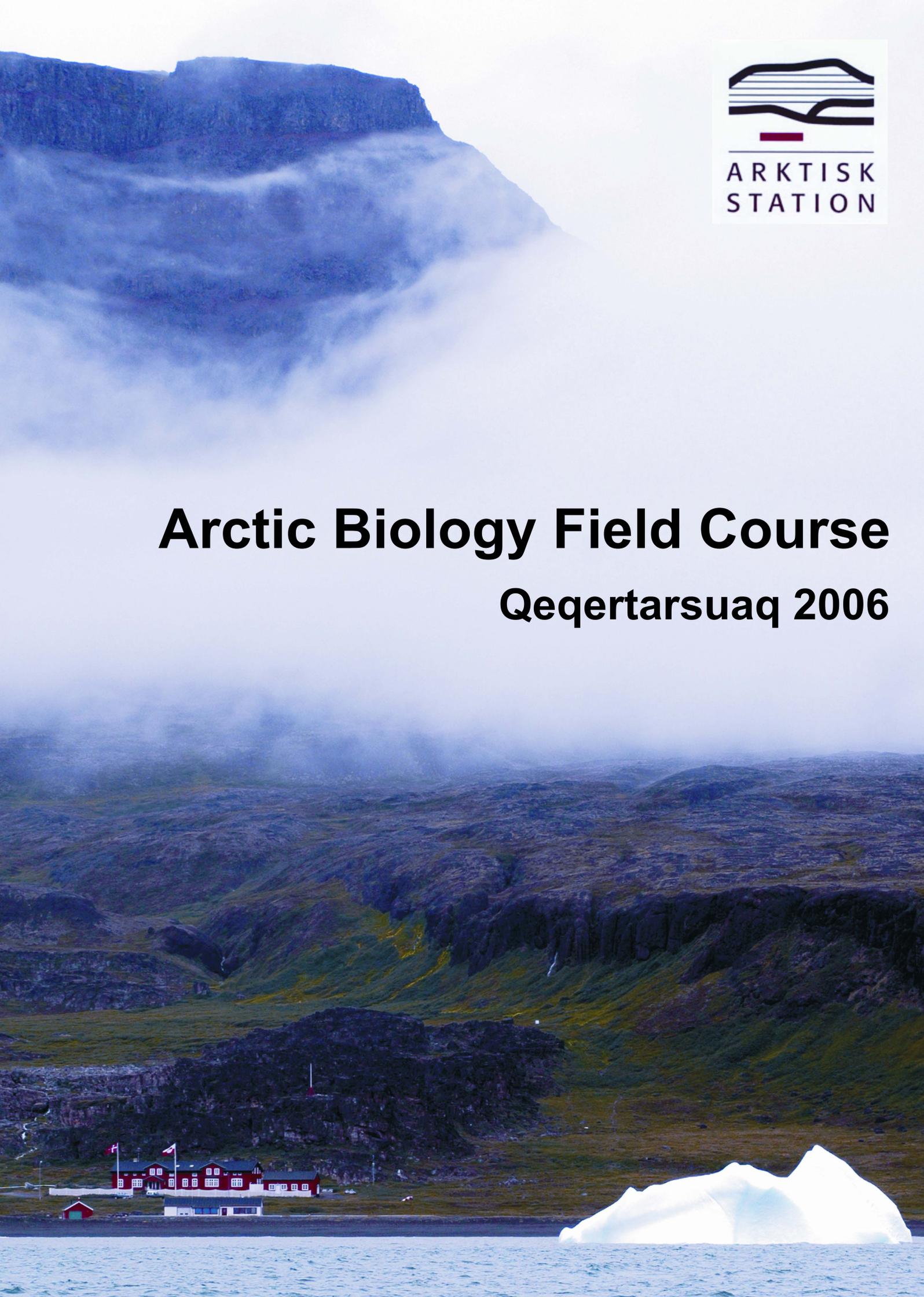




Arctic Biology Field Course

Qeqertarsuaq 2006



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Arctic Station

University of Copenhagen

Sea angel or *Clione limacina* (Gastropoda, opisthobranchia) - a small pelagic marine sea slug widely distributed in the Arctic. Photo: Jonas Thormar

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Cover Photo: Arctic Station in the shadow of Østerlien with the homothermic warm springs and in the towering presence of the 800 m tall Lyngmarksfjeldet covered in mist. Photo by Martin O. Macnaughton.

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Preface

Reinhardt Møbjerg KRISTENSEN & Poul Møller PEDERSEN

The Board of Arctic Station, Faculty of Science, University of Copenhagen, Denmark



The arctic field courses from the University of Copenhagen have been held regularly since 1973 at Arctic Station, Qeqertarsuaq (Godhavn), Disko Island, West Greenland. The station was founded in 1906 by the Danish botanist Morten Porsild, and it is thus the oldest research institution north of the Polar Circle. The 4th of August 2006 the station celebrated its 100 years anniversary. Today, the station is owned by the Faculty of Science, and it is open all year round for international and national scientists as well as graduate students. For a more comprehensive introduction to the Arctic Station, please consult the address: <http://www.nat.ku.dk/as/>.

The Arctic Biology Field Course, Qeqertarsuaq 2006, was organised by the Biological Institute and Natural History Museum, University of Copenhagen. It was carried out between the 11th and 30th of August 2006 at the Arctic Station. The field course addressed exclusively marine issues, but a few terrestrial excursions were made, e.g. the hiking trips to Ilulissat (Jakobshavn) to the enormous Ilulissat Icefjord on the 31st August and in Kangerlussuaq (Sdr. Strømfjord) on the 1st of September. The botanical teachers were Niels Daugberg and Poul Møller Pedersen, and the zoological teachers were Reinhardt Møbjerg Kristensen and Andreas Wanninger. The scientific leader of the station during the course, Henrik Sulsbrück Møller, and the whale researcher Outi Tervo (the present scientific leader) assisted the teachers with several talks, and we were allowed to use the laboratory and the microscopes with digital camera and video recorder. The dean of the Faculty of Science, Nils O. Andersen and his wife Jette Brøns, participated in the beginning of the course.

Twelve master students from the University of Copenhagen participated in the field course. They had all successfully passed the course in arctic biology, and they were chosen to carry out their proposed field projects after an evaluation by their supervisors. Furthermore, two German Ph.D.-students, Henrike Semmler and Nora Brinkmann, participated and collected various species of marine plankton larvae as part of their Ph.D.-programmes for subsequent studies in Copenhagen using confocal laser scanning microscopy. Furthermore, they helped to supervise the Danish students. Finally, the tidal tardigrade *Echiniscoides* was studied by, Søren Faurby, who is Ph.D.-student from the University of Aarhus.

The proposed field projects and selected students were:

- 1). Marine phytoplankton (Iben Rønn Veland & Maria Hastrup Jensen)
- 2). Marine, benthic macroalgae (Mads Birkeland, Christian Nielsen & Helle Wilken-Jensen)
- 3). Identification of arctic marine invertebrate larvae from West Greenland near Disko and description of their muscular and neural anatomy by confocal laser scanning microscopy (Louise Holst Hemmingsen, Payana Henriksen & Mette Liebst-Olsen).
- 4). Marine Meiofauna – Gastrotricha, Kinorhyncha and Polychaeta (Jonas Thormar & Martin O. Macnaughton).
- 5). Osmotic stress tolerance and *de novo* protein synthesis in the Arctic tardigrade (water bear), *Halobiotus crispae* (Kenneth A. Halberg & Dennis Persson).

The stay on Disko Island was general successful from a scientific point of view and this report summarises the results. All contributions published in this report are also available as pdf-files at the homepage of the Arctic Station: <http://www.nat.ku.dk/as/>.



Arctic Station. Photo: Martin O. Macnaughton.

The weather was not equivalent to the so-called “Godhavn summer”, however, the wind conditions allowed us to visit both Hareøen with an overnight camp in Nordfjord and Mudderbugten with an overnight camp at Flakkerhuk. The field course deviated from standard procedures by not using camp sites for longer periods of time, as the students needed the laboratory facilities such as microscopes, refrigerators and the cooling room, but all students were allowed to join several excursion *e.g.* to Nipisat, Skansen, and Ippik. During nearly all excursions humpback whales were observed; sometimes even observed up close from a dinghy (small boat) - this was a new and fascinating experience for both senior scientists and students.

Acknowledgements. First we want to thank the entire staff at the Arctic Station for providing logistic support, especially the crew onboard “Porsild”, Frederik Grønvold, Søren Fisker and Erik Wille, and the station manager Kjeld (Akaaraq) Mølgaard. Thanks are also given to a number of colleagues at the University of Copenhagen for assisting the students to process their field data and samples. Especially, we acknowledge the secretary of Arctic Station, Gitte Henriksen, who solved all the logistic problems with transportation also related to the station’s anniversary.

The Faculty of Science covered travel expenses for 12 students and two teachers, and additional support was given by the Arctic Station. Substantial support to the students’ accommodation was given by the Danish Botanical Society and the Carlsberg Memorial Grant for Brewer J.C. Jacobsen. Finally, we are indebted to Kenneth Agerlin Halberg for editing this report.

Dedication. We dedicate this report to stud. scient. Mette Liebst-Olsen, who after the return to Copenhagen was attacked by severe illness with an effect on the rest of her life. We give her our warmest sympathy.



The participants of the Arctic Biology Field course 2006 along with the scientific leader and technical staff.
Photo: Martin O. Macnaughton.



1. K. A. MØLGAARD **2.** N. DAUGBJERG **3.** S. FAURBY **4.** M. J. BIRKELAND **5.** H. SEMMLER **6.** M. O. MACNAUGHTON **7.** A. WANNINGER **8.** K. A. HALBERG **9.** P. M. PEDERSEN **10.** H. N. WILKEN-JENSEN **11.** D. PERSSON **12.** H. S. MØLLER **13.** M. H. JENSEN **14.** I. R. VELAND **15.** M. LIEBST-OLSEN **16.** N. BRINKMANN **17.** C. K. NIELSEN **18.** L. H. HEMMINGSEN **19.** P. HENDRIKSEN **20.** J. THORMAR **21.** R. M. KRISTENSEN

A Survey of Marine Dinoflagellates in the Waters Surrounding Disko Island, Western Greenland

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Abstract. The Global tendency towards an increase in atmospheric temperature seems to be especially pronounced in the Arctic regions, where dramatic alterations are predicted to occur within the next 40 years. The present survey was conducted in order to pursue indications of possible Global change induced alterations of the dinoflagellate species composition in the vicinity of Disko Island. In addition, the aim was to establish a base line of the species diversity for future comparison. From August 11th to the 30th, 2006, net samples were collected at nine different stations in the vicinity of Disko Island, from Mudderbugten to Hareøen. Using LM and SEM techniques we identified 50 dinoflagellate species belonging to 22 genera, of which four are new recordings for this area. These results indicate a slight change in the species composition in comparison to recordings from previous surveys; changes that may be associated with alterations of the Arctic marine food web. In addition, we have isolated single cells from the samples and obtained molecular data on 7 species of the family Dinophysiaceae. Surprisingly, the phylogenetic results support a re-instatement of the genus *Phalacroma* which, in recent literature, is considered to be synonymous with *Dinophysis*.

Keywords. Marine Dinoflagellates, Global change indication, Western Greenland, species diversity, phylogeny, *Dinophysis*, *Phalacroma*.

1. INTRODUCTION

The material for this project was collected during the Arctic field course at Arctic Station Qeqertarsuaq/Godhavn, Disko Island, Greenland, from the 11th of August to the 1st of September, 2006. This introduction contains three main paragraphs; the first concerning global changes in the Arctic environment and the role of the marine phytoplankton in this ecosystem, while the second paragraph contains descriptions of some general features of the phytoplankton group of interest: the dinoflagellates. The third paragraph presents an overview of some of the problems within the *Ceratium* (*C. arcticum* and *C. longipes*) complex.

1.1. Global warming and the Arctic marine environment

Together with climate reconstructions of the past millennia, several observations made over the last 100 years have all shown changes in the global climate, the tendency being an increase in atmospheric temperature [DICKSON, 1999; HUGHES, 2000; HANNA & CAPPELEN, 2003; ACIA, 2005]. According to the 2001 report from the Intergovernmental Panel on Climate Change (IPCC) this temperature rise has been 0.06 °C per decade during the 20th century; however, since the 1950s the increase has escalated to *ca.* 0.12 °C per decade [HANNA & CAPPELEN, 2003]. Even though general patterns are difficult to determine, the tendency is a higher temperature increase in Arctic regions in comparison to a global scale [AAGAARD *et al.*, 1999; HUGHES, 2000; ACIA, 2005]. Geographically, the Arctic can be defined as the area above the Polar circle; the 66th Northern latitude. Another definition is the area on the Northern hemisphere, apart from the Alpine regions, where the mean annual air temperature never rises above 10 °C and the annual cycle can be divided into a light and dark period [BORN & BÖCHER, 1999].

In some Arctic regions the temperature appears to be decreasing. In the period from 1958 to 2001 West Greenland has experienced a significant decrease in temperature of 1.29 °C.

This decrease is correlated with an increase in the North Atlantic oscillation (NAO) [EDWARDS *et al.*, 2001; HANNA & CAPPELEN, 2003]. NAO is a climate phenomenon that forces the atmospheric air masses between the high-pressure system of the Azores and the low-pressure system of Iceland, and is responsible for one third of the variance in sea level pressure in this region [DICKSON, 1999; EDWARDS *et al.*, 2001]. The pressure differences are stated on the NAO index and are either positive or negative. During the last decades this index has been positive due to an unusually strong sub tropic high-pressure system over the Azores and a corresponding low pressure over Iceland. This has caused stronger Western winds and colder winters in West Greenland [DICKSON, 1999; EDWARDS *et al.*, 2001; HANNA & CAPPELEN, 2003; ACIA, 2005].

The temperatures of aquatic environments are correlated with the atmospheric air temperature; hence changes in atmospheric temperature will imply changes the aquatic environments [BORN & BÖCHER, 1999; HUGHES, 2000; HANNA & CAPPELEN, 2003; ACIA, 2005]. Especially in the Arctic marine environment, which is characterised by great amounts of ice, a change in the temperature will have dramatic consequences for the entire ecosystem. This is, in part, due to the fact that the temperature determines the length of the ice-free period, the thickness and the distribution of the ice [AAGAARD *et al.*, 1999; CHRISTENSEN & RYSGAARD, 1999; NIELSEN, 2005]. Since 1980 the extent of the ice in the Arctic has been reduced with *ca.* 10% per decade [HOLLAND *et al.*, 2006]. This reduction is mainly in multi-annual ice, which influences the ratio between this and the annual ice [ACIA, 2005]. Furthermore, the temperature influences the extent of freshwater entering the marine environment, either as precipitation or melt water from icebergs and the terrestrial systems [CHRISTENSEN & RYSGAARD, 1999; ACIA, 2005; NIELSEN, 2005]. An increase in freshwater entry will reduce the salinity of the surface layer and hence strengthen the water column stratification [BORN & BÖCHER, 1999; CHRISTENSEN & RYSGAARD, 1999; ACIA, 2005]. Thus, a change in atmospheric temperature will lead to changes in the salinity and temperature of the water column, which will further influence the stability of the pycnocline (thermo -and halocline). A consequence of this is a change in the vertical heat flux in the water column, which might influence both global and regional water currents and hence the distribution of water masses and sea ice [AAGAARD *et al.*, 1999; ACIA, 2005].

A change in the ratio between ice, snow and open sea will have a feedback mechanism though changes in the albedo. Newly fallen snow has a high albedo of 70-90%, which means that most of the incoming radiation is reflected. Depending on the radiation angle, the albedo for water lies in the range of 3-5% and 50-80% [BORN & BÖCHER, 1999]. An earlier seasonal ice and snow melt due to a temperature increases will result in a positive feedback due to larger amounts of solar radiation being absorbed by the ocean and hence a further temperature increase in the marine environment [AAGAARD *et al.*, 1999; BORN & BÖCHER, 1999; HOLLAND *et al.*, 2006].

These temperature and irradiation changes in the marine environment will have a dramatic impact on the organisms that form the base of the entire food web, the phytoplankton. Some of these effects are discussed in the following Paragraphs.

Whether the temperatures rise or fall, the Arctic environment is strongly influenced by changes in the global climate, and is therefore an important area for evaluation of the consequences of these changes.

1.1.1. The Arctic marine phytoplankton

Approximately 40 % of the global net photosynthesis stems from marine pelagic primary producers [ACIA, 2005]. This production forms the base of the entire marine food web from protists to polar bears, including also the microbial and viral loops. In addition, the primary

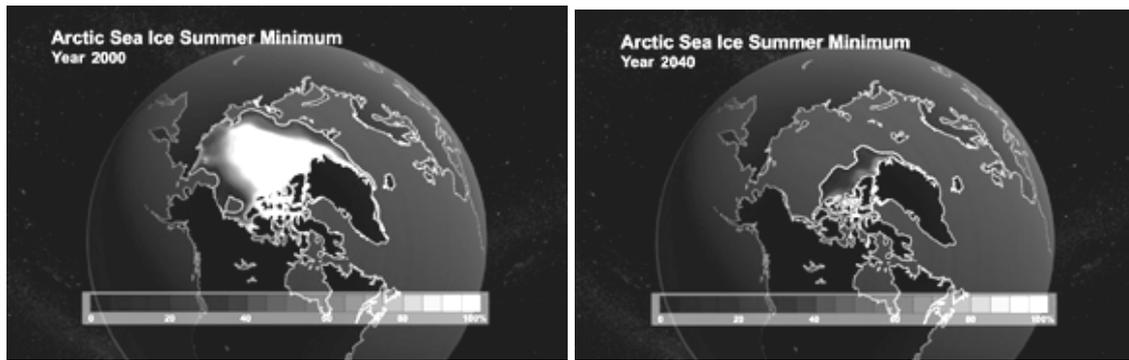


Figure 1.1. The distribution of Arctic sea ice. The first picture illustrates the present distribution of sea ice in the Arctic with a permanent ice cap. The second picture presents the same area as predicted for 2040, a situation with no permanent ice cap during the summer period [[HTTP://WWW.UCOR.EDU/NEWS/RELEASES/2006/ARCTIC_SHTML](http://www.ucor.edu/news/releases/2006/ARCTIC_SHTML)].

producers seem to serve as very sensitive indicators of changes in the environment, since the autotrophic plankton reacts with a non-linear response to even small changes in their surroundings [HAYS *et al.*, 2005]. Thus, in a time when the global climate and especially the Arctic climate is changing, a focus on the primary producers is highly relevant.

In the Arctic oceans the phytoplankton is responsible for 90-95% of the net photosynthesis [AAGAARD *et al.*, 1999; BORN & BÖCHER, 1999]; the phytoplankton comprises the autotrophic or mixotrophic organisms free floating in the water column. In the Arctic environment, which is one of the most extreme environments in the world, only *ca* 250 species of dinoflagellates are known [OKOLODKOV & DOGDE, 1996]. In addition to the pelagic phytoplankton is a group of micro-algae in the ice biota, which is specialised to low radiation, temperatures below 0 °C and highly saline environments in the brine channels within the ice and between the ice crystals [HORNER, 1989; BORN & BÖCHER, 1999; CHRISTENSEN & RYSGAARD, 1999].

The marine Arctic environment is, as mentioned above, characterised by the presence of great amounts of ice, which, in combination with the low radiation levels, has enormous consequences for the carbon-fixating organisms and hence the entire Arctic marine food web. This is in part due to a short growth period, *i.e.* when solar radiation is sufficient to support net photosynthesis, and to a winter period with the complete absence of a euphotic zone. Low levels of primary production, low input of nutrients and low turnover rates of these are other characteristics for this marine system [AAGAARD *et al.*, 1999; HUGHES, 2000; ACIA, 2005; NIELSEN, 2005]. The light and nutrients available for the marine phytoplankton are indirectly influenced by the temperature, *i.e.* though the length of the ice-free periods, the level of freshwater run-off, *etc.* Nevertheless, these factors, light and nutrients, seem to be more limiting for the primary production than the low temperature itself [TILZER & DUBINSKY, 1987; BORN & BÖCHER, 1999; ACIA, 2005; MOCK & HOCH, 2005; NIELSEN, 2005].

The Arctic marine phytoplankton is adapted to the above-mentioned abiotic factors, which characterise this environment. Apart from the indirect influence, as outlined above, extremely low temperatures also have various direct consequences for all organisms being part of the Arctic marine food web; essentially because the temperature is the determining factor for the pace of any given reaction in most biochemical processes [MATHEWS *et al.*, 2000].

The adaptations to low temperatures have been studied in various groups of phytoplankton and revealed that the adaptational mechanisms are usually adjustment of structural flexibility in many photosynthetically essential proteins complexes, such as RUBISCO and Photo system II, and altered composition of lipids in the membrane systems [DEVOS *et al.*, 1989;

DAVIDSON, 1991; SMITH *et al.*, 1994; WILLEM *et al.*, 1999; GAO *et al.*, 2000; MORGAN-KISS *et al.*, 2002; MOCK & HOCH, 2005; D' AMICA *et al.*, 2006].

1.1.2. Consequences of a temperature increase in the marine Arctic environment

Recent studies predict dramatic changes in the global climate, especially in Arctic regions [ACIA, 2005; HOLLAND *et al.*, 2006]. According to several model simulations a nearly ice-free September could be reached within approximately 30 to 50 years (Figure 1.1). This dramatic change over a relatively short time period is explained by the Arctic summer ice cover exhibiting the signature of the abrupt retreat rather than being linearly related to the global temperature. Not only the predicted total loss of multi annual ice, but also the gradual melting of the ice cap will have several climatic consequences. The ice-free period will further delay the onset of ice growth in the autumn period; in part due to the albedo feedback mechanism (Paragraph 1.1), which causes additional warming of the surface layer due to absorption of solar radiation. Furthermore, a rapid increase in ocean heat transport to the Arctic might lead to and possibly trigger the occurrence of ice-free summers [HOLLAND *et al.*, 2006].

The primary production. Major analyses of marine primary production show that the patterns in quantity and density of the phytoplankton can mainly be explained by a number of correlated factors, such as wind-induced mixture of the water column, the temperature of the surface layers as well as other hydrodynamic parameters, all influenced by the NAO [REID *et al.*, 1998; DICKSON, 1999; EDWARDS *et al.*, 2001]. Globally, the tendency is a higher primary production. However, a fall in primary production has been observed in areas with temperature decreases, for instance West of Greenland [HUGHES *et al.*, 2000; AAGAARD *et al.*, 2001; HANNA & CAPPELEN, 2003; ACIA, 2005].

As mentioned above, the growth period for the marine phytoplankton is nearly identical to the ice-free period, which is both directly and indirectly determined by the temperature. However, the length of the growth period is also limited by the totally dark winter period. In the Arctic an almost linear proportionality between the pelagic production and the length of the ice-free period has been observed [CHRISTENSEN & RYSGAARD, 1999]. Hence, the predicted temperature increase will indirectly lead to a higher level of primary production, due to longer growth periods.

The amount of light, as stated earlier, is not the only limiting factor for the primary producers; the level of nutrients also has an essential role. Thus, a rise in primary production as a consequence of higher temperatures will only be observed in areas where this can be supported by adequate nutrient levels. The availability of nutrients depends in part on the stability of the pycnocline, which is determined by wind-caused turbulence and the amount of fresh water entering the surface layer. These hydrodynamic effects are influenced by the NAO and, hence, to some degree correlated with the temperature. In areas where the availability of nutrients is insufficient to support an increase in carbon fixation a consequence of a temperature rise will be an early onset but shorter duration of the spring bloom. This can have dramatic consequences for the entire food web (Paragraph 4.1.6) [HANSEN *et al.*, 2003; ACIA, 2005; NIELSEN, 2005].

The primary producers. In the Arctic there is a tendency towards higher growth rates of marine phytoplankton with increasing temperature. For some species a linear proportionality between the Q_{10} (The increase in metabolic rates when the temperature is increased 10 °C and temperature has been observed, apparently to an upper limit where the cell cycle is arrested due to thermic stress [DAUGBJERG & MOESTRUP, 1992; SMITH *et al.*, 1994]. Due to the

mechanisms explained in 1.1.1. this limit may be lower for the cold adapted than the temperate algae. Hence, the cold adapted Arctic algae may not be able to maintain growth if the temperatures rise to levels that seem relatively low for mesophile algae, *i.e.* algae adapted to a temperate climate.

The predicted changes in the temperature will most likely influence the stability and pace of the biochemical and physiological processes and have serious consequences for the survival and competitiveness of the phytoplankton [DEVOS *et al.*, 1989; DAVISON, 1991; SMITH *et al.*, 1994; WILLEM *et al.*, 1999; GAO *et al.*, 2000; MORGAN-KISS *et al.*, 2002; HAYS *et al.*, 2005; MOCK & HOCH, 2005; D'AMICA *et al.*, 2006]. Increasing temperatures will probably allow invasion of temperate species in the phytoplankton community, leading to changes in the composition and species diversity. However, the length of the photoperiod in the Arctic exerts a serious selection pressure on the invading species, which may favour the already adapted phytoplankton.

Thus, with higher temperatures and longer growth periods in the Arctic, lower species diversity is to be expected. The species that will survive under this new condition will be the ones that can adapt to the climatic changes and to the light levels in the Arctic; and these adaptations need to occur rather fast in order to encounter the changes [HOLLAND *et al.*, 2006].

1.1.3. Conditions in the Disko Bay

Most data represented in this project are based on samples from Disko Bay, which is a *ca.* 10.000 km² bay in West Greenland (Figure 2.1), 300 km north of the Polar circle. Some areas of the bay are up to 600 metres deep [NIELSEN, 2005].

The hydrography determines the distribution of the phytoplanktonic organisms, and is an important aspect to consider. The hydrography of the surface layer in Disko Bay is highly influenced by the weather conditions and changes throughout the year. During winter the temperature of the surface layers decreases severely and the water column is mixed down to a depth of 150 meters. Normally, the bay is ice-covered two to five months during the winter. In the early spring, the surface temperature increases in accordance with the amount of freshwater influx from the terrestrial environment, *i.e.* the melting of sea ice, icebergs and the especially the Ilulissat glacier. This temperature increase strengthens the pycnocline. During the summer period, the water column consists of three layers (Figure 1.2): An upper layer of

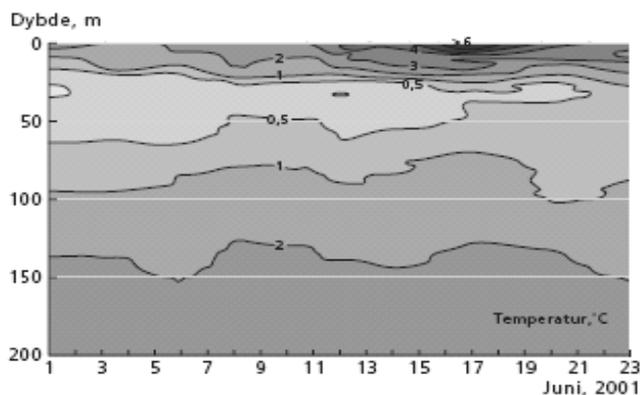


Figure 1.2. The vertical temperature distribution in the water column at Qeqertarsuaq June 2001. The remains of the cold surface layer from the winter period are located between the warm surface layer and the warm bottom layer [NIELSEN 2005].

20-30 metres that is heated by the sun and contains considerable amounts of freshwater, an intermediate cold water layer of 100-150 metres that stems from the winter surface layer, and a lower layer with higher temperatures; probably from the West Greenlandic Current (WGC) from the South (Figure 1.3) [HERMAN, 1971]. During autumn, a reduction in the outflow of melt water occurs and this, in combination with less solar heating and stronger winds, creates a water column with uniform salinity and uniformly low temperatures in the upper 80 metres [ANDERSEN, 1981].

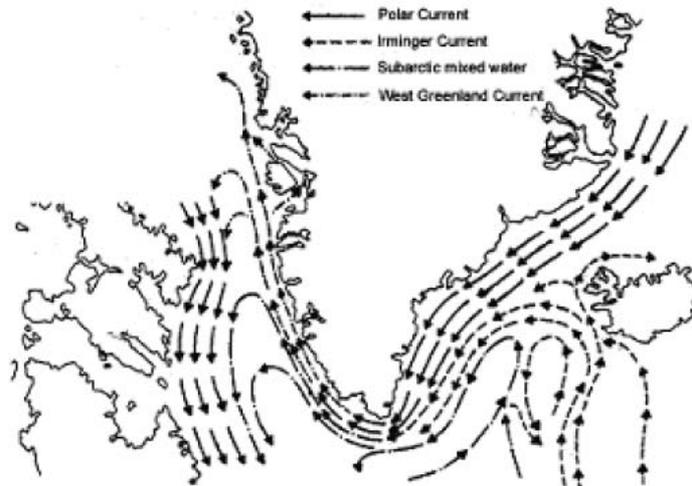


Figure 1.3. Most important currents in the waters around South Greenland [HERMANN in NØRREVANG *et al.*, 1971].

The WGC that comes from the south is warm and saline. Part of the water continues, as just described, beneath the fresher and cooler surface layer into Disko Bay. The WGC consists of a mixture of the relatively cold and fresh East Greenland Current and the relatively warm and saline Irminger Current (Figure 1.3). Another aspect in the Disko Bay area is the tidal fluctuations. The rising tides can halt outgoing currents, which are then either forced back into the bay or towards Kronprinsens Ejlande [ANDERSEN, 1981].

1.2. Dinoflagellates

Diatoms and dinoflagellates constitute the two major groups of net plankton represented in the Arctic marine environment, as is the case for all other marine environments. In this report we focus on the dinoflagellates, since they have previously been studied to a limited extent only and, hence, the knowledge on Arctic marine dinoflagellates is scarce [GRØNTVED & SEIDENFADEN, 1938]. In addition, dinoflagellates are highly important for the ecology of an area, since they can occur on different trophic levels and are found as both nano- and net plankton. Furthermore, around 70 marine dinoflagellate species are known to be toxin-producing, which can result in accumulation of toxins in larger organisms of the marine system and affect the entire food web [GRAHAM & WILCOX, 2000; [HTTP://WWW.BI.KU.DK/IOC/ GROUP2.ASP](http://www.bi.ku.dk/ioc/group2.asp)].

1.2.1. Phylogeny

Molecular data places the dinoflagellates within a monophyletic clade, the Alveolata, together with Ciliophora, Apicomplexa, Perkinsozoa and Colpodellidae. Synapomorphies for the members of Alveolata are the cortical alveoli, mitochondria with tubular cristae and the presence of diverse forms of tricolocysts [CAVALIER-SMITH, 1993; HAUSMANN *et al.*, 2003]. The dinoflagellates form a monophyletic clade with the Perkinsozoa as the sister group [HAUSMANN *et al.*, 2003].

1.2.2. Characteristics

All species of dinoflagellates have at least one stage (Paragraph 1.2.4.) as a unicellular flagellate with two heteromorphic flagella. The flagellate can be desmokont, where the flagella emerge apically, or dinokont with the flagella emerging laterally close to the equator of the cell (Figure 1.4 A, B). For the dinokont dinoflagellates, the transverse flagellum usually

lies within the cingulum, the transverse furrow which surrounds the cell; whereas the longitudinal flagellum runs along the longitudinal furrow; the sulcus. The two flagella emerge from two pores at the intersection between cingulum and sulcus [TAYLOR, 1980; VAN DEN HOEK *et al.*, 1995; STEIDINGER & TANGEN, 1997]. The transverse flagellum, which gives the dinoflagellate its characteristic, rotating movement, has a helical axoneme and, running in parallel, a contractile string composed of centrin. The longitudinal flagellum is flattened and controls the direction of movement. The flagella are anchored, below the plasma membrane, in a complex rooting system composing at least 3 types of microtubule-based roots. Near the flagellar base lies the pusule, which is a characteristic organelle for dinoflagellates. It is formed by several invaginations of the plasma membrane and of yet unknown function (Figure 1.4 C) [VAN DEN HOEK *et al.*, 1995].

In addition to the pusule, another characteristic membrane-associated feature of the dinoflagellates is the amphiesma, consisting of membrane-enclosed polygonal vesicles beneath the plasma membrane. Their number, shape and size vary among genera, and sometimes even during the life cycle of a species [MORRILL & LOEBLICH III, 1983; STEIDINGER & TANGEN, 1997]. For a large fraction of the present dinoflagellates, the amphiesmal vesicles contain 100-1000 nm thick polysaccharide plates mainly composed of cellulose, mannose and galactose; the thecal plates (Figure 1.5). Expansion of thecal plates takes place by addition of polysaccharide material along the plate margin and causes intercalated bands at the sutures; the space between adjacent plates. Species possessing such thecal plates are referred to as armoured or thecate [MORRILL & LOEBLICH III, 1983; VAN DEN HOEK *et al.*, 1995; GRAHAM & WILCOX, 2000].

The cingulum divides the dinoflagellate into an apex and an antapex, the epicone and hypocone, respectively (Figure 1.4) [VAN DEN HOEK *et al.*, 1995], and along with plate ornamentation, the number and arrangement of the thecal plates in the epicone and hypocone form a valuable base for identification of thecate dinoflagellate species (for nomenclature, see Appendix 1).

About 40% of the dinoflagellates possess a pellicula, a fibrous layer within the amphiesmal vesicles, beneath the thecal plates. The pellicula provides the cell with stability during ecdysis (extrusion of the theca) or development of a hypnozygote ("cysts", Paragraph 1.2.4). The pellicular material is known as dinosporin [TAYLOR, 1980; GRAHAM & WILCOX, 2000], since it highly resembles the sporopollenin of gymno- and angiosperms [MORRILL & LOEBLICH III, 1983; STEIDINGER & TANGEN, 1997].

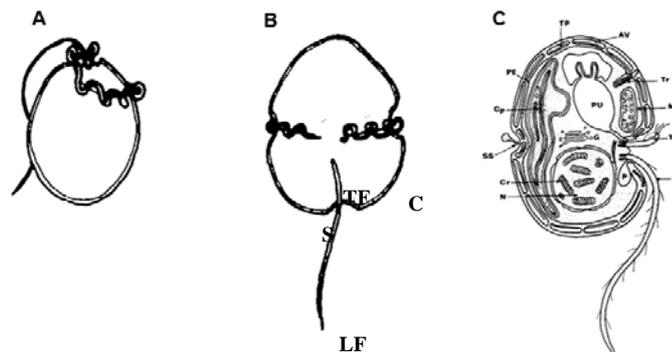


Figure 1.4. Morphological types and morphological features. **A.** Lateral view of a desmokont cell with two dissimilar flagella at the anterior end. **B.** A dinokont cell in ventral view; the two dissimilar flagella are housed in each their furrow. **C.** cingulum, S, sulcus, LF, longitudinal flagellum, TF, transverse flagellum [STEIDINGER & TANGEN, 1997]. **C.** Cross-section of a dinokont cell with typical dinoflagellate organelles. Thecal vesicles (AV) with thecal plate material (TP), chloroplast (Cp), chromosomes (Cr), Golgi apparatus (G), mitochondrion (M), nucleus (N), pellicle layer (PE), pusule (PU), striated strand (SS) of transverse flagellum (TF), trichocysts (Tr) [TAYLOR, 1980].

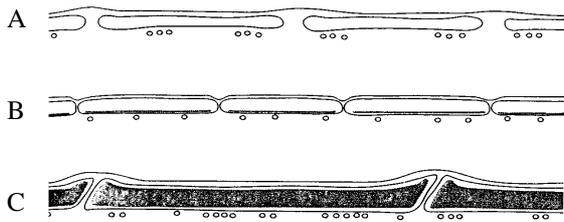


Figure 1.5. Amphiesmal vesicles with (A) none, (B) thin or (C) thick thecal plates. Below these lie microtubules [GRAHAM & WILCOX, 2000].

An almost universal feature among dinoflagellates is trichocysts. These are ejectile proteinaceous rods that lie in the periphery of the cell. If the species is thecate, the location is beneath a pore in the theca [TAYLOR, 1980; VAN DEN HOEK *et al.*, 1995; GRAHAM & WILCOX, 2000]. Some genera possess nematocysts, which are larger and more elaborate than trichocysts, and arranged radially or subradially in the cell [TAYLOR, 1980; STEIDINGER & TANGEN, 1997; GRAHAM & WILCOX, 2000].

Some dinoflagellate genera possess a discrete red eyespot located freely in the cytoplasm close to the site of flagellar origin. Other genera are equipped with eyespots consisting of a series of spherical globules within the chloroplast. Some members of one family, the Warnowiaceae have a complex eyespot; an ocellus. This feature, which highly resembles the metazoan eye, consists of a lens-like refractile hyalosome and a reddish-brown or black pigment cup backing a para crystalline retinoid [TAYLOR, 1980; VAN DEN HOEK *et al.*, 1995; GRAHAM & WILCOX, 2000].

1.2.3. Trophic conditions and chloroplasts

Plastids are found in approximately half of the extant dinoflagellates, making them capable of performing photosynthesis. The chloroplast is usually surrounded by three membranes discontinuous with the endoplasmatic reticulum, and the thylakoid membranes are usually arranged in stacks of three without a girdle lamella (Figure 1.4 C). [VAN DEN HOEK *et al.*, 1995]. Uniquely, about half of the photosynthetic dinoflagellates possess the xanthophyll-like carotenoid peridinin, which is thought to have developed in an early dinoflagellate form [YOON *et al.*, 2002]. Being the main light-harvesting complex with an absorption spectrum in the blue-green area, 470-550 nm, peridinin forms a water soluble and highly effective protein complex with chlorophyll *a* (chl *a*) within the thylakoid lumen; the transfer efficiency is nearly 100% [GRAHAM & WILCOX, 2000]. In addition, various species-specific accessory pigments, such as chl *c*, carotenoids, and xanthofylls, are found among the dinoflagellates. This diversity is due to the multiple occurrences of secondary and tertiary endosymbiosis of photosynthetic protists throughout dinoflagellate evolution [GRAHAM & WILCOX, 2000; MORDEN & SHERWOOD, 2002]. The peridinin-containing chloroplast, which also contains the proteobacterial enzyme RUBISCO II [SCHNEPF & ELBRÄCHTER, 1999], is believed to result from secondary endosymbiosis of a rhodophyte ancestor [TAKISHITA *et al.*, 2002] but dinoflagellate species with a green alga as secondary endosymbiont are also known [MORDEN & SHERWOOD, 2002]. In addition, tertiary endosymbiosis is known to have occurred with a diatom, a haptophyte, and a cryptophyte [SCHNEPF & ELBRÄCHTER, 1999; MORDEN & SHERWOOD, 2002].

Kleptochloroplasts, *i.e.* temporary endosymbionts, are found in some dinoflagellate species, for instance in the genus *Dinophysis* [TAKISHITA *et al.*, 2002]. Still being a controversial subject, it is believed that the kleptochloroplasts are usually found as only partially reduced and within a food vacuole (see below) in which they can function in photosynthesis from days to months before eventually being digested [SCHNEPF & ELBRÄCHTER, 1999]. The various

types of endosymbionts lead to great variation in dinoflagellate chloroplast colour; from typically yellow-brownish to bright yellow, green and even blue [SCHNEPF & ELBRÄCHTER, 1999; HAUSMANN *et al.*, 2003].

Hetero- and mixotrophy. About half of the known dinoflagellates lack plastids and are thus obligate heterotrophs. Since many dinoflagellate species are relatively large the surface:volume ratio might be insufficient for osmotrophy. This helps to explain why phagotrophy, where the food item is taken up in a food vacuole, is the most common feeding strategy among heterotrophic dinoflagellates [VAN DEN HOEK *et al.*, 1995; GRAHAM & WILCOX, 2000]. In addition to smaller organisms, potential prey comprises ciliates and diatoms even in the same size range as the predating dinoflagellates themselves. To avoid expansion of their cell to this extent, many dinoflagellate species possess a special organelle for food uptake; a pallidum or a peduncle, which is localised close to the sulcus and can be extended out of the cell. The exact function of the organelle and the size of food items vary in between species [GRAHAM & WILCOX, 2000; NIELSEN, 2005].

Mixotrophy, a combination of phototrophy and heterotrophy, is also well known, and many phototrophic dinoflagellates are considered as potential osmo- or phagotrophs. This is based on multiple ultra-structural analyses of plastid containing species revealing microtubular root-structures of a peduncle, although these species have never been observed having food vacuoles. In addition, many of the photosynthetic species are auxotrophs and thus strictly dependent on osmotrophy [VAN DEN HOEK *et al.*, 1995; SCHNEPF & ELBRÄCHTER, 1999; GRAHAM & WILCOX, 2000].

The most common storage product in the photosynthetic dinoflagellates is starch, positioned in grains outside of the chloroplast. Coloured droplets containing long-chained poly-unsaturated fatty acids are found in both phototrophic and heterotrophic dinoflagellates [VAN DEN HOEK *et al.*, 1995; SCHNEPF & ELBRÄCHTER, 1999].

1.2.4. Life history

The most common dinoflagellate life cycle is shown in Figure 1.6. The dinoflagellate nuclear envelope is permanent, resulting in endonuclear mitosis with the mitotic spindle placed outside of the nucleus [TAYLOR, 1980; STEIDINGER & TANGEN, 1997].

The dinoflagellate nucleus, the dinokaryon, is characteristic in having chromosomes that are

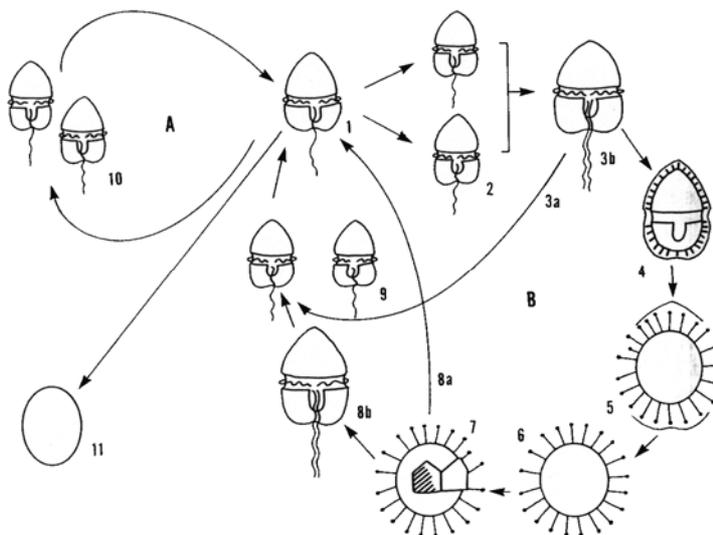


Figure 1.6. The general life cycle of a hypnozygote-producing dinoflagellate [STEIDINGER & TANGEN, 1997].

condensed throughout the cell cycle and, unlike any other eukaryote, it possesses no histones [TAYLOR, 1980; HAUSMANN *et al.*, 2003]. The vegetative cell (1 in Fig. 1.6) is motile, planktonic and haploid, and can divide asexually into two identical cells by binary fission (A in Fig. 1.6) or it can form a non-motile temporary cyst (11 in Fig. 1.6). Sexual reproduction has been observed for several species and in cultures it can be induced in by depletion of nutrients, especially nitrogen [STEIDINGER & TANGEN, 1997]. The sexual reproduction begins with the formation of gametes (2 in Fig. 1.6), which look like the vegetative cells but are typically slightly smaller. Two gametes can fuse by iso- or anisogami to produce a planozygote (3b in Fig. 1.6), a motile cell similar to the vegetative stage but with two longitudinal flagella. The planozygote can produce motile vegetative cells through meiosis (3a in Fig. 1.6) or cyst formation (4 in Fig. 1.6). The meiosis can take place in the resting stage, the hypnozygote (6 in Fig. 1.6), and produce motile vegetative cells (8a in Fig. 1.6); or excystment of the hypnozygote can result in a new planozygotic stage (8b in Fig. 1.6), which produces motile vegetative cells by meiosis. Sexual reproduction can be both homothallic and heterothallic [GRAHAM & WILCOX, 2000]. Formation of a haploid cyst stage (11) can result from a variety of environmental changes, *e.g.* available nutrients, intensity of radiation, length of the photoperiod or temperature [CHAPMAN & PFIESTER, 1995; STEIDINGER & TANGEN, 1997]. In most cases, dinoflagellate cysts are surrounded by a thick wall, contain many storage products, and are thus considered as survival stages. However, temporary division cysts during vegetative growth are known from some species, while other, heterotrophic, species form digestion cysts [STEIDINGER & TANGEN, 1997; GRAHAM & WILCOX, 2000]. Much knowledge of the history of the dinoflagellates relies on the presence of preserved cysts from marine fossil sediments [HAUSMANN *et al.*, 2003].

1.3. *Ceratium arcticum* and *C. longipes*

The dinoflagellate genus *Ceratium* is found all over the world in limnic and marine environments. According to recent literature [DODGE, 1982; STEIDINGER & TANGEN, 1997; THRONSDEN *et al.*, 2003] two of the most frequently occurring *Ceratium* forms in Northern waters can be identified as *C. arcticum* (Ehrenberg) Cleve 1901 and *C. longipes* (Bailey) Gran 1902, illustrated in Figure 1.7 A, B. However, *C. arcticum* and *C. longipes* have been considered as form variations under a former species definition of *C. tripos* (O. F. Müller) Nitzsch 1817; *C. tripos* has, nevertheless, been taxonomically revised not to include these two [VANHÖFFEN, 1897; OSTENFELD, 1899; STEIDINGER & TANGEN, 1997].

Whether these two species are genuine or rather variants of the same species has been debated several times [GRAN, 1902; DODGE, 1982; KLEKOWSKI & WESLAWSKI, 1995; STEIDINGER & TANGEN, 1997; THRONSDEN *et al.*, 2003], since the cell body-shape, length and orientation of the apical horn and the plate tabulation are identical (Figure 1.7 and Paragraph 3.1) [OSTENFELD, 1899; GRAN, 1902]. In addition, intermediate forms (Figure 1.7 C) have been observed by Gran 1902.

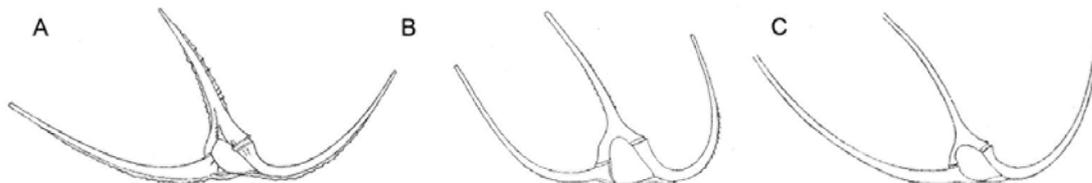


Figure 1.7. *Ceratium* forms. **A.** *Ceratium arcticum* **B.** *Ceratium longipes* **C.** Intermediate between *C. arcticum* and *C. longipes*. [GRAN, 1902].

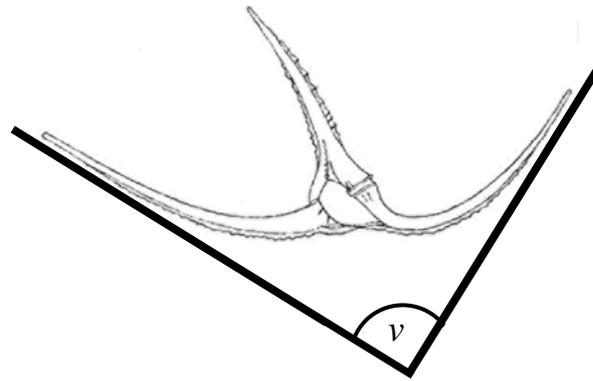


Figure 1.8. The method of Gran for measurement of the angle between the horns of *Ceratium* specimens. Illustrated is the measuring of *C. arcticum*. Angles below 180° are denoted positive, and angles above 180° negative. Modified from Gran (1902).

Concerning the separation of the two species despite their close morphological resemblance, it is important to compare the original descriptions of the species in question. Unfortunately, the original descriptions of *C. arcticum* and *C. longipes* as *Peridinium arcticum* and *P. longipes* (EHRENBERG, 1840 and BAILEY, 1855, respectively) have not been accessible to us for comparison.

In 1902, Gran determined that *C. arcticum* and *C. longipes* are in fact two species, on behalf of significant differences in the angle between the terminal ends of the anterior horns (Figure 1.8). The angles were measured on fully developed specimens since the direction of the horns might change during growth. Angles were set positive if the horns diverged and negative if the horns were convergent. The results clearly showed a division of the measured specimens into two groups with a slight overlap: the antapical horn-tips of *C. arcticum* were highly diverging ($+40^\circ$ to $+140^\circ$, usually $+70^\circ$ to $+120^\circ$) whereas *C. longipes* had weakly diverging to converging horns (-20° to $+40^\circ$, usually -10° to $+30^\circ$). The intermediate form was conferred to *C. longipes*, since this species showed the largest extent of variation in anterior horn angles [GRAN, 1902].

In addition, no statistically significant difference in the distribution of horn angles was revealed from the investigation, although Gran based his data on *Ceratium* specimens collected from the 63th to the 74th latitude in a temperature range of -11.1 to $+9.7$ °C. Hence, the morphological differences are not simply form variations caused by abiotic factors [GRAN, 1902].

The occurrence of the two species shows quite an overlap in distribution pattern (Figure 1.9) with *C. arcticum* usually coexisting with *C. longipes*, although *C. longipes* has been registered alone in more southern temperate regions [OCEAN BIOGEOGRAPHIC INFORMATION SYSTEM, 2007]. To some authors this simultaneous occurrence indicates that *C. arcticum* and *C. longipes* are both "good" species [THRONDSEN *et al.*, 2003].

In order to supplement the existing biogeographic and morphometric information, it would be useful to obtain molecular sequence data on *C. arcticum* and *C. longipes* to determine whether these *Ceratium* species should be kept separate or are in fact conspecific.

1.4. Aim of study

The aim of this study is to perform a floristic survey of the marine dinoflagellates in Disko Bay, West Greenland. As already mentioned, the Arctic represents a unique environment for assessing an indication of global climate changes and their consequences. Due to the dramatic changes in the climate already observed and the even more dramatic consequences predicted (Paragraph 1.1.2) it is especially important to focus on the phytoplankton, both as biological

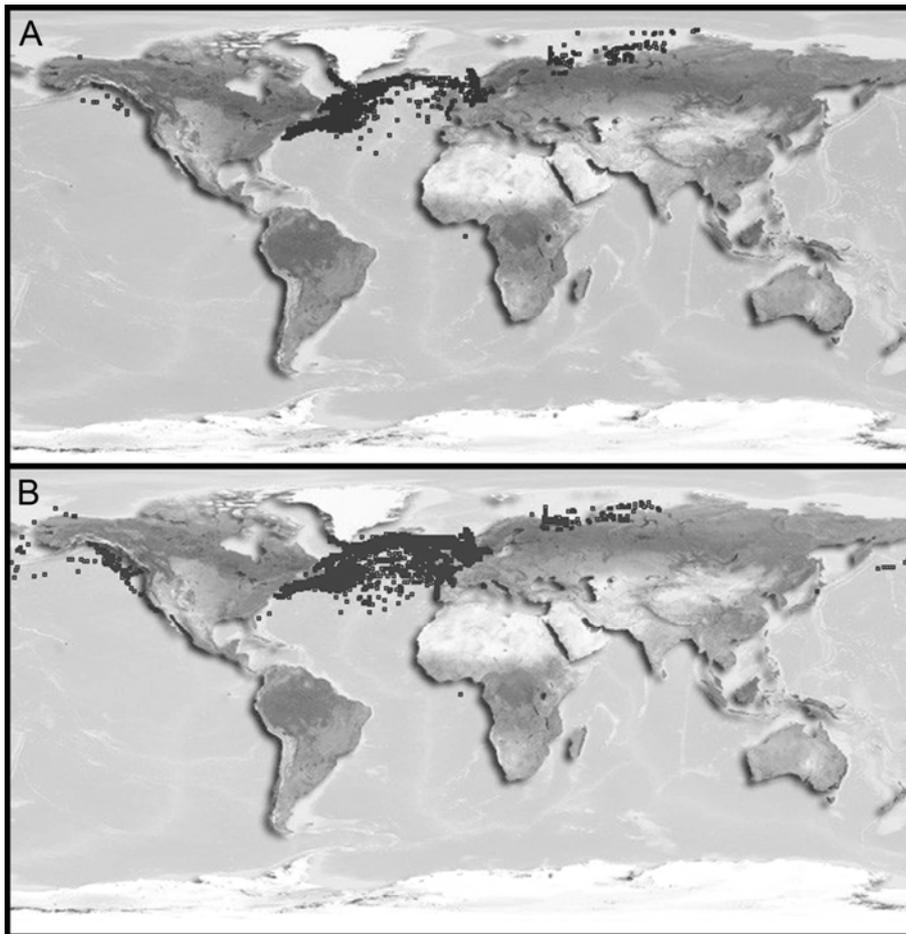


Figure 1.9. The World-wide occurrences of *Ceratium arcticum* and *C. longipes*. **A.** *C. arcticum*. **B.** *C. longipes* [Ocean biogeographic information system]. Data retrieved 16th of January 2007 from WWW.IOBIS.ORG.

indicators and to establish a *status quo* on the current species diversity, *i.e.* a baseline to which future observations on species diversity can be compared.

Similar surveys have been conducted in this area in 1928, 1994, 1996 and 2002 [GRØNTVED & SEIDENFADEN, 1938; CLAUSEN *et al.*, 1994; TRIER, 1998; JØNSSON *et al.*, 2002]. Hence, a substantial amount of information about the composition of the dinoflagellate community is available for comparison to our results.

- We have collected and studied live material from the waters around Disko Island, from the 69.11th to the 70.22nd latitude. With this project, we hope to contribute to the knowledge on the dinoflagellate species diversity, concerning both thecate and athecate species, in the sub- and high Arctic regions.
- For future comparisons we would like to contribute with light microscopic (LM) and scanning electron microscopic (SEM) photos of the dinoflagellates that are likely to be encountered in the waters surrounding Disko, and other parts of the Arctic.
- In addition, the large ribosomal subunit gene (LSU) was sequenced for *Phalacroma* cfr. *braarudii*, *Ceratium arcticum* and *C. longipes* to help clarify the taxonomic problems concerning these (Paragraph 1.3). The sequences can furthermore be used as a genetic fingerprint for comparison of populations, *e.g.* to determine if the *Ceratium* populations in Denmark are genetically similar to the one encountered in Greenland. For population studies ITS sequences are good molecular markers.
- Finally, the isolated cells can contribute to the already existing DNA sequence data of dinoflagellates.

Station	Coordinates	Description	Weather	Depth*	Date
1. Udkiggen	-	Heavy waves Samples were taken with plankton net from the coast and in rock pools	Sunshine and a clear sky.	1 m.	13.08.06
2. Plankton station	69°11 N, 53°30W	Calm waters Net samples at <i>ca.</i> 12 meters depth. Pycnocline at 17 meter. Water samples at 2.5, 10 and 20 metres	Sunshine and a clear sky	-	14.08.06
3. The harbour	-	At the entrance to the harbour. Rocks on each side not open waters. Heavy waves. Only net samples.	Strong wind and many clouds	-	15.08.06
4. Fortune Bay	69°15'446'N, 53°43.864' W	Isolated and very quiet bay area. Only net samples.	Cold and misty	20 m.	17.08.06
5. Ippik	69°17'102" N, 53°13'646" W	Relatively calm waters but a strong current. Net samples. Water samples at 2.5 and 14 m.	Cloudy		20.08.06
6. Nordfjord	69°56.777'N; 54°27.114'W and 69°56.827'N; 54°26.040'W	Net samples, surface water sample from 2.5 m.	Cold and misty	103 m.	22.08.06
7. Hareøen between Niaqua and Talerua	70°22.223'N; 54°49.780'W	Strong current. Many wing snails in the surface layer. Net samples.	Misty and raining	21.9 m.	22.08.06
8. Between Hareøen and Disko	70°17.336'N; 54°51.486'W	Open waters and strong current. Water samples from 2.5, 10 and 20m.	Misty and raining	259 m.	22.08.06
9. Mudderbugten	69°37.147 N 51°52.031 W	Net samples	Clear and quiet	-	25.08.06

Table 2.1. Descriptions of the 9 stations where the samples were collected. From the stations 2, 3, 5, and 9 no position data or vertical profile of the water column could be obtained due to CTD-errors (Paragraph 2.1.1) *) Depth refers to the total depth at the respective locality; not equivalent to sampling depth.

2. MATERIALS AND METHODS

2.1. Stations

Samples were collected at 9 different stations in the area around Disko Island, West Greenland (Figure 2.1), from Hareøen (7) to Mudderbugten (9). Apart from the first sample, which was collected from the seashore, all sampling was performed at open sea; station 3 from a speed boat and the remaining from Porsild, the research vessel of Arctic Station. Table 2.1 gives an overview of sample sites and conditions at the time of sampling.

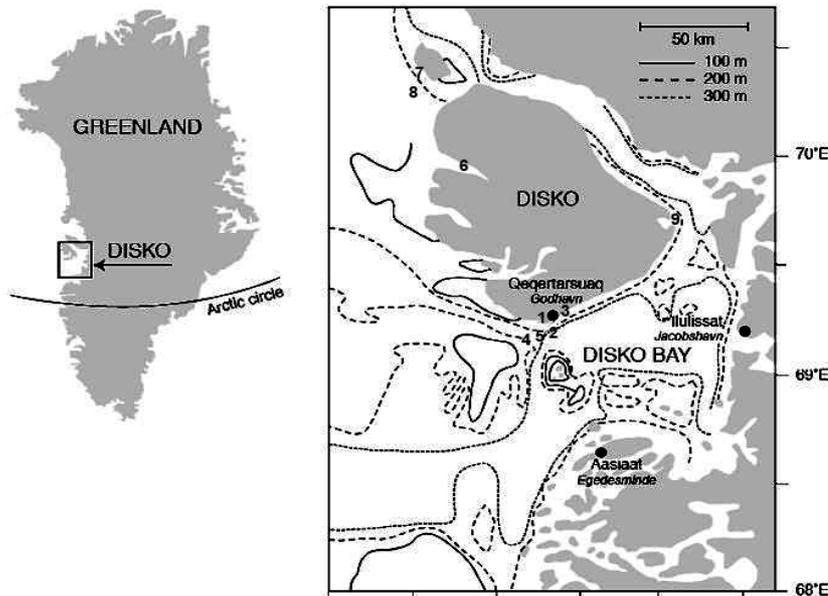


Figure 2.1. Map over Disko Island and Disko Bay. The numbers correspond to the sampling stations described in table 2.1. Modified from Hansen *et al.*, 2003.

2.2. Sampling

Dinoflagellates were collected either with plankton net or by using a Niskin bottle. Initially, the plan was to collect water at three different depths corresponding to the surface layer (2.5 m), the depth of maximal fluorescence and just above the pycnocline. The depths of maximal fluorescence and the position of the pycnocline would have been determined using a CTD.

2.2.1. CTD measurements

The CTD (Conductivity, Temperature, and Depth recorder) directly measures three parameters in the water column: conductivity, temperature and pressure. The salinity measure is derived from water's conductivity, and since pressure and depth are directly related, the pressure measurements can be converted into a depth in metres. Additional sensors can be placed on the CTD, for the measuring of dissolved oxygen, pH and fluorescence. [WWW.WINDOWS.UCAR.EDU/TOUR/LINK=/EARTH/WATER/CTD.HTML&EDU=HIGH].

During the sampling from Porsild the CTD scanner did, for unknown reasons, not function properly, making it impossible to obtain data for the depth profiles.

2.2.2. Sampling of net plankton (> 20 μm)

Sampling was performed by pulling a 20 μm plankton net through the surface layer, until the desirable concentration of organisms was obtained. The colour of the water samples (yellow-brownish) was used as indicator of the concentration. Attached to the net was a glass or plastic flask in which the organisms, withheld from the 20 μm net (Aquanet), were collected. A 20 meter rove was attached to the net, but the current highly influenced the depth of the water column that was intergraded during the sampling. If necessary, the samples were concentrated by filtering some of the water through the net.

2.2.3. Handling and storage of samples

In order to minimise the degree of thermic stress on the collected organisms, the samples were transported in a thermo flask or a thermo box with Blue Ice. After returning to Arctic Station, the samples were kept cool and without lid to allow air circulation. The samples were

analysed within 24-36 hours after sampling since little or almost no activity was observed beyond this time period.

2.3. Concentration of dinoflagellates in water samples

By using a continuous centrifuge it was possible to obtain and investigate the fraction of dinoflagellates that was not withheld by the 20 µm plankton net.

Different sample volumes were centrifuged using the continuous centrifuge and the centrifuged samples were collected with a plastic pipette and kept in 50 ml plastic containers. Only two continuous centrifuges have ever been made, and we were very pleased to have the opportunity to use one of these.

2.4. Light microscopy (LM)

Live samples were examined at temperatures of 8-10°C and LM image acquisition was performed on cells at 10, 20, 40 or 60 times magnification using an Olympus Provis BX 60 light microscope and an Olympus C5050 digital camera. The photos were taken with a resolution of either 1300×1030 or 2600×2060 pixels and subsequently edited in Photo Shop version 8.0 CS.

Video sequences were recorded with a Sony High definition video camera. To identify some of the thecate dinoflagellates we stained cells from the fixed samples with Calco flour/white, which is a fluorescent agent that binds to the cellulose in the thecal plates. After staining the cells are analysed with UV fluorescence light and photographed as described above.

2.5. Scanning electron microscopy (SEM)

On Disko Island, cells were fixed in 5% alkaline Lugol and kept cool and dark, until further analysis. Only thecate dinoflagellates are well preserved in Lugol, and hence the athecate dinoflagellates cannot be analysed using this method. To ascertain proper conservation of both thecate and athecate dinoflagellates, part of the subsequent dehydration procedure (until the 70% ethanol step, see below) should have been performed instantly.

The following steps were carried out at Section of Phycology, Institute of Biology, University of Copenhagen. To wash the cells, the fixed material (*ca.* 1 ml, depending on the concentration of cells) was poured through an 8.0 µm Millipore polycarbonate filter in a Swinnex13 filter holder and washed with double-distilled water for about 60 min. without letting the filter dry out. Subsequently, the cells were dehydrated by washing them with increasing ethanol concentrations (30%, 50%, 70%, and 96%) for at least 10 minutes at each concentration, and then 2×30 min, first with 99.9% followed by absolute ethanol (99.9% with molecular sieves).

Critical point drying (CPD) with CO₂ was performed hereafter using a Balzer CPD. The dried filters were mounted on SEM stubs with double-adhesive stickers.

At the Zoological Museum, University of Copenhagen, the filters with the fixed cells were coated with a 10-20 nm thick palladium layer and placed in a JEOL JSM 6335-F field emission electron microscope. Photos were taken with a digital camera and subsequently edited in Photo Shop version 8.0 CS.

2.6. Filtration and counting of *Ceratium longipes* and *C. arcticum*

Volumes of water samples were filtrated as described in Paragraph 2.7. A small sample volume was withheld in the filtering chamber and collected with a plastic pipette, and subsequently fixed with alkaline lugol (5%). The number of *Ceratium longipes* and *C. arcticum* (Paragraph 1.3) was counted at 40 times magnification using a stereo microscope.

2.7. Isolation of cells

The water samples for the isolation were homogenised by turning the bottle upside down 20 times. 500 ml of sample was filtered through a 2 µm filter (Millipore, cat# TTTPO4700) with a Millipore filtration system.

Using a stereo microscope (Olympus SXZ12) single cells were isolated with a capillary pipette. Individual cells were washed in drops of 0.22 µm-filtered seawater from Disko Bay (West Greenland). All isolated cells (one in each drop) were video filmed for future reference with a Sony High Definition video camera (HDR-HC1) and finally transferred to a 0.2 µl PCR tube. Isolation of cells was performed at approximately 9 °C in a temperature controlled room. The PCR tubes with isolated cells were kept frozen at -18 °C until further processing in the laboratory in Copenhagen.

2.8. DNA amplification and determination of the sequences encoding the LSU

Amplification of DNA sequences partially encoding the large ribosomal subunit (LSU), approximately 1500 base pairs, was performed in 50 µl reaction volumes using the same PCR amplification conditions (*e.g.* chemicals and temperature profile) as in DAUGBJERG *et al.* [2000]. PCR bands of the correct length using primers D1R-F and 28S-ND1483 were cleaned with a Nucleofast kit (Macherey-Nagel) by following the recommendations of the manufacturer. Following this step PCR samples were prepared for cycle-sequencing with the PCR amplification primers in addition to 3 internal primers (*viz.* D2C-R, D3A-F and D3B-R; see DAUGBJERG *et al.* 2000 for all primer sequences). Hence, LSU rDNA sequences were obtained in both directions and the cycle-sequencing reactions were executed on an automated sequencer (model 3130XL Genetic Analyzer from Applied Biosystems).

2.9. LSU rDNA determination and phylogenetic inferences

All sequence reactions were annotated by eye using Chromaspro (ver. 1.34) and added to an alignment comprising 46 LSU rDNA sequences available in GenBank. In order to polarise the in-group we selected two ciliates assigned to the genus *Tetrahymena* (*viz.* *T. pyriformis* and *T. thermophila*); hence, the ciliates constituted the out-group taxa. The alignment was performed using MACCLADE ver. 4.08 and ambiguous sites were excluded prior to phylogenetic inferences. For the selection of dinoflagellates, particular interest was paid to species belonging to the genus *Dinophysis*. However, the drawback with LSU rDNA sequences from *Dinophysis* in GenBank is that for most species less than 700 base pairs (bp) have been determined (in this context it should be mentioned that until recently even the autotrophic species of *Dinophysis* could not be cultured). These approximately 700 bp correspond to domains D1 and D2 of the LSU gene, but domain D2 is highly variable and therefore virtually impossible to align with any confidence when compared to dinoflagellates other than species of *Dinophysis*. Hence, we had to omit this domain leaving 1125 base pairs for the phylogenetic analyses. This limited number of nucleotides and the fact that for many of the species of *Dinophysis* we had to add Ns (for unknown nucleotide) to the end of the sequences, are likely to have had an impact on the power of resolution for the relationships between the more divergent lineages included. To increase the level of homology for the aligned nucleotide sites information from the secondary structure of the LSU rDNA was included as suggested by de RIJK *et al.* (2000).

Two different methods for phylogeny inference were applied, namely Maximum Parsimony (MP) and Bayesian Analysis (BA). For MP we used the heuristic search option with 1000 random additions and a branch-swapping algorithm (TBR) available in PAUP* ver. 4b10 [SWOFFORD, 2003]. All characters were unordered, weighted equally, and gaps treated as missing data. To examine the robustness of the tree topology we did a MP bootstrap

analysis with 1000 replications. Using the program MRBAYES (ver. 3.1.2) by RONQUIST & HUELSENBECK [2003], Bayesian analysis invoked the GTR substitution model with base frequencies and substitution rate matrix estimated from the data matrix. Four simultaneous Monte Carlo Markov chains (MCMC) [YANG & RANNALA, 1997] were run from random trees for a total of 500.000 generations (Metropolis-coupled MCMC). A single tree was sampled every 50 generations and the “burn-in” was evaluated for stationarity by examining the plateau in log-likelihood over generations using an Excel spreadsheet. For the LSU rDNA data set the “burn-in” of the chains occurred in fewer than 6050 generations so the first 121 trees were discarded, leaving 9880 trees for estimating posterior probabilities (PP). Thus, PP values were obtained from the 50% majority rule consensus of the kept trees.

3. RESULTS

This Paragraph contains the results obtained from the samples collected during the Arctic Field course 2006 at Arctic Station, Disko Island, West Greenland. Photo plates and descriptions of the species encountered constitute the first of four Paragraphs. The second contains a list of species, which were sampled at the different stations. The third Paragraph concerns the phylogeny of some of the dinoflagellates which were isolated for molecular analyses based on single cell PCR, while the fourth and last Paragraph concerns the results concerning the *Ceratium* species (*C. arcticum* and *C. longipes*) complex.

3.1. Species descriptions

This Paragraph contains descriptions of the orders, families, genera and species encountered. The morphometric data presented are taken from the illustrated specimens and should not be taken as fully representative for each species, since the measurements are based on to few cells. Furthermore, all of the photo documentation is presented in this Paragraph. Not all details about the encountered species could be derived through LM and SEM; in these situations the descriptions have been supplied with information from the literature.

Phylum Alveolata Cavalier-Smith, 1992

This phylum constitutes three large and old taxa, the Dinoflagellates, the Ciliophorans and the Apicomplexans. Many members of this phylum produce large cells with a high level of complexity, of which much can be attributed to varied use of the cortical alveoli; a homologous character. Thus, the amphiesmal vesicles of the biflagellate dinoflagellates, the inner membrane of the non-flagellated apicomplexans and the alveoli of the ciliated Ciliophora represent a system inherited from a single ancestor (Paragraph 1.2.1) [CAVALIER-SMITH, 1993; HAUSMANN *et al.*, 2003].

3.1.1. Order Prorocentrales Lemmermann, 1910

The cells are thecate with a desmokontr flagella orientation, *i.e.* the flagella emerge at the anterior end. The two flagella emerge from the larger of two pores (Figure 3.1.A). The smaller pore may be the opening to the pusule. Neither a cingulum nor sulcus is present. The theca consists of two valves, lateral plates, which interlock in a large suture; these two valves may be interpreted as homologues to the epicone and hypocone of the typically dinokont cell. Cell mobility is forward-directed through a spiralling motion. [DODGE, 1982; FENSOME *et al.*, 1993; VAN DEN HOEK *et al.*, 1995].

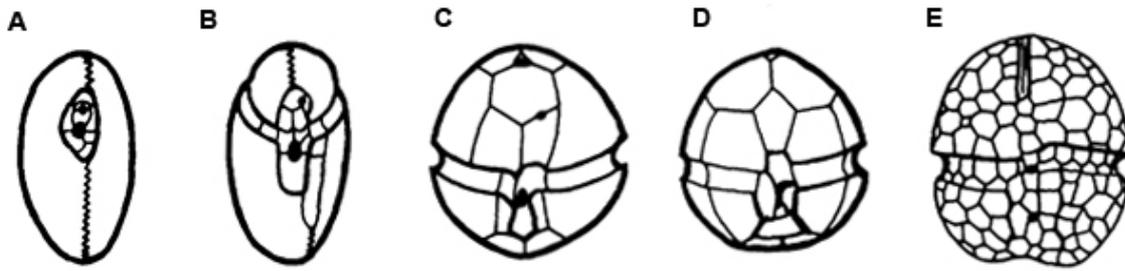


Figure 3.1. The basic dinoflagellate thecal types. **A** Prorocentroid (in apical view). **B** Dinophysoid. **C** Gonyaulacoid. **D** Peridinioid. **E** Gymnodinoid. [TAYLOR, 1980].

Family Prorocentraceae Stein, 1883

All species possess several periflagellar plates surrounding the flagellar pore complex where a normal and a helical flagellum emerge [FENSOME *et al.*, 1993].

Genus *Prorocentrum* Ehrenberg, 1833

The two valves are rotundate, elliptic to heart-shaped. The nucleus is located posteriorly in the cell. Two chloroplasts are present, sometimes together with a pyrenoid. This genus has a world-wide distribution in marine environments [DODGE, 1982]. The cells may possess apical spines and the posterior end either rounded or elongated into a spine [DODGE, 1982].

Species *Prorocentrum micans* Ehrenberg, 1833

Prorocentrum micans is the type species of this genus, and was the only desmokont dinoflagellate species observed during our stay at Arctic Station. The cell has a rounded anterior end and a pointed posterior end. The cell is widest around the middle (Plate 1 A, B). The cell is 25 μm wide and 43 μm long (Plate 1), and possesses a prominent anterior spine with wings (Plate 1 A). The theca is often perforated by numerous tubular trichocyst pores arranged in radial rows [DODGE, 1982]; these are difficult to recognise on plate 1 B, but can be faintly seen on 1 A. The large nucleus is located in the posterior end of the cell (Plate 1 B). Besides from the cell possessing green chloroplasts, information concerning the chloroplasts and the pyrenoid is difficult to obtain from plate 1. This species is cosmopolitan in the marine environment and can cause toxic red tides [VAN DEN HOEK *et al.*, 1995].

3.1.2. Order Dinophysiales Kofoid, 1926

The cells are laterally flattened with a dinokont flagella orientation and a premedian cingulum (Figure 3.1.B). Cingulum and sulcus often have wide lists supported by ribs and the single flagellar pore is typically located within the sulcus [STEIDINGER & TANGEN, 1997]. The number and arrangement of thecal plates are highly conservative and most species of this order lack chloroplasts [FENSOME *et al.*, 1993].

Family Dinophysiaceae Stein, 1883

Dinophysis was the only genus in this family until 1883 when Stein proposed a new genus, *Phalacroma* to include those species that have a prominent and clearly visible epitheca [DODGE, 1982]. However, it has several times been questioned whether this separation is acceptable, since the members of the two genera are very similar. This problem has not yet been resolved (Paragraph 3.3).

Genus *Dinophysis* Ehrenberg, 1840

Comprises cells with a small epitheca and a hypotheca that normally represents 3/4 or more of the cell length. The epitheca may be flattened or convex, and the hypotheca may be ornamented with spines or lists. The cingulum is wide and not descending. The anterior

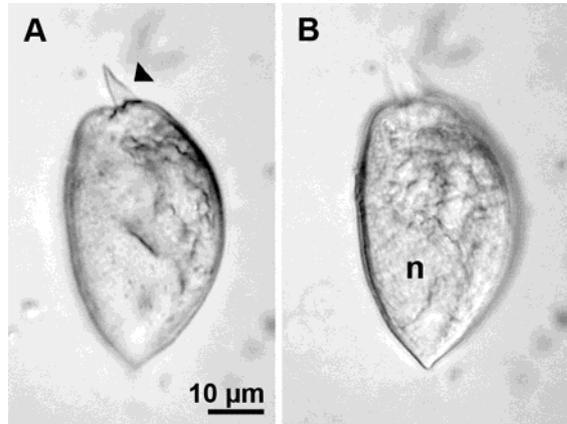


Plate 1. *Prorocentrum micans*. A-B: LM. A. High focus showing the left valve with a prominent spine (arrowhead) at the anterior end. B. Low focus on the same cell showing the large, posteriorly located nucleus (n). Same scalebars for A and B.

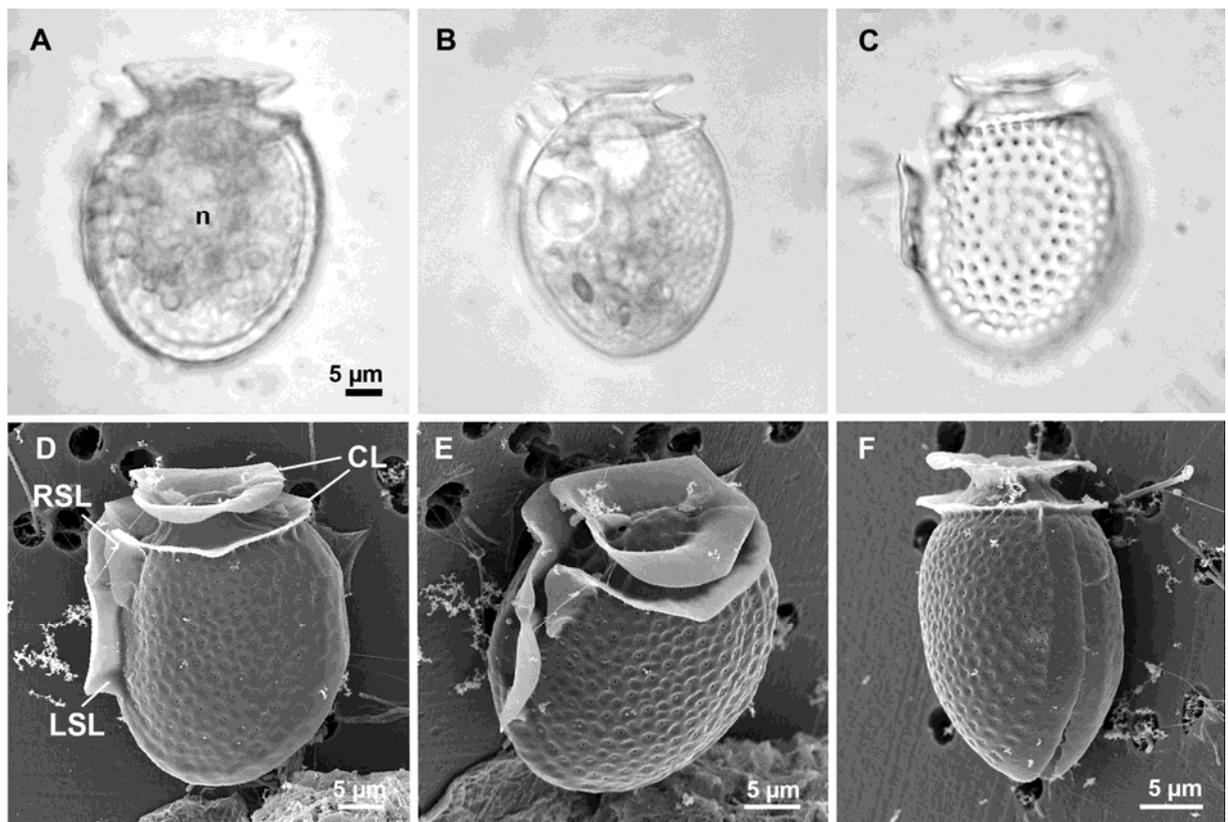


Plate 2. *Dinophysis acuminata*. A-C: LM. D-F: SEM. A. Low focus on cell viewed from the left side, showing the nucleus (n). B. Higher focus on the same cell showing the cell shape and theca. C. High focus on empty theca showing the circular areolae with pores. Same scalebar for A-C. D. Cell viewed from the left side showing the large sulcal list (LSL), the small right sulcal list (RSL) and the two well-developed cingular lists (CL). E. Oblique view showing the small epitheca. F. Left dorsal view revealing a rupture at the antapical end.

cingular list i angled into a distinctive funnel shape. The sulcus is located on the hypotheca but can extent into the epitheca and has both a left and a right list. The theca may be porate with aeroles and reticulations. The nucleus is large and located in the hypotheca. Both a pusule and chloroplasts may be present [DODGE, 1982; STEIDINGER & TANGEN, 1997]. Species within the *Dinophysis* genus produce the toxin DSP, which causes diarrhetic shellfish poisoning [THRONDSEN *et al.*, 2003].

Species *Dinophysis acuminata* Claparède and Lachmann, 1859

Cells are thecate with a small epicone and a large hypocone. Cell size ranges: 30-35 µm in length and 20-25 µm in dorso-ventral width (widest near middle of cell). The shape is oval to elliptic with a rounded antapex (Plate 2 A-F). A well-developed left sulcal list (LSL) extends beyond the midpoint of the cell (Plate 2 C-E). The thick thecal plates are covered with prominent circular areolae, each containing a pore; the pore size seems to vary (Plate 2 C-F). The epitheca is slightly convex and inclines ventrally (Plate 2 A-D, F). Two well-developed lists border the cingulum: A broad anterior cingular list with ridges, and a smooth posterior cingular list (Plate 2 D-F). The plates of the cingulum are smooth with pores (Plate 2 D). The LSL, supported by three ribs, is rather narrow with the third rib being the longest (Plate 2 C, D). The right sulcal list (RSL) is small, only one third of the LSL. *Dinophysis acuminata* is a photosynthetic species with large brown chloroplasts and a centrally located nucleus (Plate 2 A and data not shown).

Species *Dinophysis acuta* Ehrenberg, 1839

Dinophysis acuta is the type species for this genus. Cells are armoured and laterally compressed with a small epitheca and a much larger hypotheca (Plate 3 A-F and H). The cells are large, robust and oblong with a rounded posterior end (Plate 3). Cell size ranges: *ca.* 50 µm in length and 40 µm in dorso-ventral width, being widest below the middle (Plate 3). The LSL extends beyond the midpoint of the cell (about two thirds of the cell length) ending at or above the widest portion of the cell (Plate 3 H). The thick thecal plates of the hypotheca are slightly aerolated, each areole with a central pore (Plate 3 E, H, I). The epitheca is flat or slightly convex and not visible in lateral view (Plate 3 A, H). A large anterior and a smaller posterior cingular list are present, both being smooth (Plate 3 E-H). The LSL, supported by three ribs that radiate outwards, is rather broad with a convex ventral margin (Plate 3 H). The hypotheca is ovoid with a rounded antapex (Plate 3 A, H). The large nucleus is centrally located (Plate 3 B).

Dinophysis acuta is a mixotrophic species and both specimens with green and red chloroplasts were observed (Plate 3). The dramatic changes in cell shape of the specimens on 3 B, C, and D are most likely a result of phagotrophic food-uptake (Paragraph 1.2.3). These alterations in cell shape are also observed on plate 3 E, F, G, and I, where the sutures of the hypotheca have enlarged extensively; a feature we interpret as being a consequence of eating. This species is considered the most toxic of the DSP-producing members of *Dinophysis* [THRONDSEN *et al.*, 2003].

Species *Dinophysis norvegica* Claparède & Lachmann, 1859

The cells are thecate, laterally compressed with a small epitheca and a large hypotheca. The cells are large, ovoid and robust. Cell size lies within the range of 50-60 µm in length and 40-45 µm in width (Plate 5 A-F). Characteristically, the posterior end of the cell has an almost triangular shape (Plate 4 A and 5 A-F); a shape which, however, seems to vary slightly (Plate 5 A-F). The antapex is pointed (Plate 4, 5 A) or slightly rounded (Plate 5 C). The cells are widest at or slightly above the middle of the cell (Plate 4 A and 5 A-F). The thick thecal plates

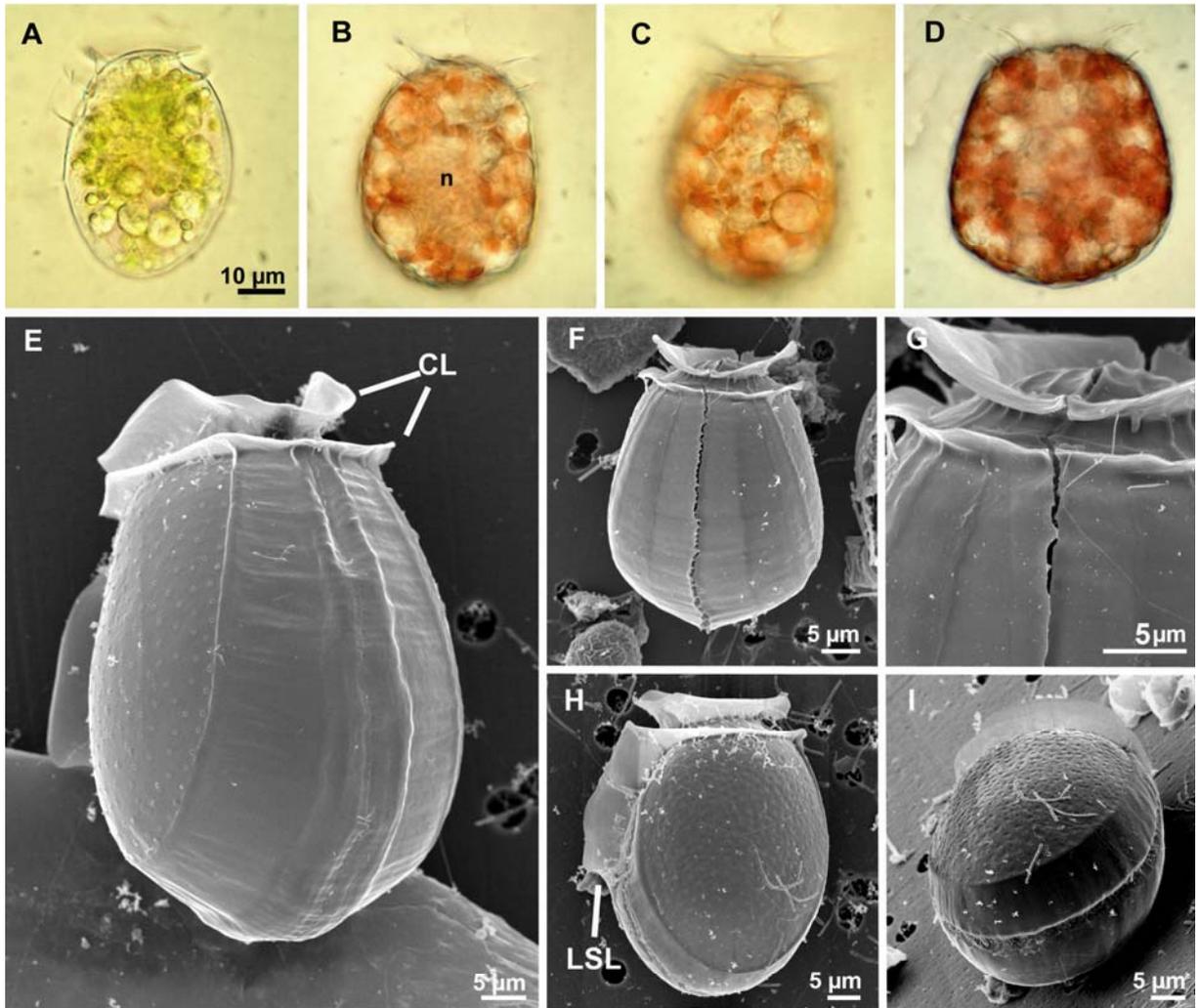


Plate 3. *Dinophysis acuta*. A-D LM, E-I SEM. **A** Low focus of cell view from the left side with greenish plastids. **B-D** Low focus, supposedly from the dorsal side since no sulcal lists can be observed, the cells contain different amounts of red plastids and have a centrally located nucleus (n). Same scale bar for A-D. **E-F** Dorsal view of a cell showing the large suture and the prominent cingulum lists (CL). **G** Detail of the dorsal part of the cingulum and the epitheca. The cell has enlarged severely, probably as a consequence of eating. **H** Cell viewed from the left side, showing the large left sulcal list (LSL). **I** Cell viewed from the antapex.

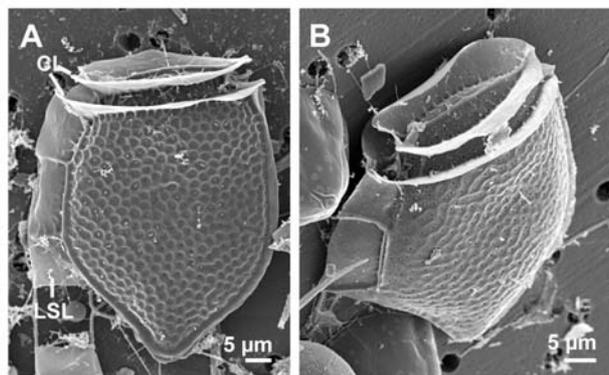


Plate 4. *Dinophysis norvegica*. SEM. **A** Cell viewed from the left side, showing the cell shape, the cingulum lists (CL) and the left sulcal list (LSL). **B** Apical view of the cell showing the small and narrow epitheca. Further more the aerolated theca with pores can be observed.

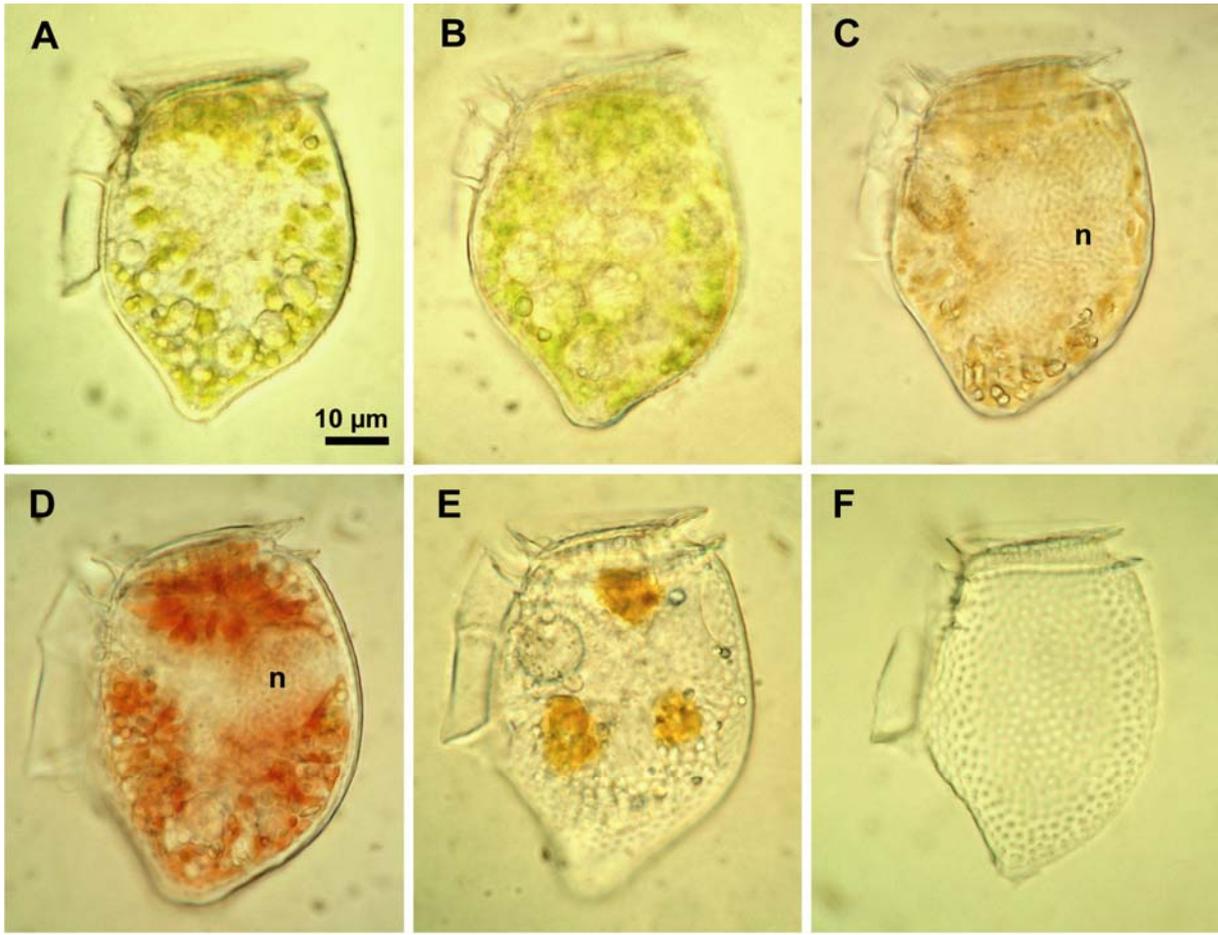


Plate 5. *Dinophysis norvegica*. LM. A-F. Cells viewed from the left side. A-E. Low focus of cells with different coloured chloroplasts or with only small fragments of chloroplasts (E), the nucleus (n) is large and dorsally located in the cell. F. High focus on an empty theca showing the porated aerola. Same scale bar for A-F.

