red* was not mimicking *Blue* or *Yellow* either, as concluded from the equal frequencies. Perhaps the heterozygotic *Yellow/Blue* was super-fit in this new combination. There were only two *Y/Y* singletons and one *B/B* clone of four, demonstrating that this marker was anyway segregating.

In the locus µs4137, the allele frequencies were 159 *Yellow* and 257 *Blue*, suggesting that the paternal *Red* was hiding among *Blue* (allelic mimicry). Aberrant allelic distribution was observed also in µs4315. The *Blue* from the farm strain was next to missing and the alleles classified as *Red* or *Green* from the paternal strain were in majority. The length differences were clear, so it was not a question of resolution.

In Fig. 5, all other loci seem to be unlinked but µs21946 A and B which were amplified by a common forward primer. Their repetitive segments were only 324 bp apart (Supplement A). Due to the low number of clones (27), estimating the crossing over distances and linkage disequilibrium makes no sense. The original phase of *B* and *Y* alleles in the parents of the *RBT* clone is not known. According to Aisala et al. (2026), at least five of the six chromosomes are marked. Some of the 11 loci must be in same chromosomes, but the crossing over had separated all but the µs21946 A+B.

Surplus of heterozygotes in all nuclear loci

Overall, there was an excess of heterozygotes calculated over individuals (N = 244; mean over loci H_e = 0.608, H_o = 0.883), and over clones (N = 27; H_e = 0.658, H_o = 0.788). The two most abundant clones were all-heterozygous, but up to seven loci were homozygous in some clones sampled latest, in Vuokinjoki 2012. The increased number of homozygous loci may suggest that the homozygosity increased between 2001 and 2012, due to loss of heterozygosity caused by hypothetical meiosis and automixis within clones. Another model could be that a local paternal strain participated still to the population. However, the extreme samples were small and from different rivers and are thus not comparable.

Locus-wise expected and observed heterozygosity values were compiled in Table 2. The mean of the expected heterozygosity (= allelic variance) calculated over individuals was H_e = 0.608. In all loci, the frequency of the observed heterozygotes was higher than expected, in average H_o = 0.883. Calculated over clones, it was slightly lower (H_o = 0.788), due to higher heterozygosity of the large clones.

Clonality was the mode of propagation.

After repeated hybridisations *Y/B* × wild *R/R*, the sexual recombination was liberated among the fusion population. The pure *RBT* strain or 'wild type' *G. salaris* were not observed at all on Kuitozero salmon. The missing wild type may indicate that the hybridisation occurred elsewhere. Another possibility is that the local *G. salaris* population was utilised to exhaustion, and no natives were left.

The clones were compiled from three rivers Kurzhma, Pisto and Vuokinjoki over the five sampling years and on 55 infected salmon parr. Of the parasite specimens, 244 were assigned to 27 genotypes. Thirteen genotypes were found as more than one worm, i.e., as clones. Fourteen genotypes were observed as singletons (hyperviviparous triplets!). Sixteen specimens out of 260 were not assigned to any clone because of empty cells (technical failures), but they were used in estimating the locuswise variation parameters in Table 2.

The most numerous clone in whole material was the *1Pi1*, which carried the maternal *Yellow/Blue* for eight loci, *Red*/*Blue* in one and *Red*/*Yellow* in three loci. This clone was found on 15 fish, in 2005, 2008, 2009 and 2012, in Pisto and Vuokinjoki rivers, demonstrating the shared parasite pool in the lake (Table 3).

The clone *4P2* (Pisto 2008) was also numerous, on seven fish and 32 specimens, carrying in five loci *Red*/*Yellow* and in two *Red*/*Blue* genotypes, but two *Yellow*/*Blue* genotypes showing that it was not a primary hybrid (like the clone *2Pi3*) but had segregated further. The homozygous *Blue*/*Blue* in µs4137 in this clone may be a hidden heterozygote.

Due to the fish to fish sampling discipline, clonality was easy to detect also because the clones were not freely dispersed among the fish but had multiplied on host (Tables 3, 4). The segregation was clear. Total expected heterozygosity was H_T = 0.564, and the mean in individual fish (parr) H_S = 0.111 leading to F_{ST} = 0.803. Spatial divergence of the clones among the rivers was apparent (Table 3), but it is not to be emphasised because the effective sample size was small due to clonality, and the samples were also temporally distinct. Definitely, the three spawning rivers share a common hybrid swarm of *G. salaris*.

