# Arctic charr (*Salvelinus alpinus*) is a suitable host for *Gyrodactylus salaris* (Monogenea, Gyrodactylidae) in Norway

G. ROBERTSEN*, H. HANSEN, L. BACHMANN *and* T. A. BAKKE

*Natural History Museum, Department of Zoology, University of Oslo, P.O. Box 1172, N-0318 Oslo, Norway*

(*Received 21 April 2006; revised 7 June and 14 July 2006; accepted 14 July 2006; first published online 23 October 2006*)

SUMMARY

*Gyrodactylus* specimens infecting both anadromous Arctic charr (*Salvelinus alpinus*) from River Signaldalselva (northern Norway) and resident Arctic charr from Lake Pålsbufjorden (southern Norway) were identified as *G. salaris* using molecular markers and morphometrics. The infection in Pålsbufjorden represents the first record of a viable *G. salaris* population infecting a host in the wild in the absence of salmon (*Salmo salar*). *G. salaris* on charr from Signaldalselva and Pålsbufjorden bear different mitochondrial haplotypes. While parasites infecting charr in Signaldalselva carry the same mitochondrial haplotype as parasites from sympatric Atlantic salmon, *G. salaris* from charr in Pålsbufjorden bear a haplotype that has previously been found in parasites infecting rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon, and an IGS repeat arrangement that is very similar to those observed earlier in parasites infecting rainbow trout. Accordingly, the infection may result from 2 subsequent host-switches (from salmon via rainbow trout to charr). Morphometric analyses revealed significant differences between *G. salaris* infecting charr in the 2 localities, and between those on sympatric charr and salmon within Signaldalselva. These differences may reflect adaptations to a new host species, different environmental conditions, and/or inherited differences between the *G. salaris* strains. The discovery of *G. salaris* on populations of both anadromous and resident charr may have severe implications for Atlantic salmon stock-management as charr may represent a reservoir for infection of salmon.

Key words: Arctic charr, Atlantic salmon, *Gyrodactylus salaris*, host-switch, molecular markers, morphometry, rainbow trout.

## INTRODUCTION

Species of the monogenean genus *Gyrodactylus* von Nordmann, 1832 are ectoparasites of fish with a varying level of host specificity (Bakke *et al*. 2002). The short generation time and hyperviviparity of gyrodactylids are considered ideal prerequisites for host switching (Cable and Harris, 2002; Boeger *et al*. 2003). Indeed, host switching is considered common for *Gyrodactylus* species (Ziętara and Lumme, 2002; Huyse and Volckaert, 2002, 2005) and has been put forward as an explanation for the speciation and radiation within the genus as well as for its large biodiversity (Brooks and McLennan, 1993; Boeger and Kritsky, 1997; Ziętara and Lumme, 2002, 2003; Meinilä *et al*. 2004).

The identification of gyrodactylids is traditionally based on the morphology of the hard parts of the posterior attachment apparatus (opisthaptor) (Malmberg, 1970), but a high degree of plasticity has been reported (Shinn *et al*. 2004). Environmental factors such as, for example, temperature may cause substantial variability in size and, to a lesser extent, in shape of the opisthaptoral hard parts and must be considered in taxonomic studies (Mo, 1991*a*, *b*, *c*, 1993; Dávidova *et al*. 2005). Several molecular markers, such as the internal transcribed spacers 1 and 2 (ITS-1 and ITS-2) and the intergenic spacer (IGS) of the nuclear ribosomal gene cluster as well as the mitochondrial cytochrome oxidase I (COI) have also been applied to study the taxonomy and systematics of gyrodactylids (see e.g. Matějusová *et al*. 2001; Ziętara and Lumme, 2002; Sterud *et al*. 2002; Cunningham *et al*. 2003; Hansen *et al*. 2003, 2006, Meinilä *et al*. 2004).

*G. salaris* Malmberg, 1957 is a pathogen of Norwegian Atlantic salmon stocks (*Salmo salar* L.) (Johnsen *et al*. 1999) and farmed rainbow trout (*Oncorhynchus mykiss* (Walbaum)) (see Mo, 1991*c*; Meinilä *et al*. 2004), and will experimentally infect and reproduce on other salmonids including anadromous Arctic charr (*Salvelinus alpinus* (L.)) (Bakke *et al*. 1996, 2002). *G. salaris* is difficult to discriminate morphologically from the closely related *G. thymalli* Žitnaň, 1960. Also molecular markers such as the nuclear ribosomal ITS (Ziętara and Lumme, 2003) and the mitochondrial COI

* Corresponding author. Tel: +47 22851677. Fax: +47 22851837. E-mail: grethe.robertsen@nhm.uio.no

 
doi:10.1017/S0031182006001223 Printed in the United Kingdom

Table 1. Details on the Norwegian Arctic charr (*Salvelinus alpinus*) and Atlantic salmon (*Salmo salar*) specimens used in this study

(All fish were screened for *Gyrodactylus* infection.)

| Sampling locality | Geographical coordinates | Water temp. (°C) | Sampling date | Host species | No. of fish | No. of *Gyrodactylus* |
|---|---|---|---|---|---|---|
| Lake Pålsbufjorden | 60°27′00N, 8°39′00E | 16 | 13–15.08.03 | *Salvelinus alpinus* | 30 | 1 |
| ,, | ,, | 10–12 | 8–12.09.03 | ,, | 22 | 3 |
| ,, | ,, | 15–8 | 15.08–10.10.03 | ,, | 24 | 67 |
| ,, | ,, | 8–7 | 19.10.03 | ,, | 15 | 10 |
| ,, | ,, | — | 27–28.10.04 | ,, | 10 | >50 |
| Lake Tinnsjøen | 59°54′00N, 8°55′00E | 7 | 03.11.03 | ,, | 10 | 0 |
| ,, | ,, | — | 18.10.05 | ,, | 111 | 0 |
| River Signaldalselva | 69°15′58N, 19°55′31E | — | 21.09.01 | ,, | 10 | >120 |
| ,, | ,, | 5·8 | 6–8.09.04 | ,, | 24 | 192 |
| ,, | ,, | 5·8 | 6–8.09.04 | *Salmo salar* | 15 | >10 000 |

sequences (Hansen *et al*. 2003, 2006) fail to unambiguously discriminate both species. IGS has been suggested to discriminate *G. salaris* and *G. thymalli* (Sterud *et al*. 2002). However, Hansen *et al*. (2006) challenge this interpretation but agree that specific arrangements of 23bp repeats in the IGS may in part be useful to discriminate parasites infecting salmon from those infecting rainbow trout.

The fish genus *Salvelinus* has a circumpolar distribution and includes several species and subspecies (Brunner *et al*. 2001), but Arctic charr is the only *Salvelinus* species with a natural distribution in Norway. Resident populations of Arctic charr occur in freshwater all over the country, but anadromous populations are restricted to northern Norway (Klemetsen *et al*. 2003). Until now, 9 *Gyrodactylus* spp. have been recorded on *Salvelinus* species worldwide (Harris *et al*. 2004). In northern Norway, with heavy *Gyrodactylus*-infected anadromous Arctic charr have been reported from the Rivers Skibotnelva and Signaldalselva. Both these rivers drain into the same fjord system and hold Atlantic salmon stocks (Mo, 1988; Johnsen *et al*. 1999; Knudsen *et al*. 2004; Kristoffersen *et al*. 2005). In Skibotnelva, *Gyrodactylus* on Arctic charr have been identified as *G. salaris* (see Mo, 1988), while those on Arctic charr in Signaldalselva have not yet been properly characterized.

In Buskerud County (southern Norway) *G. birmani* Konovalov, 1967 has been reported on resident Arctic charr in one locality (Sterud, 1999), while a pilot study revealed another *Gyrodactylus* sp. infection on the same host species in Lake Pålsbufjorden.

