Studies on the mitochondrial genome and rDNA genes from the salmon louse, *Lepeophtheirus salmonis*.

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor scientiarum of the University of Bergen

by

Kjersti Tjensvoll

Department of Biology

University of Bergen

Norway

2005

CONTENTS

ACKNOWLEDGEMENTS

The studies included in this thesis have been conducted at the Department of Biology, University of Bergen, in the period October 2000 – December 2005. The project has been funded by a fellowship from the Research Council of Norway.

I want to express my warmest thanks to my supervisor Are Nylund for his guidance, for being so supportive and for believing in me. Your office door is always open, and you are always available for questions and discussions. Your good mood and hospitality create a really pleasant working environment.

I am deeply grateful to Kjartan Hodneland and Frank Nilsen for good discussions, and guidance during both laboratory work and manuscript/thesis preparation. Your interest in this field has been very inspiring, and your help has been valuable and very much appreciated. I am also deeply grateful to Kevin Glover for his enthusiasm, guidance within the field of population genetics and for the many, many "fruitful" discussions we have had. Your guidance, and effort in correcting my manuscript and thesis, has been very valuable and much appreciated.

I am very grateful to all my colleagues in the Fish Disease Group at the Department of Biology for the many social occasions, and all the good laughs. You are enthusiastic, and create an inspiring working environment. It has been a privilege for me to work with you.

Special thanks to the salmon lice group at the Institute of Marine Research (Bjørn Olav, Lars, Rasmus, Heidi, Morten, Petter and Frank) for making me feel part of the group despite different physical locations. The dinner parties, discussions and technical assistance have been very valued.

Thanks to Heidi Kongshaugen, Lars Hamre, Øivind Øines, Mike Snow, Mark Fast, Valentina Antonova and Shigehiko Urawa for sampling salmon louse for me throughout the world.

Thanks to Gry Sjøholt for reading one of my manuscripts, and this thesis. Your comments were very much appreciated. I would also like to thank Kenneth Meland for valuable

comments on this thesis. Thanks to Marius Karlsen for all the phylogenies you have been running for me in PAUP. Lindsey Moore, thank you for correcting my English grammar.

I also wish to thank the Department of Medical Genetics and Molecular Medicine, Haukeland Universityhospital, for making their sequence facility accessible for use in a very hectic period.

Last but not at least a sincere thanks to my parents, sister and brother for always being there for me, for taking interest in my work and believing in me. Bjarne, you have encouraged, and inspired me from day 1. Your support and love means everything to me! My warmest thanks to all my friends for their support, and fun during all of these years.

Kjersti
Bergen 06.12.05

LIST OF PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals:

I. Tjensvoll K, Hodneland K, Nilsen F, Nylund A (2005)

Genetic characterization of the mitochondrial DNA from *Lepeophtheirus salmonis* (Crustacea; Copepoda). A new gene organization revealed.

Gene 353: 218-230.

II. Tjensvoll K, Glover KA, Nylund A (In press)

Sequence variation in four mitochondrial genes of the salmon louse, *Lepeophtheirus salmonis*.

Diseases of Aquatic Organisms.

III. Tjensvoll K, Hodneland K, Nylund A (submitted)

The phylogenetic position of *Lepeophtheirus salmonis* (Copepoda: Siphonostomatoida) in relation to other crustaceans based on the 28S ribosomal RNA sequence.

Manuscript submitted to Journal of Crustacean Biology.

INTRODUCTION

Historical perspectives

The salmon louse, *Lepeophtheirus salmonis* (*L. salmonis*), was first mentioned in the literature by the Norwegian bishop Pontoppidan in 1753 (Pontoppidan 1753). However, without reference to several earlier accounts the female louse was first figured, and described by Krøyer in 1837 (Krøyer 1837). Salmon lice are natural parasites on salmonids, and have usually occured in low numbers on wild fishes (Wootten et al. 1982, Berland 1993). Still, White did report heavy infestations of wild salmon already in 1940, but this was considered to be an extreme case (White 1940). The first serious outbreaks of *L. salmonis* infections occurred on Norwegian Atlantic salmon farms during the 1960s, soon after cage culture began (Pike & Wadsworth 1999). Since then the increase in salmon production has been dramatic in the North Atlantic (Pike & Wadsworth 1999), and *L. salmonis* has become a major fish pathogen causing severe skin damage by feeding on mucus, epidermis and blood (Figure1) (White 1940, Brandal et al. 1976, Wootten et al. 1977, Kabata 1979, Wootten et al. 1982). A further consequence of *L. salmonis* infections can be osmoregulatory breakdown, secondary virus or bacterial infections followed by death (Wootten et al. 1982, Nylund et al. 1993). It has been estimated that *L. salmonis* annually causes economical losses for the farming industry in Norway by 500 million NOK (Pike & Wadsworth 1999, Boxaspen & Næss 2000). However, *L. salmonis* is also a major problem in several other countries with salmon farming industry as Scotland, Ireland, Shetland, Faeroe Islands and Canada (Wootten et al. 1982, Todd et al. 1997, Pike & Wadsworth 1999, Boxaspen & Næss 2000, Mustafa et al. 2001).

Since the introduction of salmon farming, a decline in wild salmon and sea trout stocks have become noticeably, and a link between the fish farming activity and the high *L. salmonis* prevalences on wild salmonids have been suggested (Tully & Whelan 1993a, Pike & Wadsworth 1999, Tully et al. 1999, Finstad et al. 2000, Bjørn et al. 2001, Heuch & Mo 2001,

Bjørn & Finstad 2002, Butler 2002, Skilbrei 2004). These assumptions are based on infections of wild salmonids coinciding with *L. salmonis* epidemics on farmed fish following the industry development (Tully et al. 1999, Finstad et al. 2000, Bjørn et al. 2001, Bjørn & Finstad 2002, Butler 2002). Especially sea trouts, which have residence in coastal water, are believed to be under an extremely high *L. salmonis* infection pressure (Tully & Whelan 1993a, Tully et al. 1993b, Birkeland 1996, Skilbrei 2004). Heavily infested sea trout post-smolts have been observed to return to rivers within few weeks after their smolt migration, a phenomenon called premature return (Tully et al. 1993b, Birkeland 1996, Bjørn et al. 2001). This behavior is probably a consequence of the delousing effect achieved in freshwater, and a maintenance of their osmotic balance (Birkeland 1996). Since wild salmon do not have residence in coastal areas, but migrate to the feeding ground in the North Norwegian Sea, it was believed that these smolts perhaps were less exposed to infestations by *L. salmonis* (Skilbrei 2004). However, studies have indicated that migrating salmon smolts become infected by *L. salmonis* in coastal waters, before they reach the open sea, and that subsequent re-infestations also occur in the open ocean (Finstad et al. 1994, Jacobsen & Gaard 1997, Finstad et al. 2000, Todd et al. 2000, Skilbrei 2004). In Norway it is assumed that louse epizootics can cause mortality of 30-50% of all emigrating sea trout smolts, and 48-86% of all wild salmon smolts (Bjørn et al. 2001, Butler 2002).

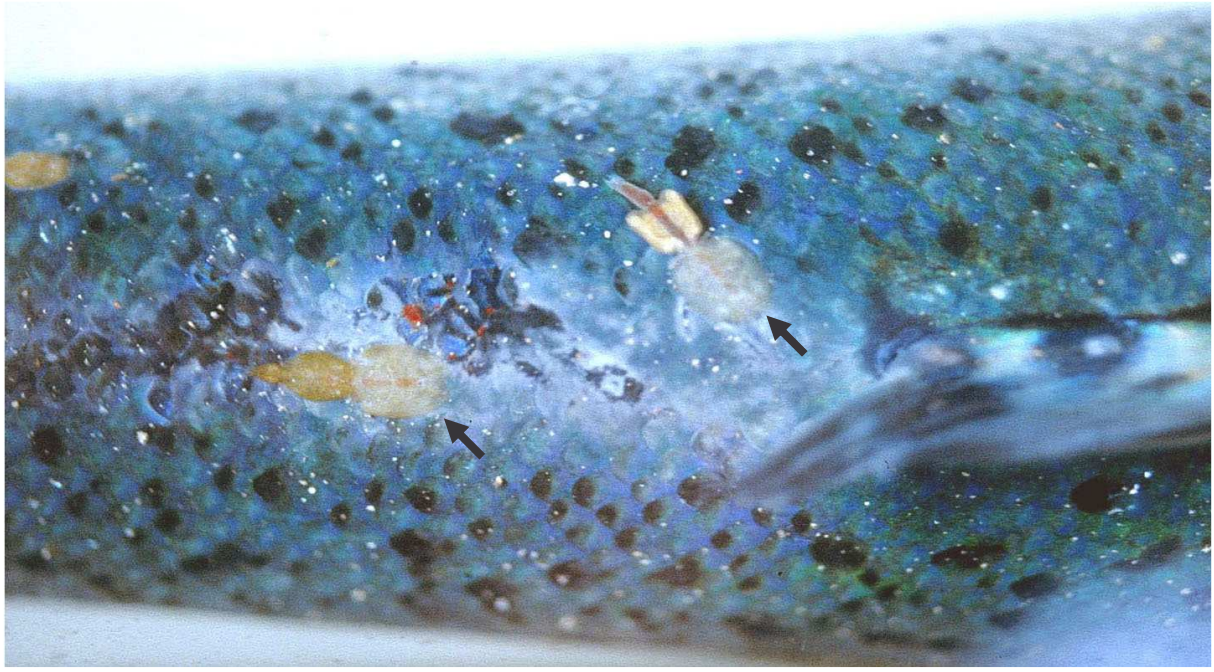

Figure 1: An Atlantic salmon infected with *Lepeophtheirus salmonis* feeding on mucus, epidermis and blood.

Copepod systematics

Lepeophtheirus salmonis is an arthropod belonging to the phylum Crustacea, subclass Copepoda (Siphonostomatoida, Caligidae). In addition to the crustaceans the arthropods also include the hexapods, chelicerates and myriapods (NCBI taxonomic database, http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/). Within Arthopoda it has long been claimed that the hexapods constitute a monophyletic group, and that their closest relatives are found within the myriapods (Nardi et al. 2003). More recently, molecular studies have rejected this relationship in favor of a closer affinity between Hexapoda and Crustacea (e.g. Friedrich & Tautz 1995, Boore et al. 1998a, Garcia-Machado et al. 1999, Giribet et al. 2001, Regier & Shultz 2001, Nardi et al. 2003, Mallatt et al. 2004, Giribet et al. 2005, Regier et al. 2005), also called Pancrustacea (Zrzavy & Stys 1997). Some morphological evidence also support this relationship (Friedrich & Tautz 1995, Giribet et al. 2001). The monophyletic status of the Hexapoda has also been questioned. This is due to the position of the Collembola

(the wingless hexapods) outside the Pancrustacean clade, in some cases, resulting in a paraphyletic Hexapoda (e.g. Nardi et al. 2003, Bitsch & Bitsch 2004, Bitsch et al. 2004, Lavrov et al. 2004).

An extensive fossil record suggests that the crustaceans arose and diversified during Cambrium (ca. 570 mya), and have subsequently undergone a long period of independent evolution (Spears & Abele 1997). Hence, the Crustacea has evolved into a group with extreme morphological diversity (Martin & Davis 2001, Regier et al. 2005). Most crustaceans are aquatic, and today the phylum is classified into the six classes Branchiopoda, Remipedia, Cephalocarida, Maxillopoda, Ostracoda and Malacostraca (Martin & Davis 2001). Copepoda is placed within the Maxillopoda and comprises approximately 11500 species placed in about 200 families and 1650 genera (Humes 1994). The aquatic copepods are found in various habitats from freshwater to marine or hypersaline inland waters, and from polar waters to hot springs (Huys & Boxshall 1991). It is considered to be the largest and most diverse group within Crustacea with life histories ranging from free-living to benthic and parasitic (Kabata 1970, Huys & Boxshall 1991). The copepods are usually small in size, with most species having body lengths of 0.5 to 5mm (Huys & Boxshall 1991). In terms of their size, diversity and abundance they can be regarded as the insects of the seas.

Copepod taxonomy is primarily based on morphological characters relating to modifications of the cephalic feeding structure (mandibles), and body segmentation (Kabata 1979, Huys & Boxshall 1991). It was Kabata (1979) who formally reassessed the phylogenetic relationships of the families of copepods parasitic on fishes, which later resulted in a revised classification for the entire subclass Copepoda (Huys & Boxshall 1991). Kabata (1979) suggested a monophyletic Copepoda with the ancestral “archiecopepod” forms living on or near the sea bottom, which in time split into two copepod groups (Figure 2). One group

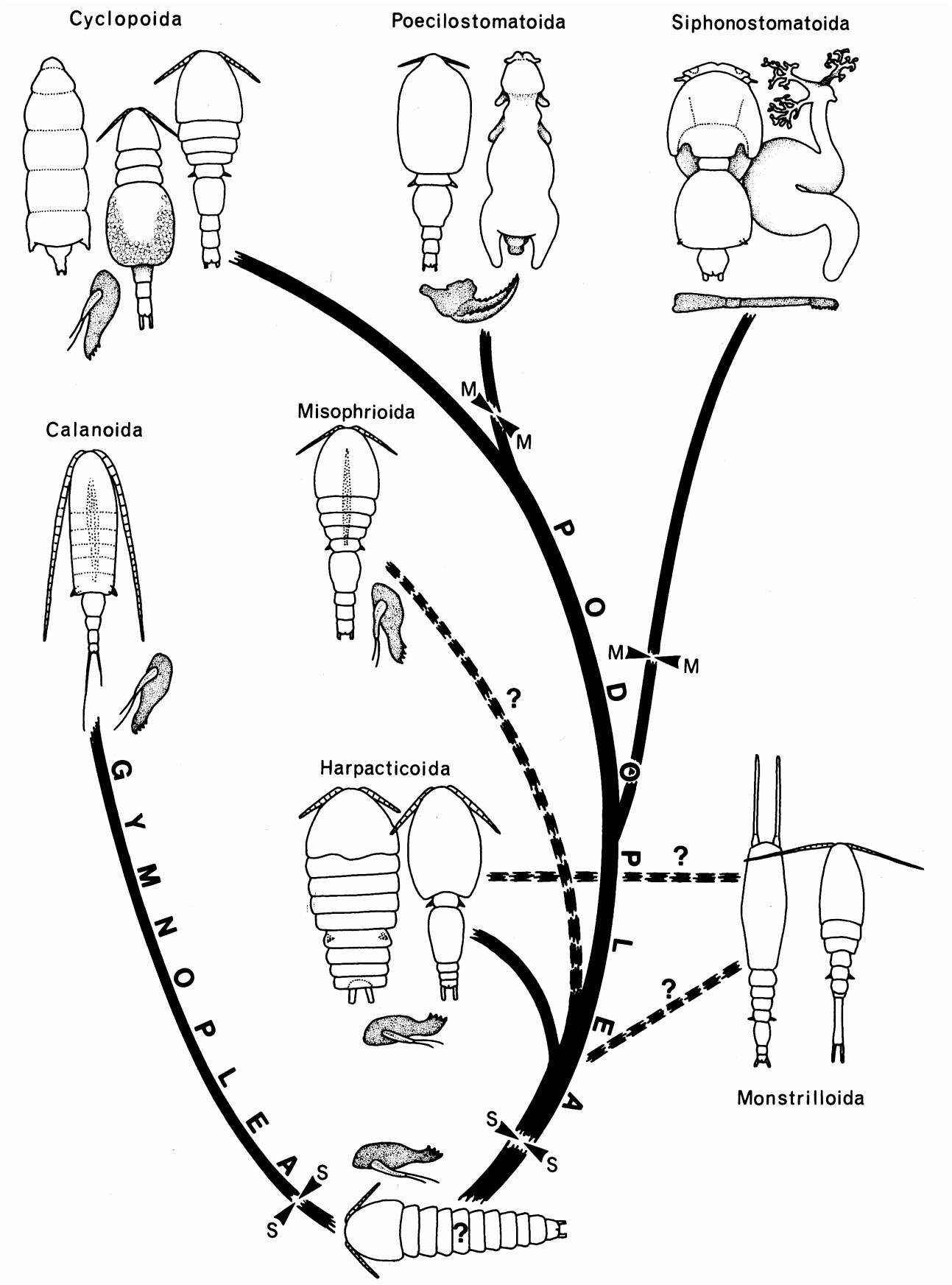

Figure 2: Copepod relationships proposed by Kabata (1979).

