A METHOD OF ILLUSTRATING THE MORPHOLOGICAL EVOLUTION OF COCCOLITHS WITH 3D ANIMATIONS APPLIED TO CALCIDISCUS LEPTOPORUS

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ABSTRACT

Animated diagrams are illustrated to show the complex microevolutionary trends and phylogeny of morphotypes in the Miocene to Recent coccolithophorid plexus of **Calcidiscus leptoporus** (calcareous nannoplankton). The present animations are supplementary information to an extended data set about the same nannoplankton group which recently appeared in a printed article (Knappertsbusch, 2000). The animations illustrate the complex microevolutionary behaviour through time of these algae in three dimensions, which was previously not possible. Watching with red-green glasses anaglyphs of the animations can be experienced in three dimensions, which makes the microevolutionary patterns even more accessible to a general audience. The aim of the present contribution is to document the methods to arrive at such graphical representations, which may be applied to future morphological and evolutionary studies with similar data sets. The microevolutionary patterns seen in these animations in 3D confirm the results seen in the previously published two-dimensional printed version. The article contains supplementary, hitherto unpublished data and software, that are useful for further analyses about the evolution of **C. leptoporus.**

INTRODUCTION

Electronic media greatly enhance the ability to visualize multidimensional observations arising from paleontological studies. Lyons and Head (1998) were the first to illustrate the power of multimedia and interactive graphical tools for visualization of microfossils in taxonomic research (and more examples have become available since then). The study presented herein is an example where the advantages of animated scenes are exploited for a set of morphometric measurements, in this case of the Miocene to Recent coccolithophorid **Calcidiscus leptoporus.** For this organism, an extended set of morphometric data representing the past 23 million years exists (Knappertsbusch 2000). In that study, the hypothesis of punctuated equilibrium (Eldredge and Gould 1972) was evaluated. Nine morphotypes of **C. lepto-**

porus were distinguished on the basis of coccolith size and number of radial elements on the distal side of the coccoliths. During time-series analysis of these data an interesting microevolutionary pattern emerged showing several intervals of frequency-mode separation (=cladogenetic events) in the morphospace of the measurements through time, including phases of unidirectional phyletic change and periods of stasis.

The interpretation of these data, however, was difficult, and comprehensive visualization of the observed microevolutionary trends to a wider audience persisted as a real challenge. Showing the data in two dimensions or treatment of the data with traditional statistical procedures always led to a loss of interesting details of the phylogenetic relationships between morphotypes. Early attempts to illustrate these data with an interactive graphics and data-plotting program capable of display in three dimensions (MacSpin) were premature (in the early 1990's) because of the lack of technology for electronic mediation. At that time, the only possibility was to document the pattern in printed media with the risk that many interesting aspects of **C. leptoporus** morphological evolution would remained obscure.

In Knappertsbusch (2000) various techniques were explored to document the morphological evolution of **C. leptoporus** in printed form. Although the morphological variability of this species through time could be illustrated, these techniques are rather abstract and remain less understandable to non-specialists. With the advent of graphical software tools for publication of animated scenes in the internet, such difficulties have now vanished. Complex data sets can now be illustrated in a more comprehensive way. The goal of the present contribution is to exploit the electronic medium to illustrate the evolutionary history of **C. leptoporus** with the help of animated scenes and to document their construction in order to demonstrate alternative ways of presenting morphometric data. Some of these illustrations are also prepared as stereo diagrams (anaglyphs), and can be watched in three dimensions with the help of red-green glasses. The source of the raw data, together with the derived data and software tools (all in the public domain), that were necessary for the construction of the animations, are given so that specialists can experiment and explore the **C. leptoporus** data set further. In this context the present contribution is not a duplication of existing results, but provides useful supplementary information about the evolution of **C. leptoporus** that is not otherwise available to the scientific community.

REVIEW OF PREVIOUS TAXONOMIC CONCEPTS AND THE PHYLOGENETIC RECONSTRUCTION OF C. LEPTOPORUS

In order to better explain the construction of the **C. leptoporus** animated phylogenetic scenes, a brief summary of the taxonomic concept and the morphometric measurements that were developed in Knappertsbusch (2000) is provided below. The motivations of the entire study, descriptions of core and sample locations, measurement techniques and precision are discussed in detail in that reference and are not repeated here. The calculations of numerical ages of all samples follows the integrated chronology of Berggren et al. (1995) and is also documented in that reference.

