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PALEOBIOLOGICAL APPLICATIONS OF THREE-DIMENSIONAL BIOMETRY ON LARGER BENTHIC FORAMINIFERA: A NEW ROUTE OF DISCOVERIES

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Abstract

Four specimens of larger benthic foraminifera (the Recent *Palaeonummulites venosus* and *Operculina ammonoides*, and the phylogenetically related Paleogene *Nummulites fabianii* and *N. fichteli*) were investigated by X-ray tomography. The resulting three-dimensional measurements enabled a comprehensive, quantitative study of shell morphology to interpret cell growth without specific shell preparation and/or destruction. After segmentation and extraction of all scanned lumina, the following characters were measured on all chambers of each specimen: chamber volume, septal distance, chamber height, and chamber width. The sequence of chamber lumina follows either a logistic function (*Palaeonummulites*, *Operculina*), where the deceleration in growth rate of the latest chambers could mark the onset of reproduction, or it can be modeled by a series of stepwise functions with differing constants (*Nummulites*). Variations around the growth model are either periodic, following external cycles, or random as expressed by abrupt deviations. Therefore, they may reflect the response of the cell to environmental changes in terms of cyclic changes (e.g., seasonality) or single events (e.g., predator attack). Correlations between chamber volume and the other chamber parameters show that septal distance always matches the sequence in chamber volume and can therefore be used as a proxy for environmental analyses in both growth models. Chamber height and width often remain constant around their function and rarely deviate drastically to accommodate the needed lumen for retaining test size and shape. Chamber width may vary according to chamber volume in involute specimens, whereas both chamber height and width correlate with volume in those tests following an Archimedean spiral. X-ray-tomography shows particular promise in determining which parameters that can be assessed routinely in two dimensions primarily reflect environmental conditions vs. parameters best used for taxonomic identification and for systematic lineage reconstruction.

INTRODUCTION

Larger benthic foraminifera (LBF) have hosted endo-symbiotic photosynthetic microalgae for >300 million years. Accordingly, they have always provided enough light to their partners by living within the photic zone and by building a shell able to host as many

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symbionts as necessary (Hohenegger, 2009). While solar energy is abundantly available near the sea surface, light energy declines rapidly with depth, and yet many LBF taxa thrive near the lower limits of sufficient light penetration for photosynthesis. Living in shallow water means dealing with hydrodynamics, and LBF build their shells to resist suspension and entrainment (Briguglio and Hohenegger, 2011). Therefore, shape analyses of LBF tests can reveal pivotal information for ecologic and environmental reconstructions. Moreover, over geological time, LBF have experienced biogeographic changes (Renema and others, 2008), environmental stresses (Hallock, 2000), and repeated extinctions and diversifications (Hottinger, 2001). Successful biological adaptations have allowed foraminifera to survive such events and have enabled LBF to be one of the most prominent carbonate producers in shallow-water environments (Hohenegger, 2006) and to be precise index fossils for biostratigraphic purposes (e.g., Serra-Kiel and others, 1998). The life and growth strategies, environmental adaptations, biology, and the taxonomic differentiation of LBF are fascinating, highly complex, and poorly understood topics that require test-shape analysis.

Many methods and techniques have been proposed during the last decades to address these questions. The results demonstrate the complexity and beauty of these protists (e.g., Hottinger, 1960; Schaub, 1981; Less, 1987; Drooger, 1993), which build their tests as greenhouses (Hallock, 1981; Hallock and others, 1991; Hohenegger and others, 1999; Renema, 2005; Hohenegger, 2009) that resist water motion (Briguglio and Hohenegger, 2009, 2011).

The quantification of such phenomena becomes more complex by including ontogenetic shell variation, life cycles (with extreme morphological differences between generations), and intraspecific variability. Despite interesting results, the actuopaleontological approach is insufficient to completely understand the paleobiology of fossil LBF. The greater complexity in morphogenesis and bauplan in fossil taxa requires fully defining an array of complex parameters to effectively model test geometry (Hohenegger, 2011; Hohenegger and Briguglio, 2012).

Methods to more effectively describe paleobiological adaptations of LBF, including those that survived extinction events, would help reconstruct iterative evolutionary tendencies and extreme proliferation at certain times and locations in geologic history. Previous studies in this direction are based primarily on biometric observation of two-dimensional parameters of oriented test sections. Such measurements are the starting point for species definition and environmental interpretation. Nonetheless, this immense body of work is constrained by its two-dimensional character. Each measurement is made from thin sections, which may not be representative of the whole test. Moreover, destruction of the test is required to obtain these sections, resulting in the loss of data that could be obtained from specimens cut in other directions.