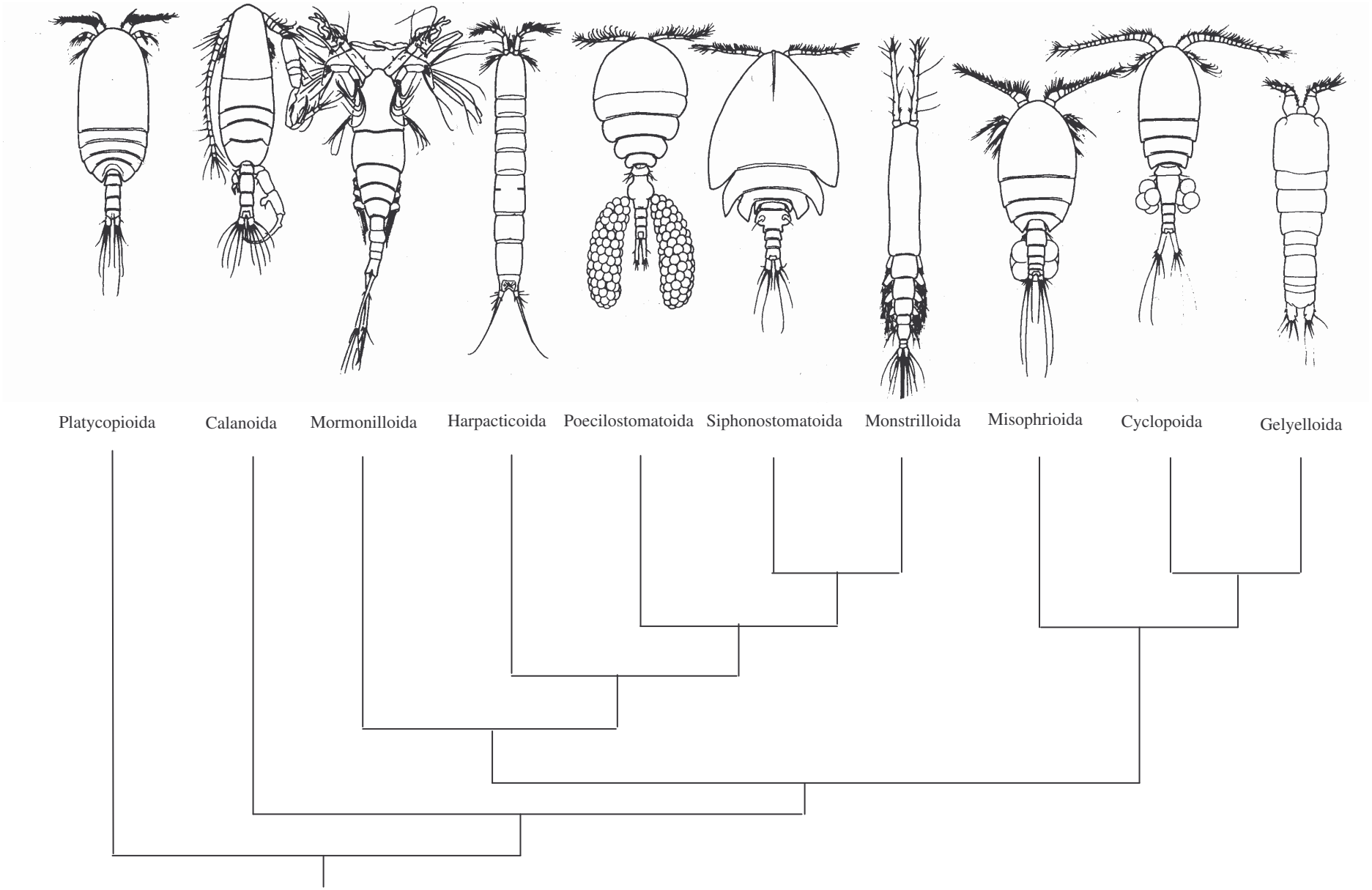

Figure 3: Copepod relationships proposed by Huys and Boxshall (1991).

consisting of the planktonic order Calanoida, while the main group consisted of active swimming copepods with the most primitive branches represented by the orders Monstrilloida, Harpacticoida and Misophrioida (Kabata 1979). Within the main group Siphonostomatoida was believed to be of a more ancient origin than the Poecilostomatoida and Cyclopoida, an assumption that was based on the development of the siphon-like mandibles (Kabata 1979). The Poecilostomatoida was believed to branch off from the Cyclopoida not very far back in the evolutionary past, while the Cyclopoida was considered the most recently derived order (Kabata 1979). In 1991 Huys and Boxshall erected several new orders and proposed a new copepod phylogeny suggesting the Mormonilloida, Harpacticoida, Poecilostomatoida, Siphonostomatoida and Monstrilloida as monophyletic (Figure 3), with the Siphonostomatoids as a sister-group to the Monstrilloida (Huys & Boxshall 1991). The cyclopoids belonged to a clade constituting a sister-group to the Siphonostomatoida-Poecilostomatoida clade. Moreover, the Calanoida formed a separate group also in this analysis.

Today, the ten copepod orders suggested by Huys and Boxshall (1991) are still recognized (Martin & Davis 2001). Copepods parasitic on fishes are mostly found within the orders Cyclopoida, Poecilostomatoida and Siphonostomatoida (Kabata 1992). The cyclopoids are the most abundant group of copepods in freshwater. They are primarily planktonic, but some parasitic freshwater cyclopoids are also found as well as free-living marine species (Huys & Boxshall 1991). In contrast, virtually all poecilostomatoids are parasites, and the majority are marine. It is a morphologicaly diverse group of copepods, containing a great number of families with large body sizes and peculiar morphology (Kabata 1979, Huys & Boxshall 1991). The third order, Siphonostomatoida, contains exclusively parasitic species, and about 75% of the parasitic copepods found on fishes belong to this order. These copepods are mostly marine in distribution, but a small number of species are also found in freshwater.

Many highly transformed types of copepods are found within the Siphonostomatoida, i. e. members of the family Nicothoidae that have lost all their appendages (Huys & Boxshall 1991).

Lepeophtheirus salmonis is placed within the Siphonostomatoida, family Caligidae (Kabata 1979). All Caligidae have similar morphology, characterized by five tagmata (cephalothorax, the fourth leg-bearing segment, genital complex, the abdomen and the tail). However, the caligids have also acquired several morphological traits as an adaptation to their life as parasites. They have a flattened body and prehensile appendages allowing them to attach to the host surface, as well as making them capable of free movement. Of the 31 Caligidae genera *Caligus* is acknowledged as the largest, whereas *Lepeophtheirus* is the second largest genus. These two genera are morphologically very similar, but one feature distinguish *Lepeophtheirus* spp from *Caligus* spp and that is the lack of lunules on the anterior margin of the adult parasites (Kabata 1979).

The biology and life cycle of L. salmonis

Lepeophtheirus salmonis has a circumpolar distribution in the northern hemisphere, occurring on most species in the genera *Salmo, Oncorhynchus* and *Salvelinus* (e.g. Kabata 1979, Wootten et al. 1982, Johnson & Albright 1991a). The life cycle of *L. salmonis* consists of 10 stages, each separated by a moulting phase (Figure 4) (Kabata 1979, Johnson & Albright 1991b, Schram 1993). The first three stages are free-living, including two planktonic naupliar stages (nauplius I and II) and one infective copepodid stage. The copepodid attaches to the host by the second antennae, and once attached it uses the maxillipeds to move on the host surface to find a suitable place to settle (Schram 1993). Most copepodids prefer to settle on the skin and fins, although gills have also been reported as

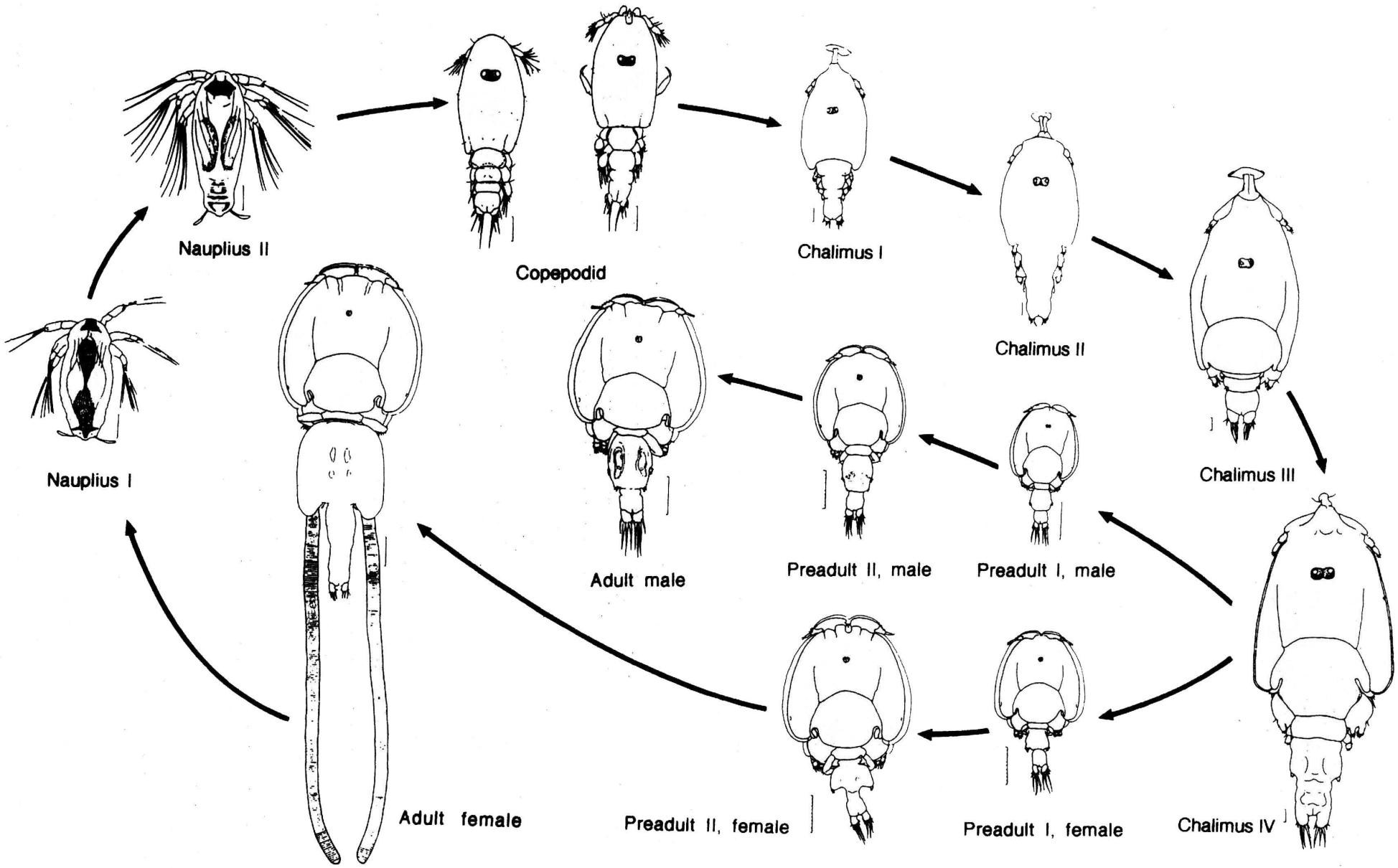

Figure 4: Life cycle of *Lepeophtheirus salmonis* (Schram 1993). Scale bars: nauplius-chalimus = 0.1 mm, preadult-adult = 1 mm.

settlement sites (Wootten et al. 1982, Johnson & Albright 1991b, Pike & Wadsworth 1999, Tucker et al. 2000, Treasurer & Wadsworth 2004). After moulting the copepodid transforms into chalimus I. All four chalimus stages (chalimus I-IV) are fixed to the body surface of the host by a frontal filament, and a new filament is produced between each moulting. After molting to the pre-adult stage the parasite is, however, able to move freely on the host surface. It is then attached to the host by the second antennae and the cephalothorax, which now may function as a cupping glass (Schram 1993).

Development, growth and survival of *L. salmonis* are greatly influenced by water temperature. Laboratory experiments have shown that development from the first nauplius to the infectious copepodid takes around 9.3 days at 5°C, 3.6 days at 10°C and only 1.9 days at 15°C (Johnson & Albright 1991a). The duration of the copepodid stage can, however, last as long as 10 days at 10°C (Johnson & Albright 1991a). Moreover, Boxaspen (2005) demonstrated that the last successful settlement of copepodids on salmon at 8°C occured 23 days after the copepodids had been introduced into the tank, and after 30 days at 6°C respectively (Boxaspen 2005). Development from egg to adult male takes around 40 days (5-6 weeks), while the time from egg to adult female takes 52 days (7.4 weeks) at 10°C (Wootten et al. 1982, Johnson & Albright 1991a). Normal development of embryos and larvae may take place at temperatures as low as 4°C (Boxaspen & Næss 2000).

The combat against L. salmonis

Today, two different approaches are used in the combat against *L. salmonis* (Evensen et al. 2004). These include the use of bath treatment with synthetic pyrethroids (Cypermethrin and deltamethrin), as well as oral treatment where in-feed chemicals are being used (Pike & Wadsworth 1999, Roth 2000, Stone et al. 2002, Grave et al. 2004, Westcott et al. 2004). Until recently organophosphates (trichlorfon (Neguvon), dichlorvos (Nuvan) and azamethiphos

(Salmosan)) were the most used pesticides against *L. salmonis* (Ramstad et al. 2002). However, the last three years the use of bath-administered neurotoxins in general (organophosphates and synthetic pyrethroids) have been in decline, while the use of the oral preparation emamectin benzoate (SLICE) has increased considerably (Evensen et al. 2004). The reason for this is that SLICE has a suitable toxic effect on all life stages of *L. salmonis*, while the synthetic pyrethroids have less effect on the chalimus stages (Pike & Wadsworth 1999, Ramstad et al. 2002, Stone et al. 2002, Westcott et al. 2004). Still, synthetic pyrethroids are the predominant treatment for larger fishes (>1 kg), due to the higher costs and the required quarantine period when using SLICE on large fish (Evensen et al. 2004, Grave et al. 2004)**.** Wrasse, or cleaner-fish, is also used as treatment, but in a smaller scale (Frost & Nilsen 2004).

A major concern when using chemicals in the combat against *L. salmonis* is the development of resistance. Most chemicals used against *L. salmonis* are insecticides, and resistant insect populations do exist for these drugs (Waldstein & W.H. 2000, Ahmad et al. 2003, Burgess 2004, Ffrench-Constant et al. 2004). Similar effects have also been observed for *L. salmonis*, particular regarding organophosphates, where reduced treatment efficiency has been reported several times (Jones et al. 1992, Devine et al. 2000, Tully & McFadden 2000, Denholm et al. 2002, Ramstad et al. 2002). In time it is also expected that reduced treatment efficiency will emerge from the use of synthetic pyrethroids (Sevatdal & Horsberg 2003).

Epizootic studies of L. salmonis

Several attempts have been made to obtain polymorphic genetic markers for studying the population genetic structure of *L. salmonis* (Isdal et al. 1997, Todd et al. 1997, Nolan et al. 2000, Tully & Nolan 2002, Dixon et al. 2004, Todd et al. 2004). In Norway, allele frequency

data from four allozymes indicated that differences might exist between northern and southern *L. salmonis* samples (Isdal et al. 1997). However, allozymes did not differentiate between *L. salmonis* collected in different locations throughout Scotland, while random amplification of polymorphic DNA (RAPD) did (Todd et al. 1997). Farm specific markers were also found in this study (Todd et al. 1997). Based on these results an expanded RAPD study was performed on farmed, and wild salmon from Scotland (Dixon et al. 2004). This study did not confirm the genetic differentiation previously found between *L. salmonis* samples in Scotland, although some degree of genetic differentiation was observed (Dixon et al. 2004). Another study, using microsatellites, showed differences between *L. salmonis* collected in Ireland, Scotland and Norway (Nolan et al. 2000). On the other hand, a recent study also using microsatellite markers did not demonstrate genetic differentiation between samples of *L. salmonis* collected on wild and farmed salmonids in Scotland, or between *L. salmonis* collected on salmonids from Scotland, Norway and Canada (Todd et al. 2004). However, significant genetic differentiation between *L. salmonis* from the North Atlantic versus the North Pacific Ocean was observed (Todd et al. 2004).

The rRNA genes coding for 18S rRNA and 5.8S rRNA of *L. salmonis*, in addition to the intergenic spacers (ITS-1 and ITS-2), have also been characterized for use in a population genetic study (Hodneland et al., unpublished). However, no genetic difference was found within any of these sequences when *L. salmonis* from Norway and Japan were compared.

Candidate genes for studies of epizootiology and phylogeny

Mitochondrial genes and the ribosomal DNA unit have been used extensively in phylogeny, and population genetic studies (e.g. Hale & Singh 1987, Hillis & Dixon 1991, Garcia-Machado et al. 1999, Saito et al. 2000, Schwenk et al. 2000, Umetsu et al. 2002, Yamauchi et al. 2002, Gantenbein & Largiader 2003, Papetti et al. 2005). While conserved regions of the

rDNA unit can be used in studies of more ancient stages of evolution, other faster evolving regions are used to infer closer genetic relationships (Grechko 2002). The mitochondrial genes are found to evolve at different rates, and thus have been classified according to their properties in resolving phylogenetic relationships among distantly related taxa (Zardoya & Meyer 1996). However, the mitochondrial genes are believed to be most suited for population genetic studies since they accumulate substitutions up to 10 times faster than nuclear genes (Shearer et al. 2002, Ballard & Whitlock 2004). Moreover, due to maternal inheritance the mitochondrial genome is haploid (Moritz et al. 1987), resulting in a population size equal to 1/2 of that for nuclear genes. Mitochondrial DNA (mtDNA) is therefore more subjected to genetic drift and fixation than nuclear DNA (Altukhov & Salmenkova 2002, Ballard & Whitlock 2004). Altogether, analyses of the mtDNA often prove useful in assessing genetic structure, gene flow or phylogenetic relationships among populations, or closely related species, when nuclear markers fail (Shoemaker et al. 2003).

Mitochondrial genes

The mitochondrial genome is a circular DNA molecule. It codes for proteins that, together with nuclear encoded products, form enzyme complexes involved in the production of ATP in oxidative phosphorylation, as well as other biochemical functions (Cummins 2001, Saccone et al. 2002). To date 102 mitochondrial genomes of arthropods have been characterized (http://www.ncbi.nlm.nih.gov/genomes/ORGANELLES/mztax_short.html), and of these 30 are within the phylum Crustacea (see Appendix, Table 1). The length of the mtDNAs, within the metazoan, have been reported in the range of 14-42 kb (Crease 1999). Despite this large size variation all metazoan mtDNAs, with few exceptions, contain the same 37 genes; 13 protein-coding genes, two rRNAs and 22 tRNAs in addition to a non-coding control region (table 1) (Boore 1999).

Table 1: The genes found in metazoan mitochondrial genomes; 13 protein-coding genes, two ribosomal RNAs and 22 tRNA genes in addition to a control region.

Genes and regions	Designation
Cytochrome oxidase subunit I, II, III	*COI*, *COII*, *COIII*
Cytochrome b	*Cyt B* or *Cyt b*
NADH dehydrogenase subunits 1-6, 4L	*ND1-6, ND4L*
ATP synthase subunits 6, 8	*A6*, *A8* or *ATP6, ATP8*
Large ribosomal RNA subunit	16S rRNA or *lrRNA*
Small ribosomal RNA subunit	12S rRNA or *srRNA*
18 transfer RNAs each specifying a single amino acid	Corresponding one-letter amino acid or amino acid abbreviation (e.g. tRNA-A or tRNA-Ala)
Two transfer RNAs specifying leucine	tRNA-L (CUN), tRNA-L (UUR) or tRNA-Leu (CUN), tRNA-Leu (UUR)
Two transfer RNAs specifying serine	tRNA-S (AGN), tRNA-S (UCN) or tRNA-Ser (AGN), tRNA-Ser (UCN)
Non-coding control region	D-loop

In some species all genes are coded from one mtDNA strand, whereas in others the genes are distributed between the two strands (Boore 1999). As opposed to genomic DNA the mitochondrial genes do not contain introns, the genes often overlap, but if not, the intergenic regions are very short (Wolstenholme 1992b). Although most protein-coding genes are relatively conserved, both rRNA genes and the tRNA genes are variable both in size and structure (Wolstenholme 1992a). Gene order may vary between metazoan lineages, but a conservation of gene order is expected among closely related species and genera (Boore 1999, Saccone et al. 2002). A gene organization representative of the Arthropoda is shown in figure 5.

