Biometry of Distephanus medianoctisol (Silicoflagellata) in the sea-ice covered environment of the central Arctic Ocean, summer 2004

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Biometry of *Distephanus medianoctisol* (Silicoflagellata) in the sea-ice covered environment of the central Arctic Ocean, summer 2004

Hideto Tsutsui and Kozo Takahashi*

Abstract

Samples for living marine phytoplankton *Distephanus medianoctisol* (Silicoflagellata with seven-sided basal ring) were obtained from the central Arctic Ocean during Integrated Ocean Drilling Program (IODP) Arctic Coring Expedition (ACEX) 302 which took place during summer 2004. Biometric and statistical analyses were performed on three different sample types: (1) Sea water, (2) Sea-ice, and (3) Diatom mat samples. The objectives of this study are to clarify morphological characteristics of *D. medianoctisol* with biometrics and statistics in three respective sample types. Out of 1226 total specimens of *D. medianoctisol*, 491 skeletons were measured for their length of four representative parts: radial spine, basal side, lateral rod, and apical side.

The differences in environmental characteristics depending on the samples types were reflected in the length of radial spines: specimens in Sea water Group represented 1.16 times longer spines than those in Diatom mat Group. Specimens belonging to Sea-ice Group represented an intermediate spine length between those of Sea water Groups and Diatom mat. As one of the possible explanations, the difference in spine length may be due to difference in space availability in sea water and diatom mat habitats, respectively. Thus, there is a future possibility to employ the spine length of *D. medianoctisol* for assessing environmental conditions in which they grew.

Keywords: Silicoflagellate, *Distephanus, D. medianoctisol*, biometry, morphology, the Arctic Ocean, IODP ACEX.

1. Introduction

Takahashi et al. (in press) described a seven-sided silicoflagellate as *Distephanus medianoctisol* based on samples collected in the central Arctic Ocean including at the North Pole during Integrated Ocean Drilling Program (IODP) Arctic Coring Expedition (ACEX) 302 which took place in summer 2004. The samples included three types: Sea water, Sea-ice, and Diatom mat. They noted a significant difference in radial spine length of *D. medianoctisol* between Sea water and Diatom mat samples.

However, the detailed and comprehensive morphometric understanding of the taxon was beyond the scope of that paper and thus it remained to be pursued in a future study. It was also partially due to limited sample population size of the skeletons for the measurements (Takahashi et al., in press).

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Therefore, the objectives of this study are: (1) to verify the biometric observations pursued in Takahashi et al. (in press) with an established biometric method, statistical processing, and increased number of specimens; and (2) to discuss the relationships between skeletal variation of *D. medianoctisol* and the differences in environmental conditions in which they grew.

Many biometric studies were conducted on various microfossil groups (de Meuter and Symons, 1975; Lazarus, 1986a, b, c; Kurihara and Takahashi, 2002; and Schmidt et al., 2004). As a biometric observation with a large number of specimens for living microplankton, for example, Kurihara and Takahashi (2002) performed over 39,000 measurements on diatom frustules during an eight-year long-term sediment trap monitoring at fixed stations, and discussed the changes in frustules size and oceanographic conditions.

In particular, there has been several biometric studies for silicoflagellates including the works on extinct genus *Corbisema* (McCartney and Loper, 1992), modern species belonging to genus *Dictyocha* and genus *Distephanus* (McCartney, 1988), and a large number of living *D. speculum* specimens in a modern ocean (Tsutsui et al., submitted). While the biometric method requires substantial processing time, high-resolution morphometric information can provide a wealth of information for several applications and thus overriding such a demerit.

Silicoflagellates are unicellar marine phytoplankton, commonly found in the modern oceans. They are assigned to Kingdom Protista, Division Heterokontophyta, Class Dictyophyceae (Van den Hoek et al., 1995). They have two flagella of un-equal length (Moestrup and Thomsen, 1990). Their skeletons are represented by basket-like framework forms, which are made of the skeletons with a pipe-like hollow cavity (for example, Haq and Boersma, 1978; Tappan, 1980). In general, two genera of silicoflagellates, *Dictyocha* and *Distephanus*, commonly occur in the modern oceans. The criteria for genera *Dictyocha* and *Distephanus* are based on the central attachment structure of the basal ring; genus *Dictyocha* has an apical bar and genus *Distephanus* has an apical ring. Their primary reproduction mode is considered as asexual, but Locker and Martini (1986) and McCartney (1987) suggested the possibility of having a sexual reproduction phase as well. Boney (1973) observed the reproduction process of *Dictyocha fibula*: first the basal ring formed, other skeletal elements developed, then finally the daughter skeleton as a mirror image of the parent skeleton formed.