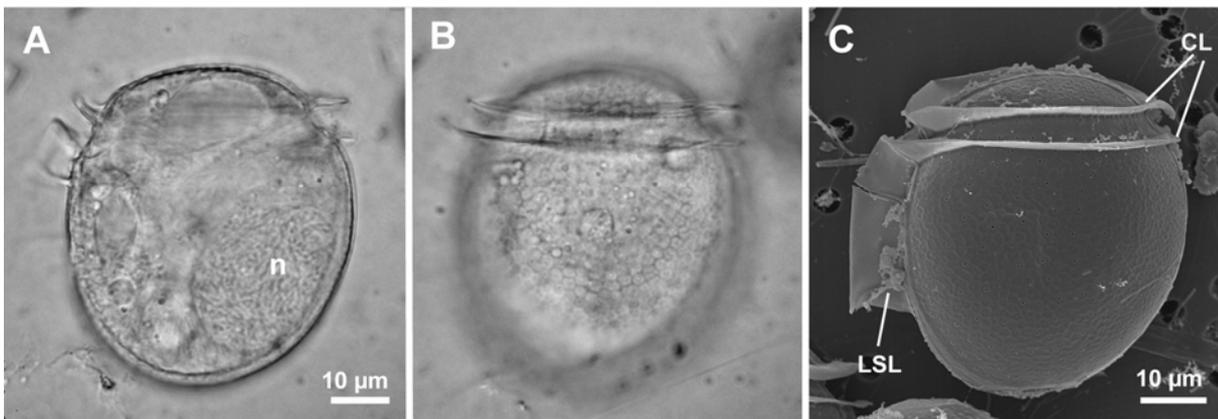


Plate 6. *Dinophysis rotundata*. A, B: LM C: SEM. A-C: Cells viewed from the left side A. Low focus showing the large dorsally located nucleus (n). B. High focus showing the thecal plates. C. Showing the cingular lists (CL) and the left sulcal list (LSL) Same scale bar for A and B.

are coarsely aerolated with the areolae being large and each containing a pore (Plate 4 A and B). The small epitheca is low, flat or weakly convex, and is obscured by cingular lists (4 B). The cingular plates have pores (Plate 4 B). The cingulum bears two well-sculptured lists, an anterior and a posterior (Plate 4 A), which are both projected anteriorly (Plate 4 A, B and 5 A-F). The LSL extends about 2/3 of the cell length (Plate 4 A and 5 A-F). This list, supported by three ribs that radiate outward, is relatively narrow (Plate 4 A and 5 A-F). The third rib is located at the mid-point of the cell or just above it (Plate 4 A and 5 A-F). The sulcal lists have delicate surface ornamentation (Plate 4 A, B).

D. norvegica is a mixotrophic species and has kleptochloroplasts [TAKISHITA *et al.*, 2002]. These were observed with many different colours, ranging from light green to brownish and red (Plate 5 A-E). The nucleus is large and dorsally located (Plate 5 C, D).

Species *Dinophysis rotundata* Claparède & Lachmann, 1859

The cells are thecate, laterally compressed with a small epitheca and a much larger hypotheca. Cell size ranges: 45-50 µm in length and 35-40 µm in width (Plate 6 A-C). Cells are broadly rounded in side view (Plate 6 A-C). The thecal surface is slightly aerolated and with scattered pores (Plate 6 B, C). The epitheca is visible in a side view; it is a small convex cap above the cingulum, low and fairly evenly rounded (Plate 6 A-C). The cingular lists (CL) are smooth and slightly anteriorly inclining (Plate 6 A-C). The LSL extends over *ca.* 3/4 of the cell length (Plate 6 C). This list is supported by three ribs and is relatively narrow but widening posteriorly (Plate 6 C). The first two ribs are situated more closely together than the second and third ribs. *Dinophysis rotundata* is a heterotrophic species without chloroplasts. The nucleus is posteriorly positioned and the protoplasm is clear with numerous food vacuoles (Plate 6 A).

Genus *Phalacroma* Stein, 1883

The cells are medium to large and typically without chloroplasts. Very identical to *Dinophysis* except from the anterior cingular list, which is not funnel shaped but horizontal, and the epitheca is clearly visible in lateral view [DODGE, 1982; STEIDINGER & TANGEN, 1997].

Species *Phalacroma* cfr. *braarudii* Nordli, 1951

The cells are thecate with a small epicone and a large hypocone. Cell size is *ca.* 15 µm in width and 20-25 µm in length (Plate 7). From a ventral or dorsal view the cell is ovoid to elliptical (Plate 7 A-C). In a side view the cell appears almost round and at the antapex the hypothecal plates are approximately as wide as the cingulum (Plate 7 E). The cingulum is excavated and very wide (Plate 7 A-C, E) and the sulcus also appears wide (Plate 7 D). The epicone is convex and visible from the side (Plate 7 A-C, E). The CLs are small and straight (Plate 7). The thecal plates of the epicone, hypocone and cingulum have pores but lack ornamentation (Plate 7 D-E). This species is heterotrophic and the protoplasm is highly vacuolated (Plate 7 A-C).

3.1.3. Order Gonyaulacales Taylor, 1980

This order comprises species with strongly asymmetrical tabulation, especially in the antapical and anterior ventral regions. The cingulum is usually descending, displaced by a cingulum width or more, and often with overhang (Figure 3.1.B). The distinct apical pore complex (APC), which consists of a small, outer pore plate and a smaller, inner cover plate, is non-symmetric as is the first of the four apical thecal plates. The basic pattern in plate tabulation is: 4', 6'', 6c, 6''', 1p, 1''''', 5s (Appendix 1, Figure 3). In many species a ventral pore is present, located at the right anterior margin of the first apical plate. Variations in

tabulation pattern, including the presence of anterior intercalary plates, form the base of subdivision within Gonyaulacales (Appendix 1, Figures 1 and 3). Especially important is the shape and position of the first apical plate [FENSOME *et al.*, 1993].

Formation of both vegetative and resting cysts occurs within this order, and the cysts may be fossilisable [FENSOME *et al.*, 1993].

Family Ceratiaceae Lindemann, 1928

Gonyaulacaulids having more than two, usually 3-4 horns with one or two of these formed by postcingular plates [FENSOME *et al.*, 1993].

Genus *Ceratium* Schrank, 1793

The cells are slightly dorso-ventrally compressed and apically extended into a horn with an APC at the tip. In addition the cells have up to two postcingular and one antapical horn with the tips being either closed (pointed) or open. The horns are all hollow, comprising cytoplasm and may possess "thorns"; intraspecific variation between number and length may occur due to environmental changes. The cingulum is slightly declining on the right, ending in a depressed ventral area with an invagination to the left; a modified sulcus, from which the longitudinal flagellum emerges [DODGE, 1982; FENSOME *et al.*, 1993; STEIDINGER & TANGEN, 1997]. The thecal plates, except for those in the ventral area, are rather thick with several trichocyst pores [DODGE, 1982; FENSOME *et al.*, 1993] and may be smooth to highly reticulated [STEIDINGER & TANGEN, 1997]. Numerous yellow-brownish chloroplasts of discoid shape are usually found in the cytoplasm, and starch grains, lipid droplets as well as food-vacuoles may be present [STEIDINGER & TANGEN, 1997]. The nucleus is large and centrally positioned. The thecal plates are shared during division [DODGE, 1982]. Some species reproduce sexually, through anisogami, with the zygote normally being similar in shape to the vegetative cells. Cyst formation is only known for freshwater species [DODGE, 1982].

Species *Ceratium arcticum* (Ehrenberg) Cleve, 1901

Very similar to *C. longipes* (see below), and sometimes regarded as a form of this species (Paragraph 1.3) [DODGE, 1982]. The dimensions of the cell are 385-400 μm in width and *ca.* 200 μm in length including the horns (Plate 8 A, C). The cell body is gonyaulacaulid with the apical horn leaning to the right (Plate 8 A, C). The antapical horns are open, only slightly curved and are directed outwards from the cell body. Below the straight posterior body margin, the horns are only vaguely curved (Plate 8 A-C). According to Gran (1902) the angle between the horns in dorsal view lie within the range of 40-140°, usually 70-120° (Paragraph 1.3); however angle measurements were not conducted in the present survey. The nucleus is centrally placed and the cell possesses yellow-brownish chloroplasts (Plate 8 A, B). The cells are only vaguely ornamented, but pores are found on the cell body as well as the antapical horns (Plate 8 C, D). Thorns are found along the horn margins (Plate 8 D). The species is mainly found in cold waters [STEIDINGER & TANGEN, 1997] (Figure 1.9).

Species *Ceratium fusus* (Ehrenberg) Dujardin, 1841

The cell is elongated and fusiform, *ca.* 150-600 μm long, 30-40 μm wide, with one pointed horn drawn out from both the epi- and hypotheca (Plate 9). A small or rudimentary antapical horn may exist and both of the long horns can be slightly bent or curved (Plate 9). Linear markings may be apparent on the surface [STEIDINGER & TANGEN, 1997]. Information about the chloroplasts and the nucleus is difficult to obtain from Plate 9. The species is cosmopolitan [STEIDINGER & TANGEN, 1997].

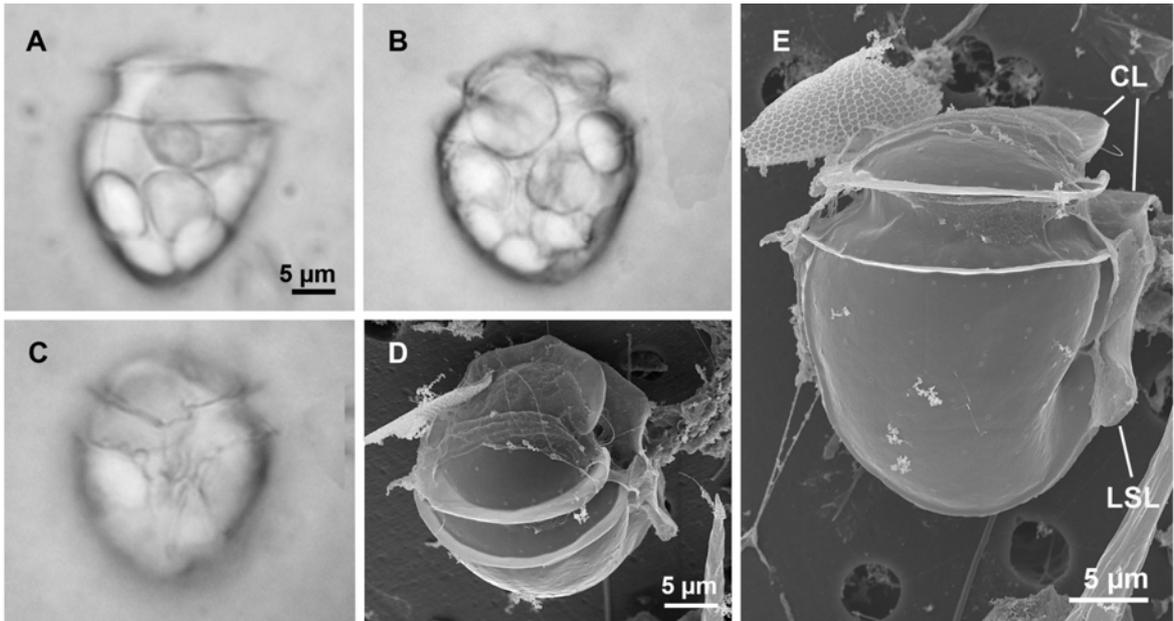


Plate 7. *Phalacroma* cfr. *braarudii* A-C: LM. D-E: SEM. **A.** High focus on a cell from a dorsal view showing the wide cingulum. **B.** Low focus of a cell, showing the highly vacuolated protoplasm. **C.** High focus on a cell view from the ventral side, showing the wide sulcus. **D.** Cell viewed from the apex, showing the epitheca. **E.** Cell view from the right side showing the narrow left sulcal list (LSL) and the cingulum lists (CL). Same scale bar for A-C.

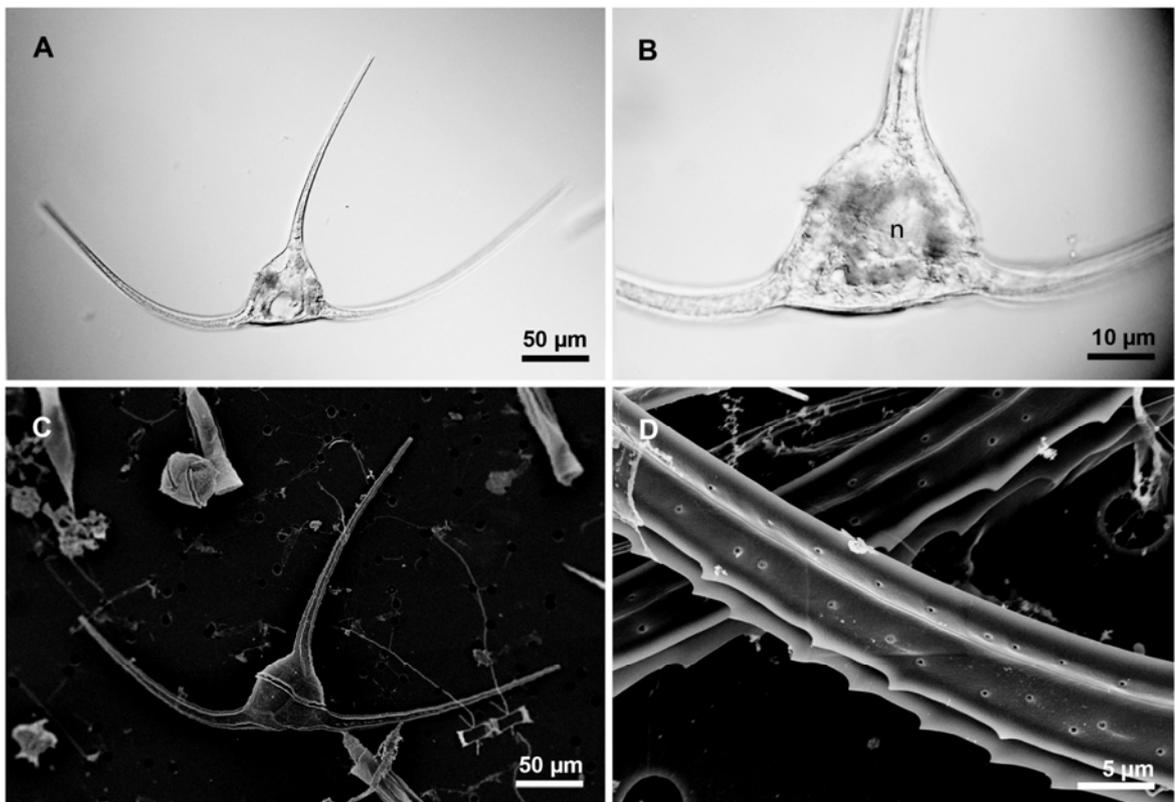


Plate 8. *Ceratium arcticum*. A-B: LM. C-D: SEM. **A.** High focus of cell viewed from the dorsal side showing the shape. **B.** Low focus of cell showing the nucleus (n) and the chloroplasts. **C.** Dorsal view showing the thecal plates and the cingulum. **D.** Close up of an antapical horn with small horns.

Species *Ceratium longipes* (Bailey) Gran, 1902.

The cell is *ca.* 230-240 μm long and 220-240 μm wide including the horns (Plate 10 A, B, F). The cell body is subpyriform to triangular with the epitheca tapered into an apical horn, which is slightly bent to the right. The hypotheca appears straight but is angled posteriorly (Plate 10 A-F). The right antapical horn emerges just beneath and approximately in parallel to, the cingulum, whereas the left emerges slightly lower and in an angle of *ca.* 45° (Plate 10 A, B, D-F). Both horns are open, of equal thickness and bent anteriorly (Plate 10 A, B, F). The right horn may be nearly parallel to and of similar length as the apical horn (Plate 10 A, B), whereas the left one is most commonly shorter and more curved (Plate 10 A, B, F). In dorsal view, the angle between the horns may vary within a range of up to 60° [GRAN, 1902]. The thecal plates are substantial and highly reticulated (Plate 10 E, F), and thorns may be found along the margins of the apical (Plate 10 C) and the antapical horns (Plate 10 G). In dorsal view the nucleus is centrally located (Plate 10 B) but appears slightly displaced in ventral view (Plate 10 D). The chloroplasts are numerous and yellow-brownish (Plate 10 A-D). This species is found in Arctic to cold, temperate waters [STEIDINGER & TANGEN, 1997] (Figure 1.9).

Family Goniodomataceae Lindemann, 1928

The cells may be antero-posteriorly compressed and, in most genera, the principal life stage is motile and thecate. Typically for this family are the three posterior-most plates, of which the first antapical homologue has a characteristic pentagonal shape. The number and position of the thecal plates display some variation, especially in the cingular and sulcal regions, but tabulation is basically: 3-4', 6'', 6c, 3-5s, 5-7''', 3'''''. Plate growth occurs only at overlapping margins. Species of this family are photosynthetic and found in both cold and warm temperate marine environments [FENSOME *et al.*, 1993].

Genus *Alexandrium* Halim, 1960

The cells are armoured and spherical to oval or slightly biconic, lacking horns and spines. The cingulum is median and descending, displaced 1-1.5 times its width but without any overhang. The surface may be reticulated, vermiculated and/or have pores. The thecal plates can be very delicate to substantial. A ventral pore is found in many species. The nucleus is elongated, sometimes C-shaped, and all species possess brown, net shaped chloroplasts. In some species, the dorso-ventral positioning of the apical pore complex together with the age of the theca, may result in apical protuberance [STEIDINGER & TANGEN, 1997; THRONDSSEN *et al.*, 2003]. The distinction of species is based on shape and position of the pore plate and the ventral pore, cell dimensions, and the shape and size of the plates near the sulcal area [STEIDINGER & TANGEN, 1997]. Species of this genus have a pore with a distinct pore plate, four apical plates, six precingular plates, five postcingulars and 2 antapical plates.

Species *Alexandrium minutum* Halim, 1960

The cell is ovoid of shape and 30 μm in length and width (Plate 11 A). Viewed from the apex the cell appears circular (Plate 11 C). The wide cingulum descends *ca.* one width and is bordered by lists. Both the left and right border of the sulcus have distinct lists made by extensions of the 1''' and 5''' (Plate 11 A, B). The thecal plates are smooth with scattered pores (Plate 11 B). The rhomboid 1' has a small ventral pore and does not appear connected to the Po (Plate 11 B). *A. minutum* is known to produce PSP toxins, which cause paralytic shell fish poisoning; an illness that can be lethal even to humans [HANSEN *et al.*, 2003b; THRONDSSEN *et al.*, 2003]. This species has a wide distribution in temperate waters [HANSEN *et al.*, 2003b].

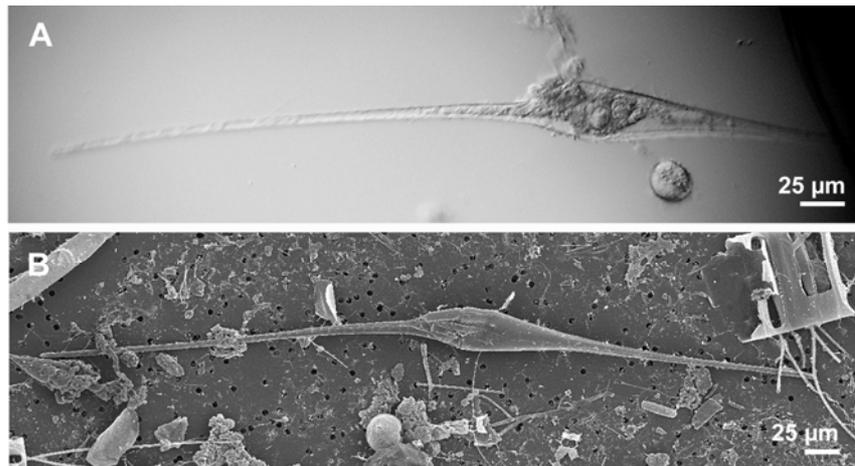


Plate 9. *Ceratium fusus*. A: LM, B: SEM. A. Low focus of cell, showing some of the apical horn and the left hypotheca horn, the chloroplasts extend into the horns. B. Cell from a ventral view showing the sulcal area.

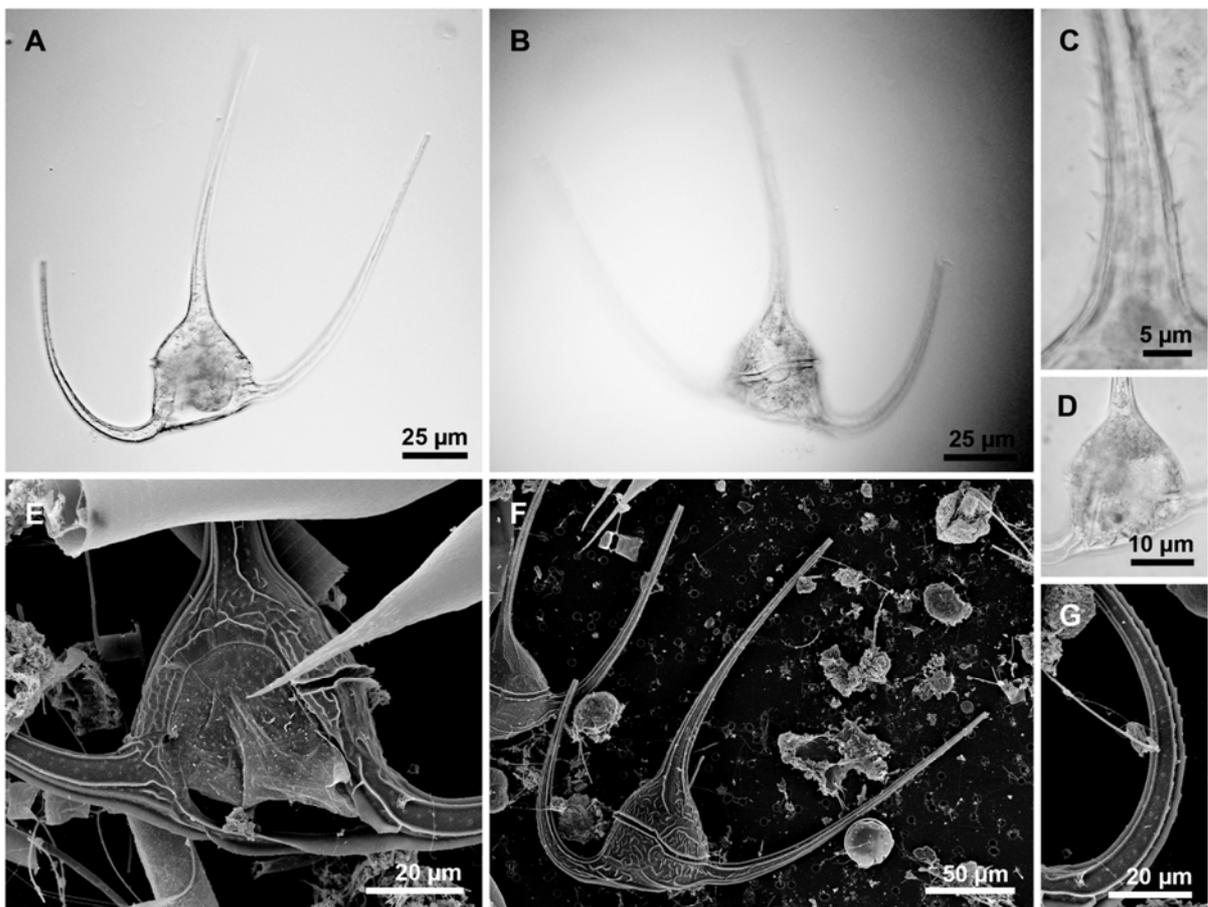


Plate 10. *Ceratium longipes*. A-D: LM. E-G: SEM. A. High focus of cell view from the ventral side showing the cell shape. B. High focus of cell viewed from the dorsal side showing the cingulum. C. Close up of the apical horn, showing chloroplasts and the small spines. D. Closeup of the cell body showing the nucleus (n) and the chloroplasts. E. Ventral view. F. Dorsal view showing some thecal plates and the cingulum. G. Close up of antapical horn showing the small spines.

Species *Alexandrium tamarensense* (Lebour) Balech, 1992

The cells are small to medium sized, *ca.* 30 µm in width and length, and the cell appears globular. The cingulum is displaced *ca.* one time its width (Plate 12 A, B), and both the sulcus and the cingulum are bordered with prominent lists (Plate 12 A, B). The sulcus broadens towards the antapex (Plate 12 B). The epitheca is broadly conical with slight shoulders (Plate 12 A, B). The hypotheca is trapezoidal and posteriorly concave. The hypotheca appears skewed because the left side is less angled than the right (Plate 12 A). The comma-shaped Po has a strongly developed callus (Plate 12 D). This plate is connected to the 1', which has a small ventral pore located proximately at the 4' (Plate 12 B, C). The 6'' is wide (Plate 12 A, B; compare with *A. minutum* plate 11 A, B). Since we only identified this species in SEM we could not obtain information on the chloroplasts and the nucleus. However, according to the literature this species possesses a sausage-shaped nucleus and yellow-brown chloroplasts [THRONDSEN *et al.*, 1993]. This species is cosmopolite and produces PSP toxins [THRONDSEN *et al.*, 1993].

Family Gonyaulacaceae Lindemann, 1928

The cells are subspheroidal, subovoidal, subpolygonal or fusiform in shape. Dorso-ventral compression is observed rarely, and the antapical outline is almost symmetrical. An apical horn as well as 1-2 antapical spines is present, but the cells have neither postcingular horns nor thecal processes. The sulcus is more or less midventral and can be straight, crossing from the upper right to lower left, or is sigmoidally shaped; usually within a groove in extant species. Cyst formation is common, and great morphological variations are found among these [FENSOME *et al.*, 1993]. Species in this family usually have 6 precingular plates (Appendix 1, Figure 1) [STEIDINGER & TANGEN, 1997; FENSOME *et al.*, 1993].

Genus *Amylax* Meunier, 1910

The cells are armoured and small to medium sized. The epitheca tapers into an apical horn with an apical pore complex and a round pore opening. The hypotheca extends into at least one antapical spine. The cell is slightly dorsoventrally compressed. Chloroplasts are present. Plate formula: Po, 3', 3a, 6'', 6c, 7 or 8s, 6''', 2'''''. The 1' is narrow with a ventral pore at the posterior right side [STEIDINGER & TANGEN, 1997].

Species *Amylax triacantha* (Jørgensen) Sournia, 1984

The cell is subpyriform to ovoid with one apical horn and three antapical spines (Plate 13). Not including the antapical spines, the cell length is *ca.* 35 µm and the width 20 µm (Plate 13). The antapical profile is inverted trapeze-shaped and the epitheca slightly triangular (Plate 13). Cingulum displacement is approximately two times its widths, leaving hardly any overhang (Plate 13 C). Thecal ornamentations are reticulate with varying distinctiveness; possibly related to cellular age (Plate 13 C, D). The chloroplast are multiple and yellow-brownish, with some being located in the apical horn (Plate 13 A, B).

Amylax triacantha is a coastal coldwater-species, found in the Pacific, Atlantic and Arctic Oceans [STEIDINGER & TANGEN, 1997; THRONDSEN *et al.*, 2003].

Genus *Gonyaulax* Diesing, 1866

The shape of the cells varies from spheroidal to polygonal and the cingulum is equatorial, displaced up to seven times its width. The sulcus is mainly on the hypotheca, but often extends to the epitheca [STEIDINGER & TANGEN, 1997; GRAHAM & WILCOX, 2000]. Thecal plate thickness is often substantial with or without ornamentation, and the theca is either shed before division or shared following cytokinesis. Many species have cyst stages, and these

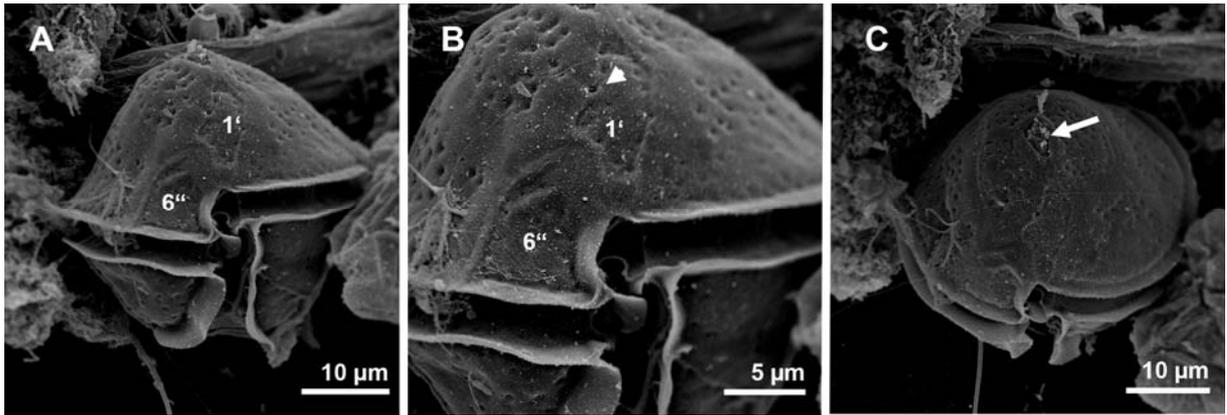


Plate 11. *Alexandrium minutum*. SEM. **A.** Ventral view showing the cell shape, the 1', sulcus and cingulum. **B.** Close-up of the ventral side showing the 1', the ventral pore (arrowhead) and the narrow 6''. **C.** Apical view of the specimen, displaying the position and shape of the Po (arrow).

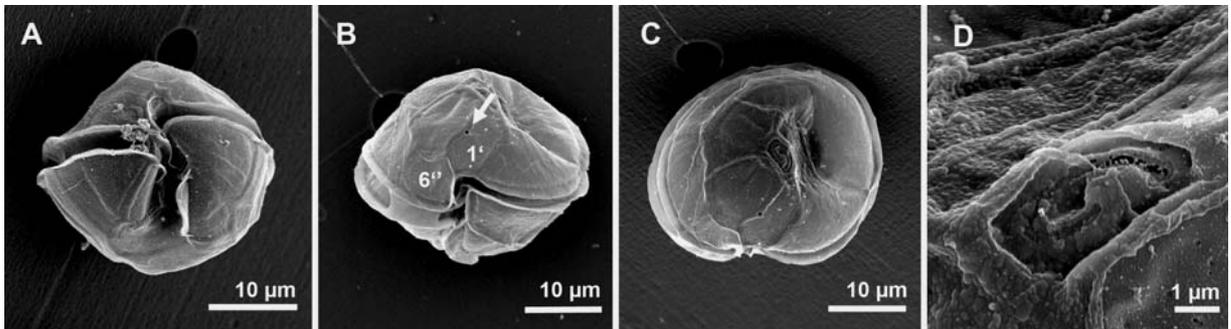


Plate 12. *Alexandrium tamarense*. SEM. **A.** Ventral view showing cell shape, the sulcus and cingulum. **B.** Ventral view showing the 1' with ventral pore (arrow) and the wide 6''. **C.** Apical view showing the epithecal plates. **D.** Close-up of apical pore area.

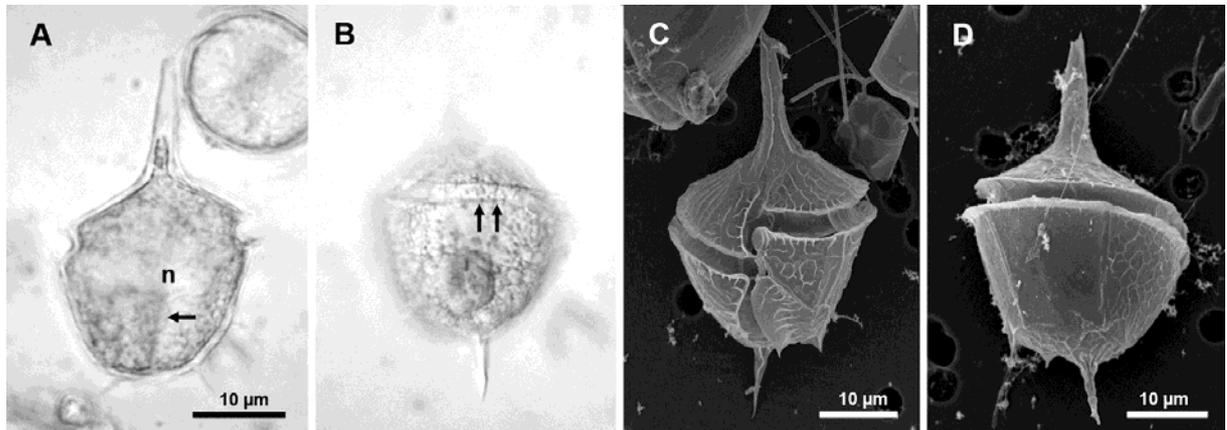


Plate 13. *Amylax triacanta*. **A-B:** LM. **C-D:** SEM. **A.** Low focus of cell from dorsal view showing the ventral side with sulcus (arrow) and the centrally located nucleus (n). **B.** High focus of the same cell showing the dorsal side and the cingulum (arrows). **C.** Ventral view of a specimen showing the cingular overhang and the sulcus. **D.** Dorsal view showing the descending cingulum and the antapical spines. Same scale bar for A and B.

species can be identified mainly on behalf of cyst morphology, due to the great form-variation between species. Apart from cyst morphology, *Gonyaulax* species can be distinguished on behalf of size and shape, plate tabulation and pattern, APC, cingulum displacement and overhang, development of apical horn, and ornamentation (Figure 3.2) [STEIDINGER & TANGEN, 1997].

Species *Gonyaulax* cfr. *alaskensis* Kofoid, 1911

The cells are broadly ovoid in shape, *ca.* 45 μm in width and 40 μm in length. The epitheca appears triangular leading into a very short apical horn (Plate 14 A, B). In an apical view, the cell appears almost circular (Plate 14 B). The hypotheca is straight, leading into a short dorsally located antapical spine (Plate 14 A). According to the literature, two short spines are present on 1'' and 5'' [DODGE, 1982]; however, these are difficult to recognise on plate 14. The cingulum is relatively narrow and displaced *ca.* 3 widths with a large overhang. Narrow lists border the cingulum (Plate 14 A, B). The sulcus, which does not reach the antapex, is sigmoidal and deeply impressed (Plate 14 A, B). The thecal plates are delicately reticulated (Plate 14). An elliptic to droplet-shaped apical pore is present, being 5 μm in length and 2 μm in width (Plate 14 C). The Po is lancet-shaped (Plate 14 B, C) and according to the literature it has stripes [DODGE, 1982].

Species *Gonyaulax* cfr. *digitale* (Pouchet) Kofoid, 1911.

Without the spines the cells is *ca.* 35 μm wide and 30 μm long (Plate 15 A). In an apical view the cell is circular with a centrally located Po (Plate 15 D, E). The epitheca leads into a large apical horn, which is almost half the length of the epitheca (Plate 15 A-C). The hypotheca has large and curved dorsal antapical spines (Plate 15 A-C). The cingulum is bordered with lists and displaced *ca.* 2 to 2.5 widths with an overhang (Plate 15 A, B). The sulcus is deeply impressed in the epitheca (Plate 15 A). The sulcal list leads into two spines on 1'' and 5''. The thick thecal plates are heavily reticulated and large sutures are present; there seems to be some intraspecific variation in reticulation on the apical horn (Plate 15, Compare A and B). The concave Po is lancet shaped without any reticulation and *ca.* 3 μm long and 1 μm wide (Plate 15 D-F).

Species *Gonyaulax* cfr. *spinifera* (Claparde & Lachmann) Diesing, 1866.

This is the type species for *Gonyaulax*. Excluding the antapical spines the cell is *ca.* 40 μm wide and 45 μm long. The cell is top-shaped and the epitheca appears lightly twisted in a ventral view (Plate 16 A). The epitheca leads into a short apical horn. The hypotheca is rounded with two small antapical spines (Plate 16 A). In apical view the cell appears somewhat circular with a centrally located APC (Plate 16 B). The cingulum is wide, displaced *ca.* 2.5 widths and is without lists. The thecal plates are thick without any ornamentation beside pores, which appear unevenly scattered (Plate 16). The Po is concave without ornamentation (Plate 16 C). On plate 16 C the Po is lifted from the pore, probably as a consequence of the washing and dehydration during SEM preparation. According to the literature this species has yellow-brown chloroplasts [DODGE, 1982].

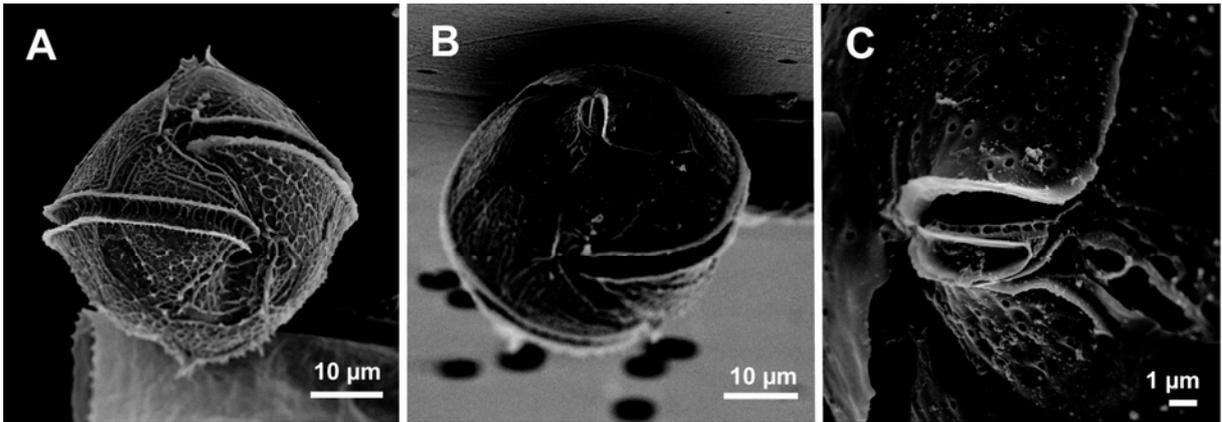


Plate 14. *Gonyaulax* cfr. *alaskensis*. SEM. A. Ventral view of the cell, showing the cell shape, the sulcus and the cingulum. **B.** Apical view, showing the epithelial plates and the location of the apical pore complex (APC). **C.** Close-up of the APC.

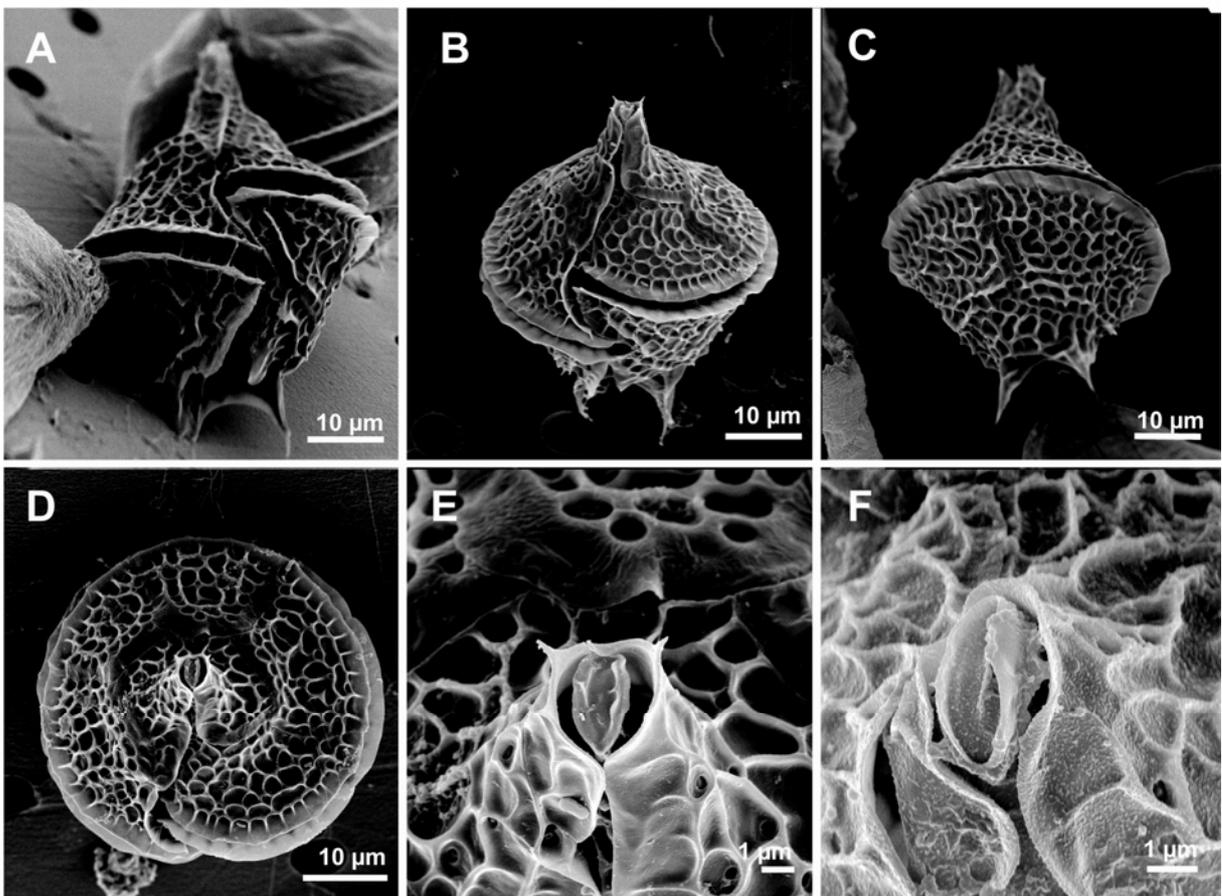


Plate 15. *Gonyaulax* cfr. *digitale*. SEM. A. Ventral view, showing the sulcus, cingulum and the antapical spines. **B.** Ventral view, showing the cingulum lists and the apical horn. **C.** Dorsal view, showing two antapical spines and the dorsal plates of the hypotheca. **D.** Apical view, showing the circular shape of the cingulum and the lists bordering this furrow. **E.** Close-up of the apical horn with the apical pore. **F.** Close-up of the apical pore showing the Po.

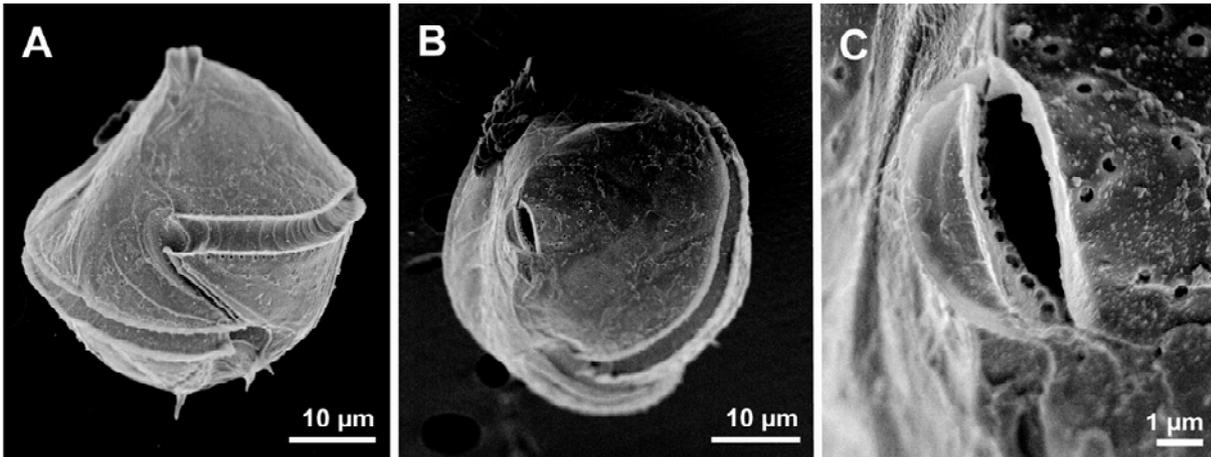


Plate 16. *Gonyaulax* cfr. *spinifera*. A-C: SEM. D-E: LM. **A.** Ventral view, showing the declining sulcus, the cingulum and the cell shape. Two small antapical spines are present. **B.** Apical view of the twisted apex. **C.** The apical pore with the Po.

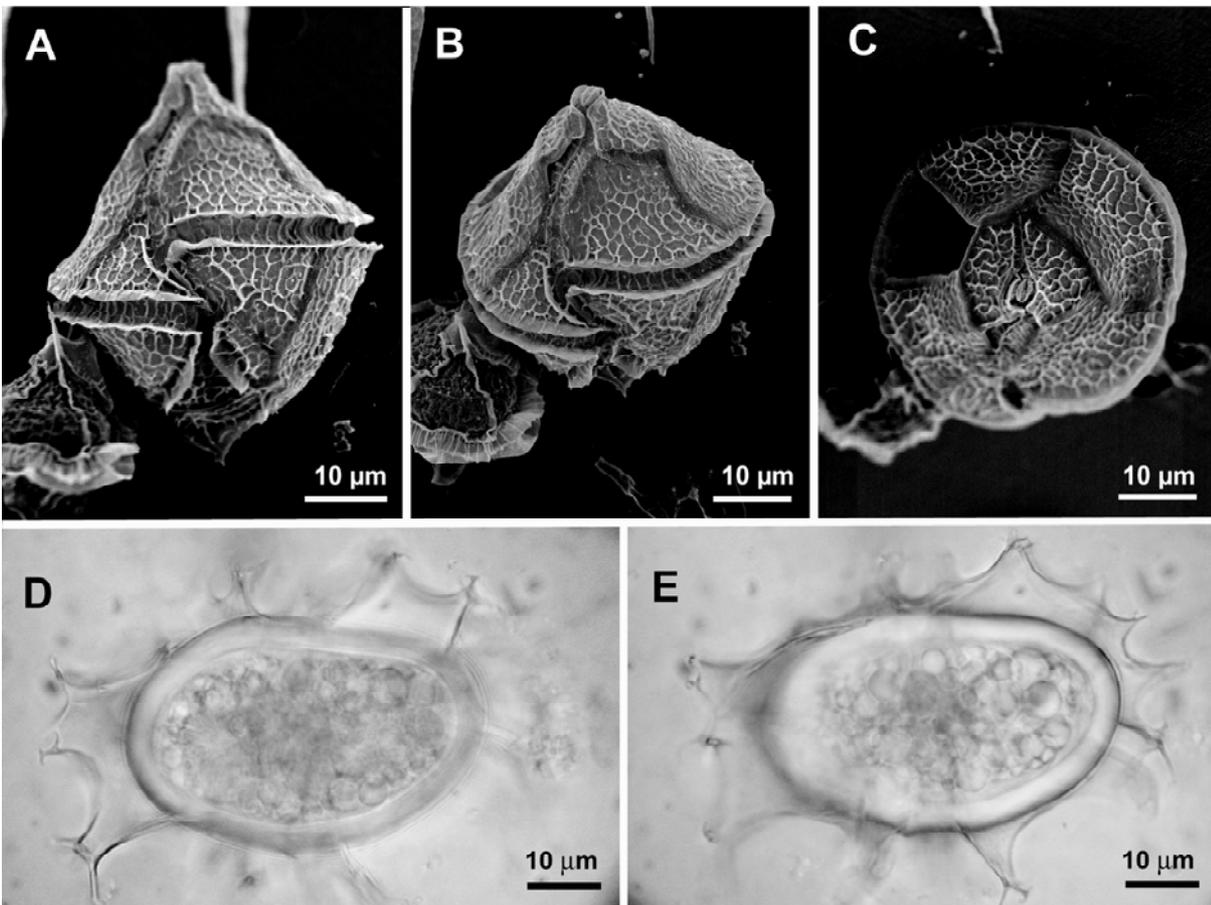


Plate 17. *Gonyaulax elongata*. SEM. **A.** Ventral view, showing the cell shape, the sulcus, the cingulum and the single antapical spine. **B.** Ventral apical view, showing the apical horn and the cingular overhang. **C.** Apical view showing the position of the Po and the plates of the epitheca. "4" is missing, which may be a consequence of the dehydration. **D.** High focus of cysts, showing the processes. **E.** Lower focus of cysts showing shape and processes. Note the thick cyst wall.

Species *Gonyaulax elongata* (Reid) Ellegaard *et al.*, 2003.

Including the apical horn and the antapical spine the cell is *ca.* 30 µm wide and 40 µm long. The cell is elongated with a tapered epitheca. The epitheca is triangular with a moderately broad apical horn (Plate 17 A). Due to the antapical horn and triangular hypotheca the cell appear rhomb shaped in ventral view. In an apical view the epitheca appears circular with the apical horn and the APC located in the centre (Plate 17 B, C). The Po is elliptic and appears in contact with the 1' (Plate 17 B, C). The hypotheca is rounded with a broad dorsally located antapical spine (Plate 17 A). The wide cingulum is displaced *ca.* one cingulum width with a small overhang, is deeply impressed and bordered with narrow lists (Plate 17 A). The sulcus runs into the epitheca and widens towards the antapex. Relatively small lists are formed from the 1''' and the 5''' (Plate 17 A). The cell is heavily reticulated (Plate 17). The cysts appear elongated in a lateral view. The outer layer is thin and slightly coarse while the inner layer is smooth (Plate 17 D, E). The paratabulation is not visible on plate 17, however according to the literature is the formula 4', 6'', 6c, ? s, 1 p, 6''', 1'''' [ELLEGAARD *et al.*, 2003]. The processes in the middle of the cell, at the place of the paracingulum are shorter than the ones located at the ends (Plate 17 D, E)

Genus *Peridiniella* Kofoid & Michener, 1991 *emend.* Balech, 1977

Morphologically, this genus is an intermediate between the orders Gonyaulacales and Peridinales (see below). The hypocone and sulcal area is typically gonyaulacaulid, but the epitheca displays protoperidinian plate tabulation and pattern (see below): Po, X, 4', 3-4a, 7'', 6c, 6-7s, 6''', 2'''' [FENSOME *et al.*, 1993; STEIDINGER & TANGEN, 1997]. The cingulum is descending and displaced about one width, and the thecal plates have reticulate ornamentation. Chloroplasts are present [STEIDINGER & TANGEN, 1997]. Some *Scrippsiella*-species have been found to match the original description of *Peridiniella* [FENSOME *et al.*, 1993].

Species *Peridiniella catenata* (Levander) Balech, 1977

A small species with cells being solitary or catenated. Cellular shape may correspondingly vary from ovoid [THRONDSEN *et al.*, 2003] to subspherical, with small but substantial hyposomal spines (Plate 18). Each cell is *ca.* 25-26 µm wide and 22-23 µm long (Plate 18). The cingulum is wide and displaced about 1.5 cingulum widths (Plate 18 A). On the contrary to other species of this genus, *P. catenata* possesses 4 intercalary plates with the 2nd and 3rd being very small as if a division of a single plate had occurred [STEIDINGER & TANGEN, 1997]. The thecal plates on both epi- and hypotheca are reticulatedly ornamented (Plate 18 B). The nucleus is centrally located (Plate 18 A). The chloroplasts are yellow-brownish [STEIDINGER & TANGEN, 1997]. *P. catenata* is mainly found in cold, brackish waters and can perform blooms [STEIDINGER & TANGEN, 1997; THRONDSEN *et al.*, 2003].

Genus *Protoceratium* Berg, 1881

The cells are small and oval to broadly biconical [STEIDINGER & TANGEN, 1997]. The cingulum is descending less than one width with no overhang, and the sulcus is nearly straight [FENSOME *et al.*, 1993]. The thecal pattern is obscured by the ornamentation, which is usually substantial and heavily reticulated. The APC separates this genus from *Gonyaulax* and *Alexandrium*, with the pore plate being circular and the pore crescent-shaped. The tabulation pattern is: Po, cp, 3', 6'', 6c, 6s, 6''', 2'''''. Chloroplasts are present. [STEIDINGER & TANGEN, 1997].

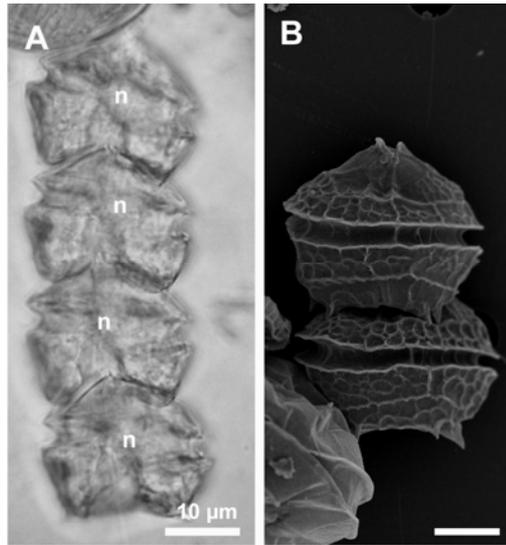


Plate 18. *Peridiniella catenata*. A: LM. B: SEM.
A. Low focus of 4 catenated cells with centrally located nuclei (n), presenting cingulum and sulcus. **B.** Dorsal view of two catenated cells, showing the cingulum lists and the reticulation. Same scale bar for A & B.

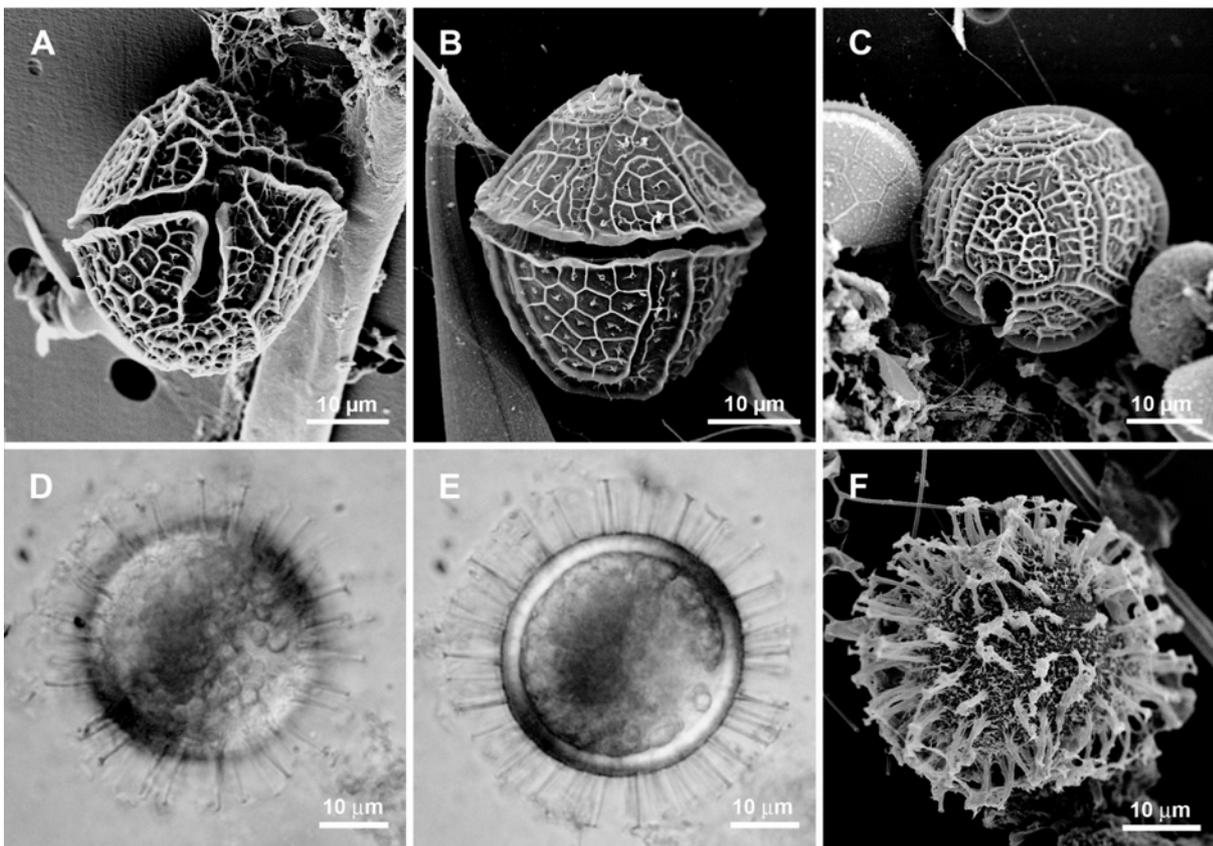


Plate 19. *Protoceratium reticulatum*. A-C, F: SEM. D-E: LM. **A.** Ventral view showing the cell shape, the sulcus and the cingulum. **B.** Dorsal view of the cell, showing the difference in ornamentation density in comparison to A and C. **C.** Antapical view showing the hypothecal plates. **D.** High focus of cyst, showing the processes. **E.** Lower focus of cyst, showing the nagel-like processes and the shape. **F.** Cysts with nagel-like processes.

Species *Protoceratium reticulatum* (Claparède & Lachmann) Bütschli, 1885

The cell is pentagonal with an overall spherical to ovoid appearance (Plate 19). The cell is *ca.* 35 µm long and 35 µm wide. The epitheca is slightly smaller than the hypotheca, and the cell tends to be antapically flattened (Plate 19, A). The ornamentation is characteristic in being highly reticulate in a polygonal pattern but the intensity seem to vary between specimens; most likely the density of reticulation is correlated with cellular age (Plate 19 –Compare A, B, C). The cingulum is displaced up to one width without overhang (Plate 19 A).

The spherical cysts are *ca.* 35 µm in diameter with numerous nail-shaped processes approximately 10 µm in length. The highly vacuolated and light brown protoplasm is surrounded by a thick colourless wall. (Plate 19 D-F).

Since the motile stage of this species was only photodocumented in SEM, information concerning the plastids and the nucleus is difficult to obtain. According to the literature the chloroplasts have a pale brownish colour [THRONDSEN *et al.*, 2003]. *P. reticulatum* produces yessotoxins (YTX). The precise effect of these are uncertain, but accumulations of the toxin has been detected in blue mussels [THRONDSEN *et al.*, 2003].

3.1.4. Order Gymnodiniales Apstein, 1909

Dinoflagellates with numerous amphiesmal vesicles without any cellulose content or with little osmophilic material. Some taxa possess a thin and flexible pellicula (Paragraph 1.2.2 and Figure 1.5), often with a fine surface ornamentation. The members of gymnodiniales are defined on the basis of the arrangement of amphiesmal vesicles, which is non-serial as opposed to Gonyaulacales and Peridinales (Paragraphs 3.1.3. and 3.1.5.). The number of amphiesmal vesicles range in numbers from a few hundreds to thousands (Figure 3.1. E). The cells are motile and both a distinct cingulum and a sulcus are present. The Gymnodiniales comprises species containing ocelli or an internal skeleton, and species either possessing or lacking plastids are found in this order. Cells are usually free-living but some species may possess a peduncle adapted to parasitism [FENSOME *et al.*, 1993; VAN DEN HOEK *et al.*, 1995; STEIDINGER & TANGEN, 1997].

Family Actiniscaceae Kützing, 1844

The cells are unarmoured and gymnodinoid with internal star-shaped siliceous skeletal elements [FENSOME *et al.*, 1993; STEIDINGER & TANGEN, 1997; THRONDSEN *et al.*, 2003]. The internal skeletal elements are composed of two structures, which enclose the perinuclear strengthening membrane and the nucleus. Species of this family are marine planktonic and non-phototrophic [FENSOME *et al.*, 1993].

Genus *Actiniscus* Ehrenberg, 1841

The cingulum is median and descending about one cingulum width. The sulcus slightly indents the epitheca [STEIDINGER & TANGEN, 1997].

Species *Actiniscus pentasterias* Ehrenberg, 1854

Since no micrograph of the ventral side was obtained, information about the cell size is difficult to obtain. However the diameter of the cell is *ca.* 50 µm (Plate 20 A). The cells are unarmoured (Plate 20, A-E), small and spherical but appear antero-posteriorly compressed (Plate 20 A-D). In each cell, a siliceous internal skeleton of two convex, five-armed star-shapes (pentasters) surrounds the nucleus (Plate 20, A, E and Plate 21). Each arm of the pentaster is *ca.* 10 µm long and the diameter is *ca.* 10 µm (Plate 20 E and Plate 21). The sulcus extends from the antapex to the epicone (Plate 20 D). Since the cell was not photographed from the ventral side, no information about cingulum can be obtained. The

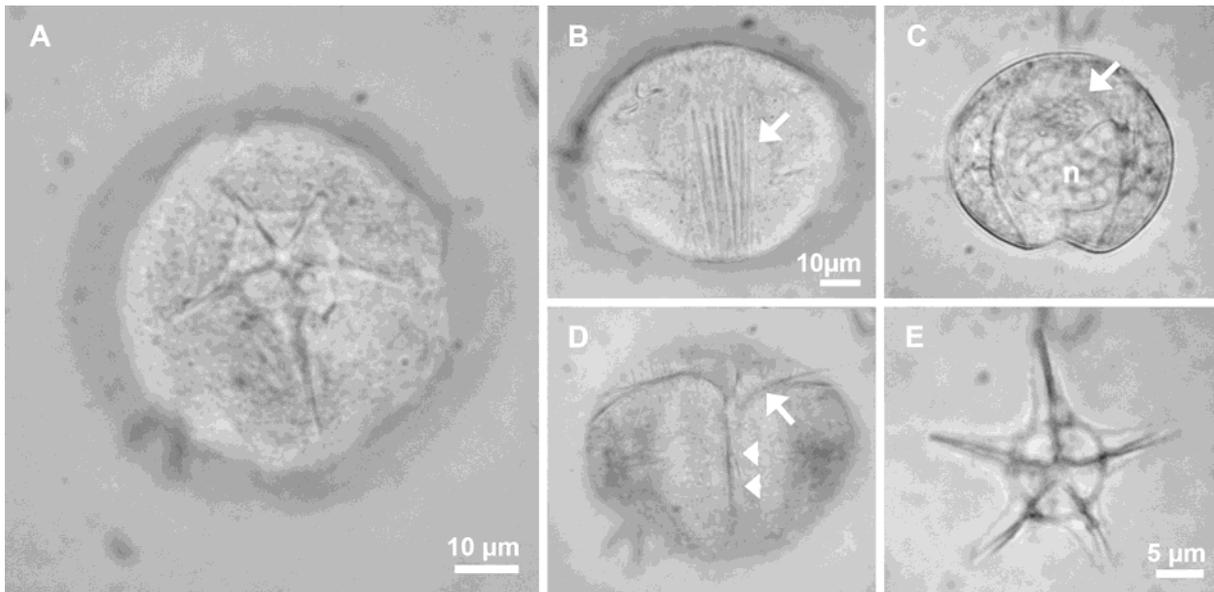


Plate 20. *Actiniscus pentasterias*. LM. **A.** High focus of cell in lateral view showing one pentaster. **B.** High focus of cell in dorsal view, showing the docidosomes (arrow). **C.** Low focus on the antapex of the same cell in dorsal view, showing the docidosomes in cross-section (arrow) and the nucleus (n) with numerous conspicuous chromosomes. **D.** High focus on cell in antapical view, showing the cingulum (arrow) and sulcus (arrowheads). **E.** A single pentaster. Same scale bar for B-D.

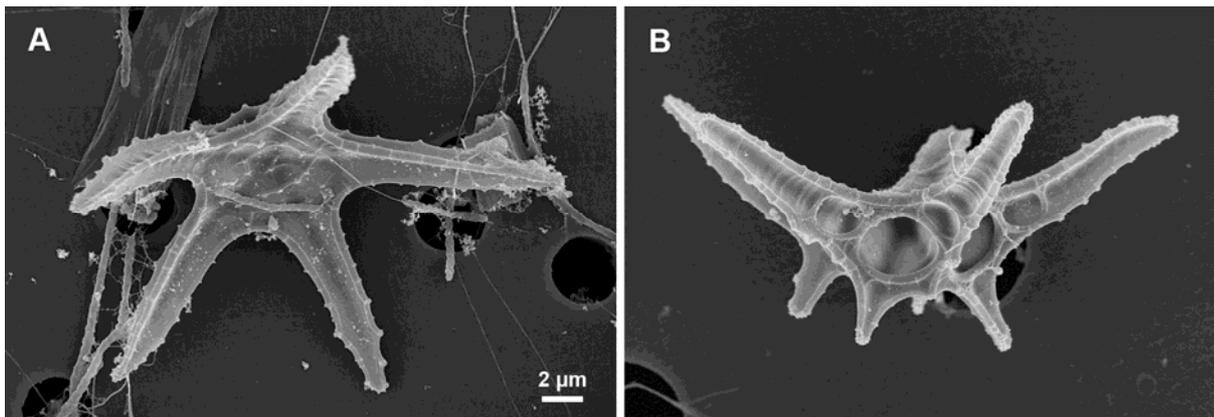


Plate 21. *Actiniscus pentasterias*. SEM. **A.** A single pentaster seen from the inside; the convex shape is probably an artefact caused during SEM preparation. **B.** A single pentaster in lateral view. Same scale bar for A and B.

nucleus is kidney-shaped and surrounded by a perinuclear capsule and contains numerous conspicuous chromosomes (Plate 20 C). A bundle of long, narrow rods are located in proximity of the nucleus, oriented in parallel with the longitudinal axis of the cell (Plate 20 D). These rods comprise the docidosomes, a sort of extrusome [HANSEN, 1993]. Chloroplasts are absent and, hence, the species is heterotrophic (Plate 20).

Family Gymnodiniaceae (Bergh, 1881) Lankester, 1985

Cells without an internal skeleton, ocelli and with a single nucleus. Normally, taxa of this family are considered athecate, but some do possess thin thecal plates. The cingulum may be more or less equatorial or approaching either of the poles. The cingulum can be planar or helical and encircle the cell up to four times. Chloroplasts may be absent or present and a peduncle has been observed in some taxa [FENSOME *et al.*, 1993].

Species *Gymnodiniaceae* sp

This species, containing red droplets, was observed at several stations; we were, however, not able to identify neither the genus nor the species. The cell is unarmoured with an elliptical shape and a rounded apex (Plate 22 A, B). The cell is *ca.* 40 μm in width and 50 μm in length (Plate 22 A). The cingulum appears to be displaced almost half a cell length but only encircles the cell once (Plate 22 A, B). The sulcus broadens towards the antapex (Plate 22 A). The surface is smooth without stria. A feature, which we interpret as an apical groove [DAUGBJERG *et al.*, 2000], is observed at the apex (Plate 22 C). This species is heterotrophic and the protoplasm is filled with several small and bright red droplets (Plate 22). No information about the nucleus was obtained. Single cells of this species were isolated several times (data not shown), making further phylogenetic analysis possible.

Genus *Cochlodinium* Schütt, 1896

This genus comprises athecate and gyrodinoid dinoflagellates having a cingulum that forms a descending left-handed spiral of 1.5 turns or more with a considerable displacement of the two ends. Frequently, the sulcus is contorted halfway or more around the cell. The cell body may appear twisted due to the cingular torsion. The nucleus is centrally or posteriorly located. Neither nematocysts nor ocelli have been observed. Within this genus the protoplasm is colourless or contains pigmentation. Only one *Cochlodinium* species contains plastids. Pusules are observed in association with the flagellar pore. Species within this genus are differentiated on the basis of shape, size, amount of cingular rotation or number of turns, and on the presence of stria or ribs [DODGE, 1982; STEIDINGER & TANGEN, 1997].

Species *Cochlodinium* sp. 1

An athecate cell, which is *ca.* 23 μm wide and 30 μm long. The shape is almost spherical (Plate 23). The cingulum is descending *ca.* 1/3 of the body length and is encircling the cell body *ca.* 1.5 turns (Plate 23 A). From the micrographs it is difficult to determine the extent of sulcal contortion. A tentacle is present (Plate 23 C). The peri-apical region contains surface ornamentation (Plate 23 A). This species is heterotrophic and contains a large pink food item in the otherwise colourless protoplasm (Plate 23). The nucleus is medianly located in the left side of the cell (Plate 23 C).

Species *Cochlodinium* sp. 2

The cell is athecate, but due to lack of scale bars the cell size cannot be determined. The shape is elongated to elliptical. The cingulum seems to encircle the cell 2.5 times, and is descending 3/4 of the body length (Plate 24). The sulcus does not appear twisted (Plate 24 B). A ribbon-shaped ornamentation encircles the upperpart of the epicone (Plate 24 A). The nucleus is located medianly in the cell (Plate 24 C). This species is heterotrophic and no chloroplasts are observed in the colourless protoplasm (Plate 24).

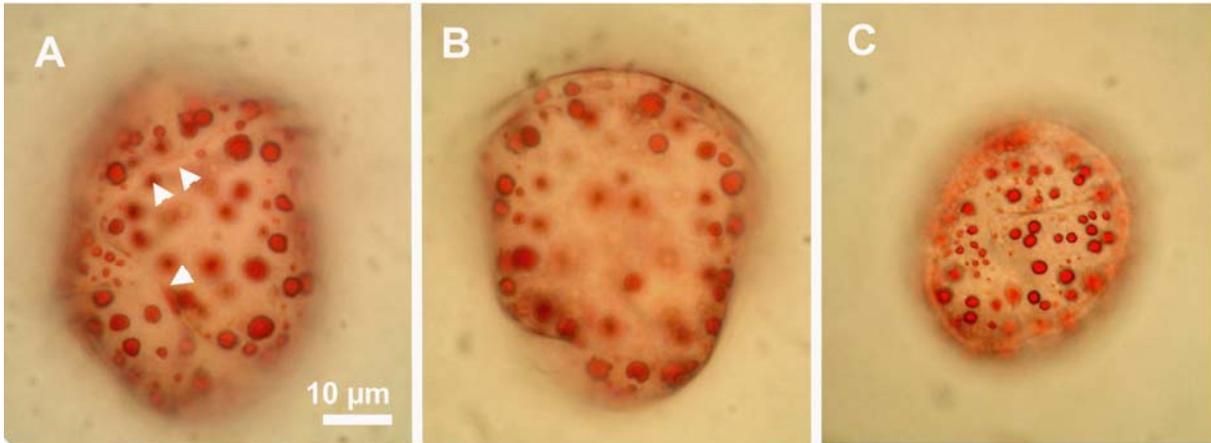


Plate 22. *Gymnodiniacea* sp. LM. **A.** High focus of a cell view from the ventral side of the antapex, showing the sulcus (arrowhead) and the beginning of the cingulum (arrowheads). **B.** High focus of a cell viewed from the dorsal side showing the cingulum. **C.** High focus on the cell from the apex showing presumable apical groove. Same scale bar for A-C.



Plate 23. *Cochlodinium* sp. 1. LM. **A.** High focus of cell in a ventral view showing the surface ornamentation. **B.** Lower focus of a cell from a ventral view, displaying a pink food item (arrowhead). **C.** Low focus of the cell, showing the large nucleus (n), which is located in the left side of the cell and the small tentacle (arrow). Same scale bar for A-C.

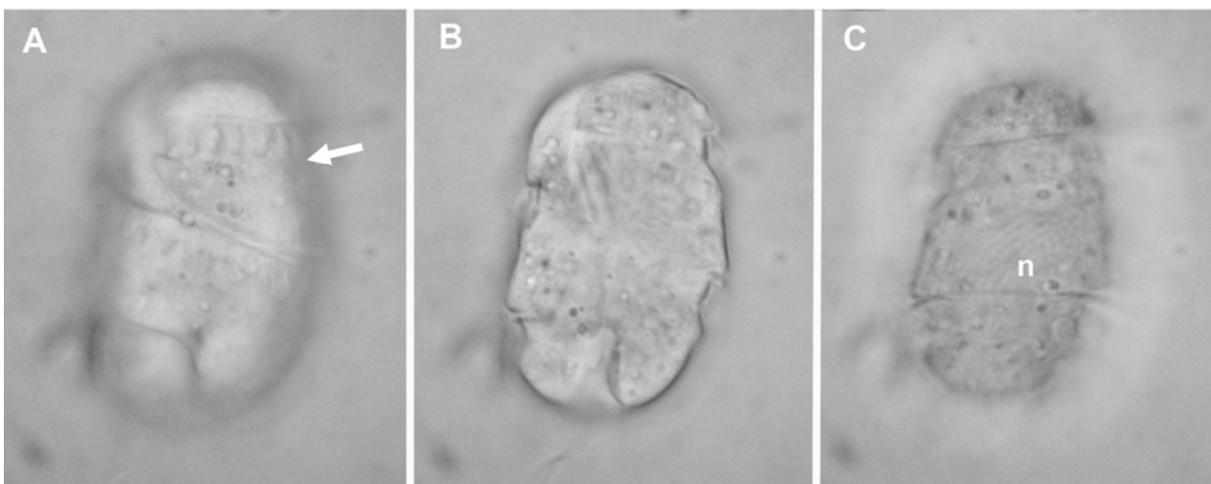


Plate 24. *Cochlodinium* sp. 2. LM. **A.** High focus from the ventral view, showing the sulcus, cingulum and a ribbon shaped ornamentation that encircles the upperpart of the epicone (arrow). **B.** Low focus from a ventral view. **C.** Low focus from the dorsal side, showing the cingulum and the large nucleus (n).

Genus *Gymnodinium* Stein, 1878

Unarmoured cells that can appear in solitary or form colonies. All members of this genus have a horseshoe-shaped apical groove running in an anticlockwise direction. The cingulum is more or less centrally positioned, and displaced one or more cingulum widths. The sulcus may extend from the antapex to the apex or be restricted to the hypocone. Members of this genus may have chloroplasts and the nucleus is centrally located. A nuclear envelope and nuclear or dorsal fibrous connective are present. The cell surface may be striated, smooth or punctated [DODGE, 1982; DAUGBJERG *et al.*, 2000].

Species *Gymnodinium gracile* Bergh, 1881

The cells are *ca.* 120-130 μm long and 50-60 μm wide around the cingulum. The cells are large with a subvoidal to elliptic shape; however the shape can change dramatically as a consequence of food uptake (Plate 25 D-F). Furthermore, the cells are capable of altering their shape through contractions around the cingulum, which is especially apparent when comparing plate 25 A and B; these micrographs were taken with only seconds apart. The narrow cingulum is premedian and displaced *ca.* 2 widths. The sulcus extends almost from the apex to the antapex, where it widens (Plate 25 C). The surface appears to have delicate stria (Plate 25 C). This species is heterotrophic, with a highly vacuolated and colourless protoplasm (Plate 25). According to the literature the protoplasm can be coloured [KOFOID & SWEZY, 1921] and this was also observed in Greenland (data not shown). In the cells on Plate 25 D-F food items can be observed; especially on F, where the food item is enormous and has altered the cell shape dramatically. The nucleus is located in the hypocone, and in the apical end of the cell several vacuoles are observed (Plate 25 A-C).

Species *Gymnodinium* sp. 1.

This species is athecate. The cell is entirely spherical, *ca.* 40 μm long and 40 μm wide. The cingulum is narrow, median located and almost not displaced (Plate 26), but with a slight overhang (Plate 26 A). The sulcus extends to the middle of the epicone (Plate 26 A, B). The surface appears smooth (Plate 26 A). This species is heterotrophic with a colourless protoplasm and many large vacuoles. The nucleus is centrally located (Plate 26 C).

Genus *Gyrodinium* Kofoid & Swezy, 1921

Naked and heterotrophic dinoflagellates. The cingulum is descending and displaced more than 1/5 of the body length forming a characteristically left-handed spiral. The apical groove is elliptical. The cell shape is highly variable but frequently ovoid to fusiform or droplet-shaped. The surface has longitudinal stria [STEIDINGER & TANGEN, 1997; DAUGBJERG *et al.*, 2000].

Genus *Gyrodinium crassum* (POUCHET) KOFOID & SWEZY, 1921

The cell is large, *ca.* 45 μm in width and 90 μm in length. The cell is very elongated and elliptical of shape, with the epicone being more slender than the hypocone (Plate 27 B). Both the apex and the antapex are rounded (Plate 27 B). The cingulum is displaced *ca.* 1/3 of the cell length and only encircles the cell once. The sulcus broadens towards the apex (Plate 27 A) and is displaced more than a half turn. Information concerning the sulcal shape near the antapex cannot be obtained from the micrographs. Narrow stria are present on both epicone and hypocone (Plate 27 A). The nucleus is enclosed by a capsule and located in the hypocone (Plate 27 B). This species is heterotrophic with a colourless protoplasm.

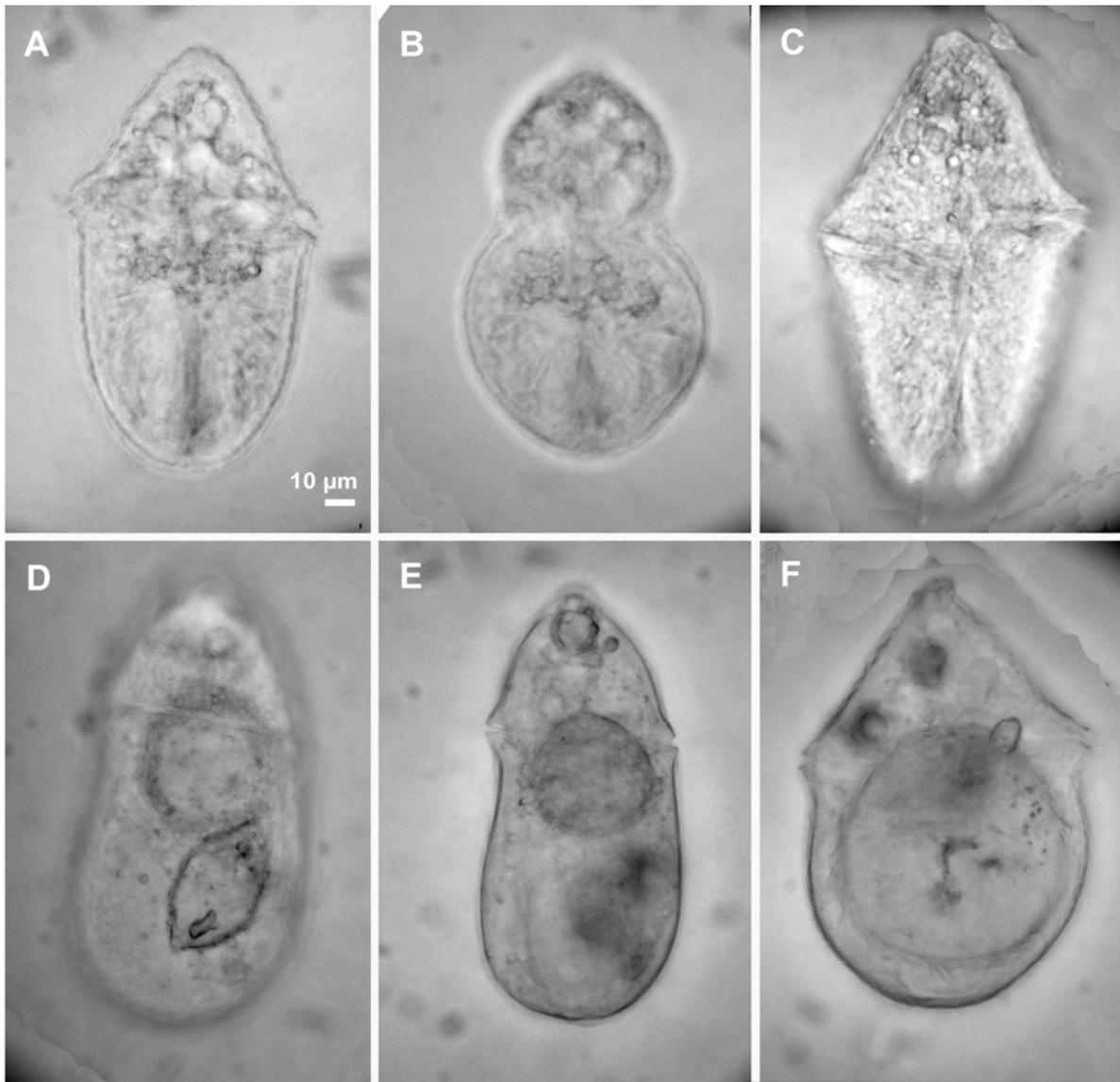


Plate 25. *Gymnodinium gracile*. LM. A. Low focus showing the typical cell shape. B. Low focus of the same cell with the cingulum being contracted. C. High focus in ventral view, showing the surface, the sulcus and cingulum. D. High focus in dorsal view showing the cingulum and a food item. E. Low focus of the same cell as D, showing another food item located in the cingular area. F. Low focus of cell with a large food item. Same scale bar for A-F.

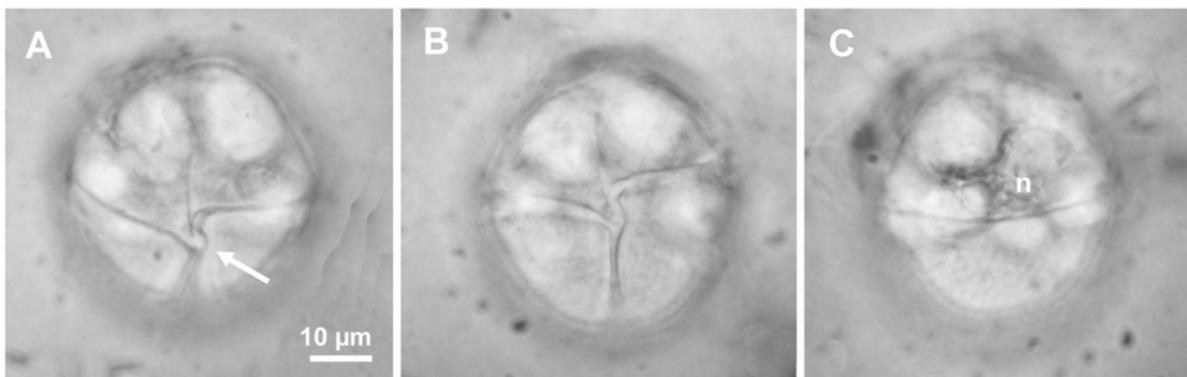


Plate 26. *Gymnodinium* sp 1. A. High focus of a cell viewed from the ventral side, showing the cingulum, which have a slight overhang (arrow) and the sulcus. B. Lower focus from the ventral view. C. High focus from dorsal view showing the cingulum and the centrally located nucleus (n). Same scale bar for A-C.

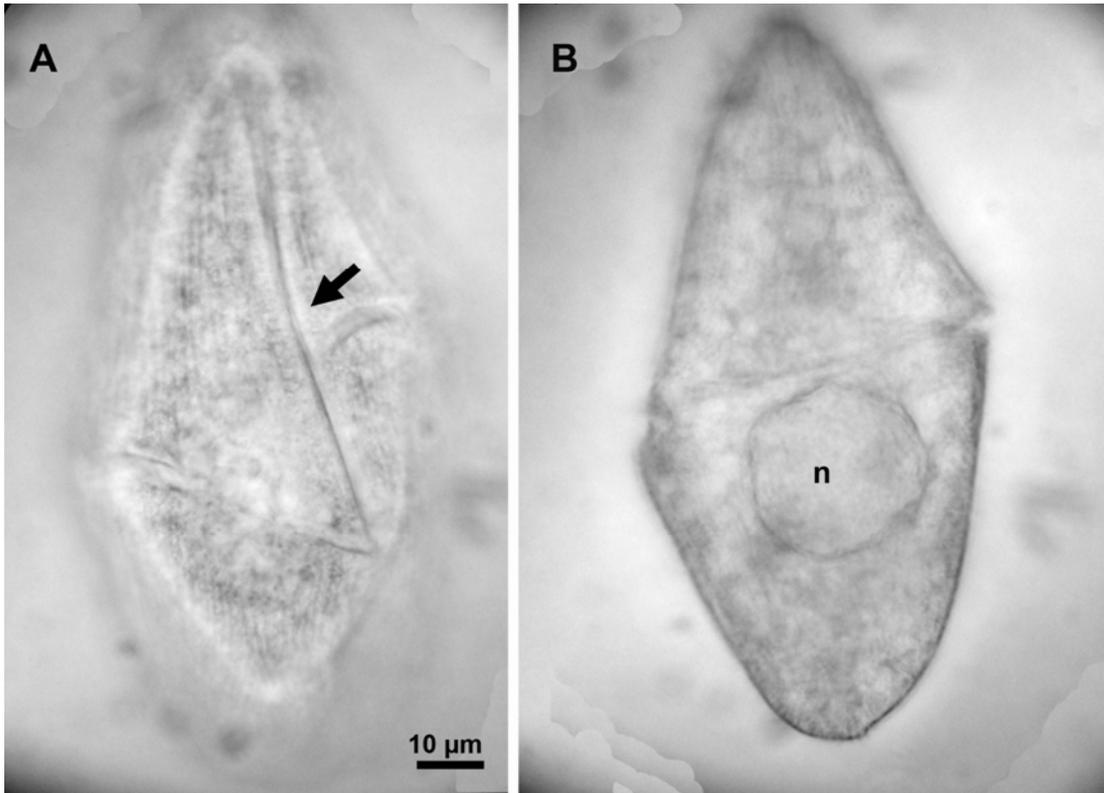


Plate 27. *Gyrodinium crassum*. LM. A. High focus of a cell viewed from the ventral side, showing the sulcus (arrow), the cingulum and the surface stria. B. Low focus showing the capsule enclosed nucleus (n). Same scale bar for A-B.

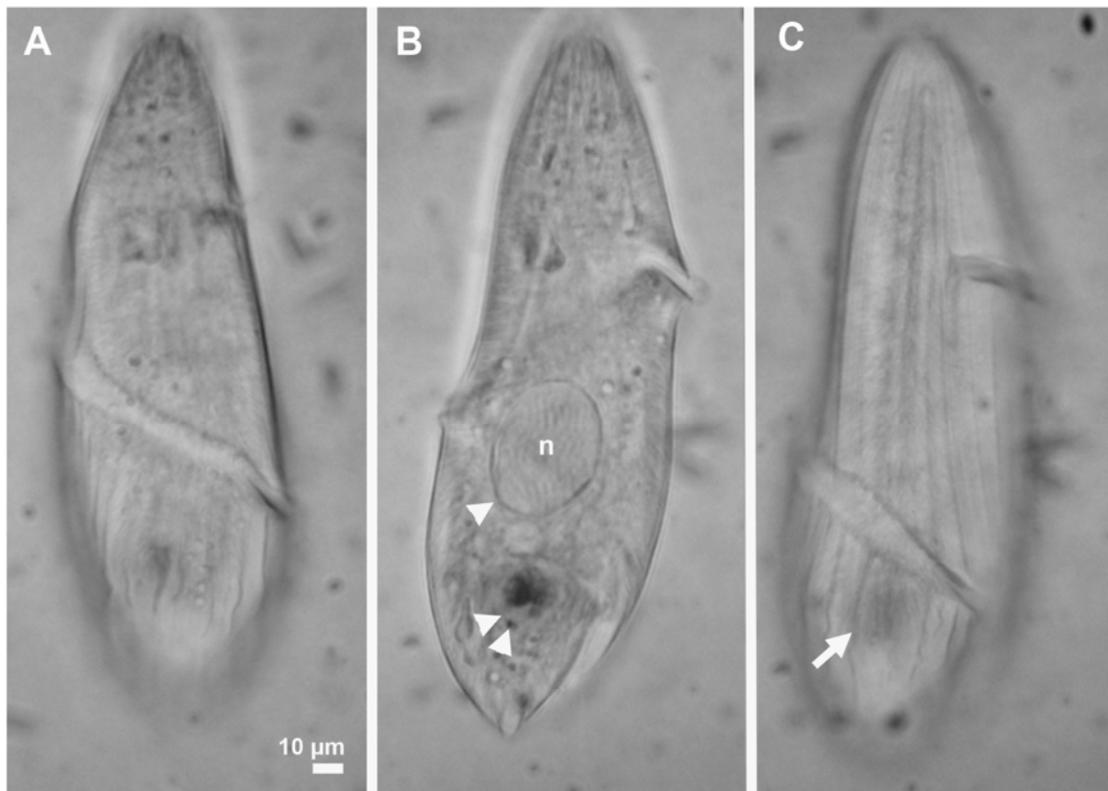


Plate 28. *Gyrodinium spirale*. LM. A. High focus of a cell viewed from the ventral side, showing the cingulum. B. Low focus of the cell, showing the centrally located nucleus (n), which is enclosed by a capsule (arrowhead) and remains of a food vacuole (arrowheads). C. High focus of the cell viewed from the ventral side, showing the broad stria on the cell surface (arrow). The sulcus can be seen on the left side of the cells hypocone on B and C. Same scale bar for A-C.

Species *Gyrodinium spirale* (Bergh) Kofoid & Swezy, 1921

This species is the type of the genus, with medium to large sized cells, *ca.* 60 μm wide and 210 μm long. The cell is slender and asymmetrically spindle-shaped with a slight longitudinal twist (Plate 28). Cingulum is displaced *ca.* 1/3 of the body length (plate 28 A, C). From the micrographs on plate 28 it is difficult to retrieve any information about the sulcus. According to the literature the sulcus extends from the apex to the antapex, is narrow at the antapex but slightly widens towards the anterior end [DODGE, 1982]. The epicone appears straight and has a slightly rounded apex. The hypocone is curved to the right with a pointed antapex (Plate 28). The surface of the cell is ornamented with longitudinal stria situated *ca.* 5 to 10 μm apart. This species has colourless protoplasm and is heterotrophic; a small food vacuole can be observed in the epitheca (Plate 28 B). The nucleus is elongated, surrounded by a capsule and median located (Plate 28 B). This cosmopolitan species is found in polar to subtropical waters [STEIDINGER & TANGEN, 1997].

Species *Gyrodinium* cfr. *undulans* Hulburt, 1957

This species is athecate. The cell is small, *ca.* 25 μm wide and 30 μm long, with an ovoid shape (Plate 29). The cingulum is wide and displaced one width (Plate 29 B). The sulcus extends from the apex to the antapex and has a sigmoidal shape on the epicone (Plate 29 A). The surface of the cell appears smooth (Plate 29 A). This species is heterotrophic with a colourless and highly vacuolated protoplasm (Plate 29 B, C). The nucleus is centrally located (Plate 29 C).

Genus *Gyrodinium* sp. 1

The cell is *ca.* 20 μm wide and 25 μm long. The shape is ovoid to elliptic with a deeply impressed cingulum (Plate 30 B-D). Both the epitheca and hypotheca are slightly conical (Plate 30 C, D). The wide cingulum is median and displaced by *ca.* one width (Plate 30 B). The sulcus appears to run from the apex to the antapex (Plate 30 B). The surface of the cell is ornamented with broad stria, which lie relatively far from each other and do not reach the apex (Plate 30 A, B). The protoplasm is colourless and the nucleus is centrally located, just above the cingulum (Plate 30 D).

Genus *Gyrodinium* sp. 2

The cells are large, straight, elongated and with rounded apex and antapex (Plate 31). The cell is *ca.* 70 μm long and 35 μm wide. The cingulum is displaced about 7 cingulum widths (Plate 31 A), and the sulcus appears to extend from the apex to the antapex where it broadens (Plate 31 A). The surface is ornamented with narrow stria (Plate 31 A). The protoplasm is colourless and highly vacuolated (Plate 31 B). The nucleus is located below the cell median and is surrounded by a capsule (Plate 31 B).

Genus *Katodinium* Fott, 1957

Cells of this genus can be armoured or unarmoured. They are small gymnodinoid cells with a postmedian cingulum. Cells appear pear shaped or inverted top shaped, pendulate, club- or mushroom-shaped. The theca can be with or without ridges or ribs. The length of the epitheca exceeds that of the hypotheca about two times, and is wider than the hypotheca. The outer membrane is covered with characteristic triangular scales with ribs or spines. Chloroplasts may be absent or present [DODGE, 1982; STEIDINGER & TANGEN, 1997].

