Global Phylogeography of Salmonid Ectoparasites of the Genus *Gyrodactylus*, with an Emphasis on the Origin of the Circumpolar *Gyrodactylus salmositica* (Platyhelminthes: Monogenea)

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ABSTRACT: The *wageneri* species group of *Gyrodactylus* contains the following molecularly confirmed salmonid parasites in Asia: *Gyrodactylus taimeni* Ergens, 1971, *Gyrodactylus magnus* Konovalov, 1967, *Gyrodactylus brachymystacis* Ergens, 1978, and *Gyrodactylus derjavini* Mikhailov, 1975; in Europe it contains the following: *Gyrodactylus derjavinoides* Malmberg, Collins, Cunningham, and Jalali, 2007, *Gyrodactylus truttae* Gläser, 1974, *Gyrodactylus teuchis* Lautraite, Blanc, Thiery, Daniel, and Vigneulle, 1999, *Gyrodactylus lavareti* Malmberg, 1956, *Gyrodactylus salvelini* Kuusela, Ziętara, and Lumme, 2008 (presented herein as a junior synonym of *Gyrodactylus salmositica* Yin and Sproston, 1948), and *Gyrodactylus salaris* Malmberg, 1957, with the lone confirmed North American exception being *G. salmositica*. The mitochondrial DNA (*cox1*, 1545 bp) of this group shows a star-like phylogenetic expansion that began 2.05 ± 0.4 million years ago (mya), estimated from the mean distance of the *cox1* gene ($d_{MCL} = 0.267$) using a tentative, potentially high-end, divergence rate of 0.13/Myr. European *G. salaris* on *Thymallus thymallus* and Asian *G. magnus* on *Thymallus arcticus* have been separated for 1.95 Myr ($d_{MCL} = 0.253$). The nuclear ITS rDNA region (1,245 bp) of *G. salmositica* was nearly uniform among North American populations of *Oncorhynchus mykiss*, *Oncorhynchus clarkii*, *Oncorhynchus nerka*, *Salvelinus fontinalis*, and *Salmo salar* (and non-native *Salmo trutta*) as well as on *Salvelinus alpinus* (under the synonym *G. salvelini*) from Lake Inari, Finland. *Gyrodactylus salmositica* is distal in a monophyletic subclade labeled by an apomorphic 56 bp insertion in the ITS1, shared by the European parasites *G. lavareti* (host: *Coregonus lavaretus*), *Gyrodactylus pomeraniae* Kuusela, Ziętara, and Lumme, 2008 (host: *Rutilus rutilus*), and *Gyrodactylus bliccensis* Gläser, 1974 (host: *Alburnus alburnus*). This subphylogeny suggests that a particular host switch from cyprinids to salmonids may have occurred less than 1.8 mya in the Old World [$d_{MCL} = 0.234$ *G. pomeraniae* vs (*G. salmositica*, *G. lavareti*)] and possibly again among coregonine hosts and *Salvelinus* 1.2 mya ($d_{MCL} = 0.156$). Although hypothetical, a transition from coregonines to charr (notably the widely distributed and adaptable *Salvelinus alpinus*) potentially could have occurred in a proglacial refugium leading to circumpolar distribution of *G. salmositica* and a secondary transition to other North American hosts. The maximum *cox1* genetic distance within *G. salmositica* on all hosts was $d_{MCL} = 0.032$, at the same level as in multihosted European *G. salaris* ($d_{MCL} = 0.032$), suggesting circa 250,000 yr of population expansion with these parasites since a temporal, coinciding bottleneck.

KEY WORDS: *Gyrodactylus salmositica*, circumpolar, Monogenea, North America, Finland

Monogenean fish parasites within the genus *Gyrodactylus* are generally inconspicuous. In evolutionary equilibrium, the host and parasite appear generally coadapted, typically not causing harm to the host fish, and while their presence at fish farms may garner attention, the clinical signs are often mild (Cone and Cusack, 1988; Rokicka et al., 2007). However, when a parasite species infects a suitable naïve host, there can be serious consequences, such as the infamous invasion of *Gyrodactylus salaris* Malmberg, 1957, of Baltic origin on wild Atlantic salmon (*Salmo salar*) populations in Norway (Johnsen and Jensen, 1991). In this case, 3 separate invasions were identified by mtDNA and traced to commercial activity

(Hansen et al. 2003). Prompted by these incidents, *G. salaris* became the scientific flagship for the genus (e.g., Bakke et al., 2007; Lumme et al., 2015), and the associated pragmatic needs rejuvenated research within this group (e.g., Gilmore et al., 2010, 2012 in North America). Still, the invasions that occurred in Norway, as well as a single instance in the White Sea basin, are the only clinically recognized pathological outbreaks of these “Russian Dolls” (Bakke et al., 2007) among wild salmon populations.

