Taxonomy and systematics of *Gyrodactylus salaris* (Monogenea, Gyrodactylidae) infecting wild populations of Arctic charr (*Salvelinus alpinus*) in Norway

Cand. scient. thesis in Zoology

by

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Forord

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1. Content Page

1. Abstract

Extensive sampling of anadromous Arctic charr (*Salvelinus alpinus*) from River Signaldalselva (Troms County) and resident Arctic charr from Lake Pålsbufjorden (Buskerud County) revealed *Gyrodactylus* sp. infections in both localities. By use of both molecular and morphometric methodology the parasite species in both localities were identified as *G. salaris*. The discovery of *G. salaris* on Arctic charr in Pålsbufjorden is the first observation of this species infecting and maintaining a population on another salmonid species in the wild without co-occurring Atlantic salmon. The molecular analyses revealed that *G. salaris* on Arctic charr from Signaldalselva and on Arctic charr from Pålsbufjorden represent different mitochondrial haplotypes, respectively the type previously detected on Atlantic salmon from Signaldalselva, and the type previously detected on Atlantic salmon from the Rivers Drammenselva, Lierelva, Lærdalselva, and rainbow trout from Lake Bullaren, Sweden. Subsequently, a morphological study was performed to compare the *G. salaris* populations over a range of hosts and geographic locations. Hence, the morphometry of the ophisthaptoral sclerites of *G. salaris* from Arctic charr from Pålsbufjorden and Signaldalselva were compared with one another. In addition the Pålsbufjorden population on Arctic charr was compared with a sample of *G. salaris* from Atlantic salmon from Drammenselva and a sample from rainbow trout from Bullaren, and *G. salaris* on Arctic charr in Signaldalselva was compared to *G. salaris* from sympatric Atlantic salmon. Using statistical methods morphometrical dissimilarities were revealed between all *G. salaris* populations. There were only found minor morphometric differences between *G. salaris* on Arctic charr and Atlantic salmon in Signaldalselva, while the dissimilarities in morphometry between the populations from the other localities were more extensive. The observed variance is in part interpreted as being related to environmental conditions; in part it is believed to indicate an influence of the host species on the morphology of the parasite ophisthaptoral sclerites. Alternatively, there may be specific morphometric differences between the *G. salaris* strains as defined by the mitochondrial haplotype. The present discovery of *G. salaris* on wild populations of resident Arctic charr in the absence of Atlantic salmon and on anadromous Arctic charr may have important implications for the management of Atlantic salmon stocks in Norway.

2. Introduction

Gyrodactylids are monogenean ectoparasites on the skin and gills of most freshwater and marine fish species. *Gyrodactylus* von Nordmann, 1832 is one of the most species-rich gyrodactylid genera (Kritsky and Boeger, 2003) and so far approximately 400 species have been described from nearly 400 host species (Harris et al., 2004). With the ~24 000 known fish species in mind, this suggests that the biodiversity of *Gyrodactylus* is greatly underestimated (Bakke et al., 2002). Typically *Gyrodactylus* species have narrow host specificity, i.e. they infect only one or a few host species. The lifestyle of gyrodactylids (hyperviviparity, ectoparasitism) makes host switching a frequently used explanation for the parasites' speciation and radiation (Brooks and McLennan, 1993; Boeger and Kritsky, 1997). In addition to allopatric speciation, host switching may be the major cause of the large biodiversity within this genus (Zietara and Lumme, 2002, 2003; Meinilä et al., 2004).

One particular species, *Gyrodactylus salaris* Malmberg, 1957, has been a severe pathogen of Norwegian stocks of Atlantic salmon (*Salmo salar* L.) for more than 25 years. The spreading of the parasite has declined but new rivers are still being infected (Johnsen et al., 1999; Mo et al., 2004). To prevent further spread of *G. salaris* to new Atlantic salmon stocks a more detailed knowledge of the biology, morphology and genetics of populations and strains of the parasite is required.

The species identification of gyrodactylids is traditionally based on morphology, first of all the size and shape of the hard parts (sclerites) of the posterior attachment apparatus (opisthaptor) (Malmberg, 1970). However, size and shape variations in these structures between species are often so subtle that the measurement of many worms from different populations must be analysed statistically before assigning them to species (Shinn et al., 2004). On the other hand, environmental factors (e.g. temperature) may cause large variability in several phenotypic morphological "traits" such as size and, to a lesser extent, shape of the ophisthaptoral sclerites of e.g. *G. salaris* specimens collected from wild Atlantic salmon (Mo, 1991a, b, c). This must be taken into account when using morphological measurements as a taxonomic tool to differentiate between salmonid gyrodactylid species (Tanum, 1983; Mo, 1991a, b, c; Shinn et al., 2004).

Molecular techniques have become increasingly important in identifying parasite species (McManus and Bowles, 1996) and several molecular markers have been

developed for gyrodactylid identification and phylogenetic studies. The most widely used markers are the internal transcribed spacer regions of the nuclear ribosomal DNA (ITS-1 and ITS-2) (Cunningham et al., 1995; Cunningham, 1997; Matejusova et al., 2003; Zietara and Lumme, 2003). These molecular markers have proven to be suitable for a discrimination of most of the valid morphologically recognized species. Further, the intergenic spacer (IGS) of the nuclear ribosomal DNA cluster which separates the tandemly repeated units of the rDNA genes from *G. salaris* has been characterized by Collins and Cunningham (2000). This molecular marker is more variable than the ITS, and has been used to differentiate also subspecies within platyhelminths (Kaye et al., 1998). Thus, the IGS can be used to discriminate between salmon, rainbow trout (*Onchorhyncus mykiss* Walbaum) and grayling (*Thymallus thymallus* L.) forms of *G. salaris* (Sterud et al., 2002; Cunningham et al., 2003). The mitochondrial cytochrome oxidase I (COI) is also a suitable marker for discriminating *Gyrodactylus* populations and in the study of phylogenetic relationships (Hansen et al., 2003; Matejusová et al., 2003; Zietara and Lumme, 2003; Meinilä et al., 2004).

The fish genus *Salvelinus* has a circumpolar distribution and includes several species and subspecies (Brunner et al., 2001). However, Arctic charr (*Salvelinus alpinus* L.) represent the only *Salvelinus* species with a natural distribution in Norway and occur in both freshwater resident and anadromous forms (Pethon, 1985). Resident populations of Arctic charr are found all over the country, while anadromous populations are restricted to northern Norway.

Several *Gyrodactylus* spp. have been recorded on *Salvelinus* species worldwide (Harris et al., 2004). So far only two *Gyrodactylus* species are described from *Salvelinus* in the Palearctic: *G. bohemicus* on brook charr (*Salvelinus fontinalis*) in Czech Republic (only found in hatcheries) (Ergens, 1992) and *G. birmani* on Arctic charr (probably *S. malma*, Dr. O. Pugachev, pers. comm.) in Kamchatka, Russia (Konovalov, 1967; Ergens, 1983). No *Gyrodactylus*-species has so far been described from Arctic charr in the Neactic. In addition, there are reports on unidentified *Gyrodactylus* spp. from Arctic charr in England (Lake Ennerdale) (Shinn et al., 1995) and in Finland (Dr. Jaakko Lumme, pers. comm.).

Reports of gyrodactylid infections on Arctic charr in Norway are scarce. The first report was from River Skibotnelva in North-Norway, where Atlantic salmon was reported infected with *G. salaris* in 1979 (Johnsen et al., 1999). The anadromous Arctic charr population have also been found relatively heavily infected with *G. salaris* (Mo,

1988). In the years 1988 and 1995, this river was treated with rotenone in order to eradicate the parasite. It is likely that *G. salaris* survived on both occasions either on resident or anadromous Arctic charr (see Johnsen et al. 1999). This is not surprising, as the ability of *G. salaris* to reproduce on Arctic charr has been demonstrated experimentally on both anadromous and freshwater resident populations (Tanum, 1983; Bakke et al., 1996). Heavily infected Arctic charr have also recently been reported from River Signaldalselva, a river in the vicinity of Skibotnelva located in the same fjord system (Knudsen et al., 2004). The colonization of *G. salaris* in Signaldalselva may be caused by anadromous fish migrating in brackish water from Skibotnelva (Johnsen et al., 1999; Hansen et al., 2003). The *Gyrodactylus* infection on Arctic charr which co-occurs with Atlantic salmon in Signaldalselva is suggested to be *G. salaris* (see Knudsen et al., 2004), however not taxonomically settled by use of the necessary methodology. In southern Norway, Sterud (1999) reported *G. birmani* on Arctic charr in Buskerud County, however, without any closer molecular or morphological description.

The main aim of the present study is to taxonomically characterize *Gyrodactylus* sp. recovered on anadromous Arctic charr from Signaldalselva (Troms County) and *Gyrodactylus* sp. on resident Arctic charr from Lake Pålsbufjorden (Buskerud County). In addition, a closer study of the variability between gyrodactylids infecting different host species and on different geographical populations was performed by the use of both morphological and molecular tools on the following populations: (i) *Gyrodactylus* sp. from anadromous Arctic charr and *G. salaris* from the sympatric Atlantic salmon in Signaldalselva; (ii) *Gyrodactylus* sp. from anadromous Arctic charr in Signaldalselva and *Gyrodactylus* sp. from resident Arctic charr in Pålsbufjorden; (iii) *Gyrodactylus* sp. on resident Arctic charr from Pålsbufjorden and two selected populations of *G. salaris* from Atlantic salmon in River Drammenselva and rainbow trout from a hatchery in Lake Bullaren, Sweden.

3. Material and methods

3.1. Fish and Parasites

In southern Norway, Arctic charr were collected with fishing nets in the lakes Pålsbufjorden, Tunhovdfjorden, Skurdalsfjorden, and Tinnsjøen, Buskerud County, during 2001- 2003 (Table 2). In northern Norway, Arctic charr and Atlantic salmon were collected concurrently by electro-fishing in Signaldalselva, Troms County, in 2001 and 2004. In addition, Atlantic salmon were collected by electro-fishing in Drammenselva, Buskerud County in 2002, and rainbow trout (*Onchorhyncus mykiss*) were collected from a fish farm in Bullaren, Sweden in 2002 (Table 2).

The fins of the adult fish were clipped immediately after the fish was killed by a blow to the head and subsequently fixed in 80% ethanol. Parr were fixed in 96% ethanol. The collected fish and fins were screened for *Gyrodactylus*-infection under a stereo-microscope (at 40X) at the Department for Zoology, Natural History Museum, University of Oslo.

3.2. Preparation of gyrodactylid specimens

The Gyrodactylids detected on fish or fins were removed by a pipette after dislodgement and put into separate Eppendorf-tubes containing 80% ethyl alcohol and stored in a refrigerator. For morphological examinations the attachment organs (ophisthaptors) of the parasites were excised from the remaining parasite body and prepared as described in 3.3.1. The bodies were stored in Eppendorf-tubes in a refrigerator until molecular analyses were performed. Doing so allows for both morphological and molecular analyses of one single parasite.