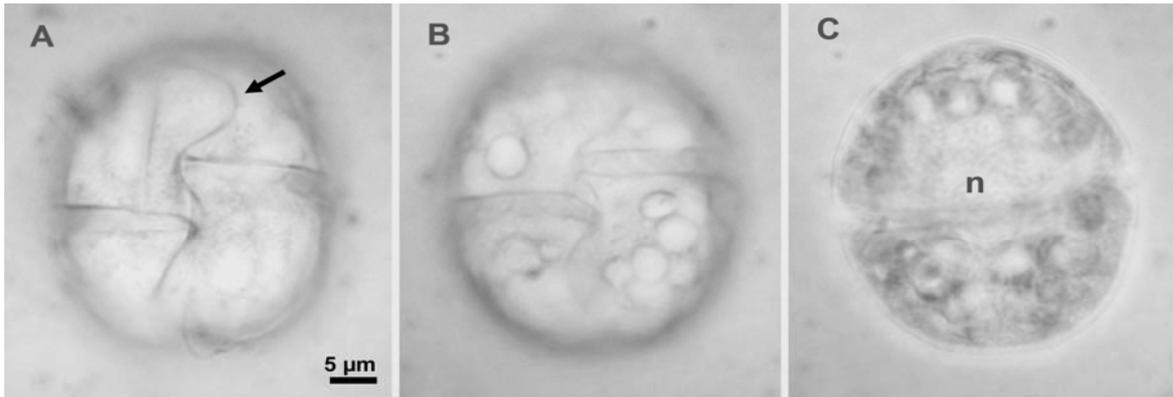


Plate 29. *Gymnodinium* cfr. *undulans*. LM. A. High focus of a cell viewed from the ventral side, showing the sigmoidal sulcus (arrow). B. Lower focus in a ventral view showing the wide cingulum. C. Low focus showing the centrally placed nucleus (n) and the colourless and vacuolated protoplasm. Same scale bar for A-C.

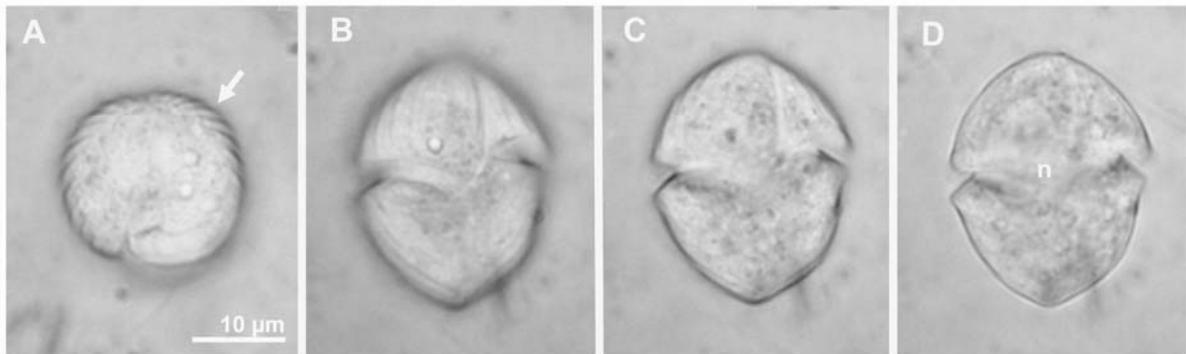


Plate 30. *Gyrodinium* sp. 1. LM. A. High focus in apical view showing the stria (arrow) on the cell surface, that do not reach the apex. B. High focus of a ventral view showing the sulcus which runs from the apex to the antapex, the median cingulum and the stria on the cell body. C. Lower focus, showing the cell shape. D. Low focus showing the centrally located nucleus (n). Same scale bar for A-D.

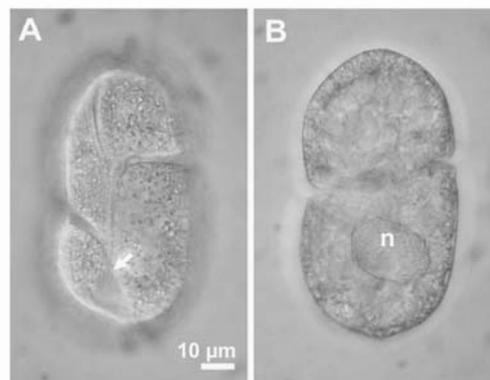


Plate 31. *Gyrodinium* sp. 2. LM. A. High focus of a cell viewed from the ventral side, showing the sulcus (arrow) and cingulum, furthermore the surface stria are seen. B. Low focus of a cell, showing the vacuolated and colourless protoplasm and the nucleus (n). Same scale bar for A and B.

Species *Katodinium glaucum* (Lebour) Loeblich III, 1965

Cells are armoured and spindle-shaped (Plate 32 A-C). The epitheca has several longitudinal ribs while only a few of these are observed on the hypotheca (Plate 32 C). The epitheca is both longer and wider than the hypotheca, and the cell is *ca.* 42 μm in length and 18 μm in width (Plate 32). The cingulum is broad and descending *ca.* three cingulum widths (Plate 32 B). The nucleus is located in the epitheca (Plate 32 A). No chloroplasts are found and the cytoplasm is colourless with a yellow body at the anterior end (Plate 32). This body is suggested to be a product of food uptake [DODGE, 1982; STEIDINGER & TANGEN, 1997].

Family Warnowiaceae Lindemann, 1928

The cells are unarmoured. Cingulum has one or two turns and the cell body may appear twisted. Furthermore, the sulcus may be contorted. The motile cell may possess one or more ocelli with red or black pigment masses, located on the left side of the sulcus. Ocelli are elaborate, light-receiving organelles, and their presence is probably related to a phagotrophic life-style (Paragraph 1.2.3.). Plastids are usually absent, but the protoplasm usually appears coloured. Nematocysts and tentacles are found in some taxa [SCHILLER, 1933; FENSOME *et al.*, 1993]

Genus *Nematodinium* Kofoid & Swezy, 1921

The cells are small to large. The cingulum encircles the cell more than once and is descending more than 0.5 cingulum width. The sulcal torsion creates a posterior loop. Nematocysts are present. The ocellus can be dispersed in small spherical lenses or in one. The pigmentation of the protoplasm is highly variable but no chloroplasts are present [SCHILLER, 1933; STEIDINGER & TANGEN, 1997].

Species *Nematodinium* sp.

The cell is athecate and located inside some sort of mucous layer (Plate 33 C). The cell is *ca.* 40 μm wide and 50 μm long (Plate 33 A-C). From Plate 33 information on the cingulum and sulcus it is difficult to retrieve, but the cingulum appears to be displaced several widths and encircles the cell more than once (Plate 33). The protoplasm is light brown and the nucleus is centrally located (Plate 33 C). Several nematocysts are present in the epicone, orientated perpendicular to the cell membrane (Plate 33 B). In the posterior part of the heterotrophic cell, a large brown body is present, presumably a food vacuole. The cell appears to contain some delicate pigmentation near the cell surface; probably oil droplets (Plate 33 A).

Genus *Proterythropsis* Marshall, 1925

The cell has a spiralling cingulum and a curved sulcus. Species of this genus have an ocellus consisting of a lens with concentric ring and capsules. The presence of nematocysts and a posterior ventral tentacle distinguishes this genus from *Nematodinium* [DODGE, 1982].

Species *Proterythropsis vigilans* Marshall, 1925

The cell is ovoid to elliptical, *ca.* 50 μm in width and 65 μm in length. The cell surface appears delicately ornamented and with pigmented areas (Plate 34 A, C). The cingulum encircles the cell by one and a half turn, continuing into the posterior ventral tentacle (Plate 34 A, C). From the plate it is difficult to retrieve information about the sulcus, but according to the literature the sulcus extents half way around the cell [DODGE, 1982]. An ocellus with red pigment and a globular lens is present in the posterior end of the cell (Plate 34 B). The large

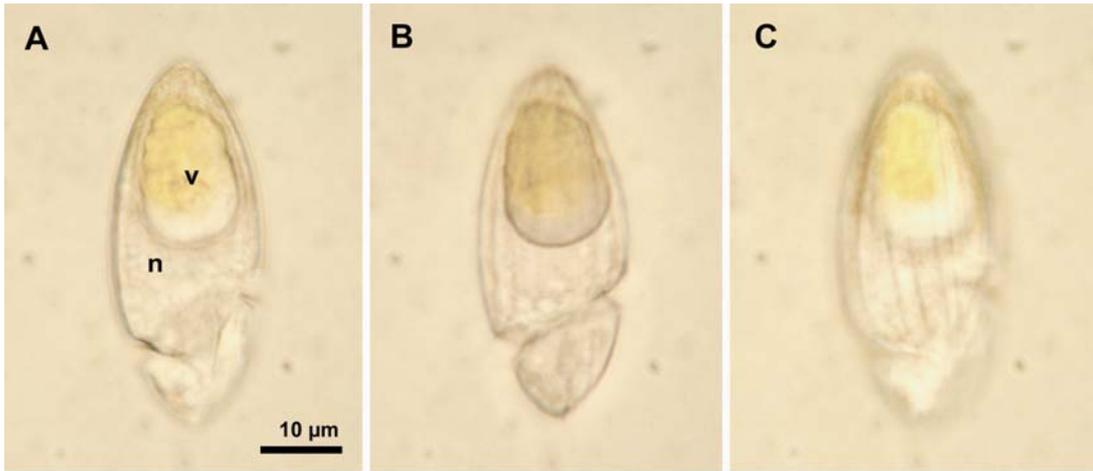


Plate 32. *Katodinium glaucum*. LM. A. Low focus showing the nucleus (n) and a food vacuole (v). B. Through focus from a ventral view, showing the cingulum from the dorsal side. C. High focus from the ventral view, showing the sulcus and the surface ribs. Same scale bar for A-C.

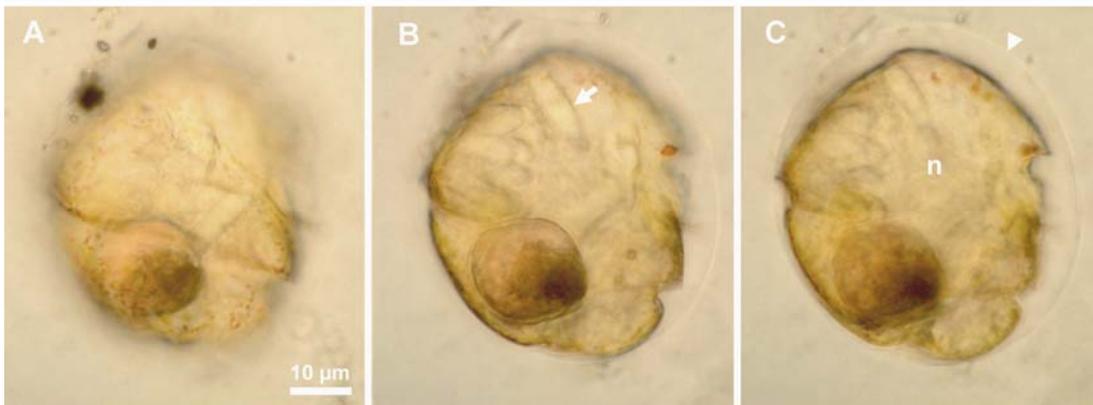


Plate 33. *Nematodinium* sp. LM. A. Through focus on the cell in ventral view, showing the surface, the cingulum. B. Lower focus from a ventral view showing the large nematocysts (arrow). C. Low focus showing the surrounding mucus layer (arrowhead) and the centrally located nucleus (n). Same scale bar for A-C.

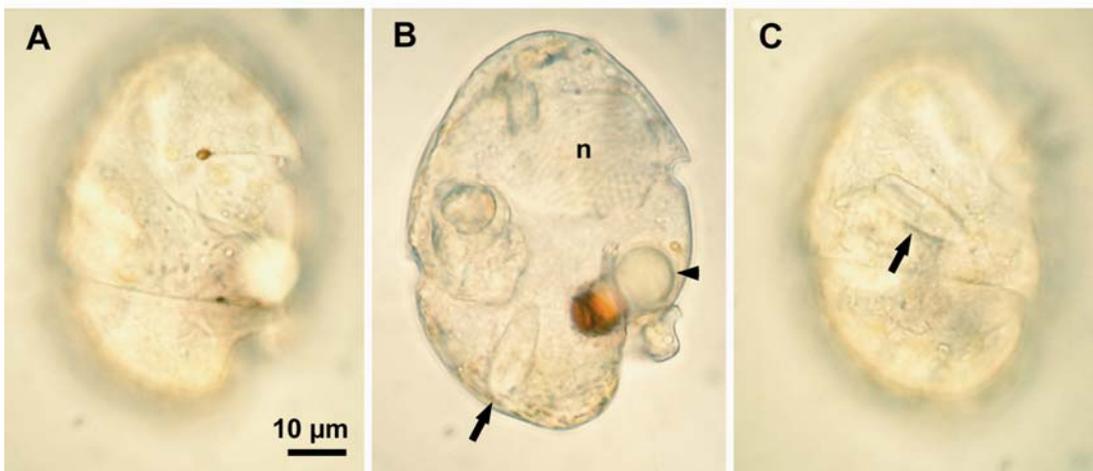


Plate 34. *Proterothropsis vigilans*. LM. A. High focus of a cell viewed from the ventral side, showing the surface and the highly displaced cingulum. B. Low focus, showing the large nematocysts (arrow), the large anteriorly located nucleus (n) and the large ocell (arrowhead) with the red pigment cup. C. High focus of a cell viewed from the dorsal side, showing cingulum and nematocysts (arrow). Same scale bar for A-C.

nucleus is anteriorly located (Plate 34 B). Nematocysts are visible on plate 34 B and C. No chloroplasts are present but a vacuole, perhaps a food vacuole, seems to be present in the cytoplasm (Plate 34 B).

Genus *Warnovia* Lindemann, 1928

Small to large cells with the cingulum encircling the cell one or two times, which may cause the cell body to appear twisted. In some species the sulcal torsion creates a posterior loop. An apical groove is present. Species of the *Warnovia* possess an ocellus with a large hyaline lens and red or black pigment masses, the location being median to posterior. Nematocysts and chloroplasts are absent [DODGE, 1982; STEIDINGER & TANGEN, 1997].

Species *Warnovia* sp.

An ovoid to elliptical cell being *ca.* 35 μm wide and 45 μm long. The cell is surrounded by a mucous layer (Plate 35 C). The cingulum is descending *ca.* 1/3 of the cell body length (Plate 35 A). An ocellus is observed in the posterior end of the cell, on the left side of the sulcus (Plate 35 A-C). The ocellus consists of a large hyaline lens but no proximal pigment mass can be observed on the micrograph (Plate 35 B). The large nucleus is located in the epicone just above the cingulum (Plate 35 C). The protoplasm is yellowish, with some darker pigmentation observed on the cell surface. Black pigment grains are located in the sulcal region at the posterior end of the cell (Plate 35 A, C), possibly originating from ocellar pigment mass.

3.1.5. Order Peridiniales Haeckel, 1894

Comprises cells with amphiesmal vesicles which contain polygonal cellulose plates, usually with a bilateral-symmetrical plate tabulation. The shape is spherical, ovoid or pyramidal and the cells are usually dorsoventrally compressed (Figure 3.1 D). Both a distinct cingulum and sulcus are present. Plate tabulation is used for diagnostics and is based on the first apical plate, which is diamond to rhomb-shaped (Appendix 1, Figure 3), in combination with the two antapical plates which are placed more or less symmetrically around the midventral/middorsal plane. A small plate, the canal plate, is commonly found on the ventral side of the APC, and may elongate ventrally towards the first apical plate. The following plate formula is characteristic: 3-6', typically only 3 or 4', 1-3a, 6 or 7". 4-6c, 4-6 s, 5''' and 1 or 2''', but typically 2''' (Appendix 1, Figure 2). Commonly, a transitional cingular-sulcal plate is found at the proximal end of the cingulum, to the left of the anterior sulcal plate. Both photosynthetic and non-photosynthetic members are found within this order [FENSOME *et al.*, 1993; vVAN DEN HOEK *et al.*, 1995; STEIDINGER & TANGEN, 1997].

Subfamily Calciodinelloideae (Taylor) Fensome *et al.*, 1993

Peridinioid cells with bipesoid tabulation on the epicone; the second anterior intercalary plate is hexa, *i.e.* six-sided (Appendix 1, Figure 4). Extant genera have a conical episome and a subsphaeroidal hyposome. The subfamily is characterised by the formation of calcerous cysts [FENSOME *et al.*, 1993].

Genus *Pentaphasodinium* Indelicato & Loeblich III, 1986

Small, thecate cells with following plate formular: Po, X, 4', 3a (2a), 7", 5c (4+t), 4s, 5''' and 2'''. The sp plate does not contact the cingulum. The cells are egg-shaped [STEIDINGER & TANGEN, 1997; THRONDSSEN *et al.*, 2003] and can easily be confused with *Scrippsiella*. The two genera can be separated on behalf of the number of cingular plates [STEIDINGER & TANGEN, 1997; THRONDSSEN *et al.*, 2003].

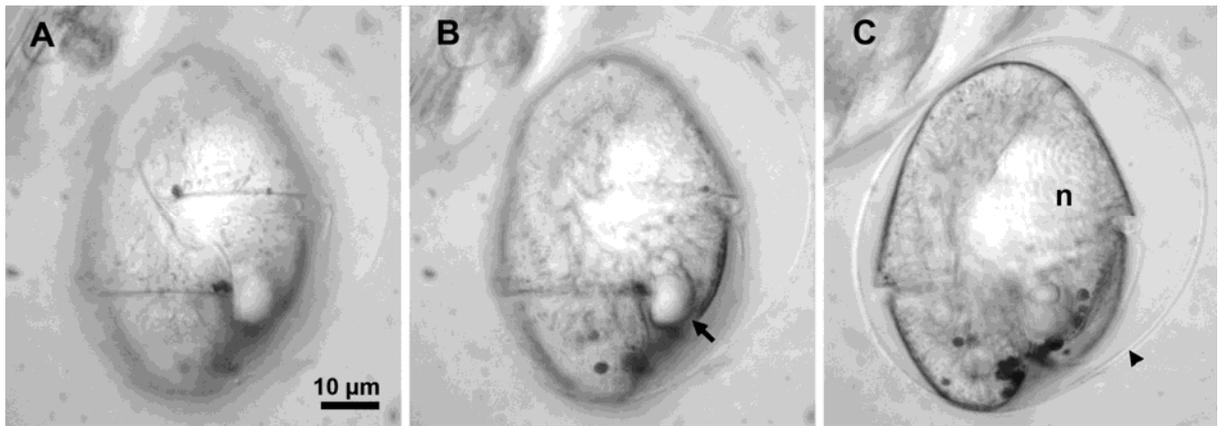


Plate 35. *Warnovia* sp. LM. A. High focus of a specimen in ventral view showing the surface and the cingulum. B. Lower focus showing the ocellus (arrow). C. Low focus showing the large and almost centrally located nucleus (n) and the mucous layer (arrowhead) that surrounds the cell. Same scale bar for A-C.

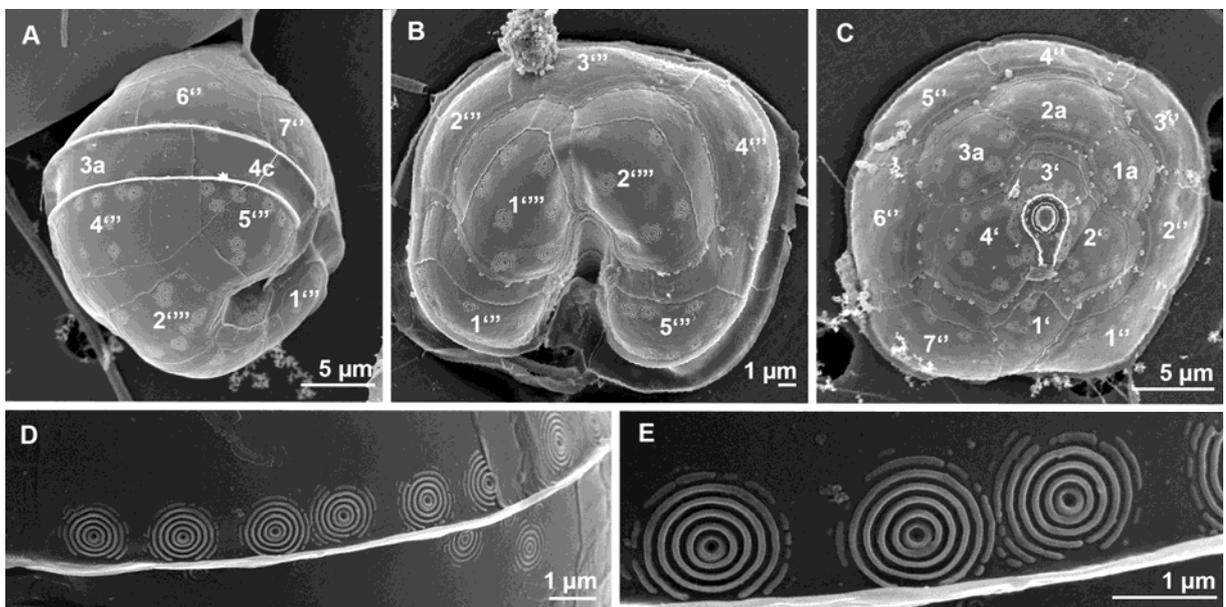


Plate 36. *Pentapphasodinium dalei*. SEM. A. Right side of the cell showing the cell shape and the wide cingulum. B. Antapical view showing the hypothecal plates and the deeply impressed sulcus. C. Cell viewed from the apex showing the epithecal plates and the APC. D and E. Close-up of cingular area showing the concentric ring surrounding each thecal pore. See Appendix 1 for the plate tabulations.

Species *Pentapphasodinium dalei* Indelicato & Loeblich III, 1986

Thecate pyriform cells in the size of *ca.* 20 µm in length and width (Plate 36 A-C). The cingulum is wide and median and bordered with narrow lists (Plate 36 A, B). The sulcus does not indent the antapex (Plate 36 A, B). The theca is smooth with distinctive thecal pores surrounded by concentric rings, *ca.* 1 µm in diameter (Plate 36 D-E). The narrow first apical is ortho and rhomb shaped (Appendix 1, Figure 3). The three other apical plates are five-sided and surround the apical pore complex. The three anterior intercalary plates form a half circle and are all five-sided. The seven precingulars have four or five sides (Plate 36 C). The hypotheca has seven large plates. The first antapical is slightly smaller than the second antapical, and both plates are five-sided (Plate 36 B). This species is difficult to recognise in the light microscope and therefore no information about the plastids and nucleus was

obtained. According to the literature the nucleus is large, median and spherical to ovoid, and the chloroplasts brown to yellowish-green [DOGDE, 1982].

Genus *Scrippsiella* Balech ex Loeblich III, 1965

The cells are thecate and small (< 50 µm). Species of this genus are photosynthetic with some species being mixotrophic and possessing a peduncle. Thecal ornamentation can be reticulate, striated or with papillae, and pores may occur. Can be distinguished on behalf of the APC and the plate tabulation: Po, X, 4', 3a, 7'', 6c, 4-5s, 5''', 2'''''. Species within *Scrippsiella* are differentiated on the basis of cell size and shape, the shape of 1' and 2a, the number of precingulars, surface ornamentation, the presence of stigma and the habitat [STEIDINGER & TANGEN, 1997].

Species *Scrippsiella* sp. 1.

The cells are ca. 15-20 µm wide and 20-25 µm long. Both in ventral and lateral view, the cell appears droplet-shaped (Plate 37 A-C, E). The shape of the epitheca is conical with an apical process (Plate 37 D). The 1' is ortho, very narrow and contacts the apical process. The hypotheca is rounded (Plate 37 A-C, E, F). The cingulum is median, deeply impressed and displaced ca. one width (Plate 37). The sulcus does not reach the antapex and is deeply impressed (Plate 37 F). Both furrows are wide and without lists (Plate 37). The thecal plates are smooth with some delicate pearl-like ornamentation along the borders of the thecal plates (Plate 37). This species contains light yellow-brown chloroplasts and have a large centrally located nucleus (Plate 37 A, B).

Family Heterocapsaceae Fensome *et al.*, 1993

Peridinioid cells with five apical plates. The cells are subovoidal with a round episome and rounded to pointed hyposome. The hyposomal tabulation is the same as described for Peridinales, the episome usually having seven precingulars. The first apical plate is asymmetrically positioned at the ventral midline while the third and second are dorsally located [FENSOME *et al.*, 1993].

Genus *Heterocapsa* Stein, 1883

The cells are small (< 20 µm) and thecate but can appear unarmored under the light microscope. The epitheca is rounded to conical and the hypotheca is rounded to attenuated. The cingulum is median and slightly descending. The thecal plates are thin and display some variation with the typical tabulation pattern: Po, cp, X, 6', 3a, 7'', 6c, 5s, 5''', 0-1p, 2'''''. Species of this genus are photosynthetic with numerous brown chloroplasts and a large pyrenoid [DOGDE, 1982; STEIDINGER & TANGEN, 1997].

Species *Heterocapsa triquetra* (Ehrenberg) Stein, 1883

This species is the type species of the genus [FENSOME *et al.*, 1993]. The plate number may vary [FENSOME *et al.*, 1993]. Both epitheca and hypotheca are conically shaped with the epitheca being apically rounded (Plate 38 A). The surface is smooth without ornamentation, resulting in visible thecal plates (Plate 38). The hypotheca tapers into a horn-like structure (Plate 38 B, C) and a posterior intercalary plate is present (Plate 38 B, C). The cingulum is displaced about one width without any overhang, and the cingulum lists are distinct; especially the lower one (Plate 38 B, C). Since this species was only photodocumented in SEM it was not possible to obtain informations concerning the chloroplasts and the nucleus.

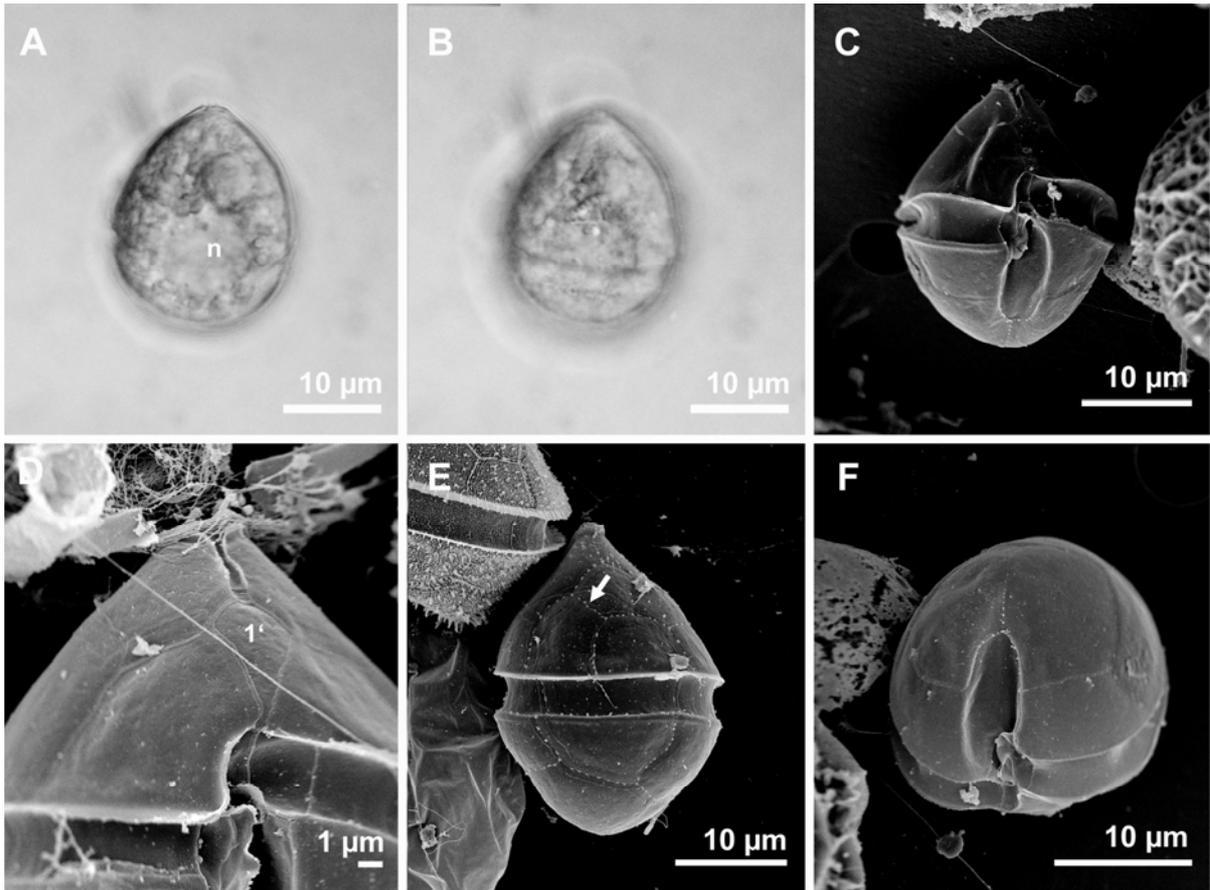


Plate 37. *Scrippsiella* sp. A-B LM, C-F SEM. A. Low focus, showing cell shape, colour and the large centrally located nucleus (n). B. High focus from the dorsal side, showing the cingulum. C. Ventral view showing the cingulum and sulcus. D. Close-up of the epitheca in a ventral view, showing the narrow 1' and the apical process. E. Lateral view of cell showing the pearl-like ornamentation between the thecal plates (arrow). F. Antapical view, showing the plates of the antapex and the sulcus.

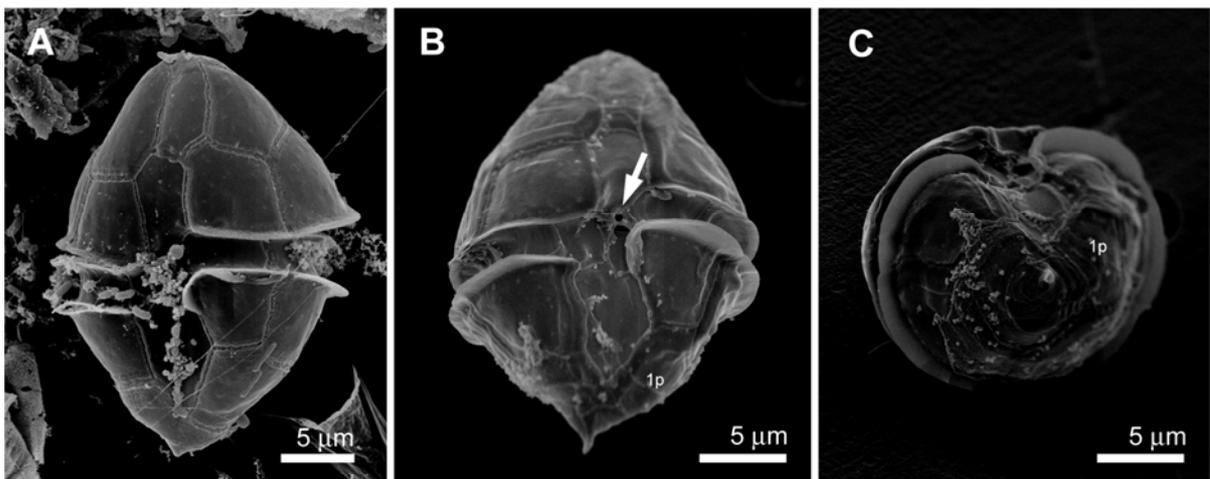


Plate 38. *Heterocapsa triquetra*. SEM. A. Cell viewed from the ventral side, showing thecal plates, sulcus and cingulum. B. Cell viewed from the ventral side, showing the flagellar pores (arrow) and the posterior intercalary plate 1p. C. Cell viewed from the antapex showing the posterior spine and the posterior intercalary plate 1p.

According to the literature the chloroplasts are brown and a pyrenoid is present [TRONDSSEN *et al.*, 2003]. The occurrence of *H. triquetra* is worldwide in neritic, estuarine, brackish and low salinity as well as marine waters [STEIDINGER & TANGEN, 1997].

Family Congruentidiaceae Schiller, 1935

Peridinales with a distinct cingulum containing three cingular plates; no intracingular margins are found on the dorsal surface. Extant species are peridinoid (Figure 3.1 D) in shape with one apical horn and two antapical spines. The general plate tabulation is peridinian, except for the subfamily Diplopsalioideae, which has a reduced number of plates. There may be one or three Kofoidian anterior intercalary plates (Appendix 1, Figure 2) in symmetric or asymmetric arrangement around the dorsal midline. Extant genera are nonphotosynthetic and marine [FENSOME *et al.*, 1993].

Genus *Preperidinium* Mangin 1913

The cells are small to medium sized and antero-posteriorly compressed resulting in a sublenticular to subglobular shape. The left sulcal lift is substantial. The cingulum is median and non-descending, with distinct, rib-supported lists. Pores are scattered over the surface. Chloroplasts are absent. The plate formula is: Po, X, 4', 1a, 7'', 4c, 5s, 5''', 1''''', with an alternative formula being 3' and 2a. The 1' is ortho (Appendix 1, Figure 3) [STEIDINGER & TANGEN, 1997].

Species *Preperidinium meunieri* (Pavillard) Elbrächter, 1993

This is the type species of the genus. The cell is small to medium sized and the shape is lenticular. In apical view the cell appears almost circular (Plate 39 A), and the epitheca has a small apical projection. The first apical plate is ortho and narrow. The third apical plate is very large and appear almost half circular. The 2' and 4' are almost similar in size and shape (Plate 39 A). Very little information concerning the precingular plates can be derived from the micrograph, but according to the literature, seven precingular plates are present [DOGDE, 1982; STEIDINGER & TANGEN, 1997]. Both the sulcus and cingulum are wide, the cingulum being median and without displacement while the sulcus is straight and running towards the antapex (Plate 39) [TRONDSSEN *et al.*, 2003]. The cingulum lists are prominent with radial ribs (Plate 39). A large wing-like sulcal list is present from the cingulum to the antapex (Plate 39 B). The antapex has only one very large and broad antapical plate, which covers almost the entire antapex (Plate 39 B). Five large postcingulars are present (Plate 39 B). Since this species was only identified in SEM no information about the nucleus could be obtained, but according to the literature *P. meunieri* is heterotrophic and the protoplasm typically red [TRONDSSEN *et al.*, 2003].

Genus *Protoperidinium* Bergh, 1881

Small to large heterotrophic cells with varying cell shape. Several species have apical and antapical spines. The cingulum is typically equatorial with or without displacement, descending or ascending, and sometimes with a cingular overhang. Surface markings vary from poroids to areolae or spines. The typical plate formula is: Po, 4', 2 or 3a, 7'', (3+t)c, 6s, 5''' and 2'''. Typically an APC present and can be used for diagnostic as can the shape of 1' (Appendix 1, Figure 3) and 2a (Appendix 1, Figure 4). Further characteristic features are cell size and shape, body contour, cingulum displacement, the presence of horns and spines, APC type and surface markings [DODGE, 1982; STEIDINGER & TANGEN, 1997].

Species *Protoperidinium bipes* (Paulsen) Balech, 1974

The cell is very small, 25 μm wide and 30 μm long, and triangular to inverted heart-shaped (Plate 40). The epitheca tapers into an apical horn, and the sides of the epicone are slightly convex both in ventral and dorsal view (Plate 40 A, B). The hypocone is shorter and biloped, each lobe having a long antapical spine (Plate 40). The cingulum is broad, non-displaced and bordered by lists (Plate 40); the extreme dorsal widening on Plate 40 C is an artifact caused by the SEM preparation, and the cell is collapsed. The sulcus extends to the antapex and broadens posteriorly [DODGE, 1982]. The thecal plates are very delicate and smooth without ornamentation (Plate 40). The first apical plate is of the meta type whilst the second intercalary plate is penta [DODGE, 1982]. Due to its size, this species is easily overlooked in the microscope. The cytoplasm is colourless with a centrally placed nucleus and without chloroplasts (Plate 40 A, B). The distribution is neretic, possibly cosmopolitan [DODGE, 1982; THRONSDEN *et al.*, 2003].

Species *Protoperidinium cerasus* (Paulsen) Balech, 1973

The cell is pear-shaped and *ca.* 52 μm in length and width, excluding the spines. The epitheca tapers into a short, apical horn (Plate 41). The cingulum is very shallow, displaced about one time its width (Plate 41 A) and bordered by substantial, rib-enforced lists (Plate 41). The deeper sulcus extends slightly onto the epitheca and is broader towards the antapex (Plate 41 A); a LSL is present as are two antapical spines of equal length (Plate 41) and with small wings (Plate 41 B). The thecal plates are delicately reticulated and the 1' is meta (Plate 41). The micrographs reveal no information about the 2a, but according to the literature it is quadra [DODGE 1982]. Since the species was not detected in LM, no information about the protoplasm could be obtained; however, this should be colourless without chloroplasts [THRONSDEN *et al.*, 2003]. The species is cosmopolitan [DODGE, 1982; THRONSDEN *et al.*, 2003].

Species *Protoperidinium brevipes* (Paulsen) Balech, 1974

The cells are small, *ca.* 22-33 μm wide and 21-31 μm long (Plate 42 A-C, E-J). The cell shape is pentagonal in overview (Plate 42 A-C, E-I) but rounded triangular to heartshaped in apical or antapical view (Plate 42 D, J, K). The epitheca is triangular with a short process (Plate 42 B, C, F, G, I) formed by the 2' and 4' apical plates and surrounding the apical pore (Plate 41 J). The hypotheca is indented by the sulcus and appears biloped (Plate 42 A, B, F-H, K) with two short antapical spines, one on each side of the sulcus (Plate 42 A-C, F, H, I). The broad cingulum is deep and displaced up to 0.5 widths (Plate 42 A-C, E, F, I), while the sulcus is very deep and extending to the antapex and widening posteriorly (Plate 42 F, H, K). Surface ornamentation is punctate with short spines or papillae, which can be fine to rather substantial; compare F, G and H. The first apical plate is meta (Plate 42 C, F, H, J), whilst the 2a is quadra (Plate 42 D, I). This species is heterotrophic with a colourless protoplasm and a large, centrally positioned nucleus (Plate 42 B). *P. brevipes* is a coastal cold water species [STEIDINGER & TANGEN, 1997; THRONSDEN *et al.*, 2003], and was detected in large numbers at all stations (data not shown).

Species *Protoperidinium conicum* (Gran) Balech, 1974

The cell is *ca.* 65 μm wide and 55 μm long (Plate 43). The cell is rhomb-shaped with straight (Plate 43 A, B) to concave sides (Plate 43 C); however, on C this is exaggerated due to thecal collapse during SEM preparation. The antapex is divided into two cone-shaped horns (Plate 43). The cingulum is excavated and only slightly displaced (Plate 43 A, C). The sulcus is very deep and distally broadened (Plate 43 B, C). The thecal plates are reticulately ornamented

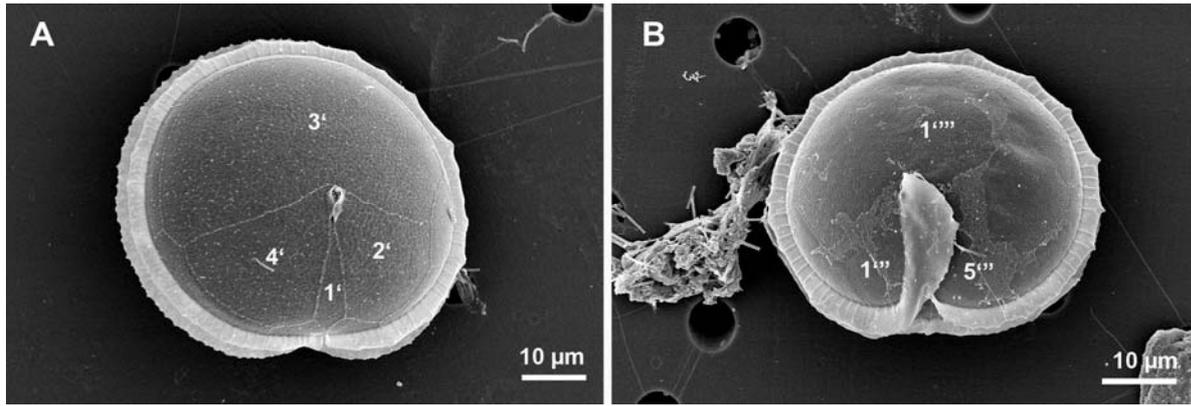


Plate 39. *Preperidinium meunieri*. SEM. **A.** Apical view showing the narrow 1', the large 3' and the wide cingular lists. **B.** Antapical view showing the large 1'''' and the large sulcal lists. For plate tabulation, see Appendix 1.

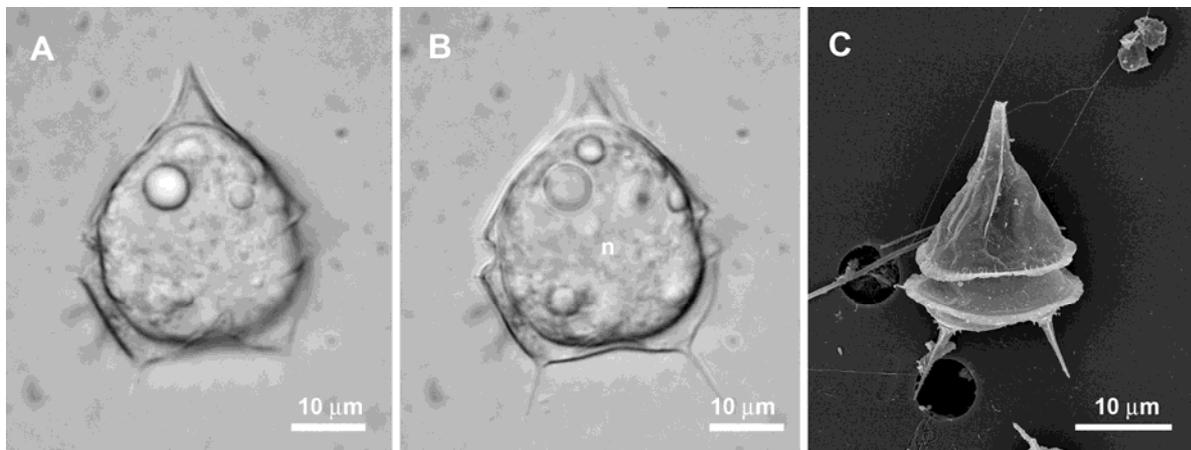


Plate 40. *Protoperidinium bipes*. A-B: LM. C: SEM. **A.** High focus on cell in ventral view showing the apical horn. **B.** Lower focus showing the cell shape, the two antapical spines and the nucleus (n). **C.** Dorsal view of a specimen showing the cingular lists and the smooth thecal plates. The cell has collapsed somewhat during the SEM preparation procedure.

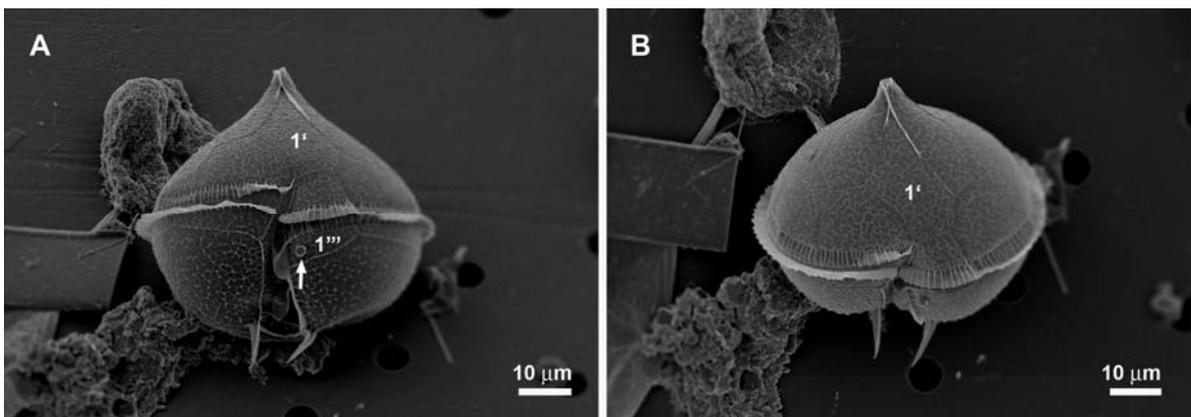


Plate 41. *Protoperidinium cerasus*. SEM. **A.** Ventral view showing the cell shape, the cingulum, sulcus and the curved antapical thorns. The 1''' possesses a distinct pore structure (arrow). **B.** Apical view showing the meta 1' and the apical horn.

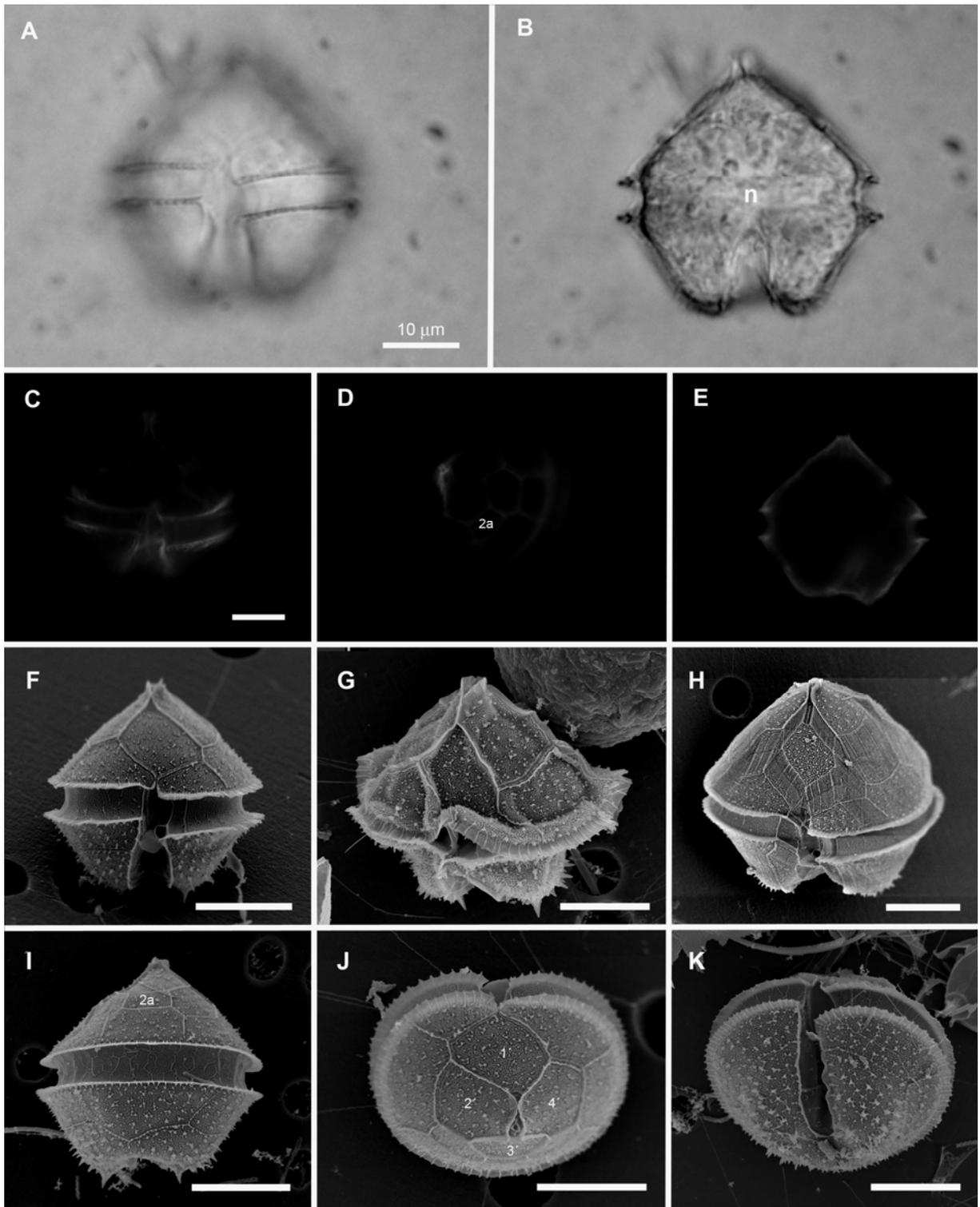


Plate 42. *Protoperidinium brevipes*. **A-B:** LM. **C-E:** Epifluorescence. **F-K:** SEM. **A.** High focus in ventral view, showing the sulcus and the median cingulum. **B.** Low focus, showing the cell shape and the centrally located nucleus (n). **C.** High focus showing the meta 1' and the distinct cingular lists. **D.** Dorsal view of the epicone, showing the characteristic 2a. **E.** Low focus showing the cell shape. **F-H.** Ventral view of three specimens showing the thecal plates and displaying the diversity in thecal ornamentation; the cell in H has very large sutures. **I.** Dorsal view showing the quadra 2a. **J.** Antapical view showing the hypothecal plates and the sulcus that reaches the antapex. Scale bars = 10 μ m.

(Plate 43 A, C), also within the cingulum and sulcus (Plate 43 C). The sutures surrounding 1', 1'' and 7'' are substantial and resemble straight lines (Plate 43 C). The protoplasm is colourless and without chloroplasts (Plate 43 A, B). This species is cosmopolitan [THRONDSEN *et al.*, 2003].

Species *Protoperidinium depressum* (Bailey) Balech, 1974

This species is very large and, hence, proper information on cellular length can not be obtained from the micrograph; however, the cells are approximately 100 µm wide (Plate 45). A long apical horn (Plate 45 A, C-F) with an apical pore (Plate 45 E, F) and two substantial but hollow antapical spines (Plate 45 B, C, D) are present. The cell is slightly dorso-ventrally flattened and rounded to heart-shaped in apical view (Plate 45 E). The cingulum has broad lists supported by ribs (Plate 45 A, C-E), and is descending (Plate 45 C) more than 2 times the cingular width [STEIDINGER & TANGEN, 1997]. The sulcus extends to the antapex beyond the antapical spines (Plate 45 D), is deep and bordered by lists (Plate 45 B, C). The thecal plates are thick with reticulated ornamentation on both epi- and hypotheca (Plate 45). The 1' is ortho (Plate 45 A, C, E) and the 2a is penta (Plate 45 F). The protoplasm is bright pink without chloroplasts (Plate 45 A, B) and the species is phagotrophic [STEIDINGER & TANGEN, 1997] and luminiscent [DODGE, 1982]. The distribution is World-wide [DODGE, 1982].

Species *Protoperidinium ovatum* Pouchet, 1883

The cell is 40 to 45 µm long and 55 to 60 µm wide. The shape is lenticular and slightly anteroposteriorly flattened. The epitheca has a small apical horn (Plate 44 B, C) The cingulum is median, displaced one width and bordered with lists (Plate 44 C). The sulcus only runs on the hypotheca and broadens towards the antapex. Distinct sulcal lists are present on 1''' and 5'''. The 1' is meta and appear twisted due to the size differences between 1'' and 7'' (Plate 44 C). This species has two large and equally sized antapical plates, which each have a winged spine (Plate 44 D). The thecal plates are sculptured with delicate reticulation. This species is heterotrophic with a pale pink protoplasm and the nucleus located in the hypotheca. The species is cosmopolitan [DODGE, 1982]

Species *Protoperidinium pallidum* (Ostenfeld) Balech, 1973

In dorsal view the shape appears rhomboid and is slightly longer than broad. The size is 70-100 µm long and 66-85 µm wide [DODGE, 1982]. The cell is dorso-ventrally flattened with a triangular epitheca (Plate 46 B). An apical process and two large antapical horns are present (Plate 46). The antapical spines have wings (Plate 46 A), which can appear as four. The radiating sutures between the apical plates have distinct lists (Plate 46 B). The cingulum appears somewhat deep and is displaced *ca.* one width; and bordered with large rib-supported lists (Plate 46). The 1' is large and para (Plate 46 B). The hypotheca is slightly convex. The protoplasm is yellow to greenish [THRONDSEN *et al.*, 2003]. Specimens of this species have been reported to be luminescent This species is cosmopolitan and have been recorded from Arctic to Antarctic seas [DODGE, 1982; THRONDSEN *et al.*, 2003]

Species *Protoperidinium pellucidum* Bergh, 1881

The cell is *ca.* 38 µm wide and 43 µm long (Plate 47 A). The epitheca is triangular and tapered into an apical horn, while the hypotheca is longer and invertedly trapez-shaped, with two short antapical spines (Plate 47 A). The cingulum is shallow, displaced less than half a width (Plate 47 A, B) and bordered by rib-supported lists (Plate 47). Likewise, the sulcus is shallow and broadens towards the antapex. Lists are bordering both sides of this furrow; however, the list formed by the first postcingular plate is largest and wing-like (Plate 47 A).

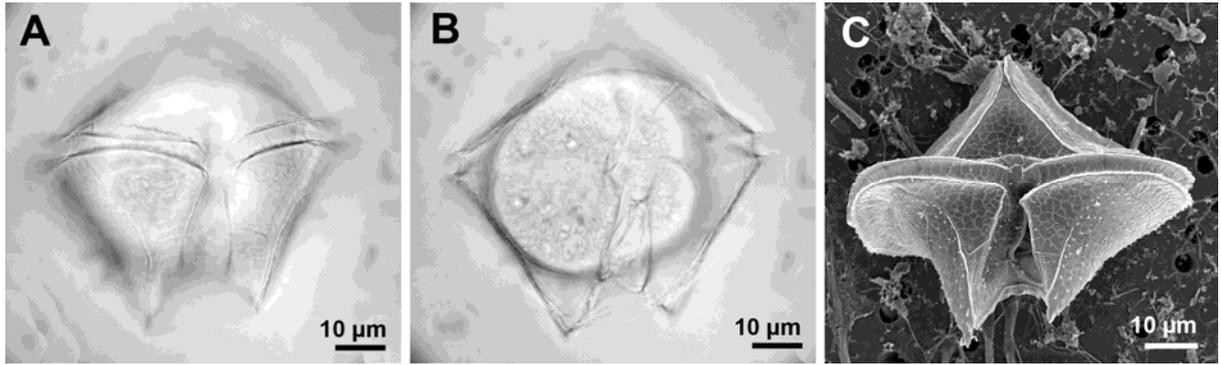


Plate 43. *Protoperidinium conicum*. A-B: LM. C: SEM. A. High focus in ventral view showing the cingulum and sulcus, which broadens towards the antapex. B. Low focus showing the cell outline and protoplasm, which is not extended into neither the apical or antapical horns. C. Ventral view showing surface ornamentation. The cell is somewhat collapsed due to the SEM preparation.

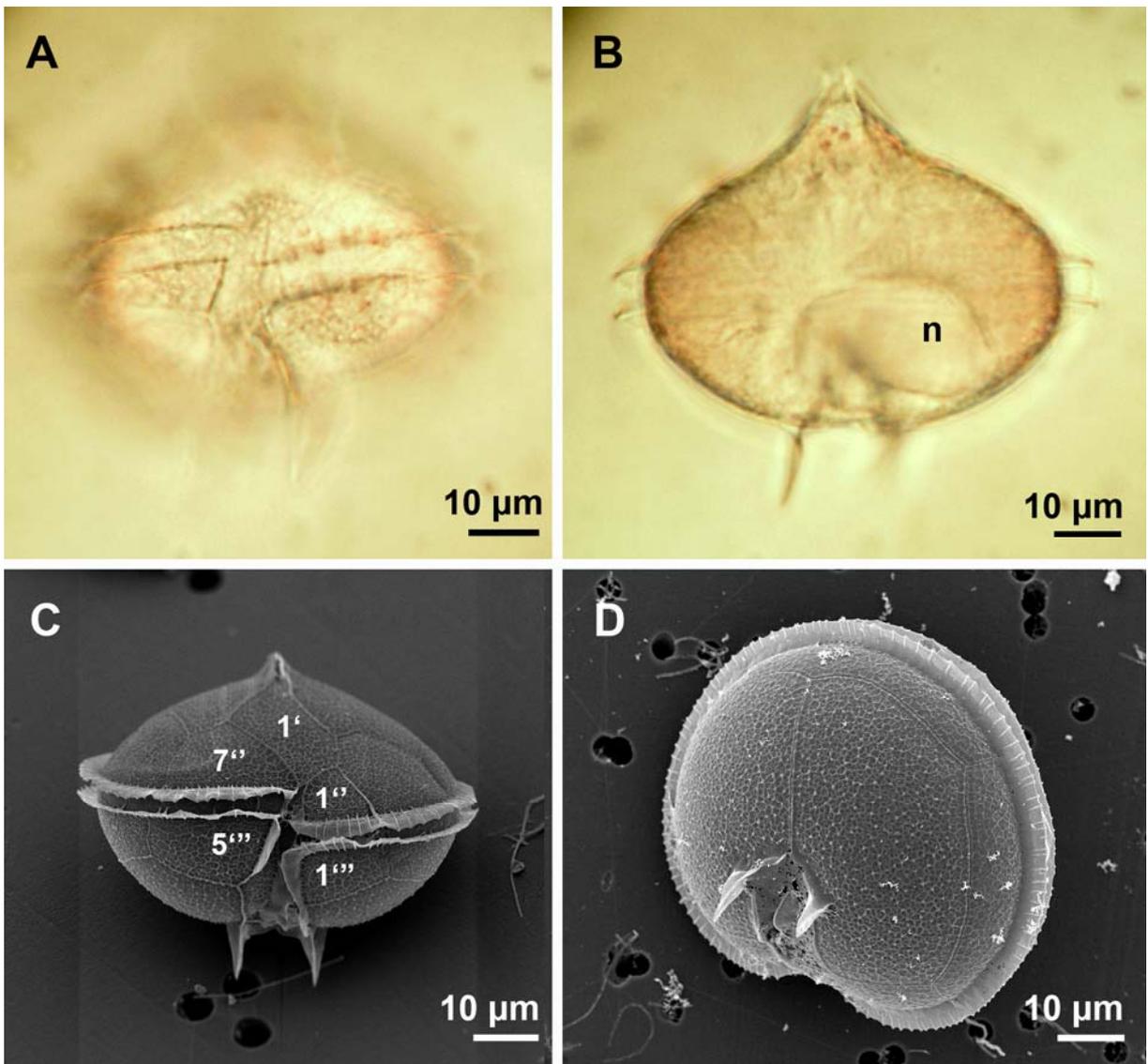


Plate 44. *Protoperidinium ovatum*. A-B: LM. C-D: SEM. A. High focus in ventral view showing the displaced and median cingulum. B. Low focus of the cell showing the pink protoplasm and distal nucleus. C. Ventral view showing the meta 1' and distinct cingular and sulcal lists. D. Antapical view of antapical plates with posterior thorns.

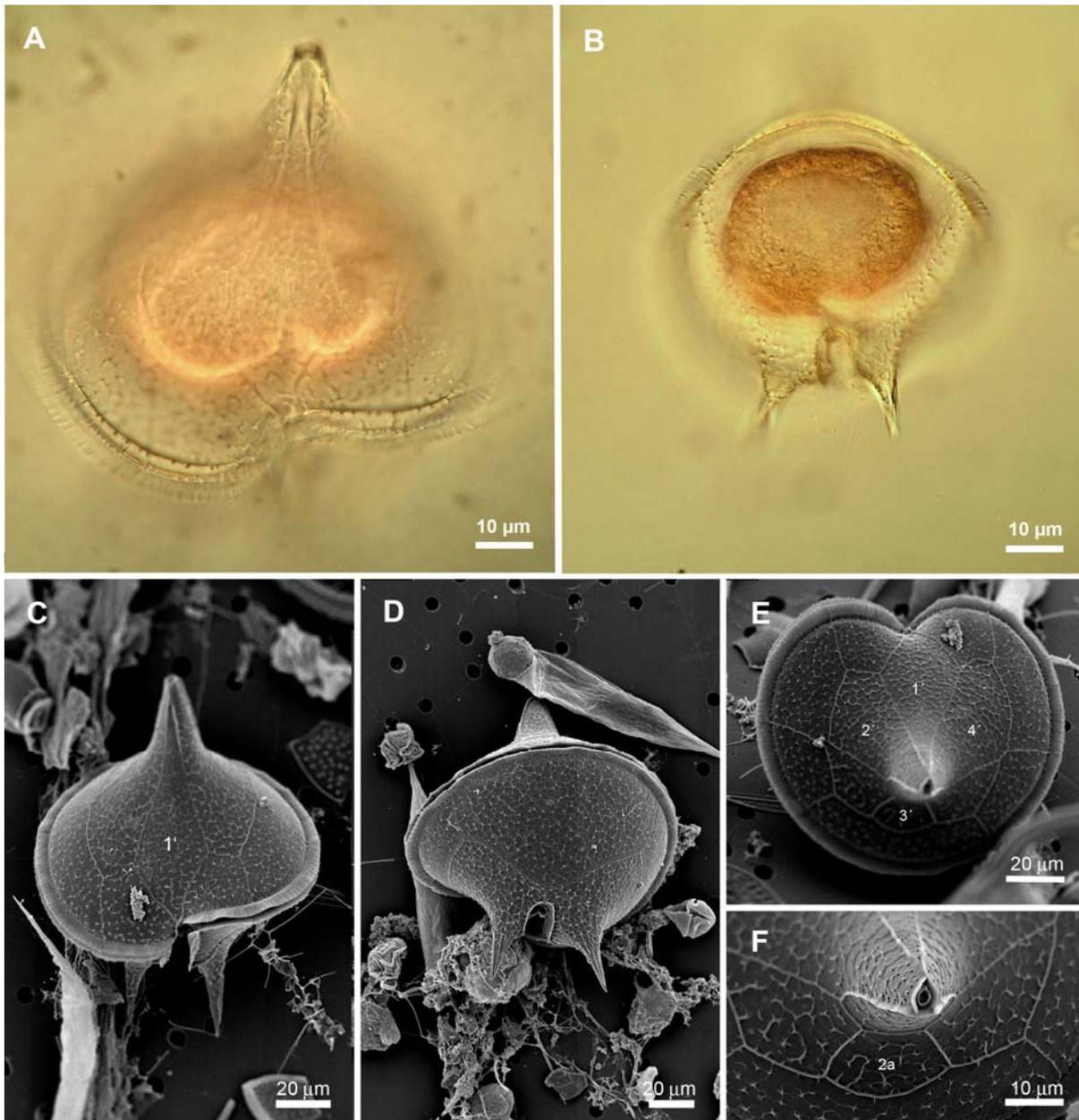


Plate 45. *Protoperidinium depressum*. A-B: LM. C-F: SEM. A. High focus on the epitheca in ventral view showing the ortho 1'. B. Low focus showing the pink protoplasm which does not extend into the large antapical horns. C. Ventral view showing the large apical horn, the median cingulum and the cingular lists. D. Dorsal view of the antapex showing some of the hypothecal plates and the antapical horns. E. Apical view showing the epithecal plates. F. Close-up of the apical horn, the APC and the 2a.

Pores associated with lightly reticulated ornamentation are scattered over the surface and along the cingular borders (Plate 47 B, C). A large, ornamented opening with numerous pores is situated on the 1''' (Plate 47 B, C); the function of this structure is unknown, but is similarly observed on *P. cerasus* (Plate 41). The 1' is para (Plate 47 A) and the 2a hexa [DODGE, 1982].

Species *Protoperidinium pentagonum* (Gran) Balech, 1974

The cell is very large and the epitheca broadly triangular (Plate 48 A). It is *ca.* 70 μm wide (Plate 48 A, B). Information about the height of the cell can not be derived from the micrograph, but according to the literature the height is within the range 75-110 μm

[THRONDSEN *et al.*, 2003]. The cingulum is deep and bordered by rib-supported lists. The radiating sutures have distinct lists (Plate 48), which are also surrounding the APC (Plate 48 C). Thecal plates are highly porated with extensive reticulations (Plate 48). The 1' is ortho (Plate 48 B), and the 2a is penta (Plate 48 A). This species can have a yellow to greenish protoplasm [THRONDSEN *et al.*, 2003], but we did not identify the species in LM. *P. pentagonum* is a temperate to tropical coastal cosmopolitan, also in eustarine to brackish waters [STEIDINGER & TANGEN, 1997; THRONDSEN *et al.*, 2003].

Species *Protoperidinium steinii* (Jørgensen) Balech, 1974

Cells of this species are pear-shaped (Plate 49) and dorsoventrally flattened (Plate 49 E). The cells are *ca.* 40-45 μm wide and 45-55 μm long (Plate 49 B-E). The epitheca extends into an apical horn, while the hypotheca is semi-globular with two long antapical spines (Plate 49). Both cingulum and sulcus are bordered with rib-supported lists; the LSL being more prominent and wing-like (Plate 49 C-E). Each of the antapical spines have three lists (compare C-E). Thecal plates are finely reticulated and delicate stria are found in the sutures (Plate 49 C-E). The 1' is meta (Plate 49 D). The cytoplasm is colourless and vacuolated without chloroplasts (Plate 49 A, B) but, according to the literature, may appear yellowish to pale pink [THRONDSEN *et al.*, 2003]. The nucleus is located above median (Plate 49 B) and the species is heterotrophic; a large food vacuole is located in the hyposome, just beneath the cingulum (Plate 49 B). *P. steinii* is a cosmopolitan species [THRONDSEN *et al.*, 2003].

Species *Protoperidinium subinermis* (Paulsen) Loeblich III, 1970

The cell is overall rhomboid in ventral and lateral view, *ca.* 60 μm wide and 55 μm long, with a triangular epitheca and a more rounded hypotheca (Plate 51 A, B). The cingulum is slightly excavated, non-displaced and bordered by lists (Plate 51) that are rib-enforced (Plate 51 D). The sulcus is deep and widens posteriorly (Plate 51 A). Thecal ornamentation is reticulate with many pores (Plate 51); also within the furrows (Plate 51 A, B, D). Lists are found along the sutures (Plate 51 A-C), as are the sutural stria (Plate 51 A, C). The 1' is ortho and slightly skewed (Plate 51 A), while 2a is quadra (Plate 51 C). No cytological information can be derived from from plate 51, but the protoplasm is usually greyish to colourless [THRONDSEN *et al.*, 2003]. This species is a coastal and oceanic cosmopolitan in temperate to tropic waters [STEIDINGER & TANGEN, 1997; THRONDSEN *et al.*, 2003].

Species *Protoperidinium* sp. 1.

Excluding the antapical horns the cell is *ca.* 25 μm long and wide. The shape is almost ovoid with convex plates, and the epitheca tapered into a short apical process (Plate 50 A). The 1' is meta and appears twisted due to the cingular displacement and the size difference of 1" and 7" (Plate 50 A). The wide cingulum is shallow, displaced *ca.* two widths and bordered with very broad rib-supported lists (Plate 50 A, B). The shallow sulcus extends into the epitheca, is slightly twisted and without lists. The hypotheca appears flattened, with two substantial and curved, wingless antapical spines of *ca.* 10 μm . The thecal plates are delicately reticulated and porated, also within the sulcus and cingulum (Plate 50 B).

Species *Protoperidinium* sp. 2

This species is very similar to *P. pallidum* (Plate 46), however the size of the cell is considerably smaller, being *ca.* 35 to 40 μm wide and 40 to 45 μm long. This species appears round in an apical view (Plate 52 B) as opposed to *P. pallidum*, which is dorso-ventrally flattened. A very similar specimen was observed by Gert Hansen in Danish waters (p. 133 and

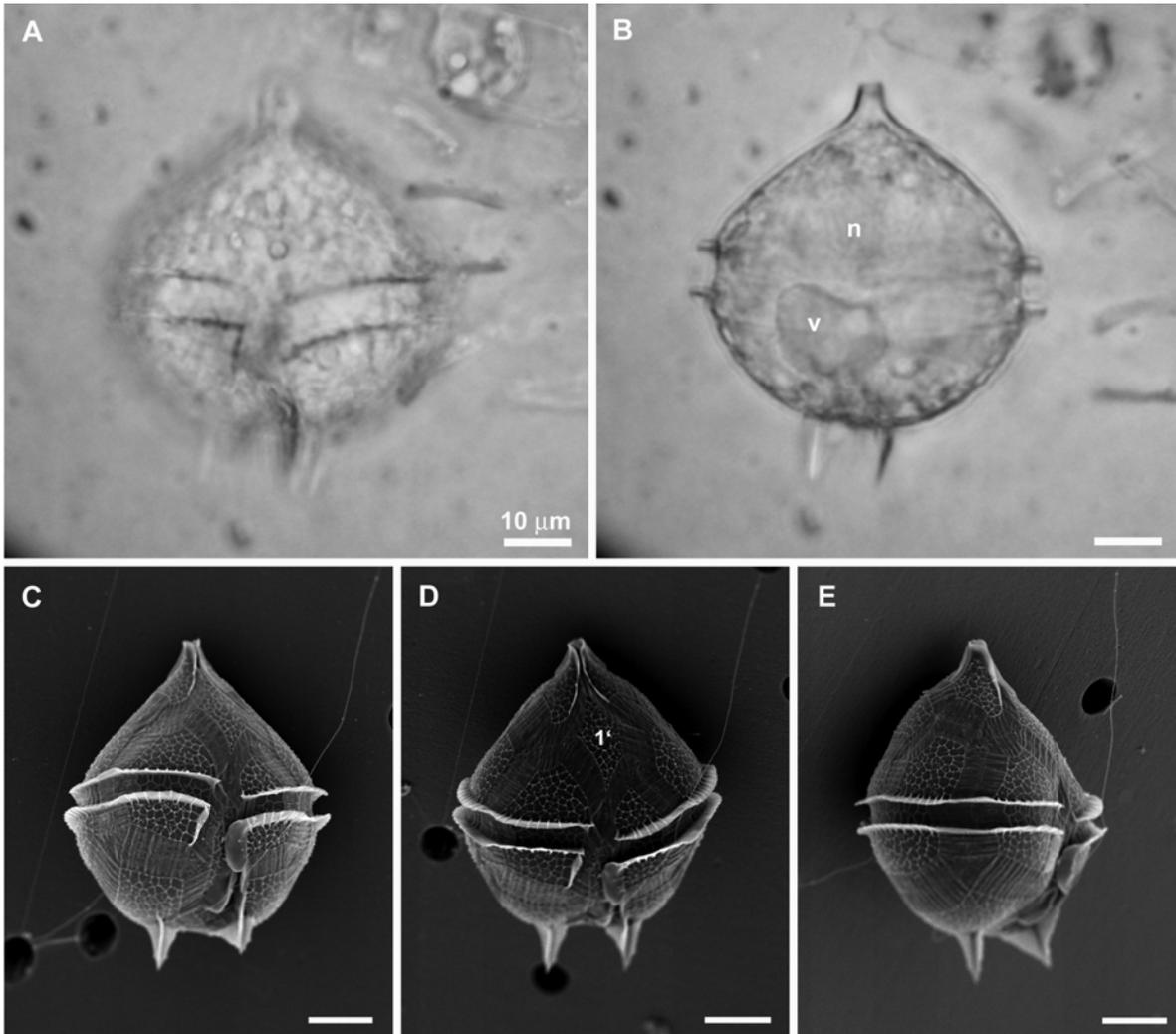


Plate 49. *Protoperidinium steinii*. A-B: LM. C-E: SEM. A. High focus in a ventral view, showing the median cingulum and the sulcus. B. Low focus, showing the cell shape with the apical horn and the two antapical spines. Furthermore the nucleus (n) and a food vacuole (v) can be observed. C. Ventral view, showing the cell shape, the sulcus, cingulum and the antapical spines. D. Ventral view, showing the 1', the apical horn and the large sutures. E. Lateral view, showing the dorsoventral flattened shape of the cell. All scale bars are 10 µm.

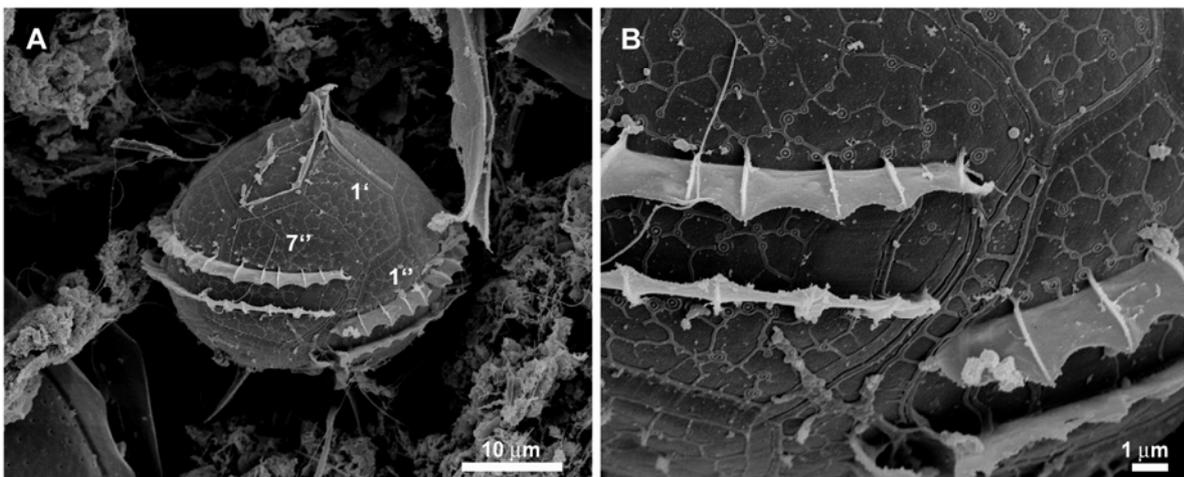


Plate 50. *Protoperidinium* sp 1. SEM. A. Ventral view showing the cell shape, the displaced cingulum, the sulcus, the distinct cingular lists, the meta 1', the apical horn and the two antapical horns. B. Close-up of the meeting of cingulum and sulcus, showing the radiating ornamentation on the cingular lists and some of the sulcal plates.

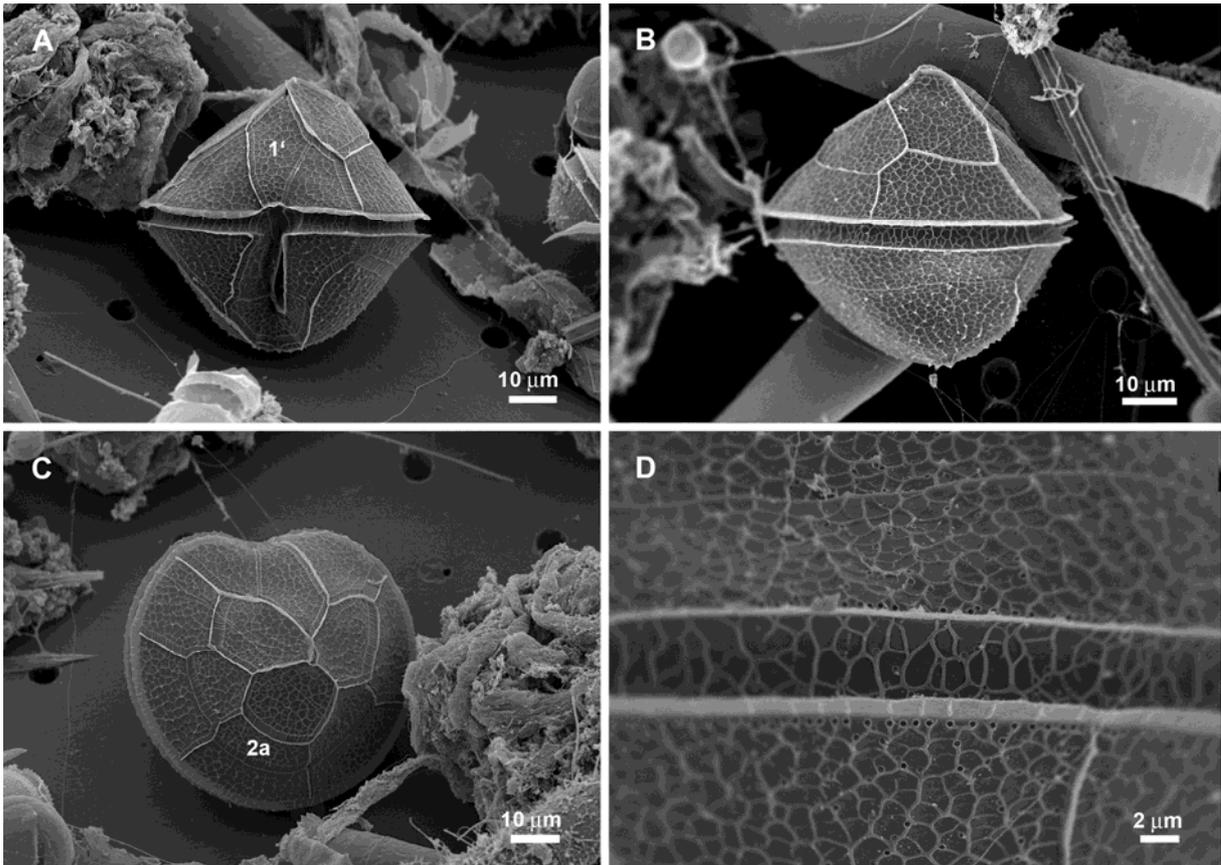


Plate 51. *Protoperidinium subinermis*. SEM. **A.** Ventral view showing the cell shape, the impressed sulcus and the cingulum bordered by lists. **B.** Lateral view showing the cell shape and some of the thecal plates. **C.** Apical view showing the epithecal plates, the ortho 1' and the quadra 2a. **D.** Close-up on cingular region revealing reticulation within this furrow.

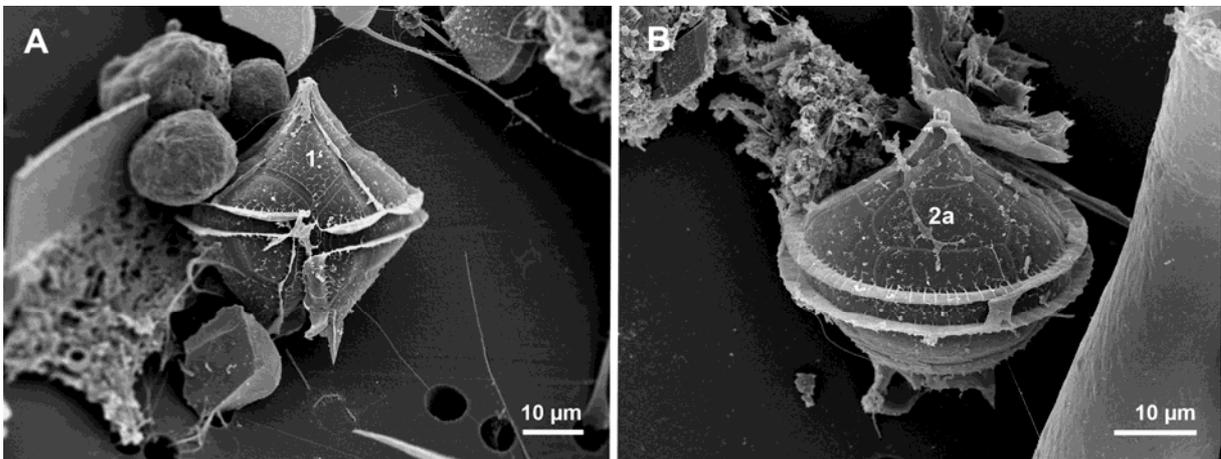


Plate 52. *Protoperidinium* sp. 2. SEM. **A.** Ventral view showing the cell shape, the cingulum with broad lists, the sulcus with wing-like left list, and the ortho 1'. **B.** Dorsal view showing the rib-supported cingular lists and the 2a.

135, *P.* sp B) [HANSEN in THOMSEN, 1992]. The epitheca is triangular and tapered into an apical process. The 1' is relatively large and ortho (Plate 52 A). In the radiating sutures between the apical plates are lists which extend from the apex to the cingulum (Plate 52). The 2a is large and hexa (Plate 52 B). The cingulum is wide, shallow, displaced one width and bordered with rib-supported lists (Plate 52). The sulcus is shallow and bordered by lists, the

one on 1" being large and wing-like (Plates 52 A). The hypotheca is triangular and has two antapical spines with small wings (Plate 52).

3.2. Species list

During the visit at Arctic Station and following in Copenhagen 50 taxa of dinoflagellates belonging to 22 genera were identified and photo documented (Paragraph 3.1 and Table 3.1). Of the 50 taxa 11 could only be identified to genus level; 8 of these taxa were athecate. During the investigation of the living material multiple other species were encountered, however several of these were not identifiable since insufficient information was retrievable from the photos. 15 of the 50 identified taxa were athecate. Several taxa could only be identified to species level in SEM: *Pentapphasodinium dalei*, *Protoperidinium pentagonicum*, *P. cerasus*, *P. pallidum*, *P. pellucidum*, *P. subinermis*, the two species of *Alexandrium*, the three *Gonyaulax* species, *Protoceratium reticulatum*, *Heterocapsa triquetra*, *Preperidinium meunieri* (Paragraph 3.1). Since the fixation and dehydration during SEM preparation damaged the athecate cells, only *Actiniscus pentasterias* could be identified in SEM due to the siliceous pentasters (Plate 21). Most taxa were encountered at several stations (Table 3.1); however, the following were only reported at one station: *Nematodinium* sp, *Alexandrium minutum*, the two species of *Cochlodinium*, *Prorocentrum micans*, *Gonyaulax* sp 1, *Katodinium glaucum*, *Protoperidinium pentagonicum*, *P. subinermis*, *P. sp 1* and *P. sp 2*. Eight of the encountered species have been reported to be toxin-producing [THRONSDEN *et al.* 2003, [HTTP://WWW.NMNH.SLEU.EDU/BOTANY/PROJECTS/DINOFLAG/TAXA.HTM](http://www.nmnh.sleu.edu/botany/projects/dinoflag/taxa.htm)]. At the 9 different stations, various numbers of taxa were identified. The highest number of species, 29, were recorded at station 4 while the lowest number, 17, were found at stations 1 and 2. On average *ca.* 25 taxa were identified at each station. The pattern seemed to be that more athecate taxa were identified at the last stations visited in comparison to the first ones (Table 3.1).

3.3. Phylogeny

During the visit at Arctic Station, 185 dinoflagellates were isolated (Appendix 3). Of these the DNA of 40 single cells was amplified with PCR, but unfortunately none of the PCR reactions resulted in products suitable for sequencing. In Copenhagen more cells were attempted amplified and sequenced resulting in the data described below.

A data matrix comprising 648 nucleotides of the domains D1 and D2 of the LSU rDNA gene was compiled for 49 taxa (sequences made accessible by Niels Daugbjerg and GenBank) in order to elucidate the phylogeny of the genera *Phalacroma* and *Dinophysis*. The topology of the phylogenetic tree (Figure 3.2.) was obtained from a Bayesian analysis and was rooted with the two ciliates *Tetrahymena pyriformis* and *T. thermophila*. Posterior probabilities for the branches were calculated. Maximum parsimony was performed resulting in 43 equally parsimonious trees, each requiring 5729 steps (trees not shown). Bootstrap values were based on 1000 replications. Values for posterior probabilities and bootstrap are nearly the same for most branches; both the deep branches and the clades for the terminal taxa are well-supported (Figure 3.2). The branch pattern and branch lengths of the resolved tree reveal that the dinoflagellates form a well-supported monophyletic group, which has diverged much from a common ancestor shared with the ciliates (Figure 3.2; see scale bar). Within the dinoflagellates several well-supported groups, containing species belonging to the same genus or order, can be observed, for instance *Amphidinium*, *Gymnodinium*, *Alexandrium* and *Gonyaulax*, which are all members of the Gonyaulacales.

The tree with associated posterior probability and bootstrap values support the monophyly of the members of Dinophysiales. The phylogenetic analysis, where *Phalacroma* is sister group to the nine species of *Dinophysis*, supports that *Phalacroma* is a good genus within the



Figure 3.2. Phylogeny of *Phalacroma cfr. braarudii* and 46 other dinoflagellates based on partial nuclear-encoded LSU rDNA sequences. The tree topology was obtained from Bayesian analysis and it was rooted with two ciliates assigned to the genus *Tetrahymena*. Maximum Parsimony analyses were also performed and resulted in 43 equally parsimonious trees, each requiring 5729 steps (trees not shown). In Parsimony analyses the consistency index was 0.351 and the retention index was 0.564. Posterior probabilities from Bayesian analyses are written as the first numbers to the left of the internodes. The second numbers are parsimony bootstrap values based on 1000 replications. The branch lengths are proportional to the number of changes, see scale bar.

	<i>Phalacroma</i>	<i>D. norvegica</i>	<i>D. acuminata.</i>	<i>D. acuta</i>	<i>D. caudata</i>	<i>D. dens</i>	<i>D. fortii</i>	<i>D. odiosa</i>	<i>D. rotundata</i>	<i>D. sacculus</i>	<i>D. tripos</i>
<i>Phalacroma</i>		24,8	25,0	25,2	24,3	25,4	25,0	24,1	26,6	25,0	24,10
<i>D. norvegica</i>	21,0		0,47	2,04	1,90	0,94	1,88	1,42	18,8	0,47	1,10
<i>D. acuminata.</i>	21,1	0,46		2,20	2,06	0,47	2,04	1,58	18,6	0,00	1,26
<i>D. acuta</i>	21,3	2,01	2,17		2,21	2,52	0,16	1,73	19,4	2,20	1,41
<i>D. caudata</i>	20,7	1,87	2,03	2,18		2,54	2,05	1,10	19,6	2,06	0,78
<i>D. dens</i>	21,4	0,93	0,46	2,47	2,49		2,52	2,06	18,6	0,47	1,74
<i>D. fortii</i>	21,1	1,96	2,01	0,16	2,02	2,47		1,57	19,4	2,04	1,25
<i>D. odiosa</i>	20,5	1,46	1,56	1,71	1,09	2,03	1,55		19,4	1,58	0,31
<i>D. rotundata</i>	22,2	16,5	16,3	17,0	17,1	16,3	17,0	17,0		18,56	19,0
<i>D. sacculus</i>	21,1	0,46	0,00	2,17	2,03	0,46	2,01	1,56	16,34		1,26
<i>D. tripos</i>	20,5	1,10	1,25	1,40	0,78	1,71	1,24	0,31	16,65	1,25	

Table 3.2. Percentual sequence divergence in 10 species of *Dinophysis* and one species of *Phalacroma*, based on 648 bp of the LSU rDNA, D1-D2 region. Uncorrected distances are given below the diagonal and above are distance estimations based on the Kimura-2-parametre model.

Dinophysiales. Most surprisingly, *Dinophysis rotundata* forms a sister group to the other *Dinophysis* species and to *Phalacroma*; this indicates that *Dinophysis rotundata* does not belong to either of the genera. The "real" *Dinophysis* clade is unresolved and has very short branches, which indicates that the LSU rDNA sequences determined contain insufficient information to resolve the relationship within this genus.

The sequence divergence estimates (Table 3.2) support the phylogeny, that neither *Phalacroma* nor *Dinophysis rotundata* belongs to the *Dinophysis* genus. From table 3.2 it is observed that the *Phalacroma* LSU sequence differs more from all of the *Dinophysis* species than the species within this genus differ from one another. The results lie in the range of 20.5% to 22.2% uncorrected distances and 24.1% to 26.6% distance estimations based on the Kimura-2-parametre model. This means that, on average, the *Phalacroma* LSU sequence differs from the other sequences at every fourth base pair. Furthermore, it is observed that *D. rotundata* also varies greatly in comparison to the other *Dinophysis* species; the data lie within a range of 16.4% to 17.1% uncorrected distances and 18.6% to 19.6% distance estimations based on the Kimura-2-parametre model. *Phalacroma* and *Dinophysis rotundata*, however, differ more from each other with 22.4% uncorrected distances and 26.6% distance estimations based on Kimura-2-parametre model, than each of the species differs from the rest of the *Dinophysis*-sequences. Between the remaining *Dinophysis* species the variation is much lower and lies in the interval of 0 to 2.49% uncorrected distances and 0 to 2.54% distance estimations based on Kimura-2-parametre model.

3.4. *Ceratium arcticum* and *C. longipes*

During the stay at Arctic Station the two *Ceratium* species depicted below, *C. arcticum* and *C. longipes*, were encountered with high frequency at all stations (Table 3.1 and Chart 3.1). Due to limitation in time no morphometric data were obtained.

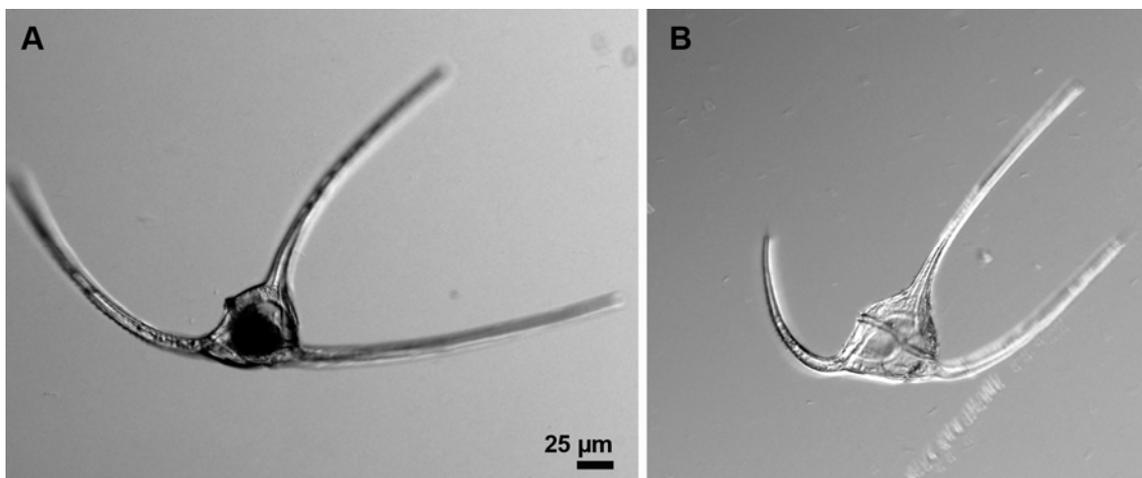


Figure 3.2. A. *Ceratium arcticum* B. *Ceratium longipes*. Lugol-fixed material. Same scale bar for A and B.

3.4.1. The morphological differences between *C. arcticum* and *C. longipes*

When comparing the two *Ceratium* species several morphological features, both similar and differing can be observed (Figure 3.2.). The apical horn of both species is open and bends to the right, and the size and shape of the cell body is similar for the two species. Furthermore, both are autotrophic and have chloroplasts located within the apical and antapical horns. The morphological differences which have been used to separate the two species are the shape and angle between the antapical horns (Paragraph 1.3). For *C. longipes* the right horn appears to be almost parallel to the apical horn, whereas the left horn is more curved and substantially shorter. The antapical horns of *C. arcticum* are only slightly curved and the angle between them is very large.

3.4.2. Occurrence of *Ceratium arcticum* and *C. longipes*

Water samples were taken at locations 2, 4, and 5-9 (Figure 2.1 and table 2.1, Paragraph 2.1) samples were filtered and the number of fully developed specimens of *Ceratium arcticum* and *C. longipes* was counted, as described in section 2.6. The results are depicted in chart 3.1.