The main goal of this study was to identify the *Gyrodactylus* spp. infections found on anadromous Arctic charr in River Signaldalselva and on resident Arctic charr in Lake Pålsbufjorden in Norway. We therefore characterized and compared the *Gyrodactylus* spp. in both localities using molecular and morphometric methods. Further, we compared parasite specimens from the anadromous Arctic charr with those recovered from Atlantic salmon in the same river.

## MATERIALS AND METHODS

### *Collection of fish and parasites*

In southern Norway, Arctic charr were collected with gill nets in Lake Pålsbufjorden (Buskerud County) during the autumn of 2003 and 2004, and in Lake Tinnsjøen (Telemark County), during the autumn of 2003 and 2005. In northern Norway, Arctic charr and Atlantic salmon were collected concurrently by electro-fishing in Signaldalselva, Troms County, in 2001 and 2004 (Table 1).

The fish were killed by a blow to the head and the fins of adults were cut off with a pair of scissors and fixed in 96% ethanol (EtOH) immediately on capture. Parr were fixed whole in 96% EtOH. Later, the fish and fins were screened for *Gyrodactylus* infection under a stereo-microscope. Parasites were removed from as many fish specimens as possible from each locality and transferred into Eppendorf-tubes containing 96% EtOH and stored at −20 °C for later processing.

### *DNA extraction, amplification and sequencing*

The opisthaptors of the *Gyrodactylus* parasites were excised from the body. The remaining bodies of 1–3 parasites per population were individually used for molecular analyses. DNA was extracted according to Cunningham *et al*. (2001). The primer pair ITS1A (5′-GTAACAAGGTTTCCGTAGGTG-3′) and ITS2 (5′-TCCTCCGCTTAGTGATA-3′) (Matějusová *et al*. 2001) was used to amplify a fragment spanning the 3′ end of the 18S gene, the

https://doi.org/10.1017/S0031182006001223 Published online by Cambridge University Press

ITS1, the 5.8S gene, the ITS2, and the 5′ end of the 28S gene. The IGS repeat region was amplified using the primers IGSV3 (5′-CTGGCTATAAT-CACGTAAGACTGC-3′) IGSV4 (5′- AAGATA-CTCATTTGACTCGGTGTG-3′) designed by Collins and Cunningham (2000). The mitochondrial cytochrome oxidase I gene (CO1) was amplified using the primer pairs of Hansen *et al*. (2003). PCR reactions were carried out using the amplification protocols published along with the primer sequences. The PCR-products were purified using a QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's recommendations. Both DNA strands were sequenced using BigDye chemistry Version 1.1 (Applied Biosystems) and an ABI3100 automatic sequencer (Applied Biosystems). For sequencing of ITS, the PCR primers and the internal primers ITS4.5 (5′-CATCGGTCTCTCGAAC-G-3′) and ITSR3A (5′-GAGCCGAGTGATC-CACC-3′) (Matějusová *et al*. 2001) and ITS28F (5′-TAGCTCTAGTGGTTCTTCCT-3′) (Ziętara and Lumme, 2003) were used. Both IGS and CO1 were sequenced using the PCR primers. All sequences were submitted to a BLASTN (Altschul, 1991) search in GenBank to establish possible identity.

### *Morphometric analyses*

After excision, the opisthaptors to be analysed by both light and scanning electron microscopy (SEM) were prepared according to a slightly modified version of Harris *et al*. (1999). A Leica DC 500 camera mounted on a Leica DM 6000B stereo microscope was used to take digitalized light microscope photographs of the opisthaptoral hard parts (at magnifications of 1000, 1250 or 1600). All distances were measured with the help of the Leica IM1000 software system using a digital calliper and a point-to-point tool.

Preparations of opisthaptoral hard parts for SEM were 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.

Only slides containing all 3 opisthaptoral hard parts: hamuli, ventral bridge, and marginal hooks were used for morphological analyses. Fifteen to 30 specimens from each population were measured. Thirty-three different linear measurements and 1 angular measurement converted to cosine values (most measurements have been described by Shinn *et al*. (2004)) were applied (see Fig. 1 and Table 2).

Principal component analysis (PCA) was employed to analyse the multivariate morphometric datasets. Having identified the axes of maximal variance, the principal components scores (PCA-scores) were compared by Mann-Whitney U tests. By negating PC1, the component that often best expresses size variation, the effects of having a between-group bias in size was assumed minimal. In addition the PCA-scores from all components, both including and excluding PC1, were subjected to Two-group permutation tests. Kruskal-Wallis and Mann-Whitney U tests were employed to explore differences in single measures between populations. All calculations and graphical illustrations were performed with the programme PAST *vs* 1·29 (Hammer *et al*. 2001).

## RESULTS

### *Infection data*

Both Arctic charr from Lake Pålsbufjorden and River Signaldalselva, and Atlantic salmon from Signaldalselva were infected with *Gyrodactylus* (Table 1). The abundance of *Gyrodactylus* sp. on Arctic charr was 0·9 in Pålsbufjorden in 2003 and 8·0 in Signaldalselva in 2004. All specimens of Atlantic salmon in Signaldalselva in 2004 were infected. No parasites were observed on Arctic charr from Lake Tinnsjøen.

### *Identification of* Gyrodactylus *sp. from Arctic charr in Signaldalselva and comparison with* G. salaris *on sympatric Atlantic salmon*

The ITS sequences obtained from the *Gyrodactylus* specimens from both Arctic charr and Atlantic salmon matched GenBank Accession number AF328871 and were thus identified as *G. salaris*. The mitochondrial COI sequences from Arctic charr were identical to the haplotype reported earlier from *G. salaris* on Atlantic salmon from Signaldalselva (GenBank Accession number AY486497) and Skibotnelva (GenBank Accession number AY486525) (Hansen *et al*. 2003).

The individual measurements taken of *G. salaris* from both Arctic charr ($n=23$) and Atlantic salmon ($n=23$) showed that only 6 out of 34 measures of the opisthaptoral hard parts differed significantly (Mann-Whitney U tests, $P<0{\cdot}05$; see Table 2). A PCA-plot demonstrates a high degree of overlap in the first and the second principal components (PC1 and PC2; see Fig. 2) . The loadings of PC1 were mostly negative, and PC1 were therefore interpreted as a component representing variance related mainly to size. The variances of the PC2-7 were interpreted as reflecting shape because of both negative and positive loadings. There were no significant differences between *G. salaris* from Arctic charr and Atlantic salmon along PC1-7 (Mann-Whitney U test, $P>0{\cdot}05$, Table 3) which collectively accounted for 70% of the variation in the dataset. Neither were there significant differences between *G. salaris* on Arctic charr and salmon when all PCA components

https://doi.org/10.1017/S0031182006001223 Published online by Cambridge University Press