The application of molecular methods advanced taxonomic research in regard to *Gyrodactylus*, with the first successful genetic markers being the 18S and ITS (ITS1–5.8S–ITS2) region of the nuclear ribosomal RNA gene cassette (Cunningham et al. 1995; Cunningham 1997; Matějusková et al., 2001; Ziętara and Lumme 2002). Later the mitochondrial marker *cox1*

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improved resolution within species (Meinilä et al., 2002; Hansen et al., 2003, 2007). The ITS region and *cox1* gene segments represent the gold standard for clinical and taxonomic “barcoding” as well as phylogenetic reconstructions of the genus (Vanhove et al., 2013). The primers available for the ITS segment are rather universal and widely used, systematically connecting parasites on different continents, including diverse subgenera (Ziętara et al., 2012; Hansen et al., 2012; Reyda et al., 2019). The published primers for mitochondrial DNA (*cox1*) are more specific, developed for and targeting the species in subgenus *Gyrodactylus* (*Limnephrotus*), and in particular, the *wagneri* species group (Malmberg, 1970). Although potentially coincidental, all *Gyrodactylus* species that infect salmonids, except *Gyrodactylus colemanensis* Mizelle and Kritsky, 1967 (You et al., 2011), belong to this group. This subgenus is primarily restricted to freshwaters of the Old World, with the only continental outliers confirmed through molecular methods being *Gyrodactylus salmonis* Yin and Sproston, 1948, on salmonids and *Gyrodactylus crysoleucas* Mizelle and Kritsky, 1967, from a commercially farmed cyprinid, Golden Shiner *Notemigonus crysoleucas* (Leis et al., 2016). Morphologically identified North American species *Gyrodactylus nerkae* Cone, Beverley-Burton, Wiles, and McDonald, 1983 (Cone et al., 1983) and *Gyrodactylus lucii* Kulakovskaya, 1952 (Cone and Dechtiar, 1986) have not yet received molecular confirmation regarding their placement within this clade.

The *wagneri* group has exceptional diversity of host species within the phylogenetic time scale, with infections occurring on at least 7 fish families, although the species and strains remain quite host specific within the ecological time scale (Ziętara and Lumme, 2002). Host specificity has been thoroughly evaluated in Norway among strains of *G. salaris* (including the synonym *Gyrodactylus thymalli* Žitňan, 1960) on grayling (*Thymallus thymallus*), Arctic charr (*Salvelinus alpinus*), rainbow trout (*Oncorhynchus mykiss*), and Atlantic salmon (*S. salar*). All shared nearly identical ITS region sequences (1 to 4 divergent nucleotides) and were accepted as belonging to the same *Gyrodactylus* species, most recently by Fromm et al. (2014), despite differences in mitochondrial haplotype and host affinity (Bakke et al., 2007; Hansen et al., 2007; Olstad et al., 2007).

Herein, we focus on *G. salmonis*, which has been observed on several North American salmonid species within the genera *Oncorhynchus*, *Salvelinus*, and *Salmo* and frequently identified from aquaculture en-

vironments (Cusack and Cone 1986; Cone and Cusack 1988). The first molecular identification of this species by Gilmore et al. (2010) showed that *G. salmonis* and *Gyrodactylus salvelini* Kuusela, Ziętara & Lumme, 2008, described from Lake Inari in Finland on *Salvelinus alpinus* (Kuusela et al., 2008), shared identical ITS as well as levels of mitochondrial DNA variation typically observed within a species. This situation appeared corollary to that of the aforementioned *G. salaris* (synonym: *G. thymalli*).

In this study, we show that *G. salvelini* is a junior synonym of *G. salmonis*, and then we describe the phylogeographic framework for this parasite through analysis of new ITS and mitochondrial DNA data. Specimens were collected from 8 rainbow trout hatcheries in 3 states in the United States, complementing earlier observations in Canada (Gilmore et al., 2010, 2012) and Mexico (Rubio-Godoy et al., 2012). Data were also collected from 2 additional salmonid infecting *Gyrodactylus* spp. from eastern Asia. This geographic range expansion increases our understanding of the evolution within host-parasite communities while emphasizing the Holarctic circumpolar faunal divisions and connections. Additionally, a recent global evolutionary presentation of the genus *Salvelinus* (Esin and Markevich, 2018) offers a comprehensive framework for further research on *G. salmonis* as well as other salmonid parasites and their hosts in the phylogeographic theater of the Holarctic.

MATERIALS AND METHODS

Samples of *G. salmonis*, preserved in 70% ethanol, were obtained in 2011 from 8 rainbow trout farms in the United States, 5 in Washington (Eells Springs SFH [State Fish Hatchery]), Naselle SFH, Spokane SFH, Ford SFH, and a private farm on the Olympic Peninsula), 2 in Montana (Giant Springs SFH, Big Springs SFH), and 1 in Pennsylvania (Lamar Fish Technology Center; USFWS). All samples were collected from subclinical infections. We also used sequences presented in previously published studies from Europe by Kuusela et al. (2008), the United States, and Canada by Gilmore et al. (2010, 2012), as well as Mexico by Rubio-Godoy et al. (2012). Samples of *Gyrodactylus magnus* Konovalov, 1967 (3 specimens) and *Gyrodactylus taimeni* Ergens, 1971 (7 specimens) were obtained from an *O. mykiss* farm in Bàn Khoang village, Sapa District, Lào Cai Province, northern Vietnam. Two specimens of *G. magnus* from Vladivostok, Russia, were also sequenced (courtesy of A. Ermolenko and M. Ziętara).