DISCUSSION

In the present study, we have a narrow scope. The high host diversity and conservative morphological and ecological evolution in the hyperviviparous genus *Gyrodactylus* awaits analysis and explanation: how the host-specific parasites can invade new hosts? The analysis here is about one example, but it displays well the opportunities in the contemporary world. The glacial perturbations have been replaced by experimental trafficking of fish and parasites into new combinations. The Kuitozero population of *Gyrodactylus salaris* was actually a progeny from a large Mendelian crossing experiment, conducted by the resources of the fisheries industry.

The industrial scale transitions of *Gyrodactylus* between fish species or between tolerant and susceptible hosts have been observed. The first reports were from Norway, and the serious pathogenic invasions observed there led to the advent of molecular population analysis. The first molecular tool to identify the Norwegian stocks and species of *Gy-*

rodactylus was the internal transcribed spacer segment of the nuclear ribosomal operon (ITS1, 5.8S, ITS2), which was introduced in 1997 by Carey Cunningham. This marker is still a valid 'barcoding' molecule for identifying and even defining species hypotheses of the genus *Gyrodactylus*.

More than 200 species have been tagged by ITS from the Barents Sea to the Antarctica, supporting the global faunistic inventory. Tens of thousands named or nameless species are still without a barcode. The ITS was finally not separating the salmon pathogens from benign parasites, but it helped to define the species *G. salaris* in numerous hosts: Atlantic and Baltic salmon, American rainbow trout, Arctic charr, European grayling, American brook trout and lake trout, and Ohrid trout (Stentiford et al. 2014). From the beginning, the ITS separated species like *Gyrodactylus derjavinoides* Malmberg, Collins, Cunningham et Jalali, 2007, *G. truttae* Glaser, 1974 and *G. teuchis* Lautraite, Blanc, Thiery, Daniel et Vigneulle, 1999 from serious pathogens, and finally helped to reconstruct the global systematic framework for salmonid ectoparasites included in the subgenus *Gyrodactylus* (*Limnonephrotus*) (Leis et al. 2021).

The next mitochondrial marker COI (Meinilä et al. 2002) was then central in detecting the host transitions, e.g., the three separate invasions of A, B and F strains into the Norwegian wild salmon stocks (Hansen et al. 2003, Ziętara et al. 2008b). Also, the infection in Kuitozero was first identified by the COI marker as a farm introduction (Ziętara et al. 2006).

The third level of markers utilised here are 12 microsatellite loci, which were developed by Heidi Aisala (Aisala et al. 2026), by searching from a draft genome of *G. salaris* and tested as variable using the parasites from rainbow trout farms. This is the first study reporting a population structure utilising this set of markers.

There will be next generations of molecular tools for analysing the parasites. Updated molecular methods derived from whole genome sequencing were used by Konczal et al. (2020, 2021). By utilising 673,104 SNPs derived from whole genome sequencing, it was demonstrated that the heterozygosity in Tobago populations of *Gyrodactylus turnbulli* Harris, 1986 on the common aquarium fish guppy (*Poecilia reticulata* Peters) was higher than in Trinidad, measured as nucleotide diversity 0.0028 *vs* 0.0012. It was associated with widely spread frozen hybrid clones, not a general uprising of the variation.

For controlling and monitoring *G. salaris*, which is the main challenge for the Northern salmon stocks, the molecular markers displayed here are cost-effective and satisfactory toolset.

The American import *Oncohynchus mykiss* is the transmission hub

Rainbow trout is cultured worldwide. In North America and in Mexico, it is infected by *G. salmonis* Yin et Sproston (Leis et al. 2021) described from *Oncorhynchus clarkii* (Richardson), but this species has not been found in Europe. In exile, the farms necessarily have contacts with native species, and numerous salmonid-specific *Gyrodactylus* species have been recorded by ITS and COI, in addition to the *G. salaris* RBT clone studied here. A heterozygotic strain of *G. salaris* imported with fish from Ossetia (Black Sea) was found in Ladoga farm in 2011 (Blue clade in Fig. 1, COI KJ941020, ITS KM192262, Ieshko et al. 2015). A frozen hybrid *Gyrodactylus pomeraniae* Kuusela, Ziętara et Lumme, 2008 × *G. lavareti* Malmberg, 1957 was identified by ITS in Finland and Russia (Koski and Malmberg 1995, Kuusela et al. 2008, Ieshko et al. 2016).