Coccospheres of **Calcidiscus leptoporus** (Figure 1) are covered by circular to sub-circular calcite platelets. On their surfaces (=distal side) these coccoliths are ornamented by sinistrally imbricated elements with sinusoidally shaped sutures. Using the diameter of the coccoliths and the number of elements per cycle in these
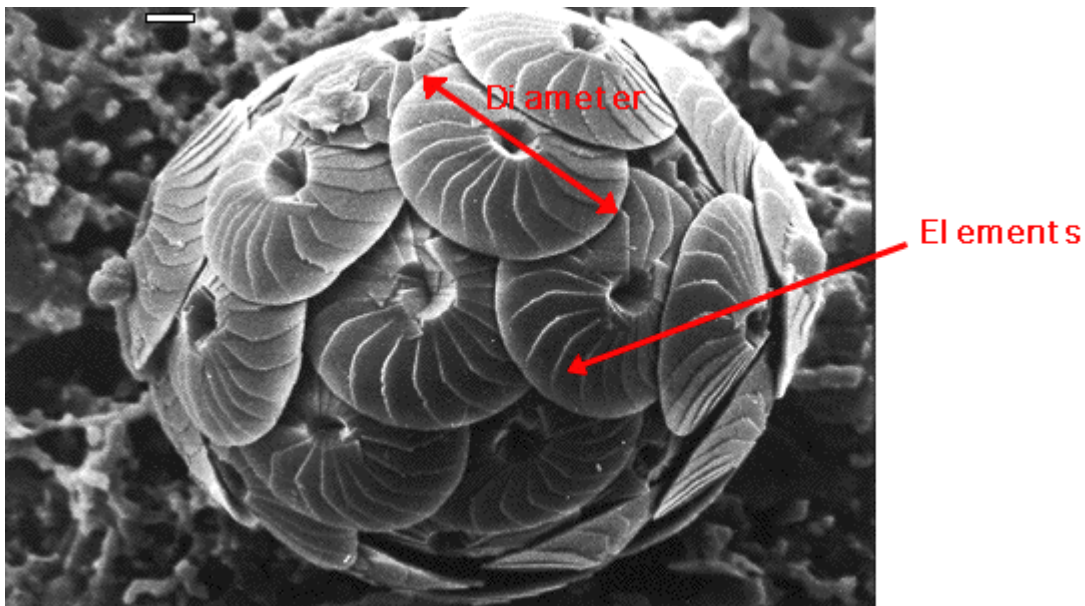
Figure 1. **Calcidiscus leptoporus** (Murray and Blackman). Coccosphere of an intact cell of from the living plankton. Scale-bar indicates 1 µm. Mediterranean Sea.

shields, various authors have distinguished several morphological variants of **C. leptoporus** in both living and fossil assemblages (Bukry and Bramlette 1969; Janin 1981; Baumann 1990; Knappertsbusch et al., 1997). The subspecific, and in some cases the specific, positions of these morphotypes in the diameter versus elements space are, however, still not yet firmly established because patterns of accepted variation within these morphotypes exhibits substantial overlap. As a result, additional criteria must be applied for reliable diagnosis. **Calcidiscus macintyrei**, for example, which is a very large extinct morphovariant, is considered a separate species by stratigraphers, whereas other investigators treated it as an informal morphovariant (Janin, 1981; Knappertsbusch et al. 1997; Knappertsbusch 2000).

The characterization of **C. leptoporus** by coccolith size and number of distal shields was, however, found useful for morphotype separation in **C. leptoporus**. This distinction was especially clear when contoured bivariate diagrams of coccolith frequencies were constructed (see exam-

ple in Figures 2 and 3). Based on this technique Knappertsbusch (2000) elaborated a taxonomic scheme with nine morphotypes from a global set of living, Holocene, and Miocene to Pleistocene assemblages (Figure 4). The interpretation of the stratigraphic distribution of these morphotypes led to the putative phylogenetic reconstruction of **C. leptoporus**
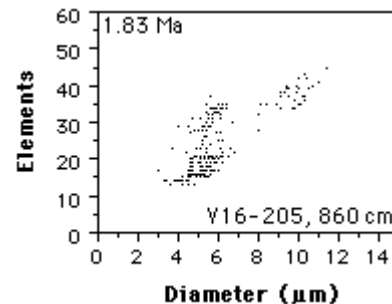

Figure 2. Contoured scatter diagrams as exploration tool for morphotype distinction: An example with a sample from core V16-205, 860 cm. Scatter plot of measurements of the coccolith diameter versus number of elements in the distal shield. The figure shows clustering of the data points into three different groups. Using a gridding algorithm Figure 3 was constructed so that frequency changes of coccoliths per grid-cells can be better followed. The contoured diagrams are easier to interpret for evolutionary changes of the coccoliths .
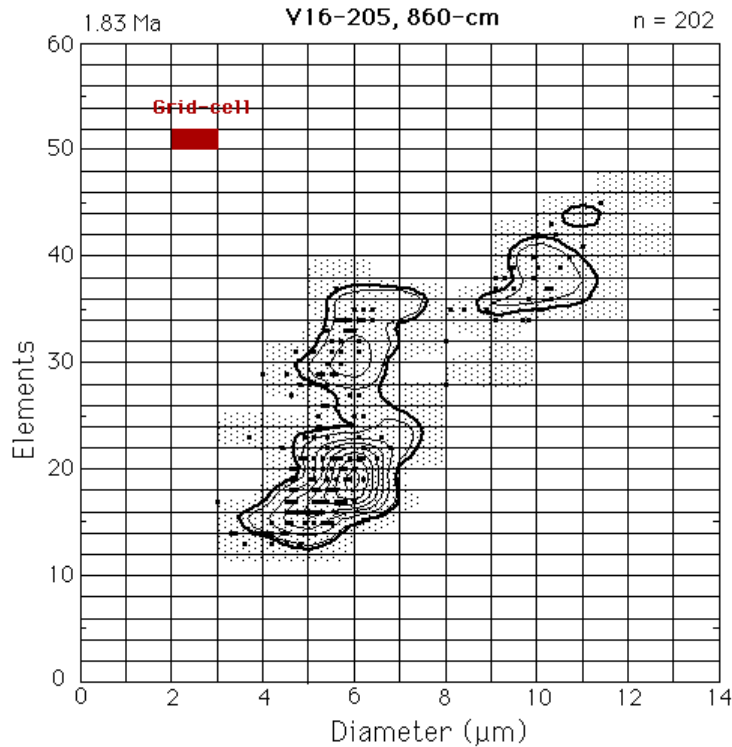
3