Morphological analysis

Gyrodactylus, preserved in 70% ethanol, from Naselle SFH were briefly rehydrated in tap water, stained in Gomori's trichrome, cleared in clove oil, and mounted in Canada balsam. Morphometric evaluation was completed following Malmberg (1970). Voucher slides were submitted to the Smithsonian National Museum of Natural History (Washington, D.C.) under Accessions USNM 1655828-1655834. Photographs and measurements of *G. salmonis* (fixed in formalin and mounted in glycerin jelly) collected from cutthroat trout *Oncorhynchus clarkii* from the Nanaimo River Hatchery in 1981 were used for morphological comparison (courtesy of Dr. David Cone). Photographs of type specimens for *G. salvelini* were also examined. Measurements are reported in μm .

DNA extraction

Parasite DNA was extracted by digesting individual specimens in 10 μl of lysis solution with a final concentration as follows: 1 $\times$ PCR buffer, 0.45% (v/v) Tween 20, 0.45% (v/v) NP 40, and 60 $\mu\text{g/ml}$ proteinase K (Lumme et al., 2017). The tubes were incubated at 65°C for 25 min to allow for proteinase K digestion, followed by 95°C for 10 min to denature the proteinase and then cooled to 4°C.

PCR amplification of the ITS region and direct sequencing

The entire ITS region of the ribosomal DNA array (spanning ITS1–5.8S–ITS2 and flanked by fragments of 18S and 28S) was amplified with *ITS1F* (5'-GTTTC CGTAG GTGAA CCT) (Ziętara et al., 2000; Rokicka et al., 2007) and *ITS2R* (5'-GGTAA TCACG CTTGA ATC) primers (Ziętara et al., 2000; Ziętara and Lumme, 2002). The PCR reaction contained 2 μl of lysate, 1 $\times$ PCR buffer, 2 mM MgCl_2 , 1 μM of each primer, 200 μM of each dNTP, and 0.4 units of *Taq* polymerase (Fermentas) in final volume of 20 μl . Thermocycler parameters were as follows: 3 min at 95°C, 37 cycles of 94°C, 48°C, and 72°C for 1 min each, followed by 7 min at 72°C and concluding with cooling to 4°C. The amplified fragments were purified from the agarose gel using ExoSAP following the manufacturer's procedure and sequenced directly with *ITS1F* and *ITS2R* as well as with 2 internal primers in 5.8S region, *ITS1R* (5'-ATTTG CGTTC GAGAG ACCG) and *ITS2F* (5'-TGGTG GATCA CTCGG CTCA), as described earlier (Ziętara and Lumme, 2003, 2004; Ziętara et al., 2008).

Amplification and sequencing of mitochondrial DNA

The analysis of mitochondrial DNA was conducted as previously described (Meinilä et al., 2002; Ziętara et al., 2006). The 20 μl PCR reaction consisted of 1 $\times$ PCR buffer, 0.2 mM dNTPs, 2 mM MgCl_2 , 1 μM of each primer, 0.5 U of Fermentas *Taq* DNA polymerase, and 2 μl of extracted DNA. The *cox1* gene was amplified either as a single fragment or as 2 overlapping fragments with *Trp2F* 5'-TTTTA GACGA TTTGT TTTCA and *Thr2R* 5'-ATAGA TTGCT TG-GTA TTACA primers. The 3' end was amplified with *FCox7* 5'-TTTTC AATAG GTATG GACGT and *Thr2R*. Thermocycler parameters were as follows: 94°C for 3 min, 38 cycles of 94°C for 30 sec, 48°C for 1 min, and 72°C for 1 min 50 sec, followed by 72°C for 7 min, and a final cooling to 4°C.

Data analysis

The phylogenetic hypotheses presented for the ITS region were constructed using Kimura's 2 parameter distance (K2P) and analysis of the Maximum Composite Likelihood distance (d_{MCL}) for the mitochondrial gene was completed with the MEGA7 program package (Kumar et al., 2016). Final checking and additional data handling were completed in MEGA X (Kumar et al., 2018). Phylogenetic trees were constructed with the Neighbor Joining or Maximum Likelihood methods. The optimal model for distance estimates and mitochondrial phylogenetic trees was GTR + G + I, with shape parameter $\alpha = 1.28$. The trees produced were quite robust as all alternative methods and options produced the same topology along with similar bootstrap values.

In order to transform the mitochondrial genetic distance estimates (d_{MCL}) to reflect more illustrative geological times, we used the value of 0.13 divergence per million years, which was calculated for *G. salaris* (Kuusela et al., 2007) and recalibrated through the stickleback parasite *G. arcuatus* from the previously glaciated region of northern Europe (Lumme, Mäkinen, et al., 2016). The value 0.13 may represent the upper end of the divergence values and could be replaced if reliable calibration points can be presented in the future.

