Stockholm University

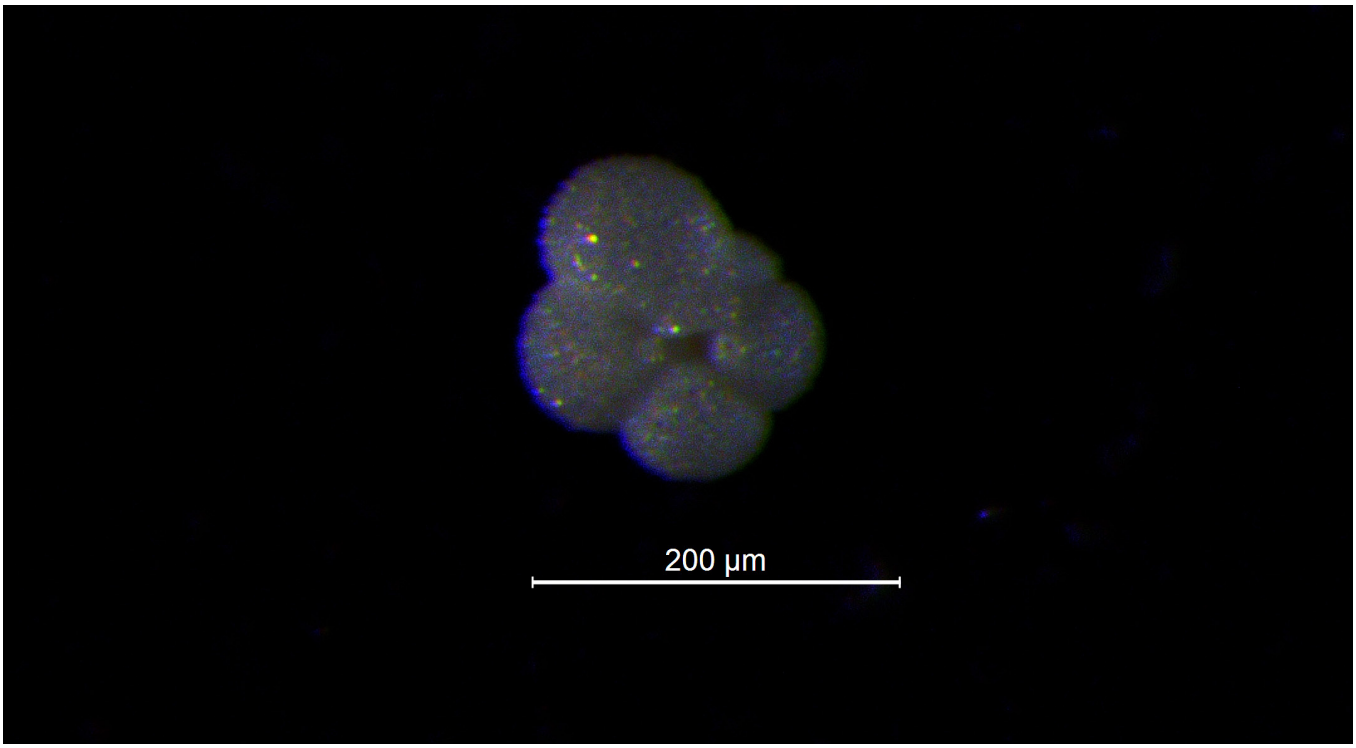
Bachelor Thesis

Degree Project in
Marine Geology 15 hp

Palaeoenvironmental significance of the planktonic foraminiferal genus *Turborotalita* in the central Arctic Ocean

Step 1: morphology

James Carter



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Department of Geological Sciences
Stockholm University
SE-106 91 Stockholm

Abstract

Turborotalita quinqueloba is a species of planktonic foraminifera that exists at high latitudes. In times of warming such as Marine Isotope Stage 11 (MIS 11) in the Pleistocene it is thought that *Turborotalita quinqueloba*, or a possible related species *T. egelida,* occupied higher latitudes in greater abundance in Polar seas. Was this part invasion from lower latitudes and part anagenetic evolution of *Turborotalita quinqueloba* to fill a new ecological niche in response to changing environmental conditions? So far this is unknown. This study compared the morphometrics of two samples of *T. quinqueloba* from the Nordic and Central Arctic seas at the time of MIS 11. This was achieved by selecting individuals for light microscopy and then photographing them. Image J software and Microsoft Excel were used to record measurements of various indices related to the shell morphology. The study found that these samples were significantly different from each other and therefore came from two different populations. Of particular note although individuals were of similar size in the two samples, the Arctic specimens were less rounded but had a more rounded final chamber and a more open umbilicus. The study is of relevance for modern assemblages in light of recent climate change. Will similar morphometric changes occur in *T. quinqueloba* as a result of global warming? Is the *Turborotalita* in MIS 11 Arctic sediments of this study a distinct morphotype *T. egelida*, a polymorph of *T. quinqueloba* or a new species?

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1 Introduction

This study concerns foraminifera or more technically foraminiferida. They are testate, amoeboid protozoa that exist in their most evolved state with having a fully mineralised test. They are present globally and the planktonic forms (as they can be both benthic and planktonic) can be very useful in stratigraphic correlation (Allaby 2013).

Foraminifera are also very good proxies for environmental conditions at the time of their life as their geochemistry and morphology reflect prevailing conditions at the time of their shell construction and sedimentation on the sea floor (Stanley 2009). Different species of foraminifera are likely to be present under different conditions and their evolution is directed by the niche to which they are most adapted (Schiebel et al 2018).

My study concerns the stratigraphic level Marine Isotope Stage 11 which was a warm interglacial in the Pleistocene epoch of the Cenozoic era 424-374K years ago (St. John et al 2012)(Figure 1).

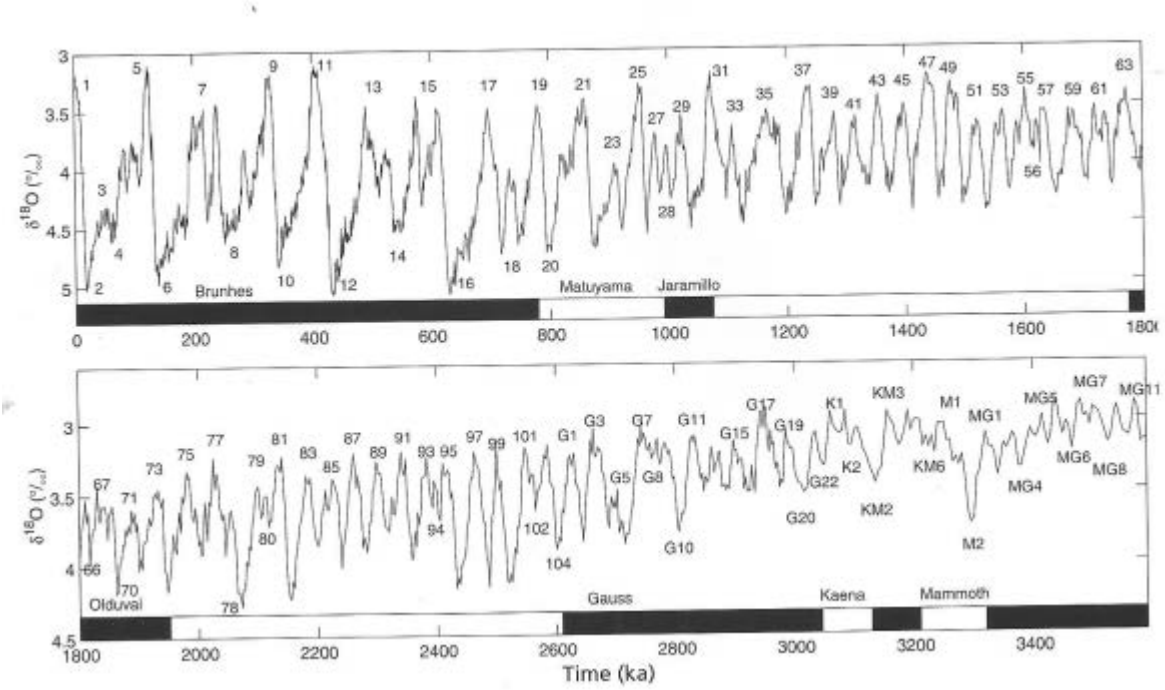


Figure 1: Marine Isotope Stages.δ^{18} reflects ice volume. From St. John et al. (2012).

Turborotalita quinqueloba and *Neogloboquadrina pachyderma(sin)* are two species that exist in Arctic waters in present times with *N. pachyderma* (sin.) being the predominant species in the coldest waters of the Central Arctic Ocean (Volkmann 2000). However it is hypothesised that *T. quinqueloba* is more present in polar waters in times of increased warmth where it is normally a predominantly subpolar species (Schiebel et al 2017). The *Turborotalita* genus is more delicate than *N. pachyderma* having a thinner wall but is spinose. Therefore it is not so easily preserved thus making attempts for complete study very difficult (Volkmann 2000). Whether the individuals of *Turborotalita* found in the interior Arctic Ocean have evolved in an indigenous way or has been advected by water currents from the South is a matter of debate (Bauch 1999).

This present study is based on the work of O'Regan et al (2019) which proposed that the less known morphotype of *T. quinqueloba, T. egelida* is an important biostratigraphic marker for MIS 11 on the Lomonosov ridge in the Arctic Ocean. Yet a morphological and taxonomic definition of *T. egelida,* which is not recognised among living planktonic foraminifer populations or genetic pools is lacking (Darling and Wade 2008) (Schiebel and Hemleben 2017). Therefore establishing the difference between *Turborotalita* specimens in the Arctic Sea and subpolar regions such as the Nordic Sea is an important one and the focus of this study (Figure 2).

In this study morphometrics are used to compare a sample of *Turborotalita* from the Nordic Sea (M23063) and a sample from the Lomonosov ridge of the Arctic Sea (LOMROG07-4PC). The question is are these two samples from the same population of foraminifera and if not are they somehow different, perhaps even different species suited to different conditions? Is the *Turborotalita* in the Arctic Sea in MIS 11 *T. quinqueloba,* a related species or *T. egelida*? Finally does this study have relevance for the present climate changes as an important indicator?

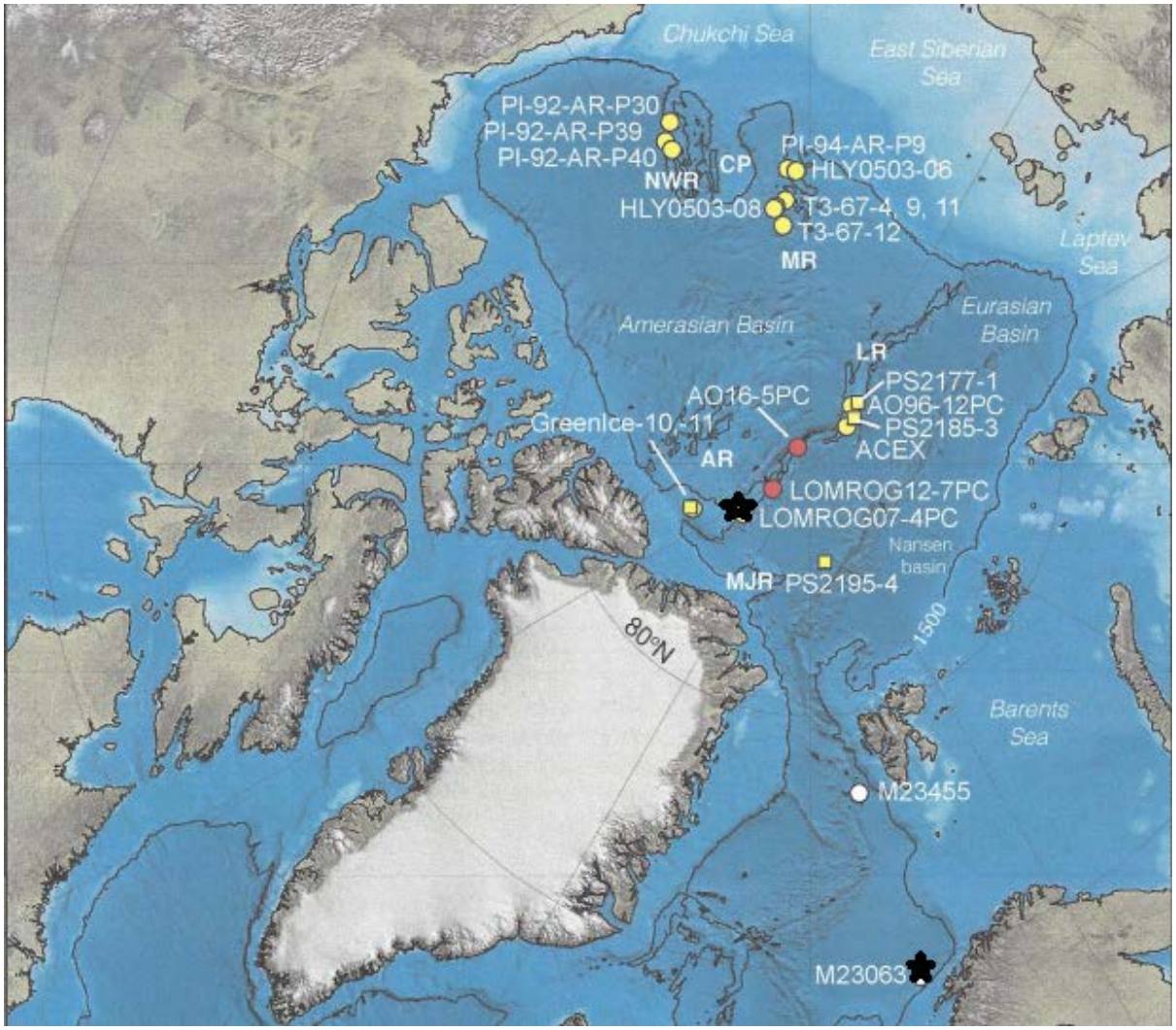


Figure 2: Key areas of coring and geography of the area of study. Samples were taken from coresM23063 (Nordic Sea, sample courtesy of A. Zhuravleva and H. Bauch) and LOMROG07-4PC (Arctic Ocean, Lomonosov Ridge –Greenland end)- black stars. This map, which is from O'Regan et al (2019), shows additional sites which were investigated in the latter author's study but not discussed here.

2 Background

2.1 A previous study of *Turborotalia*

One major study that is worthy of attention in considering this one is that of evolution and speciation of the Eocene genus *Turborotalia* (Pearson and Ezard 2014). Although *Turborotalia* is unrelated to *Turborotalita,* the approach is relevant. This Eocene study concerns the problems of ascertaining if speciation took place and used morphometrics to determine this through multivariate analysis. Foraminifera are especially useful in the stratigraphic record because they are present in large numbers in continuous successions. Evolution is sometimes considered to occur in discrete areas with stasis elsewhere but in the case of foraminifera due to circulating oceanic waters this is not the case (Pearson and Ezard 2014).

The history of morphometry and stratigraphy in marine microfossils date back to Hays in 1970 when he studied radiolarians from the Pacific and Southern Oceans (Pearson and Ezard 2014). These studies show a change in size of specimens and lineage branching in radiolarians. Planktonic foraminifera were first studied in this way in 1981 when Malmgren and Kennett studied different species over some millions of years (Pearson and Ezard 2014).

An important point to note is that changes in oceanic circulation can lead to geographical variations in the specimens studied over time. This can complicate theories of site specific evolution (Pearson and Ezard 2014). Another important point in studies of this nature is that a subjective discernment of different characteristics in morphotypes might be carried over into subsequent studies causing artificial discernment of key traits. Also of note, and this is applicable to my study, is that small sample sizes may not account for evolutionary change in the same way as large sample sizes (Pearson and Ezard 2014).

In comparing individuals it is assumed that once all various factors such as ontogenic stage or sex of the individuals are taken into account the genotypic or phenotypic traits will be more similar in two individuals of species X than in one of species X and one of species Y (Ezard et al 2010). An important consideration however is that intra species variation can be greater than inter species variation due to homology. This can confound morphometric studies (Ezard et al 2010).

Although genetic studies have allowed species to be delineated in detail, morphometrics is still a very useful technique for the following reasons. Sometimes morphometrics are the only data readily available and they can be integrated with genetic data to allow species delineation. Morphometrics allow the multi-locus aspect of a phenotype to be quantified whereas genetic studies very often are restricted to a short length of DNA. This is very important for specifying species type. In addition if the evolution is divergent these morphological traits that show the difference in specimen lines may be crucial to defining the two species (Ezard et al 2010).

