Master's Thesis

THE KINORHYNCHA OF SOUTHERN NORWAY

ESPEN STRAND

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2014

The Kinorhyncha of Southern Norway

Espen Strand

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Preface

Originally, my wish for the subject of my Master's thesis in marine biology was marine mammals, particularly whales. I wished to do something on their behavior, be it social behavior, migratory patterns or something similar. How, then, did I end up with a subject on the completely opposite side of the spectrum? After all, the microscopic Kinorhyncha bear little resemblance to the giants I originally wished to work on.

For starters, marine mammals are a difficult group to work with, as they can be elusive, and the sample size needed to find statistically significant results makes it difficult to conduct a full project over the <two years intended for a master's degree. The best I could hope for was to join a group of scientist already conducting a project and using some data already obtained. I looked for a while, and had no luck finding any possible supervisors on this particular subject. Finally, I sent an email to professor Lutz Bachmann at the Natural History Museum of Oslo, inquiring about the possibilities of him supervising me on a thesis about marine mammals. The reply I received was optimistic, and a meeting was scheduled.

On this meeting however, I found out that the only project on whales I could be given was a project on ancient genetic material. While this project did sound interesting, the prospect of little to no fieldwork made me a bit disconcerted. Additionally, however, Lutz proposed a completely different project, which seemed to be more or less based on data obtained from fieldwork. This project was looking at the species diversity of the animal phylum Kinorhyncha in Norway, something that had never been done before. The idea of a project where we went in with little to no prior knowledge fancied my interest, and the prospect of maybe being able to further the knowledge in my field of choice significantly made me make up my mind.

My thesis on this subject would be part of a larger project focusing on the entire Norway, with my focus staying on the southern coast.

Acknowledgments

The thesis is part of the Norwegian species project «Kinorhyncha, a poorly known and neglected phylum» granted by artsdatabanken.no to Lutz Bachmann

I would like to thank my supervisor Lutz Bachmann for the possibility to participate in the Norwegian Kinorhyncha species project, for valuable input along the way, and for organizing most of my fieldwork.

Jonas Thormar, my co-supervisor, has helped immensely with Kinorhyncha morphology, with training me on sampling methods and locating the animals in the samples and teaching me morphological structures.

Eve Zeyl, the technician on the project, has been invaluably helpful on every step on the way. Her help on genetics, both on performing the procedures and interpreting the results has been immensely helpful. This, in addition to input along the way, both on the project itself and in the process of writing this thesis, has made her one of my most valuable supporters, of which I am extremely grateful.

Additionally, my thanks go out to Karl Ugland and Rita Amundsen from the department of marine biology at the University of Oslo for helping me organize some of my earlier fieldwork, and allowing my presence on their fish expeditions. Similarly, the crew on the F/F Trygve Braarud helped with finding suitable sampling locations on said excursions.

I would also like to thank the people at the SEM- laboratory at Blindern, for allowing me to use their facilities for this project, and for teaching me how to use their scanning electron microscope.

Big thanks to my family for their continued support, and to my friends and co-students for fantastic years together.

Finally, a massive thank you to my dear Monica, without whom I do not know what I would have done. Thank you for providing love, support and the distractions necessary to keep me sane, and thank you for being patient with me during this stressful period. It is finished now; we finally have time to spend together!

1. Abstract

The phylum Kinorhyncha is, despite being known for more than 150 years, one of the lesser-studied animal phyla in the world, and knowledge of Norwegian Kinorhyncha biodiversity is practically non-existent. Data is presented from the first strategic Kinorhyncha sampling in Norway, with focus on the southern coast, most of which are from shallow waters (<100 meters deep). Specimens were examined morphologically, and their DNA were sequenced. The survey indicates great Kinorhyncha diversity within at least five different genera, some of which include several different species. Most speciose from this sampling is the homalorhagid genus Pycnophyes. Also documented are the cyclorhagid genera Echinoderes, Semnoderes, Centroderes, Condyloderes and Tubulideres.

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3. Introduction

3.1. General introduction

Kinorhyncha is a phylum of microscopic, free-living, benthic, interstitial animals that can be found in marine and inland sea habitats (e.g. Black Sea (Higgins and Adrianov, 1991)) all over the world. The name of the phylum originates from the Greek word *Kineo*, meaning to move or to stir; and the suffix *-runchus*, meaning snout. The name refers to the way the animals move in the sediment (Neuhaus, 2013). They perform their movement by continuously ejecting and retracting their proboscis (see figure 1), anchoring the scalids of their head in the sediment, and pulling the rest of the body forward (Neuhaus and Higgins, 2002). In English, the phylum is also known as mud dragons, likely due to the spinose middorsal protrusions we can find in several of the species (see figures 2 and 3).These are reminiscent to those often portrayed on dragons from medieval and ancient mythology, as well as more recent fantasy literature. Their general, elongated shape is also similar to the traditional Oriental dragons.

Despite the fact that Kinorhyncha were discovered more than 150 years ago (Dujardin, 1851), nothing was known of their phylogeny or anatomy until Karl Zelinka (1928) published his monograph on the phylum. In this work, he described 48 species of Kinorhyncha, but several of them were later revised. One example is the sexual dimorphic species *Paracentrophyes quadridentatus,* which he originally described as two species (*Pycnophyes quadridentatus* and *P. flagellatus;* female and male, respectively) (Higgins, 1983; Sørensen et al. 2010).

To this day, the phylum remains poorly studied. There are currently approximately 205 species of Kinorhyncha described from adult specimens, and some 50 described from juveniles (Sørensen, 2013b). Several new species are described every year, with a total of 19 in 2013 (Dal Zotto et al., 2013; Herranz and Pardos, 2013; Herranz et al., 2013; Sánchez et al., 2013; Sørensen, 2013a; Sørensen et al., 2013; Thomsen, 2013). Many scientists performing meiobenthic surveys denote Kinorhyncha presence merely as *Kinorhyncha sp.*, resulting in a knowledge gap when it comes to Kinorhyncha biodiversity.

The Kinorhyncha seem to inhabit very diverse habitats; they can be found at all depths, ranging from the deep sea at 7800 meters (Danovaro et al., 2002) up to the surface, even extending into the intertidal zone (Zelinka, 1928). They have a global distribution, and can be found in all marine habitats in the world, and they are often reported to have a high tolerance for variations in sediment size, salinity and hypoxia levels (Horn, 1978; Modig and Ólafsson, 1998).

3.2. Morphological characteristics

All described adult specimens in the phylum have 11 trunk segments, a neck segment called the placid, and a head segment (Zelinka, 1928). Traditionally the head was described as segment 1, the placid as segment two, and the trunk as segments 3-13. However, Neuhaus and Higgins (2002) proposed a change to this. Accordingly, today the first trunk segment is regarded as segment 1, whilst the head and placid are described independently. For this reason, it is important to keep in mind that earlier literature differ in their description of the specimens. This thesis will follow the terminology proposed in Neuhaus and Higgins (2002).

The phylum consists of two orders, Homalorhagida (Figure 2) and Cyclorhagida (Figure 3), which are easily distinguishable by their overall body shape. Seen from a top-down perspective, the Homalorhagida have a cigar-like shape, while the Cyclorhagida tend to narrow a lot more towards their posterior few segments. In cross sections, most Cyclorhagida are rounded in an arc dorsally, and flattened ventrally. The Homalorhagida often have a slight groove ventrally, and they appear almost triangular in cross sections with the ventral side somewhat indented. The spines are also often good characters to look at, as Cyclorhagida often have lateral spines, a characteristic that Homalorhagida lack. Trunk size is also an important difference; Homalorhagida can easily be twice the size of Cyclorhagida, and the largest known homalorhagid species, *Pycnophyes greenlandicus* reaches a trunk length of more than 1000µm (Higgins and Kristensen, 1988). Comparably, the largest Cyclorhagida, *Echinoderes rex* was described as up to 528 µm in length (Lundbye et al., 2010).

Male and female Kinorhyncha are often difficult to distinguish. The main difference is the presence of penile spines in males (see figures 2 and 3). Penile spines appear in two or three

pairs on segments 10 and 11. Other than the presence or absence of these penile spines, there is little known external sexual dimorphism in most Kinorhyncha species.