The sequences produced in this study were deposited in GenBank with accession numbers (MN854069–78 for the *cox1* gene; MN850538–48 for the ITS region). Previously published sequences included in the phylogenetic analyses were from

Kuusela et al. (2007) (*G. salaris*, including the synonym *G. thymalli*), Kuusela et al. (2008) (*G. salmonis* clade, including the synonym *G. salvelini*), Ziętara et al. (2010) (*G. lucii*, *G. salaris* “alien” *cox1*), Hahn et al. (2015) (*Gyrodactylus teuchis* Lautraite, Blanc, Thiery, Daniel, and Vigneulle, 1999, and *Gyrodactylus truttae* Gläser, 1974), Ieshko et al. (2015) (*G. teuchis*), and Lumme, Anttila, et al. (2016) (*G. salaris*). The phylogenetic reconstructions were generally based on complete 1,545 bp sequences of the *cox1* gene, although some shorter sequences (e.g., *G. teuchis* [1,355 bp] and *G. truttae* [1,349 bp]; Hahn et al., 2015) were included using pairwise deletion options.

RESULTS

Gyrodactylus salvelini is a junior synonym of *Gyrodactylus salmonis*

Measurements, reported in μm as a range, were obtained from 7 specimens from Naselle SFH. The parasites were identified as *G. salmonis*, sharing consistent morphology and characteristics with the report by Mueller (1936), although slightly larger than measurements reported by Cone et al. (1983). Anchors were 62–70 long, point 32–34, and shaft 52–59. Marginal hooks were 40–48 in total length, with a sickle 7–9 long. Ventral bar featured minute bump-like anterolateral processes, 25–26 in transverse width and 6–8 in medial length, and a total length (including the posterior membrane) of 21–25. Overall the morphology and measurements were very similar to *G. salmonis* collected from *O. clarkii* at the Nanaimo River Hatchery: anchors (61–69 long, 31–37 point, shaft 48–55), marginal hook (45–48 total length, 8–9 sickle length), and ventral bar (25 in transverse width; 9–12 in medial length; 18–28 in total length). The most significant morphological finding was that the size and shape of the taxonomically important marginal hook sickle were essentially identical among the specimens of *G. salmonis* identified during this study, those collected from *O. clarkii* during the redescription by Cone et al. (1983), as well as those from the description of *G. salvelini*. While the anchors of North American specimens (66 μm) were smaller than those described for *G. salvelini* (87 μm ; Kuusela et al. 2008), considerable seasonal variation in anchor size, apparently influenced by water temperature, has been noted with other *Gyrodactylus* species (Mo et al. 1991, 1993; Ondračková et al. 2020).

Molecular analysis revealed that the ITS sequence data was nearly identical (99.84–100% similarity)

among all specimens, including *G. salvelini*, which was identical to *G. salmonis* sequenced from Montana, Pennsylvania, and Washington. The variation of the *cox1* gene ($d_{\text{MCL}} = 0.032$) for all specimens, including *G. salvelini*, was within a range typically observed for a species sampled across geographical regions. We conclude that *G. salvelini* Kuusela, Ziętara & Lumme, 2008, is a junior synonym of *G. salmonis* Yin and Sproston, 1948. The GenBank accessions of the ITS and *cox1* submitted as *G. salvelini* have been changed by the authors to *G. salmonis* (EF113106, EF524572). This conclusion allows us to present *G. salmonis* as the first molecularly confirmed circumpolar species of freshwater *Gyrodactylus*.

ITS2 phylogeny

A phylogenetic hypothesis based on the nuclear ITS2 fragment for species in the *wagneri* group, including *Gyrodactylus crysoleucas*, the only other North American species confirmed to be in the *wagneri* group, is presented in Figure 1. A noteworthy observation from this phylogeny is that the salmonid parasites do not form a common clade but appear scattered despite their substantial representation in the database. The basal portion of the phylogenetic hypothesis in Figure 1 is rather unresolved, indicating rapid splitting and allopatric isolation into independently developing lineages. The separate clade of *G. pomeraniae*, *G. lavareti*, and *G. salmonis* is moderately supported in Figure 1 (74%) and featured a systematically informative insertion in the ITS region that deserved further analysis.

The nuclear ITS region shows the origin of *G. salmonis*

A phylogenetic hypothesis displaying the origin of *G. salmonis* is presented based on the sequenced regions of the ITS1–5.8S–ITS2 (~1,250 bp; Fig. 2). In the phylogeny leading to *G. salmonis*, there is a unique, apomorphic 56 bp insertion observed within the hypervariable segment of the ITS1 (also see Kuusela et al., 2008). This insertion was identified in *G. salmonis* (all hosts), *G. lavareti* Malmberg, 1956 (host: *Coregonus lavaretus*; several localities in Finland), *Gyrodactylus pomeraniae* Kuusela, Ziętara, and Lumme, 2008 (host: *Rutilus rutilus*; Poland and Belgium), and *Gyrodactylus bliccensis* Gläser, 1974 (host: *Alburnus alburnus*; Czech Republic, River Morava; Matějusová et al., 2001) and in the hybrid of *G. pomeraniae* × *Gyrodactylus lavareti* (host: *O. mykiss*, Finland and Russian Karelia). The cyprinid parasite *Gyrodactylus*

Figure 1. A phylogenetic hypothesis of the ITS2 region for *Gyrodactylus* species within the *wageneri* group, including all known to infect salmonids from Europe, Asia, and North America (marked red). The tree is rooted with non-*wageneri* members of subgenus *Gyrodactylus* (*Limnephrotus*). Note that the North American *Gyrodactylus crysoleucas* is included. Neighbor Joining, K2P distances.