3.3. Morphological analyses

3.3.1. Digestion of organic matter

To examine morphologically the haptoral hamuli, marginal hooks and ventral bar (sclerites) the excised ophisthaptors were purified according to a modified method of Harris et al. (1999). The ophisthaptors were placed on a slide and any excess ethanol was

removed or allowed to evaporate. Thereafter the whole opisthaptor was embedded in 0.5µl of digestion solution consisting of 75 mM Tris, 10 mM EDTA pH 8.0 containing, 5% SDS and proteinase K (100µg/ml). The digestion was allowed to continue until the soft tissue of the ophisthaptor was dissolved and the ophisthaptoral sclerites which are un-digestible were released. The digestion solution was then gently removed by rinsing the sclerites with distilled water.

3.3.2. Light microscopy

After digestion and rinsing of the released sclerites a droplet of 0.1-0.3 µl ammonium picrate glycerine was added and a cover slip (diameter 10mm) was placed on the sclerites. Finally, the cover slips were sealed with Eukitt. A Leica DC 500 camera mounted on a Leica DM 6000B stereomicroscope was used to photograph the ophisthaptoral sclerites at magnifications of 1600, 1250, or 1000 X. All sclerites were photographed and measured by the Leica IM1000 software system purchased from Tamro MedLab AS, Norway.

3.3.3. Morphological measurements

Only slides containing all three ophisthaptoral structures (hamuli, ventral bridge and marginal hooks) were used for the morphological analyses. Fifteen to 30 specimens from each population were measured. The measurements were based on an optimal number of landmarks selected on the basis of the taxonomical literature on gyrodactylids (Shinn et al., 2004). Some additional measurements that were considered likely to pick up further differences between the populations were included. In total, 34 different measurements were applied (see Table 1 and Fig. 1). The measurements were taken using a digital calliper or a point-to-point tool. In addition, one angle measurement (converted to cosines values) was used (see Fig. 1).

Table 1. List of 34 morphometric characters measured on the ophisthaptoral sclerites: hamuli, ventral bar and marginal hook. (Parentheses = character abbreviation).

Hamuli	
1	Aperture length (HAL)
2	Point length1 (HPL1)
3	Distal shaft width1 (HDSW1)
4	Shaft length1 (HSL1)
5	Aperture angle (HAA)
6	Inner curve length1 (HICL1)
7	Proximal shaft width (HPSW)
8	Root length (HRL)
9	Total length (HTL)
10	Distal shaft width2 (HDSW2)
11	Point length2 (HPL2)
12	Shaft length2 (HSL2)
13	Inner curve length2 (HICL2)
Ventral Bar	
14	Total length (VBTL)
15	Process to mid-length (VBPML)
16	Basal median length (VBBML)
17	Membrane length (VBML)
18	Central length (VBCL)
19	Lateral length (VBLL)
20	Process to process width (VBPPW)
21	Width (VBW)
22	Maximum membrane width (VBMMW)
23	Process length (VBPL)
Marginal Hook	
24	Total length (MHTL)
25	Shaft length (MHSHAL)
26	Sickle length (MHSL)
27	Sickle distal width (MHSDW)
28	Sickle heel length (MHSHL)
29	Sickle proximal width (MHSPW)
30	Sickle toe length (MHSTL)
31	Instep height (MHIH)
32	Aperture distance (MHAD)
33	Sickle toe height (MHSTH)
34	Sickle width (MHSW)

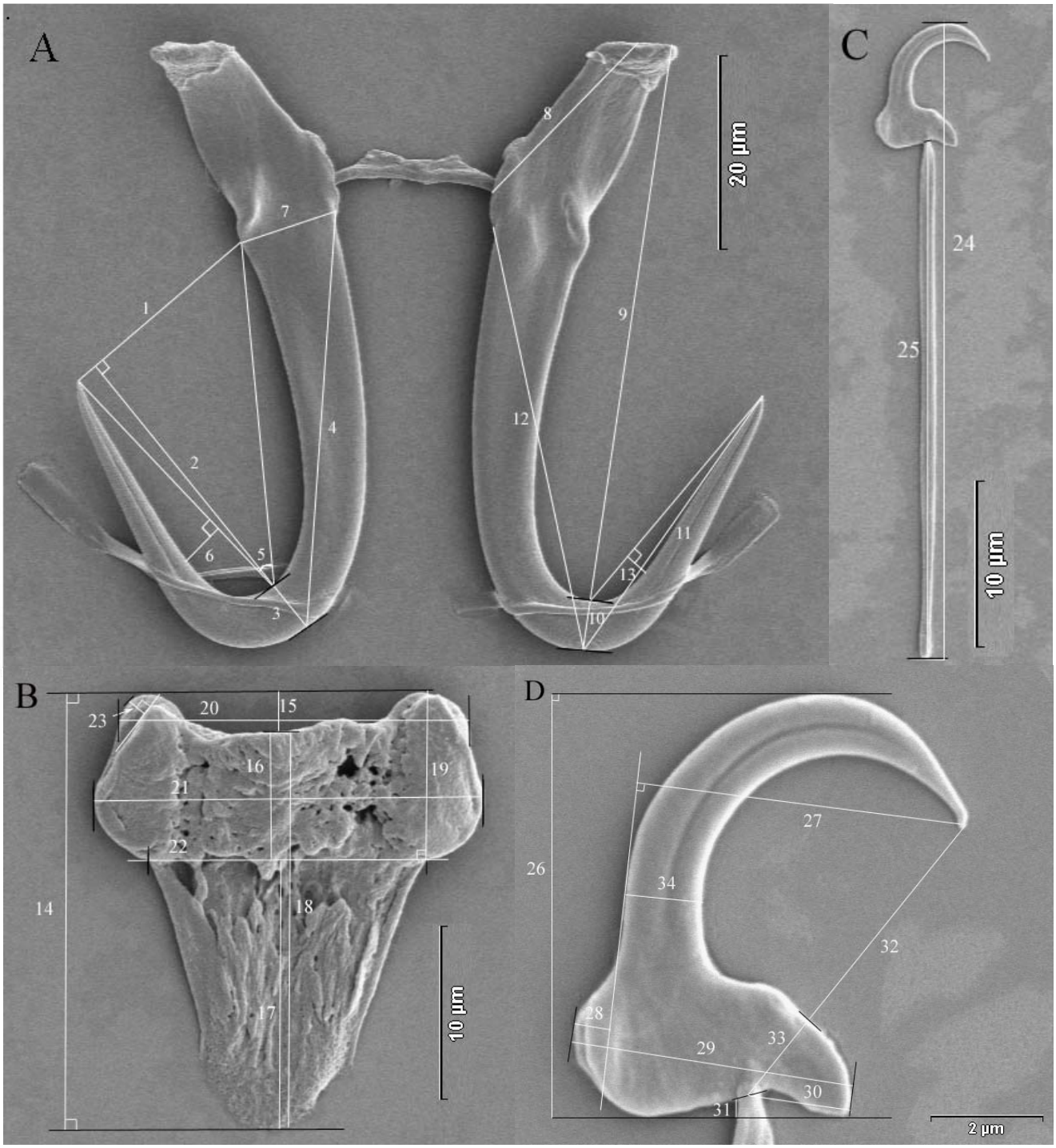

Fig. 1 A-D. Scanning electron micrographs (SEM) of the ophisthaptoral sclerites from *Gyrodactylus* sp. from Arctic charr (*S. alpinus*) in Signaldalselva illustrating the morphometric parameters used. The numbers refer to the parameters listed in Table 1. – **A**, hamuli. – **B**, ventral bridge. – **C**, marginal hook. **–** **D**, marginal hook sickle

3.3.4. Statistical analyses

Principal component analysis (PCA) was employed to analyse the multivariate datasets of the morphological measurements of the ophisthaptoral sclerites. This was done in order to project the multivariate dataset down to a reduced number of dimensions while making sure that as much as possible variance will be preserved and that visualisation of the results will be facilitated. The original variables were thus transformed into new variables that define independent patterns of variation and are not inter-correlated.

The axes of maximal variance (principal components) in the datasets were identified and interpreted. When analysing morphometric datasets the first component is frequently interpreted as the one representing size variation, while the other components are usually interpreted as representing shape variation (Jolicoeur and Mosiman, 1960; Reyment et al., 1984). The correlations of variables with the component scores were determined. Such correlation indicates the contribution of a variable to a particular component (Blackith and Reyment, 1971) and is referred to as PCA loading. This parameter can also be employed to evaluate the morphological significance of the components. When interpreting the principal components it is important to view the loadings collectively. If the loadings have the same signs, the implication is that all variables are increasing together (positive signs) or decreasing together (negative signs). Such a component is often interpreted as a component consisting of variance related to size. A component with both positive and negative loadings is interpreted as a component consisting of variance related to shape.

The scores on the principal components of the datasets were then used in subsequent Analyses of Variance (ANOVA). By negating the component that best expresses size variation, the effects of having a between-group bias in the sizes of parasites in different samples was assumed minimal.

To explore differences in single measures between populations directly without taking variation relating to size into account, Kruskal-Wallis and Mann-Whitney U tests were employed. All calculations and graphical illustrations were done with the programme PAST (ver 1.29, http://folk.uio.no/ohammer/past).

3.3.5. Scanning Electron Microscopy (SEM)

The preparation of sclerites for the scanning electron microscopy is the same as described in 3.3.1, with the only exception that the digestion was performed on a cover slip. After digestion and rinsing, the cover slip was transferred to a SEM stub and sputter-coated with

a gold-palladium mixture using a Polaron E5000 SEM coating unit for later examination in a JEOL JSM-6400 scanning electron microscope.

3.4. Molecular analyses

3.4.1. DNA extraction

After removal of the ophisthaptor, the remaining bodies of 3 - 6 parasites per population were used for molecular analyses. To extract the DNA from the bodies they were placed individually in 30µm lysis solution (proteinase K 60- 180 g/ml, Tween 20 0.45%, and TE-buffer (Tris- HCL 10 mM, EDTA 1 mM, pH 8.0)) and incubated at 65ºC overnight. Subsequently, the temperature was raised to 95ºC for ten minutes to inactivate the proteinase K. No further purification was done in order to avoid loss of DNA.

3.4.2. PCR amplification of nuclear ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA)

The primer pairs from Matejusova et al. (2001):

- ITS1A (5´-GTAACAAGGTTTCCGTAGGTG-3´)
- ITS2 (5´-TCCTCCGCTTAGTGATA-3´)

were used to amplify a fragment partially spanning the 18S gene, the internal transcribed spacer I, the 5,8S gene, the internal transcribed spacer II and partially the 28S gene by PCR.