In general, the two species seem to co-occur; the number of specimens varied considerably at the different stations but never exceeded 45 specimens per litre. The highest density was encountered at station 2, and the lowest at the stations 8 and 9 (Chart 3.1 and Appendix 2). The density of cells does not appear to be connected to the latitude or longitude; for comparison, large variation in the number of each species was found at stations 7 and 8, although the samples were taken within 1 hour at the two very closely located stations (Figure 2.1 and table 2.1).

In general, the density of *C. longipes* was higher than for *C. arcticum*. Especially on stations 2, 4, 5, and 6 the number of *C. longipes* encountered was substantially higher than the number of *C. arcticum* (Chart 3.1). Only at station 7 did *C. arcticum* account for the majority of the specimens but the difference between the two species was not pronounced. On station 8 and 9 the concentration of specimens was low and the densities of *C. arcticum* and *C. longipes* were almost similar.

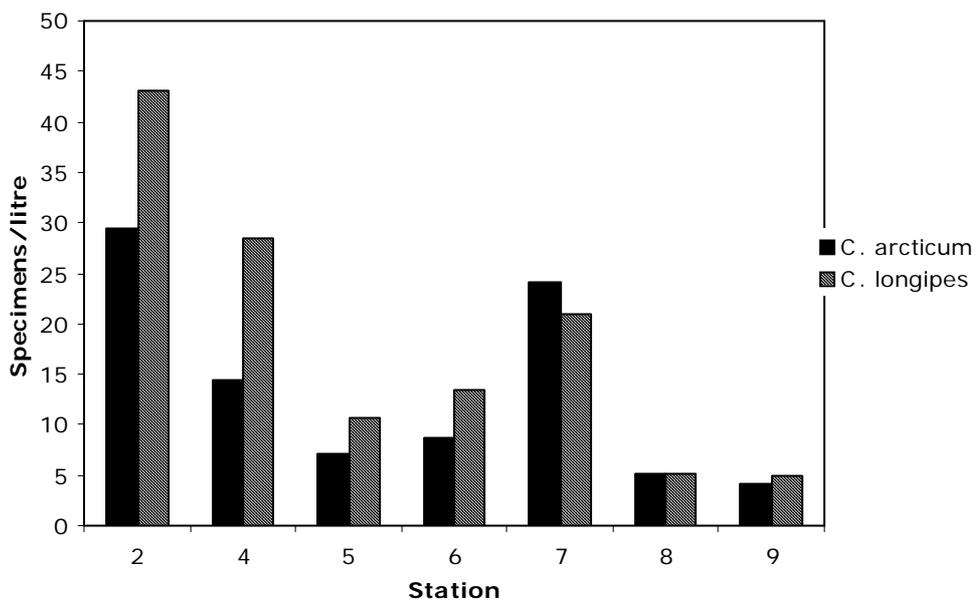


Chart 3.1. The occurrence of *C. arcticum* and *C. longipes* as number of specimens counted per litre at 7 different stations.

4. DISCUSSION

In the present survey 50 marine pelagic dinoflagellates were identified. This is the highest number of dinoflagellate species ever encountered in the vicinity of Disko (Paragraph 3.1 and 3.2) [GRØNTVED & SEIDENFADEN, 1938; CLAUSEN *et al.*, 1994; TRIER, 1998, JØNSSON *et al.*, 2002]. Of the 50 taxa several genera and species have not previously been recorded in these waters (Table 4.1). The following Paragraphs will contain comparative analyses including the results of former investigations. These analyses are followed by a discussion of the changes in the species diversity, what they might indicate and which consequences this could have on a long-term basis.

The exciting phylogenetic results concerning the members of Dinophysiacea, which reinforce the genus *Phalacroma* and indicate a third genus within the family, are discussed in the second Paragraph. Furthermore, the taxonomic chaos within the Dinophysiacea is illustrated in a historic perspective, underlining the need for a taxonomic revision of the genera of this family.

The results concerning the *Ceratium arcticum* and *C. longipes* are discussed together with a proposal for solving the problem involving these two morphological similar species.

The last Paragraph of the discussion contains methodical considerations and suggestions for improval of methods on future surveys in Greenlandic marine waters.

4.1. Survey

To compare the results of the present study to prior investigations of the marine dinoflagellate community in the vicinity of Disko is suboptimal since all of the former investigations had different aims and did not exclusively investigate the composition of the dinoflagellate diversity in the water column. Most importantly, there has been no consistency concerning the time of year for conduction of the surveys (see below).

During the Godthaab Expedition in 1928, Gunnar Seidenfaden collected phytoplankton material both vertically and horizontally at *ca.* 250 different station in the waters West of Greenland, also in Disko Bay and South of Hareøen [GRØNTVED & SEIDENFADEN, 1938]. The sampling material was not studied during the expedition, but fixated in paraformaldehyde for preservation. Since this procedure only conserves thecate dinoflagellates while the athecate specimens are slowly degraded, no information about the athecate dinoflagellates of the high Arctic was obtained during the Godthaab Expedition 1928. The subsequent surveys were all performed on live material, and includes observations of both thecate and athecate species.

In 1994, a major pelagic sampling programme was performed during the Arctic field course, with most of the sampling executed at the plankton station outside Godhavn (Table 1 in 1.3.1). Both the physical and chemical water conditions were described together with the diversity and vertical distribution of the pelagic community [CLAUSEN *et al.*, 1994].

The study conducted by Trier in 1996 was part of a major investigation of the plankton production in West Greenland, and contained both a quantitative and qualitative analysis of the phytoplankton composition.

The main focus in 2002 was the biomass of flagellates. Sampling took place at several locations from Disko Fjord to Mudderbugten, and both species diversity and biomass composition of the phytoplankton were studied [JØNSSON *et al.*, 2002].

In general, the amount of species identified seems to be connected to the time spent with identifications, the prior knowledge about the different genera and species, and on the equipment available. Table 4.1 contains a complete list of the dinoflagellate species observed in the vicinity of Disko Island from 1928 to 2006.

		Jensen & Veland, 2007						Jensen & Veland, 2007					
		Jensson <i>et al.</i> , 2002						Jensson <i>et al.</i> , 2002					
		Trier, 1998						Trier, 1998					
		Clausen <i>et al.</i> , 1994						Clausen <i>et al.</i> , 1994					
		Seidenfaden, 1938						Seidenfaden, 1938					
Thecate													
<i>Prorocentrum</i>	<i>P. micans</i>												
	<i>P. minimum</i>			x									
<i>Dinophysis</i>	<i>D. acuminata</i>		x	x	x	x							
	<i>D. acuta</i>										x		
	<i>D. norvegica</i>		x	x	x	x	x						
	<i>D. rotundata</i>		x	x	x	x							
<i>Phalacroma</i>	<i>P. cfr braarudii</i>										x		
<i>Ceratium</i>	<i>C. arcticum</i>		x	x	x	x	x						
	<i>C. fusus</i>		x								x		
	<i>C. horridum</i>										x		
	<i>C. longipes</i>		x	x	x	x	x						
<i>Alexandrium</i>	<i>A. minutum</i>										x		
	<i>A. ostenfeldii</i>										x		
	<i>A. tamarense</i>										x		
<i>Amylax</i>	<i>A. triacantha</i>		x	x							x		
<i>Gonyaulax</i>	<i>G. cfr alaskensis</i>										x		
	<i>G. cochlea</i>										x		
	<i>G. cfr. digitalis</i>										x		
	<i>G. scrippsae</i>										x		
	<i>G. cfr. spinifera</i>		x	x							x		
	<i>G. cfr elongata</i>										x		
<i>Peridinella</i>	<i>P. catenata</i>										x		
<i>Protoceratium</i>	<i>P. reticulatum</i>		x	x							x		
<i>Pentapharsodinium</i>	<i>P. dalei</i>										x		
<i>Scrippsiella</i>	<i>S. sp.</i>										x		
<i>Heterocapsa</i>	<i>H. triquetra</i>										x		
<i>Preperidinium</i>	<i>P. meunieri</i>										x		
<i>Protoperidinium</i>	<i>P. bipes</i>		x	x	x	x							
	<i>P. brevipes</i>		x	x							x		
	<i>P. cerasus</i>		x								x		
	<i>P. conicoides</i>		x								x		
	<i>P. conicum</i>		x								x		
	<i>P. depressum</i>		x								x		
	<i>P. ovatum</i>		x								x		
	<i>P. pallidum</i>		x	x	(x)						x		
	<i>P. pellucidum</i>		x								(x)		
	<i>P. pentagonium</i>										x		
	<i>P. roseum</i>		x										
	<i>P. steinii</i>										x		
	<i>P. subinermis</i>										x		
	<i>P. thorianum</i>		x										
	<i>P. sp. 1</i>										x		
	<i>P. sp. 2</i>										x		
	Athecate												
	<i>Gymnodiniaceae</i>	<i>G. sp.</i>											x
<i>A. pentasterias</i>											x	x	
<i>Amphidinium</i>	<i>A. crassum</i>										x		
	<i>A. operculatum</i>											x	
	<i>A. hadai</i>										x		
	<i>A. sphenoides</i>										x		
	<i>Cochlodinium</i>	<i>C. brandtii</i>		x									x
		<i>C. helicoides</i>		x									
<i>C. pulchellum</i>			x										
<i>C. sp. 1</i>												x	
<i>C. sp. 2</i>												x	
<i>Gymnodinium</i>	<i>G. elongatum</i>										x		
	<i>G. gracile</i>		x									x	
	<i>G. perpavum</i>		x										
	<i>G. rubrum</i>											x	
	<i>G. cfr undulans</i>											x	
<i>Gyrodinium</i>	<i>G. sp. 1</i>											x	
	<i>G. crassum</i>										x	x	
	<i>G. mitra</i>										x		
	<i>G. rhomboides</i>		x	x									
	<i>G. spirale</i>		x	x	x							x	
	<i>G. sp. 1</i>											x	
	<i>G. sp. 2</i>											x	
<i>Katodinium</i>	<i>K. glaucum</i>		x	x	x						x		
<i>Nematodinium</i>	<i>N. sp.</i>										x		
<i>Proterothropsis</i>	<i>P. vigilans</i>		x								x		
<i>Togula</i>	<i>T. jolla</i>										x		
<i>Thorodinium</i>	<i>T. robustum/teredo</i>										x		
<i>Warnovia</i>	<i>W. sp.</i>											x	

Table 4.1. List of all the 72 dinoflagellate species detected in the vicinity of Disko Island from 1928 to 2006. Colour code: white = genus/species detected in more than one survey, black = genus/species detected for the first time during the present survey, grey = detected in another survey than the present (Table 3.1). [GRØNTVED & SEIDENFADEN, 1938; CLAUSEN *et al.*, 1994; TRIER, 1998; JØNSSON *et al.*, 2002].

The time of the year at which the sampling was conducted has probably influenced the species composition of the phytoplankton to some extent. In general, variation in the diversity of the net plankton exist between the surveys from 1994, 1998, 2002 and 2006, respectively; whereas we mainly detected dinoflagellates and a few chrysophytes, the prior investigations have observed large numbers of diatoms and chrysophyte species. However, the former surveys have all been conducted during June-July, whereas the Arctic Field course 2006 took place in August. As described in Paragraph 1.1.3, the hydrography and, hence, the phytoplankton composition varies greatly throughout the year in Disko Bay [NIELSEN, 2005]. An upper layer of 80 metres with uniform salinity and temperature is formed in the water column during the autumn, through a combination of reduced freshwater input and decreased heating together with stronger winds. This is opposed to the three-layered structure of the water column during the summer. Since the hydrodynamic changes have a direct impact on the composition of the marine phytoplankton (Paragraph 1.1.3), the seasonal variation might, in part, explain some of the variation among the dinoflagellate species encountered in the different surveys.

4.1.1. Comparison with the findings from the Godthaab Expedition 1928

During the Godthaab Expedition in 1928 Seidenfaden identified 21 thecate dinoflagellate species by sampling in Disko Bay [GRØNTVED & SEIDENFADEN, 1938]. In the same area, we have encountered 15 of these species as well as 21 thecate dinoflagellates not detected by Seidenfaden (Table 4.1). Seidenfaden recognised 11 *Protoperidinium* species and we have encountered the eight of these. In addition we have identified five *Protoperidinium* species that were not recorded in 1928 by Grøntved & Seidenfaden (1938). Thus, both in 1928 and the present survey several more *Protoperidinium* species were observed than in 1994, 1998 and 2002 (seven, five, and six, respectively). One factor that may account for the differences in *Protoperidinium* species detected is the amount of time spent with identification. This is underlined by the fact that seven of our 13 identified species have been detected in SEM only (Paragraph 3.2). In the material sampled by Seidenfaden during the Godthaab expedition all the way around Disko Island, *Amylax triacanta*, *Gonyaulax digitalis* and *G. spinifera* were also detected; all species that we have encountered both in the Disko Bay, in Nordfjord and at Hareøen (Figure 2.1 and Table 3.1).

In the high Arctic region (above the 70th latitude) Seidenfaden detected relatively few dinoflagellates; only three *Dinophysis* species, one *Ceratium* and one *Protoperidinium* [GRØNTVED & SEIDENFADEN, 1938].

4.1.2. Comparison with Clausen *et al.* 1994

The only genus detected in 1994 but not in the present study is *Amphidinium*, which is a benthic marine dinoflagellate [KOFOID & SWEZY, 1921; STEIDINGER, 1997]; due to the time limits the diversity of benthic dinoflagellates was not investigated in 2006. However, as described below (Paragraph 4.1.6), several species and even genera that are included in the 2006 survey were not encountered by Clausen *et al.* (1994).

In total, 45 dinoflagellate taxa were encountered during the survey in 1994, of which 20 were athecate specimens. Of these, 22 of the 25 thecate specimens were identified to species level, whereas the same taxonomical level was only reached for 14 athecate dinoflagellates [CLAUSEN *et al.*, 1994]. Specimens only identified to genus level complicate the comparison between surveys. One possibility is comparison of micrographs and descriptions, but unfortunately, photo documentation and descriptions do not exist for all of the specimens included the species list from the 1994 survey. During the 2006 survey at least 18 more than the 15 described athecate species were encountered (data not shown). However, due to time

and technical limitations (Paragraph 4.4) these specimens were not included in the results section, which only consists of photo-documented species supplied with descriptions.

The most interesting differences between the surveys of 1994 and 2002, concerning the thecate species, is the recording of the toxic *Alexandrium ostenfeldii* [CLAUSEN *et al.*, 1994] which is a common species in the waters surrounding Greenland [ØJVIND MOESTRUP, pers. comm.]; however, this species has not since been observed in this area. Clausen *et al.* also encountered a *C. horridum* and made the only report of this species in the vicinity of Disko.

4.1.3. Comparison with Trier 1998

Trier's investigation was based on Lugol-fixed material sampled in June and July 1996 along a transect in Disko Bay and Disko Banke; the results of another transect survey are not included in this comparison since it was conducted further South.

In total, 11 athecate and 13 thecate species were recorded; however, seven of the unarmoured dinoflagellates are identified to the genus level only, whereas a single one is unidentified beyond "athecate".

Trier recorded two genera which we did not encounter during the 2006 survey, *Amphidinium* (see Paragraph 4.1.2 and 4.1.4.) and *Torodinium* Kofoid & Swezy, 1921, which are cosmopolitan and planktonic genera [DODGE, 1982]. In addition, two *Gymnodinium* species, undetected in 2006 were abundant in 1996. Unfortunately, the work of Trier (1996) is based on quantitative observation, and contains no photo documentation of the encountered dinoflagellate species. This makes a direct comparison impossible.

4.1.4. Comparison with Jønsson *et al.* 2002

In 2002, 26 different dinoflagellate taxa were observed. Of these, 18 were identified to species level belonging to 12 genera [JØNSSON *et al.*, 2002]. Among the 26 species encountered, 11 were athecate. The following species were found in the 2002 survey but not in 2006: Four unidentified *Gyrodinium* species, *Gymnodinium rubrum*, *Cochlodinium* cfr. *brandtii* Wulff, 1916, *Cochlodinium helicoides* Lebour, 1925, *Togula jolla* Flø Jørgensen *et al.*, 2002, and *Amphidinium* cfr. *operculatum* Claparède & Lachmann, 1859 [JØNSSON *et al.*, 2002]. When comparing our LM data with the obtained micrographs of unidentified *Gyrodinium* specimens from the survey of 2002, no obvious similarities are apparent. Hence, there are several *Gyrodinium* species which remain to be identified in the waters near Disko Island.

Two genera, *Togula* and *Amphidinium*, were observed in 2002 but not in 2006. However, as is the case with *Amphidinium*, *Togula* is a marine benthic dinoflagellate [FLØ JØRGENSEN *et al.*, 2004], and since no sampling of microbenthos was performed in the current survey, observations of benthic dinoflagellates were of course not expected.

No members of Warnoviaceae or Actiniscaceae were detected in the 2002 survey, neither were species of the genera *Pentaparsodinium* or *Preperidinium*. The absence of thecate genera may be explained by the fact that SEM was not conducted in 2002; in LM species of these genera are difficult to distinguish from species of *Scrippsiella* and *Diplopsalis*, respectively.

4.1.5. Below and above 70° N

The highest number of athecate species encountered, eight, were recorded at Hareøen (Figure 2.1, Table 2.1). On average, 4.5 unarmoured species were identified at each station; however, this number would have been exceedingly higher, had all of the unidentified athecate dinoflagellates been included in the results (Paragraph 4.1.2). In general, more species were recognised and identified at the last three stations (Table 3.1). This, however, does not necessarily mean that more athecate dinoflagellate species are found near Hareøen, but may

simply reflect that our LM skills concerning identification and photo documentation improved during the field course.

There does not appear to be any difference in the thecate species observed in sub Arctic and in high Arctic region (Table 3.1). More or less the same species were encountered in the two regions (Table 3.1, compare with Figure 2.1 and table 2.1), with similar frequencies (data not shown). However, as it appears from Figure 1.3, all of the nine stations are influenced by the waters transported by the West Greenland current. Hence, all samples for this survey have been collected from the same water mass, and although the dinoflagellates are motile, their distribution is predominantly controlled by the currents and movements of the water masses.

In the samples taken near Hareøen as well as in Mudderbugten, more diatoms were present both quantitatively and qualitatively than was observed in the material collected at the other stations (data not shown). We confer these variations in phytoplankton composition to different concentrations of nutrients availability, since a diatom occurrence is usually associated with a higher nutrient content than for the dinoflagellates [NIELS DAUGBJERG, pers. comm.].

An investigation for comparison of the phytoplankton composition in the high- and sub Arctic regions should include material collected at a higher latitude, thereby improving the probabilities that variations in the length of the photo- and ice free periods are of significant importance for the species diversity.

4.1.6. New species encountered

Four previously unrecorded genera in the vicinity of Disko were observed during the 2006 survey: *Preperidinium*, *Pentapharsodinium*, *Nematodinium* and *Warnovia*. In addition, eight species have been detected for the first time in this area: *Prorocentrum micans*, *Dinophysis acuta*, *Alexandrium minutum*, *A. tamarense*, *Gonyaulax alaskensis*, *G. elongata*, *Protoperidinium subinerme*, and *Gyrodinium undulans*. Among the specimens only identified to genus level, we have seven potential new recordings for the area between Mudderbugten and Hareøen.

One of the *Gonyaulax* species, *G. elongata*, was identified in LM on behalf of its cysts only, whereas the vegetative cells were detected in SEM. None of the previously conducted surveys have included studies of dinoflagellate cysts, which presumably could have increased the number of reported species from Disko Bay, especially of the genus *Gonyaulax* (Paragraph 3.1.3).

Several of the new encountered species were identified in SEM only, *i.e.* the two *Alexandrium* species, *Gonyaulax alaskensis*, *Pentapharsodinium dalei*, *Preperidinium meunieri*, and *Protoperidinium subinerme*. Hence, it can not be determined whether these species are in fact new-occurring in these waters, or simply have not been detected previously due to restrictions in the use of equipment available.

Indications of altered species composition

A comparison of the species lists in table 4.1 reveals some indications of changes in the species composition over the years. These variations might be explained by alterations in the environment due to the Global change (Paragraph 1.1). This is, nevertheless, a very delicate subject to draw conclusions about, in part since a high degree of uncertainty is connected to the species identification; especially in cases when no photo documentation of the observed species exists. Also, an element of subjectivity is assumed to have affected the reported species lists to some extent. This is illustrated by the different techniques applied for detection of the same *Protoperidinium* species in 1928 and 2006 (Table 4.1); whereas several of these species were only observed in SEM in the present survey, Grøntved & Seidenfaden (1938)

conducted all of their investigations using a light microscope [GRØNTVED & SEIDENFADEN, 1938]. In order to minimise the effects of the above mentioned subjectivity, the comparison concerning alterations in species composition is based on the thecate and easily recognisable species such as *Prorocentrum micans*, *Alexandrium minutum* and *Ceratium fusus* (Plates 1, 9 and 11, respectively). The two first have not previously been observed in this area, whereas *C. fusus* has been detected at a single sampling station by Seidenfaden in 1928 [GRØNTVED & SEIDENFADEN, 1938]. The occurrence of these three temperate species contradicts our expectations, since the annual temperatures West of Greenland have decreased 1.29°C over the past five decades (Paragraph 1.1) [HANNA & CAPPELEN, 2003]. Even though this decrease in temperature is significant [HANNA & CAPPELEN, 2003] several phytoplanktonic organisms are documented capable of sustaining viability within a wide temperature range [TILZER & DUBINSKY, 1987; DAUGBJERG & MOESTRUP, 1992; DEVOS *et al.*, 1998; MOCK & HOCH, 2005]; this is presumptively true for dinoflagellates as well [NIELS DAUGBJERG, pers. comm.].

Thus, the alterations in species composition can not be explained simply through temperature changes; rather, an explanation may be sought in the effects of the climate changes on the ocean currents (Paragraph 1.1). These currents might carry resting cysts of the temperate species (Paragraph 1.2.4) with them to the Arctic regions. However, the present survey is only a reflection of the conditions at a given moment in time, and further investigations need to be performed in the area.

Potential consequences for the food web

Some of the species that represent the first observations in the waters near Disko are heterotrophic, for instance the species of *Warnovia* and *Nematodinium* together with *Gyrodinium undulans*, *Preperidinium meunieri* and potentially the three previously undetected *Protoperidinium* species. The heterotrophic dinoflagellates are secondary producers and can phagocytise food items within their own size range or even larger as for *Protoperidinium* spp. Thus, they are competitors to the much larger zooplankton, which generally consume prey one tenth of their body size. [NIELSEN, 2005]. The protozooplankton, which also includes the heterotrophic dinoflagellates, can react instantly to increased food availability whereas the response in the zooplankton is delayed due to their much more complicated life cycles [HANSEN *et al.*, 2003a]. This is underlined by a study on *Calanus* conducted by HANSEN *et al.* [2003a] in Disko Bay, where introduction of an extra trophic level resulted in a smaller *Calanus* biomass and hence less export of organic material to the deeper layers of the water column and benthos. This benthic community, in turn, is suggested to have an impact on the higher trophic levels of the marine food web [NIELSEN, 2005]. It is tempting to speculate that similar competition mechanisms occur within the food web during the late summer and autumn months.

Toxin-producing species

Four of the first new records of marine dinoflagellates from Disko Bay species are potential or confirmed toxin producers (Table 3.1).

According to HANSEN *et al.* [2001], *Prorocentrum micans* is capable of forming "red tides" *i.e.* a bloom of carotenoid-containing cells, which can lead to harmful events such as mass death of invertebrates and fish [GRAHAM & WILCOX, 2000; HANSEN *et al.*, 2001]. Many species of the genus *Prorocentrum* are toxin-producing [HALLEGRAEFF *et al.*, 2003, WWW.BL.KU.DK/IOC/GROUP2.ASP]; however, the toxicity of *P. micans* remains to be confirmed [HANSEN *et al.*, 2001].

Alexandrium minutum has not previously been detected in the polar marine ecosystem [HANSEN *et al.*, 2003b]; hence, the present survey includes the Northern-most registration of

this species. Secondary metabolites produced by *A. minutum* as well as *A. tamarense* are known to cause paralytic shellfish poisoning, an illness that is caused by a large group of naturally occurring and potent neurotoxins collectively known as PSP toxins. These act through the blocking of Na⁺ channels in excitational cells of the muscles and nervous system, and thereby cause paralysis *etc.* of the consumers of PSP-contaminated shellfish. The devastating effects can be seen on both marine animals as well as humans [HALLEGRAEFF *et al.*, 2003].

Dinophysis acuta, another of the first records for the area, is considered the most toxic species within the DSP-producing *Dinophysis* genus [HANSEN *et al.*, 2001; THRONDSSEN *et al.*, 2003]. DSP, diarrhetic shellfish poison, is a complex of organotoxins causing symptoms similar to gastric bacterial infections. The poisoning is not lethal but some of the toxic components are suspected to induce stomach cancers [HALLEGRAEFF *et al.*, 2003].

In summary, the changes in dinoflagellate species composition may exert several effects on the Arctic marine food web, since it might distort the balance between the lower trophic levels and lead to higher accumulation of toxins in the marine invertebrates through an increased diversity of toxin-producing species; especially if the toxic dinoflagellates form massive blooms as in other parts of the world, including Denmark.

4.2. Suggested re-instatement of the genus *Phalacroma*

The species *Phalacroma* cfr. *braarudii* was encountered at several stations during the present survey (Table 3.1) as well as in 1994 and 2002 (Table 4.1). However, in both of the previous surveys the same species was described as belonging to the genus *Dinophysis*, *i.e.* *D.* cfr. *braarudii* Nordli, 1951. This illustrates the confusion that has surrounded these genera over the last 125 years [SCHILLER, 1933; GRØNTVED & SEIDENFADEN, 1938; TAYLOR, 1976; DODGE, 1982; STEIDINGER & TANGEN, 1997; THRONDSSEN, 2003].

In 1883, Stein proposed *Phalacroma* as a second genus within the Dinophysiaceae (Paragraph 3.1.2) with *P. porodictyum* as the type species. The erection of this genus was based on the presence of a more prominent epitheca than what is observed for species of the *Dinophysis*. Another genus within this family, *Pseudophalacroma*, was added in 1923 by Jorgensen, who also divided *Dinophysis* and *Phalacroma* into ten sections [DODGE, 1982], further supported by the observations by SCHILLER [1933]. The morphological characteristics used by SCHILLER to distinguish *Phalacroma* from *Dinophysis* were the size and angle of the cingular lists, whereas the shape and size of the epitheca as well as other features could vary considerably [SCHILLER, 1933]. Based on the plate formula the mentioned generic separation was questioned by TAI & SKOGSBERG in 1934: "It is far from impossible that future investigators will decide upon the union of all these forms under a single genus, *Dinophysis*" [TAYLOR, 1976]. According to DODGE [1982], all of the previously described *Phalacroma* species were included in the genus *Dinophysis* by ABÉ in 1967 [DODGE, 1982].

The confusion is maintained in recent literature, and many *Dinophysis* species are supplied with *Phalacroma*-synonyms [STEIDINGER & TANGEN, 1997; THRONDSSEN *et al.*, 2003]; in general, the former *Phalacroma* species are non-photosynthetic [TAYLOR, 1987]. According to STEIDINGER & TANGEN [1997] the genera *Dinophysis* and *Phalacroma* can be distinguished on behalf of the development and direction of the cingular lists, since the anterior list is distinctly funnel-shaped for species of the *Dinophysis*. Another character in which the genera differ is the height and shape of the epicone, which is more prominent and semi-globular in *Phalacroma* [STEIDINGER & TANGEN, 1997].

4.2.1. Phylogeny

The phylogenetic analysis revealed *Phalacroma* as forming a sister taxon to a well-supported clade of *Dinophysis* species (Figure 3.2). This taxonomical relationship was further supported by estimates of sequence divergence between the LSU D1 and D2 domains of *Phalacroma* and all of the taxa constituting the *Dinophysis* clade. Among the 648 bp comprising the D1 and D2 domains of the LSU gene, the D2 is usually hypervariable but appears highly conservative within the *Dinophysis* clade (Table 3.2 and data not shown). Depending on the model applied, the divergence of *Phalacroma* and the *Dinophysis* clade lies within a range of 20.5-26.6 % (Paragraph 3.3). Since a 20 % difference between LSU genes is enough to indicate a higher taxonomic level, *i.e.* two different genera [NIELS DAUGBJERG, pers. comm.], we hereby suggest the re-instatement of the genus *Phalacroma*.

Most surprisingly, the species *D. rotundata* is not included in the *Dinophysis* clade, but forms a sister taxon to *Phalacroma* and the "genuine" *Dinophysis* species (Figure 3.1); hence, this species has diverged as the earliest in the evolution of the *Dinophysis*-containing clade. The position of *D. rotundata* is also indicated by the sequence divergence, with estimates within the range of 16.4-19.6% depending on the model applied. In comparison to the distance estimations for *Phalacroma* and the "true" *Dinophysis*, it appears that *Phalacroma* has separated from *Dinophysis* before *D. rotundata* (Table 3.2). However, application of the Bayesian analysis which takes the evolutionary distances into account by weighting nucleotide transversions over transitions, reveals that the divergence of *D. rotundata* has occurred prior to the separation of *Phalacroma* from the "true" *Dinophysis* (Figure 3.2).

Despite these exciting issues it must be taken into consideration that the results concerning *D. rotundata* are based on a single sequence of 648 bp; hence, confirmation of this indication is required in the form of additional molecular sequence data, *i.e.* inclusion of additional and longer DNA sequences of *D. rotundata* in order to validate that the differences are not simply due to intraspecific variation. The conduction of a taxonomical revision demands more than molecular data, but the morphology of the members of Dinophysiaceae has already been studied intensively without resolving the taxonomical issues [SCHILLER, 1933; GRØNTVED & SEIDENFADEN, 1938; TAYLOR, 1976; DODGE, 1982; STEIDINGER & TANGEN, 1997; THRONDSSEN, 2003]. A supplementation of the existing data with comparison of ultra-structural features might help to sort out the taxonomical problems concerning *D. rotundata*, *Phalacroma* and *Dinophysis*.

4.2.2. Comparative morphology

When comparing the plates 2-7 (Paragraph 3.1.2), a distinct character that separates *Phalacroma* and *D. rotundata* from the 'true' *Dinophysis* species is the size of the epitheca; a feature, which is visible both in LM and SEM. Also the cingular region seems to vary. The "true" *Dinophysis* have wide, inclining to funnel-shaped cingular lists, which gives the impression of a narrow and excavated transverse furrow (Plates 2.5). In contrast, the cingular lists are more narrow and angled to a lesser extend for both *D. rotundata* and *Phalacroma* (Plates 6 and 7). This is interesting in the context that KOFOID & SKOGSBERG [1928], who adopted the *Phalacroma* sections suggested by Jorgensen (1923, see above), refer to the second of these sections as *Rotundatum*, comprising 12 species [SCHILLER, 1933]. Even in recent literature, *D. rotundata* can be found described as *Phalacroma rotundata* [STEIDINGER & TANGEN, 1997].

One of the morphological characteristics previously used as delimitation between genera within the Dinophysiaceae was the size ratio between the epicone and hypocone, excluding the cingulum [TAYLOR, 1976]. Apparently, the authors of the past and present who consider

Dinophysis as the only genus of Dinophysiaceae have underestimated the importance of the size and shape of the epicone as an identification-feature.

In summary, molecular data support STEIN [1883] in his perceptions concerning the existence of two genera within the Dinophysiaceae. Furthermore, we believe to have found indications of yet another genus in this family. Additional studies addressing this issue are clearly needed.

4.3. *Ceratium arcticum* versus *longipes*

Several times during the Arctic field course 2006 did the question arise, of whether a *Ceratium* specimen under the microscope was a *Ceratium arcticum* or *C. longipes*. Hence, different approaches were attempted to address the question on the form variations between these species.

Apparently, there is a higher density of *C. longipes* than *C. arcticum* in the waters of this area (Chart 3.1); however, the number of specimens counted was not high enough for a difference in frequency to be supported by statistics. In general, both of the species showed a patchy distribution, which is typical for pelagic planktonic organisms since they are mostly controlled by water currents and the availability of nutrients [NIELS DAUGBJERG, pers. comm.].

Due to the markedly similar morphology (Paragraph 3.4.1) the identification and counts are associated with a degree of uncertainty, since many specimens could not readily be conferred as belonging to either of the two species. As described in paragraph 1.3 GRAN [1902] determined that great variation in anterior horn-angles occur within *C. longipes* (Figure 1.8). If, as GRAN suggested, all of the diverging specimens had been conferred to *C. longipes*, the frequency and distribution of the two species would appear much more uneven, *i.e.* with a higher ratio of *C. longipes* : *C. arcticum*.

Morphometrical data including the two species and the diverging specimens should have been obtained to either confirm or reject the statement of Gran (1902) that the angles between the antapical horns are normal distributed for both of the two *Ceratium* species [GRAN 1902]. These data might have contained indications on whether the specimens encountered were highly diverging or rather two types of *Ceratium* with many intermediate forms, *i.e.* to revive the question of whether *Ceratium longipes* and *C. arcticum* are in fact two different species or rather form variations of the same (Paragraph 1.3). Unfortunately, time did not permit a thorough morphometrical analysis.

Molecular data on *Ceratium* was not obtained, since the PCR resulted in the amplification of more than one DNA fragment, which made sequence determination impossible. This could be explained through contamination of the samples, but is highly unlikely due to the careful isolation procedure (Paragraph 2.7). Another possibility is amplification of DNA fragments from possible food items of dinoflagellate origin. In general, single cell PCR is a difficult technique, often with a low success rate. DNA sequence data for the *Ceratium* species from Disko can, nevertheless, still be obtained, since single-cell PCR and sequence determination can be performed by isolating cells from the Lugol-fixed material.

4.4. Methods

Overall, more species have been identified in this survey compared to those conducted previous in this area, but apart from the arguments given in Paragraph 4.1, the 50 listed species are clearly an underestimate of the species diversity in the vicinity of Disko Island; neither dinoflagellates forming part of the microbenthic community nor the nanoplankton (<20 µm) have been examined.

Compared to the surveys of 1994, 1998 and 2002 we have identified relatively few athecate dinoflagellates in comparison to thecate dinoflagellates (Table 4.1). However, several more athecate specimens were encountered but due to technical difficulties insufficient such as LM photo documentation we have been unable to identify these. In particular, information on the nucleus and cingulum is missing for many specimens, and these as well as the shape of an apical groove, if present, are essential characters for identification of athecate dinoflagellates. Characterisation of the apical groove requires SEM, but, unfortunately, we were not able to identify any athecate species in SEM either; simply because the Lugol fixation deforms the unarmoured cells. To avoid this and achieve optimal preparations, part of the dehydration step for SEM preparation should have been performed immediately after sampling [GERT HANSEN, pers. comm.].

Many of the thecate species detected in this survey were identified in SEM only: *Alexandrium minutum*, *A. tamarense*, *Pentaparsodinium dalei*, *Gonyaulax alaskensis*, *G. digitalis*, *Preperidinium meunieri*, *Protoperidinium pentagonum*, and *P. subinermis*. In addition, two unidentifiable *Protoperidinium* species were detected with this technique. Thus, the application of SEM has expanded the species list for the 2006 survey by almost 20%.

The use of Calco flour/white would have eased the identification of thecate species in Greenland and on the fixed materials and, hence, decreased the fraction of species identified in SEM only. For instance, *Alexandrium minutum*, of which few specimens were detected at a single station, might have been encountered in LM combined with calco flour white, since the narrow 6'' and the overlapping growth zones (Plates 11 and 12) would hence have been readily apparent. Unfortunately, due to limitations of the digital camera used in Greenland the Calco flour/white-technique could not be applied on live material.

A practical explanation for the variations in species diversity and the species encountered at the various stations (Table 3.1) may be found in the different amounts of time invested in analysis of the samples. For instance, material from stations 2 and 8 were never analysed in SEM, which is reflected by the composition of the species lists (Table 3.1). Also, the variation in the viability of the live material after sampling was a limiting factor for the LM analysis in Greenland, and was highly connected to the transport time, density of cells in the samples and the storage temperature. Clearly, the facilities at Arctic Station were not adjusted for cooling the laboratory to the desired 4°C, especially with 4-5 persons analysing samples in it; this became evident when the air conditioning broke down and the room temperature increased to 13°C within a few minutes.

The morphometric measurements data in Paragraph 3.1. are insufficient results and can not be used as representative for the described species without other features, since only the photographed specimens were measured. Hence, the naturally occurring variation in morphometric parameters within a species was not taken into account. In addition to the intraspecific variation, several abiotic factors are known to influence the pace of cell division, and since newly divided cells are typically smaller than older ones, this is another element that makes the morphometric data insufficient. [DAUGBJERG & MOESTRUP, 1992; CHAPMAN & PFIESTER, 1995]. In addition, many of the species that are capable of reproducing sexually (Figure 1.9) form gametes which closely resemble the vegetative stage but are usually smaller. Also, phagotrophic behaviour is most likely to result in marked size variation of heterotrophic dinoflagellates. Special attention should be taken when morphometric data are obtained from some of the larger athecate species, such as *Gymnodinium gracile* (Paragraph 3.1.4 and Plate 25), since their shape may change due to the pressure from the coverslip. Hence, morphometric measurements ought to be obtained from several specimens from each sample, and without coverslips when measuring larger cells.

Although we have worked intensively with this survey, we did not accomplish everything we had in mind, especially concerning the phylogeny of the Arctic marine dinoflagellates. More molecular data are required for support of the separation of *Phalacroma* and especially *D. rotundata* from the *Dinophysis*, but also to help elucidating the problems concerning *Ceratium arcticum* and *C. longipes*. The phylogenetic analysis comparing the dinoflagellate populations in Danish waters and in the vicinity of Disko Island based on ITS-sequences has not been conducted, but time did not permit us to amplify and sequence more DNA. However, we still have 100+ tubes with single cells, including *D. rotundata*, on which PCR and sequence determination of the LSU and ITS sequences can be performed.

5. FUTURE PERSPECTIVES

This survey is the first attempt to perform systematics in the vicinity of Disko Island within a modern context. Several of the species encountered are previously undetected in this area, which we, as mentioned, perceive as indications on variation in the species composition of the dinoflagellates. These alterations could be indicative of global climate change, hence it is most important to continue the surveillance of the phytoplankton community within this area. An understanding of the profound changes in the marine ecosystem in Greenland is crucial, since the Greenlandic main export consists of fish and seafood. Hence, a change in structure of the marine Arctic food web together with introduction of additional toxin producing species could have dramatic consequences for the entire Greenlandic society. Further quantitative and qualitative investigations are required.

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- [HTTP://WWW.UCOR.EDU/NEWS/RELEASES/2006/ARCTIC.SHTML](http://www.ucor.edu/news/releases/2006/arctic.shtml)
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APPENDIX 1

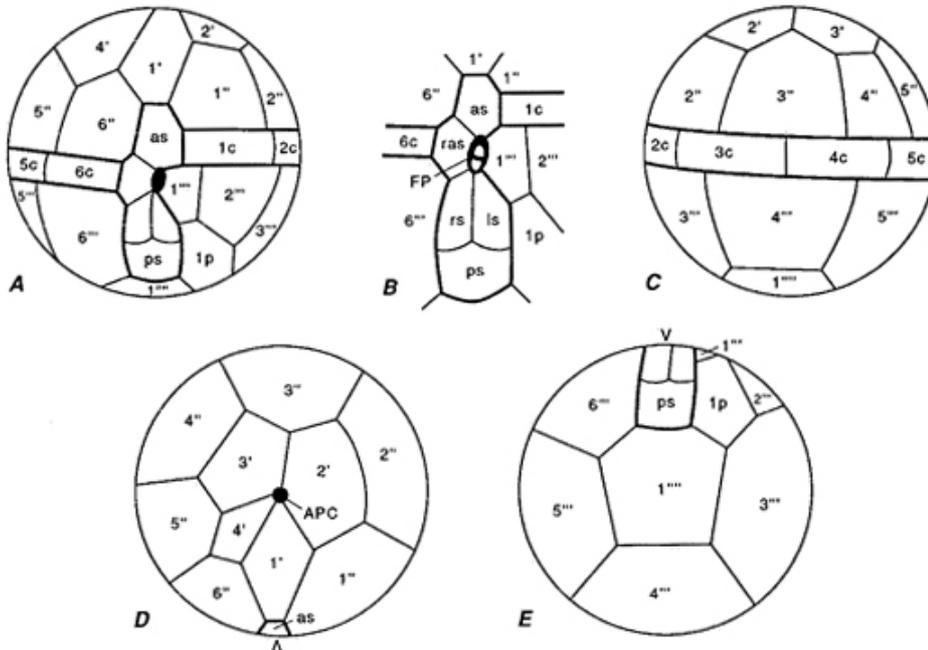


Figure 1. The standard gonyaulaclean Kofoid tabulation. **A.** Ventral view. **B.** Midventral detail. **C.** Dorsal view. **D.** Apical view. **E.** Antapical view. APC-apical pore complex, FP-flagellar pore, VP-ventral pore. ' -apical plate, a-anterior intercalary plate, ''-precingular plate, s-sulcal plate, c-cingular plate, '''-postcingular plate, p-posterior intercalary plate, ''''-antapical plate.

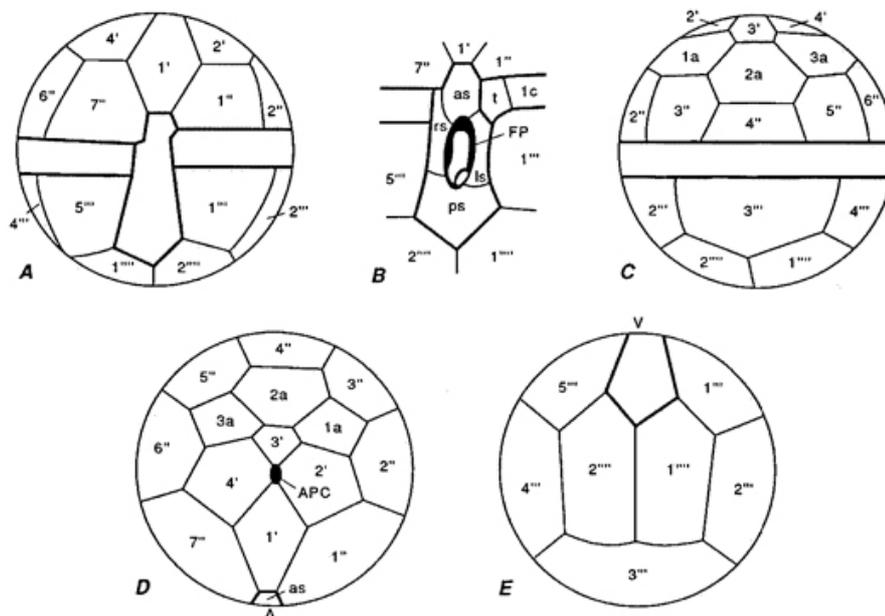


Figure 2. The standard peridinialean Kofoid tabulation. **A.** Ventral view. **B.** Midventral detail. **C.** Dorsal view. **D.** Apical view. **E.** Antapical view. APC-apical pore complex, FP-flagellar pore, VP-ventral pore. ' -apical plate, a-anterior intercalary plate, ''-precingular plate, s-sulcal plate, c-cingular plate, '''-postcingular plate, p-posterior intercalary plate, ''''-antapical plate.

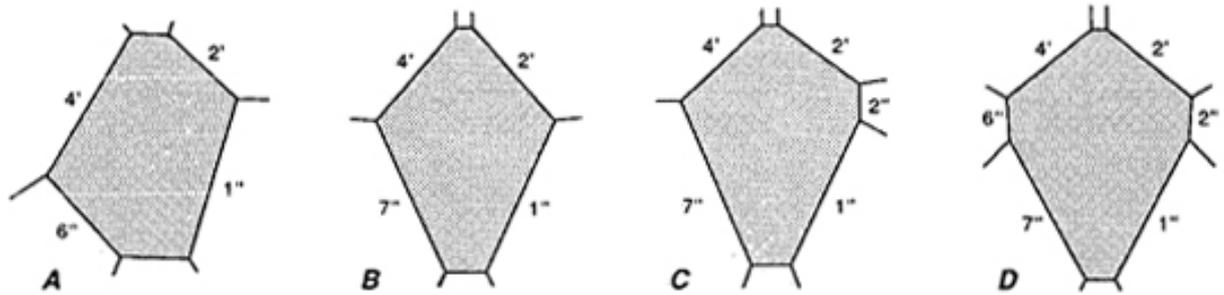


Figure 3. The first apical plates and its homologues. **A:** Gonyaulacales. **B-D:** Peridinales. **B.** Ortho, four-sided plate which contacts two precingulars. **C.** Meta, five-sided plate which contacts three precingulars. **D.** Para, six-sided plate which contacts four precingulars.

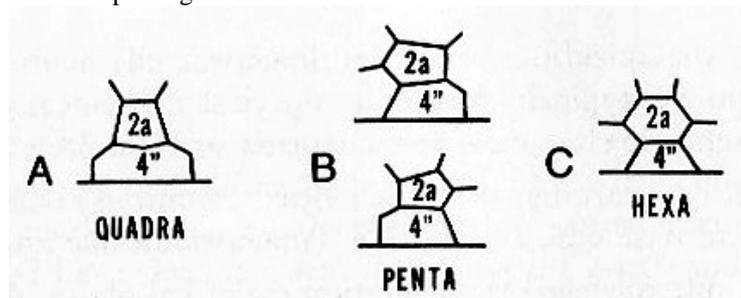


Figure 4. Variations of the second anterior intercalary plate (2a). **A.** Quadra; four-sided plate. **B.** Penta; five-sided plate, which can be twisted to the left or right side. **C.** Hexa; six-sided plate.

APPENDIX 2

The number of *Ceratium* specimens collected per sample volume at the various stations.

Station	<i>C. longipes</i>	<i>C. arcticum</i>	Intermediate	Sample volume (ml)
2	134	83	55	3745
4	44	16	25	1980
5	56	35	15	5990
6	43	26	10	3550
7	35	41	12	1950
8	17	17	7	3990
9	21	16	16	5840

The Rhodoliths of Disko Fjord, Greenland: First visual record of the *Lithothamnion glaciale/tophiforme* (Corallinales, Rhodophyta) aggregation in Disko Fjord, 69°N, Greenland

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Abstract. Unattached calcified red algae, often termed ‘maerl’ or ‘rhodoliths’ are present on the sea floor near the settlement in Disko Fjord, Disko Island, Greenland. Despite occasionally being caught by fishermen, and a few scientific samplings over the years by the use of Van Veen grabs, this Greenlandic occurrence have not received much attention in the literature. In August 2006, these aggregations were inspected by SCUBA diving for the first time. A habitat with a high abundance of rhodoliths and heterogeneity in their distribution was observed. The morphology, distribution, and fauna are compared with rhodolith beds at similar latitudes and these habitats are generally alike. The size of these branched rhodoliths is among the largest in the world and may indicate an area with ideal conditions for their growth and survival.

Keywords. Rhodolith, maerl, *Lithothamnion*, Disko Fjord, Greenland

1. INTRODUCTION

In 1962, a porous and calcareous boulder-like structure was collected in Disko Fjord, Greenland, and it have been kept at the “Arctic Station” with its original note stuck to it, saying “Coral”? It turned out to be an unnoticed variety of the encrusting red algae *Lithothamnion glaciale* Kjellman, 1883 or *L. tophiforme* (Esper) Unger, 1858 forming these ball-shaped structures with a diameter of more than 10 cm.

The existence of such unattached non-geniculate coralline algae is not new to science, as they for more than a hundred years have been known to cover large areas of the sea bed [KJELLMAN, 1883; WEBER-VAN BOSSE & FOSLIE, 1904]. They are known from sub-tidal sea-beds all over the world, from the tropics to the high Arctic [BOSENCE, 1983a; FOSTER, 2001]. For a thorough overview of the topic, see the reviews by BOSENCE [1983a; 1983b] and FOSTER [2001] combined with the extensive study by FREIWALD & HENRICH [1994] on morphology, sedimentary dynamics, growth, carbonate production, distribution and associated fauna, as well as a supplemental issue of Aquatic Conservation: Marine Freshwater Ecosystems [2003] with the main focus on conservation.

The terms “maerl”, “rhodoliths” and various spellings thereof have been applied to these free-living coralline nodules, depending on whether they had a non-algal core, but also differing between geologists and biologists as well as Europeans and Americans. Today, ‘rhodolith’ is widely used for any nodule comprised predominantly of coralline algae, and is partly synonymous with maerl, which is used mostly for unattached branched crust of irregular shape that consists wholly of coralline algae [FOSTER, 2001; DONNAN & MOORE, 2003a].

1.1. Rhodolith formation

Rhodoliths can be formed by several genera in the Rhodophyta (fx. *Lithothamnion*, *Phymatolithon* and *Litophyllum*) [ADEY & MACINTYRE, 1973] and several species may be present in the same area or even on the same rhodolith [RIOSMENA-RODRÍGUEZ *et al.*, in press; FOSTER, 2001]. To understand the formation of rhodoliths, it is necessary to know about the

growth of non-geniculate coralline algae. The species have different growth forms, but the plasticity also varies greatly within the species, depending on the environment and age [RIOSMENA-RODRIGUEZ *et al.*, 1999].

The three overall forms are: a smooth flat crust, crusts with knobby protuberances, or a variety of branched forms. Species may thus progress from a smooth crust to one with protuberances that may later develop into branches [KJELLMAN, 1883; FREIWALD & HENRICH, 1994; ADEY *et al.*, 2005] or it may remain in an intermediate stage. They all grow faster along their margins, as they here get the best surface to energy investment ratio, which is important for their photosynthesis. For those with three-dimensional growth form (as opposed to flat) this means outward and upward [FREIWALD & HENRICH, 1994]. Branching forms have the risk of breaking, and in these situations they respond by increased intercalary growth of the branches near breaking point [FREIWALD & HENRICH, 1994]. This is important in increasing the sphericity of the rhodoliths.

Free-living rhodoliths may have different origins and the formation go through several stages. If coralline spores settle on a mobile substrate such as shells, coral rubble or small pebbles, they may form nucleated or “massive” rhodoliths by overgrowing their substrate [KJELLMAN, 1883]. The early stages of nucleated rhodoliths takes shape according to their nucleus and gradually become spheroidal or discoidal as crust thickness increase [SCOFFIN *et al.*, 1985]. The resulting shape often depends on how much they tumble around.

If the coralline spores instead settle on a stable substrate such as rocks, they may grow and gradually develop into branched grows, often as hemispheroid “heads” [FREIWALD & HENRICH, 1994]. Strong wave action, such as during storms, may cause these heads or branches to break. The breaking may be facilitated by larger size, *i.e.* larger surface compared to the size of the basal attachment. Or if the most basal branches are dead and have been affected by bioerosion. They become rhodoliths at time of detachment, and as they are photosynthetic, nothing directly hinders them in continuing to live the place they are redeposited to.

A variation of this method of formation is at places with a reefal framework of non-geniculate coralline algae. These create “build-up derived” rhodoliths whose formation is fairly identical, but the subsequent facies distribution differ as the hydrographic regimes are dissimilar [FREIWALD & HENRICH, 1994]. Such reefal coralline algal build-ups are rare though.

A third method of formation is by fragmentation of the rhodoliths themselves. During physical disturbance of the rhodoliths, through wave action or perhaps bioturbation, some of their branches may break. Such a newly detached branch will often continue its growth and gradually grow towards a more spheroid shape. This type of internal recruitment may be a very important, or perhaps major, contributor of rhodoliths in a rhodolith bed [FOSTER, 2001].

Several factors affect the shape and survival of rhodoliths. Light penetration through the water may affect both rhodolith shape as well as maximum depth for survival. Rhodoliths are generally more openly branched where light levels are low, in order to reduce self-shading, while a dense structure is possible where light is plenty [FREIWALD & HENRICH, 1994; FOSTER, 2001].

Rhodoliths are very vulnerable to being covered by fine sediments. A study showed that it was more likely due to restricted gas exchange than to lack of light, as total darkness did not have as detrimental effects [FREIWALD & HENRICH, 1994]. Rhodoliths thus require a sufficient level of water motion to prevent their thalli from being smothered with silt or being permanently stuck in the substrate. Moderate or strong currents, or sometimes wave action, are therefore a prerequisite to prevent or remove sediments fouling or burying the rhodolith [BOSENCE, 1976; FOSTER *et al.*, 1997; MARRACK, 1999].

Spheroid rhodoliths are even able to maintain living tissue all around, possibly due the prevailing currents constantly redepositing the sediments around them [SCOFFIN *et al.*, 1985]. This may even rotate them in small increments [FREIWALD, 1995] although tidal currents in general does not move the rhodoliths [FREIWALD & HENRICH, 1994; MARRACK, 1999] except perhaps in habitats of intertidal rhodoliths. Bioturbation or occasional storms, however, assist in the turning and replacement of rhodoliths over time so that the sides change their exposure to optimal and adverse conditions of light and water exchange [MINNERY, 1990; FREIWALD & HENRICH, 1994; FREIWALD, 1995; MARRACK, 1999]. Rhodoliths contain large starch reserves, possibly supporting them during winter at high latitudes or other unfavourable conditions [FREIWALD & HENRICH, 1994] and reallocation/transport within the thallus to support unfavourable areas of growth has also been suggested [LITTLER *et al.*, 1991].

Another important factor of water movement is the risk of physical damage. Frequent and strong wave action will effectively break the branches too often for the rhodoliths to ever increase in size. These places will instead have kelp beds or other tolerant communities. Weaker and less frequent waves may have small and dense rhodoliths, while protected or deeper sites have a reduced wave impact and allows more openly branched forms. This last hydrodynamic regime is where we most commonly find rhodoliths. Here the occasional storm may result in fragmentation and rotation, beneficial to the development of the rhodolith beds.

The largest reported massive (nucleated with smooth or protuberant crust) rhodoliths have a diameter of up to 20 centimetres [MINNERY, 1990] while the largest branched forms may reach the same size in arctic habitats [KJELLMAN, 1883] or even 0.5 m as reported by FOSLIE [1890; 1929].

The reported growth rate of branched rhodoliths in subarctic and temperate waters is a branch extension of 0.05 – 1.5 mm yr⁻¹, which is up to five times lower than the rate from branched tropical rhodoliths [FOSTER, 2001]. But a calcium carbonate fixation rate of 30–1423 g CaCO₃ m⁻² yr⁻¹ is similar to, or even higher than that of subtropical or tropical rhodolith bed, and comparable to the lower rates from tropical coral reefs. This high fixation rate makes temperate rhodolith beds one of the fastest carbonate-producing marine environments in temperate and subarctic waters, facilitated by a very high standing crop when present [FREIWALD & HENRICH, 1994; BOSENCE & WILSON, 2003; BLAKE & MAGGS, 2003; Wilson *et al.*, 2004].

1.2. Distribution and formation of rhodolith beds

Because of these requirements for formation and survival, rhodoliths are only found in places with certain physical conditions and hydrographical regimes, and are usually distributed in different gradients of shape and density, forming so called “rhodolith beds” or “maerl beds”. These may be either pavements or biostromal bank-like structures. Their need for light confines them to coastal areas, at depths depending on a trade-off between water transparency/turbidity, wave exposure, and risk of burial/sedimentation.

Living rhodoliths have been found on intertidal flats [SCOFFIN *et al.*, 1985] and are actively growing down to depths of at least 90 m, with partly living rhodoliths down to 290 m [LITTLER *et al.*, 1991]. These large depths can only be obtained in areas with exceptionally clear water. Around tropical reefs, a high abundance is often found at or below the lowest depth limit of corals, and may perhaps set the lower limit for coral growth by being an unstable substrate, unsuitable for coral spat [FRICKE & MEISCHNER, 1985]. The opposite, that corals exclude rhodoliths from shallower depths, could also be the case. In temperate and colder waters, living rhodoliths are most common at depths of less than 20 m [ADEY & ADEY, 1973; FREIWALD *et al.*, 1991; KONAR *et al.*, 2006]. But the depth limits are species-specific,

depend on many other factors, and extensive live beds have been found deeper than 40 meters [KJELLMAN, 1883; ADEY *et al.*, 2005]

A certain distance to areas with high run-off of terrigenous material, such as fjords, is necessary, but a complex topography in the area will facilitate the settling of *e.g.* silt, and shallow sills are especially good at retaining material that could have an adverse effect [FREIWALD & HENRICH, 1994]. Headlands, narrow channels, or shallow platforms with relatively strong currents are often ideal for extensive beds, as well as sheltered but open areas with some wave action, such as inlets or coastal bays [BOSENCE, 1979; DONNAN & MOORE, 2003a]. Arctic and sub-arctic corallines have proven to be well adapted to the light regimes of the north [FREIWALD & HENRICH, 1994; WILSON *et al.*, 2004] and maximum depth of the beds may thus be determined by lack of water motion, in addition to diminishing light levels, while the shallowest is set by exposure to wave action.

The size of rhodolith beds is usually in the order of hectares [FREIWALD & HENRICH, 1994; PILLER & RASSER, 1996; DONNAN & MOORE, 2003a] although an extensive bed runs along the coast of Brazil, from 2°N to 23°S [KEMPF, 1970].

1.3. Bioerosion and Sediments

The substrate under dense bed of branched rhodolith is often comprised of poorly sorted sediments as the branched framework limits water movement across the sediment layer. A mixture of gravel, mud, skeletal carbonates and other CaCO₃ sediments is often found below and within the lower branches of rhodoliths. The beds are host to many carbonate-secreting invertebrates and together with bioerosion of the inner dead parts by boring sponges, annelids, bivalves *etc.* makes rhodoliths important contributors of calcareous sediments, and this may over time lead to the formation of thick gravel banks [MINNERY, 1990; FREIWALD & HENRICH, 1994; PERRY, 2003; DONNAN & MOORE, 2003a]. For a detailed compositional analysis of sediments within a rhodolith framework, see FREIWALD & HENRICH [1994].

1.4. Fauna

The porous and three dimensional structures of branched rhodoliths create a very heterogenous habitat and are known to host a high diversity, especially of cryptofaunal species living within the interstices [STELLER *et al.*, 2003]. In northern waters there is relatively little epiphytic growth on living algal tissue, but a dense coverage on the inner dead thalli by all types of filter feeders: polychaetes, cirripedes, molluscs and bryozoans. Diatoms and foraminiferans also colonise both living and dead tissue. A dense vagile epifauna is also present, and especially polyplacophorans, sea urchins and brittle stars are abundant, along with gastropods, ostracods, and other crustaceans [FREIWALD & HENRICH, 1994]. In addition, the sediments within and below the rhodoliths contains a rich meiofauna [HIGGINS & KRISTENSEN, 1988; THORSEN *et al.*, 1989]. The absence of larger structures may exclude predators that are usually found at rocky reefs, *e.g.* some fish species [FOSTER, 2001], but it is nevertheless more heterogeneous than sandy or muddy bottoms. They host endemic and rare species [see STELLER *et al.*, 2003] and are also efficient nursery grounds for several echinoderms and molluscs [KAMENOS *et al.*, 2004].

1.5. Conservation

Rhodolith beds have received less scientific attention than the three other major benthic communities covered by marine macrophytes: coralline reefs, kelp beds and forests, and sea grass beds [FOSTER, 2001]. This fourth major habitat may be under great threat due to their strong susceptibility to sedimentation [WILSON *et al.*, 2004] combined with slow growth and

unique requirements for formation. Even small anthropogenic disturbances, whether it is physical destruction, eutrophication, or altered sedimentation, may therefore quickly affect these faunistically rich communities [DONNAN & MOORE, 2003b]. Commercial harvesting of “maerl” for use as soil fertilizers does not represent a sustainable use due to the slow growth, and fisheries trawling may bury them by causing heavy siltation [STELLER *et al.*, 2003].

1.6. Previous studies of rhodoliths in Greenland

Now and then the rhodoliths of Disko Fjord have been caught in nets of the local fishermen but were just regarded as a curiosity. A few studies have looked closer at these algae as well as the fauna of the area, all by the use of a Van Veen grab for sampling. HIGGENS and KRISTENSEN [1988] studied the meiofauna and found a new kinorhynch species, *Echinoderes peterseni*, inside the rhodoliths. THORSEN *et al.*, [1989] studied the Rhodolith growth forms and all macrofauna as well as some meiofauna, while Düwel and Wegeberg [1992; 1994] focused on the *Lithothamnion* morphology and systematics. L. K. Rosenvinge [1883] reports rhodoliths near the settlement Ikamiut in Disko Bay and a few other places, but other Greenlandic occurrences are rare.

2. MATERIALS AND METHODS

A known rhodolith bed is present outside the settlement Kangerluk (Disko Fjord), Disko Island, West Greenland, at 69°29.100N, 53°56.210W (Fig. 1). It was visited using R/V Porsild on the 16 August 2006 as part of the Arctic Field Course 2006 by the University of Copenhagen. A qualitative survey of the area was done by SCUBA diving at depths of 5-14 m. Visual sampling was done by photography and video recording. Physical samples were collected by hand in sealable plastic bags. Additional samples were collected by the use of a Van Veen grab at depths of 8-20 m. Rhodoliths and associated fauna and sediment were treated by various methods (no treatment, freshwater shock or MgCl sedation) and fixated (ethanol, formalin, glutaraldehyde).

All material is stored at the Zoological Museum, University of Copenhagen, Denmark, for further taxonomic studies. A thorough identification and quantification of the fauna was not the aim of this study due to restricted logistics.

3. RESULTS

3.1. Rhodolith distribution

Only a small area in proximity to the Kangerluk settlement was surveyed, and it is therefore difficult to give a confident estimation of the extent of the rhodolith bed. Based on the visual observations and Van Veen grabs from this study, combined with data and positions from previous studies, it appears to be in the order of hectares. Water surface temperature was 10°C with 6°C at 10 meters depth. Salinity was 33‰ and pH 8-9. A strong pycnocline was present in the upper few metres. The bottom was gently sloping down to 10 meters depth where it became almost platform-like with a depth increase of about one meter for each 20 meters further distance from shore.

The substrate underneath and around the rhodoliths consists of mud and gravel topped with an organically rich thin brown layer (Fig. 2a). Many were covered partly by sediments – others not at all. There were many small rocks (< 10 cm) at depths above 8 m, and larger rocks (10-20 cm) in the areas with dense rhodolith cover. Other areas in the 10-20 m depth zone were barren for rocks as well as rhodoliths (Fig. 3a) The rocks were mostly devoid of protuberant crusts and only had “flat” *Phymatolithon*-like crusts, while “foreign” debris, like charcoal (Fig. 4f) and broken glass (Fig. 2a), carried protuberant forms of *Lithothamnion*.

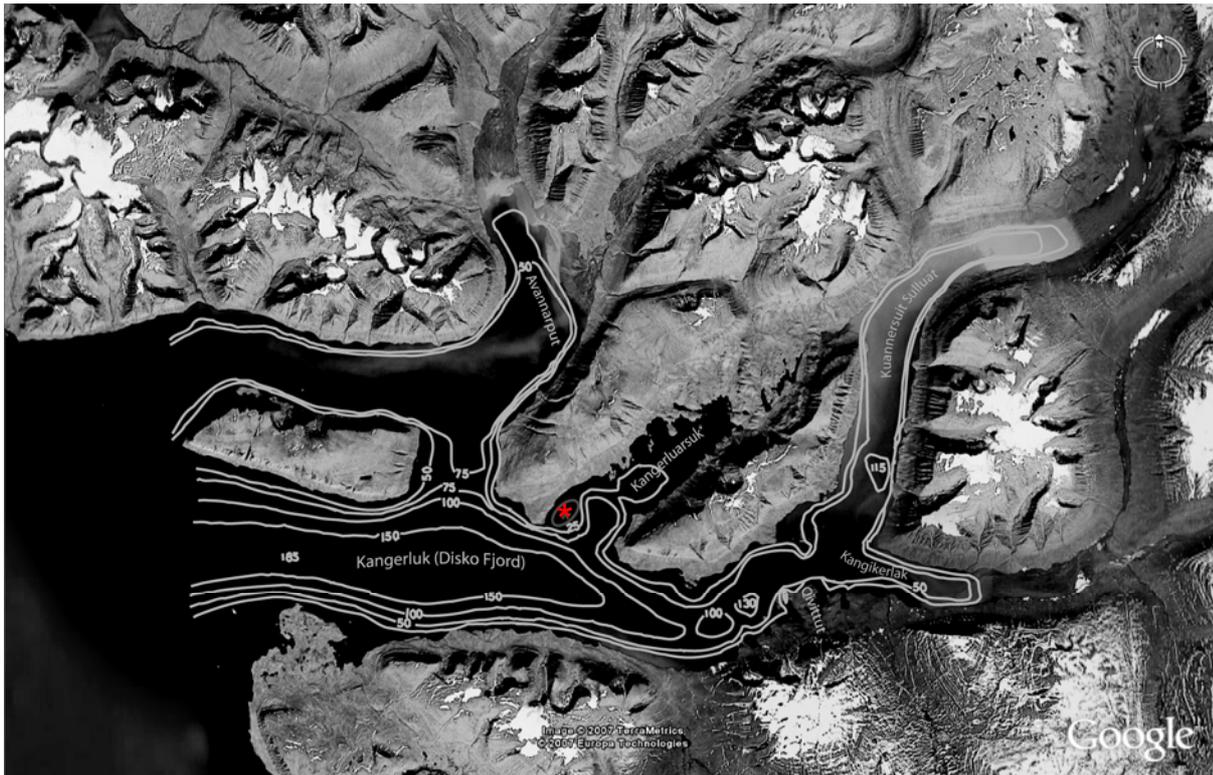


Figure 1. Map of Disko Fjord (Kangerluk). The rhodolith bed is marked by the asterisk *. Bathymetry reproduced from Schmid and Piepenburg 1993, with depth curves in meters. Map reproduced from Google (TerraMetrics).

Rhodoliths were found within a depth range of 6-20 m, but by far the highest abundance was between 9 and 13 m depth. Here they covered most of the seabed (Fig. 2c), although rarely multilayered. But some of these areas with dense cover were merely narrow bars 1-2 m wide and 20-30 m long and adjacent to barren patches with only one or two live rhodoliths per m² and a little amount of dead fragmented thalli. Rhodolith cover declined gradually above 9 m and below 13 m depth.

A large rock, one meter wide and 70 cm tall, was present within the rhodolith bed at 13 meters depth. It had a barren patch next to it with no rhodoliths in a half circle a little smaller than itself. But the same pattern was not observed next to a slightly smaller one. Another observation was an almost one meter deep depression at about 13 meters depth (Fig. 2b). It had a half circle formed ridge, about ten centimetres higher than the adjacent rhodolith bed, on which there are no rhodoliths. A little patch of rhodoliths appear to have tumbled down the side. From the depression and towards deeper water, there was an elongated track devoid of rhodoliths.

3.2. Rhodolith morphology

Morphology and size changes gradually with depth: Small irregular rhodoliths ($d < 5$ cm) and fragmented live thalli are found in the shallowest depths. Large spherical ($d = 5-13$ cm) dense but still porous rhodoliths at 7-13 m, often hollow (Fig. 4a). Between the spherical rhodoliths there are many small branch fragments. At greater depths, the rhodoliths are smaller (3-7 cm) and more fragile openly branched forms (Fig. 4b). Only a few of the “spheroid” rhodoliths were truly spherical – shape varied, but within certain limits and most were of similar size. They were round overall but could have some irregularity in their shape, or be a little elongated or discoidal.

The largest rhodolith collected was an unattached plate of protuberant crust with a diameter of 17 cm (Fig. 4c) while the largest spheroid rhodolith was 13 cm at its longest dimension. The surfaces of the rhodoliths showed some variation. Most had live tissue all around, and only a few larger openings towards their centre, while others apparently were dead on one of their sides - usually the one facing or partly submerged in the substrate. The thallus of larger rhodoliths formed a crust of closely spaced branches, one to one and a half centimetres thick, surrounding a hollow interior with a volume in tens of cubic centimetres. The inner side was usually dead or at least bleached of pigmentation. Large holes towards the interior were common and appeared to be from physical destruction. The only exception from having a hollow interior was specimens with live branches growing inwards, often towards a hole on the other side of the rhodolith.

The combined volume of “protected space” in this habitat thus seem much larger than between the holdfasts in kelp forests, rocks and pebbles in stone reefs, or any seagrass bed or sediment flat. Coral reefs have an enormous standing stock with a vertical extension far beyond of the flat rhodolith beds. But live corals rarely offer a matrix with such small pores, and are often aggressive towards epibionts. The layers of coral rubble can provide a lot of habitat, but perhaps with a longer distance to the free water mass.

3.3. Fauna

A brief characterisation of the fauna observed and collected is here presented. As observations are sparse, they may not be representative of the entire rhodolith bed, but are nevertheless an accurate account of the area surveyed. See *e.g.* THORSEN *et al.*, [1989] for an extensive species list from a previous study.

The macrofauna is dominated by echinoderms and molluscs inhabiting the internal voids as well as being on top and in between rhodoliths. Brittlestars (primarily *Ophiopholis aculeata* L., 1967) are very conspicuous in the areas with large rhodoliths, extending their arms upwards thereby creating what appears to be a forest of arms (front-page). They are still present in areas with small rhodoliths and crust, but in low numbers and not lifting their arms from the substrate. The sea urchins *Strongylocentrotus droebachiensis* Müller, 1776 and *S. pallidus* Sars, 1871 are omnipresent in high densities on all substrates, but a barren patch showed an even higher density of large specimens (Fig. 3a), while there is a high number of small sea urchins among the rhodoliths. Sea stars and sun stars (*Leptasterias polaris* Müller and Troschel, 1842 and *Solaster papposus* L., 1767) are also common, and juveniles are found between rhodoliths. The chitons *Tonicella rubra* L., 1767 and *T. marmorea* Fabricius, 1780 were all over the rhodoliths and many were juveniles. Two species of large sea anemones were present within the dense bed, *Urticina* sp. Ehrenberg, 1834 (front-page) and *Metridium senile* L., 1761 (Fig. 3b) while the tube-dwelling anemone *Cerianthus* sp. Delle Chiaje, 1830 was in the adjacent areas. The bivalve *Hiatella arctica* L., 1767 was often fixed inside the rhodoliths (Fig. 5b), while other species of bivalves and gastropods were less abundant. The area also contains plenty of empty bivalve shells. Although sparsely investigated, the crustaceans were not very conspicuous, and only a large spider crab *Hyas araneus* L., 1758 were observed at 6 m depth, while amphipods were present in the collected rhodoliths. Each rhodolith usually contained a few sipunculids (Fig. 5c) and one or two species of large nemertean (Fig. 5d). A diverse polychaete fauna was found inhabiting the rhodolith surfaces, with Polynoidae being the most conspicuous family. The framework is host to a large variety of sessile growth forms such as sponges and colonies of bryozoans and hydrozoans mainly inhabiting the dead parts. In addition, the surfaces are rich in epiphytic foraminiferans and diatoms but no further studies were done. The meiofauna of the sediments within and beneath the rhodoliths have not yet been identified.

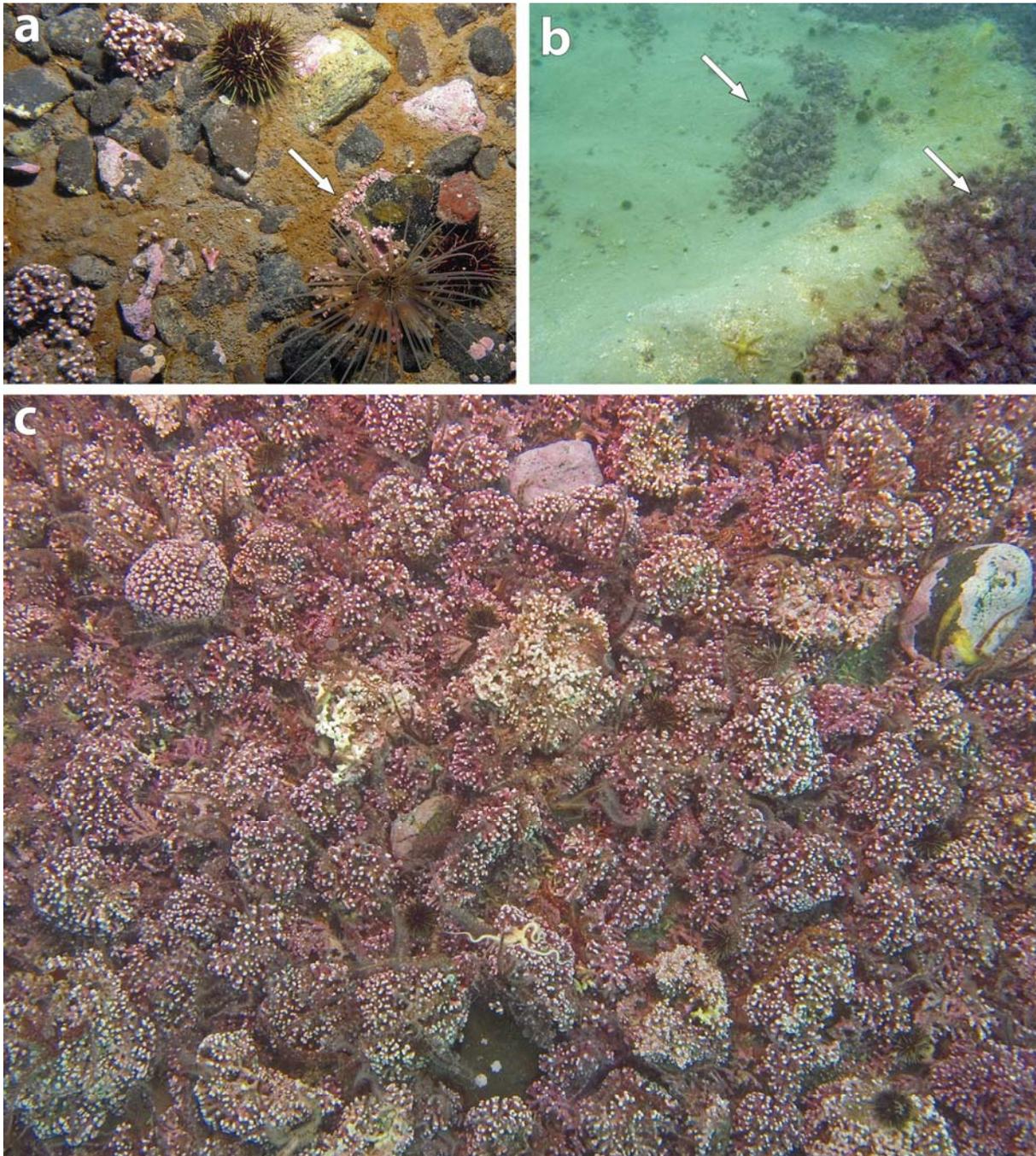


Figure 2. Rhodolith habitats. **A.** Shallowmost area of the rhodolith bed (6-7 m). The sediment is covered with a thin brown layer of organically rich material. Small rhodoliths are present, while a thin laminate algal crust is present on some of the pebbles. A piece of glass carries a more protuberant algal crust (arrow). **B.** A hollow in the sea bed within the area of dense rhodolith cover, about 2 m wide. A little patch of rhodoliths have tumbled down the side (arrow). The other arrow indicate a rhodolith with its dead area (white) facing upwards. **C.** Dense rhodolith cover at 11 m depth, with many partly broken rhodoliths.

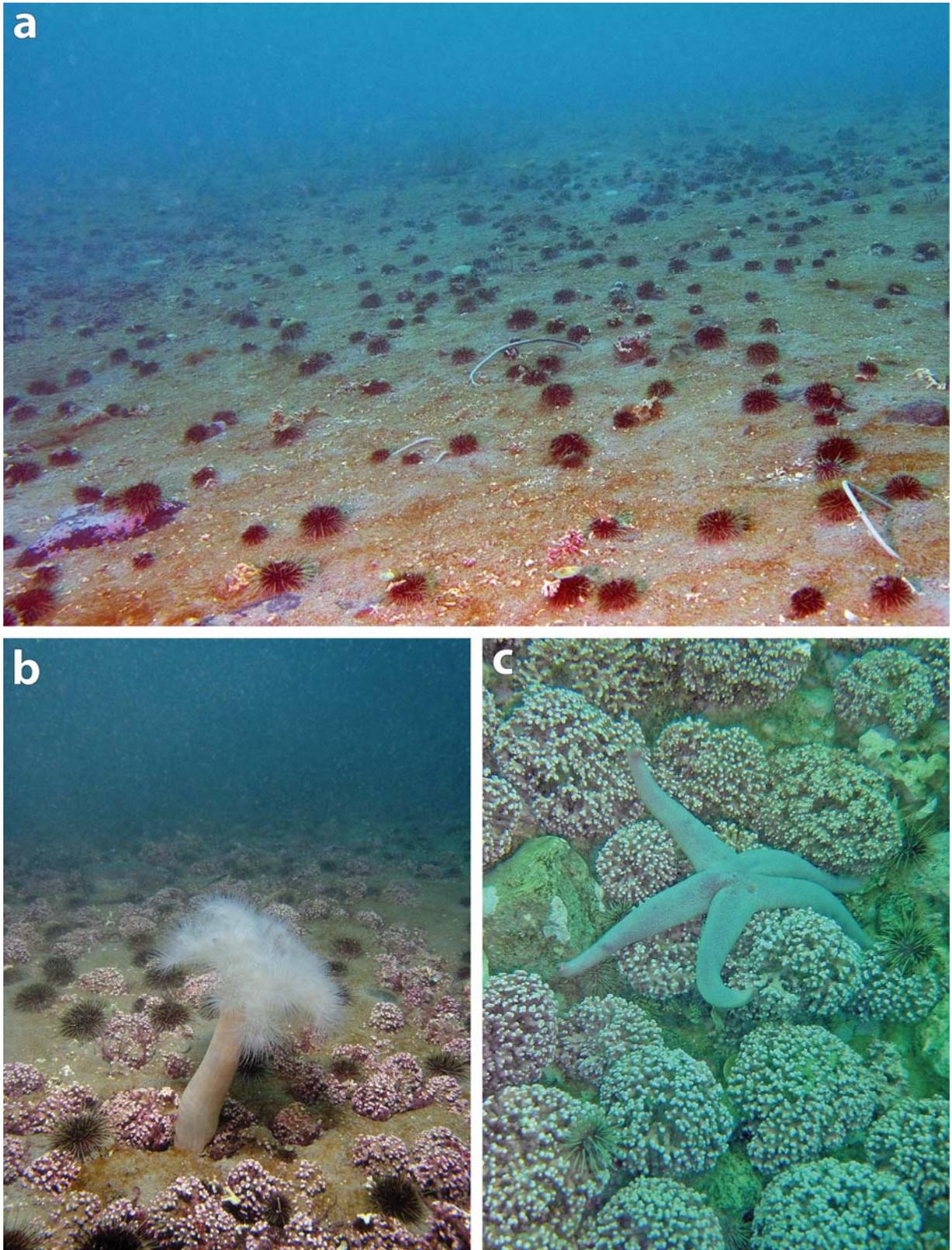


Figure 3. Rhodolith habitats. **A.** Area adjacent to the dense rhodolith aggregations at 10 m depth. It is barren of rhodoliths, and heavily populated by the sea urchins (*S. droebachiensis*). **B.** Rhodolith bed at 8 m depth, with rhodoliths partly submerged in the sediment, and a large sea anemone (*Metridium senile*). **C.** A rhodolith bed near Qaqortoq (Julianehåb). Photo by Agnes Mols Mortensen.

An Atlantic or Greenland cod, *Gadus morhua* L., 1758 or *G. ogac* Richardson, 1836, and an Atlantic Halibut, *Hippoglossus hippoglossus* L., 1758, were swimming in the area and a Butterfish, *Pholis* cf. *gunnelus* L., 1758, was caught within a rhodolith.

4. DISCUSSION

The rhodolith beds in Greenland are greatly understudied, and almost nothing is known about their fauna and distribution. Their presence seems to be unnoticed in the scientific community working with such habitats, and is exemplified by the absence of any Greenlandic records on the map updated by Foster [2001] or any recent mentions in the literature. A reason is that the studies on these rhodolith beds have not drawn attention to themselves, as the rhodoliths are not stated in the abstracts or summaries, making it difficult to find them when searching the literature. HIGGENS and KRISTENSEN [1988] only mention it shortly in the body text of their monograph on kinorhynchs, while the other studies have been published in ISBN-numbered university reports in Danish. Several rhodophyte taxonomists are aware of the rhodoliths in Disko Fjord and are often consulted regarding taxonomic questions on nongeniculate coralline algae by other rhodolith scientists, but apparently only very few are aware of the Greenlandic presence still. The accidental discovery of the first rhodolith bed in Alaska in 2004 [see SHIRAYAMA *et al.*, 2004; EUREKALERT.ORG, 2004] received much media attention, and in that way made itself visible to the community. The press release mentions that rhodoliths can be found “near Greenland” but without giving any reference. The subsequent publication [KONAR *et al.*, 2006] omits any rhodoliths “near Greenland” altogether.

4.1. Rhodolith morphology at different latitudes

The term rhodolith encompass a wide range of shapes and species of free-living nongeniculate coralline algae, whose formation and habitat may differ a lot. The rhodoliths of this study belong to the branched forms which are rarely nucleated. Massive nucleated rhodoliths with a smooth crust will thus be disregarded in the discussion below. Although there are some similarities with the fauna and formation of their habitats, the added weight of an internal rock, and the lack of a large interstitial space makes it a very different habitat.

Not all literature is good at stating the physical dimensions of the rhodolith in their studies. Taxonomic papers often omit the gross morphology and focus at the cell level, while many geological papers concentrate on the facies, but don't mention the sizes of their constituents.

4.1.1. Tropical and subtropical branched rhodoliths

Tropical and subtropical branched rhodoliths are difficult to generalise as there are a great number of species and may be found from the intertidal [SCOFFIN *et al.*, 1985] and at great depths of more than a hundred meters [KEMPF, 1970; LITTLER *et al.*, 1991]. Rhodoliths in the Gulf of California have been extensively studied and are dense and spheroid, and most commonly in the size range of 2-7 cm with an average below 5 cm [STELLER & FOSTER, 1995; FOSTER, 2001], similar to the intertidal ones from Rarotonga [SCOFFIN *et al.*, 1985]. But all shapes can be found in the tropics as shown on the plates by Foslie [1929]. The Red Sea even have rhodoliths that are somewhat similar in both shape and size to those of this study – being up to 15 cm at the longest dimension [PILLER & RASSER, 1996].

4.1.2. The Mediterranean

The Mediterranean is a kind of intermediate between the tropics and North-East Atlantic. Its lack of coral reefs makes the rhodoliths more important as carbonate fixators, while the depth

range is similar to the tropics – from less than six meters depth and down to 80 meters or more in the west, or 180 meters in the east [BASSO, 1996]. It has the maerl-type rhodoliths, *i.e.* open irregularly branched crusts that are generally shorter than 5 centimetres, and also small spheroid and nodular rhodoliths as well as large irregular and multispecific lumps with sedimentary fillings [BASSO, 1998].

4.1.3. The North-East Atlantic and other temperate waters

The North-East Atlantic and other temperate waters are in some areas dominated by the maerl-type rhodoliths which form beds along the western coasts of Spain [BÁRBARA *et al.*, 2004], France [GRALL & HALL-SPENCER, 2003], Ireland [DE GRAVE & WHITAKER, 1999], Scotland and England, and Norway [ADEY & ADEY, 1973; FREIWALD *et al.*, 1991]. Larger, and more spheroid rhodoliths are more common in the northern parts, most notably along the west coast of Norway [FREIWALD *et al.*, 1991], and a few places along the coast of Ireland [DE GRAVE & WHITAKER, 1999], Scotland and England. Both growth forms (maerl-type and spheroid-type) are most likely present several other places, as indicated by the distribution of the rhodolith forming species [ADEY & MACINTYRE, 1973; ADEY & ADEY, 1973; ADEY *et al.*, 2005], although the actual growth form (lamine vs. rhodolithic) is rarely indicated in these records.

The depth of such beds are usually less than 30 meters, although confirmed reports exist from down to 50 meters [DE GRAVE & WHITAKER, 1999; ADEY *et al.*, 2005]. There are several reports of beds down to 100 meters in Ireland, but these are still unconfirmed [BRIAND, 1991]. The size of temperate maerl-type and spheroid rhodoliths is less than 10 cm at the longest dimensions, usually in the range of 3-5 cm [ADEY & MACINTYRE, 1973; BOSENCE, 1983b; ADEY *et al.*, 2005].

4.1.4. Subarctic and Arctic rhodolith beds

Subarctic and Arctic rhodolith beds are not rare, but reports are somewhat clustered from scattered localities in the North Atlantic Ocean and the Arctic Ocean. There is an apparent lack of records from the Kara Sea, East Siberian Sea, Chukchi Sea, Beaufort Sea and Bering Sea, while they are common in the Norwegian Sea, Barents Sea and eastern part of the Norwegian Sea [see KJELLMAN, 1883; FOSTER, 2001]. Most well studied are those along the west coast of northern Norway, especially in the Troms Area [KJELLMAN, 1883; FOSLIE, 1890; 1891; 1895; ADEY, 1971; ADEY & MACINTYRE, 1973; FREIWALD *et al.*, 1991; FREIWALD & HENRICH, 1994].

The morphological diversity is great, from the maerl-type to regular and irregular shapes of more spheroid rhodoliths of all sizes, as shown best on the plates by FOSLIE [1929]. The recent discovery of an Alaskan bed at 60°N is the northernmost Pacific record, and had a 50% cover of very openly branched ping-pong ball sized rhodoliths, and similar sized maerl-type rhodoliths (*Phymatolithon calcereum* and an unknown Sporolithacean) at depths of 8 to at least 18 meters [KONAR *et al.*, 2006, RIOSMENA-RODRIGUEZ, pers. comm.]. Kjellman [1883] reports of extensive beds of *L. glaciale* on the west and north coast of Spitzbergen up to 79°56'N, on the coast of Russian Lapland, and on the west coast of Novaya Zemlya. They were usually 15-20 cm large spheroids, sometimes nucleated, and most abundant at depths of 15-35 meters. He also describes *L. soriferum* (today *L. tophiforme*) to be smaller (up to 8 cm), never nucleated, and with early ramification, which resembles other specimens from this study (Fig.4b). It is found in 15-30 meters of water, and according to him, not yet at latitudes above 71°N.



Figure 4. Rhodolith morphology. **A.** Hollow rhodolith from the dense bed at 11 m depth. **B.** Smaller and more openly branched rhodolith from 18 m depth. **C.** Lower side of a 17 cm wide flat plate of *Lithothamnion* sp. from 11 m depth. A growth form common on large rocks. Clear distinction between the dead white thalli and the live red thalli of its upper layers. **D.** Close-up of a fragment broken off C, showing the thickness of the plate (≈ 2 cm) and its dense branching pattern. **E.** Partly broken 13 cm wide rhodolith from 11 m depth, seen from 'above'. Notice the dead white thalli of the inner lumen, and the live thalli, also on the inner side, where the side is broken (arrow). **F.** Charcoal (≈ 5 cm) with a slightly protuberant crust.



Figure 5. Makrofauna from rhodoliths. **A.** Cryptic colouration of the chiton *Tonicella rubra*. **B.** Siphon of the bivalve *Hiatella arctica*. Notice its close resemblance to the knobby ends of the coralline algae. **C.** Sipunculid (*Golfingia* sp.) living within the rhodolith branch matrix with its introvert towards the outer perimeter. Total length \approx 5 cm. **D.** Nemertean common inside the rhodoliths. Total length \approx 12 cm.

Foslie reports a similar depth range for *L. glaciale* in northern Norway and adds that it have occasionally been found at 5-9 meters depth. He also mentions an Icelandic presence, referring to “Strömfelt”. The sizes mentioned for rhodoliths are astonishing, and rarely referred to in the present day literature: a diameter of at least 50 cm for *L. glaciale* in the Arctic sea [FOSLIE, 1890; 1895] and of more than 40 cm for *L. fornicatum* [FOSLIE, 1891] from Troms. Specimens larger than 20 cm at the longest dimension have not been reported since.

There are numerous records of rhodoliths in Norway – from more southern records of dense spheroid specimens in Kristiansund 63°N [FOSLIE, 1895; SNELI, 1968] to the extensively studied areas in Troms, and the northernmost Finnmark [KJELLMAN, 1883; FOSLIE, 1895]. Most characteristic of the Norwegian rhodoliths is that they are rarely of the maerl-type but more often spheroids of sizes around 5 cm, occasionally 10 cm, or fragments of these.

4.1.5. Reports of rhodoliths in Greenland

Reports of rhodoliths in Greenland are sparse except of the few recent mentions. KJELLMANN [1883] refers to specimens collected in West Greenland by Th. M. Fries, while ROSENVINGE [1883; 1898] mentions rhodoliths of *L. fruticosum* and *L. tophiforme* from Sukkertoppen and Qaqortoq (Julianehaab), *L. botryoides* (now *L. glaciale*) from Ikamiut and Aasiaat (Egedesminde) in the Disko Bay, and *L. glaciale* collected by N. Hartz from Danmarks-Ø in Scoresbysund 70°27'N, East Greenland (but whether they formed rhodoliths is not mentioned). Other localities of *L. glaciale* in Greenland are to be found in ROSENVINGE [1883] but so far it has not been possible to obtain this reference. But some of the the specimens mentioned in ROSENVINGE [1883] are included in the Herbarium of Foslie [WOELKERLING *et al.*, 2005] and the plates of FOSLIE [1929] which states them to be from Ikamiut.

The neotype of *L. tophiforme* is on of those collected by Ryberg in Qaqortoq (Julianehåb) and is a 5 cm long specimen of the maerl-type [ADEY *et al.*, 2005]. A recent observation of rhodoliths south of Qaqortoq (Julianehåb) in the Southwest of Greenland, reported here for the first time, is on a rockier substrate (A. M. MORTENSEN, pers. comm.). No studies have been done on these, but their presence is well-known to the locals who find the area to be a nice diving spot due to its lack of macro algae. The underwater photos obtained from the area shows some small areas with more than a fifty percent cover of live spheroid rhodoliths, resting among similar sized rocks interspersed with sediment (Fig. 3c). Large bedrock surfaces are also present in the area and carry a few large plates of protuberant coralline crust, several centimetres thick. These protuberant plates appear to only have a few places of attachment and thus likely to be detached by physical disturbance.

4.1.6. The rhodoliths of Disko Fjord

The rhodolith of Disko Fjord are generally irregularly rounded, with some constructional voids which appears to be from branches breaking of towards their base. Most common size was just below 10 cm at the longest dimension. None of the collected specimens contained a nucleus, and there were no evident signs of them being formed on one.

THORSEN *et al.*, [1989] investigated the rhodolith bed of Disko Fjord, which was also the focus of the present study. They regarded the openly branched rhodolith as *L. tophiforme*, and the spheroid ones with a dense surface and internal cavity as *L. glaciale*. These last ones were reported to have a size of up to 20 cm, although the largest specimen in their measurement of interstitial space is 10 cm.