The Eocene study chose a location for a core to be taken at Site 865 of the Ocean Drilling Programme. This site is within the North Pacific gyre in the tropics. A gyre is particularly suitable for study as it circulates water continuously within itself but is continuous with the rest of the ocean. The site they used was a guyot which although within the gyre has been gradually moving through plate movement (Pearson and Ezard 2014).

The Eocene team studied the extinct species *Turborotalia cerroazulensis* which was a particularly abundant and widely distributed Eocene foraminifer species. It can be shown over time using traditional visual species classification that there were six morphospecies showing gradual evolution. Earlier forms were more rounded whereas the shape of later forms were more angular with a keel around the edge (Ezard et al 2010)

Despite there being more than one morphospecies in the time series it was thought that only one morphospecies was present at a particular time. Two time intervals were assessed statistically to see

if there were morphological clusters of traits. Both upper and lower Eocene times were analysed (Ezard et al 2010).

There was also a change in chirality of specimens, sinistral becoming more dominant, plus an increase in the number of chambers in the final whorl and a general size increase (Ezard et al 2010).

This could be a wonderful example of evolutionary change in a species even if the species were coexisting. In their statistics the authors assumed Gaussian distribution of traits and used Principle Component Analysis. They note that if there is a hidden change in morphology, mean and standard deviations are unlikely to show any difference although it may be real. Therefore a Bayesian model was used which analysed the cluster i.e. its volume and shape etc. Stratigraphic successions were tested for clusters and stratophenetic linkage was applied. If there was an overlap of clusters an ancestral relationship is implied (Pearson and Ezard 2014).

If the species are cryptic it is believed that over time morphometrics will eventually be able to detect a significant difference between them (Pearson and Ezard 2014).

The change in morphospecies might be explained either by anagenetic change or as a result of geographic factors (Pearson and Ezard 2014).

However the alternative hypothesis that more than one species was present at a time was supported for the upper Eocene sample implying evolutionary divergence had taken place. This was not the case for the middle Eocene sample (Ezard et al 2010)(Figure 3).

The authors presume that the morphology selected was important for feeding, buoyancy or protection. Perhaps of relevance to my study is the suggestion that more compressed test shapes are less buoyant and therefore cooling temperatures of the Eocene meant tests could occupy a more suitable depth for survival (Pearson and Ezard 2014).

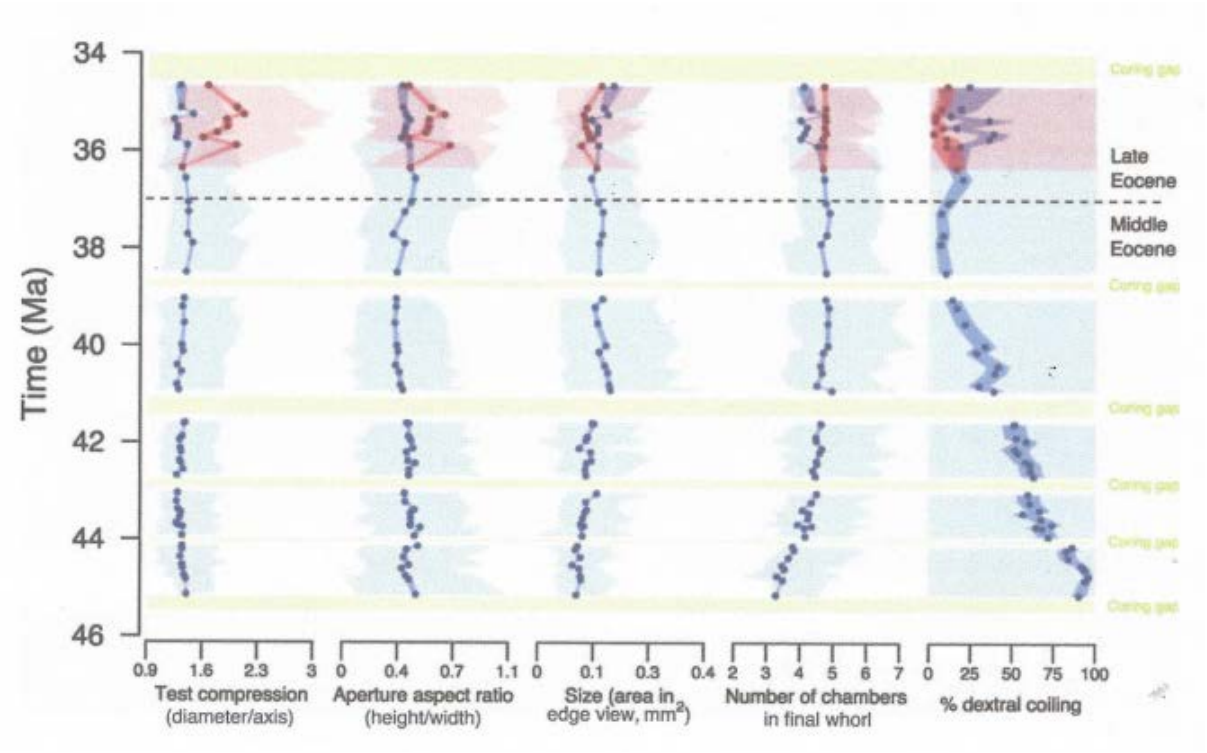


Figure 3: Evolutionary divergence of traits in Eocene *Turborotalita*. From Pearson and Ezard 2014.

2.2 Recent observations of *Turborotalita* on the Lomonosov ridge

My study is based on the work of O'Regan et al (2019) where *Turborotalita egelida* has been found pre MIS 5 in the Arctic Ocean and has been proposed as a marker for MIS 11. This would imply conditions which were out of the ordinary in MIS 11 in the oceanographic system of polar waters (O'Regan et al 2019).

O'Regan et al (2019) describe how correlation has been difficult in the Arctic because microfossils are erratic in occurrence and diversity. Also geomagnetic polarity reversals are common in the Arctic but appear anomalous and are difficult to synthesise with known geomagnetic stratigraphic levels according to the global framework. Furthermore stable isotope records are difficult to interpret

because of the sporadic occurrence of shells. This is why the finding of *T. egelida* is important (O'Regan et al 2019).

Moreover, Pleistocene sediments before the last interglacial in core LOMROG12-7PC found the presence of *Turborotalita* which makes their relationship to *T. egelida* and *T. quinqueloba* taxonomically important for stratigraphic reasons. From the cores LOMROG12-7PC and AO16-5PC specimens from MIS 5.1 and 5.2 look like *T. quinqueloba* which is found in the Nordic Seas and not the *T. egelida* found from MIS 11 (O'Regan et al 2019)(see figure 2).

Morphological distinctions between shells is the basis of foraminiferal classification. Using traditional visual approaches the *T.* quinqueloba morphology for instance has a final chamber that is ampulate and extends into the umbilicus, the coiling is enrolled and the wall texture implies a spinose phenotype. These features are found in the Lomonosov Ridge assemblages, however the specimens there are usually small (<125 microns) with smooth walls. This is different from the specimens of *T. quinqueloba* found in subpolar regions which are not translucent under light microscopy i.e. have gametogenic calcite. The Lomonosov Ridge *T. quinqueloba* are also smaller than *T. egelida* which typically has an open umbilicus and a rounded final chamber. Adding to the confusion is that small specimens presumably *T. quinqueloba* have a rounded final chamber in the Fram Strait core of MIS 5 (O'Regan et al 2019).

The relationship between *T. quinqueloba* and *T. egelida* remains unclear although molecular studies have shown two *Turborotalita* genotypes in cool Nordic and North Atlantic Seas. O'Regan et al (2019) speculate that Arctic *Turborotalita* is unlikely to have been brought by advection from waters further south because of predominantly counter clockwise circulation of ocean waters in the Arctic and also the fact that such immature tests that were advected would be dissolved on their way to the seafloor.

The most reasonable argument therefore is that warming conditions allowed *Turborotalita* to survive further north. It is queried whether the small size of these specimens perhaps indicates a different form of reproduction i.e. asexual (Kate Darling, personal communication). This commonly occurs in benthic foraminifera. In other words nature found yet another way of filling a biological niche. Maybe *Turborotalita* evolved to reproduce asexually to cope with more extreme climatic conditions and limited time for growth (O'Regan et al 2019).

The present study was useful in determining morphometrics of >125 micron specimens in Arctic waters as this area at this time usually contained small specimens. Also what is the relationship between *T. quinqueloba* and *T. egelida*? Did *T. quinqueloba* habitually enter Arctic waters in interglacials or was it just at certain times? Further more extensive work to be carried out on the Lomonosov ridge might answer these questions (O'Regan et al 2019).

2.3 Distribution of *T. quinqueloba.*

Before I go on to my method and results I thought it expedient to say what is known of the distribution of *T. quinqueloba* in the Arctic sea.

Studies show that the sea floor sediments of the Arctic Ocean are dominated by foraminifera of two species *N. pachyderma* (sin.) and *T. quinqueloba* (Volkmann 2000). Volkmann (2000) confined her study to the Fram Strait (81-82°N) where there is an inflow of warm Atlantic water in the East and out flow of cold surface water to the Nordic Sea in the West and the Outer Laptev Sea (76-80°N) where freshwater influx from Siberia gives low saline surface waters.

The Arctic sea is well stratified which means there is little vertical mixing and water movement is mainly by advection. Plankton tow studies show that in the Fram Strait individuals of *N. pachyderma* (sin.) and *T. quinqueloba* show maximum abundance at 0-100m depth. *N. pachyderma* (sin.) in its

juvenile stage is distinguished from *T. quinqueloba* by having a thicker wall without spines. However sometimes *T. quinqueloba* shed their spines. Juvenile forms of both species are commonly smaller than 125 microns (Volkmann 2000).

In the Outer Laptev sea *N. pachyderma* (sin.) is ten times more abundant than *T. quinqueloba* and the latter is at peak abundance between 100 and 200m water depth. When there is permanent ice coverage *N. pachyderma* (sin.) migrates to shallow water 0-50m in depth (Volkmann 2000).

In the Fram Strait *N. pachyderma* (sin.) shows a maximum abundance between temperatures of -1.5 and -1.8°C and of salinity between 32.6 and 34.0. *N. pachyderma* (sin.) prefers fresh cold water in the Arctic. *T. quinqueloba* in contrast occupies a wide temperature range of -0.7 to 3.4°C and narrow salinity range of 34-35. Therefore it occupies slightly lower water at the ice sea margin where salinities are higher (Volkmann 2000).

There is a strong seasonal fluctuation of numbers of foraminifera with highest numbers in late summer and autumn. Summer with its warmer temperatures causes water to be more stratified. *N. pachyderma* (sin.) is present at 4 individuals per m^3 in the upper 500m, *T. quinqueloba* only 0.5 per m^3 (Figure 4).

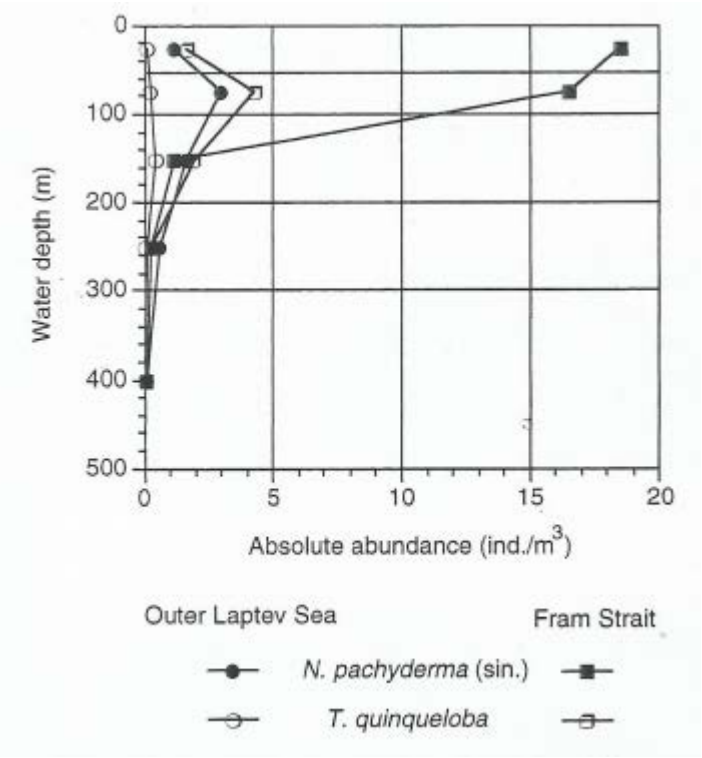


Figure 4: Living foraminifera average abundances from plankton tows (125-250 microns). From Volkmann (2000).

Studies have shown that planktonic foraminifers from other oceans are most abundant near the chlorophyll maximum at 30m water depth (Fairbanks and Wiebe 1980). However Volkmanns study showed *N. pachyderma* (sin.) and *T. quinqueloba* to exist below this chlorophyll maximum. The food source of *T. quinqueloba* is not known however they frequently occur with Atlantic copepods (*Calamus finmarchicus)* - (Volkmann 2000).

It is thought that reproduction of *N. pachyderma* (sin.) follows the synodic cycle and that in early ontogenic stages chambers grow very quickly. High numbers of individuals >250 microns in size are found at the time of the full moon with small numbers at the time of the new moon (Volkmann 2000). At the full moon high numbers of kummerform individuals are found (having an odd shaped final chamber)i.e. reproducing forms. A lunar cycle of reproductivity is believed to be the norm for *T. quinqueloba* as well (Volkmann 2000).

Isotope composition is important in the enquiry into the depth habitat of these foraminifers (Bauch et al 1997) but this is often hindered in *T. quinqueloba* by the dissolution of delicate calcite before sedimentation. This is not the case for *N. pachyderma* (sin.) which has a thicker shell (Vincent and Berger 1981).

3 Method

Samples were taken from the core of sediment from the two locations in the Nordic sea (M23063, 733cm) and Arctic sea (LOMROG07 PC04, 298-299cm) (see Figure 2). The foraminifera were sieved through a mesh to separate individuals greater than 125 microns. This was done because it is easier to be more confident with the species identification in slightly large specimens compared to small ones, in which it is possible to mix species. An aluminium 'microsplitter' was used to help reduce the sample to a manageable size for extracting a representative population of specimens for preparation. For Site M23063, 733cm all specimens were extracted from the >125 micron fraction, resulting in a foraminifera sample size of 215 individuals of *Turborotalita.* In the LOMROG07 PC04, 298-299cm sample the same sieving and splitting approach was taken, however even after splitting the sample there were many more than 200 individuals, therefore specimens were taken at random to achieve a sample size of 252 individuals.

Specimens of *Turborotalita* were identified by light microscopy amongst other forams by visual recognition. The main characters used to identify *T. quinqueloba* were the shiny delicate-looking shell wall, coiled shape with an ampulate final chamber. They co-occurred with a variety of other planktonic foraminifera including abundant *N. pachyderma* (sin.), rare *G. bulloides,* rare *Orcardia reideli,* and rare *Globigerinita uvula*. The interior Arctic *Turborotalita* morphospecies were similarly smooth walled but these lacked the ampulate final chamber and instead had a wide open umbilicus. Individuals were mounted on a glass slide, using water soluble paper glue, scored with

compartments, four to a compartment . They were laid on their spiral side so that the ventral surface (umbilical side) of the foraminifera was apparent.

Photography software and hardware was used to photograph each specimen from the microscope at magnification ×363 (×16 coarse magnifier on microscope). All specimen images were catalogued using Microsoft Excel. Distinguishing features were noted as well as number of chambers in the final (visible) whorl and also the chirality of coiling-dextral or sinistral.