Internal wall characters (e.g., trabeculae transversae in nummulitids), septal width, and chamber height are impossible to obtain from one oriented section. Commonly, three-dimensional structures, as well as complex chamber shapes, are misunderstood in two-dimensional analyses. The splitting of nummulitid shells may help in some cases, but

quantifying chamber parameters on half tests still neglects the third dimension. This is especially relevant in differentiating between involute and evolute coiling.

In recent years, several instruments and software have been developed to tackle some of these problems. Computed tomography, although well-known and broadly used for many years in (bio)medical investigations and material sciences, involved huge initial costs that made it unaffordable for academia. This method has now become available for biological purposes, with advanced and upgraded techniques yielding impressive, increasingly more precise and faster results at a more affordable price. The possibility to quantify any measurable geometric parameter of an organism's interior is a great opportunity to tackle LBF structures, architectures, bauplan, and the many other questions addressed above (Briguglio and Benedetti, 2012). The digital resolution at the micrometer level is the only solution to the many controversies about the phylogenies and evolutionary trends in fossil LBF.

The present work was designed to show how computed tomography can be used to overcome previous shortcomings (bi-dimensionality, oriented sections, specimen destruction) to solve problems involving the functional morphology, environmental dependence, and ontogeny of LBF. Several results presented here provide insight into how this technology can be used to study paleobiology, including ontogeny, life cycle, and paleoenvironmental reconstructions. Particular attention is given to cell growth and its apparent reaction to environmental changes, and to the correlation among parameters describing chamber shape and the resulting volume variations. Several insights about the biology and the ontogeny of recent and fossil LBF can be inferred by measuring the test volume occupied by protoplasm during growth steps (Briguglio and others, 2011), as this may reveal how the cell reacts to environmental variations and to local stress. The measurement of the chamber volume for LBF geometry is quite time consuming, as each chamber is connected to the next one in different locations (i.e., foramen, stolons), and this requires manual corrections, for each chamber, of all the two-dimensional slices obtained by the scanning process. However, 3-D technology and software engineering is speeding up the segmentation process, and in the near future it will be possible to scan and measure many chambers in a relatively short time. This study examines only four specimens, each belonging to a different species, with the goal of demonstrating the potential of microCT and its application to LBF, with some interesting results.

Because test volume is a combination of several geometric characters (chamber height, width, and length; Fig. 1), a correlation between volume and these variables is here given to demonstrate when and how intensely each cell reacted to various environmental changes.

MATERIALS AND METHOD

Single specimens of *Palaeonummulites venosus*, *Operculina ammonoides*, *Nummulites fabianii*, and *N. fichteli* were investigated with micro-computed tomography at the University of Vienna, Austria. The scans were done at the Department of Theoretical Biology, with image processing at the Department of Palaeontology. Detailed description of this method and its possible application to foraminifera have been described by Speijer and

others (2008), Briguglio and others (2011), and Görög and others (2012). The list of scanned specimens is reported in Table 1 with information on the sample and scan properties.

Samples were scanned in small cylindrical plastic containers (a polypropylene pipette tip). Most plastics are relatively transparent to X-rays and thus suitable to scan mineralized specimens. The specimens were scanned in vertical position to reduce thickness crossed by X-ray radiation, thus yielding more contrast. Paperboard was used to maintain them in position during rotation.

The reconstruction process, which creates the black and white X-ray stack from X-ray images provided by the CT scanner, aligns pixels from the top of the scanned object to the bottom. This procedure may take several hours and produces a stack of slices normal to the equatorial plane. To reconstruct the geometry of scanned specimens, the sequence of virtual axial sections requires a huge number of slices, which increases the visualization time even for advanced computer stations; the image stack can reach 20–35 Gb depending on the resolution and scan quality. The reconstructed stacks are normally smaller (~10–15 Gb), but still too large to run fast three-dimensional visualizations and elaborations. To reduce the number of slices, a dedicated program allows re-slicing along arbitrarily defined planes. An exact re-slicing along the equatorial plane drastically reduces the stack size and speeds up the working process, segmentation, and successive rendering of the volumes. Such size reduction affects neither data quality nor image resolution; it only reduces the amount of data without information. The computer used for manipulating the image stacks was equipped with an Intel®Core (TM) 2 Quad CPU Q9400 at 2.66 GHz, 8 GB of RAM with a Microsoft Windows XP Professional x64 system, provided by the Department of Palaeontology, University of Vienna, Austria.

