

**Taxonomy and systematics in *Gyrodactylus*
von Nordmann, 1832 (Monogenea):
studies on a problematic species complex parasitizing salmonids**

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Oslo, December 2007

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List of papers

The thesis is based on the following papers. They will be referred to in the text by their Roman numerals.

- I. Olstad, K., Cable, J., Robertsen, G. & Bakke, T.A. 2006. Unpredicted transmission strategy of *Gyrodactylus salaris* (Monogenea: Gyrodactylidae): survival and infectivity of parasites on dead hosts. *Parasitology* 133, 33-41.
- II. Olstad, K., Robertsen, G., Bachmann, L. & Bakke, T.A. 2007. Variation in host preference within *Gyrodactylus salaris* (Monogenea): an experimental approach. *Parasitology* 134, 589-597.
- III. Olstad, K., Shinn, A.P., Bachmann, L. & Bakke, T.A. 2007. Host-based identification is not supported by morphometrics in natural populations of *Gyrodactylus salaris* and *G. thymalli* (Platyhelminthes, Monogenea). *Parasitology* 134, 2041–2052.
- IV. Olstad, K., Bachmann, L. & Bakke, T.A. Phenotypic plasticity of opisthaptor hard parts in *Gyrodactylus* spp. (Monogenea) from salmonids. (Manuscript).
- V. Olstad, K., Bachmann, L. & Bakke, T.A. Shape variation in natural populations of *G. salaris* and *G. thymalli* analysed using geometric morphometrics. (Manuscript).

Summary

The two monogenean species *G. salaris* and *G. thymalli*, are almost identical at the nuclear molecular level. There is also no support from mtDNA (*cox1*) sequences for monophyly of all *G. salaris* or *G. thymalli* haplotypes. It has therefore been suggested that these taxa represent a case of incipient speciation. Based on this, whether ultimately considered one or more species, these taxa together are referred to as the *G. salaris* / *G. thymalli* species complex. It is argued in the present thesis that a total-evidence approach to the taxonomy of the *G. salaris* / *G. thymalli* species complex is at lower risk of oversimplifying the taxonomic conclusions than is a single-criterion approach for species delimitation. One aim is therefore to apply a comprehensive all-inclusive approach to the taxonomy of the *G. salaris* / *G. thymalli* species complex.

The morphology of the opisthaptor hard-parts, is considered taxonomically informative, and therefore representing a non-linear approach to taxonomy in *Gyrodactylus*. However, traditional linear measurements are not necessarily the optimal approach in studies of morphology. Furthermore, environmental factors may influence on the size and possibly also the shape of the opisthaptor hard-parts. Accordingly, one aim in the present thesis is to improve the methodology in studies of systematics in *Gyrodactylus* based on morphology. In the analytical approach to study morphology, shape descriptors from studies of geometric morphometrics proved to work well. It is therefore reasonable to assume that in future studies the application of geometric shape descriptors is justified.

The presented results indicated that taxonomic revisions in the *G. salaris* / *G. thymalli* species complex based on a single species criterion, despite advantages of comparability, are unlikely to lead to an overall satisfactory delimitation of species. All methods applied so far for assessing the taxonomy and systematics of the *G. salaris* / *G. thymalli* species complex document the importance of evolutionary relationship as the absolute basic grouping criterion. However, for example species definition exclusively based on mtDNA sequence data fails to reflect the significant differences in host preferences and pathogenicity. It is therefore concluded that the knowledge of phylogenetic relationships in the species complex should be supplemented with information on morphology and even more important, on ecology, when it comes to defining the boundaries between the taxa, whether on a species level or below.

1 Introduction

'Species' in biology is a category which represents from a human viewpoint, a recognizable group of recurrently appearing populations of organisms that is believed to represent a more or less coherent evolutionary group (Hey, 2001). A reliable estimate of species boundaries is of central importance not only to the large body of research that concerns this taxonomic level, but also to biodiversity related management (Greene, 1994). However, there is presently no universally applicable, operational definition of the biological unit, the species: the literature is saturated with 'species concepts' promoting a combination of delimitation criteria. Mayden (1997) listed 24 different species concepts and since then, new ones have continuously appeared. The diversity of concepts reflects the diversity of events associated with the speciation process and the differing research interests of authors (Cracraft, 2000; Hey, 2001).

Most species descriptions in the genus *Gyrodactylus* Normann, 1832 are based on morphology of the opisthaptor hard-parts, although they often also contain important information about ecology, including for example host, life-history or locality (Bakke et al., 2007). Over 400 species of *Gyrodactylus* have been described (Harris et al., 2004) but from only ~200 predominantly teleost hosts (Bakke et al., 2002). An extrapolation to the ~24,000 teleost species would suggest some 20,000 gyrodactylid species. The theoretical framework underlying species criteria in general have been developed extensively throughout the years (see e.g. Wheeler and Meier, 2000). However, only few specific operational methods have been proposed for the practical delimitation of these species among *Gyrodactylus* (but see Ziętara and Lumme, 2003). This lack of criteria for species delimitation has been a source of controversy regarding the taxonomic status of the closely related *Gyrodactylus salaris* Malmberg, 1957 and *G. thymalli* Žitňan, 1960.

1.1 Species concepts

According to Bakke et al. (2007), one of the currently most interesting questions regarding *Gyrodactylus* concerns species concepts; i.e. what is the relationship between operational taxonomic units (OTU) that we currently regard as valid species? In the literature on *Gyrodactylus*, a species description usually does not refer to any specific concept. The characteristics for delimitation and recognition of a species (i.e. diagnostics) are in most

instances not necessarily a part of its definition, and are traditionally assumed to be best sought in phenotypic characters and qualities. The most widely applied morphological method of delimiting species in a broader context is based on the presence of fixed or non-overlapping character differences between geographically allopatric samples (see Wiens and Servedio, 2000).

One important clue to species identification is the reflection of the evolutionary history of which they are a part, and that formed them (Hey, 2001). Lineage-based concepts recognize species on the basis of reciprocal monophyly of gene genealogies and as such seem to ultimately fulfill the demand to reflect evolutionary history (Mishler and Donoghue, 1982; Donoghue, 1985). Opponents to lineage-based concepts, however, will claim that tree-based species diagnoses are associated with a number of problems. For example, gene trees may not always be congruent with species trees due to e.g. lineage sorting of ancestral polymorphisms (Moore, 1995). Alternatively, there are also non-lineage based methods. One example is multivariate analysis of generalized phenotypes that can be used to identify groupings (phenetic clusters) which are considered species in the absence of intermediates (Mallet, 1995). Although relatively few concepts promote ecological criteria (vanValen, 1976), ecology is without doubt an important topic of current research on speciation processes. Some authors have argued that species delimitation should be treated independently from investigations of the speciation process due to a risk of circularity (Goldstein and DeSalle, 2000). However, given that both sympatric and allopatric populations are more likely to speciate in the context of adaptive divergence (Marchetti, 1993; Schluter, 2001), ecological compatibility may provide a useful indication of whether two closely related populations have the potential to hybridize or not (Schluter, 2001; Templeton, 2001).

The biological species concept of Mayr (1942) is, at least in theory, the gold standard for discrimination of species in *Gyrodactylus*, as it is among zootaxa in general. However, as it is non-operational for practical purposes according to its definition, the biological species concept is not used directly or explicitly. To date, the only work that deals explicitly with species concept within *Gyrodactylus* is the paper by Ziętara and Lumme (2003), in which the authors explore a combined solution including molecular, typological, and biological concepts. According to Ziętara and Lumme (2003) the currently most applied species concept for *Gyrodactylus* has traditionally been a typological one (as described by Mayr (1963), but see also an alternative definition by Cracraft (2000)).