Image J software was used to measure morphometric details of each specimen and recorded in the Excel spreadsheet. Measurements recorded were:-

1. Apertural lip width
2. Final whorl maximum diameter
3. Final whorl maximum breadth passing through the umbilicus
4. Final whorl whole area
5. Final whorl perimeter
6. Final whorl circularity
7. Final chamber area excluding lip
8. Final chamber perimeter excluding lip
9. Final chamber circularity excluding lip
10. Final + penultimate chamber area excluding lip
11. Final + penultimate chamber perimeter excluding lip
12. Final + penultimate chamber circularity excluding lip
13. Umbilicus area

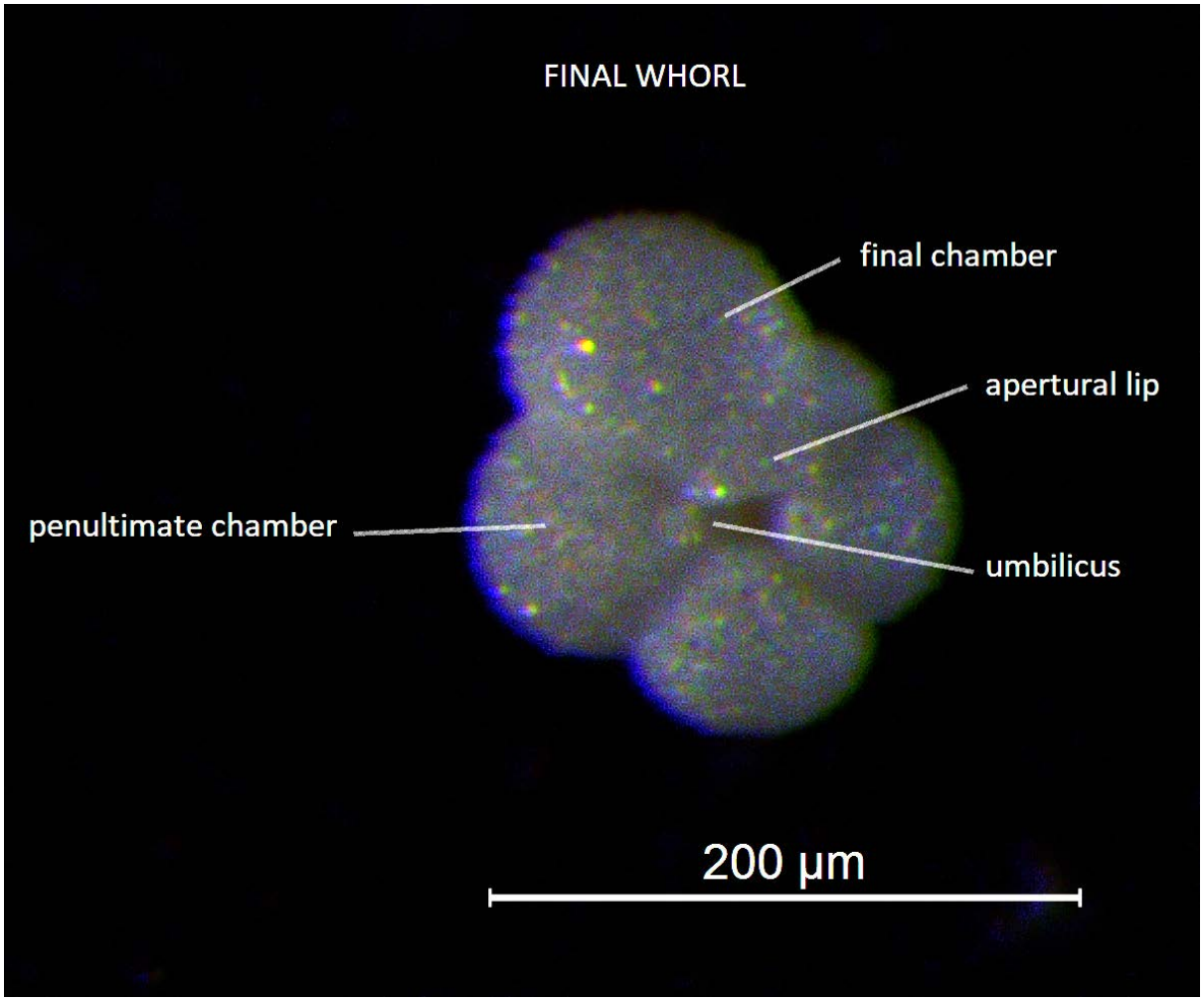


Figure 5: Principle parts of the final whorl of a specimen of *Turborotalita quinqueloba* from Core sample M23063 (Nordic Seas)

Microsoft Excel was used to calculate the mean, sample standard deviation, sample variance and range of values for each measurement for both samples. Equal sample variance was assumed in both samples and a Student t-test was used to compare the samples for each measurement. A probability of 0.05 was taken as significant and the P value for each t-test (two tailed) is recorded. If there proved to be a significant difference in the measurements taken on the two samples then it would be reasonable to infer that the two samples came from different populations of foraminifera.

4 Results

Of the shells catalogued there was a slight difference in chirality in the spirals of individuals between the two samples. From core LR07_PC04, 298-299cm 56% of individuals were sinistral and 44% dextral. In core M23063, 733cm 52% were sinistral and 48% dextral.

For the measurements recorded results were divided into class sizes and a histogram was plotted. Class boundaries were taken as the same for both samples for each measurement and plotted on the same histogram for comparison-series 2 and series 1. This allowed visualisation of a subsequent Student t test to compare the two samples. I generally felt that all the distributions were essentially Gaussian and this was a prerequisite for the test (Figure 6).

Equal variance was assumed between the two samples in the Student t test and probabilities for both one tailed and two tailed test were calculated based on the t statistic.

For apertural lip width (6a) the LR07_PC04 core sample had a mean of 12.84 microns, range 1.97-43.75; the M23063 sample mean was 12.59 microns, range 0-62.35. The two tailed t test showed test probability P of 0.656. Therefore there was no significant difference between samples.

For final whorl diameter (6b) LR07_PC04 core sample had a mean of 206.69 microns, range 156.8-322.19; the M23063 sample had a mean of 201.58 microns, range 146.57-292.22. Two tailed t-test showed probability P of 0.01468. Therefore the samples were significantly different.

For final whorl maximum breadth through the umbilicus (6c) LR07_PC04 core sample specimens had a mean of 160.46 microns, range 114.3-270.31; M23063 core sample mean was 168.24 microns range 122.15-233.96. The Student t test P value for a two tailed test was 5.24×10^{-5}. Therefore the two samples were significantly different from each other. In other words although the Arctic individuals had a greater diameter than the Nordic individuals they had less breadth.

For final whorl area (6d) LR07_PC04 core sample specimens had a mean of 25322.49 microns squared, range 16028.93-61381.25; M23063 mean 25361.99 microns squared range 16876.3-51152.92. Student t test P value (two tailed) was 0.940232. In other words there was no difference in area of specimens between samples.

For final whorl perimeter (6e) LR07_PC04 core sample mean was 630.21 microns, range 498.64-1003.55; M23063 sample mean 619.81 microns, range 496.84-914.87. Student t test (two tailed) P value was 0.108827 i.e. no difference statistically between samples.

For final whorl circularity (6f) LR07_PC04 core sample mean was 0.793, range 0.707-0.865; M23063 core sample mean was 0.823, range 0.721-0.904. T test P value (two tail) was 3.25×10^{-26}. Therefore statistically the Nordic Sea specimens were more circular than those from the Arctic Ocean.

For final chamber area excluding the apertural lip (6g) LR07_PC04 core sample mean was 6735.69 microns squared range 2390.99-17957.91; M23063 core sample mean 7103.22 range 2209.26-13841.97. P value (two tailed test) was 0.08243. Therefore, no significant difference between samples.

For final chamber perimeter excluding apertural lip (6h) LR07_PC04 sample mean was 294.09 microns range 183.13-492.75; M23063 core sample mean was greater 307.29, range 180.55-435.51. T test P value (two tail) was 0.003709 i.e. a significant difference.

Final chamber circularity excluding apertural lip (6i) showed LR07_PC04 core sample mean to be 0.954 range 0.826-0.98; M23063 mean 0.915 range 0.666-0.971. P value (two tail) was 1.57×10^{-28} i.e. the final chamber of Arctic specimens was significantly rounder than Nordic Sea specimens.

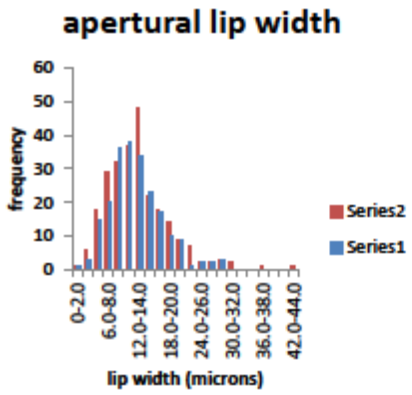
For final+penultimate chamber area excluding the apertural lip (6j) LR07_PC04 sample mean was 12839.53 microns squared, range 6514.81-30164.4; M23063 mean 13005.11 microns squared, range 6702.29-23627.39. T test (two tail) P value was 0.547304 i.e. no significant difference.

For final+penultimate chamber perimeter excluding the apertural lip (6k) the LR07_PC04 core sample mean was 485.62 microns, range 336.54-802.18; M23063 core sample mean 469.06 microns, range 322.16-668.63. P value (two tailed) was 0.003669 i.e. the Arctic Ocean measurements were significantly greater than the Nordic Sea ones.

For final+penultimate chamber circularity excluding the apertural lip (6l) LR07_PC04 sample mean was 0.681, range 0.506-0.89; M23063 sample mean was 0.741, range 0.461-0.926. P value (two tailed) was 4.13×10^{-18}. Therefore the Nordic Sea specimen measurements were more circular than those for the Arctic Ocean specimens.

For umbilicus area (6m) LR07_PC04 core sample mean was 895.73 microns squared, range 45.02-5569.21; M23063 sample mean 698.227 microns squared, range 14.24-2599.68. P value (two tailed) was 7.23×10^{-5}. Therefore the Arctic specimens had a larger umbilical area.

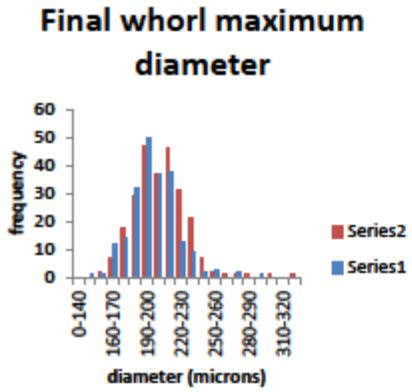
There was also a significant difference in number of chambers in the final whorl of these two samples (6n). LR07_PC04 sample mean was 4.385, range 3.5-5.6; M23063 sample mean was 4.482, range 3.8-5.5. P value (two tailed) was 8.43×10^{-5}. Therefore Nordic Sea individuals had more chambers in their final whorl than Arctic Ocean individuals.



		Series 2	Series 1
n		252	215
Mean		12.84	12.59
Sample SD		6.097	6.09
Sample variance		37.17	37.09
Minimum value		1.97	0
Maximum value		43.75	62.35

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	12.8435	12.59132
Variance	37.16731	37.08985
Observations	252	215
Pooled Variance	37.13166	
Hypothesized Mean Difference	0	
df	465	
t Stat	0.445757	
P(T<=t) one-tail	0.32799	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.65598	
t Critical two-tail	1.965079	

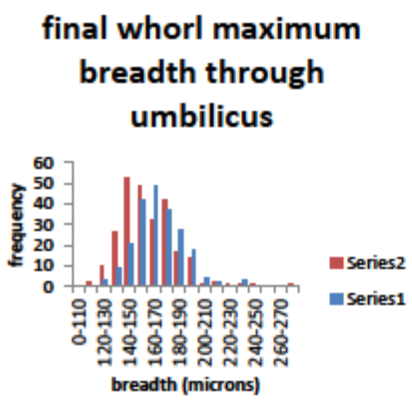
Fig.6a:Apertural lip width (microns). Series 2:core LR07_PC04,298-299 cm; series 1: core M23063, 733 cm



		Series 2	Series 1
n		252	215
Mean		206.69	201.58
Sample SD		23.29	21.42
Sample variance		542.38	458.9
Minimum value		156.8	146.57
Maximum value		322.19	292.22

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	206.6875	201.5826
Variance	542.3801	458.9018
Observations	252	215
Pooled Variance	503.9621	
Hypothesized Mean Difference	0	
df	465	
t Stat	2.449335	
P(T<=t) one-tail	0.00734	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.01468	
t Critical two-tail	1.965079	

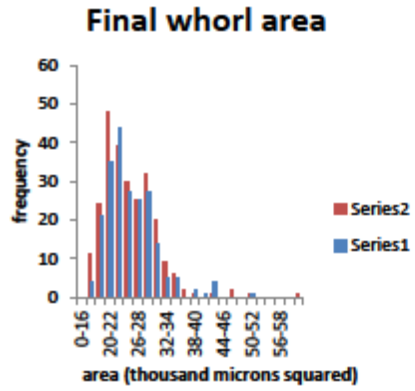
Fig.6b: Final whorl maximum diameter (microns). Series 2:core LR07-PC04, 298-299cm; series 1:core M23063, 733 cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	160.4606	168.2382
Variance	470.3873	363.2263
Observations	252	215
Pooled Variance	421.0702	
Hypothesized Mean Difference	0	
df	465	
t Stat	-4.08253	
P(T<=t) one-tail	2.62E-05	
t Critical one-tail	1.648137	
P(T<=t) two-tail	5.24E-05	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		160.46	168.24
Sample SD		21.69	19.06
Sample variance		470.39	363.23
Minimum value		114.3	122.15
Maximum value		270.31	233.96

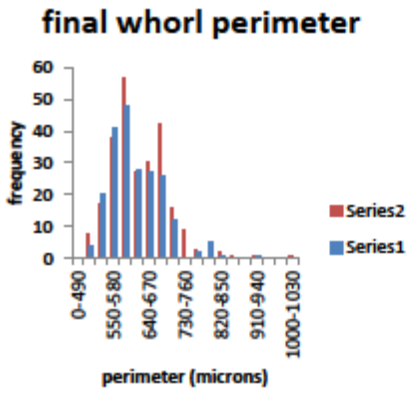
Fig.6c: Final whorl maximum breadth through umbilicus (microns). Series 2: core LR07_PC04, 298-299cm; series 1: core M23063, 733 cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	25322.49	25361.99
Variance	35011379	28826655
Observations	252	215
Pooled Variance	32165076	
Hypothesized Mean Difference	0	
df	465	
t Stat	-0.07502	
P(T<=t) one-tail	0.470116	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.940232	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		25322.49	25361.99
Sample SD		5917.04	5369.05
Sample variance		35011379	28826655
Minimum value		16028.93	16876.3
Maximum value		61381.25	51152.92

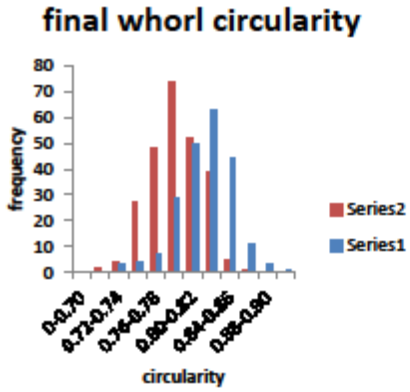
Fig.6d: Final whorl area (microns squared). Series 2:core LR07_PC04, 298-299cm; series 1: core M23063, 733 cm



		Series 2	Series 1
n		252	215
Mean		630.21	619.81
Sample SD		72.63	66.12
Sample variance		5275.3	4372.38
Minimum value		498.64	496.84
Maximum value		1003.55	914.87

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	630.2052	619.8073
Variance	5275.299	4372.375
Observations	252	215
Pooled Variance	4859.76	
Hypothesized Mean Difference	0	
df	465	
t Stat	1.606574	
P(T<=t) one-tail	0.054413	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.108827	
t Critical two-tail	1.965079	

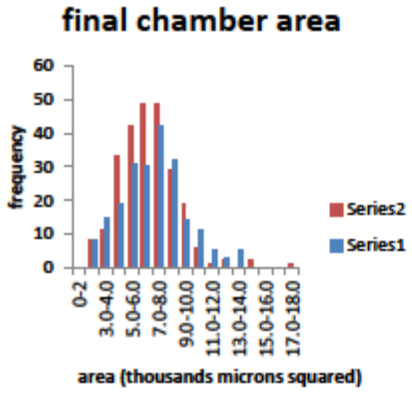
Fig.6e: Final whorl perimeter (microns). Series 2:core LR07_PC04,298-299 cm; series 1: core M23063, 733cm



		Series 2	Series 1
n		252	215
Mean		0.793	0.823
Sample SD		0.0277	0.0292
Sample variance		0.00077	0.00085
Minimum value		0.707	0.721
Maximum value		0.865	0.904

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	0.792901	0.822558
Variance	0.000766	0.00085
Observations	252	215
Pooled Variance	0.000805	
Hypothesized Mean Difference	0	
df	465	
t Stat	-11.2616	
P(T<=t) one-tail	1.76E-26	
t Critical one-tail	1.648137	
P(T<=t) two-tail	3.52E-26	
t Critical two-tail	1.965079	

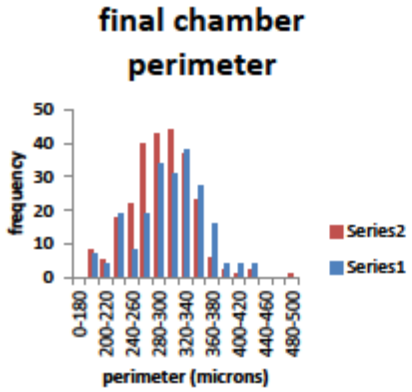
Fig.6f: Final whorl circularity (a/1). Series 2:core LR07_PC04, 298-299 cm; series 1: core M23063, 733cm