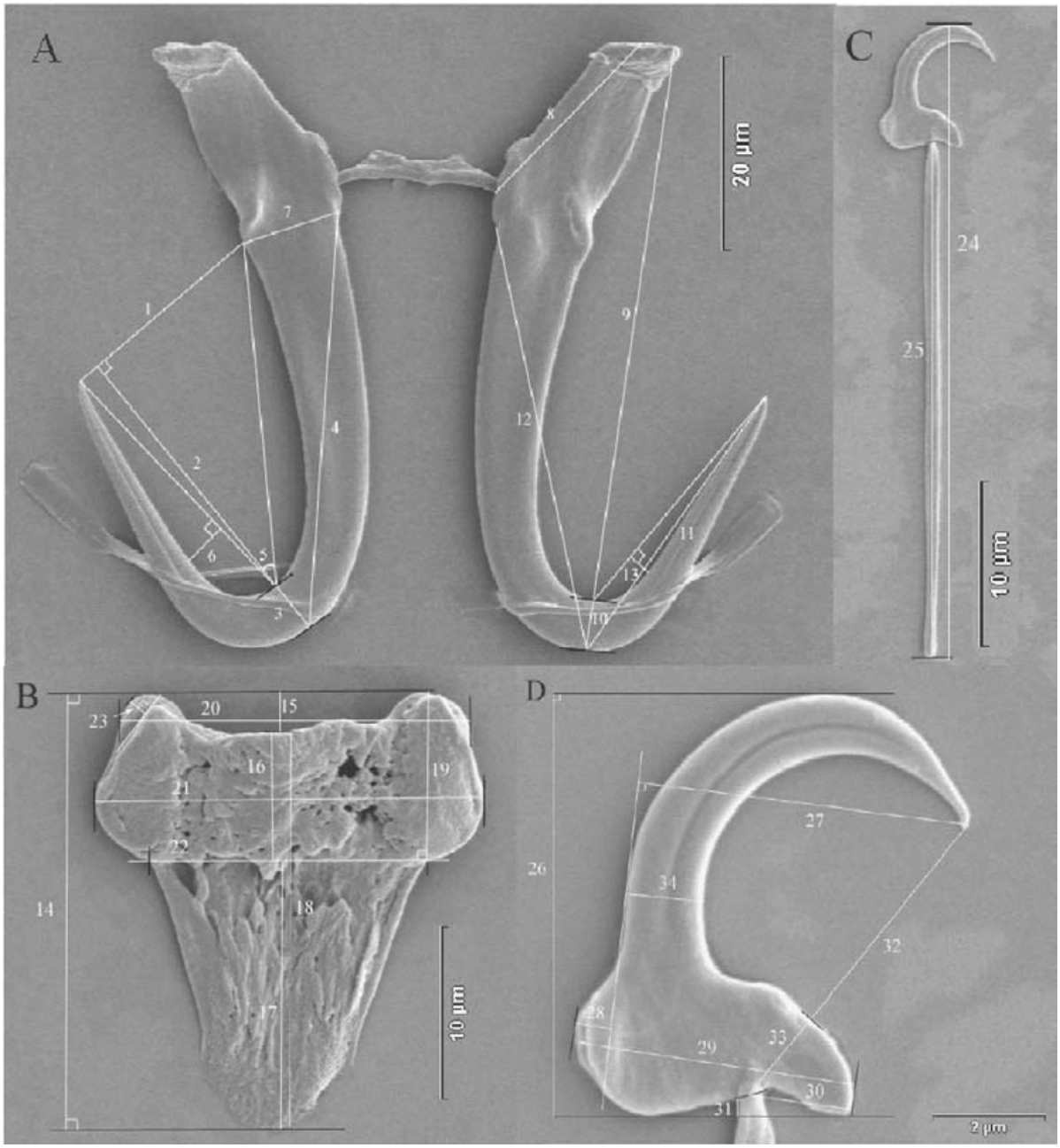


Fig. 1. (A–D) Scanning electron micrographs of the opisthaptoral hard parts from *Gyrodactylus* sp. from Arctic charr (*Salvelinus alpinus*) in Signaldalselva illustrating the morphometric characters used. The numbers refer to the characters listed in Table 1. (A) Hamuli; (B) ventral bar; (C) marginal hook; (D) marginal hook sickle.

were considered collectively, nor when PC1 was excluded from the analyses (Two-group permutation tests, $P > 0{\cdot}05$).

*Identification of* Gyrodactylus *sp. from Arctic charr in Pålsbufjorden and comparison with* Gyrodactylus *sp. from Arctic charr in Signaldalselva*

The ~1250 bp ITS sequences (GenBank Accession number DQ898302) obtained from 5 *Gyrodactylus* specimens from Arctic charr from Pålsbufjorden (3 collected in 2003 and 2 collected in 2004) were identified as *G. salaris*. Only one G→A transition at position 288 of the ITS 2 region was detected consistently in all individuals when compared to GenBank Accession number AF328871. This particular G→A transition has never been observed before. The 720 bp mitochondrial COI sequence (GenBank Accession number DQ923578) was identical to the haplotype reported from *G. salaris* on hatchery reared rainbow trout and on Atlantic salmon from the Norwegian rivers Drammenselva, Lierelva and Lærdalselva (Hansen *et al.* 2003; Meinilä *et al.* 2004). The 685 bp of the IGS repeat region (GenBank Accession number DQ898303)

https://doi.org/10.1017/S0031182006001223 Published online by Cambridge University Press

Table 2. The morphometric results of 34 characters measured on the opisthaptoral hard parts: hamuli, ventral bar and marginal hook of *Gyrodactylus salaris* from Arctic charr (*Salvelinus alpinus*) ($N=30$) from Pålsbufjorden, and Arctic charr ($N=23$) and Atlantic salmon (*Salmo salar*) ($N=23$) from Signaldalselva

(Each measure is given as micrometer ($\mu$m) $\pm$standard deviation (S.D.), range in parentheses. The results of a Mann-Whitney U-test based on the different individual measurements are also presented (statistically significance, $P<0{\cdot}05$).)