1.2 An all-inclusive view

Approaches to species delimitation that combine as many independent sources of data as possible have been suggested by numerous authors (e.g. Mishler and Donoghue, 1982; Puerto et al., 2001; Wiens and Penkrot, 2002; Sanders et al., 2006). The classical paper by Mishler and Donoghue (1982) provides the basis for what has later been referred to as the Phylogenetic species concept *sensu* Mishler and Theriot (Mishler and Theriot, 2000). In this work, the authors emphasize a two-fold theoretical platform, namely (i) that organisms should be grouped into species on the basis of evidence for monophyly, and (ii) that criteria used to assign species rank to certain monophyletic groups must vary among different organisms (but might well include ecological criteria to the presence of breeding barriers in particular cases) (Mishler and Donoghue, 1982). As to point (ii), the authors argue that a narrowing of species delimitation criteria is at risk of oversimplifying the complexity of variation patterns in nature. As in the paper by Mishler and Donoghue (1982), it is argued in the present thesis for a pluralistic approach that is at lower risk of oversimplifying the taxonomic task than is a single-criterion approach for species delimitation. Here, however, the focus is restricted to the species within the genus *Gyrodactylus*. Furthermore, the idea of pluralism in general and the two theoretical criteria of Mishler and Donoghue (1982) in particular, will serve as guidelines throughout the present thesis.

1.3 Speciation in *Gyrodactylus*

Gyrodactylus is one of several hyperdiverse monogenean genera and may provide important insights into parasite speciation processes in general due to its particular mode of reproduction (Cable and Harris 2002; Bakke et al., 2007). Two mechanisms of speciation are normally recognised as specific for *Gyrodactylus* (see Bakke et al. 2002; see also recent review on speciation in parasites in general by Huys et al. 2005). The first is host-parasite co-evolution, in which the gradually accumulating evolutionary divergence of hosts leads to isolation and subsequent evolution of their parasites. If these parasites with time become more closely adapted to, and more dependent on their specific host, they may also lose the general potential to infect a range of hosts. The second mode of speciation may occur when hosts acquire parasites from taxonomically unrelated organisms inhabiting the same environment. In literature, this is referred to as host-switching or

ecological transfer. There is no implication about the length of time over which evolution of host specificity has been taking place. Originally, parasite speciation was viewed predominantly from the perspective of co-evolution. In a speciation context, this would thus be referred to as co-speciation. The concept of co-speciation was the speciation mode underlying e.g. the work on the systematics of gyrodactylids by Malmberg (1970). At present, very few convincing cases of co-speciation have been demonstrated (see Page et al., 1996). For example, Bakke et al. (2002) could find little convincing evidence for any co-evolutionary relationships between gyrodactylids and their fish hosts. On the contrary, molecular work has shown the importance of host-switching especially based on failure to identify co-evolutionary trends between gyrodactylids and their fish hosts (Ziętara et al., 2002; Boeger et al., 2003; Huysse et al., 2003). Thus, the speciation mode in *Gyrodactylus* is assumed to be predominantly based on host-switching.

Recently, Ziętara et al. (2007a,b) have suggested that hybridization between clonal lines is an important, but until now underestimated, mode of speciation within *Gyrodactylus*. However, the actual relative impact of these mechanisms in *Gyrodactylus* speciation is yet a matter to be resolved. The suggestion by Ziętara et al. (2007a,b) is based on *Gyrodactylus* having a quite unique strategy of reproduction (see e.g. Cable and Harris 2002) which may play an important role in the speciation processes. In a manner of sequential viviparity, they give birth to almost fully developed young which already contain developing embryos *in utero*. The first-born offspring develops at the centre of its still embryonic parent (Cable and Harris 2002). This physical origin at the centre of an immature embryo has been taken as evidence that the first-born daughter arises asexually. Only after the second daughter begins to develop the male reproductive system becomes fully functional (Harris, 1985). Subsequent daughters develop either by parthenogenesis or sexually (Harris, 1993). It has been demonstrated by e.g. Kathariner's (1904), and subsequently repeated with *G. gasterostei* by Harris (1998) that reproduction (at least of the first-born daughters) can continue for up to 30 generations without the need for sexual reproduction. However, detailed knowledge concerning the intraspecific frequency of sexual versus asexual reproduction is for most species not available. Nevertheless, the frequency of sexual versus asexual reproduction is of importance, since (i) most of the applied species concepts today are based on grouping organisms according to gene-pools as a direct result of sexual reproduction, and (ii) it may play a major role for the rate of genetic differentiation.

1.4 The *G. salaris* / *G. thymalli* species complex

G. salaris and *G. thymalli* are very closely related and there is a body of papers on the taxonomy and possible synonymy of the two. At the molecular level, using nuclear ribosomal gene sequences as markers, *G. salaris* and *G. thymalli* are almost identical (Cunningham, 1997; Ziętara and Lumme, 2002). According to sequence data of the mitochondrial cytochrome oxidase I gene (*cox1*) there is no support for monophyly of all *G. salaris* or *G. thymalli* haplotypes (Hansen et al., 2003; 2006, 2007a; Meinilä et al., 2004; illustrated in fig. 1). Based on these analyses, Hansen et al. (2003) presented three alternative taxonomic scenarios: (i) *G. salaris* and *G. thymalli* represent two polytypic species, (ii) *G. salaris* and *G. thymalli* represent one polytypic species or (iii) *G. salaris* and *G. thymalli* refer to a complex of more than two sibling species. However, Bakke et al. (2007) consider *G. salaris* and *G. thymalli* a case of incipient speciation with the sibling taxa representing either two semispecies or a superspecies, reproductively more or less isolated by host preference. Based on this, Bakke et al., 2007 use the annotation “*G. salaris* / *G. thymalli* species complex”. In the present thesis, this is acknowledged as an appropriate provisional annotation, regardless whether ultimately considered one or more species, and it will therefore be used consistently throughout the text when referring to the mentioned taxa. The “*G. salaris* / *G. thymalli* species complex” will also include the Danish rainbow trout (*Oncorhynchus mykiss*) variants described by Lindenstrøm et al., 2003 (named Gx) and by Jørgensen et al. (2007), as well as the *G. salaris* parasitizing Arctic charr (*Salvelinus alpinus*) in lake Pålbufjord, Norway (Robertsen et al., 2007). Although *G. bohemicus* Ergens, 1992 described from rainbow trout and brook trout (*Salvelinus fontinalis*), admittedly is likely to be closely related to this group (see Bakke et al., 2007), it was not included in the present study.

1.4.1 Lineage-based methods in studying evolutionary relationships in the *G. salaris* / *G. thymalli* species complex

In recent years, the application of molecular markers in the taxonomy and systematics of *Gyrodactylus* species has increased. The sequencing of the internal transcribed spacers (ITS-1 and ITS-2) of the nuclear ribosomal DNA (rDNA) showed that many *Gyrodactylus* species can be discriminated by these sequences (Ziętara and Lumme 2002). However, *G. salaris* cannot be differentiated from its closest relative *G. thymalli* by means of ITS-1 and

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ITS-2 (Cunningham, 1997, Ziętara and Lumme, 2002). Currently the mitochondrial *cox1* gene is the only marker allowing for a genetic discrimination of populations or strains of *G. salaris* and *G. thymalli* (see e.g., Hansen et al. 2003, 2006, 2007b; Meinilä et al. 2004) (illustrated in fig. 1). Populations of the *G. salaris* / *G. thymalli* species complex can be grouped into several well supported clades based on *cox1* sequences. However, there is no support for the monophyly of either *G. salaris* or *G. thymalli* (Hansen et al., 2003, 2006, 2007b; Meinilä et al., 2004). Based on this, Meinilä et al. (2004) suggest *G. thymalli* being a junior synonym of *G. salaris*, and that all forms of this taxon from rainbow trout, Atlantic salmon (*Salmo salar*) or grayling (*Thymallus thymallus*) therefore should be referred to as the *G. salaris* cluster or *G. salaris sensu lato*. This synonymisation is not formal according to the International Code of Zoological Nomenclature (ICZN, 1999), and has not yet been accepted in the general literature in the field (see Bakke et al. 2007).