		Series 2	Series 1
n		252	215
Mean		6735.69	7103.22
Sample SD		2133.61	2429.15
Sample variance		4552274	5900777
Minimum value		2390.99	2209.26
Maximum value		17957.91	13841.97

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	6735.694	7103.215
Variance	4552274	5900777
Observations	252	215
Pooled Variance	5172875	
Hypothesized Mean Difference	0	
df	465	
t Stat	-1.74051	
P(T<=t) one-tail	0.041215	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.08243	
t Critical two-tail	1.965079	

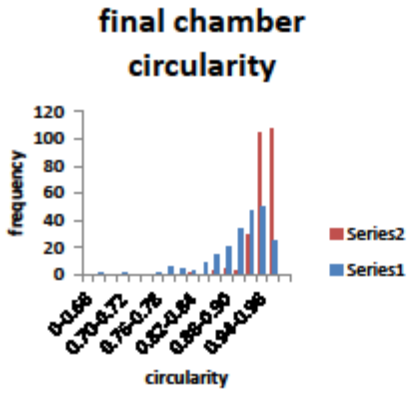
Fig.6g: Final chamber area (microns squared). Series 2:core LR07_PC04, 298-299cm; series 1:core M23063, 733cm



		Series 2	Series 1
n		252	215
Mean		249.09	307.29
Sample SD		45.8	52
Sample variance		2097.53	2704.28
Minimum value		183.13	180.55
Maximum value		492.75	435.51

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	294.0902	307.2915
Variance	2097.532	2704.283
Observations	252	215
Pooled Variance	2376.768	
Hypothesized Mean Difference	0	
df	465	
t Stat	-2.91665	
P(T<=t) one-tail	0.001855	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.003709	
t Critical two-tail	1.965079	

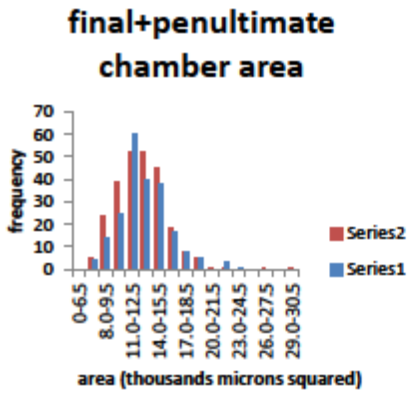
Fig.6h: Final chamber perimeter (microns). Series 2: core LR07_PC04, 298-299cm: series 1:core M23063, 733 cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	0.954127	0.915181
Variance	0.00036	0.002297
Observations	252	215
Pooled Variance	0.001251	
Hypothesized Mean Difference	0	
df	465	
t Stat	11.85987	
P(T<=t) one-tail	7.87E-29	
t Critical one-tail	1.648137	
P(T<=t) two-tail	1.57E-28	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		0.954	0.915
Sample SD		0.019	0.0479
Sample variance		0.00036	0.0023
Minimum value		0.826	0.666
Maximum value		0.98	0.971

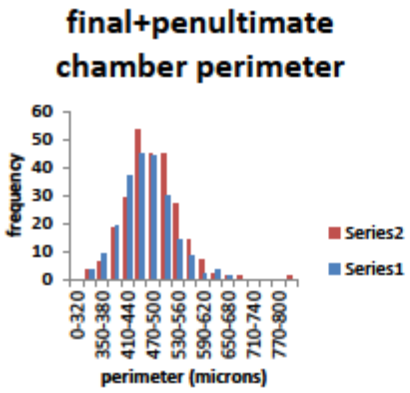
Fig.6i: Final chamber circularity (a/1). Series 2:core LR07_PC04, 298-299 cm; series 1: core M23063, 733 cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	12839.53	13005.11
Variance	9299574	8148650
Observations	252	215
Pooled Variance	8769901	
Hypothesized Mean Difference	0	
df	465	
t Stat	-0.60225	
P(T<=t) one-tail	0.273652	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.547304	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		12839.53	13005.11
Sample SD		3049.52	2854.58
Sample variance		9299574	8148650
Minimum value		6514.81	6702.29
Maximum value		30164.4	23627.39

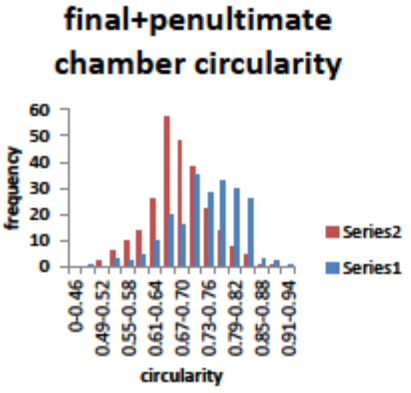
Fig.6j: Final+penultimate chamber area (microns squared). Series 2:core LR07_PC04, 298-299cm; series 1:core M23063, 733cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	485.6182	469.0622
Variance	4106.333	3287.063
Observations	252	215
Pooled Variance	3729.293	
Hypothesized Mean Difference	0	
df	465	
t Stat	2.920129	
P(T<=t) one-tail	0.001834	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.003669	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		485.62	469.06
Sample SD		64.08	57.33
Sample variance		4106.33	3287.06
Minimum value		336.54	322.16
Maximum value		802.18	668.63

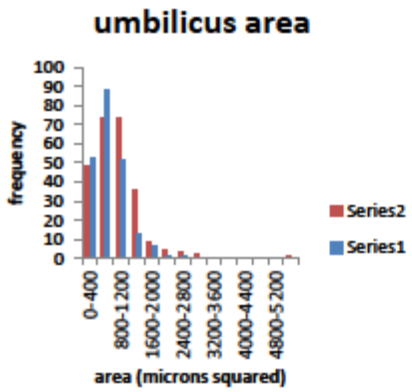
Fig.6k:Final+penultimate chamber perimeter (microns). Series 2: core LR07_PC04, 298-299cm; series 1:core M23063, 733cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	0.681218	0.74073
Variance	0.004345	0.005821
Observations	252	215
Pooled Variance	0.005024	
Hypothesized Mean Difference	0	
df	465	
t Stat	-9.0434	
P(T<=t) one-tail	2.07E-18	
t Critical one-tail	1.648137	
P(T<=t) two-tail	4.13E-18	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		0.681	0.741
Sample SD		0.0659	0.0763
Sample variance		0.00435	0.00582
Minimum value		0.506	0.461
Maximum value		0.89	0.926

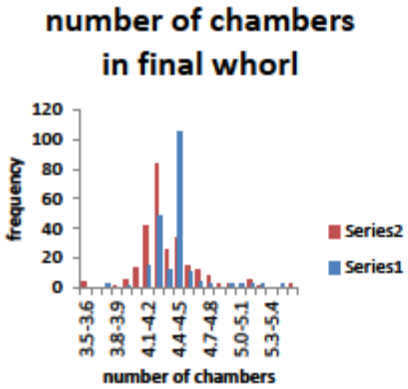
Fig.6l:Final+penultimate chamber circularity (a/1). Series 2: core LR07_PC04, 298-299cm; series 1: core M23063, 733cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	895.7314	698.2265
Variance	367701	181935.9
Observations	252	215
Pooled Variance	282209.1	
Hypothesized Mean Difference	0	
df	465	
t Stat	4.004546	
P(T<=t) one-tail	3.62E-05	
t Critical one-tail	1.648137	
P(T<=t) two-tail	7.23E-05	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		895.73	698.277
Sample SD		606.38	426.54
Sample variance		367701	181936
Minimum value		45.02	14.24
Maximum value		5569.21	2599.68

Fig.6m: Umbilicus area (microns squared). Series 2:core LR07_PC04, 298-299 cm; series 1: core M23063, 733 cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	4.385317	4.48186
Variance	0.074644	0.061772
Observations	252	215
Pooled Variance	0.06872	
Hypothesized Mean Difference	0	
df	465	
t Stat	-3.96679	
P(T<=t) one-tail	4.22E-05	
t Critical one-tail	1.648137	
P(T<=t) two-tail	8.43E-05	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		4.385	4.482
Sample SD		0.273	0.249
Sample variance		0.0746	0.0618
Minimum value		3.5	3.8
Maximum value		5.6	5.5

Fig.6n: Number of chambers in the final whorl. Series 2:core LR07_PC04, 298-299cm; series 1: core M23063, 733cm

5 Discussion

Out of the 14 variables measured in the two MIS 11 samples from the interior Arctic and Nordic seas 9 showed significant difference between the samples that imply consistent morphological differences that might be relevant to biological and taxonomic differences. Before exploring those ideas further the limitations of the method must be discussed.

5.1 Limitations

Visual morphometrics by its very nature is subjective but it is hoped observers would get similar results as a result of the sheer weight of statistical probability. Sources of variation among the pictures include orientation of the specimen-in three dimensions, as some chambers lie on top of others; the angle of specimens to the vertical is different along with the angle to the vertical and horizontal of constituent chambers. For instance the apertural lip looks different when viewed from different angles, which can make identifying its limits problematic at times.

Statistical analysis is limited by the small sample sizes. It is difficult to decide whether there was a speciation event as only samples from MIS 11 are given. Earlier studies such as the Eocene study (Pearson and Ezard 2014) give an idea as to when speciation took place in *Turborotalia* by sampling over a wide time frame, i.e. over a time series of 4-5 million years.

Also the present study is limited to comparison using only simple statistics and the Student t test. More involved methods for larger studies use principle components analysis to detect different clustering of traits between species (Ezard et al 2010).

The Eocene study used slightly different measurements to the ones I have used but is very similar in those recorded. Umbilical angle I have not been able to measure in my study for instance.

5.2 Findings

My findings nevertheless are interesting for the following reasons. Applying the Student t test to measurements of final whorl area, final whorl perimeter, final chamber area and final + penultimate chamber area resulted in P values above 0.05 (two tailed test assuming equal variance) between samples. This implies that the null hypothesis is proved for these measurements, i.e. that there is no difference in the populations from which these samples were drawn.

However final whorl maximum diameter, final whorl maximum breadth passing through the umbilicus, final whorl circularity, final chamber perimeter, final chamber circularity, final + penultimate chamber perimeter, final + penultimate chamber circularity and umbilicus area were all significantly different between the two samples (P value less than 0.05 for two tailed test assuming equal variance between samples). This proves the alternative hypothesis that there is a difference between these samples implying that they come from different populations.

By viewing the final chamber alone it is subjectively quite visible without taking measurements that the Arctic sample is more circular: this was proved statistically. The results would seem to infer that although measured areas are similar between samples ie size, the shape of the individuals in the two samples is different.

Bauch (1994) in his study of test size in the Norwegian-Greenland Sea based his measurements on the maximum diameter of the test on the ventral aspect. This was the same measurement that I used. He found that test size decreased in colder water to the West compared to water in the East of the study. He speculates whether this is related to smaller size specimens being brought in by Atlantic currents, perhaps juvenile varieties of *Turborotalita* (Bauch 1994). In contrast my study showed that the maximum whorl diameter increased in the colder water of the interior Arctic station, however the whorl breadth decreased. Although my study is hampered by only looking at

one size fraction of shells (>125 microns) my result tends to imply that there is no difference in test sizes between the two sites at this MIS 11 interval. However the shape of the shells is different as is the relative size of the umbilicus, which is larger in the interior Arctic sample.

Furthermore in my study number of chambers in the final whorl is significantly different between the two samples with the Lomonosov Ridge *Turborotalita* having on average 4.39 in the final whorl compared to 4.48 in the Nordic Seas supporting the alternative hypothesis that the samples come from two different populations.

The apertural lip shows no significant difference between samples confirming the null hypothesis that there is no difference in the samples for this measurement. Importantly, the measured variables are relevant to taxonomic separation of species and might be useful in future species definitions.

Interesting to note is that although *N. pachyderma* shows a significant predilection for the sinistral form in cold Polar waters, in my study sinistral and dextral forms were fairly equal in both samples of *Turborotalita* ; 52% sinistral in the Nordic Sea, 56% sinistral in the Arctic Sea. Also worthy of mention is that the Lomonosov ridge sample only contained one species of foraminifera i.e. no *N. pachyderma* (sin.); is there an environmental cause for this?

5.3 Evolution

The biological history of planktonic foraminifera is a history of extinctions and radiations in which the same basic patterns tend to emerge. Biologists try to predict the rate of evolution and how oceanographic changes affect this evolution of species (Pearson 1993).

Homeomorphy is present across the species of planktonic foraminifera as individuals compete for the same niches- this makes deciding which species an organism belongs to difficult (Pearson 1993).

Anagenesis is common amongst planktonic foraminifera where there is a progressive linkage of intermediate stages in a gradual evolution. This may be the case in my study of two populations of *Turborotalita*- both samples representing intermediate forms. Chronoclines illustrate this well although my study is taken from one time frame-MIS 11. Actual cladogenesis where one genus or higher taxa splits into another may occur in this way but is not anagenetic (Pearson 1993).

This makes taxonomy difficult as the theory of biological evolution and its Linnean classification supports the idea of discrete species rather than a gradual imperceptible transition. Pearson suggests that this 'punctuated equilibrium' is not applicable to planktonic foraminifera or other marine microfossils (Pearson1993).

Sheldon in 1987 had this problem with Ordovician trilobites; the individuals defied categorization into discrete species; this is the same with marine microfossils (Pearson 1993). Simpson in 1953 noted that taxonomic evolution is different to structural evolution as the rates of change of both are quite different (Pearson 1993).

Therefore the term morphospecies has become useful. This may be a central form within the population or represent a polymorphism, ontogenic state or a geographical form (Pearson 1993).

This would appear to be the case with my two samples which maybe represent different morphospecies. An ontogenic stage would be unlikely because of the similarity in size of the sample specimens but them being geographical variants is a possibility.

5.4 Insights

On a deeper level than morphometrics genetic variation among individuals is important in establishing whether they belong to different genotypes. Homeomorphy can be confusing in this

respect. The emergence of the new genetics has enabled taxonomic debates to be solved (Schiebel et al 2018).

Compared to morphometrics, such results are not subjective. Homeomorphy amongst planktonic foraminifera is very common. In the Tertiary there was an initial radiation of foraminifera from the individuals that had survived the Cretaceous. Possibly certain benthic forms also made the jump to becoming planktonic (Schiebel et al 2018). Nature is very resourceful and if a particular morphotype is successful in a particular niche it will be selected by evolution. This is the case for the MIS 11 and also the present and is perhaps an environmental case for my two samples being different.

It has been discovered that many morphospecies may be comprised of different genetic types and that therefore many 'cryptic' species are apparent (Schiebel et al 2018). It would seem logical that the next step in this research would be to genetically type the species. Molecular data is telling us that polyphyletic origins of planktonic foraminifera are derived from two or more benthic lines after the Cretaceous-Tertiary extinction (Schiebel et al 2018).

A further approach not achieved in this study would be the analysis of isotopes within the shell substance. For instance marine carbon cycling can be interpreted through carbon-13 estimations as well as vital effects within organisms. Furthermore abiotic forcings such as temperature, salinity and pH are important in the pelagic zone and oxygen isotope and carbon isotope study is of vital importance (Schiebel et al 2018).

For many planktonic foraminifera many cryptic species are being discovered. This is the same for *Turborotalita quinqueloba* as well as *N. pachyderma* and *G. bulloides*. High throughput sequencing (HTS) allows large volumes of water to be sampled and offers hope of yet more information on defining cryptospecies (Schiebel et al 2018). In the near future this will allow the ecologies of these cryptic species to be studied in detail along with their geographical preferences (Schiebel et al 2018).

6 Conclusion

Foraminifera are numerous in the stratigraphic record and geographically ubiquitous. It is obvious that at high latitudes planktonic foraminifera are affected by climatic change like the rest of nature and are therefore worthy of serious study (Schiebel et al 2018). This is especially true of organisms such as *Turborotalita quinqueloba* in the warm interglacial of MIS 11 and the present day. Studying the past is the key to the future!

Nature always finds a way and the two samples in my study may constitute an anagenetic difference related to the warming climate at the poles.

The fact that individuals between the two samples had a different shape is interesting and seems relevant even though there was a lack of evidence for one being an earlier ontogenic stage as both samples had similar sized specimens. Perhaps the shape difference was related to different buoyancy requirements to inhabit different depths of water for feeding or temperature requirements etc.

Likewise it is difficult to determine whether individuals were transported into Arctic waters by advecting currents or were indigenous dwellers.