2. Materials and Methods

2.1. Samples

Takahashi et al. (in press) obtained Sea water, Sea-ice and Diatom mat samples at four sampled locations (Fig. 1), which included *D. medianoctisol*. Logistics of samples are listed (Table 1). Since the central Arctic Ocean at the times of sampling was in the pack ice condition the ice-breaking was necessary for generating open water space for water sampling. The ice-breaking was performed by I/B & R/V *Oden* belonging to the Royal Swedish Academy of Science. The sampling was performed at the stern of the vessel with a bucket (ca. 15 liters), a weight, and a rope by aiming at open water just generated by the I/B. The bucket sampling was repeated twice to obtain ca. 30 liters as a total sample volume of sea water and sea-ice. The Sea water samples were collected at three occasions whereas Sea-ice and Diatom mat samples were captured in two occasions only (Table 2: Takahashi et al., in press).

The obtained samples were treated with hydrogen peroxide, and then mounted on microslides with Mount Media® of the Wako Chemicals Co. Ltd in the laboratory on I/B & R/V *Oden* immediately after sampling. The method for sample identification for types and dates follows that of Takahashi et al. (in press). That is, the sample types are designated as SW, SC, and DM for sea

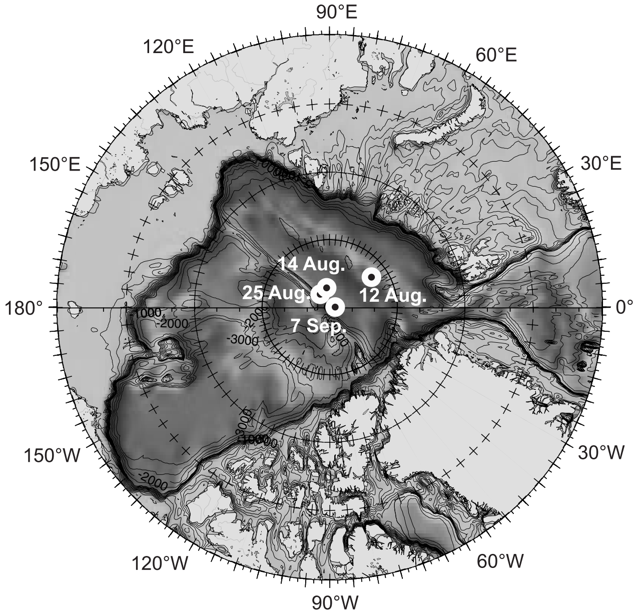

Fig. 1. Sampled locations (four circles) in the central Arctic Ocean. Map is from the Collaborative Research Center (SFB) 574 site: http://www.ifm-geomar.de/.

Table 1. Logistics of sampled locations (Takahashi et al., in press).

Date in 2004	12 August	14 August	25 August	7 September
Latitude	87°03.2'N	87°54.8'N	87°56.0'N	89°59.0'N
Longitude	40°11.0'E	138°32.6'E	140°43.3'E	63°11.9'E
Air temperature (°C)	-1.6	-0.4	-1.5	-8.2
Water temperature (°C)	-1.7	-1.8	-1.2	-1.6
Water depth (m)	3550	1220	1172	4458
Ice thickness Old/Fy: Average (cm)	150*	250/100	120/200	200/120
Ice thickness Old/Fy: Max (cm)	300*	300/150	180/300	400/180

water, sea-ice, and diatom mat, respectively. The sample date and month follows the sample type and hence, for example, the sea water sample obtained on 14 August 2004 is designated as SW14Aug (Table 2).

The microslides were brought back to the shore-based laboratory located at the Kyushu University. The microscope magnification at x600 throughout this study was employed for investigating specimens and photomicrography. Such a high magnification is needed in order to prevent erroneous measurements of the skeletons because that otherwise the skeletons cannot be clearly seen sometimes due to their low contrast or near transparent appearance. Furthermore, silicoflagellates have skeletal frameworks in three-dimensional manner rather than being flat. Their ideal skeletal form and terminology are illustrated (Fig. 2). When focused on their apical ring in apical view (Fig. 2A), the basal ring becomes out of focus. This is because that apical ring and basal ring are connected with lateral rod (Fig. 2B) and that the distance between the two planes of apical ring and basal ring exceeds the focal depth at x600 magnification. Figure 2C represents an oblique view of the specimens illustrated in Figs. 2A and 2B. Therefore, two frames of light micrographs

Table 2. Numbers of counted individuals or pairs of double skeletons of silicoflagellates. The unit employed here represents number of individuals per microslide.