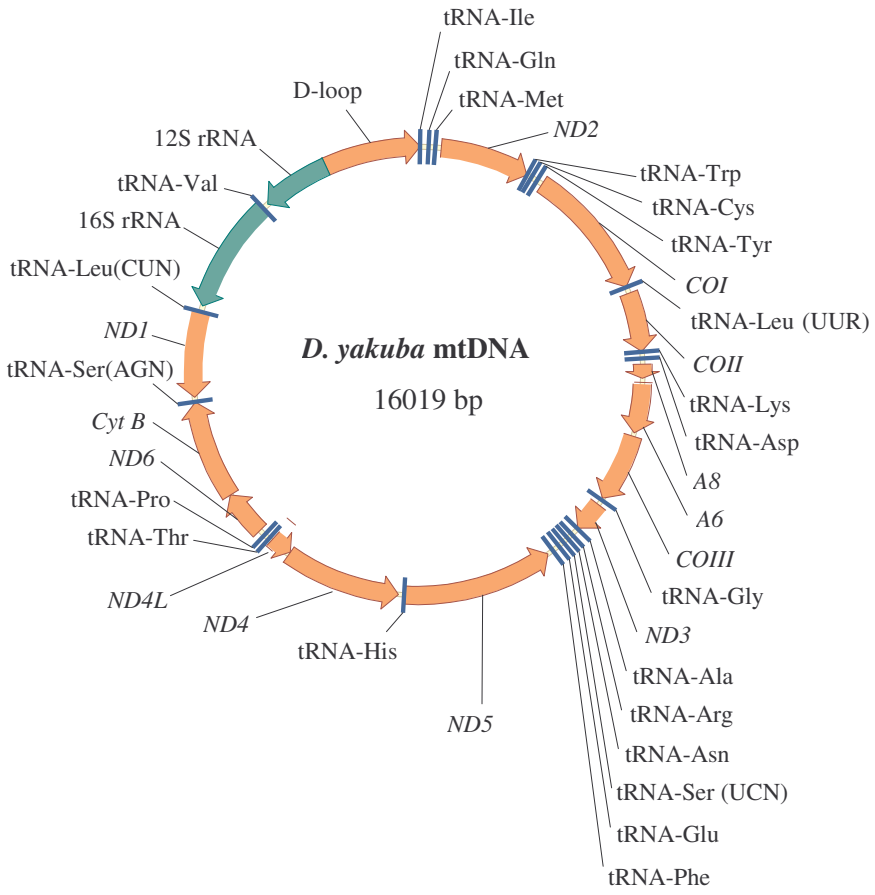

Figure 5: Mitochondrial gene organization representative of the Arthropoda, here presented by the genome of *Drosophila yakuba*. Genes coded on the complementary strand are shown by counter-clockwise arrows.

The four mitochondrial genes *COI*, 16S rRNA, *A6* and *Cyt B* are the most often used in population genetic studies, also within Crustacea (e.g. Bucklin et al. 1997, Lee 2000, Gopurenko & Hughes 2002, Jarman et al. 2002, Hurwood et al. 2003, Baratti et al. 2005). Another region considered to be informative for studies of population structure is the control region (D-loop). In a number of metazoan species tandemly arranged repeated sequences are found within the D-loop, and the number of repeated copies have been found to vary between individuals of a species (Wolstenholme 1992b, Saccone et al. 2002). However, it has quite recently been reported that the variability in this region is extremely high, even when

considering members of the same species, making alignment of the D-loop difficult to perform (Altukhov & Salmenkova 2002, Saccone et al. 2002).

rRNA genes

Ribosomal RNA (rRNA) constitutes 80-90% of the total cellular RNA in both eukaryotes and prokaryotes. It is represented in the genome by multiple genes where the number varies from 100-200 copies in lower eukaryotes up to several hundred in higher eukaryotes (Lewin 1997). The rRNA genes are arranged as tandem repeats of a nuclear ribosomal gene cluster (rDNA) encoding 18S rRNA, 5.8S rRNA and 28S rRNA, which are separated by the transcribed spacers ITS-1 and ITS-2 (Figure 6) (Hillis & Dixon 1991). An external transcribed spacer (ETS) is located upstream of the 18S rRNA gene (Figure 6). The transcribed spacers (ITS-1, ITS-2 and ETS) contain the processing signals for the rRNA transcripts, as the genes are transcribed as parts of a larger precursor molecule that is subsequently processed in several steps to yield the mature rRNAs (Hillis & Dixon 1991). A nontranscribed spacer (NTS), also called the intergenic spacer (IGS), is located at the 5` end of the rDNA unit separating the 18S-28S tandem repeats (Figure 6).

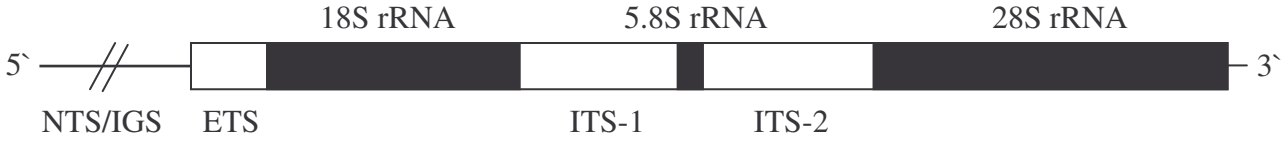

Figure 6: Organization of an eukaryotic rDNA tandem repeat. The rRNA genes 18S, 5.8S and 28S are transcribed as a large precursor molecule, also including two internal transcribed spacers (ITS-1 and 2) and the external transcribed spacer (ETS). The nontranscribed spacer (NTS), or the intergenic spacer (IGS), separates the repeated units.

Ribosomal RNA genes consist of both core regions, as well as expansion regions (Clark et al. 1984). Core segments have very conserved nucleotide sequences, which fold into secondary structures that are common to all eukaryotes (Linares et al. 1991). In contrast, the expansion segments can vary considerably in length and sequence between different eukaryotic organisms (Linares et al. 1991). This may be due to the presence of repetitive motifs, insertions or an accumulation of single nucleotide polymorphisms (Hassouna et al. 1984, Hancock & Dover 1988, Tautz et al. 1988, Hillis & Dixon 1991). Of the rRNA genes 18S rRNA is most used in phylogeny, due to its relatively slow evolving sequence (Hillis & Dixon 1991). The 28S rRNA gene, on the other hand, is larger and the expansion regions are much more variable, both in length and sequence than those of 18S rRNA (Hancock et al. 1988). Hence, 28S rRNA has been used in population genetic studies, as well as within molecular systematics (e.g. Crandall et al. 2000, Jarman et al. 2000, Remigio & Hebert 2000, Babbitt & Patel 2002, Stevens et al. 2002, Taylor et al. 2002, Sawabe et al. 2003, Schnabel & Hebert 2003, Duan et al. 2004, Mallatt et al. 2004).

In addition to the rRNA genes, the spacer regions can also be used to infer phylogenetic and population genetic relations (Hillis & Dixon 1991). Among the spacers, the NTS evolves most rapidly, while the transcribed spacers (ITS-1, ITS-2 and ETS) are somewhat more conserved (Hillis & Dixon 1991). A tandemly repeated sequence comprises also part of the NTS region. This sequence generally varies in length of 100-200 bp, resulting in a variable overall length of the NTS region (Jorgensen & Cluster 1988). The number of sub-repeating elements differ among individuals, but it has also been found differences in the NTS repeat within individuals (Jorgensen & Cluster 1988). Despite this, several population genetic studies have been performed using the NTS region (e.g. Cunningham et al. 2003, De Arruda et al. 2003, Printzen et al. 2003, Gupta et al. 2004, Huguet et al. 2004).

AIMS OF THE STUDY

The main goal of this project was to characterize both mitochondrial and nuclear genes, and use them to study the population genetic structure of *L. salmonis* along the Norwegian coast and throughout the North Atlantic. The ability to provide such data should prove useful in analysing; a) the dispersal potential of *L. salmonis* larvae, b) gene flow between *L. salmonis* populations, c) recurrent infestation on salmon farms, d) the impact of these infestations on wild salmonids and e) development of resistance in *L. salmonis* populations to chemoterapeutants. Since the mitochondrial genes *A6, COI, Cyt B* and 16S rRNA have been reported to be highly informative for population genetic studies in other crustaceans, and arthropods, these genes were chosen for the present study. The nuclear 28S rRNA gene also contains variable regions, and the nontranscribed spacer (NTS) includes tandemly repeated sequences that could be informative for population genetic studies. Furthermore, both mitochondrial and rDNA genes have proved useful for inferring phylogenetic relationships.

Specific aims were to:

1. Characterize the mitochondrial genome of *L. salmonis*.
2. Characterize 28S rRNA, and the nontranscribed spacer (NTS), of *L. salmonis*.
3. Use the mitochondrial genes *A6, COI, Cyt B* and 16S rRNA, in addition to 28S rRNA and the NTS region, to study the population genetic structure of *L. salmonis* along the Norwegian coast and in the North Atlantic.
4. Use sequence information from mitochondrial and rDNA genes to find the phylogenetic position of *L. salmonis*.

SUMMARY OF RESULTS

Paper I

Genetic characterization of the mitochondrial DNA from *Lepeophtheirus salmonis* (Crustacea; Copepoda). A new gene organization revealed.

The mtDNA from *L. salmonis* is 15 445 bp. It contains 13 protein-coding genes, two ribosomal RNA genes and 22 tRNA genes in addition to a non-coding control region (D-loop). Whereas tRNA-Cys was not identified in the *L. salmonis* mtDNA, two copies of tRNA-Lys were characterized. This has not previously been reported in any crustacean species.

The mitochondrial gene order in *L. salmonis* differs significantly from the gene order found in the three copepods (*Tigriopus japonicus*, *Eucalanus bungii, Neocalanus cristatus*), and the other crustaceans previously characterized. Among the exceptions are the organization of *ND4*/*ND4L* and *A8*/*A6*, which are usually transcribed as one bicistronic mRNA, but are separated by several genes in the *L. salmonis* mtDNA. Furthermore, the two rRNA genes are encoded on opposite strands in *L. salmonis*, and this has not previously been found in any other arthropods. Despite these differences a phylogenetic analysis, based on the mitochondrial protein sequences, did group *L. salmonis* together with *T. japonicus*.

Paper II

A study of single nucleotide polymorphisms (SNPs) in four mitochondrial genes of the salmon louse, *Lepeophtheirus salmonis*.

The four mitochondrial genes *A6, COI, Cyt B* and 16S rRNA were used to examine the genetic variation in *L. salmonis* collected from seven locations; Norway (Finmark, Sogn og Fjordane and Øst-Agder), Scotland, Canada, Russia and Japan. All genes showed an extremely high level of polymorphisms, leading to an intraspecific variation of 17.5% in *A6*,

15.9% in *COI*, 14.4% in *Cyt B* and 10.6% in 16S rRNA. The majority of the polymorphisms were only observed within single individuals, resulting in a high number of private haplotypes within each gene.

Sequence variation found in the four mitochondrial genes did not reveal genetic differentiation among the three Norwegian samples from Finmark, Sogn og Fjordane and Øst-Agder. Furthermore, no genetic differentiation was observed between *L. salmonis* sampled in Norway, Scotland and Russia. However, pairwise sequence comparisons indicated that a weak degree of differentiation might exist between *L. salmonis* sampled in the northeast Atlantic, and *L. salmonis* from the east coast of Canada. All samples collected in the Atlantic were clearly different from the Pacific sample, as expected. Extensive gene flow due to passive transport of *L. salmonis* larvae along the Norwegian coast, and the migratory pattern of the salmonid host is suggested to explain the lack of distinct populations in the North Atlantic.

Paper III

The phylogenetic position of *Lepeophtheirus salmonis* (Copepoda, Siphonostomatoida) in relation to other crustaceans based on the 28S ribosomal RNA sequence.

Two separate phylogenetic analyses, based on the sequence of 28S rRNA, were performed in order to find the position of *Lepeophtheirus salmonis* (Copepoda, Siphonostomatoida, Caligidae) in relation to a selection of other copepod, crustacean and arthropod species. The arthropod phylogeny shows monophyly of several accepted groups like Copepoda, Hexapoda (Insecta and Collembola) and Branchiopoda, giving support to the phylogenetic analyses performed. Furthermore, the Hexapoda is placed as sister-group to the Copepoda. The three orders within the Copepoda (Siphonostomatoida, Poecilostomatoida and Cyclopoida) are each monophyletic, with the Poecilostomatoida being the closest relative to Siphonostomatoida.

Within the Siphonostomatoida, *L. salmonis* group together with *Lepeophtheirus pollachius* with 100% support. The examined *Caligus* species constitute the sister-group to *Lepeophtheirus* spp., thus making the Caligidae monophyletic. However, members of the family Lernaeopodidae do not constitute a monophyletic group in our analysis.

DISCUSSION

Characterization of the L. salmonis *mitochondrial genome*

Very little is known about the population structure of *L. salmonis* (Tully & Nolan 2002), despite the economical losses this pathogen causes for the Atlantic salmon farming industry (Pike & Wadsworth 1999, Mustafa et al. 2001). Several population genetic studies have been performed, but these studies have resulted in contradictory conclusions based on the use of different genetic markers (Isdal et al. 1997, Todd et al. 1997, Nolan et al. 2000, Tully & Nolan 2002, Dixon et al. 2004, Todd et al. 2004). This contributes to a great confusion about the existence of distinct *L. salmonis* populations, and new genetic approaches are therefore needed. Since mitochondrial genes are used extensively in population genetic studies, the mitochondrial genome of *L. salmonis* was characterized (AY625897) (Paper I). The mtDNA contains the usual 37 genes found in metazoan mitochondrial genomes, including 22 encoding tRNAs, but a completely new gene organization was revealed (Figure 7). tRNA-Cys was not identified, neither by the tRNAscan-SE program nor manually anticodon/motifs searches, and this was probably due to large deviations in the secondary structure. However, two copies of tRNA-Lys are present in the mitochondrial genome of *L. salmonis*. Moreover, 12S and 16S rRNA has opposite transcriptional polarity in the *L. salmonis* mtDNA (Paper I).

To date, a total of 30 crustacean mitochondrial genomes have been characterized (see Appendix, Table 1). Within the copepods the mtDNA from *L. salmonis* (Paper I) and *T. japonicus* (Machida et al. 2002) have been completely characterized, while *Eucalanus bungii* and *Neocalanus cristatus* are only partly characterized (Machida et al. 2004). Even if the differences in locations of tRNA genes are ignored, since tRNA genes are frequently involved in gene rearrangements (Wolstenholme 1992b), very limited similarities in gene order are demonstrated between the four copepods (Paper I). In comparison, 21 of the other 28 characterised crustacean mtDNAs have similar gene organization as the mitochondrial

genome of *D. yakuba* (see figure 2 in Paper I; Lavrov et al. 2004, Yamauchi et al. 2004, Miller et al. 2005, Segawa & Aotsuka 2005, Miller et al. Unpublished, Swinstrom et al. Unpublished). These include all the four branchiopods characterized (*Daphnia pulex*, *Artemia franciscana*, *Triops cancriformis* and *Triops longicaudatus*), eight of eleven decapods (*Penaeus monodon*, *Panulirus japonicus*, *Portunus trituberculatus*, *Callinectes sapidus*, *Marsupenaeus japonicus*, *Pseudocarcinus gigas*, *Macrobrachium rosenbergii* and *Geothelphusa dehaani*), five stomatopod (*Squilla mantis*, *Harpiosquilla harpax*, *Squilla empusa, Lysiosquillina maculata* and *Gonodactylus chiragra*), two of three cirripeds (*Tetraclita japonica* and *Pollicipes polymerus*), one cephalocarid (*Hutchinsoniella macracantha*) and one pentastomid (*Armillifer armillatus*). Hence, it is quite conspicuous that the mtDNAs from the four copepods all have different gene organizations (see figure 2 in Paper I). One feature that is shared between *L. salmonis*, *T. japonicus* and *N. cristatus*, but not *E. bungii*, is the separation of *ND4* and *ND4L* by several genes (see figure 2 in Paper I). In vertebrates, *ND4* and *ND4L* are localized together due to transcription of one bicistronic mRNA (Wolstenholme 1992b). This is probably also the general rule for the crustaceans, with exception of the three copepods and one cirriped, *Megabalanus volcano* (Paper I; Begum et al. 2004, Lavrov et al. 2004, Yamauchi et al. 2004, Miller et al. 2005, Segawa & Aotsuka 2005, Sun et al. 2005, Miller et al. Unpublished, Swinstrom et al. Unpublished). Furthermore, *A6 and A8* are also transcribed as one bicistronic mRNA among higher invertebrates, as *A6* has an internal start codon within *A8* (Wolstenholme 1992b, Hickerson & Cunningham 2000). Overlap of *A6* and *A8* are found in all crustacean mtDNAs characterized, with *L. salmonis* being the only exception (Paper I; Begum et al. 2004, Lavrov et al. 2004, Yamauchi et al. 2004, Miller et al. 2005, Segawa & Aotsuka 2005, Sun et al. 2005, Miller et al. Unpublished, Swinstrom et al. Unpublished).