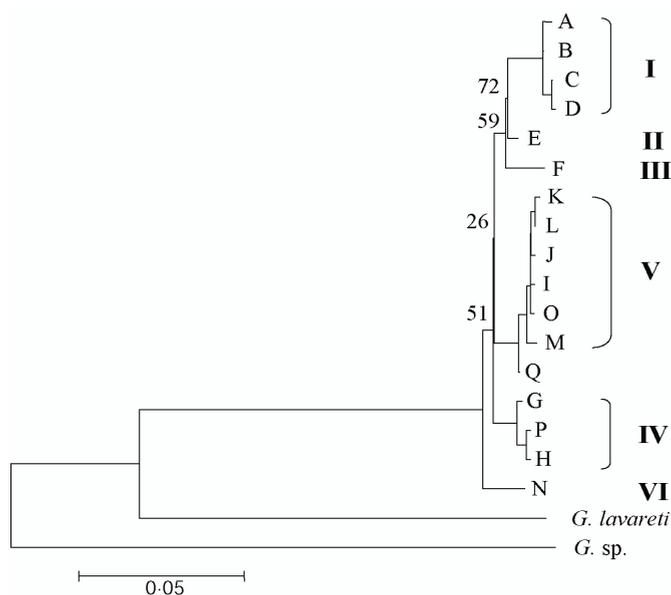


Fig. 1: Illustration of the low support for the basal nodes in the systematics based on mitochondrial Cytochrome Oxidase I (*cox1*) sequences of *Gyrodactylus salaris* and *G. thymalli*: Neighbor-joining dendrogram (Kimura's two parameter) of mitochondrial haplotypes modified after Hansen et al. (2006). The depicted haplotypes and clades (Roman capitals) constitute samples as follows: **I** - *G. salaris* from a number of localities in Norway and Sweden; **II** - *G. salaris* from river Göta älv, Sweden; **III** - haplotypes often referred to as the rainbow trout variant of *G. salaris*; **IV** - *G. thymalli* from river Trysilelva, Norway; **V** - *G. thymalli* from a number of localities in the river Glomma drainage system, Norway; **VI** - *G. thymalli* from river Hnilec, Slovak Republic. Bootstrap support as percentages (1000 replicates) is included for the basal nodes. Scale bar refers to a genetic distance of 0.05.

1.4.2 Non-lineage-based methods in studying evolutionary relationships in the *G. salaris* / *G. thymalli* species complex

Biology and ecology. Despite *G. salaris* and *G. thymalli* being closely related, their host-species preferences as observed from laboratory infection experiments, are different (Soleng and Bakke, 2001; Bakke et al. 2002; Sterud et al. 2002). The host specificity of different *G. salaris* populations or strains has previously been examined in detail: rivers Drammenselva / Lierelva populations (e.g. Bakke et al. 1990, 1991, 1996, 1999; Cable et al. 2000); river Steinkjerelva population (Bakke and MacKenzie, 1993), and Batnfjordelva population (Bakke et al. 2002) (see also Bakke et al., 2007). *G. salaris* is pathogenic to Eastern Atlantic salmon, whereas *G. thymalli* appears to be non-pathogenic to its primary host, grayling, or any other of its potential host (Soleng and Bakke, 2001; Bakke et al. 2002; Sterud et al. 2002). According to these experiments on host preference, *G. salaris* is only to a limited extent able to exploit grayling as a host. The host specificity of *G. thymalli* has also been examined, although in less detail. This parasite utilises Atlantic salmon even less effectively (Bakke et al., 2002, Sterud et al. 2002; O.G. Øvstaas, personal information) than *G. salaris* can exploit grayling. Other members of the *G. salaris* / *G. thymalli* species complex that have been tested experimentally are the rainbow trout variants of *G. salaris* isolated by Lindenstrøm et al. (2003) and Jørgensen et al. (2007) from Danish rainbow trout and *G. salaris* parasitizing Arctic charr in lake Pålbufjord, Norway (Robertsen et al. 2007; Paper II, present thesis). These parasites from Danish rainbow trout and Norwegian Arctic charr failed to infect stocks of Eastern Atlantic salmon successfully but reproduced on rainbow trout (Lindenstrøm et al. 2003; Jørgensen et al., 2007; Paper II, present thesis) and Arctic charr (Paper II, present thesis).

Morphology. Gyrodactylid alpha taxonomy is based on morphology, i.e. mainly morphometrics of the opisthaptor hard parts consisting of marginal hooks, hamuli and a ventral bar (see e.g. Malmberg, 1957, 1970, 1993; McHugh et al. 2000; Shinn et al., 1993, 1996, 1995b, 2000, 2001, 2004; *Gyrodactylus* opisthaptor hard-parts illustrated in fig. 2), although it may also implicitly rely heavily on the host identity (Bakke et al. 2007). Originally Malmberg (1970) found that the shape of the marginal hooks is the taxonomically most informative structures. In the *G. salaris* / *G. thymalli* species complex, in order to obtain increased resolution to the analyses, more emphasis has also been put on the other hard-parts of the opisthaptor (see e.g. Kay et al., 1999 Shinn et al. 2004). The opisthaptor hard-parts in *Gyrodactylus* are composed of keratin-like proteins (Kayton,

1983; Shinn et al., 1995a) and are considered to provide substantial taxonomically useful information. The structures are relatively stable in shape and for the most fully formed already at birth (but see alternative hypothesis forwarded in Paper IV, present thesis).

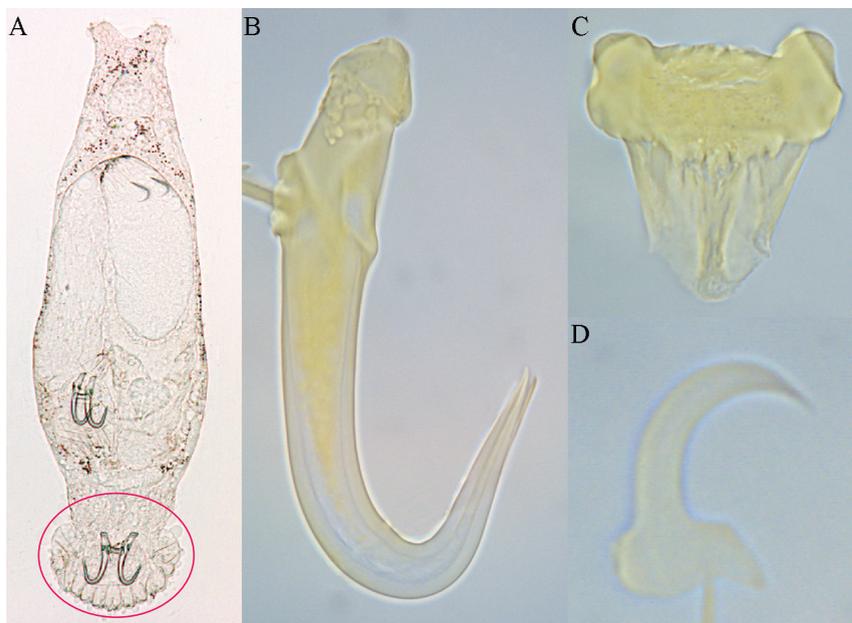


Fig. 2: Pictures illustrating **A**: a live individual of *Gyrodactylus salaris*. The opisthaptor with the hamuli, ventral bars and marginal hooks of the parental individual is highlighted by a circle. Opisthaptoral hard-parts of the F1 and F2 individuals can be seen the parent. **B - D**: Opisthaptoral hard-parts from a *G. salaris* individual depicted after digestion of all soft tissue; **B**: hamulus; **C**: ventral bar; **D**: marginal hook sickle. The size of the pictures does not reflect the relative size of the structures.