| Character measured | *G. salaris* Arctic charr Pålsbufjorden | Mann-Whitney *P* | *G. salaris* Arctic charr Signaldalselva | Mann-Whitney *P* | *G. salaris* Atlantic salmon Signaldalselva |
|---|---|---|---|---|---|
| Hamulus (H) | | | | | |
| 1 Aperture length | 24·15 ± 2·90 (20·66–32·87) | >0·05 | 24·03 ± 1·11 (21·19–25·63) | >0·05 | 23·81 ± 1·28 (21·53–27·28) |
| 2 Point length1 | 39·34 ± 0·95 (36·66–41·44) | <0·01 | 34·78 ± 1·11 (32·54–36·68) | >0·05 | 35·25 ± 0·86 (33·47–36·86) |
| 3 Distal shaft width1 | 7·58 ± 0·69 (6·76–9·34) | <0·01 | 6·20 ± 0·21 (5·85–6·66) | <0·05 | 6·02 ± 0·37 (5·23–6·8) |
| 4 Shaft length1 | 46·54 ± 1·28 (44·55–49·13) | <0·01 | 42·27 ± 1·24 (38·96–44·09) | >0·05 | 42·55 ± 1·73 (38·12–45·11) |
| 5 Aperture angle | 0·77 ± 0·06 (0·57–0·84) | <0·01 | 0·74 ± 0·02 (0·71–0·78) | >0·05 | 0·76 ± 0·03 (0·70–0·81) |
| 6 Inner curve length1 | 3·94 ± 1·01 (1·63–6·24) | <0·01 | 5·82 ± 1·23 (3·52–8·99) | >0·05 | 5·62 ± 0·93 (4·13–7·29) |
| 7 Proximal shaft width | 12·11 ± 0·75 (10·07–13·72) | <0·01 | 10·81 ± 0·55 (10·05–12·28) | >0·05 | 10·56 ± 0·44 (9·67–11·57) |
| 8 Root length | 27·47 ± 1·19 (24·58–29·87) | <0·01 | 23·30 ± 1·90 (20·79–29·32) | >0·05 | 22·88 ± 1·02 (20·42–24·74) |
| 9 Total length | 75·88 ± 1·77 (72·47–79·41) | <0·01 | 68·15 ± 2·08 (64·00–72·57) | >0·05 | 67·72 ± 2·24 (61·28–71·92) |
| 10 Distal shaft width2 | 7·41 ± 0·65 (6·46–9·27) | <0·01 | 5·74 ± 0·26 (5·17–6·09) | >0·05 | 5·60 ± 0·28 (4·85–5·94) |
| 11 Point length2 | 38·33 ± 0·87 (36·73–40·48) | <0·01 | 32·93 ± 1·37 (30·03–35·21) | >0·05 | 33·64 ± 0·91 (31·79–35·45) |
| 12 Shaft length2 | 49·38 ± 1·27 (46·55–51·77) | <0·01 | 46·52 ± 1·40 (43·55–48·78) | >0·05 | 46·25 ± 1·79 (40·50–48·53) |
| 13 Inner curve length2 | 2·59 ± 0·59 (1·46–3·72) | >0·05 | 2·47 ± 0·65 (1·37–3·87) | <0·05 | 2·82 ± 0·50 (1·99–4·06) |
| Ventral Bar (VB) | | | | | |
| 14 Total length | 30·71 ± 2·16 (28·16–40·21) | <0·01 | 27·66 ± 1·85 (25·03–34·30) | >0·05 | 27·29 ± 1·48 (24·46–30·97) |
| 15 Process to mid-length | 2·10 ± 0·76 (0·40–3·75) | >0·05 | 2·25 ± 0·84 (0·37–3·51) | <0·05 | 2·73 ± 0·70 (0·53–3·54) |
| 16 Basal median length | 11·07 ± 1·65 (8·37–15·39) | <0·01 | 8·58 ± 1·08 (6·45–10·78) | <0·05 | 7·64 ± 1·02 (5·72–10·32) |
| 17 Membrane length | 17·47 ± 2·30 (13·02–24·98) | >0·05 | 16·82 ± 1·37 (15·28–21·69) | >0·05 | 16·91 ± 1·37 (13·99–19·65) |
| 18 Central length | 28·70 ± 2·33 (25·19–37·81) | <0·01 | 25·42 ± 1·73 (22·98–31·19) | >0·05 | 24·56 ± 1·53 (22·28–27·75) |
| 19 Lateral length | 11·63 ± 1·13 (9·58–15·22) | >0·05 | 11·20 ± 0·83 (9·71–12·52) | >0·05 | 11·06 ± 0·70 (9·36–12·14) |
| 20 Process to process width | 27·55 ± 2·14 (25·59–36·48) | <0·01 | 23·43 ± 1·43 (21·15–28·75) | <0·05 | 22·56 ± 1·16 (20·33–24·36) |
| 21 Width | 29·18 ± 1·84 (27·04–37·24) | <0·01 | 25·56 ± 1·45 (23·88–31·19) | >0·05 | 24·67 ± 1·20 (21·73–26·43) |
| 22 Maximum membrane width | 21·14 ± 1·51 (19·14–26·82) | <0·01 | 17·75 ± 1·33 (15·93–22·79) | >0·05 | 17·09 ± 1·47 (14·26–19·75) |
| 23 Process length | 2·01 ± 0·36 (1·49–2·94) | <0·01 | 1·35 ± 0·28 (0·85–2·04) | >0·05 | 1·27 ± 0·28 (0·75–1·92) |
| Marginal Hook (MH) | | | | | |
| 24 Total length | 40·18 ± 1·02 (38·19–42·58) | 0·025 | 39·49 ± 0·93 (37·62–40·73) | >0·05 | 39·30 ± 0·92 (37·21–40·64) |
| 25 Shaft length | 32·80 ± 0·92 (30·85–34·37) | >0·05 | 32·44 ± 0·80 (30·59–33·56) | >0·05 | 32·27 ± 0·80 (30·55–33·53) |
| 26 Sickle length | 7·93 ± 0·22 (7·59–8·4) | <0·01 | 7·61 ± 0·19 (7·33–7·89) | <0·05 | 7·48 ± 0·19 (7·07–7·8) |
| 27 Sickle distal width | 6·05 ± 0·27 (5·63–6·73) | >0·05 | 5·93 ± 0·21 (5·41–6·34) | >0·05 | 5·99 ± 0·16 (5·60–6·26) |
| 28 Sickle heel length | 0·78 ± 0·11 (0·57–0·99) | >0·05 | 0·75 ± 0·13 (0·51–0·99) | >0·05 | 0·71 ± 0·09 (0·57–0·88) |
| 29 Sickle proximal width | 5·29 ± 0·31 (4·95–6·23) | >0·05 | 5·16 ± 0·16 (4·86–5·41) | >0·05 | 5·15 ± 0·20 (4·69–5·51) |
| 30 Sickle toe length | 2·01 ± 0·20 (1·74–2·77) | >0·05 | 1·99 ± 0·12 (1·82–2·32) | >0·05 | 1·98 ± 0·13 (1·69–2·15) |
| 31 Instep height | 0·61 ± 0·13 (0·43–0·87) | <0·01 | 0·42 ± 0·10 (0·27–0·60) | >0·05 | 0·45 ± 0·09 (0·28–0·6) |
| 32 Aperture distance | 6·42 ± 0·20 (6·09–7·2) | <0·01 | 6·20 ± 0·12 (6·00–6·46) | >0·05 | 6·19 ± 0·16 (5·84–6·52) |
| 33 Sickle toe height | 1·76 ± 0·20 (1·20–2·43) | <0·01 | 1·67 ± 0·09 (1·50–1·80) | >0·05 | 1·62 ± 0·11 (1·41–1·79) |
| 34 Sickle width | 1·51 ± 0·13 (1·28–1·9) | <0·01 | 1·39 ± 0·07 (1·29–1·55) | >0·05 | 1·38 ± 0·10 (1·08–1·53) |

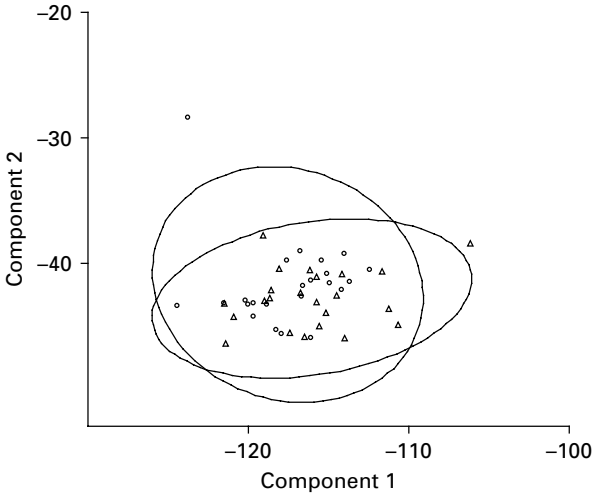


Fig. 2. PCA plot of the morphometric data of all measurements (see Table 1) of *Gyrodactylus* sp. from Arctic charr (*Salvelinus alpinus*) (circle) and *G. salaris* from Atlantic salmon (*Salmo salar*) (triangle) from Signaldalselva in the two first planes (Component 1 *vs* Component 2) of the PCA plot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

contained a particular arrangement of 23 bp repeats (ABBABBBBBB – PPPSURVQ) that is identical to that of some clones obtained for *G. salaris* from hatchery reared rainbow trout (GenBank Accession numbers AY490400-AY490402) (Hansen *et al.* 2006). Such a repeat arrangement has never been reported in Norwegian *G. salaris* from salmon (Hansen *et al.* 2006).

A comparison of the morphology of *G. salaris* from Arctic charr from Pålsbufjorden ($n=30$) (see Fig. 3) and Signaldalselva ($n=23$) (see Fig. 1) showed that 24 out of 34 measures of the opisthaptoral hard parts differed significantly (Mann-Whitney U tests, $P<0{\cdot}05$; see Table 2). In a PCA-plot there is almost no overlap of the morphological measurements of *G. salaris* from Arctic charr in Pålsbufjorden and Signaldalselva along PC1 (Fig. 4). The loadings of PC1 were mostly positive so the variance in this axis seems mainly to originate from size-differences. The 2 *G. salaris* populations were significantly different in PC1-3 (Mann-Whitney U tests, $P<0{\cdot}05$; Table 4), which collectively account for 89% of the variance. In PC4-6, which collectively account for 8% of the variance, the morphometric differences between these populations are small and not statistically significant (Mann-Whitney U tests, $P>0{\cdot}05$; Table 4). The morphometry of the 2 *G. salaris* populations was significantly different when all components were considered collectively (Two-group permutation test, $P<0{\cdot}05$). When PC1 were excluded from the analysis, there were no significant differences (Two-group permutation test, $P>0{\cdot}05$).