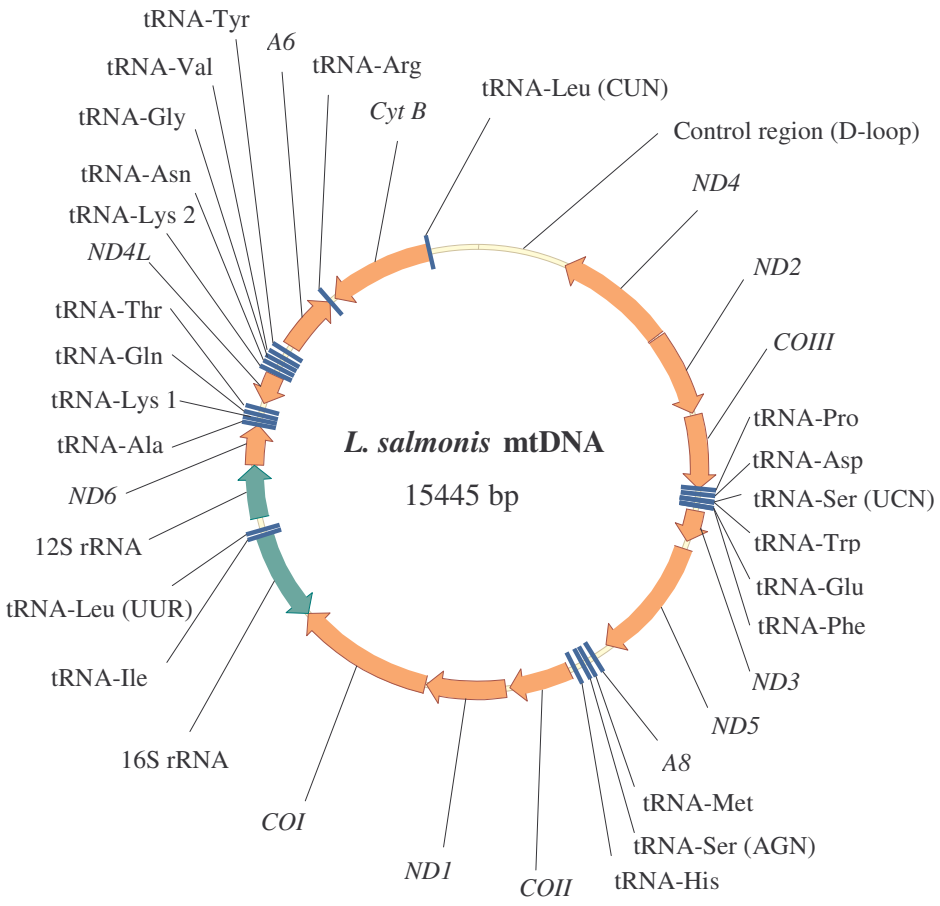

Figure 7: The mitochondrial genome of *Lepeophtheirus salmonis* contains 13 protein-coding genes, two rRNA genes and 22 tRNA genes in addition to a control region (AY625897). Two copies of tRNA-Lys are present, while tRNA-Cys was not identified. Both DNA strands contain coding regions, but very few genes overlap.

At present, it is not clear which mechanisms are responsible for the deviating gene organization seen in *L. salmonis* compared to the other crustaceans (see figure 2 in Paper I; Begum et al. 2004, Lavrov et al. 2004, Yamauchi et al. 2004, Miller et al. 2005, Segawa & Aotsuka 2005, Sun et al. 2005, Miller et al. Unpublished, Swinstrom et al. Unpublished). Three mechanisms have, however, been proposed for mitochondrial gene rearrangements 1) the duplication-random loss mechanism (e.g. Moritz et al. 1987, Boore & Brown 1998b, Boore 1999, Lavrov et al. 2002) 2) transposition of genes (e.g. Moritz et al. 1987, Boore &

Brown 1994, Macey et al. 1997, Boore & Brown 1998b, Groth et al. 2000, Saccone et al. 2002, Tomita et al. 2002) and 3) intramitochondrial recombination (e.g. Dowton & Campbell 2001, Machida et al. 2002, Miller et al. 2004). The duplication-random loss mechanism is the most accepted model to explain mitochondrial gene rearrangements (Moritz et al. 1987, Boore & Brown 1998b, Boore 1999, Lavrov et al. 2002). In this model, the gene duplication is either a result of errors in the replication or strand slippage and misparing, followed by a deletion of one gene copy from the genome. The deleted gene copy has then often been inactivated (e.g. Moritz et al. 1987, Stanton et al. 1994, Arndt & Smith 1998, Kumazawa et al. 1998, Boore & Brown 1998b, Lavrov et al. 2002). The second mechanism proposed for mitochondrial gene rearrangements involves transposition of genes that are flanked by two tRNAs (Saccone et al. 2002). The genes are then considered to be similar to a transposable element, with the two tRNAs corresponding to the long terminal repeats (LTRs) (Saccone et al. 1990). Three genes (*Cyt B*, *A6* and *ND4L*) are flanked by tRNAs in the *L. salmonis* mtDNA, and thus might be similar to transposable elements (see Saccone et al. 2002). Intramitochondrial recombination, as well as transposition, may lead to inversion of genes (see Machida et al. 2002, Miller et al. 2004). In *L. salmonis* mtDNA the two rRNA genes have inverted orientation, compared to each other (Figure 7). This phenomenon has not earlier been reported in any crustaceans, but has been found in five starfishes (Smith et al. 1989, Asakawa et al. 1995, Matsubara et al. 2005). Inversion of a fragment containing 16S rRNA has been suggested to result in the different transcriptional directions, of the two rRNA genes, seen in these cases. In conclusion, the ancestral caligid mtDNA organization is at the present time unknown, and it is therefore impossible to say which mechanisms that have resulted in the mitochondrial gene order observed in *L. salmonis*.

Two tRNA-Lys genes present in the L. salmonis *mtDNA*

Two copies of tRNA-Lys, possessing the same anticodon (UUU), are present in the mtDNA of *L. salmonis* (Paper I). A sequence comparison revealed that only 45% of the nucleotides were identical between the two *L. salmonis* tRNA-Lys sequences. Moreover, both sequences gave high tRNA scores, by the tRNAscan-SE program, and distinct secondary cloverleaf structures were proposed (Paper I). Considering this and the fact that the algorithm in the tRNAscan-SE program is far too strict to account for the structural diversity observed in mitochondrial tRNA genes, it reduces the possibility of one tRNA-Lys being a false positive (Paper I).

Generally, two tRNA-Leu (CUN or UUR) and two tRNA-Ser (AGN or UCN) genes are present in animal mitochondrial genomes (Boore 1999). However, within arthropods duplication of other tRNA genes have also been found (e.g. Hoffmann et al. 1992, Gissi & Pesole 2003). Dissimilar anticodons were observed in these cases, and it was therefore concluded that the use of different genetic codes were the reason for these tRNA duplications (Hoffmann et al. 1992, Gissi & Pesole 2003). In the demosponge *Axinella corrugata* (*A. corrugata*) two copies of tRNA-Ala have been described (Lavrov & Lang 2005). Both tRNAs possess the same anticodon (UGC), distinct secondary structures were predicted and the nucleotide sequences had only an identity of 52% (Lavrov & Lang 2005). This is analogous to the tRNA-Lys situation found in the *L. salmonis* mtDNA. In the study published by Lavrov and Lang (2005) a hypothesis called tRNA gene recruitment was introduced. This hypothesis was based on the result from a tRNA phylogeny indicating that one tRNA-Ala, in *A. corrugata*, originated from tRNA-Thr rather than from tRNA-Ala (Lavrov & Lang 2005). An explanaition involving duplication of tRNA-Thr followed by a mutation in the anticodon was implied (see also Higgs et al. 2003, Rawlings et al. 2003). Since mtDNAs from closely related caligid species have not been characterized, it is at present not possible to perform a tRNA

phylogeny to find the origin of the two tRNA-Lys genes in *L. salmonis*. A sequence comparison of all the tRNA genes in the *L. salmonis* mtDNA did, on the other hand, demonstrate that the tRNA- Lys 1 gene is most similar to tRNA-Tyr (50% identity), whereas tRNA-Lys 2 has highest similarity to tRNA-Met (62% identity). Still, the similarity is not particularly high, and no conclusion regarding tRNA gene recruitment can therefore be made from these results.

The duplication of tRNA-Lys in *L. salmonis* might also be explanied by a duplication-random loss mechanism (e.g. Moritz et al. 1987, Boore & Brown 1998b, Boore 1999, Lavrov et al. 2002). This implies that tRNA-Lys has been duplicated, but one gene copy has not yet been deleted from the genome. Moreover, three genes separate the tRNA-Lys copies in the *L. salmonis* mtDNA (Figure 7). It is therefore likely that a transposition of one tRNA-Lys gene must have followed the duplication event, if this is the expected scenario (Moritz et al. 1987, Boore & Brown 1994, Macey et al. 1997, Boore & Brown 1998b, Groth et al. 2000, Tomita et al. 2002). This speculation is based on the suggestion that tRNA genes could be considered as mobile elements, due to the frequently observed tRNA rearrangements seen in mitochondrial genomes (Moritz et al. 1987, Saccone et al. 2002). The fact that the two sequences are very divergent does, however, not support a tRNA-Lys duplication hypothesis. On the other hand, the duplication event could be ancient, and the initial sequence resemblance could therefore have been eroded by substitutions.

In marsupial the mitochondrial tRNA-Lys is not funtional, and a nuclear-encoded version is therefore imported into the mitochondria (Dörner et al. 2001). Several unusual features did, however, indicate that the mitochondrial tRNA-Lys gene found in marsupials was a pseudogene. An alignment of the mitochondrial tRNA-Lys gene sequences revealed that several marsupials do not possess the anticodon (UUU) for lysine-tRNA (Dörner et al. 2001). Furthermore, loss of several conserved nucleotides in the inferred tRNA secondary

structure was also observed. Although this situation is in marked contrast to that found in *L. salmonis* we cannot at present exclude the possibility that one tRNA-Lys gene might not be functional in *L. salmonis*.

Population structure of L. salmonis

Our study on the population genetic structure of *L. salmonis* revealed an extremely high level of intraspecific variation in the four mitochondrial genes *A6, COI, Cyt B* and 16S rRNA (Paper II). Despite this, no genetic differentiation was observed between *L. salmonis* sampled along the Norwegian coast, or between *L. salmonis* from Norway, Scotland and Russia (Paper II). Passive transport of *L. salmonis* larvae by ocean currents and the migratory pattern of the salmonid hosts are the two factors assumed to contribute to the high gene flow observed in the North Atlantic. A weak indication that *L. salmonis* sampled in Canada might be different from *L. salmonis* in the northeast Atlantic was, however, observed when the sequences were pairwise compared (Paper II). Furthermore, *L. salmonis* from the Pacific Ocean (Japan) was clearly distinct from the six Atlantic samples (Paper II).

Data from previous studies have indicated that *L. salmonis* displays population genetic differentiation in both Norway (Isdal et al. 1997) and Scotland (Todd et al. 1997, Dixon et al. 2004). *Lepeophtheirus salmonis* sampled from Norway, Scotland and Ireland have also been demonstrated to be genetically different (Nolan et al. 2000). Phenotypic plasticity where enzymes are differently expressed during different life-stages, or as a consequence of environmental factors may perhaps explain the results obtained by Isdal et al. (1997). Moreover, the possibility for contamination from the epidermal host mucus, host blood, bacterias or from epibionts on *L. salmonis* must be regarded as high in the RAPD studies which differentiated Scottish samples (Todd et al. 1997, Dixon et al. 2004). Contamination of *L. salmonis* samples with foreign DNA certainly would influence on the resulting analysis,

and this could also explain why the RAPD results published by Todd et al. (1997) have not been possible to reproduce (Dixon et al. 2004). One of the two informative microsatellites published by Nolan et al. (2000) has also been used in a more extensive population genetic study, including six microsatellite markers (Todd et al. 2004). The results from this study contradict the existence of distinct *L. salmonis* populations in the North Atlantic (Todd et al. 2004), results that are also supported by our findings using mitochondrial genes (Paper II).

Data from the present study suggests that a high level of genetic exchange exist between *L. salmonis* samples along the Norwegian coast (Paper II). This supports the deduction commonly made for marine invertebrates in that the inclusion of a planktonic larva in the life cycle confers dispersal potential, and extensive gene flow between samples resulting in genetic homogeneity (e.g. Johnson & Black 1982, Hunt & Ayre 1989, Liu et al. 1991, Hunt 1993, Williams & Benzie 1993, Silberman et al. 1994, Ayre 1995). *Lepeophtheirus salmonis* has three free-living stages in its life cycle (Schram 1993), and the copepodids can survive up to 23 days at 8°C and 30 days at 6°C (Boxaspen 2005). Furthermore, both the nauplia and copepodids are presumed to be centered in the upper meters of the water column (Heuch et al. 1995, Costelloe et al. 1996), and the *L. salmonis* larvae are therefore expected to be dispersed over considerable distances due to passive transport by ocean currents (see Costelloe et al. 1996, Costelloe et al. 1998, Asplin et al. 1999, Bucklin et al. 2000, Pedersen et al. 2001, Tully & Nolan 2002). Two streams are dominating along the Norwegian coast, the Norwegian Atlantic Current and the Norwegian Coastal Current (Figure 8). The Norwegian Atlantic Current has one branch that deflects and enters through the Faroe-Shetland channel encountering the Norwegian Coastal Current, while another branch deflects west of Møre going southwards (Figure 8) (Poulain et al. 1996, Fosså 2001, Pedersen et al. 2001). The Norwegian Coastal Current, on the other hand, originates

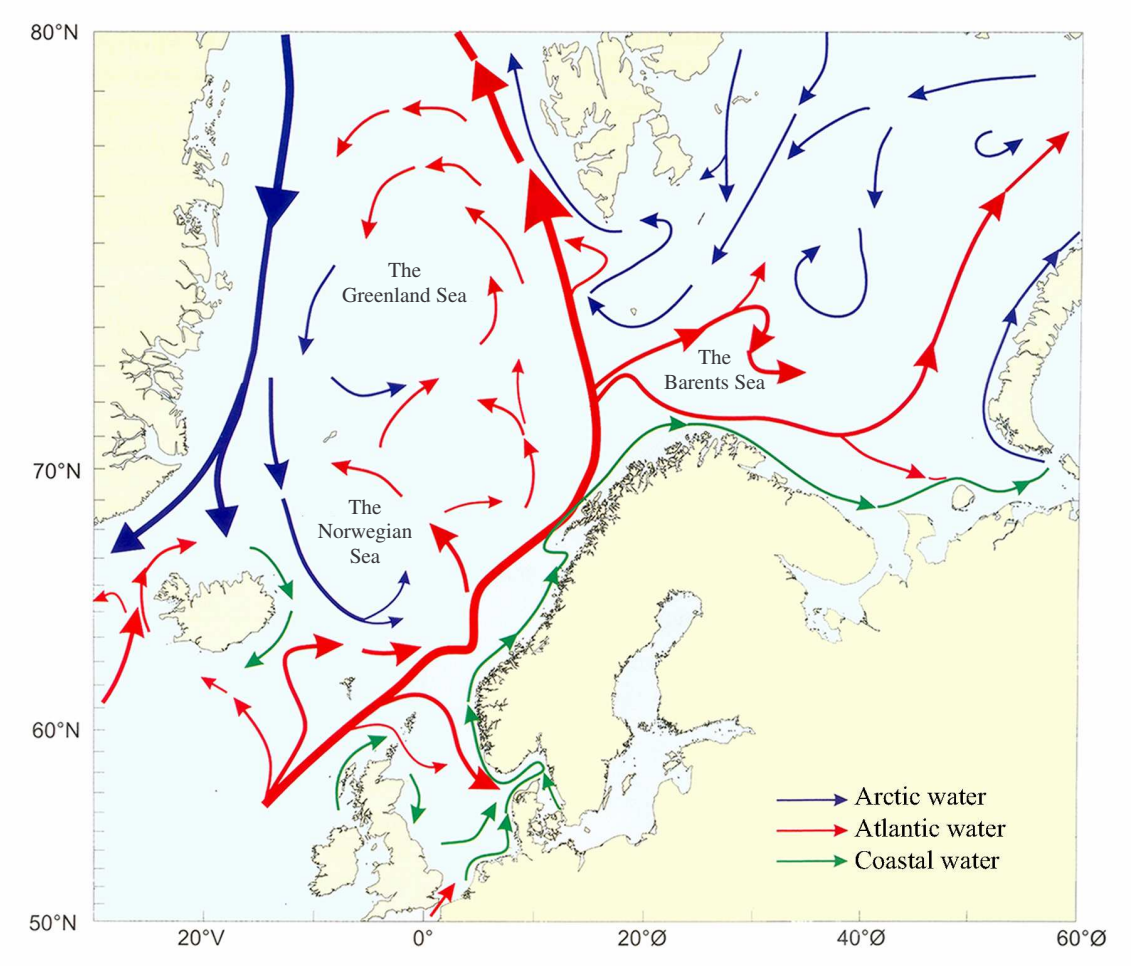

Figure 8: The mean current system of the Norwegian Sea, Greenland Sea and Barents Sea (Fosså 2001).

from the Baltic Sea transporting water from Skagerrak northward along the Norwegian coast (Figure 8) (Pedersen et al. 2001, Asplin et al. 2002). It is characterized as a stream being very variable in direction, resulting in a high exchange of water in the fjords (Asplin et al. 2002). Consequently, *L. salmonis* larvae from different fjords are likely to be mixed, and an accumulation of larvae may be achieved by the many gyres resulting from this stream (see Kaartvedt 1993). The Norwegian Coastal Current is also characterized as a very strong current. However, the speed varies extensively, depending on the weather conditions, but can range from 20 cm/s to 1 m/s (Pers. com. Lars Asplin, Institute of Marine Research, Bergen, Norway). In conclusion, the three free-living stages of the *L. salmonis* can last 3-4 weeks making it reasonable to assume that passive transport of nauplius and copepodid larva by the

Norwegian Coastal Current, and the Norwegian Atlantic Current, contributes greatly to the high gene flow observed between *L. salmonis* samples along the Norwegian coast.

Another aspect of *L. salmonis* dispersion to be considered is the contribution from wild salmonids, including escaped farm salmon. Wild salmon are assumed to contribute extensively to the spreading of *L. salmonis* throughout the North Atlantic (Todd et al. 2004). In Norway, *L. salmonis* infestations of wild salmon occur when the post-smolts migrate through the fjords and coastal areas on their way to the feeding grounds (Finstad et al. 2000, Todd et al. 2000). In contrast to this, the migratory pattern of escaped farm salmon is poorly known. Studies indicate that many return to their release location, but a large number (20-40%) also migrate to the oceanic feeding grounds (Hansen et al. 1987, Hansen et al. 1993, Jacobsen & Gaard 1997, Butler 2002). However, prior to seaward migration escaped farm salmon may spend a considerable amount of time in coastal waters, which can result in heavy infestations with *L. salmonis* (Jacobsen & Gaard 1997, Heuch & Mo 2001, Butler 2002). This further leads to the assumption that escaped farm salmon transfer increasing numbers of *L. salmonis* to wild salmon in the open seas (see Jacobsen & Gaard 1997), since high burdens of lice have been found on salmon in this area (Holst et al. 1993, Jacobsen & Gaard 1997). Salmon from most countries bordering the North Atlantic utilize the area north of the Faroe Islands during their oceanic feeding phase (Jacobsen & Gaard 1997). A study examining the distribution, migratory pattern and origin of wild salmon caught outside the Faroes reported that 40% of the recaptured salmon were of Norwegian origin, while 20% originated from both Scotland and Russia (Hansen & Jacobsen 2003). Salmon from Canada were also caught in this same feeding area (Hansen & Jacobsen 2003). This suggests an explanatory mechanism for the overall genetic similarity found, in our study, between *L. salmonis* sampled from Norway, Scotland, Russia and Canada (Paper II). The presence of both chalimus and pre-adult stages of *L. salmonis* on wild salmon throughout the winter months, and the increasing

abundance and density of *L. salmonis* with the sea age of the wild salmon indicate that infestation occurs at the feeding ground (Jacobsen & Gaard 1997). Furthermore, salmon returning from the sea in summer and autumn re-infect the coastline, and in this way contribute to the high gene flow observed between *L. salmonis* samples throughout the North Atlantic (Paper II).