Intraspecifically, hamuli and bars appear largely invariant. This was demonstrated by Harris (1998) for *G. gasterostei* who found the variance of hamular dimensions in natural populations not greater than that within inbred laboratory lines. This result also implies that the morphology of the hamuli and bars is tightly controlled genetically. However, it is well known that at least the size of the opisthaptoral hard-parts varies according to environmental conditions (Malmberg 1970; Kulemina, 1977; Ergens and Gelnar, 1985; Mo, 1991a,b,c, 1993; Dmitrieva and Dimitrov, 2002; Huyse and Volckaert, 2002; Dávidová et al., 2005; Paper IV, present thesis). An important environmental factor affecting hamulus and the marginal hook size is temperature. According to a rule of thumb, increased temperature results in smaller hamuli and marginal hook sizes (Malmberg 1970;

Kulemina, 1977; Ergens and Gelnar, 1985; Mo, 1991a,b,c, 1993; Dmitrieva and Dimitrov, 2002; Dávidová et al., 2005). The mechanism behind this effect is attributed to growth rates being dependent on temperature-sensitive embryo development rather than fecundity (Kulemina, 1977). Reproductive rate at different temperatures has also been examined in *G. gasterostei* (Harris, 1982) and in *G. salaris* (Jansen and Bakke, 1991). In the latter, the generation time and time between successive births were negatively correlated with temperature whereas the reproductive rate increased with increasing temperature. The negative correlation between temperature and size has been considered as a general rule as it has been supported in most relevant studies so far (but see Paper IV, present thesis). The environmental effect of temperature is known to result in non-overlapping size ranges in samples from the same population taken at the warmest and coldest periods of the year (Mo, 1991b).

Other environmental factors such as e.g. host identity (Robertsen et al., 2007) and site of attachment (Huysse and Volckaert, 2002) can also affect haptor morphology. Dmitrieva and Dimitrov (2002) also showed an effect of salinity on hamulus and marginal hook size, although in this case parasites were not characterised genetically and may have represented different genotypes. Both Ergens and Gelnar (1985) and Mo (1991c) explored experimentally the effect of temperature on the morphology of the opisthaptor hard-parts. However, the sets of measurements used for morphometrics have been largely extended since these studies (McHugh et al., 2000; Shinn et al., 2000; Shinn et al., 2004; Paper III, present thesis). Furthermore, the studies by Ergens and Gelnar (1985) and Mo (1991c) included full-range variation in experimental populations, and could thus not address phenotypic plasticity *per se*, but rather intraspecific genotypic- along with phenotypic variation. To include more species in comparative morphometric studies is also important because, according to Bakke et al. (2007), particularly in the *G. salaris* / *G. thymalli* species complex, there is evidence that morphology may change following host-switching and isolation. This conclusion is based on the observations that populations of *G. salaris* from salmon (and rainbow trout) are morphologically different to specimens from grayling (Lindenstrøm et al., 2003; Shinn et al., 2004; Papers III, V, present thesis), despite their assumed very recent origin by host-switching (see Meinilä et al., 2004). In the opinion of Bakke et al. (2007), these observations suggest that in addition to environmental factors host-shifts may change the rate of morphological evolution in gyrodactylids.

The opisthaptor hard-parts of *Gyrodactylus* have few specific landmarks that can be used for morphometric point-to-point distance analyses. Inaccuracy in the description of

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landmark positions for linear measurements in gyroductylid taxonomy therefore inevitably occurs. However, the use of standardized sets of measurements between established landmarks allowed Kay et al. (1999) to apply rapid semi-automatic identification of gyroductylids by even relatively untrained operators. Subsequently, this technique was developed further to utilise the power of PC neural networks to learn identification of single gyroductylids (McHugh et al., 2000; Shinn et al., 2000). However, these methods of semi-automated identification are basically diagnostic, and not designed for delimitation of species borders and definition of species.

Linear measurements doubtlessly have the potential to capture shape. However, to what extent such measurements capture shape, and not only size, is a matter of debate. The power of shape analyses has not been largely explored in *Gyroductylus*. Malmberg (1970) noted that shape, particularly of the marginal hook sickles, can be useful to discriminate individuals if linear dimensions fail because their structural information is represented mostly by outlines. One approach to address this potential is geometric morphometrics using the sliding semilandmark method capturing outlines (Green, 1996; Bookstein, 1997). Such an approach, in which initially non-homologous landmarks along outlines are mathematically made homologous from subject to subject, was applied for the first time for *Gyroductylus* in the present study (Papers IV, V).

2 Main aims of the thesis

In the *G. salaris* / *G. thymalli* species complex lineage-based methods such as e.g. phylogenetic analyses of homologous molecular sequences basically serves best the theoretical requirements, whereas non-lineage-based methods such as e.g. morphology and ecology serves best conservation management and applied fields of study (Bakke et al., 2007). For example, lineage-based methods like phylogenetic analyses of mitochondrial DNA sequences give some resolution in the *G. salaris* / *G. thymalli* species complex (Hansen et al., 2003, 2006, 2007a; Meinilä et al., 2004). However, the groups (in the particular literature annotated haplogroups or clades) are not coherent with pathogenicity, an important aspect in management (Hansen et al., 2007b).

Based on the works by e.g. Malmberg (1957, 1970, 1993), McHugh et al. 2000 and Shinn et al. (1993, 1996, 1995b, 2000, 2001, 2004), the morphology of the opisthaptoral hard-parts, is likely to be highly taxonomically informative, and therefore representing a non-linear approach to taxonomy in *Gyrodactylus*. However, traditional linear measurements are not necessarily the optimal morphometric approach in studies of morphology. Furthermore, it is well established that environmental factors may influence on the size and possibly also shape of the opisthaptoral hard-parts. Accordingly, one main aim in the present thesis is to analyse and improve the methodology in morphological-based studies of systematics in *Gyrodactylus*.

A second main aim in the present thesis is to apply a comprehensive all-inclusive approach to the taxonomy of the *G. salaris* / *G. thymalli* species complex as stated in chapter 1.2. It is argued that such an approach is at lower risk of oversimplifying the taxonomic conclusions than a single-criterion approach for species delimitation. Essential for an all-inclusive view is the implementation of the available knowledge from both lineage-based and non-lineage-based methods. Accordingly, the present study explore how the taxonomy in the *G. salaris* / *G. thymalli* species complex may result from non-lineage-based approaches such as morphometrics and studies of ecology and biology in combination with systematics based on molecular markers as reported in the literature.

3 Summary of papers

Paper I

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

A multidisciplinary approach (survival experiments, infection experiments and electron microscopy) was used to address the importance of infected dead hosts as a significant source for the spread of *Gyrodactylus* parasites in infected host populations. Temperature-dependent survival and re-infection potential of *G. salaris*, both on and off dead hosts was investigated. In contrast to previous assumptions, the present study showed that individuals of *G. salaris* do not necessarily leave a host immediately after its death. The experimental laboratory infections demonstrated that the establishment and subsequent population growth of parasites previously maintained on dead hosts for 3 days was similar to that of parasites transferred between living hosts. Furthermore, parasites that remained on a dead host survived and maintained their infectivity for longer periods than detached worms. Ultrastructural evidence indicated that *G. salaris* fed on the dead hosts, and the laboratory experiments demonstrated that the lifespan of worms on dead hosts was more than twice as long as of detached individuals; at 18°C, survival off the host was 1d at 3°C, 4d. Thus, the potential time interval for transmission to live hosts may be significantly longer than previously considered. Although renowned for their conserved morphology, it was indicated that gyrodactylids employ a variety of different life-history traits and behaviours related to the ecology of their primary host. For *G. salaris*, remaining with a dead host might be a specialized behavior attributed to a combination of the high risk related to transmission in running water and the increased likelihood of contacting a new host due to nipping, cannibalism or scavenging by a susceptible host.

