

EVOLUTIONARY DIVERGENCE IN PHILONEMA (NEMATODA;
PHILOMETRIDAE) PARASITES OF B.C. SALMONIDS

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIERMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in the Department
of
Zoology

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA
September, 1990

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ABSTRACT

Philonema (Nematoda; Philometridae) from anadromous hosts, sockeye salmon (Oncorhynchus nerka), and non-anadromous hosts, rainbow trout (Oncorhynchus mykiss), were studied in order to determine if hosts with different life histories were infected with the same or different species of Philonema. Worms from the two host species were morphologically indistinguishable. However, electrophoretic banding patterns produced by restriction enzyme digestion of DNA extracted from Philonema demonstrated the presence of two genetic types corresponding to the two host species. This supports the idea that at least two species of Philonema are endemic in British Columbia. Philonema oncorhynchi Kuitunen-Ekbaum, 1933 is a parasite of sockeye salmon which undergo a long ocean migration before returning to freshwater to spawn, while P. agubernaculum Simon and Simon, 1936 is a parasite of rainbow trout (and other salmonids) which live in lakes.

Kokanee (O. nerka kennerlyi), a non-anadromous offshoot of sockeye, were infected with the same worm as sockeye probably because the two hosts have similar life histories. Steelhead smolts, anadromous O. mykiss, contained worms identified as P. agubernaculum. This likely represented an accidental infection because steelhead do not usually contact Philonema.

Philonema were examined from various localities in B.C. Philonema agubernaculum showed population divergence corresponding to the different geographic localities from which it was collected. This likely reflects the isolation of these parasite populations in unconnected watersheds. Philonema oncorhynchi showed polymorphisms spread throughout many of the populations. The lack of population divergence probably results from gene flow between parasite populations brought about by wandering hosts.

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ACKNOWLEDGEMENTS

The author would like to thank Dr. Martin Adamson for suggesting and supervising this study and for help in revising this manuscript. The author would also like to thank Dr. Leo Margolis of the Pacific Biological Station, for organizing several sockeye collections and for his interest in this project. Thanks are extended to Dr. Don Moerman for his assistance in teaching the author techniques involved with DNA extraction and southern blotting.

The author would like to thank Mr. Stu Barnettson of the Fulton River Salmon Project, for his assistance in the collection of fish on several occasions. The author is grateful to Mrs. Karen Beckenbach, in Dr. John Webster's lab at Simon Fraser University, for probing southern blots in this study. Thanks are extended to Bob Carveth for his help in revising this manuscript. Finally, thanks are extended to Stewart "the Chief" Noble for his assistance in field collections.

INTRODUCTION

Philonema spp. (Dracunculoidea; Philometridae) are nematode parasites in the body cavity of salmonids (Salmoniformes; Salmonidae). Mature ovoviviparous females leave the host by the reproductive system and release larvae when they contact freshwater. Larvae, if eaten by a suitable copepod intermediate host, develop to become infective to the final host. Transmission occurs when infected copepods are ingested by a salmonid host. Larvae leave the copepod, penetrate through the hosts gut, and enter the swimbladder (Platzer and Adams, 1967). Larvae then leave the swimbladder and enter the coelom where they will develop into adults. In the coelom worms mate, after which males will die.

The genus contains nine nominal species; the first three were described from North America (Kuitunen-Ekbaum, 1933; Simon and Simon, 1936; Richardson, 1937) and the remaining six were described from Asia (Fujita, 1939; Fujita, 1940; Bauer, 1946; Fukui, 1961; Rumyantsev, 1965). The nine nominal species of Philonema are morphologically difficult to distinguish since females become little more than bags filled with larvae and morphological characters used for identification show a high degree of overlap between species. Positive identification of species is often not possible because worms can not be reliably

diagnosed from their morphology. Therefore, the number of species is not clear since many species may represent synonyms for previously described material (Baylis, 1948, Akhemerov, 1955, Platzer, 1964, Vismanius et al., 1987). A method independent of morphology is therefore needed to resolve taxonomic problems within the genus Philonema.

Curran et.al. (1985) suggested a molecular technique which uses restriction fragment length differences in repetitive DNA sequences to identify nematode species not easily separated by morphological characters. Extracted DNA is cleaved with restriction enzymes which recognize specific sequences and DNA fragments are separated using agarose gel electrophoresis. Repetitive DNA sequences, corresponding to highly repetitive genes (e.g. ribosomal and histone genes), can then be visualized by staining with ethidium bromide. The technique is rapid, relatively inexpensive and may have an advantage over protein electrophoresis since proteins are frequently influenced by environmental and ontogenetic factors making their patterns more difficult to interpret (Curran et.al., 1985).

Two species of Philonema have been reported from British Columbia. Philonema oncorhynchi Kuitunen-Ekbaum, 1933, is a parasite of anadromous sockeye salmon (Oncorhynchus nerka) and P. agubernaculum Simon and Simon, 1936 supposedly infects non-anadromous lake dwelling salmonids. Many lakes contain sockeye and non-anadromous salmonids infected with Philonema spp., therefore B.C.

represents an ideal location for collection and identification of material. In this study Philonema was collected from sockeye salmon and rainbow trout (Oncorhynchus mykiss), a non-anadromous salmonid known to carry Philonema, in the same lake (Babine Lake) and the technique described by Curran et al. (1985) was used to determine if worms from these two hosts represented one or two species of Philonema.

An intriguing situation also existed in the fact that kokanee (O. nerka kennerlyi), a non-anadromous freshwater offshoot of sockeye, from Babine Lake were infected with an unidentified species of Philonema. Conversely, steelhead trout, anadromous O. mykiss, were also infected with an unidentified species of Philonema.

Furthermore, Philonema samples in both O. mykiss and O. nerka were collected from various B.C. localities and the same technique was used to determine if worms from geographically separate areas had undergone population divergence. The freshwater fish fauna of B.C. represents a recent recolonization, since much of the province was glaciated 14,000 years ago. During glacial retreat, fish reinvaded from areas of refugia in the north and south (McPhail and Lindsey, 1986). Since this time many watersheds have become isolated, restricting gene flow in fish and their parasites. Sockeye, on the other hand, are not restricted to freshwater. Their anadromous behaviour may take them many miles off shore and mix them with other

sockeye populations; but they show a strong homing tendency when returning to their natal nursery lakes and spawning streams. This homing behaviour may restrict gene flow in Philonema because it is transmitted in nursery lakes.

MATERIALS AND METHODS

Sample Collection

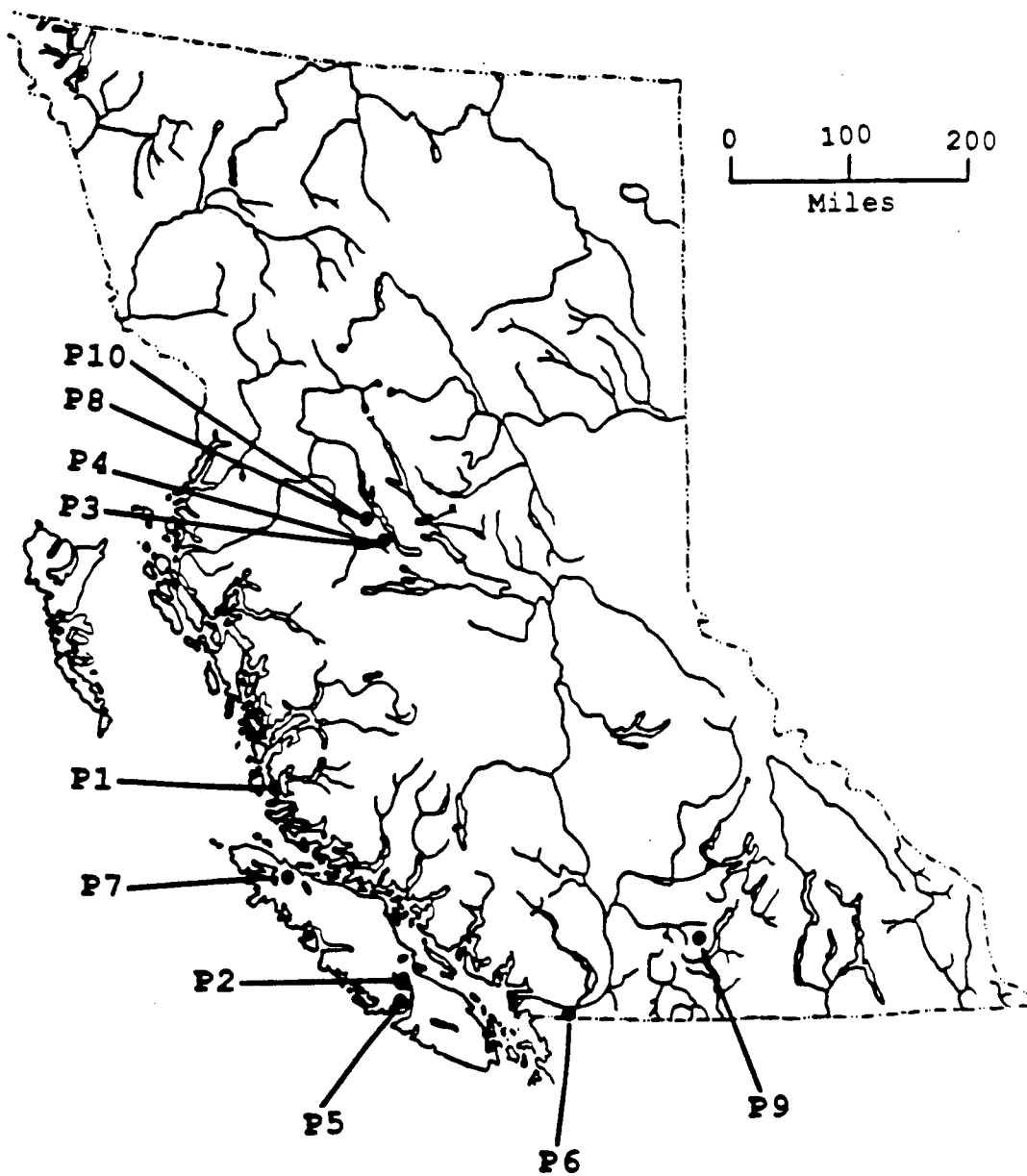
Prespawning sockeye salmon, kokanee, and rainbow and steelhead trout were examined from various British Columbia localities (fig 1.). Sockeye were collected from Rivers Inlet, Sproat River, Pierre Creek, Henderson Lake, Cultus Lake and Fulton River. Kokanee were collected from Pierre Creek. Rainbow trout were collected from Fulton River and Pennask Lake; Steelhead smolts were collected from Lake O'Connor. Fish were collected by seine or dip net and examined in the field or packed on ice and shipped to the lab for examination.

Fish were dissected by making a mid-ventral incision from anus to pectoral girdle. Philonema spp. were found lying free throughout the coelom, entangled in pyloric caeca and within gonads of some females. Using a bent probe, worms were carefully removed to prevent cuticle rupture, placed individually in 1.2 ml Nalgene cryovials, and immediately frozen at -196°C in liquid nitrogen for transport. Later, worms were transferred to a -70°C freezer for storage.

Figure 1. Collection localities for Philonema spp.

from various salmonid hosts in British Columbia.

P1-sockeye, Rivers Inlet, P2-sockeye, Sproat River, P3-sockeye, Pierre Creek (Babine Lake), P4-kokanee, Pierre Creek (Babine Lake), P5-sockeye, Henderson Lake, P6-sockeye, Cultus Lake, P7-steelhead smolts, Lake O'Connor, P8-rainbow trout, Fulton River (Babine Lake), P9-rainbow trout, Pennask Lake, P10-sockeye, Fulton River (Babine Lake).



Adult female Philonema spp. used for light microscopy were fixed in cold 95% ethanol to prevent bursting (Platzer, 1964), and transferred to 70% ethanol for storage. Adult males were fixed in the same manner, or in hot glycerine alcohol (5% glycerine, 70% ethanol).