The proposal of O' Regan et al that the *Turborotalita* of MIS 11 Arctic waters was a less known *Turborotalita egelida* morphotype is supported in this study by the fact that the final chamber is more circular and the umbilicus more open than the Nordic Sea specimens (O'Regan et al 2019).

Worthy of consideration I feel is that the two samples in the Nordic Sea and Arctic Sea were intimately related- they had the same size of apertural lip for instance. Perhaps they represent intermediates or morphospecies. Perhaps they are an example of anagenetic evolution in planktonic foraminifera. I hope this study is beneficial to the outcome of this ongoing research.

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Stockholm University

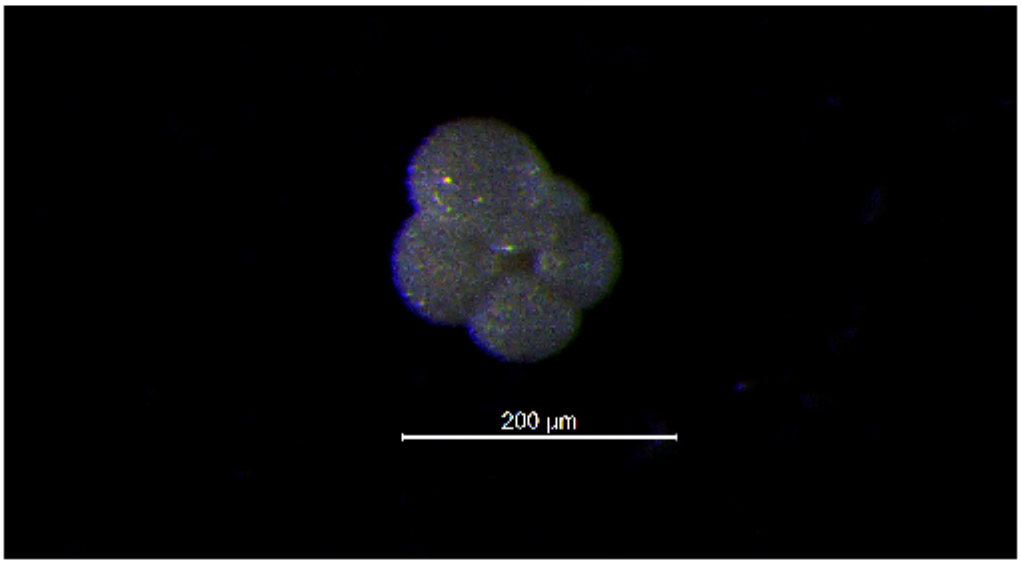
Bachelor Thesis

Degree Project in
Marine Geology 15 hp

Palaeoenvironmental significance of the planktonic foraminiferal genus *Turborotalita* in the central Arctic Ocean

Step 1: morphology

James Carter



Stockholm 2020

Department of Geological Sciences
Stockholm University
SE-106 91 Stockholm

Abstract

Turborotalita quinqueloba is a species of planktonic foraminifera that exists at high latitudes. In times of warming such as Marine Isotope Stage 11 (MIS 11) in the Pleistocene it is thought that *Turborotalita quinqueloba*, or a possible related species *T. egelida,* occupied higher latitudes in greater abundance in Polar seas. Was this part invasion from lower latitudes and part anagenetic evolution of *Turborotalita quinqueloba* to fill a new ecological niche in response to changing environmental conditions? So far this is unknown. This study compared the morphometrics of two samples of *T. quinqueloba* from the Nordic and Central Arctic seas at the time of MIS 11. This was achieved by selecting individuals for light microscopy and then photographing them. Image J software and Microsoft Excel were used to record measurements of various indices related to the shell morphology. The study found that these samples were significantly different from each other and therefore came from two different populations. Of particular note although individuals were of similar size in the two samples, the Arctic specimens were less rounded but had a more rounded final chamber and a more open umbilicus. The study is of relevance for modern assemblages in light of recent climate change. Will similar morphometric changes occur in *T. quinqueloba* as a result of global warming? Is the *Turborotalita* in MIS 11 Arctic sediments of this study a distinct morphotype *T. egelida*, a polymorph of *T. quinqueloba* or a new species?

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1 Introduction

This study concerns foraminifera or more technically foraminiferida. They are testate, amoeboid protozoa that exist in their most evolved state with having a fully mineralised test. They are present globally and the planktonic forms (as they can be both benthic and planktonic) can be very useful in stratigraphic correlation (Allaby 2013).

Foraminifera are also very good proxies for environmental conditions at the time of their life as their geochemistry and morphology reflect prevailing conditions at the time of their shell construction and sedimentation on the sea floor (Stanley 2009). Different species of foraminifera are likely to be present under different conditions and their evolution is directed by the niche to which they are most adapted (Schiebel et al 2018).

My study concerns the stratigraphic level Marine Isotope Stage 11 which was a warm interglacial in the Pleistocene epoch of the Cenozoic era 424-374K years ago (St. John et al 2012)(Figure 1).

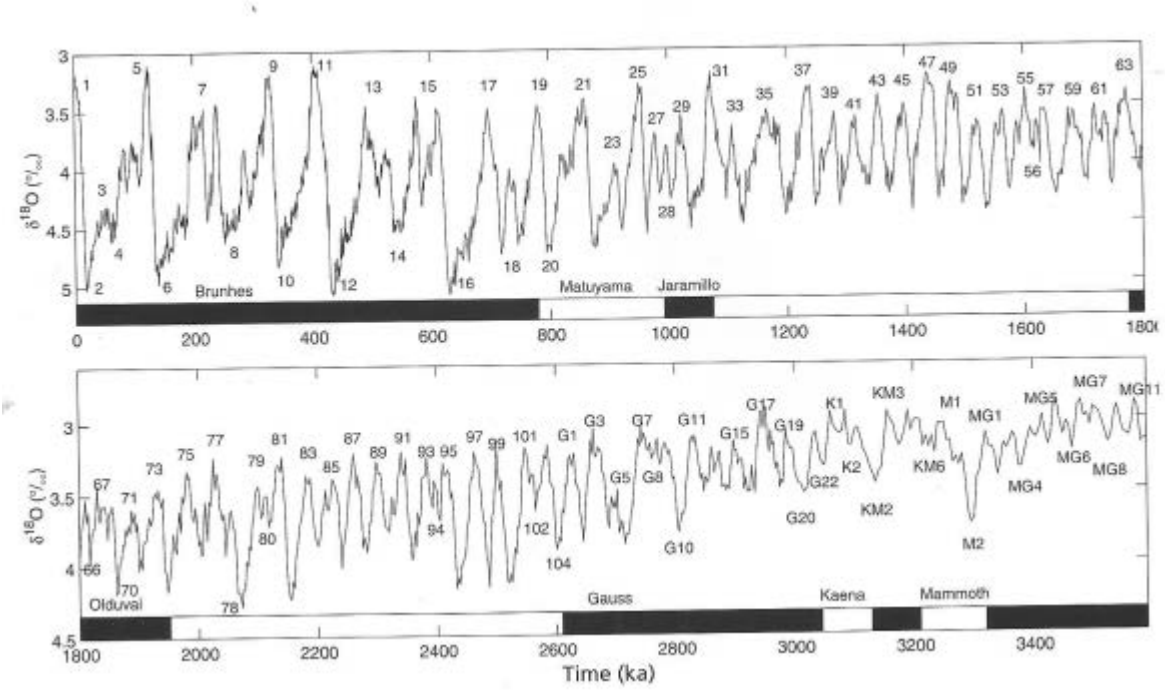


Figure 1: Marine Isotope Stages.δ^{18} reflects ice volume. From St. John et al. (2012).

Turborotalita quinqueloba and *Neogloboquadrina pachyderma(sin)* are two species that exist in Arctic waters in present times with *N. pachyderma* (sin.) being the predominant species in the coldest waters of the Central Arctic Ocean (Volkmann 2000). However it is hypothesised that *T. quinqueloba* is more present in polar waters in times of increased warmth where it is normally a predominantly subpolar species (Schiebel et al 2017). The *Turborotalita* genus is more delicate than *N. pachyderma* having a thinner wall but is spinose. Therefore it is not so easily preserved thus making attempts for complete study very difficult (Volkmann 2000). Whether the individuals of *Turborotalita* found in the interior Arctic Ocean have evolved in an indigenous way or has been advected by water currents from the South is a matter of debate (Bauch 1999).

This present study is based on the work of O'Regan et al (2019) which proposed that the less known morphotype of *T. quinqueloba, T. egelida* is an important biostratigraphic marker for MIS 11 on the Lomonosov ridge in the Arctic Ocean. Yet a morphological and taxonomic definition of *T. egelida,* which is not recognised among living planktonic foraminifer populations or genetic pools is lacking (Darling and Wade 2008) (Schiebel and Hemleben 2017). Therefore establishing the difference between *Turborotalita* specimens in the Arctic Sea and subpolar regions such as the Nordic Sea is an important one and the focus of this study (Figure 2).

In this study morphometrics are used to compare a sample of *Turborotalita* from the Nordic Sea (M23063) and a sample from the Lomonosov ridge of the Arctic Sea (LOMROG07-4PC). The question is are these two samples from the same population of foraminifera and if not are they somehow different, perhaps even different species suited to different conditions? Is the *Turborotalita* in the Arctic Sea in MIS 11 *T. quinqueloba,* a related species or *T. egelida*? Finally does this study have relevance for the present climate changes as an important indicator?

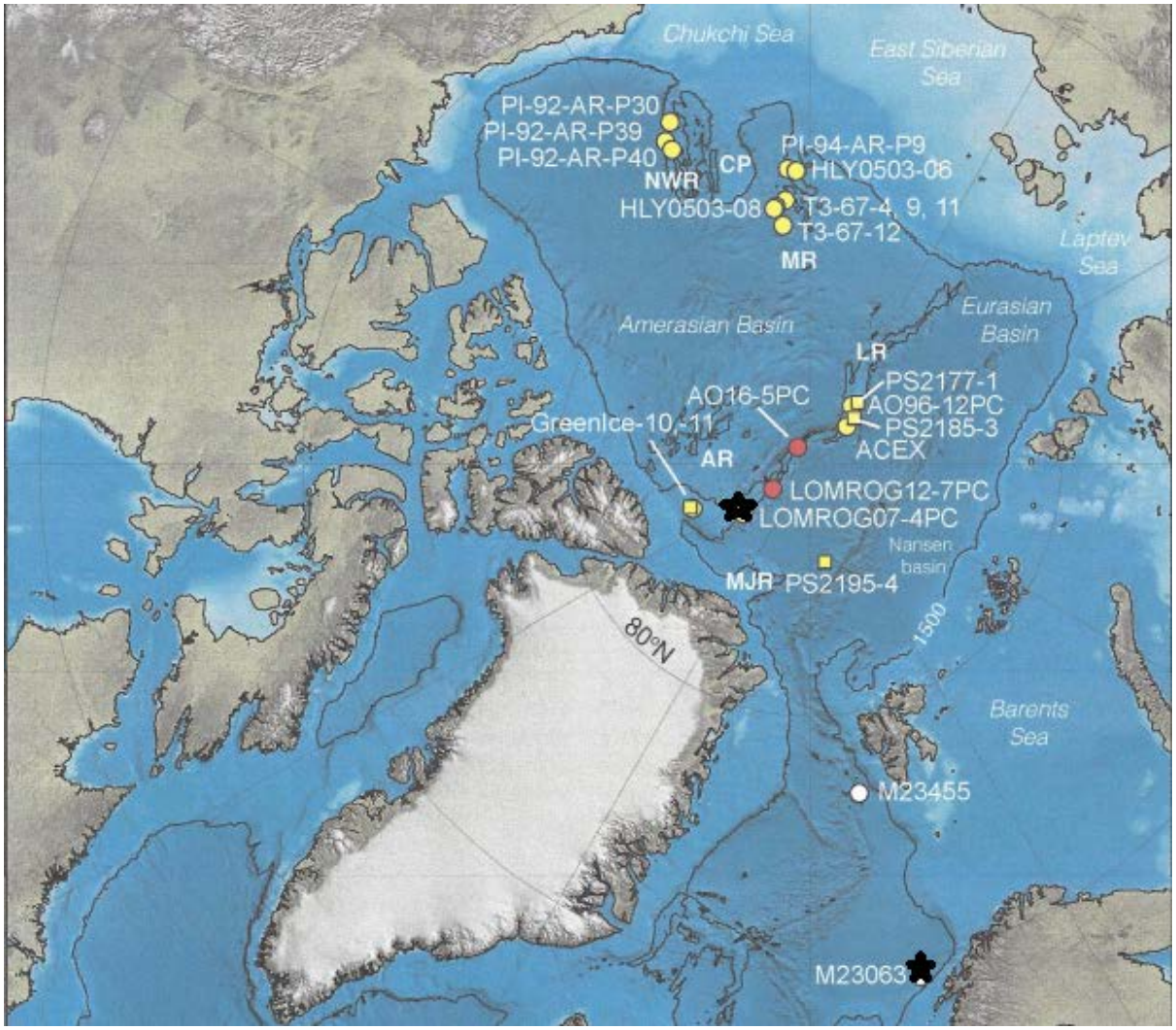


Figure 2: Key areas of coring and geography of the area of study. Samples were taken from coresM23063 (Nordic Sea, sample courtesy of A. Zhuravleva and H. Bauch) and LOMROG07-4PC (Arctic Ocean, Lomonosov Ridge –Greenland end)- black stars. This map, which is from O'Regan et al (2019), shows additional sites which were investigated in the latter author's study but not discussed here.

2 Background

2.1 A previous study of *Turborotalia*

One major study that is worthy of attention in considering this one is that of evolution and speciation of the Eocene genus *Turborotalia* (Pearson and Ezard 2014). Although *Turborotalia* is unrelated to *Turborotalita,* the approach is relevant. This Eocene study concerns the problems of ascertaining if speciation took place and used morphometrics to determine this through multivariate analysis. Foraminifera are especially useful in the stratigraphic record because they are present in large numbers in continuous successions. Evolution is sometimes considered to occur in discrete areas with stasis elsewhere but in the case of foraminifera due to circulating oceanic waters this is not the case (Pearson and Ezard 2014).

The history of morphometry and stratigraphy in marine microfossils date back to Hays in 1970 when he studied radiolarians from the Pacific and Southern Oceans (Pearson and Ezard 2014). These studies show a change in size of specimens and lineage branching in radiolarians. Planktonic foraminifera were first studied in this way in 1981 when Malmgren and Kennett studied different species over some millions of years (Pearson and Ezard 2014).

An important point to note is that changes in oceanic circulation can lead to geographical variations in the specimens studied over time. This can complicate theories of site specific evolution (Pearson and Ezard 2014). Another important point in studies of this nature is that a subjective discernment of different characteristics in morphotypes might be carried over into subsequent studies causing artificial discernment of key traits. Also of note, and this is applicable to my study, is that small sample sizes may not account for evolutionary change in the same way as large sample sizes (Pearson and Ezard 2014).

In comparing individuals it is assumed that once all various factors such as ontogenic stage or sex of the individuals are taken into account the genotypic or phenotypic traits will be more similar in two individuals of species X than in one of species X and one of species Y (Ezard et al 2010). An important consideration however is that intra species variation can be greater than inter species variation due to homology. This can confound morphometric studies (Ezard et al 2010).

Although genetic studies have allowed species to be delineated in detail, morphometrics is still a very useful technique for the following reasons. Sometimes morphometrics are the only data readily available and they can be integrated with genetic data to allow species delineation. Morphometrics allow the multi-locus aspect of a phenotype to be quantified whereas genetic studies very often are restricted to a short length of DNA. This is very important for specifying species type. In addition if the evolution is divergent these morphological traits that show the difference in specimen lines may be crucial to defining the two species (Ezard et al 2010).

The Eocene study chose a location for a core to be taken at Site 865 of the Ocean Drilling Programme. This site is within the North Pacific gyre in the tropics. A gyre is particularly suitable for study as it circulates water continuously within itself but is continuous with the rest of the ocean. The site they used was a guyot which although within the gyre has been gradually moving through plate movement (Pearson and Ezard 2014).

The Eocene team studied the extinct species *Turborotalia cerroazulensis* which was a particularly abundant and widely distributed Eocene foraminifer species. It can be shown over time using traditional visual species classification that there were six morphospecies showing gradual evolution. Earlier forms were more rounded whereas the shape of later forms were more angular with a keel around the edge (Ezard et al 2010)

Despite there being more than one morphospecies in the time series it was thought that only one morphospecies was present at a particular time. Two time intervals were assessed statistically to see

if there were morphological clusters of traits. Both upper and lower Eocene times were analysed (Ezard et al 2010).