Sea trout spend from 1 to 5 years in freshwater before migrating to coastal waters to feed. Once in sea, the trout are largely coastal in their habitat, but some fish have the ability to migrate over variable distances (Pemberton 1976, Butler 2002). For instance, in Scotland tagged sea trout have been recaptured 126 km from their starting point (Butler 2002). Hence, the possibility that infected sea trout migrating at large distances can contribute to the high gene flow observed between *L. salmonis* samples in the North Atlantic cannot be excluded.

Although the overall result show that there is no differentiation between *L. salmonis* samples throughout the North Atlantic a pairwise sequence comparison indicated that a weak, but significant, differentiation might exist between the European samples and *L. salmonis* sampled from the east-coast of Canada (Paper II). These results contrast with the results published by Todd et al. (2004) based on data from six microsatellite markers. It is, however, expected when applying markers with a high mutation rate, like the mtDNA, that some individual low frequency alleles might appear (see Neigel 1997), and this may explain why a weak difference was observed between *L. salmonis* collected from Canada and several locations in Europe (Paper II). This explanation is supported by the insignificant values displayed when the analysis was performed on haplotypes based only upon the most frequent polymorphisms (Paper II).

Despite the genetic similarity, it is clear that the mitochondrial genes used in this study can detect barriers to gene flow where they exist (Paper II). *Lepeophtheirus salmonis* sampled from wild chum salmon (*Oncorhynchus keta*) in the Pacific Ocean (Ishikari, Japan) was

clearly distinct from the Norwegian, Scottish, Russian and Canadian samples, when all four mitochondrial genes were aligned (Paper II). This suggests geographic isolation or strongly reduced gene flow between *L. salmonis* in the Pacific Ocean and the North Atlantic, as expected, but a possible adaptation to the host *Oncorhynchus* could also partly explain this (Paper II). Wild salmonids found in the North Atlantic are in the genera *Salmo* and *Salvelinus*, while the species present in the Pacific Ocean is mostly within the genera *Oncorhynchus* (e.g. Nagasawa & Takami 1993, Nagasawa 2004)

High intraspecific variation in* L. salmonis *mitochondrial genes

The four mitochondrial genes *A6, COI, Cyt B* and 16S rRNA of *L. salmonis* contained extremely high levels of genetic variation (Paper II). Highest intraspecific variation was found within *A6* where 17.5% of the nucleotides were polymorphic followed by 15.9% in *COI*, 14.4% in *Cyt B* and 10.6% in 16S rRNA. This high variation is most likely a consequence of a large *L. salmonis* population size caused by the higher accessibility of hosts, introduced by the salmon farming industry (Paper II). A large population size will result in a higher amount of low frequency haplotypes being present within the sample, and thereby lead to a higher genetic variation. When we compare the intraspecific variation within each of the six locations in the North Atlantic, large differences are observed among them. In *A6* the variation ranged from 5.6-7.1%, in *COI* from 5.2-6.8%, in *Cyt B* from 3.6-5.8% and in 16S rRNA from 2.9-4.6%. However, a comparison of the North Atlantic samples with *L. salmonis* from wild chum salmon (*Oncorhynchus keta*) caught in the Pacific Ocean revealed an intraspecific variation that seems to be noticeably lower (2.5% in *A6*, 1.4% in *COI*, 4.9% in *Cyt B* and 0.9% in 16S rRNA). This support the assumption that the high intraspecific variation found in the North Atlantic is due to a large *L. salmonis* population size. Although *L. salmonis* occurs on farmed coho salmon (*Oncorhynchus kisutch*) and rainbow trout

(*Oncorhynchus mykiss*) in Japan, it is not a serious problem for the farming industry (Nagasawa 2004). This is partly explained by the fact that these salmonids seem less susceptible to *L. salmonis* infestations (Nagasawa 2001, 2004). Still, *L. salmonis* is a common parasite of wild chum and pink salmon, and it is believed that these are the most important hosts for *L. salmonis* in the Pacific Ocean (Nagasawa 2001, 2004). The abundance of the wild salmonids varies, however, greatly from year to year resulting in a large influence on the *L. salmonis* population in this region (Nagasawa 2001).

On the other hand, it cannot be excluded that the high level of intraspecific variation observed within the four mitochondrial genes of *L. salmonis* could also be a consequence of negative selection (Paper II). That is, the high level of low frequency haplotypes present within the *L. salmonis* samples could be a result of an accumulation of slightly deleterious polymorphisms (Fry 1999, Blier et al. 2001, Fay et al. 2001). The use of delousing pesticides, in the combat against *L. salmonis*, may result in an artificial selection where negative, rather than positive traits, could be selected for.

Phylogenetic studies of L. salmonis

The expansion regions within 28S rRNA are highly variable, and sequence variations could therefore be present within this gene from *L. salmonis*. This was, however, not the case. Amplification of 3692 bp of 28S rRNA from *L. salmonis* sampled in Norway and Japan did not reveal any sequence variation, the same as found using 18S rRNA (Hodneland et al. unpublished). Furthermore, characterization of the NTS region, by screening a *L. salmonis* genomic library, was unsuccesful.

The 28S rRNA was used in a phylogenetic study to find the position of *L. salmonis* in relation to other crustacean, and arthropod, species (Paper III). Since 18S rRNA is often used to analyse arthropod relationships (Martin & Davis 2001) two phylogenies based on this gene

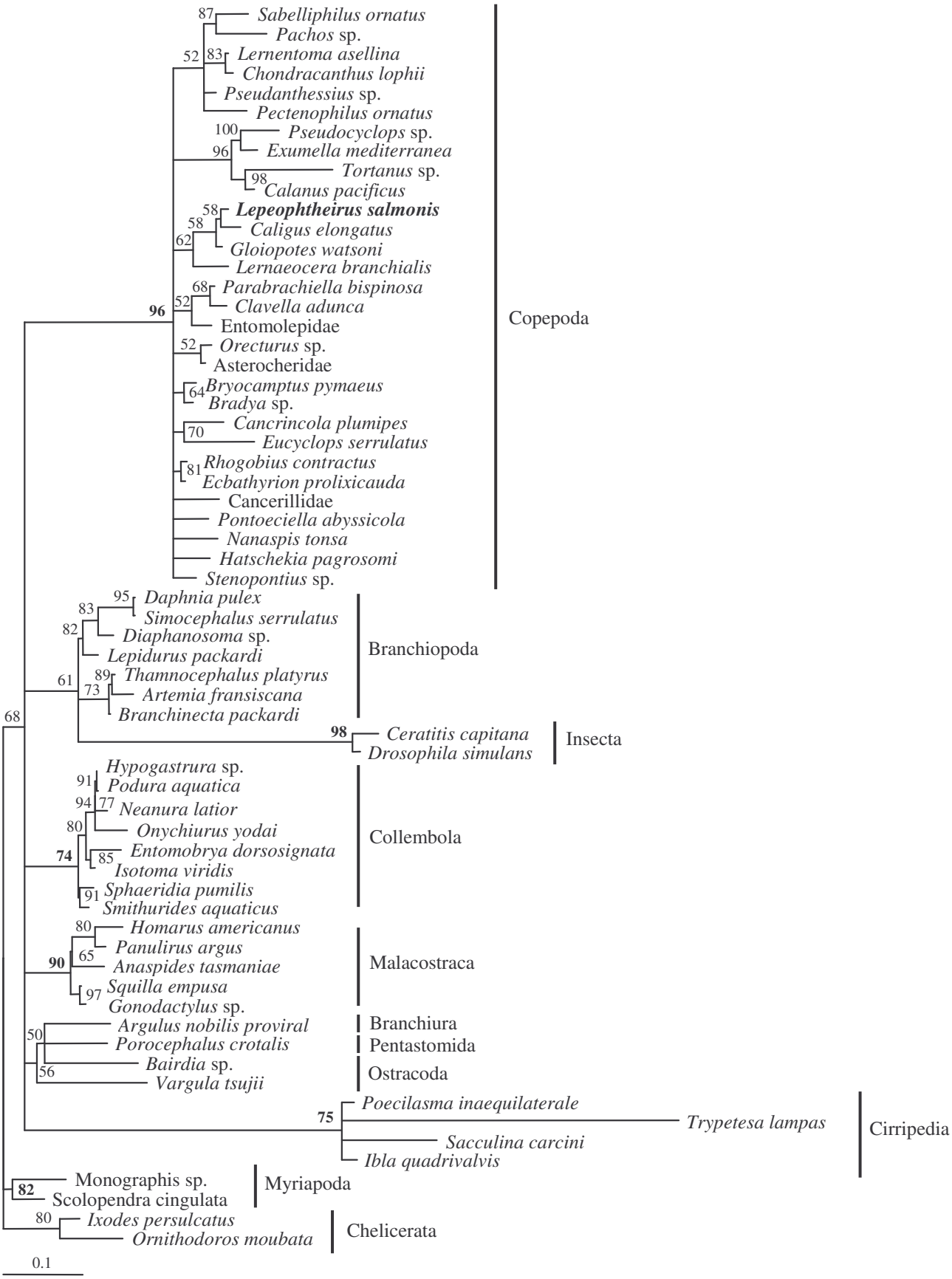

Figure 9: Arthropod phylogeny based on 18S rRNA, with Chelicerata as outgroup. The maximum-likelihood tree was constructed in Tree-Puzzle 5.2 using HKY with gamma distribution, and 2500 puzzling steps (values presented above branches). The Copepoda form a highly supported monophyletic group, but their relationship to other arthropods included is not resolved. Other supported groups are the Cirripedia, Malacostraca, Collembola, Insecta and Myriapoda.

were performed to compliment the 28S rRNA phylogeny. Moreover, *COI* has been classified as a reliable marker in resolving distant phylogenetic relationships among vertebrates (Zardoya & Meyer 1996), and a *COI* phylogeny was thus also performed. The 18S rRNA phylogeny included 64 species representative of the four arthropod groups (see Appendix, Table 2), but the phylogeny is not well resolved (Figure 9). Despite this poor resolution it is clearly demonstrated that the copepods included constitute a highly supported monophyletic group with a quartet puzzle (QP) bootstrap value of 96. In addition to this, several other groups are also supported (QP>70%). These include Cirripedia (QP bootstrap=75), Malacostraca (QP bootstrap=90), Collembola (QP bootstrap=74), Insecta (QP bootstrap=98) and Myriapoda (QP bootstrap=82). The position of the copepods in relation to the other arthropods included is, however, not resolved.

To better resolve the relationships within the Copepoda a new 18S rRNA phylogeny was performed (Figure 10) where only members from the Copepoda are included. This phylogeny place all the caligids in one highly supported group (QP bootstrap=84). *Lepeophtheirus salmonis* group together with *C. elongatus* (QP bootstrap=90), with *G. watsoni* as a sister-group to them. However, the relationships within the Siphonostomatoida are not resolved, as is also the case for Cyclopoida, Harpacticoida and Poecilostomatoida. On the other hand, the Calanoida form a group with high support (QP bootstrap=85).

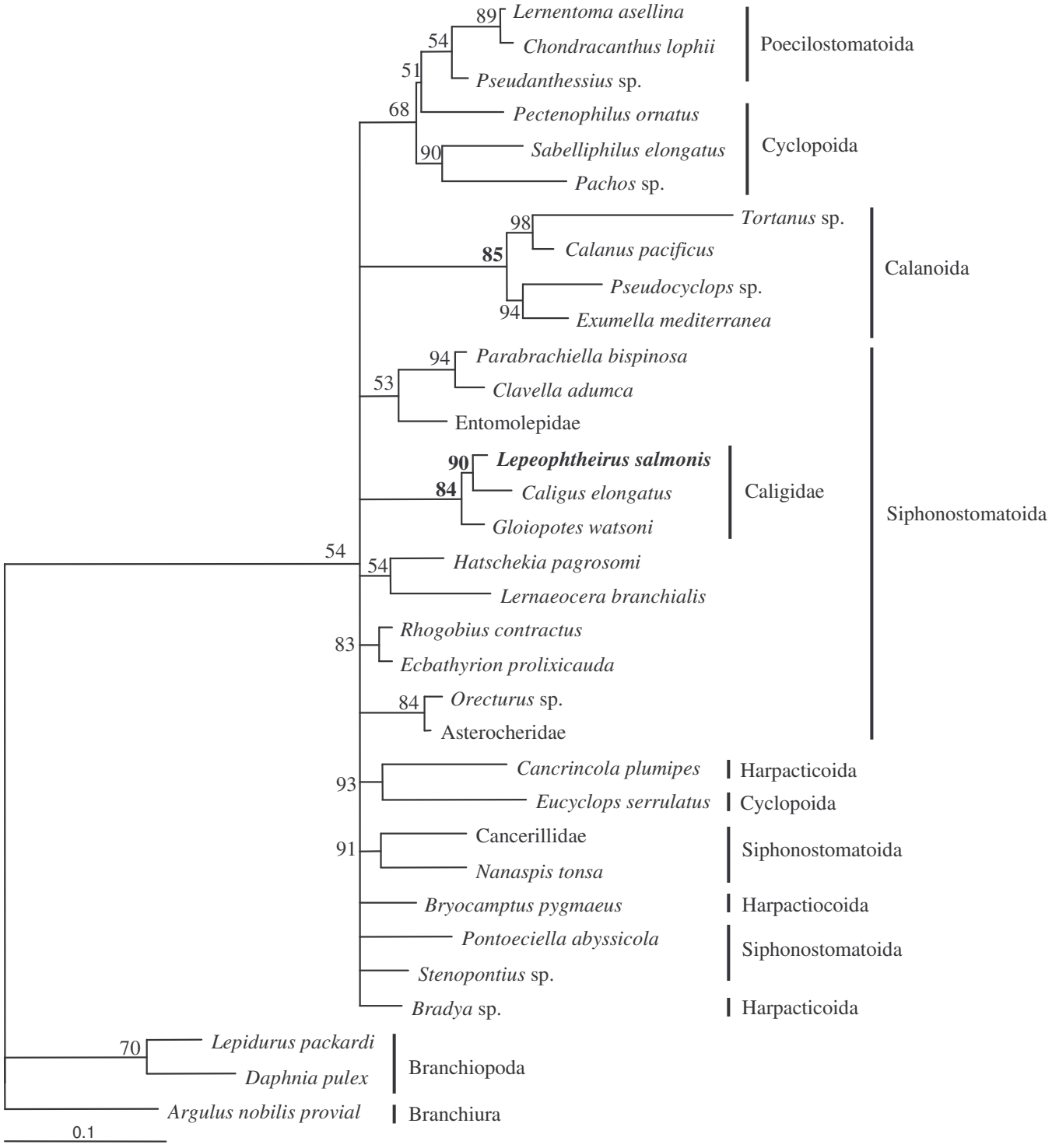

Figure 10: Copepod relationships inferred from 18S rRNA, with Branchiura and two branchiopods as outgroups. The maximum-likelihood tree was constructed in Tree-Puzzle 5.2 using the HKY model with gamma distribution, and 10 000 puzzling steps (values presented above branches). The tree is not well resolved, but the three caligids are placed in one distinct group. Moreover, the Calanoida constitute a highly supported group.

Low resolution have also been the result of previous phylogenetic studies using 18S rRNA (e.g. Turbeville et al. 1991, Abele et al. 1992, Spears & Abele 1997, Mallatt et al. 2004). Within Crustacea a phylogenetic analysis of 18S rRNA from selected maxillopodan suggested that the copepods are more closely related to the cirripeds than previously thought (Abele et al. 1992). However, the data set were analyzed in different ways, and only one of the four trees supported this relationship (Abele et al. 1992). Another phylogenetic study using 18S rRNA made it impossible to speculate in maxillopodan, crustacean or arthropod relationships due to low resolution (Spears & Abele 1997). Still, species from the same crustacean taxa grouped together with high support, as was also the case in this study (see figure 9). Consequently, the phylogenetic performance of the rRNA genes has been questioned (e.g. Spears & Abele 1997, Giribet & Ribera 2000). On the other hand, it has often been concluded within arthropod phylogeny, based on rDNA sequences, that inclusion of more taxa or more data per taxon might result in improved resolution (Spears & Abele 1997). This was, however, not the case in our analysis where inclusion of a substantial number of arthropod species in the 18S rRNA phylogeny did not improve the resolution (Figure 9). In comparison the copepod and arthropod phylogenies based on 28S rRNA were much better resolved (Paper III). Both 28S rRNA phylogenies place *L. pollachius* as the closest relative to *L. salmonis*. *Caligus elongatus* and *C. curtus* constitute a sister-group to the two *Lepeophtheirus* species, forming a monophyly of the Caligidae. Siphonostomatoida is also supported using 28S rRNA, with the Poecilostomatoida being the closest relative of the taxa represented followed by Cyclopoida. The Copepoda form a monophyletic group, with the Hexapoda as their closest relative. This phylogeny also supports the morphological copepod phylogeny proposed by Huys and Boxshall (1991) (Figure 3) in placing the Cyclopoida as a sister-group to Siphonostomatoida and Poecilostomatoida. In contrast, the copepod phylogeny proposed by Kabata (1979) is not supported. They placed the Siphonostomatoida as the most

acient group, which constituted a sister-group to the Poecilostomatoida-Cyclopoida clade (Figure 2).

COI did not give any phylogenetic resolution for the arthropods included in this study (see Appendix, Table 3). This applied to phylogenies based on both nucleotide and amino acid sequences (data not presented), although the latter did group the copepods as monophyletic with a support of 82%.