A hybrid of *G. teuchis* with a close but distinct relative was detected by ITS in Jänisjärvi farm (AJ249350 *vs* KM192263, Ieshko et al. 2015). This hybrid strain was found (by RFLP) also in Polish farms both on rainbow trout and brown trout (Rokicka et al. 2007). *Gyrodactylus taimeni* Ergens, 1971 from *Hucho taimen* (Pallas) and *G. magnus* Konovalov, 1967 from Arctic graylling *Thymallus arcticus* (Pallas) were recorded in Vietnamese rainbow trout farms (Leis et al. 2021). *Gyrodactylus derjavinoides* replaced the name *G. derjavini* Mikailov, 1975 in European waters (Malmberg et al. 2007). Specimens from Norway and Sweden represented identical heterozygotic microsatellite genotype, suggesting a single clone (unpublished data). The original *G. derjavini* was found in rainbow trout farms on Iranian coast of the Caspian Sea (Malmberg et al. 2007).

Gyrodactylus salaris RBT strain has been confirmed by different molecular markers persistently in rainbow trout (*Oncorhynchus mykiss*) farms in Denmark, Norway, Sweden, Finland, Poland, Italy, and Russia (Koski and Malmberg 1995, Buchmann and Bresciani 1997, Hansen et al. 2003, 2007, Jørgensen et al. 2007, 2016, Kania et al. 2007, 2022, Rokicka et al. 2007, Evseyeva 2009, Paladini et al. 2009). The earliest molecular marker useful for detecting the *RBT* clone was the single nucleotide heterozygosity ITS-1068^{R}, reported in Norwegian non-pathogenic *F strain* on *Salvelinus alpinus* in Norway and in *O. mykiss* farms in Denmark (Kania et al. 2007).

The primary hybridisation

This first-step hybridisation creating the European *RBT* strain combined most probably haploid genomes from two inbred populations separated since preglacial era (d_{MCL} = 0.025) to an evolutionary stable situation H_e = 0.5 and H_o = 1, maintained for ever in abstinence. The *RBT* clone was heteroplasmic for two distant COI genes from the beginning. The hybridisation was a unique event, and the clone was derived from a single hybrid daughter. In retrospect, and as a self criticism, we believe that the mitochondrial heteroplasmy described first time in this paper was also overlooked or neglected, as an aberration of the paradigm of uniparental inheritance of mitochondrial DNA. However, the heteroplasmy opens the secret of the primary hybridisation. The red and purple clades in the mitochondrial tree (Fig. 1) can be hypothetically localised, one in the Balkans, and the second on the southern Baltic Basin, but anyway, they are very distant.

The d_{MCL} = 0.025 shows that the matrilines have diverged early, predating the latest glaciation. The suggested values of molecular divergence rate for COI in *Gyrodactylus* (subgenus *Limnonephrotus*) vary between 3.6 % to 13.7 % per million years, depending on the selected calibration points

(Kuusela et al 2007, Hahn et al. 2011, Mieszkowska et al. 2018). This leads to a range of 700 000 to 180 000 years of separation of the parents of the *RBT* strain. The distance of about 2.5–3.5 % is standard (Fig. 1) between the isolated parasite lineages on grayling in Europe, from Caucasus via Danube to England, and to the Baltic and the White Sea basins (Hansen et al. 2007, 2022). The phylogeography of the host, European graylings, was presented by Weiss et al. (2002), and it fits well to the diverged and isolated spatial of the parasite.

All 12 microsatellite loci of the *RBT* strain were permanently heterozygotes. This was a convincing proof that the strain is a frozen hybrid, derived from a single fertilisation, never reproducing by recombination and living in abstinence. The extant specimens are connected by millions of fissions. The parthenogenesis occurs without recombination. It might be mitotic (apomictic), or via premeiotic replication (Blanc et al. 2025). The *RBT* differs fundamentally from other *G. salaris* populations, where the parthenogenesis clearly leads to loss of heterozygosity (Tornio River, Lumme et al. in preparation).