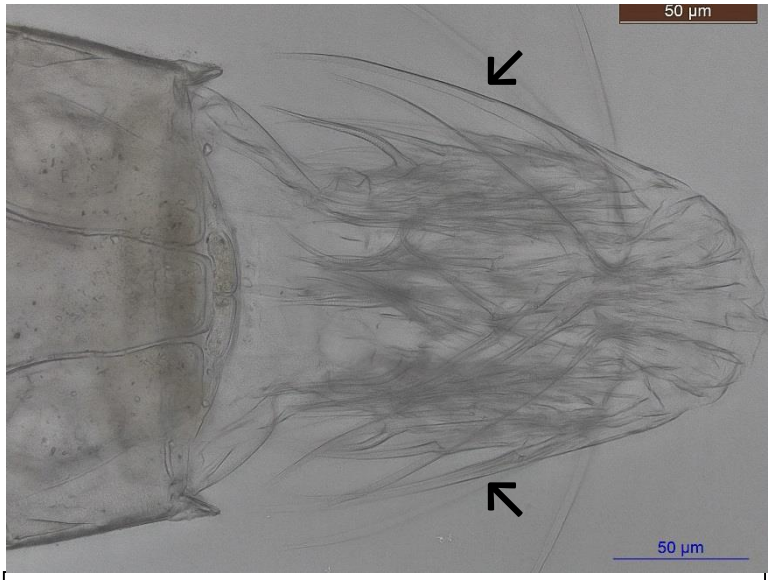

Figure 1: Ejected proboscis of *Pycnophyes sp.* Scalids (black arrows) are used as anchoring in the sediment for locomotion.

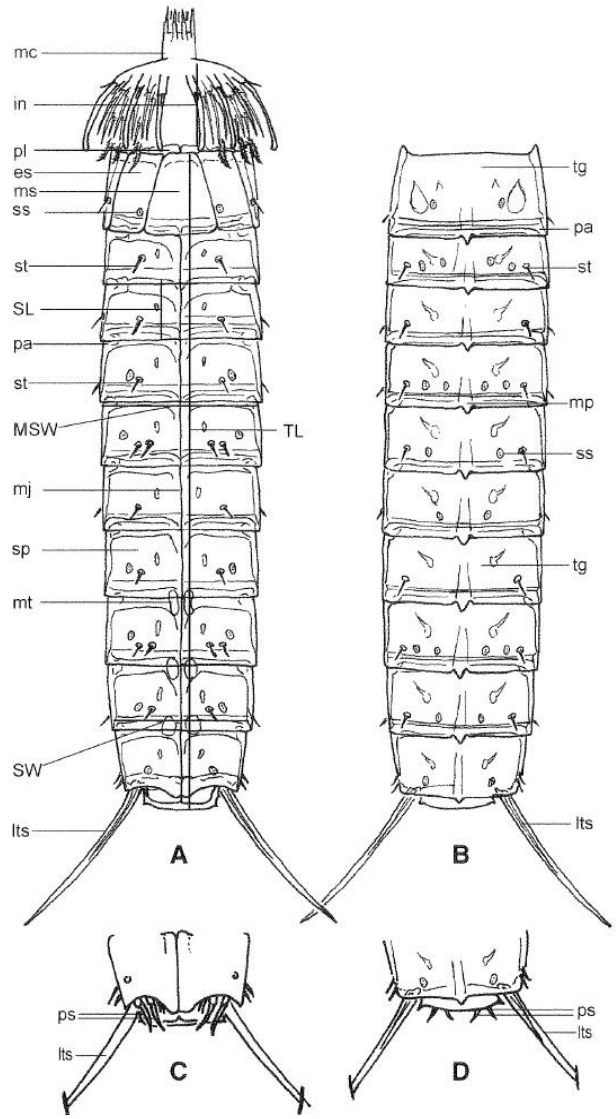

Figure 2: Homalorhagid morphology exemplified with the genus Pycnophyes. **A:** Female, ventral view. **B:** Female, dorsal view. **C:** Male, ventral view; terminal segment. **D:** Male, dorsal view; terminal segment. **Abbreviations**: **es**, episternal plate; **in**, introvert; **lts**, lateral terminal spine; **mc**, mouth cone; **mj**, mid sternal junction; **mp**, middorsal process; **ms**, mid sternal plate; **MSW**, maximum sternal width; **mt**, mid ventral thickenings; **pa**, pachycyclus; **pI**, placid; **ps**, penile spines; **SL**, segment length; **sp**, sternal plate; **ss**, sensory spot; **st**, seta; **SW**, standard width; **tg**, tergal plate.

Sørensen and Pardos (2008), redrawn from Kristensen and Higgins (1991).

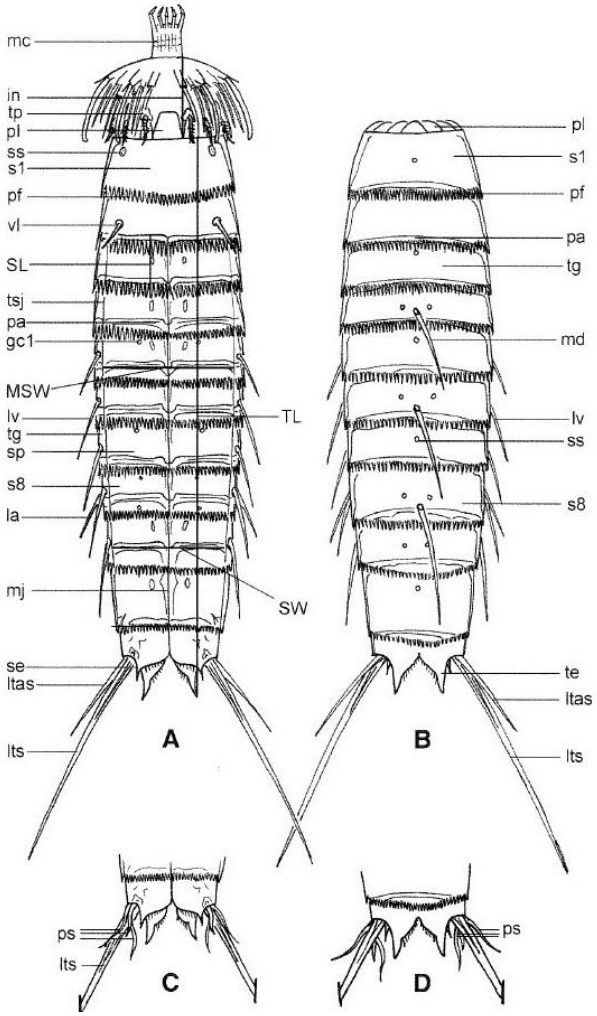

Figure 3: Cyclorhagid morphology exemplified with the genus Echinoderes. **A:** Female, ventral view. **B:** Female, dorsal view. **C:** Male, ventral view; terminal segment. **D:** Male, dorsal view; terminal segment. **Abbreviations: gc1**, glandular cell opening type 1; **in**, introvert; **la**, lateral accessory spine; **ltas**, lateral terminal accessory spine; **lts**, lateral terminal spine; **lv**, lateroventral spine; **mc**, mouth cone; **md**, middorsal spine; **mj**, midsternal junction; **MSW**, maximum sternal width; **pa**, pachycyclus; **pf**, pectinate fringe; **pl**, placid; **ps**, penile spines; **s**, segment (followed by segment number); **se**, sternal extension; **SL**, segment length; **sp**, sternal plate; **ss**, sensory spot; **SW**, standard width; **te**, tergal extension; **TL**, trunk length; **tg**, tergal plate; **tp**, trichoscalid plate; **tsj**, tergosternal junction; **vt** ventrolateral spine.

Sørensen and Pardos (2008), redrawn from Kristensen and Higgins (1991).

3.3. Aims of the current work

The aims for the present work is to provide a first survey of the Norwegian Kinorhyncha biodiversity. Prior to this project, very few studies had been done on the Kinorhyncha diversity in Norway. Merely a handful of scientists have ever recorded the presence of Kinorhyncha in Norwegian waters, including Svalbard (Schepotieff, 1907; Adrianov, 1995). Additionally, these studies covered only a limited geographic range. There has never been a full systematic Kinorhyncha survey performed in Norway.

As documented in surveys recently performed in the Iberian Peninsula (Sánchez et al. 2011; Sánchez et al. 2012; Sánchez et al. 2014), intensive surveying campaigns can yield significant results. During these surveys of the waters around the peninsula, ten Kinorhyncha species new to the region and eight undescribed species were discovered. Similarly, a strategic surveying of the Korean coast has been performed (Sørensen et al., 2010a; Sørensen et al., 2010b; Sánchez et al., 2013; Sørensen et al., 2013).