Extraction of DNA

Only female Philonema spp. were used for DNA extraction because of their larger size. Vials containing worms were removed from the -70°C freezer and placed in a small thermos of liquid nitrogen. Individual frozen worms were transferred to 1.5 ml Eppendorfs and ground under liquid nitrogen using a disposable pestle. A 0.7 ml aliquot of proteinase K buffer [100 mM Tris (hydroxymethyl) methylamine (Tris) pH 8.0, 50 mM ethylenediaminetetraacetic acid (EDTA), 1% sodium dodecyl sulfate (SDS), 200 mM NaCl] was added to each Eppendorf, followed by approximately 2 mg of Proteinase K. Eppendorfs were incubated in a dry bath for 16-20 hours at 65°C to maximize digestion. DNA was extracted three times, allowing at least 0.5 hours between extractions. The aqueous layer was collected by spinning Eppendorfs for 2-3 minutes and transferring the viscous supernatant to a clean Eppendorf using a wide-mouth plastic pipette. Extractions proceeded as follows:

1. equal volume redistilled phenol saturated with TE (10 mM Tris pH 7.5, 1 mM EDTA)

2. equal volume 1 phenol:1 sevag (24 chloroform:1 isoamyl alcohol).
3. equal volume sevag.

Following extraction, 3/5 volume of 20% polyethyleneglycol 8000 in 2.5 M NaCl was added and DNA was precipitated overnight at 4°C. Eppendorfs were spun in a microcentrifuge for 15 minutes to pellet DNA. The DNA pellet was washed twice with cold 70% ethanol and vacuum dried for 5-10 minutes before being resuspended in 200 µl of TE.

DNA Electrophoresis

Extracted DNA was examined by running all samples on minigels at 100 volts for about 20 minutes. A known quantity of Hind III cut lambda DNA was included as a size standard and samples approximately 20 kb containing 20 µg or more of DNA were selected for digestion with restriction endonucleases.

DNA was sampled from at least 5 worms from each collection site. The following restriction enzymes (supplied by BRL) were used to digest each sample; Bam HI, Eco RI, Hae III, Hind III, Hinf I, Hpa I, Msp I, Pst I, Xba I and Xho I. Digestion reactions involved 2-8 µg of DNA suspended in 18 µl of TE, 2.5 µl 10X buffer (supplied with the enzyme by the vendor), 1.0 µl bovine serum albumin (BSA) (2 mg/ml), 10 µl RNase A (10 mg/ml) and 2.5 µl of

restriction endonuclease. The reaction mixture was incubated at 37°C, in a water bath, for 1-5 hours, depending on activity of enzymes used. Reactions were halted by adding 0.5 M EDTA to a final concentration of 10 mM (Maniatis et al., 1982). Loading buffer with marker dye (15% ficoll, 0.25% bromophenol blue, 0.25% xylene cyanol) was added to a final concentration of 3%.

DNA samples were loaded into 0.7% agarose gel containing 2.5 mg/ml ethidium bromide for DNA staining. Lambda Hind III was used as a size standard to calculate restriction fragment sizes. Gels were run in a horizontal gel box, containing TBE buffer (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA), at 30-40 volts for 18-20 hours to separate DNA restriction fragments. Gels were destained for 0.5 hours in distilled water and photographed using a UV illuminator box and Kodak Polaroid camera. Kodak type 55 film (positive/negative) was used and repetitive DNA banding patterns were traced from negatives.

Initially, restriction digests of single samples from all collection areas were run together. Later, repetitive samples from various sites digested with selected enzymes were run.

Southern Transfer

Photographed gels were placed in 0.25 M HCl for 10-15 minutes to depurinate DNA and were then soaked in transfer

buffer (0.02 M NaOH and 1 M ammonium acetate) for 5-10 minutes prior to preparation of southern transfer apparatus. Transfer apparatus consisted of a glass reservoir for buffer and a Plexiglass plate over the reservoir to support gels. Two pieces of Whatman 3 MM paper were inserted into the transfer buffer as wicks. Gels were placed on wicks and a piece of Hybond-N hybridization transfer membrane (Amersham) was cut to size and placed over the gel. Two pieces of Whatman 3 MM paper were cut to size and placed over the transfer membrane. Air bubbles were removed at each stage by rolling them out with a long glass rod. A piece of Handi-Wrap, with a window around the gel, was used to cover the apparatus. A weighted stack of Scott paper towels was placed over the gel. DNA transfer was allowed to proceed overnight.

Following transfer, membranes were removed and soaked in 0.4 M NaOH for 20 minutes to denature DNA (Broad et al., 1988). Membranes were rinsed with 2X SSC (0.02 M sodium citrate and 0.3 M NaCl) and air dried for 1 hour. Membranes were wrapped in Handi-Wrap and exposed to UV light for 3 minutes to cross-linked DNA. Membranes were then stored at room temperature until used.

Hybridization

Membranes were probed with a cluster of ribosomal genes which included the 5.8, 18s and 28s genes, and a

nontranscribed region. The ribosomal cluster (pBx 2) was a 7.2 kb Eco R1 fragment isolated from the pinewood nematode Bursaphelenchus xylophilus (Nematoda; Tylenchida) and cloned in Puc 19. The probe (supplied by Dr. J. Webster's lab at Simon Fraser University) was labelled by nick translation (Maniatis et al., 1982) using ^{32}P ATP. The reaction contained 400 ng of probe DNA in a final volume of 50 μl and was set up as follows: 5 μl 10X nick translation buffer (NTB) [500 mM Tris pH 7.5, 100 mM magnesium sulfate, 10 mM dithiothreitol (DTT), 500 $\mu\text{g/ml}$ BSA], 37 μl distilled water, 2 μl -ATP (10 mM GTP, 10 mM TTP, 10 mM CTP), 1 μl diluted DNase I [dilute stock DNase I (1 mg/ml in 50 mM Tris pH 7.5, 10 mM MgSO_4 , 1 mM DTT, 50% glycerol) to 1/10000 in 1X NTB], 1 μl DNA polymerase I (approximately 2-4 units), 2 μl pBx 2 (400 ng), 2 μl ^{32}P ATP (20 μCi). The reaction mixture was incubated at 12-15°C for 2 hours and was halted using 5 μl of 0.2 M EDTA and 2% SDS. The probe was purified (to remove free nucleotides) in a Sephadex (G25 fine) spin column constructed from a 1 ml pipet tip plugged at one end with glass wool.

Membranes were prehybridized for 2 hours at 62°C in sealed freezer bags containing 100 ml of prehybridization solution [5X SSPE (5 mM EDTA, 0.06 M $\text{NaH}_2\text{PO}_4\text{-H}_2\text{O}$, 0.7 M NaCl), 0.3% SDS, 2.5X Denharts (0.05% BSA, 0.05% ficoll 400, 0.05% polyvinyl pyrrolidone 40)]. Bags containing membranes were drained and 4 ml of hybridization solution (purified probe, 5X SSPE, 0.3% SDS, 2.5X Denharts) per 100 cm^2 of

transfer membrane was added to each bag. Membranes were hybridized at 62°C overnight, washed once for 5 minutes and three times for 10 minutes each in 2X SSPE and 0.2% SDS. Washed membranes were blotted dry, wrapped with Saran Wrap and placed in a developing cassette with a single intensifying screen and X-ray film. Cassettes were placed in a -70°C freezer and film was exposed for 5 days.

Microscopy

Adult male and female Philonema spp. from Babine Lake and Pennask Lake were examined and drawn using a Nikon microscope equipped with drawing tube. Measurements were calculated using a digitizing pad (Hi-pad Texas Instruments) and software from Sonnet-Gap.

RESULTS

Morphology of Worms

Philonema sp. from O. mykiss

Adult male and female Philonema sp. were found in prespawning rainbow trout from Fulton River and the Pennask Lake egg collection site. Worms apparently caused little or no pathology. Worm burdens (# of worms per host) in trout were generally lower than those in sockeye (Table 1) and many trout contained no adult worms (fish were not examined for larvae).

Steelhead smolts from Lake O'Connor were infected with mature Philonema sp. and worm burdens were often high. Worms caused severe pathological effects such as greatly distended, fluid filled bellies and thin abdominal walls. Worms were frequently found protruding from the anus. Dead fish were observed floating at the surface of net pens.

Table I

HOST	LOCALITY	# EXAMINED	# INFECTED* (PREVALENCE)	INTENSITY
sockeye	Rivers In.	10	10 (100%)	+++
sockeye	Sproat Rv.	14	14 (100%)	++
sockeye	Pierre Ck.	10	10 (100%)	++
kokanee	Pierre Ck.	16	16 (100%)	+
sockeye	Henderson	12	12 (100%)	++
sockeye	Cultus Lk.	18	15 (83%)**	++
steelhead	O'Connor	13	13 (100%)	+++
rainbow	Fulton Rv.	17	6 (35%)	+
rainbow	Pennask Lk.	23	11 (48%)	+
sockeye	Fulton Rv.	11	11 (100%)	++

* # infected refers to adult worms only, fish were not examined for larvae.

** Cultus lake fish were collected on two occasions, once in October, 1987 and again in November, 1988. On the second occasion fish were collected from Lyndell beach and some may have spawned and released worms; thus 83% may be an underestimate of prevalence.

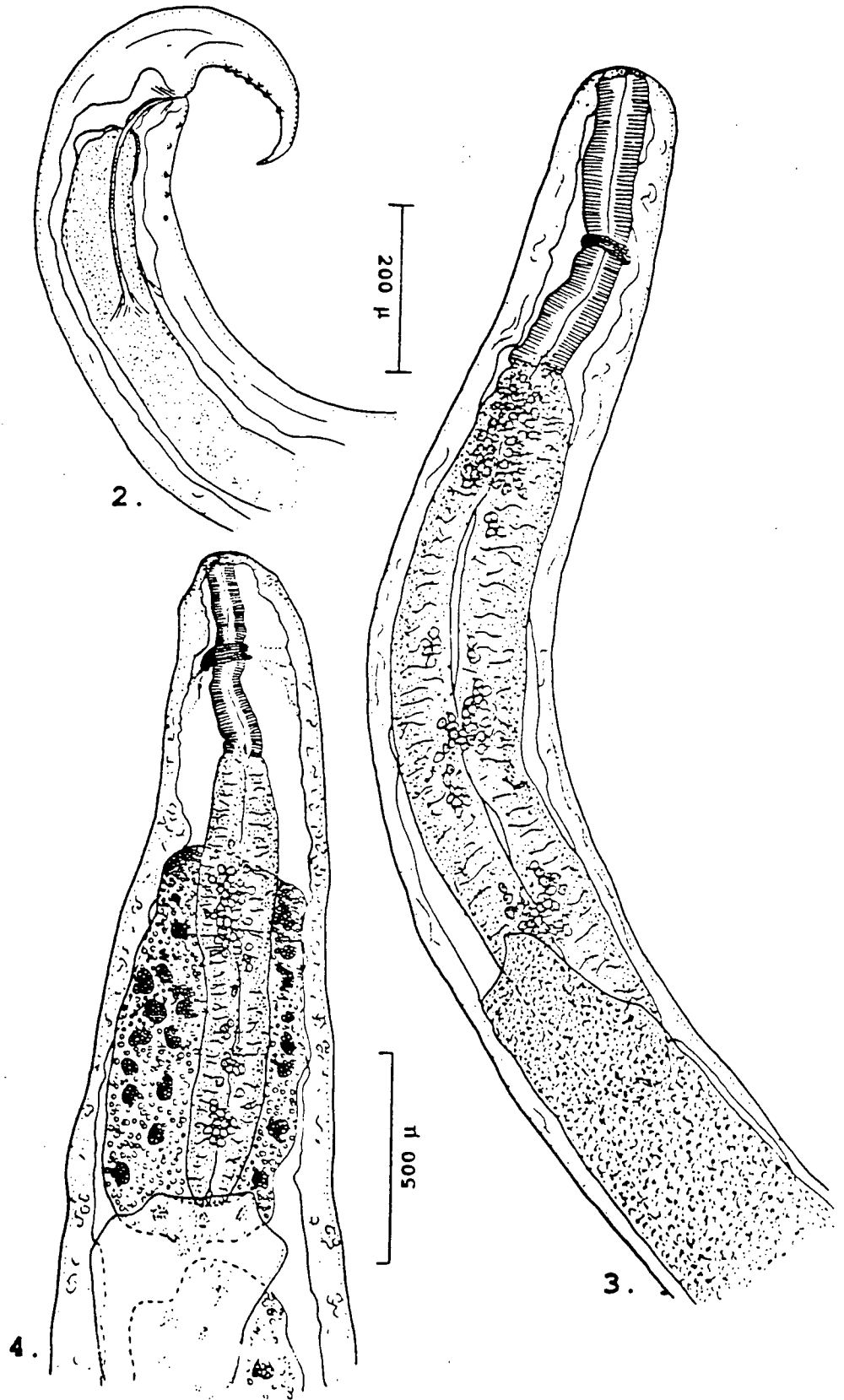
Description: Male (3 specimens) (figs. 2 and 3): Length 14.64 mm (12.28 to 18.68 mm). Maximum width 286 μ m (234 to 371 μ m). Oesophagus consisting of anterior muscular portion 407 μ m (345 to 488 μ m) long, and a posterior glandular portion, 1000 μ m (891 to 1175 μ m) long, emptying into intestine. Nerve ring 226 μ m (185 to 278 μ m) from anterior end, near midpoint of muscular oesophagus. Single large testis beginning 1.11 mm (1.06 to 1.16 mm) from anterior end, near oesophago-intestinal junction, extending posteriorly to cloacal junction just anterior to anus. Slender, arcuate spicules equal and similar, 315 μ m (299 to 333 μ m) long. Gubernaculum absent. Tail conical, 345 μ m (310 to 402 μ m) long, with six to ten pairs of papillae. Five to eight pairs of preanal papillae were also observed.