		D. medianoctisol				*D. speculum*						
Sample Type	Sample Date	Normal form (Single)	Double skeleton	Double skeleton (normal+aberrant)	Aberrant form (Single)	Normal form (Single)	Double skeleton	Double skeleton (normal+aberrant)	Aberrant form (Single)	Aberrant double skeleton	Aberrant double skeleton (not mirror image)	Ohter Silicoflagellates
Sea Water	12 August		No sample									
	SW14Aug	139			20	35			2			2
	SW25Aug	147	54	2	27	49	10	4	4	4		6
	SW7Sept	272	100	36	64	12	4		3			1
Sea-Ice	12 August		No sample									
	SC14Aug	90	26		18	16	8				2	1
	SC25Aug	12	6			1	2					2
	7 September		No sample									
Diatom Mat	DM12Aug	94			5	4						
	DM14Aug	68	4	2	40	9			1			
	25 August		No sample									
	7 September		No sample									
TOTAL		822	190 (95 pairs)	40 (20 pairs)	174	126	24 (12 pairs)	4 (2 pairs)	10	4 (2 pairs)	2 (1 pair)	12

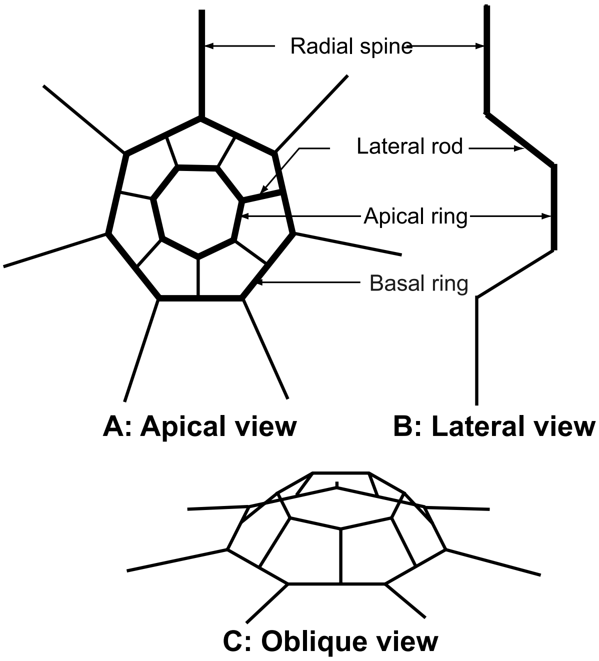

Fig. 2. Terminology and an ideal skeletal form of genus *Distephanus*.

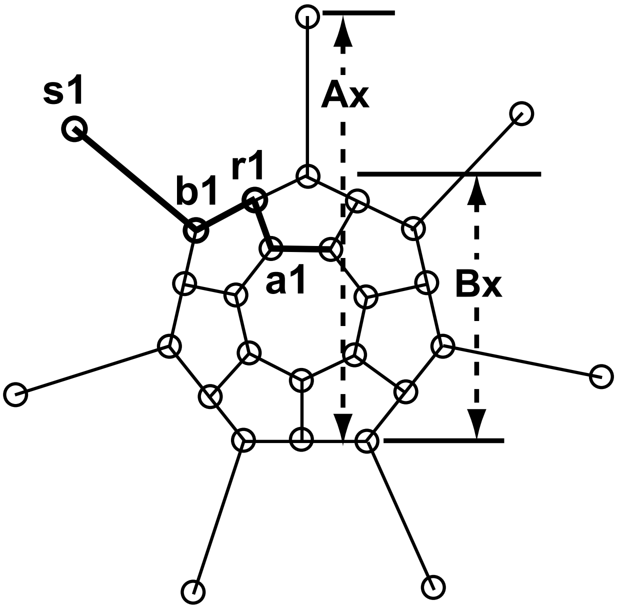

Fig. 3. Numbering method of skeletal elements for the measurements.

were taken for one specimen: one is focused on apical ring, and the other is focused on basal ring. An actual measurement is based on such a synthetic photograph of the two frames.