The software ImageJ (<http://rsbweb.nih.gov/ij/>), perhaps the most popular open-source imaging software in neuroscience, was used to measure 2-D images and to basically visualize a 3-D dataset through plug-in, including Volume Viewer (<http://rsb.info.nih.gov/ij/plugins/volume-viewer.html>) and VolumeJ (<http://webscreen.ophth.uiowa.edu/bij/vr.htm>). We used Image Surfer (another open-source program; <http://cismm.cs.unc.edu/>) for volume rendering, quantifications, slicing at arbitrary orientations, measurements in 2-D and 3-D, and taking snapshots suitable for publication.

Scan resolution depends on several variables that reflect the distance of the object from the X-ray source and detector; the scanner type also plays a role. Scan quality depends on scan intensity (kV and μ A) and the density of imaging.

After calibration, all chambers were segmented and extracted for each specimen. The following parameters were measured for each chamber: chamber volume, septal distance, chamber width, and chamber height, as displayed in Figure 1. For further statistical analyses, the volume data were linearized using the cubic root. To determine potential significant correlation between volume increase and the two-dimensional parameters measured on each chamber, correlations were calculated for each specimen and are represented as correlation matrices.

To recognize and quantify periodic deviations and cyclic variations of the cell growth around the regression function

$$\hat{y}=ax+b \quad (1)$$

or the constant

$$\hat{y}=a_j, \quad (2)$$

where j indicates the different stepwise functions, we calculated residuals r from the linear function for each measured value y_i as

$$r_i=y_i - (ax_i+b) \quad (3)$$

and from stepwise functions as

$$r_{ij}=y_{ij} - a_j, \quad (4)$$

where

$$a_j=1/n_j \sum_{i=1}^{i=n_j} y_{ij} \quad (5)$$

Because the linear regression increases due to chamber number x , deviations from the linear functions are small in earlier growth stages and large in later stages. Therefore, all residuals r_i have to be standardized by

$$r_{s,i}=(y_i - ax_i - b) / (ax_i+b) \cdot 100 \quad (6)$$

to make the intensity of deviations comparable for all growth stages i (chamber number). Standardized residuals have also been calculated in case of stepwise functions as

$$r_{s,ij}=(y_{ij} - a_j) / a_j \cdot 100. \quad (7)$$

The three-dimensional chamber models of the scanned specimens, the data measured on each chamber, the residuals calculated for the measured parameters and their corresponding standardized values are reported for *P. venosus* (Figs. 2, 3), *O. ammonoides* (Figs. 4, 5), *N. fabianii* (Figs. 6, 7) and *N. fichteli* (Figs. 8, 9). The latter two are fossil species commonly believed to be in an ancestor (*N. fabianii*)-descendant (*N. fichteli*) relationship (e.g., Schaub, 1981). As the standardized residuals were calculated in percentages and all ordinate axes were constructed identically, comparisons among the measured parameters are possible. For all investigated specimens, the proloculus and deuteroecolus measurements were excluded to avoid incorrect data interpretation due to their exceptionally large dimensions compared with the following chambers.

RESULTS

Palaeonummulites venosus

The chamber-volume sequences can be fitted by logistic growth (Fig. 2D). Two major lower peaks are evident when the chamber-lumina sequence is linearized by cubic roots (Fig. 2E): the first at chamber 30 and the second at chamber 40. At the same locations, strong deviations from the linear trend are visible in the septal-distance sequence (Fig. 2F). Moreover, the only deviation in the chamber-width sequence is visible at chamber 40 (Fig. 2G) and in chamber-height sequence only at chamber 30 (Fig. 2H).

The correlation matrix (Fig. 2I) indicates that the volume sequence highly correlates with septal distances and secondarily with chamber widths. Consequently, septal distance and chamber width are also significantly correlated.