Female (1 specimen) (fig. 4): Length 59.7 mm. Maximum width 772.7 μ m. Oesophagus consisting of anterior muscular portion 474.3 μ m long, and posterior glandular portion, 1067.6 μ m long, joining a flattened, empty intestine. Nerve ring encircling muscular oesophagus near its middle, 241.4 μ m from anterior end of worm. Small ovary present at both ends of worm. Anterior ovary 703.5 μ m from anterior end of worm and posterior ovary 550.5 μ m from tip of tail. Ovaries emptying into large uterus filling most of worms body. Uterus containing many small embryos appearing to be in latter stages of blastulation (no first stage larvae observed). Neither anus nor vulva observed and both assumed to have atrophied.

Figures 2-4. Philonema sp. from O. mykiss. 2.

Posterior end of male. 3. Anterior end of male.

4. Anterior end of female.



Philonema sp. from O. nerka

Adult male and female Philonema sp. were found in prespawning sockeye and kokanee from all sample sites. Worm burdens were often high (Table 1) but pathological effects were not observed with the exception of occasional thin walled, fluid filled cysts (containing dead males and/or female worms) on the liver or body wall. Philonema sp. collected from O. nerka tended to be larger than those from O. mykiss but otherwise worms were morphologically indistinguishable.

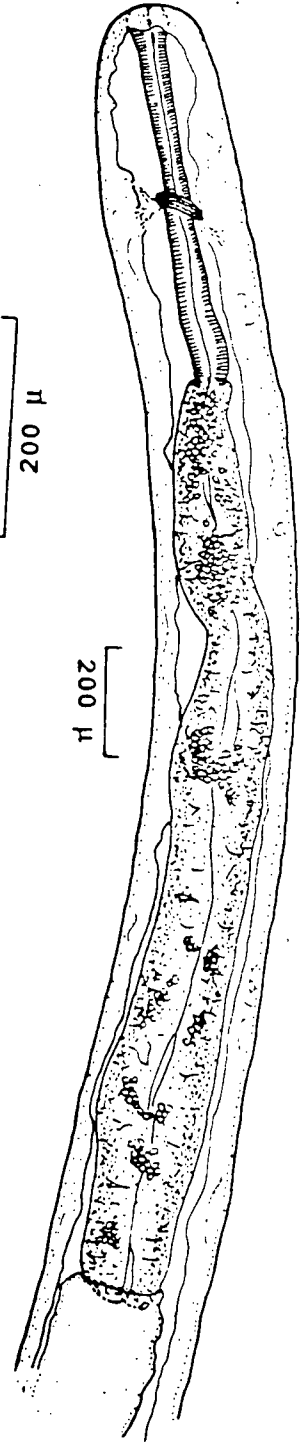
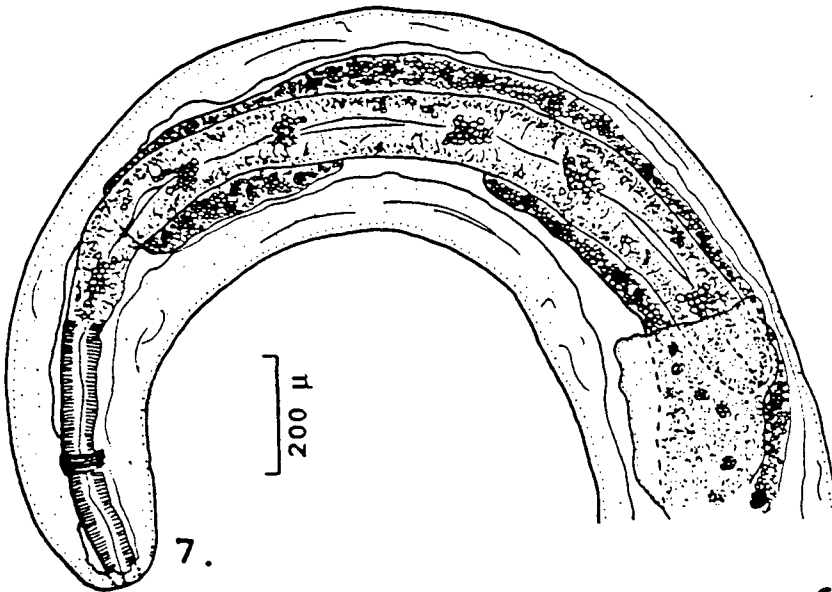
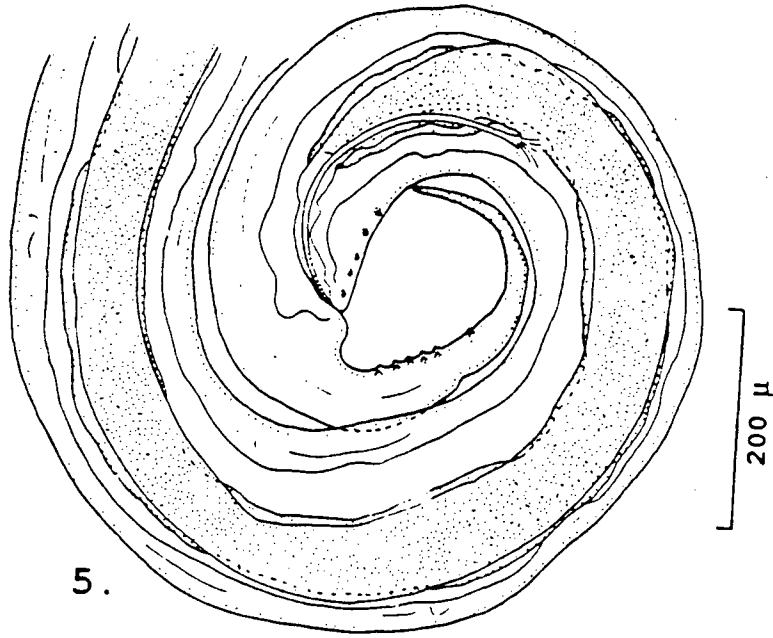
Description: Male (2 specimens) (figs. 5 and 6): Length 29.1 mm (27.24 to 30.95 mm). Maximum width 297 μ m (274 to 320 μ m). Oesophagus consisting of anterior muscular portion, 649 μ m (637 to 660 μ m) long, and posterior glandular portion 1824 μ m (1723 to 1925 μ m) long, emptying into intestine. Nerve ring 325 μ m (311 to 339 μ m) from anterior end of worm, encircling musculo-oesophagus near its midpoint. Single large testis beginning 2.55 mm (1.06 to 4.04 mm) from anterior end and extending posteriorly to the cloacal junction just anterior to anus. Slender, arcuate spicules equal and similar, 339 μ m long in one of specimens. Gubernaculum absent. Conical tail, 505 μ m long in one specimen, with six pairs of postanal papillae. Five pairs of preanal papillae were also observed.

Female (2 specimens) (fig. 7): Length 89.79 mm (48.96 to 130.62 mm). Maximum width 725 μ m (495 to 955 μ m). Oesophagus consisting of anterior glandular portion 1752 μ m (1580 to 1923 μ m) long, joining flattened, empty intestine. Nerve ring 286 μ m (227 to 345 μ m) from anterior end, encircling muscular oesophagus near its middle. Reproductive system consisting of small anterior ovary, 676 μ m (550 to 802 μ m) from anterior end, and small posterior ovary, 760 μ m (720 to 799 μ m) from posterior extremity. Ovaries emptying into large uterus filling much of body. Uterus containing single cells, early cleavage embryos and early gastrulae, but no first stage larvae were observed. Vulva and anus not observed and both were assumed to have atrophied.

Figures 5-7. Philonema sp. from O. nerka. 5.

Posterior end of male. 6. Anterior end of male.

7. Anterior end of female.



Restriction Endonuclease Digestion

Banding patterns produced by restriction endonuclease digestion clearly separated Philonema sp. into two groups corresponding to the two host species from which they were collected (figs. 8-13). Thus, worms from rainbow and steelhead trout (O. mykiss) had similar banding patterns and could be distinguished from those in sockeye (O. nerka) and kokanee (O. nerka kennerlyi). Of 10 restriction endonucleases used, only 3 revealed bands shared by the two groups; Hae III and Hpa I produced 0.4 kb bands (figs. 9 and 11), and Xho I a 3.2 kb band (fig. 13).

Figure 8. Eco R1 digestion of Philonema spp. (P) and C. elegans (N2) DNA. Philonema spp. are from the following hosts and localities; P1-sockeye, Rivers Inlet, P2-sockeye, Sproat River, P3-sockeye, Pierre Creek, P4-kokanee, Pierre Creek, P5-sockeye, Henderson Lake, P6-sockeye, Cultus Lake, P7-steelhead, Lake O'Connor, P8-rainbow trout, Fulton River, P9-rainbow trout, Pennask Lake, P10-sockeye, Fulton River.

Eco R1

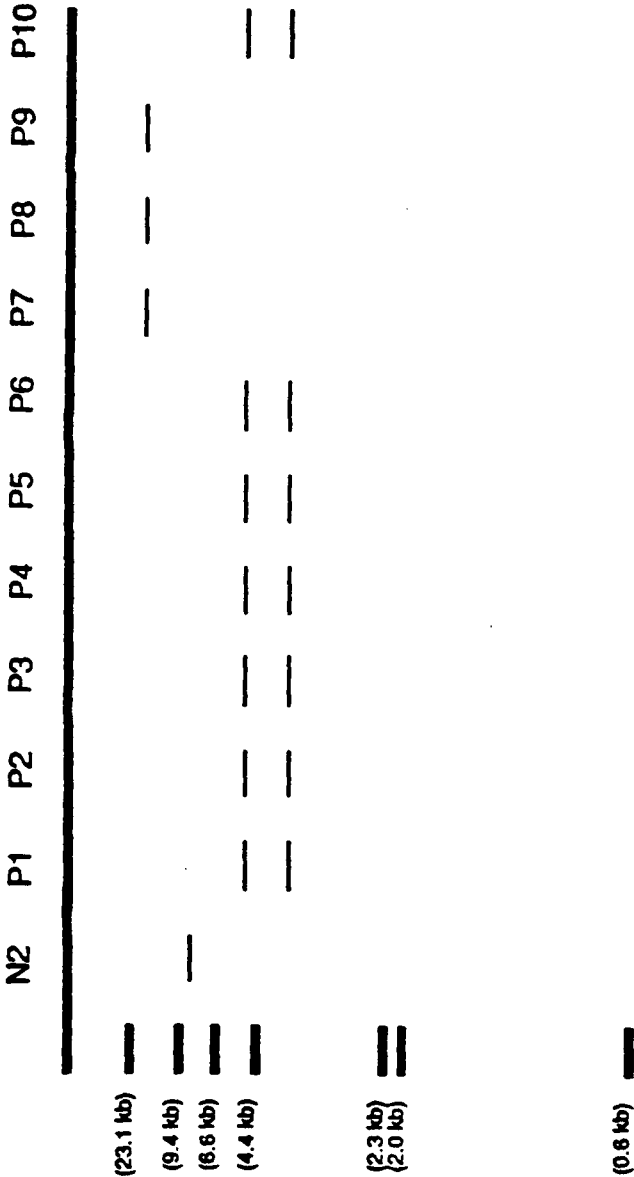


Figure 9. Hae III digestion of Philonema spp.(P) and C. elegans (N2) DNA. Philonema spp. are from the following hosts and localities; P1-sockeye, Rivers Inlet, P2-sockeye, Sproat River, P3-sockeye, Pierre Creek, P4-kokanee, Pierre Creek, P5-sockeye, Henderson Lake, P6-sockeye, Cultus Lake, P7-steelhead, Lake O'Connor, P8-rainbow trout, Fulton River, P9-rainbow trout, Pennask Lake, P10-sockeye, Fulton River.