Figure 3. Gridded and contoured data derived from the scatterplot shown in Figure 2. The original scatter data are indicated by black points. The vertical and horizontal lines indicate the grid with a grid-cell size of 1 μm by two elements. Coccoliths that fall within a grid-cell were counted using program Grid.f, which resulted in coccolith frequencies per grid-cell. The coccolith frequencies were the slight smoothing algorithm in Surface III and contour intervals of two coccoliths per grid-cell. The shaded area indicates the distribution of non-zero frequencies of less than two coccoliths per grid-cell. Note, that some shaded grid-cells contain no coccoliths which is due to smoothing and boundary effects (a coccolith, that falls onto a grid-line is encountered as half a coccolith). The thick contour line encircles areas with two or more coccoliths per grid-cell. This contour-line was chosen in order to construct the animated diagrams of coccolith changes through geological time as shown in Figure 6A-6B, and Figures 8 to 9.
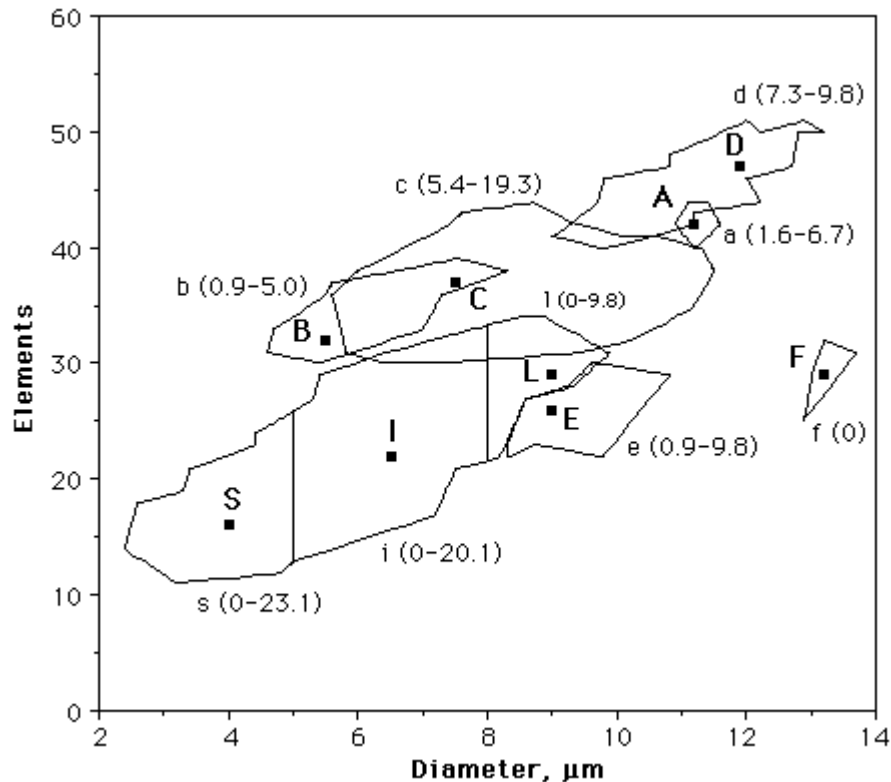