There was also a change in chirality of specimens, sinistral becoming more dominant, plus an increase in the number of chambers in the final whorl and a general size increase (Ezard et al 2010).

This could be a wonderful example of evolutionary change in a species even if the species were coexisting. In their statistics the authors assumed Gaussian distribution of traits and used Principle Component Analysis. They note that if there is a hidden change in morphology, mean and standard deviations are unlikely to show any difference although it may be real. Therefore a Bayesian model was used which analysed the cluster i.e. its volume and shape etc. Stratigraphic successions were tested for clusters and stratophenetic linkage was applied. If there was an overlap of clusters an ancestral relationship is implied (Pearson and Ezard 2014).

If the species are cryptic it is believed that over time morphometrics will eventually be able to detect a significant difference between them (Pearson and Ezard 2014).

The change in morphospecies might be explained either by anagenetic change or as a result of geographic factors (Pearson and Ezard 2014).

However the alternative hypothesis that more than one species was present at a time was supported for the upper Eocene sample implying evolutionary divergence had taken place. This was not the case for the middle Eocene sample (Ezard et al 2010)(Figure 3).

The authors presume that the morphology selected was important for feeding, buoyancy or protection. Perhaps of relevance to my study is the suggestion that more compressed test shapes are less buoyant and therefore cooling temperatures of the Eocene meant tests could occupy a more suitable depth for survival (Pearson and Ezard 2014).

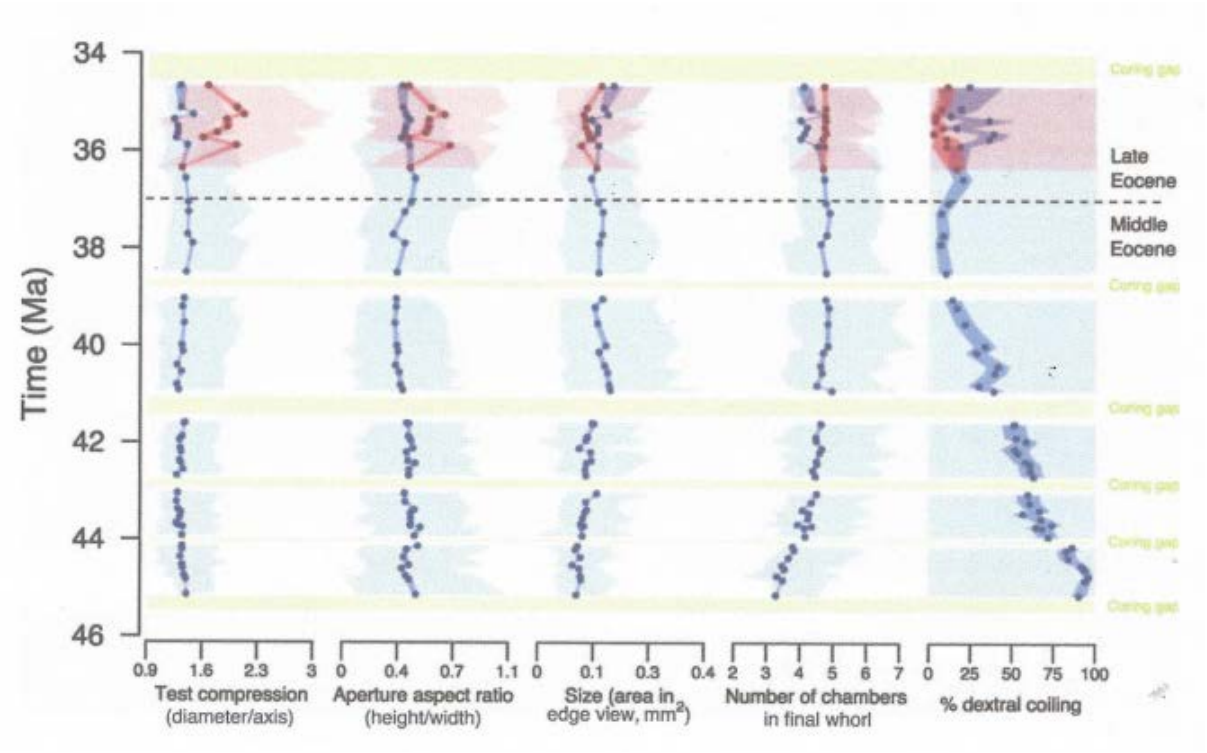


Figure 3: Evolutionary divergence of traits in Eocene *Turborotalita*. From Pearson and Ezard 2014.

2.2 Recent observations of *Turborotalita* on the Lomonosov ridge

My study is based on the work of O'Regan et al (2019) where *Turborotalita egelida* has been found pre MIS 5 in the Arctic Ocean and has been proposed as a marker for MIS 11. This would imply conditions which were out of the ordinary in MIS 11 in the oceanographic system of polar waters (O'Regan et al 2019).

O'Regan et al (2019) describe how correlation has been difficult in the Arctic because microfossils are erratic in occurrence and diversity. Also geomagnetic polarity reversals are common in the Arctic but appear anomalous and are difficult to synthesise with known geomagnetic stratigraphic levels according to the global framework. Furthermore stable isotope records are difficult to interpret

because of the sporadic occurrence of shells. This is why the finding of *T. egelida* is important (O'Regan et al 2019).

Moreover, Pleistocene sediments before the last interglacial in core LOMROG12-7PC found the presence of *Turborotalita* which makes their relationship to *T. egelida* and *T. quinqueloba* taxonomically important for stratigraphic reasons. From the cores LOMROG12-7PC and AO16-5PC specimens from MIS 5.1 and 5.2 look like *T. quinqueloba* which is found in the Nordic Seas and not the *T. egelida* found from MIS 11 (O'Regan et al 2019)(see figure 2).

Morphological distinctions between shells is the basis of foraminiferal classification. Using traditional visual approaches the *T.* quinqueloba morphology for instance has a final chamber that is ampulate and extends into the umbilicus, the coiling is enrolled and the wall texture implies a spinose phenotype. These features are found in the Lomonosov Ridge assemblages, however the specimens there are usually small (<125 microns) with smooth walls. This is different from the specimens of *T. quinqueloba* found in subpolar regions which are not translucent under light microscopy i.e. have gametogenic calcite. The Lomonosov Ridge *T. quinqueloba* are also smaller than *T. egelida* which typically has an open umbilicus and a rounded final chamber. Adding to the confusion is that small specimens presumably *T. quinqueloba* have a rounded final chamber in the Fram Strait core of MIS 5 (O'Regan et al 2019).

The relationship between *T. quinqueloba* and *T. egelida* remains unclear although molecular studies have shown two *Turborotalita* genotypes in cool Nordic and North Atlantic Seas. O'Regan et al (2019) speculate that Arctic *Turborotalita* is unlikely to have been brought by advection from waters further south because of predominantly counter clockwise circulation of ocean waters in the Arctic and also the fact that such immature tests that were advected would be dissolved on their way to the seafloor.

The most reasonable argument therefore is that warming conditions allowed *Turborotalita* to survive further north. It is queried whether the small size of these specimens perhaps indicates a different form of reproduction i.e. asexual (Kate Darling, personal communication). This commonly occurs in benthic foraminifera. In other words nature found yet another way of filling a biological niche. Maybe *Turborotalita* evolved to reproduce asexually to cope with more extreme climatic conditions and limited time for growth (O'Regan et al 2019).

The present study was useful in determining morphometrics of >125 micron specimens in Arctic waters as this area at this time usually contained small specimens. Also what is the relationship between *T. quinqueloba* and *T. egelida*? Did *T. quinqueloba* habitually enter Arctic waters in interglacials or was it just at certain times? Further more extensive work to be carried out on the Lomonosov ridge might answer these questions (O'Regan et al 2019).

2.3 Distribution of *T. quinqueloba.*

Before I go on to my method and results I thought it expedient to say what is known of the distribution of *T. quinqueloba* in the Arctic sea.

Studies show that the sea floor sediments of the Arctic Ocean are dominated by foraminifera of two species *N. pachyderma* (sin.) and *T. quinqueloba* (Volkmann 2000). Volkmann (2000) confined her study to the Fram Strait (81-82°N) where there is an inflow of warm Atlantic water in the East and out flow of cold surface water to the Nordic Sea in the West and the Outer Laptev Sea (76-80°N) where freshwater influx from Siberia gives low saline surface waters.

The Arctic sea is well stratified which means there is little vertical mixing and water movement is mainly by advection. Plankton tow studies show that in the Fram Strait individuals of *N. pachyderma* (sin.) and *T. quinqueloba* show maximum abundance at 0-100m depth. *N. pachyderma* (sin.) in its

juvenile stage is distinguished from *T. quinqueloba* by having a thicker wall without spines. However sometimes *T. quinqueloba* shed their spines. Juvenile forms of both species are commonly smaller than 125 microns (Volkmann 2000).

In the Outer Laptev sea *N. pachyderma* (sin.) is ten times more abundant than *T. quinqueloba* and the latter is at peak abundance between 100 and 200m water depth. When there is permanent ice coverage *N. pachyderma* (sin.) migrates to shallow water 0-50m in depth (Volkmann 2000).

In the Fram Strait *N. pachyderma* (sin.) shows a maximum abundance between temperatures of -1.5 and -1.8°C and of salinity between 32.6 and 34.0. *N. pachyderma* (sin.) prefers fresh cold water in the Arctic. *T. quinqueloba* in contrast occupies a wide temperature range of -0.7 to 3.4°C and narrow salinity range of 34-35. Therefore it occupies slightly lower water at the ice sea margin where salinities are higher (Volkmann 2000).

There is a strong seasonal fluctuation of numbers of foraminifera with highest numbers in late summer and autumn. Summer with its warmer temperatures causes water to be more stratified. *N. pachyderma* (sin.) is present at 4 individuals per m^3 in the upper 500m, *T. quinqueloba* only 0.5 per m^3 (Figure 4).

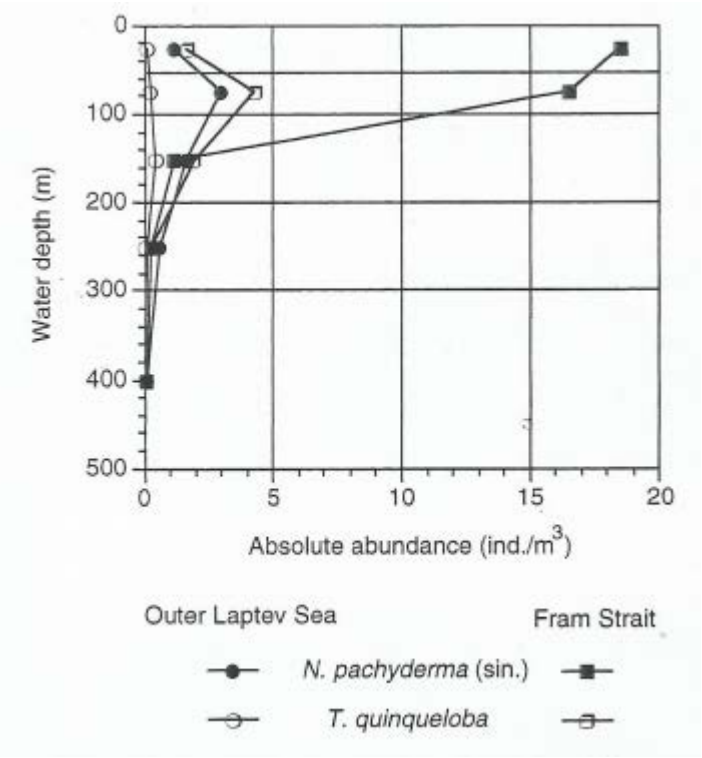


Figure 4: Living foraminifera average abundances from plankton tows (125-250 microns). From Volkmann (2000).

Studies have shown that planktonic foraminifers from other oceans are most abundant near the chlorophyll maximum at 30m water depth (Fairbanks and Wiebe 1980). However Volkmanns study showed *N. pachyderma* (sin.) and *T. quinqueloba* to exist below this chlorophyll maximum. The food source of *T. quinqueloba* is not known however they frequently occur with Atlantic copepods (*Calamus finmarchicus)* - (Volkmann 2000).

It is thought that reproduction of *N. pachyderma* (sin.) follows the synodic cycle and that in early ontogenic stages chambers grow very quickly. High numbers of individuals >250 microns in size are found at the time of the full moon with small numbers at the time of the new moon (Volkmann 2000). At the full moon high numbers of kummerform individuals are found (having an odd shaped final chamber)i.e. reproducing forms. A lunar cycle of reproductivity is believed to be the norm for *T. quinqueloba* as well (Volkmann 2000).

Isotope composition is important in the enquiry into the depth habitat of these foraminifers (Bauch et al 1997) but this is often hindered in *T. quinqueloba* by the dissolution of delicate calcite before sedimentation. This is not the case for *N. pachyderma* (sin.) which has a thicker shell (Vincent and Berger 1981).

3 Method

Samples were taken from the core of sediment from the two locations in the Nordic sea (M23063, 733cm) and Arctic sea (LOMROG07 PC04, 298-299cm) (see Figure 2). The foraminifera were sieved through a mesh to separate individuals greater than 125 microns. This was done because it is easier to be more confident with the species identification in slightly large specimens compared to small ones, in which it is possible to mix species. An aluminium 'microsplitter' was used to help reduce the sample to a manageable size for extracting a representative population of specimens for preparation. For Site M23063, 733cm all specimens were extracted from the >125 micron fraction, resulting in a foraminifera sample size of 215 individuals of *Turborotalita.* In the LOMROG07 PC04, 298-299cm sample the same sieving and splitting approach was taken, however even after splitting the sample there were many more than 200 individuals, therefore specimens were taken at random to achieve a sample size of 252 individuals.

Specimens of *Turborotalita* were identified by light microscopy amongst other forams by visual recognition. The main characters used to identify *T. quinqueloba* were the shiny delicate-looking shell wall, coiled shape with an ampulate final chamber. They co-occurred with a variety of other planktonic foraminifera including abundant *N. pachyderma* (sin.), rare *G. bulloides,* rare *Orcardia reideli,* and rare *Globigerinita uvula*. The interior Arctic *Turborotalita* morphospecies were similarly smooth walled but these lacked the ampulate final chamber and instead had a wide open umbilicus. Individuals were mounted on a glass slide, using water soluble paper glue, scored with

compartments, four to a compartment . They were laid on their spiral side so that the ventral surface (umbilical side) of the foraminifera was apparent.

Photography software and hardware was used to photograph each specimen from the microscope at magnification ×363 (×16 coarse magnifier on microscope). All specimen images were catalogued using Microsoft Excel. Distinguishing features were noted as well as number of chambers in the final (visible) whorl and also the chirality of coiling-dextral or sinistral.