Figure 10. Hinf I digestion of Philonema spp.(P) and C. elegans (N2) DNA. Philonema spp. are from the following hosts and localities; P1-sockeye, Rivers Inlet, P2-sockeye, Sproat River, P3-sockeye, Pierre Creek, P4-kokanee, Pierre Creek, P5-sockeye, Henderson Lake, P6-sockeye, Cultus Lake, P7-steelhead, Lake O'Connor, P8-rainbow trout, Fulton River, P9-rainbow trout, Pennask Lake, P10-sockeye, Fulton River.

Hinf I

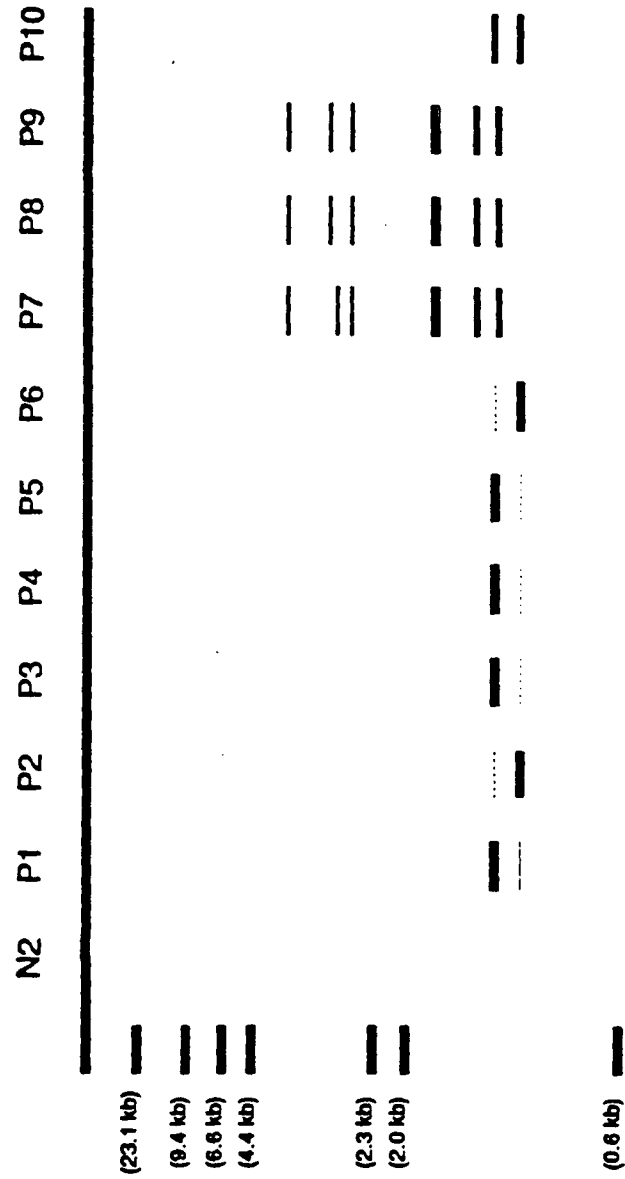


Figure 11. Hpa I digestion of Philonema spp. (P) and C. elegans (N2) DNA. Philonema spp. are from the following hosts and localities; P1-sockeye, Rivers Inlet, P2-sockeye, Sproat River, P3-sockeye, Pierre Creek, P4-kokanee, Pierre Creek, P5-sockeye, Henderson Lake, P6-sockeye, Cultus Lake, P7-steelhead, Lake O'Connor, P8-rainbow trout, Fulton River, P9-rainbow trout, Pennask Lake, P10-sockeye, Fulton River.

Hpa I

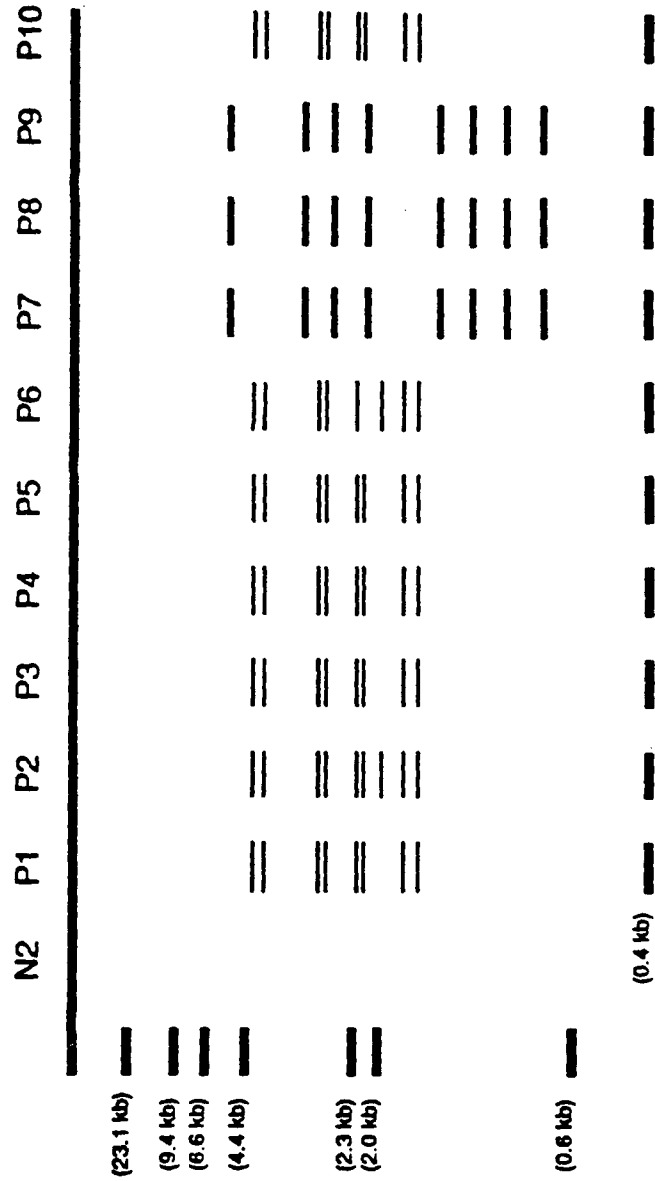


Figure 12. Xba I digestion of Philonema spp. (P) and C. elegans (N2) DNA. Philonema spp. are from the following hosts and localities; P1-sockeye, Rivers Inlet, P2-sockeye, Sproat River, P3-sockeye, Pierre Creek, P4-kokanee, Pierre Creek, P5-sockeye, Henderson Lake, P6-sockeye, Cultus Lake, P7-steelhead, Lake O'Connor, P8-rainbow trout, Fulton River, P9-rainbow trout, Pennask Lake, P10-sockeye, Fulton River.

Xba I

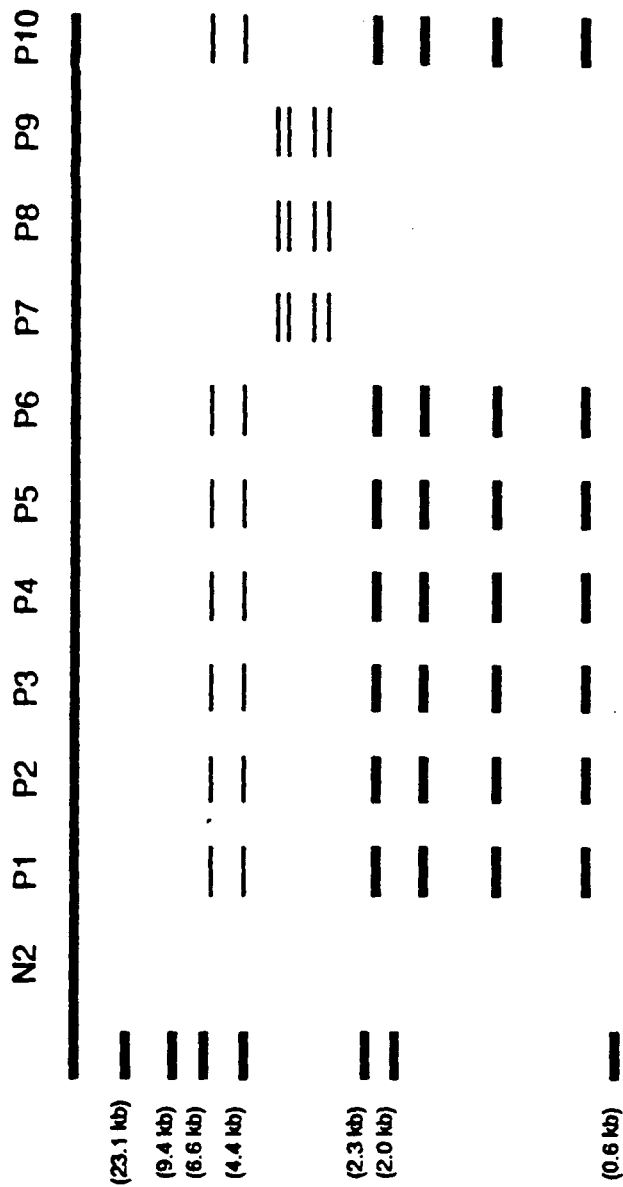
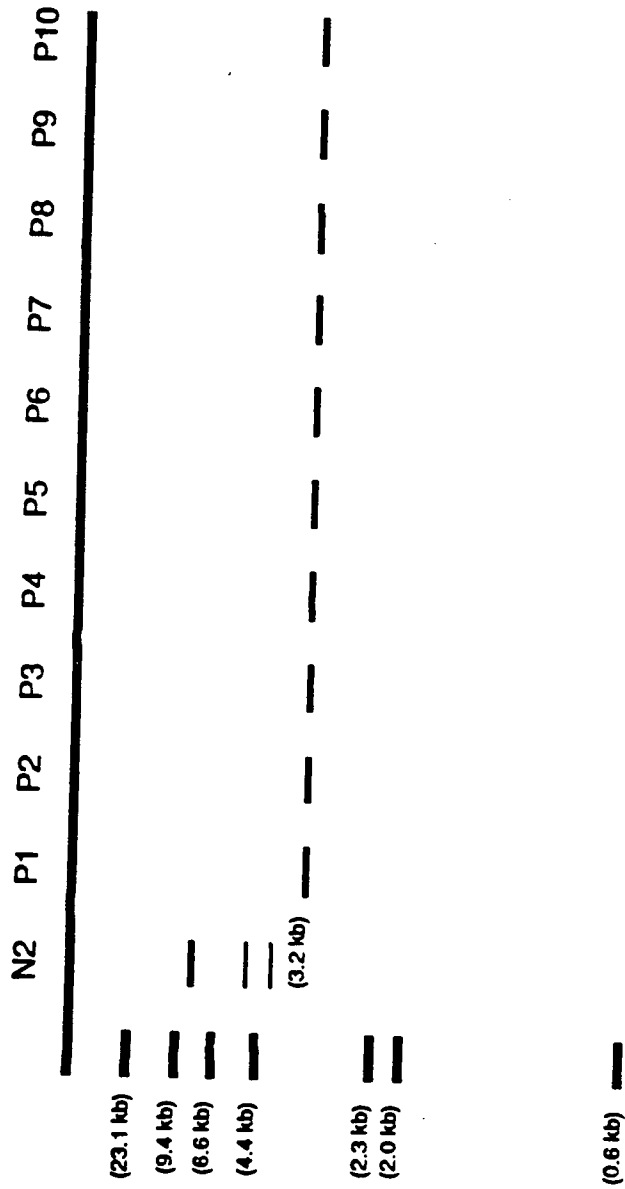


Figure 13. Xho I digestion of Philonema spp. (P) and C. elegans (N2) DNA. Philonema spp. are from the following hosts and localities; P1-sockeye, Rivers Inlet, P2-sockeye, Sproat River, P3-sockeye, Pierre Creek, P4-kokanee, Pierre Creek, P5-sockeye, Henderson Lake, P6-sockeye, Cultus Lake, P7-steelhead, Lake O'Connor, P8-rainbow trout, Fulton River, P9-rainbow trout, Pennask Lake, P10-sockeye, Fulton River.