Figure 2. Left: Phylogenetic hypothesis of *Gyrodactylus salmonis* and the nearest relatives based on alignments of the long ITS1-5.8S-ITS2 sequence. The scale bar represents the number of base substitutions per site (K2P). Right: Mitochondrial Maximum Likelihood tree of the subclade that contains the 56 bp insertion in the ITS1 of *G. salmonis*.

Figure 3. A phylogenetic hypothesis showing the clade-specific evolution of the 56 bp insertion in the ITS1 region.

ouluensis Kuusela, Ziętara, and Lumme, 2008 (host: *R. rutilus*) belongs to the same clade but was basally located and, interestingly, lacked this 56 bp insertion. The ITS tree was rooted with a sister outgroup consisting of the following: *Gyrodactylus leucisci* Žitňan, 1964 (host: *Leuciscus idus*), an undescribed *Gyrodactylus* sp. (host: *R. rutilus*), and *Gyrodactylus osmeri* Rumyantsev, Shul'man et Ieshko, 1998 (host: *Osmerus eperlanus*).

The 56 bp insertion in the hypervariable segment of the ITS1 apparently originated from the Intergenic Spacer (IGS) segment of the ribosomal DNA cassette, a sequence that has previously been identified from *G. salaris* (see Kuusela et al., 2008). The fragment was localized in the nonrepeated segment, position 889–948, in the 2.62 kb PCR product of the IGS (GenBank AJ276032; Collins and Cunningham 2000). The phylogenetic evolution of the 56 bp fragment is illustrated in Figure 3.

The genotype among all isolates of *G. salmonis*, including the Finnish sample, was generally uniform. There were also a few shorter published ITS sequences from *G. salmonis* on *Salvelinus fontinalis* (Nova Scotia), *Salmo salar* (Maine), *O. mykiss* (Washington, British Columbia), and *O. clarkii* (British Columbia; Gilmore et al., 2010), as well as on *Onchorhynchus nerka* (British Columbia; Gilmore et al., 2012), which were identical with the standard C¹⁶ A⁸⁹⁸. A heterozygous variant M¹⁶ W⁸⁹⁸ of the ITS region, differing by 2 transversions, was observed in 3 rainbow trout farms in the North American samples (Big Springs SFH in Montana and Naselle SFH and Ford SFH in Washington). The heterozygous clone in 3 separate North American facilities appears to be a cross between the standard *G. salmonis* and a derived strain A¹⁶ T⁸⁹⁸, which did not appear in our study. Site 898 was re-

ported as either T or A in the 13 sequences from Mexico, but site 16 on the 5' end was missing from these 1,168 bp-long samples (Rubio-Godoy et al., 2012). In our material, the M¹⁶ W⁸⁹⁸ heterozygote was combined with more than 1 mitochondrial haplotype (Fig. 2), suggesting this was not a single clone but was produced through repeated hybridizations.

Mitochondrial phylogeny of salmonid parasites in the *wageneri* group

We used a mitochondrial phylogenetic hypothesis of 16 named species within the *wageneri* group for rooting and scaling the phylogenetic clade containing *G. salmonis* (Fig. 4). The key finding, observed in both nuclear and mitochondrial DNA analyses, was the lack of a salmonid specific clade. A moderately supported but early diverged clade (77% bootstrap support in NJ, only 54% in ML tree) included the mitochondrial lineages of *G. magnus* and *G. salaris*. The type host of *G. magnus* is Arctic grayling (*Thymallus arcticus*) in eastern Russia; it was also found in an *O. mykiss* farm in Vietnam and sequenced on Manchurian trout *Brachymystax lenok* as *Gyrodactylus brachymystacis* Ergens, 1978, by Gilmore et al. (2010). The *G. magnus* clade differs from *G. salaris* by $d_{MCL} = 0.253$, with both lineages infecting *Thymallus* spp. on opposite regions of the continent suggesting almost 2 Myr of allopatric separation. The allopatric variation of *G. salaris* on European grayling (*T. thymallus*) suggested grayling was the original host of *G. salaris*, which then transferred to *S. salar* via hybridization after the Eemian interglacial period 130 thousand years ago (kya) in the primordial refugium maintaining the later-evolving Baltic salmon stocks (Kuusela et al., 2008). The maximum divergence measured within the

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Figure 4. A mitochondrial phylogenetic hypothesis of selected species of *Gyrodactylus* within the *wagneri* species group. Salmonid parasites shown in red. *Gyrodactylus lucii* on Northern Pike (*Esox lucius*) and 4 allopatric species on Eurasian Minnow (*Phoxinus phoxinus*) were included for scaling, displaying the early divergence of the salmonid parasites. The Neighbor Joining tree was based on maximum composite likelihood distances (d_{MCL}) of 135 complete *cox1* sequences (1,545 bp). The rate variation among sites was modeled with GTR model with gamma distribution (shape parameter 1.28).