Figure 4. Taxonomic scheme of C. leptoporus morphotypes (from Knappertsbusch, 2000), that was applied during the present study. Black squares indicate the positions of frequency modes of individual morphotypes (labelled with large letters). The closed lines encircle the morphological variability for each morphotype (labelled with small letters). The numbers in parentheses indicate the temporal range of morphotypes in millions of years.
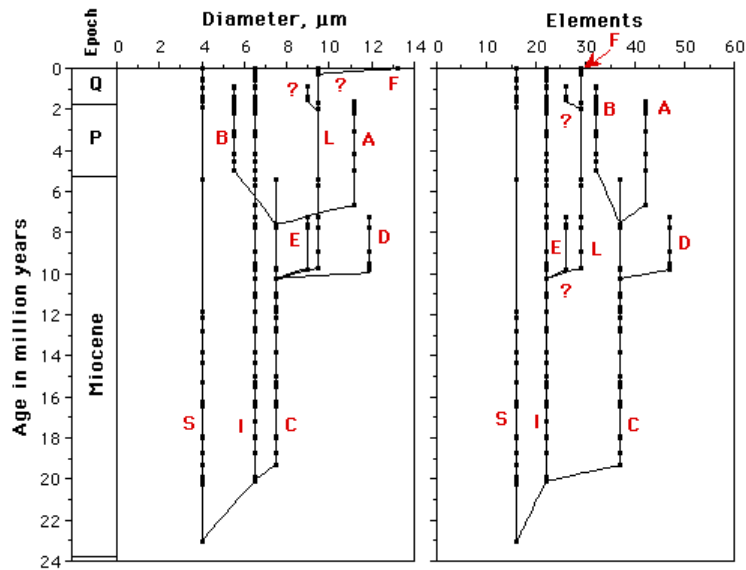
Figure 5. Reconstruction of the bivariate phylogenetic relationships among C. leptoporus morphotypes (designated with red letters) from the Miocene to Recent, that resulted from Approach II described in Knappertsbusch (2000). The diagrams were derived by application of the taxonomic scheme illustrated in Figure 4.

from the Miocene to Recent shown in Figure 5.

The phylogenetic reconstruction obtained in this way was compared with a semi-continuous mathematical data model, that interpolated between coccolith densities per unit areas of diameter versus elements (=grid-cells, see Figure 3) in samples from one time-level to the next. This approach avoided application of the artificial classification scheme shown in Figure 4 (the details of this model are explained under "Approach 3" and in Figures 8 and 9 of Knappertsbusch 2000).

DATA AND METHODS

The used steps to construct the animated diagrams were as follows:

1. Get bivariate raw data (x,y).

2. Grid the x,y data and determine bivariate frequency distributions.

3. Construct contour maps of coccolith frequencies per grid cell.

4. Extract pre-selected contour lines (two coccoliths per grid-cell).

5. Construct bivariate time-frequency diagram in 3D (addition of time).

6. Construct animated scenes from suites of rotated views.

Raw data:

The original measurements of diameters and element measurements are electronically deposited at the **Supplemental Database of Journal of Paleontology** or directly at the URL http://www.journalofpaleontology.org/datasets/knappertsbusch/Server/Supplement.html (follow the links for raw data). For each sample, there exists an ASCII text file, with the bivariate measurements for each coccolith given as X (diameter, in µm) and Y (number of elements), separated by a comma.

Preparatory steps: Gridded data

Inter- and intrasample morphological variability was analyzed by gridding the 'diameter versus number of elements' space of each sample into rectangular cells 1 µm long and two elements wide, and counting the frequency (subsequently designated as F) of specimens per grid-cell. This was done using FORTRAN program **Grid.f,** which was written by the author. The results are bivariate frequency distributions for every sample with X, Y, and F representing categories of 1 µm coccolith diameter, two elements in the distal shield and coccolith density per grid-cell. These frequency distributions formed

the basis for the construction of the contour plots illustrated in Knappertsbusch (2000) under the URL http://www.journalofpaleontology.org/datasets/knappertsbusch/Server/Supplement.html (follow the links under 'Raw Data' and then contour plots). The values for the graphs of bivariate frequency distributions per sample are supplied with the present study and can be downloaded as text files (see Appendix: Downloading Instructions).

Preparatory steps: contour plots:

Contour plots proved to be most practical to document the intra- and inter-sample changes by observation of the position of modes of the morphotypes. These were constructed by importing the matrices of bivariate frequency distributions that were obtained with Grid.f to SURFACE III vers. 2.6plus software from the Kansas Geological Survey. SURFACE III is in the shareware domain and is available from the Technical Information Services page under the URL http://www.kgs.ukans.edu/Tis/surf3/. Surface III draws contour lines from X, Y, Z data at user-defined intervals and exports these in the form of Cartesian coordinates. In the present case a contour interval of two coccoliths per grid-cell was found to be most practical to map the frequency modes of morphotypes. The Cartesian coordinates (x,y) for all contour lines that were constructed per sample can be downloaded as text files (see Appendix: Downloading Instructions).

Construction of a phylogenetic model in 3D from contour plots:

For visualization of the evolutionary pattern of **C. leptoporus**, contour lines were exported as Cartesian coordinates using the 'export/contours to ArcInfo' tool in Surface III. In a next step the coordinates of those contours indicating the density of F>2 coccoliths per grid-cell were chosen in every sample. This was done

with a conventional spreadsheet and data plotting program (e.g., Cricket Graph) where individual contours could be plotted separately and then selected by repeated plot-and-cut operations. Next, the obtained X,Y couples of the selected contour lines were assigned the geological age of the particular sample. This resulted in X,Y,Z triplets in ASCII format, Z indicating the sample age in millions of years. The values were then organized in text blocks per sample and per morphotype ready for viewing with the 3D graphical display tool Rotater 3.0 from Craig Cloeden. Rotater spins spatial data in 3D in real time and is freely available under the URL http://raru.adelaide.edu.au/rotater/ for Macintosh computers, and under the URL http://www.silicon-alley.com/cat/rotate.html for Windows computers. Numerical codes were used to assign each morphotype a different color in Rotater. The data for **C. leptoporus** prepared in this way can be downloaded and watched with Rotater 3.0 (see Appendix: Downloading Instructions. Rotater 3.0 must be installed first).