2.2. Biometric method of Distephanus medianoctisol

Measurements for *D. medianoctisol* are based on light micrographs as stated above. Basic measurement method and algorithm follow that of Tsutsui (2000). The transfer algorithm from a *D. speculum* microscopic image to a wire-frame data on this biometric system follows the following two rules. (1) A terminal point on radial spine is defined as 's' and other points are defined as 'b' for a point in basal ring, as 'r' for a point in lateral rod, and as 'a' for a point in apical ring (Fig. 3). After the labeling procedure is completed, (2) each point is replaced on the coordinate data for computer graphics (Fig. 3). When the initial characteristics of a specimen of *D. medianoctisol* cannot be assigned, then the biometric algorithm will assign temporarily numbers for all the skeletal parts along the clockwise perimeter. In default configuration, the starting number begins at ten O'clock: s1, b1, r1 and a1 points.

Because that *D. medinoctisol* has three-dimensional structure, true length of lateral rod has to be calculated from the length in apical view and the distance between apical and basal ring planes. However, in this study, the length of lateral rod was measured as two-dimensional length in apical view due to the lack of focus depth gauge belonging to the light microscope. The observation code has two data structure: (1) identification/observation code with database structure, and (2) length data for each skeletal part. Such observational data can readily be converted in spreadsheet for further statistical analysis application.

3. Results

3.1. Count results of D. medianoctisol and other silicoflagellate taxa

Count results of *D. medianoctisol* and other silicoflagellate taxa are tabulated (Table 2). Takahashi et al. (in press) provided the results of specimens' counts, but they did not offer coordinates (ordinates and abscissa) on microslide stage for each of the silicoflagellate specimens. Although the count data in this study are based on the results of Takahashi et al. (in press), we had to re-spot the specimens for biometrics and hence the counts were not exactly identical but more or less the same within an acceptable allowance.

Subsequently, a total of 1226 individuals of *D. medianoctisol* and 170 individuals of *D. speculum* were counted from all three-sample types. In addition to ordinary specimens, the count results included aberrant forms and paired individuals, the latter were counted as two skeletons for a paired number. Other silicoflagellates than *D. medianoctisol* and *D. speculum* included were twelve individuals. The target specimens employed in this study were selected when fulfilled the following two prerequisites: (1) the specimens lying flat on a micro slide so that the measured distance is close to reality without substantial errors; and (2) other than aberrant forms. The observational system employed in this study does not allow measurements for aberrant form. When one or more of the following criteria are satisfied they are defined as aberrant forms: (a) all or any skeletal parts are deformed (e.g., twisted, absent, possessing extra-structure); (b) any skeletal parts are not symmetric in apical view; and (c) any parts ordinarily supposed to be present are absent.

3.2. Measured results for D. medianoctisol

A total of 491 specimens were determined to be suitable for biometric measurements and the following subtotals for each of the sample types were examined: (1) 305 specimens from Sea water;

(2) 87 from Sea-ice; and (3) 99 from Diatom mat samples. As a basic set of statistics for each type of samples the followings are listed: minimum (hereafter Min), maximum (Max), mean (Mean), standard deviation (SD), cumulative variance (CV), kurtosis (Kurt) and skewness (Skew) (Table 3).

The SD for radial spine was 4.83 µm, and the CV was 33.1 (Table 3). These values were much higher than other skeletal parts: basal side, lateral rod and apical side. The values in Kurt and Skew provide a good measure for a normal distribution. The Kurt radial spine was -0.96. In these cases, variables must be checked by plotting in a graph. All skeletal parts of the Skew were confirmed to be greater than 0: it means that values are skewed towards the right side. Since the values for the Kurt were less than 3, thus it means the distribution of many of rather similar values with extraordinary small number of dissimilar values.

Table 3. Summary biometric statistics for the measured lengths in total specimens.