Baltic salmon specific clade of *G. salaris* was $d_{MCL} = 0.011$ (85 kya).

DISCUSSION

The taxonomic history of *Gyrodactylus salmonis*

Gyrodactylus salmonis was originally described as “*Gyrodactylus elegans*, variety B” from various trout species examined from Ithaca, New York, U.S.A., as well as the state of Washington, mentioning that forms of the parasite collected from cutthroat trout, brown trout, brook trout, and rainbow trout all shared “uniform characters” (Mueller, 1936). Then Yin and Sproston (1948), with whom the taxonomic authority rests, mentioned measurement data from Mueller (1936) for *G. elegans* but also included the subspecies *salmo-*

nis from “various trout in the U.S.A.” The most thorough description was completed by Cone et al. (1983), where this parasite was redescribed as *G. salmonis* Yin and Sproston, 1948, using specimens collected from hatchery raised cutthroat trout. Unfortunately, the Nanaimo River Fish Hatchery, where Cone et al. (1983) redescribed *G. salmonis*, no longer produces cutthroat trout that could have potentially hosted *G. salmonis* for comparison. In this study, we collected specimens identified as *G. salmonis* from rainbow trout from the state of Washington, both of which were mentioned by Mueller (1936), to compare with *G. salvelini*.

Gilmore et al. (2010) mentioned differences in anchor size regarding why they did not synonymize *G. salmonis* and *G. salvelini*. However, several studies have shown that variation in anchor size for

Gyrodactylus spp. can occur seasonally and be quite substantial (Mo, 1991, 1993; Ondračková et al. 2020), with differences up to ~ 20 μm in the case of *G. salaris* (Mo, 1991). Furthermore, Ondračková et al. (2020) found significant differences among the anchor sizes of *Gyrodactylus* specimens from different continents. While synonymization based on morphology alone would not be convincing in this case, with the only impediment being incongruent anchor sizes, the molecular data clearly connect *G. salmonis* and *G. salvelini*. The ITS data were identical among *G. salvelini* and several North American *G. salmonis* specimens. The ITS region is the most widely used sequence in *Gyrodactylus* taxonomy because of its high level of variability even among closely related species. The authors are unaware of any phylogenetic analysis where 2 *Gyrodactylus* samples shared identical ITS1/5.8S/ITS2 sequences and were still considered separate species. Additionally, the similarity of the mitochondrial sequences was consistent with a single species sampled across a geographic region, further supporting the conspecific nature of *G. salmonis* and *G. salvelini*. It is important to note that molecular data for *G. salmonis* were unavailable when *G. salvelini* was described, making it difficult to conceive that the parasite could have been found on continents separated by a marine environment. If the molecular data had been available at that time, the species would have been identified as *G. salmonis*. We conclude, based on morphological and genetic data, that *G. salvelini* is a junior synonym of *G. salmonis*.

Mitochondrial phylogeny of salmonid parasites in the *wagneri* group

The global phylogenetic hypothesis of selected *wagneri* group species (Fig. 5) separated most salmonid parasites as ancient lineages with very long “barcoding gaps,” extending well beyond the $4\times$ rule to separate species (Birky and Barraclough, 2009). The *cox1* tree suggested a star-like expansion (Fig. 5) from the Most Recent Common Ancestor (MRCA) for the *wagneri* group with a nearly simultaneous separation of numerous lineages 1.64 to 2.48 million years ago (mya; $d_{\text{MCL}} = 0.213$ to 0.322) leading to the extant species presently observed. The mean genetic distance between the distal OTUs was $d_{\text{MCL}} = 0.267$, suggesting the timing of the MRCA as 2.05 mya.

The range of the mitochondrial variation within *G. salmonis* on all hosts (Fig. 4; max $d_{\text{MCL}} = 0.032$) represents the latest wave of expansion since a possible glacial bottleneck. It is similar to the variation of *G.*

salaris on European hosts, including the original hosts *S. salar*, *Salmo letnica*, and *T. thymallus*, as well as the imported *O. mykiss* and *S. fontinalis* (max $d_{\text{MCL}} = 0.032$). The circumpolar *Gyrodactylus arcuatus* Bychowsky, 1933, on *Gasterosteus aculeatus* displays a similar level of mitochondrial variation (max $d_{\text{MCL}} = 0.040$; Lumme, Mäkinen, et al., 2016). The variation observed with *G. arcuatus* apparently originated on the Atlantic side of the globe because only a single strayed haplotype of the Barents Sea cluster was found in samples collected in the Pacific, from Vladivostok, Russia, as well as Puget Sound, Washington, U.S.A. (Lumme Mäkinen, et al., 2016). It is important to note that *G. arcuatus* is not a *wagneri* group species and is capable of spreading in marine environments, although the latest expansion with this species almost certainly occurred within continental, freshwater refugia.