To amplify IGS, the primers from Collins and Cunningham (2000) were used:

- IGSV3 (5´-CTGGCTATAATCACGTAAGACTGC-3´)
- IGSV4 (5´- AAGATACTCATTTGACTCGGTGTG-3´)

To amplify overlapping ~ 400 bp segments of the mitrochondrial COI gene, the primer-pairs defined by Hansen et al. (2003) were used:

- ZMO1 (5´-GCGMCTAAATGCTTTAAGGGCTTG-3´)
- ZMO2 (5´- CCAAAGAACCAAAATAAGTGTTG-3´)
- ZMO3 (5´- TGTCYCTACCAGTGCTAGCCGCTGG-3´)
- ZMO4 (5´- GAGGATAGCACTATCCCTGTCAC-3´)

The amplification reaction contained 1 µl of the DNA template, PCR reaction buffer (Roche), 200 mM dNTPs, 1mM of each primer and 1U *Taq* polymerase (Roche) in a total volume of 25 ml. The PCR reaction was performed in a PCR system 9700 (Applied

Biosystems) using the following protocol: 4 minutes at 95 ºC, followed by 35 cycles of 1 min at 95 ºC, 1 min 50 ºC and 2 min 72 ºC. PCR products were visualized on a 1.0% agarose gel stained with ethidium bromide. The PCR-products were purified by using the QIAquick PCR Purification Kit (Quiagen) according to the manufacturer's protocol.

3.4.3. DNA sequencing of ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA)

Both strands of the purified PCR-products were sequenced according to the chain-termination method (Sanger et al., 1977) using the BigDye chemistry (Applied Biosystems) and an ABI 3100 automatic sequencer. For ITS the PCR-primers and the internal primers were used from Matejusova et al. (2001) and from Zietara and Lumme (2003):

- ITS4.5 (5´-CATCGGTCTCTCGAACG-3´)
- ITS3A (5´-GAGCCGAGTGATCCACC-3´)
- ITS28F (5´-TAGCTCTAGTGGTTCTTCCT-3´)

For the sequencing of the IGS the same primers as for the PCR amplification were used. To sequence the mitochondrial CO1 gene, the primers from Hansen et al. (2003) were used:

- ZMO2 (5´-CCAAAGAACCAAAATAAGTGTTG-3´)
- ZMO3 (5´-TGTCYCTACCAGTGCTAGCCGCTGG-3´)

4. Results

4.1. Sampling results

Arctic charr and Atlantic salmon from both northern and southern Norway were infected with the ectoparasitic monogeneans. *Gyrodactylus* infections were detected on fish from the lakes Pålsbufjorden, Tunhovdfjorden and Bullaren, and the rivers Signaldalselva and Drammenselva. Fish from the lakes Skurdalsfjorden and Tinnsjøen were not infected (Table 2). *Gyrodactylus* specimens were particularly prevalent on adult Arctic charr in Pålsbufjorden in the autumn 2003 (August to October) and hence used for the morphological analyses. Arctic charr collected by ice fishing in December 2002 and March 2003 in Tunhovdfjorden were uninfected. Specimens of *Gyrodactylus* were also recovered from parr of Arctic charr collected by electro-fishing in Signaldalselva. The obtained parasites were characterized morphometrically and molecularly and compared to *G. salaris* specimens infecting the concurrently occurring Atlantic salmon parr.

The abundance of *Gyrodactylus* on the fins of Arctic charr in Pålsbufjorden was estimated to 0.89, which is much less than the estimated 8 *Gyrodactylus* per examined Arctic charr from Signaldalselva. *G. salaris* was also found on the fins and skin of Atlantic salmon parr collected in Drammenselva in June 2002, and on fins of adult rainbow trout in a hatchery in Bullaren in May 2002. These parasites were used for comparison with *Gyrodactylus* specimens recovered from Arctic charr in Pålsbufjorden in the autumn 2003.

Table 2. Details on the sampled *Salvelinus alpinus , Salmo salar, Salmo trutta, and Oncorhyncus mykiss.* All fish were screened for G*yrodactylus* infection.

Sampling locality (Country)	Geographical coordinates	Water temp. (ºC)	Sampling date	Host species examined	No. fish examined	No. of *G.* specimens
Lake Pålsbufjorden (Norway)	60º 27' 00 N, 8º 39' 00 E	16	13-15.08.03	*S. alpinus*	30	1
"	"	10-12	8-12.09.03	*S. alpinus*	22	3
"	"	15-8*	08.-10.10.2003	*S. alpinus*	24	67
"	"	8-7*	19.10.2003	*S. alpinus*	15	10
Lake Tunhovdfjorden (Norway)	60º 25' 00 N, 8º 53' 00 E	-	12.04.2003	*S. alpinus. + S. trutta*	78	0
"	"	-	05.-08.2003	*S. alpinus*	30	0
"	"		05.-08.2003	*S. alpinus + S. trutta*	15	0
"	"	-	11.09.2003	*S. alpinus*	12	1
Lake Skurdalsfjorden (Norway)	60º 27' 00 N, 8º 23' 00 E	6,8	10-19.10.03	*S. alpinus*	92	0
Lake Tinnsjøen (Norway)	59º 54' 00 N, 8º 55' 00 E	7	03.11.2003	*S. alpinus*	10	0
River Signaldalselva (Norway)	69º 15' 58 N, 19º 55' 31 E	-	21.09.01	*S. alpinus*	Ca. 10	Numerous
"	"	5,8	6-8.09.04	*S. alpinus*	24	192
"	"	5,8	6-8.09.04	*S. salar*	15	Numerous
River Drammenselva (Norway)	59º 46' 35 N, 9º 54' 04	14-15**	18.06.2002	*S. salar*	10+	Numerous
Lake Bullaren (Sweden)	58º 39' 24 N, 11º 32' 36 E	-	13.05.2002	*O. mykiss*	3 fins	Numerous

* on 5.08.03 the temperature in Lake Pålsbufjorden was ca 15 ºC in most depths and there was still a thermocline. In September the thermocline had almost disappeared and the temperature was between 12-13 ºC (10.09.03) (approximate values found in Brabrandt et al., 2004). Early in October there was most likely complete circulation of the water column and the temperature probably exceeded 7 ºC.
** Approximate values calculated by Ånund Sigurd Kvambekk, NVE.

4.2. Gyrodactylus sp. from Arctic charr and Atlantic salmon in Signaldalselva

4.2.1. Molecular characterization (CO1)

Two specimens of *Gyrodactylus* from Arctic charr were identified as *G. salaris* of haplotype B according to Hansen et al. (2003). Haplotype B has previously been found in *G. salaris* infecting Atlantic salmon from Signaldalselva and Skibotnelva in Norway, and rivers Vindelälven and Torneälven in Sweden (Hansen et al., 2003). Hence, the *Gyrodactylus* population on Arctic charr in Signaldalselva is considered identical to that of *G. salaris* on Atlantic salmon in Signaldalselva based on COI sequences.

4.2.2. Morphological analyses and comparisons

G. salaris from Arctic charr in Signaldalselva (Figure 1 A-D) were characterized morphometrically and compared with parasites from the sympatric Atlantic salmon parr. The mean values, standard deviations, and ranges of the 34 measures (Fig. 1 A-D, Table 1) are based on the morphometric measurements of 23 individual *G. salaris* specimens from Arctic charr and compared to a similar number of worms from Atlantic salmon

(Table 3). The individual measurements taken of *G. salaris* from both Arctic charr and Atlantic salmon showed that 6 out of 34 measures of the hamuli (HDSW1 and HICL2), the ventral bar (VBPML, VBML and VBPPW) and the marginal hooks (MHSL) differed significantly (Mann-Whitney U tests, $p < 0.05$) between *G. salaris* from the two host species (measurements that differ significantly are marked with asterisks in Table 3).

Table 3. Measurements of the *G. salaris* specimens measured from Arctic charr (N = 23) and from Atlantic salmon (N = 23) in Signaldalselva, North Norway. Each measure is given as micrometer (µm) ± standard deviation (SD), range in parentheses. Statistically significant differences ($p < 0.05$, Mann-Whitney U-Test) between the two metapopulations of *G. salaris* are indicated with *

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Character measured	***G. salaris*** **Arctic charr**	***G. salaris*** **Atlantic salmon**
Hamulus (H)		
1 HAL	24.03 ± 1.11 (21.19-25.63)	23.81 ± 1.28 (21.53-27.28)
2 HPL1	34.78 ± 1.11 (32.54-36.68)	35.25 ± 0.86 (33.47-36.86)
3 HDSW1*	6.20 ± 0.21 (5.85-6.66)	6.02 ± 0.37 (5.23-6.8)
4 HSL1	42.27 ± 1.24 (38.96-44.09)	42.55 ± 1.73 (38.12-45.11)
5 HAA	0.74 ± 0.02 (0.71-0.78)	0.76 ± 0.03 (0.70-0.81)
6 HICL1	5.82 ± 1.23 (3.52-8.99)	5.62 ± 0.93 (4.13-7.29)
7 HPSW	10.81 ± 0.55 (10.05-12.28)	10.56 ± 0.44 (9.67-11.57)
8 HRL	23.30 ± 1.90 (20.79-29.32)	22.88 ± 1.02 (20.42-24.74)
9 HTL	68.15 ± 2.08 (64.00-72.57)	67.72 ± 2.24 (61.28-71.92)
10 HDSW2	5.74 ± 0.26 (5.17-6.09)	5.60 ± 0.28 (4.85-5.94)
11 HPL2	32.93 ± 1.37 (30.03-35.21)	33.64 ± 0.91 (31.79-35.45)
12 HSL2	46.52 ± 1.40 (43.55-48.78)	46.25 ± 1.79 (40.50-48.53)
13 HICL2*	2.47 ± 0.65 (1.37-3.87)	2.82 ± 0.50 (1.99-4.06)
Ventral Bar (VB)		
14 VBTL	27.66 ± 1.85 (25.03-34.30)	27.29 ± 1.48 (24.46-30.97)
15 VBPML*	2.25 ± 0.84 (0.37-3.51)	2.73 ± 0.70 (0.53-3.54)
16 VBBML*	8.58 ± 1.08 (6.45-10.78)	7.64 ± 1.02 (5.72-10.32)
17 VBML	16.82 ± 1.37 (15.28-21.69)	16.91 ± 1.37 (13.99-19.65)
18 VBCL	25.42 ± 1.73 (22.98-31.19)	24.56 ±1.53 (22.28-27.75)
19 VBLL	11.20 ± 0.83 (9.71-12.52)	11.06 ±0.70 (9.36-12.14)
20 VBPPW*	23.43 ± 1.43 (21.15-28.75)	22.56 ± 1.16 (20.33-24.36)
21 VBW	25.56 ± 1.45 (23.88-31.19)	24.67 ± 1.20 (21.73-26.43)
22 VBMMW	17.75 ± 1.33 (15.93-22.79)	17.09 ± 1.47 (14.26-19.75)
23 VBPL	1.35 ± 0.28 (0.85-2.04)	1.27 ± 0.28 (0.75-1.92)
Marginal Hook (MH)		
24 MHTL	39.49 ± 0.93 (37.62-40.73)	39.30 ± 0.92 (37.21-40.64)
25 MHSHAL	32.44 ± 0.80 (30.59-33.56)	32.27 ± 0.80 (30.55-33.53)
26 MHSL*	7.61 ± 0.19 (7.33-7.89)	7.48 ± 0.19 (7.07-7.8)
27 MHSDW	5.93 ± 0.21 (5.41-6.34)	5.99 ± 0.16 (5.60-6.26)
28 MHSHL	0.75 ± 0.13 (0.51-0.99)	0.71 ±0.09 (0.57-0.88)
29 MHSPW	5.16 ± 0.16 (4.86-5.41)	5.15 ±0.20 (4.69-5.51)
30 MHSTL	1.99 ± 0.12 (1.82-2.32)	1.98 ± 0.13 (1.69-2.15)
31 MHIH	0.42 ± 0.10 (0.27-0.60)	0.45 ± 0.09 (0.28-0.6)
32 MHAD	6.20 ± 0.12 (6.00-6.46)	6.19 ± 0.16 (5.84-6.52)
33 MHSTH	1.67 ± 0.09 (1.50-1.80)	1.62 ± 0.11 (1.41-1.79)
34 MHSW	1.39 ± 0.07 (1.29-1.55)	1.38 ± 0.10 (1.08-1.53)

A PCA- plot shows a high degree of overlap in the first principal component (PC1) (see Fig. 2), which account for 34% of the variance in the dataset. The loadings of PC1 were mostly negative, and PC1 were thus interpreted as a component representing variance related mainly to size (some variables (HAA, VBPML, MHSDW, MHIH and MHSW) had a vague positive correlation with the component scores). Along PC1 there were not found any significant differences between *G. salaris* from Arctic charr and Atlantic salmon (ANOVA, p> 0.05, Table 4). Accordingly *G. salaris* on salmon and charr in Signaldalselva do not differ from each other with respect to size. There is also a high degree of overlap of morphological measurements of *G. salaris* from Arctic charr and Atlantic salmon along PC2 in the PCA- plot (Fig. 2).