The nuclear markers are not elucidating anything about the prehistory of the *RBT* clone, because there are no data from the hypothetical parents. Instead, the microsatellites are useful in understanding the *post coitum* history after the next host switch step in Kuitozero. The frozen hybrid clone on the industrially trafficked novel host is a kind of textbook example of the evolutionary role of asexual propagation. The main advantage is in transition and dispersal. Avoiding sex also means maintaining the evidently successful epistatic interactions, e.g., a possibly multigenic host recognition system. Sex within such a luckily preadapted clone equals selfing and may destroy the adaptive combinations, while creating new but untested (Peck and Waxman 2000). The successful farm history of the RBT clone shows that *G. salaris* is no slave of sex, if the genes happen to be in good position to each other and with the host (Schön and Martens 2003).

Introduction of the third genome: sexual recombination opens new opportunities

The secondary hybridisation occurred either in the Lake Kuitozero, or during the chain of transport and stocking the farm fish either in Russia or in Finland. An infection by *G. salaris* is rather asymptomatic both on rainbow trout farms and on the Baltic salmon, so transferring the parasite was not an indication of serious recklessness, only lack of knowledge and understanding. Both rainbow trout and landlocked salmon have been transferred in the upwaters of Kuitozero in the Finnish side of the border. The Russian authors (Shulman et al. 2007) explicitly suggested this as the source of infection: " [In the upper lakes in] Pista [watercourse] was released 9907 pcs. 2-year-old lake salmon from Lake Saimaa, where *G. salaris* can live (Annex № 6 to the Protocol of the 35th session of the Joint Russian-Finnish Commission on the use of trans- boundary water systems). Unreasonable releases of juveniles of unknown origin were also conducted by Finnish fishermen in 1993, 1995, 1996, 1998." (Report on Fish and Wild Animals No. 178)

After first shock, the infection in Kuitozero was not considered as seriously pathogenic by experienced Russian parasitologists (Shulman et al. 2007, Ieshko et al. 2012). If the source of Kuitozero infection was the stocked lake salmon from Saimaa, the list of possible paternal ancestry contacts is long, because the Saimaa salmon stock is maintained in a hatchery and rejuvenated by adults grown from juveniles stocked in diverse parts of the Saimaa water system. However, this chain was never confirmed, and the parasite was never studied in the Enonkoski hatchery maintaining the stock.

The male parent of the hybrid swarm of *G. salaris* in Kuitozero remains unknown. *Gyrodactylus salaris* is a persistent but low-frequency parasite on graylings (*Thymallus thymallus*) in the White Sea basin as well as in the Baltic basin (Meinilä et al. 2004, Kuusela et al. 2007, Hansen et al. 2007). On the White Sea salmon (*S. salar*) *G. salaris* was observed earlier only in the Keret' river, as the fatal infection which was introduced directly from Lake Onega (Kudersky et al. 2003, Kuusela et al. 2009).

It is not excluded that *G. salaris* existed on Kuitozero salmon, as an undetected commensal. The missing pure paternal genotypes and COI support this option. On the tolerant Baltic salmon host in the lakes Onega and Ladoga, the prevalence of *G. salaris* was extremely low (Ziętara et al. 2008a), and in normal fishing or fisheries practices it goes undetected.

Heterosis and balancing selection facilitates the genomic rejuvenation

The RBT clone was born in rainbow trout farm as a hybrid. Above we listed three other cases of adoption, and they all were hybrids as well. Considering the magnitude of the industry, the number of such transitions is low. The relative rarity of *Gyrodactylus* in rainbow trout industry suggests that a successful clone needs a rare combination of epistatic interactions preadapting it on just this host. It is not an accident that the succesful invaders are hybrids. Without sex, a parasite is not making very many experimental changes in the host recognition system, and even with sex, it may be rare.

As long as the clone is propagating asexually without recombination like the *RBT*, this lucky combination remains fixed. If the clone starts self sex, it will lose some of the beneficial interactions, but also the heterozygosity (genetic variation) via inbreeding (Peck and Waxman 2000). Frozen hybrid keeps the recessive harmful mutation silent. This is most important when the number of progeny is low, due to expensive hyperviviparity. The frozen hybrid is protected from the inbreeding erosion. The deleterious recessives are not expressed, but with time, their number only increases, a process called Muller's ratchet (Gabriel et al. 1993).