Paper II

Variation in host preference within Gyrodactylus salaris (Monogenea): an experimental approach. (Olstad, K., Robertsen, G., Bachmann, L. & Bakke, T.A. 2007., Parasitology 134, 589-597).

In lake Pålbufjorden, southern Norway, upstream the anadromous stretches of the river Numedalslågen which is densely populated with Atlantic salmon, a resident Arctic charr population has been reported to be infected with *G. salaris* (Robertsen et al. 2007). This population of *G. salaris* is reported viable in the absence of its primary host, Atlantic salmon (Robertsen et al., in press). In addition, there is no record of *G. salaris* infecting Atlantic salmon in the anadromous sections of the river Numedalslågen. Previously, observations of Arctic charr infected with *G. salaris* have been reported only from river systems in which Arctic charr occur sympatrically with Atlantic salmon which is also infected with *G. salaris*. The infectivity and reproductive capacity of the *G. salaris* population on Arctic charr from lake Pålbufjorden was studied experimentally on wild and hatchery-reared Atlantic salmon as well as on Arctic charr and rainbow trout. Arctic charr and rainbow trout were moderately susceptible, whereas the two Atlantic salmon stocks from the rivers Drammenselva and Numedalslågen, respectively, were innately resistant to only slightly susceptible. These results were significantly different to that of the control group, the *G. salaris* strain from Atlantic salmon in Drammenselva. Thus, the *G. salaris* population from Arctic charr in lake Pålbufjorden was considered non-pathogenic to Atlantic salmon. But most importantly, these results demonstrated that host preferences and pathogenicity may significantly differ within the same haplotype (here: *G. salaris* haplotype F, according to Hansen et al., 2003; Robertsen et al. 2007), and that the present *G. salaris* as identified by molecular or morphological means cover both strains that are malign and benign towards Atlantic salmon. Furthermore, it was pointed out that the apparent ease with which species and strains closely related to *G. salaris* may undergo host switching could ultimately be an avenue for a significant expansion of the geographical range of pathogenic variants of *G. salaris*.

Paper III

Host-based identification is not supported by morphometrics in natural populations of Gyrodactylus salaris and G. thymalli (Platyhelminthes, Monogenea). (Olstad, K., Shinn, A.P., Bachmann, L. & Bakke, T.A. 2007., Parasitology, 134, 2041–2052).

This study was designed to explore whether there is a consistent pattern of morphometric variation between different *G. salaris* and *G. thymalli* populations or populations bearing different mitochondrial haplotypes. The morphometric variation between the natural populations was studied in the context of “diagnostic realism”, i.e. of individuals from natural populations without considering differences in environmental parameters. Ten sample locations were chosen to comprehensively represent the variation in *cox1* genealogy. In addition, the original type-material of both species was also included in the analyses. Twenty-seven point-to-point measurements from the opisthaptor hard parts were analyzed by digital image processing and uni- and multivariate statistics. A substantial intra- and interpopulation variation in hook morphometry was noticed. However, almost all populations resembled the type-material in accordance with the *a priori* species designations based on host species. Only *G. thymalli* on grayling from the Norwegian river Trysilelva did not follow the general pattern but was more similar to the *G. salaris* type material from Atlantic salmon than to *G. thymalli*. Therefore, it was concluded that there is no unambiguous support in the morphometric variation of the *G. salaris* and *G. thymalli* populations for an *a priori* host based species delineation. Furthermore, the observed morphometric variation in the measured characters for *G. salaris* and *G. thymalli* did not support an assumption of more than two sibling species. The study also indicated that the species determination of gyrodactylids from natural populations of fish is not straightforward when based solely on morphometrics. However, the study demonstrated an urgent need for more detailed knowledge on the potential impact of environmental factors on the phenotype of gyrodactylid populations.

Paper IV

Phenotypic plasticity of opisthaptor hard parts in Gyrodactylus spp. (Monogenea) from salmonids. (Olstad, K., Bachmann, L. & Bakke, T.A., Manuscript).

These laboratory experiments were designed to analyse the effect of some specific environmental variables on the shape and size of the taxonomically important hard parts of *Gyrodactylus salaris* and *G. thymalli*. Linear and geometric morphometrics using sliding semilandmarks was applied on isogenic strains of both species. Thus, genetically based variation was kept at a minimum. The experiments produced a number of specific results: (i) the ventral bar of *G. salaris* appeared unique as it did not follow the expected pattern according to previously published results of size being inversely correlated with temperature; (ii) there was a clear overlap in size between the largest hamuli and marginal hooks from *G. salaris* (at 5°C) and their respective smallest counterparts from *G. thymalli* (at 12°C); (iii) the hard parts of *G. salaris* grown on grayling, rainbow trout and Arctic charr were larger to similar in size with individuals from the primary host Atlantic salmon; (iv) there was no consistent trend of shape differences among hamuli and marginal hooks from *G. salaris* grown at 5, 12, or 18°C; (v) there was a stronger signal for shape differences between *G. thymalli* individuals grown at 5 and 12°C than among *G. salaris* individuals grown in the temperature range of 5 to 18°C; and (vi) generally, the shape of hamuli and marginal hooks from *G. salaris* differed when grown on the secondary hosts as compared to the primary host, Atlantic salmon. However, there was no consistent trend in the shape differences among the hard structures. The present results demonstrated that there is a significant phenotypic plasticity in the *G. salaris* / *G. thymalli* species complex dependent on variation in environmental factors that hampers or prevents unambiguous morphometric species identification of individuals from natural populations.

Paper V

Shape variation in natural populations of G. salaris and G. thymalli analysed using geometric morphometrics. (Olstad, K., Bachmann, L. & Bakke, T.A., Manuscript).

Size of the *Gyrodactylus* opisthaptor hard-parts, constituting taxonomically informative structures, is highly dependent on environment, whereas shape is less so. Shape alone should therefore be expected to give a less disturbed, and thus more likely correct, taxonomically informative signal. The aim of this study was to explore whether there is a consistent pattern of difference between *G. salaris* and *G. thymalli* in analyses of geometric shape variables (pure shape) and to compare the results with previous analyses of traditional linear measurements (size and shape) on the same material. Therefore, the present study re-analysed the individuals included in Paper III (present thesis) and incorporated the knowledge of the impact of environmental factors on size and shape (Paper IV, present thesis). Based on the findings in the present analyses, it is concluded that shape of marginal hooks is likely to represent an important taxonomic structure in the discrimination of species in *Gyrodactylus*. Variation in shape was fully in line with *a priori* species assignments based on host species and mitochondrial DNA systematics among the analysed populations of *G. salaris* and *G. thymalli*. Further resolution to the analyses was provided by shape of hamuli, but not ventral bars. Thus, unlike traditional morphometrics, the present study provided morphometric support for a previously launched hypothesis that *G. salaris* and *G. thymalli* represent two polytypic species.