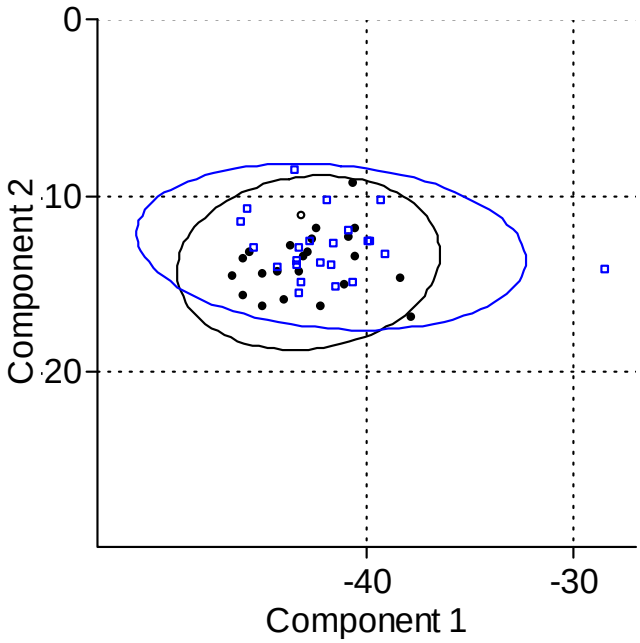

Fig. 2. PCA plot of the morphometric data of all measurements (see Table 1) of *Gyrodactylus* sp from Arctic charr (in blue) and *G. salaris* from Atlantic salmon (in black) from River Signaldalselva in the two first planes (Component 1 vs Component 2) of the PCA plot. (ellipses represent 95% confidence intervals about the mean).

The variances of the PC2-7 were interpreted as reflecting shape due to the occurrence of both negative and positive loadings. The PCA-scores of PC2-4 and PC6-7, which collectively accounted for 50% of the variation described, showed no significant differences along these components (ANOVA, p>0.05, Table 4). However there is a significant (ANOVA, p=0.024) difference between *G. salaris* from salmon and charr in

axis 5 but this component accounted for only 6 % of the total variation in the morphological traits. Consequently, there are found some small shape-differences in *G. salaris* between these two host species.

Table 4. The percent variation described by the seven first components of the PC analyses of *G. salaris* from charr and salmon in Signaldalselva. The results of an ANOVA test based on the PCA-scores of the different components are also presented.

PCA		**ANOVA**
Component	% Variation	p
1	34.357	0.144
2	25.557	0.216
3	9.578	0.094
4	7.003	0.429
5	6.323	0.024
6	3.996	0.392
7	3.635	0.995

4.3. Gyrodactylus sp. from Arctic charr in Pålsbufjorden, Atlantic salmon in Drammenselva and rainbow trout in Bullaren

4.3.1. Molecular characterization (ITS, CO1, IGS)

The ITS sequences of *Gyrodactylus* specimens from Arctic charr from Pålsbufjorden were identical to those of *G. salaris* (and also *G. thymalli*).

The COI sequence of the *Gyrodactylus* from Arctic charr in Pålsbufjorden is identical to that of *G. salaris*, haplotype F according to Hansen et al. (2003). Haplotype F has previously been detected in the following *G. salaris* populations on Atlantic salmon: Drammenselva, Lierelva and Lærdalselva, and in addition in specimens recorded from rainbow trout in a hatchery in Bullaren, Sweden (Hansen et al., 2003).

The analysis of the IGS of the specimens from Arctic charr in Pålsbufjorden revealed highest similarity in sequence and structure to *G. salaris* specimens previously analysed from the rainbow trout in the hatchery in Bullaren (Hansen et al., in prep.). The obtained IGS sequence did not match those found in other specimens with haplotype F, i.e. *G. salaris* from the Drammenselva, Lierelva and Lærdalselva. Hence, the *Gyrodactylus* population on Arctic charr from Pålsbufjorden is according to the molecular markers ITS, COI and IGS regarded identical to that of *G. salaris* found on hatchery reared confined rainbow trout (Bullaren, Sweden).

4.3.2. Morphological analyses and comparisons

G. salaris from Arctic charr from Pålsbufjorden (Fig. 3 A-D) were characterized morphometrically and compared with parasites of the same mitochondrial haplotype (F) collected from Atlantic salmon in Drammenselva and rainbow trout in Bullaren (Fig. 4 A-F).

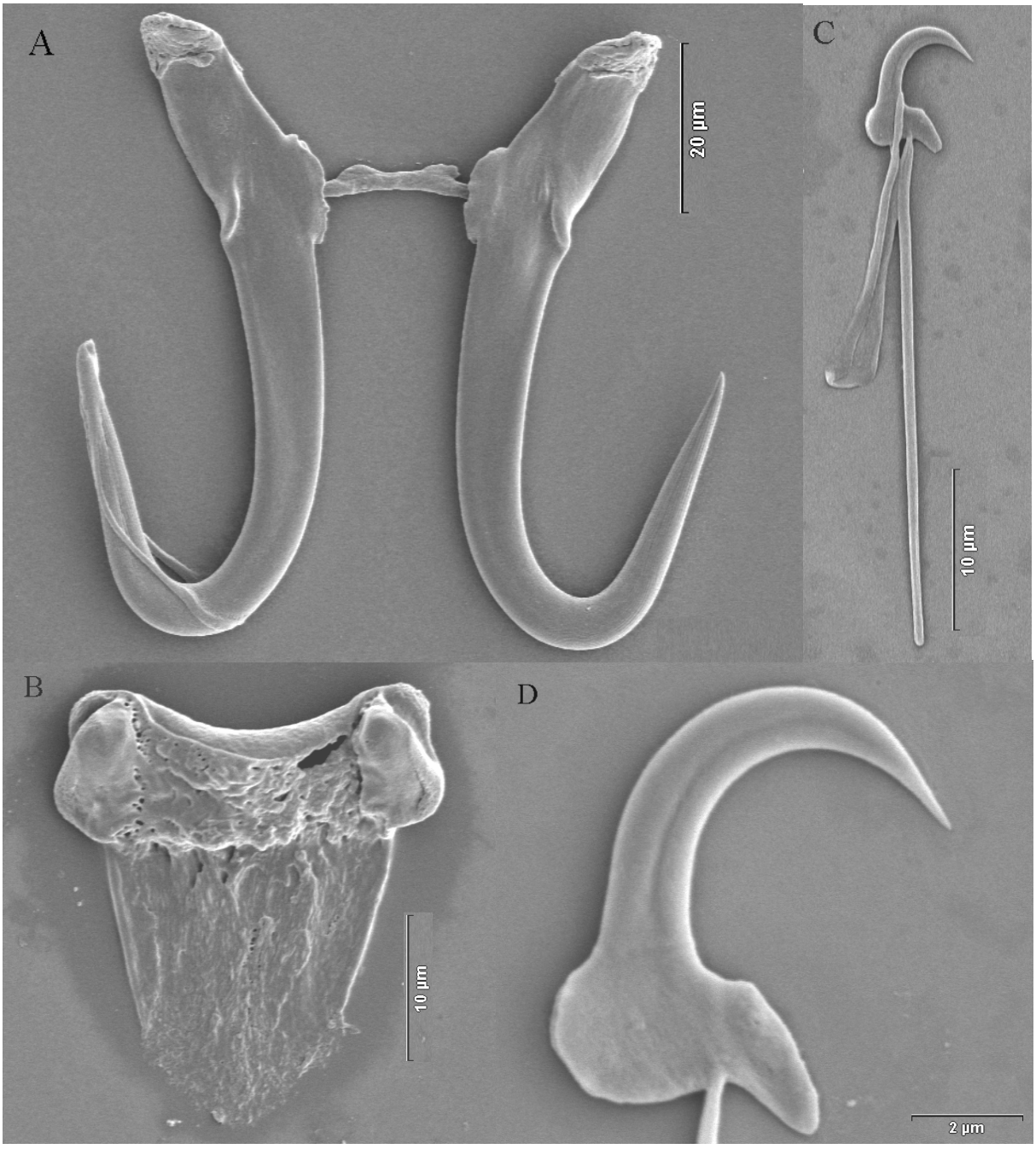

Fig. 3 A-D. Scanning electron micrographs (SEM) of the ophisthaptoral sclerites of *G. salaris* from Arctic charr in Pålsbufjorden. – **A**, hamuli. – **B**, ventral bridge. **– C**, marginal hook. **– D**, marginal hook sickle.

