# The Biodiversity of Mud Dragons (Kinorhyncha) in the Fjords of Møre og Romsdal, Norway

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# Abstract

The poorly studied phylum Kinorhyncha (mud dragons) consists of small, benthic invertebrates inhabiting marine environments at depths ranging from the intertidal- to abyssal zones worldwide. Kinorhyncha are members of the meiofauna, inhabiting the upper layers of oxygenated sediment on the ocean floor. This study aimed at assessing the biodiversity of Kinorhyncha in five selected fjords on the Norwegian Northwest coast in the Møre og Romsdal county; Ålvundfjord, Sunndalsøra, Øksendal, Eidsvåg and Eresfjord. In total, 166 Kinorhyncha specimens were identified to species/genus levels through sequencing parts of the nuclear 18S gene. The identified Kinorhyncha belong to the six genera *Pycnophyes*, *Paracentrophyes*, *Kinorhynchus*, *Echinoderes*, *Semnoderes* and *Condyloderes*. A significant differentiation between number of specimens per species in each fjord was detected. There was also discovered trends that different kinorhynch species prefer different microenvironments (depths). High boat traffic and affiliated port activity, as taking place in Sunndalsøra, likely reduces the diversity and abundance of kinorhynch communities.

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# 1. Introduction

## 1.1 Mud dragons (Kinorhyncha)

The Ecdysozoan phylum Kinorhyncha includes species that are part of the marine meiofauna with a global distribution (Neuhaus and Higgins, 2002). The kinorhynch species prefer the upper oxygenated layers (Vidaković, 1984) of mud or sand in marine sediments from shallow waters down to depths reaching up to 7800m in the Atacama Trench (Danovaro et al., 2002). They can endure high fluctuations in salinities (Horn, 1978), pH and temperatures (Neuhaus and Higgins, 2002), indicating their capacity to adapt to various environments.

In 1841, the French naturalist Felix Dujardin was the first to discover Kinorhyncha on the north coast of the Bretagne, France (Huys and Coomans, 1989). Almost 180 years later, the phylum is still poorly studied. But with new technologies and an increased fascination for life too small to be seen with the naked eye, new discoveries are being made at an increasing pace. This is documented by the growing number of scientific publications in recent years. A quick search on google scholar (30.04.2020) revealed that since the first recognition of Kinorhyncha in 1841 until the end of the 20th century, 948 articles were published. During the last 20 years the number of articles concerning Kinorhyncha has increased by 730%, to a total of 7860 publications.

The phylum Kinorhyncha is divided into two main orders, the Homalorhagida and the Cyclorhagida, which constitutes of two and five families, respectively. Morphologically, Homalorhagida and Cyclorhagida can be distinguished easily based on the overall body shape. The cross-section body plan of homalorhagid species appears triangular, while the cross-section of a cyclorhagid constitute a more rounded shape (Figure 1b and c). The Cyclorhagida normally have several trunk spines together with a mid-terminal spine that is not present in the species of the Homalorhagida order, which contrarily have two distinct lateral terminal spines (Brusca et al., 2016). The World Register of Marine Species currently documents a diversity of 298 species, with 24 genera within the order of Cyclorhagida and 18 genera within the order of Homalorhagida (WoRMS, 2020). However, the biodiversity of Kinorhyncha is far from being comprehensively assessed, and the number of species in the phylum is expected to rise considerably within the years to come.

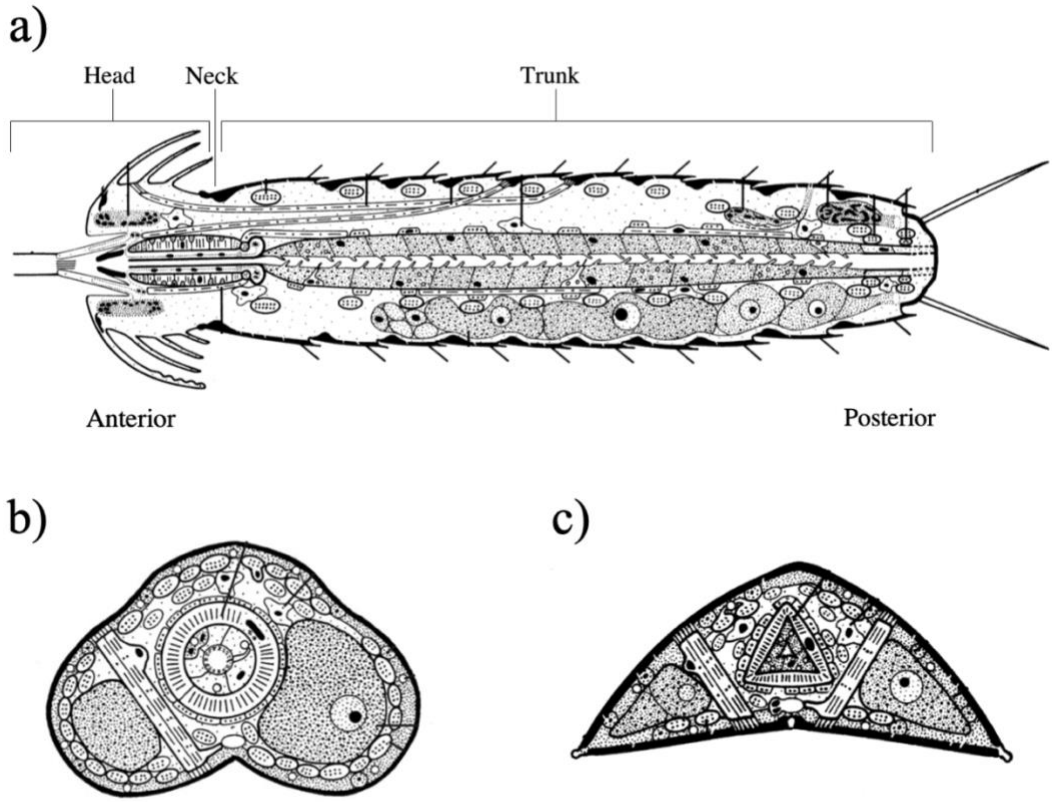


**Figure 1**: Overall body plan of Kinorhyncha and cross sections illustrating the conspicuous first glance of morphological difference between orders. a) typical ventral kinorhynch body plan constituting 13 segments divided into head, neck and trunk (Homalorhagida), the cross section of b) the Cyclorhagida and c) the Homalorhagida (Neuhaus and Higgins, 2002).

The typical kinorhynch body plan as described by Neuhaus and Higgins (2002) consists of 13 segments, divided into head, neck and trunk (Figure 1a). Movement is achieved by extending and retracting the introvert (Figure 2), a segmented mouth cone comprised with scalids, creating a forward movement (Shirley, 2009). The kinorhynch diet is discussed usually under the *a priori* assumption that Kinorhyncha are decomposers of organic material and diatoms (Neuhaus and Higgins, 2002). Based on the muscular structures in the head and mouth cone Hirose and Yamasaki (2015) found that some Kinorhynch species may feed on cellular material, bacteria and other small particles. Brusca et al. (2016) provide a list of major characteristics that separates Kinorhyncha from the other segmented ecdysozoan clades. According to the list, the key features include that Kinorhyncha are triploblastic with a bilateral segmentation, the species are either blastocoelomate or acoelomate, absence of locomotory cilia, a complete gut-system and that Kinorhyncha are gonochoristic with directly developed juveniles from embryo. As is typical for gonochoristic organisms, there is no obvious differentiation between male and female, except the gonads and in some species

posterior segments. When morphologically distinguishing species, the number and placement of spines and placids (constituting the neck) along with oral styles in the mouth cone are the key diagnostic features.

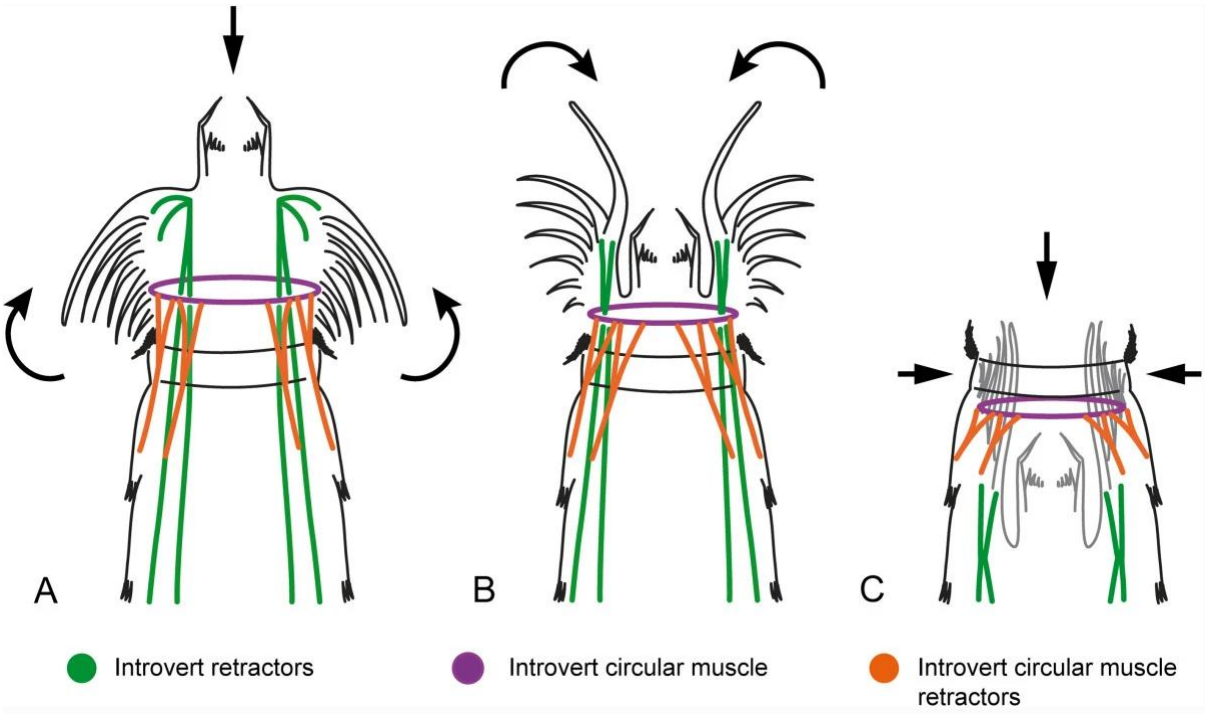


**Figure 2**: Schematic overview of introvert extension and retraction, creating a forward movement with colour coded muscular systems involved in the process (Herranz et. al., 2014).

Kinorhyncha belongs together with Priapulida and Loricifera to the Scalidophora, which constitutes a monophyletic clade within the Ecdysozoa in sister group relationship to Nematoida and Panarthropoda (Giribet and Edgecombe, 2017). There is currently only limited molecular data, and in particular genomic data, available for Loricifera, Priapulida and Kinorhyncha. Accordingly, the Scalidophora is only a weakly supported clade in recent phylogenetic and phylogenomic studies (Borner et al., 2014). The lack of molecular support for the relationship within Scalidophora has led to a taxonomic subclade that is still largely based on morphological characters (Giribet and Edgecombe, 2017).