Until now only very few studies used molecular methods for addressing the taxonomy, systematics, and phylogeny of the Kinorhyncha (exception being Yamasaki et al., 2013). However, a reference database of DNA barcodes may be very helpful for species identification based on molecular markers. The current study attempts to take the initial steps towards a DNA barcoding of Norwegian Kinorhyncha.

4. Materials and methods

4.1. Sampling methods

Sediment sampling methods were adjusted according to depth; for shallow waters (<1m) mud and sand samples were manually collected directly in a bucket. Sediments from deeper waters were sampled by using a small and manually manageable van Veen- Grab, which collects approximately 0,5-1 kg sediment at a time. The grab, attached to a rope, can be lowered into the sea either from a pier or a boat. The locking mechanism of the grab will release when it hits the sea floor, thus snapping the grab shut and capturing sediment that can subsequently be brought to the surface.

The preferred method of extracting Kinorhyncha from the mud has been Robert Higgins' "Bubble-and-Blot method" (1964). This method takes advantage of the hydrophobic nature of the Kinorhyncha's cuticle. Decanting a bucket containing approximately 1-3kg sediment and a few litres of seawater into a clean bucket with some force creates turbulence. After doing this eight to ten times, many of the Kinorhyncha, along with certain other meiofauna species can be collected from the water surface, where they are held by the surface tension. They are collected by placing a sheet of paper on the water, and subsequently washing them from the paper into a collection tube using 96% ethanol for temporary storage.

4.2. Sampling locations

For the purpose of this thesis, the southern Norwegian coast was defined as ranging from Hvaler as the southeastern limit to Mandal as the western limit. The focus for sampling has been in Hvaler and the Oslo Fjord. This was because the Hvaler area soon turned out to hold great Kinorhyncha diversity, and because the close proximity of the Oslo Fjord allowed for field trips on a relatively short notice. Both locations also provided opportunities to resample at different times of the year.

Sample number	Date	Coordinates	Location name	Depht
1	18.06.2013	59.598191,10.654211	Hvitsten	< 1m
2	18.06.2013	59.598191,10.654211	Hvitsten	2.5m
3	18.06.2013	59.518727,10.681847	Son	< 1m
4	18.06.2013	59.515871,10.678794	Son	20-22m
5	18.06.2013	59.421639,10.611982	Alby	< 1m
6	18.06.2013	59.421639,10.611982	Alby	< 1m
7	18.06.2013	59.488285,10.644099	Nes	< 1m
8	18.06.2013	59.426570,10.642168	Moss	2.5m
9	19.06.2013	59.091893,11.233881	Sponvika	ca 1m
10	19.06.2013	59.104133,11.222357	Stensvik	ca 4m
11	19.06.2013	59.104133,11.222357	Stensvik	< 1m
12	19.06.2013	59.177644,11.160759	Ullerøy	ca 9m
13	19.06.2013	59.078871,10.938191	Hvaler	2.5m
14	19.06.2013	59.081824,10.933734	Hvaler	17-18 m
15	19.06.2013	59.084593,11.069318	Hvaler	7m
16	19.06.2013	59.083515,11.068793	Hvaler	5m
17	19.06.2013	59.038030,11.052114	Hvaler	40 cm
18	21.06.2013	59.731485,10.299683	Lier	< 1m
19	21.06.2013	59.710712,10.376587	Hyggen	< 1m
20	21.06.2013	59.611561,10.415211	Klokkarstua	< 1m
21	21.06.2013	59.610280,10.415597	Klokkarstua	< 1m
22	21.06.2013	59.540301,10.555029	Tofte	< 1m
23	21.06.2013	59.571367,10.617735	Filtvet	< 1m
24	21.06.2013	59.641718,10.606009	Sætre	< 1m
25	21.06.2013	59.664284,10.600605	Sætre	< 1m
26	21.06.2013	59.762826,10.498553	Nærsnes	< 1m
27	01.07.2013	59.515871,10.678794	Son	20- 25m
28 and 29	01.07.2013	59.426570,10.642168	Moss	2.5m
30 and 31	01.07.2013	59.177644,11.160759	Ullerøy	ca 9m
32, 33, 34, 35 and 37	01.07.2013	59.084593,11.069318	Hvaler	7m
38 and 39	01.07.2013	59.081824,10.933734	Hvaler	17-18 m
40	02.07.2013	59.044195,10.043287	Larvik	12m
41	02.07.2013	59.033754,10.066585	Larvik	2m
42	02.07.2013	59.037480,10.226833	Tjodalyng	3 m
43	02.07.2013	59.125556,10.224444	Sandefjord	2m
44	02.07.2013	59.350827,10.472003	Åsgårdstrand	3m
45	02.07.2013	59.481422,10.331848	Holmestrand	2m
46	04.07.2013	59.125556,10.224444	Sandefjord	2m
47	04.07.2013	58.999423,10.043176	Stavern	
48 and 49	04.07.2013	59.106300,9.702123	Porsgrunn	2m
50	04.07.2013	59.046278,9.701570	Stathelle	11-12 m

51	04.07.2013	58.953807,9.617322	Stathelle	10-11m
52	04.07.2013	58.868591,9.416505	Kragerø	ca 27m
53	04.07.2013	58.622425,8.931989	Tvedestrand	ca 11m
54	04.07.2013	58.339185,8.596682	Grimstad	ca 17m
55 and 56	05.07.2013	58.008985,7.546211	Mandal	ca 11m
57	05.07.2013	58.099096,7.933539	Kristiansand	ca 7m
58	05.07.2013	58.255069,8.393616	Lillesand	13-14m
59	05.07.2013	58.469722,8.798856	Arendal	6-7m
60	05.07.2013	58.718056,9.240654	Risør	10m
61	12.07.2013	59.890465,10.634592	Snarøya, Rolfsflua	ca 2m
62	12.07.2013	59.833945,10.476895	Vettre	ca 4.5 m
63	12.07.2013	59.759929,10.501941	Nærsnes	ca 2,5 m
64	12.07.2013	59.667264,10.571312	Sætre	
97	20.08.2013	59.731057,10.727343	Vinterbro	28m
98	20.08.2013	59.722734,10.718889	Vinterbro	20m
99	20.08.2013	59.724800,10.725807	Vinterbro	14m
100	20.08.2013	59.723943,10.727854	Vinterbro	15m
101	22.08.2012	59.085717,10.938520	Hvaler	24m
102	22.08.2013	59.088211,10.940979	Hvaler	25m
103	22.08.2013	59.069448,10.927181	Hvaler	ca 16m
104	22.08.2013	59.056658,10.909055	Hvaler	ca 5m
105	22.08.2013	59.043521,10.902272	Hvaler	32m
106	22.08.2013	59.082090,10.931602	Hvaler	< 1m
107	26.08.2013	59.880484,10.762466	Oslo, Bunnefjord	ca 19m
108	26.08.2013	59.876207,10.744139	Oslo, Bunnefjord	33 m
109	26.08.2013	59.877528,10.721705	Oslo, Bunnefjord	46m
110	26.08.2013	59.869056,10.698920	Oslo, Bunnefjord	74-77m
111	26.08.2013	59.867385,10.678897	Oslo, Bunnefjord	20-27m
112	27.08.2013	59.729460,10.567144	Oslofjord	ca 41m
113	27.08.2013	59.725314,10.561987	Oslofjord	100-130m
114	27.08.2013	59.715482,10.545422	Oslofjord	ca 40m
115	27.08.2013	59.718100,10.539855	Oslofjord	ca 80 m
116	27.08.2013	59.717418,10.530491	Oslofjord	ca 90m
117	27.08.2013	59.732000,10.523169	Oslofjord	ca 20m
152	27.09.2013	59.086807,10.940888	Hvaler	14m
153	27.09.2013	59.095497,10.971236	Hvaler	40m
154	27.09.2013	59.090675,10.989088	Hvaler	ca 38m
155	27.09.2013	59.063252,10.963991	Hvaler	ca 58m
156	27.09.2013	59.030467,10.937157	Hvaler	ca 40m
157	27.09.2013	59.048475,10.910099	Hvaler	18m
158	27.09.2013	59.081761,10.935523	Hvaler, Sand Marina	14m
159	07.06.2013	59.07884,10.78835	Hvaler, Seikrakk	10-20m
160	07.06.2013	59.07884,10.78835	Hvaler, Seikrakk	10-20m
161	07.06.2013	59.07884,10.78835	Hvaler, Seikrakk	10-20m

