Three dimensional reconstructions of *Nummulites* tests reveal complex chamber shapes

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Larger benthic foraminifera (LBF) are an important tool for the biostratigraphy of (sub)tropical shallow marine deposits. In Paleogene the genus Nummulites is an important genus for biostratigraphical zonation schemes. However, classification is Europe centered and based on external characters and equatorial thin sections. New results from regions outside the northern Tethys shows that a more rigid framework for the classification of nummulites is needed.

Here we present a new tool for achieving this goal. We visualise 3D chamber shape of *Nummulites djodjokartae* and compare these to traditional morphometrical characters. To achieve this goal we use computed microtomography of well preserved Nummulites tests. We find that despite the regular shape in equatorial and axial thin section 3D chamber shape is not predicted by these sections. We argue that 3D reconstructions of Nummulites tests will be a great aid in improving our understanding lineages within the genus Nummulites, and to elucidate its evolutionary and biogeographical history.

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Introduction

Larger benthic foraminifera (LBF) are an important tool for the biostratigraphy of (sub)tropical shallow marine deposits. The ranges of species of nummulitids, alveolinids, and orthophragminids form a basis for the shallow marine zonation (SBZ) for the Paleogene of the western Tethys (Serra-Kiel et al., 1998). The genus *Nummulites* is particularly abundant in many Eocene deposits and contains over 500 described species (e.g. Schaub, 1981). However, differing priorisation of characters, species concepts, and reference frameworks have resulted in a taxonomic maze. The most frequently used reference work is the revision of *Nummulites* by Schaub (1981), who classified the genus into lineages (which he called 'phyla') based on externally visible characters (e.g., shape of the septal trace, number, size, and distribution of pillars), and internal characters from equatorial thin sections (e.g., chamber length and septal shape). Within these lineages the species are separated by biometrical characteristics, primarily the size of the first chamber (proloculus) in the A-form, secondarily by ontogenetic increase of the whorl radius, both measured in equatorial thin sections. Within most lineages the species are ordered by increasing proloculus size, but also by the number of whorls, the diameter, and increasing size difference between the sexual and a-sexual generation. The youngest members of these lineages are the most distinct, but the ancestral members of the lineages are often difficult to separate. Many smaller, more simple shaped specimens cannot be placed in these lineages other than by geographical association. Furthermore, most of the material examined by Schaub (1981) is from southwest Europe, with few samples from central and eastern Europe. Recently, the paradigm that morphological change in LBF occurs at the same time and rate over large geographical distances has been questioned (Renema, 2015; Cotton, Pearson & Renema, in review), highlighting the need for a reference framework in which also non-European occurrences fit.

Bottlenecks in our understanding of the evolution of *Nummulites* have arisen partially because the majority of characters used are derived from looking at specimens in equatorial thin section and external view only. Vertical sections are not widely used. For example, in Schaub's (1981) revision of western Tethyan *Nummulites* no vertical sections were figured. It was not until 1991 that Kleiber added the vertical sections to most of Schaub's species. Based on external observations, Adams (1988) emphasized that the shape of the septal filaments is an important character, and he provided a reference framework for categorising them. Racey (1992) assessed the degree of interdependence of morphological characters and concluded that ?internal characters evaluated in equatorial and axial thin section were the most important. However, contrary to Schaub (1981), he valued characters in axial thin section as important as equatorial sections.

The recent advance of high resolution computed tomography in palaeontology has enabled the reconstruction of the three-dimensional morphology of foraminifera tests (Speijer et al. 2008; Briguglio, Metscher & Hohenegger, 2011; Görög et al. 2012). Here we present 3D-models of *Nummulites* tests with the purpose of increasing our understanding of growth and morphology of complex *Nummulites* in order to assess whether 2 dimensional analyses are truly representative of the test and whether key morphological traits are being lost during this preparation technique. Using *N. djokdjokartae* as a model organism, we will demonstrate that chamber shape is not well represented in either equatorial or axial thin sections, but is an important character to understand *Nummulites* growth and as a derivative evolution.

Methods

Eocene *Nummulites djokdjokartae w*ere collected from four stratigraphic levels within the Nanggulan Formation at Kali Watupuruh, Nanggulan, Central Java. The Nanggulan Formation is a sequence of overall deepening upwards marine mudstones, sandstones, and conglomerates. At the top of the sampled interval the presence of abundant large *Discocyclina* indicates that this stratigraphic level correlates with the middle Bartonian Ta-Tb boundary (see Renema, 2007; Lunt, 2013)., The samples are therefore considered to be early Bartonian (38–40Ma) in age.