Construction of animated scenes:

The last step was to compile an animated scene of the evolution of **C. leptoporus** viewed with Rotater. This was performed using suites of bitmap pictures every 4.7 degree of rotation around the time-axis (= Z) in mono and stereo (red-green) mode. The pictures were then imported into GifBuilder 0.5 for Macintosh. GifBuilder is freeware and available at the CNET freeware/shareware site under the URL http://www.download.com (direct ftp download is possible at ftp://ftp.cmp.com/pub/filemine/gifbuilder-0.5.sit.hqx).

RESULTS

Figures 6A-6B illustrate animated evolutionary models for **C. leptoporus** from
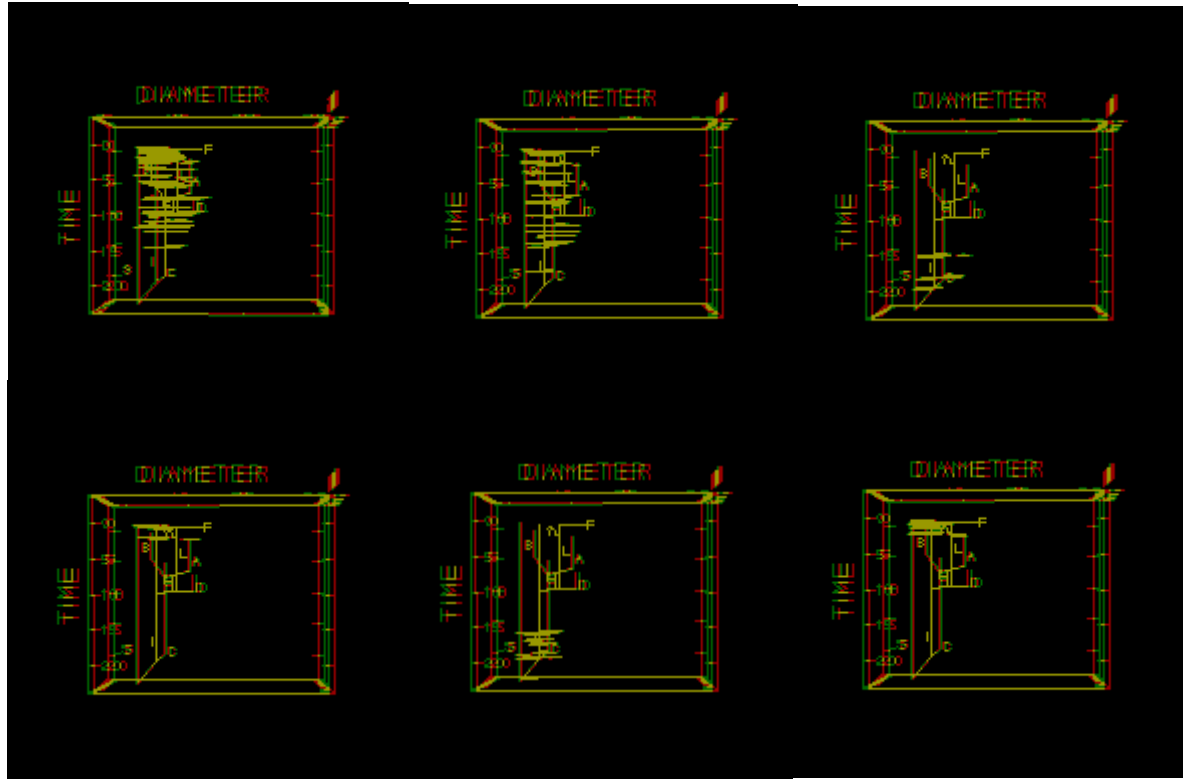
A. Mono
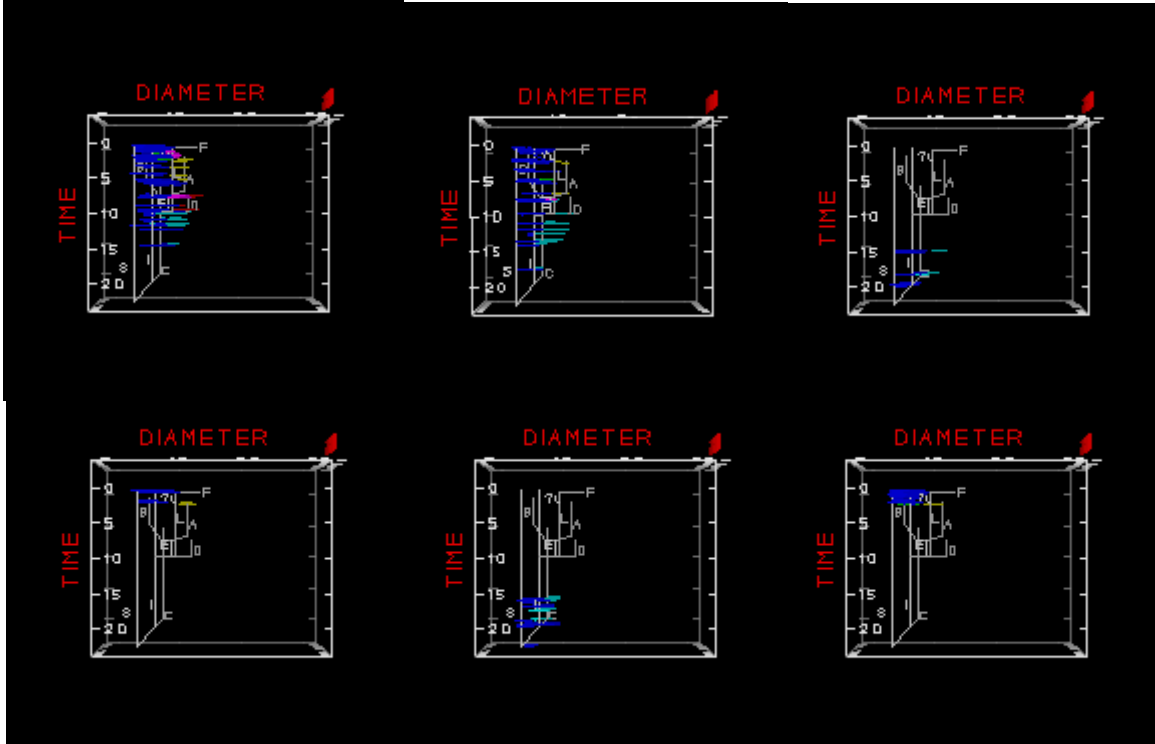
B, Stero