COI also resulted in relatively low-resolution phylogeny when only the copepods were included in the analysis (Figure 11). However, the caligids are placed in one group with high support (QP bootstrap=97), where *L. salmonis* group together with *L. pectoralis* (QP bootstrap=92). Furthermore, the three *Caligus* species (*C. curtus*, *C. centrodonti* and *C. elongatus*) constitute a sister-group to *Lepeophtheirus* spp. The closest relative to the Caligidae is the Harpacticoid species, *Cletocamptus helobius*, but the support for this relationship low (QP bootstrap=65). Two other harpacticoids is placed as outgroup to the Calanoida, also with low support (QP bootstrap=57), resulting in a polyphyletic Harpacticoida. Still, Calanoida forms a group with high support (QP bootstrap=91). Due to low support for many of the branches, it is not possible to compare this analysis with the copepod relationships proposed by Kabata (1979) (Figure 2), or Huys and Boxshall (1991) (Figure 3). The caligid phylogeny proposed by Øines et al (2005), based on *COI*, is not well supported, and many of the species were not identified. Hence, a comparison with this study is of little value.

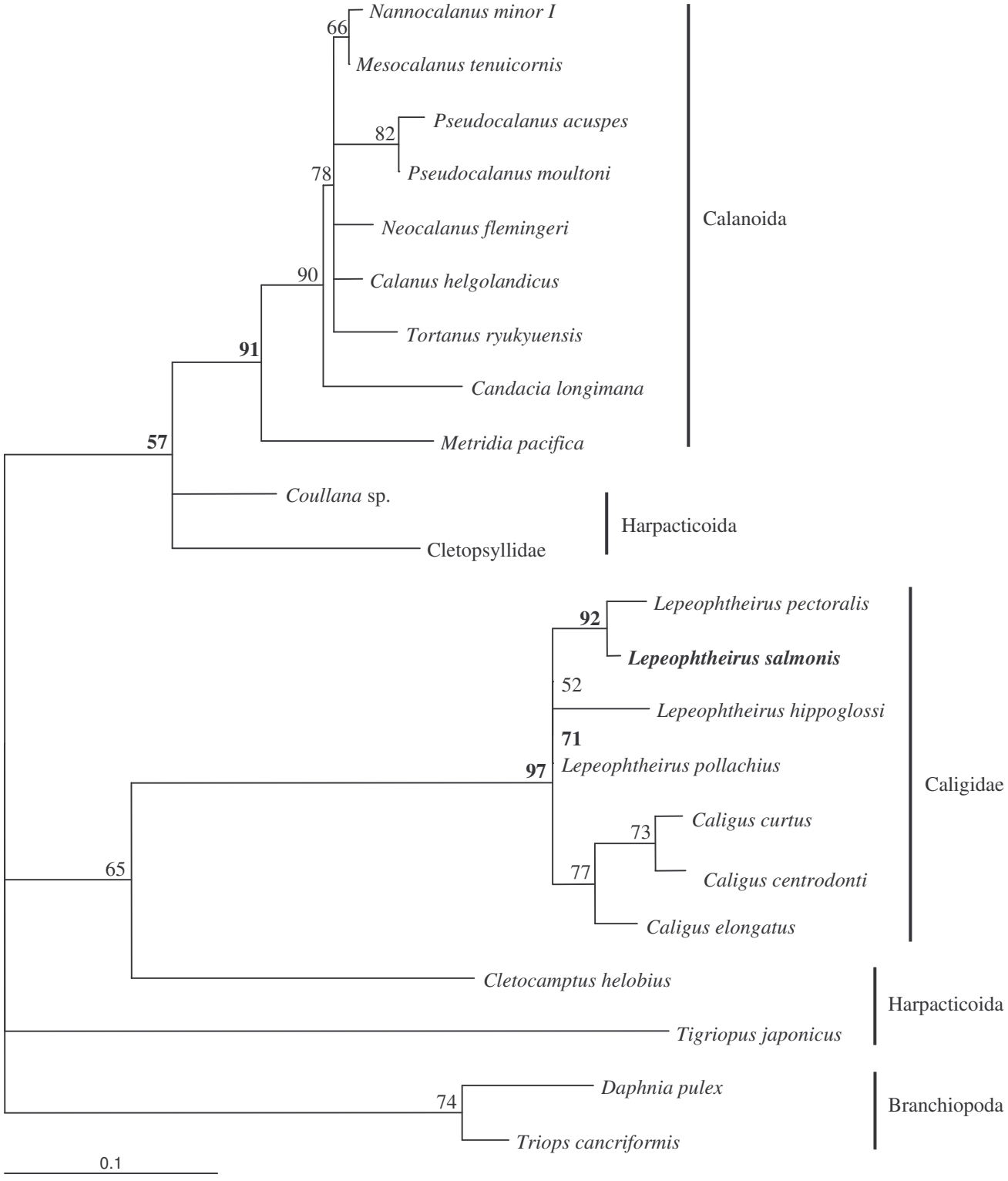

Figure 11: The relationships within Copepoda inferred from the amino acid sequences of *COI* with two branchiopods as outgroup. The maximum-likelihood tree was constructed in Tree-Puzzle 5.2 using the HKY model with gamma distribution, and 1000 puzzling steps (values presented above branches). The phylogeny groups *L. salmonis* together with *L. pectoralis*, being close relatives to *Caligus* spp.

For more than a century, phylogenetic analyses of crustaceans have shown a high degree of diversity. It has therefore been implied that crustacean phylogeny can only be sensibly discussed in the framework of arthropod relationships (Giribet et al. 2005). However, only a few of the molecular phylogenies that deal with arthropods use a broad sample of crustacean taxa (Giribet et al. 2001, Regier & Shultz 2001, Nardi et al. 2003, Bitsch et al. 2004, Lavrov et al. 2004, Mallatt et al. 2004, Regier et al. 2004, Giribet et al. 2005, Regier et al. 2005). Within Copepoda very few molecular systematic studies have been published (Bucklin et al. 1992, Bucklin et al. 1995, Braga et al. 1999, Bucklin et al. 1999, Hill et al. 2001, Bucklin et al. 2003, Øines & Heuch 2005), and the position of copepods in relation to other crustaceans have only been inferred in a few cases (Abele et al. 1992, Spears & Abele 1997). Furthermore, the molecular phylogenies based on 18S rRNA (Figure 9 and 10) and *COI* (Figure 11) presented in this thesis, in addition to 28S rRNA (Paper III), showed quite different results. Although *COI* is considered to be a very conserved protein, and has been classified as a good phylogenetic marker for resolving distant vertebrate relationships (Zardoya & Meyer 1996), a high intraspecific variation resulting in several amino acid substitutions was found within this gene in *L. salmonis* (Paper II). It was therefore not unexpected that the phylogenies based on *COI* resulted in low resolution, since this gene seems to be too polymorphic to resolve deeper copepod relationships (Figure 11). While the analysis performed on 18S rRNA was not able to resolve the relationships within Copepoda or copepods relationship to other crustaceans (Figure 9 and 10), the analysis of 28S rRNA resulted in a better resolution (Paper III). This is in agreement with a previous phylogenetic study where the performance of 18S and 28S rRNA was compared, and higher resolution was achieved using 28S rRNA due to the contribution of more phylogenetic signal (Mallat et al 2004). The copepod phylogeny based on 28S rRNA (Paper III) also supported the morphological phylogeny proposed by Huys and Boxshall (1991). However, the support for

Cyclopoida is low, and more taxa must be included to better infer the relationships within the Copepoda.

CONCLUSION

The main goal of this project was to characterize both mitochondrial and nuclear genes, and use them to study the population genetic structure of *L. salmonis* throughout the North Atlantic. Whereas the nuclear 28S rRNA gene was not suited for detecting any differentiation between *L. salmonis* samples, the four mitochondrial genes *A6, COI, Cyt B* and 16S rRNA clearly differentiated between *L. salmonis* sampled in the North Atlantic and the Pacific Ocean, as expected. No genetic differentiation was, however, demonstrated between *L. salmonis* sampled throughout the North Atlantic (Norway, Scotland, Russia and Canada). The migratory pattern of the salmonid hosts and passive transport of *L. salmonis* larvae by ocean currents are believed to contribute to this high gene flow. These results have implications for the salmon farming industry in different ways. In order to use pesticides in the combat against *L. salmonis*, delousing must be synchronised over a broader geographic area than what is currently done. The most important implication is, however, that the potential for spreading of genes associated with possible resistance is extensive, considering that *L. salmonis* can be dispersed over large geographic distances.

FUTURE PERSPECTIVES

Based on the results presented in this thesis, several aspects warrant further investigation:

- Characterization of the *L. salmonis* mitochondrial genome revealed a completely new gene organization within Crustacea (Paper I). It is currently difficult to specify which mechanisms are responsible for this novel gene order, but characterization of more mitochondrial genomes from closely related caligid species may provide some answers.
- Two tRNA-Lys genes are present within the mitochondrial genome of *L. salmonis* (Paper I). One way of investigating whether the tRNA-Lys duplication is caused by duplication of tRNA-Lys itself, or if it is a result of tRNA gene recruitment, would be to perform a phylogentic comparison of mitochondrial tRNAs from several closely related caligid species. Knowledge about the origin of the two tRNA-Lys genes found in *L. salmonis* may give insight into the mechanisms resulting in the frequently observed mitochondrial tRNA rearrangements. Furthermore, it will also give support to previously proposed mechanisms involved in tRNA evolution.
- In this study, weak genetic differentiation was observed between the European and the Canadian *L. salmonis* samples (Paper II). This indication contrast with the recent data published by Todd et al. (2004) using microsatellites. It would therefore be interesting to use their microsatellite markers on our *L. salmonis* samples, for an evaluation of these results. Markers associated with genes that are targets for development of resistance could perhaps reveal differentiation between *L. salmonis* samples. There are already some indications that a sodium channel gene, which is the target gene for pyrethroids, might differentiate between North Atlantic *L. salmonis* samples (Pers. com Anders Fallang, Norwegian School of Veterinary Science, Oslo, Norway), and this approach should be tested.

- The 28S rRNA gene seems very promising for resolving copepod relationships (Paper III). Nevertheless, this needs to be verified by including more copepods representative of several orders, families and genera. Moreover, the mitochondrial gene order has been found to vary across the Metazoa, generating interest in using this for phylogenetic inference (see Boore et al. 1998a, Boore & Brown 1998b, Wilson et al. 2000, Lavrov et al. 2004). So far, different mitochondrial gene orders have been found within the four copepods characterized, or partially characterized. A phylogeny based on mitochondrial gene orders from different copepods could therefore be interesting to perform, for inferring copepod relationships, when more genomes have been sequenced.

REFERENCES

Abele LG, Spears T, Kim W, Applegate M (1992) Phylogeny of selected Maxillopodan and other crustacean taxa based on 18S ribosomal nucelotide-sequences - a preliminary-analysis. Acta Zool 73:373-382

Ahmad M, Arif MI, Ahmad Z (2003) Susceptibility of *Helicoverpa armigera* (Lepidoptera : Noctuidae) to new chemistries in Pakistan. Crop Prot 22:539-544

Altukhov YP, Salmenkova EA (2002) DNA polymorphism in population genetics. Russ J Genet 38:989-1008

Arndt A, Smith MJ (1998) Mitochondrial gene rearrangement in the sea cucumber genus *Cucumaria*. Mol Biol Evol 15:1009-1016

Asakawa S, Himeno H, Miura K, Watanabe K (1995) Nucleotide sequence and gene organization of the starfish *Asterina pectinifera* mitochondrial genome. Genetics 140:1047-1060.

Asplin L, Boxaspen K, Sandvik AD (2002) Lakselus - en trussel for villaksen. Havets miljø, Fisken og havet særnr.2-2002:144-149

Asplin L, Salvanes AGV, Kristoffersen JB (1999) Nonlocal wind-driven fjord-coast advection and its potential effect on plankton and fish recruitment. Fish Oceanogr 8:255-263

Ayre DJ (1995) Localized adaptation of sea-anemone clones - evidence from transplantation over two spatial scales. J Animal Ecol 64:186-196

Babbitt CC, Patel NH (2002) Phylogenetic relationships within the Malacostraca (Crustacea) based on 18S and 28S rDNA sequences. Integr Comp Biol 42:1189-1189

Ballard JWO, Whitlock MC (2004) The incomplete natural history of mitochondria. Mol Ecol 13:729-744

Baratti M, Goti E, Messana G (2005) High level of genetic differentiation in the marine isopod *Sphaeroma terebrans* (Crustacea Isopoda Sphaeromatidae) as inferred by mitochondrial DNA analysis. J Exp Mar Biol Ecol 315:225-234

Begum RA, Tsuchida K, Yamaguchi T, Nishida M, Watabe S (2004) Complete mitochondrial genome of the sessile barnacle *Tetraclita japonica*. Mar Biotechnol in press

Berland B (1993) Salmon lice on wild salmon (*Salmo Salar*) in western Norway. In: Boxshall GA, Defaye D (eds) Pathogens of Wild and Farmed Fish: Sea lice. Ellis Horwood, Chichester, p 179-187

Birkeland K (1996) Consequences of premature return by sea trout (*Salmo trutta*) infested with the salmon louse (*Lepeophtheirus salmonis* Krøyer): Migration, growth, and mortality. Can J Fish Aquat Sci 53:2808-2813

Bitsch C, Bitsch J (2004) Phylogenetic relationships of basal hexapods among the mandibulate arthropods: a cladistic analysis based on comparative morphological characters. Zool Scr 33:511-550

Bitsch J, Bitsch C, Bourgoin T, D'Haese C (2004) The phylogenetic position of early hexapod lineages: morphological data contradict molecular data. Syst Entomol 29:433-440

Bjørn PA, Finstad B (2002) Salmon lice, *Lepeophtheirus salmonis* (Kroyer), infestation in sympatric populations of Arctic char, *Salvelinus alpinus* (L.), and sea trout, *Salmo trutta* (L.), in areas near and distant from salmon farms. ICES J Mar Sci 59:131-139

Bjørn PA, Finstad B, Kristoffersen R (2001) Salmon lice infection of wild sea trout and Arctic char in marine and freshwaters: the effects of salmon farms. Aquac Res 32:947-962

Blier PU, Dufresne F, Burton RS (2001) Natural selection and the evolution of mtDNA-encoded peptides: evidence for intergenomic co-adaptation. Trends Genet 17:400-406

Boore JL (1999) Animal mitochondrial genomes. Nucleic Acids Res 27:1767-1780

Boore JL, Brown WM (1994) Complete DNA-sequence of the mitochondrial genome of the black chiton, *Katharina tunicata*. Genetics 138:423-443

Boore JL, Brown WM (1998b) Big trees from little genomes: mitochondrial gene order as a phylogenetic tool. Curr Opin Genet Dev 8:668-674.

Boore JL, Lavrov DV, Brown WM (1998a) Gene translocation links insects and crustaceans. Nature 392:667-668

Boxaspen K (2005) Experimental infection success of salmon lice (*Lepeophtheirus salmonis*) on salmon (*Salmo salar* L.). The effect of temperature (6° to 14°C) and age of copepodids. ICES J Mar Sci In press

Boxaspen K, Næss T (2000) Development of eggs and the planktonic stages of salmon lice (*Lepeophtheirus salmonis*) at low temperatures. Contrib Zoo 69:51-55

Braga E, Zardoya R, Meyer A, Yen J (1999) Mitochondrial and nuclear rRNA based copepod phylogeny with emphasis on the *Euchaetidae* (Calanoida). Mar Biol 133:79-90

Brandal PO, Egidius E, Romslo I (1976) Host blood: A major food component for the parasitic copepod *Lepeophtheirus salmonis* Krøyeri, 1838 (Crustacea, Caligidae). Norw J Zool 24:341-343

Bucklin A, Frost BW, Bradford-Grieve J, Allen LD, Copley NJ (2003) Molecular systematic and phylogenetic assessment of 34 calanoid copepod species of the Calanidae and Clausocalanidae. Mar Biol 142:333-343

Bucklin A, Frost BW, Kocher TD (1992) DNA sequence variation on the mitochondrial 16S rRNA in *Calanus* (Copepoda; Calanoida): intraspecific and interspecific patterns. Mol Mar Biol Biotechnol 1:397-407

Bucklin A, Frost BW, Kocher TD (1995) Molecular systematics of six *Calanus* and three *Metrida* species (Calanoida: Copepoda). Mar Biol 121:655-664

Bucklin A, Guarnieri M, Hill RS, Bentley AM, Kaartvedt S (1999) Taxonomic and systematic assessment of planktonic copepods using mitochondrial COI sequence variation and competitive, species-specific PCR. Hydrobiologia 401:239-254

Bucklin A, Kaartvedt S, Guarnieri M, Goswami U (2000) Population genetics of drifting (*Calanus* spp.) and resident (*Acartia clausi*) plankton in Norwegian fjords. J Plankton Res 22:1237-1251

Bucklin A, Smolenack SB, Bentley AM, Wiebe PH (1997) Gene flow patterns of the euphausiid, *Meganyctiphanes norvegica*, in the NW Atlantic based on mtDNA sequences for cytochrome b and cytochrome oxidase I. J Plankton Res 19:1763-1781

Burgess IE (2004) Human lice and their control. Annu Rev Entomol 49:457-481

Butler JR (2002) Wild salmonids and sea louse infestations on the west coast of Scotland: sources of infection and implications for the management of marine salmon farms. Pest Manag Sci 58:595-608; discussion 622-629.

Clark CG, Tague BW, Ware VC, Gerbi SA (1984) *Xenopus-Laevis*-28S ribosomal-RNA - a secondary structure model and its evolutionary and functional implications. Nucleic Acids Res 12:6197-6220

Costelloe M, Costelloe J, Coghlan N, O'Donohoe G, O'Connor B (1998) Distribution of the larval stages of *Lepeophtheirus salmonis* in three bays on the west coast of Ireland. ICES J Mar Sci 55:181-187

Costelloe M, Costelloe J, Roche N (1996) Planktonic dispersion of larval salmon-lice, *Lepeophtheirus salmonis*, associated with cultured salmon, *Salmo salar*, in western Ireland. J Mar Biol Ass UK 76:141-149

Crandall KA, Harris DJ, Fetzner JW (2000) The monophyletic origin of freshwater crayfish estimated from nuclear and mitochondrial DNA sequences. Proc Roy Soc Lond B Bio Sci 267:1679-1686

Crease TJ (1999) The complete sequence of the mitochondrial genome of *Daphnia pulex* (Cladocera: Crustacea). Gene 233:89-99.