The rhodolith forming species encountered in this study was *Lithothamnion cf. glaciale* and or *L. cf. tophiforme* but the taxonomic resolution of the species have been obscured by

intermediary forms and unclarity between the species of *Lithothamnion tophiforme* and *L. glaciale*, partly because the type material of *L. glaciale* have not been reinvestigated recently [DÜWEL & WEGEBERG, 1992; 1994; WEGEBERG, pers. comm.]. A recent study [ADEY *et al.*, 2005] may have solved the confusion by the use of SEM but this has not yet been applied to the rhodoliths of Disko Fjord.

ADEY *et al.*, [2005] states *L. tophiforme* as being golf- to tennis ball sized, and often with early ramification and no nucleus as seen in (Fig.4b) but the chosen neotype have a morphology that were not observed in this study. KJELLMANN [1883] described *L. glaciale* as being larger, and often with an internal void from a former nucleus, a morphology similar to the larger specimens of this study. A mixture of both species is common in many sub arctic rhodolith beds [ADEY *et al.*, 2005] and may also be the case in this arctic aggregation. Rhodoliths with a size of 20 cm as found by THORSEN *et al.*, [1989] are by far the largest recently found rhodoliths and would underline the rareness of this site. But branched rhodoliths of 13 cm as found in this study are also very uncommon and are among the largest reported from anywhere in the world, save for the old records by KJELLMAN [1883] and FOSLIE [1929].

4.2. Morphological aspects of the Disko Fjord rhodoliths

A few morphological characteristics of the rhodoliths from this study are worth discussing. Although most rhodoliths were more or less spheroid, any unattached growth of non-geniculate coralline algae is per definition a rhodolith, including perfectly flat growths. Secondly, many of the larger rhodoliths exhibited unpigmented areas, in most cases on the side that faced the substrate. The most interesting feature of the rhodoliths is the internal void present in the large specimens from 9-14 meters depth, and the centimetre sized holes they often have into it. The origin of such internal void may have different explanations, some very hypothetical, others more plausible. They will all be discussed as it facilitates the reasoning, and may differ between species and habitats.

4.2.1. A flat rhodolith?

The large flat plate of a two centimetres thick crust of densely clustered branches (Fig. 4c) obviously originate from growth on a large rock or similar flat substrate (in this area, trash is an actual possibility). But apart from its flat shape, the morphology of the crust itself was similar to that of the rounded rhodoliths. Branching or protuberant crust was not observed on the rocks in the area, but cannot be excluded – only the laminate crust of *Phymatolithon* sp. was seen.

4.2.2. Dead or unpigmented areas on live rhodoliths

The rhodoliths which had a side buried one or two centimetres into the sediment were clearly affected by this, with that area being bleached or dead. But dead sides were not restricted to buried rhodoliths. Most larger ones had one or two dead areas, which most likely stem from having that side facing the substrate for too long [WILSON *et al.*, 2004]. Smaller rhodoliths did not seem to suffer from this, except perhaps at a few branch tips. They are more openly branched which gives better water exchange and light conditions, while their lower density makes them more susceptible to tumbling, thus preventing long-term burial.

Most rhodoliths in areas with dense cover were not buried and were on a sediment of coarse particles. This indicates ample currents along the bottom to prevent or remove fine sediments, but rhodoliths lying slightly shallower, at 7 meters depth, were partly buried (Fig. 3b). Explanations could be increased sedimentation or reduced current energy from an

extremely local variation in the bottom currents. Perhaps due to interaction with the pycnocline. But no distinctive wave- or current ripples were observed, and a further investigation of the area is necessary.

4.2.3. Formation of internal cavities

One hypothesis is whether a hollow rhodolith can be formed from a thick plate of protuberant or branched crust that become detached from a rock due to a storm event and/or bioerosion of its lower layers. Its only way to become spheroid would be through increased and directed marginal growth. If the live side of the flat rhodolith faced the substrate, growth would be directed towards the margins and upwards, due to their phototactic growth response. This could theoretically give a somewhat rounded structure if their margins were able to merge together. If the live side instead faced the surface, no void would be formed, and it would most likely grow towards a dense hemispheroid. And tumbling to the “right” side for growth would be less likely for a flattened structure. All in all a very speculative hypothesis, but it is an important step in reasoning that the large rhodoliths are unlikely to stem from a well developed flat crust on rocks, such as the one seen in Fig. 4c.

Another is given by KJELLMAN [1883, p.94] who writes about large *L. glaciale* rhodoliths that “On their lower side, which is turned towards the bottom, such individuals are often furnished with a large opening, through which the originally included object has fallen out. According to him, the internal lumen is mainly the leftover space of the rock or shell that the algae originally settled on. Why it falls out is not made clear, but an effect of worms and boring mussels in producing surface pores and cavities is mentioned. Worth noting in the citation is the word “often” which either implies that some: a) still have the object inside, b) are not formed on a foreign object and instead have their own coralline branches inside or c) have a cavity without having a large opening to the exterior.

How an internal nodule can get out also depends on its substance. If it is a small rock, it would have to leave before it becomes fully overgrown or alternatively break its way through from the inside during storms. Another possibility exists if the nucleus is a shell. Over time a dead shell may degrade, largely due to the boring of sponges and worm, and the crust it carried may grow to form larger rhodoliths [ADEY *et al.*, 2005]. Although this didn't directly explain the formation of a cavity or opening into it, it gives a clue that bioerosion may be important in rhodolith beds.

Several of the rhodoliths of this study had an internal cavity without having an opening into it, and with no signs of a nucleus. This is also commonly observed in Norwegian rhodoliths near Kristiansund (N. AUKAN, pers. comm.). If these rhodoliths initially had a rock nucleus, the exit hole would have to be closed secondarily by thallus growth. If it used to have a shell as nucleus, subsequent bioerosion could theoretically leave a cavity. Finding intermediate stages of these processes would render them the best support. No nucleated rhodoliths were found in this study, and the closest resemblance to one was small and living *Hiatella arctica* with a maximum distance from the surface within “a siphons reach”. Although this does not exclude a nucleus from having a role, it clearly shows that it is not the predominant cause in this rhodolith bed.

As all smaller rhodoliths had a core of their own branches, it is reasonable to expect this situation to be the stage prior to the hollow form. The core will stay alive as long as it receives sufficient light and water exchange, but may change when growth increases the radius of the spheroid rhodolith. An open branching can counteract this, but run a higher risk of breaking its fragile branches. This is probably why the most openly branched rhodoliths are found deeper than more dense shapes as in this study and *e.g.* those by MINNERY [1990], PILLAR and RASSER [1996], and ATABAY [1998]. Likewise were the small rhodoliths of this study more

densely branched at shallow depths, where light conditions are better and wave impact higher. It is likely that the light levels cause an inherent response in the branching density, while water motion may control directly by physically breaking long and thin branches [FREIWALD & HENRICH, 1994; FOSTER, 2001]. And that the rhodolith subsequently responds to this by increased intercalary growth [FREIWALD & HENRICH, 1994] which increases density.

At some stage the rhodolith will attain a size or density at which it is impossible or uneconomical to keep the core alive. While corraline thalli is efficient at preventing fouling by epiphytes and infestation by endolithic organisms when alive, partly by attracting species that feed on them [FREIWALD, 1993], their dead parts are severely affected. A continuous bioerosion by algae, fungi, sponges and larger boring species will over time destruct the dead thalli core [FREIWALD & HENRICH, 1994]. As the rhodolith continues its radial growth more of the inner thalli will die, followed by erosion, thus forming an internal cavity without large openings.

This “radial bioerosion” is observed in most of the rhodoliths where the internal dead thalli curves along with outer live thalli (Fig. 4e). If bioerosion was not important you should easily find dead rhodoliths of similar shape and size underneath the live ones, potentially forming banks of fragmented and dead thalli over time. No such banks were found in this study, but are common in other rhodolith beds [FOSTER, 2001; BOSENCE & WILSON, 2003; ADEY *et al.*, 2005].

4.2.4. Small and large holes in spheroid rhodoliths

Among the rhodoliths collected there were also several which did have openings larger than two centimetres, sometimes as much as 7 centimetres. Openings in the size of one or two centimetres are most likely from *Hiatella arctica*, which was also observed frequently by FREIWALD [1991] in rhodoliths larger than 5 centimetres. Similar sized indentations in the surface may occur where a branch with several outer ramifications have broken off near its basis. I suspect the large holes to be a result of physical destruction combined with localised bioerosion. When the rhodoliths grow larger they grow in weight and diameter, but crusts thickness usually stay the same. It is normally between a half and two centimetres in *L. glaciale*, and varies within the rhodolith [KJELLMAN, 1883]. When tumbling during *e.g.* storms, the chance is thus higher that the crust will break. The slightly discoidal rhodolith in Fig. 4e is larger than ten centimetres, and have a large hole in both its flattened upper and lower side. It clearly had an upper and lower side at the time of collection, as indicated by less pigmentation in the lower half which was still alive. The marginal areas around the opening contain dead thalli, and the openings were unfortunately enlarged slightly by the physical handling during collection. I expect the initial openings to be a result of that side facing or being buried in the substrate for too long. The effects of reduced light and water exchange will be most pronounced towards the middle of that area, which may die and be eroded by bioerosion. During a storm event it may have tumbled, and would be most likely to break in this weakened area. Two holes should be explained by two such events. Another interesting feature of this rhodolith is the area of live thalli where the side is broken (Fig. 4c, arrow). This inner area would normally be dead but the breaking of one side has improved the living conditions. A similar thing was observed in another rhodolith which only had a large opening in the upper side. Underneath this hole, an upward growth was observed from the lower crust of this hollow structure, *i.e.* into the lumen. As this growth was taller than a centimetre, it could indicate that the rhodolith had been in a stable position for a long time.

Holes of intermediate size (2-3 cm) were also observed, and their edges were often live and with a thick crust that continued a little around the inner edge. The cause of the hole could

be boring or tumbling, and their current structure simply appears to be a result of subsequent growth.

4.2.5. Rhodoliths at intermediate depths are dense and large

The strong difference in rhodolith size and branch density at various depths may have one or more explanations. One explanation for the increased density of large specimens, and which may be valid for all rhodolith forming species, is that their increased weight makes the branches break more easily. But other aspect, such as light and water movement can also be important in explaining how small specimens of the same size can have strikingly different branch densities. The possibility of having two species present, *L. glaciale* and *L. tophiforme*, could theoretically account for the differences if their growth form and optimal growth conditions are dissimilar.

4.2.6. Morphological aspects – a summary

The modes of formation and causes of internal voids and external openings can be manifold. Some clearly have or have had a nucleus, others have not. Bioerosion and physical damage and burial all are important processes, while light, current and sedimentation have different effect on the many different species. Both formation from spores and by fragmenting occur within the bed, and internal voids may be from a previous nucleus (primary formation) or from bioerosion (secondary formation). The best evidence for or against the various theories is the lack or presence of intermediate stages, but while present in some areas, they may be absent in others. They different species have a set of environmental requirements and responses but no strict bauplan for their growth and this may account for the variation within species and between habitats.

4.3. Bioturbation

Bioturbation have often been suggested to be important for the formation or survival of rhodoliths, by rotating them, thereby reducing the effect of sedimentation and risk of burial [FOSTER, 2001]. ROSENVINGE [1898, p.225] shortly assume that rhodoliths are commonly moved by the fauna. FREIWALD and HENRICH [1994] speculate that the rhodoliths may be moved or temporarily picked up when carnivorous fishes, such as the Cod (*Gadus morhua*) or the wolf fish (*Anarhicas lupus*) feed on the brittlestar arms that extends from their hide on or within the rhodoliths. In a rhodolith bed in Kristiansund, Norway, the gaping file-shell (*Limaria hians*) have been observed to actively force itself under the rhodoliths where they are normally found, thereby rotating them quite often (N. AUKAN pers. comm.). The study by MARRACK [1999] showed several fish species picking up rhodoliths in order to forage for invertebrates below, while other fish made burrows, displacing the rhodolith. Sea urchins were also observed digging into the sediment, and crabs disturbed by walking below and among them. The rhodolith bed in Disko Fjord contained several large carnivorous fish, and plenty of brittlestars for them to feed on, while the sheer abundance of sea urchins may cause minor disturbance along with the occasional but large crabs that are present in the area.

4.4. Distribution of rhodoliths at regional and local scales

The habitats in which we encounter specimens included in the collective term ‘rhodoliths’ are surprisingly similar in many hydrodynamic and marine topographical aspects, despite consisting of many different species all over the world. Although the habitats initially may sound very different, e.g. “Open shores and sheltered places” [FOSLIE, 1895] or “...at headlands or between two islands” [BOSENCE & WILSON, 2003], they are characterized by

moderate currents, and little wave actions. On exposed coast, larger depths will reduce the wave disturbance.

Due to their unattached state, the distribution of the rhodoliths on the sea bed is governed by the topography and hydrodynamic regime which can make them aggregate in depressions, in finger-like patches or bars, and sometimes ridges and ripples are formed by waves and currents.

4.4.1. Physiogeography of Kangerluk (Disko Fjord)

The study area resides in the outer end of the fjord Kangerluarsuk next to the settlement of Kangerluk (Fig. 1) Most other studies have overlooked this inlet, and instead focused on all the other areas: The main fjord Kangerluk and its two innermost parts, Kuannersuit Sulluat, and Kangikerlak [SCHMID & PIEPENBURG, 1993; Gilbert *et al.*, 1998; MØLLER *et al.*, 2001; GILBERT *et al.*, 2002; DESLOGES *et al.*, 2002]. Both of these have an input of glacial meltwater with suspended sediments, but a great amount of this is trapped by a sill near Qivittut, around the confluence of these two inner branches. Kangerluarsuk receives no direct glacial runoff and is about ten kilometres from the sill at Qivittut, and sediment input is thus expected to be minor in this area. Wind-induced wave disturbance of the rhodolith bed can be evaluated based on the wind fetch at different wind directions. The bed is situated on the leeward side of a point and is relatively protected from upfjord winds, but may be quite exposed under certain rare wind conditions. The wind fetch for upfjord winds have been calculated for Disko Fjord [GILBERT *et al.*, 1998] and show a fetch of up to a hundred kilometres at maximum near the rhodolith bed. But the general pattern is a fairly protected area with less than ten kilometres fetch, but with the rare possibility of large waves that may affect the rhodoliths.

4.4.2. Distribution of rhodoliths in Kangerluk (Disko Fjord)

The rhodolith distribution on the seabed exhibited a graduated change in density and morphology with depth, and elongated patches of rhodoliths were present, but not dominating. The seabed appeared to consist of mud and gravel rather than a bank of dead rhodolith thalli. Uncalcified macrophytes were virtually absent.

The site of initial formation of the Disko Fjord rhodoliths has not been observed with certainty. I believe that recruitment from within the bed by fragmentation or settling of spores is the origin of the majority of the rhodoliths in the bed, but there are other possibilities. As no nucleated rhodoliths were observed, the formation would have to be in a different place if they are indeed initially formed with a nucleus. They could also have been formed elsewhere, at similar or shallower depths closer to the headland or further into the fjord, and subsequently transported to the surveyed area, *e.g.* a storm-triggered redeposition onshore. The formation at such remote places could either be from a fixed algal crust on rocky outcrops, or a fixed algal build-up. No such sites were observed near to the rhodolith bed in Disko Fjord, but no directed attempt was done to locate any.

The little depression in the seabed observed in this study (Fig. 2b), with its ridge and elongated patch without rhodoliths, seemed to be caused by a physical alteration, and not like the polygon depressions observed in Troms [FREIWALD *et al.*, 1991]. Although only a few small icebergs normally enter Disko Fjord [ANDERSEN, 1981; GILBERT *et al.*, 1998], the area right south of Kangerluarsuk has a high frequency of iceberg scours [DESLOGES *et al.*, 2002] and could potentially be a cause. Another possibility is anchoring by a large vessel, but this happens rarely, and would be more likely to cause a barren patch than a deep depression.

4.5. Comparison with other rhodolith beds

The physical conditions of the Disko Fjord rhodolith bed are similar to those of the Norwegian beds in the Troms area. They are both in fjord areas at about 70° latitude. They are physically sheltered from strong waves except during storms from certain wind directions. They have a relatively low input of terrigenous material due to a certain distance from the source, sills in between, and currents running across their platforms may prevent sediment from settling. Surface temperature in summer is around 10°C-14°C in the inner part of Disko Fjord [ANDERSEN, 1981; DESLOGES *et al.*, 2002; pers. obs.] and may reach 15-16°C in Troms [SOOT-RYEN 1934, cited in FREIWALD & HENRICH, 1994]. DESLOGES *et al.*, [2002] describes that a strong pycnocline is evident in the upper 5 m of the inner area of Disko Fjord, which then weakens and thins to less than 2 m at intermediate sites, corresponding well with the observations of the present study.

The tide in inner Kangerluk has a maximum of 2.3 m [DESLOGES *et al.*, 2002] while the mean tidal range measured close to Tromsøy is 1.78 m and the maximum range is 2.93 m [SÆTRE 1972 cited in: FREIWALD *et al.*, 1991]. The rhodoliths have their highest coverage in the depth range of 9-20 meters at both sites [FREIWALD *et al.*, 1991], with a graduation between of bars and belts of rhodolith pavements. Some of the Troms sites have megaripples with wave lengths of 2 to 3 m and heights of approximately 0.5 meters, created by strong tidal currents that are commonly found in the sound channels [FREIWALD *et al.*, 1991]. No such ripples were observed in Disko Fjord. Both sites are also dominated by rounded rhodoliths instead of the maerl-type, but the Greenlandic rhodoliths were generally larger. Almost all rhodoliths in Troms exceeding a size of 5 cm reveal one or more borings by *Hiatella arctica* [FREIWALD *et al.*, 1991], which is partly true for the Greenlandic ones (have not been investigated thoroughly).

The rhodolith beds of Spitsbergen, Novya Zemlaya, and other sites in northern Norway are likely to resemble the one in Disko Fjord, with large rhodoliths of *L. glaciale* and *L. tophiforme*, but virtually no investigations have been done since their original discovery. Only very few sites are known in the arctic part of North America, but there are no evident reasons why they should be absent. The Alaskan rhodolith bed [KONAR *et al.*, 2006] is in the Pacific and does not resemble the more arctic ones, as it was primarily the maerl-type rhodolith *Phymatolithon calcereum*, although the depth range was very similar (8-18 meters or deeper). Considering the size of Greenland, its latitudinal extension, and that it have fjord-systems facing all cardinal directions, the lack of rhodolith beds is stunning and maybe not entirely true. I expect that a directed attempt at locating rhodolith beds will reveal them within a wide latitudinal range in the fjords of Greenland, especially along the west coast. Although by no means a dominant habitat, they may be important as host and rearing ground for a diverse fauna.

4.6. Fauna

Where the rhodolith aggregations are dense they form a unique habitat with some similarity to coral reef structures in having large interstitial space, giving an ideal habitat for many organisms. The species richness within the rhodolith bed is often greater than in the adjacent areas with rocks or sediment, most likely due to the more heterogenous substrate it provides. The rhodolith bed of Disko Fjord was host to a large numbers of young and juvenile macrofauna such as the sea stars, sun stars, chitons and brittlestars. This is in accordance with reports from other Atlantic beds [KAMENOS *et al.*, 2004] which are reported to be important rearing grounds for a range of echinoderms, as well as economically important molluscan species. Besides being excellent food grounds, it is likely that the habitat offers them increased physical protection against large predators. Protection may also be an important

factor for many of the other organisms that are often very cryptic, *i.e.* camouflaged or hiding within the network of branches. The red colour of the brittlestars and chitons conceals them very well among the rhodoliths (Fig. 5a) and for some species it is a likely result of grazing the coralline red algae, or indirectly from feeding on other organisms that contain these pigments, although some pigments may be self-synthesized [BANDARANAYAKE, 2006]. The interstitial space of the rhodoliths in the area is on average sixty percent of their total volume [THORSEN *et al.*, 1989] and thus leaves much space to be inhabited by fauna and other flora. The sipunculans and the boring bivalve *Hiatella arctica* both peak out from within the rhodolith with their introvert and siphon, respectively. The siphon of *H. arctica* has an astounding similarity to the knobby endings of rhodolith branches (Fig. 5b) and is thus well camouflaged. The chitons are usually on the live surface of the rhodolith, but still with branches extending above them, while the brittle stars are underneath or between the rhodoliths. From here they extend their extremities for suspension feeding (front-page), and still protect their vital bodypart – the central disk (calyx). Other species live or feed inside the rhodoliths without a need for the outer environment.

The faunal community of this rhodolith bed is similar to those reported from other Atlantic and Arctic beds [SNELI, 1968; FREIWALD & HENRICH, 1994] and even similar to tropical beds where the same faunal groups dominate, although the actual species differ [STELLER *et al.*, 2003; HINOJOSA-ARANGO & RIOSMENA-RODRIGUEZ, 2004]. The species richness is often high within the many faunal groups present, strongly facilitated by the heterogeneity of these habitats. The resulting high biodiversity is generally is fairly similar among rhodolith bed in very different parts of the world and also gives a robust functional diversity of the communities in the beds [BARBERA *et al.*, 2003].

Meiofauna collected in this study have not yet been analysed, and only a few other studies have looked at it previously. The coarse carbonate sediment within the beds is very suitable for interstitial organisms, and is so far the type locality for two species of kinorhynchs [HIGGINS & KRISTENSEN, 1988]. They also have an unusually rich fauna of interstitial ostracods, perhaps one of the richest known in Greenland, of which many may be new species [PENNEY, 1992], although there have been no publications since.

FREIWALD and HENRICH [1994] analysed the internal sediments of the rhodolith framework cavities, and the adjacent sediment deposits. The component analyses showed coralline algae to constitute half of it, and with bivalve, gastropod, foraminiferan and echinoderm fragments to constitute equal amounts of the rest. The coralline fragments come from physical damage such as storms and bioturbation, and from the faecal pellets of grazing macrofauna. Ophiroid ossicles and bryozoans also contribute a little. Ostracods are lighter particles and are primarily found within the framework, as the open flats have more current which results in well sorted sands of heavier particles. Almost nothing is known about the meiofauna of rhodolith beds anywhere in the world, but they appear to be a promising habitat to conduct such studies in.

4.7. Growth rate

The growth rate of most rhodoliths is very low, rarely exceeding 1 mm branch extension in a year [FOSTER, 2001; BOSENCE & WILSON, 2003]. In addition to this, rhodoliths have high risk of breaking their branches at times of strong hydrodynamic action. A spheroid rhodolith with a longest dimension of 10 cm would theoretically be at least 50 years old (radial growth of 1 mm y^{-1} from the centre). This is without doubt a minimum age and the rhodolith of 50 cm would therefore be at least 250 years old, most likely much more as its outer branches are likely to have broken off at least a few times in this period. Such a slow rate of growth is also very important in terms of conservation and how they cope with different anthropogenic impacts.

4.8. Summary

The rhodolith beds here presented are the only recent records from Greenland since those mentioned by ROSENVINGE [1898]. This study includes the first visual observation of the rhodolith bed in Disko Fjord, as well as photos from rhodoliths near Qaqortoq. The investigation were preliminary and in no way extensive, but nevertheless revealed interesting aspects of a rather unknown habitat of Greenland. It reemphasises the century old findings of Kjellman, Foslie and others, which have been somewhat overlooked in recent distribution maps, and the 50 cm large rhodoliths would be interesting to relocate in future studies. The size of the rhodoliths of this study is among the largest in the world, although considerably smaller than those at even higher latitudes. They are home to a species rich and scientifically interesting community.

Due to the morphology with a large interstitial space and cavities, it provides a habitat for a high biomass and species richness. The meiofauna among rhodoliths is greatly understudied all over the world, despite its potential for hosting extraordinary specimens in the rhodolith-derived carbonate gravel. The bioturbation of rhodolith beds by the gaping file-shell (*Limaria hians*) is reported here for the first time, but is most likely common wherever these two species live together.

With a growth rate reported to be less than 1 mm a year for *Lithothamnion glaciale* and related species with rhodoliths, this would mean that some are at minimum 65 years old. The Rhodolith bed in Disko Fjord is yet a fairly pristine rhodolith habitat, and thus interesting to compare with more affected beds. Although there at the present are no direct threats to them in the form of dredging, collection or increased local pollution, protection of this unique habitat should be a consideration for the future. Further studies, general awareness and contact with local people may undoubtedly reveal new sites, but so far this habitat is still very rare in Greenland.

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A Macroalgal Investigation, Disko Island 2006, with a Description of a New Genus and a New Species of Brown Alga (Phaeophyceae)

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Abstract. During Arctic Field Course 2006, macroalgae were sampled at 11 different locations at Disko Island, West Greenland. Five samplings were done with a triangle dredge in the sublittoral zone and 6 were collected by hand in the littoral zone. Seventy-six species were identified in total. This result was compared to previous investigations from 1990 and 2002. Overall 106 species have been identified and 20 stations visited during these 3 surveys. Three unknown species were found at LORAN, Ippik and Satut. Morphological investigations were done with microscopy of sliced material, from LORAN and Ippik, using the paraffin technique. Morphology was also studied in frozen and fresh material. Cultures from the reproductive organs were inoculated for further investigations. The species from LORAN and Ippik proved morphologically identical. Phylogenetic analyses of sequences of the chloroplast-encoded *rbcL* gene were performed. The DNA-sequence from the Ippik and LORAN specimen was unreadable. Work with the species from Satut proved successful and showed 2 % sequence divergence from the sister taxa. This, together with the morphological investigations, supports that the specimens from LORAN and Satut are two new species, and that the LORAN specimen is also a new genus.

Keywords. Brown algae, *Phaeophyceae*, *Ectocarpales*, *Chordariaceae*, Phylogeny, *rbcL*-gene

1. INTRODUCTION

The following report is based on macroalgae survey during Arctic Field Course, Arctic Station, University of Copenhagen, August 11th to September 1st, 2006. The aim of the study was primarily to gain personal knowledge about the arctic marine macro flora around Greenland. Secondly we hoped that our survey could add information and details to the existing knowledge about diversity and distribution of macroalgae on Disko Island.

1.1. Introduction to Disko Island

Disko is a large island, located about 200 km north of the Arctic Circle and 100 km from the west coast of Greenland (Fig. 1). It has an area of approximately 8,600 km², which makes it one of the 100 largest islands in the world. The island is part of the Tertiary volcanic province of West Greenland and is mainly made up by lavas. The climate on Disko is arctic, hence from December until May the sea around Godhavn is ice-covered, while the surrounding fjords are navigable from June to November (Fig. 4) (The folder: Research Station of Natural History in Greenland).

1.2. Macroalgae and the arctic marine environment

There are approximately 200 different species of macroalgae in Greenland, in comparison to the 400 species found in Danish waters. Greenlandic macroalgae are not well adapted to cold growth temperature. They often show a temperature optimum for growth around 15°C. This is a lot more than the natural growth conditions in Greenlandic waters [PEDERSEN, 2006]. Another important and limiting factor is the short growth period, which prevents a great number of species from migrating further north since they do not have enough time to produce the reproductive organs. Furthermore, an effect of the short growth period is that a marked seasonal variability in species composition is not detectable in the arctic summer [PEDERSEN, 2006a]. In other words, the Greenlandic macro flora is still consisting of pioneers in an ongoing migration and adaptation.

Overall, going north in Greenland means: Shorter and shorter growth period, reduction in biomass, and reduction in species diversity, especially in the littoral zone. The species that cope with the hard conditions in the northernmost areas are opportunistic species with short generation time and great and effective reproductive potential, such as *Blidingia minima* (Nägeli ex Kützing) Kylin, *Pylaiella littoralis* (Linnaeus) Kjellman, *Rhodochorton purpureum* (Lightfoot) Rosenvinge [PEDERSEN, 2006a].

2. MATERIALS AND METHODS

2.1. Sampling

Algae were collected from 11 different locations around Disko Island, West Greenland, in August 2006 (Fig. 1, Fig. 2, and Fig. 3). Sampling from the sublittoral zone (5-10 meters) was done with a triangle dredge either dragged after the research vessel "Porsild" or a speedboat. The rest of the sampling was handpicked from the littoral zone and the top of the sublittoral zone. The gathered material was kept in seawater. The material was sorted out in the lab on Arctic Station and when possible determined to species level, using different literature [RUENESS, 1977; PEDERSEN, 1976; ROSENVINGE, 1893; LUND, 1959], stereoscopes (SZX12) and light microscopes (Olympus BH2). Pictures were taken with Nikon DSFi1 using the program NIS-Elements mounted on a light microscope (Olympus BH2). Species of interest were cultured by inoculation of fertile fragments and/or frozen for further investigation. The cultures were kept in the Phycology department standard medium MV30 [CHRISTENSEN, 1982], at 4°C. The light:dark period was 16:8 hours at a light intensity of $11\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

2.2. Locations in the littoral zone

1) Exposed side near Udkiggen:

The algae were collected at low tide in the littoral zone by hand. In an exposed place as this, the algae are not only subjected to the regular dehydration/drying-out, but also wave exposure and ice scouring. The perennial algae were only found in the small cracks between the rocks, where they were protected from ice.

2) A small laguna between Udkiggen and Qeqertarsuaq:

This place was protected from severe wave exposure and ice scouring, which resulted in a domination of perennial macroalgae in the littoral zone. Fucales dominated the vegetation in the tidal zone, due to their drying tolerance. Because of the protected locality, a lot of organic material/mineral sediment had settled on the bottom of the laguna. The depth at low tide was approximately one and a half meters.

3) Qeqertarsuaq – the Peninsula:

A number of tidal rock pools were found on Qeqertarsuaq. Here the species are not exposed to waves and ice scouring. The species that live here have other factors affecting their growth. The temperature usually gets higher than in the sea because of the limited water volume and restriction in water flow. The salinity can rise, when water evaporates or fall because of precipitation.

4) Lyngmarksbugten:

Lyngmarksbugten is a protected, small bay. The substrate was rocks on a sandy sea bed. The depth was less than 5 meters.

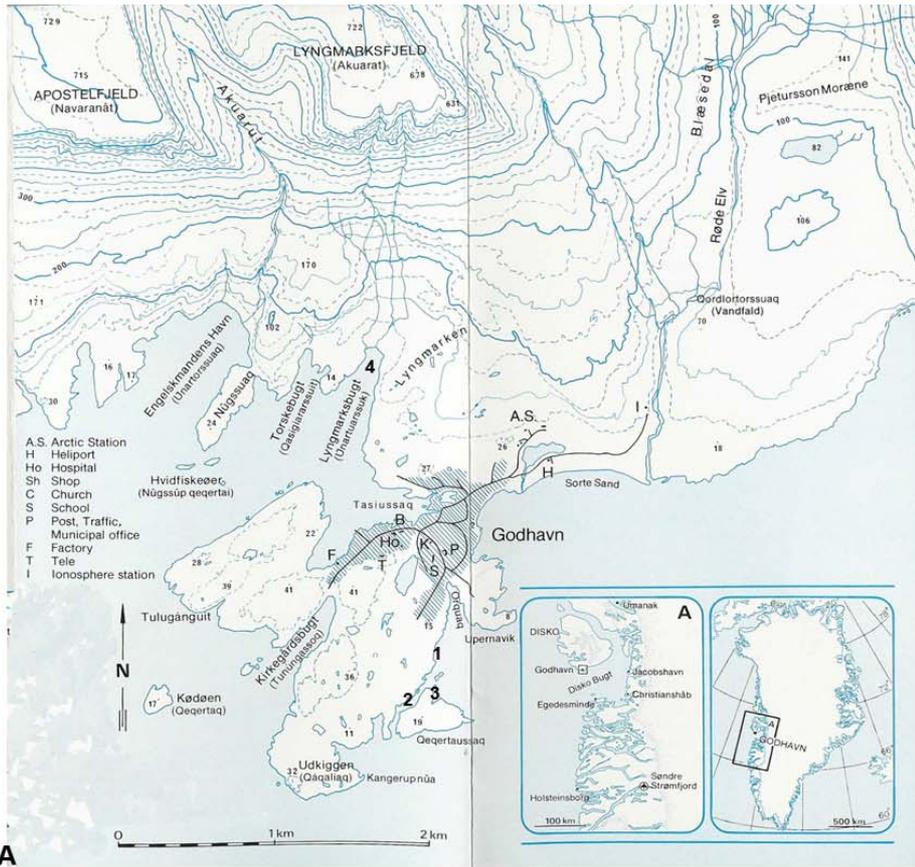


Fig. 1. The area around Quequertarsuaq, where many of the littoral zone samples were gathered. The numbers shows the sampling sites and corresponds to the numbers in the text and on the species list.

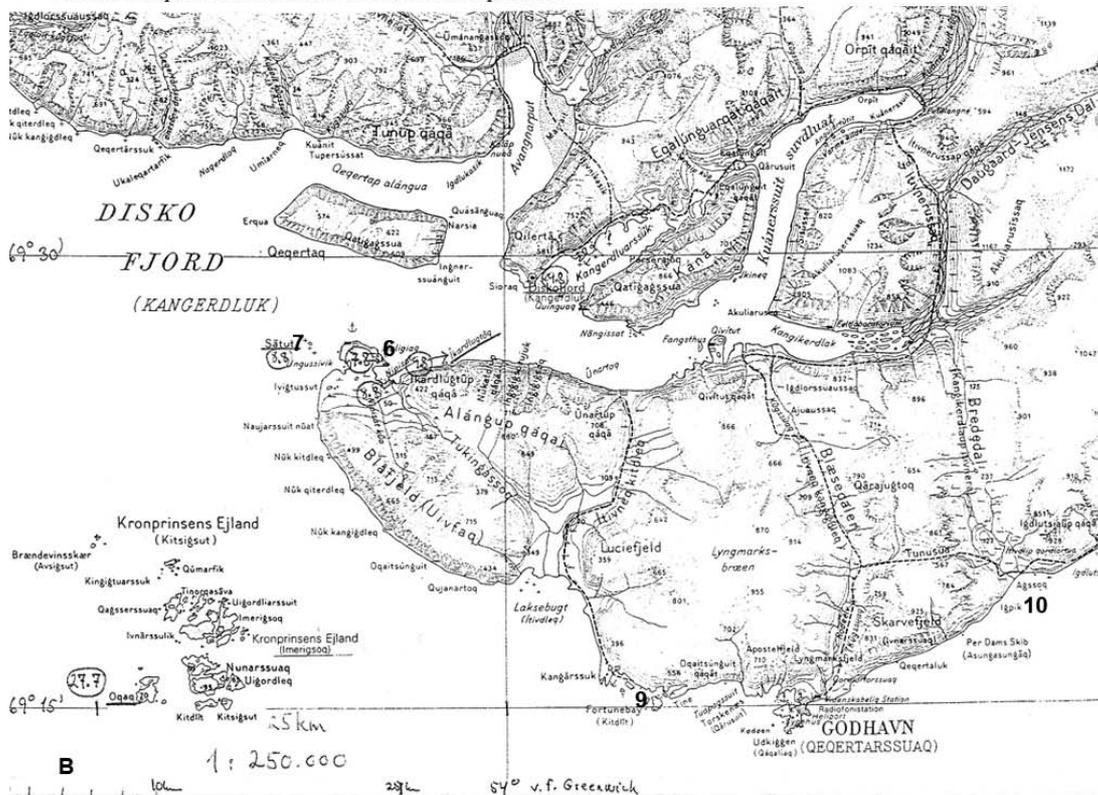


Fig. 2. The locations where many of the sublittoral samples were gathered. The numbers shows the sampling sites and corresponds to the numbers in the text and on the species list.

5) Hareøen:

This location was very exposed, to both waves and ice, and there were only algal growth between the large boulders, which lay just near the shore.

6) LORAN:

Near LORAN station, Nipisat the substrate was large boulders, with small muddy areas in between. The location was exposed to waves and ice scouring.

2.3. Locations in sublittoral zone7) Nordfjord:

Samples were collected close to a stone beach. The substrate was medium sized rocks with sandy areas in between. The current was strong.

8) Satut:

The substrate was mostly rocks and sand, but the first two pulls with the dredge were non-successful because of sea urchins. There was a strong current around Satut, probably due to stone reefs.

9) Kangerlussuak (Fortunebay):

This location was a protected bay. The substrate was large rocks and sand. The current was medium and there was sign of ice scouring in the littoral zone.

10) Ippik:

The substrate on this location was a mixture of mud and gravel. The current was medium.

11) HareØ:

On this location there was an entire forest of large *Saccharina longicuris* (Bachelot de la Pylaie) Kuntze and *Laminaria nigripes* J. Agardh. The substrate was large rocks with sandy areas in between. The current was medium and great numbers of pelagic organisms were observed. The combination of kelp forest and a rich pelagic fauna indicates that this area is a highly productive ecosystem.

2.4. Nomenclature of the species list

The nomenclature on the species list (Appendix 1) is based on <http://www.algaebase.org>. There are discrepancies between the classification found on <http://www.algaebase.org>, and classifications found elsewhere in the literature.

Ectocarpales is one of the orders in doubt. According to CHRISTENSEN [1982], the formation of longitudinal walls is restricted to plurilocular sporangia. [Http://www.algaebase.org](http://www.algaebase.org) and FRITSCH [1935 & 1945] use a much broader circumscription of the Ectocarpales including both uniseriate (haplostichous) and parenchymatous (polystichous) thalli, e.g. *Gononema aecidioides* (Rosenvinge) Pedersen and *Trachynema mortenseni* (Lund) Pedersen).

Furthermore, Christensen argues that four species belonging to Ectocarpales *s.l.* should have an order of their own. This is *Petalonia fascia* (Müller) Kuntze, *Petalonia zosterifolia* (Reinke) Kuntze, *Scytosiphon complanatus* (Rosenvinge) Doty, and *Scytosiphon lomentaria* (Lyngbye) Link. They should belong to the order of Scytosiphonales, because of parenchymatic thalli, a different life history, a single parietal chloroplast and one large pyrenoid [CHRISTENSEN, 1982].

Eudesme virescens (Carmichael ex Berkeley) Agardh and *Elachista fucicola* (Velley) Areschoug are also placed in the Ectocarpales, even though Pedersen (pers. ref.) has placed them in Chordariales because of a syntagmatic basal system in *Elachista* and *Eudesme* has a multiaxial syntagmatic macrothallus.

In this survey a broad perception of the Ectocarpales is chosen, which means that Chordariaceae is placed in the order Ectocarpales.

<i>Colouring</i>	<i>Coloured cell components</i>	<i>Colour</i>
Safranin	Nucleus, incl. nucleolus	Red
	Lignin in cell walls	Red
	Protein bodies	Reddish
Fast-Green	Cytoplasm	Green/Blue
	Primary cell walls (cellulose pectin)	Green/Blue

Table 1. A table of the cell components coloured by safranin and Fast-Green and the resulting colour of the cell component.

Step	Temperature	Time (min)
1	94 C°	3
2	94 C°	1
3	50 C°	1
4	72 C°	3
5	72 C°	6

Table 2. The PCR-amplification temperature and time. Steps 2-4 are repeated for 35 cycles.

2.5. Microscopy and paraffin technique

This work was performed at Botanical Laboratory, University of Copenhagen, Denmark. Small pieces from the main axis and first order branches were taken from undefinable Chordariaceae from LORAN and Ippik. These were cut into pieces of approximately 5 mm. The pieces were then fixated in formalin-aceto-alcohol (FAA) composed of 2% formaldehyde, 5% acetic acid and 58% ethanol. Dehydration, with ethanol, was done by increasing concentration (30%, 50%, 70%, 96%, abs. ethanol) and the material was placed in estisol before mounted in paraffin.

A Leitz rotation-microtome was used to slice the material. Slices were cut to a thickness of 10 µm and placed on a slide with eggwhite-glycerol and distilled water. For the hydration, histoclear was applied to remove paraffin. Ethanol was added in decreasing concentration (abs. ethanol, 96%, 70%) and finally distilled water to complete hydration. Safranin-Fastgreen 2-step colouring was used to stain all material, which was mounted with DePeX. Colouring of the different cell components can be seen in Table 1 [JØRGENSEN & FREDERIKSEN, 2006].

Pictures were taken with Nikon DSFi1 using the program NIS-Elements. Microscopy of the frozen material was performed on light microscope (Olympus BH2) and pictures were taken with Nikon DSFi1 using the program NIS-Elements.

2.6. DNA

The phylogenetic analysis was based on the *rbcL*-gene according to studies by SIEMER & PEDERSEN [1998]. DNA was extracted using the Sepagene RV-R kit (Sepagene RV-R, Sanko Junyaku Co., Ltd., Tokyo, Japan). The PCR was performed on the PCR Machine Peltire-Effect Cycling PTC-100™, with the primers BLSrbcL3F and BLSrbcS3R or nested PCR with one primer combination of BLSrbcL3F and NDrbcL8R, and a combination of BLSrbcL773F with BLSrbcS3R (Table 2). The PCR-fragments were checked for length, quality and quantity on a 1.5% agarose gel.

PCR-purification was made using the kit NucleoFast® 96 PCR-Clean Up, according to the manufacturer's instructions. Following this step PCR samples were prepared for cycle sequencing with the PCR amplifications primers and additional 3 internal primers. The primers used were 1) BLSrbcL3F, 2) BLSrbcL773F, 3) BLSrbcS3R, 4) *rbc11R*, and 5) NDrbcL8 (Table 3). Hence, *rbcL* sequences were obtained in both directions and the cycle sequencing reactions were run on an automated sequencer (model 3130XL Genetic Analyzer from Applied Biosystems).

Primer	Sequence (5'-3')	Position
BLSrbcL3F	GGCRCCGGAGAATCTATATG	IGS/ <i>rcbL</i> 17 → 3
BLSrbcL773F	GAGCAAAYGYAYGAACGTGC	<i>rbcL</i> 754 → 773
BLSrbcS3R	AAACATCCTTGTGTAACCTCTC	<i>rbcS</i> 23 → 3
<i>rbc11R</i>	AACGTTT ^A / _T ^T / _C ^T / _G CCAACGC	<i>rbcL</i> 665 → 648
NDrbcL8	CCAATAGTACCACCACCAAAT	<i>rbcL</i> 1232 → 1212

Table 3. The primer sequences and their position on the *rbcL* gene. Primer sequences by courtesy of Daugbjerg and from Siemer [1998].

2.7. Phylogeny

The phylogenetic analysis was based on the *rbcL*-gene. This gene was chosen on the basis of earlier phylogenetic analyses performed by SIEMER *et al.*, [1998], and since it is a protein-coding gene, it is easier to align.

Sequence fragments were assembled by eye using the program Chromaspro ver. 1.34 (Technelysium Pty. Ltd.) and added to an alignment comprising 128 *rbcL* sequences available in GenBank (App. 2). Alignments were conducted using MEGA version 3.1 [KUMAR, TAMURA, & NEI, 2004] and ClustalW. Parsimony and bootstrapping analyses were also made in Mega version 3.1. Heuristic searches were performed with CNI (level=1) with initial tree by random addition (10 reps). Close-Neighbor-Interchange (CNI) is a branch swapping method that begins with a given initial tree. The trees were then gathered in a strict consensus tree with consensus value of 50. Statistical support for the individual branches was measured by bootstrapping (500 replicates with CNI (level=1) with initial tree by Random addition (10 replicates).

On the basis of earlier studies made by DAUGBJERG & ANDERSEN [1997], members of the Vaucheriaceae (*Vaucheria bursata* (Müller) Agardh), Tribonemataceae (*Tribonema intermixtum* Pascher), and Phaeothamniaceae (*Phaeothamnion confervicola* Lagerheim) were selected as an out-group in order to polarize the in-group.

3. RESULTS

3.1. Species list

76 species were identified. 50 species were found in the littoral zone and 63 species were found in the sublittoral zone. In appendix 1, all the observed species are compiled into a species list together with the respective localities, at which they were found. In addition, pictures of selected species are presented in plate 4. In table 4, numbers of different species within the present classes are presented for the different localities.

As seen in App. 1 and table 1, Ippik, Nordfjord and Satut showed the highest species diversity. Phaeophyceae was by far the dominant class and observed for all localities.

Pylaiella littoralis (Linnaeus) Kjellman (Phaeophyceae), *Dictyosiphon foeniculaceus* (Hudson) Greville (Phaeophyceae), *Chordaria flagelliformis* (Müller) Agardh (Phaeophyceae), *Polysiphonia stricta* (Dillwyn) Greville (Rhodophyceae), and *Acrosiphonia centralis* (Lyngbye) Kjellman (Chlorophyceae) are the species found at most localities.

3.2. Species of special interest

Some of the specimens found, needed closer examination. Unknown specimens of brown algae were found at LORAN, Ippik and Satut. An attached specimen of *Ascophyllum nodosum* (Linnaeus) Le Jolis was found in Disko fjord, which is the northernmost position, where this species has been found. The plant was found on a wave exposed rocky shore near

Locality	Phaeo-phyceae	Rhodo-phyceae	Chloro-phyceae	Total
1) Exposed side near Udkiggen	10	4	7	21
2) Small creek near Udkiggen	12	3	6	21
3) Qeqertarsuaq	16	2	5	23
4) Lyngmarksbugten	10	3	5	18
5) HareØ (littoral)	6	1	6	13
6) LORAN	8	6	7	21
7) Nordfjord	16	6	8	30
8) Satut	14	8	5	27
9) Kangerlussuaq	9	0	7	16
10) Ippik	18	8	5	31
11) HareØ (sublittoral)	13	9	2	24

Table 4. Number of species found on the different locations.

the LORAN station (69°27,054 N/54°13,133W). It was attached to a stable rock and protected from severe wave action. The plant was approximately 5 cm tall and grew solitarily. Beside the single attached specimen, plenty of drifting *A. nodosum* were found in the area.

A sample of the rare *Chordaria chordaeformis* (Kjelmann) Kawai & Kim was found in the rock pools at Qeqertarsuaq. Fragments of the plant were inoculated in crude cultures and have now developed into macro thallus. The cultures are kept at the Phycology department, Biological Institute, University of Copenhagen.

3.3 Morphology of the LORAN and Ippik specimens

During field trips to Nipisat in Disko Fjord and Ippik, unknown plants were found. In Nipisat the specimen was found free floating. It was collected by hand near the LORAN station (69°27,064 N/54°13,133W).

The Ippik specimen was found in a dredge sample at approximately 10 meters depth (69°17,102N/53°13,646W). The plants resembled swollen ecotypes of *Chordaria flagelliformis* and *Sphaerotrichia divaricata* (Agardh) Kylin. A close examination showed that these plants had a multiaxial syntagmatic medulla, densely covered with a cortex composed of uniseriate assimilators, ending in a swollen apical cell several times bigger than the mother cell below. Both plants had unilocular sporangia embedded among the assimilators on the main axis, the Ippik specimen on the branches as well. The LORAN specimen had no unilocular sporangia on the laterals. Instead we found plurilocular sporangia.

3.3.1. Description (App. 5)

Macrothallus is up to 17 cm long, but the length is a bit uncertain as only two specimens were found. The main axis is cylindrical and approximately 1 mm wide and irregular laterally branched. First order side branches are cylindrical 2 mm thick and 5 cm long and appear swollen (Plate 4, A, B). No phaeophycean hairs have been detected on the studied material, but structures, which resemble hair bases, are recognized.

The plant is composed of a multiaxial syntagma with three sets of tissues: The 1st layer, the medulla, in both main axis and branches is composed of slender elongated hyaline cells, longitudinally arranged. The medullar cells are stretched by growth and leave the central part of the filament partially hollow (Plate 1, A & D). The 2nd layer, the intermediate layer is composed of larger more rounded cells which are longer than wide, connecting the medullar

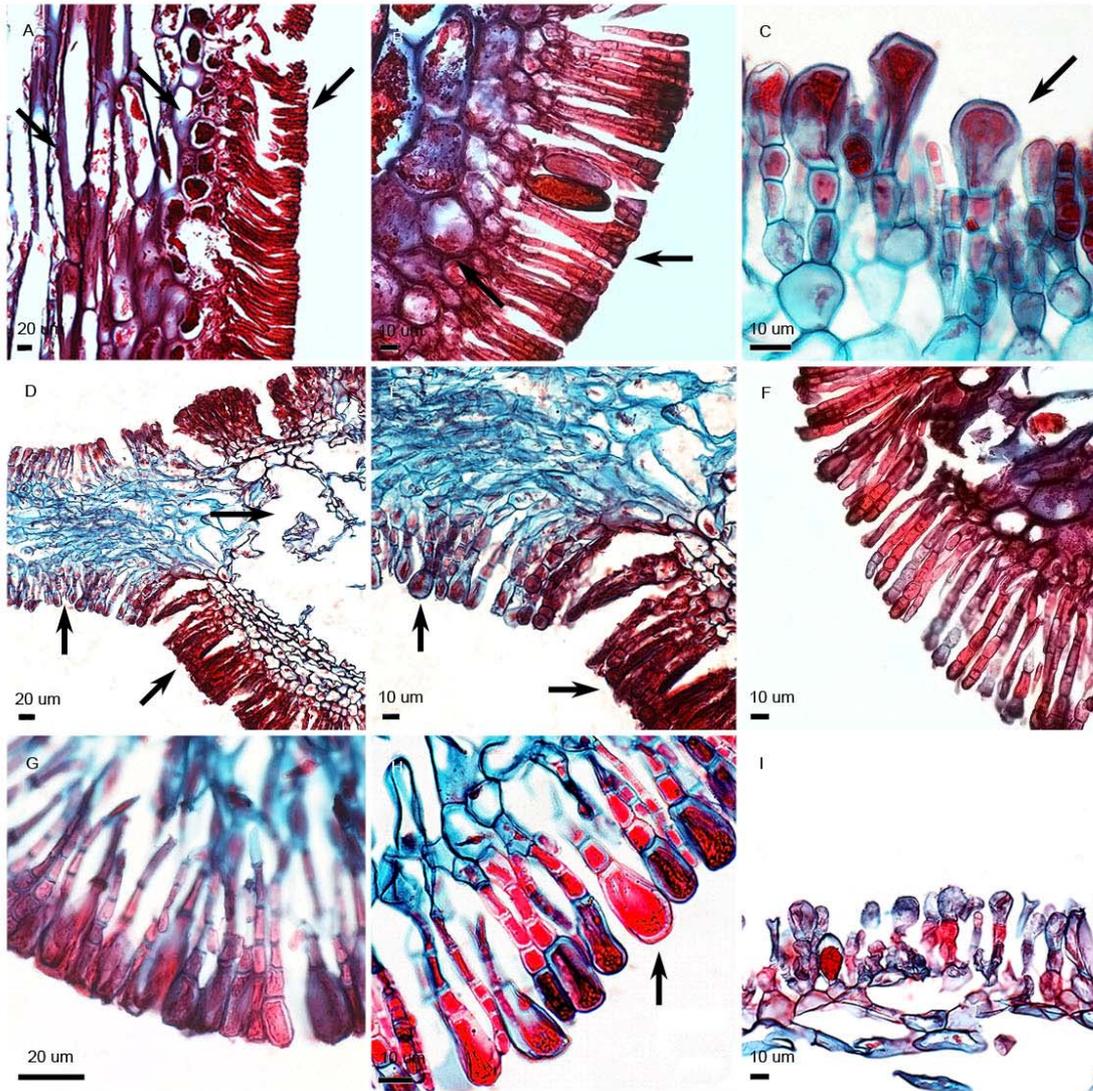


Plate 1 (Paraffin technique)

- A: Main axis. Multi-axial syntagma with 3 layers; medulla, intermediate and cortical layer (arrows) (specimen from the LORAN locality).
- B: Main axis. Cortical layer of assimilating filaments (right arrow) growing out from an intermediate layer of rounded cells (left arrow) (specimen from the LORAN locality).
- C: Cortical layer of assimilating filaments (arrow) (specimen from the LORAN locality).
- D: Dissection of main axis and side branch. Cortical layer on side branch (left arrow) differs from cortical layer on main axis (right arrow). Partially hollow medulla (arrow) (specimen from the Ippik locality).
- E: Cortical layer on side branch (left arrow) differs from cortical layer on main axis (right arrow) (specimen from the Ippik locality).
- F: Assimilating filaments of main axis (specimen from the Ippik locality).
- G: Assimilating filaments of side branch axis (specimen from the LORAN locality).
- H: Assimilating filaments of side branch, with large, rounded apical cells (arrow), growing out from intermediate cell layer (specimen from the LORAN locality).
- I: Multi-axial syntagmatic composition of the thallus (specimen from the Ippik locality).

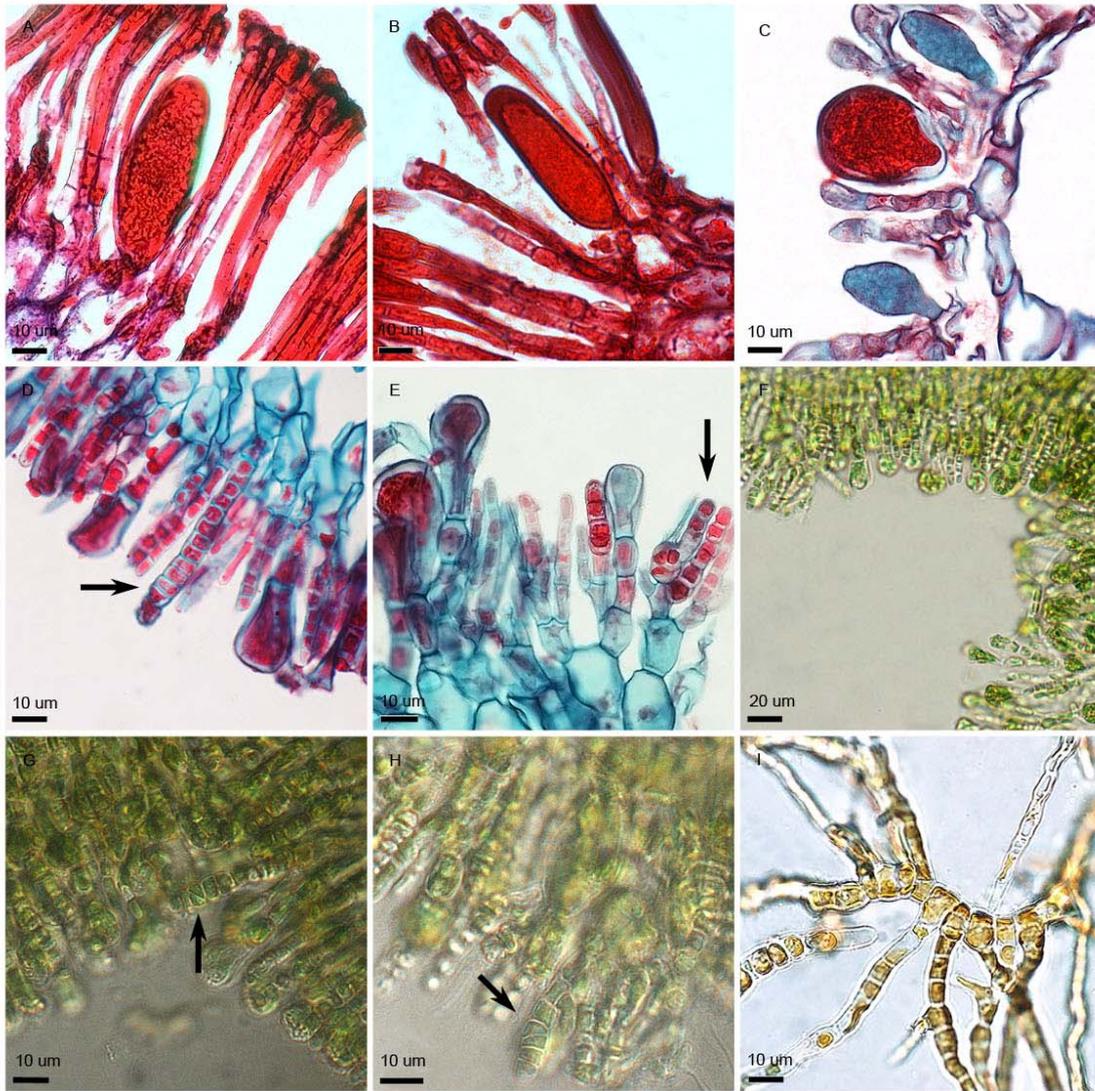


Plate 2 (A-E: paraffin technique, F-H: frozen material, I: living material)

- A: Unilocular sporangium embedded between assimilating filaments on main axis (specimen from the Ippik locality).
 B: Unilocular sporangium embedded between assimilating filaments on main axis (specimen from the LORAN locality).
 C: Unilocular sporangium embedded between assimilating filaments on side branch (specimen from the Ippik locality).
 D: Uniseriate plurilocular sporangia between assimilating filaments (arrow) (specimen from the LORAN locality).
 E: Uniseriate branched plurilocular sporangium embedded in cortical layer of assimilating filaments (arrow) (specimen from the LORAN locality).
 F: Numerous uniseriate plurilocular sporangia embedded in the cortical layer of assimilating filaments (specimen from the LORAN locality).
 G: Cells in plurilocular sporangium with oblique cell walls (arrow) (specimen from the LORAN locality).
 H: Cells in plurilocular sporangium with oblique cell walls (arrow) (specimen from the LORAN locality).
 I: Micro thallus. Creeping system of uniseriate, branched filaments.

layer with the cortical layer (Plate 1, B & C). The 3rd layer, the cortical layer is diverging between main axis and branches (Plate 1, D & E). The cortical layer of the main axis is composed of densely packed unbranched anticlinal uniseriate assimilating filaments, branching of from one of the cells from the intermediate layer (Plate 1, B, F, & G). The assimilating filaments are varying in length from 2 to 8 cells increasing in size towards the apical cell (Plate 1, B, & F). On the side branches, the cortical 3rd layer branches of from the intermediate 2nd layer. The assimilating filaments of side branches are varying in length from 2-4 cells. The apical cells on the filaments are enlarged and rounded and several times bigger than the mother cell below (length/width: mean value = 24,64/11,95 μm , s.d. = 4,12/2,88 μm , n = 28) (Plate 1, C, H, & I). Cells of the assimilating filaments are longer than wide (Plate 1, C, H, & I). The assimilating filaments on side branches are less densely packed than the assimilating filaments on the main axis. It has not been possible to detect a meristematic zone in our material but, according to the definition of Chordariaceae proposed by SETCHELL & GARDNER [1925], subapical growth is characteristic for species belonging to the Chordariaceae.

Unilocular sporangia, found on both main axis and side branches, are embedded in the cortical layer between the assimilating filaments and grow out from the intermediate cell layer as well. Unilocular sporangia, on main axis, are large and ovoid (length/width: mean value = 51,57/18,87 μm , s.d. = 4,85/3,07, n = 13) (Plate 1, B & plate 2, A & B). Unilocular sporangia, on branches are shorter and more rounded (length/width: mean value = 37,22/23,55 μm , s.d. = 4,26/3,36, n = 8) (Plate 1, I & Plate 2, C) Unilocular sporangia are only found on the main axis on the LORAN specimen while appearing on both main axis and branches on the Ippik specimen.

Uniseriate, sometimes branched, plurilocular sporangia are also present, embedded in the cortical layer as well. The uniseriate plurilocular sporangia are composed of up to 12 cells (length/width: mean value = 4,10/5,80 μm , s.d. = 0,70/0,75, n = 10) and do not end up in a swollen apical cell (Plate 2, D-H). Cells in the plurilocular sporangia are irregularly divided, with oblique walls, rounded and irregular in shape (Plate 2, G & H). Plurilocular sporangia are only found on side branches from the LORAN specimen.

A number of the crude cultures made on Greenland, have resulted in the growth of identical micro thalli. The micro thalli are composed of inconspicuous creeping systems of filaments, which are uniseriate and branched and grow by the division of apical cells (Plate 2, I). We still need to confirm the actual identity of the micro thalli. Ongoing DNA and autecological studies will hopefully reveal the origin of the micro thalli.

3.4. Morphology of the Satut specimen

A close examination of the material collected at Satut, revealed the presence of an interesting specimen. The specimen was found by Poul Møller Pedersen, and grew epilithic in the sublittoral zone.

The plant showed some similarities with *Delamarea attenuata* (Kjellmann) Hariot. The surface of mature thalli was covered with swollen, elongated cells, often mentioned as paraphyses, with numerous discoid chloroplasts. Among these, unilocular sporangia and phaeophycean hairs are found. The phaeophycean hairs are endogenous and thus provided with a basal sheath. The initial macrothallus is a uniseriate filament. By longitudinal division the cortex is divided into segments (like the pericentral cells on *Polysiphonia*) and the thallus becomes twisted by this process.

3.4.1. Description (App. 5)

Macrothallus is up to 1 cm long. The filaments are up to 200 μm wide and unbranched. The filaments get thicker toward the end and have the shape of a club (Plate 3, A). Young

filaments are uniseriate (Plate 3, C). The cells undergo series of longitudinal divisions and ultimately develop into a parenchyma of two entities: A medullar layer with somewhat elongated cells and a cortex formed by repeated anticlinal and periclinal cell divisions in the initial parenchymatous thallus (Plate 3, G). The cortical cells form a spirally wound, one cell layer thick, cortex (Plate 3, A, B, & G). It is obvious that the growth is diffuse, since there is no sign of a meristem, which is in accordance with FRITSCH [1945]. On the young filaments the cortical cells lie close to the medullar cells and to one another, but later on they become bigger (Length/width: Mean value = 64,03/35,13 μm , s.d. = 7,65/2,85 μm , n = 8) and pyriform and are loosely arranged (Plate 3 A, B). All the rounded cells seem to be able to develop into unilocular sporangia (Plate 3, B). The release of zoospores was observed in the light microscopy (Plate 3, D). No plurilocular sporangia were observed.

Each filament ends with a phaeophycean hair (Plate 3, F). Hairs are also present growing out from the cortical cells, in a regular distance from one another along the side of the filaments (Plate 3, E). The bases of the phaeophycean hairs are embedded in hair sheaths (Plate 3, F).

3.5. Phylogeny

rbcL sequence was successfully obtained for the Satut specimen. No stop codons appeared within the *rbcL* sequences (available upon request). 128 species were used for the phylogenetic analyses and 94 equal parsimony trees were found and gathered in a strict consensus tree (App. 3). It was not possible to obtain readable sequences for the LORAN and Ippik specimens, so further investigations are needed in order to determine if the molecular data match the LORAN specimen.

The Satut specimen branched out with *Delamarea attenuata* (Delamareaceae) with a bootstrap value on 97%. *Punctaria plantaginea* (Roth) Greville (Punctariaceae) branched out as a sister group to this clade with a bootstrap value of 97% (App. 4).

4. DISCUSSION

4.1. Survey

In earlier studies from 1990 and 2002, 73 and 69 species were found respectively [HANSEN & SCHLÜTTER, 1990; CHRISTENSEN *et al.*, 2002]. Thirty of these species were not found in this survey. 20 localities in total for the three surveys were investigated and a total of 106 species found. 137 different species has been reported from Disko Bay [WILCE, 1964]. It is a relatively small difference, which could be caused by differences in the sampling method, difficulties in determination of the different species, and the applied nomenclature.

4.2. Morphology of the LORAN and Ippik specimens.

The multiaxial syntagmatic composition of the medulla, a cortex composed of assimilators ending in swollen apical cell and unilocular sporangia on macro thallus, are all in agreement with the syntagmatic species in the order Ectocarpales family Chordariaceae. The presence of a 2nd intermediate layer, of smaller branched cells, between the medulla and cortex, is also a characteristic feature, seen in e.g. *Saundersella simplex* (Saunders) Kylin, *Chordaria flagelliformis* and *Sphaerotrichia divaricata* [KYLIN, 1947]. The medullar cells are rectangular and elongate like in *S. simplex*, *C. flagelliformis* and *S. divaricata* [KYLIN, 1947; VAN DEN HOEK, 1995]. The differentiation between cortex on branches and main axis, as seen in these plants has not, as far as we know, been reported from known species.

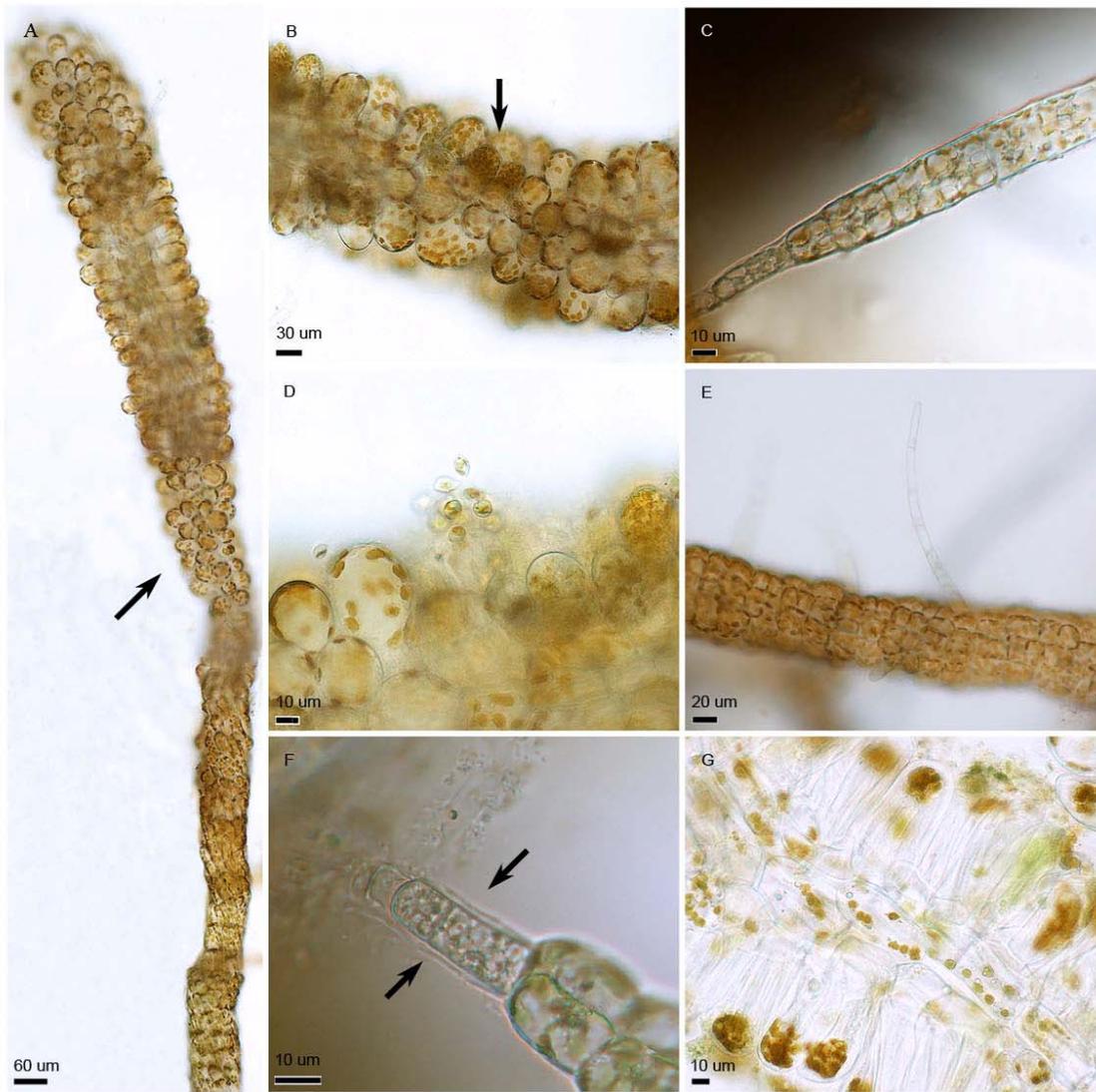


Plate 3 (Living material)

- A: The minute parenchyma broadens towards the apex and has the shape of a club. Surface cells develop into paraphyses with numerous discoid chloroplasts (arrow).
- B: Two unilocular sporangia (arrow) between paraphyses.
- C: Young filament, initially uniseriate, later on with longitudinal cell divisions, which eventually form a terete parenchyma.
- D: Filaments with segmented and spirally wound cortex.
- E: Lateral phaeophyceyan hair.
- F: Phaeophyceyan hair growing out from the apex of a filament with a basal sheath (arrows).
- G: Parenchyma with paraphyses.



Plate 4 (Living material)

A: New species, specimen from LORAN locality. Main axis with numerous branches that appear swollen.

B: New species, specimen from Ippik locality.

C: *Ceramium rubrum*.

D: *Ptilota serrata*.

E: *Ptilota serrata*.

F: *Stictyosiphon tortilis*.

G: *Haplospora globosa*.

H: *Urospora peniciliformis*.

I: *Pylaiella varia*.

The compact composition of the cortex on the main axis has similarities with cortex from *Chordaria magellanica* Kylin [KYLIN, 1947]. The cortex has similarities with the cortex in *S. divaricata*, *C. flagelliformis*, and *S. simplex*, but assimilating filaments in *S. divaricata* and *C. flagelliformis* have different number of cells, composition and arrangement [KYLIN, 1947; VAN DEN HOEK, 1995]. Assimilating filaments from *S. simplex* are quite similar to our plants, but since the macrothallus of *S. simplex* is indistinguishably unbranched this species is out of the question. In many species belonging to Ectocarpales *s.l.*, rich numbers of unilocular ovoid sporangia are found embedded between assimilators in cortex. This is in accordance with our material. Uniseriate plurilocular sporangia, as found on the LORAN specimen, are, to our knowledge, never described in any of the mentioned species. Therefore we consider this as a new genus belonging to the order Ectocarpales and the family Chordariaceae.

4.3. Morphology of the Satut specimen.

The morphology of the Satut specimen indicates that this might be a new species within the genus *Delamarea*. *Delamarea* is an unbranched genus in agreement with the Satut specimen. Several thalli develop from the same prostrate microthallus. They are parenchymatic, with distinct medullar and cortical layer. Furthermore, they both have phaeophycean hairs with basal sheaths and characteristic club-shaped paraphyses [ROSENVINGE, 1893]. The Satut specimen differs from *D. attenuata*, in respect to the cortical layer of cells, which are spirally wound and clearly divided into distinct segments created by longitudinal cell divisions.

On the Satut specimen, development of paraphyses and unilocular sporangia starts from the apex of the thallus. This results in the creation of a thallus getting thicker towards the end, thus having the shape of a club (Plate 3, A). The apical appearance of paraphyses and unilocular sporangia and the club-shaped morphology of the mature thallus are not in agreement with *D. attenuata*. Based on these morphological similarities and differences we argue that this is a new species within the genus *Delamarea*.

4.4. Phylogeny

In order to use the phylogenetic data to confirm if the two investigated species are new species, it is necessary to study the sequence divergences intraspecifically and interspecifically in the family Chordariaceae. The molecular phylogenetic analyses showed a sequence divergence of 2% between the Satut specimen and *D. attenuata*. This divergence is higher than what is normally seen within a genus in the Chordariaceae. This could indicate that the specimen is in fact a new species especially in comparison with the morphological observations. There are problems though: Different strains of the same species branches out in different clades, which could be due to interspecific variation or perhaps identification of the species is not completely correct.

4.5. *Ascophyllum nodosum* (Linnaeus) Le Jolis

The distribution of *A. nodosum* in Greenland, have been reported to spread toward north [OLSEN, 2001]. Until 2006 the northernmost population was reported from Udkiggen, Godhavn (69°15'N, 53°34'W). This population was found in 1998 under a survey during Arctic Field Course 1998. On this year's field course, a careful examination of several localities resulted in the finding of a specimen of *A. nodosum* in Disko Fjord. The plant was found on a wave exposed rocky shore near the LORAN station. It was attached to a stable rock and protected from severe wave action. The plant was approximately 5 cm tall and grew solitarily.

Beside the single attached specimen, plenty of drifting *A. nodosum* were found in the area. Direct experiments of dispersal distances of furoid algae have shown that gamete dispersal is

limited to 1-5 m from the mother plant. Long-distance dispersal is therefore mediated by detached, floating and fertile thalli [OLSEN, 2001].

The fact that only a single specimen was found makes the existence of a population in Disko Fjord very uncertain. But since the presence of a specimen is in accordance with the increasing distribution of *A. nodosum*, combined with the finding of floating plants, we find it reasonable to expect the existence of a population in the vicinity of our finding.

The development in abundance of *A. nodosum* has been explained in connection with increasing annual temperature in West Greenland. Changes in sea temperature in West Greenland water are a complex matter. Beside the atmospheric influence, the North Atlantic Oscillation (NOA) has a great influence on the East Greenlandic and the Irminger current and thereby on the marine climate (salinity and temperature) and also on the atmospheric conditions in western Greenland [http://www.dmi.dk/dmi/fortsat_varmt_over_vestgroenlandske_fiskebanker].

In spite of the general global warming, temperatures in western Greenland have been decreasing since 1968 [STERN & HEIDE-JØRGENSEN, 2003] and sea temperatures have only showed a relatively limited increase since the mid 1990's [http://www.dmi.dk/dmi/fortsat_varmt_over_vestgroenlandske_fiskebanker]. Based on these data we find it reasonable to argue that the ongoing migration of *A. nodosum* is not only caused by changes in temperature. The measured migration is probably also a natural continuation of the migration that has been going on since the last glacial maximum, which occurred between 18-10,000 years ago [OLSEN, 2001]. How the expected changes in temperature will effect the diversity, distribution and migration patterns of macroalgae, is hard to say, but *A. nodosum* can be a valuable and informative key species in the future studies of climate effects on the Greenlandic algal flora.

4.6. *Chordaria chordaeformis* (Kjellmann) Kawai & Kim

In the tidal rock pools at Qeqertaussauq a specimen of *C. chordaeformis* was found during Arctic Field Course 2006. Rosenvinge first registered *C. chordaeformis* in Greenland in 1893. *C. chordaeformis* is characterised by a simple, unbranched (or very few branches) thallus, which looks like a combination of *C. flagelliformis* and *Chorda filum* (Linnaeus) Stackhouse. Its geographical distribution is limited to cold-water areas and it has been recorded in Greenland [ROSENVINGE, 1893], The Arctic Sea [KJELLMAN, 1877] and from the North Pacific [TOKIDA, 1934]. Initially, *C. chordaeformis* was described as *C. flagelliformis* f. *chordaeformis* by KJELLMAN [1877]. This was done due to the overlapping and plastic morphology of the species.

The following studies of the life history of *C. flagelliformis* and *C. flagelliformis* f. *chordaeformis* proved it hard to elucidate the taxonomic relationship. No complete life history was obtained and no sexual reproduction observed [SAUVAGE, 1929; CARAM, 1955; KORNMANN, 1962; KAWAI & KUROGI, 1982].

In a molecular phylogenetic analysis from 2002, Kim and Kawai sequenced nearly the complete *rbcL* gene and its spacer region between *rbcL* and *rbcS*, in order to compare *C. flagelliformis* and *C. flagelliformis* f. *chordaeformis*. The analysis proved the existence of three genetic groups of *C. flagelliformis*. Group 1 was defined as f. *chordaeformis* from North Pacific, group 2 as f. *chordaeformis* from other areas and group 3 as f. *flagelliformis*. Based on this study, *Chordaria chordaeformis* (Kjellmann) Kawai & Kim is now recognized as an independent species.

5. CONCLUSION

The macroalgal survey 2006 resulted in the determination of 76 species. Together with surveys from 1990 and 2002, samples have been taken at 20 different localities and a total of 106 species identified. This provides information of the macroalgal diversity and distribution around Disko, for future studies.

Samplings at the LORAN and Ippik localities resulted in the finding of a, presumably, new genus. Sampling at Satut resulted in the finding of a, presumably, new species. The existence of the new *Delamarea* species is supported by morphological and molecular investigations. Morphological investigations support that the plants from LORAN and Ippik localities is a new genus. Molecular investigations are still ongoing. At sampling in Disko fjord an attached specimen of *A. nodosum* was found. This is the northernmost finding of *A. nodosum*, which underlines the importance of *A. nodosum* as a species for monitoring the effect of possible climate changes. The findings prove that even though macroalgal studies have been going on for more than a century, it is still possible to uncover new ground.

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	1)	2)	3)	4)	5)	6)	7)	8)	9)	10)	11)
Phaeophyceae											
"Ukendt brunalge"								x	x	x	
"Ukendt kofleformet brunalge"								x			
Laminariales											
<i>Agarum clathratum</i>			x				x	x		x	x
<i>Alaria esculenta</i>			x	x				x			x
<i>Chorda filum</i>			x	x				x	x	x	
<i>Saccharina latissima/Laminaria saccharina</i>			x	x				x	x	x	
<i>Saccharina longicirris/Laminaria longicirris</i>											x
<i>Laminaria nigripes</i>											x
Fucales											
<i>Ascophyllum nodosum</i>											x
<i>Fucus evanescens</i>			x	x							x
<i>Fucus vesiculosus</i>			x	x							x
Desmarestiales											
<i>Desmarestia aculeata</i>								x	x	x	x
<i>Desmarestia viridis</i>								x	x	x	x
Ectocarpales											
<i>Chordaria flagelliformis</i>			x	x				x	x	x	x
<i>Chordaria chordaeformis</i>								x			
<i>Dictyosiphon foeniculaceus</i>			x	x				x	x	x	x
<i>Ectocarpus fasciculatus</i>								x			
<i>Ectocarpus siliculosus</i>								x			
<i>Elachista fucicola</i>			x	x				x	x	x	x
<i>Eudesme virescens</i>								x			
<i>Gononema acidioides</i>								x			
<i>Isthmoplea sphaerophora</i>								x			
<i>Laminariocolax tomentosoides</i>											x
<i>Omphalophyllum ulvaceum</i>								x			
<i>Petalonia fascia</i>			x	x					x		
<i>Petalonia zosterifolia</i>											
<i>Phaeostroma longisetum</i>											x
<i>Pogotrichum filiforme</i>											x
<i>Pylaiella littoralis</i>			x	x				x	x	x	x
<i>Pylaiella sp.</i>											x
<i>Pylaiella varia</i>								x			
<i>Seytosiphon complanatus</i>											x
<i>Seytosiphon lomentaria</i>			x	x							x
<i>Stictosiphon tortilis</i>											x
<i>Trachynema mortensenii</i>											x
Tilopteridales											
<i>Halosiphon tomentosus</i>								x	x		x
<i>Haplospora globosa</i>											x
<i>Saccorhiza dermatodea</i>				x					x		
Sphaecelariales											
<i>Sphaecelaria arctica</i>								x			x
<i>Sphaecelaria plumosa</i>								x	x		x
Rhodophyceae											
Acrochaetiales											
<i>Acrochaetium microscopicum</i>								x		x	x
Ceramiales											
<i>Antithamion sp.</i>											x
<i>Ceramium tenuicorne</i>			x								
<i>Ceramium virgatum/Ceramium rubrum</i>								x			
<i>Ceramium sp.</i>								x			
<i>Ceramium strictum</i> eller måske <i>Ceramium tenuicorne</i>									x		
<i>Membranoptera denticulata</i>									x		x
<i>Polysiphonia arctica</i>								x	x	x	x
<i>Polysiphonia stricta</i>			x	x				x	x	x	x
<i>Ptilota serrata</i>								x			x
<i>Rhodomela lycopodioides</i>			x					x			x
Bangiales											
<i>Bangia fuscopurpurea</i>									x		
<i>Porphyra miniata</i>			x					x	x	x	x
<i>Porphyra purpurea</i>								x			
Gigartinales											
<i>Callotryphion cristata</i>											x
<i>Pimbrofolium dichotomum</i>											x
Palmariales											
<i>Devaleraea ramentacea</i>			x	x							x
<i>Palmaria palmata</i>											x
Corallinales											
<i>Lithothamnion compactum</i>									x		
<i>Lithothamnion topiforme</i>											x
<i>Lithothamnion glaciale</i>									x		
Chlorophyceae											
Codiolales											
<i>Acrosiphonia centralis</i>			x	x	x			x	x		x
<i>Acrosiphonia sonderi</i>			x	x				x	x	x	x
<i>Ulvaria fusca/Monostroma fuscum</i>								x	x	x	x
<i>Monostroma grevillei</i>								x	x	x	x
<i>Spongomorpha aeruginosa</i>											x
<i>Urospora penicilliformis</i>											x
Chaetophorales											
<i>Bolbocoleon piliferum</i>											x
<i>Syncoryne reinkei</i>											x
Cladophorales											
<i>Cladophora sp.</i>			x	x	x			x		x	x
<i>Chaetomorpha linum</i>								x			
<i>Chaetomorpha melagonium</i>								x			x
<i>Rhizoclonium riparium</i>			x								
<i>Rhizoclonium tortuosum/Chaetomorpha capillaris</i>			x	x						x	x
Chlorococcales											
<i>Chlorochytrium cohnii</i>			x								
<i>Chlorochytrium dermatocolax</i>											x
<i>Chlorochytrium inclusum</i>			x								
Ulvales											
<i>Ulva intestinalis/Enteromorpha intestinalis</i>									x	x	
<i>Ulva prolifera/Enteromorpha prolifera</i>									x		
<i>Enteromorpha sp.</i>											x
<i>Ulva lactuca</i>			x								
<i>Ulvaria fusca</i>			x	x							x
Ultrichales											
<i>Ullothrix speciosa</i>									x	x	
<i>Ullothrix pseudoflaccida</i>									x	x	

Appendix 1.

The nomenclature on the species list is according to Algaebase.org. Names on the left side of a slash are the current name and on the right side is the synonym. The numbers over the columns corresponds to the different locations: 1) Exposed side near Udkiggen, 2) A small creek between Qeqertaussaq (the "fake" island) and Udkiggen, 3) Qeqertaussaq – the "fake" island, 4) Lyngmarksbugten, 5) HareØ, 6) LORAN, 7) Nordfjord, 8) Satut, 9) Kangerlussuak (Fortunebay), 10) Ippik, 11) HareØ.

App. 2

Taxon and authority	Algal class	Reference	Genbank accession no.
<i>Tribonema intermixtum</i>	Tribonemataceae	Zuccarello, G.C. and Lokhorst, G.M	AY682444.
<i>Pelagophycus porra</i>	Laminariaceae	Yoon <i>et al.</i> (2001)	AF319000
<i>Macrocystis pyrifera</i>	Laminariaceae	Yoon <i>et al.</i> (2001)	AF318998.
<i>Macrocystis integrifolia</i>	Laminariaceae	Yoon <i>et al.</i> (2001)	AF318997.
<i>Lessoniopsis littoralis</i>	Alariaceae	Yoon <i>et al.</i> (2001)	AF318996.
<i>Postelsia palmaeformis</i>	Laminariaceae	Yoon <i>et al.</i> (2001)	AF318995
<i>Nereocystis luetkeana</i>	Laminariaceae	Yoon <i>et al.</i> (2001)	AF318994
<i>Lessonia vadosa</i>	Lessoniaceae	Yoon <i>et al.</i> (2001)	AF318993.
<i>Thalassiohyllum clathrus</i>	Costariaceae	Yoon <i>et al.</i> (2001)	AF318989.
<i>Agarum turneri</i>	Costariaceae	Yoon <i>et al.</i> (2001)	AF318987
<i>Agarum clathratum</i>	Costariaceae	Yoon <i>et al.</i> (2001)	AF318986.
<i>Laminaria sinclairii</i>	Laminariaceae	Yoon <i>et al.</i> (2001)	AF318974
<i>Laminaria hyperborea</i>	Laminariaceae	Yoon <i>et al.</i> (2001)	AF318972
<i>Laminaria digitata</i>	Laminariaceae	Yoon <i>et al.</i> (2001)	AF318971
<i>Eckloniopsis radicata</i>	Lessoniaceae	Yoon <i>et al.</i> (2001)	AF318969
<i>Nereocystis luetkeana</i>	Laminariaceae	Cho <i>et al.</i> (2006)	DQ372565.
<i>Macrocystis pyrifera</i>	Laminariaceae	Cho <i>et al.</i> (2006)	DQ372564
<i>Hedophyllum sessile</i>	Laminariaceae	Cho <i>et al.</i> (2006)	DQ372562
<i>Halosiphon tomentosus</i>	Halosiphonaceae	Kawai and Sasaki (2000)	AB037137
<i>Chorda rigida</i>	Chordaceae	Kawai <i>et al.</i> (2001)	AB035788
<i>Chorda filum</i>	Chordaceae	Kawai <i>et al.</i> (2001)	AB035783
<i>Agarum clathratum</i>	Costariaceae	Sasaki <i>et al.</i> (unpubl.)	AB087236
<i>Saccorhiza dermatodea</i>	Phyllariaceae	Sasaki <i>et al.</i> (2001)	AB045252
<i>Phyllariopsis brevipes</i>	Phyllariaceae	Sasaki <i>et al.</i> (2001)	AB045245
<i>Thalassiohyllum clathrus</i>	Costariaceae	Kawai <i>et al.</i> (2001)	AB035793
<i>Haplospora globosa</i>	Tilopteridaceae	Kawai and Sasaki (2000)	AB037138
<i>Halosiphon tomentosus</i>	Halosiphonaceae	Kawai and Sasaki (2000)	AB037136
<i>Turbinaria conoides</i>	Sargassaceae	Rohfritsch <i>et al.</i> (2007)	DQ448836
<i>Cystoseira osmundacea</i>	Sargassaceae	Harvey and Goff (2006)	AY545475
<i>Halidrys dioica</i>	Sargassaceae	Harvey and Goff (2006)	AY545469
<i>Fucus vesiculosus</i>	Fucaceae	Pearson <i>et al.</i> (unpubl.)	DQ307680
<i>Sargassum muticum</i>	Sargassaceae	Draisma <i>et al.</i> (2001)	AJ287854
<i>Ascophyllum nodosum</i>	Fucaceae	Draisma <i>et al.</i> (2001)	AJ287853
<i>Fucus spiralis</i>	Fucaceae	Phillips <i>et al.</i> (2005)	AY246553
<i>Sargassum polyphyllum</i>	Sargassaceae	Phillips <i>et al.</i> (2005)	AY518391
<i>Hizikia fusiformis</i>	Sargassaceae	Phillips <i>et al.</i> (2005)	AY449537
<i>Carpoglossum confluens</i>	Sargassaceae	Phillips <i>et al.</i> (2005)	AY183896
<i>Silvetia babingtonii</i>	Fucaceae	Lee <i>et al.</i> (unpubl.)	AF132473
<i>Pelvetiopsis limitata</i>	Fucaceae	Lee <i>et al.</i> (unpubl.)	AF106514
<i>Papenfussiella kuromo</i>	Chordariaceae	Kawai <i>et al.</i> (unpubl.)	AB079010
<i>Sphaerotrichia divaricata</i>	Chordariaceae	Kim <i>et al.</i> (unpubl.)	AB077167
<i>Papenfussiella laxa</i>	Chordariaceae	Kawai <i>et al.</i> (unpubl.)	AB079016
<i>Myriogloea simplex</i>	Chordariaceae	Kawai <i>et al.</i> (unpubl.)	AB079015
<i>Tinocladia crassa</i>	Chordariaceae	Kawai <i>et al.</i> (unpubl.)	AB079014
<i>Punctaria tenuissima</i>	Chordariaceae	Siemer and Pedersen (unpubl.)	AF207816
<i>Petalonia fascia</i>	Scytosiphonaceae	Siemer and Pedersen (unpubl.)	AF207815
<i>Elachista stellaris</i>	Chordariaceae	Siemer and Pedersen (unpubl.)	AF207812
<i>Scytosiphon lomentaria</i>	Scytosiphonaceae	Siemer and Pedersen (unpubl.)	AF207811
<i>Phaeostroma pustulosum</i>	Unclassified Ectocarpales	Siemer and Pedersen (unpubl.)	AF207808
<i>Kuckuckia spinosa</i>	Ectocarpaceae	Siemer and Pedersen (unpubl.)	AF207804
<i>Hecatonema sp.</i>	Chordariaceae	Siemer and Pedersen (unpubl.)	AF207802
<i>Feldmannia irregularis</i>	Acinetosporaceae	Siemer and Pedersen (unpubl.)	AF207800
<i>Chordaria flagelliformis</i>	Chordariaceae	Siemer and Pedersen (unpubl.)	AF207798
<i>Asperococcus fistulosus</i>	Chordariaceae	Siemer and Pedersen (unpubl.)	AF207796
<i>Acinetospora crinita</i>	Acinetosporaceae	Siemer and Pedersen (unpubl.)	AF207795
<i>Chordaria chordaeformis</i>	Chordariaceae	Kim and Kawai (unpubl.)	AB066072
<i>Acrothrix pacifica</i>	Acrotrichaceae	Kim and Kawai (unpubl.)	AB066060
<i>Striaria attenuata</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055415
<i>Stictyosiphon soriferus</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055413
<i>Sorocarpus micromorus</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055411
<i>Punctaria plantaginea</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055410
<i>Litosiphon pusillus</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055406
<i>Leptonematella fasciculata</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055405
<i>Laminariocolax tomentosoides</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055404
<i>Isthmoplea sphaerophora</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055403

<i>Humnia onusta</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055402
<i>Haplospora globosa</i>	Tilopteridaceae	Siemer <i>et al.</i> (unpubl.)	AF055400
<i>Giraudia sphacelarioides</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055399
<i>Elachista fucicola</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055398
<i>Dictyosiphon foeniculaceus</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055397
<i>Delamarea attenuata</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055396
<i>Acrothrix pacifica</i>	Acrotrichaceae	Kim and Kawai (unpubl.)	AB066060
<i>Halothrix lumbricalis</i>	Chordariaceae	Lee <i>et al.</i> (2002)	AY079430
<i>Polytretus reinboldii</i>	Chordariaceae	Siemer and Pedersen unpubl.)	AF207809
<i>Striaria attenuata</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055415
<i>Coelocladia arctica</i>	Unclassified Ectocarpales	Siemer <i>et al.</i> (unpubl.)	AF055395
<i>Desmarestia tabacoides</i>	Desmarestiaceae	Kawai and Sasaki (2000)	AB037140
<i>Desmarestia latifrons</i>	Desmarestiaceae	Kawai and Sasaki (2000)	AB037139
<i>Himantothallus grandifolius</i>	Desmarestiaceae	Draisma <i>et al.</i> (2001)	AJ287850
<i>Desmarestia viridis</i>	Desmarestiaceae	Draisma <i>et al.</i> (2001)	AJ287849
<i>Desmarestia ligulata</i>	Desmarestiaceae	Draisma <i>et al.</i> (2001)	AJ287848
<i>Scytosiphon lomentaria</i>	Scytosiphonaceae	Cho <i>et al.</i> (unpubl.)	AB265738
<i>Spermatochnus paradoxus</i>	Chordariaceae	Cho and Boo (unpubl.)	AY996366
<i>Leathesia difformis</i>	Chordariaceae	Cho and Boo (unpubl.)	AY996365
<i>Petrospongium rugosum</i>	Chordariaceae	Cho and Boo (unpubl.)	AY996364
<i>Scytosiphon dotyi</i>	Scytosiphonaceae	Cho <i>et al.</i> (unpubl.)	DQ239771
<i>Hydroclathrus tenuis</i>	Scytosiphonaceae	Cho <i>et al.</i> (unpubl.)	DQ239770
<i>Chnoospora minima</i>	Scytosiphonaceae	Cho <i>et al.</i> (unpubl.)	DQ239768
<i>Ectocarpus siliculosus</i>	Ectocarpaceae	McCauley and Wehr unpubl.)	AY307410
<i>Pleurocladia lacustris</i>	Chordariaceae	McCauley and Wehr unpubl.)	AY307409
<i>Bodanella lauterborni</i>	Unclassified Ectocarpales	McCauley and Wehr unpubl.)	AY307408
<i>Heribaudiella fluviatilis</i>	unclassified Ectocarpales	McCauley and Wehr unpubl.)	AY307406
<i>Sauvageaugloia ikomae</i>	Chordariaceae	Ajisaka <i>et al.</i> (unpubl.)	AB199642
<i>Heterosaunderella hattoriana</i>	Chordariaceae	Ajisaka <i>et al.</i> (unpubl.)	AB199641
<i>Eudesme virescens</i>	Chordariaceae	Ajisaka <i>et al.</i> (unpubl.)	AB199640
<i>Cladosiphon umezakii</i>	Chordariaceae	Ajisaka <i>et al.</i> (unpubl.)	AB199638
<i>Cladosiphon okamuranus</i>	Chordariaceae	Ajisaka <i>et al.</i> (unpubl.)	AB199632
<i>Soranothera ulvoidea</i>	Chordariaceae	Cho <i>et al.</i> (unpubl.)	AY986515
<i>Colpomenia sinuosa</i>	Scytosiphonaceae	Cho <i>et al.</i> (unpubl.)	AY398469
<i>Colpomenia phaeodactyla</i>	Scytosiphonaceae	Cho <i>et al.</i> (unpubl.)	AY398467
<i>Colpomenia durvillei</i>	Scytosiphonaceae	Cho <i>et al.</i> (unpubl.)	AY398466
<i>Pylaiella littoralis</i>	Acinetosporaceae	Assali <i>et al.</i> (1990)	X55372
<i>Ectocarpus siliculosus</i>	Ectocarpaceae	Valentin and Zetsche (1990)	X52503
<i>Laminarionema elsbetiae</i>	Chordariaceae	Peters (2003)	AJ439858
<i>Microspongium tenuissimum</i>	Chordariaceae	Peters (2003)	AJ439847
<i>Elachista antarctica</i>	Chordariaceae	Peters (2003)	AJ439841
<i>Austrofilum incommodum</i>	Chordariaceae	Peters (2003)	AJ439838
<i>Stschapovia flagellaris</i>	Chordariaceae	Kawai and Sasaki (2004)	AB117920
<i>Tilopteris mertensii</i>	Tilopteridaceae	Sasaki <i>et al.</i> (2001)	AB045260
<i>Phaeosiphoniella cryophila</i>	Tilopteridaceae	Sasaki <i>et al.</i> (2001)	AB045259
<i>Carpomitra costata</i>	Sporochneaceae	Sasaki <i>et al.</i> (2001)	AB045257
<i>Saccorhiza polyschides</i>	Phyllariaceae	Sasaki <i>et al.</i> (2001)	AB045256
<i>Phyllariopsis purpurascens</i>	Phyllariaceae	Sasaki <i>et al.</i> (2001)	AB045250
<i>Hecatonema sp.</i>	Chordariaceae	Siemer <i>et al.</i> (unpubl.)	AF055401
<i>Pogotrichum filiforme</i>	Acinetosporaceae	Siemer <i>et al.</i> (unpubl.)	AF055409
<i>Stypocaulon scoparium</i>	Stypocaulaceae	Draisma <i>et al.</i> (unpubl.)	AJ287898
<i>Sphacelaria californica</i>	Sphacelariaceae	Draisma <i>et al.</i> (unpubl.)	AJ287893
<i>Sphacelaria tribuloides</i>	Sphacelariaceae	Draisma <i>et al.</i> (unpubl.)	AJ287891
<i>Sphacelaria yamadae</i>	Sphacelariaceae	Draisma <i>et al.</i> (unpubl.)	AJ287890
<i>Sphacelaria divaricata</i>	Sphacelariaceae	Draisma <i>et al.</i> (unpubl.)	AJ287889
<i>Sphacelaria arctica</i>	Sphacelariaceae	Draisma <i>et al.</i> (unpubl.)	AJ287881
<i>Sphacelaria nana</i>	Sphacelariaceae	Draisma <i>et al.</i> (unpubl.)	AJ287875
<i>Halopteris filicina</i>	Stypocaulaceae	Draisma <i>et al.</i> (unpubl.)	AJ287895
<i>Cladostephus spongiosus</i>	Cladostephaceae	Draisma <i>et al.</i> (2001)	AJ287863
<i>Choristocarpus tenellus</i>	Choristocarpaceae	Draisma <i>et al.</i> (2001)	AJ287861
<i>Phaeothamnion confervicola</i>	Phaeothamniaceae	Bailey (1998)	AF064746
<i>Vaucheria bursata</i>	Vaucheriaceae	Andersen and Bailey (2002)	AF476940
<i>Heterosigma akashiwo</i>	Raphidophyceae	Ki and Han (In press, 2006)	DQ470668

App. 3

***Chordaria* var. from LORAN**

Holotype: LORAN specimen.