Studies on taxonomy and phylogenetic relationships that are based on morphological characters frequently have to cope with significant challenges that certainly also apply to the Scalidophora in general and to the Kinorhyncha in particular. Three issues will briefly be assessed. First, morphological features that discriminate species may be minute and difficult

to assess. Further they may challenge or even break during sampling and/or fixation. Additionally, the morphological features may differ between developmental stages. For Kinorhyncha, Sørensen and Pardos (2008) provided a fairly good key for morphological identification of kinorhynch species, but most of the species are still not easily distinguishable and/or the determination of the morphological character stages may require sophisticated equipment such as e.g. access to electron microscopes. Second, morphologically based phylogenies are generally considered less reliable than molecular based ones, often in cases where convergent evolution is present as discussed by Zou and Zhang (2016) or when phenotypically similar species are impossible to discriminate based on morphological traits (Correa et al., 2011). The latter certainly is a challenge with Kinorhyncha. Importantly, molecular data are static, where scientists have a framework consisting of the nucleotide arrangement for analysis. The morphological assessment can differ depending of authors and/or time of evaluation. In that respect, molecular phylogenies are interpretations of universal codes, in contrast to more subjective interpretations of morphological characters used for phylogeny. Third, the lack of kinorhynch fossils poses a particular difficulty for the morphological based assumption of the clade Scalidophora (Zhang et al., 2015) and accordingly, the divergence time of Kinorhyncha, Priapulida and Loricifera within Scalidophora remains unresolved. However, recently Zhang et al. (2015) described a fossilized kinorhynch ancestor *Eokinorhynchus rarus* from the early Cambrian. A careful morphological analysis of the specimen might shed new light on the phylogenetic relationships within the subclade Scalidophora.

Molecular species identification of Kinorhyncha is by now possible to some extent, and largely relies on nucleotide sequence data for two molecular markers, i.e. the nuclear ribosomal 18S gene and the mitochondrial cytochrome oxidase subunit 1 (CO1) gene (Yamasaki, 2017; Herranz et al., 2018; Randsø et al., 2019). Kinorhyncha phylogenies have been reconstructed by comparing molecular data with morphological data to interpret interrelationships within the kinorhynch taxon (Sørensen et al., 2015) and molecular data have also been used in attempts to the discovery of new species (Varney et al., 2019; Yamasaki and Fujimoto, 2014). Nevertheless, the use of 18S and CO1 sequences as molecular markers for species as tiny as Kinorhyncha is not necessarily straightforward. The 18S and CO1 target genes are found in all species, and usually highly conserved regions are most suitable for designing universal primers. Accordingly, PCR based amplification and

sequencing approaches are highly susceptible for cross-contamination of DNA from other sources.

In a recent study Schratzberger and Somerfield (2020) reviewed the impacts of human activity that endangers the meiofauna community. They found that anthropogenic activities such as bottom-fishing, introduction of invasive species and climate change reshape the ocean floor, creating both abiotic (sediment texture, bed forms, oxygenation) and biotic (biofilm, organic matter, community interactions) alterations. The Kinorhyncha are distinctive due to the fact that they inhabit both the coastal zones and the deep seas. Consequently, disturbances that happens in both coastal areas such as tourism, settlement, industry, transport, agriculture, fishing and trade, and perturbations in the deep seas such as bottom trawling, deep-sea mining and dumped waste (IPBES, 2019) mainly due to population and economic growth, all affect the ecosystems the Kinorhyncha inhabit. The life history of meiofauna generally constitutes short generation times and multiple offspring, which is believed to make them less vulnerable to timing of disturbance and the rate of recolonization can be rapid (Schratzberger and Somerfield, 2020). Nevertheless, lowered survival rate and fitness due to habitat changes might exceed the tolerance limit threshold making ecosystem recovery unfeasible. As mentioned above, Kinorhyncha prefer the oxygenated layers of the sediments but with increasing temperatures of the water due to global warming, the ability to hold oxygen decreases (Helm et al., 2011), this may directly affect the kinorhynch ability to thrive and consequently decreases the potential for resistance. Climate change and other anthropogenic activities disrupt ecosystems, fragments or eliminate habitats through increased water temperatures, creates oxygen deficiency, acidification, eutrophication, upwelling and intensification of storm activities (IPBES, 2019), which in turn affect both the life histories of species and the fundamental structure of their habitat. These changes to the meiofaunal community may lead to a turnover of species composition from a meio- and macrofauna dominated habitat, to a homogenic habitat largely consisting of microbes able to survive in anaerobic environments (Franco et al., 2008). The listed factors of human disturbances on the marine environment, represent major menaces to the ecosystem services the meiofauna community serves.

## 1.2 Aim of the thesis

There is until today no comprehensive assessment of kinorhynch biodiversity in Norway, and reports of kinorhynch occurrence along the Norwegian coastline have only been circumstantial. The most comprehensive survey has been performed in the artsdatabanken.no founded artsprosjekt "*Kinorhyncha – a poorly known and neglected animal phylum*" in 2013-14 (project number knr. 56-12, pnr. 70184227, assigned to Prof. Lutz Bachmann, Natural History Museum Oslo). The project delivered a first rough overview on Kinorhyncha diversity and identified, although not exhaustively assessed, the inner fjords of Møre og Romsdal, Norway, as conspicuously species rich (personal information).

This thesis aimed at an in-depth assessment of the kinorhynch biodiversity in five selected fjords, i.e. Sunndalsøra, Øksendal, Ålvundfjorden, Eidsvåg and Eresfjorden in Møre og Romsdal, Norway. Particular emphasis was on seasonal variation and depth zonation of the kinorhynch meiofauna community. The selected fjords differ strikingly in the extent of human activities that may impact local ecological parameters, and thus, also kinorhynch diversity and abundance.

# 2. Materials and methods

## 2.1 Sampling locations

Fieldwork to collect Kinorhyncha specimens was conducted in May, July and September 2019 at five fjord localities on the Norwegian northwest coast of Møre og Romsdal, in order to monitor seasonal variation in species abundance and diversity. In total, 81 environmental samples were taken from the five selected fjords (Figure 3 and 4); Tingvollfjorden at Sunndalsøra and Øksendal, Trongfjorden at Ålvundfjord, Langfjorden at Eidsvåg and Eresfjord. Sampling depths ranged from ca. 5m to ca. 160m (Table 1). Ålvundfjorden, Eidsvåg and Øksendal were sampled at all three points in time, whereas Sunndalsøra and Eresfjorden were only sampled in July and September.

Knowledge from a previous monitoring of Kinorhyncha biodiversity (personal communication) led to the decision of which fjords to sample. For the localities Ålvundfjorden, Øksendal and Eidsvåg the previous survey had shown that Kinorhyncha species could be commonly found at these localities. Although the five fjords are located within short geographical distances, they are affected by different kinds and extent of human activities; ranging from the almost non-impacted Eresfjorden with mainly agriculture and mostly local transit, to the highly impacted Sunndalsøra, a regional center with the largest aluminium factory in Europe and associated port facilities (Table 2).

The number of people connected to the fjords and associated settlements/towns was expected to represent an important factor on how the fjords' ecology is affected. In the sampling area of this thesis, the amount of sewage and other waste certainly increases with large populations as in the Sunndalsøra and Eidsvåg communities, but also the agricultural landscape in Ålvundfjord, Øksendal and Eresfjord cause runoff that might affect the physicochemical balance in the fjords. Other activities such as transportation of large cargo and boats in the fjords cause upwelling of sediments that might also disturb the meiofauna including Kinorhyncha. The fjords in Sunndalsøra and Eidsvåg are in this thesis considered fjords with substantial human impact due to population numbers, the town's status as commercial centers in the area and the corresponding association to ports and utilization of the fjords. The Øksendal settlement has the smallest population number of the targeted fjords

but was first of all selected due to its location in respect to Sunndalsøra. The side fjord of Tingvollfjorden at Øksendal might also be affected by heavy traffic of large cargo ships due to the factory located in Sunndalsøra, although the valley at Øksendal itself is rural. Ålvundfjord was selected as a fjord only moderately impacted by human activities. Eresfjord is regarded as the least impacted fjord from human activities with only a small local harbour and low population numbers.

The fjords were targeted to address if the extent of local human impact in the fjords affects the abundance and species diversity of Kinorhyncha.

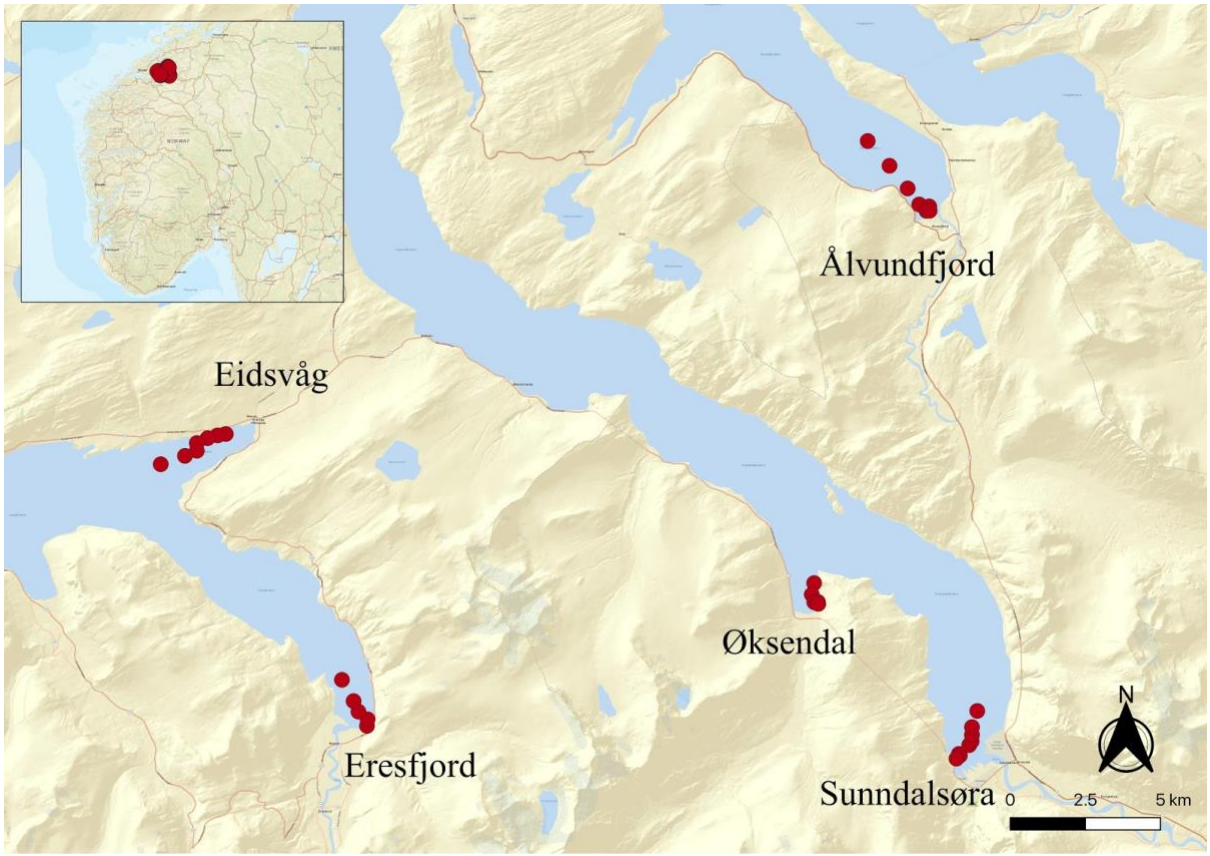


**Figure 3**: Overview map of the sampled fjords Eidsvåg, Eresfjorden, Øksendal, Sunndalsøra and Ålvundfjord in the Møre og Romsdal county, Norway, in 2019.