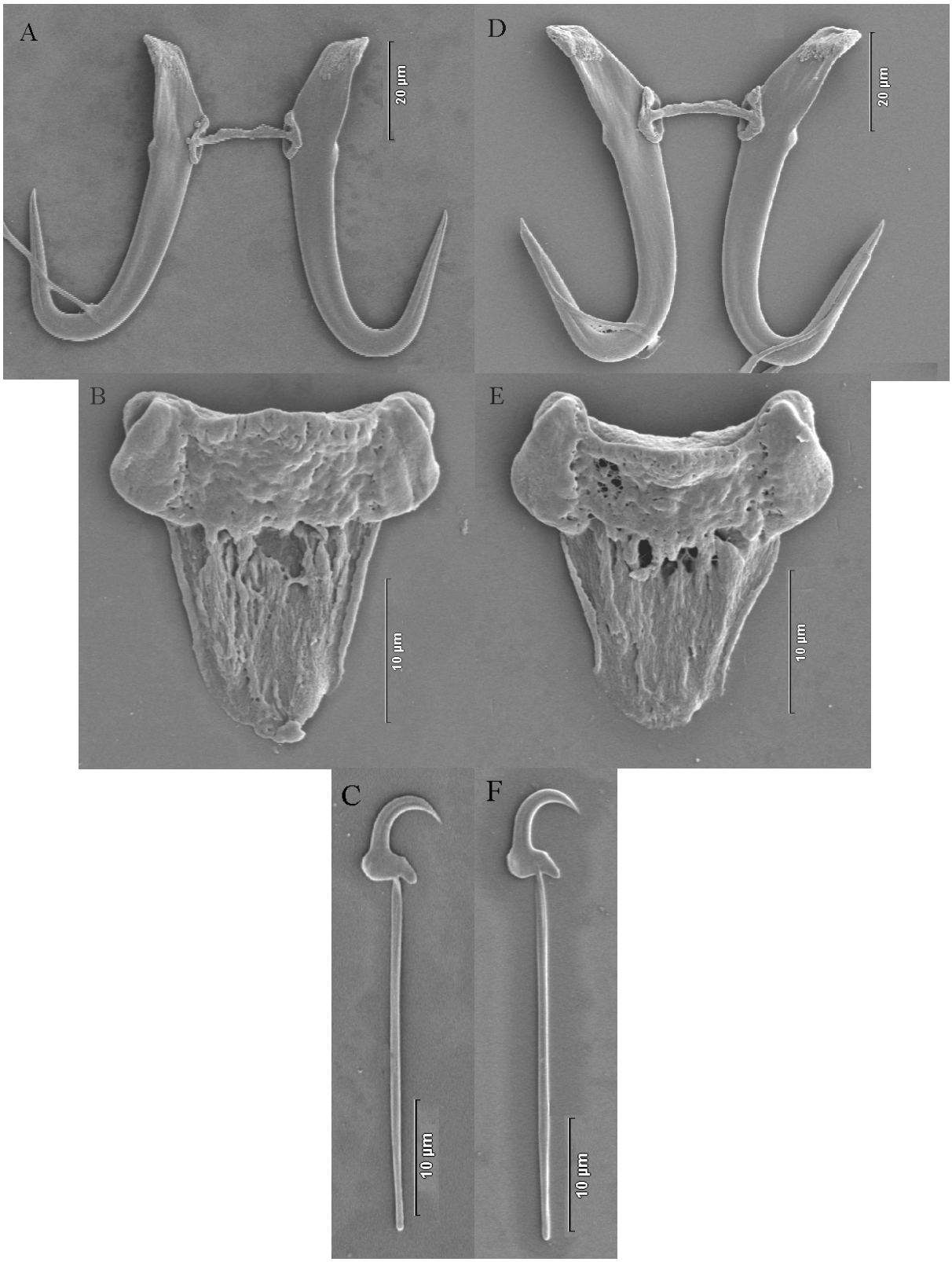

Fig. 4. A-F. Scanning electron micrographs (SEM) of the ophisthaptoral sclerites of *G. salaris* from Atlantic salmon in Drammenselva and from rainbow trout from a hatchery in Bullaren. Drammenselva: - A, hamuli. **– B**, ventral bridge. **– C**, marginal hook. Lake Bullaren: - **D**, hamuli. **– E**, ventral bridge. – **F**, marginal hook.

The mean values, standard deviations, and ranges of the 34 measures (Fig. 1, Table 1) are based on the morphometric measurements of 30 *G. salaris* specimens from Arctic charr (Pålsbufjorden), 15 specimens from Atlantic salmon (Drammenselva) and 15 specimens from rainbow trout in Bullaren (Table 5).

Table 5. Measurements of the *G. salaris* from Arctic charr (N = 30) from Pålsbufjorden, Atlantic salmon (N = 15) from Drammenselva, south Norway, and rainbow trout (N = 15) from a hatchery in Bullaren, Sweden. Each measure is given as micrometer (µm) ± standard deviation (SD), range in parentheses.

Character measured	***G. salaris* (Arctic charr) Pålsbufjorden**	***G. salaris* (Atlantic salmon) Drammenselva**	***G. salaris* (rainbow trout) Bullaren**
Hamulus			
1 HAL	24.15 ± 2.90 (20.66-32.87)	25.66 ± 1.18 (23.78-27.81)	23.84 ± 1.32 (19.9-25.15)
2 HPL1	39.34 ± 0.95 (36.66-41.44)	35.02 ± 1.32 (32.53-37)	37.23 ± 1.05 (35.42-39.48)
3 HDSW1	7.58 ± 0.69 (6.76-9.34)	6.58 ± 0.50 (5.7-7.54)	6.81 ± 0.29 (5.95-7.23)
4 HSL1	46.54 ± 1.28 (44.55-49.13)	42.60 ± 1.96 (38.74-45.44)	44.31 ± 1.24 (41.77-46.04)
5 HAA	0.77 ± 0.06 (0.57-0.84)	0.71 ± 0.03 (0.64-0.74)	0.77 ± 0.02 (0.74-0.83)
6 HICL1	3.94 ±1.01 (1.63-6.24)	4.87 ± 1.10 (2.79-6.38)	4.67 ± 0.81 (3.28-6.35)
7 HPSW	12.11 ± 0.75 (10.07-13.72)	11.25 ± 0.86 (8.95-12.34)	11.40 ± 0.42 (10.8-12.48)
8 HRL	27.47 ± 1.19 (24.58-29.87)	24.13 ± 1.87 (19.23-26.48)	26.60 ± 1.65 (22.66-28.78)
9 HTL	75.88 ± 1.77 (72.47-79.41)	69.88 ± 3.91 (61.97-74.73)	72.07 ± 2.65 (65.79-74.97)
10 HDSW2	7.41 ± 0.65 (6.46-9.27)	6.17 ± 0.46 (5.44-7.14)	6.49 ± 0.37 (5.63-7.2)
11 HPL2	38.33 ± 0.87 (36.73-40.48)	33.03 ± 1.25 (30.27-35.02)	35.98 ± 1.14 (33.65-38.13)
12 HSL2	49.38 ± 1.27 (46.55-51.77)	46.56 ± 2.44 (42.33-50.71)	47.45 ± 1.49 (43.85-49-28)
13 HICL2	2.59 ± 0.59 (1.46-3.72)	2.23 ± 0.55 (1.52-3.43)	2.68 ± 0.56 (1.92-3.71)
Ventral Bar			
14 VBTL	30.71 ± 2.16 (28.16-40.21)	28.30 ± 1.69 (25.16-30.99)	28.69 ± 0.80 (27.27-29.78)
15 VBPML1	2.10 ± 0.76 (0.40-3.75)	1.86 ± 0.42 (1.12-2.64)	2.02 ± 0.96 (-0.89-3.31)
16 VBBML	11.07 ± 1.65 (8.37-15.39)	10.32 ± 1.77 (6.29-12.89)	10.38 ± 1.55 (7.4-12.9)
17 VBML	17.47 ±2.30 (13.02-24.98)	16.29 ± 1.68 (12.46-18.78)	16.26 ± 1.23 (14.39-17.93)
18 VBCL	28.70 ± 2.33 (25.19-37.81)	26.86 ± 2.45 (23.22-33.62)	26.66 ± 1.43 (24.27-29.72)
19 VBLL	11.63 ± 1.13 (9.58-15.22)	11.55 ± 1.34 (9.78-13.67)	11.43 ± 0.53 (10.55-12.47)
20 VBPPW	27.55 ± 2.14 (25.59-36.48)	24.57 ±1.93 (19.77-27.67)	26.24 ± 1.73 (24.32-29.28)
21 VBW	29.18 ± 1.84 (27.04-37.24)	25.93 ± 1.63 (21.76-28.23)	26.69 ± 0.76 (25.22-28.3)
22 VBMMW	21.14 ± 1.51 (19.14-26.82)	17.89 ± 0.92 (16.33-20.4)	18.41 ± 0.74 (16.67-19.19)
23 VBPL	2.01 ± 0.36 (1.49-2.94)	1.92 ± 0.52 (1.05-2.81)	1.92 ± 0.32 (1.13-2.28)
Marginal Hook			
24 MHTL	40.18 ± 1.02 (38.19-42.58)	37.80 ± 0.87 (36.3-39.23)	38.87 ± 1.15 (37.12-40.4)
25 MHSHAL	32.80 ± 0.92 (30.85-34.37)	30.77 ± 0.90 (29.48-32.17)	31.63 ± 1.11 (29.84-33.15)
26 MHSL	7.93 ± 0.22 (7.59-8.4)	7.42 ± 0.27 (6.6-7.74)	7.83 ± 0.24 (7.32-8.33)
27 MHSDW	6.05 ± 0.27 (5.63-6.73)	5.73 ± 0.26 (5.27-6.03)	5.67 ± 0.28 (5.16-6.14)
28 MHSHL	0.78 ± 0.11 (0.57-0.99)	0.68 ± 0.13 (0.5-0.9)	0.67 ± 0.16 (0.46-0.99)
29 MHSPW	5.29 ± 0.31 (4.95-6.23)	5.01 ± 0.34 (4.07-5.4)	5.14 ± 0.24 (4.72-5.65)
30 MHSTL	2.01 ± 0.20 (1.74-2.77)	1.91 ± 0.14 (1.59-2.11)	1.95 ± 0.13 (1.75-2.29)
31 MHIH	0.61 ± 0.13 (0.43-0.87)	0.39 ± 0.12 (0.23-0.67)	0.59 ± 0.09 (0.46-0.79)
32 MHAD	6.42 ± 0.20 (6.09-7.2)	6.13 ± 0.16 (5.61-6.32)	6.10 ± 0.15 (5.89-6.48)
33 MHSTH	1.76 ± 0.20 (1.20-2.43)	1.62 ± 0.11 (1.4-1.79)	1.80 ± 0.12 (1.61-2.01)
34 MHSW	1.51 ± 0.13 (1.28-1.9)	1.38 ± 0.10 (1.16-1.52)	1.57 ± 0.12 (1.36-1.73)

The individual measurements taken of *G. salaris* from Arctic charr, Atlantic salmon and rainbow trout showed that 26 out of 34 measures of the hamuli (HAL, HPL, HDSW, HSL, HAA, HICL, HPSW, HRL, HTL, HDSW2, HPL2 and HSL2), the ventral bar (VBML2, VBCL, VPPW, VBW and VBMW) and the marginal hooks (MHTL, MHSHAL, MHSL, MHSDW, MHSHL, MHIH, MHAD, MHSTH and MHSW) differed significantly (Kruskal-Wallis tests, $p < 0.05$, Table 6). Of the measurements found to differ significantly between the tree populations, 19 proved to be different between *G. salaris* from Pålsbufjorden and Bullaren, 25 were significantly different between *G. salaris* from Pålsbufjorden and Drammenselva and 15 measurements were significant different between *G. salaris* from Drammenselva and Bullaren (Mann-Whitney U tests, $p<0.05$, Table 6).

Table 6. Kruskal-Wallis *post hoc* tests (Mann-Whitney U tests) for each morphometric character of the *G. salaris* populations from Pålsbufjorden, Drammenselva and Bullaren. Only those variables that were statistically significant different ($p<0.05$) between the populations are listed (abbreviations see Table 1).