N=491	Radial Spine	Basal Side	Lateral Rod	Apical Side
Min (µm)	4	8	3	3
Max (µm)	25	13	6	7
Mean (µm)	14.6	10.7	5.0	5.3
SD (µm)	4.83	0.95	0.57	0.57
CV	33.05	8.85	11.25	10.72
Skew	0.11	0.28	0.26	0.29
Kurt	-0.96	-0.65	-0.33	-0.17

4. Discussion

4.1. Reproduction of Distephanus medianoctisol and % contribution to Distephanus

The populations of *D. medianoctisol* are prosperously growing in the study area, which is suggested as having many double skeletons counted representing their reproduction phase. This has already been documented by Takahashi et al. (in press: Table 2). The silicoflagellates reproduction involves division, resulting in two daughter cells of a mirror image from a parent cell (e.g., Boney, 1973; Tappan, 1980). The fact that 230 individuals (=115 pairs) of double skeletons were found, illustrating significant productivity (see, for example, Takahashi and Blackwelder (1992) for a comparison with the middle latitude).

In other areas, the sinking silicoflagellate flora at sediment trap Station PAPA (50ºN, 145ºW; 3800 m trap depth) in the North Pacific was contributed nearly 90% by *D. speculum* (Takahashi, 1985). Moreover, the dominant species of silicoflagellates was *D. speculum* with the relative abundance of 96% in total specimens at the sea surface at Station 2-2 (57.3ºS, 150ºE) in the Pacific Sector of the Southern Ocean (Kawabata and Nishida, 1992). However, the relative abundance of *D. speculum* in this study accounted for only twelve % of total specimens, perhaps responding to the specific environmental conditions of the Arctic Ocean with the perennial sea-ice.

4.2. Normal distribution curve of all skeletal parts

Frequency distributions of the measured data are shown (Fig. 4). The Frequency data for basal side (Fig. 4B), lateral rod (Fig. 4C) as well as apical side (Fig. 4D) all showed nearly a single modal normal distribution pattern in all the sample type Groups. On the other hand, radial spine of Sea water Group (Fig. 4A, solid line) showed a bi-modal distribution which could not be clearly split into two modes. Especially, the smaller mode (i.e., left peaks, hereafter with this explanation) of Sea water and Sea-ice (dotted line) are located nearly at 13 µm, however, the right side peak in Sea

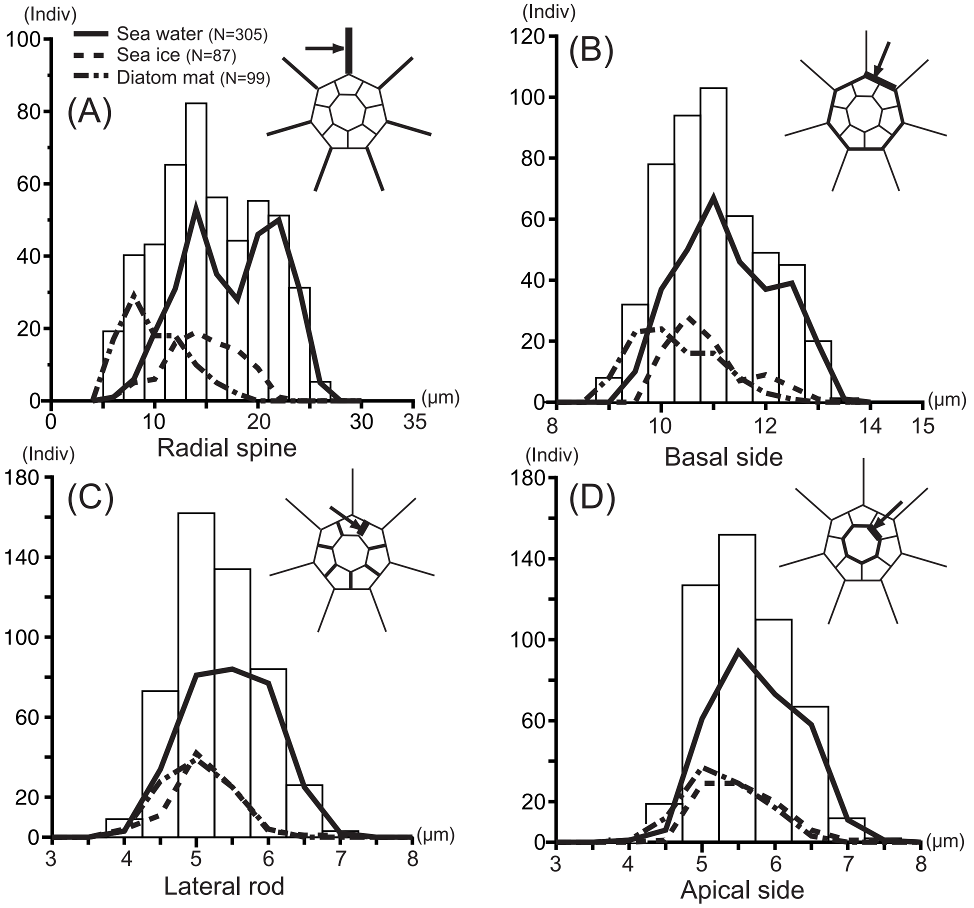

Fig. 4. Histograms for size distribution in total specimens and distribution curves on lengths of each of the skeletal parts for three sample types.