Xho I



Banding patterns within groups showed little variation for most enzymes (figs. 14-17), but Philonema populations from O. mykiss differed with respect to patterns produced by Bam H1, Hae III and Hinf I. These differences corresponded to different collection localities.

Bam H1 digestion produced a 2.1 kb band that was present in Fulton River and Pennask Lake worms and absent in Lake O'Connor worms (fig. 15). Two bands, 3.1 and 2.8 kb, produced by Hinf I digestion were present in Fulton and Pennask populations and absent in Lake O'Connor worms, while a 2.6 kb present in Lake O'Connor worms was absent from Fulton and Pennask worms (fig. 16).

Banding patterns produced by Hae III digestion distinguished Philonema from the 3 O. mykiss populations (fig. 17). Lake O'Connor worms had 2 bands, 3.5 kb and 1.1 kb, not present in either the Fulton or Pennask populations. Fulton River worms had a 2.4 kb band not present in the other two populations, and lacked 2.8 kb band which was present in the other two.

Banding patterns produced by the enzymes Eco R1, Hpa I (fig. 14) and Xho I were identical for all O. mykiss collection localities.

Figure 14. Hpa I DNA digest of Philonema taken from O. mykiss. Philonema sp. are from the following hosts and localities; P7-steelhead, Lake O'Connor, P8-rainbow trout, Fulton River, P9-rainbow trout, Pennask Lake.

Figure 15. Bam H1 DNA digest of Philonema taken from O. mykiss. Philonema sp. are from the following hosts and localities; P7-steelhead, Lake O'Connor, P8-rainbow trout, Fulton River, P9-rainbow trout, Pennask Lake. The 2.1 kb band is shared by the Fulton and Pennask populations but is not present in the Lake O'Connor population.

Bam H1

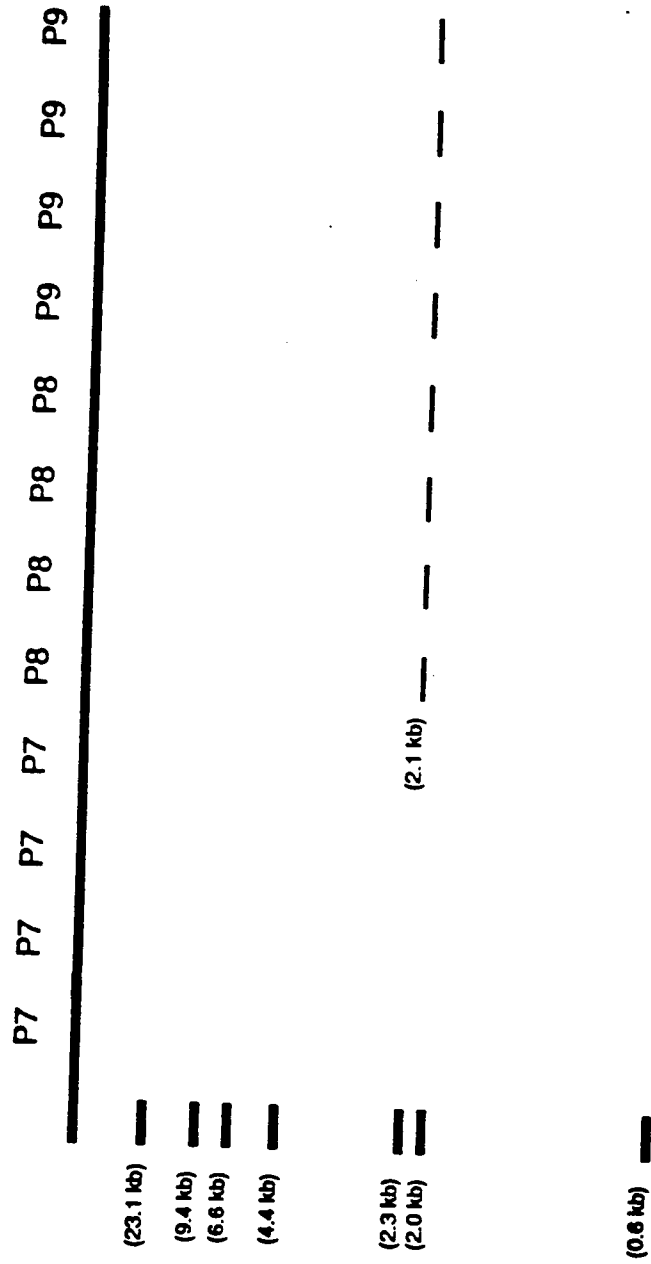


Figure 16. Hinf I DNA digest of Philonema taken from O. mykiss. Philonema sp. are from the following hosts and localities; P7-steelhead, Lake O'Connor, P8-rainbow trout, Fulton River, P9-rainbow trout, Pennask Lake. The 3.1 and 2.8 kb bands are shared by the Fulton and Pennask populations but are not present in the Lake O'Connor population. The Lake O'Connor population has a 2.6 kb band not present in the other two populations.

Hinf I

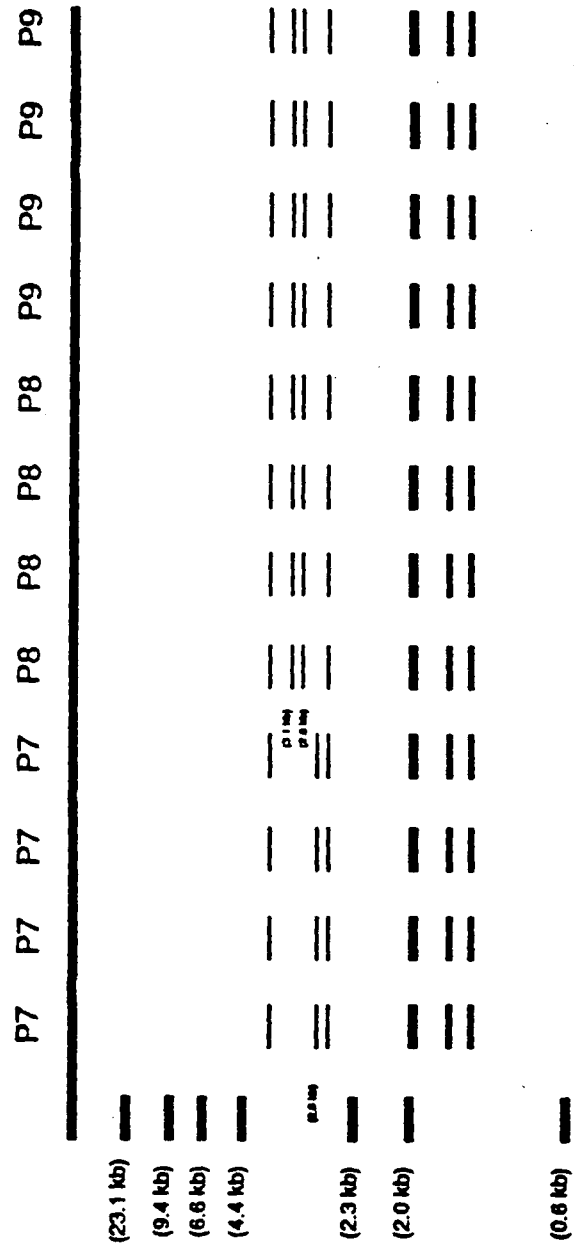


Figure 17. Hae III DNA digest of Philonema taken from O. mykiss. Philonema sp. are from the following hosts and localities; P7-steelhead, Lake O'Connor, P8-rainbow trout, Fulton River, P9-rainbow trout, Pennask Lake. The Fulton and Pennask populations share the 3.6 kb band which Lake O'Connor worms lack. The Lake O'Connor population has two bands, 3.5 and 1.1 kb, not seen in the other two populations. The 2.8 kb band is shared by the Pennask and Lake O'Connor populations and the Fulton River population has a 2.4 kb band not seen in the other populations.

Philonema populations from O. nerka showed banding pattern differences for the enzymes Bam H1, Hinf I and Hpa I, but these differences were not restricted to any population (figs. 18-20).

Bam H1 digestion produced a 2.6 kb band that was present in Cultus Lake worms, most Sproat Lake worms, one worm from Henderson Lake, one worm from Pierre Creek, and in much fainter form in worms from Fulton River (fig. 18). Two bands, 1.0 and 0.9 kb, produced by Hinf I digestion were polymorphic. In Cultus Lake worms, most Sproat River worms, one worm from Henderson Lake and one worm from Pierre Creek the 0.9 kb band was more prominent. In the remainder of the samples from Henderson Lake, Pierre Creek, Sproat River and samples from Rivers Inlet, the 1.0 kb band is of greater intensity. However, in samples from Fulton River these bands were nearly equal in intensity. Finally, a 1.9 kb band produced by Hpa I digestion was present in Cultus Lake worms, most Sproat River worms and one worm from Pierre Creek (fig. 20) (the Henderson Lake and Fulton River populations were not examined).

Banding patterns produced by the enzymes Eco R1, Hae III, Xho I and Xba were identical for all O. nerka collection localities.

Figure 18. Bam H1 DNA digest of Philonema from O. nerka. Philonema sp. are from the following hosts and localities; P1-sockeye, Rivers Inlet, P2-sockeye, Sproat River, P3-sockeye, Pierre Creek, P4-kokanee, Pierre Creek, P5-sockeye, Henderson Lake, P6-sockeye, Cultus Lake, P10-sockeye, Fulton River. The 2.6 kb band corresponds to pBx 2, a cluster of ribosomal genes isolated from B. xylophilus.

Bam H1

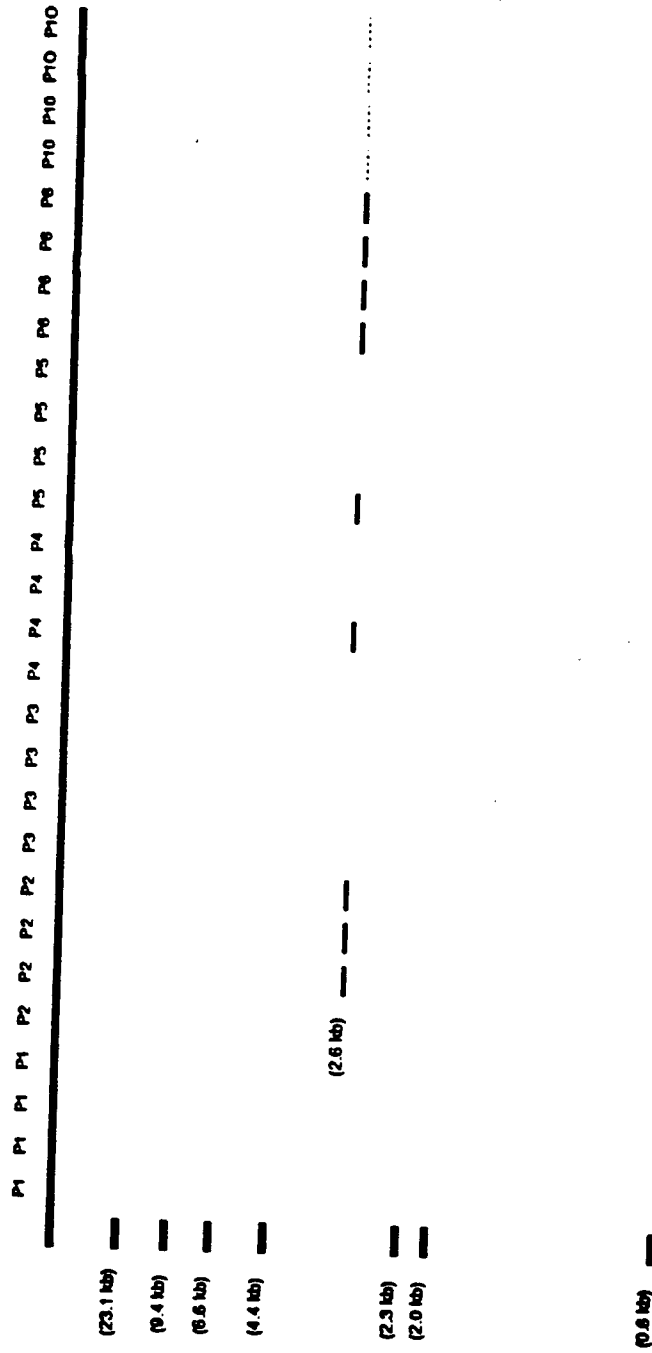


Figure 19. Hinf I DNA digest of Philonema from O.
nerka. Philonema sp. are from the following hosts
and localities; P1-sockeye, Rivers Inlet, P2-
sockeye, Sproat River, P3-sockeye, Pierre Creek,
P4-kokanee, Pierre Creek, P5-sockeye, Henderson
Lake, P6-sockeye, Cultus Lake, P10-sockeye, Fulton
River.