Cummins JM (2001) Mitochondria: potential roles in embryogenesis and nucleocytoplasmic transfer. Hum Reprod Update 7:217-228

Cunningham CO, Collins CM, Malmberg G, Mo TA (2003) Analysis of ribosomal RNA intergenic spacer (IGS) sequences in species and populations of *Gyrodactylus* (Platyhelminthes: Monogenea) from salmonid fish in northern Europe. Dis Aquat Organ 57:237-246

De Arruda MCC, Ferreira MASV, Miller RNG, Resende MLV, Felipe MSS (2003) Nuclear and mitochondrial rDNA variability in *Crinipellis perniciosa* from different geographic origins and hosts. Mycol Res 107:25-37

Denholm I, Devine GJ, Horsberg TE, Sevatdal S, Fallang A, Nolan DV, Powell R (2002) Analysis and management of resistance to chemotherapeutants in salmon lice, *Lepeophtheirus salmonis* (Copepoda: Caligidae). Pest Manag Sci 58:528-536.

Devine GJ, Denholm I, Horsberg TE (2000) Chemoterapeutant resistance in sea lice: what is it and what can be done about it? Caligus:12-14

Dixon BA, Shinn AP, Sommerville C (2004) Genetic characterization of populations of the ectoparasitic caligid, *Lepeophtheirus salmonis* (Krøyer 1837) using randomly amplified polymorphic DNA. Aquac Res 35:730-741

Dowton M, Campbell NJH (2001) Intramitochondrial recombination - is it why some mitochondrial genes sleep around? Trends Ecol Evol 16:269-271

Duan Y, Kerdelhue C, Ye H, Lieutier F (2004) Genetic study of the forest pest *Tomicus piniperda* (Col., Scolytinae) in Yunnan province (China) compared to Europe: new insights for the systematics and evolution of the genus *Tomicus*. Heredity 93:416-422

Dörner M, Altmann M, Paabo S, Morl M (2001) Evidence for import of a lysyl-tRNA into marsupial mitochondria. Mol Biol Cell 12:2688-2698

Evensen Ø, Breck O, Hjeltnes B, Nilsen F, Schrøder MB, Håstein T (2004) Helse-/sykdomsproblemer hos norske oppdrettsarter, Norges forskningsråd, Oslo

Fay JC, Wyckoff GJ, Wu CI (2001) Positive and negative selection on the human genome. Genetics 158:1227-1234

Ffrench-Constant RH, Daborn PJ, Le Goff G (2004) The genetics and genomics of insecticide resistance. Trends Genet 20:163-170

Finstad B, Bjørn PA, Grimnes A, Hvidsten NA (2000) Laboratory and field investigations of salmon lice (*Lepeophtheirus salmonis* Krøyer) infestation on Atlantic salmon (*Salmo salar* L.) post-smolts. Aquac Res 31:795-803

Finstad B, Johnsen BO, Hvidsten NA (1994) Prevalence and mean intensity of salmon lice *Lepeophtheirus salmonis* Krøyer infection on wild Atlantic salmon, *Salmo salar* L, postsmolts. Aquacult Fish Manag 27:761-764

Fosså JH (2001) Fisken og havet. Havets miljø, p 4-6

Friedrich M, Tautz D (1995) Ribosomal DNA phylogeny of the major extant arthropod classes and the evolution of myriapods. Nature 376:165-167.

Frost P, Nilsen F (2004) Lakselus-vaksineutvikling, Havforskningsinstituttet, Bergen

Fry AJ (1999) Mildly deleterious mutations in avian mitochondrial DNA: Evidence from neutrality tests. Evolution 53:1617-1620

Gantenbein B, Largiader CR (2003) The phylogeographic importance of the Strait of Gibraltar as a gene flow barrier in terrestrial arthropods: a case study with the scorpion *Buthus occitanus* as model organism. Mol Phylogenet Evol 28:119-130

Garcia-Machado E, Pempera M, Dennebouy N, Oliva-Suarez M, Mounolou JC, Monnerot M (1999) Mitochondrial genes collectively suggest the paraphyly of crustacea with respect to insecta. J Mol Evol 49:142-149

Giribet G, Edgecombe GD, Wheeler WC (2001) Arthropod phylogeny based on eight molecular loci and morphology. Nature 413:157-161

Giribet G, Ribera C (2000) A review of arthropod phylogeny: New data based on ribosomal DNA sequences and direct character optimization. Cladistics 16:204-231

Giribet G, Richter S, Edgecombe GD, Wheeler WC (2005) The position of crustaceans within Arthropoda-Evidence from nine molecular loci and morphology. In: Jenner SKR (ed) Crustacea and Arthropod Relationships. CRC publishers, p 307-352

Gissi C, Pesole G (2003) Transcript Mapping and Genome Annotation of Ascidian mtDNA Using EST Data. Genome Res. 13:2203-2212

Gopurenko D, Hughes JM (2002) Regional patterns of genetic structure among Australian populations of the mud crab, *Scylla serrata* (Crustacea: Decapoda): evidence from mitochondrial DNA. Mar Freshwater Res 53:849-857

Grave K, Horsberg TE, Lunestad BT, Litleskare I (2004) Consumption of drugs for sea lice infestations in Norwegian fish farms: methods for assessment of treatment patterns and treatment rate. Dis Aquat Organ 60:123-131

Grechko VV (2002) Molecular DNA markers in phylogeny and systematics. Russ J Genet 38:851-868

Groth C, Petersen RF, Piskur J (2000) Diversity in organization and the origin of gene orders in the mitochondrial DNA molecules of the genus *Saccharomyces*. Mol Biol Evol 17:1833-1841

Gupta PK, Sharma S, Kumar S, Balyan HS, Beharav A, Nevo E (2004) Adaptive ribosomal DNA polymorphism in wild barley at a mosaic microsite, Newe Ya'ar in Israel. Plant Sci 166:1555-1563

Hale LR, Singh RS (1987) Mitochondrial DNA variation and genetic structure in populations of *Drosophila melanogaster*. Mol Biol Evol 4:622-637

Hancock JM, Dover GA (1988) Molecular coevolution among cryptically simple expansion segments of eukaryotic 26S/28S rRNAs. Mol Biol Evol 5:377-391

Hancock JM, Tautz D, Dover GA (1988) Evolution of the secondary structures and compensatory mutations of the ribosomal RNAs of *Drosophila melanogaster*. Mol Biol Evol 5:393-414.

Hansen LP, Doving KB, Jonsson B (1987) Migration of farmed adult Atlantic salmon with and without olfactory sense, released on the Norwegian coast. J Fish Biol 30:713-721

Hansen LP, Jacobsen JA (2003) Origin and migration of wild and escaped farmed Atlantic salmon, *Salmo salar* L., in oceanic areas north of the Faroe Islands. ICES J Mar Sci 60:110-119

Hansen LP, Jacobsen JA, Lund RA (1993) High numbers of farmed Atlantic salmon, *Salmo Salar* L., observed in oceanic waters north of the Faroe Islands. Aquacult Fish Manag 24:777-781

Hassouna N, Michot B, Bachellerie JP (1984) The complete nucleotide-sequence of mouse 28S ribosomal-RNA gene- implications for the process of size increase of the large subunit ribosomal-RNA in higher eukaryotes. Nucleic Acids Res 12:3563-3583

Heuch PA, Mo TA (2001) A model of salmon louse production in Norway: effects of increasing salmon production and public management measures. Dis Aquat Organ 45:145-152.

Heuch PA, Parsons A, Boxaspen K (1995) Diel vertical migration -a possible host-finding mechanism in salmon louse (*Lepeophtheirus salmonis*) copepodids. Can J Fish Aquat Sci 52:681-689

Hickerson MJ, Cunningham CW (2000) Dramatic mitochondrial gene rearrangements in the hermit crab *Pagurus longicarpus* (Crustacea, Anomura). Mol Biol Evol 17:639-644

Higgs PG, Jameson D, Jow H, Rattray M (2003) The evolution of tRNA-Leu genes in animal mitochondrial genomes. J Mol Evol 57:435-445

Hill RS, Allen LD, Bucklin A (2001) Multiplexed species-specific PCR protocol to discriminate four N. Atlantic *Calanus* species, with an mtCOI gene tree for ten *Calanus* species. Mar Biol 139:279-287

Hillis DM, Dixon MT (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. Q Rev Biol 66:411-453.
Hoffmann RJ, Boore JL, Brown WM (1992) A novel mitochondrial genome organization for the blue mussel, *Mytilus- Edulis*. Genetics 131:397-412
Holst JC, Nilsen F, Hodneland K, Nylund A (1993) Observations of the biology and parasites of postsmolt Atlantic salmon, *Salmo salar*, from the Norwegian Sea. J Fish Biol 42:962-966
Huguet V, Mergeay M, Cervantes E, Fernandez MP (2004) Diversity of Frankia strains associated to *Myrica gale* in Western Europe: impact of host plant (*Myrica* vs. *Alnus*) and of edaphic factors. Environ Microbiol 6:1031-1041
Humes AG (1994) How many copepods? Hydrobiologia 292-293:1-7
Hunt A (1993) Effects of contrasting patterns of larval dispersal on the genetic connectedness of local-populations of 2 intertidal starfish, *Patiriella-Calcar* and *P-Exigua*. Mar Ecol-Prog Ser 92:179-186
Hunt A, Ayre DJ (1989) Population-structure in the sexually reproducing sea-anemone *Oulactis-Muscosa*. Mar Biol 102:537-544
Hurwood DA, Hughes JM, Bunn SE, Cleary C (2003) Population structure in the freshwater shrimp (*Paratya australiensis*) inferred from allozymes and mitochondrial DNA. Heredity 90:64-70
Huys R, Boxshall AG (1991) Copepod evolution. The Ray Society, London
Isdal E, Nylund A, Nævdal G (1997) Genetic differences among salmon lice (*Lepeophtheirus salmonis*) from six Norwegian coastal sites: evidence from alloenzymes. Bull Eur Assn Fish P 17:17-22
Jacobsen JA, Gaard E (1997) Open-ocean infestation by salmon lice (*Lepeophtheirus salmonis*): Comparison of wild and escaped farmed Atlantic salmon (*Salmo salar* L.). ICES J Mar Sci 54:1113-1119
Jarman SN, Elliott NG, Nicol S, McMinn A (2002) Genetic differentiation in the Antarctic coastal krill *Euphausia crystallorophias*. Heredity 88:280-287
Jarman SN, Nicol S, Elliott NG, McMinn A (2000) 28S rDNA evolution in the Eumalacostraca and the phylogenetic position of krill. Mol Phylogenet Evol 17:26-36.
Johnson MS, Black R (1982) Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp. Mar Biol 70:157-164
Johnson SC, Albright LJ (1991a) Development, Growth, and survival of *Lepeophtheirus salmonis* (Copepoda, Caligidae) under laboratory conditions. J Mar Biol Ass UK 71:425-436
Johnson SC, Albright LJ (1991b) The developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda, Caligidae). Can J Zoolog 69:929-950
Jones MW, Sommerville C, Wootten R (1992) Reduced sensitivity of the salmon louse, *Lepeophtheirus salmonis*, to the organophosphate dichlorvos. J Fish Dis 15:197-202
Jorgensen RA, Cluster PD (1988) Modes and tempos in the evolution of nuclear ribosomal DNA - New characters for evolutionary studies and new markers for genetic and population studies. Ann Mo Bot Gard 75:1238-1247
Kabata Z (1970) Crustacea as enemies of fishes. T.F.H. Publications, Inc., Jersey
Kabata Z (1979) Parasitic copepoda of british fishes. Ray Society, London
Kabata Z (1992) Copepods Parasitic on Fishes. London: Published for the Linnean Society of London and the Estuarine and Brackish-Water Sciences Association by Academic Press.
Krøyer H (1837) Om Snyltekrebsene, især med Hensyn til den danske Fauna. Nat hist tidsskr, Kjøbenhavn 1:605-628
Kumazawa Y, Ota H, Nishida M, Ozawa T (1998) The complete nucleotide sequence of a snake (*Dinodon semicarinatus*) mitochondrial genome with two identical control regions. Genetics 150:313-329
Kaartvedt S (1993) Drifting and resident plankton. B Mar Sci 53:154-159
Lavrov DV, Boore JL, Brown WM (2002) Complete mtDNA sequences of two millipedes suggest a new model for mitochondrial gene rearrangements: Duplication and nonrandom loss. Mol Biol Evol 19:163-169
Lavrov DV, Brown WM, Boore JL (2004) Phylogenetic position of the Pentastomida and (pan)crustacean relationships. Proc Roy Soc Lond B Bio Sci 271:537-544
Lavrov DV, Lang BF (2005) Transfer RNA gene recruitment in mitochondrial DNA. Trends Genet 21:129-133
Lee CE (2000) Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate "populations". Evolution 54:2014-2027
Lewin B (1997) Genes. Oxford University Press Inc., New York
Linares AR, Hancock JM, Dover GA (1991) Secondary structure constraints on the evolution of *Drosophila* 28-S ribosomal-RNA expansion segments. J Mol Biol 219:381-390
Liu LL, Foltz DW, Stickle WB (1991) Genetic population-structure of the southern oyster drill *Stramonita* (= Thais) *haemostoma*. Mar Biol 111:71-79
Macey JR, Larson A, Ananjeva NB, Fang Z, Papenfuss TJ (1997) Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. Mol Biol Evol 14:91-104

Machida RJ, Masaki MU, Mutsumi N, Shuhei N (2004) Large-scale gene rearrangements in the mitochondrial genomes of two calanoid copepods *Eucalanus bungii* and *Neocalanus cristatus* (Crustacea), with notes on new versatile primers for the srRNA and COI genes. Gene 332:71-78

Machida RJ, Miya MU, Nishida M, Nishida S (2002) Complete mitochondrial DNA sequence of *Tigriopus japonicus* (Crustacea: Copepoda). Mar Biotechnol 4:406-417

Mallatt JM, Garey JR, Shultz JW (2004) Ecdysozoan phylogeny and Bayesian inference: first use of nearly complete 28S and 18S rRNA gene sequences to classify the arthropods and their kin. Mol Phylogenet Evol 31:178-191

Martin JW, Davis GE (2001) An updated classification of the recent Crustacea. Natural History Museum of Los Angeles County, Los Angeles

Matsubara M, Komatsu M, Araki T, Asakawa S, Yokobori S, Watanabe K, Wada H (2005) The phylogenetic status of Paxillosida (Asteroidea) based on complete mitochondrial DNA sequences. Mol Phylogenet Evol 36:598-605

Miller AD, Burridge CP, Austin CM (Unpublished) The complete mitochondrial DNA sequence of the mantis shrimp *Harpiosquilla harpax* (Crustacea. Stomatopoda: Squillidae).

Miller AD, Murphy NP, Burridge CP, Austin CM (2005) Complete mitochondrial DNA sequences of the decapod crustaceans *Pseudocarcinus gigas* (Menippidae) and *Macrobrachium rosenbergii* (Palaemonidae). Mar Biotechnol 7:339-349

Miller AD, Nguyen TT, Burridge CP, Austin CM (2004) Complete mitochondrial DNA sequence of the Australian freshwater crayfish, *Cherax destructor* (Crustacea: Decapoda: Parastacidae): a novel gene order revealed. Gene 331:65-72.

Moritz C, Dowling TE, Brown WM (1987) Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Ann Rev Ecol Syst 18:269-292

Mustafa A, Rankaduwa W, Campbell P (2001) Estimating the cost of sea lice to salmon aquaculture in eastern Canada. Can Vet J 42:54-56.

Nagasawa K (2001) Annual changes in the population size of the salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) on high-seas Pacific Salmon (*Oncorhynchus* spp.), and relationship to host abundance. Hydrobiologia 453:411-416

Nagasawa K (2004) Sea lice, *Lepeophtheirus salmonis* and *Caligus orientalis* (Copepoda: Caligidae), of wild and farmed fish in sea and brackish waters of Japan and adjacent regions: A review. Zool Stud 43:173-178

Nagasawa K, Takami T (1993) Host utilization by the salmon louse *Lepeophtheirus salmonis* (Copepoda, Caligidae) in the sea of Japan. J Parasitol 79:127-130

Nardi F, Spinsanti G, Boore JL, Carapelli A, Dallai R, Frati F (2003) Hexapod origins: Monophyletic or paraphyletic? Science 299:1887-1889

Neigel JE (1997) A comparison of alternative strategies for estimating gene flow from genetic markers. Annu Rev Ecol Syst 28:105-128

Nolan DV, Martin SAM, Kelly Y, Glennon K, Palmer R, Smith T, McCormack GP, Powell R (2000) Development of microsatellite PCR typing methodology for the sea louse, *Lepeophtheirus salmonis* (Kroyer). Aquac Res 31:815-822

Nylund A, Wallace C, Hovland T (1993) The possible role of *Lepeophtheirus salmonis* (Krøyer) in the transmission of infectious salmon anaemia. In: Boxshall GA, Defaye D (eds) Pathogens of Wild and Farmed Fish: Sea lice. Ellis Horwood Limited, Chichester, p 367-373

Papetti C, Zane L, Bortolotto E, Bucklin A, Patarnello T (2005) Genetic differentiation and local temporal stability of population structure in the euphausiid *Meganyctiphanes norvegica*. Mar Ecol-Prog Ser 289:225-235

Pedersen OP, Tande KS, Slagstad D (2001) A model study of demography and spatial distribution of *Calanus finmarchicus* at the Norwegian coast. Deep-Sea Res PT II 48:567-587

Pemberton R (1976) Sea trout in north-Argyll-sea lochs, population, distribution and movements. J Fish Biol 9:157-179

Pike AW, Wadsworth SL (1999) Sealice on salmonids: their biology and control. Adv Parasitol 44:233-337.