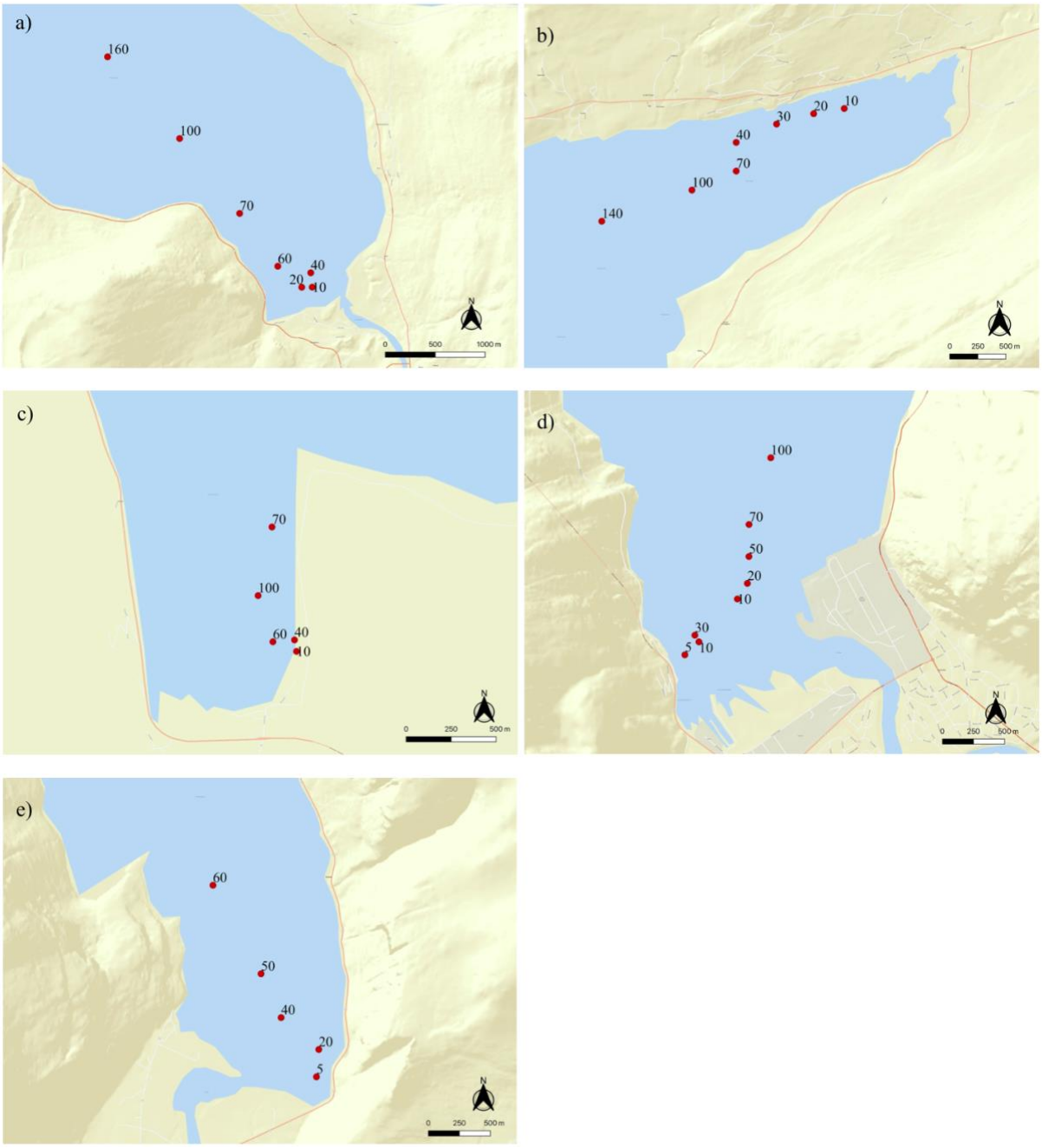


**Figure 4**: Detailed map of the sampling locations with sampling depth (meters) plotted for each fjord, a) Ålvundfjord, b) Eidsvåg, c) Øksendal, d) Sunndalsøra and e) Eresfjorden.

**Table 1**: Sampling locations, sample ID, geo coordinates, depth and date for each sampling site on the northwest coast of Møre og Romsdal, Norway 2019.

| Sampling site | Sample ID | Latitude | Longitude | Depth (m) | Date |
|---|---|---|---|---|---|
| Ålvundfjord | 500 | 62.83957 | 08.50640 | 40 | 14.05.19 |
| | 501 | 62.84041 | 08.50010 | 60 | 14.05.19 |
| | 502 | 62.84512 | 08.49270 | 70 | 14.05.19 |
| | 503 | 62.85209 | 08.48115 | 100 | 14.05.19 |
| | 504 | 62.85922 | 08.46732 | 160 | 14.05.19 |
| | 505 | 62.83861 | 08.50675 | 10 | 14.05.19 |
| Eidsvåg | 506 | 62.77223 | 08.05484 | NA | 14.05.19 |
| | 507 | 62.77226 | 08.04996 | 10 | 14.05.19 |
| | 508 | 62.77189 | 08.04495 | 20 | 14.05.19 |
| | 509 | 62.77110 | 08.03842 | 30 | 14.05.19 |
| | 510 | 62.76949 | 08.03181 | 40 | 14.05.19 |
| | 511 | 62.76741 | 08.03133 | 70 | 14.05.19 |
| | 512 | 62.76571 | 08.02402 | 100 | 14.05.19 |
| Øksendal | 513 | 62.72167 | 08.43465 | 10 | 15.05.19 |
| | 514 | 62.72167 | 08.43465 | 10 | 15.05.19 |
| | 515 | 62.72215 | 08.43208 | 60 | 15.05.19 |
| | 516 | 62.72435 | 08.43039 | 100 | 15.05.19 |
| | 517 | 62.72787 | 08.43184 | 70 | 15.05.19 |
| | 518 | 62.73174 | 08.42332 | NA | 15.05.19 |
| Eidsvåg | 519 | 62.77222 | 08.05005 | 10 | 09.07.19 |
| | 520 | 62.77180 | 08.04473 | 20 | 09.07.19 |
| | 521 | 62.77097 | 08.03827 | 30 | 09.07.19 |
| | 522 | 62.76951 | 08.03124 | 40 | 09.07.19 |
| | 523 | 62.76723 | 08.03123 | 70 | 09.07.19 |
| | 524 | 62.76571 | 08.02352 | 100 | 09.07.19 |
| | 525 | 62.76322 | 08.00780 | 140 | 09.07.19 |
| Sunndalsøra | 526 | 62.67696 | 08.52595 | 30 | 09.07.19 |
| | 527 | 62.67648 | 08.52659 | 10 | 09.07.19 |
| | 528 | 62.67957 | 08.53263 | 10 | 09.07.19 |

| | | | | | |
|---|---|---|---|---|---|
| | 529 | 62.68070 | 08.53420 | 20 | 09.07.19 |
| | 530 | 62.68264 | 08.53444 | 50 | 09.07.19 |
| | 531 | 62.68495 | 08.53446 | 70 | 09.07.19 |
| | 532 | 62.68976 | 08.53787 | 100 | 09.07.19 |
| | 533 | 62.67554 | 08.52438 | 5 | 09.07.19 |
| Øksendal | 534 | 62.72786 | 08.43198 | 70 | 09.07.19 |
| | 535 | 62.72445 | 08.43048 | 100 | 09.07.19 |
| | 536 | 62.72215 | 08.43208 | 60 | 09.07.19 |
| | 537 | 62.72224 | 08.43443 | 40 | 09.07.19 |
| | 538 | 62.72167 | 08.43465 | 10 | 09.07.19 |
| Ålvundfjord | 539 | 62.85918 | 08.46674 | 160 | 10.07.19 |
| | 540 | 62.85185 | 08.48090 | 100 | 10.07.19 |
| | 541 | 62.84512 | 08.49270 | 70 | 10.07.19 |
| | 542 | 62.84037 | 08.50019 | 60 | 10.07.19 |
| | 543 | 62.83978 | 08.50667 | 40 | 10.07.19 |
| | 544 | 62.83848 | 08.50490 | 20 | 10.07.19 |
| | 545 | 62.83849 | 08.50695 | 10 | 10.07.19 |
| Eresfjord | 546 | 62.68532 | 08.14172 | 5 | 10.07.19 |
| | 547 | 62.68728 | 08.14209 | 20 | 10.07.19 |
| | 548 | 62.68956 | 08.13616 | 40 | 10.07.19 |
| | 549 | 62.69270 | 08.13301 | 50 | 10.07.19 |
| | 550 | 62.69904 | 08.12543 | 60 | 10.07.19 |
| Ålvundfjord | 551 | 62.85916 | 08.46663 | 160 | 25.09.19 |
| | 552 | 62.85198 | 08.48106 | 100 | 25.09.19 |
| | 553 | 62.84505 | 08.49264 | 70 | 25.09.19 |
| | 554 | 62.84035 | 08.50018 | 60 | 25.09.19 |
| | 555 | 62.83979 | 08.50671 | 40 | 25.09.19 |
| | 556 | 62.83850 | 08.50490 | 20 | 25.09.19 |
| | 557 | 62.83853 | 08.50696 | 10 | 25.09.19 |
| Sunndalsøra | 558 | 62.68875 | 08.53786 | 100 | 25.09.19 |
| | 559 | 62.68491 | 08.53447 | 70 | 25.09.19 |
| | 560 | 62.68268 | 08.53453 | 50 | 25.09.19 |

| | | | | | |
|---|---|---|---|---|---|
| | 561 | 62.68067 | 08.53422 | 20 | 25.09.19 |
| | 562 | 62.67955 | 08.53265 | 10 | 25.09.19 |
| | 563 | 62.67648 | 08.52665 | 10 | 25.09.19 |
| | 564 | 62.67696 | 08.52579 | 30 | 25.09.19 |
| Øksendal | 565 | 62.72784 | 08.43202 | 70 | 25.09.19 |
| | 566 | 62.72444 | 08.43053 | 100 | 25.09.19 |
| | 567 | 62.72215 | 08.43202 | 60 | 25.09.19 |
| | 568 | 62.72224 | 08.43443 | 40 | 25.09.19 |
| | 569 | 62.72167 | 08.43465 | 10 | 25.09.19 |
| Eresfjord | 570 | 62.69903 | 08.12543 | 60 | 26.09.19 |
| | 571 | 62.69269 | 08.13296 | 50 | 26.09.19 |
| | 572 | 62.68958 | 08.13619 | 40 | 26.09.19 |
| | 573 | 62.68729 | 08.14207 | 20 | 26.09.19 |
| | 574 | 62.68533 | 08.14175 | 5 | 26.09.19 |
| Eidsvåg | 575 | 62.77218 | 08.04998 | 140 | 26.09.19 |
| | 576 | 62.77180 | 08.04480 | 100 | 26.09.19 |
| | 577 | 62.77096 | 08.03834 | 70 | 26.09.19 |
| | 578 | 62.76954 | 08.03128 | 40 | 26.09.19 |
| | 579 | 62.76724 | 08.03123 | 30 | 26.09.19 |
| | 580 | 62.76544 | 08.02304 | 20 | 26.09.19 |
| | 581 | 62.76325 | 08.00780 | 10 | 26.09.19 |

**Table 2**: Population numbers and local economy in the settlements connected to the targeted fjords. Population numbers collected from Statistisk Sentralbyrå (Statistisk sentralbyrå, 2020).

| **Settlement** | **Population** | **Municipality** | **Economy*** |
|---|---|---|---|
| Sunndalsøra | 4140 | Sunndal | Industry and regional commercial center |
| Øksendal | 370 | Sunndal | Agriculture |
| Ålvundfjord | 470 | Sunndal | Agriculture and small industry |
| Eidsvåg | 950 | Molde | Small industry and local commercial center |
| Eresfjord | 360 | Molde | Agriculture |

* the assessment is based on a rough overall impression by the author

## 2.2 Sampling methods

Sampling was conducted from a small boat. A van Veen grab was used to collect approximately 1-2 kg sediment per haul (Figure 5). Meiofauna was extracted from the sediments by means of the bubble-and-blot method (Giere, 2009), and stored in 96% ethanol in plastic test tubes for downstream processing and storage. In brief, the bubble-and-blot method makes first a homogenous blend of seawater and mud by pouring the sample from one bucket into another until a homogeneous mixture are obtained (Figure 6). At the same time air bubbles are produced that drive meiofauna to the surface, where they can readily be collected with a sheet of paper. The meiofauna specimens were washed from the paper into the test tube with 96% ethanol. The test tubes were marked with sampling number associated with coordinates and depth of the sample site.