We scanned 74 tests of *Nummulites djokdjokartae* from four the four levels. For the scanning we used a Bruker/Skyscan 1172 at 5-7 µm pixel size. These specimens were used to characterise virtual horizontal thin sections comparable to those used in traditional descriptions of *Nummulites* populations. In these sections we measured proloculus size, number of whorls, number of chambers per whorl, whorl radius, chamber height, and proximal and distal angles of the septa.

Seven specimens were used to reconstruct the 3D chamber shape. To do this, the reconstructed image stack was imported into Avizo, in which we segmented all succesive chamber cavities (chamber lumen and alar prolongations). In most cases chamber shape is obvious and could be reconstructed by selecting within the chamber and allowing Avizo to automatically extrapolate the chamber. In rare occasions one of several problems could arise: 1) The chamber is very small, and has no alar prolongations (Fig. 1). Such a chamber was included as a full chamber when it appeared as a chamber in the virtual equatorial section; 2) The chamber is highly assymetrical, and only has a single alar prolongation (Fig. 1x). Such a chamber was included as a full chamber when it appeared as a chamber in the virtual thin section. Otherwise it was included in the chamber to which it connected in the chamber lumen.; 3) Especially in later whorls, chamber shapes could become complex, and the presence of pillars rendered them discontinuous. In most cases the connections between chambers were , but in rare occasions the law of super position was used: older chambers are overlain by younger chambers (Fig. 1x). In one specimen (06KW01_05) four lateral parts of chambers could not be related to a growth increment with certainty(Fig. 2). Following segmentation the volume of each chambers were calculated using the material statistics option in Avizo 8.1.

Results

Morphology in 2D thin sections. No morphological differences were found between the four samples examined, hence all specimens are treated as coming from a single population. In nearly all specimens square chambers formed less than a whorl, resulting in a straight line in the whorl diagram (Fig 3). Based on total number of whorls and proloculus diameter two groups can be recognized, however, group A1 with a proluculus size of smaller than 400 µm, and more than 5 whorls, and group A2 with a proloculus larger than 400 µm and fewer than 5 whorls (Fig 4). The general morphology in both groups includes several initial chambers that are as high as long, followed by 3-5 whorls with chambers that are up to 3 times longer than high (Fig. 5). Overall this results in an increase in chamber length and a comparable number of chambers per whorl in whorls 3-5, despite the increase in radius (Fig. 6). Proximal and distal angle of the septa is comparable in all specimens (Fig.7). Total number of chambers differ between the A1 and A2 group, Specimens in A1 have ~130-150, in A2 90-110 chambers.

Chamber shape in 3D. Despite the regular shape in equatorial and axial thin section, chamber morphology becomes increasingly more complex in each whorl. Chamber shape is typical for *Nummulites* in the first one to three whorls, with alar prolongations extending to the central part of the test, and are symetrical on either side of the equatorial plane. In rare occasions small chambers without alar prolongations occur. In most cases these chambers are not recognisable in equatorial thin section other than by their very short lengths. In succesive whorls this shape becomes more irregular as the result of extending the alar prolongations. Simultaneously the alar prolongations become narrower and assymetrical. They form at an angle to the line from the septum to the central area of the test. In whorl three and four most alar prolongations are not interrupted. Alar prolongations are narrowed by pustules which are visible in successive chambers and lie in the chamber, not on the septal filaments. With increasing chamber number the length of the alar prolongations becomes longer, their shape more irregular, and the asymmetry over the equatorial plane increases. Usually succesive chambers have one long and one short alar prolongation on the same side of the test, followed by a few chambers where this pattern is mirrored. In the first 50 chambers this pattern is comparable between specimens from group A1 and A2. Specimens in group A1 have more chambers, and the chambers in the last whorl become increasingly more irregular and more dificult to reconstruct due to partitioning of the (long) alar prolongation. These alar prolongations curve around the central part of the test. The longest alar prolongations can make a 270 degree rotation (Fig. 2). The stacking of these thin alar prolongations results in the very regular axial section which almost has the appearance of lateral chamberlets as observed in *Spiroclypeus*.

Discussion

Our results provide new insights into the evaluation of morphological characters in two versus three dimensions. We argue that when only using external views and oriented thin sections characters can be misinterpreted and that understanding the 3D structure is needed to come to a better understanding of *Nummulites* evolution, and as a consequence their use in biostratigraphy.