water Group appeared from ca. 22 μm. Moreover, there is one clear minimum frequency value seen between the two modes in the histogram, whose characteristics can only be seen in Sea water Group.

In addition, the modal peaks for radial spine of Diatom mat Group are located from 7 to 9 μm (Fig. 4A, partial broken/dotted line). Takahashi et al. (in press) suggested that Diatom mat Group has short radial spines and basal sides compared to those of Sea-ice or Sea water Groups. However, other skeletal parts except for radial spine did not show a clear difference like that shown by radial spine. It seems that the variation in radial spine can appear readily compared to other skeletal parts.

4.3. T-test results for axis length in Sea water and Diatom mat Groups

Sea water versus Diatom mat Groups represent opposite trends in their radial spine length, but other statistical characters do not clearly show such an opposite trend. Therefore, not only radial spine but also basal ring is the best combination for checking skeletal characters with statistics (Table 4). A T-test has been performed between Sea water and Diatom mat Groups. The variables employed here are the ratios between the length from spine tip to opposite attachment of basal side (hereafter Ax) and basal ring diameter (hereafter Bx) (Fig. 3). Since Takahashi et al. (in press) pointed out earlier the difference in their radial spine length between Sea water and Diatom mat

Table 4. The T-test results of spine axis (Ax: maximum of spine axis length) and basal ring diameter (Bx: max. of basal ring diameter). The class values are the Sea water and the Diatom mat group.

	T-value	F-value	Prob.>F'
Spine axis max. (Ax)	-15.04*	1.86	0.0004 (<1%)
Basal axis max. (Bx)	-10.87*	1.44	0.0329 (<5%)
Ratio (Ax : Bx)	-15.28*	1.67	0.0033 (<1%)
			Prob.<|T| 0.01*

Groups, the t-test should be very useful.

Ax values are the most convenient observed values reflecting radial spine length difference. The computed results for Ax and Bx lengths suggest a clear morphological difference with <1% or <5% probabilities, in particular, indicating that the length of radial spine reflects the sample type difference. However, Bx does not clearly show the difference relative to Ax. As a result, spine length is the best for expressing environmental form in Sea water and Diatom mat Groups.

4.4. Morphological characters for sample types

For a reference, the t-test results are shown for all skeletal parts with Sea water and Diatom mat Groups (Table 5). Of course the length of radial spines shows a clear difference between Sea water and Diatom mat Groups, but the length of lateral rod also show a secondary significant value. However, a clear skeletal difference cannot be recognized in the normal distribution curve (Fig. 4C). These morphological differences in radial spine and lateral rods reflect difference in the environmental conditions in which they grew, namely the eco-type of genetic expression. That is, *D. medianoctisol* has a layout of radial spines freely in the environmental conditions of sea water which does not limit the extension of radial spines. On the other hand, *D. medianoctisol* in Diatom mat is hard to extend their radial spine length because of the following reasons. (1) The known high ice-algae diatom population density (Katsuki et al., in press) provides a negative effect in extending thier radial spines as the physical space is limited in sea-ice (e.g., brine channels); (2) Long extended radial spines as plankton are no longer needed in Diatom mat (epiphytic habitat, not planktonic); (3) It can conserve energy by building minimal amount of silica skeletons and thus avoiding a possible competition with co-habiting diatoms for nutrient adsorption; and (4) It is also possible that co-habiting diatoms as a competitor emit chemically/biologically suppressing compounds to *D.*

Table 5. The T-test results for all skeletal parts. The class values are the Sea water and the Diatom mat group.