**Figure 5**: Van Veen grab used to collect seafloor sediment samples, by manually hauling the grab into boat.

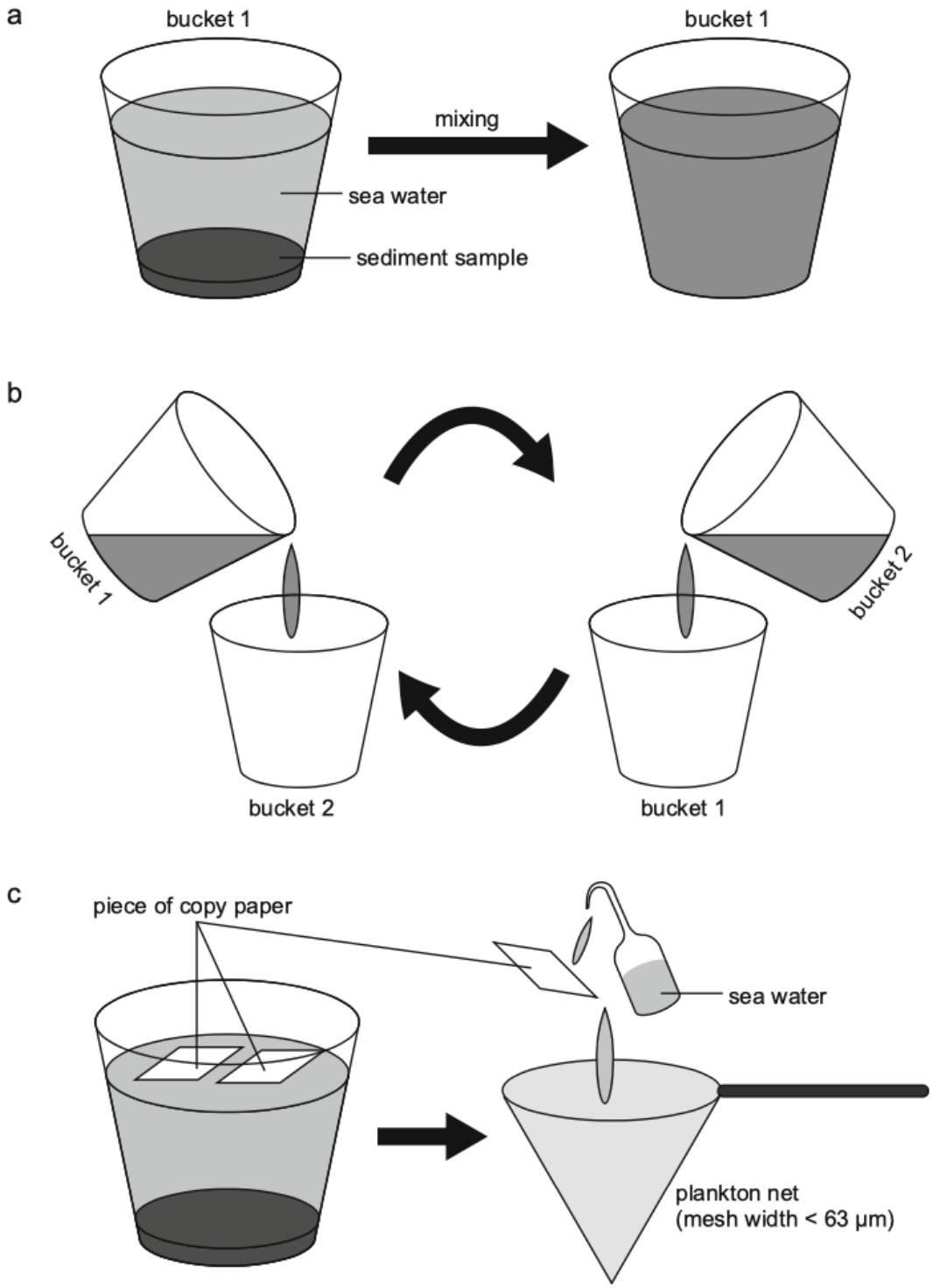


**Figure 6**: Schematic overview of bubble-and-blot method as described by Yamasaki (2017).
a) seawater and sediment are mixed by pouring substance from one bucket into another, b) creating a homogenous blend by multiple repetitions of pouring, c) collecting specimens from sheet of paper.

The environmental samples were thoroughly examined in lab at the Natural History Museum in Oslo with a stereo microscope at 10x magnification. Kinorhyncha specimens were identified based on overall morphology, and individually hand-picked from the meiofauna samples and stored in 1.5ml reaction tubes containing 30 µl 96% ethanol for downstream DNA analysis. The specimens were also sorted as either Homalorhagida or Cyclorhagida during the morphological identification process. However, some of the very small specimens, suspected of being juvenile stages, could not be unambiguously identified as homalorhagids or cyclorhagids. The number of segments was then counted, and overall body structure was examined in order to determine if the specimens belonged to the phylum of Kinorhyncha. In case of uncertainty, the respective specimens were nevertheless picked for downstream molecular analyses as 'suspected' Kinorhyncha. In addition, the presence of microplastics in the environmental samples was noted.

## 2.3 Molecular analysis

For DNA extraction of individual kinorhynch specimen the protocol of the QIAamp DNA Micro Kit (Qiagen) for "Isolation of genomic DNA from tissues" was used, resulting in 30µL of isolated total genomic DNA. Subsequently, two molecular markers were targeted for by Polymerase Chain Reaction (PCR) employing the thermostable AmpliTaq Gold™ DNA Polymerase (ThermoFisher Scientific). The experimental conditions are presented in Table 3. For the nuclear ribosomal 18S, the primer pairs S30/5FB, 4FB/1806R and 18S-323dir/18S-823rev were used, and for the mitochondrial Cytochrome Oxidase Subunit 1 (CO1) the primer pair LCO1490/HCO2198 were used (Table 4).

**Table 3**: Cycling conditions during PCR amplification of 18S and CO1 markers of individual Kinorhyncha specimens.

| | | 34 cycles | | | |
|---|---|---|---|---|---|
| | **Initial denaturation** | **Denaturation** | **Annealing** | **Extension** | **Final extension** |
| Temp (°C) | 94 | 94 | 56/55 | 72 | 72 |
| Duration | 5,00 | 0,30 | 0,45 | 1,00 | 7,00 |

During PCR amplification of DNA, the denaturation, annealing temperature and extension are important factors. If annealing temperature is set too low the primers are susceptible for cross amplification by non-specific annealing to other locations than the targeted ones on the DNA strand. In contrast, a too high annealing temperature will lead to a reduced reaction efficiency as the possibility of the primer to anneal is significantly reduced (Rychlik et al., 1990). An optimization test of annealing temperature for primer pair 18S-323dir/18S-823rev was conducted and optimal $T_a$ was found to be 55°C. Following amplification, PCR products were visualized using standard agarose gel electrophoresis.

**Table 4**: Primers used in PCR for amplification of 18S and CO1 targeted regions.

| | | | |
|---|---|---|---|
| **18S** | S30 | Forward | GCTTGTCTCAAAGATTAAGCC |
| | 5FK | Reverse | TTCTTGGCAAATGCTTTCGC |
| | 4FB | Forward | CCAGCAGCCGCGGTAATTCCAG |
| | 1806R | Reverse | CCTTGTTACGACTTTTACTTCCTC |
| | 18S-323dir | Forward | ATAACGGGTAACGGYGAATCAGGG |
| | 18S-823rev | Reverse | TTATTCCATGCACCACTATWCAGGC |
| **CO1** | LCO1490 | Forward | GGTCAACAAATCATAAAGATATTGG |
| | HCO2198 | Reverse | TAAACTTCAGGGTGACCAAAAAATCA |

The obtained PCR products were purified and subsequently Sanger sequenced. The actual DNA-sequencing was outsourced to StarSEQ Mainz, Germany. The sequencing results were received digitally both as text-files including the sequences after base-calling and as ab1 formatted trace files containing the DNA sequence electropherogram as well as raw data along with some other 'run' information.

The electropherograms were viewed using the MEGAX software (Kumar et al., 2018), and ambiguous positions were either trimmed or edited manually. The edited sequences were screened against GenBank using BLAST (Basic Local Alignment Search Tool) function

(Johnson et al., 2008) of the National Center for Biotechnology Information (NCBI). The highest scoring database entries were used to determine the Kinorhyncha specimens to species or at least to genus level.

The specimens that were molecularly identified to species/genus were then registered to the Corema (Collection and Research management) database of the Natural History Museum in Oslo to ensure that the information will be available with open access to scientists and enthusiasts interested in the meiofauna.

## 2.4 Statistics

The Kruskal Wallis test with a following Bonferroni post hoc test, was run to assess the statistical support for possible variation in the three key questions of this thesis. Firstly, if the distribution of specimens per species is the same across each fjord. Secondly, if the distribution of number of specimens is the same across depth zonation. And thirdly, if there is statistical support for seasonal variation in number of specimens at each sampling site. The statistical analysis was conducted in SPSS Statistics (available from https://programvare.uio.no/produkt/6861.html).

A relative abundance curve (RAD) was generated using Microsoft Excel to detect variation in species richness and species evenness of both the whole dataset and for the distribution between Homalorhagida and Cyclorhagida. The species richness is defined as the number of species found in a habitat or ecosystem and the species evenness is defined as the mathematical independent variable drawn from the species richness (Gosselin, 2006). The species evenness shows the distribution of species based on the species richness across the ecosystem of interest.

# 3. Results

In total, 394 Kinorhyncha individuals were morphologically identified in the meiofauna samples collected in the five selected fjords in Møre og Romsdal, Norway, by screening with stereo microscope. Additionally, plastic particles were noticed in all environmental samples collected. A breakdown of the identified specimens according to sampling period, sampling depth and geographic origin is provided in Figure 7. Overall, the highest number of specimens were identified from Eidsvåg, and the least in Sunndalsøra.

All individual specimens were subject to molecular species identification using either parts of nuclear 18S ribosomal gene and/or the mitochondrial cytochrome oxidase I gene. Of the 394 Kinorhynch specimens, 166 (42%) individuals were successfully determined to either species (96 specimens) or genus level (70 specimens) using molecular markers. The compiled sequence data are provided as supplementary information S1. The identified Kinorhyncha species belonged to six genera; three genera belonging to the order Homalorhagida and three to the order Cyclorhagida. A list of species is presented in Table 5.

**Table 5**: Kinorhyncha species found in selected fjords in Møre og Romsdal, Norway (2019).

| **Order** | **Genera** | **Species** |
|---|---|---|
| Homalorhagida | *Pycnophyes* | *rugosus* |
| | | *zelinkaei* |
| | | sp. |
| | *Paracentrophyes* | *quadridentatus* |
| | *Kinorhynchus* | *giganteus* |
| Cyclorhagida | *Echinoderes* | *capitatus* |
| | | *setiger* |
| | | sp. |
| | *Semnoderes* | *armiger* |
| | *Condyloderes* | sp. |

The relative species frequency of each molecularly identified Kinorhyncha species compiled for the whole sampling period and area was calculated (Figure 8). *Pycnophyes rugosus* was found to be most common (25%) relative to the other species. Specimens only identified to

the genus *Pycnophyes* made up for 35%. The least abundant species was *Kinorhynchus giganteus*, represented with only one specimen collected in Eidsvåg.