Hinf I

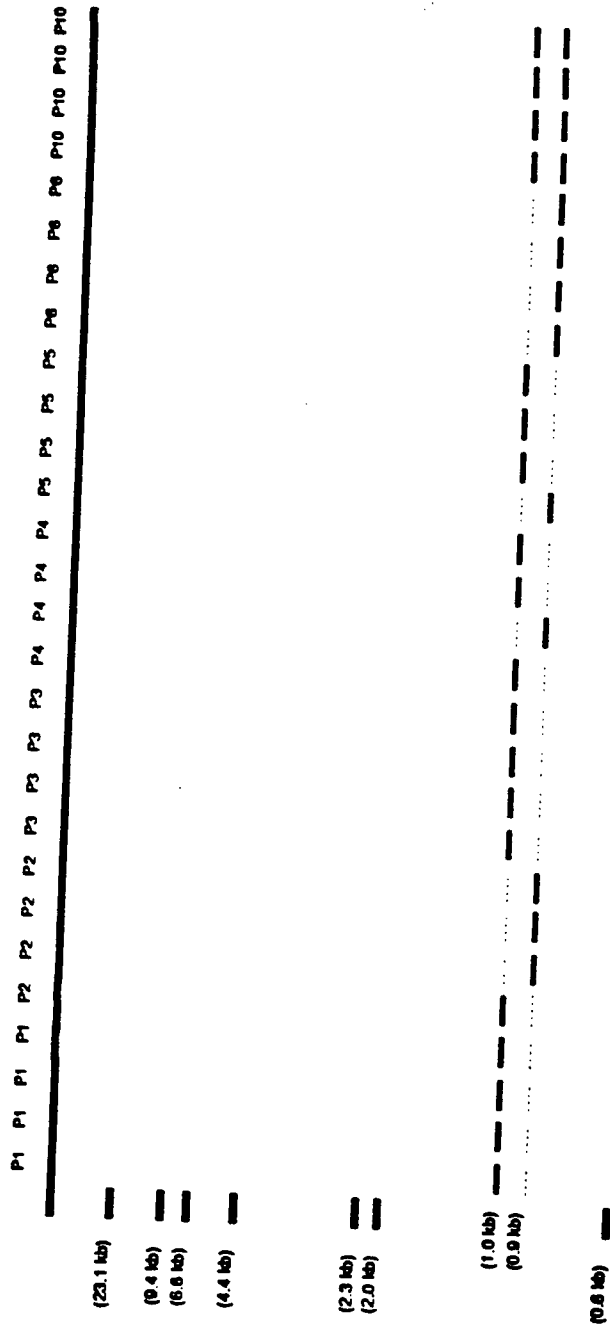
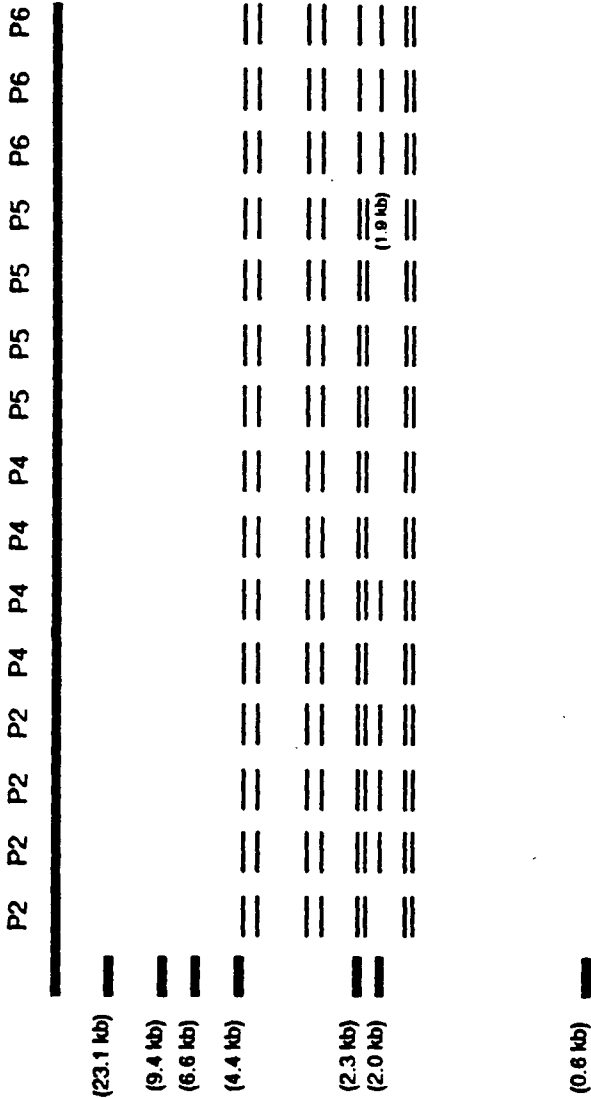


Figure 20. Hpa I DNA digest of Philonema from O.
nerka. Philonema sp. are from the following hosts
and localities; P2-sockeye, Sproat River, P4-
kokanee, Pierre Creek, P5-sockeye, Henderson Lake,
P6-sockeye, Cultus Lake.

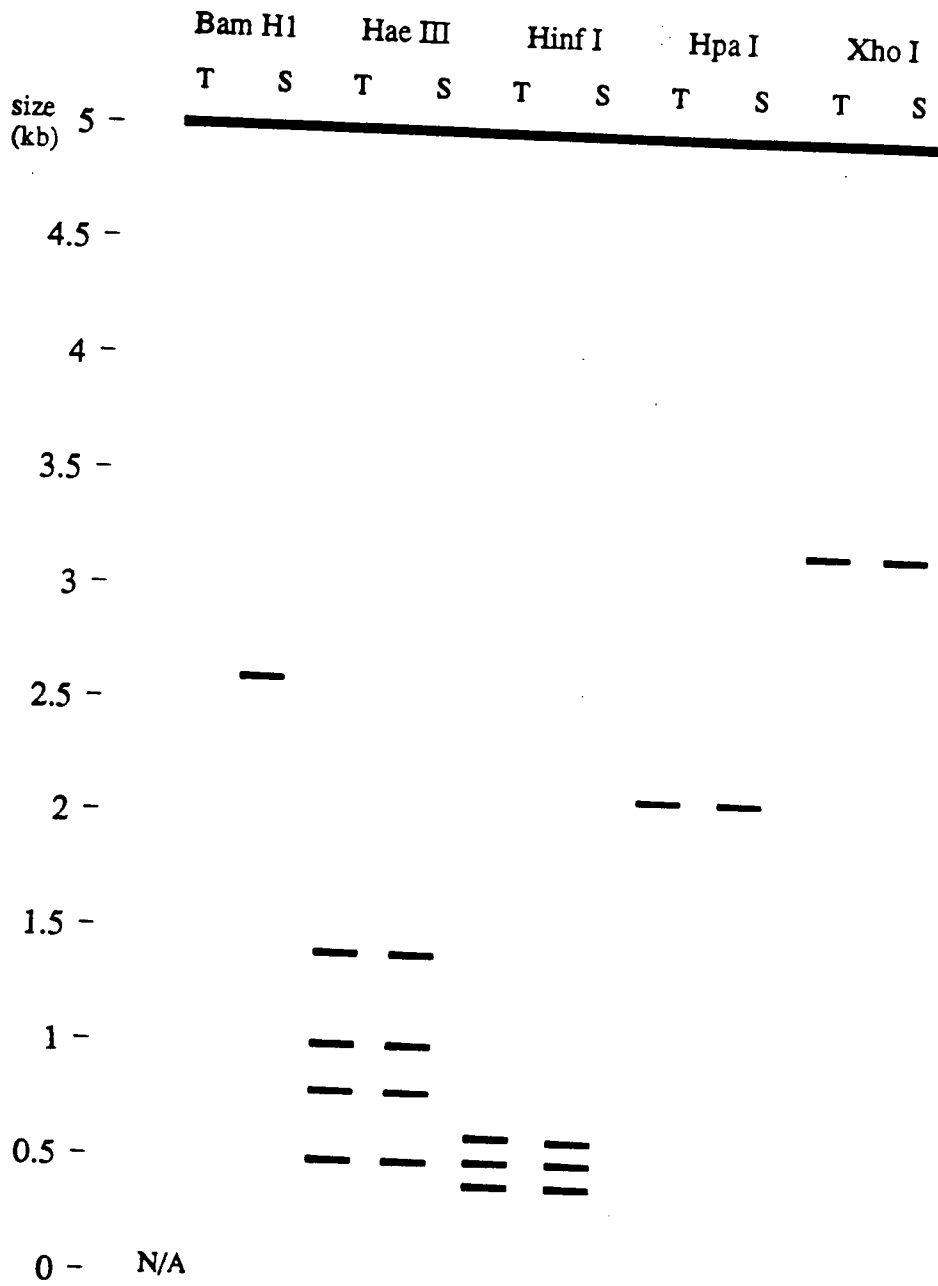
Hpa I



Hybridization with pBx 2 Probe

Southern blots taken from gels were probed with pBx 2 from Bursaphelenchus xylophilus (Nematoda; Tylenchida). Bands resulting from hybridization with the pBx 2 probe are summarized in fig. 21. Banding patterns produced by the enzymes Hae III, Hpa I, Hinf I and Xho I were identical for Philonema from O. mykiss and O. nerka. Southern blots from restriction digests with other enzymes were not available since heavy background contamination made bands impossible to discern. The 3.2 kb pBx 2 fragment produced by Xho I digestion may correspond to a band of similar size seen in fig. 13, Xho I digestion of extracted Philonema DNA. Hae III digestion produced 4 pBx 2 fragments; the 1.4 kb fragment is similar in size to a band seen in Hae III digestion of DNA from worms in O. mykiss (figs. 9 and 17) but it has no corresponding band in worms from O. nerka. Similarly, the 2.1 kb pBx 2 fragment produced by Hpa I digestion is similar in size to a band seen in Hpa I digestion of worms from O. mykiss (figs. 11 and 14) but a similar band is absent in worms from O. nerka.

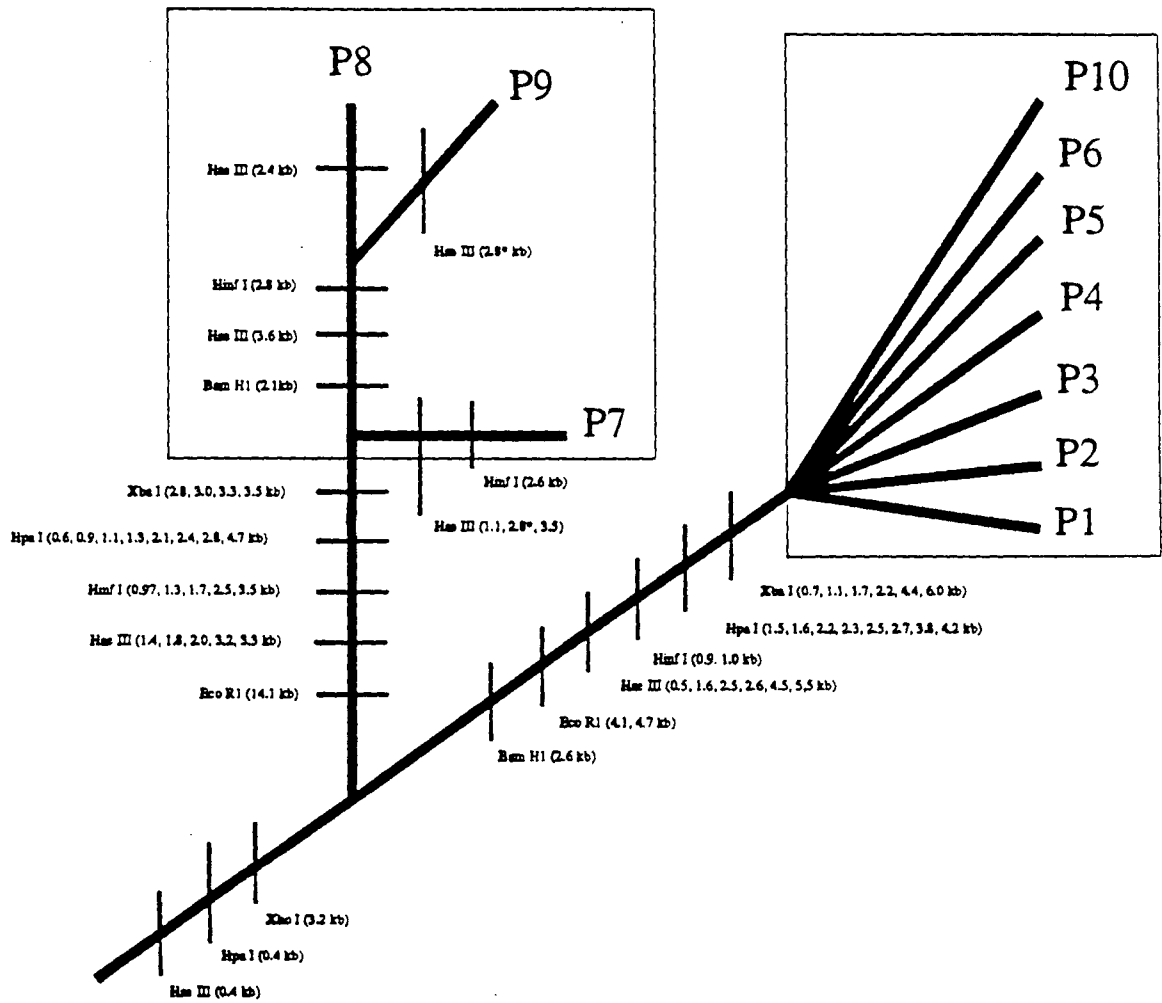
Figure 21. Summary of all bands resulting from hybridization with the pBx 2 probe, a cluster of ribosomal genes isolated from B. xylophilus. T - Philonema sp. from O. mykiss, S - Philonema sp. from O. nerka.