Image J software was used to measure morphometric details of each specimen and recorded in the Excel spreadsheet. Measurements recorded were:-

1. Apertural lip width
2. Final whorl maximum diameter
3. Final whorl maximum breadth passing through the umbilicus
4. Final whorl whole area
5. Final whorl perimeter
6. Final whorl circularity
7. Final chamber area excluding lip
8. Final chamber perimeter excluding lip
9. Final chamber circularity excluding lip
10. Final + penultimate chamber area excluding lip
11. Final + penultimate chamber perimeter excluding lip
12. Final + penultimate chamber circularity excluding lip
13. Umbilicus area

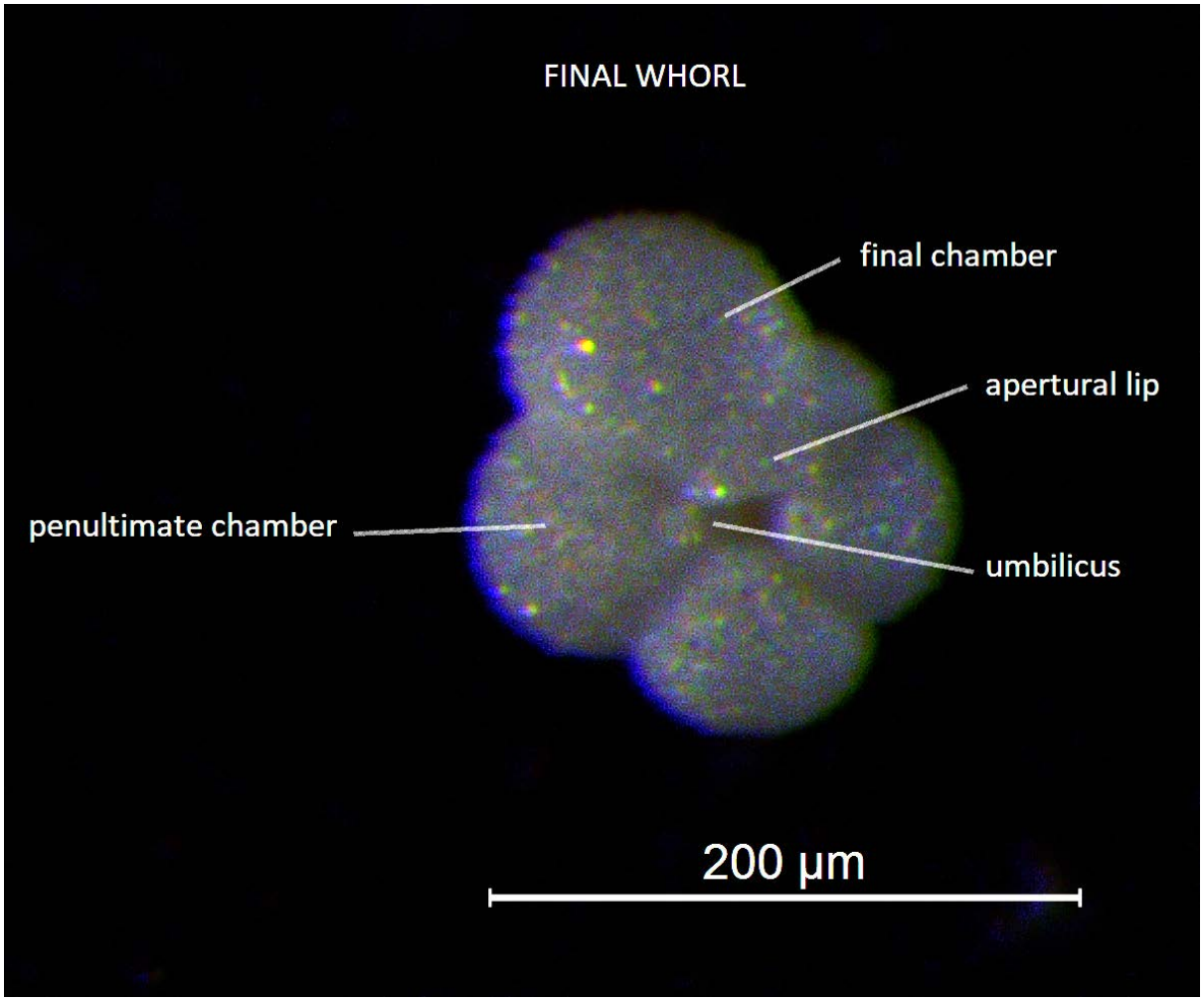


Figure 5: Principle parts of the final whorl of a specimen of *Turborotalita quinqueloba* from Core sample M23063 (Nordic Seas)

Microsoft Excel was used to calculate the mean, sample standard deviation, sample variance and range of values for each measurement for both samples. Equal sample variance was assumed in both samples and a Student t-test was used to compare the samples for each measurement. A probability of 0.05 was taken as significant and the P value for each t-test (two tailed) is recorded. If there proved to be a significant difference in the measurements taken on the two samples then it would be reasonable to infer that the two samples came from different populations of foraminifera.

4 Results

Of the shells catalogued there was a slight difference in chirality in the spirals of individuals between the two samples. From core LR07_PC04, 298-299cm 56% of individuals were sinistral and 44% dextral. In core M23063, 733cm 52% were sinistral and 48% dextral.

For the measurements recorded results were divided into class sizes and a histogram was plotted. Class boundaries were taken as the same for both samples for each measurement and plotted on the same histogram for comparison-series 2 and series 1. This allowed visualisation of a subsequent Student t test to compare the two samples. I generally felt that all the distributions were essentially Gaussian and this was a prerequisite for the test (Figure 6).

Equal variance was assumed between the two samples in the Student t test and probabilities for both one tailed and two tailed test were calculated based on the t statistic.

For apertural lip width (6a) the LR07_PC04 core sample had a mean of 12.84 microns, range 1.97-43.75; the M23063 sample mean was 12.59 microns, range 0-62.35. The two tailed t test showed test probability P of 0.656. Therefore there was no significant difference between samples.

For final whorl diameter (6b) LR07_PC04 core sample had a mean of 206.69 microns, range 156.8-322.19; the M23063 sample had a mean of 201.58 microns, range 146.57-292.22. Two tailed t-test showed probability P of 0.01468. Therefore the samples were significantly different.

For final whorl maximum breadth through the umbilicus (6c) LR07_PC04 core sample specimens had a mean of 160.46 microns, range 114.3-270.31; M23063 core sample mean was 168.24 microns range 122.15-233.96. The Student t test P value for a two tailed test was 5.24×10^{-5}. Therefore the two samples were significantly different from each other. In other words although the Arctic individuals had a greater diameter than the Nordic individuals they had less breadth.

For final whorl area (6d) LR07_PC04 core sample specimens had a mean of 25322.49 microns squared, range 16028.93-61381.25; M23063 mean 25361.99 microns squared range 16876.3-51152.92. Student t test P value (two tailed) was 0.940232. In other words there was no difference in area of specimens between samples.

For final whorl perimeter (6e) LR07_PC04 core sample mean was 630.21 microns, range 498.64-1003.55; M23063 sample mean 619.81 microns, range 496.84-914.87. Student t test (two tailed) P value was 0.108827 i.e. no difference statistically between samples.

For final whorl circularity (6f) LR07_PC04 core sample mean was 0.793, range 0.707-0.865; M23063 core sample mean was 0.823, range 0.721-0.904. T test P value (two tail) was 3.25×10^{-26}. Therefore statistically the Nordic Sea specimens were more circular than those from the Arctic Ocean.

For final chamber area excluding the apertural lip (6g) LR07_PC04 core sample mean was 6735.69 microns squared range 2390.99-17957.91; M23063 core sample mean 7103.22 range 2209.26-13841.97. P value (two tailed test) was 0.08243. Therefore, no significant difference between samples.

For final chamber perimeter excluding apertural lip (6h) LR07_PC04 sample mean was 294.09 microns range 183.13-492.75; M23063 core sample mean was greater 307.29, range 180.55-435.51. T test P value (two tail) was 0.003709 i.e. a significant difference.

Final chamber circularity excluding apertural lip (6i) showed LR07_PC04 core sample mean to be 0.954 range 0.826-0.98; M23063 mean 0.915 range 0.666-0.971. P value (two tail) was 1.57×10^{-28} i.e. the final chamber of Arctic specimens was significantly rounder than Nordic Sea specimens.

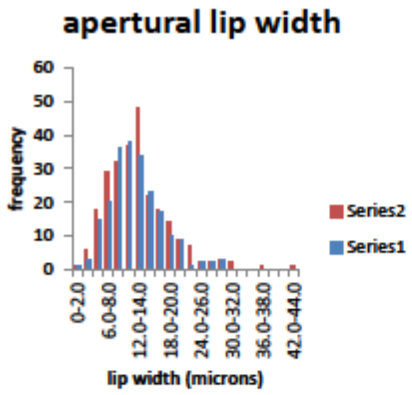
For final+penultimate chamber area excluding the apertural lip (6j) LR07_PC04 sample mean was 12839.53 microns squared, range 6514.81-30164.4; M23063 mean 13005.11 microns squared, range 6702.29-23627.39. T test (two tail) P value was 0.547304 i.e. no significant difference.

For final+penultimate chamber perimeter excluding the apertural lip (6k) the LR07_PC04 core sample mean was 485.62 microns, range 336.54-802.18; M23063 core sample mean 469.06 microns, range 322.16-668.63. P value (two tailed) was 0.003669 i.e. the Arctic Ocean measurements were significantly greater than the Nordic Sea ones.

For final+penultimate chamber circularity excluding the apertural lip (6l) LR07_PC04 sample mean was 0.681, range 0.506-0.89; M23063 sample mean was 0.741, range 0.461-0.926. P value (two tailed) was 4.13×10^{-18}. Therefore the Nordic Sea specimen measurements were more circular than those for the Arctic Ocean specimens.

For umbilicus area (6m) LR07_PC04 core sample mean was 895.73 microns squared, range 45.02-5569.21; M23063 sample mean 698.227 microns squared, range 14.24-2599.68. P value (two tailed) was 7.23×10^{-5}. Therefore the Arctic specimens had a larger umbilical area.

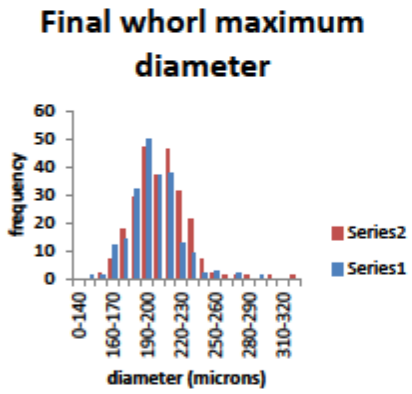
There was also a significant difference in number of chambers in the final whorl of these two samples (6n). LR07_PC04 sample mean was 4.385, range 3.5-5.6; M23063 sample mean was 4.482, range 3.8-5.5. P value (two tailed) was 8.43×10^{-5}. Therefore Nordic Sea individuals had more chambers in their final whorl than Arctic Ocean individuals.



		Series 2	Series 1
n		252	215
Mean		12.84	12.59
Sample SD		6.097	6.09
Sample variance		37.17	37.09
Minimum value		1.97	0
Maximum value		43.75	62.35

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	12.8435	12.59132
Variance	37.16731	37.08985
Observations	252	215
Pooled Variance	37.13166	
Hypothesized Mean Difference	0	
df	465	
t Stat	0.445757	
P(T<=t) one-tail	0.32799	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.65598	
t Critical two-tail	1.965079	

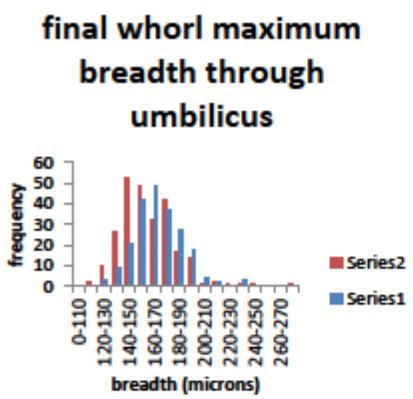
Fig.6a:Apertural lip width (microns). Series 2:core LR07_PC04,298-299 cm; series 1: core M23063, 733 cm



		Series 2	Series 1
n		252	215
Mean		206.69	201.58
Sample SD		23.29	21.42
Sample variance		542.38	458.9
Minimum value		156.8	146.57
Maximum value		322.19	292.22

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	206.6875	201.5826
Variance	542.3801	458.9018
Observations	252	215
Pooled Variance	503.9621	
Hypothesized Mean Difference	0	
df	465	
t Stat	2.449335	
P(T<=t) one-tail	0.00734	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.01468	
t Critical two-tail	1.965079	

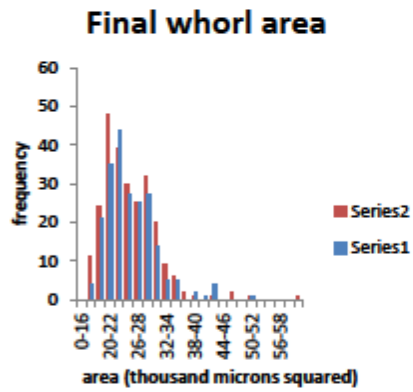
Fig.6b: Final whorl maximum diameter (microns). Series 2:core LR07-PC04, 298-299cm; series 1:core M23063, 733 cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	160.4606	168.2382
Variance	470.3873	363.2263
Observations	252	215
Pooled Variance	421.0702	
Hypothesized Mean Difference	0	
df	465	
t Stat	-4.08253	
P(T<=t) one-tail	2.62E-05	
t Critical one-tail	1.648137	
P(T<=t) two-tail	5.24E-05	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		160.46	168.24
Sample SD		21.69	19.06
Sample variance		470.39	363.23
Minimum value		114.3	122.15
Maximum value		270.31	233.96

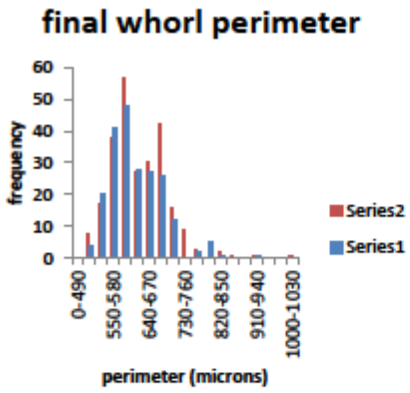
Fig.6c: Final whorl maximum breadth through umbilicus (microns). Series 2: core LR07_PC04, 298-299cm; series 1: core M23063, 733 cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	25322.49	25361.99
Variance	35011379	28826655
Observations	252	215
Pooled Variance	32165076	
Hypothesized Mean Difference	0	
df	465	
t Stat	-0.07502	
P(T<=t) one-tail	0.470116	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.940232	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		25322.49	25361.99
Sample SD		5917.04	5369.05
Sample variance		35011379	28826655
Minimum value		16028.93	16876.3
Maximum value		61381.25	51152.92

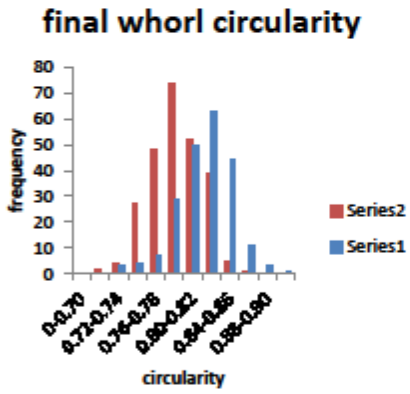
Fig.6d: Final whorl area (microns squared). Series 2:core LR07_PC04, 298-299cm; series 1: core M23063, 733 cm



		Series 2	Series 1
n		252	215
Mean		630.21	619.81
Sample SD		72.63	66.12
Sample variance		5275.3	4372.38
Minimum value		498.64	496.84
Maximum value		1003.55	914.87

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	630.2052	619.8073
Variance	5275.299	4372.375
Observations	252	215
Pooled Variance	4859.76	
Hypothesized Mean Difference	0	
df	465	
t Stat	1.606574	
P(T<=t) one-tail	0.054413	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.108827	
t Critical two-tail	1.965079	

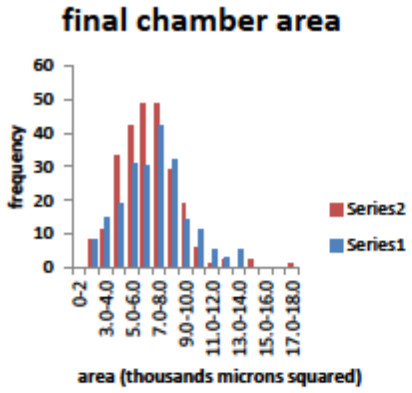
Fig.6e: Final whorl perimeter (microns). Series 2:core LR07_PC04,298-299 cm; series 1: core M23063, 733cm



		Series 2	Series 1
n		252	215
Mean		0.793	0.823
Sample SD		0.0277	0.0292
Sample variance		0.00077	0.00085
Minimum value		0.707	0.721
Maximum value		0.865	0.904

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	0.792901	0.822558
Variance	0.000766	0.00085
Observations	252	215
Pooled Variance	0.000805	
Hypothesized Mean Difference	0	
df	465	
t Stat	-11.2616	
P(T<=t) one-tail	1.76E-26	
t Critical one-tail	1.648137	
P(T<=t) two-tail	3.52E-26	
t Critical two-tail	1.965079	

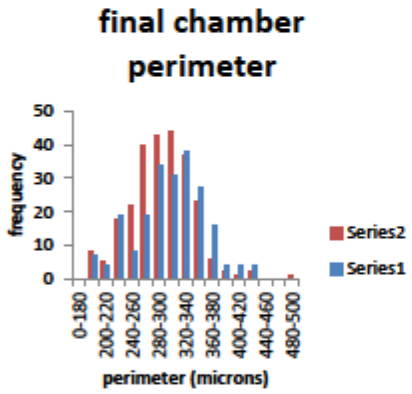
Fig.6f: Final whorl circularity (a/1). Series 2:core LR07_PC04, 298-299 cm; series 1: core M23063, 733cm



		Series 2	Series 1
n		252	215
Mean		6735.69	7103.22
Sample SD		2133.61	2429.15
Sample variance		4552274	5900777
Minimum value		2390.99	2209.26
Maximum value		17957.91	13841.97

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	6735.694	7103.215
Variance	4552274	5900777
Observations	252	215
Pooled Variance	5172875	
Hypothesized Mean Difference	0	
df	465	
t Stat	-1.74051	
P(T<=t) one-tail	0.041215	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.08243	
t Critical two-tail	1.965079	