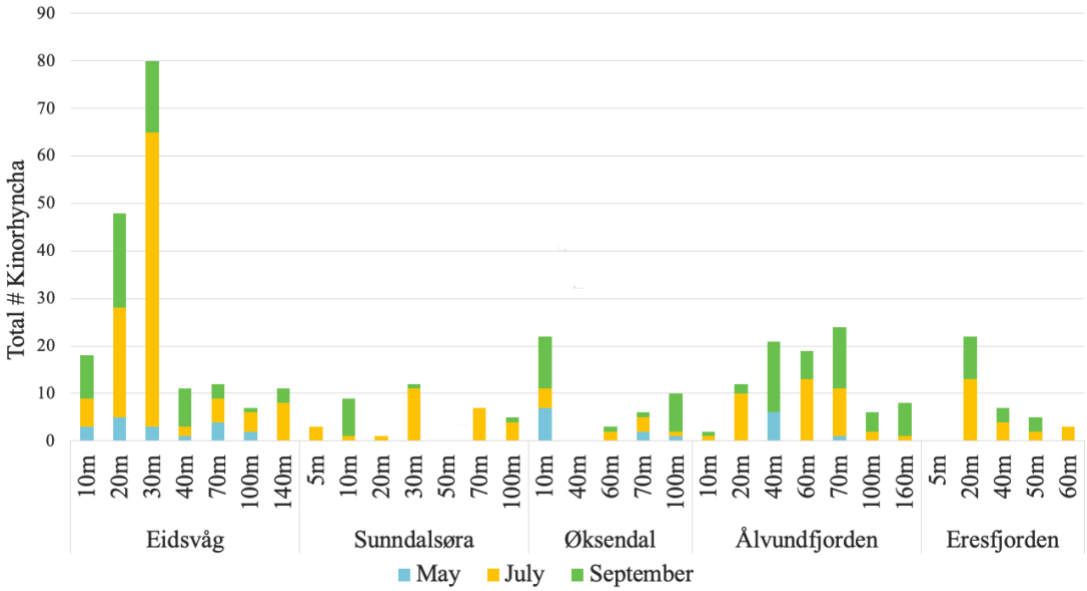


**Figure 7**: Total numbers of morphologically identified Kinorhyncha specimens collected in 2019 from five selected fjords in Møre og Romsdal, Norway. The sampling depth and season is indicated.

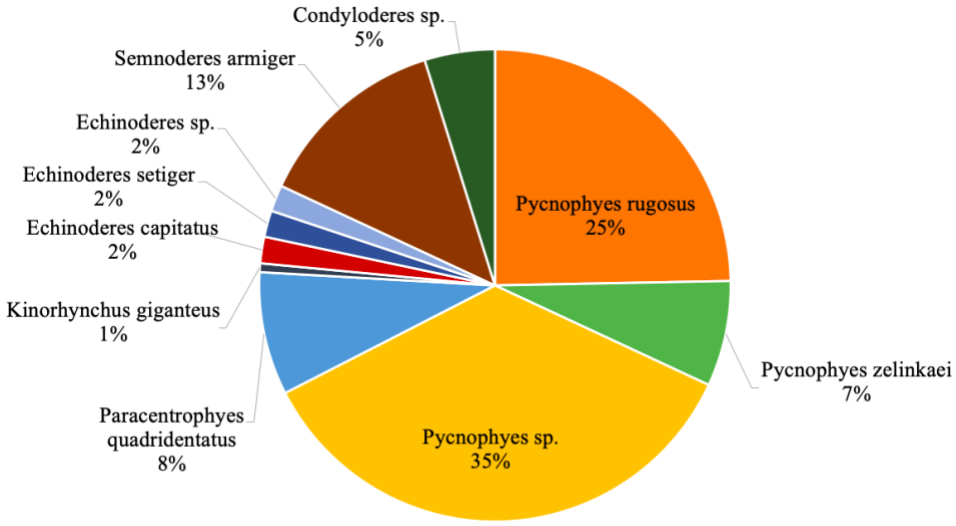


**Figure 8**: Relative frequency of molecularly determined Kinorhyncha species collected in five selected fjords in Møre og Romsdal, Norway 2019.

A relative abundance curve analysis was performed to interpret the species richness and species evenness (Figure 9, Table 6). The species richness in Møre og Romsdal proved to be 10. Based on the steep slopes observed, the species evenness proved to be low for both the whole sampling set as well as for the classes Homalorhagida and Cyclorhagida separately.

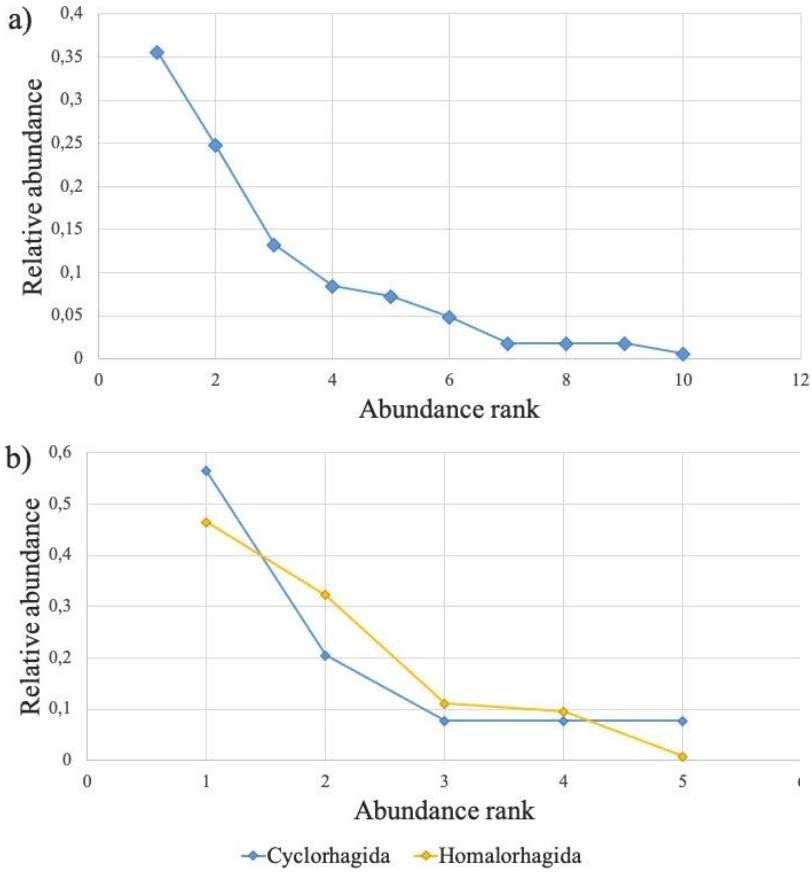


**Figure 9**: Rank abundance curves for the Kinorhyncha specimens collected in Møre of Romsdal, Norway 2019, a) the whole Kinorhyncha dataset and b) for Cyclorhagida and Homalorhagida separately.

**Table 6**: Rank abundance values of Kinorhyncha species identified in Møre og Romsdal, 2019.

| Rank | Species | Order | # individuals | Proportion |
|---|---|---|---|---|
| 1 | *Pycnophyes* sp. | Homalorhagida | 59 | 0.35 |
| 2 | *Pycnophyes rugosus* | Homalorhagida | 41 | 0.25 |
| 3 | *Semnoderes armiger* | Cyclorhagida | 22 | 0.13 |
| 4 | *Paracentrophyes quadridentatus* | Homalorhagida | 14 | 0.08 |
| 5 | *Pycnophyes zelinkaei* | Homalorhagida | 12 | 0.07 |
| 6 | *Condyloderes* sp. | Cyclorhagida | 8 | 0.05 |
| 7 | *Echinoderes capitatus* | Cyclorhagida | 3 | 0.02 |
| 8 | *Echinoderes setiger* | Cyclorhagida | 3 | 0.02 |
| 9 | *Echinoderes* sp. | Cyclorhagida | 3 | 0.02 |
| 10 | *Kinorhynchus giganteus* | Homalorhagida | 1 | 0.01 |

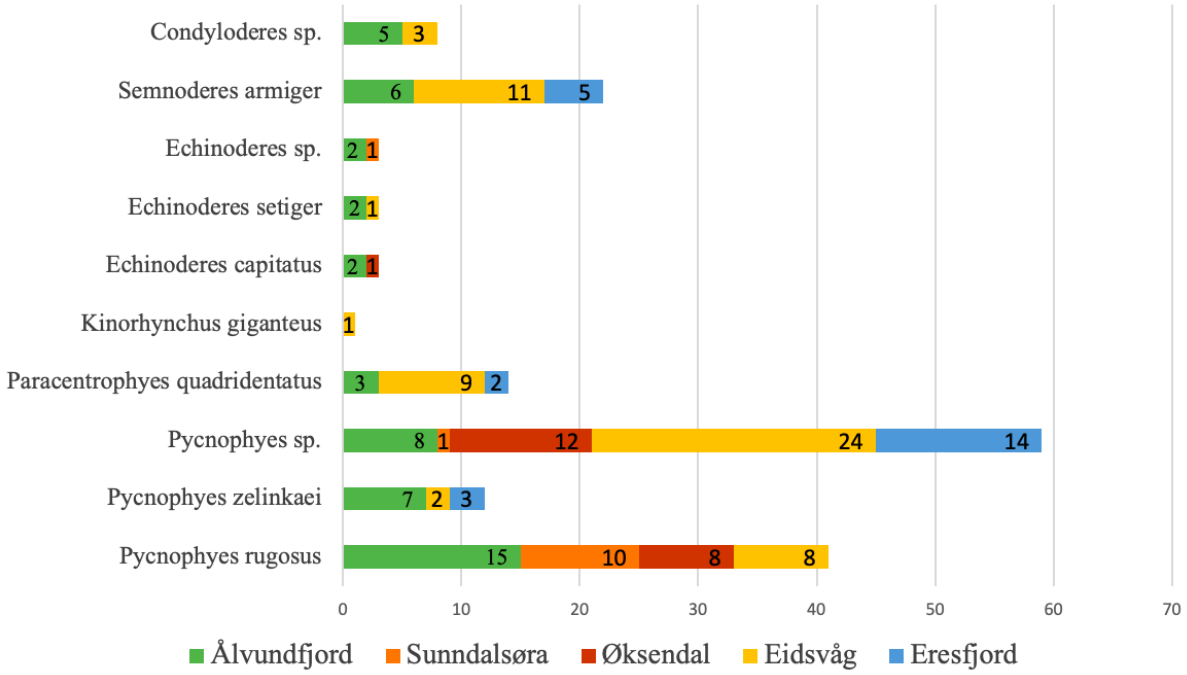


**Figure 10**: Number of molecularly identified Kinorhyncha species in each of the five targeted fjords Ålvundfjord, Sunndalsøra, Øksendal, Eidsvåg and Eresfjord in Møre og Romsdal, Norway.

A seasonal increase of the number of sampled and molecularly determined specimens (Figure 10) is observed in Ålvundfjord with seven specimens in May, 16 specimens in July and 27 specimens molecularly identified in September (Figure 11 a). The depth with the highest number of individuals in Ålvundfjorden were found to be at 70 meters in July and at 40 meters in May and September. For Eidsvåg the highest species richness of all sampled fjords was found, where 59 specimens could be molecularly determined (Figure 11 b). The highest abundance of kinorhynch individuals in Eidsvåg was found at 30 meters depth in July. In total, there were 6 specimens in May, 33 in July and 20 in September. Øksendal revealed a lower species richness, with a total of 21 specimens molecularly identified (Figure 11 c). The highest number of specimens was found in September with 11 specimens. Most specimens were found at a depth of 10 meters in both May and September. Sunndalsøra proved to be the fjord with least number of kinorhynch specimens with 12 individuals molecularly determined (Figure 11 d). All specimens were sampled at only two locations, at 70 meters and 100 meters. Eresfjorden revealed a relatively high number of specimens with 17 in July and 7 specimens in September. The highest number of specimens were found at a depth of 20 meters in both July and September (Figure 11 e).