	G. salaris **(rainbow trout, Bullaren)**	***G. salaris*** **(salmon, Drammenselva)**
G. salaris **(Arctic charr, Pålsbufjorden)**	HPL1 (p<0.01) HDSW1 (p<0.01) HSL1 (p<0.01) HICL1 (p=0.019) HPSW (p<0.01) HTL (p<0.01) HDSW2 (p<0.01) HPL2 (p<0.01) HSL2 (p<0.01) VBML (p=0.024) VBCL (p<0.01) VBPPW (p=0.018) VBW (p<0.01) VBMMW (p<0.01) MHTL (p<0.01) MHSHAL (p<0.01) MHSDW (p<0.01) MHSHL (p=0.043) MHAD (p<0.01)	HAL (p<0.01) HPL1 (p<0.01) HDSW1 (p<0.01) HSL1 (p<0.01) HAA (p<0.01) HICL1 (p<0.01) HPSW (p<0.01) HRL (p<0.01) HTL (p<0.01) HDSW2 (p<0.01) HPL2 (p<0.01) HSL2 (p<0.01) VBCL (p<0.01) VBPPW (p<0.01) VBW(p<0.01) VBMMW (p<0.01) MHTL (p<0.01) MHSHAL (p<0.01) MHSL (p<0.01) MHSDW (p<0.01) MHSHL (p=0.022) MHIH (p<0.01) MHAD (p<0.01) MHSTH (p<0.01) MHSW t (p<0.01)
G. salaris **(rainbow trout, Bullaren)**		HAL (p<0.01) HPL1 (p<0.01) HSL1(p=0.014) HAA (p<0.01) HRL (p<0.01) HDSW2 (p=0.025) HPL2 (p<0.01) VBPPW (p=0.03) VBMMW (p=0.019) MHTL (p=0.018) MHSHAL (p=0.04) MHSL (p<0.01) MHIH (p<0.01) MHSTH (p<0.01) MHSW (p<0.01)

A PCA- plot shows that there is not a complete overlap of morphological measurements of the tree *G. salaris* populations in PC1 (Fig. 5 A).

The loadings for PC1 all have positive signs except HICL1 which is not directly related to size but describes the curvature of the hamuli. Accordingly, PC1 captures variance mainly related to size. All populations differed significantly from each other in PC1 (ANOVA and Tukey`s pairvise comparisons, $p<0.05$, Table 7). This together with the high variation (57%) captured in PC1 implies that the three *G. salaris* populations differ in sizes. In PCA- plots showing the scores of PC2-3 there seems to be high degree of overlap of morphological measurements in the three *G. salaris* populations (Fig. 5 A- B).

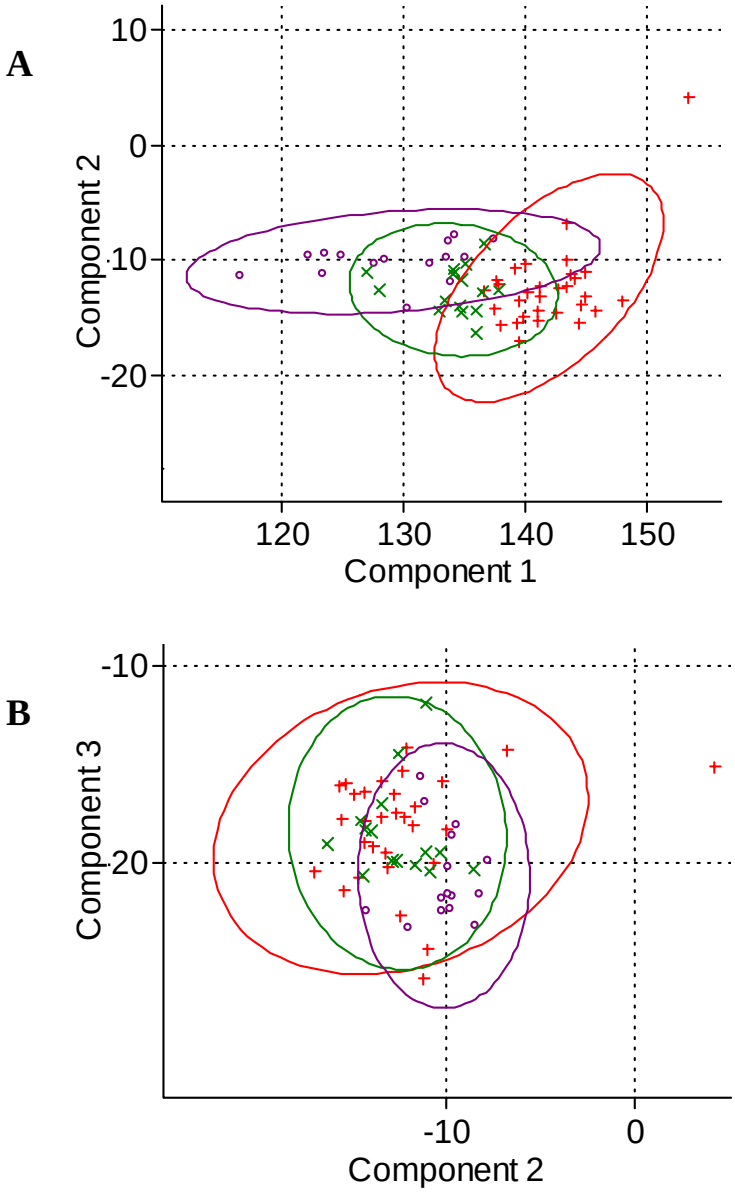

Fig. 5 A, B. PCA plots of the morphometric data of all measurements (see Table 1) of *G. salaris* from Arctic charr in Pålsbufjorden (in red), Atlantic salmon from Drammenselva (in purple) and rainbow trout from Bullaren (in green). – **A**, the two first planes (Component 1 vs Component 2) of the PCA plot. – **B**, the second and third plane (Component 2 vs Component 3) of the PCA plot (ellipses represent 95% confidence intervals about the mean).

These components account for 26-9% of variance in the dataset. The loadings for PC2-5 all have a mix of negative and positive signs and are therefore interpreted as consisting of variance related to shape. In PC4 and PC5 (collectively capturing 21% of the variance) there were no significant differences between morphometry of *G. salaris* from the three populations (ANOVA and Tukey`s pairvise comparisons, $p>0.05$, Table 7). In PC2, however, *G. salaris* from Drammenselva is significantly different from *G. salaris* from Bullaren. In PC3 *G. salaris* from Drammenselva is significantly different from *G. salaris* from Pålsbufjorden as well as *G. salaris* from Bullaren (Tukey`s pairvise comparisons, $p=0.02$ and $p=0.04$, respectively). This component captures 9.4% of the variance in the dataset, which implies that there are moderate significant differences in shape, in addition to differences in size, between the ophisthaptoral sclerites of *G. salaris* from Drammenselva and *G. salaris* both from Pålsbufjorden and Bullaren.

Table 7. The percent variation described by the first five components of the PC analyses. The results of an ANOVA test based on the PCA-scores of the different components in addition to post-hoc tests based on Tukey`s pairvise comparisons of the populations are also given.

PCA		**ANOVA**	**Post-hoc tests**		
Component	% Variation	p(same)	Pålsbu-Drammen	Pålsbu-Bullaren	Bullaren-Drammen
1	57.379	1,718 E-13	0.0001189	0.0001198	0.002412
2	11.68	0.037	0.064	0.982	0.042
3	9.352	0.015	0.020	0.959	0.041
4	5.196	0.965	0.993	0.965	0.989
5	4.294	0.135	0.724	0.140	0.482

4.4. Comparison between the morphology of G. salaris on Arctic charr from Pålsbufjorden and Signaldalselva

A comparison between the morphology of *G. salaris* from Arctic charr from Pålsbufjorden and from Signaldalselva showed that 24 out of 34 measures of the hamuli (HPL, HDSW, HSL, HAA, HICL, HPSW, HRL, HTL, HDSW2, HPL2 and HSL2), the ventral bar (VBTL, VBBML, VBCL, VPPW, VBW, VBMW and VBPL and the marginal hook (MHTL, MHSL, MHIH, MHAD, MHSTH and MHSW) differed significantly (Mann-Whitney U tests, $p<0.05$, Table 8).

Table 8. The results of a Mann-Whitney U tests for each morphometric variable of the two *G. salaris* populations from Arctic charr in Pålsbufjorden and Signaldalselva. Only those variables that were statistically significant different ($p<0.05$) between the populations are listed (abbreviations see Table 1).

Measure	**p(same)**
2 HPL1	<0.01
3 HDSW1	<0.01
4 HSL1	<0.01
5 HAA	<0.01
6 HICL1	<0.01
7 HPSW	<0.01
8 HRL	<0.01
9 HTL	<0.01
10 HDSW2	<0.01
11 HPL2	<0.01
12 HSL2	<0.01
14 VBTL	<0.01
16 VBBML	<0.01
18 VBCL	<0.01
20 VBPPW	<0.01
21 VBW	<0.01
22 VBMMW	<0.01
23 VBPL	<0.01
24 MHTL	0.025
26 MHSL	<0.01
31 MHIH	<0.01
32 MHAD	<0.01
33MHSTH	<0.01
34 MHSW	<0.01

In a PCA- plot there seems to be almost no overlap of the morphological measurements of *G. salaris* from Arctic charr in Pålsbufjorden and Signaldalselva along PC1 (Fig. 6 A). The loadings of PC1 are all positive as expected if the bulk of variance in the dataset originated from size-differences. The only measure that does not follow this trend is MHIH (marginal hook) that was found not directly correlated to size. The variance in PC1 is accordingly interpreted as resulting from differences in size between the populations. Thus, the two populations proved to be significant different in PC1 (ANOVA, $p<0.01$, Table 9), which means that they are different in size. In PC2-3 there seems to be a high degree of overlap (Fig. 6 A- B).