Figure 6. Animated scenes of the C. leptoporus coccolith morphospace through the Neogene with frequencies >2 coccoliths per grid-cell. **A.** (mono) and **B.** (stero). All diagrams follow the integrated time-scale of Berggren et al. (1995).
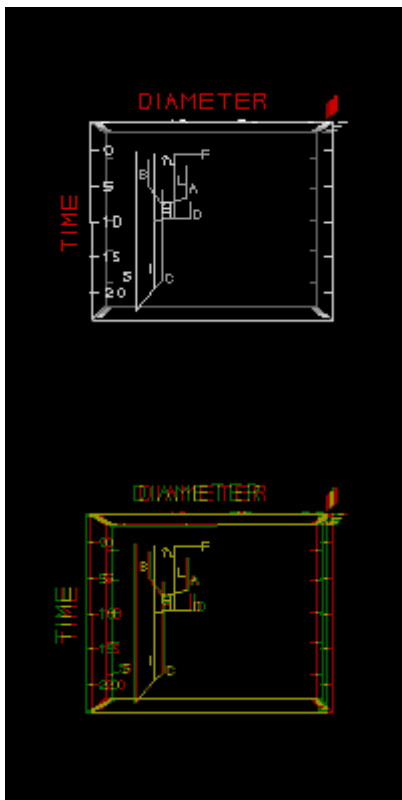
Figure 7. Reconstruction of the phylogenetic relationships in **C. leptoporus.**

Figure 8. Global phylogeny of **C. leptoporus**.

the early Miocene to Recent at individual sites (note, that the diagrams can be watched in mono and stereo views; for stereo vision use red-green glasses to experience the -3D effect in an optimal way. -3D glasses are available from Philip Kurzenberger GMBH under the URL http://www.3d-brillen.de/. The animations are combined with the phylogenetic tree (Figure 7), that was derived from the "morphotype classification" approach in Knappertsbusch (2000). Figure 8 shows composites in mono- and stereo views, where the data from all cores are included to arrive at a global impression of the morphological evolution of **C. leptoporus**.