The amount of interspecific variation in Asian *G. taimeni* (native hosts: *Hucho taimen*, *B. lenok*), Asian *G. magnus* (hosts: *T. arcticus*, *Brachymystax lenok*), Caspian *Gyrodactylus derjavini* Mikhailov, 1975 (host: *Salmo trutta caspius*), and European *Gyrodactylus derjavinioides* Malmberg, Collins, Cunningham, and Jalali, 2007 (host: *S. trutta*) is unknown, because these species have not been extensively sampled in their native range. The parasite strains “adopted” by the globally trafficked *O. mykiss* may be single, widely spread clones, thereby producing a deflated impression of the variation among the parasite species in the wild as some of these farm strains have not been observed in wild populations. Conversely, the variation among the 4 *cox1* haplotypes in *G. salmonis* from *O. mykiss* farms in the United States suggests these infections were not spread from 1 source, but the parasite was introduced to farms during several independent events, probably with several fish provenances. In Europe, the almost invariable *G. truttae* was analyzed on many *S. trutta* populations, suggesting complete geographical sampling coverage. However, the effects of modern human-facilitated dispersion are difficult to exclude, even when sampling among wild populations (Hahn et al., 2015).

The *wagneri* species group of parasites is freshwater bound

The distribution of *G. salmonis* is circumpolar and connected with species of *Salvelinus* and *Onchorhynchus*, which both have anadromous and landlocked forms. The continuous distribution of *S. alpinus* from Scandinavia to the Bering Strait and North America suggests a continuous distribution

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Figure 5. A radial tree based on the mitochondrial *cox1* gene of selected distal (extant) taxonomic units within the *wagneri* group. The data were reduced from Figure 4 for clarity and supplemented with a few unpublished taxa. Mean distance between the distal tips $d_{MCL} = 0.267$.

of *G. salmonis* as well, but due to freshwater dependency of the *wagneri* species group, we predict the parasite to be found exclusively in freshwaters (e.g., Lake Inari) and likely on the numerous Siberian relict isolates of *Salvelinus* (e.g., *Salvelinus boganidae* Berg, 1926, *Salvelinus czerskii* Drjagin, 1932, *Salvelinus drjagini* Logasschev, 1940, *Salvelinus jacuticus* Borisov, 1932, *Salvelinus lepechini* (Gmelin 1788), *Salvelinus taimyricus* Michin, 1949, *Salvelinus taranetzi* Kaganowsky, 1955, *Salvelinus tolmachoffi* Berg, 1926, and *Salvelinus elgyticus* Viktorovsky et Glubokovsky, 1981; all described as separate species in the *Atlas of Russian Freshwater Fishes* by Reshetnikov, 2003). These Russian charr species are likely periglacial relicts dispersed along

the continuous freshwater habitats associated with the continental ice during the last glacial period. A recent comprehensive review suggested that dispersion of *S. alpinus* from a Siberian refugium both westwards and eastwards may have left behind the numerous isolates (Esin and Markevich, 2018). This may also be the case regarding the origin and spread of *G. salmonis*. The periglacial lake system in North America has been well illustrated by Power (2002), and the ice age lakes in northeastern Europe and Asia have been described by Mangerud et al. (2002), among others. Despite the scientific interest in charr evolution (Magnan et al., 2002), very little is known about the “parasite-supported phylogeography” of the genus. Parasite sampling among the salmonid populations of

Coregonus, *Thymallus*, *Oncorhynchus*, and *Salvelinus* isolated along the northern edge of the Eurasian and North American continents would have the potential to be especially productive, likely shedding light on the general processes of biogeography. Additionally, the recent observation of *Gyrodactylus birmani* Konavolov, 1967, and *Gyrodactylus* sp. on *Salvelinus albus* and *Salvelinus schmidtii* in Kronotsky Lake, Kamchatka Peninsula (Sokolov and Gordeev, 2014) are quite interesting and warrant molecular confirmation.

Herein, we suggest the wide distribution and limited variation of *G. salmonis* is connected with a relatively late transition to *S. alpinus*, possibly in a continental European refugium where paleolithic fossils of both *Coregonus* and *Salvelinus*, in addition to *Thymallus*, *Salmo*, *Esox*, and *Cottus*, have been found in sediments produced during the Last Glacial Maximum (Lõugas et al., 2013), followed by a subsequent spread along the northern continental margin of freshwater habitat associated with the genus *Salvelinus* (see Esin and Markevich, 2018). The pattern observed in the parasites on grayling, *G. salaris* (synonym: *G. thymalli*), on *T. thymallus* in Europe and *G. magnus* (possibly synonymous with *G. brachymystacis*) on *T. arcticus* in eastern Asia is similar. However, the allopatric clades apparently separated much earlier ($d_{MCL} = 0.253$) and were not mixed during the continent-wide perturbation of the host genus observed in the Lake Baikal area (Koskinen et al., 2002).

Other freshwater species of *Gyrodactylus* with the potential to have a circumpolar distribution, although awaiting molecular confirmation, include *Gyrodactylus lotae* Gusev, 1953 (host: *Lota lota*), *Gyrodactylus lucii* Kulakovskaya, 1952 (host: *Esox lucius*), and *Gyrodactylus spathulatus* Mueller, 1936 (hosts: *Catostomus commersonii* [North America]; *Catostomus catostomus* [eastern Asia]; see Leis et al., 2021).