Cladogram

The most parsimonious arrangement of shared bands described from figs. 8-20 is indicated by a cladogram (fig. 22). The cladogram indicates the two groups of Philonema corresponding to the host species from which they were removed (i.e.. P1, P2, P3, P4, P5, P6 and P10 are from O. nerka and P7, P8 and P9 are from O. mykiss). Worms from O. nerka resulted in an unresolved polychotomy for all populations. Worms from O. mykiss indicated Fulton River (P8) and Pennask (P9) populations were more closely related to one another than either was to Lake O'Connor (P7) worms.

Figure 22. Cladogram representing hypothetical relationships of Philonema spp. samples based on repetitive bands from figs. 8-20. Philonema spp. are from the following hosts and localities; P1-sockeye, Rivers Inlet, P2-sockeye, Sproat River, P3-sockeye, Pierre Creek, P4-kokanee, Pierre Creek, P5-sockeye, Henderson Lake, P6-sockeye, Cultus Lake, P7-steelhead, Lake O'Connor, P8-rainbow, Fulton River, P9-rainbow, Pennask Lake, P10-sockeye, Fulton River.

*O. mykiss**O. nerka*

DISCUSSION

The genus Philonema contains nine nominal species in anadromous and non-anadromous salmonids distributed throughout the Northern hemisphere. Three species of Philonema have been described from North America, including the type P. oncorhynchi Kuitunen-Ekbaum 1933. Philonema oncorhynchi was originally described from prespawning Oncorhynchus nerka captured in English Bay, Vancouver, British Columbia (Kuitunen-Ekbaum, 1933). The species has subsequently been recorded in O. nerka from various localities along coastal British Columbia, Vancouver Island and Alaska (Bangham and Adams, 1954, Margolis, 1963, Pennel et. al., 1973, Bailey and Margolis, 1987), Akhmerov (1955) reported P. oncorhynchi in O. nerka from Kamchatka. Philonema oncorhynchi has been found in the lower Fraser River drainage (Cultus Lake) but is apparently absent from the upper Fraser (Bailey and Margolis, 1987). Margolis (pers. comm.) indicated that of 75 adult sockeye examined from the lower Columbia only three infected fish were found and a total of 4 P. oncorhynchi worms were recovered; no worms were found in 48 smolts examined from lakes in the Columbia drainage. It seems likely that Philonema oncorhynchi is not present in the Columbia River and reported infections may represent wandering fish since

collection data from this study suggests prevalence of P. oncorhynchi approaches 100% in endemic areas.

Philonema agubernaculum Simon and Simon, 1936 was the second species of Philonema to be described from Prosopium williamsoni, Salvelinus fontinalis and Salmo shasta (= Oncorhynchus mykiss) from Green River Lakes, Mill creek, and Freemont Lake in the Wyoming National Forest. It has since been reported from S. fontinalis from Newfoundland (Sandeman and Pippy, 1967), from S. fontinalis, S. namaycush, S. alpinus, Coregonus clupeaformis and Prosopium cylindracium from coastal Labrador (Hicks and Threlfall, 1973), from S. fontinalis and landlocked Salmo salar from Maine (Meyer, 1954), and from S. alpinus from the Koukdiuak River, Baffin Island (Dick and Belosevic, 1981). A third North American species, P. salvelini Richardson, 1937, was described from S. fontinalis in Lake Edward, Quebec.

The remaining species of Philonema were described from Asia. Philonema sibirica (Bauer, 1946) Rumyantsev, 1965, originally described from Coregonus albula, was redescribed in the same host from Lake Kuita, U.S.S.R. Rumyantsev (1965) suggested that P. sibirica might be a synonym of P. agubernaculum. Two species of Philonema were described from Japan: P. ochotense Fujita in Fukui, 1961 (host and locality unrecorded) and P. elongata Fujita, 1940 in Oncorhynchus kawamurae from Lake Tazawa. Finally, three species were described from Kamchatka, U.S.S.R.; P. kondai Fujita, 1939 in Oncorhynchus keta, P. tenuicauda Fujita,

1939 in O. nerka and P. salvelini Fujita, 1939 in Salvelinus leucomaenis. Fujita was not aware that P. salvelini had been preoccupied by Richardson's (1937) species.

The species can be divided into two groups based on host biology: P. oncorhynchi, P. kondai, P. tenuicauda and P. elongata occur in anadromous salmonids of the genus Oncorhynchus. These salmonids typically undergo long ocean migrations and spawn only once in their lifetimes. On the other hand, P. agubernaculum, P. salvelini Richardson 1937, P. salvelini Fujita 1939 and P. sibirica occur in non-anadromous salmonids belonging to diverse genera (Coregonus, Oncorhynchus, Prosopium, Salmo and Salvelinus) which usually breed several times during their life.

Of the anadromous Oncorhynchus, O. nerka is most commonly infected with Philonema. The few reports of Philonema in other anadromous Oncorhynchus may well represent cross-infections from sockeye. The reason for this restriction presumably has to do with the fact that sockeye remain for at least a year in nursery lakes. This is presumably the site of transmission for Philonema. The copepod Cyclops bicuspidatus is an abundant plankton in many B.C. lakes (Foerster, 1968) and is an important food source for planktivorous salmonids. Platzer and Adams (1966) demonstrated that C. bicuspidatus is a suitable intermediate host for Philonema. This may explain why salmonids that live in streams and rivers, and do not have access to the abundant lake plankton, are rarely infected with Philonema.

Philonema species are morphologically very similar and this has led to taxonomic problems in the genus. Baylis (1948) suggested P. agubernaculum and P. oncorhynchi were identical and the smaller size of the former was attributable to host differences or degree of maturity of worms. Akhmerov (1955) considered P. agubernaculum, P. elongata, and P. sibirica synonyms of P. oncorhynchi, and a recent Russian key to the Nematoda (Vismanius et.al., 1987) synonymizes all of Fujita's species with P. oncorhynchi, recognizing only one other species, P. sibirica, in waters of the U.S.S.R.

In this study, Philonema spp. from O. mykiss and O. nerka were morphologically indistinguishable. Characters such as number of caudal papillae in males have been used in the diagnosis of new Philonema species (Fujita, 1939). However, variability in both preanal and postanal caudal papillae was noted in the population of Philonema from Pennask Lake. This character probably has little value for species identification.

Simon and Simon (1936) suggested that P. agubernaculum could be differentiated from P. oncorhynchi by its smaller size and by the ratio of anterior muscular oesophagus to posterior glandular oesophagus, given as 1:2.7 for P. agubernaculum and 1:1.1 for P. oncorhynchi. No difference between Philonema from O. mykiss or O. nerka was noted for oesophageal ratios in worms examined and values ranged from

1:2.3 to 1:3.1. Although Philonema from O. mykiss tended to be smaller than those from O. nerka, morphometric analysis by Platzter (1964) showed a high degree of overlap in length measurements for Philonema spp. from O. nerka, O. keta, O. mykiss, landlocked S. salar, Salvelinus malma, S. fontinalis and P. williamsoni, making size a questionable character for distinguishing Philonema species. Platzter (1964) demonstrated that size of adult Philonema varied depending on host species as well as host sex, and further illustrated overlap in variation of such characters as distance from anterior end to nerve ring, muscular and glandular oesophagus, number of caudal papillae, and tail and spicule length, and concluded that P. agubernaculum was morphologically identical with P. oncorhynchi.

Bashirullah (1966) decided the two worms were distinct species based on differences in life history. He suggested P. oncorhynchi is a parasite of anadromous salmonids and undergoes slower development, taking 32-35 months to reach maturity. Sockeye do not return to fresh water for two or more years and Bashirullah (1966) showed that elevated host hormones prior to spawning stimulated female worms to undergo their final maturation. Bashirullah (1966) suggested that P. agubernaculum, a parasite of non-anadromous salmonids, develops more quickly reaching maturity in 6-12 months. He suggested that these worms matured independently of host hormones but had no

experimental evidence to support this. Developmental differences are not host determined because cross-infection experiments (Bashirullah, 1966) show that developmental rate was unaffected when worms were in different hosts. Bashirullah (1966) further supported his thesis that the species were distinct with protein electrophoresis. However, sample sites for P. agubernaculum (O. mykiss from Kootenay Lake) and P. oncorhynchi (O. nerka from Cultus Lake) were geographically separated, raising the possibility that differences resulted from population variation in a single, widely distributed species.

This study examined restriction fragment banding patterns in repetitive sequences of DNA and supports Bashirullah's interpretation that P. agubernaculum and P. oncorhynchi represent distinct species. Results from this study demonstrate that Philonema spp. fall into two genetically distinct groups, corresponding to the two host species from which they were collected. Banding patterns were different for most enzymes used. Only 3 enzymes (Hae III, Hpa I and Xho I) revealed shared bands. Results from hybridization with the cluster of ribosomal genes (pBx2) probe failed to show any differences between Philonema species. This may be because the pBx 2 probe represents a cluster of ribosomal genes which may be highly conserved and therefore show no differences between closely related species.

Even though P. oncorhynchi and P. agubernaculum were collected from sites where their respective hosts were sympatric (e.g. Babine Lake), the two groups of worms still maintained their genetic identity, implying no hybridization occurs even when the opportunity presumably exists. The two Philonema groups probably represent good biological species and are hereafter referred to as P. agubernaculum (infecting O. mykiss) and P. oncorhynchi (infecting O. nerka).

Curran et al. (1985) demonstrated the feasibility of using restriction fragment length differences of repetitive DNA sequences (such as ribosomal and histone genes) as a tool for rapid identification of nematode species. Many nematode groups include species that are difficult to distinguish morphologically making identification difficult. Using Eco R1, Curran et. al. (1985) demonstrated restriction fragment length differences for repetitive genes among selected species and populations of nematodes belonging to the genera Caenorhabditis, Heterorhabditis, Meloidogyne, Romanonmermis, Steinernema and Trichinella.

Restriction endonucleases recognize and cleave specific sequences of nucleotides in double-stranded DNA. The size and number of resulting fragments vary depending on the number and location of cut sites. Restriction fragments of repetitive genes are visible on agarose gels because they are represented in high numbers. Bands of equal size are assumed to be homologous, and similar banding patterns produced by a given enzyme reflect relatedness. It is

unlikely that the same enzyme will produce repetitive bands of the same size in unrelated stretches of DNA. These assumptions are supported by empirical data. Curran et al. (1985) noted that the number of shared bands decreased as more distantly related groups were compared. For example, transgeneric comparisons showed no shared bands while comparisons of closely related species revealed only a small percentage of shared bands; within species, most or all bands were shared. This implies that shared bands indicate recent shared ancestry, and supports use of this technique in examining population and species relationships in morphologically indistinguishable groups. Restriction fragment length differences are of little use in investigating relationships among distantly related taxa because few, if any, shared bands occur.