## DISCUSSION

*Gyrodactylus* specimens from Arctic charr from both the north Norwegian Signaldalselva and the south Norwegian Pålsbufjorden were identified as *G. salaris* by means of molecular and morphometric methods. The parasites from the 2 localities belong to 2 different mitochondrial lineages. In addition, the morphometric measurements of the parasites from both Signaldalselva and Pålsbufjorden fall within the relatively wide range of measurements for the up to 15 characters for *G. salaris* published earlier by Mo (1991 *a*, *b*, *c*) and Shinn *et al.* (2001, 2004) (except for 1 individual from Pålsbufjorden that had an atypically large ventral bar).

Our finding of *G. salaris* on wild anadromous Arctic charr in Signaldalselva confirms the suggestion of the presence of this species made by Knudsen *et al.* (2004) and is in line with observations of *G. salaris* on Arctic charr in the nearby River Skibotnelva (Mo, 1988; Kristoffersen *et al.* 2005). Our data also corroborate earlier experimental data which showed that Arctic charr from some anadromous populations can serve as a suitable host for *G. salaris* (strain from Atlantic salmon in the river Lierelva) (see Bakke *et al.* 1996). The infections of both Atlantic salmon and Arctic charr in the same locality with *G. salaris* bearing the same mitochondrial haplotype strongly indicate that both parasite metapopulations belong to the same suprapopulation. It is therefore a reasonable assumption that anadromous Arctic charr can acquire *G. salaris* from co-occurring infected Atlantic salmon (see Knudsen *et al.* 2006). Transmission of the parasite may occur through direct contact between infected fish, indirectly from the substrate, or via drift in the water column (Bakke *et al.* 1992; Soleng *et al.* 1999). Atlantic salmon parr are frequently found in deeper parts of rivers with strong water currents, whereas parr of Arctic charr are usually found in shallow waters near the shore (Heggberget, 1984). Hence, indirect transmission of *G. salaris* from salmon to Arctic charr is probably the most important transmission route. Further support for this assumption comes from the observation of Olstad *et al.* (2006) that *G. salaris* can survive for up to 2·5 days off its host. It is of course also possible that heavily infected and moribund salmon parr may display abnormal behaviour and move into more shallow waters, thereby facilitating transmission between the host species (see Bakke *et al.* 1992; Knudsen *et al.* 2006). However, the relatively high average infection and prevalence of *G. salaris* on Arctic charr in Signaldalselva also indicates *in situ* reproduction on Arctic charr (Knudsen *et al.* 2006).

*G. salaris* from Arctic charr and Atlantic salmon in Signaldalselva are morphologically almost indistinguishable, which may be taken as further support

https://doi.org/10.1017/S0031182006001223 Published online by Cambridge University Press

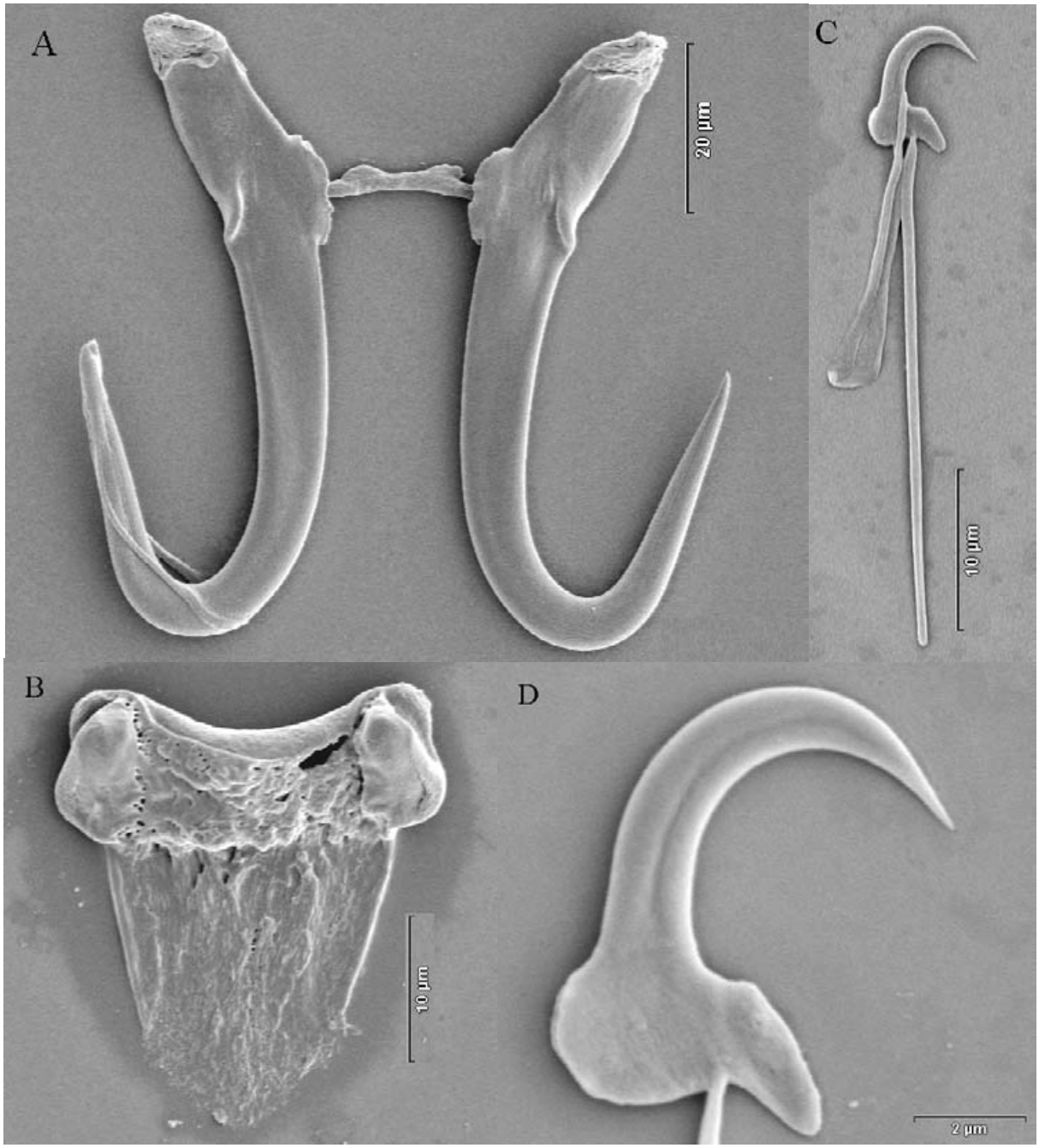


Fig. 3. (A–D) Scanning electron micrographs of the opisthaptoral hard parts of *Gyrodactylus salaris* from Arctic charr (*Salvelinus alpinus*) in Pålsbufjorden. (A) Hamuli; (B) ventral bar; (C) marginal hook; (D) marginal hook sickle.

for both populations belonging to the same gene pool. The minor morphometric differences may represent host-induced differences since the macro-environment is identical. Previously, host-dependent morphometric differences have not been observed for *G. salaris* (see Mo, 1991*c*) or other *Gyrodactylus* species (e.g. Mo, 1993; Geets *et al.* 1999). This was, however, suggested by Huyse and Volckaert (2002) who found significant morphometric differences within *G. rugiensoides* infecting *Pomatoschistus pictus* and *P. minutus*. The epidermis of different fish species provide different host-specific micro-environments (Buchmann and Lindenstrøm, 2002) which may affect the phenotype of the opisthaptoral hard parts.