162	07.06.2013	59.07884,10.78835	Hvaler, Seikrakk	10-20m
163	19.02.2013	59.881667,10.646167	Lysakerfjorden	80m
164	02.04.2013	59.870117,10.604517	Snarøya	31m
165	02.04.2013	59.85645,10.56315	Oslofjorden	34m
166	02.04.2013	59.843333,10.578667	Oslofjorden	71m
167	03.04.2013	59.755217,10.54385	Oslofjorden	33m
168	03.04.2013	59.73715,10.54385	Oslofjorden	44m
169	03.04.2013	59.711283,10.5365	Oslofjorden	110m
170	22.04.2013	59.6518,10.620767	Oslofjorden	83m
171	22.04.2013	59.633483,10.617683	Oslofjorden	150m
172	22.04.2013	59.623883,10.623417	Oslofjorden	195m
173	07.05.2013	59.670467,10.6088	Oslofjorden	98m
174	07.05.2013	59.641267,10.608017	Oslofjorden	119m
175	07.05.2013	59.6025,10.6239	Oslofjorden	152m
176	02.10.2013	59.89024,10.706461	Lindøya	1m
177	02.10.2013	59.891639,10.698035	Nakholmen	8m
178	02.10.2013	59.898883,10.730760	Hovedhøya	4m
179	02.10.2013	59.901802,10.740802	Vippetangen	ca 7m
180	02.10.2013	59.886450,10.724897	Gressholmen	ca 5m
181	04.10.2013	59.907862,10.697292	Bygdøy	4m
182	04.10.2013	59.904226,10.701212	Bygdøy	10m
183	04.10.2013	59.905921,10.719406	Aker Brygge	19m
188	07.02.2014	59.516389,10.679152	Son	20m
189	07.02.2014	59.426570,10.642168	Moss	2.5m
190	07.02.2014	59.177644,11.160759	Ullerøy	ca 9m
191	07.02.2014	59.084603,11.06924	Hvaler	7 m

Table 1: Dates, locations and depths of samplings for Kinorhyncha performed on the southern Norwegian coast. Samples omitted are from other locations in Norway, performed for the Kinorhyncha species project. Depths denoted as <1m refer to beach samplings.

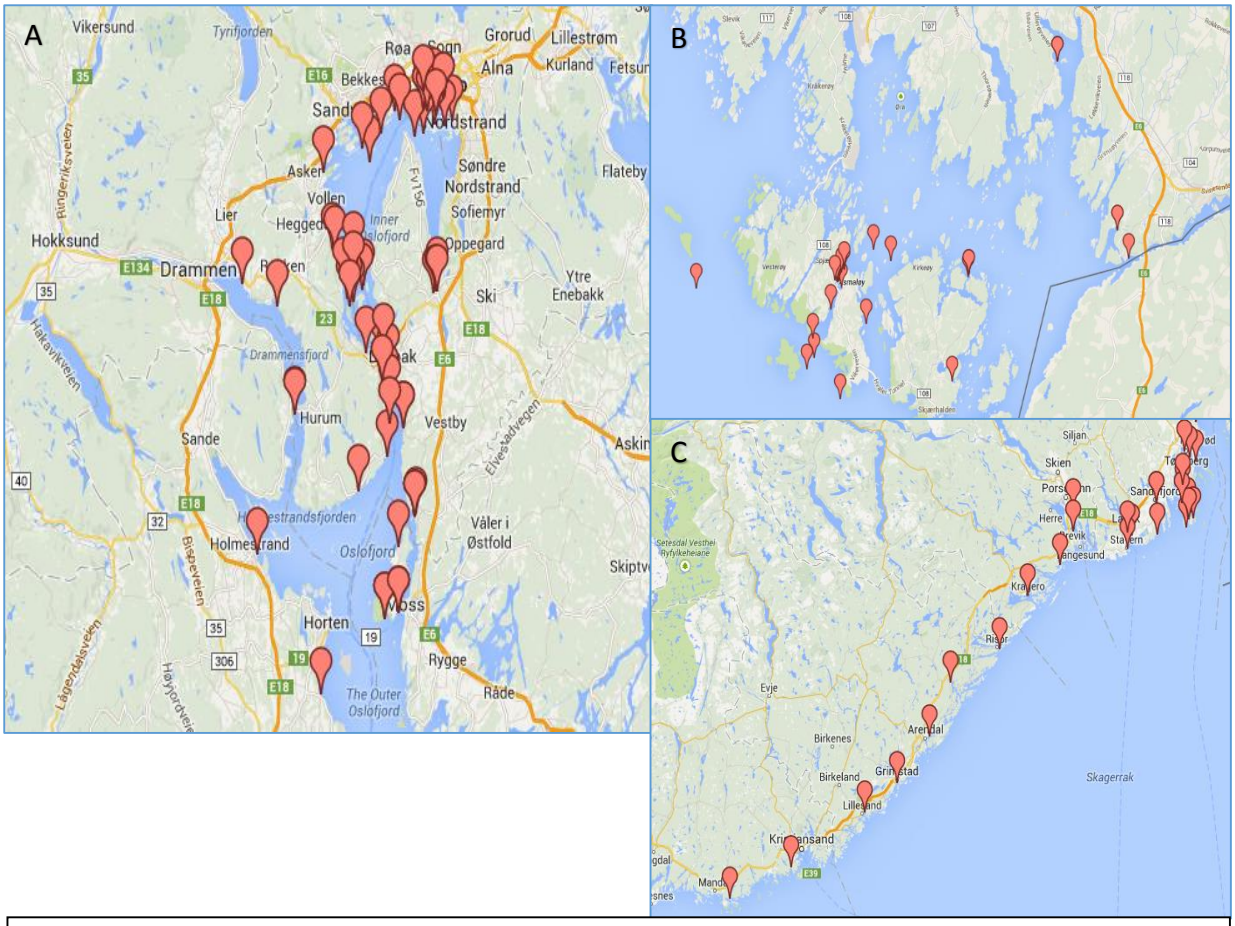

Figure 4: Maps of locations in southern Norway where Kinorhyncha samplings were performed. **A:** The Oslo Fjord. **B:** The Hvaler region. **C:** The coastline from Tønsberg to Mandal.

4.3. Sample processing

The meiofauna samples were usually examined within one week of sampling with a stereomicroscope. The recovered Kinorhyncha were roughly sorted by order, as the Cyclorhagida and the Homalorhagida are easily distinguishable even at low magnification. Some exuvia were included, despite being unusable for any morphologic species identification. The reason they were included was to test if they could be usable for molecular analyses. All specimens were individually labelled and databased.

4.4. Morphology

After the initial sorting, the Kinorhyncha specimens were examined with a LEICA DM6000B light microscope mounted with a LEICA DFC420 digital camera (LEICA: Solms, Germany). The standard approach to mounting Kinorhyncha for photography is to mount them in the non-fluorescent glycerol based gel Fluoromount G® (Southern Biotechnology Associates, Inc., USA), as recommended in Sørensen and Pardos 2008. This was also done for some specimens, but since the specimens were to be used for genetic analyses, the majority of them were not permanently mounted. The two approaches for temporary mounting were to put the specimens in water or in an SDS solution. Both methods would preserve DNA integrity. However, there was a risk of the specimens drying out if kept too long for the morphological analyses in the microscope's concentrated light and heat.

In the SDS solution, the Kinorhyncha specimens would appear more transparent in micrographs, allowing easier analysis of subcuticular structures. Transparency of the specimens was further increased if incubated in a wet chamber for a few days prior to inspection. The need to work fast leaves little time to perform characteristics diagnosis at the time. Thus, all morphological analyses of Kinorhyncha also subject to molecular analyses were conducted on the micrographs rather than on physical specimens. This procedure renders species determination much more difficult.

Photographs of the whole animals were taken, as well as more detailed pictures of the various body parts; these usually included the head (first few segments), the middle part of the animal, and the last few segments. On some larger specimens the middle was covered by 2-4 sections. Occasionally, there would also be individuals with their introvert ejected (see figure 1), and these structures were also documented individually.

Photographing included multi-focus stacks, i.e. series of pictures with different focal points. Each section would be photographed with 10 to 70 different focal points, depending on animal transparency and size. Pictures with the different focal points were combined into composite images.