4 Discussion

4.1 The theory of species: Evolution *versus* observation

Ten years ago, Mayden (1997) listed 24 different species concept. Today, there is still no sign of an agreement among biologists on just one single concept. Despite the long history of dispute, de Queiroz (1998) noted that most species concepts agree fundamentally that species are lineages, and for sexual organisms, they are lineages that are united through the process of gene flow (Mayr, 1942). What authors have disagreed about, and what assumedly have motivated the numerous different species definitions, are the best criteria for recognizing these lineages (de Queiroz, 1998). However, whether or not species are real entities that exist in nature regardless of definition, they represent categories of organisms as the outcome of two different processes, namely: (i) the evolutionary processes behind biological diversity; and (ii) the human observation, recognition and naming of patterns of recurrence (Hey, 2001). This dichotomy between diversity *per se* and the human made pattern and naming of it also reflects the approaches of species studies throughout time, and to some extent also the taxonomic controversies. For example, studies of phylogenetic relationships either through morphological or molecular approaches will have the potential to trace the evolutionary history of the operational taxonomic units (OTU) directly. Although recurring patterns may also potentially reflect evolutionary processes, they are always at risk of representing plesiomorphies, leading to false phylogenetic signals. However, false phylogenetic signals, or the lack of phylogenetic signals, are not unique to observational methods. Also molecular approaches used to phylogeny have its caveats. For example, gene trees may not always be congruent with species trees due to e.g. lineage sorting of ancestral polymorphisms (Moore, 1995). The dichotomy (evolution *versus* observation) of what species are will be the primer of discussion concerning the taxonomy and systematics of what is here referred to as the *G. salaris* / *G. thymalli* species complex.

G. salaris and *G. thymalli* are according to the data available by today almost identical at the nuclear molecular level (Cunningham, 1997; Ziętara and Lumme, 2002). There is no support from mitochondrial *cox1* sequences for the monophyly of either all *G. salaris* or *G. thymalli* haplotypes (Hansen et al., 2003, 2006, 2007a; Meinilä et al., 2004). These mitochondrial sequence data do not provide the full story, as there are some

differences in the nuclear IGS sequences (Sterud et al., 2002; Cunningham et al., 2003; Collins et al., 2004; Hansen et al., 2006). Mitochondrial haplotypes spread through a population at different rates than nuclear alleles because of their maternal mode of transmission. For example, due to the predominantly asexual and parthenogenetic reproductive strategy in *Gyrodactylus*, Bakke et al. (2007) expect this to potentially explain the fixed mitochondrial haplotype F (annotation by Hansen et al. 2003; found in e.g. rivers Drammenselva, Lierelva, Lærdalselva and lakes Pålbufjord and Bullaren). As to whether the phylogeny based on the *cox1* marker correctly represents the species tree of the *G. salaris* / *G. thymalli* species complex is probably too preliminary to assess. At present, the inclusion of any new *cox1* sequence can lead to a different representation of the inferred phylogenetic relationships within the *G. salaris* / *G. thymalli* species complex as there is basically no statistical support for the basal nodes of the phylograms. Recent inclusions of haplotypes from new sampling localities have only resulted in an increased number of distinct haplogroups (see Hansen et al. 2007a). However, in parts of the following discussion, focus is put on how the present observations match the proposed phylogenetic relationships in the *G. salaris* / *G. thymalli* species complex according to Hansen et al. (2003; 2006; 2007a) and Meinilä et al. (2004).

4.2 The *G. salaris* / *G. thymalli* species complex: what do we observe?

To date, the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) is the only marker allowing for a genetic discrimination of populations of *G. salaris* and *G. thymalli* (see e.g. Hansen et al., 2003, 2006, 2007a,b; Meinilä et al., 2004). Hansen et al. (2003) suggested that *G. salaris* and *G. thymalli* either are (i) two polytypic species, (ii) one polytypic species, or (iii) a complex of more than two sibling species, without favouring one hypothesis over the others due to the limited empirical data. Meinilä et al. (2004), in contrast, favoured the hypothesis of *G. salaris* and *G. thymalli* being a one-species complex based on the lack of monophyly of the mitochondrial haplotypes of the two species. The synonymisation is, however, not formally justified according to the International Code of Zoological Nomenclature (ICZN, 1999), and neither does it seem to have been accepted in the general literature in the field. The use of mtDNA sequence data in species delimitation is controversial, and some authors have argued that species should not be delimited based on these data alone (e.g. Moritz, 1994; Sites and Crandall, 1997; Puerto et al., 2001). Such data may e.g. be problematic in that all mitochondrial genes are

inherited as a single linkage group. As a result, any mismatch between gene and population histories caused by ancestral polymorphism or gene flow between species will simultaneously affect all mitochondrial genes (Moore, 1995).

In the *G. salaris* / *G. thymalli* species complex as well as in the entire genus *Gyrodactylus*, alpha taxonomy is traditionally based on morphology, predominantly on the morphometrics of the attachment apparatus (see e.g. Malmberg, 1970). However, in practice, species diagnostics also relies heavily on host identity. The frequently observed strict host specificity of *Gyrodactylus* species is taken as support for such a pragmatic approach (see e.g. Bakke et al., 1992, 2002, 2007). Therefore, in an “evolution *versus* observation” context as sketched above, morphometrics of the opisthaptor and species preference and -specificity constitute the major focus for methods related to “observation”.

4.2.1 Size and shape in taxonomy based on the morphology of opisthaptor hard parts

Size *versus* shape

According to the results in the present thesis (Paper III, V), in some instances in analyses of traditional morphometrics, size gives a stronger signal than shape. As also indicated in these papers, this might be problematic because size is highly dependent on environmental parameters such as e.g. temperature, whereas shape is less affected (Malmberg, 1970; Kulemina, 1977; Ergens and Gelnar, 1985; Mo, 1991a,b,c; Dmitrieva and Dmitriov, 2002; Dávidová et al., 2005; Paper II, present thesis). Although size in itself may contain a significant taxonomic signal, its variation caused by environmental parameters may be difficult to interpret. For the *G. salaris* / *G. thymalli* species complex, the results presented in Paper IV (present thesis) clearly indicated that differences in the temperature at sampling may alone be the cause for an observed extensive range-overlap between the two species.

Environmental impact on the morphology of hard-parts

Previously, there have been reports on variation in size but only subtle variation in shape over gradients of temperature for *Gyrodactylus* opisthaptor hard parts (Ergens and Gelnar, 1985; Malmberg, 1970; Kulemina, 1977; Mo, 1991a,b,c; Dmitrieva and Dmitriov, 2002; Dávidová et al., 2005). These studies were based on the traditional morphometry using linear point-to-point measurements or visual analysis or a combination of both. Although statistically significant differences in shape were found between groups from

different environmental regimes in the present work (Paper IV), the differences were considered minute and not biologically significant.

Marginal hooks and hamuli. From the findings in Paper IV (present thesis) it was suggested that for the marginal hooks and hamuli the length of the period of embryogenesis is the main reason for size differences among otherwise identical individuals of *Gyrodactylus*. One suggested explanation for this is that a longer duration of embryogenesis would result in longer period of growth for the opisthaptor hard-parts (Kulemina, 1977). Since *Gyrodactylus* have prolonged embryogenesis at lower temperature, duration of embryogenesis could thus explain why the hard-parts grow bigger at lower temperature and *vice versa*. Similarly, parasites from less favoured hosts could be expected to grow bigger opisthaptor hard-parts due to an increased period of embryogenesis. This could be explained by e.g. suboptimal food sources, immunological host reactions, or a combination of both (Paper IV, present thesis). Such an interpretation is also supported by recent experimental observations showing that the generation time of the same strain of *G. salaris* is longer on grayling than on Atlantic salmon (8.1 *versus* 5.5 days, respectively; O.G. Øvstaas, personal information). However, the reason why the size of the marginal hooks from the secondary hosts was not largest in the present data despite longer development period remains unsolved under the above tentative explanation.

Ventral bars. In the experiments with isogenic parasites grown at different temperatures (Paper IV, present thesis) it was found that the size of *G. salaris* ventral bars responded oppositional to the remaining hard-parts structures - i.e. that they grew bigger at higher temperatures. Hence, a hypothesis of ventral bars post-birth growth was therefore forwarded (Paper IV, present thesis). Jackson and Tinsley (1995) reported post-birth growth in the hamuli of *Gyrdicotylus gallieni*. However, it was only the “internal parts”, i.e. the roots of the hamuli that grew bigger in older individuals. According to Shinn et al. (1995a) age-related changes or growth could also be expected in the hamulus roots in *Gyrodactylus*. Since the ventral bar is also an “internal structure”, the post-birth growth hypothesis of this structure might seem plausible.