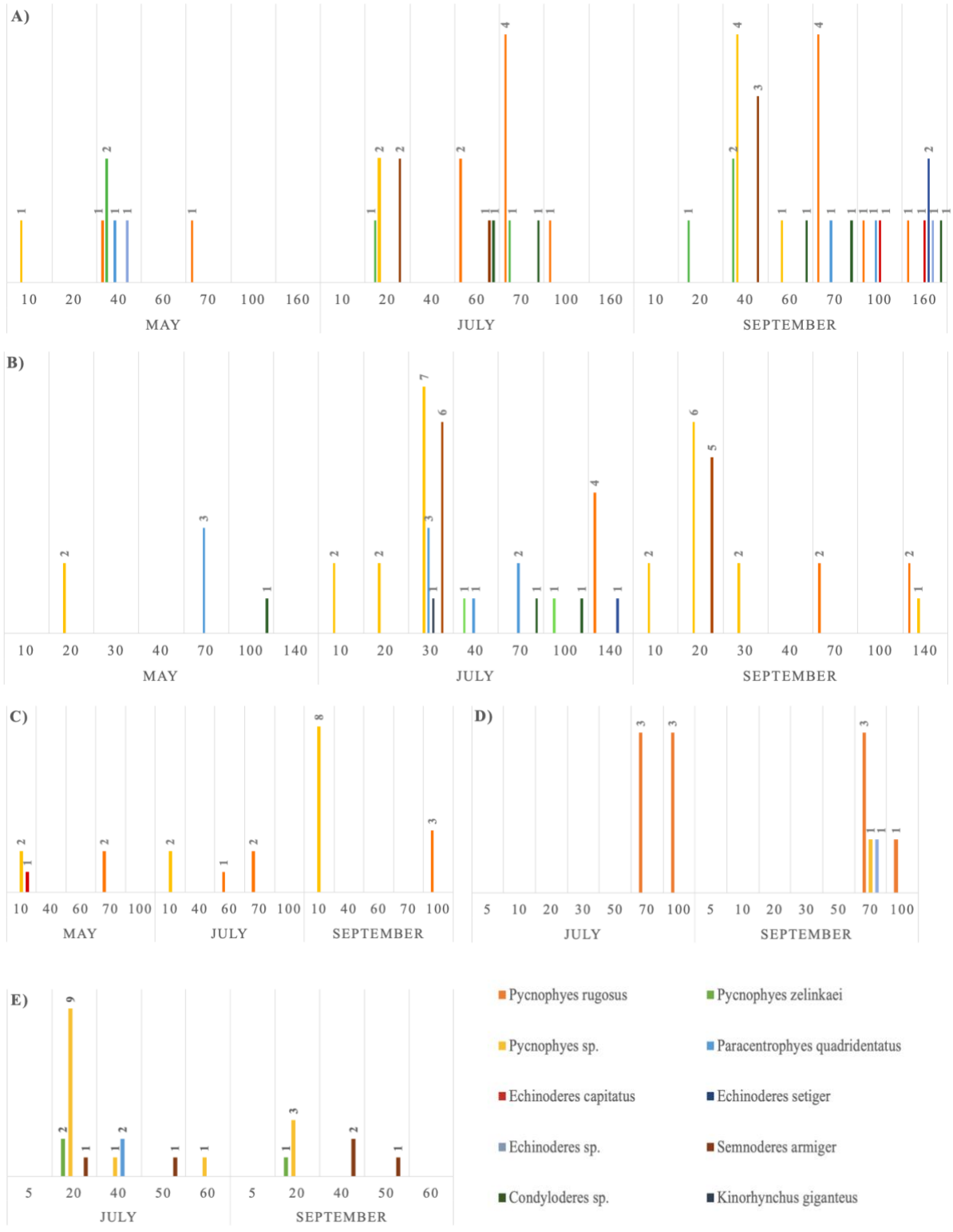


**Figure 11**: Number of Kinorhyncha species and genus identified broken down according to sampling depth, season and sampling location; a) Ålvundfjord b) Eidsvåg c) Øksendal d) Sunndalsøra and e) Eresfjord.

A Kruskal Wallis test revealed statistical differentiation in the distribution of specimens per species across each targeted fjord ($\chi_2(4) = 11.979$, $p = 0.018$). The Bonferroni post hoc test revealed differentiations between *Pycnophyes* sp. and *Kinorhynchus giganteus*, *Condyloderes* sp., *Echinoderes* sp., *Echinoderes setiger* and *Echinoderes capitatus*. A distinction was also found between *Pycnophyes rugosus* and *Kinorhynchus giganteus*, *Condyloderes* sp., *Echinoderes* sp., *Echinoderes setiger* and *Echinoderes capitatus*. The significant differentiation between the targeted fjords were found to be Eidsvåg relative to Sunndalsøra and Øksendal, and Ålvundfjord relative to Sunndalsøra, Øksendal and Eresfjord.

A significant seasonal differentiation of Kinorhyncha abundance could not be supported by a Kruskal Wallis test ($\chi_2(2) = 5.565$, $p = 0.062$), and a subsequent post hoc test was therefore not conducted. There was no obvious seasonal peak in species abundance, but a clear population growth was noticed between May and July sampling. In Eidsvåg and Eresfjord the highest abundance of Kinorhyncha was found in July with 33 and 17 specimens respectively. Ålvundfjorden and Øksendal showed the highest abundance in September with 27 and 11 specimens molecularly determined. No seasonal variation was observed in Sunndalsøra based on the molecularly identified specimens (Figure 12).

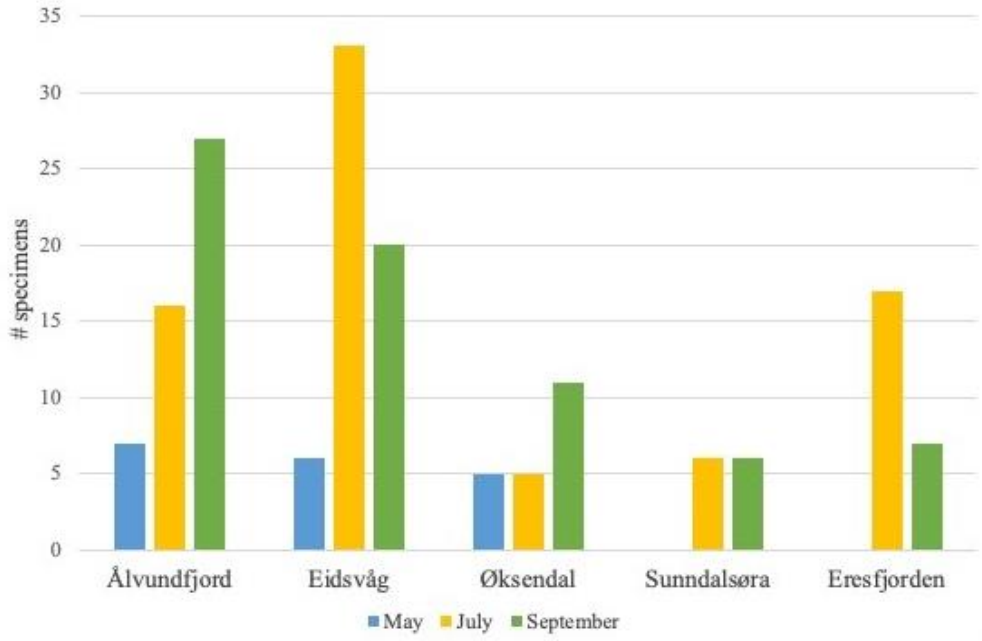


**Figure 12**: Variation in seasonal abundance of Kinorhyncha in each sampled fjord based on molecularly determined specimens observed in May, July and September on the northwest coast of Norway, Møre og Romsdal 2019.

There was no statistical support for another key hypothesis of this thesis, i.e. the of number of specimens across depth zonation did not differ significantly ($\chi_2(10) = 14.371$, $p = 0.157$). The Bonferroni post hoc test was subsequently not conducted as no significant results were found in the Kruskal Wallis test. Nevertheless, trends can be seen in the dataset (Figure 13). Species of the genus *Echinoderes* tend to prefer depths deeper than 70 meters. As a consequence of a small dataset, all the *Echinoderes* specimens were clustered together. *Semnoderes armiger* and *Paracentrophyes quadridentatus* seem to inhabit almost the same depths, at 20-60 meters and 30-70 meters respectively. The genus of *Condyloderes* were only found between depths of 60 and 100 meters, whereas the genus of *Pycnophyes* were found at almost all sampled depths. *Pycnophyes zelinkaei* were found at depths between 20 and 40 meters, *Pycnophyes* sp. at depths of 10 to 60 meters and *Pycnophyes rugoses* between depths of 60 and 160 meters. For *Kinorhynchus giganteus* nothing can be stated in this report as only one specimen could be detected.

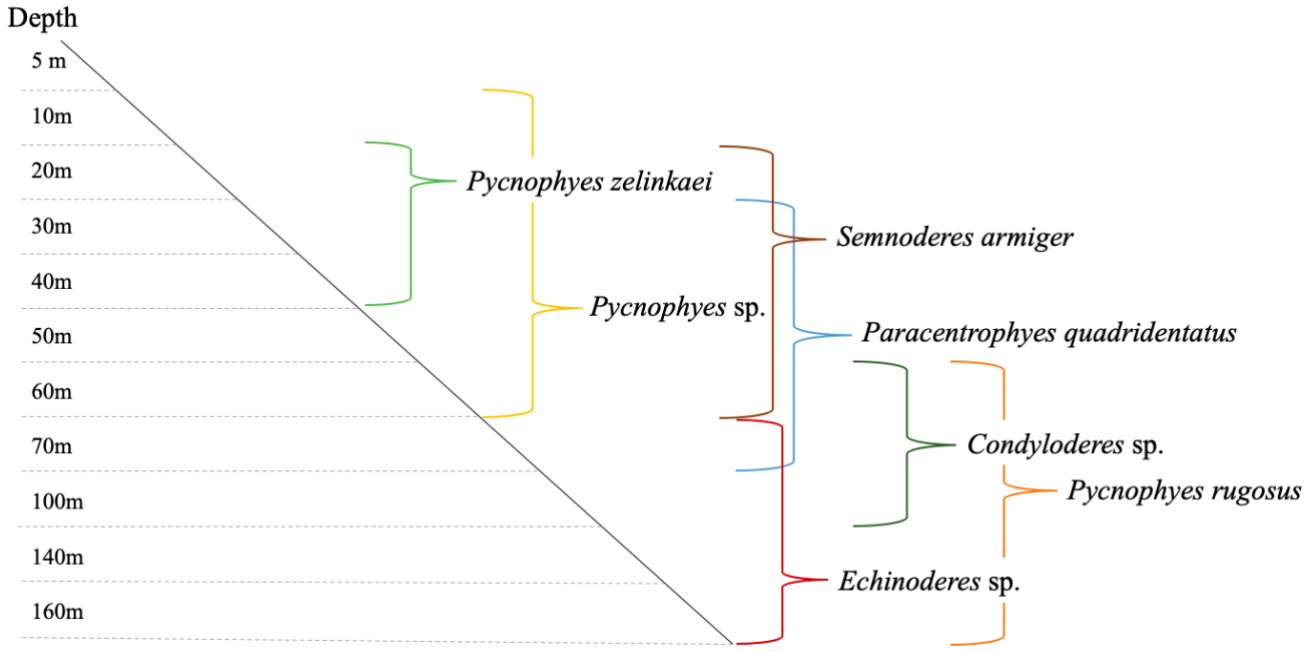


**Figure 13**: Trends in depth zonation of Kinorhyncha species in Møre og Romsdal, Norway 2019.

In order to assess the quality of the molecular species identification, a quick heuristic maximum likelihood (ML) analyses with nearest-neighbour-interchange (NNI) was generated with MEGAX, for the 18S sequences obtained for the sampled kinorhynch sequences (Figure 14). The resulting dendrogram makes phylogenetic sense and illustrates the

phylogenetic relationships between the Kinorhyncha species collected in the five sampled fjords Ålvundfjorden, Eidsvåg, Øksendal, Sunndalsøra and Eresfjord.