4.5. Genetic analyses

DNA was extracted from the specimens using the "Isolation of genomic DNA from tissues" protocol for the QIAamp DNA Micro Kit (Qiagen). The extracted genetic material was then amplified using PCR. The segments amplified were the mitochondrial cytochrome oxidase subunit I (COI) and the ribosomal 18s RNA.

Sequencing was performed by the custom sequencing service starSEQ. Completed sequences were edited using the software MEGA V.5.2. Neighbour joining dendrogram was also created using this software.

5. Results

Great Kinorhyncha diversity was found during this survey. While few species have been reported from Norwegian waters earlier, a total of six Kinorhyncha genera were found during this thesis on the southern Norwegian coast alone. One of these belonged to the Homalorhagida, and five of them to the Cyclorhagida. Kinorhyncha were found along the entire coastline (see figure 5).

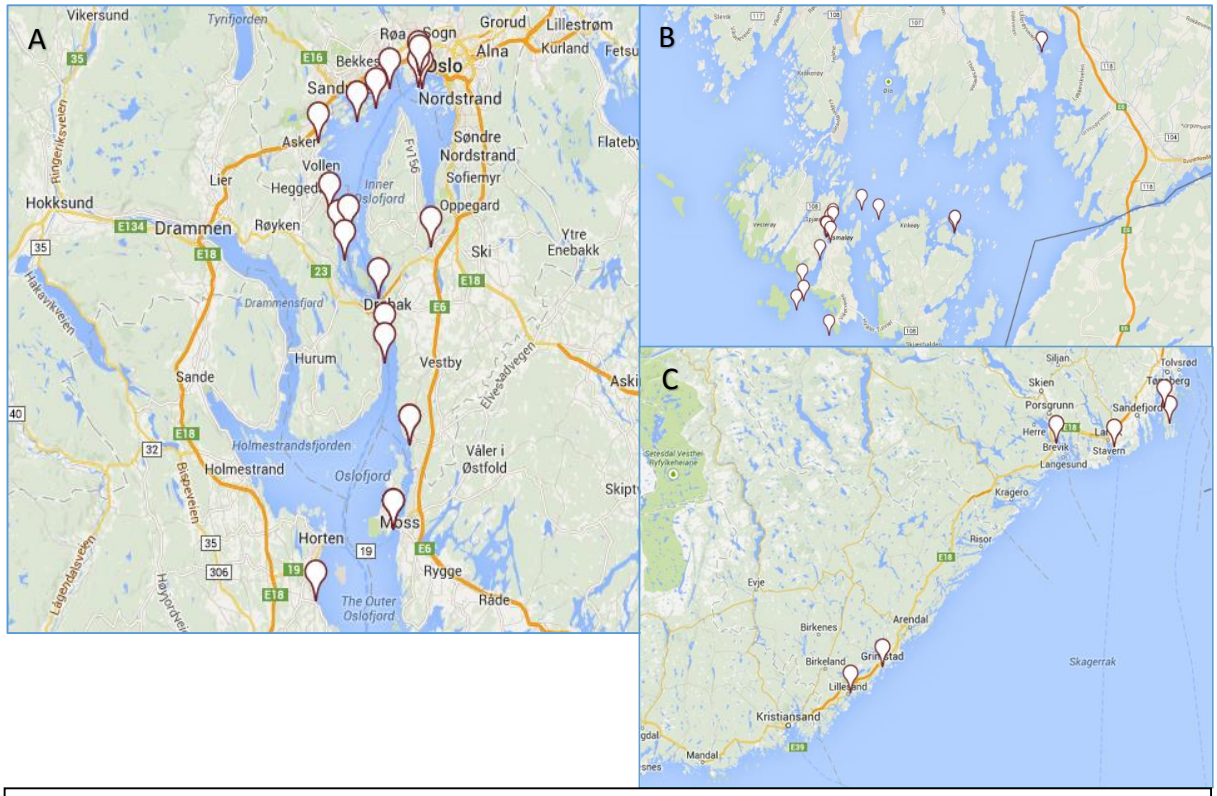

Figure 5: Maps of locations where Kinorhyncha were found in southern Norway, including locations with only exuvia. **A:** The Oslo Fjord. **B:** The Hvaler region. **C:** The Coastline from Tønsberg to Mandal.

5.1. Morphology

All diagnostic characters are taken from Sørensen and Pardos (2008).

5.1.1. Pycnophyes (Zelinka, 1907)

This represents the only genus of the order Homalorhagida present in the samples from southern Norway. Species of Pycnophyes were also the most common ones in the entire survey. Pycnophyes is distinguishable from other homalorhagid genera by the characteristic trapezoid-shaped midsternal plate on the first segment, whilst also having lateral terminal spines.

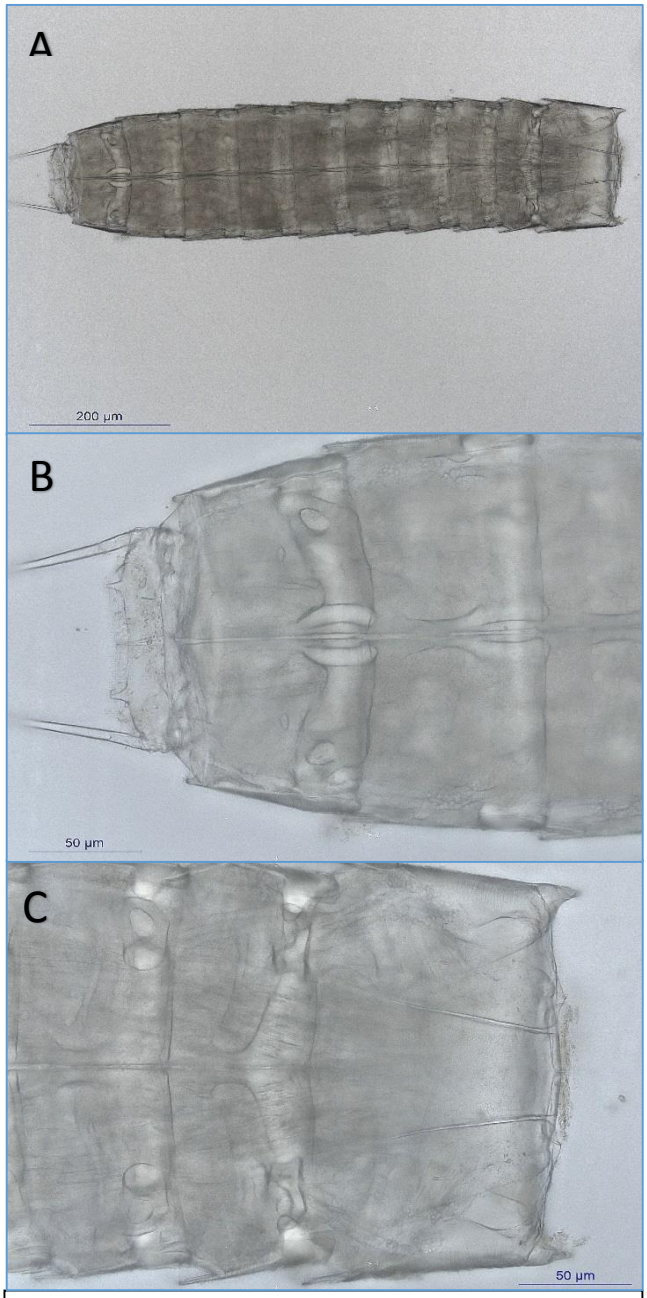

Figure 6: Specimen 105-1_08. Multi-focus light micrographs of *Pycnophyes sp.* **A:** Full trunk of entire animal, dorsal view **B**: Segments 8-11. **C:** Segments 1-3.

5.1.2. Echinoderes (Claparéde, 1863)

Echinoderes is the most common cyclorhagid genus found in this survey, and is the most species rich Kinorhyncha genus. Each of the first two trunk segment consist of one closed ring each. This, in addition to the paired lateral terminal spines and the characteristic tergal extensions found on segment 11 makes the genus quite easily recognizable.