In the experiments presented in Paper IV (present thesis), the clones of *G. salaris* and *G. thymalli* were founded on just one single gravid, asexually reproduced, specimen in order to minimize variation caused by parameters other than environmental ones. Such a set-up was considered close to the optimal when analysing the range of phenotypic plasticity. However, the variation in the two clones was then not representing the range of variation in the populations they originated from. Therefore, the present results could not

render itself to any comprehensive taxonomical comparisons between *G. salaris* and *G. thymalli*. It should also be mentioned that in these analyses the population used to generate the isogenic strain of *G. thymalli* originated from river Trysilelva which during the experiments turned out to be most atypical among the *G. thymalli* populations when analysed by both traditional (Paper III, present thesis) and geometric morphometrics (Paper V, present thesis). Furthermore, this population also bears a different mitochondrial haplotype than the other Norwegian *G. thymalli* populations in this study (see Hansen et al., 2007a). The incongruent and to some extent complex results obtained with a rather simple experimental set-ups in Paper IV (present thesis) implies that studies of pure shape (i.e. geometric morphometrics) should have the potential to render taxonomically more reliable and useful information than size itself and size incorporated in linear measurements.

4.2.2 Host specificity - ecology and biology

One obvious emerging question is whether there are clear boundaries of host specificity in the *G. salaris* / *G. thymalli* species complex. Host species has always been an important ecological character in delimiting and assigning species in the genus *Gyrodactylus* (Bakke et al., 1992). One of the effects of this can be seen in the many species in the genus bearing names according to their type-host. Due to its pathogenicity towards Atlantic salmon, the host preferences and specificity of *G. salaris* has been studied extensively (see Bakke et al., 2002) whereas only limited data have been produced for *G. thymalli* in this respect. The Norwegian *G. thymalli* populations from the rivers Gudbrandsdalsågen and Glomma are so far the only ones that have been subjected to host preference tests (Soleng and Bakke, 2001; Bakke et al., 2002; Sterud et al., 2002). Their congeners throughout the genus are generally assumed to be host-specific (see Bakke et al., 1992, 2002). However, *G. salaris* appear to have a wider host-range than most other *Gyrodactylus* species (see Bakke et al., 1992, 2002). *G. salaris* is generally pathogenic, causing heavy infections on the east Atlantic group of Atlantic salmon but appear benign, causing mild infections on the Baltic salmon populations (Bakke et al., 2002; Dalgaard et al., 2003; 2004). There is, however, probably exceptions from this general rule as Bakke et al. (2004) demonstrated that the susceptibility of salmon from the Baltic river Indalsälva almost corresponded to that observed in control groups with east Atlantic salmon. Relatively little attention has been paid to possible variation in infectivity and reproductive potential among different

populations of *G. salaris* (Bakke et al., 2002). Lindenstrøm et al. (2003) described a variant of *G. salaris* (annotated Gx) from a rainbow trout culture in Denmark, which performed better on rainbow trout than on any of the experimentally infected Atlantic salmon stocks. This discovery complicated the picture of *G. salaris* as the malign species on one hand and *G. thymalli* as the benign parasite on the other. With the subsequent observations of yet another non-pathogenic *G. salaris* strain parasitizing rainbow trout in Denmark (Jørgensen et al., 2007) and one parasitizing Arctic charr in Norway (Paper II, present thesis), the picture was even further complicated. The results presented in Paper II (present thesis) demonstrated that the *G. salaris* population in Lake Pålbufjorden was not only non-pathogenic to Atlantic salmon, but also that both Arctic charr and rainbow trout were suitable hosts. This has direct practical implications on work concerning systematics. For example, in the *G. salaris* / *G. thymalli* species complex, leading work in establishing evolutionary relationships based on mtDNA *cox1* markers for extensive numbers of samples has been carried out by Hansen et al. (2003, 2006, 2007a) and Meinilä et al. (2004). However, in these studies, representatives of populations were basically assigned a species status according to their respective host-species. Thus, the taxonomic status of the OTU's in this systematics is based almost exclusively on identity of the host-species. Due to the complicated host preference situation as mentioned above, this situation is far from optimal. Furthermore, it is at conflict with the currently undisputed principle that type specimens of species appropriately described according to the International Code of Zoological Nomenclature constitute the reference material for species identification. To date, Paper III (present thesis) is the only reported work in which wild populations of *G. salaris* and *G. thymalli* have been compared with the respective type-materials, thus assigning samples to species according to their original descriptions.

Although renowned for their conserved morphology, gyrodactylids display great variation in their ecological traits. In addition to the above mentioned variation in host specificity and level of pathogenicity, life history traits and behaviour related to the ecology of their hosts, also show great variation (Paper I, present thesis). For example, one type of transmission strategy employed by individuals of *G. turnbulli*, is migration from recently dead hosts onto the water film (Cable et al. 2002). This behaviour is expected to increase the chances of parasites contacting a new host since the main host species of *G. turnbulli* Harris, 1986, the guppy (*Poecilia reticulata*), is a surface feeder. Contrarily, the results on behaviour of *G. salaris* in Paper I (present thesis) demonstrated that on the stream-dwelling Atlantic salmon they largely remained with their dead hosts. Thus, for *G.*

salaris, to remain with a dead host might be a specialized behavior attributed to a combination of the high risk related to transmission in running water and the increased likelihood of contacting a new host due to nipping or scavenging by a susceptible host (Paper I, present thesis). Unfortunately, similar knowledge concerning other closely related members of the *G. salaris* / *G. thymalli* species complex is presently not available. Knowledge of the potential variety in the ecology and biology of gyrodactylids could shed light on interesting similarities / dissimilarities in the evolution of ecological traits among relevant taxonomical groupings or OTU's. It could also shed light on potential differences in the rate of evolution in ecological traits compared to other traits that are considered taxonomically informative, like e.g. morphology of the marginal hooks or *cox1* sequences.

From an Atlantic salmon conservation-management perspective, it is not the parasite itself but the infectious disease it causes that is the focus of concern. Gyrodactylosis is regarded as one of the most important threats to wild European Atlantic salmon populations, and *G. salaris* is listed by the 'Office International des Épizooties' (OIE) in the Aquatic Animal Code (OIE, 2004). At present, however, the disease is linked to the species taxonomically referred to as *G. salaris*. This is for management purposes not satisfactory, as the results in the present thesis (Paper II) have demonstrated the existence of a non-virulent *G. salaris* strain (lake Pålbufjord) for which Atlantic salmon is resistant although the parasite apparently is very closely related both by means of morphometry and *cox1* sequence data, to the virulent *G. salaris* populations of the same haplotype parasitizing Atlantic salmon in the neighbouring rivers Drammenselva, Lierelva and Lærdalselva (sequences from Hansen et al., 2003).

Known pathogens are important in a conservation-management perspective. However, it should also be kept in mind that rapidly evolving groups of parasites may have a potential to become pathogens. The results presented in Paper I (present thesis), might give indications of a mechanism promoting the evolution of pathogenicity in this group: in the interaction *G. salaris* - Norwegian Atlantic salmon, host death is a frequent end-point of the infection. As discussed in Paper I (present thesis), transmission from a dead host via post-mortem cannibalism is likely to be a very successful transmission strategy in rivers. Transmission via dead hosts may thus act as a positive feedback mechanism promoting the evolution of pathogenicity, at least in a short-term perspective. Furthermore, host switches are considered common for species of *Gyrodactylus* in general (see Ziętara and Lumme, 2002; Huyse and Volckaert, 2002, 2005) and even more so for the *G. salaris* / *G. thymalli* species complex as e.g. indicated by the diversity in host preference (see e.g. Bakke et al.,

2002; Lindenstrøm et al., 2003; Jørgensen et al., 2007; Paper II, present thesis) and the highly polymorphic nature in the *cox1* gene (Hansen et al., 2003; Meinilä et al., 2004). In a management context, therefore, the findings of several apparently non-pathogenic strains of *G. salaris* in Denmark and Norway (see e.g. Lindenstrøm et al., 2003; Jørgensen et al., 2007; Paper II, present thesis) are all but comforting since a transmission strategy via dead hosts combined with frequent host switches will potentially promote the evolution of pathogenicity. Ultimately, this could also be an avenue for a significant expansion of the geographical range of pathogenic variants of *G. salaris*.