	T-value	F-value	Prob.>F'
Radial spine	-15.95*	2.16	0.0000 (<1%)
Basal side	-10.63	1.36	0.0707
Lateral rod	-8.67*	1.68	0.0030 (<1%)
Apical side	-7.18	1.23	0.2249
			Prob.<|T| 0.01*

medianoctisol and thus *D. medianoctisol* is stimulated to produce shorter spines. If such chemical reactions work for shorter radial spine of *D. medianoctisol*, it can be considered as allelopathy. Essentially the allelopathy is the act by a species trying to control the competitor with secreting toxin (allelochemicals) on land plant (Rice, 1984). One of micro algae produced and released a toxin for controlling other competitors in specific environmental conditions (e.g., Granéli et al., 2008).

A schematic illustration is presented for the relationships between different Ax lengths of ideal three sample types; Sea water, Sea-ice and Diatom mat (Fig. 5). The Ax value for Sea water Group is 1.83, Sea-ice is 1.75, and Diatom mat is 1.57, demonstrating the distinctive environmental difference. The results are also reflected in the histogram curves and the statistics of the normal distribution. Except for the physical space reason mentioned earlier, the nutrition problem for silicoflagellates probably results in significant influence for Sea water and Diatom mat Groups, respectively. That is, sea-ice diatoms continuously compete with silicoflagellates in nutrient consumption. Therefore, not only the abundance of double skeleton numbers but also the radial spine lengths in Diatom mat samples are significantly different compared to those in Sea water samples. Summarizing the above, diatoms in Sea water or Sea-ice are not as dense as in Diatom mat samples and hence *D. medianoctisol* in Sea water can build and extend freely of their radial spine length without a possible inhibitory act by diatoms.

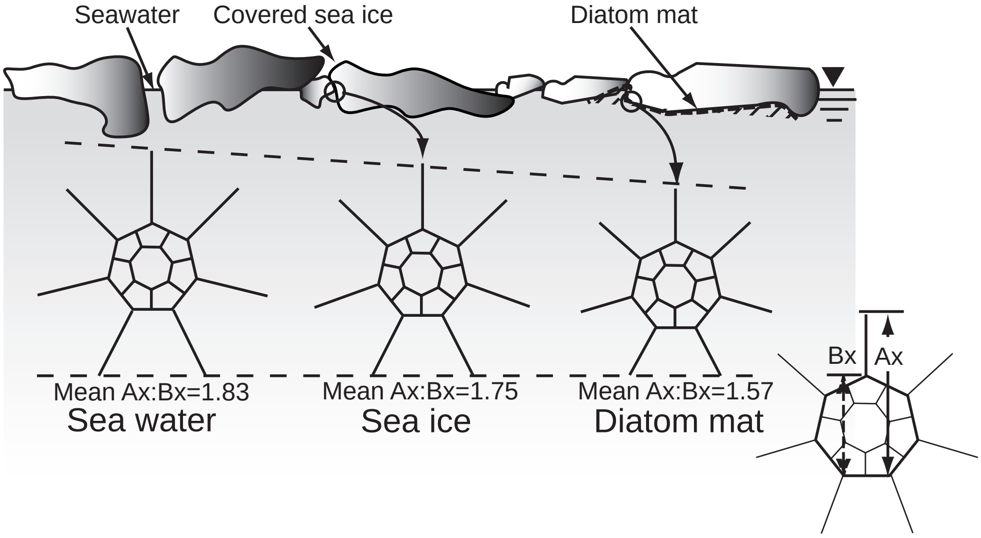

Fig. 5. Mean ratios of Ax/ Bx for each of sample types.

4. Summary

1. A total of 1226 specimens of *Distephanus medianoctisol* were observed in the samples collected from the central Arctic Ocean (summer 2004). The 491 measured specimens belong to three different environmental types: Sea water (N = 305), Sea-ice (N = 87), and Diatom mat (N = 99).

2. Dominant species and the relative abundances in total are very different between this study and the previous studies at Station PAPA (50ºN, 145ºW; 3800 m trap depth) in the North Pacific or

Station 2-2 (57.3ºS, 150ºE, 0 m depth) in the Southern Ocean.

3. Radial spines of *D. medianoctisol* represent two morphological characteristics: Diatom mat Group represents short radial spines whereas Sea water Group represents long radial spines.

4. *Distephanus medianoctisol* can possibly change the length of radial spines while they grow, according to the ambient environmental conditions. One of the plausible reasons for the short radial spine length in Diatom mat relative to those in Sea water are possibly due to the poor physical space availability. Although not proven here and thus topics for future studies, competition or allelopathy for *D. medianoctisol* by diatoms are also discussed.

5. Acknowledgements

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