Pontoppidan E (1753) Det første Forsøg paa norges Naturlige Historie, forestillende dette Kongeriges Luft, Grund, Fielde, Vande, Væxter, Metaller, Mineraler, Steen-arter, Dyr, Fugle, Fiske og omsider Indbyggernes Naturel, samt Sædvaner og levemaade. Det kongelige Waysenhuses Bogtrykkerie, Kiøbenhavn

Poulain PM, WarnVarnas A, Niiler PP (1996) Near-surface circulation of the Nordic seas as measured by Lagrangian drifters. J Geophys Res-oceans 101:18237-18258

Printzen C, Ekman S, Tonsberg T (2003) Phylogeography of *Cavernularia hultenii*: evidence of slow genetic drift in a widely disjunct lichen. Mol Ecol 12:1473-1486

Ramstad A, Colquhoun DJ, Nordmo R, Sutherland IH, Simmons R (2002) Field trials in Norway with SLICE (R) (0.2 % emamectin benzoate) for the oral treatment of sea lice infestation in farmed Atlantic salmon *Salmo salar*. Dis Aquat Organ 50: 29-33

Rawlings TA, Collins TM, Bieler R (2003) Changing identities: tRNA duplication and remolding within animal mitochondrial genomes. Proc Natl Acad Sci U S A 100:15700-15705

Regier JC, Shultz JW (2001) Elongation factor-2: A useful gene for arthropod phylogenetics. Mol Phylogenet Evol 20:136-148

Regier JC, Shultz JW, Kambic RE (2004) Phylogeny of basal hexapod lineages and estimates of divergence times. Ann Entomol Soc Am 97:411-419

Regier JC, Shultz JW, Kambic RE (2005) Pancrustacean phylogeny: hexapods are terrestrial crustaceans and maxillopods are not monophyletic. Proc Roy Soc Lond B Bio Sci 272:395-401

Remigio EA, Hebert PD (2000) Affinities among anostracan (Crustacea: Branchiopoda) families inferred from phylogenetic analyses of multiple gene sequences. Mol Phylogenet Evol 17:117-128.

Roth M (2000) The availability and use of chemotherapeutic sea lice control products. Contrib Zoo 69:109-118

Saccone C, Attimonelli M, De Giorgi C, Lanave C, Sbisa E (1990) The role of tRNA genes in the evolution of animal mitochondrial DNA. In: Quagliarello E, Papa S, Palmieri F, Saccone C (eds) Structure, function and biogenesis of energy transfer systems. Elsevier, Amsterdam, p 93-96

Saccone C, Gissi C, Reyes A, Larizza A, Sbisa E, Pesole G (2002) Mitochondrial DNA in metazoa: degree of freedom in a frozen event. Gene 286:3-12

Saito M, Kojima S, Endo K (2000) Mitochondrial COI sequences of brachiopods: Genetic code shared with protostomes and limits of utility for phylogenetic reconstruction. Mol Phylogenet Evol 15:331-344

Sawabe K, Takagi M, Tsuda Y, Tuno N (2003) Molecular variation and phylogeny of the *Anopheles minimus* complex (Diptera: Culicidae) inhabiting Southeast Asian countries, based on ribosomal DNA internal transcribed spacers, ITS1 and 2, and the 28S D3 sequences. Southeast Asian J Trop Med Public Health 34:771-780

Schnabel KE, Hebert PDN (2003) Resource-associated divergence in the Arctic marine amphipod *Paramphithoe hystrix.* Mar Biol 143:851-857

Schram TA (1993) Supplementary descriptions of the developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). In: Boxshall GA, DeFaye D (eds) Pathogens of Wild and Farmed Fish: Sea lice. Ellis Horwood Limited, Chichester, p 31-47

Schwenk K, Posada D, Hebert PDN (2000) Molecular systematics of European *Hyalodaphnia*: the role of contemporary hybridization in ancient species. Proc Roy Soc Lond B Bio Sci 267:1833-1842

Segawa RD, Aotsuka T (2005) The mitochondrial genome of the Japanese freshwater crab, *Geothelphusa dehaani* (Crustacea: Brachyura): Evidence for its evolution via gene duplication. Gene 355:28-39

Sevatdal S, Horsberg TE (2003) Determination of reduced sensitivity in sea lice (*Lepeophtheirus salmonis* Krøyer) against the pyrethroid deltamethrin using bioassays and probit modelling. Aquaculture 218:21-31

Shearer TL, Van Oppen MJ, Romano SL, Worheide G (2002) Slow mitochondrial DNA sequence evolution in the *Anthozoa* (Cnidaria). Mol Ecol 11:2475-2487

Shoemaker DD, Keller G, Ross KG (2003) Effects of Wolbachia on mtDNA variation in two fire ant species. Mol Ecol 12:1757-1771

Silberman JD, Sarver SK, Walsh PJ (1994) Mitochondrial -DNA variation and population-structure in the spiny lobster *Panulirus-Argus*. Mar Biol 120:601-608

Skilbrei O (2004) Negative virkninger av lakselus på laks i havet, Havforskningsinstiuttet, Bergen

Smith MJ, Banfield DK, Doteval K, Gorski S, Kowbel DJ (1989) Gene arrangement in sea star mitochondrial DNA demonstrates a major inversion event during echinoderm evolution. Gene 76:181-185.

Spears T, Abele LG (1997) Crustacean phylogeny inferred from 18S rDNA. In: Fortey RA, Thomas RH (eds) Arthropod relationships. Chapman & Hall, London, p 169-187

Stanton DJ, Daehler LL, Moritz CC, Brown WM (1994) Sequences with the potential to form stem-and-loop structures are associated with coding-region duplications in animal mitochondrial-DNA. Genetics 137:233-241

Stevens JR, Wall R, Wells JD (2002) Paraphyly in Hawaiian hybrid blowfly populations and the evolutionary history of anthropophilic species. Insect Mol Biol 11:141-148

Stone J, Roy WJ, Sutherland IH, Ferguson HW, Sommerville C, Endris R (2002) Safety and efficacy of emamectin benzoate administered in-feed to Atlantic salmon, *Salmo salar* L., smolts in freshwater, as a preventative treatment against infestations of sea lice, *Lepeophtheirus salmonis* (Krøyer). Aquaculture 210:21-34

Sun H, Zhou K, Song D (2005) Mitochondrial genome of the Chinese mitten crab *Eriocheir japonsinensis* (Branchyura: Thoracotremata: Grapsoidea) reveals a novel gene order and two target regions of gene rearrangements. Gene 349:207-217

Swinstrom KS, Caldwell R, Fourcade HM, Boore JL (Unpublished) The First Complete Mitochondrial Genome Sequences for Stomatopod Crustaceans: Implications for Phylogeny.
Tautz D, Hancock JM, Webb DA, Tautz C, Dover GA (1988) Complete sequences of the rRNA genes of *Drosophila melanogaster*. Mol Biol Evol 5:366-376.
Taylor DJ, Ishikane CR, Haney RA (2002) The systematics of *Holarctic bosminids* and a revision that reconciles molecular and morphological evolution. Limnol Oceanogr 47:1486-1495
Todd CD, Walker AM, Hoyle JE, Northcott SJ, Walker AF, Ritchie MG (2000) Infestations of wild adult Atlantic salmon (*Salmo salar* L.) by the ectoparasitic copepod sea louse *Lepeophtheirus salmonis* Kroyer: prevalence, intensity and the spatial distribution of males and females on the host fish. Hydrobiologia 429:181-196
Todd CD, Walker AM, Wolff K, Northcott SJ, Walker AF, Ritchie MG, Hoskins R, Abbott RJ, Hazon N (1997) Genetic differentiation of populations of the copepod sea louse *Lepeophtheirus salmonis* (Kroyer) ectoparasitic on wild and farmed salmonids around the coasts of Scotland: Evidence from RAPD markers. J Exp Mar Biol Ecol 210:251-274
Todd CM, Walker AM, Ritchie MG, Graves JA, Walker AF (2004) Population genetic differentiation of sea lice (*Lepeophtheirus salmonis*) parasitic on Atlantic and Pacific salmonids: analyses of microsatellite DNA variation among wild and farmed hosts. Can J Fish Aquat Sci 61:1176-1190
Tomita K, Yokobori S, Oshima T, Ueda T, Watanabe K (2002) The cephalopod *Loligo bleekeri* mitochondrial genome: Multiplied noncoding regions and transposition of tRNA genes. J Mol Evol 54:486-500
Treasurer JW, Wadsworth SL (2004) Interspecific comparison of experimental and natural routes of *Lepeophtheirus salmonis* and *Caligus elongatus* challenge and consequences for distribution of chalimus on salmonids and therapeutant screening. Aquac Res 35:773-783
Tucker CS, Sommerville C, Wootten R (2000) The effect of temperature and salinity on the settlement and survival of copepodids of *Lepeophtheirus salmonis* (Kroyer, 1837) on Atlantic salmon, *Salmo salar* L. J Fish Dis 23:309-320
Tully O, Gargan P, Poole WR, Whelan KF (1999) Spatial and temporal variation in the infestation of sea trout (*Salmo trutta* L.) by the caligid copepod *Lepeophtheirus salmonis* (Kroyer) in relation to sources of infection in Ireland. Parasitology 119:41-51.
Tully O, McFadden Y (2000) Variation in sensitivity of sea lice [*Lepeophtheirus salmonis* (Kroyer)] to dichlorvos on Irish salmon farms in 1991-92. Aquac Res 31:849-854
Tully O, Nolan DT (2002) A review of the population biology and host-parasite interactions of the sea louse *Lepeophtheirus salmonis* (Copepoda: Caligidae). Parasitology 124 Suppl:S165-182.
Tully O, Poole WR, Whelan KF (1993b) Infestation parameters for *Lepeophtheirus salmonis* (Krøyer) (Copepoda: Caligidae) parasitic on sea trout (*Salmo trutta* L.) off the west coast of Ireland during 1990 and 1991. Aquacult Fish Manag 24:545-555
Tully O, Whelan KF (1993a) Production of nauplii of *Lepeophtheirus salmonis* (Krøyer) (Copepoda: Caligidae) from farmed and wild salmon and its relation to the infestation of wild sea trout (*Salmo trutta* L.) off the west coast of ireland in 1991. Fish Res 17:187-200
Turbeville JM, Pfeifer DM, Field KG, Raff RA (1991) The phylogenetic status of arthropods, as inferred from 18S ribosomal-RNA sequences. Mol Biol Evol 8:669-686
Umetsu K, Iwabuchi N, Yuasa I, Saitou N, Clark PF, Boxshall G, Osawa M, Igarashi K (2002) Complete mitochondrial DNA sequence of a tadpole shrimp (*Triops cancriformis*) and analysis of museum samples. Electrophoresis 23:4080-4084.
Waldstein DE, W.H. R (2000) Synergism of tebufenozide in resistant and susceptible strains of obliquebanded leafroller (Lepidoptera: Tortricidae) and resistance to new insecticides. J Econ Entomol 93:1768-1772
Westcott JD, Hammell KL, Burka JF (2004) Sea lice treatments, management practices and sea lice sampling methods on Atlantic salmon farms in the Bay of Fundy, New Brunswick, Canada. Aquac Res 35:784-792
White HC (1940) "Sea lice" (*Lepeophtheirus*) and death of Salmon. J Fish Res BD Can 5:172-175
Williams ST, Benzie JAH (1993) Genetic consequences of long larval life in the starfish *Linckia-Laevigata* (Echinodermata, Asteroidea) on the great-Barrier-reef. Mar Biol 117:71-77
Wilson K, Cahill V, Ballment E, Benzie J (2000) The complete sequence of the mitochondrial genome of the crustacean *Penaeus monodon*: are malacostracan crustaceans more closely related to insects than to branchiopods? Mol Biol Evol 17:863-874.
Wolstenholme DR (1992a) Genetic novelties in mitochondrial genomes of multicellular animals. Curr Opin Genet Dev 2:918-925.
Wolstenholme DR (1992b) Animal mitochondrial DNA: structure and evolution. Int Rev Cytol 141:173-216.
Wootten R, Smith JW, Needham EA (1977) Studies on the salmon louse, *Lepeophtheirus*. Bull Off Int Epiz 87:521-522

Wootten R, Smith RJ, Needham EA (1982) Aspects of the biology of the parasitic copepods *Lepeophteirus salmonis* and *Caligus elongatus* on farmed salmonids, and their treatment. Proc Roy Soc Edinb B 81:185 - 197

Yamauchi M, Miya M, Nishida M (2002) Complete mitochondrial DNA sequence of the Japanese spiny lobster, *Panulirus japonicus* (Crustacea: Decapoda). Gene 295:89-96

Yamauchi MM, Miya MU, Machida RJ, Nishida M (2004) PCR-based approach for sequencing mitochondrial genomes of decapod crustaceans, with a practical example from kuruma prawn (*Marsupenaeus japonicus*). Mar Biotechnol 6:419-429

Zardoya R, Meyer A (1996) Phylogenetic performance of mitochondrial protein-coding genes in resolving relationships among vertebrates. Mol Biol Evol 13:933-942

Zrzavy J, Stys P (1997) The basic body plan of arthropods: Insights from evolutionary morphology and developmental biology. J Evol Biol 10:353-367

Øines Ø, Heuch PA (2005) Identification of sea louse species of the genus *Caligus* using mtDNA. J Mar Biol Ass UK 85:73-79

APPENDIX

Table 1: Mitochondrial genomes characterized within the phylum Crustacea.

Species	Accession number
Argulus americanus	NC_005935
Armillifer armillatus	NC_005934
Artemia franciscana	NC_001620
Callinectes sapidus	NC_006281
Cherax destructor	NC_011243
Daphnia pulex	NC_000844
Eriocheir sinensis	NC_006992
Geothelphusa dehaani	NC_007379
Gonodactylus chiragra	NC_007442
Harpiosquilla harpax	NC_006916
Hutchinsoniella macracantha	NC_005937
Lepeophtheirus salmonis	NC_007215
Lysiosquillina maculata	NC_007443
Macrobrachium rosenbergii	NC_006880
Marsupenaeus japonicus	NC_007010
Megabalanus volcano	NC_006293
Pagurus longicarpus	NC_003058
Panulirus japonicus	NC_004251
Penaeus monodon	NC_002184
Pollicipes polymerus	NC_005936
Portunus trituberculatus	NC_005037
Pseudocarcinus gigas	NC_006891
Speleonectes tulumensis	NC_005938
Squilla empusa	NC_007444
Squilla mantis	NC_006081
Tetraclita japonica	NC_008974
Tigriopus japonicus	NC_003979
Triops cancriformis	NC_004465
Triops longicaudatus	NC_006079
Vargula hilgendorfii	NC_005306

Table 2: Sequences included in the phylogenies based on 18S rRNA, in alphabetical order.

Species	**Accession numbers**	**Species**	**Accession numbers**
Anaspides tasmaniae	L81948	*Lepidurus packardi*	L34048
Argulus nobilis proviral	M27187	*Lernaeocera branchialis*	AY627030
Artemia franciscana	AJ238061	*Lernentoma asellina*	AY627003
Asterocheridae sp.	AY627018	Monographis sp.	AY596371
Bairdia sp.	L81943	*Nanaspis tonsa*	AY627029
Bradya sp.	AY627016	*Neanura latior*	AY037172
Branchinecta packardi	L26512	Orecturus sp.	AY627017
Bryocamptus pygmaeus	AY627015	*Onychiurus yodai*	AY037171
Calanus pacificus	L81939	*Ornithodoros moubata*	L76355
Caligus elongatus	AY627020	Pachos sp.	AY627014
Cancerillidae sp.	AY627021	*Panulirus argus*	AY743955
Cancrincola plumipes	L81938	*Parabrachiella bispinosa*	AY627027
Ceratitis capitata	AH006961S1	*Pectenophilus ornatus*	AY627032
Chondracanthus lophii	L34046	*Podura aquatica*	AF005452
Clavella adunca	AY627028	*Poecilasma inaequilaterale*	AY520654
Daphnia pulex	AF014011	*Pontoeciella abyssicola*	AY627031
Diaphanosoma sp.	AF144210	*Porocephalus crotali*	M29931
Drosophila simulans	AY037174	Pseudanthessius sp.	AY627007
Ecbathyrion prolixicauda	AY627024	Pseudocyclops sp.	AY626994
Entomobrya dorsosignata	AY596360	*Rhogobius contractus*	AY627023
Entomolepidae sp.	AY627025	*Sabelliphilus elongatus*	AY627010
Eucyclops serrulatus	L81940	*Sacculina carcini*	AY520656
Exumella mediterranea	AY629259	*Scolopendra cingulata*	U29493
Gloiopotes watsoni	AY627019	*Simocephalus serrulatus*	AF144216
Gonodactylus sp.	L81947	*Sminthurides aquaticus*	AY596364
Hatschekia pagrosomi	AY627026	*Sphaeridia pumilis*	AY145140
Homarus americanus	AY743945	*Squilla empusa*	L81946
Hypogastrura sp.	AY596362	Stenopontius sp.	AY627022
Ibla quadrivalvis	AY520655	*Thamnocephalus platyurus*	AF144218
Isotoma viridis	AY596361	Tortanus sp.	AY626995
Ixodes persulcatus	AY274888	*Trypetesa lampas*	L26520
Lepeophtheirus salmonis	AF208263	*Vargula tsujii*	DQ096577

Table 3: Protein sequences included in the phylogeny of *COI*, in alphabetical order.

Species	**Accession numbers**	**Species**	**Accession numbers**
Artemia parthenogenetica	AAX54683	*Gonodactylaceus caldwelli*	AAG29090
Calanus helgolandicus	AAT99460	*Heterosaccus lunatus*	AAY59885
Caligus centrodonti	AAW81049	*Ixodes persulcatus*	BAC22597
Caligus curtus	AAW81045	*Lepeophtheirus hippoglossi*	AAW81041
Caligus elongatus	AAW81050	*Lepeophtheirus pollachius*	AAW81042
Candacia longimana	AAN16096	*Lepeophtheirus salmonis*	YP_271852
Ceratitis capitata	AAY34455	*Lirceolus bisetus*	AAW32901
Cherax destructor	AAR37034	*Lithobius forficatus*	CAC69937
Cletocamptus helobius	AAK63000	*Mesocalanus tenuicornis*	AAL68664
Cletopsyllidae sp.	AAQ97371	*Metridia pacifica*	AAL84604
Coullana sp.	AAK63001	*Nannocalanus minor I*	AAG53450
Cyamus erraticus	AAZ05871	*Neocalanus flemingeri*	AAG53442
Dahlella caldariensis	AAC47571	*Neoverruca brachylepadoformis*	BAD98495
Daphnia magna	AAV67773	*Ornithodoros moubata*	BAC22581
Daphnia pulex	AAQ90458	*Panulirus japonicus*	NP694520
Daphniopsis pusilla	AAM47518	*Penaeus monodon*	NP038289
Drosophila yakuba	CAC14066	*Pseudocalanus acuspes*	AAL68665
Gammarus oceanicus	AAX22163	*Pseudocalanus moultoni*	AAG53453
Gomphiocephalus hodgsoni	AAO43659		

Paper I

Paper II

Paper III