4.3 An all-inclusive view in the *G. salaris* / *G. thymalli* species complex

Following Meinilä et al. (2004), the lack of monophyly of *G. salaris* and *G. thymalli*, based on *cox1* sequences indicate that they should be considered the same species with *G. thymalli* as the junior synonym. According to Bakke et al. (2007), this may also be the case for *G. bohemicus*. However, based on the principal of conservatism, and taken the contradictory signals also from potentially phylogenetically informative markers, Bakke et al. (2007) conclude to maintain *G. salaris*, *G. thymalli* and *G. bohemicus* as separate species (quote) “until further clarification using new molecular markers, knowledge of the impact of different microenvironment factors on *Gyrodactylus* morphology and hybridisation experiments”. The present results do not contradict this conclusion. However, the results in this thesis provided some important knowledge to this difficult task: information that should also be useful in an all-inclusive view as sketched up in chapter 1.2.

Morphology. Since the works of Malmberg (1957, 1970, 1993), marginal hooks have been considered a taxonomically informative structure among the opisthaptor hard-parts. The shape of the marginal hooks was in the present work (Paper V) found to be a taxonomically important feature. In the analyses, there was, however, no indication of separation of the population parasitizing Arctic charr in lake Pålbufjord from the remaining *G. salaris* populations parasitizing Atlantic salmon and rainbow trout. On the other hand, the resolution provided by the present analyses of hamuli-shape indicated that the populations from lakes Pålbufjord and Bullaren (both *G. salaris*) as well as from river Trysilelva (*G. thymalli*) could be regarded as somewhat aberrant. The analyses of traditional linear morphometric measurements (Paper III, present thesis) were not consistent with grouping based on host species: the population from river Trysilelva was

closer to *G. salaris* than to *G. thymalli* (Paper V, present thesis). However, since size was found to be highly dependent on temperature, whereas shape less so (Paper IV, present thesis), shape is expected to potentially capture taxonomically more reliable and useful information than size incorporated in linear measurements.

Ecology and biology. Host preference and host specificity are observed to vary between *G. salaris* and *G. thymalli* (see Soleng and Bakke, 2001; Bakke et al., 2002; Sterud et al., 2002). Sterud et al. (2002) considered these ecological differences to be significant and concluded that *G. salaris* and *G. thymalli* are two distinct species. The results presented in Paper II indicated that the *G. salaris* population in lake Pålbufjord was almost unable to reproduce on Atlantic salmon, but that Arctic charr and rainbow trout are suitable hosts. Corresponding results of *G. salaris* strains for which Atlantic salmon stocks could not be regarded as primary host have been reported in Denmark by Lindenstrøm et al. (2003) and Jørgensen et al. (2007). However, an unexpected and highly significant observation in Paper II (present thesis) was that two *G. salaris* populations with identical *cox1* haplotypes had diametrically different host preference: the *G. salaris* strain from river Drammenselva (haplotype F according to Hansen et al. (2003)) was pathogenic to Atlantic salmon whereas *G. salaris* from lake Pålbufjord (also haplotype F) was almost unable to reproduce on the same host stock. Accordingly, host preference and specificity should no longer be an overall and conclusive argument for considering *G. salaris* and *G. thymalli* two species.

How the *G. salaris* / *G. thymalli* species complex should eventually be grouped on a species level is still an open question. In a management perspective there is a need for a nomenclatural system reflecting significant biological and ecological differences. Based especially on e.g. the results in Paper II (present thesis) two *G. salaris* populations with identical *cox1* haplotypes but diametrically different host preference, is difficult to coordinate when delimiting species within the nomenclatural concept of the International Code of Zoological Nomenclature (ICZN, 1999). Therefore, a question whether there is reason to apply other relevant taxonomic levels may be raised. The formal category of subspecies is the lowest rank defined by the Code of Zoological Nomenclature. It is generally referred to as a taxonomic subdivision of a species consisting of an interbreeding, usually geographically isolated population of organisms. For potentially incipient species like *G. salaris* and *G. thymalli*, another possible level is semispecies. These are populations that have acquired some attributes of species rank: organisms constituting a borderline between species and a subspecies. Bakke et al. (2007) consider *G. salaris* and *G. thymalli*

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a case of incipient speciation with the sibling taxa representing either two semispecies (or one polytypic species) or a superspecies (several sibling species), reproductively more or less isolated by host preference. One possible scenario could be to apply the level of semispecies or subspecies to capture the potential differences in host preference under defined conditions. Methods providing further insight into patterns of genetic exchange are likely to be available in the near future, e.g. through development of high-resolution nuclear markers. The question of which taxonomic level is relevant in this context should await the development of such methods.

5 Future perspectives

The assessment of biodiversity through molecular tools has provided important insights for the genus *Gyrodactylus* in general and the *G. salaris* / *G. thymalli* species complex in particular. Nevertheless, the present work documents that a pure DNA-based taxonomy do not satisfactorily cope with the particular taxonomic problems of the *G. salaris* / *G. thymalli* species complex. Species definition exclusively based on mtDNA sequence data fails to reflect the significant differences in host preferences and pathogenicity (see e.g. Hansen et al. 2007b). However, there is at least theoretically no absolute ecological boundary between *G. salaris* parasitizing Atlantic salmon and Arctic charr and *G. thymalli* parasitizing grayling. Therefore, Bakke et al. (2007) suggested the *G. salaris* / *G. thymalli* species complex as an example of ongoing but not yet completed speciation following host switches. During this process the parasites are developing new patterns in host preferences. The major taxonomic question under such a scenario would be to agree on criteria as to when a speciation process is accomplished. Taken the rather unique situation of studying ongoing or very recent speciation the *G. salaris* / *G. thymalli* species complex provides an excellent opportunity to extend the knowledge on speciation processes in general.

On a quite general level the present study has shown that the evolutionary rates vary for different traits that may be used as markers and determinants in taxonomic and systematic studies. As such, also this study illustrates the discordance between species boundaries inferred from different pattern-based criteria and therefore underscores the dichotomy between evolutionary groups and the categories that we use to define them (Hey, 2001). The presented results indicate that taxonomic revisions in the *G. salaris* / *G. thymalli* species complex based on a single species criterion, despite advantages of comparability, are unlikely to lead to an overall satisfactory delimitation of species.

The reasons why some populations of *G. salaris* can be so damaging, while the closely related congener *G. thymalli* is not, still remain obscure. Also, the mechanisms behind the differences in host preference and host specificity are still not understood. Certainly, much additional research is needed to better understand these mechanisms and, thus, also contribute to knowledge on speciation.

In the present analytical approach to study morphology, shape descriptors proved to work well. It is reasonable to assume that in future studies the application of geometric shape descriptors is justified. Whether sliding semilandmarks, as used in the present

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studies, is the optimal choice or if other variants perform better cannot be answered today and may differ from case to case.

All methods applied so far for assessing the taxonomy and systematics of the *G. salaris* / *G. thymalli* species complex document the importance of evolutionary relationship as the absolute basic grouping criterion. It is, however, concluded that the knowledge of phylogenetic relationships in the species complex should be supplemented with information on morphology and even more important, on ecology, when it comes to defining the boundaries between the taxa, whether on a species level or below.

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