In *P. venosus*, the residuals of the linearized volume (Fig. 3A) and their standardized values (Fig. 3B) show that, besides the deviations at chambers 30 and 40, the first chambers of this specimen were characterized by strong deviations. Although they are completely hidden in the volume sequence by the logistic function, they are visible in the residuals sequence and correctly displaced in size in the standardized volume sequence. Also, documented by the standardized residuals of chamber width and height (Figs. 3F, H), these deviations are due to larger initial values in the test. Standardized chamber volume (Fig. 3B) oscillates around the expected trend, never varying more than 10–15%, whereas the initial deviations and at chambers 30 and 40 reach 40%.

The sequence of the standardized septal-distance residuals (Fig. 3D) shows how strongly such parameters may vary, with deviations up to 40–60% from the expected trend. The strong deviation in chambers 30 and 40 is reflected in septal-distance residuals and in their standardized values. In the sequence of chamber-width residuals (Fig. 3E), the major deviation at chamber 40 is visible, and in the standardized sequence (Fig. 3F) an additional deviation appears at chamber 10, which is represented neither in the volume sequence nor in its residuals. The deviation at chamber 30 is also visible in the chamber-height residuals (Fig. 3H). The standardized residuals of chamber heights (Fig. 3F) and widths (Fig. 3H) show that these parameters remain constant around the calculated linear function and do not vary by >20% of the expected trend (except for the deviations in the initial chambers).

Operculina ammonoides

The volume sequence shows logistic growth in *O. ammonoides* (Fig. 4D). Major deviations from the logistic growth function are visible around chambers 14–18 for the linearized volumes (Figs. 4D, E). Strong deviations from the linear function are visible at the same life stages in the septal-distance sequence (Fig. 4F), although much less prominent in the chamber-width sequence (Fig. 4G). Chamber-height values are extremely variable (Fig. 4H). A major disturbance is visible in the growth of this specimen from chambers 15–35 (see fig. 2 in Briguglio and others, 2011). This disturbance produced chambers with reduced height, resulting in an irregular spiral. Notably, such extreme deviations, which can reach –60% from the expected values, do not result in visible volume deviations. Because of this morphological characteristic producing a negative disturbance on the cell growth in the

investigated specimen, the calculated linear function has been obtained by omitting chambers 16–34 (Fig. 4H). The correlation matrix (Fig. 4I) shows that the septal-distance sequence is the only character that mirrors the chamber-volume sequence.

Residual statistics better show the deviations of chamber volumes from the linear function (Fig. 5A) compared with the linearized sequence (Fig. 4E), and the deviations at chambers 14–18 are easily recognizable. The rest of the standardized volume sequence oscillates around the expected values (deviation <10%).

The septal-distance residuals show several deviations, which may attain negative values 50% of those expected and positive values at chambers 14–18 exceeding 100% of the expected values (Fig. 5D). The strong volume deviation at chamber 14 is partly accommodated by a modified septal-distance parameter.

Chamber-width residuals show positive peaks at chambers 18 and 32 (Fig. 5E), exceeding 30% of the expected values (Fig. 5F). The first part of the sequence is characterized by very high positive deviations for the first two chambers. The terminal part of the standardized chamber-width residuals constantly oscillates around 10–15% of the expected values.

The deviations in chamber-height residuals are enormous and divert very prominently from chambers 14–35 (Fig. 5G). The standardized-values sequence points to a strong deviation in the initial chambers, attaining negative peaks 60% where the growth disturbance took place (Fig. 5H).

Nummulites fabianii

In *N. fabianii* the logistic function, as reflected in the chamber volume, is not representative of growth. Rather, a sequence of linear functions with insignificant multiplicative constants remains more or less parallel to the x-coordinate. After 25 chambers (Figs. 6D, E) the additive constant of the linear function changes instantaneously, producing a distinctive step that is also repeated after chamber 50. This particular geometric trend cannot be expressed by a linear regression line; a sequence of three constant functions $y = a_j$ (j = function number) must, therefore, be calculated (Fig. 6E) and applied to obtain consistent residuals (Figs. 7A, B).

The stepwise trend is well documented in the septal-distance sequence (Fig. 6F) and visible in both other parameters (Figs. 6G, H). This sequence is the character that best mirrors the volume sequence, as documented in the correlation matrix (Fig. 6I).