Worms collected from kokanee in Babine Lake were identical with those in sockeye from the same region and are identified as P. oncorhynchi. This is not surprising since kokanee represent a self sustaining offshoot, derived from an anadromous sockeye population in the lake (Scott and Crossman, 1973, Foote et.al., 1989), that forgo ocean migration and remain in the nursery lake. Kokanee, like sockeye, spawn once in their lifetime, usually in their fourth year. Philonema oncorhynchi is probably carried into the kokanee population when the latter arises from the sockeye population which are or were in the same nursery lake. Material from steelhead smolts from net pens in Lake

O'Connor was identified as P. agubernaculum. Steelhead are an anadromous derivative of rainbow trout, but smolts are not usually exposed to Philonema because the freshwater stage of their life cycle usually involves stream or river residence (Withler, 1966). These smolts were probably carrying worms that circulate in resident salmonids.

Philonema spp. are normally non-pathogenic, but severe pathological effects were noted in steelhead smolts. Smolts likely became infected when they were placed in the lake which presumably contains an endemic salmonid population carrying P. agubernaculum and pathology probably resulted because these hosts are immunologically naive. Sakanari and Moser (1990) studied pathological changes induced by pleurocercoids of the cestode Lacistorhynchus dollfusi in east and west coast striped bass (Morone saxatilis). They showed that these parasites induced more intense pathological changes in east coast striped bass. They suggested that because east coast striped bass do not contact L. dollfusi they are immunologically naive and this results in a stronger pathological response. On the other hand, Sakanari and Moser (1990) suggested that west coast striped bass, introduced from the east coast, have adapted to L. dollfusi because they have been associated with the parasite for more than 20 generations.

Dracunculoids are tissue dwelling parasites of vertebrates and their cycles typically involve use of copepods as intermediate hosts. This life style presents a

problem because first stage larvae must reach the external environment to be ingested by the intermediate host. Many dracunculoids undergo a migration to subcutaneous tissues where they breach the host tegument to release their larvae. However, Philonema spp. make use of the host's reproductive system and pass to the external environment with the hosts roe during spawning (Platzer, 1964). Philonema spp. use copepods of the genus Cyclops as intermediate hosts (Vik, 1964, Bashirullah, 1966, Platzer and Adams, 1966, Ko and Adams, 1969) and larvae undergo development to the infective third stage within these hosts. Since P. agubernaculum and P. oncorhynchi are known to occur in sympatry (e.g.. Babine Lake) and both species use the same intermediate host, natural cross-infections should occur, and this could lead to hybridization and possible breakdown of species barriers.

Cross-infection experiments by Bashirullah (1966) demonstrate that P. agubernaculum and P. oncorhynchi are capable of infecting a variety of salmonids. A sub-adult P. agubernaculum was recovered from an experimentally infected sockeye after 161 days, and a rainbow trout experimentally infected with P. oncorhynchi survived 346 days after which time a fourth stage worm was recovered from the swimbladder. There is also evidence for natural cross-infections. Migrant sockeye from Shushwap lake and Seton Lake were found to be infected with immature Philonema spp. (Bashirullah, 1966, Margolis, pers. comm.). Since P. oncorhynchi is absent from the upper Fraser and Columbia Rivers these

infections likely represent cross-infection with P. agubernaculum. While cross-infection apparently occurs, no mature P. agubernaculum was recovered from the 35 sockeye and kokanee examined in the present study, yet all were presumably sympatric with salmonids infected by P. agubernaculum. Similarly, no P. oncorhynchi were recovered from the 5 Fulton River rainbow trout known to be sympatric with sockeye harboring Philonema.

Bashirullah (1966) argued that hybridization is prevented because P. agubernaculum has a yearly life cycle and can not survive the extended migration of sockeye. Furthermore, he believed that unlike P. oncorhynchi, P. agubernaculum develops directly to adulthood independent of cues from host hormones. He showed that P. agubernaculum develops more rapidly than P. oncorhynchi regardless of host but never recovered gravid females of P. agubernaculum. Even after six months, worms were subgravid. This is consistent with the hypothesis that P. agubernaculum requires host hormonal cues before undergoing its final maturation. Circumstantial evidence suggests P. agubernaculum is tied to host hormonal changes since Platzer (1964) only recovered mature worms from reproductively mature hosts in the wild.

It seems more likely that P. agubernaculum and P. oncorhynchi have similar life histories; both can reproduce only when the host reproduces. Development in the final host is probably diphasic; worms develop to adulthood

directly but require host hormonal cues to undergo final maturation. The fact that P. oncorhynchi develops more slowly than P. agubernaculum merely reflects its adaptation to a host which breeds only once usually at four years. If it were not true, then when P. agubernaculum entered hosts which may not mature for several years or hosts such as charr (genus Salvelinus) which breed every other year, worms would be at a reproductive dead-end. Furthermore, kokanee spend their entire lives feeding on plankton and benthic invertebrates (Scott and Crossman, 1973) and should be exposed throughout their life to Philonema spp. Therefore, at least those P. agubernaculum picked up a year prior to spawning should survive to host spawning. Bashirullah (1966) examined 200 kokanee examined from Kootenay Lake and only one contained an adult Philonema; P. oncorhynchi is absent in this area and the infection probably represents P. agubernaculum.

Rainbow trout generally take 3-5 years to mature and may live as long as 8 years (Scott and Crossman, 1973); therefore, P. oncorhynchi acquired early should have ample time to grow and reach maturity. No cross-infections were observed, but sample size was small because only the 5 worms examined from Fulton River were known to be sympatric with P. oncorhynchi.

It seems likely that successful cross-infections by Philonema spp. are prevented by a host immune response which encapsulates and destroys worms before they can mature.

Bashirullah (1966) observed cysts containing Philonema in the body cavity of sockeye from Shushwap Lake. Philonema agubernaculum and P. oncorhynchi have undergone molecular divergence, as evidenced by different banding patterns, and this divergence may be associated with antigenic changes which result in rejection when Philonema spp. enters an inappropriate host. This host immune response may prevent hybridization between the two species of Philonema by destroying one species prior to maturation and mating. However, it is also possible that cross-infections and hybridization are prevented because worms are unable to mature in unsuitable hosts due to physiological differences in the host species.

Fish restricted to isolated freshwater drainage systems often undergo genetic divergence from neighboring populations in other watersheds because land masses and salt water act as barriers to dispersal. Philonema agubernaculum samples showed banding patterns specific to geographic localities from which they were collected probably because there is no gene flow between populations since their hosts are probably restricted to their respective drainage basins. Pennask Lake and Fulton River populations were nearly identical (distinguished by only two Hae III bands), while Lake O'Connor worms appear to have undergone greater divergence from the two mainland populations since different banding patterns were observed for three enzymes (Bam H1, Hae III and Hinf I). Curran et al. (1985) demonstrated

banding pattern differences between species but failed to show differences between populations of Caenorhabditis elegans. This led them to speculate that restriction fragment length differences may represent markers for populations which can no longer freely interbreed and they may be associated with speciation, when genetic isolation allows rapid fixation of novel genome characteristics. While banding pattern differences seem to reflect genetic isolation, there is no reason to conclude that each population represents a different species. It seems more likely that, like morphological differences, differences in banding patterns are likely to occur among potentially interbreeding populations in widely distributed species.

Minchella et. al. (1989) studied restriction fragment length differences in urban and sylvatic populations of Trichinella spiralis (Nematoda; Trichinellidae). All 12 restriction enzymes used successfully separated sylvatic (wild) Trichinella from urban (swine) Trichinella. Most enzymes gave similar banding patterns for sylvatic populations. However, the enzyme Cla I distinguished all sylvatic isolates, suggesting that sylvatic T. spiralis is organized into more or less distinct populations.

Most of B.C. was glaciated as recently as 14,000 years ago. Melting and subsequent retreat of glaciers resulted in formation of large lakes and streams (Briggs, 1986), and facilitated reinvasion of freshwater fish from areas of refugia in the north and south. The Fraser River system was

principally recolonized from the Columbia River via glacial lakes in the Okanagan valley. The Skeena system has a fish fauna similar to the Columbia River, indicating a previous connection via the Fraser, probably while the Skeena river was blocked and its tributaries flowed east into the Fraser (McPhail and Lindsey, 1986). Thus, the close relationship between Pennask Lake and Fulton River P. agubernaculum may represent their common origin via a Columbia River dispersal event. On the other hand P. agubernaculum from Lake O'Connor may have diverged much earlier, following an early postglacial migration across the Strait of Georgia. Alternatively, Lake O'Connor worms may represent a population which survived in a central coast refugia during the most recent Pleistocene glaciation. There is increasing evidence, mainly botanical, that an area in the Queen Charlottes and northern Vancouver Island was unglaciated during the most recent Pleistocene glaciation (McPhail and Lindsey, 1986, Ogilvie, 1989). If P. agubernaculum from Lake O'Connor represents a population which survived in this refugia, then it should prove to be more similar to worms from the Queen Charlottes. Philonema agubernaculum from southern Vancouver Island should be more similar to the southern mainland since it was probably colonized from this area following glacial retreat.

Results from Bam H1, Hinf I and Hpa I digestion appear to indicate two genetic types of P. oncorhynchi, however, these two types occurred within the same population (e.g.

Sproat River, Henderson Lake, Fulton River and Pierre Creek) and are best interpreted as polymorphisms. Sockeye salmon are anadromous and undergo an ocean migration which takes them off shore before they return to spawn in fresh water. Like other salmonids, sockeye show a strong homing tendency for their nursery lake (and natal stream). If homing were perfect, it would restrict gene flow in P. oncorhynchi populations and possibly lead to geographic variation because worms are transmitted in nursery lakes. A study by Quinn et al. (1987) using protein electrophoresis and parasite prevalence data suggested sockeye may stray less than 1% of the time. However, this small amount of straying may be sufficient to prevent population divergence in their parasites or may result in mixing if changes do occur.

Sockeye introductions may also have contributed to mixing of populations of P. oncorhynchi. Sockeye have been transplanted into many B.C. watersheds in recent times. If infected smolts were transplanted they could seed uninfected lakes with P. oncorhynchi or mix with an existing population. Supporting evidence for this hypothesis is difficult to find since many introductions are unrecorded and when they are, method of transplant is often not stated. However, most fish are transplanted as eggs (Foerster, 1968) which would not carry Philonema.

The technique used in this study allows easy identification of Philonema spp. in B.C. and should facilitate further studies on host and geographic

distributions of the group. Philonema agubernaculum has been reported from a wide variety of salmonid hosts but our specimens were collected from O. mykiss; it remains to be seen whether a single species of Philonema infects all non-anadromous salmonids in B.C. It may be relatively easy to characterize the geographic distribution of P. oncorhynchi and resolve misidentifications for this species because it appears to lack geographic variation. However, it will be more difficult to characterize the geographic distribution of P. agubernaculum. This species has been reported from various localities across North America and may also be present in Asia. Isolates from B.C. show divergence and those from more distant geographic areas may show degrees of divergence that argue against their inclusion in a single species.

P. oncorhynchi is probably restricted to O. nerka. Rarely, it has been reported from O. keta (Akhemerov 1955, Platzer, 1964) but it is probably not a common parasite of chum and other Pacific salmon for ecological reasons. Sockeye are unique in that smolts spend 1-3 years feeding in a nursery lake which is the focus of the Philonema infection. If O. nerka is the only natural host for P. oncorhynchi then worms should only be found within this hosts range. Reports of P. oncorhynchi outside the range of sockeye are likely misidentifications.

Pacific salmon (genus Oncorhynchus) represent a recent lineage, 2-3 million years old (Thomas et al., 1986), and sockeye diverged from this lineage about 2 million years ago. Philonema oncorhynchi may have coevolved with sockeye from an ancestor found in more primitive non-anadromous salmonids, in response to a change in the host's life history. Philonema oncorhynchi probably arose sometime after this event since the worm is not present throughout the whole host range; it is absent from the Upper Fraser and Columbia Rivers. Philonema oncorhynchi may have arisen on the Asiatic or Alaskan coast during Pleistocene glaciation and colonized the B.C. coast when sockeye populations reinvaded from areas of refugia in Beringia (McPhail and Lindsey, 1986).

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