The morphometric differences between *G. salaris* infecting Arctic charr in Signaldalselva and in Pålsbufjorden exceed the small morphometric differences observed between the parasites infecting Arctic charr and Atlantic salmon in Signaldalselva. The opisthaptoral hard parts of *G. salaris* from Arctic charr in Pålsbufjorden are larger than those from Arctic charr in Signaldalselva and there are also some differences in shape. Earlier investigations have shown that the size of the opisthaptoral hard parts of *G. salaris* is negatively correlated with water

https://doi.org/10.1017/S0031182006001223 Published online by Cambridge University Press

Table 3. The percentage variation described by the 7 first components of the PCA analyses of *Gyrodactylus salaris* from Arctic charr (*Salvelinus alpinus*) and Atlantic salmon (*Salmo salar*) in Signaldalselva

(The results of a Mann-Whitney U-test based on the PCA-scores of the different components are also presented.)

| PCA | | Mann-Whitney U | |
|---|---|---|---|
| Component | % Variation | t = ub | *P* |
| 1 | 34·357 | 216 | 0·29 |
| 2 | 25·557 | 218 | 0·31 |
| 3 | 9·578 | 198 | 0·15 |
| 4 | 7·003 | 244 | 0·66 |
| 5 | 6·323 | 179 | 0·06 |
| 6 | 3·996 | 228 | 0·43 |
| 7 | 3·635 | 261·5 | 0·06 |

Table 4. The percentage variation described by the first 6 components of the PCA analyses of *Gyrodactylus salaris* from Arctic charr (*Salvelinus alpinus*) in Pålsbufjorden and Signaldalselva

(The results of a Mann-Whitney U-test based on the PCA-scores of the different components are also shown.)

| PCA | | Mann-Whitney U | |
|---|---|---|---|
| Component | % Variation | t = ub | *P* |
| 1 | 65·421 | 0 | 6·31E-10 |
| 2 | 13·577 | 216 | 0·02 |
| 3 | 5·833 | 224 | 0·03 |
| 4 | 3·744 | 324·5 | 0·72 |
| 5 | 2·587 | 322 | 0·69 |
| 6 | 1·987 | 281 | 0·25 |

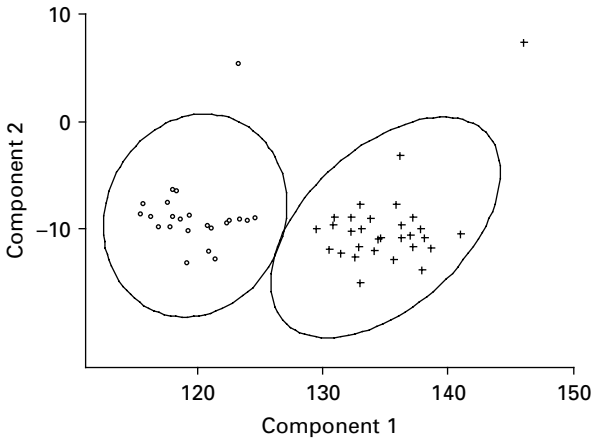


Fig. 4. PCA plots of the two first planes of the morphometric data of all measurements (see Table 1) of *Gyrodactylus salaris* on Arctic charr (*Salvelinus alpinus*) from Pålsbufjorden (cross) and Signaldalselva (circle) (ellipses are 95% confidence intervals about the mean).

temperature (Mo, 1991*a*, *b*, *c*). This has also been observed for several other *Gyrodactylus* species (e.g. Ergens, 1976, 1981; Ergens and Gelnar, 1985; Mo, 1993; Appleby, 1996). Since *G. salaris* in Signaldalselva was collected at a lower temperature than *G. salaris* in Pålsbufjorden its opisthaptoral hard parts are expected to be larger. However, the opposite has been observed. Although more comprehensive sampling is required to satisfactorily answer this question, our observation may be taken as an indication that the size differences of *G. salaris* infecting Arctic charr in Signaldalselva and in Pålsbufjorden may depend on other factors than water temperature.

*G. salaris* from Arctic charr in Pålsbufjorden and in Signaldalselva belong to different mitochondrial haplogroups. The parasites from Pålsbufjorden carry the same mitochondrial haplotype as parasites from Atlantic salmon in the River Drammenselva (Hansen *et al*. 2003). Mo (1991*c*) hypothesized that *G. salaris* on Atlantic salmon in Drammenselva has larger opisthaptoral hooks than usually observed from parasites on other Atlantic salmon stocks. The morphology of *G. salaris* from Pålsbufjorden is also slightly different from other *G. salaris* populations with the same mitochondrial haplotype, i.e. from *G. salaris* populations on Atlantic salmon in Drammenselva and farmed rainbow trout in western Sweden (Robertsen, 2005). Although these differences may be linked to the macroenvironment, it can currently not be ruled out that *G. salaris* from Pålsbufjorden already shows adaptation to the new host species, the Arctic charr. However, other explanations for the observed morphological differences between parasites infecting the two Arctic charr populations may be possible.

The finding of *G. salaris* on resident Arctic charr in Pålsbufjorden in southern Norway is the first record of a viable and sustained infection of *G. salaris* on another wild salmonid in the absence of salmon and is therefore of particular interest. The mitochondrial haplotype of this *G. salaris* is the same as that of *G. salaris* on Atlantic salmon from the Norwegian rivers Drammenselva, Lierelva and Lærdalselva, and of *G. salaris* on farmed rainbow trout throughout Fennoscandia (Hansen *et al*. 2003; Meinilä *et al*. 2004). However, *G. salaris* from salmon in Lierelva was unable to reproduce on resident Arctic charr from Lake Korssjøen, Southern Norway (Bakke *et al*. 1996). This may indicate host or parasite specific differences that are not reflected by the currently used genetic markers. The IGS sequences of *G. salaris* from Pålsbufjorden were most similar to sequences found in specimens from farmed rainbow trout (see Sterud *et al*. 2002; Cunningham *et al*. 2003, Hansen *et al*. 2006). Thus, both CO1 and IGS sequences are congruent with a hypothesis of rainbow trout being the source of the infection of Arctic charr in Pålsbufjorden. Originally, Arctic charr were

https://doi.org/10.1017/S0031182006001223 Published online by Cambridge University Press

introduced into Pålsbufjorden from Lake Tinnsjøen between 1910 and 1919 (Aass, 1970). *G. salaris* may have been introduced simultaneously, however, no gyrodactylids on Arctic charr from Tinnsjøen have been observed yet. The Arctic charr could also have been infected when arriving in Pålsbufjorden, but *G. salaris* has not been found on brown trout, which is the only native salmonid in the lake (unpublished observations). It is thus more likely that *G. salaris* was introduced into Pålsbufjorden more recently. Rainbow trout were imported from Jutland (Denmark) and kept in various fish farms in southern Norway before they were introduced to Pålsbufjorden on several occasions during 1962–1964, and to the nearby and connected lake Tunhovdfjorden between 1962 and 1967 (Per Aass, personal communications). According to this line of argument, rainbow trout must have acquired the *G. salaris* infection in Danish or Norwegian fish farms. A 'rainbow trout strain' of *G. salaris* may subsequently have switched to the resident Arctic charr population in Pålsbufjorden. This hypothetical scenario implies at least 2 host-switches: the first from Atlantic salmon to rainbow trout in Danish or Norwegian fish farms and the second from rainbow trout to Arctic charr in Pålsbufjorden. After the second host switch, the parasite must have adapted rapidly to the new host species, as the rainbow trout vanished from fish catches within only 4 years after its last introduction (Per Aass, personal communications). This hypothesis implies a remarkably rapid host switch, as rainbow trout and Arctic charr probably co-occurred only for about 6 years. The reproductive mode of *Gyrodactylus* facilitates a rapid speciation by isolation and subsequent genetic diversification after a successful host-switch (Cable and Harris, 2002; Ziętara and Lumme, 2002; Meinilä *et al*. 2004) since a single worm can give rise to a viable population. In addition, recurrent host-switching has been put forward to promote rapid host-specific adaptation (Cribb *et al*. 2002; Poulin, 2002; Ziętara and Lumme, 2002).