Residual chamber-volume statistics based on their function constants show that deviations rarely attain $\pm 40\%$ of the expected values (Fig. 7B), but are normally confined between the limits of $\pm 20\%$. The stepwise growth trend in these calculations is not visible because residuals have been calculated according to their corresponding function constant a . Septal distances are much less predictable than chamber volumes; they often reach values $\pm 40\%$ than expected (Fig. 7D). The residuals of chamber widths and chamber heights are highly variable (Figs. 7E, G), but the deviations are not significant. Standardized residuals for these characters remain constant within the limits of $\pm 20\%$ (Figs. 7F, H).

Nummulites fichteli

The chamber-volume sequence of the *N. fichteli* specimen (Fig. 8D) is similar to *N. fabianii*. Though less visible, stepwise growth is recognizable in all measured parameters. Almost 90 chambers are divided into four steps with constant functions (Fig. 8E) that are used to subdivide the chamber-volume, septal-distance, chamber-height, and chamber-width sequences.

Major deviations from the expected volume trends are visible at chambers 40, 46, 70, and 87 (Fig. 8E). The septal-distance sequence deviates prominently at the same locations where volume deviations were also observed (Fig. 8F). The chamber-width sequence does not deviate strongly from the function constants, and the only significant deviation corresponds to chamber 70 (Fig. 8G). Chamber-height sequence shows an oscillation around the function constants and deviates slightly more than chamber width. Major deviations are visible at chambers 40 and 46 (Fig. 8H). A very high correlation was found between volume and septal distance in this specimen. Chamber heights and widths also highly correlate with the volumes (Fig. 8I).

The residuals statistics highlight the most prominent deviations in the volume sequence (Fig. 9A), and the normalization of these values shows how they deviate by 40% of the expected values (Fig. 9B). The deviations at chambers 40, 46, 70, and 87 are again evident. In septal-distance residuals and in their standardized values, the major deviations are clearly visible; they deviate by >60% of the expected value. Other values are still far from the expected constant, oscillating around 40% in both directions (Figs. 9C, D). Chamber-width and chamber-height residuals are much less saw-toothed than septal-distance ones (Figs. 9E, G), and their standardized values do not deviate by >10–20% from the expected values. The four major deviations observed in the volume sequence are visible, and they deviate 50% in standardized residuals for chamber width and height (Figs. 9F, H). The procedure used here to calculate separated linear functions for the stepwise growth (instead of one single regression line) has been statistically checked for both fossil specimens (Fig. 10).

DISCUSSION

Ontogenetic changes in chamber shape (e.g., volumes) represent the reaction of the cell to internal and external factors, the growth program, and the limitations due to environmental conditions. Thus, all disturbances, deviations, or interruptions of the genetically controlled growth program are manifested in the test morphology and chemistry.

External factors operate either constantly, such as temporary environmental fluctuations (temperature, illumination, etc.), or instantaneously, as in catastrophic events (in the sense of Poston and Stewart, 1978) such as storms, unsuccessful food capture, or the presence of competitors. Internal factors, which may be related to the morphogenetic program, may abruptly alter growth similarly, as demonstrated in ammonites (Kullmann and Scheuch, 1970).

The calculation of test growth in nummulitids is a good example to show how growth reacts to external factors. The method of standardized residuals visualizes variations from given

growth functions and yields interesting results such as consistent (periodic) or chaotic (instantaneous) growth deviations.

Two questions concerning cyclicity can be asked. What external phenomena induce periodicity, and what parameter or combination of parameters reacts to these external variations? Finding such correlations is crucial in paleobiological studies of LBF to understand their ontogeny and life cycles. Some attempts have been made to study the shell chemistry (Wefer and Berger, 1980; Sarswati, 2012), and others are trying to tackle LBF cyclicity by laser-ablation-plasma mass spectrometry (Evans and others, 2011, 2012), yet without published results. Coupling chamber-volume measurements with such data, once available, will provide an interesting direction for further research.

Chamber-volume cyclicity reflects the interaction between growth (modeled by a mathematical function) and environment (subjected to regular and abrupt changes). If a factor forces a growing cell to deviate from its normal growth function pattern, then the cell itself will build a new chamber to accommodate the new volume of protoplasm. In this process, the cell may modify one or more of the three parameters defining the chamber: septal distance, chamber height, and chamber width. Biologically, changing the septal distance is the easiest solution to accommodate a defined volume. The modification of chamber height or chamber width, in contrast, will result in a more complicated bauplan deviation, such as in a change from an involute to evolute coiling system (or vice versa). Therefore, slight modifications of chamber volumes (e.g., cyclicity) are accommodated by septal-distance modifications, while abrupt volume deviations (e.g., resulting from predation, longer environmental stress, competition), may also require modification of chamber height and width.