Macrothallus ad 17 cm altus et axis principalis ad 1mm latus cum ramis primariis ad 2mm crassus et 5 cm longus. Thallus multiaxialis syntagma est. Medulla e cellulis magnis composita. Stratus intermedius minorum cellularum rotundatarum medullam a cortice separat. Cortex axis principalis fillis arcte contiguis assumptibus e 2-8 cellulis compositus, modice crescens magnitudine versus cellulam apicalem. Cortex ramorum laxius constructus, filla assumptia breviora sunt, 2-4 cellulae, cum cellulis terminalibus compluries maioribus quam cellula matricialis (longitudo/lattitudo : aestimatio media = 24,64/11,95 μm , s.d.= 4,12/2,88 μm ,n=28). Sporangia unilocularia ovoidea sunt et e stratu intermedio orta et modo in axe principale. Sporangia plurilocularia uniseriata usque ad 12 cellulas composita. Sporangia plurilocularia inter assimilatores et modo in ramis crescunt.

The macrothallus is up to 17 cm long and main axis up to 1 mm wide, with first order laterals up to 2 mm thick and 5 cm long. Thallus is a multiaxial syntagma. The medulla is composed of large cells. An intermediate layer of smaller more rounded cells separates the medulla from the cortex. The cortex of the main axis is composed of densely packed assimilating filaments of 2-8 cells, lightly increasing in size toward the apical cell. The cortex of the laterals is more loosely constructed, the assimilating filaments are shorter, 2-4 cells, with terminal cells several times bigger than the mother cell (Length/width: Mean value = 24,64/11,95 μm , s.d. = 4,12/2,88 μm , n = 28). Unilocular sporangia are ovoid and originate from the intermediate layer and only on the main axis. Plurilocular sporangia are uniseriate composed of up to 12 cells. Plurilocular sporangia develop among assimilators and only on branches.

***Delamarea claviformis* sp. nov.**

Macrothallus ad 1 cm longus, usque ad 200 μm latus et sine ramis est. Attenuatus versus basem (est) et formam spiraliter claviformis vulneratis habet. Macrothalli juvenes uniseriati et phaeophyceanis veris pilis cum vagina basale terminati (sunt). Primae divisiones longitudinales distinctae et regularibus intervallis formatae (sunt). Postremo parenchyma tere crescit. Cellulae corticales saepe spiraliter formatae. Ante fertilitatem cellulae corticales proximaliter in paraphyses amplificunt et sporangia unilocularia (longitudo/lattitudo: aestimatio media = 64,03/35,13 μm , s.d.=7,65/2,85 μm , n=8) e base paraphysum crescentia. In macrothallis nulla sporangia plurilocularia observata.

The macrothallus is up to 1 cm long, up to 200 μm wide and unbranched. It attenuates toward the base and has the shape of a spirally wounded club.

Young macrothalli are uniseriate and are terminated by true phaeophycean hairs with a basal sheath. The initial longitudinal divisions are distinct and formed at regular intervals. Ultimately a terete parenchyma develops. The cortical cells are often spirally arranged. Before fertility cortical cells enlarge proximally into paraphyses and unilocular sporangia (Length/width: Mean value = 64,03/35,13 μm , s.d. = 7,65/2,85 μm , n = 8) develop at the base of the paraphyses. No plurilocular sporangia observed on macrothalli.

Taxonomic Analysis of Greenlandic *Echiniscoides* (Heterotardigrada)

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Abstract. As part of an analysis of the biogeography and phylogeny of the marine *Echiniscoides* material of the two Greenlandic species (Heterotardigrada) was collected at Arktisk Station. Preliminary analyses show that *E. sigismundi groenlandicus* is very closely related to *E. s. sigismundi* and that *E. hoepneri* is related to a number of taxa from warmer waters. The biogeography of Greenlandic and Non-Greenlandic *Echiniscoides* as well as a few morphological and cryptobiotic observations are discussed.

Keywords. Tardigrada, *Echiniscoides*, *E. sigismundi*, *E. hoepneri*, phylogeny, COI, 28S, H3, biogeography

1. INTRODUCTION

The genus *Echiniscoides* is under the present tardigrade systematics one of only 2 marine genera within the otherwise terrestrial order Echiniscoida. The number of species within this group is unknown. Until 1980 it was believed that the genus contained only one species, *E. sigismundi*, which was assumed to be cosmopolitan based on reports from several continents in both hemispheres. In 1976 a southern hemisphere subspecies *E. s. polynesiensis* was described by Renaud-Mornant and in 1980 a second species *E. hoepneri* and a number of subspecies of *E. sigismundi* was described by KRISTENSEN & HALLAS [1980]. After this a number of additional species has been described and according to the newest checklist of tardigrades the genus contains eight species one of them *E. sigismundi* has eight subspecies [GUIDETTI & BERTOLANI 2005]. Most of the species appear to have small distributions and many are known only from their type locality.

Two taxa of this genus are known from Greenland and the waters around Arktisk Station are the type locality for both of them. Both species live in tight association with barnacles of the species *Semibalanus balanoides*. *E. hoepneri* lives as a parasite on the barnacles in the lower tidal zone and *E. s. groenlandicus* lives commensally mainly in the higher tidal zone. *E. hoepneri* is only known from the island of Disko whereas *E. s. groenlandicus* has the largest known distribution of any taxon in the genus and is reported from several places in Greenland as well as the Atlantic coast of North America and arctic and subarctic Norway and Russia [KRISTENSEN & HALLAS, 1980].

As part of my Ph.D., I am trying to elucidate the phylogeny and biogeography of the genus *Echiniscoides* and I was therefore very happy to get the opportunity to go to Arktisk Station during the Arctic Biology field course since it enabled me to incorporate the Greenlandic taxa in my analysis. Neither the collection of materials nor the analyses of already collected materials for these analyses have been completed and therefore I am only able to report the results from the preliminary results here.

As another part of my Ph.D., I am looking at the evolution of cryptobiosis by comparing anhydrobiotic ability between different taxa in *Echiniscoides*. *E. s. groenlandicus* is known to be able to survive anhydrobiosis whereas *E. hoepneri* can not [KRISTENSEN & HALLAS, 1980]. For this analysis I collected barnacles with *E. s. groenlandicus* and dried them in Greenland so the analysis of anhydrobiotic ability could be made upon my return to Denmark, but unfortunately the *E. s. groenlandicus* did not survive this treatment well and only a single living individual was found and therefore this analysis could not be done.

Locality	COI sequences	28S sequences	H3 sequences
Lynæs	4	1	0
Kristineberg (Sweden)	4	1	1
Århus	8	1	1
Faroe Islands	9	1	1
Esbjerg	9	0	0
Roscoff (France)	1	1	0
La Coruna (Spain)	5	1	0
Lanzarote (Canary Islands)	1	0	0
Sicily	3	1	1
Thailand	5	1	1
Seychelles	1	0	0

Table 1. Locality of collected tardigrades and numbers of useable sequences from the individual localities.

2. MATERIALS AND METHODS

Description of the habitat

The tardigrades were collected at Sorte Sand near Arktisk Station living on barnacles of the species *Semibalanus balanoides* in the middle and upper tidal zone. A number of algae were living on the barnacles; these were kindly identified by Poul M. Pedersen to cyanobacteria of the species *Pleurocapsa amethysta* and a number of green algae cellagregates and *Codilium* stages.

DNA extractions

The total genomic DNA was extracted using the STE-buffer method [MANIATIS *et al.*, 1982]. Animals of both species were cleaned and removed from the substrate under a dissecting microscope. The animals were then identified to species under a light microscope and frozen in STE buffer.

Three loci were used in the analysis: The mitochondrial COI was amplified with the primers HCO and LCO [FOLMER *et al.*, 1994] and the nuclear 28S with the primers 1274F and 689R [MARKMANN, 2000]. The nuclear locus H3 was attempted amplified using the primers H3aF and H3bR [COLGAN *et al.*, 1998] which have worked on other taxa in the genus but failed for both *E. s. groenlandicus* and *E. hoepneri*. COI was amplified from individual tardigrades whereas 28S and H3 was amplified from pools of five animals. PCR reactions was made using Quigen mastermix© in volumes of 40µl. The annealing temperature was 42°C for both COI and 28S. For H3 a touch-down PCR technique was used where the annealing temperature was gradually lowered from 59 to 53°C in consecutive cycles. The product was checked on 1% agarose-gels and successful amplifications were cleaned and afterwards sequenced in both directions by Macrogen®. A few of the non Greenlandic COI sequences were only sequenced in reverse and their sequence should only be considered preliminary.

Genetic analyses

Forward and reverse sequences were compared and edited in Bioedit [HALL, 1999]. All sequences were compared to the database at NCBI using Blast [ALTSCHJUL *et al.*, 1990] Alignment was done using ClustalX [THOMPSON *et al.*, 1994] and checked manually. Tardigrades from a number of other locations, besides those obtained from Greenland, were included in the analysis (see Table 1).

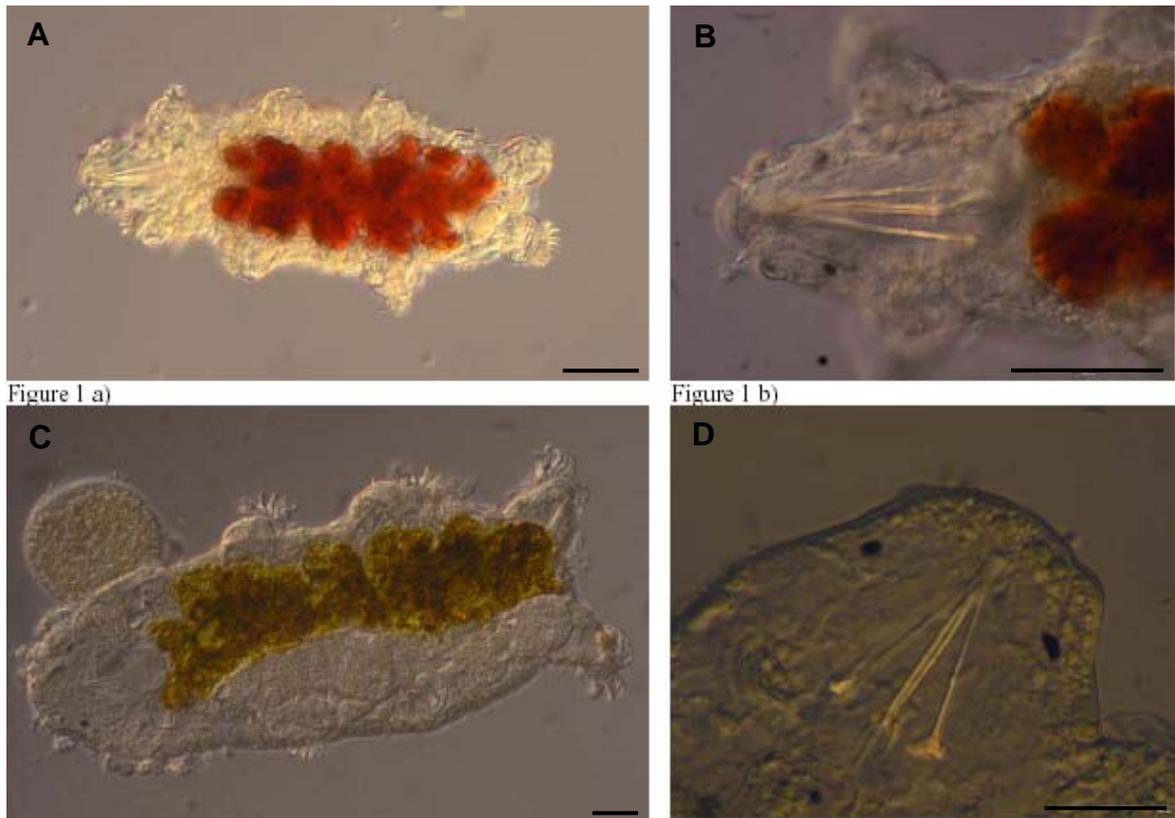


Figure 1. Pictures of entire animals and head regions of *E. hoepneri* (A+B) and *E. sigismundi groenlandicus* (C+D). Comparisons of Figure 1A and 1C clearly shows the different gut color between the two species which is reddish in *E. hoepneri* and brownish in *E. sigismundi groenlandicus*. Figure 1B and 1D shows one the main diagnostic characters between *E. hoepneri* and *E. sigismundi groenlandicus* as the stylets in the buccal region are clearly longer in *E. hoepneri*. Scale bars in A+B = 50 μ m and in C+D = 25 μ m.

All tardigrades used were collected from barnacles except the ones from Roscoff which were collected from polychaetes and is thought to be a new species [KRISTENSEN pers. comm.].

Distances between all COI sequences were analyzed with K2P using Mega v. 3.1. [KUMAR *et al.*, 2004]. The sequences fell into 9 discrete clusters with less than 2% sequence variation between sequences from the same cluster but between 8 and 26% variation between sequences from different clusters. The largest of these containing the sequences of *E. s. groenlandicus* were analyzed using a Haplotype network with the program TCS [CLEMENT *et al.*, 2000]. These clusters were all assumed to be monophyletic and in the combined analysis of all three genes only a single COI from each cluster was used. In the phylogeny based only on COI a single sequence from all clusters was used except the largest cluster which is represented by a sequence from Greenland, a sequence from Århus and a sequence from the Seychelles. A parsimony analysis of the individual loci (28S and COI) was performed using Mega v. 3.1. [KUMAR *et al.*, 2004]. A Bayesian analysis of the individual loci (28S and COI) and the total dataset containing COI, 28S and H3 was performed using MrBayes v. 3.1 [HEULSENBECK & RONQUIST, 2001]. The best models were chosen by looking at Bayes factor [KASS & RAFTERY, 1995] and the preferred models were GTR+ Γ +I for COI, K2P+I for 28S and SYM+I for H3. For the combined dataset the favored model was three partitions with individual parameters one for each gene, with the one exception being same frequencies of invariable sites for 28S and H3.

3. RESULTS

Morphological observations

Although not the focus of the investigation two interesting observations were found. The most important of these was the discovery of males in the species *E. hoepneri*. In the original description, KRISTENSEN & HALLES [1980] stated that males do not exist which would be very interesting since almost all marine tardigrades have separate sexes and parthenogenesis is virtually unknown. Unfortunately the male specimens were lost during fixation which makes a formal description impossible at this point.

The other observation was a rather consistent colour difference between *E. hoepneri* and *E. s. groenlandicus*. Heterotardigrades get rid of excrements only during ecdysis [KRISTENSEN pers. comm.] and therefore the gut region is coloured by the food and is gradually becoming darker as wastes are concentrated until next ecdysis. *E. hoepneri* gradually gets a red colour whereas *E. s. groenlandicus* becomes dark brown. The colour of *E. hoepneri* is presumably caused by barnacle larvae which is their main food. Cycliophorans develop a similar colour when they eat crustacean material [P. FUNCK pers. comm.] which supports this idea. The colour may thus be a character, which has a certain taxonomic value as different colours may indicate different food sources and may therefore help separate cryptic species which appear to be common in the genus (see results from genetic analysis). Pictures of both the head region and the entire animal can be seen in Figure 1.

Genetic analysis

DNA extractions from the Greenlandic *Echiniscoides* turned out to be more difficult than extractions from other taxa in the genus and I have so far only managed to get two COI sequences from *E. s. groenlandicus* and one 28 S sequence from *E. hoepneri*. Further extractions will be performed on the remaining material later but these will unfortunately not be possible to incorporate in this analysis. The few sequences from Greenland do however give interesting results.

The COI sequences from Greenland form part of a cluster formed by individuals from Sweden, The Faroe Islands, Lynæs, Esbjerg, Århus and interestingly the Seychelles (Hereafter referred to as *sigismundi* cluster). Not all sequences collected in the Nordic countries belong to this cluster however. Another cluster is composed of several sequences from Århus and Esbjerg (hereafter referred to as *AarhusSp*) and a third is composed of a single aberrant sequence from the Faroe Islands. Generally, sequences from the other collection sites form individual clusters with two exceptions. The proposed new species from Roscoff clusters with tardigrades from La Coruna whereas the sequences from Thailand belong to two different clusters which are, however, sister groups. The phylogeny based on the individual loci as well as the combined dataset can be seen in Figure 2a-d. The network analysis of the *sigismundi* cluster can be seen on Figure 3.

4. Discussion

Taxonomy

The sequences of *E. s. groenlandicus* clearly fall within the sequences of the *sigismundi* cluster. With the exception of the individual from the Seychelles, the remainders of the sequences in this cluster are all *E. s. sigismundi* and the status of subspecies for *E. s. groenlandicus* seems perfectly justified since the non-monophyletic status of *E. s. sigismundi* with regards to *E. s. groenlandicus* indicates current or recent gene flow.

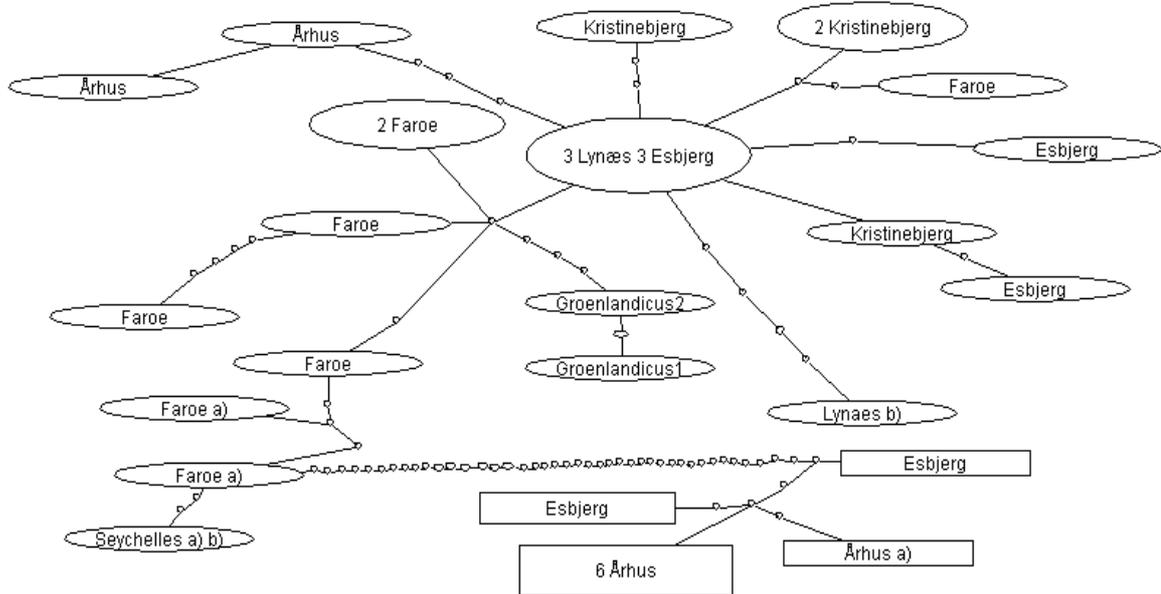
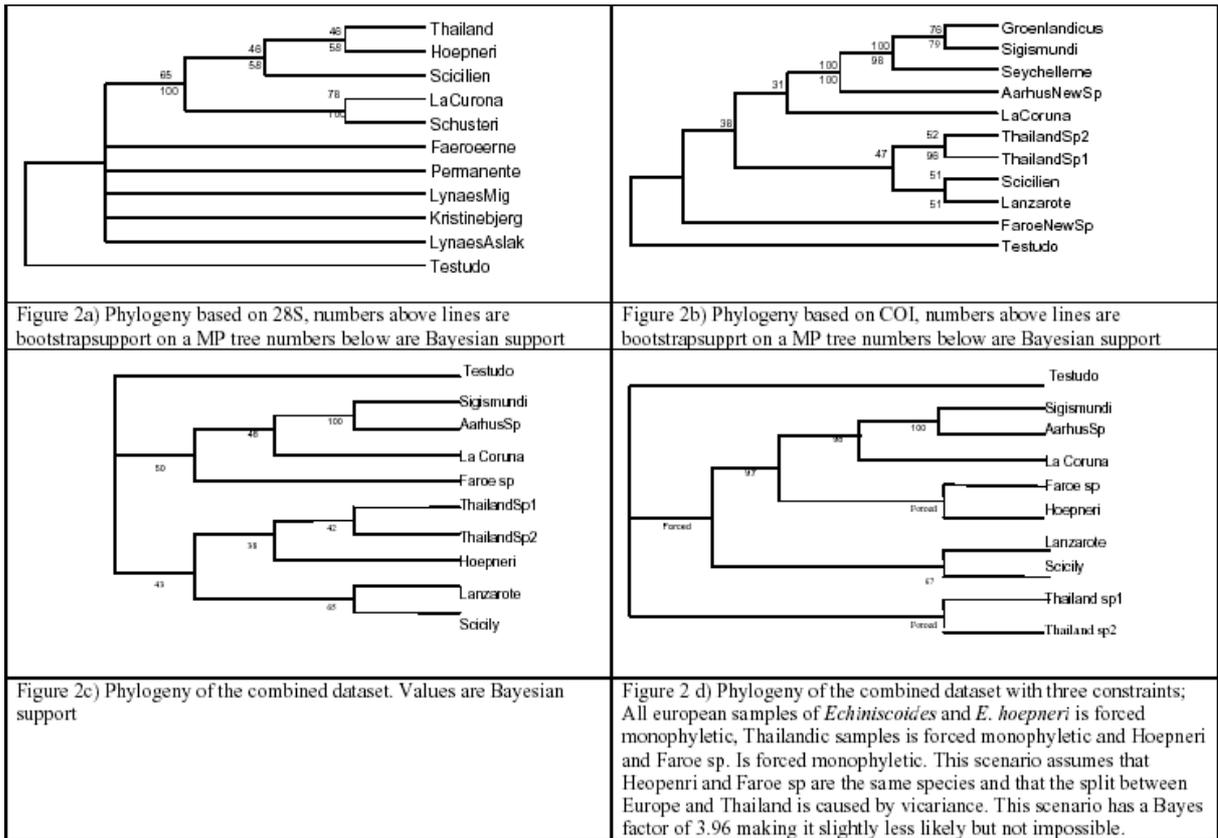


Figure 3. Networkanalysis of the *sigismundi* cluster and *Aarhus sp.* All haplotypes from the *sigismundi* cluster are placed in ovals and all haplotypes from *Aarhus sp* are placed in rectangles. The majority of haplotypes are singletons but a few haplotypes are shared by several specimens, these are put in larger rectangles/ovals and the number of sequences in each haplotype is noted. A few of the haplotypes marked with **a**) or **b**) are less reliable than the remainder. **a**) indicate that the specimen has only been sequenced in one direction which mean that one or two nucleotides may be coded wrong. **b**) indicate that he sequence comes from a pool of animals. The small circles indicate hypothetical haplotypes needed to link the sampled sequences.

The discovery of the *AarhusSp* cluster which appears to be a cryptic species within *E. s. sigismundi* may, however, have taxonomical implications and an analysis of diagnostic differences between the two species in Denmark and a reanalysis of the type material of *E. s. sigismundi* is necessary to decide whether *groenlandicus* is a subspecies of *sigismundi* or the new species. The new species has not yet been found near the locality of the neotype which indicates that *groenlandicus* probably is a subspecies of *E. sigismundi*.

The analysis of the non-Greenlandic specimens also has taxonomical implications. I believe that all clusters of COI sequences should be given species status, although there should, be taken caution to three clusters composed of only a single individual namely *ThailandSp2.*, *FaroeSp.* and *LanzaroteSp.*

It is at present not possible to give species names to all the clusters but the preliminary analyses give the following suggestions. The specimens from La Coruna are genetically very similar to the specimens from Roscoff and I find it unlikely that they represent different species. The individuals from La Coruna cluster appear to be *E. s. galliensis* based on size, cuticular sculpture and claw number and should probably be elevated to *E. galliensis*. I find it likely that *E. s. hispaniensis* previously known from La Coruna [KRISTENSEN *et al.*, 1980] but not included in this analysis represents a different species. The Sicily cluster is probably the tardigrade referred to as *E. s. sigismundi* in an analysis from the Mediterranean [GRIMALDI & D'ADDABBO, 2001] but it clearly represents a new species or the subspecies *E. s. mediraniens* although diagnostic characters remain to be found. One of the Thai clusters is likely to represent *E. andamensis* although it is unknown which one.

Biogeography

From a parsimony point of view it would be reasonable to assume that the COI sequence of *FaoreSP* could represent *E. hoepneri* since it would require one less undiscovered species in the Nordic countries but this hypothesis has to be rejected when calculating the Bayes factor for a model forcing the monophyly of *Hoepneri* and *FaroeSp* in the combined analysis which indicate *FaroeSp* represent an undescribed species. Interestingly when two more constraints, namely the forced monophyly of all European *Echiniscoidea* and the monophyly of Thai *Echiniscoidea* were placed on the tree (Figure 2d) the likelihood increased and the resulting tree is only marginally significant worse than the tree without constraints. These results are probably caused by local adaptive peaks in the search for the best tree and I do not believe that it can be concluded if *FaroeSp* and *E. Hoepneri* are the same without COI sequences from *E. hoepneri* or a 28S sequence from *FaroeSp*.

The network analysis of *E. s. groenlandicus* in an otherwise mainly Nordic cluster in my opinion indicates a recent possibly post ice age arrival of *E. s. groenlandicus* from Europe for instance possibly over the Faroe Islands and Iceland. There were no shared haplotypes which could indicate a lack of contemporary gene flow but the number of sequences in this preliminary analysis is too low to make strong conclusions. The sequence from the Seychelles in this cluster is very odd. As mentioned in the introduction, taxa of the genus generally have a very limited distribution and it therefore seems unlikely that the natural distribution of *E. sigismundi* includes the Nordic countries, Greenland and the Seychelles. I find it more likely that this is a case of human induced transport, a similar case has probably been found in the harbor of Sydney although in that case DNA sequences were not obtained so cryptic species cannot be excluded [KRISTENSEN pers. comm.].

E. hoepneri is related to a number of subtropical and tropical taxa from Thailand, Lanzarote and the Mediterranean and its occurrence in Greenland is rather odd (Figure 2d) and the separation of the two clusters is not easily understood from biogeographic theory. It is

possible that it can be resolved with the inclusion of Atlantic North American tardigrades, which I am currently collecting.

Cryptobiosis

It is difficult to interpret the fact that the survival rate of *E. s. groenlandicus* was so low that I could not make the analyses of anhydrobiotic survival, since it is nearly impossible to keep conditions identical under transport. Still, I believe that the results are a real phenomenon and not caused by such artefacts. Tardigrades from Sicily, Thailand, and Esbjerg have all survived similar treatments, albeit the Tardigrades from Thailand did have a moderate mortality rate. The tardigrades from the Faroe Islands however failed to survive transport. Sequences from the Faroe Islands have shown that 8 out of 9 belong to the *sigismundi* cluster and the 9th may or may not be a *E. hoepneri*. Based on this, it seems likely that low anhydrobiotic survival is a general phenomenon in the cluster of the *sigismundi* cluster. Further supporting this is a comparative analysis of anhydrobiotic survival in four populations, two mainly composed of *E. s. sigismundi* and two mainly composed of *AarhusSp* [FAURBY unpublished]. In this analysis both populations of *AarhusSp* had a higher survival rate than the populations of *E. s. sigismundi*.

It is very interesting that *E. hoepneri* is placed in a cluster of species with well developed cryptobiotic ability but nevertheless is non-cryptobiotic [KRISTENSEN *et al.*, 1980]. One taxon from this cluster, the species from Sicily has the greatest cryptobiotic ability of the taxa studied so far [FAURBY unpublished]. This indicates that cryptobiotic ability within the genus *Echiniscoides* is rather plastic but apparently mainly between species.

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Comments on the Physiology, Morphology and Phylogeny of *Halobiotus crispae* (Eutardigrada: Hypsibiidae)

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Abstract. *Halobiotus crispae* was initially described from Nipisat, Disko Island, Greenland in 1982. Since then several other localities throughout the northern hemisphere have been discovered. Uniquely, this species is characterized by seasonal cyclic changes in morphology and physiology known as cyclomorphosis. In the present study osmotic stress tolerance were compared in *Halobiotus crispae* collected at Nipisat Bay, Disko Island Greenland and Vellerup Vig Isefjord, Denmark. Our results demonstrate that *H. crispae* is a euryhaline species able to tolerate enormous changes in ambient salinity, greatly exceeding those encountered in nature. The ability to cope with varying salinities however is related to the cyclomorphic stage. The effect of osmotic stress on volume regulation at a whole organismal level was also investigated. Our results indicate that large volume changes takes place during exposure to very dilute as well as concentrated saltwater solutions. Differences in habitats between the two populations endorse the suggestion that the ability to overcome salinity fluctuations is a factor in determining the horizontal distribution of *H. crispae*. Experiments with freeze-tolerance on animals in pseudosimplex 1 showed a seemingly inverse relationship between survival and cooling rate. Desiccation was not tolerated in any of the cyclomorphic stages. Genetic distance between the two populations in Nipisat Bay and Vellerup Vig were investigated using the two loci COI and ITS2. Results show very little variation between the populations suggesting that they are both *H. crispae*. In addition, specimens initially identified as *Halobiotus stenostomus* from Ærø, Denmark in our study is a deep-water form of *H. crispae*. Our SEM investigation confirms this new interpretation. Musculature and nervous system of *H. crispae* were reconstructed from whole animals by immunocytochemistry and confocal laser scanning microscopy.

Keywords. Malpighian tubules, cyclomorphosis, osmotic stress, volume regulation, genetic diversity, CLSM

1. INTRODUCTION

Over the course of the past three decades numerous investigations of the tardigrade fauna has been undertaken from the Danish Arctic Station, Qeqertarsuaq, Disko Island, West Greenland. The tardigrade fauna is extremely rich in Arctic and Antarctic regions, thus 112 species have currently been described from the area in and around Arctic Station; almost half of them new to science [HEIDE-JØRGENSEN & KRISTENSEN, 1999]. The secondary marine genus *Halobiotus* contain only five members, (*H. stenostomus*, *H. appeloefi*, *H. geddesi*, *H. arcturulus* and *H. crispae*) yet two of them *H. crispae* [KRISTENSEN, 1982] and *H. arcturulus* [CRISP & KRISTENSEN, 1983] have been described from Greenland. Consequently, future studies on Tardigrada in the Arctic is very important and will undoubtedly prove as fruitful as it has been in the past, hereby contributing to the ever growing knowledge on this “smaller” phylum.

1.1. Tardigrada

1.1.1. Phylogenetic position

Ever since their discovery in the 18th Century by Eichhorn the phylogenetic position of Tardigrada has remained problematic. Resolving the classification of this “lesser-known” group of protostomes has, however, become very important in recent years due to the supposed significance in elucidating early metazoan phylogeny, in particular with respect to the arthropods [SCHMIDT-RHAESA *et al.*, 1999; GRAHAM, 2001]. Based on morphological features as well as fossil records [BERGSTRÖM & HOU, 2001] previous attempts to infer the position of the Tardigrada have resulted in conflicting conclusions because of structural similarities with

such diverse phyla as Nematoda [GAREY *et al.*, 1996], Loricifera [KRISTENSEN, 1991], Arthropoda [NIELSEN *et al.*, 1997] and Onychophora [KINCHIN, 1994], why some authors prefer to treat them as an enigmatic group. More recent molecular studies based on 18S rRNA sequences, however, succeeded in inferring tardigrades as a monophyletic sister group of the arthropods [GIRIBET *et al.*, 1996; GAREY *et al.*, 1999] thus forming a monophyletic clade; the common taxon of Tardigrada, Onychophora and Euarthropoda was later on termed Panarthropoda by NIELSEN [2001]. These new data of an inferred close relationship between Tardigrada and Arthropoda was in another study supported by the evidence for a clade of all molting animals, the Ecdysozoa [AGUINALDO *et al.*, 1997], which completely altered the traditional view of “segmented” protostomes forming a monophyletic group, *i.e.* the Articulata theory. Today, the Articulata hypothesis has fallen out of favour [MAXMEN *et al.*, 2005], yet, the topology among the Ecdysozoan phyla is being debated [SCHMIDT-RHAESA, 2001], especially with respect to the position of onychophorans [GAREY, 2001], as newer studies often show an unresolved relationship within the Panarthropoda [PARK *et al.*, 2006]. Consequently, after more than 240 years of research, the final systematic position of the Tardigrada still remains to be elucidated.

1.1.2. General morphology

Tardigrades are bilaterally symmetrical micrometazoans with a body divided into five variably distinct body segments; a cephalic segment containing a mouth, eyespots and sensory organs (*cirri and clavae*) and four trunk segments. The first three trunk segments each bear a pair of lateroventrally directed legs, while the terminal trunk bears a pair of posteroventrally directed legs, typically terminating in 4 to 13 claws or suction disks. A typical tardigrade body averages from 50-1200 μm in length and is ventrally flattened with a convex dorsal side. The tardigrade is covered by a segmented chitinous cuticle, which is periodically shed during molting over the course of its life. Body colour may be opaque, white, or such colours as brown, green, pink, red, orange or yellow depending on cuticular pigments or dissolved materials in the body fluids, and contents of the digestive tract. The digestive system consists of a foregut, midgut and a hindgut with a pair of stylets, stylet supports and stylet glands flanking the buccal tube (Fig. 1&6). They have a hemocoel-type of fluid filled body cavity, *i.e.* an open circulatory system as seen in most arthropods and molluscs, which functions in circulation and respiration. The muscle system consists of somatic, visceral, pharyngeal and stylet muscles. The somatic muscles may be either truly cross-striated, as in arthrotardigrada [KRISTENSEN, 1978], or obliquely striated; however, the stylet support muscles of the pharyngeal bulb are always cross-striated [EIBYE-JACOBSEN, 1996]. The nervous system consists of a relatively large lobed dorsal brain *i.e.* the supraoesophageal ganglion, divided into three lobes homologized with the protocerebrum, deutocerebrum and tritocerebrum of euarthropods [NIELSEN, 2001]. The trilobed brain is a clear arthropod character, and has been used in linking Tardigrada to the arthropod line. Furthermore, the supraoesophageal ganglion is joined by a pair of circumoesophageal connectives to a suboesophageal ganglion, which in turn is linked to a ventral chain of four fused paired ganglia. These correspond to the individual trunk segments, and are connected intrasegmentally by transverse commissures and intersegmentally by longitudinal connectives, thereby resembling the ladder-like central nervous system described from arthropods and annelids [MARCUS, 1929; DEWEL *et al.*, 1999]. The nervous system is therefore distinctly metameric. Additionally, an autapomorphy for all tardigrades is the commissure from the protocerebrum to the first leg ganglion.

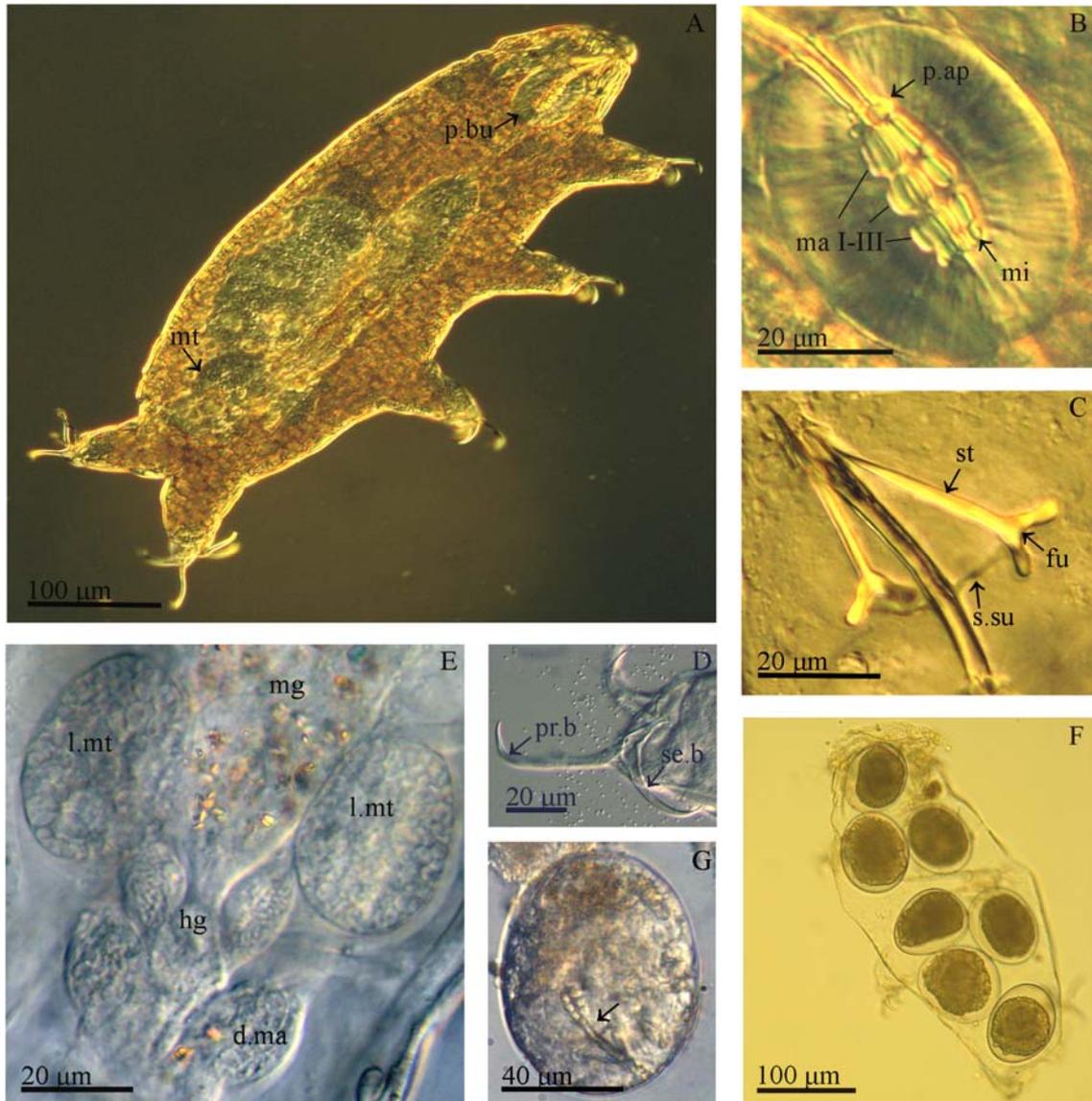


Figure 1. Light micrographs of *H. crispae* (Nomarski-technique). **A.** Overview of active male from Nipisat. **B.** Close up on pharyngeal bulb. **C.** Close up on the stylets, note the large furca. **D.** Close up of the external claw of a hind leg. **E.** Overview of the Malpighian tubules. **F.** Overview of exuvium with eggs. **G.** Close up of egg, note the microplacoid is not separated from the third macroplacoid (arrow). Abbreviations; p.bu = pharyngeal bulb; mt = Malpighian tubules; p.ap = pharyngeal apophysis; ma I-III = first to third macroplacoid; mi = microplacoid; st = stylet; s.su = stylet support; fu = furca; pr.b = primary branch; se.b = secondary branch; l.mt = lateral Malpighian tubules; d.ma = dorsal Malpighian tubules; mg = midgut; hg = hind gut.

1.1.3. Major taxa

Traditionally described as a smaller phylum, Tardigrada comprises more than 960 described species [GUIDETTI & BERTOLANI, 2005]; however, taxonomists expect that at least 10.000 species exists [RAMLØV & KRISTENSEN, 1985]. Based on morphological features two main extant lineages have been established: Heterotardigrada and Eutardigrada. A third class, Mesotardigrada, is based on only a single species *Thermozodium esakii* Rahm, 1937. However, since the type material does not exist and the type locality was destroyed in an earthquake [RAMAZZOTTI & MAUCCI, 1983] this third class is now considered dubious.

Heterotardigrada is comprised of two orders: Arthrotardigrada and Echiniscoidea - with arthrotardigrades possessing the most plesiomorphic characters [KINCHIN, 1994]. Arthrotardigrada consist almost exclusively of marine species (with the exception of *Styraconyx hallasi* Kristensen, 1977), while Echiniscoidea contain terrestrial, limnic as well as marine species. Morphologically, heterotardigrades are very diverse in body shape and overall appearance when compared to eutardigrades, and are generally smaller in size. The key taxonomic characters separating the two orders include a separate gonopore, a closed three-lobed anus, well-developed cephalic-, trunk- and leg appendages and the absence of Malphigian tubules [GUIDETTI & BERTOLANI, 2005]. The shape of the feet is often used in determination of family and genus [Hansen, 2004 M. Sc. thesis unpublished].

The class Eutardigrada includes the orders Parachela and Apochela. Parachela consists entirely of limno-terrestrial species, with the exception of the marine genus *Halobiotus*, *Ramajendas renaudi* from Antarctic and two species of *Isohypsibius*. A modest number of terrestrial species divided into 3 genera are included in Apochela. In general, eutardigrades are cylindrically shaped with more or less indistinct segmentation with a complex pharyngeal bulb supported by flexible macro- and microplaciods consisting of thickened cuticular structures (see Fig. 1A+B) [NELSON, 2002]. The key characters separating eutardigrades from heterotardigrades are open cloaca, the presence of Malphigian tubules and the strong reduction of cephalic sensory structures (see Fig. 1E,5C+D,7D&8E). The most utilised characters in relation to taxonomy are the type of bucco-pharyngeal apparatus and the form of the claws [SCHUSTER *et al.*, 1980; GUIDETTI & BERTOLANI, 2005].

1.1.4. Ecology

Tardigrades may be found from the Arctic to the Antarctic and from the tallest mountain (Himalaya 6000m) to the deep-sea (more than 8000 m below sea level) [RAMLØV & KRISTENSEN, 1985]. They occupy a diversity of ecological niches. Yet, they are exclusively aquatic animals, requiring a film of water for completion of their lifecycle; which may take between 3-30 months [NELSON, 2002]. Tardigrades are predominantly egg-laying, with both sexual and parthenogenetic modes of reproduction described [BERTOLANI, 2001]. In some eutardigrade species eggs are left behind protected inside the exuvium during molting (Fig. 1F+G&7I). Little is known about their embryology [EIBYE-JACOBSEN, 1996/1997], and at present, our understanding of the lifehistory of tardigrades is relatively limited. A few long-term studies have nevertheless been conducted, most notably by SUZUKI (2003) who provided a detailed description of moulting, lifespan and embryology of *Milnesium tardigradum* DOYÈRE, 1840. Due to scarcity of data, the biogeography of Tardigrada is also virtually unknown, but regardless of species or habitat, populations of tardigrades often show a very patchy distribution pattern, but may at the same time display an amazing species richness [HANSEN, 2005] as well as large numbers of individuals [GUIDETTI *et al.*, 1999].

Even though all tardigrades are aquatic, terrestrial (or limno-terrestrial) tardigrades are capable of entering a reversible ametabolic state of latent life called cryptobiosis in response to unfavourable environmental conditions (e.g. freezing, desiccation, salinity fluctuations and low oxygen tension [WRIGHT *et al.*, 1992; WRIGHT, 2001]). In this cryptobiotic state their total lifespan may be greatly extended by up to 20 years [JØRGENSEN *et al.*, 2007] and is generally believed to confer a high passive dispersal potential through airborne transport, which may explain some species' cosmopolitan distribution pattern. This amazing ability is shared with other well established members of the cryptic fauna *i.e.* nematodes and rotifers, and is a crucial capacity ensuring survival in a fast changing habitat, which is little exploited by other organisms.

1.2. *Halobiotus crispae* (Eutardigrada: Hypsibiidae)

Marine eutardigrades have been known for over a decade, and it is alleged that they are secondarily adapted to the marine environment [CRISP & KRISTENSEN, 1983]. The transition from freshwater to seawater must have required the possession of a large osmoregulatory capacity [MØBJERG & DAHL, 1996]. The species *Halobiotus crispae* Kristensen, 1982 (*halos* (gk): sea; *biotus* (gk): life) was initially described from Nipisat Bay, Disko Island, Greenland but has since been discovered at several other localities throughout the northern hemisphere [MØBJERG *et al.*, 2007]. *H. crispae* colonise littoral habitats (see Fig 3&4), often characterised by large fluctuations in abiotic factors such as salinity, making osmoregulation imperative to survival. The ability to cope with these fluctuations in salinity might be adaptively correlated with the enormous size of the excretory organs, the Malpighian tubules found in this genus; a specimen measuring 520 µm possessed initial segments with a width and length of 52 µm and 65 µm respectively. The size of the excretory organs, comprising up to one-third of the body cavity [KRISTENSEN, 1982; MØBJERG & DAHL, 1996], has been shown to vary with the annual cycle of cyclomorphosis [KRISTENSEN, 1982]. This suggests that the osmotic stress tolerance of *H. crispae* might similarly change with the turn of the seasons [MØBJERG & DAHL, 1996]. A seasonal cyclic change in morphology and physiology was for the first time clearly established within Tardigrada in *H. crispae* [KRISTENSEN, 1982] but has since been suggested for other tardigrades [HANSEN & KATHOLM, 2002; GUIDETTI *et al.*, 2006].

1.3. Aim of the study

In this study we expose individuals in the active stage of the species *Halobiotus crispae* from Nipisat Bay, Disko Island, W. Greenland, to gradual changes in the osmotic pressure gradient, and compare these results with similar results previously obtained by HALBERG *et al.* [2006] on different cyclomorphic stages from Vellerup Vig, Isefjord, Denmark. In general, marine invertebrates do not regulate the ionic concentration of their extracellular fluid with respect to external seawater, and they are therefore categorized as osmoconformers [ZANOTTO & WHEATLY, 2006]. This may however be different for organisms in the subtidal zone, as the large changes in the external surroundings make osmocomformaty very unfavourable. During the study, salinities in the range of 0‰ – 90‰ were examined quantitatively, while exposure to higher salinities was investigated qualitatively. In this context, the ability to tolerate desiccation and freezing were investigated as exposures to these extremities are associated with cellular dehydration and subsequent osmotic stress. Most notably was the questions concerning *i.e.* do the tardigrades enter an ametabolic state of life or do they osmoregulate during severe hypertonic exposure? Furthermore, by examining the response pattern to desiccation and freezing in the different cyclomorphic stages, important questions concerning the ecology of *H. crispae* are answered. These questions relate to how *H. crispae* cope with the profound changes in abiotic parameters taking place at the different known localities. For example at Nipisat the entire bay is frozen for up to 8 months a year.

The effect of osmotic shock on volume regulation at the organism level was investigated by using light microscopic photos taken at different times at varying salinities. This technique allowed us to elucidate whether *H. crispae* is able to counteract the osmotic water movement following sudden changes in the external salinity, and with what time course. At the same time, it permits us to address central questions concerning the physiology of the excretory system in Tardigrada. In the laboratory, most aquatic invertebrates will swell or shrink following transfer to different salinities, causing death from excessive swelling in more *stenohaline* species [OGLESBY, 1981]. Consequently, body volume regulation is generally of great ecological importance during periods of large ambient changes in salinity, as seen in sublittoral habitats.

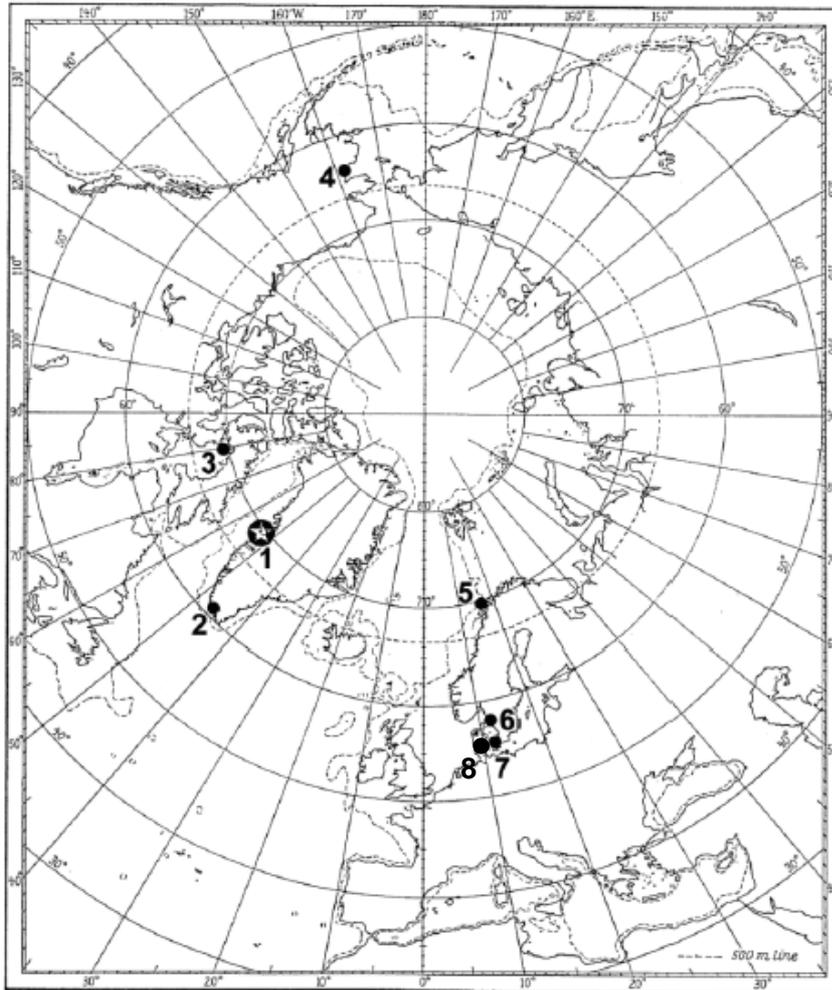


Figure 2. Distribution of *Halobiotus crispae*. **1:** The type locality at Nipisat, Disko Island, Greenland. **2:** Ikka fjord, Greenland. **3:** Igloolik, Arctic Canada. **4:** Fairbanks, Alaska, USA. **5:** Grindøy, Tromsø, Norway. **6:** Kristineberg, Sweden. **7:** Vellerup Vig, Denmark. **8:** Ærø, Denmark. Modified from: MØBJERG *et al.* 2007.

Despite a rather detailed knowledge of tardigrade morphology as well as inclusion in recent molecular phylogenetic studies, the exact relationships of tardigrades are still controversially discussed. In order to increase the morphological database, the anatomy of the nervous system and the musculature of *H. crispae* were investigated by applying fluorescence-coupled antibodies. These were raised against the neurotransmitter FMRFamide and acetylated α -tubulin, in addition to FITC-coupled phalloidin to label filamentous F-actin, in combination with confocal laser scanning microscopy.

In the present study, we also expand on the currently known molecular data on *Halobiotus crispae* in order to establish whether the populations of Nipisat Bay and Vellerup Vig, Isefjord are in fact the same species. The reason behind doing so are the profound differences observed, and recently described by MØBJERG *et al.* (2007), between the two populations, hereby raising the question whether they are the same species.

The aim of this study is to investigate the general response pattern of the sublittoral species *H. crispae* from the Disko Island, Greenland to osmotic stress, and subsequently compare the results with acquired data from the southernmost locality of Vellerup Vig, Isefjord, Denmark. Furthermore, we wish to expand on the currently known molecular data.



Figure 3. Sampling location at Nipisat. **A.** The Halobiotus Island. **B.** Overview of sampling area, note the low water depth. **C.** A specific sample site, note the mussels and filamentous algae. (Photo: Kenneth A. Halberg)

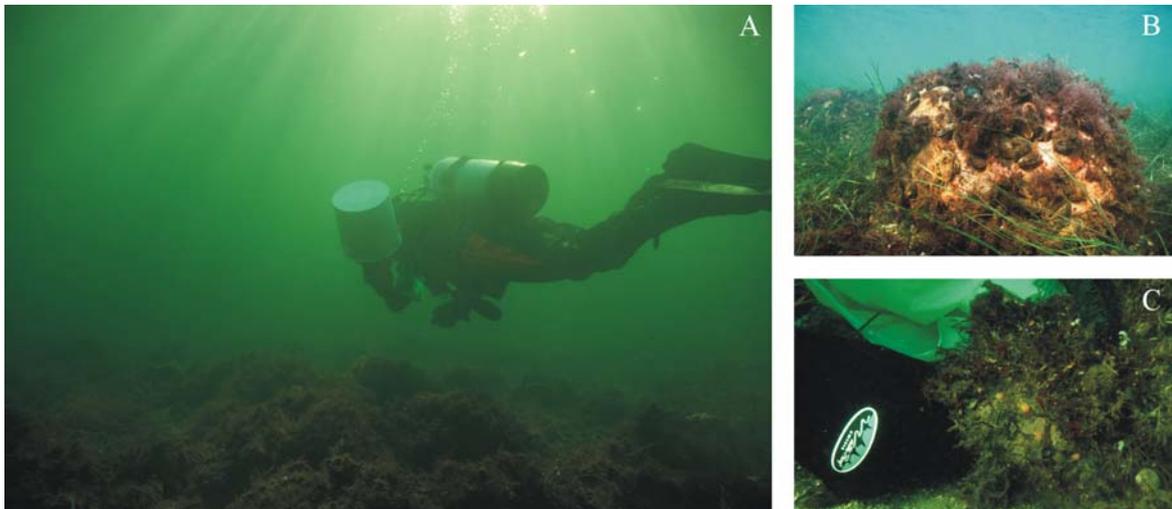


Figure 4. Sampling location at Vellerup Vig, Denmark. **A.** Scuba diver sampling approximately 70 m from the shore, at a water depth of 1.5-2 m. **B.** Close up of typical sampling site. **C.** Sampling at the rock-seabed interface. (Photo: Martin Macnoughton).

2. MATERIALS AND METHODS

2.1. Sampling and preparation

This study is partly based on material collected from Nipisat Bay (N 69° 25.934', E 54° 10.768'), Disko Island, Greenland (in the subtidal zone 3-4 cm below maximal low tide) on the 16th of August, 2006 during a field course in arctic biology, and partly from the southernmost locality Vellerup Vig, in the Isefjord (N 55° 44.209', E 11° 51.365'), Denmark (at a depth of 1.5 – 3.0 meters) in the period November 11, 2005 - September 9, 2006 as part of an ongoing investigation (see Fig. 2). At Nipisat Bay, the sediment consisted of fine sand, with pebbles and small stones and was extremely rich in meiofauna, predominantly polychaetes, nematodes, halacarids and harpacticoid copepods [KRISTENSEN, 1982]. In the Isefjord, the substrate consisted chiefly of coarse sand and the eelgrass (*Zostera marina*) with

a patchy distribution of large rocks. Rocks, algae and sediment were collected at both localities and later sorted out at the laboratory of Arctic Station, Godhavn or at the Zoological Museum, Copenhagen. Samples were freshwater-shocked and subsequently decanted into a mermaid bra (mesh size 62 μm) and transferred to Petri dishes. The tardigrades were identified under stereomicroscope ($\times 25$ - $\times 40$ magnification), and was primarily found on the proximal ("rhizoid") part of different filamentous algae (*Stictyosiphon tortilis*, *Dictyosiphon foeniculaceus*, *Sphacelaria arctica*, *Pylaiella littoralis*, *Acrosiphonia centralis*, *Rodomela lycopodieides*, *Enteromorpha ad proliferia*, *Sphacelaria cirrosa*, *Furcellaria lumbricalis*, *Ahnfeltia plicata* and *Erythrothricia carinea*) found in association with rocks and the blue mussel (*Mytilus edulis*). From the collected material more than 2500 specimens and several hundred exuvia were recovered, with adult specimens ranging in size from 360-650 μm and 320-480 μm in length from Nipisat Bay and the Isefjord respectively. The majority of these animals will be used in the near future in order to identify and quantify different protein-species relevant to osmotic tolerance. Live tardigrades were stored at 4°C in physiological seawater (20‰) supplied with substrate. The salinity at Nipisat Bay was 18 ‰ (water temp.: °C) at the time of sampling, however, with large fluctuations in salinity (4-32 ‰) occurring over both shorter (during tidal cycles) and longer (formation/thawing of ice) periods of time. The salinity at Vellerup Vig in the Isefjord was in contrast relatively constant at 18-20 ‰ year round (water temp.: 1-16°C annually) [RASMUSSEN, 1973]; pH was 8-9 at both localities. Measurements of abiotic factors *in situ* were performed using a thermometer for water- and air temperature, a refractometer (S-1 Shibuya Land) for salinity and pH-strips for pH (Cybercell Biochem, Alberta, US). Geographical positions were measured with a Magellan GPS 2000.

2.2. Confocal laser scanning microscopy (CLSM)

Specimens of *Halobiotus crispae* were relaxed using sparkling water (carbon-enriched water) and subsequently fixed in 4% paraformaldehyde (PFA) in 0.1M phosphate buffered saline (PBS) for 15 minutes at room temperature and washed 4×15 min in 0.1M PBS with 0.1% NaN_3 (PB). For filamentous actin (F-actin) staining, animals were ultrasonicated for 90 seconds and incubated in PB with 0.1% Triton X-100 (PTA) over night and stained in a 1:40 dilution of Oregon Green 488 phalloidin (Molecular Probes, Eugene OR, USA) for 18h. The specimens were thereafter rinsed thrice in PB without 0.1% NaN_3 and mounted on two coverslips in Flouromount mounting medium (Southern Biotechnology Associates, Inc. Birmingham, AL). For antibody (AB) staining, specimens were mechanically disrupted using a microscopic needle before preincubation in 6% normal goat serum (NGS) and PTA (blockPTA) over night for blocking of unspecific binding sites. The primary antibodies (pAB), mouse anti-acetylated α -tubelin (Sigma, Heidelberg, Germany; dilution 1:500 in blockPTA) and monoclonal rabbit anti-FMRFamide-peptide-gated sodium channel (Chemicon, Temecula, CA; 1:400 in blockPTA) was applied for 24-48h at room temperature. Subsequently, the specimens were washed in several changes of PB and incubated in the flourophore fluoresceinisothiocyanate (FITC)-conjugated secondary antibodies (sAB: goat-anti-mouse and goat-anti-rabbit; Sigma, Heidelberg, Germany) overnight. The sAB was diluted 1:300 in blockPTA. Finally, the specimens were mounted as described above. Image acquisition was performed on a Leica DM RXE 6 TL microscope equipped with a Leica TCS SP2 AOBS confocal laser scanning unit, using the 488 nm line of an argon/crypton laser.

2.3. Scanning Electron Microscopy

For electron microscopy the animals were fixed in 2% glutaraldehyde, 2% formalin or 2% osmium tetroxid, buffered with 0.1M sodium cacodulate adjusted to pH 7.4, for 1h at room-

Species	Locality	GenBank Accession Number	
		COI	ITS2
<i>Halobiotus crispae</i>	Vellerup Vig, Denmark	EF620413	EF620421
<i>Halobiotus crispae</i>	Nipisat, Greenland	EF620412	EF620420
<i>Halobiotus crispae</i>	Ærø, Denmark	EF620414	EF620422
<i>Isohypsibius granulifer</i>	Nivå, Denmark	-	EF620424
<i>Isohypsibius prosostomus</i>	Nivå, Denmark	EF620416	EF620423
<i>Ramazzotius oberhauseri</i>	Nivå, Denmark	EF620417	EF620425
<i>Thulinus stephaniae</i>	Sinai, Egypt/Genbank	EF620418	EF620419

Table 1. The taxon and gene sampling in the present study. After MØBJERG *et al.* 2007.

temperature. After fixation the specimens were washed thoroughly in distilled H₂O and then dehydrated in a graded series of ethanol and acetone prior to critical point drying. Following dehydration the animals were mounted on aluminium stubs and coated with a 10-20 nm layer of platinum. Scanning images were acquired with a JEOL JSM-6335-f scanning electron field microscope.

2.4. Molecular methods

Twenty-five specimens of *H. crispae* were pooled and stored in DNA-buffer prior to DNA extraction from each locality (see Table 1). Subsequently, the stored material was carefully grinded using the STE-buffer DNA extraction method [MANIATIS *et al.*, 1982]. The primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') [FOLMER *et al.*, 1994] for the mitochondrial cytochrome oxidase subunit I (COI) gene and 3SN (5'-GCG TCG ATG AAG AGC GCA GC-3') and BD2 (5'-TAT GCT TAA ATT CAG CGG GT-3') [DEJONG *et al.*, 2001] for the nuclear internal transcribed spacer 2 (ITS2) region were used for both PCR amplification and direct sequencing. The PCR reaction mix was prepared in a total volume of 4×20 µL with 0.1 µL Taq polymerase (Roche; 0.5 U/µL), 2 µL 10× buffer (Roche; 15 mM MgCl₂), 1 µL dNTP (4 mM), 1 µL of each primer (3.2 pmol/µL), 1 µL of DNA template and 14 µL double distilled H₂O. The setting for the PCR-reaction was 95 °C for 5 min., 37 cycles at 94 °C for 10 s, 42 °C for 30 s, and 72 °C for 30 s, followed by 72 °C for 10 min. Following the PCR-reaction samples were pooled in 2×40 µL samples in order to obtain sufficient DNA. Chromatograms obtained from the automated sequencer (ABI PRISM™ 310 Genetic Analyzer) were edited and consensus sequences created from the complementary strands using the Staden Package [STADEN, 1996]. The obtained sequence was aligned prior to phylogenetic analyses with ClustalX v.1.83 [THOMPSON *et al.*, 1997] using default parameters. Genetic distance as well as nucleotide composition between the COI and ITS2 sequences was calculated using MEGA 2.1 [KUMAR *et al.*, 1993]. For inferring phylogeny, distance based neighbor-joining (NJ) was performed. In this study species of *Isohypsibius* Thulin, 1928, *Thulinus* (Bertonlani, 1981) and *Ramazzotius* Binda & Pilato, 1986 were used as outgroup as MØBJERG *et al.* [2007] showed *Halobiotus* to have evolved within *Isohypsibius*, while *Ramazzotius oberhauseri* Doyère, 1840 was shown by JØRGENSEN and KRISTENSEN [2004] to be more distantly related to *Halobiotus* and *Thulinus*. It was not possible to obtain an ITS2 sequence by direct sequencing for *R. oberhauseri* probably due to length differences in the alleles.

2.5. Osmotic stress tolerance

To investigate the osmotic stress tolerance of *Halobiotus crispae* in the different cyclomorphic stages, two batches of 20 animals in each respective stage were placed in embryo dishes, and allowed to acclimate to a solution of 20‰ (physiological salinity) for a minimum of 24 hours. The definition of physiological salinity (20‰) was arbitrarily chosen on the basis of the relatively stable salinity at Vellerup Vig, Isefjord (18-20‰). At the locality of Nipisat, enormous fluctuations in salinity occur on a temporal and spatial scale (4-34‰), however to ensure the comparability of the osmotic stress exposure between the populations the same salinity of (20‰) was applied. This is important to bear in mind when analysing the results. Following the acclimation period, one group of animals was exposed to gradual increases in salinity until a maximum was reached and no movement could be discerned, while the other group was simultaneously exposed to gradual decreases in salinity to a minimum of 0‰. These changes in ambient salt concentration were performed by periodically replacing small volumes of water with prefixed solutions of higher and lower salinity respectively. The salinity was measured continuously with a refractometer (S-1 Shibuya Land) to certify that the observations were made at the same salinities. After the salinity had been changed, the animals were allowed to acclimate for a short period of time (20-40 min.) before further changes were made. After this period of acclimation the number of passive animals was counted. An animal was considered passive when no movement could be registered at $\times 80$ magnification. Throughout the experiment the animals were continuously observed in order to document their behaviour, and after all the animals were passive, the process was reversed. From Vellerup Vig, all three cyclomorphic stages were investigated, but it was only possible to obtain data from the active stage from the population in Nipisat Bay. Data on the active and pseudosimplex 2 (P2) stage from Vellerup Vig were obtained in a previous study, and are included in this investigation in order to allow us a comparison of the results. In all the experiments, the saltwater solutions used were made with natural seawater from the respective habitats in preference to artificial seawater. This was done in order to insure the normal permeability characteristics of membranes and intercellular components, hereby minimizing potential perturbations. Animals were kept at approx. 4°C during the experiments as thermal stress might influence the level of stress tolerance [pers. observation].

2.6. Cryptobiosis

The ability of *Halobiotus crispae* to tolerate desiccation (anhydrobiosis) and freezing (cryobiosis) was investigated for each cyclomorphic stage from the population at Vellerup Vig, Isefjord, as well as for the active stage from from Nipisat Bay.

2.6.1. Anhydrobiosis

Desiccation was investigated by transferring ten specimens in each cyclomorphic stage to an embryo dish containing 0.5 ml of seawater (20‰), which was allowed to dry to completion over a period of 6 days at 4°C. During this time, water continuously evaporates from the embryo dishes causing a concomitantly increase in salinity (up to app. 350‰; saturated sea water). This allowed us to monitor severe hypertonic exposure qualitatively. Following total desiccation the animals were rehydrated in seawater of very salt concentration (90‰) and gradually lowered to physiological salinity (20‰). Subsequently, the animals were monitored daily over a period of 6 days, while the number of individuals resuming activity was registered. During this period the temperature was kept at 4°C.

2.6.2. Cryobiosis

Freezing was studied by transferring ten specimens in each cyclomorphic stage to 0.5, 1 and 1.5 ml of water (20‰) respectively in Eppendorf vials. Following the transfer the sample was subjected to freezing to -18°C and furthermore kept frozen throughout a total period of 24 h. In organisms able to tolerate internal ice formation, the rate of cooling is generally very important because of the osmotically induced cellular dehydration caused by exclusion of salts from the growing ice front i.e. the freeze-concentration effect. Consequently volumes of water were employed corresponding to different rates of freezing in an attempt to investigate a correlation between cooling-rate and survival. Preceding estimations of survival, the sample was gradually thawed at 4°C and monitored over a period of 4 days.

2.7. Volume regulation

Individual adult specimens of *Halobiotus crispae* in P1 from Vellerup Vig, Denmark and active stage from Nipisat, Greenland were acclimated to 20‰ (defined relatively as 100% of physiological sea water (S.W.)) at 4°C for at least 1 h, and subsequently photographed and exposed for set time periods of 30 min, 1, 2, 4, 24 and 48 h to the various saltwater concentrations of 10% S.W., 50% S.W. and 200% S.W. (2‰, 10‰ and 40‰ respectively) at 4°C. The reason for defining the salinities as percentages of physiological salinity is to facilitate comparisons with other organisms living at different physiological salinities. At the end of each time interval, individuals were transferred, in a drop of the appropriate solution, to glass microscope slides and photographed under cover slips using a light microscope (Olympus BX 51, ×200 magnification) for subsequent estimations of body volume (see below). During photography, great care was taken to minimize the time spent by the animals under the cover slips, in order to evade potential evaporation of water, which would alter the osmotic pressure of the medium. Following photography, the individuals were retransferred to the respective salinities, until the end of the next set time period where a new photograph was taken. At each of the time intervals, minimums of 3 individuals were photographed at each of the seawater concentrations. Furthermore, a separate mortality experiment was performed in order to quantify potential mortality associated with the experimental procedure.

Body volume was estimated from photos, taken at the set time periods at the respective salinities, using the DP-software programme. The body volume was calculated assuming that the body and hind legs of *H. crispae* are cylindrical i.e. see equation (1), and while the error introduced by this assumption is considerable, it still allowed us to derive a general response pattern of *H. crispae* to osmotic stress and body volume changes. Median length (h_{Body} & h_{Leg}) and average diameter (d_{Body} & d_{Leg}) of the body and largest hind leg were measured using DP-soft, and body volume was calculated using equation (1). In this context, calculating the largest hind leg and subsequently multiplying this value with two, as opposed to a summation of the volumes of the respective legs, achieved the best estimation as telescopic withdrawal of body extremities easily made one leg longer than the other. To determine whether significant deviation occurred in the mean values over the experimental period, the obtained body volume estimations were compared to the value recorded in 100% SW using a two-sample t-test (level of significance ≤ 0.05). The obtained relative volume changes were subsequently plotted against the incubation time.

$$(1) V_{\text{Total}} = \Pi (r^2_{\text{Body}} h_{\text{Body}} + 2r^2_{\text{Leg}} h_{\text{Leg}})$$

V_{Total} is the total body volume, r is the radius derived from the average diameter while h is the median length of body and hindlegs.

A limitation of this technique involves the necessity of a standardized position of the individuals during photography, in order to enhance the comparability of the individual measurements. Considerable difficulty was experienced in achieving this because of high animal motility and was further complicated by the tardigrades' ability to telescopically withdraw their limbs into the body cavity. Anaesthetizing or other chemical modifications of the specimens to reduce motility were not employed because it might introduce additional stress and potential unpredictable side effects. The problem was alleviated by manipulating the animal into the same position each time, but it will inherently create deviant body volume estimations not attributive to osmotic stress. Consequently, in order to validate our experimental protocol, five controls were performed as well, where pictures were taken at time 0 h and 24 h of animals exposed to a constant salinity of 20‰. Significant difference between time 0 h and 24 h were tested using two-sample t-test (level of significance ≤ 0.05). Due to the difficulty of the experimental procedure, and a limited time frame, it was only possible to obtain measurements at hypertonic salinities up to 24 h from the population from Nipisat Bay, Greenland. Measurements on specimens in the active stage from Vellerup Vig, Denmark were obtained in a previous study, however, are included and described in order to provide a complete picture of volume regulation in *H. crispae*.

2.7.1. Survival

In order to quantify survival, a separate experiment was undertaken to assess the mortality of *Halobiotus crispae* exposed to the experimental treatments. The primary criterion of survival was motility; a specimen that moved when disturbed was alive. However, under conditions of osmotic stress, activity is greatly reduced. Consequently, lack of motility could not be considered a good criterion for mortality. Inactive specimens were gradually transferred from the treatment into physiological seawater and only if no recovery ensued were they considered dead. A total of 20 individuals were immersed in seawater solutions of 10‰, 50‰, and 200‰ of physiological salinity. At each time interval the percent passive and dead was calculated.

3. RESULTS

3.1. Confocal laser scanning microscopy (CLSM)

3.1.1 Nervous system

When describing our neuronal staining with anti FMRFamide and anti α -tubulin we discriminate between the central (CNS), and the peripheral (PNS) nervous system. In this context the CNS consists of the cerebral ganglion and the two ventral nerve cords while all other neural components are considered as parts of the PNS.

FMRFamide antibody staining showed the strongest immunoreactivity in the two ventral nerve cords extending almost through the entire length of the animal in anterior-posterior direction, from the suboesophageal ganglion situated in the head to the hind legs (Fig. 5A). These main nerves run approximately 14-18 μm apart from and parallel to each other, connected with commissures of which three can be discerned. In the region of the Malpighian tubules as well as the body extremities, major parts of the FMRFamidergic system were revealed. In contrast to the FMRFamide staining, immunocytochemistry involving acetylated α -tubulin produced the strongest immunoreactivity in the cerebral ganglion, which seems to form a saddle-like structure above the buccal tube (Fig 5C). Two weakly defined lobes form the lateral parts of the "saddle", and at the posterior third of each lobe, nerves at the level of the eye is directed towards the epidermis to a posterolateral sensory field. Similar nerves

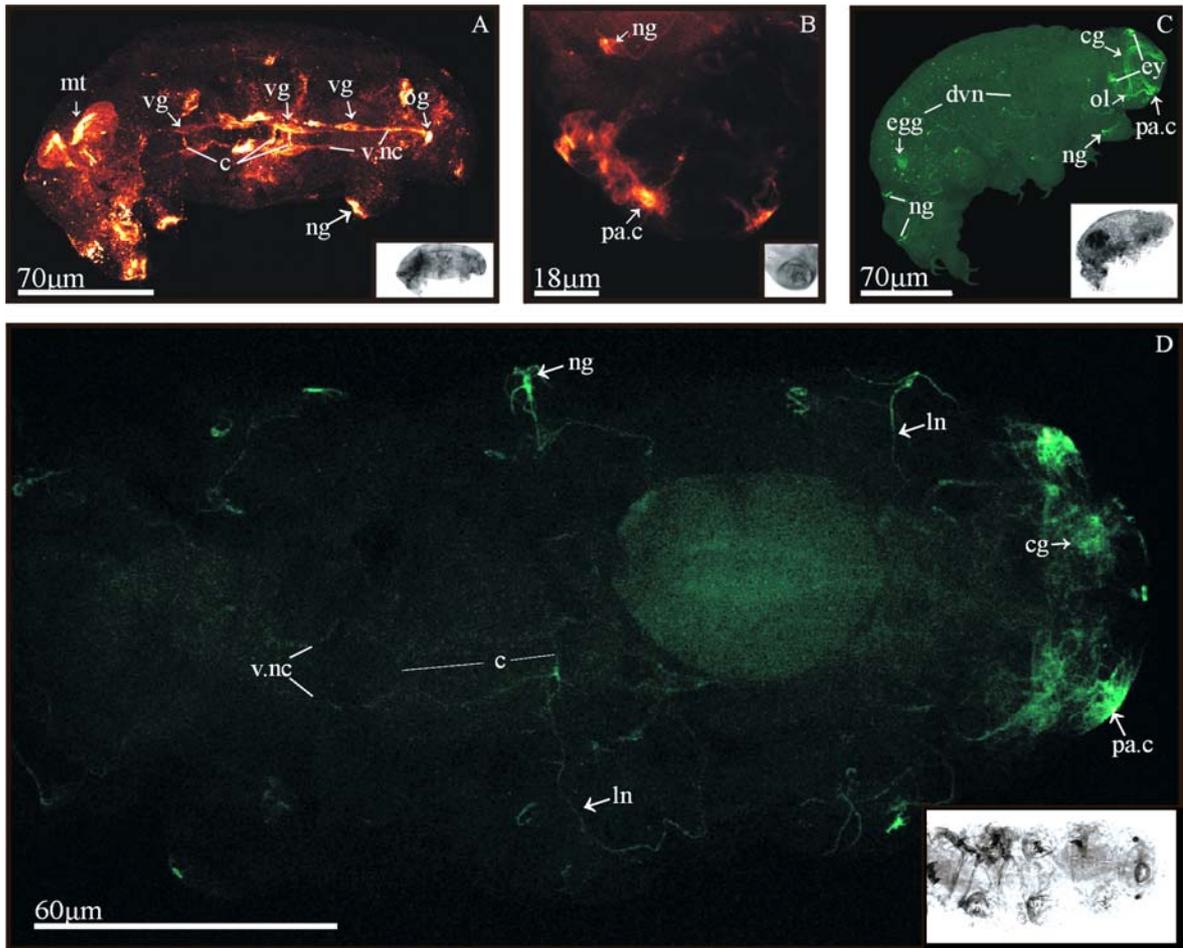


Figure 5. Legends on next page.

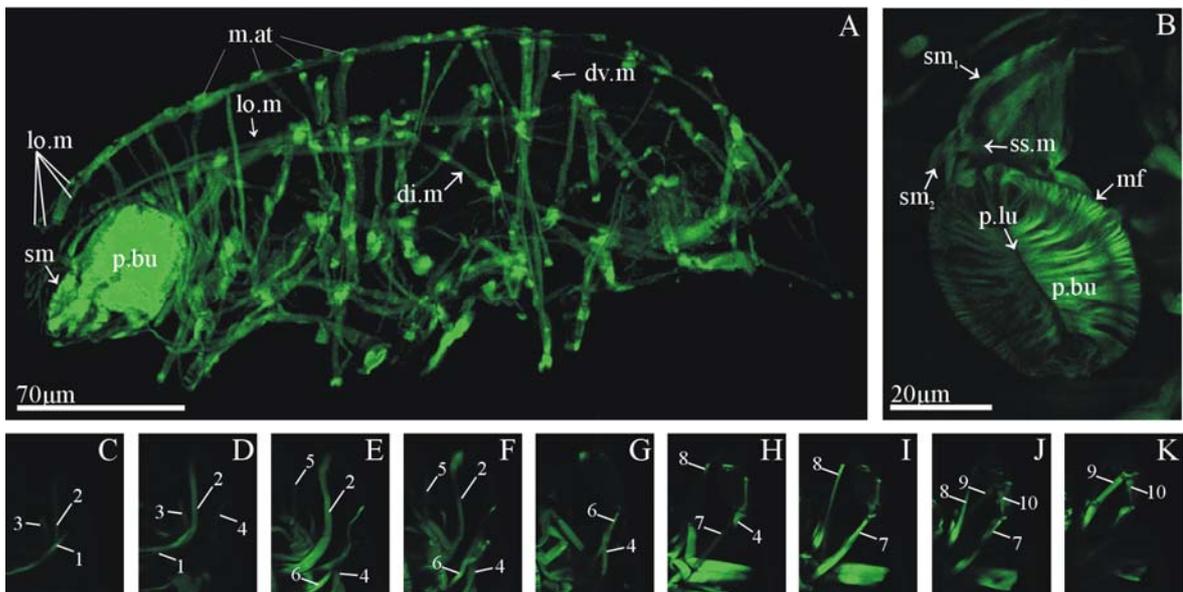


Figure 6. Legends on next page.

Figure 5. Confocal laser scanning micrographs of the nervous system of *H. crispae* with inserted lightmicrographs. **A.** Lateral overview of FMRFamide staining. **B.** Staining of α -tubulin in the head region, ventral view. **C.** Lateral overview of α -tubulin staining. **D.** Ventral overview of α -tubulin staining. Abbreviations; mt = Malpighian tubules; vg = ventral ganglion; v.nc = ventral nerve cord; og = suboesophageal ganglion; pa.c = papilla cephalica; cg = cerebral ganglion; ol = outer lobe; ey = eyes; dv.n = dorsoventral nervecord; ng = nebanganglien; c = commissure; ln = lateral nerve. **Figure 6.** Confocal laser scanning micrographs of muscle system of *H. crispae*. **A.** Lateral overview of F-actin staining. **B.** Close up of F-actin staining in the pharyngeal bulb and stylet muscles. The muscles of the pharyngeal bulb and stylets are always cross-striated, which is clearly indicated by the visible Z-lines. **C-K.** Close up on one of the front legs in different optical sections of a CLSM stack with numbers 1-10 indicating different muscles. Abbreviations; lo.m = longitudinal muscle; dv.m = dorsoventral muscle; di.m = diagonal muscle; sm_{1,2} = stylet muscles; ss.m = stylet support muscle; p.bu = pharyngeal bulb; m.at = muscle attachment; mf = muscle filaments; p.lu = pharyngeal lumen.

extend to the epidermis at the level of the papillae cephalica. Two dorso-ventral nerve cords were revealed in the lateral view. The ventral nerve cords and their commissures can be discerned in the ventral view, along with nerves extending laterally into each leg (Fig. 5B, C & D).

3.1.2. Muscle system

Concerning the musculature, the locomotory muscle system in the body and the bucco-pharyngeal muscle system in the head will be described separately. The muscles of the body can be considered as belonging to three categories: Longitudinal muscles, diagonal muscles and dorsoventral muscles. In the head the predominant muscles are those of the stylets and of the pharyngeal bulb. The longitudinal muscles are relatively thick and situated in the dorsolateral plane, extending from the head to the hind legs. From the dorsal muscles, three or four dorsoventral muscles seem to extend into each leg of the first three pairs of legs, indicating that they are the primary locomotory muscles (Fig. 6A). A closer look at a front leg reveals at least 10 different muscles (Fig. 6C-K). The diagonal muscles have dorsoventral orientation and span approximately half of the body length. These muscles are attached to the cuticle (see 7A, F+H & Fig 8A+G)

The muscles of the bucco-pharyngeal apparatus are shown in figure 6B. The pharynx is a muscular ovoid or pear shaped bulb, with arrays of radial muscles extending from the periphery of the bulb to the surface of the pharyngeal lumen. The stylet muscles are arranged around the buccal tube in a cone shaped structure consisting of two outer and some inner muscles, which seem to be connected to the buccal tube and at the dorsal and ventral anterior apophysis (sm₁ of Fig. 6B). The stylets seem to be connected to the pharyngeal bulb via several muscles extending from the area of the furca (sm₂ of Fig. 6B). Additionally, the stylet support muscles can be discerned at the basal part of the stylets (Fig. 6B). The musculatures of the pharyngeal bulb as well as the stylet-apparatus are truly cross-striated, clearly indicated by the presence of Z-lines.

3.2. Scanning electron microscopy (SEM)

SEM investigations of specimens in the active stage from Nipisat, Disko Island, Greenland and Vellerup Vig, Denmark reveal an identical outer morphology of the two populations. Fig. 7A shows a lateral overview of *H. crispae* from Vellerup Vig. Notice the long claws and the protuberant exterior part of the buccal apparatus similar to the specimen from Nipisat shown in Fig. 7G. A close-up of the head region (Fig. 7B) reveals lumps on the forehead known as the papillae cephalica, considered to be chemosensory areas. In addition a light ring lines the mouth opening and two rows of teeth can be discerned just inside the mouth opening. The first row is the small mucrones and behind them are some flat blade-like structures (Fig. 7E).

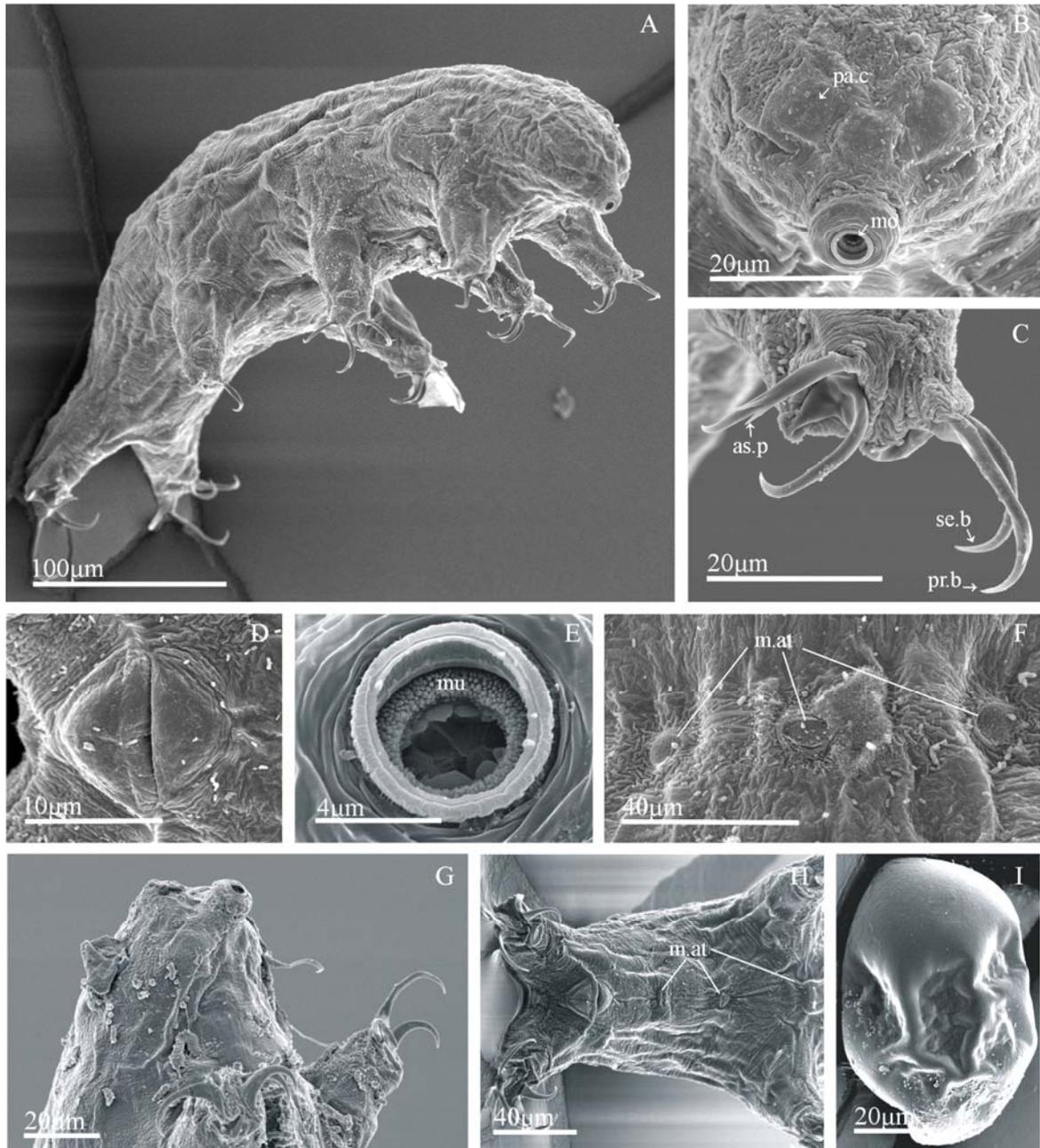


Figure 7. SEM of the active stage of *H. crispae*. **A.** Lateral overview of *H. crispae* from Vellerup Vig. **B.** Close up of forehead and mouth. **C.** Close up of claw on front leg. **D.** Cloaca. **E.** The peribuccal region. **F.** Ventral cuticular muscle attachments. **G.** Anterior overview of *H. crispae* from Nipisat. **H.** Cloaca and Ventral cuticular muscle attachments of the Nipisat specimen. **I.** Egg. Abbreviations; pa.c = papilla cephalica; mo = mouth; as.p = accessory point; pr.b = primary branch; se.b = secondary branch; mu = mucrones; m.at = muscle attachment.

A closer look at the claws (Fig. 7C), show a heteronych claw system with an internal and external double claw on each leg. The primary branches on both the external and internal double claws have small accessory points. The cloaca (Fig. 7D) is positioned ventrally between the hind legs and is closed by two lobes. On the ventral side anterior to the cloaca several muscle attachment sites are seen as more or less circular depressions in the wrinkled cuticle. Fig. 7F+H show muscle attachment sites on a specimen from Vellerup and Nipisat respectively. The eggs of *H. crispae* are plain and smooth (Fig. 7I) and do not have elaborate patterns. The wrinkled depressions in the egg are an artefact of the preparation.