If the asexual life before the remolding of the genomes had led to accumulation of deleterious recessive alleles in the chromosomes, then it might be expressed as heterosis after the liberation of the sex, i.e., as a better viability and larger clones of the heterozygotes in comparison to the homozygotes. The relative frequencies of observed and expected heterozygotes among clones (Table 3) was H_o/

H_e = 1.32, which might be used as fitness value w_{het} = 1.32 versus homozygote w_{hom} = 1.00. In the conventional beanbag genetic exercise, this might lead to equal allelic frequencies in about 30 (sexual) generations from any start frequency. Harris (1998) counted 19 asexual generations in 120 days (120/19 = 6.3) in temperatures between 0.1 to 10°C in *Gyrodactylus gasterostei* Glaser, 1974. By single fission, 2^{19} is a large number. In the Kuitozero population, the fitness differences are mainly expressed as multiplication of the clones, so the simple model may be useless. Clonality makes the effective population size small as such (244 > 27), and the family size variation (104 – 1, mean 9.3) even smaller. The heterosis is counteracting these effects.

The equal allelic frequencies is also maximising the benefits of sex (Agrawal 2006). Equal allelic frequencies translate to maximum proportion of recombination within and between the chromosomes, exposing the harmful recessives to purifying selection. Maximising the combinations offers an option for adaptive selection. Finally, shuffling the alleles may lead to combinations which are preadapted for a novel host. Evolution is blind but opportunistic.

Successful hybrid clone also means narrow bottleneck. Asexual reproduction without recombination protects from inbreeding erosion, but harmful mutations accumulate further. Asexuality is often connected with polyploidisation, which protects the clone even longer from mutational meltdown (Lokki 1976, Suomalainen 1976, 1987). However, further evolution and adaptation is blocked. In many biological examples, sexual recombination is explained as the necessary mean of evolutionary rejuvenation (Bengtsson 2003, D'Souza et al. 2006, D'Souza and Michiels 2009, Maccari et al. 2013, Hojsgaard and Hörandl 2015). A rare sexual intercourse may lead to a narrow population bottleneck and kind of saltatory evolution, even speciation, observable *a posteriori* in the phylogenetic histories (Birky and Barraclough 2009).

The *RBT* clone was a product of a single fertilisation, and it achieved a successful new life on a new host, expanded all around the old Europe, and it probably met numerous new opportunties. The farmed rainbow trouts escape, or are stocked for recreational fishing, but so far, only few transitions of the COI marker into other species are known. In Norway to Arctic charr and Atlantic salmon, and in Russia, to the salmon in Lake Kuitozero. The recently observed transitions to Barents Sea rivers need continuous monitoring, and are potentially a high risk for the most valuable Russian and Norwegian salmon stocks (Hansen et al. 2022).

The secondary hybridisation in Kuitozero has led to a production of a large collection of hopeful clones. The public satellite images from Kuitozero show a massive increase in the rainbow trout production, and the follow-up of the salmon populations is urgent. The liberated sexuality among the Kuitozero parasites indicates that the components of the sexual attractiveness, mate recognition, self-recognition, and lust are now present in optimal combinations, and the benefits, costs, advantages and joys of sex are utilised maximally as long as the situation is on (Allen and Lynch 2008, Gorelick and Carpinone 2009).

Kuitozero infection is an example of evolutionary rejuvenation

The variation of the asexual modes of reproduction and their combinations with sex is exhaustive (Bell 1985, Bengtsson 2003). It is obvious that sexuality is the primary mode of animal life, and the many forms of asexuality are derived independently, many times and in most variable modes, as responses to evolutionary challenges (Schön et al. 2009). The advantage of uniparental propagation is demonstrated when a pathogen is rapidly spreading in a virgin land, but the recombination is needed to make new beginnings. Without recombination, a clone is not adaptive, but doomed to lowering success because of evolving host resistance (including vaccinations), and ultimately to extinction, due to the accumulation of deleterious mutations (Muller's ratchet: Gabriel et al. 1993, Lynch et al. 1993, Lushai and Loxdale 2002). Among free-living and parasitic flatworms, a wide spectrum of complex life cycles and associated modifications of sexuality are found (Ramm 2017). Good examples of alternating sexual propagation and often polyploid parthenogenesis are found in the well-studied planarians (D'Souza et al. 2006, D'Souza and Michiels 2009), which are classical demonstrations of the power and elegance of the pre-molecular genetics and cytology.

Predominant clonality and rare intervening recombination are indeed common in the evolution of many parasites and pathogens, especially among viruses, bacteria, and fungi (Detwiler and Criscione 2010, Tibayrenc and Ayala 2012). *Gyrodactylus* species obviously are utilising this evolutionary strategy and the opportunities created by the fisheries industry in the modern world.

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