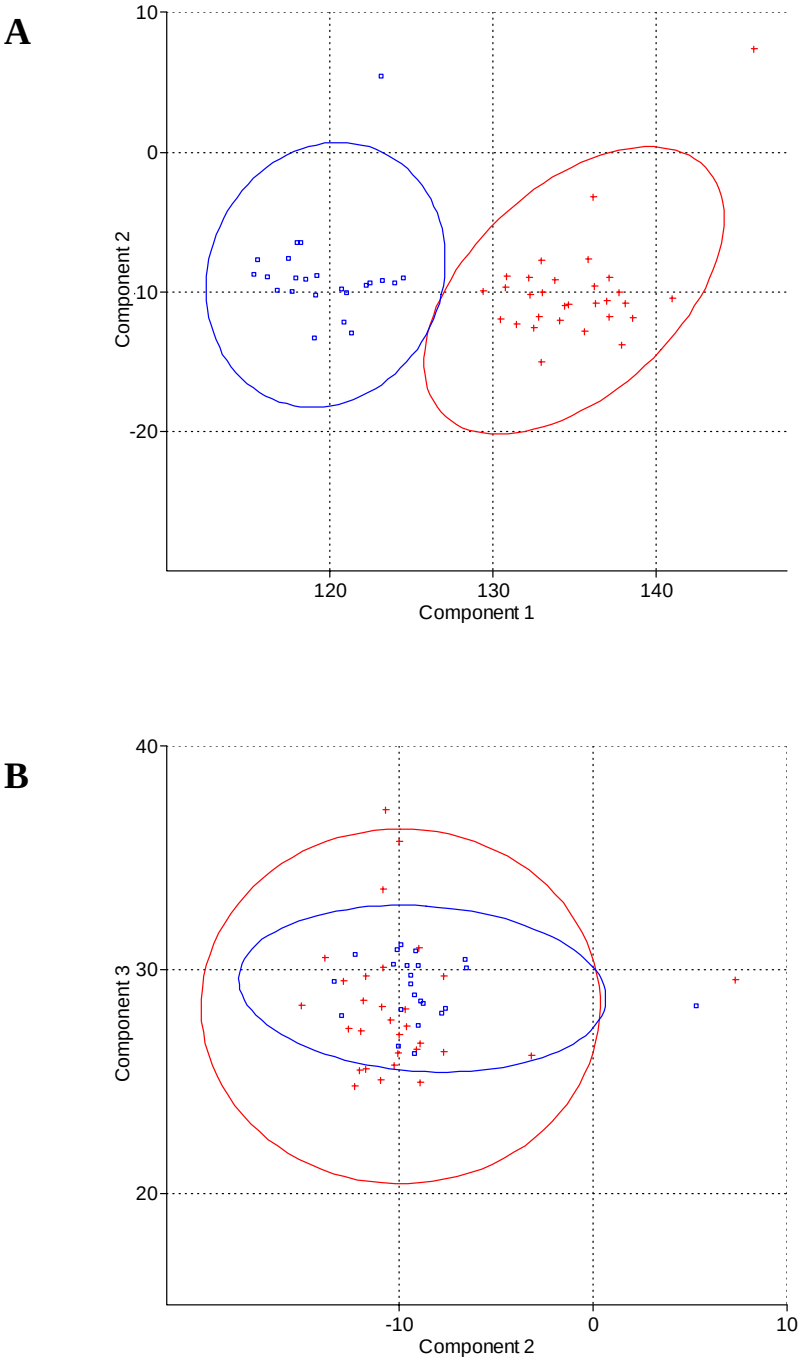

Fig. 6 A, B. PCA plots of the morphometric data of all measurements (see Table 1) of *G. salaris* on Arctic charr from Lake Pålsbufjorden (in red) and River Signaldalselva (in red). - **A**, the two first planes (Component 1 vs. Component 2) of the PCA plot. – **B**, the second and third plane (Component 2 vs. Component 3) of the PCA plot (ellipses are 95% confidence intervals about the mean).

PC2-6 is interpreted as consisting of variance related to shape due to mixed signs of the loadings. These components accounts for 13-2% of the variance (collectively capturing 28% of the variance) in the dataset, and thus the morphometrical differences between these populations are small and insignificant (ANOVA, p>0.05, Table 9). Therefore *G. salaris* infecting Arctic charr in Pålsbufjorden and Signaldalselva are not different from each other with respect to shape.

Table 9. The percent variation described by the first six components of the PC analyses of *G. salaris* from charr in Pålsbufjorden and Signaldalselva. The results of an ANOVA test based on the PCA-scores of the different components are also shown.

PCA		**ANOVA**
Component	% Variation	p(same)
1	65.421	6.184E-23
2	13.577	0.286
3	5.833	0.251
4	3.744	0.664
5	2.587	0.707
6	1.987	0.513

5. Discussion

Arctic charr from both the north Norwegian Signaldalselva and the south Norwegian Pålsbufjorden was found infected with *Gyrodactylus*. By the use of molecular and morphological methods both *Gyrodactylus* populations were identified as *G. salaris*. The morphometric measurements of the *Gyrodactylus* from both Signaldalselva and Pålsbufjorden fall within the range of *G. salaris* for the 15 characters published by Cunningham et al. (2001). However, in one of the specimens from Arctic charr in Pålsbufjorden the four measures of the ventral bridge (VBTL, VBML2, VBCL and VBPPW) were clearly out of the range described by Cunningham et al. (2001). This specimen from Pålsbufjorden is atypical in having a larger ventral bridge than the other specimens from the same locality. Otherwise, shape and size are not specifically different.

The sequences of the internal transcribed spacer (ITS) of the nuclear ribosomal gene cluster and the mitochondrial cytochrome oxidase I gene (COI) of the specimens from Arctic charr in Signaldalselva and Pålsbufjorden were identical to that of *G. salaris* from the respective clade I and clade III as described by Hansen et al. (2003). The finding that *G. salaris* is able to infect wild anadromous Arctic charr in Signaldalselva confirms the suggestions from the field-studies of Knudsen et al. (2004), and the experimental work by Bakke et al. (1996). Further, the finding that *G. salaris* infecting Atlantic salmon also infects the sympatric Arctic charr in Signaldalselva is in accordance with the observations made in the nearby river Skibotnelva (Mo, 1988; Kristoffersen et al., 2005). The observations in Skibotnelva and Signaldalselva indicate that Arctic charr co-occurring with Atlantic salmon infected with *G. salaris* most probably will acquire the infection. The finding that the freshwater resident Arctic charr from Pålsbufjorden apparently is continuously infected by *G. salaris* without any co-occurring Atlantic salmon was, however, more surprising. In addition the susceptibility of the resident Arctic charr population in Pålsbufjorden is surprising due to a previous study demonstrating that another resident stock of Arctic charr was resistant to *G. salaris* of the same haplotype (i.e. from Lierelva, Bakke et al., 1996).

The documented *G. salaris* infection on Arctic charr in Signaldalselva was compared morphologically with *G. salaris* of the same mitochondrial haplotype infecting Atlantic salmon living in sympatry with Arctic charr in Signaldalselva. The results demonstrated that the morphology of *G. salaris* on Arctic charr and Atlantic salmon was

almost indistinguishable. The minor morphometrical differences (significant differences in six out of 34 measurements and in component 5 of the PCA) between parasites detected from the two host species may be interpreted as a result of differences associated to the host-species since all environmental parameters were kept similar.

Host dependent differences in morphology of gyrodactylids infecting closely related host species have been studied both in the field and by laboratory experiments. In a laboratory study, *G. salaris* sampled from rainbow trout in a fish farm in Lake Tyrifjorden, infected both Atlantic salmon parr and rainbow trout (Mo, 1991c). The size and shape of the parasites ophisthaptoral sclerites were found to be indistinguishable when infecting salmon parr and rainbow trout at the same temperature in the laboratory. In another study concerning *G. derjavini* which normally infects brown trout, *G. derjavini* specimens prepared from concurrently sampled Atlantic salmon and trout in River Sandvikselva, did not vary with respect to morphometry (Mo, 1993). In addition, Geets et al. (1999) did not find any significant differences between the morphometry of *G.* cf. *arcuatus* specimens on two different but related host species; *Pomatoshistus minutus* and *P. lozanoi*. Thus, previous studies have shown that there is no host-dependent variation in ophisthaptoral hard parts of *G. salaris* as well as other *Gyrodactylus* species when infecting closely related hosts. The findings in the present study, on the contrary, show that *G. salaris* from the same locality can vary slightly in morphometry when infecting different salmonid host species.

G. salaris on Atlantic salmon and Arctic charr in Signaldalselva could either represent two different metapopulations or belong to the same population of parasites. If *G. salaris* on Arctic charr and Atlantic salmon in Signaldalselva consist of two different metapopopulations the differences in morphology observed could result from an early step of differentiation to a new host. However, a hypothesis of two separate metapopulations is considered unlikely as it contradicts the finding that the parasites on Arctic charr are of the same haplotype as those on salmon. Another objection to this hypothesis is that *Gyrodactylus* on sympatric fish species seems to frequently switch hosts (Bakke et al., 1992). This is in accordance with the studies performed by Mo (1983, 1993) where two host species shared a common population of parasites, i.e. *G. derjavini* infecting the primary host brown trout and Atlantic salmon in Sandvikselva. The Atlantic salmon was heavier infected in periods with low water supply in the river, yielding a higher density of fish and thus a higher infection pressure of *G. derjavini* from brown trout. Further, *G. salaris* is known to have a high potential for host switching among

salmonids (see Bakke et al., 2002). Soleng et al. (1999) has shown that *G. salaris* both in laboratory and field studies can be transmitted to new salmon hosts both by direct contact between infected live fish and/or dead fish, besides indirectly from the substrate or via drift in the water column. Both indirect and direct transmission of *G. salaris*, are probably important routes for infection of Arctic charr in Signaldalselva since parr of Arctic charr and Atlantic salmon occupy somewhat different habitats in the river. Atlantic salmon is frequently found in deep parts of the river with strong water currents, while Arctic charr is more often found in shallow waters near the shore (Heggberget, 1984). It is also possible that heavily infected or dying Atlantic salmon parr displays abnormal behaviour in moving into more shallow waters and thus may facilitate the spreading of the parasite to parr of Arctic charr. In the light of the findings made by Mo (1983) and Soleng et al. (1999) it is likely that *G. salaris* on Arctic charr and Atlantic salmon in Signaldalselva consist of a common population with origin from Atlantic salmon as opposed to two different metapopulations constrained to the host species. Consequently, it is considered unlikely that the morphometrical differences found between *G. salaris* parasitizing Arctic charr and Atlantic salmon are connected to the parasites representing separate populations.

One possible reason for the observed differences in morphometry of *G. salaris* on Arctic charr and Atlantic salmon belonging to a common population of parasites is that different host species will provide different microenvironments for monogenean parasites (see Buchmann and Lindenstrom, 2002). The microenvironment offered by the host could potentially influence the phenotype of the ophisthaptoral sclerites of gyrodactylids. Another reason to the observed dissimilarities in the morphometry of *G. salaris* on Arctic charr and Atlantic salmon may involve the variable morphology recorded between individuals of *G. salaris* (Malmberg, 1987). If some of the parasites display traits (morphological or linked to morphology) that are favourable for survival and reproduction on Arctic charr after a host-switch they may after some time dominate in the sample from Arctic charr. Cone and Wiles (1989) found that specific individuals of *G. colemanensis* would be dislodged in instances when the attachment mechanisms and the parasite morphology did not fit host epidermal architecture. This finding may support the theory of *G. salaris* specimens with particular “traits” are dominating in the sample of parasites from Arctic charr as it indicates that morphological variability within the same *Gyrodactylus* population can affect the ability of individual parasites to survive on a new host after transmission. In this context it must be mentioned that reproduction of

G. salaris on Arctic charr in Signaldalselva is considered likely due to the high intensities of parasites observed on individual Arctic charr (Knudsen et al., 2004). Such high intensities can hardly be explained solely by transfer from Atlantic salmon. Reproductions after a successful host switch may certainly enhance the effect of *G. salaris* specimens in possession of particular "traits" dominating in the gyrodactylid population on Arctic charr. Based on the various observations mentioned above the differences in the morphometry between *G. salaris* on Atlantic salmon and Arctic charr could be explained by the assumption that the particular parasites that are able to establish and reproduce on Arctic charr after transmission from Atlantic salmon (see Bakke et al. 1991) are morphologically slightly different from the mean of morphological traits of the *G. salaris* specimens infecting Atlantic salmon. If so, *G. salaris* may appear slightly different with respect to the morphometry of the ophisthaptoral sclerites in the two host species. The alternative hypothesis that seems likely to explain the observed differences in morphometry between *G. salaris* on Arctic charr and Atlantic salmon are, as mentioned above, that the host is influencing the phenotype of the parasites.