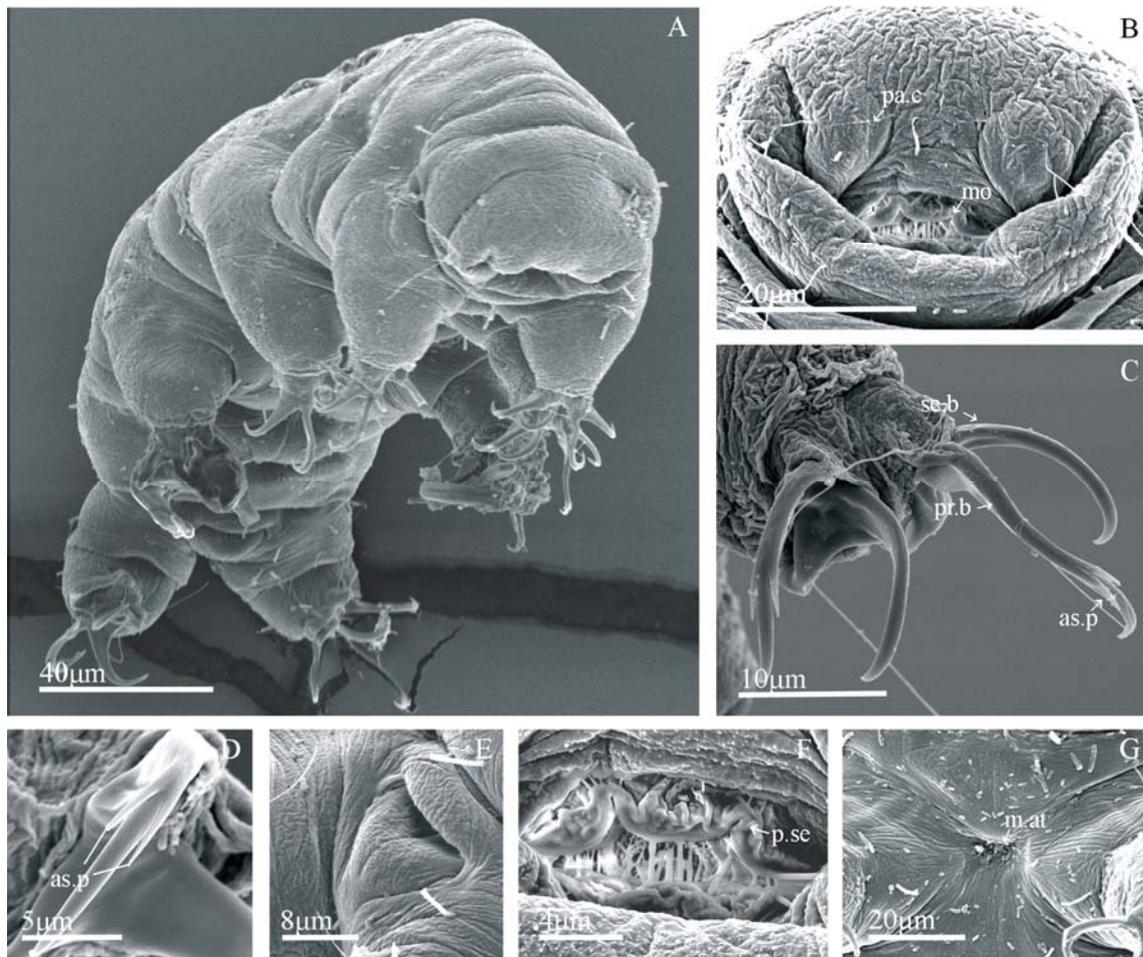


Figure 8. SEM of the pseudosimplex 1 stage of *H. crispae* from Ærø. **A.** Lateral overview, note the smooth cuticle. **B.** Close up of forehead and mouth. **C.** Close up of claw on front leg. **D.** Primary branch with accessory points. **E.** Open cloaca. **F.** The peri-buccal region. The mouth opening seems to be obstructed by some filamentous structures extending from the peribuccal sensory organ. **G.** Ventral cuticular muscle attachments. Abbreviations; pa.c = papilla cephalica; mo = mouth; as.p = accessory point; pr.b = primary branch; se.b = secondary branch; p.se = peribuccal sensory organ; m.at = muscle attachment.

Fig. 8A-G show SEM micrographs of a specimen sampled at Ærø at a depth of 7-8 meters. The lateral overview (Fig. 8A) reveals an outer morphology corresponding to that of *H. crispae* in the P1 stage. The papillae cephalica can be discerned above the invaginated mouth opening, which is blocked by cuticular bars (Fig. 8B+F). Furthermore the cuticle is not smooth but covered by a fine grid of creases. The claws are long and each leg is equipped with an internal and external double claw. Each primary branch has accessory points (Fig. 8C+D) as described for the specimens from Vellerup Vig and Nipisat. Ventrally between the hind legs is the cloaca with the two lobes (Fig. 8E). The number of muscle attachments and their position is similar to that seen in specimens from Vellerup Vig and Nipisat, though they appear as deeper depressions in the cuticle than described above (Fig. 8G).

3.3. Molecular methods

In this study we bring the first data on the genetic variation between two geographically distinct populations of *Halobiotus crispae* based on the two loci COI and ITS2. In addition we expand on the preliminary data on the phylogenetic position of *Halobiotus* using the molecular locus ITS2. Our data on the ITS2 region shows that *Halobiotus* has evolved within

a paraphyletic *Isohypsibius* (Fig. 9A+B). Data on COI and ITS2 reveal very low genetic distance between the populations at Nipisat Bay and Vellerup Vig, Isefjord, indicating that they both are *H. crispae*. A comparable low genetic variation in COI between *H. crispae* and *Halobiotus stenostomus* Richters, 1908 collected at Ærø, suggests an initial incorrect species identification of this material, as the molecular data shows it is *H. crispae* from deeper water (6–8 m).

3.3.1. Cytochrome oxidase subunit I (COI)

The COI data matrix was 584 bp for *H. crispae*. The data matrix had five variable nucleotide positions. The average nucleotide composition of COI was A (25.0%), C (22.9%), G (18.2%), T (33.9%) showing a small AT bias (AT 58.9%, CG 41.1%). The sequence variation between the two populations of *H. crispae* consisted entirely of 3rd codon substitutions, all of them being transitions. The genetic distance between *H. crispae*, Nipisat and *H. crispae*, Vellerup Vig was only (0.73%), whereas the distance between *H. crispae*, Nipisat and *H. crispae*, Ærø was (0.98%). The sequence variation between *H. crispae*, Vellerup and *H. crispae*, Ærø was also comparatively small (0.73%). Direct sequencing on individual specimens from Vellerup Vig in the Isefjord revealed two haplotypes of COI, with a haplotype sequence variation of (0.76%). A third haplotype seemed to be present, but was not independently confirmed by other sequences. Surprisingly, the genetic diversity within the population at Vellerup Vig was similar in magnitude as the sequence variation discovered between two geographically distinct populations separated by more than 3400 km. In comparison, the genetic distance between *Halobiotus* and *Isohypsibius* was 22.4%–22.9%, while being 21.7%–22.0% between *Halobiotus* and *Thulinus*. Finally, the sequence diversity between *Halobiotus* and the outgroup *Ramazzotius oberhaeuseri* was 26.3%–26.8%.

The phylogenetic inference by neighbor-joining inferred three distinct lineages for the COI data set (Fig. 9A). The three main lineages were *Thulinus*, *Isohypsibius* and a clade consisting of *Halobiotus*. The bootstrap branch supports for the clades were weak except for *Halobiotus*. Within the *Halobiotus* clade the Greenlandic haplotype was basal to the remaining haplotypes.

3.3.2. Internal transcribed spacer 2 (ITS2)

The ITS2 data matrix consisted of 489 bp for *H. crispae*. The average nucleotide composition of ITS2 was A (23.5%), C (23.0%), G (27.6%), T (25.9%), with a data matrix consisting of three variable nucleotide positions. As a result, the genetic variation between *H. crispae*, Nipisat and *H. crispae*, Vellerup was merely (0.56%). Sequences derived from individual specimens were unable to confirm the presence of different haplotypes within the population. The substitutions in *H. crispae* were all point mutations. In contrast, the genetic distance between *Halobiotus* and *I. prosostomus* was (34.7%) while being 39.2%–39.8% between *Halobiotus* and *I. granulifer*. The sequence variation between *Halobiotus* and *Thulinus* was (33.3%).

The topological resolution of the ITS2 tree inferred two divergent lineages (Fig. 9B) with *Isohypsibius granulifer* as the basal taxon. These consisted of a clade of *Halobiotus* as well as a clade of *Isohypsibius prosostomus* and *Thulinus stephaniae* thus showing a paraphyletic *Isohypsibius*. Numerous aberrant morphological characters in *Halobiotus* (including the secondary adaptation to the marine environment) however contradict the notion of a paraphyletic *Isohypsibius*, why a thorough morphological and molecular approach is needed.

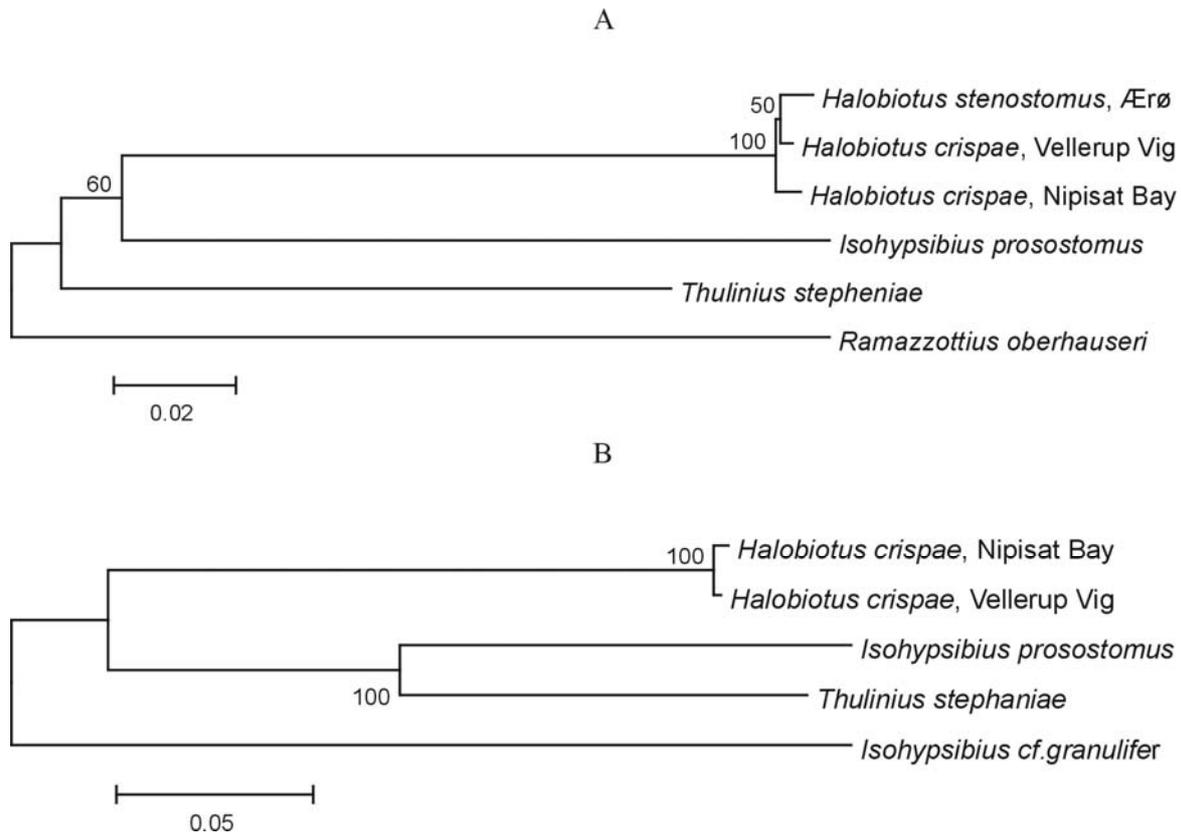


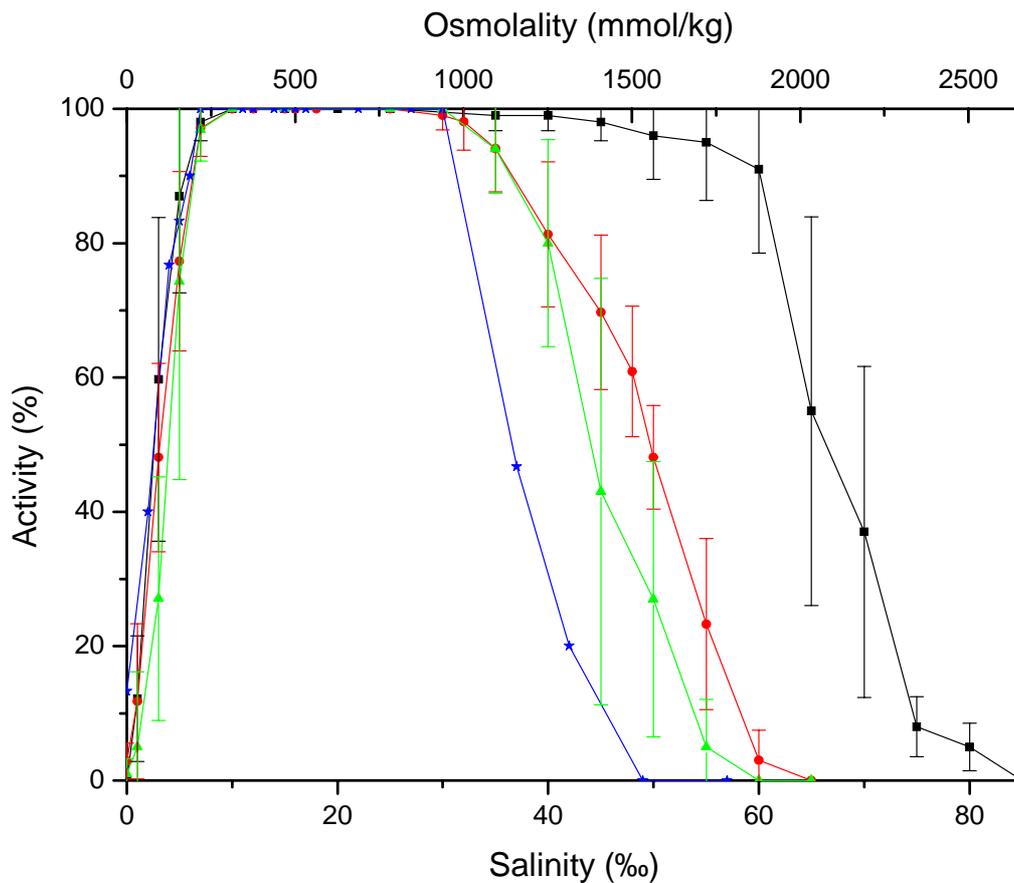
Figure 9. The phylogenetic relationships of *Halobiotus*. Neighbour-joining based on separate analysis of the mitochondrial COI gene (A) and the ITS 2 region (B). Modified from MØBJERG *et al.* 2007.

3.4. Osmotic stress tolerance

Figure 10 shows the level of activity displayed by *Halobiotus crispae* in different cyclomorphic stages from Vellerup Vig and in the active stage from Nipisat, when exposed to a continuous decrease or increase in salinity, during a four to five hour period at 4°C. The results presented are an average of five individual experiments; and from here a general response pattern to osmotic stress was evident. Animals exposed to increasing hypotonic solutions experienced reduced motility caused by the increase in hydrostatic pressure of the body cavity, while specimens immersed in increasing hypertonic solutions displayed reduced movement as a result of the massive efflux of water, causing invaginations of intersegmental cuticle and limbs. Progressive disruption of locomotory skills were generally observed as the osmotic gradient increased, but almost all specimens survived and resumed activity following a gradual retransfer to physiological salinity. The average mortality rate during the experiments were 1.5% in the population from Nipisat Bay, and 2.9% in Vellerup Vig, although the dead animals were often specimens observed to have a deviant behavior even during the preliminary changes in salinity

Active stage, Nipisat

During the preliminary hypotonic exposure ($\rightarrow 10\%$) no reduction in activity could be discerned, but at 7% a slightly reduced activity was observed as 98% on average of the animals were still active. Even at a salinity of 5%, as much as 87% were active, but their activity level was markedly affected, manifested by swollen bodies and impaired movement. As the salt concentration was decreased to 3% approximately 60% were still active, even



Active stage, Vellerup

In the first period of increasing hypotonic stress exposure ($\rightarrow 10\text{‰}$) no passive individuals were observed and the animals showed only negligible decreases in motile behavior. As the salinity continued to decline the first animals were observed passive, but as much as 97% on average of the specimens were still active at a salinity of 7‰. A mean of 77% remained active during immersion in 5‰, but important changes in behavior were now visible, as motility was clearly decreased. This tendency became progressively worse as only 48% of the individuals remained active at a salinity of 3‰. Further decreases in salt concentration resulted in dramatic reductions of activity; leaving only 12% active animals on average at a salinity of 1‰, of which most had to be provoked to move. A few individuals (a mean of 3%) were able to sustain very small levels of motility (movement of at least two legs and the head) in distilled water.

In the preliminary hypertonic salinity range ($\rightarrow 25\text{‰}$) there was no indication of any visible decrease in activity. Further increases in salinity resulted in some cases in a few individuals rendered passive, primarily smaller specimens ($\leq 250\text{-}300\ \mu\text{m}$), but in general almost all individuals (94% on average) maintained activity in the salinity range from 20‰ to 35‰. At a salinity of 40‰ we observed a mean of 81% active individuals, but the animals were now beginning to express considerable decreases in motility, clearly influencing their behavior. Whereas an average of 70% remained active at a salinity of 45‰, approx. half of the animals were inactive at 50‰ (averagely 48% active). During this increase in salinity, most individuals began displaying contractions in the anterior-posterior direction as well as invaginations of cuticle and limbs. These apparently movement-restrictive symptoms escalated as salinity was increased to 55 ‰, decreasing activity to a mean of 23%. Movement was now very slow and sluggish for the remaining specimens, and their legs were now telescopically withdrawn into their body cavity, indicating an inability to migrate or forage at this salinity. Only 3% on average could uphold activity during exposure to 60‰, and at 65‰ no activity was discernible at any time, suggesting an upper salinity barrier for activity of *H. crispae* in Vellerup Vig between 60‰-65‰.

Pseudosimplex 1 (P1), Vellerup

As with the active stage no individuals in the P1 stage was observed to be passive during the first period of exposure to hypotonic stress ($\rightarrow 10\text{‰}$), but their motility already seemed to be somewhat weakened. The motility at 7‰ was obviously diminished, even though 97% was active. At this point the animals aggregated in small clusters and seemed passive at first glance, but are easily provoked to move. A mean of 74% active animals were observed at 5‰, though movement had become even more weakened. Most animals were rendered passive when exposed to 3‰, with only 27% active individuals showing very limited and lethargic movement. Only 5% and 1% managed to remain active in 1‰ and 0‰ respectively, with motility at a minimum (head + two legs).

During hypertonic exposure a notable motility reduction was observed at 30‰, but none were passive. At 35‰ the first passive individuals were noticed (94% active), and the animals began displaying an aggregating behavior akin to the one described above. A mean of 80% were active at 40‰ and motility was decreased as invaginations of cuticle and limbs became apparent. Less than half (43%) were active at 45‰ and only 27% were active when exposed to 50‰, as contractions of the body and invaginations of cuticle and limbs became more conspicuous, thereby hampering movement even further. At 55‰ an average of 5% persisted, displaying very weak movement. No active animals were observed when solutions reached 60‰, and all individuals were curled up and legs had been telescopically withdrawn into the body.

Pseudosimplex 2 (P2), Vellerup

Our results obtained with the P2 stage only represents a single experiment and can therefore not be ascribed any statistical value and should only be viewed as showing a possible trend.

During the preliminary hypotonic exposure ($\rightarrow 10\text{‰}$) there was no discernible decrease in motility and all animals remained active. All animals remained active in 7‰ , but movement seemed slightly hampered. At 6‰ the first passive animals were observed, leaving 90% active. Motility declined even further as the salinity was reduced. A minor reduction in salinity to 5‰ reduced activity to 83% and at 4‰ one third of the animals had become passive (75% active). As much as 40% and 13% were observed to be active even at 2‰ and in distilled water respectively, showing an increased reduction in motility though not as pronounced as seen in the P1 stage.

In the first period of hypertonic immersion ($\rightarrow 30\text{‰}$) no animals were rendered passive, but as seen with the other cyclomorphic stages, a progressive reduction in their movements was evident. At a salinity of 37‰ more than half of the animals were rendered passive by the osmotic stress, while the remaining individuals displayed very limited and lethargic movements; some animals even needed provocation to move. Only 20% were active at 42‰ and most of the tardigrades had to be provoked before moving. No animals retained activity at 49‰ showing a reduced hypertonic tolerance in relation to the other cyclomorphic stages.

3.5. Cryptobiosis

3.5.1. Anhydrobiosis

When exposing *Halobiotus crispae* in each of the cyclomorphic stages to desiccation none survived (survival 0% , $N=10$ in each experiment). During the experimental period, water continually evaporated from the embryo-dishes hereby concentrating the remaining particles and decreasing the water potential. This created a concomitant reduction in viability in the exposed individuals. Already after 48h, only a small film of water remained (app. 120‰) while the animals exhibited considerable reductions in the anterior-posterior axis resembling a “tun” state. At this point, none of the specimens moved but could still be revived following gradual retransfer to physiological salinity. By day six, the animals were almost completely desiccated and no longer showing contractions in the longitudinal axis. Salt crystals were present. At this stage the animals were no longer alive, thus showing that the behavioral reduction in the anterior-posterior axis was upheld by active muscle-contraction. Following rehydration, none survived even after 6 days of post-experimental monitoring; hereby showing that desiccation is not tolerated by *H. crispae* in any of the cyclomorphic stages.

3.5.2. Cryobiosis or freeze tolerance

Exposure to freezing temperatures (-18°C) was not tolerated by specimens in either P2 from Vellerup Vig or the active stage from both localities (survival 0% , $N=10$ in each experiment). However, animals in P1 from Vellerup Vig displayed an inverse relationship between survival and freezing rate (volume of seawater) when exposed to subzero temperatures (Fig. 11). This was interesting as the P1 stage is found during the Danish summer. Following freezing, the animals that survived gradually revived over a period of days at 4°C , however no additional specimens revived after the fourth day (observed a maximum of 10 days after freezing). Surprisingly, stomach contents could frequently be seen in the surviving animals. There is no evidence for cryobiosis, however animals in the P1 stage are clearly freeze tolerant in Vellerup, and perhaps also Nipisat.

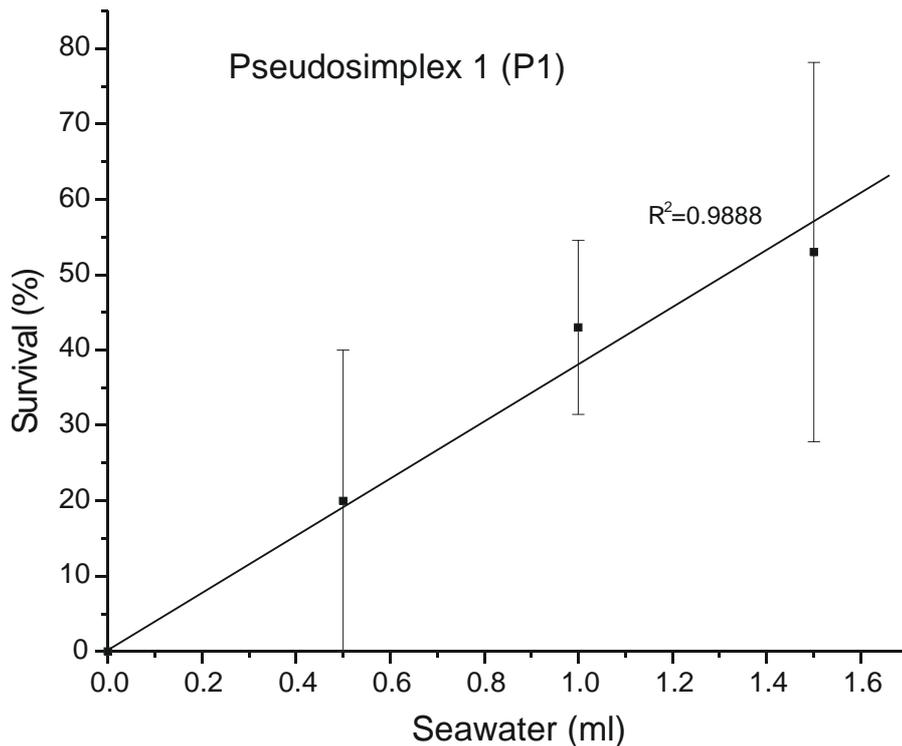


Figure 11. Survival of the Pseudosimplex 1 (P1) stage during exposure to negative temperatures showing an inverse relationship with volume of seawater (\rightarrow cooling rate).

3.6. Volumeregulation

For controls, five specimens were measured at time 0 h and 24 h. Body volume measurements of specimens immersed in 100% S.W are given in Table 2. There were no significant difference between the values recorded after 0 h and 24 h (two-sample t-test, ≤ 0.05).

Figure 12 shows the relative volume changes in *Halobiotus crispae* following direct exposure to 10%, 50% and 200% S.W corresponding to a salinity of 2‰, 10‰ and 40‰ respectively, for up to 48 h. These results provide an overall response pattern of *H. crispae* invoked during the imposition of osmotic shock. In the hypotonic solutions (10% and 50% S.W.), an initial increase in body volume was followed by stabilisation and a period of some recovery over the remaining period of time. Specimens immersed in hypertonic solutions (200% S.W.) showed an almost inverse response pattern, with an initial reduction in body volume followed by a small period of recovery, however, with a significant change in body volume over the full period of 48h (seen in both the Nipisat and Vellerup population). Overall, there seems to be a noticeable inverse relationship between the body volume of *H. crispae* and the external salinity. With the initial transfer of individuals in the active stage from the population at Vellerup Vig, Denmark to the hypotonic solutions, body water values rose abruptly, as the osmotic shock resulted in a massive water uptake along the water concentration gradient. This response was most pronounced for specimens immersed in 10% S.W., as total body volume increased to a mean of 209% during the first 30-minutes, and continued to rise to a value of 275% on average after 2 h exposure. During this period most specimens became passive. Animals immersed in 50% S.W. experienced initial increases in total body volume also (219% after 4 h), although significant changes in body volume were not detected before after 1 h exposure. The comparatively greater relative increase in body volume observed during 10% S.W. exposure must be explained by the fact that the rate of passive water movement is proportional to the osmotic gradient set up by the transfer, hereby

Total body volume (%)			
	0 h	24 h	P value
<i>H. crispae</i>	100	100 ± 3 %	1,000

Table 2. Mean body volume (%) and standard deviations of the control measurements (N=5) in 100% S.W. after 24 h.

also explaining the observed mortality (see Table 3). In addition, specimens displayed progressive reductions in mobility as the increasing hydrostatic pressure in the body cavity seemed to make movement difficult, and was generally very limited after 1-2 h in 10% S.W. Individuals immersed in 50% S.W. seemed to do better as some specimens retained a relatively high level of activity even after 4h. Following the rapid osmotic increase in body volume was a period of stabilization, as very little average variation occurred in 10% S.W. during the next 22 h, resulting in a mean total body volume of 279% after 24 h. In this period however, animal motility was completely lost, most likely resulting from impairment of locomotory skills due to increased internal hydrostatic pressure. Specimens immersed in 50% S.W. experienced a period of stabilization and recovery as total mean body volume dropped to 170% after 24 h, and most remained active in spite of swollen bodies caused by the osmotic influx of water. Up until now, all the body volume measurements performed on specimen immersed in 10% S.W. solutions, were significantly different from the values before the osmotic shock exposure. However, throughout the rest of the exposure period a recovery was observed for individuals in 10% S.W, resulting in a body volume that was not significantly different after 48 h compared to 100% S.W. The majority of the animals survived the 48 h exposure (see Table 3). Average body volume values of specimens immersed in 50% S.W. seemed to stabilize at a total of 176% with only small changes occurring in mean body volume between 24 h and 48 h immersion. Worthy of note is the fact that individuals immersed in 10% and 50% S.W. after 48 h exposure, both seemed to stabilize at a mean relative body volume of approx. 180%, which was not significantly different to the initial body volume at time 0 h, while furthermore regaining limited movements.

Exposure of active specimens of *H. crispae* from Vellerup Vig to 200% S.W. induced an initial significant reduction after 30-minutes in average body volume of over 37% due to the osmotic efflux of water. Concomitant reductions in motility following transfer were observed, but far from the degree observed for specimens immersed in hypotonic solutions. Preceding an almost total recovery of mean body volume after 4 h exposure, where body volume was not significantly different from 0 h in 100% S.W, observed values were somewhat erratic, fluctuating between a mean of 76% and 50% total body volume after 1 h and 2 h respectively. Small anterior-posterior contractions as well as progressive reductions in motility during the first 4 h exposure were observed, but with no apparent functional limitations. Throughout the rest of the exposure period total body water values continued to drop, culminating in a relative volume reduction of 42% after 48 h. No mortality was registered and animal mobility was in all cases retained throughout the full exposure period, even though body volume was significantly different after 48 h immersion compared to 100% S.W. When specimens in the active stage from Nipisat Bay, Greenland were immersed in 200% S.W., a similar general response pattern between the two populations was observed. Initially, significant reductions in body volume occurred (37% reduction after 30-minutes) however, after just 2 h exposure, body volume was restored again (90%) and was not significantly different from 100% S.W. This was as previously mentioned not demonstrated to be the case in animals from the population at Vellerup Vig, which in parallel experienced significant reductions in mean body

	S.W. solution								
	10%			50%			200%		
	V	N	P1	V	N	P1	V	N	P1
30 min	10 / 0	0 / 0	15/0	0/0	0/0	0/0	0/0	0/0	15/0
1 h	55 / 0	50/0	65/0	0/0	0/0	0/0	0/0	0/0	10/0
2 h	80 /20	60/0	90/0	0/0	0/0	0/0	0/0	0/0	5 /0
4 h	90 /34	70/0	90/0	0/0	0/0	0/0	0/0	0/0	0 /0
24 h	100/34	70/0	55/0	0/0	0/0	0/0	0/0	0/0	5 /0
48 h	65 /40	50/0	50/0	0/0	0/0	0/0	0/0	0/0	5 /0

Table 3. Passivity (%) / Mortality (%) of *H. crispae* in the different seawater solutions at the various time intervals. V, Vellerup (active stage); N, Nipisat (active stage); P1, Vellerup (pseudosimplex 1 stage).

volume of 50%. This signifies a comparatively faster and more efficient response by specimens from the population at Nipisat Bay. Over the course of the next 2 h body volume stabilized, but dropped again after 24 h immersion (72%) to similar levels exhibited by the Vellerup population (62%). At no time did the hypertonic exposure seem to affect animal motility or mortality (see Table 3). In résumé, a distinct parallel response pattern between the two populations was seen when exposed to hypertonic salinities, albeit with a more effective response in animals from the Nipisat population. However, there was considerable variation between the responses of the P1 stage and active stage of *H. crispae* from Vellerup Vig to the 10% saltwater concentration. Animals in the P1 stage were much less affected by the hypotonic exposure, experiencing significantly smaller relative changes in body volume. This difference is exemplified after 1 h immersion, as maximal average body volume reached 161% opposed to a mean total body volume of 231% observed in specimens in the active stage at the same time interval. Furthermore, following 4 h exposure a recovery was observed in specimens in the P1 stage, as average body volume was not significantly different from 100% S.W. In contrast, individuals in the active stage experienced a period of stabilization, culminating in a total mean body volume of 250%. Even after 24 h, body volume of individuals in the P1 stage was not significantly different from specimens in 100% S.W. even though further exposure to this salinity induced a significant increase in body volume (129%) compared to 100% S.W. after 48 h immersion. Even so, animal activity was greatly affected by the 10% S.W. exposure (see Table 3).

4. DISCUSSION

4.1. Confocal laser scanning microscopy (CLSM)

4.1.1. Nervous system

According to MARCUS [1929] a ventral nerve cord with four fused paired ganglia is present in the eutardigrade *Macrobiotus hufelandi* Schultze, 1834. From our results with FMRFamide we can distinguish only three ventral ganglia, while the fourth as well as the suboesophageal ganglion could not be discerned. Our immunocytochemical stainings of the cerebral ganglion using α -tubulin does not reveal all the structures presented by WIEDERHÖFT and GREVEN [1996] in the eutardigrade *Milnesium tardigradum* DOYÈRE, 1840. However, α -tubulin staining shows a collection of nerves forming what vaguely resembles a saddle-like structure, corresponding

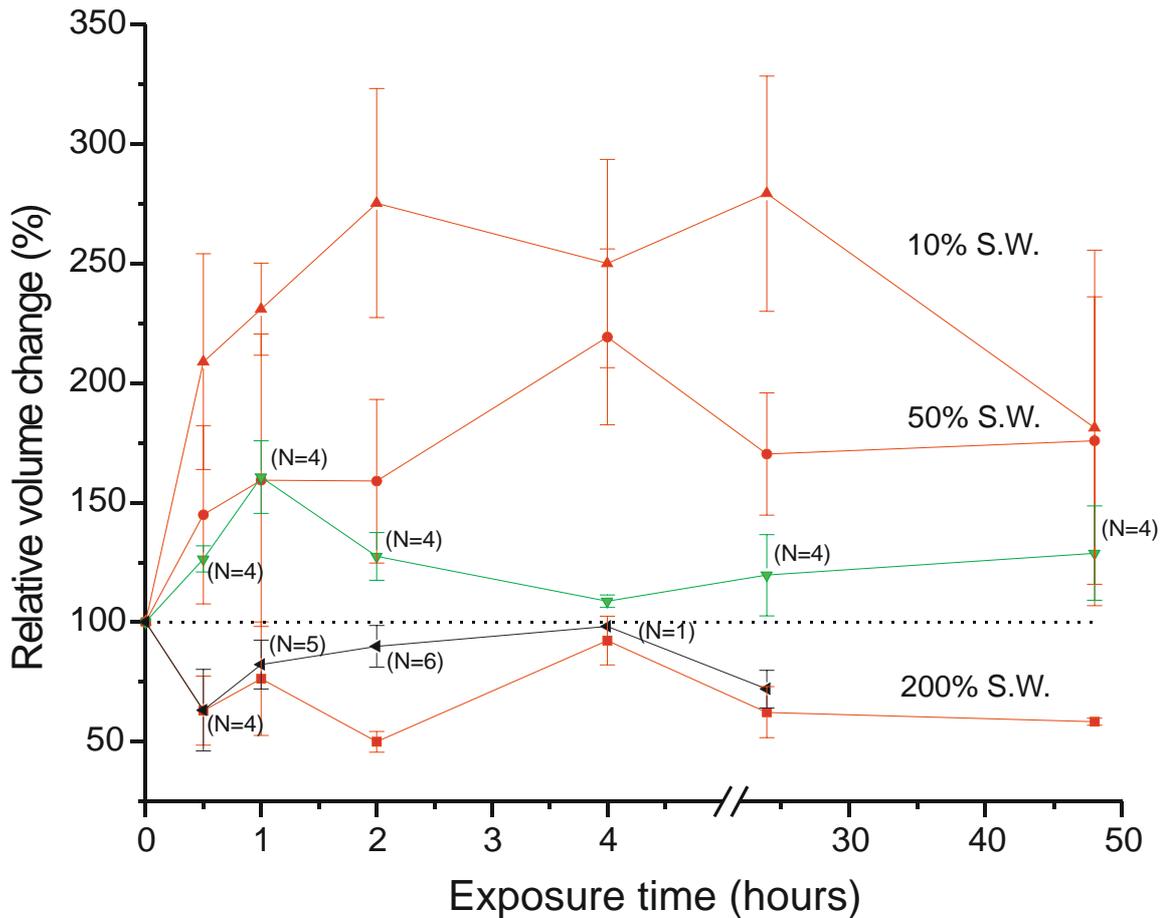


Figure 12. Graph showing the total body volume of *H. crispae* in three S.W. concentrations over a period of 48 hours. The error bars indicate \pm standard deviation. Dashed lines indicate the volume of *H. crispae* in 100% S.W. If nothing else indicated N=3 at each of the set time periods at each respective salinity. (Black) Active stage, Nipisat in 200% S.W. (Red) Active stage, Vellerup Vig. (Green) Pseudosimplex 1 stage, Vellerup Vig in 10% S.W.

to the supraoesophageal ganglion and the lateral lobes seem analogous to the outer lobes in *Milnesium tardigradum*. In connection to these lobes we seem to be able to recognize the neuronal structures described, in connection to the eyes and papillae cephalica. The suboesophageal ganglion does not show any immunoreactivity.

The deviations we find in the brain of *H. crispae*, compared to *Macrobotus hufelandi* and *Milnesium tardigradum*, must be due to artefacts of preparation and analysis, because some of the same structures have been recognized in *H. crispae* by KRISTENSEN [1982]. This is mainly the supra- and suboesophageal ganglia and the eye, which he found to be an internal structure situated in the cerebral ganglion, in the part known as protocerebrum. In addition, the papillae cephalica were shown to extend from the part of the brain known as deutocerebrum.

According to the results discussed above, FMRamide and α -tubulin are present in different parts of the CNS, but in the PNS they seem to generate immunoreactivity in the same components. This is interesting because one would expect anti α -tubulin to stain all nerves [A. Wanninger pers. comm.].

4.1.2. Musculature

Our results concerning the muscle system are generally in agreement with work done by MARCUS [1929]. He shows that *Macrobotus*, has four longitudinal dorsal muscles and one lateral on each side. Our phalloidin stainings show the four longitudinal dorsal muscles as well as several muscles extending into the head region, but the longitudinal lateral muscles are not visualized. In addition he shows 10 muscles from the dorsal and lateral muscles, and 2 from the ventral muscle, extending into each of the first pair of legs. In the second pair 14 muscles were identified, 10 from the dorsal plane and 4 from the ventral, and in the third pair he discerned 16 individual muscles (12 dorsal and 4 ventral). In contrast the movement of the hind legs are controlled by 7 muscles, 3 dorsal and 4 ventral.

From our immunocytochemical preparations it is very difficult to distinguish the number of individual muscle fibers in the legs. Though, in a thorough investigation of individual stack images of a front leg it was possible to discern at least 10 different muscles. In the lateral overview the ventral muscles and those in the legs are positioned more or less in the same plane, why it is close to impossible to separate each muscle. This problem could possibly be solved by using the IMARIS™ program, which reconstructs the muscles in 3-D based on the image stack obtained with the CLSM.

According to our CLSM results most of the muscles seem to attach to the dorsolateral muscles and this is confirmed in MARCUS (1929). In addition, SEM on *H. crispae* shows some cuticular muscle attachments between the legs in the ventral plane as well as in the lateral plane of the body (Fig. 6). The somatic muscles are attached to these characteristic zones in the cuticle by cuticular fibres, which extend from a dense layer just below the epidermis into the epicuticle [KRISTENSEN, 1978; GREVEN, 1980; KINCHIN, 1994].

In the bucco-pharyngeal apparatus one can discriminate between stylet muscles, stylet support muscles and those of the pharyngeal bulb. These muscles are true cross-striated. Stylet muscles has been described in *Macrobotus hufelandi*, also by MARCUS [1929], where he show 15 individual muscles involved with the stylets and supporting structures. From our results we can only distinguish 4 to 6 muscles around the stylets and 4 connected to the pharyngeal bulb. The musculature of the pharyngeal bulb of *H. crispae* in the active form has been investigated with TEM by EIBYE-JACOBSEN [1996]. She found that the pharynx is an ectodermal myoepithelium consisting of arrays of radial muscles. It is mentioned that the radial muscles are attached to the basement membrane of the bulb, starting as single muscle filaments fusing along their length to form muscle cells attached to the cuticle of the pharyngeal lumen. The action of these muscles contributes to the animal's suction capability, by contraction and relaxation of the radial muscles, and propels ingested nutrients along the alimentary canal, when a food item has been pierced by the stylets [KINCHIN, 1994]. Our result seems to confirm these findings, though it is impossible to define the pharynx as a myoepithelium based on the CLSM image alone.

4.2. Scanning electron microscopy (SEM)

The SEM investigation indicate that the material collected from Vellerup, Nipisat and Ærø all belong to the same species *i.e.* *Halobiotus crispae*. Nevertheless, the Ærø material has previously been described as *Halobiotus stenostomus* by JØRGENSEN & KRISTENSEN [2004]. However, based on the aberrant wrinkled cuticle, the closed invaginated mouth opening and the claw system, characters originally described from *H. crispae* from Nipisat [KRISTENSEN, 1982], the specimen clearly belong to *H. crispae* in the pseudosimplex 1 stage. Additionally, the investigation on the specimens from Vellerup and Nipisat display striking morphological similarities in the structure of cuticle, the mouth opening and the heteronych claw system.

These data support the assumption that the specimens found at the two localities indeed belong to the same species.

4.3. Molecular methods

Our molecular data show low sequence diversity between the populations at Nipisat and Vellerup in both COI (0.73%) and ITS2 (0.56%), as well as low haplotype diversity in COI (0.76%). Because COI is a fast evolving protein coding mitochondrial gene, one would not expect such low sequence diversity in an organism as wide spread as *H. crispae*. There is approximately 3400 km between the two populations [MØBJERG *et al.*, 2007]. *Milnesium tardigradum* have shown higher sequence diversity within Denmark (0.63-5.81%) [JØRGENSEN *et al.*, 2007], but *M. tardigradum* might be a species complex of cryptic species [A. JØRGENSEN pers. comm.]. In contrast a sequence diversity of 0.16-1.28% was shown for *Echiniscus testudo* DOYÈRE, 1840 which has a wide spread distribution. This can in part be explained by the fact that *E. testudo* is a parthenogenically reproducing species, whereas *M. tardigradum* is sexually reproducing; though some populations of *M. tardigradum* only contain females [JØRGENSEN *et al.*, 2007]. If one compares our results with these findings, it shows that *H. crispae* contains relatively low sequence diversity for a sexually reproducing species sampled over a vast geographical area. This indicates that *H. crispae* might have an effective dispersal potential *i.e.* by “stepping stones” and be a young species as a result of a relatively recent speciation event. Indeed, the genetic diversity between the populations in Nipisat Bay and Vellerup Vig is less than what is currently used in DNA barcoding [HERBERT *et al.* 2003]. Barcoding studies on lepidopterans as well as on arachnids have shown that genetic divergence between species often (98% of cases) is greater than 2-3% [HERBERT & BARRETT, 2005]. Given that this applies to Tardigrada, our molecular data confirm what morphology has indicated, that the two populations are the same species. Furthermore this also implicates that *H. stenostomus* from Ærø, Denmark [JØRGENSEN & KRISTENSEN, 2004] is in fact a deep water form (6-8m) of *H. crispae* which suggests that *H. crispae* is not exclusively limited to shallow-water habitats as previously believed.

4.4. Osmotic stress tolerance

It is well established that salinity is an important independent factor in shaping the horizontal and vertical community structure of marine free-living meiofauna. The gradation of communities described in estuaries bears direct evidence of this [FORSTER, 1997; GONZÁLEZ-ORTEGÓN, 2006]. Consequently, osmotic conditions are of fundamental importance for an organism to occur permanently in a habitat. However these conditions are by no means static. In subtidal habitats, large temporal and spatial fluctuations in salinity occur regularly; apart from salinity changes accompanying tidal cycles, precipitation and terrestrial surface water run-off, variations are induced by evaporative water-loss and surface ice formation. As a result, subtidal habitats can be defined as being one of the most stressful marine biotopes demanding extraordinary adaptations of the organisms that live here.

The degree of osmotic stress exerted on a population of tardigrades is dependent strongly upon the location at which it occurs; the higher in the subtidal zone the greater the exposure to factors that alter salinity. Presuming that ambient salinity is of significance to tardigrade assemblages, it should be expected that populations inhabiting localities experiencing greater fluctuations in salinity, would respond more effectively to the consequences of osmotic stress. This study provides good evidence for this. At the study site of Nipisat Bay, Disko Island, W. Greenland, *Halobiotus crispae* is a member of the upper shoreline community. This close to land, huge oscillations in salinity are taking place; indeed salinities between 4-32‰ have been measured [R.M. KRISTENSEN pers. comm.]. Although showing a similar tolerance during

hypotonic exposure, specimens from Nipisat Bay displayed a significantly higher tolerance of increasing sea water concentrations when compared to animals from the southernmost locality. Some specimens even tolerated as much as 80‰. This observation seems well in accordance with their greatly exposed habitat; and of particular importance to life in the transitional zone between land and sea. In fact, during maximum low-tide sub-populations may become temporarily trapped in small bodies extremely sensitive to the factors that alter salinity *e.g.* surface evaporation. In contrast, at the southernmost locality of Vellerup Vig, Isefjord, Denmark *H. crispae* colonise a narrow band in the littoral zone at a depth of 1.5-3.0 meters. Therefore, this community is not subject to the same extreme influences as the population at Nipisat Bay, Greenland. In fact, water samples taken at the respective depths over the course of an entire year varied no more than 18‰-20‰ [MØBJERG *et al.*, 2007], while RASMUSSEN [1973] recorded salinities varying in the range of 17.5‰-21.5‰. Accordingly, we observed that all activity ceased at a comparatively low ambient salinity (65‰) in specimens from this locality, indicating that animals from Vellerup Vig are more sensitive to hypertonic exposure. As a result, the obvious differences in habitat preference, as well as the factors affecting salinity, are reflected by the overall tolerance of osmotic stress exposure exhibited by the two populations; and most likely caused by divergent selection pressures exerted at the two localities.

As previously mentioned, tolerance of hypotonic exposure were more or less similar between the two populations. Indeed, (11.8%) and (12.2%) of the specimens from Vellerup Vig and Nipisat Bay respectively, were active when exposed to 1‰ seawater. On a parallel note, this is in some contrast to the preliminary physiological investigations performed by KRISTENSEN [1982] which showed that the active stage was intolerant of melt water. However, the frequencies as well as the severity of the changes occurring at the two localities are not the same. At Nipisat Bay, the active stage is found during the Greenlandic summer [KRISTENSEN, 1982]; a season defined by elevated temperatures and increased precipitation [MØBJERG *et al.*, 2007]. Consequently, it is easily envisioned how sudden drops in salinity may occur when considering their highly exposed habitat. In fact, 6-8‰ was measured in small bodies of water during sampling when raining. In this context it is not difficult to comprehend why tolerance of hypotonic exposure seems relevant. Conversely, at Vellerup Vig Isefjord the active stage immerses during the Danish winter/fall [MØBJERG *et al.*, 2007]. However, at a depth of 1.5-3.0 meters of water *H. crispae* is buffered from sudden, extreme changes in ambient salinity. Actually, little change seems to occur [RASMUSSEN, 1973]. So why are the exhibited hypotonic tolerances more or less similar? Especially considering that differences in habitat have resulted in changes of their hypertonic tolerances. Abilities not conferring an adaptational advantage are susceptible to accumulation of deleterious mutations over time. This could imply that different compensatory mechanisms are responsible during very high or very low salt concentrations respectively, which has been reported in several other published cases performed on related phyla like nematodes and annelids [OGLESBY, 1981]. In this regard, tolerance of hypertonic exposure could be affected without impinging on the hypotonic tolerances. However, this may be difficult to conclude on the sole basis of our preliminary results. Even so, since our molecular data suggest a recent invasion from north to south, the ability to tolerate extremely low osmotic pressures might not yet have been affected by selection.

When looking at the different cyclomorphic stages at Vellerup Vig, Denmark it becomes apparent that the osmotic stress tolerance of *Halobiotus crispae* changes with the synchronical annual cycle of cyclomorphosis. This has previously also been suggested by MØBJERG & DAHL [1996]. These variations probably relate to dissimilar requirements in the different cyclomorphic stages. At Vellerup Vig the conversion into the P1 stage is associated with the

beginning of summer (May-June) [MØBJERG *et al.*, 2007]. The P1 stage is characterized by possessing two cuticles; the outer being sclerotized and dark in colouring [KRISTENSEN, 1982]. Although retaining their locomotory skills in this stage, mechanical resistance might be imposed by the second outer cuticle. This would be of particular significance during periods of osmotic stress with ensuing changes in body volume. Consequently, given that the ability to move is impeded by a more rigid outer structure, the level of activity would be affected at any given salinity. Indeed, only (3.6%) are active at a salinity of 1‰. This might explain the reduced activity (tolerance) observed during osmotic stress exposure when compared to active specimens.

The P2 stage is very short, but indeed very interesting. Of the different cyclomorphic stages, the P2 stage is least tolerant of high salt concentrations; however, it is also the stage which appears to handle low osmotic pressures most successfully. In fact (13.3%) remain active in distilled water. At Nipisat Bay the P2 stage appear in springtime (April-May) [MØBJERG *et al.*, 2007] when the ice thaws and incredible amounts of ice meltage empties into the bay. In this context, the increased hypotonic tolerance must be seen as direct adaptation to these extreme circumstances. This is also supported on the ultrastructural level, as the size of the Malpighian tubules and therefore the ability to osmoregulate has been found to be largest in the P2 stage [MØBJERG & DAHL, 1996]. However, this does not seem to corroborate with the limited tolerance of hypertonic solutions, unless different mechanisms are responsible during high or very low salt concentrations respectively, as previously claimed.

This study demonstrates that irrespective of the cyclomorphic stage *Halobiotus crispae* is capable of tolerating a very wide range of salinities; it is a *euryhaline* species. Indeed they are able to tolerate salinities greatly exceeding what would ever occur in nature. The salinity tolerance exhibited by *H. crispae* is unparalleled within Tardigrada, except for the tidal heterotardigrade *Echiniscoides sigismundi* Schultze, 1865 which is the only species in which osmobiogenesis has been demonstrated [KRISTENSEN & HALLAS, 1980]. This is an excellent adaptation to their habitat in the subtidal zone; indeed so successful that *H. crispae* have completely replaced arthrotardigrades from the subtidal zone in the Arctic [CRISP & KRISTENSEN, 1983]. However it is not claimed on the basis of these results that *H. crispae* is an osmoregulator. Yet since the animals are able to tolerate the observed broad spectrum of salinities, and a change in internal inorganic ion concentration of app. 200 mOsm (~7‰) usually have lethal consequences in most organisms [GILLES, 1979], it seems exceedingly probable.

4.5. Cryptobiosis

4.5.1. Anhydrobiosis

Anhydrobiosis is a widespread strategy common to most cryptic fauna in response to desiccation [WRIGHT *et al.*, 1992; CAPROLI *et al.*, 2004]. The ability to enter an anhydrobiotic stage is well known in tardigrades, especially limno-terrestrial species [RAMLØV & WESTH, 2001; SCHILL *et al.*, 2004]. Anhydrobiosis involves the loss of “bulk” water as well as that bound to macromolecules and internal structures. This process leads to the formation of a tun state. Experiments have shown that anhydrobiosis is dependant upon the accumulation of polyhydroxyl compounds, such as trehalose [WESTH & RAMLØV, 1991], which has led to what is called the water replacement theory [CROWE *et al.*, 1998]. It is believed that water molecules are replaced by trehalose, thereby conferring protection against any damaging effects during desiccation [WRIGHT, 2001]. More recently anhydrobiosis has also been shown to depend on the synthesis of molecular chaperones *i.e.* heat-shock-proteins (HSP) [RAMLØV & WESTH, 2001; SCHILL *et al.*, 2004] in relation to molecular crowding and protein-protein interactions.

As previously mentioned *H. crispae* is secondarily adapted to the marine environment. Depending on how recent readaptation has occurred, one could hypothesize that a remnant ability to enter an anhydrobiotic stage could still be present. According to our results this is not the case in any of the cyclomorphic stages, as all specimens die when exposed to e.g. the atmosphere or extreme salinity (app. 300‰ saturated seawater). Indeed, when considering the different *H. crispae* localities anhydrobiosis hardly seems a likely employed survival strategy. Consequently, this implies that *H. crispae* is actively tolerating/osmoregulating during periods of severe changes in the ambient osmotic pressure gradient.

4.5.2 Cryobiosis or freezetolerance

Winter temperatures at Nipisat Bay are always well below zero, and often below the crystallization temperature of the body fluids of most organisms. This indicates that winter survival of *Halobiotus crispae* is dependent upon the tolerance of very low temperatures. At this time of year *H. crispae* is in the pseudosimplex 1 (P1) stage, which in fact has been described as a movable cyst specifically adapted to the harsh arctic winter conditions [KRISTENSEN, 1982]. Indeed, preliminary experiments on negative temperatures showed that animals in the P1 and P2 stage tolerated gradual freezing down to -20°C [KRISTENSEN, 1982]. Recently this has been reevaluated to only include the P1 stage [MØBJERG *et al.*, 2007]. Our results show that animals in the P1 stage are able to tolerate temperatures as low as -18°C , while freezing is lethal in the other cyclomorphic stages. Freeze-tolerance has been demonstrated in other tardigrades before *i.e.* *Richtersius coronifer* (RICHTERS, 1903) [RAMLØV & WESTH, 1992] and *Amphibolus nebulosus* and has been shown to depend on the presence of ice nucleating agents (INA) [WESTH & KRISTENSEN, 1992]. INA act by inducing ice-formation at relatively high temperatures thereby controlling the rate of ice growth. In the last mentioned study, the occurrence of low weight cryoprotectives such as polyols was not detected, indicating that a significant depression of the crystallization temperature was not involved in winter survival of these species. This is in contrast to what generally is reported from freeze-tolerant insects [ZACHARIASSEN, 2004]. Whether a similar situation is the case in *H. crispae* can not be answered on the basis of these results alone. However, the fact that survival is inversely correlated with the rate of freezing indicates that biochemical adaptations are needed to be immobilized in preparing for freeze-tolerance. Yet, the freezing point in *H. crispae* have been shown to be surprisingly low (-15°C) [Unpublished data], thus excluding the presence of INAs. However a similar relationship between freezing rate and survival was recently described in *R. coronifer* [KATHOLM, 2006 M. Sc. thesis unpublished]. In this study it was speculated that the synthesis of heat-shock-proteins (HSP) conferred protection against cellular dehydration induced by ice formation, and indeed this may be the case. Both freezing and dehydration have long been thought to rely on the same biochemical mechanisms. However, whether this is true has heatedly been debated [CROWE *et al.*, 1990] as experiments on trehalose levels, as well as the lack of a tun state during freezing, have suggested that this is not entirely true [WRIGHT, 2001]. In light of our results the two exposures can not rely on the same molecular basis for tolerance of these extremes, as the P1 stage has been shown not to tolerate desiccation [KRISTENSEN, 1982].

It seems peculiar that the P1 stage from Vellerup Vig is able to tolerate negative temperatures, as this stage appears during the Danish summer. Here negative temperatures never occur. Thus, while the P1 stage is an adaptation to the long arctic winter in Greenland, it might possibly enable *H. crispae* to tolerate high temperatures and oxygen depletion in Denmark [MØBJERG *et al.*, 2007]. Nonetheless, the P1 stage has retained the ability to tolerate ice formation.

4.6. Volumeregulation

Volume regulation as a phenomenon at the whole-organism level may be thought of in two contexts. One is the steady-state response of the organism to steady-state environmental conditions, and usually involves the presence of at least slight osmolality differences between the environmental medium and the body fluids of the organism. However, the second type of volume regulation involves physiological responses, which are invoked as a response to osmotic stress, such as a transfer of an organism from one salinity to another. In this context, volume regulation is thought of in terms of the ability of the animal to restore the steady state. In the present study, it was the second type of volume regulation which was investigated, although there is no clear evidence of that these two types of whole animal volume regulation involve separate mechanisms [OGLESBY, 1981].

What this study demonstrates is that both of the investigated cyclomorphic stages of *Halobiotus crispae* have the ability, albeit with some differences, to regulate body volume, while small but significant differences persist between the two investigated populations in the active stage. During hypotonic exposure, a tardigrade is exposed to an initial, rapid influx of water. In species able to osmoregulate, this increase is followed by a period of gradual reduction in body volume to levels approaching those observed in normal ambient water salinities. This was best demonstrated by specimens in the P1 stage. Conversely, when exposed to hypertonic solutions a fast reduction in body volume is followed by a continuous return to normal levels. A response best exhibited by specimens in the active stage from the population at Nipisat Bay. A similar response pattern was reported from several species of intertidal nematodes [FORSTER, 1997], however specific differences relating to the degree of water loss/gain and rates of recovery seems to entail interesting ecological consequences. Animals in the P1 stage appeared much less affected by a reduced salinity, compared to specimens in the active stage, displaying only comparatively small variations in total body volume. Although clearly regulating body volume, this is also attributed to the presence of two cuticles in this stage, which mechanically prevents large changes in body volume. As previously mentioned the second cuticle also affects animal mobility, manifested by the fact that specimens in the P1 stage become passive more quickly during the initial periods of severe hypotonic exposure. Under the same conditions, specimens in the active stage underwent much larger variations in body volume. Most specimens experienced increases in hydrostatic pressure rendering them passive, while others experienced rupture of the cuticle of which there is no recovery. Consequently, the tolerance of very large body volume changes, especially during severe hypotonic exposure, clearly involves the properties of the cuticle. Functionally this may involve either (1) resisting *i.e.* hydrostatic expulsion or (2) accommodating the large body volume changes occurring. Therefore it might be speculated if the ability of *H. crispae* to survive incidents of reduced salinity depends on whether it can regulate its body volume efficiently enough to prevent the internal pressure of reaching a critical value, upon which bursting occurs. However, when applying an integrative approach it seems enigmatic that P1 from Vellerup Vig should regulate body volume more efficiently than the active stage. At Nipisat Bay, the active stage experience greater oscillations in salinity than what would ever occur at Vellerup Vig. This is also reflected by the overall salinity tolerance but not so well in regulation of body volume, hence implying that specimens in the active stage have the ability to tolerate sustained periods of significant changes in body volume.

The responses to elevated salinity were more uniform with specimens from both populations displaying some ability to recover from the initial reduction in body volume, even if the rate at which this was achieved differed. Animals from the population at Vellerup Vig regained steady-state values within 4 h, while specimens from Nipisat Bay recovered after

just 2 h exposure. Nevertheless, beyond this time individuals from both populations experienced reductions in body volume indicating an inability to regulate body volume over longer periods of time. No mortality (or even passivity) was registered during hypertonic immersion; indeed the animals did not even exhibit major differences in behaviour compared to 100% S.W. However, the fact that specimens from Nipisat recover faster from hypertonic shock compared to animals from Vellerup is in agreement with the relatively more exposed habitat. Animals from this locality also tolerate significantly higher salinities, logically implying a connection between salinity tolerance and volume regulation.

5. CONCLUSIONS

This study concerns several aspects of the secondary marine species *Halobiotus crispae* from Nipisat, Disko Island, Greenland as well as Vellerup Vig, Isefjord, Denmark. Extensive protocol-development demonstrates that immunocytochemistry (ICC) in concert with confocal scanning laser microscopy (CLSM) can be a powerful tool in reconstructing the internal anatomy of Tardigrada. Using the antibodies anti-acetylated α -tubulin and anti-FMRF-amide it is shown that different neurotransmitters are present in different parts of the CNS, while being co-localized in the same components in the PNS. The muscle system can successfully be reconstructed using FITC conjugated phalloidin. Our molecular data on ITS2 show that *H. crispae* evolved within a paraphyletic *Isohypsibius*, and is a relatively young species. Low sequence diversity in COI and ITS2 between the populations at Nipisat and Vellerup Vig show that they are the same species. Exposure to osmotic stress demonstrated that *H. crispae* is a euryhaline species capable of tolerating fluctuations in salinity greatly exceeding those encountered in nature. This salinity tolerance is related to the cyclomorphic stage. Experiments on desiccation and cooling revealed that desiccation was not tolerated, and only animals in P1 are freeze tolerant. Exposure to osmotic shock indicates that *H. crispae* experience significant changes in body volume initially. Immersion in hypotonic solutions reveals that these changes are less pronounced in animals in P1; however, regulation of body volume was demonstrated in both stages over a period of 48 h. Exposure to hypertonic salinity revealed a faster recovery of specimens from Nipisat, but animals from both populations displayed an inability to regulate body volume during long-term exposure. Nevertheless, mobility was retained throughout the exposure suggesting a tolerance of long periods of reduced body volume. Difference in habitat, salinity tolerance and volume regulation between the two populations endorses the suggestion that the ability to overcome salinity fluctuations is a factor in determining the horizontal distribution of *H. crispae*. Future studies will be directed against elucidating the molecular basis for osmoregulation in Tardigrada.

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The Larval Muscle System of Arctic Polychaetes and Polyplacophorans

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Abstract: Marine polychaete and polyplacophoran larvae were gathered in the Disko Bay area of Greenland in August 2006. The larvae were transported to the University of Copenhagen in Denmark. There, confocal laser scanning microscopy was used in order to achieve high-resolution images of the muscular systems of the two taxa. The aim of the project was to compare the muscular systems of the taxa, with focus on the development of serially repeated organ systems in these two lophotrochozoan taxa. The analysis of the resulting images concluded that within polychaetes the development of segments takes place in the posterior end and pushes the older segments upward. The most developed segments are therefore found in the anterior part of the animal and the youngest segments, in which muscles are not entirely developed yet, are found in the posterior end. Polyplacophorans on the other hand, do not show true segmentation, as development of their musculature does not proceed in a strict anterior-posterior pattern.

Keywords. Lophotrochozoa, segmentation, muscular system, confocal laser scanning microscopy.

1. INTRODUCTION

Muscle tissues are major mesoderm-derived components of bilateral body plans, as diverse as flatworms or vertebrates [WANNINGER, 2004]. Data on muscular anatomy have shown to provide useful characters for systematic analyses on species as well as phylum level and thus allow significant insight regarding metazoan body plan evolution. This paper focuses on the analysis of polychaete (Annelida) and polyplacophoran (Mollusca) larval muscular systems and the comparison of these two taxa of metazoans.

The ciliated larvae of polychaete annelids are extraordinarily variable in form and function [YOUNG, 2002]. This diversity presents unique opportunities and challenges to those interested in the functional morphology, ecology and evolution of larvae [YOUNG, 2002]. Polyplacophora are a taxon within the phylum Mollusca. Numerous studies have been performed on both taxa, but so far, no one has compared the muscular development of polychaete and polyplacophoran larvae. In this study, the comparison is especially focused on the development of what *seems* to be segmentation within the two taxa. In the Annelida the segments develop from a pre-anal growth zone. The development of segments takes place in the posterior end and pushes the older segments upward. Therefore, the most developed segments are found in the anterior part of the animal and the youngest segments, which might not even have developed their muscles entirely yet, are found in the posterior end. Polyplacophorans on the other hand do not show true segmentation as they develop their musculature synchronously.

There are two competing theories of the evolution of segmentation. The traditional theory states that segmentation evolved in the early ancestor of Spiralia and ecdysozoans and that some taxa secondarily lost the segmentation. This statement supports the hypothesis that it is far easier to lose a specific character, than it is to develop the same character twice. A more recent hypothesis of the evolution of segmentation states that segmentation in Annelida and ecdysozoans evolved analogously.



Figure 1. Map showing Disko Island off the west coast of Greenland. Sampling sites marked by black dots.

2. MATERIALS AND METHODS

The samples were collected around Disko Island in Greenland (see Fig.1) at depths varying from 5 – 250 m., using 50 μm , 100 μm and 200 μm plankton-nets. The collections took place at several different locations, ranging from shallow waters to the open sea.

2.1. Storing and staining specimens

The specimens were isolated from the samples shortly after return to the lab (< 6 h). Hereafter they were relaxed in 3.5 % magnesium chloride (MgCl_2), fixed in 4.0 % paraformaldehyde (PFA), stored in 0.1 M phosphate buffer (PBS) with 0.1% sodium azide (NaN_3) and kept below 5°C until further treatment in Copenhagen, Denmark.

The staining of the muscular system of the larvae was carried out at the Institute of Biology, University of Copenhagen, Denmark. The larvae were washed in 0.1 M PBS. Permeabilisation was achieved by using PBT (0.1 M PBS + 0.2 % Triton X-100) for 1h. Bodipy FL phalloidin (Invitrogen/Molec.Probes, USA) diluted in PBT (1:40) was used for the staining. After incubating for 1 h the larvae were washed in 0.1 M PBS for three times 15 min. each. Subsequently, the larvae were embedded in Vectashield mounting medium on glass slides and stored in darkness below 5°C.

2.2.1. Confocal laser scanning microscopy

CLSM (Leica TCS SP2 AOBS) was used to scan the specimens. By having a confocal pinhole, the microscope is efficient at rejecting out of focus fluorescent light. The practical effect of this is that your image comes from a thin section of your sample (you have a small depth of field). By scanning many thin sections through your sample, you can build up a very clean three-dimensional image of the sample [SEMWOGERER & WEEKS, in press]. The 3D imaging is particularly useful for species below the millimetre-range, since muscle structures. Digital recordings of the fluorescence signal as well as light micrographs were produced.

Overlay pictures of the light and fluorescence micrographs were generated by using Adobe Photoshop CS.

3. RESULTS

3.1. Polychaetes

Sabellariidae.

See Figure 1-6. Two pairs of longitudinal muscles run dorso-laterally and ventro-laterally from the anterior muscle mass to the posterior end of the larva (Figures 1 & 5). In the lower anterior end of the larvae the longitudinal muscles run through an area of intense muscle mass, where long cilia fasten to the larva body. These cilia are visible in figure 4. Above the area where the cilia fasten, a very well developed apical musculature is noticeable (Figure 1). Between the muscle pairs transverse muscles form, each formation indicates the appearance of a segment (Figures 2 and 5). Within each segment, a thin muscle runs longitudinally forming small arches, thereby interconnecting the segments (Figure 3). Through the centre of the larvae, two muscles run ventrally from the anterior end to the posterior end (Figure 1). Distal ring musculature is visible on the ventral side of the larva in Figure 5.

Oweniidae.

See Figures 7 and 8. Two pairs of thick longitudinal muscles run from the region of the apical organ to the area in which the long cilia fasten, this area is visible by containing an intense muscle mass. This will be referred to as the “muscle bundle”. Between the apical organ and the buccal area the intestine is visible which is surrounded by up to 15 thin circular muscles. There is no structure of muscles in the area of the prototroch. In the anteriormost region there are several thin longitudinal muscles between the two pairs of thick longitudinal muscles.

Pectinariidae.

See Figures 9 and 10. From the buccal area there are two muscles running towards the posterior pole in the anterior part and slimming near the posterior part of the larvae. At the posterior end the muscle fibres are connected to each other. Around the intestine there are 12 slim muscle strings running longitudinally from anterior to posterior. On the dorsal side, there are two large muscles on each side running from anterior to posterior. Along these, there are four muscles on each side running from anterior to posterior, from the prototroch to the telotroch. On the ventral side of the posterior pole, there are two muscle arches. In the buccal area, several layers of muscle arches are present.

Unidentified polychaete larva.

See Figure 11. The larval muscular structure includes a muscle bundle in the anterior region that consists of several loops of fibres that create a complex structure. Underneath this structure, the prototroch is situated. A bundle of circular muscles underlies the prototroch. One pair of outer longitudinal muscle bundles and two pairs of median longitudinal muscle bundles run from anterior to posterior. The outer longitudinal muscles and one of the pairs of the median muscles each split into two in the anterior end underneath the prototroch. The second median pair also splits into two separate muscle strands in the posterior end. From each of the outer longitudinal muscle bundles the dorso-ventral musculature has started to form.

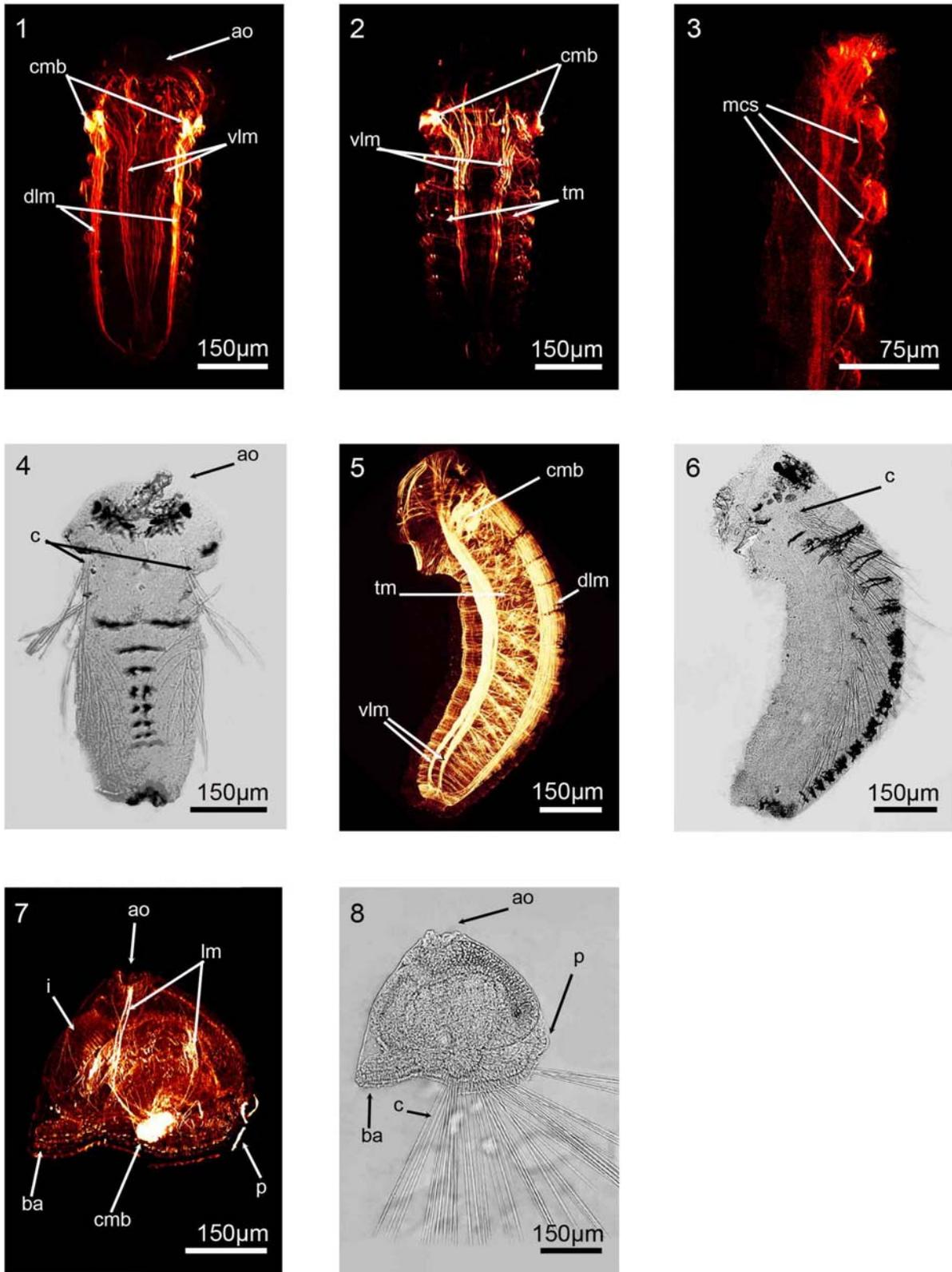


Figure 2. Legends 1-16 on next page.

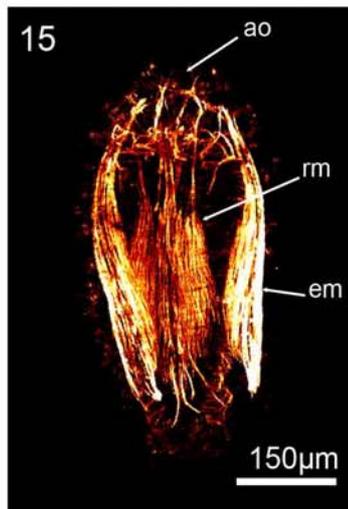
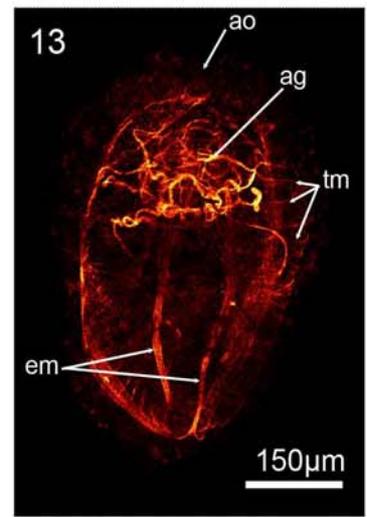
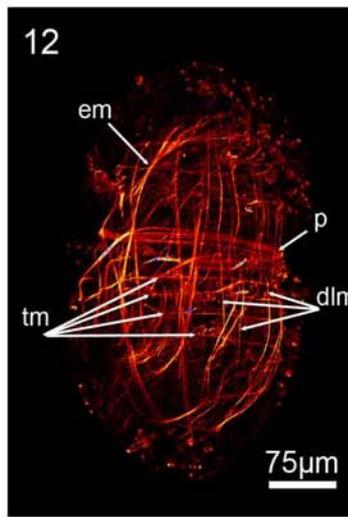
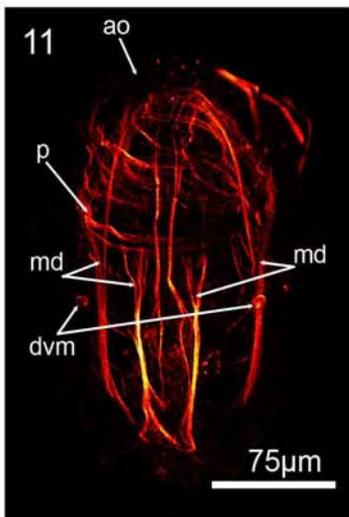
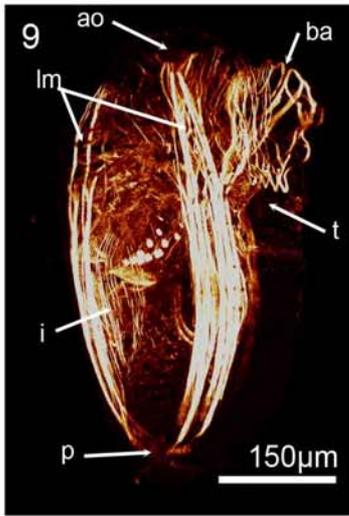


Figure 2. 1. Polychaete larva. Dorso-lateral view of a *Sabellariidae* larva visualized by phalloidin staining. dlm: one pair of dorso-lateral longitudinal muscles. ao: apical organ. vlm: one pair of ventro-lateral longitudinal muscles. cmb: muscle bundles with cilia fasten to. **2.** Polychaete larva. Dorso-lateral view of a *Sabellariidae* larva visualized by phalloidin staining. vlm: one pair of ventro-lateral longitudinal muscles. tm: transverse muscles. cmb: muscle bundles with cilia fasten to. **3.** Polychaete larva. Dorso-lateral view of a *Sabellariidae* larva visualized by phalloidin staining. Enlargement of the distal segments in image 1. mcs: muscles connecting segments. **4.** Polychaete larva. Dorso-lateral view of a *Sabellariidae* larva visualized by light microscopy. ao: apical organ. c: point at which long cilia fasten. **5.** Polychaete larva. Lateral view of a *Sabellariidae* larva visualized by phalloidin staining. vlm: one pair of ventro-lateral longitudinal muscles. dlm: one pair of dorso-lateral longitudinal muscles. cmb: muscle bundles with cilia fasten to. tm: transverse muscles. **6.** Polychaete larva. Lateral view of a *Sabellariidae* larva visualized by light microscopy. c: point at which long cilia fasten. **7.** Polychaete larva. Lateral view of an *Oweniidae* larva, visualized by phalloidin staining. ao: apical organ, ba: buccal area, cmb: cilia muscle bundles, i: intestine, lm: paired longitudinal muscles, p: prototroch. **8.** Polychaete larva. Lateral view of an *Oweniidae* larva visualized by phalloidin staining. ao: apical organ, ba: buccal area, c: long cilia, p: prototroch. **9.** Polychaete larva. Dorsolateral view from the right of a *Pectinariidae* larva visualized by light microscopy. ao: apical organ, ba: buccal area, i: intestine, lm: paired longitudinal muscles, p: prototroch, t: telotroch. **10.** Polychaete larva, dorsolateral view from the right of a *Pectinariidae* larva visualized by phalloidin staining. ba: buccal area, p: prototroch, t: telotroch. **11.** Polyplacophoran larva. Ventral view visualized by phalloidin staining. ao: apical organ, p: prototroch, md: muscles dividing, dvm: dorso-ventral longitudinal muscles. **12.** Polyplacophoran larva, dorsal view visualized by phalloidin staining. em: enrolling muscles, p: prototroch, tm: transversal muscles, dlm: dorso-lateral longitudinal muscles. **13.** Polyplacophoran larva (x), transversal view with the ventral side to the left and the dorsal side to the right, visualized by phalloidin staining. ao: apical organ, ag: apical grid, em: enrolling muscles, tm: transversal myofibrils. **14.** Polyplacophoran larva (x), transversal view with the ventral side to the left and the dorsal side to the right. Visualized by light microscopy. **15.** Polyplacophoran larva (y), dorsal view visualized by phalloidin staining. ao: apical organ, em: enrolling muscles, rm: rectus musculature. **16.** Polyplacophoran larva (y), dorsal view visualized by light microscopy.

3.2. Polyplacophora

Unidentified polyplacophoran larvae.

See Figures 12-16. In the anterior part of the larva in Figure 13, there is a complex three-dimensional structure, the apical grid. Underneath the apical grid, the prototroch ring is situated. The ventral side shows the ventrolateral longitudinal muscle that runs all the way from the anterior pole to the posterior pole. The dorsal side also shows a longitudinal muscle structure which is the dorsal rectus muscle. Transversal myofibrils can be seen in the dorsal region of the putative shell plates.

There is no clear sign of the prototroch ring muscle in Figure 15 and 16. In the anterior part, the apical grid is seen as a complex muscle structure. A pair of longitudinal muscles, called the enrolling muscles, run from anterior to posterior. In the centre of the larvae the rectus muscle has developed. Transversal myofibrils are visible. In Figure 12 enrolling musculature is visible on the left side. Beneath the prototroch ring, transversal muscles are situated. There are several longitudinal muscles running from anterior to posterior.

4. DISCUSSION

4.1. Comparison of the larval myoanatomy of polychaetes.

The polychaete taxa included in this study all show two pairs of longitudinal muscles running from anterior to posterior. These two muscle pairs are able to contract, thereby enabling the larvae to contract the entire length of the body. Apart from the two pairs of thick longitudinal muscles, the general muscular anatomy of the different polychaete families is not very similar. The shape of the larval body varies significantly, thus the muscle anatomy is different among the families as well.

Sabellariidae had a distinct body wall ring musculature. Figure 1 and 5 both show muscle stainings of *Sabellariidae*. However, ring musculature is only visible ventrally in Figure 5.

Since the larva in Figure 5 is larger than the one in Figure 1, it is likely that it is the oldest, and furthest developed, which could explain the difference in ring musculature development. A study of the annelid *Capitella sp.* showed that circular muscles appear as complete bands ventrally before they are seen dorsally [HILL, 2001]. Hence, this could explain why ring musculature is only visible on the ventral side in Figure 5. An inner layer of longitudinal muscles and an outer layer of ring musculature has been proposed as ground pattern for Annelida. Nonetheless, TZETLIN *et al.* [2002] found that absence of circular muscle fibers in polychaetes occur more frequently than generally thought, which would be in accordance with our results. Circular muscles are important for endobenthic burrowing forms but not necessary for epibenthic animals, which move with their parapodia or cilia. Thus, the lack of circular muscles could be due to either convergence or a plesiomorphic character depending on whether the ancestral polychaete was epi- or endobenthic. To describe and compare different stages of the larval development in further detail it would be necessary to rear the larvae from hatching until metamorphic competence.

4.2. Comparison of the larval myoanatomy of Polyplacophora

It has not been possible to distinguish the Polyplacophora into specific taxa. Nevertheless, there is good reason to believe that the three individuals investigated in this study can be divided into at least two different taxa. The individuals in Figure 13 and 15 have proven to be very similar and might therefore be placed in the same taxon. When comparing Figures 13 and 15 to Figure 12, there are some characters such as enrolling musculature, prototroch ring and transversal musculature, which they have in common. On the contrary, the specimen in Figure 12 differs from the others by having several dorsal longitudinal muscles. Thus, it is most likely that the specimen in Figure 12 belongs to another taxon than the specimen in Figure 13 and 15. This occurrence of several dorsal longitudinal muscles has not been described in any polyplacophoran before, which makes this phenomenon extremely interesting for future projects.

It has been argued that the serial structure of shell plates and muscles in the adult animal is a vestige of a segmented molluscan ancestor [LAKE, 1990]. However, considering the transversal musculature (Figs. 12, 13 & 15) the development occurs to be different from how segmented animals develop. Thus, polyplacophora should not be considered segmented.

4.3. Comparison of the larval myoanatomy of polychaetes and polyplacophorans

When observing the adult animals it could seem like both Polyplacophora and polychaetes are segmented. However, the development of the polyplacophoran larvae proves that the “units” of the polyplacophoran body are not developed in the same manner as segments and can thus not be defined as segments. The adult morphological arrangement of paired shell muscles of polyplacophores is a secondary condition [WANNINGER & HASZPRUNAR, 2002]. The transversal musculature under the shell plates is presumably a polyplacophoran apomorphy, which co-evolved with the shell plates [WANNINGER & HASZPRUNAR, 2002]. Contradictory, fig. 5 showing a polychaete reveals that this taxon do grow segments. The larvae grow in anterior-posterior direction; segments are therefore developed from the posterior end.

Our analysis has shown that the body wall structure of polychaete larvae mainly consists of diagonal and longitudinal muscles. A greater part of the body wall structure in some of the larvae was observed to consist of ring musculature (fig 15). This is in agreement with the description of the body wall musculature of worm-shaped groups and therefore also annelids. In the Polyplacophora, this kind of body wall structure was not found. However, it has been suggested that the apical muscle grid may be an indication of polyplacophorans having a worm-shaped molluscan ancestor, and that the rectus muscle has replaced the longitudinal fibres [WANNINGER & HASZPRUNAR, 2002]. Since there are no longitudinal muscle fibres in the

apical grid in the Polyplacophora investigated in this study, it seems that the rectus muscle has replaced these.

5. CONCLUSION

Polychaetes develop segmentation through a strict anterior-posterior mode of development while Polyplacophora do not show such an ontogenetic pattern. The Annelida is thus in general the only group within the Spiralia that show true segmentation. However, there is indication that both polychaetes and Polyplacophora origin from an unsegmented wormlike ancestor.

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A Study of Two Meiofaunal Polychaetes from Disko Island, Greenland and New Techniques for Visualization of the Jaw Apparatus

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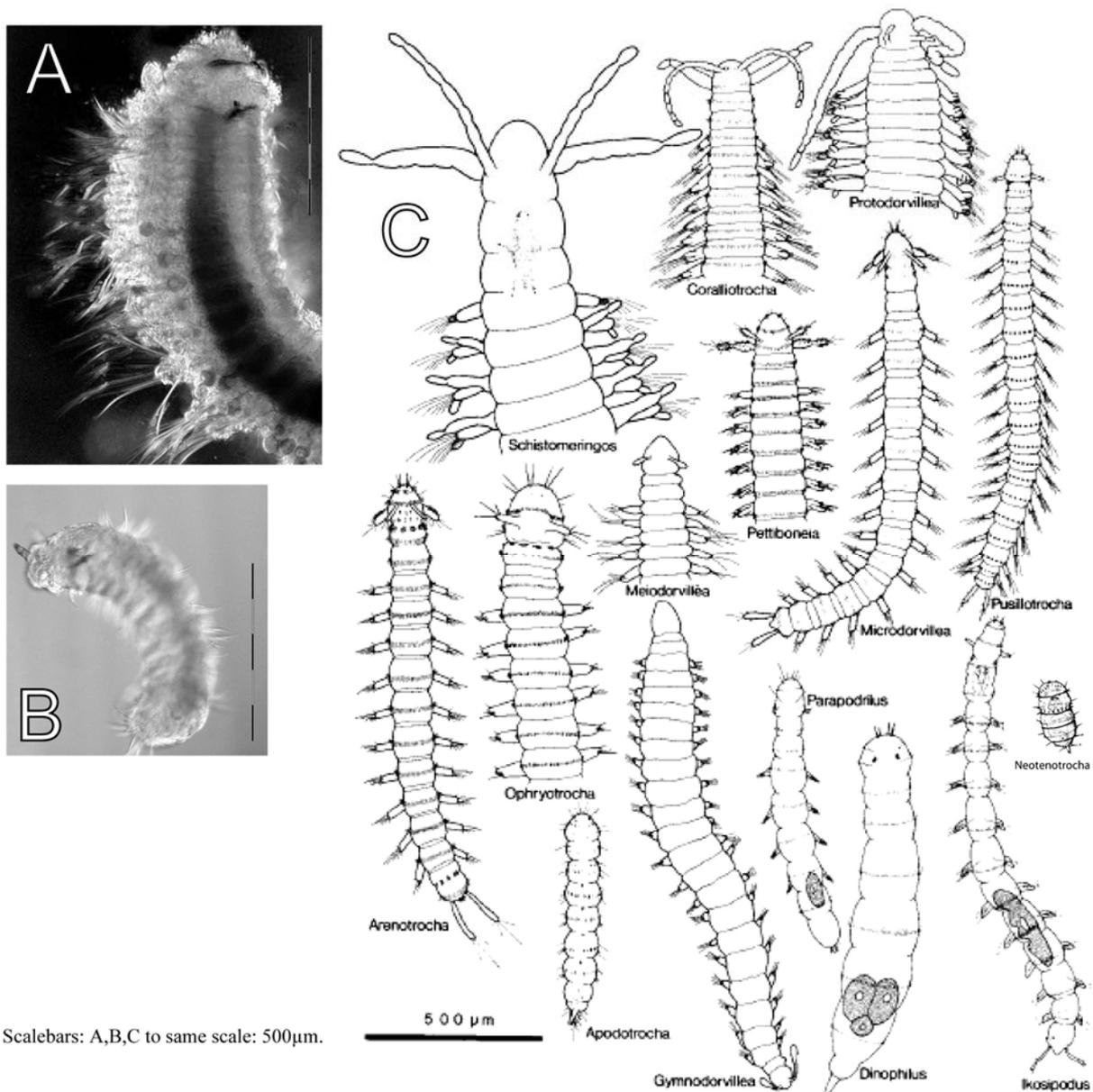
Abstract. During meiofaunal investigations of mud samples from south of Ippik, Disko Island, Greenland specimens of a dorvilleid, *Ophryotrocha* sp., and a lumbrinerid were found in several samples. Furthermore samples of *Capitella capitata* and *Psammodrillus aedificator* were recovered from shore localities on Disko Island. Various methods are tried and evaluated in order to investigate the jaw apparatus of dorvilleids.

Keywords. *Dorvilleidae*, jaw apparatus, morphology, progenesis, phylogeny

1. INTRODUCTION

1.1. Natural history

The Dorvilleidae is a family of eunicid polychaetes which includes around 120 nominal species that are best known from shallow water [ROUSE & PLEIJEL, 2001], but are also found in deep water [JUMARS, 1974; HILBIG & BLAKE, 1991]. Most dorvilleids have a complicated jaw apparatus, and while they are often considered facultative carnivores [FAUCHALD & JUMARS, 1979], they can also survive on plant matter for extended periods [ÅKESSON, 1967]. The earliest dorvilleid species was discovered in the early 1800's, but was first described as *Nereis rudolphi* Delle Chiaje, 1828 [ROUSE & PLEIJEL, 2001]. Other dorvilleids were described in the subsequent years under other polychaete families, such as *Ophryotrocha* that was described as a genus in the family Eunicidae [CLAPARÈDE & MECZNIKOV, 1869], until the family was erected by Chamberlin in his work on findings from the Albatross expedition in 1891-1905 [CHAMBERLIN, 1919]. The dorvilleids are a morphologically very heterogenous family (Fig. 1), and include some of the smallest described polychaetes [EIBYE-JACOBSEN & KRISTENSEN, 1994] as well as macrofaunal forms [ROUSE & PLEIJEL, 2001]. Many of the minute and interstitial taxa have been proposed to be neotenic/progenetic due to their resemblance to juveniles of macrofaunal forms [WESTHEIDE & RISER, 1983; WESTHEIDE, 1987]. The presence of a complex maxillo-mandibular pharyngeal armature is central in defining the extant Eunicimorph polychaetes and makes them one of the morphologically best supported higher groupings of bristleworms [ORENSANZ, 1990; STRUCK *et al.*, 2006]. This fact is important in Dorvilleidae, where the detailed jaw morphology is of significant systematic importance [PURSCHKE, 1987] and even the absence of jaws can be a source of taxonomic information [PURSCHKE, 1985]. Many jaw-bearing dorvilleids have ctenognath P-type (from German: Primitiv) jaws, but *Ophryotrocha* and a few minor genera distinguish themselves through the possible fusion of several jaw elements [PAXTON, 2004] to the formation of K-type (Kompliziert) jaws, that resemble ice-tongs, during the course of their ontogeny. This divergence can be traced back to the Jurassic Era and is one of the large dichotomies amongst the Dorvilleids [ORENSANZ, 1990]. As the dorvilleid jaw apparatus undergoes an ontogenetic transformation, study of the various jaw elements present at different times in the development can be used to compare the jaws of juvenile macrofaunal species and adult minute species, as well as for diagnostic purposes.



Scalebars: A,B,C to same scale: 500µm.

Figure 1. Increasing juvenile characters with decreasing size. **A.** Adult *Ophryotrocha* sp.; **B.** Juvenile *Ophryotrocha* sp.; **C.** In same scale examples of 15 different Dorvilleid genera (modified from [WESTHEIDE, 1987; EIBYE-JACOBSEN & KRISTENSEN, 1994]). Image **A** of individual collected on 24. Aug 2006 at Ippik taken prior to fixation in 99 % ethanol. Image **B.** of individual collected on 20. Aug 2006 at Ippik and taken prior to fixation in 2% glutaraldehyde.

1.2. Phylogenetic problems

The majority of the 36 extant dorvilleid genera have been described fairly recently, thus a generic revision by Jumars 3 decades ago stated that there were 8 genera of Dorvilleidae, and three of those were erected in the paper in question [JUMARS, 1974].

Phylogenetically, however, the morphological differences between the externally reduced taxa can be difficult to score as many characters are reduced to presence/absence scoring [STRUCK, 2006]. Much of the work that has been undertaken on dorvilleids in recent years has worked to elucidate the phylogeny of the family and its placement within the polychaetes. However the problematic use of external characters makes it difficult to construct a reliable

morphological phylogeny for the family [DAHLGREN *et al.*, 2001] and the recently undertaken molecular work on the family gives rise to some new interpretations. For instance, the formerly considered independent family Dinophilidae was included in the dorvilleids based on morphological data [EIBYE-JACOBSEN & KRISTENSEN, 1994]. More recent molecular phylogenetic investigations reinstate the group as a family [STRUCK *et al.*, 2005] and furthermore questions the internal relationships of the Dorvilleids themselves [STRUCK *et al.*, 2002; STRUCK *et al.*, 2006]. While molecular approaches, as elsewhere in systematics, seems to hold great promise for the future, the phylogenies based on the rDNA genes 18S and 28S and mitochondrial 16S rDNA often clash greatly with even well established earlier morphological trees [STRUCK, 2006; STRUCK *et al.*, 2006] leaving more questions than have been answered. The way forward, it seems, must then be to incorporate molecular work in the present phylogenies, but doing so without disregard for the well founded morphological work. Material from the present study has been sent for DNA extraction and will hopefully yield data that, in a thesis under way, combined with other dorvilleid molecular data as well as entries in GenBank, will be usable in a combined morphological/molecular phylogenetic tree.