Arctic charr (*Salvelinus alpinus*) are a competent host for several *Gyrodactylus* spp., including *G. salaris*

Gyrodactylus infections of Arctic charr (*S. alpinus*) have been reported for several species, including *Gyrodactylus birmani*, *G. derjavini*, *G. salmonis* (under synonym: *G. salvelini*), and *G. salaris* (Harris et al. 2004; Kuusela et al. 2008). In Norway, the Arctic charr has been infected by several strains of *G. salaris* separated through *cox1* barcoding, indicating the species is not only susceptible to monogenean infections, but also tolerant as the infections were not reported as pathogenic. The mitochondrial strain *cox1*^F

of *G. salaris* has spread through rainbow trout farming (Hansen et al., 2003) and been found on Arctic charr in Buskerud County, Norway (Robertsen et al., 2007; Olstad et al., 2007). Strain *cox1*^A represents the clone that caused the main Norwegian epidemic documented from *G. salaris* infecting *S. salar* and was also found on Arctic charr in Fustvatnet, Norway. In Skibotnelva, Norway, strain *cox1*^B of *G. salaris* infecting *S. salar* was treated twice with rotenone, but afterwards the parasite was still present on upstream-dwelling *S. alpinus* (Winger et al., 2008, 2012). These examples illustrate that Arctic charr appear to be a host quite tolerant of subclinical infections by several *Gyrodactylus* species, supporting the plausibility this host was involved in the circumpolar distribution of *G. salmonis*.

Rainbow trout have not introduced *G. salmonis* outside of North America

A natural host of *G. salmonis* is *O. mykiss*, a North American species that is one of the most widely introduced salmonids and commercially farmed fish worldwide (Fausch 2007). However, it is interesting that the only case of *G. salmonis* identified outside of boreal North America was reported from a feral trout population in Mexico (Rubio-Godoy et al., 2012). The *cox1* haplotype identified from Mexico was previously unique but found in this study from facilities located in Washington and Montana, U.S.A. (Fig. 2; Rubio-Godoy et al., 2012). A very plausible explanation for the lack of transmission of *G. salmonis* outside of North America involves the likelihood that only fertilized eggs were transported to other continents, thereby leaving their parasites behind.

In exile, rainbow trout have adopted numerous salmonid parasites

On new continents, *O. mykiss* has apparently lost its native parasite, *G. salmonis*, and became infected with a wide range of new parasites. The most commonly found parasites on rainbow trout, within the species or lineages studied in European farms, belong to *G. salaris sensu lato*. However, the most pathogenic Baltic salmon specific clade *G. salaris sensu stricto* has not been observed on rainbow trout, and the parasite strains spreading on *O. mykiss* have seldom infected *S. salar* (Kuusela et al., 2007). On rainbow trout, *G. salaris* is represented by several lineages identified with the *cox1* gene, including the Ossetian strain in Lake Ladoga (Ieshko et al., 2015), the Norwegian *cox1*^F mentioned above (Hansen et al., 2003), and

additional strains in Italy (Paladini et al., 2009). The hybrid *G. pomeraniae* × *G. lavareti* was found in *O. mykiss* farms and has not been identified from wild populations (Kuusela et al., 2008). Furthermore, the following species presented in Figure 5 are molecularly identified strains collected from *O. mykiss*: *G. taimeni* (Vietnam), *G. magnus* (type host ITS in Vladivostok, ITS and *cox1* in a Vietnam farm, ITS in China), *G. truttae* (Rokicka et al., 2007; Hahn et al., 2015), *G. derjavini* and *G. derjavinoidea* (Malmberg et al., 2007), *G. teuchis* (Rokicka et al., 2007; Hahn et al., 2011, 2015; Ieshko et al., 2015), and the “alien” *G. salaris* (Lindenstrøm et al., 2003; Rokicka et al., 2007; Ziętara et al., 2010).

The *wagneri* species group: Host switching capacity as an evolutionary novelty

In an early molecular study of the *wagneri* group species, it was concluded that host switching was common in the evolution of *Gyrodactylus* (Ziętara and Lumme, 2002). However, in the freshwater-restricted subgenera *G.* (*Gyrodactylus*), all European species are parasites of cyprinids. In the subgenus *G.* (*Limnephrotus*), only the *wagneri* group parasitizes diverse host families, suggesting this group is more capable of host switching; other clades are much more conservative and primarily infect cyprinids. Due to low sampling coverage, these generalizations are weakly supported, but future surveys identifying *Gyrodactylus* from various fish families to test this hypothesis would be straightforward.

The results of this study are an incomplete outline describing an intriguing case of evolution within the framework of host-parasite interactions. The difficulties associated with the interpretation and unavoidable incompleteness resulting from the circumpolar scale should only encourage researchers as the most pronounced effects resulting from climatic change are expected to occur within this northern region inhabited by *Coregonus*, *Oncorhynchus*, *Salvelinus*, and *Gyrodactylus*.

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