The observation that *G. salaris* is able to infect resident Arctic charr in Pålsbufjorden was somewhat surprising. This is the first time *G. salaris* has been recorded in a wild resident Arctic charr population over a prolonged period (recorded in 2000, 2003 and 2004) without co-occurring Atlantic salmon. In the present study, repeated transfer of *G. salaris* from Atlantic salmon can be excluded. According to Sterud (1999) *G. birmani* has been recorded on Arctic charr in Buskerud County. This species was not observed in Pålsbufjorden; however, the absence of *G. birmani* does not exclude the possibility of *G. birmani* occurring elsewhere on Arctic charr in Buskerud County.

The molecular and morphological analyses of *G. salaris* on Arctic charr in Pålsbufjorden and Signaldalselva gave the opportunity to compare the morphology of *G. salaris* of different populations and mitochondrial haplotypes infecting the same host-species. The ophisthaptoral sclerites of *G. salaris* from these two Arctic charr populations were different in size but similar in shape. The sclerites of the specimens from the sampling in Pålsbufjorden are generally larger than those of *G. salaris* from Signaldalselva. Environmental factors such as differences in water temperature could cause morphological dissimilarities as the sampling in Signaldalselva (5.8ºC) was performed at lower temperature than the temperature during the sampling period in Pålsbufjorden (7 - 15ºC). However, this contradicts several earlier investigations that

have shown that the ophisthaptoral hard parts of *G. salaris* increase in size with decreasing water temperature (Mo, 1991a, b, c). This seems to be a general trend in gyrodactylids as it is also observed in several other *Gyrodactylus*-species such as e.g. *G. aphyae, G. macronychus, G. truttae, G. katharineri, G. derjavini, G. callariatis* (Ergens (1976, 1981; Ergens and Gelnar, 1985; Mo, 1993; Appleby, 1996). Thus, the larger ophisthaptoral sclerites of *G. salaris* from Arctic charr in Pålsbufjorden than on Arctic charr in Signaldalselva are considered to be size differences between the two parasite populations that are temperature independent.

The finding that *G. salaris* from Arctic charr in Pålsbufjorden is larger than *G. salaris* from Arctic charr in Signaldalselva appear to be in accordance with the observations of Mo (1991c). He noticed that *G. salaris* found on rainbow trout in Tyrifjorden had larger ophisthaptoral hooks than *G. salaris* on Atlantic salmon usually had. In this context it is noteworthy that the COI of *G. salaris* infecting Arctic charr from Pålsbufjorden was of the same haplotype as *G. salaris* from Drammenselva. Mo (1991c) hypothesized that Drammenselva was infected with *G. salaris* from Tyrifjorden, which implies that *G. salaris* from Pålsbufjorden may have the same haplotype as the "large" *G. salaris* from rainbow trout in Tyrifjorden. The larger ophisthaptoral sclerites of *G. salaris* infecting Arctic charr from Pålsbufjorden than found in Signaldalselva might therefore be based on genetic differences between the two populations (haplotypes). However, other explanations for the observed size differences cannot be excluded. For example, the Arctic charr sampled in Pålsbufjorden were mostly adult (3-5 year old) while the Arctic charr sampled from Signaldalselva were parr (0+ and 1+). This host size differences may also affect the gyrodactylid phenotypes. For example the size of the ophisthaptoral hard parts of *G. arcuatus* has previously been found affected by the age of the host as specimens infecting older hosts had larger ophisthaptoral hard parts than those parasitizing small fish (Malmberg, 1970). This is in accordance with *G. salaris* from Pålsbufjorden having larger sclerites than *G. salaris* from Signaldalselva.

Further differences that could have led to variability in the morphology of the parasites from the two populations could be that the resident Arctic charr in Pålsbufjorden and the anadromous Arctic charr in Signaldalselva are of different fish stocks. They could therefore represent different genotypic and/or phenotypic challenges for the gyrodactylids. Bakke et al. (1996) showed that there may be distinctions in the susceptibility of different stocks of Arctic charr towards *G. salaris* since anadromous Arctic charr from the Hammerfest stock in North Norway was susceptible whereas the

resident Arctic charr from the Korssjoen stock in south-eastern Norway was not. Arctic charr is a species that has high level of molecular variation between different populations (Wilson et al., 2004). Accordingly, the observed dissimilarities in morphology between the two *G. salaris* populations on Arctic charr may be related to host population differences inducing different phenotypes. These different traits may also have been selected for in the two different host populations.

In summary, the differences in size of the ophisthaptoral sclerites of *G. salaris* infecting Arctic charr in Pålsbufjorden and Signaldalselva can be caused either by strain dissimilarities in the parasite populations, and/or microenvironmental factors such as strain dissimilarities in the Arctic charr populations or differences in age of the host (adults versus parr). However, the possibility of differences related to some macroenvironmental factors cannot be ruled out.

The sequencing of the mitochondrial COI gene of *G. salaris* from Arctic charr in Pålsbufjorden identified it to a haplotype that belongs to the mitochondrial clade III according to Hansen et al. (2003). This particular haplotype has earlier been detected in *G. salaris* from Atlantic salmon in Drammenselva /Lierelva and Lærdalselva, and rainbow trout from Bullaren (Hansen et al, 2003). The sequence of the IGS from *G. salaris* from Pålsbufjorden showed highest similarity to the sequence of *G. salaris* on rainbow trout from Bullaren. This IGS sequence has previous been found in *G. salaris* adapted to rainbow trout (Sterud et al., 2002; Cunningham et al., 2003). To explore potential differences in morphology between *G. salaris* from different host species and localities but belonging to the same mitochondrial haplotype, *G. salaris* from Arctic charr (Pålsbufjorden), Atlantic salmon (Drammenselva) and rainbow trout (Bullaren) were subjects for more detailed morphometric analyses. The results of the morphometrical analyses of *G. salaris* between the three populations showed clear differences in size. There were also slight differences in the shape of the hooks of *G. salaris* from Pålsbufjorden and Drammenselva, as well as between the specimens from Drammenselva and Bullaren. Several measures of the hamulus (HAL, HPL, HDSW, HSL, HAA, HICL, HPSW, HRL, HTL, HDSW2, HPL2, HSL2), the ventral bridge (VBML2, VBCL, VPPW, VBW, VBMW) and the marginal hooks (MHTL, MHSHAL, MHSL, MHSDW, MHSHL, MHIH, MHAD, MHSTH and MHSW) proved to be significantly different between these populations of *G. salaris*. However, these results represent most likely difference in size as proposed by the results of ANOVA performed on the PCA-scores.

The observed morphometric dissimilarities recovered may also be due to that the *G. salaris* measured from the different localities were sampled under different macroenvironmental conditions as e.g. water temperatures. Such differences may also partly be accounted to host-species induced differences in phenotypic traits of the parasites, as discussed above.

The infection history of Signaldalselva is known from molecular studies which state that Signaldalselva probably was infected via estuarine migration of Atlantic salmon from the river Skibotnelva (Hansen et al., 2003). In contrast, the spread of the rainbow-trout form of *G. salaris* to Arctic charr in Pålsbufjorden remains unclear. The Arctic charr in Pålsbufjorden originally came from Lake Tinnsjøen (Ass, 1970), and the possibility that the *G. salaris* was introduced to Pålsbufjorden concurrently with the Arctic charr cannot be ruled out. However, no gyrodactylid infection has been reported on Arctic charr from Tinnsjøen. In addition, the relatively few fish from Tinnsjøen screened for infection was uninfected. This might indicate that *G. salaris* was introduced to Pålsbufjorden in a later incidence. Another possible introduction route of *G. salaris* to Pålsbufjorden employs rainbow trout. Roe of rainbow trout from Jutland in Denmark was hatched in various fish farms in Southern Norway and the fish was introduced into Pålsbufjorden on several occasions from 1962-1964 and to Tunhovdfjorden between 1962 and 1967 (Per Aass, pers. comm.). However, within four years after the last introduction the rainbow trout had vanished from the fish catches (Per Aass, pers. comm.). Even though the introduced rainbow trout did not manage to persist in the lake, it is possible that the *G. salaris* today parasitizing Arctic charr in Pålsbufjorden was introduced concurrently with the fish and switched to Arctic charr before the rainbow trout disappeared. If this interpretation is correct, the rainbow trout must have acquired the infections in Norwegian fish farms since it was introduced to Norway as roe. This scenario is not unlikely as the host range of *G. salaris* has experimentally been found to be wide (Bakke et al., 2002). After switching from rainbow trout to Arctic charr the parasite might have adapted rapidly to the new host species. This scenario includes a remarkable fast host switch, since the transmission window from one host species to the other has been very short, as the rainbow trout and Arctic charr co-occurred probably only about six years in Pålsbufjorden. As recurrent host switching is considered to promote rapid host-specific adaptation and subsequent speciation (Cribb et al., 2002; Poulin, 2002; Zietara and Lumme, 2002), this seems to be a likely explanation of the fact that Arctic charr are infected with the rainbow-trout form of *G. salaris*. The life cycle of

gyrodactylids facilitate speciation via isolation and genetic divergence after a successful host-switching event (Cable and Harris, 2002; Zietara and Lumme, 2002; Meinilä et al., 2004) since it renders possible that one pregnant worm can give rise to a viable deme after transmission to a new host. Most probably, the finding of *G. salaris* on the resident Arctic charr in Pålsbufjorden is an excellent example of the ability of rapid host-switching of the parasite species which frequently seem to occur in the genus.

The detection of a *G. salaris* infection on resident Arctic charr in a lake, which drains into a watercourse with uninfected Atlantic salmon, raises questions on the potential for further spread of the parasite to uninfected salmon stocks. Accordingly, there may be a need for examination of the infectivity of this *G. salaris* form on salmon. Infectivity studies to reveal the potential of the *G. salaris* from Arctic charr to establish and reproduce on Atlantic salmon, Arctic charr and rainbow trout, are ongoing. Generally, the observation that stocks of Arctic charr are able to maintain infections of *G. salaris* in the absence of Atlantic salmon will certainly have implications for the Norwegian salmon management and surveillance programmes.

Regarding Signaldalselva, it would be of interest to settle whether *G. salaris* can survive on Arctic charr for prolonged periods without contact with salmon. If the *G. salaris* infecting Arctic charr in Signaldalselva also proves to be able to survive and reproduce on Arctic charr in the absence of Atlantic salmon this will have implications for the management of Atlantic salmon stocks since it indicate that Arctic charr, in addition to salmon, must in general be removed or disinfected in order to eliminate *G. salaris* from an infected water course. In addition, there is certainly a need for more detailed studies on the influence of both host species on the parasite morphology by use of e.g. isogenic lines of *G. salaris*. The present results are excellent examples of the remarkable ability of *G. salaris* to switch to new host species over very short time intervals.

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