The finding that *G. salaris* can infect resident Arctic charr in the absence of alternative hosts indicates the potential for radiation of this species complex. Further speculation about the importance of Arctic charr as a reservoir for *G. salaris* awaits detailed cross-infection experiments with different *G. salaris* strains. However, preliminary results suggest that a strain of *G. salaris* from salmon was more pathogenic to salmon than the Arctic charr strain from Pålsbufiorden (Olstad *et al*. manuscript in preparation).

The present findings have implications for the Norwegian salmon management and surveillance programmes as Arctic charr may sustain a *G. salaris* infection and hence be a potential focus for spreading of the parasite to uninfected Atlantic salmon stocks. Thus, the presence of Arctic charr must be taken into consideration along with Atlantic salmon when trying to eliminate *G. salaris* from an infected water course.

We thank Cathrine Vollelv, Henning Pavels, Finn Smestad, Kjersti Kvalsvik and Bjørn A. Bjerke, NHM, Department for Zoology, University of Oslo (UiO), and Rune Knudsen, NFH, University of Tromsø, for help with the fieldwork, Torill M. Rolfsen for support at the Scanning Electron Microscope Unit, University of Oslo, and Kjetil Olstad, NHM, University of Oslo and Jo Cable, Cardiff University for laboratory assistance and advice. This work was supported by the NRC 'Wild Salmon Program' (Project no. 145861/720) and the National Centre for Biosystematics (Project no. 146515/420, co-founded by the NRC and the NHM, UiO).

## REFERENCES

**Aass, P.** (1970). The winter migrations of charr, *Salvelinus alpinus* L., in the hydroelectric reservoirs Tunhovdfjord and Pålsbufjord, Norway. *Report of the Institute of Freshwater Research Drottningholm* **50**, 5–44.

**Altschul, S. F.** (1991). Amino acid substitution matrices from an information theoretic perspective. *Journal of Molecular Biology* **219**, 555–565.

**Appleby, C.** (1996). Variability of the opisthaptoral hard parts of *Gyrodactylus callariatis* Malmberg, 1957 (Monogenea: Gyrodactylidae) from Atlantic cod *Gadus morhua* L. in the Oslo Fjord, Norway. *Systematic Parasitology* **33**, 199–207.

**Bakke, T. A., Harris, P. D. and Cable, J.** (2002). Host specificity dynamics: observations on gyrodactylid monogeneans. *International Journal for Parasitology* **32**, 281–308. DOI:10.1016/S0020-7519(01)00331-9.

**Bakke, T. A., Harris, P. D., Jansen, P. A. and Hansen, L. P.** (1992). Host specificity and dispersal strategy in gyrodactylid monogeneans, with particular reference to *Gyrodactylus salaris* Malmberg (Platyhelminthes, Monogenea). *Diseases of Aquatic Organisms* **13**, 63–74.

**Bakke, T. A., Jansen P. A. and Harris, P. D.** (1996). Differences in susceptibility of anadromous and resident stocks of Arctic charr to infections of *Gyrodactylus salaris* under experimental conditions. *Journal of Fish Biology* **49**, 341–351.

**Boeger, W. A. and Kritsky, D. C.** (1997). Coevolution of the Monogenoidea (Platyhelminthes) based on a revised hypothesis of parasite phylogeny. *International Journal for Parasitology* **27**, 1495–1511.

**Boeger, W. A., Kritsky, D. C. and Pie, M. R.** (2003). Context of diversification of the viviparous Gyrodactylidae (Platyhelminthes, Monogenoidea). *Zoologica Scripta* **32**, 437–448.

**Brooks, D. R. and McLennan, D. A.** (1993). *Parascript: Parasites and the Language of Evolution*. Smithsonian Institution Press, Washington, DC.

**Brunner, P. C., Douglas, M. R., Osinov, A., Wilson, C. C. and Bernatchez, L.** (2001). Holarctic phylogeography of Arctic charr (*Salvelinus alpinus* L.) inferred from mitochondrial DNA sequences. *Evolution* **55**, 573–586.

**Buchmann, K. and Lindenstrøm, T.** (2002). Interactions between monogenean parasites and their fish hosts. *International Journal for Parasitology* **32**, 309–319.

**Cable, J. and Harris, P. D.** (2002). Gyrodactylid developmental biology: historical review, current status and future trends. *International Journal for Parasitology* **32**, 255–280.

**Collins, C. M. and Cunningham, C. O.** (2000). Characterization of the *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes: Monogenea) ribosomal intergenic spacer (IGS) DNA. *Parasitology* **121**, 555–563. DOI:10.1017/S0031182099006770.

**Cribb, T. H., Chisholm, L. A. and Bray, R. A.** (2002). Diversity in the Monogenea and Digenea: does lifestyle matter? *International Journal for Parasitology* **32**, 321–328.

**Cunningham, C. O., Mo, T. A., Collins, C. M., Buchmann, K., Thiery, R., Blanc, G. and Lautraite, A.** (2001). Redescription of *Gyrodactylus teuchis* Lautraite, Blanc, Thiery, Daniel & Vigneulle, 1999 (Monogenea: Gyrodactylidae); a species identified by ribosomal RNA sequence. *Systematic Parasitology* **48**, 141–150.

**Cunningham, C. O., Collins, C. M., Malmberg, G. and Mo, T. A.** (2003). Analysis of ribosomal RNA intergenic spacer (IGS) sequences in species and populations of *Gyrodactylus* (Platyhelminthes: Monogenea) from salmonid fish in northern Europe. *Diseases of Aquatic Organisms* **57**, 237–246.

**Dávidova, M., Jarkoský, J., Matějusová, I. and Gelnar, M.** (2005). Seasonal occurrence and metrical variability of *Gyrodactylus rhodei* Žitnaň, 1964 (Monogenea, Gyrodactylidae). *Parasitology Research* **95**, 398–405.

**Ergens, R.** (1976). Variability of hard parts of opisthaptor of two species of *Gyrodactylus* Nordmann, 1832 (Monogenoidea) from *Phoxinus phoxinus* (L.). *Folia Parasitologica* **23**, 111–126.

**Ergens, R.** (1981). Variability of hard parts of opisthaptor in *Gyrodactylus truttae* Gläser, 1974 (Gyrodactylidae: Monogenea). *Folia Parasitologica* **28**, 37–42.

**Ergens, R. and Gelnar, M.** (1985). Experimental verification of the effect of temperature on the size of hard parts of opisthaptor of *Gyrodactylus katharineri* Malmberg, 1964 (Monogenea). *Folia Parasitologica* **32**, 377–380.

**Geets, A., Appleby, C. and Ollevier, F.** (1999). Host-dependent and seasonal variation in opisthaptoral hard parts of *Gyrodactylus* cf. *arcuatus* from three *Pomatoschistus* spp. and *G. arcuatus* from *Gasterosteus aculeatus*: a multivariate approach. *Parasitology* **119**, 27–40.

**Hammer, Ø., Harper, D. A. T. and Ryan, P. D.** (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**, 1–9.

**Hansen, H., Bachmann, L. and Bakke, T. A.** (2003). Mitochondrial DNA variation of *Gyrodactylus* spp. (Monogenea, Gyrodactylidae) populations infecting Atlantic salmon, grayling and rainbow trout in Norway and Sweden. *International Journal for Parasitology* **33**, 1471–1478. DOI:10.1016/S0020-7519(03)00200-5.