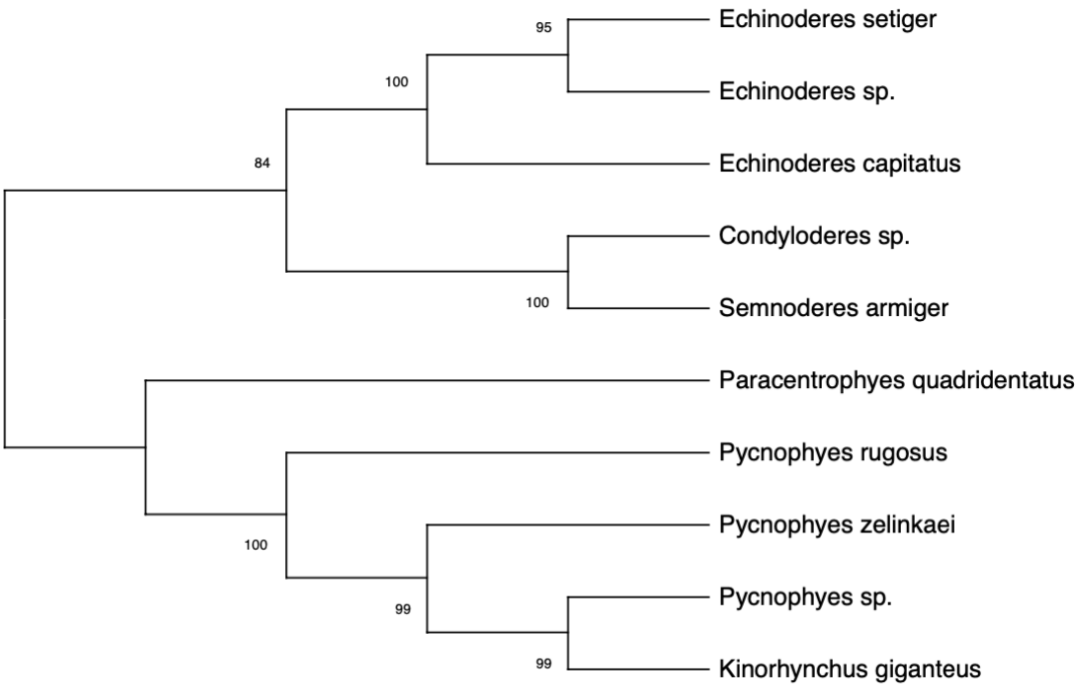


**Figure 14**: ML phylogenetic tree of 18S sequence data representing the molecularly identified Kinorhynch species collected in 2019 in selected fjords of Møre og Romsdal, Norway. Bootstrap support values based on 1000 replicates are indicated.

# 4. Discussion

The current study assessed the kinorhynch biodiversity in five targeted fjords in the Møre og Romsdal county, Norway. The selected fjords ranged from the almost non-impacted fjord Eresfjorden only experiencing local transit to Sunndalsøra, where the largest aluminium factory in Europe is based. This exploitation of the fjords as well as local environmental factors certainly affect the population structures of Kinorhyncha.

In total, 394 kinorhynch specimens were morphologically identified, but only 166 individuals could be confirmed as Kinorhyncha by molecular species identification. There are several reasons that might explain the discrepancy in the morphological and molecular approaches for kinorhynch identification. The morphological procedures applied in this study were a somewhat superficial 'quick and dirty' approach to rapidly screen the meiofauna samples for downstream analyses. There is very few obvious morphological characteristics in Kinorhyncha and juvenile stages of other meiofauna organisms can easily be misinterpreted. In addition, misinterpretation of morphological features of Kinorhyncha may have occurred more frequently in the initial phase of the thesis before extended experience with this phylum had been obtained. Indeed, the discrepancy of morphological and molecular identification was more frequent in the May and July samplings than in September. However, morphological misidentification did not affect this thesis other than leading to an increased workload for the molecular species identification. Other reasons for the observed discrepancy between morphological and molecular species identification may be due to the process of manually transferring the Kinorhyncha specimens from the environmental samples to small reaction tubes, and it cannot be excluded that some specimens got lost underway.

In the course of the molecular species identification it turned out that the less specific and universal primer pairs S30/5FK, 4FB/1806R and LCO1490/HCO2198 proved to be highly susceptible to cross-contamination. The targeted genes are present in all animals, and 18S in particular is highly conserved. While this on the one hand allows for designing universal primer pairs it also opens for amplification of contaminating DNA, a risk that is of even greater concern when using low annealing temperatures. The obtained sequence data confirmed to some extent the morphological misidentification issue and identified other meiofauna species such as e.g., nematodes, polychaetes and annelids. Nevertheless, these

primers allowed identification to species level, whereas the alternative and more specific primer pair of 18S-323dir/18S-823rev in some cases did not provide enough information to determine specimens to species level. During the molecular species identification, none of the sequences obtained with the CO1 primer pair LCO1490/HCO2198 provided good enough sequences to be implemented in the species determination. Most of the CO1 generated sequences came at best from contaminating material. Accordingly, only the 18S gene were used in the identification. It is also noteworthy that the DNA lab at the Natural History Museum in Oslo is a multi-user facility, which implies an increased risk of contamination between projects.

## 4.1 Biodiversity of Kinorhyncha in the targeted fjords

In the targeted fjords in Møre og Romsdal a relatively low Kinorhyncha species richness was observed with only seven species within six genera identified. The species evenness also proved to be relatively low, with a high abundance of *Pycnophyes* species. Even with a more specific look at the distribution of species/genera identified within the classes of Homalorhagida and Cyclorhagida, the species evenness was still low. The steeper slope of the cyclorhagids compared to the homalorhagids visualized in the RAD curve (Figure 9 b) indicates a slightly skewed distribution of more homalorhagid specimens present than cyclorhagids. The list of identified Kinorhyncha species in the course of this study includes *Pycnophyes rugosus*, *Pycnophyes zelinkaei*, *Paracentrophyes quadridentatus*, *Echinoderes capitatus*, *Echinoderes setiger*, *Semnoderes armiger* and *Kinorhynchus giganteus*. Some of the specimens could not be determined to species level, but were identified to genus level, i.e. *Pycnophyes*, *Condyloderes* and *Echinoderes*. Overall, a significant differentiation in number of specimens of each species was observed between sampled fjords.

In the targeted fjords Kinorhyncha were detected with a significant differentiation in both diversity and abundance. As suspected due to the high level of human activities particularly when regarding the port facilities, Sunndalsøra showed the lowest abundance and diversity of Kinorhyncha. All specimens molecularly identified to species level were unpredictably found at depths of 70 and 100 meters. The low abundance of Kinorhyncha found in Sunndalsøra compared to the other sampling fjords might indicate limited habitat and ecological resources for meiofauna in this area due to the heavy traffic and possible pollution associated with the

aluminium factory. The factory port facilities generate soil-, water- and air pollution from the cargo ships (Trozzi and Vaccaro, 2000) transporting aluminium, which all contributes to a less favourable habitat on the ocean floor. In addition, the propeller of large cargo boats can whirl up the sediments, creating abrupt alterations of the meiofauna habitat which is regarded as one of the major factors threatening the meiofauna community (Schratzberger and Somerfield, 2020).

The side fjord of Tingvollfjorden at Øksendal is geographically very close to the Sunndalsøra sampling localities. Initially Øksendal was expected to be an ideal location in Tingvollfjorden where Kinorhyncha prospering could be detected. But only a slightly higher species richness than in Sunndalsøra was observed. Since the fjord of Øksendal locally only is affected by small-boat transit and agriculture, the data collected may indicate that the ship activity at Sunndalsøra also impact the Kinorhyncha diversity at Øksendal.

Ålvundfjord is also geographically close to Tingvollfjorden but the inner parts of the two fjords; Tingvollfjorden and Trongfjorden, are separated from each other by an extended ridge. Here, a seasonal increase in abundance was observed. Ålvundfjord represent the most diverse sampling site with nine of the observed species/genera present. The highest abundance of kinorhynchs was found at water depths of 40 meters, closely followed by 70 meters. Given that there are minor human activity at Ålvundfjord, this fjord may reflect the most natural situation in the larger Tingvollfjord area. In that respect, Ålvundfjord could potentially reflect the kinorhynch diversity in Tingvollfjord without the human alterations observed in Sunndalsøra.

Eidsvåg represented the fjord with the highest abundance of Kinorhyncha of the targeted fjords. Although with a somewhat speculative peak of abundance in July. Eidsvåg is known from previous studies (personal communication) for being a Kinorhyncha diversity hotspot, which was confirmed in this study. Eidsvåg represented the second most impacted fjord in terms of human activities and seem to contradict the initial expectation that human impact affects the abundance of Kinorhyncha, or at least might confirm that Kinorhyncha prospering is not affected by moderate human activities as how the fjord of Eidsvåg is utilized. Whether the high number of specimens could be a result of high amounts of nutrition run-off associated with a larger town and the surrounding agriculture could be an explanation to the abundance observed. However, this seem unlikely as Eresfjord and Øksendal in that case also

would yield a higher abundance of Kinorhyncha. Therefore, the elevated abundance of kinorhynchs due to the excess input of nutrition from agriculture seem unlikely but cannot be ruled out. However, the observed abundance of Kinorhyncha in Eidsvåg suggests that the level of human activity in this fjord at least does not negatively affect the Kinorhyncha diversity. It may indicate that the differentiation between the abundance of Kinorhyncha in Eidsvåg and Sunndalsøra are caused by the port activity in Sunndalsøra. If true, one may conclude that other parameters such as population size of the town/settlement closely associated with the targeted fjord is of less importance for meiofauna diversity. Furthermore, the Eidsvåg fjord has an entirely different geographical direction than all the other fjords. Eidsvåg is located in a north-south direction, and the other targeted fjords are all located in an east-west direction. If ecological parameters are affected by this geographical direction or not would only be speculations, but it is worth noticing.

Among the targeted fjords in this study, Eresfjord is regarded the least impacted fjord of human activity. Nevertheless, species richness was found to be relatively low, with only four identified species/genera. Eresfjord and Eidsvåg should in theory be highly comparable as they are both located in the end of Langfjorden, but this study provides too little information from Eresfjord to comprehensively compare these two fjords. In addition, Eresfjord is much shallower than the other fjords with the highest Kinorhyncha abundance found at a depth of 20 meters. It must also be noted that Eresfjord, together with Sunndalsøra, was only sampled during July and September, which may represent a sampling bias.

## 4.2 Seasonal variation

When availability to light and temperatures increase, the primary production is expected to grow (Brown et al., 2004). Based on the *a priori* assumption that Kinorhyncha feed on diatoms and decompose organic material, a direct consequence of the increased availability to resources from approximately March until September, would be an increased frequency of reproductively mature Kinorhyncha (Higgins and Fleeger, 1980). In that respect, this would lead to a kinorhynch population growth during this surveys time of sampling. From sampling period in May to July an increase in abundance was observed, with a roughly fourfold increase in the number of Kinorhynch specimens. With the highest recruitment period of juvenile Kinorhyncha in May to September (Higgins and Fleeger, 1980), the population is

expected to experience a peak in population density to ensure population survival during times of scarce resources. But the seasonal variation in Kinorhyncha abundance observed in the target fjords in Møre og Romsdal, is not consistent with the expected outcome from the increased availability to resources nor population growth. The September sampling revealed a slightly lowered number of molecularly identified specimens, which in turn indicate a somewhat decrease in population abundance. This could be a consequence based on technical issues described above in both sampling and molecular species identification. However, it could also be elucidated by three possible biological explanations; the possible peak in population growth a) occurred in between sampling times, b) occurred after sampling ceased or c) there is no clear peak of population growth in the Kinorhyncha population in Møre og Romsdal. Regardless, there was not detected any statistical significance for a seasonal variation in the kinorhynch community structure in Møre og Romsdal. In addition, there was also an issue with uneven sampling as Sunndalsøra and Eresfjord were sampled only in July and September, which created an inconsistency that surely affected the information obtained to detect seasonal variation. The May sampling had to be dropped for the two localities due to issues with time management of the fieldwork, which was improved in July and September. On the other hand, the relatively small dataset turned out to be a more serious issue for this study. To fully explore the potential of seasonal variation, a more comprehensive sampling period might yield a better dataset to provide information on seasonal variation in the Kinorhyncha community in Møre og Romsdal, Norway.