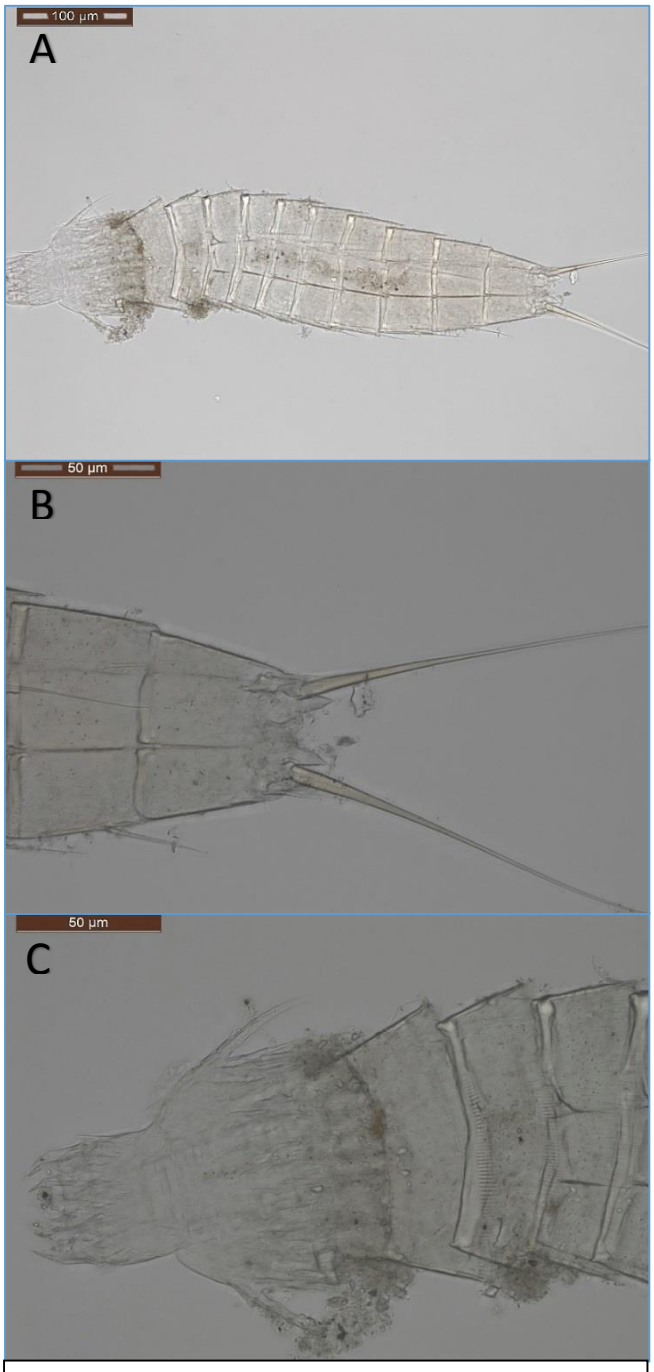

Figure 7: Specimen 103-2_03. Multi-focus stack micrographs of *Echinoderes sp*. **A:** Full trunk of entire animal, dorsal view. **B:** Segments 9-11. **C:** Segments 1-4, with the introvert ejected.

5.1.3. Tubulideres (Sørensen et al., 2007)

Only one species has ever been recorded of the cyclorhagid genus Tubulideres, and it has only been documented from Florida (Sørensen et al., 2007). The specimen documented here is of uncertain taxonomy, as both the quality of the micrograph and its relative position leave few details to observe. However, the traits visible seem to fit with those described in Tubulideres. It seems to completely lack cuspidate spines, which the genera Antygomonas, Semnoderes and Sphenoderes, with which it is easy to mistake Tubulideres, have. Sadly, it is impossible to assess in this specimen if the characteristic lateroventral and ventrolateral tubules are present on segment 2.

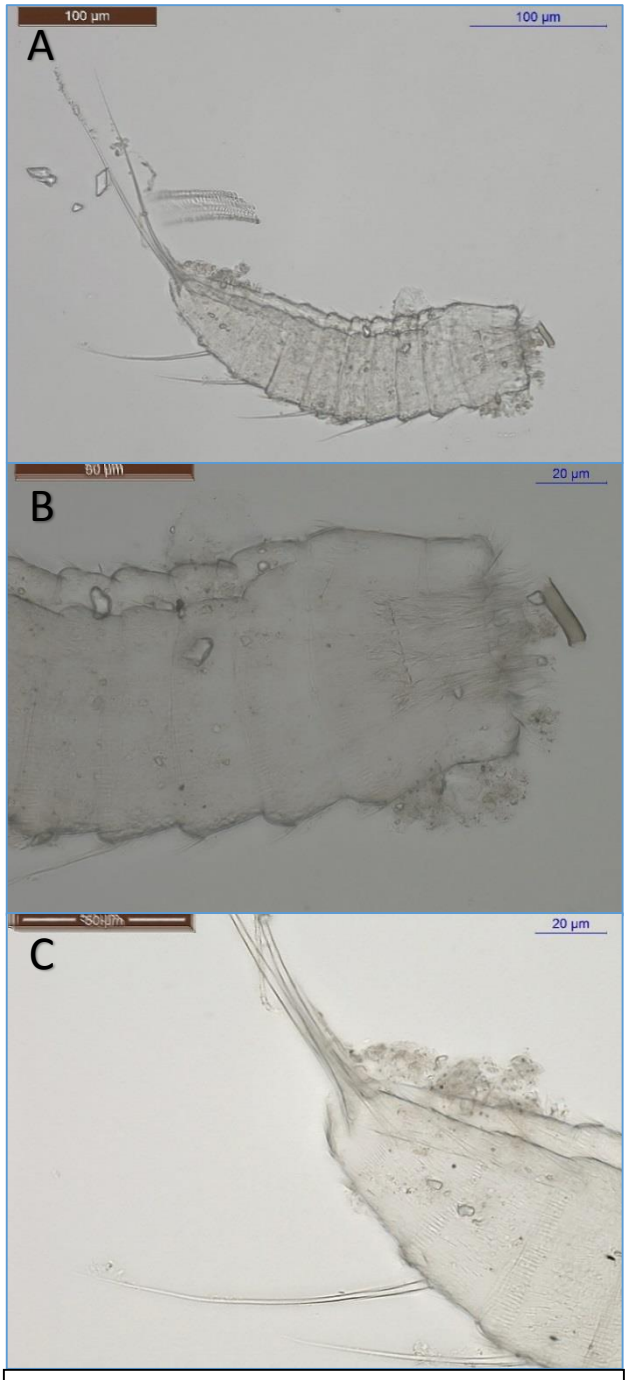

Figure 8: Specimen 104-1_01. Multi-focus light micrographs of *Tubulideres sp. conf.* **A:** Full trunk of entire animal, lateroventral view. **B:** Segments 1-5. **C:** Segments 8-11.

5.1.4. Condyloderes (Higgins, 1969)

The small cyclorhagid genus Condyloderes is distinguished from other genera on its midterminal spine. It may initially be mistaken for other cyclorhagid genera with both midterminal and lateral terminal spines, but Condyloderes is the only genus in which the former is shorter than the latter. Unfortunately, the characteristic shape of their placids is difficult to observe in this specimen.