This interpretation is supported by the present observations that for the investigated specimens the septal-distance sequence always matches the volume sequence with high significance. This means that the septal distance is the primary parameter for defining the size of each chamber. As shown, all major deviations in the volume sequence are always visible in the septal-distance sequence and at the same position.

For involutely coiled species (e.g., *P. venosus*), chamber width also clearly matches the volume sequence (Fig. 2I). The alar prolongations (in the sense of Hottinger, 2006) are thus probably used to create the needed lumen to accommodate cell growth by varying their extension around the previous whorl. Where alar prolongations are absent (e.g., *O. ammonoides*), the correlation between chamber width and volume is consequently very weak.

In all investigated specimens, negative peaks in the residuals are much more abundant than positive peaks. This is logical because, during growth, a rapid increase in protoplasm would be accommodated by increased rate of chamber formation, thereby avoiding creation of chambers with larger lumina. In contrast, under stress, there may be insufficient protoplasm to build normal-sized lumina, resulting in chambers with smaller lumina.

According to logarithmic and Archimedean spirals, however, chamber heights have fixed geometries and cannot be modified unless a major accident disturbs the growth rate. Based

on an Archimedean spiral that keeps chamber heights constant, the increase in cell volume cannot strictly follow a logistic growth connected with a logarithmic spiral. As clearly evident in *N. fabianii*, the need to grow faster and/or with larger lumina induces an incidental increase in chamber height; this increase is reflected in test growth by a sequence of linear functions with stepwise increasing additive constants, thus approximating exponential growth. Therefore, an abrupt positive shift in chamber height can be a successful solution to accommodate the increased volume for a longer time. Consequently, the correlations are good between volume residuals and chamber height and width for both fossil specimens featuring an Archimedean coiling geometry.

Stronger deviations were observed in initial chambers for almost all standardized residuals in chamber widths. Even if the proloculus and the deuterolocus are not included in the sequences, the first three or four chambers may embrace the relatively larger proloculus by increasing their width for better accommodation. This stabilizes them in creating the first part of the marginal chord where the main canal system is located.

CONCLUSIONS

The chamber sequence in nummulitids mirrors individual growth and makes such growth measurable by calculating the chamber lumina using a microCT scan. Such measurements accurately show the growth form of the foraminiferal cell and mark the deviation from a latent mathematical growth function due to external and internal factors.

Minor disturbances in cell growth and all deviations in oscillatory growth are manifested in septal distance. This is because chamber height and width strictly follow the morphogenetic program to retain test shape, with very few possibilities for stronger deviations from that shape. Observed deviations in chamber height and width are always related to growth impedances caused by major factors such as competitors, predators, or adverse local ecological conditions.

This makes studying the chamber-volume sequences—and their relationship to septal-distance, chamber-width, and chamber-height sequences—of primary interest in cell biology and shell morphology. Using residual statistics and their standardization for all measured parameters can be pivotal to assess deviations and to quantify them in relation to overall cell growth. Quantifying septal distances and chamber heights and widths on other specimens might show how they vary during growth and how much they can deviate from given mathematical growth models to accommodate the needed cell volume. These results are important to define those characters that are correlated to the volume sequence and therefore act as proxies for ecological variations and environmental reconstructions. This approach also helps separate these characters from those whose sequence is more constant and is therefore better suited for taxonomic identification and for phylogenetic lineage reconstructions, if measured on entire populations.

The results of this study show that septal distances and their sequence during the cell's life can provide much information on the paleobiology of the cell itself as they are well correlated to the volume sequence. Using three-dimensional studies to identify the optimum

parameters to study in two dimensions, including oriented thin-section measurements, can increase the speed at which multiple specimens can be studied in equatorial sections.

The study of chamber height and width can also provide important information on major environmental changes reflected in volume growth in taxa possessing a logarithmic spiral or extended alar prolongations. In shells following Archimedean spirals (i.e., almost all fossil nummulitids), chamber width and height can be used for taxonomic identifications and for systematic lineage reconstructions.

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Figure 1.
Measurements of chamber height, septal distance, and chamber width on each chamber lumen.

Figure 2.