## 4.3 Depth zonation

The Kinorhyncha have been recorded thriving at a wide variety of depths, ranging from the intertidal to the abyssal zones (McIntyre, 1962; Danovaro et al., 2002; Neuhaus and Blasche, 2006; Adrianov and Maiorova, 2015). The current study covered only depths until ca 160 meters, and there was no significant differentiation found between number of specimens relative to depth for the five targeted fjords. The minor differentiation in Kinorhyncha abundance depending depth zonation observed in this study can therefore only be seen as trends without sufficient statistical support. In an attempt to obtain some indication of optimal depth zonation in Møre og Romsdal, the depths were clustered together in ranges of 35 meters; 5-40, 45-80, 85-120, 125-160. But neither this clustering revealed any further insight, and a significant differentiation in Kinorhynch abundance was subsequently not

found. Here, a larger sample size is certainly required for a more powerful analysis. *Echinoderes* species as well as *Pycnophyes rugosus* seem to prefer depths below ca 60-70 meters whereas *Semnoderes armiger* and *Paracentrophyes quadridentatus* seem to prefer less deep habitats. Although currently only trends, the various Kinorhyncha species occurring in the fjords on the Norwegian northwest coast seem to have adapted to particular microenvironments.

## 4.4 Human impact on the kinorhynch community

The assessment of human impact has in this study been focusing on the level of utilization of the fjords in respect to fishing, passage and transport, and size of the associated settlements. Other than the aluminium factory and associated activities in Sunndalsøra, there was not recorded any heavy utilization in the other fjords, where only small-boat passage was observed, and no heavy fishing nor aquaculture occurred. As reported by Schratzberger and Somerfield (2020) heavy bottom-fishing is regarded as one of the main factors threatening the meiofauna community together with invasive species. They further state that the global ocean environment is threatened by climate change. As increased temperatures, acidic waters and aerobic habitats are rapidly created, the metabolic rates change which in turn will affect the life history of the meiofauna (Schratzberger and Somerfield, 2020). The distribution of meiofauna, at least nematodes, has been found to largely depend on temperature of habitat (Wieser and Schiemer, 1977), this would be transferrable to other meiofauna as ectotherms use the external environment to regulate body temperature. As in the case of Kinorhyncha, they have been found to endure various environmental states (Horn, 1978; Neuhaus and Higgins, 2002; Kozloff, 1972) at a large range of depths throughout the world. In other words, kinorhynchs would in that respect be quite resilient to environmental change. But as anoxic environments are created as a consequence of the increased water temperatures, the survival rate of kinorhyncha are expected to decrease as a direct effect of the new anoxic habitats (Dal Zotto et al., 2016). During analysis of the environmental meiofauna samples, plastic particles were found in all samples from all of the targeted fjords. Meiofauna has been found to ingest microfibers (Gusmão et al., 2016). Although studies on this subject are limited, it represents another potential threat to the kinorhynch prospering, not only in Møre og Romsdal but also around the globe. As the ocean temperatures rise at an increasing pace and new threats alter the habitats, it is currently impossible to predict how the kinorhynch

diversity will evolve. A long-term survey of the kinorhynch community in Møre og Romsdal would yield better knowledge and understanding on how and to what degree the kinorhynch community is affected by human activity.

# 5. Conclusion

The current study on Kinorhyncha biodiversity on the northwest coast of Norway has provided information on the kinorhynch community in the fjords of Møre og Romsdal. There was a relatively high abundance and diversity of kinorhynchs in both Eidsvåg and Ålvundfjord, and a kinorhynch richness in Øksendal, Sunndalsøra and Eresfjord. A correlation was observed between human activities and abundance of Kinorhyncha species. The port facilities and possible pollution associated with the aluminium factory in Sunndalsøra, may be a main factor for low abundance of Kinorhyncha in Sunndalsøra and the geographically close side fjord of Øksendal. More extended sampling, both in terms of time and samples, is required to properly assess the community structures of kinorhynchs. The limited dataset of the current study only allows for identifying trends in the Kinorhynch community in Møre og Romsdal, Norway, and to derive hypotheses for more comprehensive future studies.

# 6. Acknowledgements

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Needless to say, I want to thank my family and friends for emotional support and for pushing me to work even harder.

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# Supplementary Information S1 – Edited nucleotide sequences obtained for the Kinorhyncha specimens collected in Møre og Romsdal, Norway, 2019.

## Order Homalorhagida;

### *Pycnophyes rugosus*:

500A S30/5FK:
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540A S30/5FK:

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541A S30/5FK:

TGTTTTAAAGATTAAGCCATGCATGTCTAAGTACACGCTCCGGCAAAGCAAAACCGCAGAAGGCTCAGTATTT
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541B S30/5FK:

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541F S30/5FK:

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541J S30/5FK:

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542C 18S-323dir/18S-823rev:

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542K S30/5FK:

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551B 4FB/1806R:

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552C S30/5FK:

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553A 18S-323dir/18S-823rev:

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553D S30/5FK:

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553E 4FB/1806R:

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553M S30/5FK:

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558A 18S-323dir/18S-823rev:

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559C 18S-323dir/18S-823rev:

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559D S30/5FK:

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559E 18S-323dir/18S-823rev:

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566E S30/5FK:

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566G S30/5FK:

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AATTCTTGGATCGCAGCAAGACGAACAGCTGCGAAAGCTTTT

566H 18S-323dir/18S-823rev:

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575A S30/5FK:

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575C S30/5FK:

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577A S30/5FK:

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577B S30/5FK:

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## *Pycnophyes zelinkaei*:

500E 4FB/1806R

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500G 4FB/1806R:

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522B S30/5FK:

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524C S30/5FK:
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541I S30/5FK:
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544B 18S-323dir/18S-823rev:
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547E 18S-323dir/18S-823rev:
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547F S30/5FK:
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555H S30/5FK:

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555M 4FB/1806R:

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556B S30/5FK:

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573A S30/5FK:

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## *Pycnophyes* sp.:

500 A 4FB/1806R:

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508B 4FB/1806R:

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508E 4FB/1806R:

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514B 4FB/1806R:

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514D S30/5FK:

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519A 4FB/1806R:

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519C 18S-323dir/18S-823rev:

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520G 4FB/1806R:

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520Q S30/5FK

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521G 18S-323dir/18S-823rev:

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521HH S30/5FK:

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521UU S30/5FK:

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521YY S30/5FK:

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521ZZ S30/5FK:

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521ÅÅ S30/5FK:

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521DDD S30/5FK:

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538E S30/5FK:

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538F 18S-323dir/18S-823rev:

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544A S30/5FK:

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GAGGATCTATTGGAGGACAAGTCTGGTGCCAGCACCCGCGGTAATTCCAGCTCCAATAGCGTATATTAATGCT
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544I S30/5FK:

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CACTCCCGGCACGGGGAGGTAGTGACAAGAAATAACAATACAGGACTCTTTCGAGGCCCTGTAATTGGAATG
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547A S30/5FK:

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547B S30/5FK:

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547D S30/5FK:

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547G S30/5FK:

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547H S30/5FK:

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547J S30/5FK:

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547K S30/5FK:

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547L S30/5FK:

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547M S30/5FK:
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548C S30/5FK:
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550A S30/5FK:
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554C S30/5FK:
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555A S30/5FK:
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555F 18S-323dir/18S-823rev:
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555G S30/5FK:
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555I 18S-323dir/18S-823rev:
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559H 18S-323dir/18S-823rev:
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569C S30/5FK:
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569D S30/5FK:

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569F 18S-323dir/18S-823rev:

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569G S30/5FK:

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569H S30/5FK:

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569I S30/5FK:

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569J 18S-323dir/18S-823rev:
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569K 18S-323dir/18S-823rev:
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573C S30/5FK:
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573E S30/5FK:
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573I S30/5FK:
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575B 18S-323dir/18S-823rev:
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579H S30/5FK:
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579O 18S-323dir/18S-823rev:
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580D S30/5FK:
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580G S30/5FK:
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580M 18S-323dir/18S-823rev:
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580N 18S-323dir/18S-823rev:
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580P 18S-323dir/18S-823rev:
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580R 18S-323dir/18S-823rev:
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581C S30/5FK:
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581D S30/5FK:
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## *Paracentrophyes quadridentatus*:

500H S30/5FK:
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511B 18S-323dir/18S-823rev:
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511C 18S-323dir/18S-823rev:
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511D S30/5FK:
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521MM 4FB/1806R:

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521PP 18S-323dir/18S-823rev:

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521ÆÆ S30/5FK:

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522A S30/5FK:

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523F S30/5FK:

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548A S30/5FK:

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548B S30/5FK:

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552D S30/5FK:
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553B 18S-323dir/18S-823rev:
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## *Kinorhynchus giganteus*:

521Æ S30/5FK:
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CGTTTG

Order Cyclorhagida;

*Echinoderes capitatus*:

514A S30/5FK:
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551G S30/5FK:
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552A 4FB/1806R:
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GATTATTTA

*Echinoderes setiger*:

525A S30/5FK:
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551A S30/5FK:

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551F S30/5FK:

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## *Echinoderes* sp.:

500C 4FB/1806R:

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TGGCAACATGTCG

551E 18S-323dir/18S-823rev:

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559B 18S-323dir/18S-823rev:

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## *Semnoderes armiger*:

521K S30/5FK:

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521R S30/5FK:

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521Y S30/5FK:

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521Z 4FB/1806R:

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GA

521BB S30/5FK:

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A

521RR S30/5FK:

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542F S30/5FK:

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544C S30/5FK:

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544E S30/5FK:

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547C S30/5FK:

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549B S30/5FK:

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555B S30/5FK:

AGCTCCGGCACAGCGAAACCGCGAATGGCTCATTAAATCAGCTATGATTTATTGGATGAATACTTGCACATGG
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555C 18S-323dir/18S-823rev:

ATAACGGGTAACGGTGAATCAGGGTTTGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAA
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CAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGAATC
TCACGTTTTGGGTACGTGGTTCGCTATATAGCGATTACTACGTCTCCCGACGTACCTCTCCGATTTCCCTCGGT
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555L S30/5FK:

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GACTTACGAAAGGGTGCACTTGCCAGACCAAAAACCAATCGGGCGTCAGCCCGTTCAGTGGCGAACCATGGC
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571C S30/5FK:

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572A S30/5FK:

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572C S30/5FK:

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580A S30/5FK:

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580B S30/5FK:

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580I S30/5FK:

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580O S30/5FK:

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580Q S30/5FK:

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## *Condyloderes* sp.:

512B S30/5FK:

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TGCGCGTCCCGTCGTACCTCTCCGCTTTCCCTCGGTGCTCTTAACTGAGTGTCTTGGGTTACGGGACTGTTTACT
TTGAAAAAATTAGAGTGTTCAAAGCAGGCAGGCCGCCTGAATAGTGGTGCATGGAATAATGGAAGACGACCT
CGGGTCTATTTTGTTGGTTTCCGGACTCAGAGGTAATGATTAAGAGGGACTGCCGGGGGCATCCGTACTGCTG
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523E 18S-323dir/18S-823rev:

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TGGCGTACGGGCGCGCAGTTCGCTTTCACAGCGACTACTGCGCGTCCCGTCGTACCTCTCCGCTTTCCCTCGGT
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524A S30/5FK:

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CTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCACGCAAATTACCCACTCCCAGCTCGGGGAGGTAG
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541G S30/5FK:
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542J S30/5FK:
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551C S30/5FK:
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553H S30/5FK:
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554B S30/5FK:

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