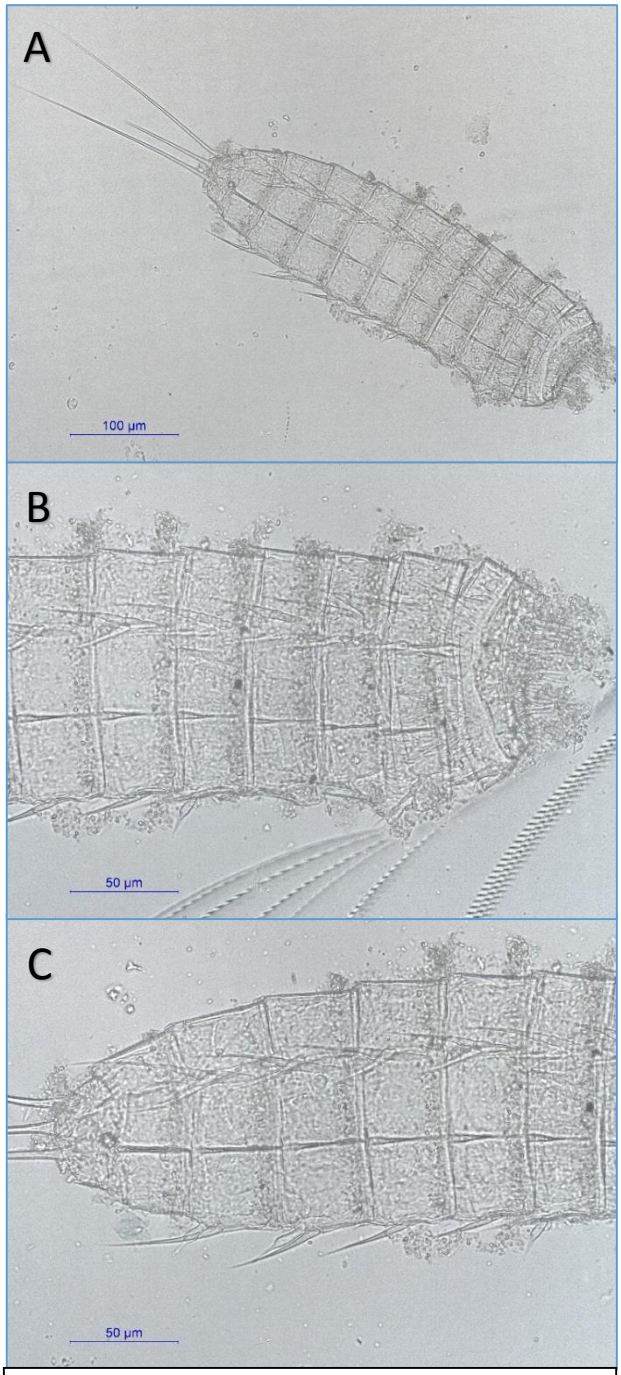

Figure 9: Specimen 175-1_01. Multi-focus light micrographs of *Condyloderes sp.* **A**: Full trunk of entire animal, lateroventral view. **B**: Segments 1-7. **C**: Segments 5-11.

5.1.5. Centroderes (Reinhard, 1881)

Centroderes is another small cyclorhagid genus, of which few specimens were found. Again, the quality of the micrograph and this specimen's position render diagnostics difficult. A few traits are still observable, however. It is possible to see the long midterminal spine, and the short and stout middorsal spines present in almost all segments. Additionally, the characteristic pair of long ventrolateral spines extending from segment 1 over several following segments are present.

On Centroderes, the midventral part of the anterior margin of segment 1 extends onto the following segments as a spinose process, but this trait is unfortunately unobservable in this specimen.

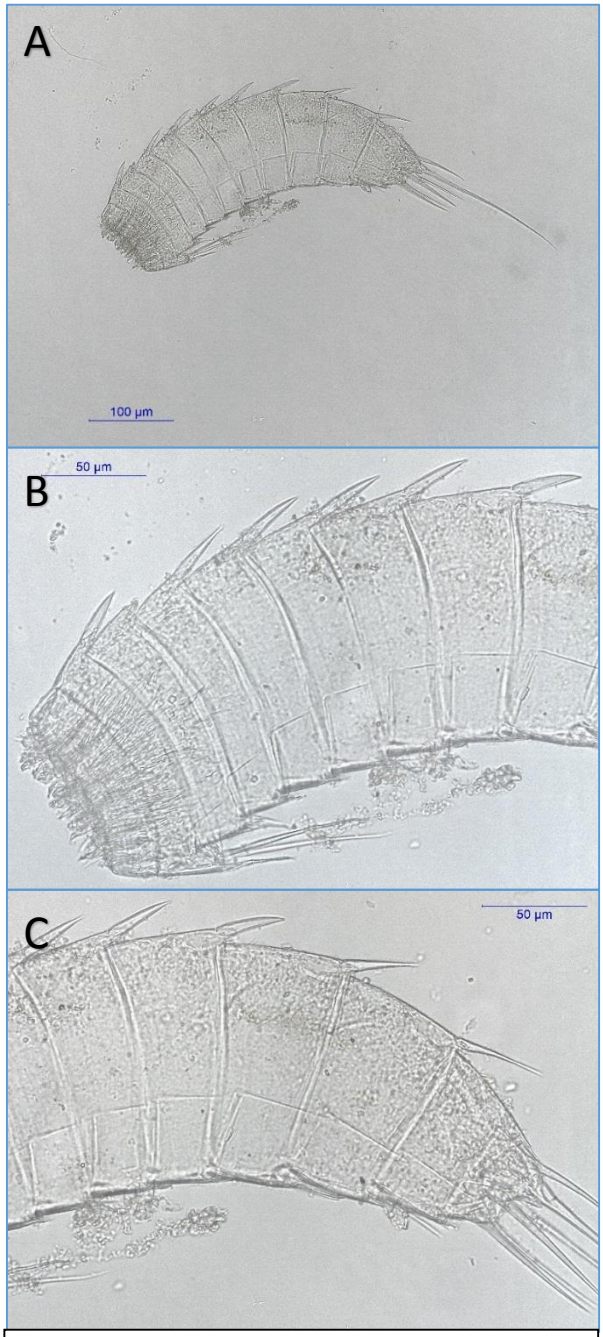

Figure 10: Specimen 157-2_07. Multi-focus light micrograph of *Centroderes sp.* **A:** Full trunk of entire animal, lateral view. **B:** Segments 1-8. **C:** Segments 5-11.

5.1.6. Semnoderes.

The final genus observed is the cyclorhagid genus Semnoderes. It can easily be mistaken for Tubulideres or Sphenoderes. Unlike Tubulideres, however, Semnoderes possesses cuspidate spines. It can be very difficult to distinguish between Semnoderes and Sphenoderes, but the latter generally has much stronger and thicker lateroventral spines. In this specimen, we unfortunately cannot easily observe the characteristic clam-shaped first segment.

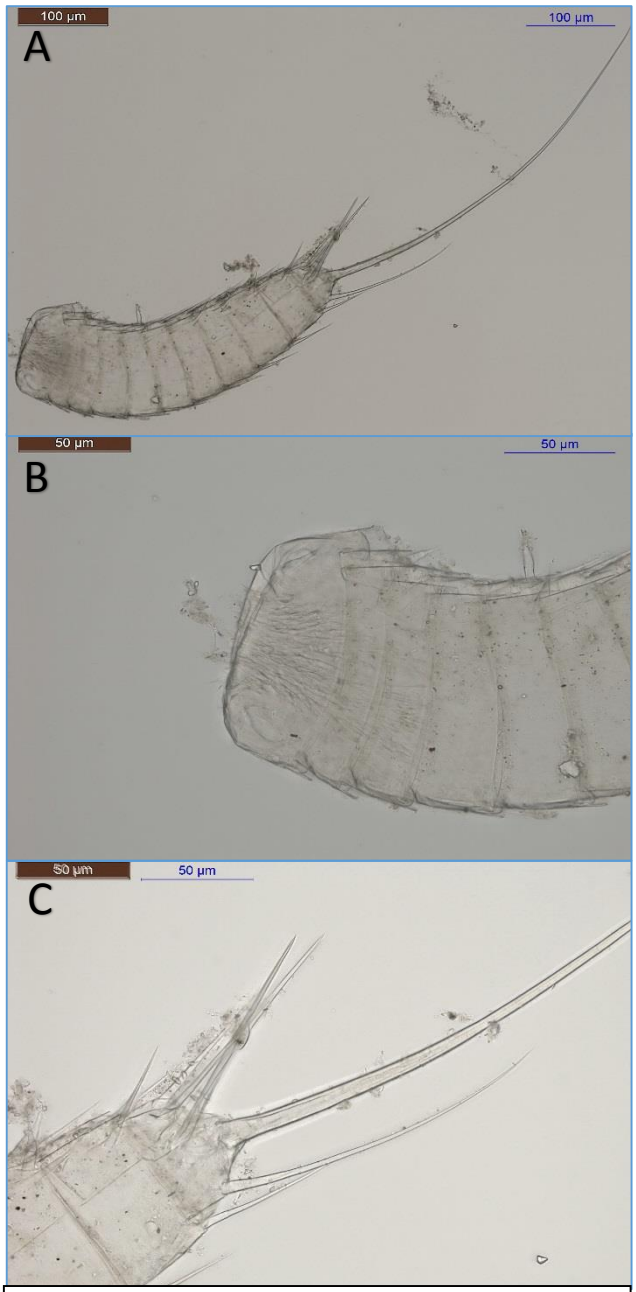

Figure 11: Specimen 156-2_1. Multi-focus light micrograph of *Semnoderes sp*. **A:** Full trunk of entire animal, lateral view. **B:** Segments 1-6. **C:** Segments 9-11.

5.2. DNA Barcoding

For entire Norway, COI sequences were obtained from 157 specimens, and 18s sequences from 173 specimens, for a total of 193 different specimens.

Neighbor joining dendrogram of COI sequences was created (see figure 12). This tree reveals at least 15 clades of Kinorhyncha from the entirety of Norway. This means that a few clades can be ignored for the purpose of this thesis, but it is still worth to note that some clades have not been found in the south yet.