These composites demonstrate a cladogenetic event occurred during the Upper Miocene, during which a prominent branch of large morphotypes (morphotype D) developed from morphotype C. A second cladogenetic event occurred during
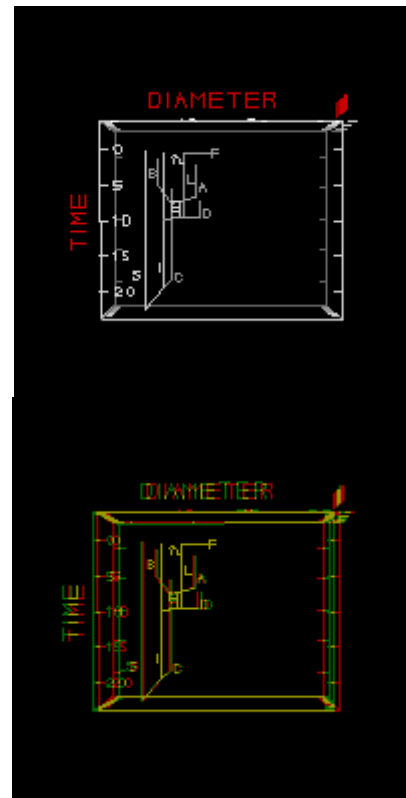
the Upper Miocene to Pliocene leading to the coccoliths of morphotype A, that stratigraphers call **C. macintyrei**. Note, that coccoliths of morphotypes C, D, and A have large areas of overlap in the bivariate classification scheme of diameters and elements illustrated in Figure 4 and it would not be possible to discriminate between them without additional information. However, these morphotypes are clearly separated from each other by time: when looking at the stratigraphic development of diameters and elements, separation patterns become apparent and morphotype D can easily be separated from morphotype A because of their different phyletic trends during different time intervals. These observations were reported in Knappertsbusch (2000) with the help of two-dimensional contour plots, but clearly, the animated diagrams presented here are more easily understood than the previously published flat diagrams. These patterns possibly indicate
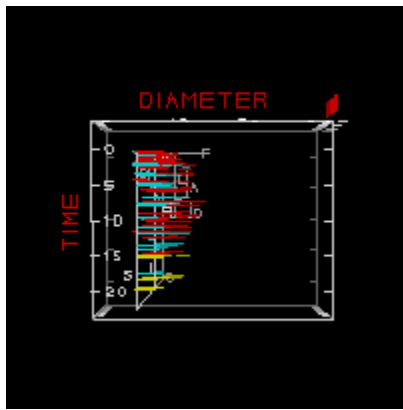
Figure 9. Environmental influence on coccolith morphology: Comparison of cold-water (DSDP Site 251 and 251A, red) with warm water (DSDP Site 236, blue, and DSDP Site 223 and 224, yellow) environments.

Figure 10. Morphotypes A and B of morphocline B from an Early Pleistocene (1.8-1.6 Ma) time-slice showing latitudinal trends in mean coccolith diameter and mean number of elements. The data included represent only coccoliths from morphocline B of Knappertsbusch et al. (1997), see Figure 11 for further explanations. The samples, that are included in this figure (labelled with ID numbers in brackets) are listed in Table 1. The vertical bars represent ±95 % univariate confidence intervals from the means. Explanation of symbols: Solid (black) = mean coccolith diameter in µm. Open (red) = mean number of elements in the distal shield.

true speciation events, and most probably are driven by paleoenvironmental changes. An environmental influence is indicated when the measurements from the southern Indian Ocean (DSDP Site 251) are compared with those from equatorial Indian Ocean DSDP sites 236, 223 and 224 (Figure 9): At the higher latitude site, large coccoliths such as morphotypes A and D appear more frequently than at the low-latitude end. This trend is confirmed in samples from the equatorial Pacific (sample DSDP 572A-3-4, 80cm), North Atlantic (DSDP 366A-3-5, 60cm; V16-205, 779 cm; and ME69-196, 471 cm) and Indian Ocean (DSDP 251-6-2, 110 cm; and DSDP 236-3-1, 120 cm) at an Early Pleistocene (1.8-1.6 Ma) time slice. At this time coccoliths, including the two extinct morphotypes A and B, show significantly higher mean coccolith diameters
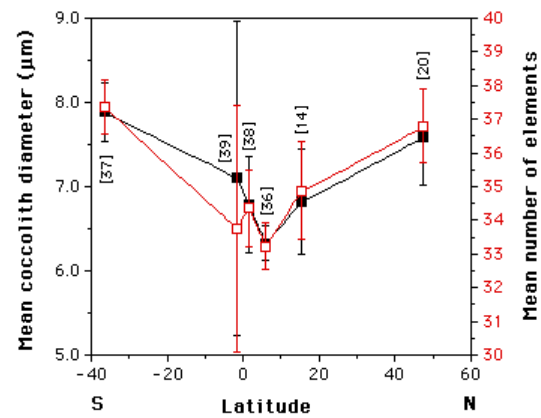
and mean numbers of elements at high latitudes than in equatorial regions (Figures 10 to 11).

Interestingly, extant representatives of **C. leptoporus** (morphotypes S, I, L, and F) show an opposite trend with respect to sea-surface temperatures. Here, large cells covered by large coccoliths (i.e. morphotypes L and F) appear to prefer waters with temperatures above the 23°C isotherm, whereas the smaller morphotypes S and I are cosmopolitan (Knappertsbusch et al. 1997). These observations suggest that a strong environmental component

Table 1. Samples and ages used for the construction of Figure 11. The ID's on the left hand side refer to the ID numbers used in Knappertsbusch et al. (1997).