1.3. Previous findings

Findings of dorvilleids in West Greenland are fairly rare in the literature and while *Ophryotrocha littoralis* Levinsen, 1879 was described from Egedesminde [LEVINSEN, 1879] and an *Ophryotrocha* sp. was found on the southeastern coast of Disko Island [KRISTENSEN & NØRREVANG, 1982] large polychaete studies have failed to record findings [WESENBERG-LUND, 1950; BLAKE & DEAN, 1973; CURTIS, 1977 & 1979]. The regional studies closest to Disko that deal with dorvilleids are Hilbig and Blake's 1991-study from the U.S. Atlantic slope and rise, and Riser 1999 on *Dinophilus* and other interstitial polychaetes from New England.

2. MATERIALS AND METHODS

2.1. Sampling

2.1.1. Dorvilleid locality sampling

Triangle-dredge samples were taken by R/V *Porsild* of the mud bottom off the southern coast of Disko Island, near Ippik (N 69°17.233', W 53°13.797') between 180 m and 200 m depth. The area is an old shrimp field, and the bottom mud is rich and well oxygenated. Onboard the ship the mud was stirred up with seawater and the solution screened with 250 and 125 µm mesh nets. The content was transferred to buckets of saltwater and ice was used to keep the samples as close to collecting temperature as possible throughout the ensuing work (see Table 1).

2.1.2. *Capitella* locality sampling

Capitella capitata Fabricius, 1780 was sought after at several locations, both at a littoral beach location south of Qeqertasuaq, as well as in the harbour itself. On the 16th of Aug. 2006 during a field trip to Disko Fjord, a shallow inlet at Nipisat, which at times was used for butchering whale carcasses, (so it was heavily organically enriched) was sampled and sediment brought back to Arctic Station.

Date	Location	Sampling gear	Position start	Position finish	Time start	Time finish	Depth start (m)	Depth finish (m)	Temp. (surface) °C	Temp. (sediment) °C
16.08	Nipisat	Shovel and Sieve	N 69° 27,054' W 54°13,133'				0,5	1		
20.08	Ippik	Triangular dredge	N 69° 17,264' W 53°13,724'	N 69° 17,233' W 53°13,797'			189	187		2,8
24.08	Ippik	Triangular dredge	N 69° 17,138' W 53°13,665'	N 69° 17,227' W 53°13,820'	11:20	11:25	222	200	7,4	2,9
24.08	Ippik	Triangular dredge	N 69° 17,114' W 53°13,636'	N 69° 17,194' W 53°13,844'	11:40	11:50	233	212	7,5	2,8
25.08	Itersla	Mini Van Veen grab	N 69° 26,989' W 52°19,688'				2,5			
25.08	Ippik	Triangular dredge	N 69° 17,314' W 53°13,251'	N 69° 17,356' W 53°13,226'	17:15	17:25	203	191	7,7	2,1

Table 1. Principal samplings during Arctic field course 2006.

2.1.3. *Psammodrillus* and *Diurodrillus* locality sampling

Psammodrillus aedifactor Kristensen & Nørrevang, 1982 was found for the first time since the original description [KRISTENSEN & NØRREANG, 1982] during a transect at Skansen/Itersla (Table 1). In the same sample *Diurodrillus westheidei*, Kristensen & Niilonen, 1982 was also found. The samples were taken with mini Van Veen grab from R/V *Porsild's* tender RIB and kept cool in labeled buckets until investigation at Arctic Station, where after both species were fixed with paraformaldehyde.

2.2. Sorting

Screened samples were washed in a 60 µm conical net in the lab and inspected alive under a Wild-Heerbrugg dissection microscope. A few samples were subjected to ultra-centrifuging in a silica sol, like BURGESS 2001, but with the addition of kaolin. This separated out meiofaunal specimens from the sediment by subjecting the sample to accelerated gravitation in the differential density of the colloidal solution. The specimens collected at the top and the sediment formed a precipitate in the bottom, while the kaolin formed a lid that prevented resuspension of fine sediment. Some specimens were photographed with an Olympus C5060 on an Olympus BX-51 compound microscope and videotaped with a JVC TK-C-1381 on an Olympus SZX-12 dissection microscope. Animals were anaesthetized in isotonic MgCl₂ solution before fixing in paraformaldehyde (2%), glutaraldehyde (2%), ETOH (99%) or formalin (4%) for cLSM, SEM/TEM, molecular or LM work respectively.

2.3. SEM

In order to facilitate study of the jaw structure of the animals, some new techniques had to be developed in order to dissect the jaws elements out of the specimens and clean them for SEM. As material from the fieldwork was scarce, cultured individuals of other dorvilleid species (*Ophryotrocha alborana*, nom. nud. [DAHLGREN *et al.*, 2001], *Dorvillea albamaculata* Åkesson & Rice, 1992) were used to try out different techniques. The best results were obtained on specimens subjected to different agents that could dissolve the soft tissues around the jaw elements. Both sodium hypochlorite (NaClO) and enzymatic digestion with pancreatin were tried each by themselves and in tandem. The sodium hypochlorite was prepared as a dilute solution of household bleach and was used in a modification of the method for preparing gnathostomulid jaws for SEM [SØRENSEN, 2000]. The pancreatin solution was prepared according to an arachnid protocol [ALVARES-PADILLA & HORMIGA, 2007 in press], in use, for the first time on polychaetes, it was found to require much shorter

digestion times than indicated for arachnids. The anterior part of the animal containing all the jaw elements was severed with a shard of scalpel blade or the tip of a hypodermic needle to allow the agents improved access to the soft tissue. After 10-15 minutes of enzyme activity at room temperature the jaw elements were left inside the remains of the anterior cuticle. They could be flushed out carefully by pipette, before being transferred to distilled water with an Irwin loop in order to rinse off remaining enzyme. They were placed in a drop of distilled water on a circular glass cover slip, the same size as an aluminium Cambridge stub, and bound to the glass when the water evaporated. Jaws were left on the stubs to dry overnight in a desiccation chamber, before sputter coating.

Whole specimens for SEM were dehydrated in a graded series of ethanol and acetone to 100% acetone at room temperature. The specimens were critical point dried with CO₂ in a BAL-TEC CPD 030. Sputter coating was a 140 s cycle with platinum-palladium in a JEOL JFC-2300HR high resolution fine coater. Thickness obtained was approx 20 nm. SEM images were made with a JEOL JSM-6335F Field Emission Scanning Electron Microscope using a 7 kV acceleration voltage and controlled by the program PC-SEM and manipulated in Adobe Photoshop CS.

2.4. Image stacking

Several Nomarski LM image-series were taken on an Olympus BX-51 compound microscope and combined to qualitative image stacks using the shareware program CombineZM (version as of 9th of February 2007) by Alan Hadley, England.

3. RESULTS

3.1. Dovilleid findings

Work on sampled target animals from Ippik recovered two polychaete species. One was found to be a species of the genus *Ophryotrocha* Claparède & Mecznikov, 1869 but it could not be identified further by the available literature, so it is referred to as *Ophryotrocha* sp. Body of one of the specimens of the species (Fig. 2A) is wide, around 1 mm long and 0.3 mm wide, it is composed of 14 setigers, the ciliation of which is indistinguished in the present study. The prostomium is bluntly triangular, about twice as broad as long, and at the posterior margin there are two antennae, that are short, digiform and could be indistinctly biarticulate, although it could be an artifact of a squat clavate palp covering the basis of the antenna. The maxilla observed (Fig. 3A) has 6 pairs of free denticles with pronounced teeth, and a carrier. However the *Ophryotrocha*-type forceps was not observed. This does not exclude the possibility that they could be present, but very delicate, in the species. The mandibles are L-shaped with slender handles subequal in length to the cutting blades, the latter are wide and in Fig. 3B they appear to be bifid or have a lateral projection, however none such is visible *in situ* in Fig. 3A. Only the handles and median edge of the cutting blades are visibly sclerotized (Fig 2D, 3A&I). The parapodia lack cirri, are uniramous, and the notopodia are missing throughout the body. The neuropodia are supported by a single neuro-acicula (Fig. 2C, 3G&H) and all supra-acicular setae are simple, serrated capillary setae (Fig. 2B). Subacicular setae are all heterogomph compound falcigerous and spinigerous setae (Fig. 2E, 3H). The pygidium has two anal cirri and a single median pygidial stylus (Fig. 2G).

Figures 2 and 3 show several plates of what is believed to be a juvenile specimen (although Fig. 3G, H are from a larger specimen) which might explain why it in Fig. 3A is not possible to see forceps type carriers amongst the maxillary elements.

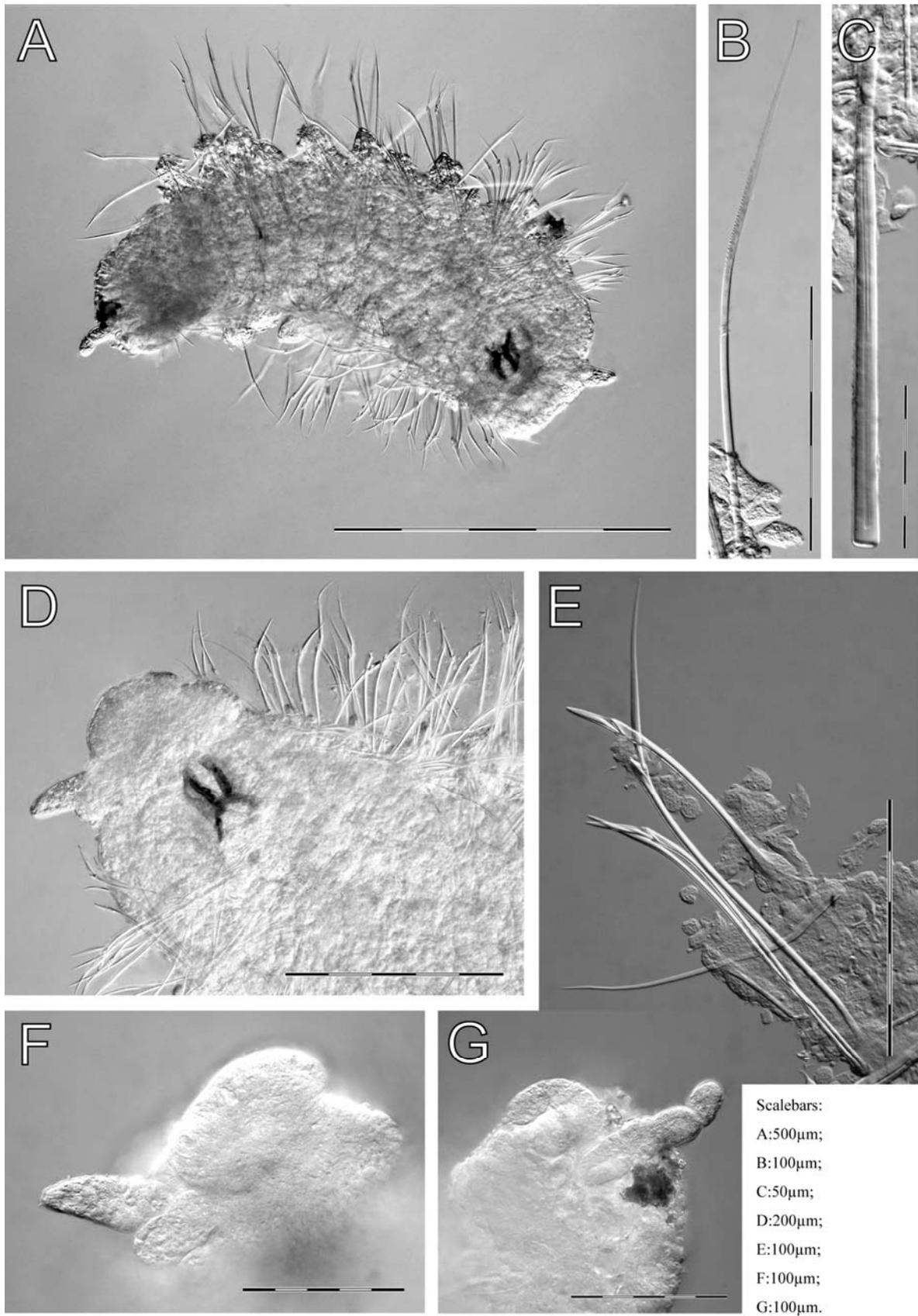


Figure 2. Legends on next page.

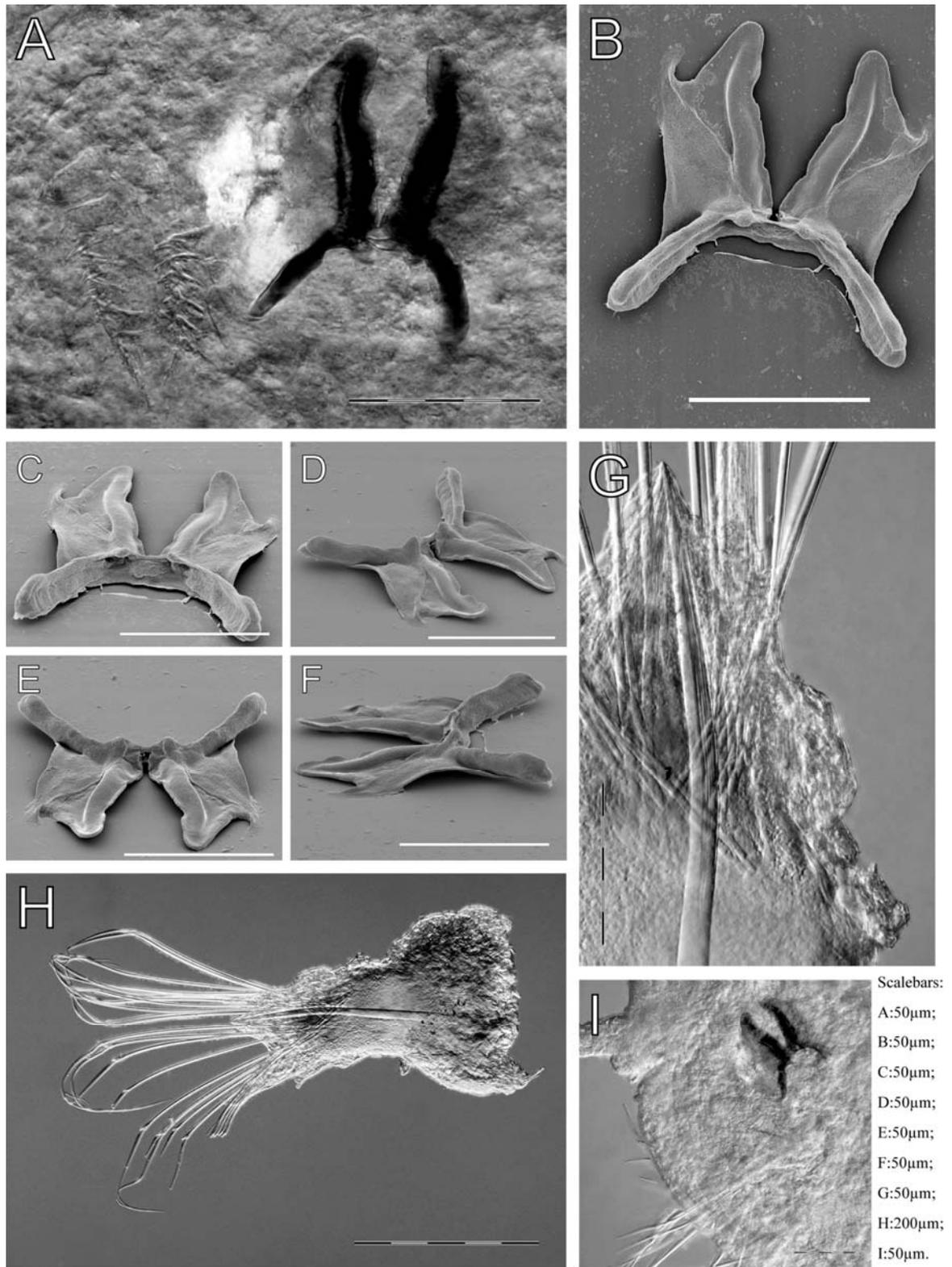


Figure 3. Legends on next page.

Figure 2. *Ophryotrocha* sp: **A.** Habitus, ventral view. **B.** Serrated capillary seta. **C.** Neuro-acicula. **D.** Anterior end with antenna, ventral view. **E.** Falcigerous and spinigerous compound setae. **F.** Prostomium and antenna. **G.** Pygidium with anal cirri and medial pygidial stylus. All images recorded from individual specimen (collected on 20.Aug 2006 at Ippik and fixed in 2% glutaraldehyde) with Olympus BX-51 microscope with Nomarski, and stacked in CombineZM. **Figure 3.** *Ophryotrocha* sp: **A.** Squash mount of mandibles and maxillary apparatus. **B.** SEM overview of mandible. **C,D,E & F.** Mandible from different angles. **G.** Acicula and setal bases of posterior neuropodium. **H.** Posterior parapodium. **I.** Peristomium, ventral view. Images A&I from individual used in Fig1. Images B-F from individual in same sample, (treated with pancreatin). Images G&H recorded from individual (collected on 24.Aug 2006 at Ippik and fixed in 99 % ethanol) with Olympus BX-51 microscope with Nomarski, and stacked in CombineZM.

Fig. 3A and B-F also show the differences in viewing mandibles in situ and when exposed for viewing by SEM. The mandibles belong to two different individuals. As can be seen, much of the mandibular detailing is satisfactorily viewed in LM, but the maxilla plates would have gained greatly by viewing in SEM. Image stacking was used in Fig. 2A,B,C,D,E,F,G; Fig. 3A,G,H,I; Fig. 4A,D. utilizing image stacks of between 2 (e.g. 2C) and 10 (e.g. 2A) single photos.

3.2. Lumbrinerid findings

Fig.4 shows two polychaetes that during the field course were mistaken for dorvilleids, but closer examination revealed a type of chaetae not found in Dorvilleidae. These are called hooded-hook setae (Fig. 4D, F) and are setae with a distally curved tip (can be either a single tip, falcate, or have few to many teeth, dentate [MERZ & WOODIN, 2006]) that, as the name implies, is enclosed in a hood (of the same β -chitin as the seta itself). The opening (Fig. 4F) of the hood is, typically for Lumbrineridae, formed as a slit in the apical end [HAUSER, 2005]. In Fig. 4D (right), one can make out how there is a structure inside the hood. Tentatively the two specimens seem to be juveniles of *Lumbrineris* cf. *minuta* Théel, 1879 which is a circumpolar species, recorded from Hudson Bay [ATKINSON & WACASEY, 1989], Long Straight [GAGAEV, 1994] in Scandinavian waters and the North Sea [HANSSON, 1998] and Southern Davis Strait and Ungava Bay [STEWART *et al.*, 1985], as well as noted from the west coast of Greenland (although not north of 70°N [WESENBERG-LUND, 1950], However *L. minuta* is difficult to distinguish from *Lumbrineris tenuis* Verrill, 1873 when dealing with small specimens [CURTIS, 1977].

3.3. Capitellid findings

Some 20 specimens of *Capitella capitata* (quite small (~10 mm)) were found during the fieldwork, and approximately half were fixed in formalin and ethanol respectively for possible use as morphological and molecular vouchers. As *C. capitata* was the earliest described species of what in the past decades has been understood to be a species complex, with regard to adult morphology, it is unlucky that the type specimens were apparently lost a long time ago. Many of the *Capitella* sp. has been found to be differing in several other features, such as life-history traits [TSUTSUMI & KAKUCHI, 1984]. The specimens collected in this study have been handed on for further work, as there is considerable international interest in obtaining *C. capitata* from a location close to the original type locality of Frederikshaab/Paamiut and possibly designating them as neotypes for the species. The original description states the type locality to be in littoral sand and under stones, but also mentions *C. capitata* living under the sandy bottom, with spiral mounds being pushed up from the burrows [Fabricius, 1780].

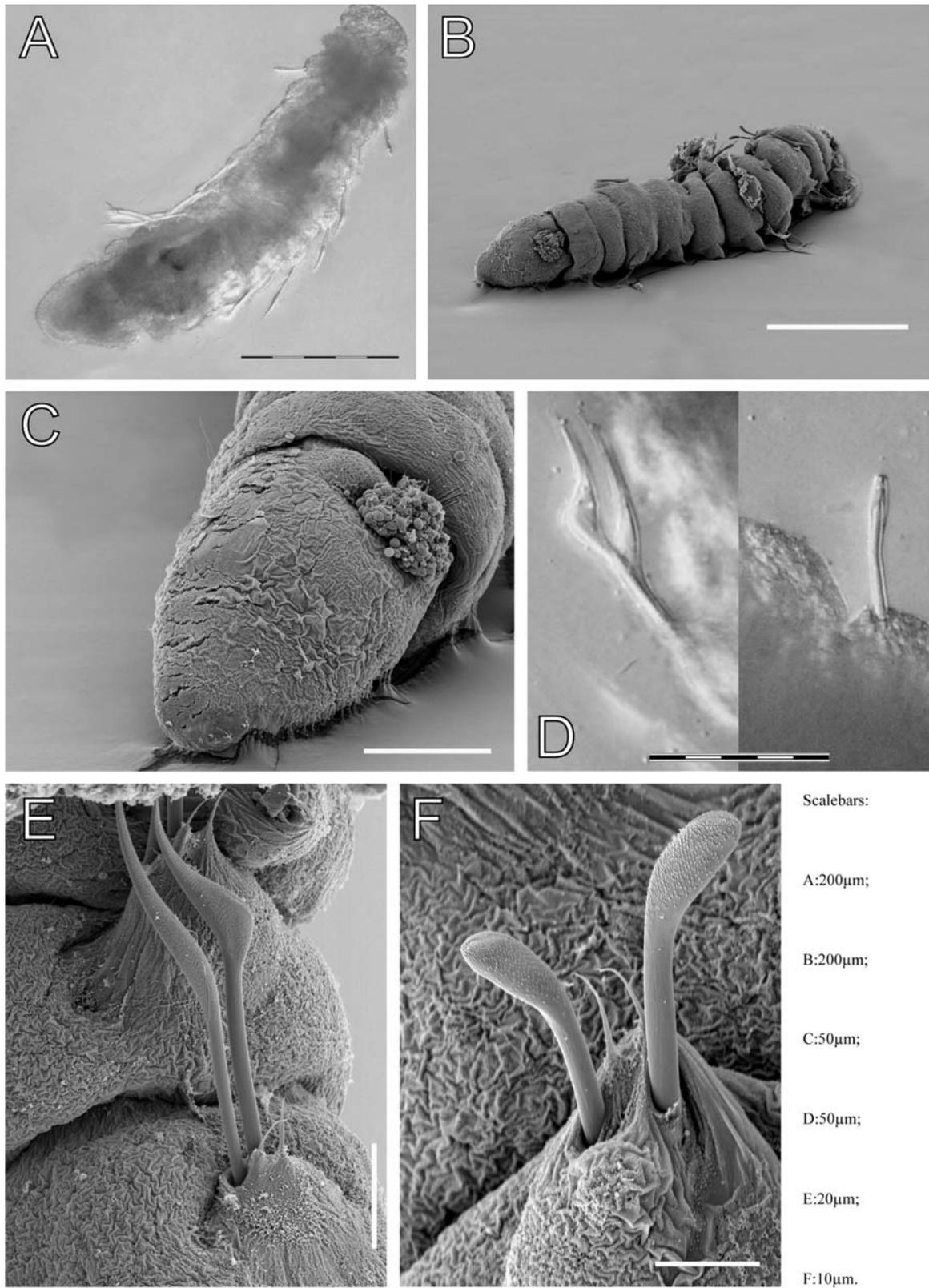


Figure 4. *Lumbrineris* cf. *minuta*: A: Habitus, LM; B: Habitus, SEM; C: Prostomium, SEM; D: Geniculate and hooded-hook setae, LM; E: Geniculate setae, SEM; F: Hooded-hook setae;SEM. Images A&D recorded from individual (collected on 24.Aug 2006 at Ippik and fixated in 99 % ethanol) with Olympus BX-51 microscope with Nomarski, and stacked in CombineZM. Images B,D,E,F collected on 20.Aug 2006 at Ippik and fixed in 4% formalin.

The type locality is approx. 1000 km south of Disko Island. However, the 0.5-1 meter deep inlet at Nipisat that the samples from this study were taken from, is nevertheless an option as a new type locality, as it contained organically enriched sandy sediment, not mud as was the case with Tsutsumi and Kakuchi in 1984, or the sublittoral 3-12 meters depth as the recordings from Godhavn harbour by Curtis in 1979.

3.3. Psammodrilid and diurodrilid findings

Psammodrilus aedificator was recorded again, for the first time since the original discovery in 1978 [KRISTENSEN & NØRREVANG, 1982]. Approximately 10 individuals were found, including two juveniles with intact anal cirri, in a sample from 2.5 meters depth off the type locality of Itersla/Skansen on the south coast of Disko Island. The present sample depth was very close to the 3 meters depth originally cited [KRISTENSEN & NØRREVANG, 1982]. The failures previously to recover the species from the type locality could be caused by not taking the local tide into consideration when measuring the depth. A previous Arctic Station study of meiofaunal species at Itersla recorded a tidal amplitude, at nearby Flakkerhuk, of 2.4 meters [SØRENSEN, 1998]. This could be compounded by the fact that bottom composition and grain size, through gradient sorting, was found to change during the course of a transect from the beach out to a depth of approx. 10 meters. *Diurodrilus westheidei*, which was originally found close to the same location, but through a “sarfarssuk” (a polynya) in the middle of the arctic winter [KRISTENSEN & NIILONEN, 1982], was also found in the sample. As the findings were made on the evening prior to the termination of the field course, no further sampling could be performed under the present course. The GPS position of the successful sample site has been noted for future investigation (see Table 1), and hopefully it will be investigated repeatedly in the future.

4. DISCUSSION

Previous studies of the dorvilleid ctenognath jaw apparatus have been conducted in detail for some taxa, but the jaws are most often viewed by LM in situ through either KOH cleared specimens or squash preparations [PAXTON, 2004]. For large numbers of macrofaunal specimens, dissection and mounting of the pharyngeal apparatus in diluted glycerine is a practical option [PAXTON, 2005], but it rapidly becomes impossible with diminishing specimen size. In this study image stacking was found to be a very effective image enhancing operation that facilitated non-destructive imaging work on jaw elements and other morphological details of the small specimens. Through use of quite simple qualitative image stacks, one could overcome many of the problems of depth-of-field limitation at the high magnifications needed for these animals. Never the less, in order to get the best view of the maxillary details, one needs to do SEM studies of the exposed jaw elements. Several different approaches are possible in order to get SEM images of the jaw elements, but many of the methods have draw-backs. In many other jaw-bearing polychaetes the growth of the maxillary apparatus is undertaken by distal division of maxillary teeth. In Dorvilleidae, however, the entire maxillary apparatus is periodically replaced [PAXTON, 1980], which means that recovering shed maxilla is theoretically possible. Unfortunately, it is very tricky to find the expelled maxillae in the holding tanks, and the method does not yield any mandibles. Another method to get the maxillary apparatus out is to make jaw preparations from dead and decomposed animals [PAXTON & AKESSON, 2007] as the jaw elements are easier to locate if still inside the cuticular envelope. These two methods obviously require specimens that are not yet fixed, and must therefore be done in the field laboratory or from cultured populations. When the collected material is scarce and cultures are not possible, more proactive methods

are needed to extract the jaws. The used approach involving partial extraction of the jaw apparatus (through decapitation) and cleansing with combined soft tissue digesting enzymes and low concentrations of sodium hypochlorite was found to yield consistent results. Yet, a problem with sodium hypochlorite was found to be the digestion of ligaments holding the maxillary plates together. When this happened the individual plates disassociated and were practically impossible to locate again. Also the numbering and placement of the plates in the maxillae holds much information that is easily lost when not in the original configuration. Accordingly, very low concentrations of sodium hypochlorite were required in order to avoid disassociation problems. Pancreatin was found to be very benign to the sample and easy to work with, but it carries some restrictions to the prior fixation method used, as *e.g.* formaldehyde and glutaraldehyde cross-link the proteins making it impossible for the enzymes to break down the soft tissues. The jaw-extraction processes are unfortunately relatively destructive methods that leave no other head material than the cuticula and the jaws. This means the destruction of the many morphological features of the prostomium and peristomium, but the decapitated body is usually left for molecular work. In some of the high-magnification SEM images of jaws, from cultured specimens, exposed with this method, small holes were observed in the surface. In order to rule out that these were artifacts caused by the digestion by pancreatin and sodium hypochlorite a trial was carried out. Jaws that had been cleaned by ciliates were compared to pancreatin and sodium hypochlorite cleaned samples, but no difference was visible and the holes seem to be part of a natural pore structure of the hollow jaw elements.

5. CONCLUSION

The inability to determine a nominal species for the *Ophryotrocha* sp. could well be due to its being a new species. The scarcity of material has not made it possible to propose such, but future investigations with more specimens should hopefully shed light over the taxonomic status of the species. Much of the preliminary work had to be carried out on dorvilleid species kept in culture, in order to build up a functional method without destroying valuable samples from Disko Island. As only species from shallow, temperate or tropical waters, have been successfully kept in culture [DAHLGREN *et al.*, 2001] the specimens used for some aspects of method development on, have had no direct relation to Disko. It could be interesting however to see if cultures could possibly be set up for the first coldwater species.

The more promising dorvilleid localities were sampled late in the field trip reducing available time for sorting. However as dorvilleid benthic distributions can be notoriously patchy, finding large numbers would have required hitting just the right place, and not using valuable time looking through unproductive samples. In the future one could try to conduct many more samplings and use ultracentrifugation technique in order to determine which samples could yield the wanted numbers.

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Baleen Whales in Disko Bay, Western Greenland

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Abstract. Whales, dolphins and porpoises are divided into two suborders, Odontoceti and Mysticeti, and species from both of them can be found in the Disko Bay area. Together with Sirenians, Cetaceans are best adapted into the aquatic lifestyle. During the Arctic Biology course in Qeqertarsuaq 2006 three species of Cetaceans were observed, which were fin whale *Balaenopeta physalus*, minke whale *Balaenoptera acutorostrata* and humpback whale *Megaptera novaeangliae*.

Keywords. Baleen whales, Qeqertarsuaq

1. INTRODUCTION

Order Cetacea, which includes whales, dolphins and porpoises, is divided into two suborders: Odontoceti and Mysticeti. Suborder Odontoceti comprises of 76 toothed whale species while the suborder Mysticeti includes 11 baleen whale species. All together 6 toothed whale species and 6 baleen whale species can be found in Disko Bay. These are beluga whale *Delphinapterus leucas*, narwhal *Monodon monoceros*, harbour porpoise *Phocoena phocoena*, killer whale *Orcinus orca*, long-finned pilot whale *Globicephala melas* and northern bottlenose whale *Hyperodon ampullatus*. Blue whale *Balaenoptera musculus*, fin whale *Balaenoptera physalus*, sei whale *Balaenoptera borealis*, minke whale *Balaenoptera acutorostrata* and humpback whale *Megaptera novaeangliae* belong to the family Balaenopteridae and bowhead whale *Balaena mysticetus* is a member of the family Balaenidae. Only bowhead whale, beluga whale and narwhal can be regarded as true arctic species since they remain in the arctic region throughout the year following the retreating and expanding ice edge [MOORE & REEVES, 1993; HEIDE-JØRGENSEN *et al.*, 2003; HEIDE-JØRGENSEN *et al.*, 2006].

Disko Bay was an important area for the commercial whaling of the great whales in the 19th century [ESCHRICHT & REINHARDT, 1861]. At that time bowhead whale was one of the preferred species but also blue whales, fin whales, humpback whales and later minke whales were harvested. Especially bowhead whale suffered greatly from the whaling and the numbers in Disko Bay reduced dramatically. Blue whale is still a very infrequent guest in Disko Bay [KALE MØLGAARD, KNAPK, pers. comm.] but the population numbers of the other species have increased. Humpback whales are numerous although no estimates of the current population size in Western Greenland exist. Minke whale and fin whales population numbers have increased sufficiently to sustain a moderate harvest again.

During the Arctic Biology course in Qeqertarsuaq 2006 three species of Cetaceans were observed which were fin whale, minke whale and humpback whale.

2. DIVING PHYSIOLOGY OF CETACEANS

Cetaceans are together with Sirenians the most adapted mammals to the aquatic world. They spend the majority of the time submerged and are capable of even sleeping under water [GNONE *et al.*, 2001]. The changes in ambient conditions with increasing depth such as increasing pressure, decreasing temperature and decreasing solar radiation set limits to the diving capacity of animals [KOOYMAN, 1989]. However, the ultimate limiting factor of the actual dive duration for marine mammals is oxygen. The basic concept in diving physiology is

the theoretic aerobic diving limit (TADL) which defines the longest possible period of time which the animal can spent submerged and during which lactate acid does not yet accumulate into the blood. Diving within the TADL limits is regarded to be efficient use of time and energy resources. However, the TADL is not necessarily determining the actual dive duration. Foraging blue and fin whales have been observed to exhibit frequent dives lasting less than the calculated TADL and the body size would give reason to expect [SCHEER & KOVACS, 1997; CROLL *et al.*, 2001] and it has been suggested that the costs of the feeding method add up tremendously to the consumption of energy during a dive [CROLL *et al.*, 2001]. Another reason for the short dives can simply be the patchy distribution of prey.

According to Boyle's law an air space is reduced to one half of its original volume every ten meters of descend resulting in doubling of the air pressure within the air sac. Adaptations to these laws of physics can be seen in the morphology, physiology and behaviour of marine mammals. All marine mammals empty their lungs prior to the dive which would collapse anyway while descending into depths [KOOYMAN, 1989]. The oxygen is stored primarily in the blood and secondarily in the muscle tissue [ELSNER, 1969; KOOYMAN, 1989]. The volume of blood in diving mammals is superior to that of the non-diving animals [ELSNER, 1969; ZAPOL, 1987; KOOYMAN, 1989]. In addition the diving mammals possess higher blood oxygen capacities than the terrestrial mammals [ELSNER, 1969]. Remarkable changes in the body function of marine mammals take place during a dive [LENFANT, 1969; ELSNER, 1969; KOOYMAN, 1989]. The heart rate decreases to half of that at the surface [ELSNER, 1969; KOOYMAN, 1989]. The blood circulation is restricted and directed to the sensitive organs such as heart and brains [ELSNER, 1969; KOOYMAN, 1989]. Muscles in general are cut off from the circulation and depend on the oxygen stored in myoglobin [KOOYMAN, 1989].

3. FEEDING METHODS OF BALEEN WHALES

Baleen whales do not possess any teeth but a row of baleen plates, which projects from the outer edges of the roof of the mouth forming a dense sieve [BERTA & SUMICH, 1999]. The baleen plates are made out of keratin-like substance resembling nails and hair of other mammals. In the inner edge of each plate there is a fringe of thin threads which make the sieve even harder to pass. The number, length and shape of the baleen plates as well of the length and density of the threads vary from species to species. Baleen whales feed mainly on planktonic and micronectonic crustaceans and small pelagic fish.

Baleen whales use three different methods for prey capture: engulfment, skimming and mud scooping [BERTA & SUMICH, 1999]. The species of the family Balaenopteridae are streamlined and fast swimmers. They are all characterised by an extremely large mouth and grooves in the ventral side of the body known as the throat grooves, which enable the mouth cavity to stretch even bigger in size. Their baleens are relatively short and generally low in number. These species utilise engulfment where they capture a large volume of water into their mouth cavity by movements of the tongue and the forward swimming motion of the animal. The prey, krill or fish, are trapped inside the mouth and get entangled to the threads of the baleen plates when the whale forces the water out through the baleens using the muscles in the mouth and especially its highly elastic and muscular tongue. The tongue is further used to scrape off the prey from the baleens and to assist in swallowing. The rorqual whales using engulfment predominantly feed alone and if in groups do not exhibit cooperative feeding. However, the humpback whale, member of the same family, is a clear exception. These whales use a very special cooperative technique called the bubble net feeding where they herd the fish into tight schools by releasing streams of air bubbles around the fish. When the

school of fish is near the surface the whales rush upwards from the depths with their mouths open and capture the prey by using engulfment method.

The slower and more robust build right whales from the family Balaenidae are characterised by arched upper jaw and extremely long and numerous baleen plates [BERTA & SUMICH, 1999]. Instead of capturing large body of water into their mouth they skim through the water column at a constant depth with relatively constant speed with their mouths open. Due to the high number of baleen plates the sieve they create is very dense and allows the whale to capture smaller particles than the rorqual whales and make this feeding method extremely efficient.

Grey whale is the only species, which exhibits mud scooping as foraging method [BERTA & SUMICH, 1999]. It has the shortest baleens of all the Mysticetes and only three to five throat grooves are present. Grey whales feed primarily on bottom invertebrates, especially small amphipod crustaceans, which it sucks into its mouth from the other side and expels the mud and water from the other side. However, there is some evidence that also bowhead whales forage at the bottom but the method they use is unclear [LOWRY, 1993]. Bowhead whales are not thought to feed on benthic fauna like the grey whales but filter pelagic fauna which lies just above the bottom. Grey whale and bowhead whale are the opposites of each other when it comes to baleens – grey whales possess the shortest and coarsest baleen plates while the ones of bowhead are the longest and finest in structure [BERTA & SUMICH, 1999].

4. DESCRIPTIONS OF OBSERVED SPECIES DURING THE ARCTIC BIOLOGY COURSE 2006

Fin whale *Balaenoptera physalus*

Fin whale is a member of the family Balaenopteridae [GAMBELL, 1985]. There has been discussion of the existence of subspecies but no firm proofs have been able to demonstrate.

Fin whale is the second largest of the whales exceeded in size only by the blue whale [GAMBELL, 1985]. They reach typically 22 – 27 m in length with females and the specimen in Southern Hemisphere being bigger. Fin whales have 50 – 100 ventral grooves. The head occupies 20-25 % of the body length and is triangular in dorsal view equipped with a single crest in the middle. The lower jaw is strongly convex laterally and protrudes 10-20 cm beyond the tip of the rostrum when the mouth is closed. The body is streamlined in shape but somewhat fuller than that of the blue whale. The fluke is broad and has a notch in the middle. The relatively large dorsal fin forms an angle of $<40^\circ$ with the back and is situated two thirds of the way along the back. Fin whales are dark grey in colour above grading gradually into white. The fluke and the flippers have also a white ventral side. The head has strikingly asymmetrical coloration, which extends to the baleens as well. The right side of the lower jaw is black with dark greyish or black baleens but the left side of the lower jaw is white with white or yellow baleens. Each side of the upper jaw hosts 260-480 baleen plates.

Fin whales feed mainly on planktonic crustaceans such as Euphasiids but utilise also fish and cephalopods [KAWAMURA, 1980; GAMBELL, 1985]. There is considerable variation in the preferred prey items in different areas and seasons [GAMBELL, 1985]. Fin whales are known to dive down to 128 m in search of prey [CROLL *et al.*, 2001]. Deep dives reaching 470 m have also been reported from the fin whales inhabiting the Ligurian Sea [PANIGADA *et al.*, 1999].

Minke whale *Balaenoptera acutorostrata*

Minke whale is the smallest of the species in the family Balaenopteridae [STEWART & LEATHERWOOD, 1985]. A lot of discussion has been carried on about the existence of

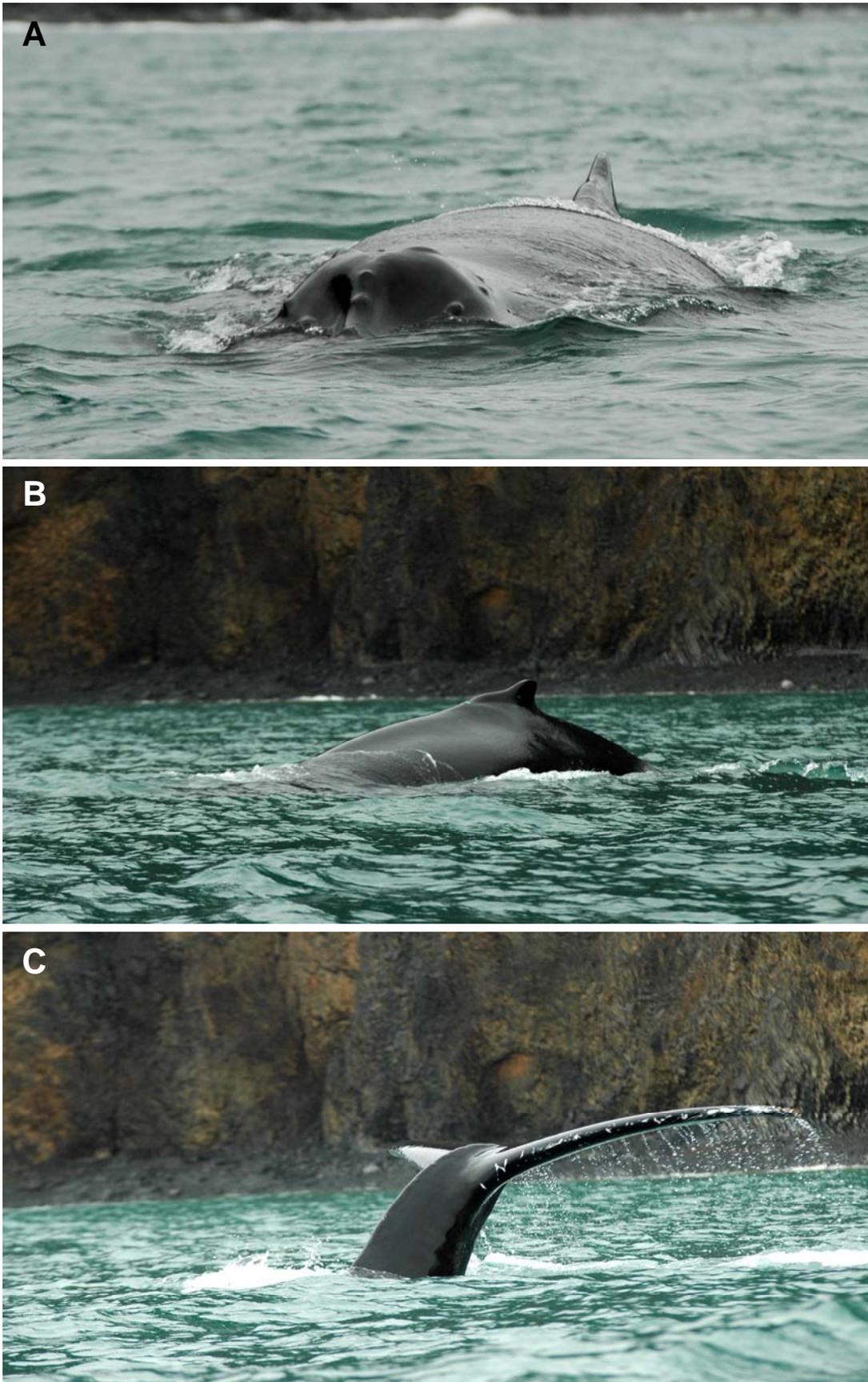


Figure 1. Diving series of a Humpback whale *Megaptera novaeangliae* (photos: Martin O. Macnaughton).

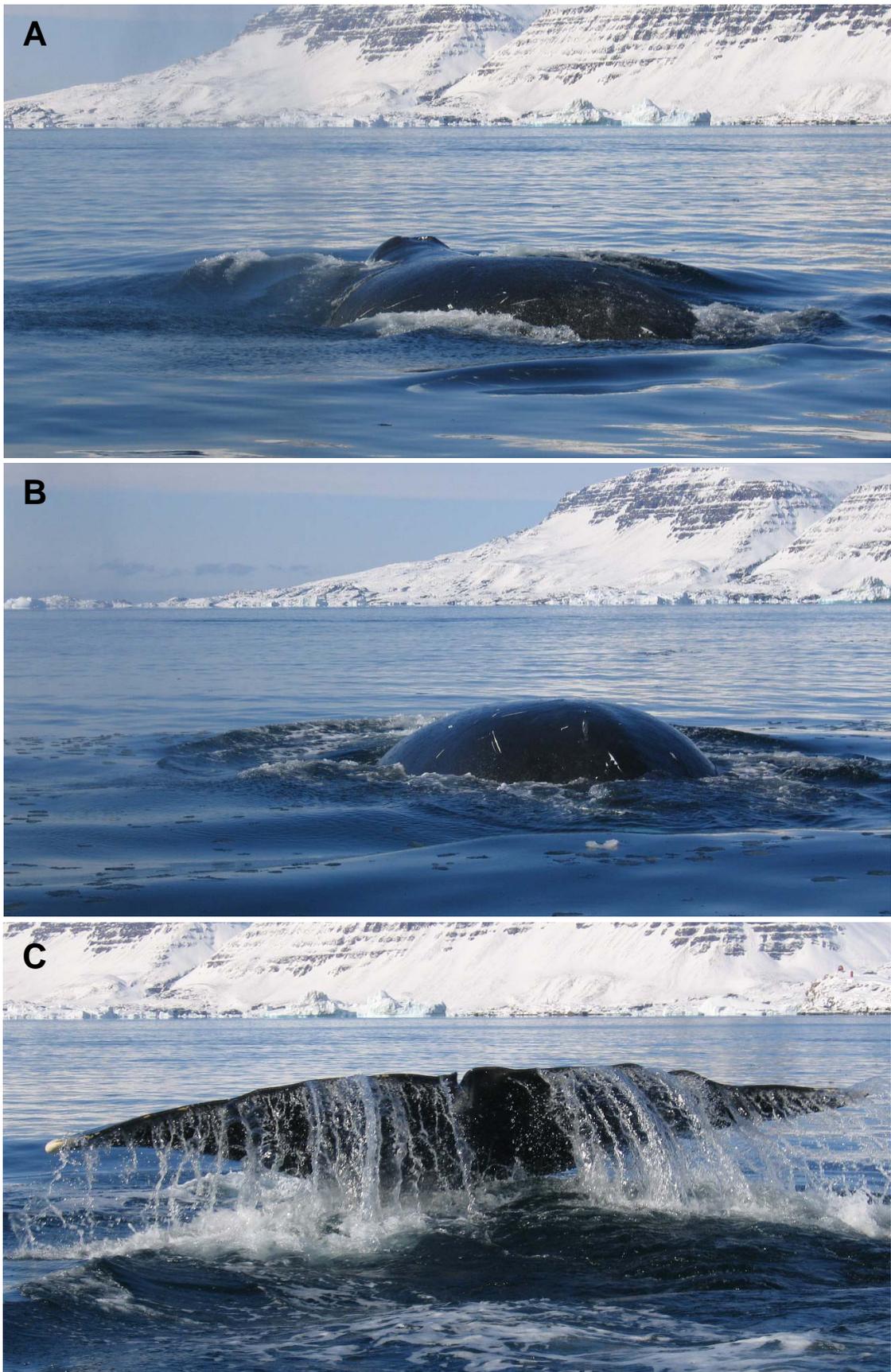


Figure 2. Diving series of a Bowhead whale *Balaena mysticetus*. (Photos: John D. Jacobsen).

subspecies. Despite of a considerable amount of individuals taken in the harvest every year in different parts of the world the question of specific, subspecific, racial and stock differences in minke whale remains unanswered.

The length of minke whales seldom exceeds 10 m and is typically 6 – 8 m [STEWART & LEATHERWOOD, 1985]. The weight varies between 2 – 2.7 t. The rostrum is tremendously pointed and narrow with a single ridge in the middle. The dorsal fin is relatively tall and located in the posterior end of the body. The fins are small, pointed and characterised by a white band on the dorsal side. The dorsal side of the animal is usually darker in colour than the ventral side. The coloration of the body and baleens and the number of baleen plates has a high degree of variability among regions as well as individuals.

The diet of minke whales is the most variable of all the baleen whales and differs greatly between areas [STEWART & LEATHERWOOD, 1985]. They have been reported to feed on Euphasiids, copepods and on several species of fish [KAWAMURA, 1980; STEWART & LEATHERWOOD, 1985].

Humpback whale *Megaptera novaeangliae*

Humpback whale (see Fig 1) is a member of the Balaenopteridae family but due to several anatomical differences it is placed in a separate genus *Megaptera* “long winged” [WINN & REICHLEY, 1985]. Although variation in colour pattern, size and other morphological features occurs a single species is accepted.

The body of the humpback whale is stouter and shorter than that of the other members of the family Balaenopteridae [WINN & REICHLEY, 1985]. They reach about 18 m in length and weigh approximately 30 t. The head is rounder in shape and characterised by smooth subcutaneous knobs that are placed on the dorsal side of the snout, chin and mandibles. On the rostrum the knobs are arranged in three lines. The coloration on the body ranges from totally black to black with white markings. The belly of some individuals can be completely white. The baleen plates are typically blackish brown or grey and number 270-400 on each side of the jaw. The longest baleen measured was 204 cm long.

The most unique features of the humpback whale are the shape of the flippers and flukes [WINN & REICHLEY, 1985]. The flippers are extremely long and narrow equalling 23-31 % of the total body length. The inner surface is usually white while the outer surface ranges from black to dark grey with white markings. The fluke is characteristic for each individual and is been used in identification. The fluke has a heart shape with a fringed outer margin. The coloration patterns on the ventral side vary while the dorsal side is usually dark in colour.

Humpback whales feed on a variety of prey items including euphasiids and a different fish species [KAWAMURA, 1980]. Humpback whales typically dive to depths of 60 – 120 m in search of prey [DOLPHIN, 1987] but deeper dives extending down to 240 m have also been reported [HAMILTON *et al.*, 1997].

5. BOWHEAD WHALE *Balaena mysticetus*

Research on baleen whales in Disko Bay has concentrated on the ecology and behaviour of bowhead whales (see Fig 2). Topics of interest have included feeding and diving behaviour of bowhead whales [LAIDRE *et al.*, in press], area usage and migration patterns of bowhead whales [HEIDE-JØRGENSEN *et al.*, 2003; HEIDE-JØRGENSEN *et al.*, 2006] and vocal behaviour of bowhead whales [TERVO, 2006].

Bowhead whale females can reach 20 m in length but lengths of 16 – 18 m are more common [NERINI *et al.*, 1984]. The males are smaller like in all the baleen whale species. The



Figure 3. Seasonal distribution of bowheads in Davis Strait and Hudson Bay. Cross hatched areas illustrate the summer distribution and the hatched area winter distribution [MOORE & REEVES, 1993].

weight of an entire specimen has never been managed to measure but it has been estimated that bowhead whales can weigh 70-100 t [REEVES & LEATHERWOOD, 1985]. Bowhead whales are dark in colour ranging from dark grey to black except for a white patch under the chin. The head is proportionally larger than in other baleen whales and the upper jaw is typically arched. The width of the fluke may reach two-fifths of the body length and it is smooth on the margins and has a deep notch in the middle. Unlike the other right whales the skin of the bowhead whale is clear from barnacles and other external parasites. Bowhead whales do not possess ventral grooves.

5.1. Bowhead whale movements in the Davis Strait

Bowhead whales are found from the north western part of Hudson Bay from mid-May to mid-September (Fig. 4) [MOORE & REEVES, 1993]. There are some observations that suggest that bowhead whales would be present in the area throughout the year [MCLAREN & DAVIS, 1982]. Bowhead whales in the Davis Strait are generally divided into Davis Strait and Hudson Bay stock but individuals from the both stocks are likely to use same wintering grounds in Hudson Strait [HEIDE-JØRGENSEN *et al.*, 2006].

Davis Strait and the northern parts of the Labrador Sea are important wintering areas for bowhead whales [RICHARDSON & FINLEY, 1989]. The northernmost point for winter distribution is thought to be Disko Bay [MOORE & REEVES, 1993]. Bowhead whales arrive to the vicinity of Qeqertarsuaq in mid-January to early February and departure in early May to early June [ESCHRICHT & REINHARDT, 1861; HEIDE-JØRGENSEN *et al.*, 2003; HEIDE-JØRGENSEN *et al.*, 2006] after which they head across Baffin Bay to Lancaster Sound and to the surrounding areas (Fig. 3) [HEIDE-JØRGENSEN *et al.*, 2006]. Approximately 250 bowhead whales occupy West Greenland between the months of March and May [HEIDE-JØRGENSEN & ACQUARONE, 2002]. The other possible route takes them up north through Smith Sound into Kane Basin [MOORE & REEVES, 1993]. The summer distribution extends from the Canadian

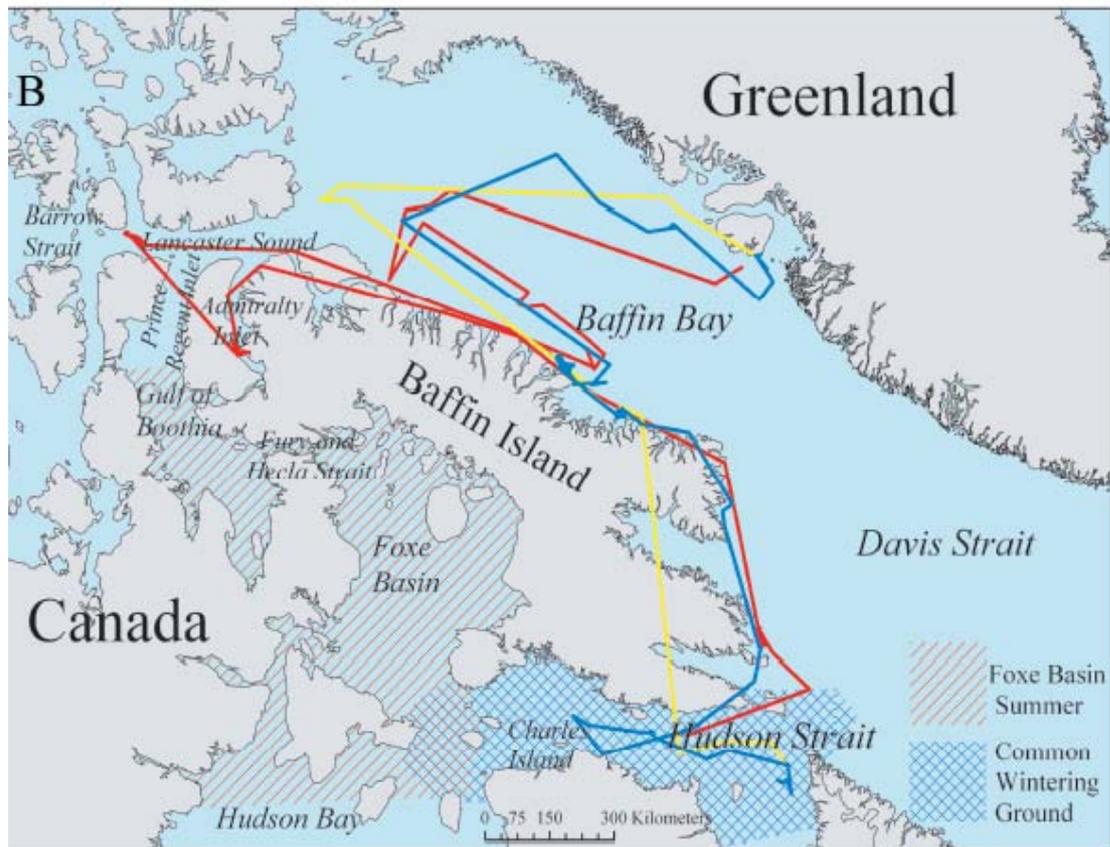


Figure 4. Tracks of three bowhead whales tagged in Disko Bay in May 2002 and 2003 [HEIDE-JØRGENSEN *et al.*, 2006].

High Arctic Archipelago southwards off the north eastern coast of Baffin Island. An autumn migration southwards along the coast of Baffin Island takes place in late September extending to October [MOORE & REEVES, 1993; HEIDE-JØRGENSEN *et al.*, 2006]. The population inhabiting Davis Strait and Hudson Bay is thought to be separated from the Bering Sea stock by heavy ice in Viscount Melville Sound and in adjacent areas [REEVES & MITCHELL, 1985].

5.2. Acoustic behaviour of bowhead whales

Bowhead whale acoustic vocalisation has been recorded from the Bering Sea population in May [LJUNGBALD *et al.*, 1982; CLARK & JOHNSON, 1984; LJUNGBLAD *et al.*, 1984; CUMMINGS & HOLLIDAY, 1987] and in September and October [LJUNGBLAD *et al.*, 1982] and from the Davis Strait population in Isabella Bay in August and September [RICHARDSON & FINLEY, 1989] and in Disko Bay from February to May [TERVO, 2006].

Simple calls have not been assigned to any particular behaviour [LJUNGBLAD *et al.*, 1984] but they, particularly simple calls with ascending and descending frequency referred often as up and down calls [RICHARDSON & FINLEY, 1989] have been recorded in the presence of socially and sometimes sexually active whales [LJUNGBLAD *et al.*, 1984; RICHARDSON & FINLEY, 1989]. Low complex AM calls with minimum frequencies starting from 25 Hz have been recorded from socially active bowhead whales in Isabella Bay in August and September [RICHARDSON & FINLEY, 1989]. Complex calls have often recorded in the presence of mildly socialising (within a body length) or actively socialising (body contact) bowhead whales [LJUNGBLAD *et al.*, 1984; WÜRSIG *et al.*, 1984] and in the presence of sexually active whale

groups [RICHARDSON & FINLEY, 1989]. Complex calls were also characteristic emissions of groups engaging in homosexual activity [RICHARDSON & FINLEY, 1989].

Humpback whale song has been suggested to function as an advertisement display [TYACK & CLARK, 2000] and the same could be the case for bowhead whale song even if the sex of the singing individuals is unknown. Songs of bowhead whale have been recorded during April and May off Point Barrow during the spring migration but this particular singing is thought to be only a remnant from the winter breeding season and therefore fail to represent the entire richness of the singing repertoire [LJUNGBALD *et al.*, 1982; LJUNGBLAD *et al.*, 1984; CLARK & JOHNSON, 1984].

The first recordings from bowhead whale winter repertoire were made in Disko Bay in February and March 2005 where a vast amount of song notes, simple FM calls and complex AM calls were present [TERVO, 2006]. Compared to April and May, February and March had a profoundly higher signalling activity of song notes, simple FM calls and complex AM calls (signals per minute), the repertoire of song notes was substantially broader and there were typically more than one animal vocalizing at the same time. These findings indicate that bowhead whales in Disko Bay engage more in sexual and social behaviour in February and March than later in the season in April and May making Disko Bay a potential mating area for the Davis Strait bowhead whales.

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Diary of the Arctic Biology Field Course 2006

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Friday August 11th

The air was thick with anticipation as a group of 12 students, three PhD.-students and supervisors met an early Friday morning at Kastrup Airport. The reason for this strange meeting was the bi-annual field course in Arctic biology, which was about to commence. Check-in progressed smoothly while everybody talked and laughed in nervous excitement, trying to get to know each other better. There was not much time; however, most took the opportunity to do a bit of shopping prior to take-off. Everyone was in high spirits as the flight Copenhagen, Denmark – Kangerlussuaq, Greenland took off.

We landed safely in Kangerlussuaq (Søndre Strømfjord) around noon local time. From here we needed a connecting flight to Ilulissat (Jakobshavn) however we had several hours until departure. In this period of time most went sight-seeing as even here, at the airport in Kangerlussuaq, the magnificence of the Greenlandic nature was evident. For most it was their first encounter with the beautiful but rough Arctic climate.

The flight with the small DASH-7 from Kangerlussuaq to Ilulissat offered incredible sights of Greenland from above (Figure 1A). Here endless rivers and lakes in every shade of blue snaked through the rocky landscape, while enormous amounts of ice covered everything. It was truly magnificent.

As we landed in Ilulissat, it was intended that we should fly directly with a hired helicopter (the large Sikorsky S-61N9) to Disko Island. However, two hikers had gone missing close to Ilulissat Isbræ, thus requiring the help of the helicopter in searching for them. This delayed our flight, why most of us went on a small hiking trip in the vicinity. Here, under the heavy influence of mosquitoes, we saw our first icebergs.

Finally our helicopter arrived. The flight over Disko Bay took only about a half hour; however, we had a great ride and a great view of Disko Island and Storbræen from the helicopter. The two other supervisors (Poul and Reinhardt) and Kjeld (Akaaraq) Emil Mølgaard (the technical leader) were at the heliport to pick us up, and to drive our equipment to Arctic Station. The walk to the station was very short because the heliport was located at Sorte Sand in the protected area (Fig 1B).

Saturday August 12th

The day started in misty conditions unfortunately postponing our scheduled hiking trip to Kuannit (Angelica-locality). Instead, time was spent establishing the respective laboratories in the individual groups, which among other things involved relocating a refrigerator and piecing together the different microscopes. Work progressed well until a large group of humpback whales was spotted in the bay outside the Arctic Station, thus putting an end to the collective working-effort. While some behaved rationally most scattered frantically in different directions in an attempt to get a closer look at these magnificent creatures. Loosing track of time in this more or less euphoric state the scheduled excursion was undertaken. Along the way we saw many warm springs (Fig. 2C), icebergs breaking off (Fig. 2A) and beautiful vegetation like the insectivorous plant, Common Butterwort (*Pinguicula vulgaris*), Arctic Cotton-grass (*Eriophorum scheuchzeri*; Fig. 6D), Narrow-leafed Labrador-tea



Figure 1. **A.** The view from aboard the DASH-7 from Kangerlussuaq to Ilulissat. **B.** the large Sikorsky S-61N9 at the landing pad near the Arctic Station. **C.** View of the Arctic Station from the helicopter landing pad. Photos: Martin O. Macnaughton.

(*Ledum palustre*), Dwarf Birch (*Betula nana*) and Viviparous Knotweed (*Polygonum viviparum*) just to name a few. Following lunch atop pillar basalt (Fig. 2B) overlooking the sea, an attempt was made to locate native species of orchids, Small White Orchid (*Leucorchis albida*) in the cold homothermic springs; however without any success. It was a fantastic but exhausting hiking trip taking more than three hours hereby leaving everyone in need of coffee. After dinner the projects were presented which was followed by cake (and in some cases a small nightcap).

Sunday August 13th

Today we started early, since the Macro-algae group (Mads, Christian and Helle) needed to sample at low tide from to “Udkiggen”. The water was the lowest at 7 a.m. and 7 p.m., and we

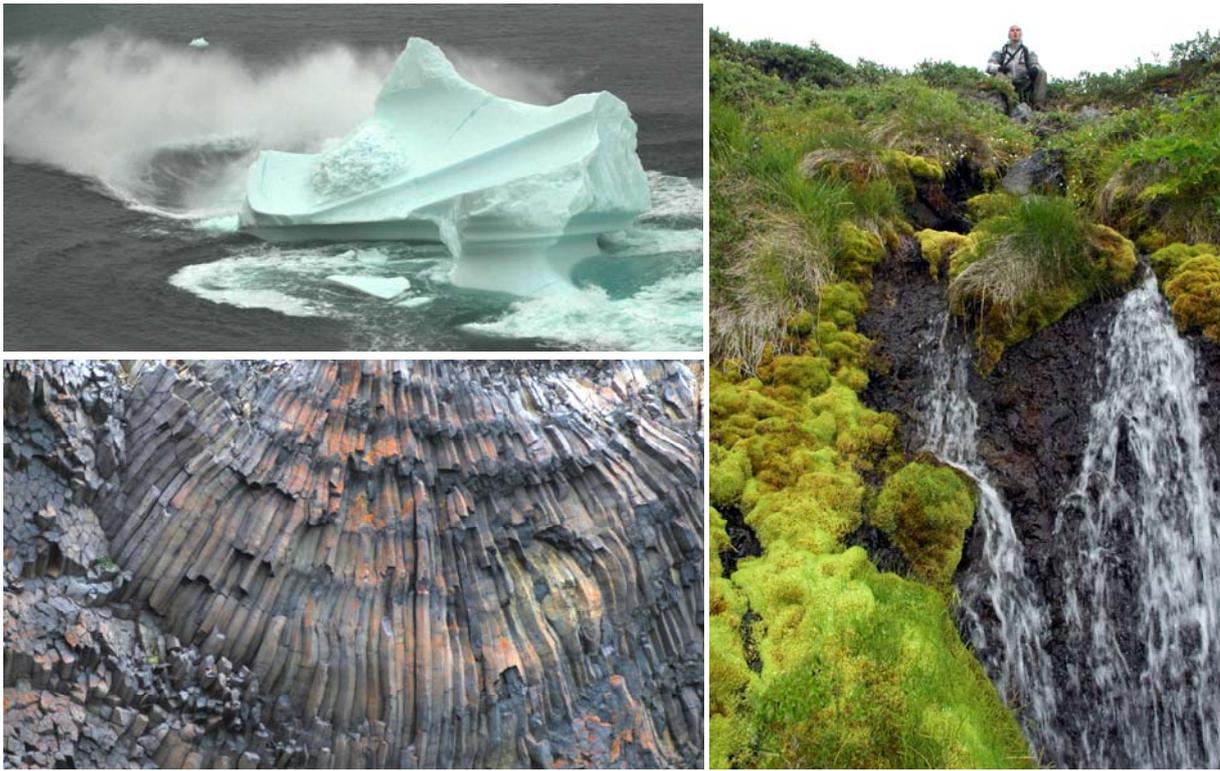


Figure 2. A. Iceberg breaking off just off the coast of Kuannit. B. Pillar basalt - a characteristic type of stone found near Kuannit. C. One of the numerous small warm springs with waterfalls near Kuannit. Photos: Martin O. Macnaughton.

wanted to be there at 9 a.m. Besides us, the phytoplankton group (Maria and Iben), the meiofauna group (Martin and Jonas) and the tardigrade group (Dennis and Kenneth) also went sampling, the last group in the hunt of the eutardigrade *Halobiotus crispae*, which had been found close to Udkiggen in 1990.

It was cold and a bit windy when we left the Arctic Station, but luckily it cleared up within an hour. This was the first real day of sampling and working on our projects. The sea was freezing cold, and after half an hour of sampling our fingers hurt -but what one doesn't do for science. Well at home again all groups searched their samples, excited to see if we had found anything out of the ordinary.

In the macro-algae group we found two different variants of *Elachista*, we were looking for. One *Elachista fucicola* var. *lubrica*, grew epiphytically on *Rhodomela*, and *E. fucicola* var. *fucicola* was found growing on a *Fucus*-species. This *E. fucicola* was morphologically similar to the first one, only with smaller unilocular sporangia and somewhat smaller cells.

Later in the afternoon people yelled whale (again) from the veranda and people went down to the water front to get a good "close-up" picture of the tale fin of a diving humpback whale (*Megaptera novaeanglia*) (Fig. 3C). Unfortunately, as we got down to the water the whales had all swum out again, but Martin and Jonas had reached the water before the rest of us and taken some pictures of them when they were near shore.

The dinner was musk-ox; a debut for the most of us. After dinner the groups presented their projects, but during the presentations whales were spotted again, which caused some disturbance. Following the last presentation we ran down to the water once more to get that one good shot of the whales. Yet again the whales were on their way out to sea, and short after the light disappeared as well. Although we were north of the Arctic circle in the middle of August, we could already feel that the days were getting shorter.

Monday August 14th

In calm weathers with sunshine and low wind, the plankton-studying groups were transported to the stationary plankton station by Porsild. The sampling procedures were observed by curious fulmars (*Fulmarus glacialis*), who also got a lot of attention themselves. Meanwhile, the macro-algae group was sampling at Udkiggen, and on their way there, they saw a large group of seals; or rather, the remains of them.

After returning to Arctic Station, the plankton-girls spent the rest of the day in the cooled laboratory, studying the fresh samples, while the macroalgae and meiofauna groups were preparing for their great expedition the following day.

Later in the evening, around the time of the spectacular macro-algae exhibition, a raven (*Corvus corax*) was observed just outside of the laboratory building. Until late in the evening, many humpback whales gathered in the bay and came so close that their white tail patterns were visible even to people standing on the porch.

Tuesday August 15th

Clouds hung over the bay as the plankton groups waved goodbye to the meiofauna and macro-algae groups, who took off for their expedition at sea. While the clouds were getting heavier and it began raining, the plankton investigators stayed inside the laboratories to analyze the samples from the previous day. There was a calm and cozy atmosphere at Arctic Station.

Excursion, Nipisat Bay

Three days with sun and warm weather had been replaced by rain and wind on the day when the first expedition at this years field course left Qeqertarsuaq/Godhavn, heading for Disko Fjord and Nipisat Bay - a voyage lasting approximately 4 hours by ship. Our goal was to collect tardigrades (*Halobiotus crispae*) at the type locality, polychaetes (Macro - and meiofauna in general), macro-algae and hopefully stomachs from the Arctic charr, *Salvelinus alpinus* (Fig. 3D).

In Godhavn, Søren, Erik and Fari (Frederik Grønvold), the Greenlandic captain and crew on Porsild, awaited us. As soon as our equipment was in the hold and everybody on board, we left Godhavn. The colourful houses of Godhavn were disappearing behind the rocks and we were on our way to Nipisat. The rain was still falling but the wind had weakened. On the starboard side the enormous black cliffs rose toward the sky. Waterfalls broke the massive black and tumbled towards the beach below. The icebergs suddenly seemed small, but then a massive white shinning wall passed nearby and the awe returned. After a while we met the first whale; a female fin whale (*Balaenoptera physalus*) with a calf. The fin whale is one of the true giants' of the ocean, reaching 24 m and a weight of nearly 80 ton. It is the second largest whale in the world. After a while the whales disappeared in the deep. The rest of the trip was quiet. The fulmars followed the boat and while passing the impressive Blåfjeld a pair of minke whales (*Balaenoptera acutostorostrata*) appeared nearby.

We arrived in Disko Ford after four hours of sailing. The camp was established on a beautiful position at the Nipisat Bay. To the north the fjord opened with mountains fainting in the low hanging clouds (Fig. 3B). On the other side of the camp the River Nipisat was cutting through the marsh and lowland. The different groups spread out in the terrain. The coast had to be searched for Tardigrada and Polychaeta substrate and barnacles. This substrate, typical sand and mud, was then to be sorted carefully back in the laboratory. Another group found the tadpole-shrimp, *Lepidurus arcticus* in a lake near the camp. The small but deep pond containing the *Lepidurus* appeared to be a dead-ice hole. The River Nipisat has a great stock of anadromous Arctic charr (*Salvelinus alpinus*) and the fish were in the river to spawn at this



Figure 3. A. Camp site at Mudderbugten. B. Mountains near Nipisat bay with the research vessel "Porsild" in the foreground. C. Diving Humpback Whale (*Megaptera novaeanglia*). D. Freshly caught Arctic charr (*Salvelinus alpinus*) from Nipisat bay. Photos: Martin O. Macnaughton.

time of year. Soon the first fish were caught; beautiful specimens at approximately 60 cm and 2.5 kg and the river seemed to be full of them.

The macro-algae-group returned to Porsild. The fjord had to be investigated with a triangle sledge. The result was disappointing, the sledge was full of sea urchins (*e.g. Strongylocentrotus droebachiensis*) an animal with a bad reputation among macro-algae people. The next attempt was on more shallow water and it turned out to be much better; great examples of *Laminaria* and *Alaria* appeared in the sledge. Nearly three meters high and still those plants are not as interesting as the small plants among the hapters. The samples were packed in plastic buckets ready to bring home, where more sophisticated equipment allowed for closer examination.

Wednesday August 16th

The phytoplankton group went to the harbour for fresh samples, and on their way they visited the local museum. Among others, the museum was featuring aquarelle paintings by the Greenlandic artist Jacob Danielsen, born in Disko Fjord, depicting traditional Greenlandic hunting methods.



Figure 4. A. Soccer team “Arctic Station” B. Soccer team Qeqertarsuaq C. Arctic bathing by Dennis Persson following the intense soccer match. Photos: Martin O. Macnaughton.

Unfortunately, the samples from the harbour were of poor quality, so two thirds of the phytoplankton researchers convinced Kjeld (Akaraaq) to take them out in his top-modern speed boat, in order to let supervisor Niels Daugbjerg almost risk his life in order to collect water samples outside of the harbour.

The zooplankton group took a day off, and went hiking up to “Skarvefjeldet”. During the 6-7 hours of climbing and balancing, they spotted both snow hares (*Lepus timidus*) and ptarmigans (*Lagopus mutus*).

Excursion, Nipisat Bay

Early in the morning the camp was brought down and everybody returned to Porsild. First stop at the day’s program was the old LOREN station in Diskofjord. It’s an awful place but a small bay has some rich organic sediment perfect for polychaetes. The location delivered a great surprise. A specimen of the brown algae *Ascophyllum nodosum* was found attached on a rock (69°27,054 N/54°13,133W). It was small, only about 8 cm and situated behind some rocks on an exposed outer coast. This is the northernmost specimen of this species ever detected. After LOREN we continued to Diskofjord bygd. At this location two divers (John and Jonas) wanted to search for the calcium carbonate-incrusted red alga, *Lithothamnion glaciale*. It’s an interesting organism but today we were looking for the fauna hidden in the calcium carbonate constructions. *Lithothamnion* covers the bottom, creating a unique environment, which indeed deserves protection and conservation. The meiofauna inside the incrusted red algae is unique – containing mud dragons (Kinorhyncha) and water bears

(Tardigrada). The last buckets and plastic bags were filled with the collected samples, but whether everybody got what they came for would only be revealed after hard work in the laboratory.

Porsild weighed anchor and the whales appeared once again. This time two humpback whales (*Megaptera novaeanglia*) breaks the surface (Fig. 3C) while Porsild left the Disko Fjord, bound for Godhavn and Arctic Station.

Thursday August 17th

The day was spent sorting and investigating the newly collected samples from the field trip to Nipisat Bay. This was done in the respective groups with varying success. For instance, all though Nipisat is the type locality of marine tardigrade *Halobiotus crispae*, this water bear remained elusive throughout the day in spite of intense searching in the collected samples. If this species was not found it would put an end to one of the projects. Luckily, at the end of the day, we found what we was looking for, however, it was not until the very last sample this fascinating animal was discovered.

Friday August 18th

The day started with a lot of insecurity about where the field-trip would take us. Because of the weather, we did not go to Hareøen as intended; instead we would be going to Kronprinsens Island or Fortune Bay. Kronprinsens Islands are very flat, but when viewed from the laboratory it looks like there are huge mountains, due to a mirage.

The waves were so high that we only went to Fortune Bay. It is called this because in the old days it was a good place for catching whales. At 10:00 we were all aboard. The passengers were the phytoplankton team (Niels and Iben – Maria stayed home because of a broken knuckle), the macro algae people (Poul, Helle, Christian and Mads) and last, but definitely not least, the great marine invertebrate larvae team (Andreas, Henrike, Nora, Payana, Mette and Louise).

On the way to Fortune Bay the weather was quite rough. The waves were, according to the captain, about 2 m high, which feels like a lot, when your boat does not have a keel! Porsild is an ice breaker, which explains the lack of a keel.

When we arrived at Fortune Bay the sea became calm, because it is a sheltered area. The GPS-coordinates for the location we decided to use for sampling was 69°15.446'N and 53°43.864'W. The depth was 20 m. Some people started taking samples, while Mads and Niels caught scorpion fish (*Myoxocephalus scopius*). At 11:00 the macro-algae team went sampling in a small speedboat. It went OK for a while, but at some point the rope from their triangular dredge got caught in the propeller. All three men in the boat had to pull to keep them from stranding. When they got back Iben was done sampling and the other plankton team was finishing. At 12:15 the course was set for Godhavn. It was a nice sampling place for invertebrate larvae; the most interesting was at 17 m depths, near the bottom.

When everyone got back, Mads and Christian made hot chocolate with whipped cream for everyone.

Saturday August 19th

The time had just passed midnight and the last pink lights on the icebergs outside had disappeared. We were looking through samples from Fortune Bay. We had found some beautiful polyplacophora eggs and were watching these when suddenly right in front of our eyes one of the eggs hatched and out came a fine little polyplacophora larvae. That was an incredible sight. The rest of the day was mainly spent in the laboratories, where everyone tried to get as much as possible out of the samples before the material would die.

At 19.00 we had a most important soccer match to play. Traditionally the local women's soccer team (Fig. 4B) play against students and staff of Arctic Station and from a historical point of view the odds were very much against us, since last year Arctic Station lost 9-0. However, bad odds were not a matter to coach Daugbjerg and in no time he had lifted the spirit and everyone was ready for the challenge (Fig. 4A). He organized some warm-up such as a bit of running; some did a little jumping but mainly stretching seemed to be the way to warm-up the body. Everyone was well equipped wearing mainly hiking or rubber boots.

1st half turned out good. We did have the need to clear out some smaller misunderstanding such as which end defense was, but besides that everything was fine. Our fantastic goalie, Poul made the most incredible savings and after 1st half Arctic Station was leading 3-0. Halftime was well used. Raising alcohol levels with beer, raising nicotine levels with cigarettes and of course – more stretching!

2nd half got started, but somehow it seemed that Arctic Station had lost the control of the game. If this had anything to do with the switch of the referee we will never know. Nevertheless the local soccer team soon had made it a tie and even though we made one more scoring before the end, the local team also scored and after 2nd half the score was 4-4. After 2 rounds of extra time (2 times Greenlandic 5 minutes) the local team had scored again and the game ended 5-4 to the local team. After the game a couple of true Viking guys (Dennis and Jonas) cooled down with a short swim and water sampling in the ocean (Fig 4C).

Sunday August 20th

Excursion, Ippik

In light of the previous day's football match, everybody moved very slowly and painfully around while packing the last things for the day's collection trip to Ippik. Eventually we got everything loaded on the station's pickup truck, and made it to the quay, so that by 10:00 AM we set to sea under grey skies. A light drizzle was wetting the people who stood on the foredeck, but within a few minutes of leaving the harbour, they were rewarded with the first whale sighting of the day. A young Fin Whale (*Balaenoptera physalus*) crossed under the boat when we passed between "Udkiggen" and "Kødøen". The sea was choppy, and while it might have influenced some decisions to head for the bunks, it seemed that people were very pragmatic about sail time, and considered it a good time to catch up on days of sleep deprivation. Once at Ippik (only about a good hours sailing from Qeqetarsuaq) the sleeping biologist sprung to life in order to get some serious sampling underway. Porsild was used to haul the triangular dredge along the bottom of the old Ippik shrimp fields, and sampled an incline at 187-200 meters depth. Poul's group meanwhile took the dingy in closer to shore to hand dredge for macro-algae. When the large triangular dredge was pulled over the shipside it contained a good quantity of rich, well oxygenated mud (even though the netting had caught on one of the steel teeth). The mud was dutifully sieved and washed on deck, which meant careful maneuvering of the ship in order not to foul up Niels' water samples and Andy's group's plankton hauls. As Poul's group was completing their dredging, a small group of 4-5 Humpback Whales (*Megaptera novaeanglia*) were drawing along the coast line. Erik, the sailor, let the dingy drift along with the whales, and from the foredeck of Porsild it appeared almost as if the group was on top of the huge cetaceans. When Erik brought the macro-algae group back to the ship, Niels persuaded him to bring a fresh group back out to the whales. The rough seas heaving the dingy up and down the side of Porsild was suddenly not a problem, as camera laden biologists scrambled aboard. This time we got even closer than before, but already at a distance the cameras whirred and clicked worse than a boatload of Japanese tourists. When the whales broke the water mere meters from the dingy, the photography

reached fever pitch as people simultaneously tried to zoom out fast, and avoid falling as the boat rocked in the giants' surface braking waves. While the whales seemed very aware of our presence (and happily never bumped into us), it seemed not to bother them. They continued to forage quite unabated, and flippers, heads and tailfins broke the surface, as a testament to the complicated underwater acrobatics their heavy bodies must perform beneath the waves. When the cameras and their tired operators could take no more, we caught up with Porsild, which was steaming homeward bound. Well back at Arctic Station, we eagerly began looking through the day's samples, although many a camera was set to transfer images to respective laptops, for a quick check of the day's pictures. Henrike baked a German speciality cake that was blissfully devoured during the screening of Niel's edited version of Andy's whale video-recordings. It could be that the "fantaaastisk" wildlife images were the reason nobody discovered the bit of cinnamon in the lucky piece of cake. The Hare Island groups packed during the evening, while others poured over their microscopes to the early hours of the morning.

Monday August 21st

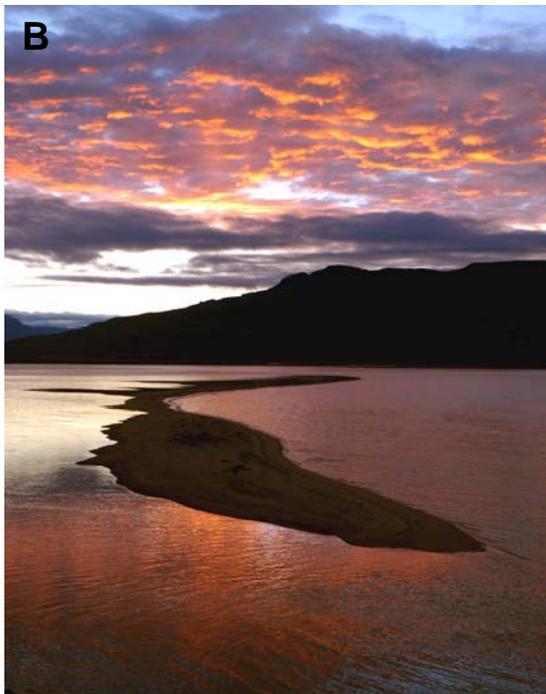
Excursion, Hare Island

Off to Hare Island; this time we succeeded in making it out of Godhavn, apparently the weather gods were on our side. The micro- and macro-algae teams as well as the zoo-plankton group left Arctic Station at 10 a.m. after having packed and scolded down a few litres of coffee. After having crammed everything onto Porsild and given the captain permission to leave, Poul remembered he had left his waders back at Arctic Station. Big mistake, no biologist with any pride would leave behind such utterly important equipment! This resulted in Poul rushing back to Arctic Station. Mean while the crew stood with their arms crossed, sighed, shaking their heads and glaring at the empty harbour, "patiently" waiting for the arrival of Poul. He did return and the venture to Hare Island could finally begin.

The time was spent in constant lookout for whales, which was rewarded by the sight of five humpback whales. Besides whales, we managed to see a few cormorants, several passing flocks of eiders, two seals (though only spotted by the crew) and many fulmars. When not watching the amazing scenery, the team of people would play games, watch movies (some of them twice and with no sound) or sleep. Our sleeping champion was Mette, who managed to sleep for more than 24 hours straight, only interrupted by the collecting of samples. At around 7 p.m. we reached our destination of the day, Nord Fjord. We found the perfect campsite, set up the tents, decided the campsite was imperfect, moved all the tents and came to the conclusion that this would not do either and moved the tents once again, this time to a rocky ground (the previous two had been more or less in the middle of a swamp). Getting back to Porsild for our dinner proved to be a bit difficult. The hunting season for eiders had set in on the 15th of august, which meant that the crew of Porsild had gone hunting. After a successful hunt (they got two eiders, though one was lost in the water), they did return to pick us up. After having scoffed down Poul's marvellous chilli con carne, the macro-algae group went off to gather samples while the rest of us rested under deck. Then we returned to the campsite. We were lucky enough to have found a place with lots of drift wood, so it did not take long before fire-master Mads had lit the perfect bonfire. The rest of the evening was spent roasting marshmallows, sipping very cold whiskey and enjoying the thought of being more than 70° north.

Tuesday August 22nd

Samples collected from the excursion to Hare Island were analyzed.



◀ **Figure 5.** **A.** Camp setup from “Mudderbugten”. **B.** Evening view from camp in A. **C.** Reinhardt Kristensen sampling for *Limnognathia maerski* at Isuungua spring, with the research vessel Porsild in the background. **D.** Icebergs on the way home from “Mudderbugten”. Photos: Martin O. Macnaughton.

Wednesday August 23rd

Preparations to the following day’s excursion to Mudderbugten were made, while the remaining groups concentrated on the samples collected during the previous trip to Hare Island.

Thursday August 24th

Excursion, Mudderbugten

We got to Porsild at 10:00 and the engine started right away. The first stop was at Ippik (formerly known as Iqqik). The position was 69°17.138’N and 53°13.665’W. Sampling was made by Reinhardt and Martin with a device called “trekantskraberen” (the triangle dredge) at depths of about 222 m. The surface water temperature was 7.5°C, but the mud they got up was only 2.8°C! At noon, Porsild went on towards Mudderbugten. People played cards, watched a movie or slept. At 17:30 we reached Mudderbugten and went ashore at Isuungua at the foot of Pingo Fjeld. Some took samples and some just watched birds such as ptarmigans, cormorants, Greenland White-fronted Goose *Anser albifrons flavirostris*, Canada Goose *Branta canadensis*, Kittiwake *Rissa tridactyla*, Wheatear *Oenanthe oenanthe*, American Pipit *Anthus spinoletta rubescens*, Purple Sandpiper *Calidris maritima* and observed tracks of foxes. Apart from the animals we also noted that the houses from the Thule-culture were about (in a matter of years) to be washed into the ocean. Reinhardt showed us a chief’s grave with human and whale bones. The reason for collecting samples at Isuungua spring (Fig. 5C) was to get new material of *Limnognathia maerski* (Micrognathozoa) -a new phylum of animals discovered during the student field course in 1994. We all left Kvandalen/ the Ramsar Area (a protected wetland) and went back to Porsild for supper.

Nora and Reinhardt were ill (the Godhavn flue) and stayed the night at Porsild. The rest of the students went ashore, set up tents and made a fire from driftwood in the cool sand on the beach of Flakkerhuk (Fig. 3A & 5A+B).

Friday August 25th

Excursion, Mudderbugten

At 05:30 Kenneth and Dennis started sampling but didn’t wake up the rest of the scientists until 8:00. At 9:00 we were all back at Porsild, where Nora had been up early, sampling for the plankton teams. First, we went to Skansen, where more plankton samples were made in the deeper waters (almost 200 m), and Martin and Reinhardt got some fantastic sand samples at low water. On the way back some more mud-sampling were made at Ippik. Most people slept on the way back. Some worked on the mud and one person was told stories by Fari, the captain. He told about both his father and younger brother sailing Porsild. He had been sailing for 43 years, mostly as a fisherman, but since 2001 as the captain of Porsild. He and the crew members Søren Fisker and Erik Wille have known each other almost all their lives. At six in the evening Reinhardt, Jonas and Martin were almost done washing the mud samples and we reached Godhavn in nice weather conditions as was characteristic for the whole trip.

Saturday August 26th

The samples collected during the field trip to Mudderbugten were investigated, and work on the respective projects continued unabated.



Figure 6. A. View over "Engelskmandens Havn". B. Liverwort (*Marchantia polymorpha*). C. Fir Club-moss (*Huperzia selago*). D. Arctic Cotton-grass (*Eriophorum scheuchzeri*) E. A mushroom. Photos: Iben Veland.

Sunday August 27th

Today everyone was going on a hiking trip to "Engelskmandens Havn" (Fig. 6A & 7F), a rather tough walk in mountainous terrain, expected to last most of the day. We started out from the Arctic Station about 10:00 and after 2-3 hours we reached the top of the ravine, which ran approximately 150-200 meters at a steep angle down to the small cove called "Engelskmandens Havn" (Fig. 6A). Of course there were several stops along the route as we passed many interesting sites, which gave Reinhardt and Poul the opportunity to share in their vast knowledge of the vegetation (Fig. 6B-E & 7A-E) and the general area.

Scaling the steep ravine proved both difficult and dangerous because of loose rocks and an entangling maze of vegetation. To make it even more difficult a homothermal cold spring (4°C) trickled through the ravine's centre, making the rocks slippery in some places. When everybody was safely at the bottom Reinhardt showed us a radioactive spring, which harboured a more "southern" fauna. Along the coastline we found the three species of orchids: Heart-leaved Twayblade (*Listera cordata*; Fig. 7D), Northern Green Orchid (*Platanthera hyperborea*) and Small White Orchid (*Leucorchis albida*). After investigating the area the group split up and some went along a coastal route to the Arctic



Figure 7. A. Crowberry (*Empetrum hermaphroditum*) B. Livelong Saxifrage C. Viviparous Knotweed D. Orchid E. Henrike Semmler climbing up from “Engelskmandens Havn” F. Sledge dogs in the mist on the Lyngmarks glacier.

Station. The rest climbed back up the ravine and took the same route we had come from. The idea was to take a detour up to the Lyngmarks Glacier (Fig. 7F), but only four daring hikers had the energy to challenge the misty heights of the glacier (Fig. 7E). It was a harsh ascent to the shelter at 805 m above sea level and moss samples were collected at intervals along the way. We were looking for the High Arctic tardigrade *Pseudechiniscus victor*, a species first found on Disko Island during the 1990 Field Course.

Monday August 28th

Today was the last day of the projects, which was spent by most running around in a frantic effort to complete the last tasks of the respective projects. However, as the last practical things concerning the projects were completed, we all began looking forward to the evening’s dinner and party. All the people involved in the field course; the crew onboard “Porsild”, Frederik Grønvold, Søren Fisker and Erik Wille, the station manager Kjeld (Akaaraq) Mølgaard, the cook and housekeeper Naja as well as the scientific leader of Arctic Station, Henrik Sulsbrück were invited. As the evening arrived we had giant snow crabs (*Chionoecetes opilio*) for dinner, which tasted exquisitely, and afterwards we all engaged in dancing to a local

Greenlandic band we had met the evening before and persuaded to come play for us. It was an incredibly fun and memorable evening.

Tuesday August 29th

As we all slowly woke up, some with hangovers after the party the day before, we began packing our equipment. Microscopes had to be packed, samples had to be taken care of, chemicals had to be stored safely etc. Work progressed slowly, however we all got it done. At lunch time all our expedition boxes were safe in the harbour – ready for shipping.

Wednesday August 30th

The day when we had to say goodbye to the Arctic Station, which had been our home for the past three weeks, had arrived. Our ship to Asiaat/Ilulissat was set to depart at 12:00. Most of us were a bit melancholic about leaving, however also excited about going home.

The sail trip offered its own incredible sights of amazing icebergs drifting in the sunset with scores of malemukker surfing the winds just over the seawater surface. Some even saw a female Fin Whale (*Balaenoptera physalus*) with her calf.

We arrived in Ilulissat at approximately 21:45 and transported to a youth hostel where we were to spend the night. We were all very hungry when we arrived, however everything was closed. Even so, we managed to find a Chinese restaurant which we convinced to reopen, just for us, and all got something to eat.

Thursday August 31st

The morning was spent by taking a small trip to the Ilulissat Isbræ. Incredibly sights of massive blocks of ice floating out into the sea filled our minds as we walked the approximately 7 km through rocky landscapes. As we got home, most of us went shopping for a quick lunch before the departure to Kangerlussuaq at 15:00. We arrived at Kangerlussuaq 15:45 where we spent our last night in Greenland. Some went for musk ox-safari, others for treasure hunting in the dunes of “Fossilsletten”, or pool-shooting, before turning in at the youth hostel.

Friday September 1st

The following morning we were all ready to go home. Check-in was at 9:30 in the relatively busy airport of Kangerlussuaq. However, logistic problems usually arise when travelling in Greenland - and this was no exception. A microscope we had been allowed to carry onboard in every other flight as hand luggage was now suddenly too big and had to be checked in separately as large freight. This situation created a mild form of panic as the plane was leaving soon and the microscope had to be checked in at a separate hanger some distance away. The situation was nonetheless quickly handled by Reinhardt and Kenneth and we all made the scheduled departure with the flight GL 782 to Copenhagen at 11:10; however with an extra freight cost of 4000 DKr. We arrived in Copenhagen at 19:30 (local time) tired, exhausted, happy and sad after a wonderful and exciting trip to Disko Island few of us will ever forget.