Running the 18s sequences through the BLAST software also revealed quite a bit of information. Certain hits were 100%, including one Echinoderes sp. from Japan

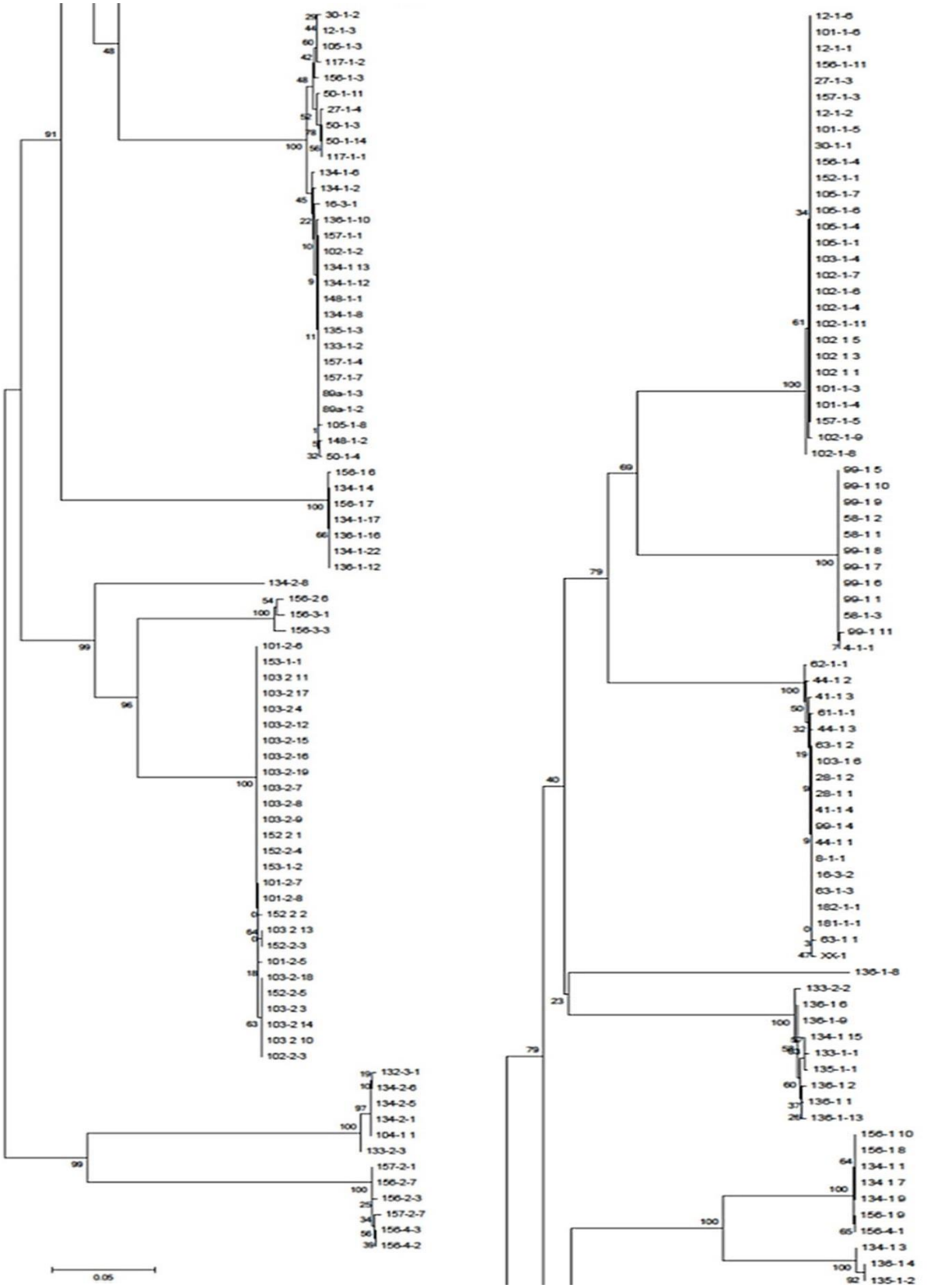

Figure 12: Neighbour joining dendrogram of COI sequences of Kinorhyncha specimens collected in Norway. Bootstrap support for the respective nodes is indicated. Samples from other locations in Norway are also included.

6. Discussion

6.1. Congruence between morphology and genetics

The genera diagnosed morphologically seem to fit well with what we see from the molecular results. Firstly, the neighbour joining dendrogram (figure 12) groups specimens of what was earlier determined to be the same genera together perfectly. The only genera that are split up in several clades are Pycnophyes and Echinoderes. Pycnophyes is split into seven clades and Echinoderes into two. Whether these clades all fully translate into good species remains to be further studied.

Preliminary BLAST results of the 18s RNA seem to correspond almost perfectly to the morphologic genera. The only genus not matching is Tubularides, where the specimens have no match at all. Additionally, these results also seem to be congruent with the phylogenetic tree.

6.2. Problems with morphology

As mentioned above, morphology based species determination of Kinorhyncha has been problematic. This is due to several factors, such as limited time spent on photographing the specimens, and general inexperience when it comes to knowing what constitutes an informative Kinorhyncha micrograph. Most of the pictures of the specimens that were combined to make multi-focus stacks tended to have too much information in them. The foci were not sharp enough, and so the final stacks were usually full of noise. This made observing certain characteristic traits in micrographs much more challenging. Method improvement was only achieved at a low pace as most individuals were subjected to molecular analyses after taking photographs, and thus no longer available for further morphological inspections.

Additionally, while identification keys to the Kinorhyncha genera (Sørensen and Pardos, 2008) have been helpful, the keys provided to help species identification are often general and to some extent subjective. An example of this is the middorsal protrusions (See figure 3),

often used as species characteristics in the genus Pycnophyes. Keys distinguish between rounded or obtuse, and pointed or horny protrusions. The protrusions in question tend to be ambiguous, and in some cases, determining whether they are rounded or pointed could be considered subjective (Sánchez et al., 2011).

Some older species descriptions of Kinorhyncha, such as that of *Pycnophyes maximus* (Reimer, 1963) can be both undetailed and ambiguous, and to complicate matters further, their holotypes are sometimes lost.

Finally, the rate with which new species of Kinorhyncha are being described quickly renders every new version of the dichotomous species keys outdated.

6.3. Additional notes

Attempts were also made to apply Scanning Electron Microscopy and CT-scanning methods to the Kinorhyncha for this project, but there was too little time to fully take advantage of them. One important thing to note, however, is that for both SEM and CT-scanning, it was still possible to extract enough DNA for sequencing from the Kinorhyncha specimens that went through these procedures. While no good scans or SEM micrographs were created for this project, the implications of this are important. It is possible to perform DNA sequencing of these specimens that have a potentially much better basis for species determination. SEM is currently standard procedure for Kinorhyncha morphological studies, and CT-scan definitely has great potential, as it allows for 3D reconstruction and easier observation of subcuticular and internal structures.

Traditionally, all Kinorhyncha taxonomy has been based on morphological approaches. One consequence of this is that when a Kinorhyncha with a characteristic that differs slightly from the norm for a species is observed, it is easy to describe it as a new species. While the difference in characteristics may be genuine, it is hard to assess whether an abnormality is a characteristic for a new species, or merely a regional variation. Neuhaus and Sørensen (2013) explore the concept of one global species instead of several in their paper on the genus Campyloderes. Interestingly, one of the specimens had a BLAST match of 100% with an Echinoderes species from Japan, further validating this possibility.

In addition to the genera described herein, a second, interesting homalorhagid genus was also observed elsewhere in Norway for the Kinorhyncha species project. This genus is Paracentrophyes, a small genus easily distinguishable from Pycnophyes.

7. Conclusions

The first survey of the southern Norwegian waters have provided insight into the rich Kinorhyncha biodiversity found there. While many genera have been documented, even greater diversity is expected to actually exist. Continued surveying of Norwegian waters will still be required, as many localities, substrates, depths, salinities and other variables have not been fully covered yet. The Norwegian Kinorhyncha species project, "Kinorhyncha, a poorly known and neglected phylum" is currently surveying the entirety of the Norwegian coast.

The basis for barcoding Norwegian Kinorhyncha has also been laid, and will provide a helpful tool in future Kinorhyncha biodiversity surveys. The road ahead to a substantial database is long however, and probably requires years of work before being fully applicable.

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