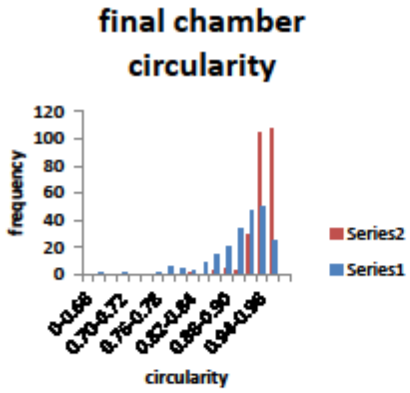
Fig.6g: Final chamber area (microns squared). Series 2:core LR07_PC04, 298-299cm; series 1:core M23063, 733cm



		Series 2	Series 1
n		252	215
Mean		249.09	307.29
Sample SD		45.8	52
Sample variance		2097.53	2704.28
Minimum value		183.13	180.55
Maximum value		492.75	435.51

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	294.0902	307.2915
Variance	2097.532	2704.283
Observations	252	215
Pooled Variance	2376.768	
Hypothesized Mean Difference	0	
df	465	
t Stat	-2.91665	
P(T<=t) one-tail	0.001855	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.003709	
t Critical two-tail	1.965079	

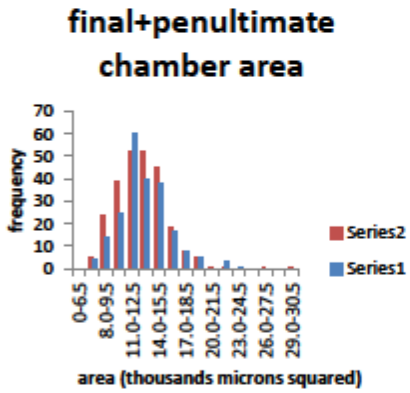
Fig.6h: Final chamber perimeter (microns). Series 2: core LR07_PC04, 298-299cm: series 1:core M23063, 733 cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	0.954127	0.915181
Variance	0.00036	0.002297
Observations	252	215
Pooled Variance	0.001251	
Hypothesized Mean Difference	0	
df	465	
t Stat	11.85987	
P(T<=t) one-tail	7.87E-29	
t Critical one-tail	1.648137	
P(T<=t) two-tail	1.57E-28	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		0.954	0.915
Sample SD		0.019	0.0479
Sample variance		0.00036	0.0023
Minimum value		0.826	0.666
Maximum value		0.98	0.971

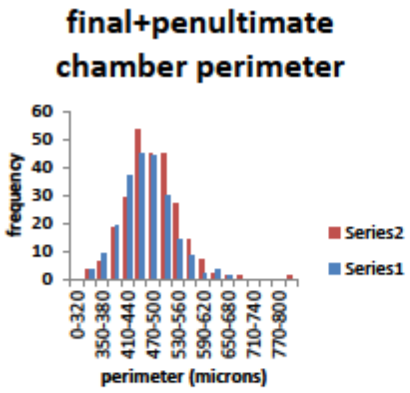
Fig.6i: Final chamber circularity (a/1). Series 2:core LR07_PC04, 298-299 cm; series 1: core M23063, 733 cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	12839.53	13005.11
Variance	9299574	8148650
Observations	252	215
Pooled Variance	8769901	
Hypothesized Mean Difference	0	
df	465	
t Stat	-0.60225	
P(T<=t) one-tail	0.273652	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.547304	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		12839.53	13005.11
Sample SD		3049.52	2854.58
Sample variance		9299574	8148650
Minimum value		6514.81	6702.29
Maximum value		30164.4	23627.39

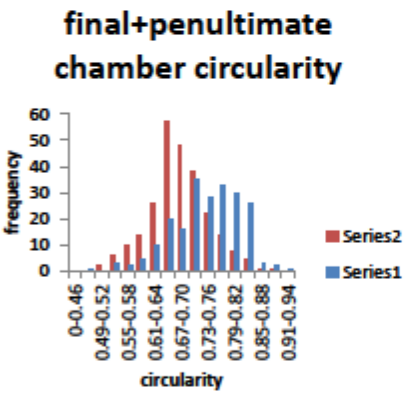
Fig.6j: Final+penultimate chamber area (microns squared). Series 2:core LR07_PC04, 298-299cm; series 1:core M23063, 733cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	485.6182	469.0622
Variance	4106.333	3287.063
Observations	252	215
Pooled Variance	3729.293	
Hypothesized Mean Difference	0	
df	465	
t Stat	2.920129	
P(T<=t) one-tail	0.001834	
t Critical one-tail	1.648137	
P(T<=t) two-tail	0.003669	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		485.62	469.06
Sample SD		64.08	57.33
Sample variance		4106.33	3287.06
Minimum value		336.54	322.16
Maximum value		802.18	668.63

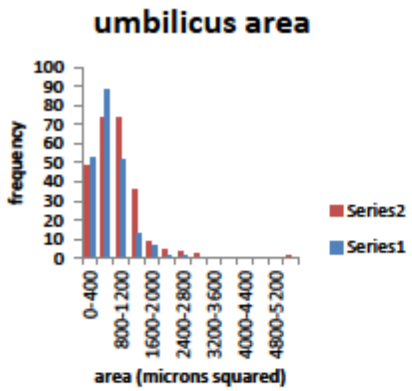
Fig.6k:Final+penultimate chamber perimeter (microns). Series 2: core LR07_PC04, 298-299cm; series 1:core M23063, 733cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	0.681218	0.74073
Variance	0.004345	0.005821
Observations	252	215
Pooled Variance	0.005024	
Hypothesized Mean Difference	0	
df	465	
t Stat	-9.0434	
P(T<=t) one-tail	2.07E-18	
t Critical one-tail	1.648137	
P(T<=t) two-tail	4.13E-18	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		0.681	0.741
Sample SD		0.0659	0.0763
Sample variance		0.00435	0.00582
Minimum value		0.506	0.461
Maximum value		0.89	0.926

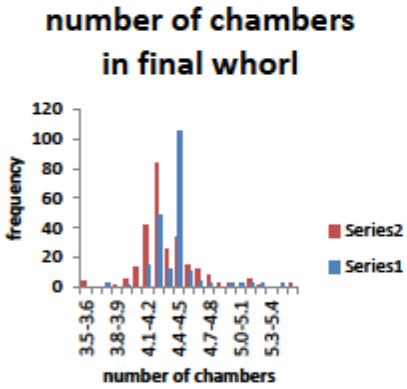
Fig.6l:Final+penultimate chamber circularity (a/1). Series 2: core LR07_PC04, 298-299cm; series 1: core M23063, 733cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	895.7314	698.2265
Variance	367701	181935.9
Observations	252	215
Pooled Variance	282209.1	
Hypothesized Mean Difference	0	
df	465	
t Stat	4.004546	
P(T<=t) one-tail	3.62E-05	
t Critical one-tail	1.648137	
P(T<=t) two-tail	7.23E-05	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		895.73	698.277
Sample SD		606.38	426.54
Sample variance		367701	181936
Minimum value		45.02	14.24
Maximum value		5569.21	2599.68

Fig.6m: Umbilicus area (microns squared). Series 2:core LR07_PC04, 298-299 cm; series 1: core M23063, 733 cm



t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	4.385317	4.48186
Variance	0.074644	0.061772
Observations	252	215
Pooled Variance	0.06872	
Hypothesized Mean Difference	0	
df	465	
t Stat	-3.96679	
P(T<=t) one-tail	4.22E-05	
t Critical one-tail	1.648137	
P(T<=t) two-tail	8.43E-05	
t Critical two-tail	1.965079	

		Series 2	Series 1
n		252	215
Mean		4.385	4.482
Sample SD		0.273	0.249
Sample variance		0.0746	0.0618
Minimum value		3.5	3.8
Maximum value		5.6	5.5

Fig.6n: Number of chambers in the final whorl. Series 2:core LR07_PC04, 298-299cm; series 1: core M23063, 733cm

5 Discussion

Out of the 14 variables measured in the two MIS 11 samples from the interior Arctic and Nordic seas 9 showed significant difference between the samples that imply consistent morphological differences that might be relevant to biological and taxonomic differences. Before exploring those ideas further the limitations of the method must be discussed.

5.1 Limitations

Visual morphometrics by its very nature is subjective but it is hoped observers would get similar results as a result of the sheer weight of statistical probability. Sources of variation among the pictures include orientation of the specimen-in three dimensions, as some chambers lie on top of others; the angle of specimens to the vertical is different along with the angle to the vertical and horizontal of constituent chambers. For instance the apertural lip looks different when viewed from different angles, which can make identifying its limits problematic at times.

Statistical analysis is limited by the small sample sizes. It is difficult to decide whether there was a speciation event as only samples from MIS 11 are given. Earlier studies such as the Eocene study (Pearson and Ezard 2014) give an idea as to when speciation took place in *Turborotalia* by sampling over a wide time frame, i.e. over a time series of 4-5 million years.

Also the present study is limited to comparison using only simple statistics and the Student t test. More involved methods for larger studies use principle components analysis to detect different clustering of traits between species (Ezard et al 2010).

The Eocene study used slightly different measurements to the ones I have used but is very similar in those recorded. Umbilical angle I have not been able to measure in my study for instance.

5.2 Findings

My findings nevertheless are interesting for the following reasons. Applying the Student t test to measurements of final whorl area, final whorl perimeter, final chamber area and final + penultimate chamber area resulted in P values above 0.05 (two tailed test assuming equal variance) between samples. This implies that the null hypothesis is proved for these measurements, i.e. that there is no difference in the populations from which these samples were drawn.

However final whorl maximum diameter, final whorl maximum breadth passing through the umbilicus, final whorl circularity, final chamber perimeter, final chamber circularity, final + penultimate chamber perimeter, final + penultimate chamber circularity and umbilicus area were all significantly different between the two samples (P value less than 0.05 for two tailed test assuming equal variance between samples). This proves the alternative hypothesis that there is a difference between these samples implying that they come from different populations.

By viewing the final chamber alone it is subjectively quite visible without taking measurements that the Arctic sample is more circular: this was proved statistically. The results would seem to infer that although measured areas are similar between samples ie size, the shape of the individuals in the two samples is different.

Bauch (1994) in his study of test size in the Norwegian-Greenland Sea based his measurements on the maximum diameter of the test on the ventral aspect. This was the same measurement that I used. He found that test size decreased in colder water to the West compared to water in the East of the study. He speculates whether this is related to smaller size specimens being brought in by Atlantic currents, perhaps juvenile varieties of *Turborotalita* (Bauch 1994). In contrast my study showed that the maximum whorl diameter increased in the colder water of the interior Arctic station, however the whorl breadth decreased. Although my study is hampered by only looking at

one size fraction of shells (>125 microns) my result tends to imply that there is no difference in test sizes between the two sites at this MIS 11 interval. However the shape of the shells is different as is the relative size of the umbilicus, which is larger in the interior Arctic sample.

Furthermore in my study number of chambers in the final whorl is significantly different between the two samples with the Lomonosov Ridge *Turborotalita* having on average 4.39 in the final whorl compared to 4.48 in the Nordic Seas supporting the alternative hypothesis that the samples come from two different populations.

The apertural lip shows no significant difference between samples confirming the null hypothesis that there is no difference in the samples for this measurement. Importantly, the measured variables are relevant to taxonomic separation of species and might be useful in future species definitions.

Interesting to note is that although *N. pachyderma* shows a significant predilection for the sinistral form in cold Polar waters, in my study sinistral and dextral forms were fairly equal in both samples of *Turborotalita* ; 52% sinistral in the Nordic Sea, 56% sinistral in the Arctic Sea. Also worthy of mention is that the Lomonosov ridge sample only contained one species of foraminifera i.e. no *N. pachyderma* (sin.); is there an environmental cause for this?

5.3 Evolution

The biological history of planktonic foraminifera is a history of extinctions and radiations in which the same basic patterns tend to emerge. Biologists try to predict the rate of evolution and how oceanographic changes affect this evolution of species (Pearson 1993).

Homeomorphy is present across the species of planktonic foraminifera as individuals compete for the same niches- this makes deciding which species an organism belongs to difficult (Pearson 1993).

Anagenesis is common amongst planktonic foraminifera where there is a progressive linkage of intermediate stages in a gradual evolution. This may be the case in my study of two populations of *Turborotalita*- both samples representing intermediate forms. Chronoclines illustrate this well although my study is taken from one time frame-MIS 11. Actual cladogenesis where one genus or higher taxa splits into another may occur in this way but is not anagenetic (Pearson 1993).

This makes taxonomy difficult as the theory of biological evolution and its Linnean classification supports the idea of discrete species rather than a gradual imperceptible transition. Pearson suggests that this 'punctuated equilibrium' is not applicable to planktonic foraminifera or other marine microfossils (Pearson1993).

Sheldon in 1987 had this problem with Ordovician trilobites; the individuals defied categorization into discrete species; this is the same with marine microfossils (Pearson 1993). Simpson in 1953 noted that taxonomic evolution is different to structural evolution as the rates of change of both are quite different (Pearson 1993).

Therefore the term morphospecies has become useful. This may be a central form within the population or represent a polymorphism, ontogenic state or a geographical form (Pearson 1993).

This would appear to be the case with my two samples which maybe represent different morphospecies. An ontogenic stage would be unlikely because of the similarity in size of the sample specimens but them being geographical variants is a possibility.

5.4 Insights

On a deeper level than morphometrics genetic variation among individuals is important in establishing whether they belong to different genotypes. Homeomorphy can be confusing in this

respect. The emergence of the new genetics has enabled taxonomic debates to be solved (Schiebel et al 2018).

Compared to morphometrics, such results are not subjective. Homeomorphy amongst planktonic foraminifera is very common. In the Tertiary there was an initial radiation of foraminifera from the individuals that had survived the Cretaceous. Possibly certain benthic forms also made the jump to becoming planktonic (Schiebel et al 2018). Nature is very resourceful and if a particular morphotype is successful in a particular niche it will be selected by evolution. This is the case for the MIS 11 and also the present and is perhaps an environmental case for my two samples being different.

It has been discovered that many morphospecies may be comprised of different genetic types and that therefore many 'cryptic' species are apparent (Schiebel et al 2018). It would seem logical that the next step in this research would be to genetically type the species. Molecular data is telling us that polyphyletic origins of planktonic foraminifera are derived from two or more benthic lines after the Cretaceous-Tertiary extinction (Schiebel et al 2018).

A further approach not achieved in this study would be the analysis of isotopes within the shell substance. For instance marine carbon cycling can be interpreted through carbon-13 estimations as well as vital effects within organisms. Furthermore abiotic forcings such as temperature, salinity and pH are important in the pelagic zone and oxygen isotope and carbon isotope study is of vital importance (Schiebel et al 2018).

For many planktonic foraminifera many cryptic species are being discovered. This is the same for *Turborotalita quinqueloba* as well as *N. pachyderma* and *G. bulloides*. High throughput sequencing (HTS) allows large volumes of water to be sampled and offers hope of yet more information on defining cryptospecies (Schiebel et al 2018). In the near future this will allow the ecologies of these cryptic species to be studied in detail along with their geographical preferences (Schiebel et al 2018).

6 Conclusion

Foraminifera are numerous in the stratigraphic record and geographically ubiquitous. It is obvious that at high latitudes planktonic foraminifera are affected by climatic change like the rest of nature and are therefore worthy of serious study (Schiebel et al 2018). This is especially true of organisms such as *Turborotalita quinqueloba* in the warm interglacial of MIS 11 and the present day. Studying the past is the key to the future!

Nature always finds a way and the two samples in my study may constitute an anagenetic difference related to the warming climate at the poles.

The fact that individuals between the two samples had a different shape is interesting and seems relevant even though there was a lack of evidence for one being an earlier ontogenic stage as both samples had similar sized specimens. Perhaps the shape difference was related to different buoyancy requirements to inhabit different depths of water for feeding or temperature requirements etc.

Likewise it is difficult to determine whether individuals were transported into Arctic waters by advecting currents or were indigenous dwellers.

The proposal of O' Regan et al that the *Turborotalita* of MIS 11 Arctic waters was a less known *Turborotalita egelida* morphotype is supported in this study by the fact that the final chamber is more circular and the umbilicus more open than the Nordic Sea specimens (O'Regan et al 2019).

Worthy of consideration I feel is that the two samples in the Nordic Sea and Arctic Sea were intimately related- they had the same size of apertural lip for instance. Perhaps they represent intermediates or morphospecies. Perhaps they are an example of anagenetic evolution in planktonic foraminifera. I hope this study is beneficial to the outcome of this ongoing research.

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