Palaeonummulites venosus. **A** equatorial and **B** axial views of chamber lumina, **C** axial view of chamber lumina covered by the test (note alar prolongations reaching the umbonal area), **D** chamber volume, **E** cubic root of chamber volume, **F** septal distance, **G** chamber width, **H** chamber height; all measurements in mm. **I** correlation matrix: correlation coefficients in the lower left portion of the matrix and probabilities of independence (no correlation) in the upper right portion of the matrix. Bold and underlined fonts represent extreme and significant correlations.

Figure 3. *Palaeonummulites venosus*. Residuals and standardized residuals (in %) for linearized chamber volume (A, B), septal distance (C, D), chamber width (E, F), and chamber height (G, H).

Figure 4.

Operculina ammonoides. **A** equatorial and **B** axial views of chamber lumina, **C** axial view of chamber lumina covered by the test (note alar prolongations reaching the umbonal area), **D** chamber volume, **E** cubic root of chamber volume, **F** septal distance, **G** chamber width, **H** chamber height; all measurements in mm. **I** correlation matrix: correlation coefficients in the lower left portion of the matrix and probabilities of independence (no correlation) in the upper right portion of the matrix. Bold and underlined fonts represent extreme and significant correlations.

Figure 5. *Operculina ammonoides*. Residuals and standardized residuals (in %) for linearized chamber volume (A, B), septal distance (C, D), chamber width (E, F), and chamber height (G, H).

Figure 6.

Nummulites fabianii. **A** equatorial and **B** axial views of chamber lumina, **C** axial view of chamber lumina covered by the test (note alar prolongations reaching the umbonal area), **D** chamber volume, **E** cubic root of chamber volume, **F** septal distance, **G** chamber width, **H** chamber height; all measurements in mm. **I** correlation matrix: correlation coefficients in the lower left portion of the matrix and probabilities of independence (no correlation) in the upper right portion of the matrix. Bold and underlined fonts represent extreme and significant correlations.

Figure 7. *Nummulites fabianii*. Residuals and standardized residuals (in %) for the linearized chamber volume (A, B), septal distance (C, D), chamber width (E, F) and chamber height (G, H).

Figure 8.

Nummulites fichteli. **A** equatorial and **B** axial views of chamber lumina, **C** axial view of chamber lumina covered by the test (note alar prolongations reaching the umbonal area), **D** chamber volume, **E** cubic root of chamber volume, **F** septal distance, **G** chamber width, **H** chamber height; all measurements in mm. **I** correlation matrix: correlation coefficients in the lower left portion of the matrix and probabilities of independence (no correlation) in the upper right portion of the matrix. Bold and underlined fonts represent extreme and significant correlations.

Figure 9.

Nummulites fichteli. Residuals and standardized residuals (in %) for the linearized chamber volume (A, B), septal distance (C, D), chamber width (E, F) and chamber height (G, H).

Figure 10.
 Comparison of residual variances between linear regression, stepwise functions, F-values,
 and probabilities of variance in *Nummulites fabianii* and *N. fichteli*.

Table 1

Scanning properties for the investigated specimens and additional information on their provenance.

	<i>Palaeonummulites venosus</i> (Fichtel and Moll, 1798)	<i>Operculina ammonoides</i> (Gronovius, 1781)	<i>Nummulites fabianii</i> (Prever in Fabiani, 1905)	<i>Nummulites fichteli</i> (Michelotti, 1841)
Code	V0	ammonoides 1	fabianii 3	fichteli 14
Camera temperature	−55°C	−55°C	−55°C	−55°C
Image size	510 × 512	504 × 512	1024 × 1024	1024 × 1024
kV	80	77	80	80
μA	46	45	50	50
Pixel size	4,258 μm	4,645 μm	4,228 μm	4,228 μm
Slices	276	178	258	268
Size	66.4 Mb	43.3 Mb	231 Mb	238 Mb
Provenance	Sesoko, Japan. 50-m water depth	Motobu Town, Motobu Peninsula, Okinawa, Japan. 18-m water depth	Baciu Quarry, Cluj-Napoca, Romania.	Biarritz, Rocher de la Vierge, France.
Age	Recent	Recent	Latest Eocene, SBZ 20	Earliest Oligocene, SBZ 21
Reference	Hohenegger, 1994	Hohenegger and others, 1999	Papazzoni and Sirotti, 1995	Boussac, 1911; Schaub, 1981; Mathelin and Sztrákos, 1993