**Hansen, H., Martinsen, L., Bakke, T. A. and Bachmann, L.** (2006). The incongruence of nuclear and mitochondrial DNA variation supports conspecificity of the monogenean parasites *Gyrodactylus salaris* and *G. thymalli*. *Parasitology* doi: 10.1017/5003//82006000655 (in the Press).

**Harris, P. D., Cable, J., Tinsley, R. C. and Lazarus, C. M.** (1999). Combined ribosomal DNA and morphological analysis of individual gyrodactylid monogeneans. *Journal of Parasitology* **85**, 188–191.

**Harris, P. D., Shinn, A., Cable, J. and Bakke, T. A.** (2004). Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. *Systematic Parasitology* **59**, 1–27.

**Heggberget, T. G.** (1984). Habitat selection and segregation of parr of Arctic charr (*Salvelinus alpinus*), brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar* L.) in two streams in North Norway. In *Biology of the Arctic Charr* (ed. Johnson, L. and Burns, B. L.), pp. 217–231. Proceedings of the International Symposium on Arctic charr, Winnipeg, Manitoba, University of Manitoba Press.

**Huyse, T. and Volckaert, F. A. M.** (2002). Identification of a host-associated species complex using molecular and morphometric analyses, with the description of *Gyrodactylus rugiensoides* n. sp. (Gyrodactylidae, Monogenea). *International Journal for Parasitology* **32**, 907–919.

**Huyse, T. and Volckaert, F. A. M.** (2005). Comparing host and parasite phylogenies: *Gyrodactylus* flatworms jumping from goby to goby. *Systematic Biology* **54**, 710–718. DOI:10.1080/10635150500221036.

**Johnsen, B. O., Møkkelgjerd, P. I. and Jensen, A. J.** (1999). The parasite *Gyrodactylus salaris* on salmon parr in Norwegian rivers, status report at the beginning of year 2000. *NINA Oppdragsmelding* **617**, 1–129 (in Norwegian, English summary).

**Klemetsen, A., Amundsen, P.-A., Dempson, J. B., Jonsson, N., O'Connell, M. F. and Mortensen, E.** (2003). Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecology of Freshwater Fish* **12**, 1–59.

**Knudsen, R., Rikardsen, A. H., Kristoffersen, R., Sandring, S. and Siikavoupio, S.** (2004). Registrations of *Gyrodactylus* spp. in the fish community in Signaldalselva and Kitdalselva in Troms county 2003. *NINA oppdragsmelding* **817**, 1–23 (in Norwegian, English summary).

**Knudsen, R., Adolfsen, P., Sandring, S., Kristoffersen, R., Rikardsen, A. and Siikavuoppio, A.** (2006). The suitability of anadromous Arctic charr as host and vector of the monogenean *Gyrodactylus salaris*. *Ecology of Freshwater Fish*. (in the Press.)

**Kristoffersen, R., Richardsen, A., Winger, A. C., Adolfsen, P. and Knudsen, R.** (2005). Arctic charr as a long-term host of *Gyrodactylus salaris* in River Skibotnelva, northern Norway. *NINA Rapport* **36**, 1–27 (in Norwegian, English summary).

**Malmberg, G.** (1970). The excretory system and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). *Arkiv für Zoologi* **23**, 1–235.

**Matějusová, I., Gelnar, M., McBeath, A. J. A., Collins, C. M. and Cunningham, C. O.** (2001). Molecular markers for gyrodactylids (Gyrodactylidae: Monogenea)

from five fish families (Teleostei). *International Journal for Parasitology* **31**, 738–745. DOI:10.1016/S0020-7519(01)00176-X.

**Meinilä, M., Kuusela, J., Ziętara, M. S. and Lumme, J.** (2004). Initial steps of speciation by geographic isolation and host switch in salmonid pathogen *Gyrodactylus salaris* (Monogenea: Gyrodactylidae). *International Journal for Parasitology* **34**, 515–526. DOI:10.1016/j.ijpara.2003.12.002.

**Mo, T. A.** (1988). Gyrodactylusundersøkelser av fisk i forbindelse med rotenonbehandlingen av Skibotnelva i august 1988. *Gyrodactylusundersøkelsene ved Zoologisk Museum, Universitetet i Oslo* 1–14. (In Norwegian.)

**Mo, T. A.** (1991*a*). Seasonal variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in River Batnfjordselva, Norway. *Systematic Parasitology* **19**, 231–240.

**Mo, T. A.** (1991*b*). Variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in laboratory experiments. *Systematic Parasitology* **20**, 11–20.

**Mo, T. A.** (1991*c*). Variations of opisthaptoral parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) in a fish farm, with comments on the spreading of the parasite in south-eastern Norway. *Systematic Parasitology* **20**, 1–9.

**Mo, T. A.** (1993). Seasonal variations of the opisthaptoral hard parts of *Gyrodactylus derjavini* Mikailov, 1975 (Monogenea: Gyrodactylidae) on brown trout *Salmo trutta* L. parr and Atlantic salmon *S. salar* L. parr in the River Sandvikselva, Norway. *Systematic Parasitology* **26**, 225–231.

**Olstad, K., Cable, J., Robertsen, G. and Bakke, T. A.** (2006). Unpredicted transmission strategy of *Gyrodactylus salaris* (Monogenea: Gyrodactylidae): survival and infectivity of parasites on dead hosts. *Parasitology* **133**, 33–41. doi: 10.1017/S0031182006000966.

**Poulin, R.** (2002). The evolution of monogenean diversity. *International Journal for Parasitology* **32**, 245–254.

**Robertsen, G.** (2005). Taxonomy and systematics of *Gyrodactylus salaris* (Monogenea, Gyrodactylidae) infecting wild populations of Arctic charr (*Salvelinus alpinus*) in Norway. *Cand. scient. thesis in Zoology, University of Oslo* pp. 1–41.

**Shinn, A. P., Gibson, D. I. and Sommerville, C.** (2001). Morphometric discrimination of *Gyrodactylus salaris* Malmberg (Monogenea) from species of *Gyrodactylus* parasitizing British salmonids using novel parameters. *Journal of Fish Diseases* **24**, 83–97.

**Shinn, A. P., Hansen, H., Olstad, K., Bachmann, L. and Bakke, T. A.** (2004). The use of morphometric characters to discriminate specimens of laboratory-reared and wild populations of *Gyrodactylus salaris* and *G. thymalli* (Monogenea). *Folia Parasitologica* **51**, 239–252.

**Soleng, A., Jansen, P. and Bakke, T. A.** (1999). Transmission of the monogenean *Gyrodactylus salaris*. *Folia Parasitologica* **46**, 179–184.

**Sterud, E.** (1999). Parasitter hos norske ferskvannsfisk. *Norsk Zoologisk Forening*. **7**, 1–22. (In Norwegian.)

**Sterud, E., Mo, T. A., Collins, C. M. and Cunningham, C. O.** (2002). The use of host specificity, pathogenicity, and molecular markers to differentiate between *Gyrodactylus salaris* Malmberg, 1957 and *G. thymalli* Zitnan, 1960 (Monogenea: Gyrodactylidae). *Parasitology* **124**, 203–213. DOI: 10.1017/S0031182001001044.

**Ziętara, M. S. and Lumme, J.** (2002). Speciation by host switch and adaptive radiation in a fish parasite genus *Gyrodactylus* (Monogenea: Gyrodactylidae). *Evolution* **56**, 2445–2458. DOI:10.1554/0014-3820(2002)056[2445:SBHSAA]2.0.CO;2.

**Ziętara, M. S. and Lumme, J.** (2003). The crossroads of molecular, typological and biological species concepts: two new species of *Gyrodactylus* Nordmann, 1832 (Monogenea: Gyrodactylidae). *Systematic Parasitology* **55**, 39–52.