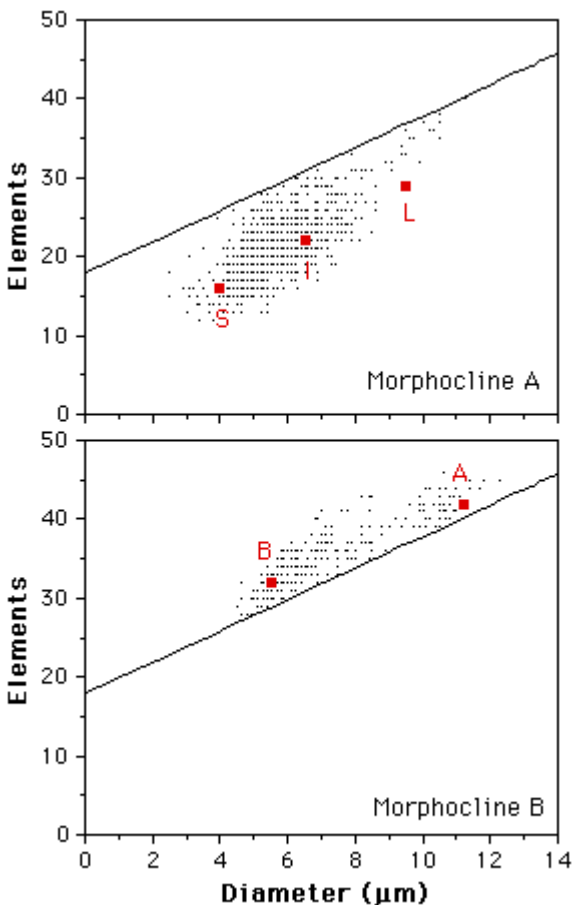| ID	Sample	Area	Age (Ma)
38	DSDP 572A-3-4, 80 cm	Equatorial Pacific	1.6
36	DSDP 366A-3-5, 60 cm	Equatorial Atlantic	1.6
14	V16-205, 799 cm	Tropical North Atlantic	1.7
20	ME69-196, 471 cm	North Atlantic	1.6
37	DSDP 251-6-2, 110 cm	Southern Indian Ocean	1.6
39	DSDP 236-3-1, 120 cm	Tropical Indian Ocean	1.8

Figure 11. Coccolith measurements from an Early Pleistocene (1.8-1.6 Ma) time-slice, as illustrated in Figure 13a and 13c of Knappertsbusch et al. (1997). The diagrams include samples DSDP 572A-3-4, 80 cm (Equatorial Pacific)), DSDP 366A-3-5, 60 cm; V16-205, 779 cm and ME69-196, 471 cm (North Atlantic); DSDP 251-6-2, 110 cm and DSDP Site 236-3-1, 120 cm (Indian Ocean) (Table 1). The positions of morphotype modes are indicated as red squares. The line separates coccoliths of morphocline A (upper figure, including morphotypes S, I, and L) from coccoliths of morphocline B (lower figure, including morphotypes A and B). The equation of the separation line is Y = 2.0 X + 17.946 (X denotes the coccolith diameter in μm, and Y the number of elements in the distal shield).

influenced size and coccolith morphology in the evolutionary history of these algae.

CONCLUSIONS

Animated diagrams were demonstrated to be of practical use for the inter-pretation of multidimensional data showing microevolutionary patterns. Usually, morphometric measurements require massive statistical treatment before evolutionary trends come to the light. In the case of **C. leptoporus** the complex microevolutionary patterns could be visualized with animated scenes and so are more easily understood to the reader than the 'flat' contour diagrams presented in Knappertsbusch (2000). Most of the conclusions can be read in that study and are not here. In that study paleoenvironmental influences were suggested to explain at least partially the changes in coccolith morphology and the observed cladogenetic events. In the present contribution morphological changes are demonstrated as a function of latitude during an Early Pleistocene (1.8 - 1.6 Ma) time-slice, which further confirms an environmental control of coccolith morphology in the case of the extinct morphotypes A and B of the taxon **C. leptoporus**.

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Lyons, Patrick D. and Laurence Head 1998. QuickTime VR: A Powerful New Illustrative Tool for Micropaleontological Research. **Palaeontologia Electronica,** 1(2): 12p., 219 KB. http://www-odp.tamu.edu/paleo/1998_2/lyons/issue2.htm

APPENDIX: DOWNLOADING INSTRUCTIONS

Each of the download sections are available in three formats.

The full set of files are available on the PE site. Click on the linked section.

The full set of files for each section are available as ZIP files. Click on the ZIP link in the section, choose the ZIP file, and use File/Save As to transfer to a directory of choice.

The full set of files for each section are available as SIT files. Click on the SIT link in the section, choose the SIT file, and use File/Save As to transfer to a directory of choice.

PE Note: The SIT files were generated with DropStuff™ 5.5 from Aladdin. The .sit files can be unstuffed with StuffIt Expander 5.5 from Aladdin (www.aladdin-sys.com). CONTOURS.SIT, 376 KB; GRID.SIT, 52 KB; GRIDDED.SIT, 92 KB; and ROTATING.SIT, 136 KB).FORTRAN program Grid.f,

GRID.ZIP

GRID.SIT

Bivariate Frequency Distributions for PC

GRIDDED.ZIP

Bivariate Frequency Distributions for Macs

GRIDDED.SIT

Contour Lines

CONTOURS.ZIP

CONTOURS.SIT

C. Leptoporus Rotating Data (Rotater 3.0 must be installed first)

ROTATING.ZIP

ROTATING.SIT