Primary, secondary, and tertiary septal filaments. Based solely on the external morphology of *Nummulites* tests, Adams (1988) categorised the septal filament traces into three groups. Primary septal filaments arise directly from the septa. Secondary filaments are the distal walls of chamberlets and develop in reticulate and sub-reticulate *Nummulites*. Tertiary filaments originate as spiral ridges or spurs from the primary or secondary septal filaments. However, he does not provide a model of how these structures form during growth. Foraminifera grow by adding chambers to the apertural face of the test. However, exactly how this method of incremental growth results in the different categories of septal filaments is not explained by Adams (1988). Our study shows that in *Nummulites djokdjokartae*, which is closely related to *N. brittanicus* used in Adams' study (Renema et al 2003), all three categories of septal filaments are present. Our data show, however, that all three have the same origin, and are the traces of the primary septal filaments. The apparent presence of secondary septal traces, resulting in branching structures, is the result of superposition of alar prolongations from within the same whorl. This superposition is not apparent in traditional growth models of *Nummulites,* and is the result of the irregular chamber shape with alar prolongations extending in variable lengths over the external surface of the test, and vary from being almost straight and extending to the polar region, to extending around the polar region for almost two-thirds of the shell. Superposition of these two chamber types results in apparent branching patterns in the traces of the septal filaments. In extreme cases chambers run almost parallel to the marginal cord, and form spiral ridges or tertiary septal filaments as defined by Schaub (1981).

Two dimensional sections overemphasise the regularity of numulitid tests. Macrospheric specimens of *Nummulites djokdjokartae* have a very regular appearance in both horizontal and vertical thin section. In horizontal section there are 4-6 whorls with chambers with almost straight septa and chamber shape which changes from being higher than long in the initial whorl to longer than high in the later whorls. In axial section there are stacks of alar prolongations of very similar height visible throughout the test, including the polar region. In traditional growth models of *Nummulites* with the chambers converging in the polar region, it is expected that alar prolongations narrow towards the center and often a polar pillar is present. Although the regularity of the stack of alar prolongations gives the impression that all chambers have long alar prolongations aligned next to each other, when the3D reconstructions of the chambers are examined, it becomes clear that frequently alar prolongations of non-successive chambers are aligned next to eachother in the central part of the test, and that sometimes the same alar prolongation transverses an axial plane multiple times (Fig. 2, Fig. 3). The presence of alar prolongations over the polar region is only possible when chambers extend over this region. In axial thin section the test looks symetrical, but apart from the first ten or so regular chambers, all chambers are asymetrical in the equatorial plane. This does not conform the description of the genus *Nummulites,* which includes involute, biconvex, planispiral coiling.

Chamber shape in equatorial thin section has been related to chamber volume, potentially a more biologically relevant metric to estimate foraminiferal growth. Chamber volume of the seven specimens segmented in this study shows a comparable, but highly irregular pattern. The initial 30-50 chambers hardly increase in volume, followed by a rapid increase in volume of the following chambers. However in the latter phase occasional small chambers are also formed . These are probably the result of geometric constraints in chamber formation and serve to increase the regularity of the apertural face to facilitate growth in subsequent chambers.

Chamber shape progressively becomes more complex, in the first whorl chambers are regularly involute with curved septal and septal filaments, followed by developing more irregular septal filaments, and extension of the alar prolongations. This comes together with increase asymmetry and chamber volume.

Conclusion

We argue that to clarify uncertainties and improve the definitions of lineages within the genus *Nummulites*, three dimensional reconstructions of chamber shape and volume has the potential to provide important additional characters, next to equatorial and axial thin sections. Furthermore, these also provide insight into how long used characters, such as the shape of septal filaments can be related to chamber formation and, hence, growth.

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Figure captions

Fig. 1. Detail of chambers 87-89 of specimen 06KW01_05 in four different perspectives. a: small chamber with a single alar prolongation. b: Superposition of alar prolongation over the alar prolongation of the previous whorl. Scale bar is 250 µm

Fig. 2. Chamber shape per whorl in specimen 06KW01_05. Note the increase in complexity of chamber shape. A: whorl 1; B: Whorl 2; C: whorl 3; D: whorl 4; E: whorl 5; G: first half of whorl6; H: second half of whorl 6. In yellow and red that could not attributed to a chamber with certainty are indicated. Note that in whorl 6 the alar prolongations of half a whorl cover up to ¾ of the test. Scale bar is 250 µm.

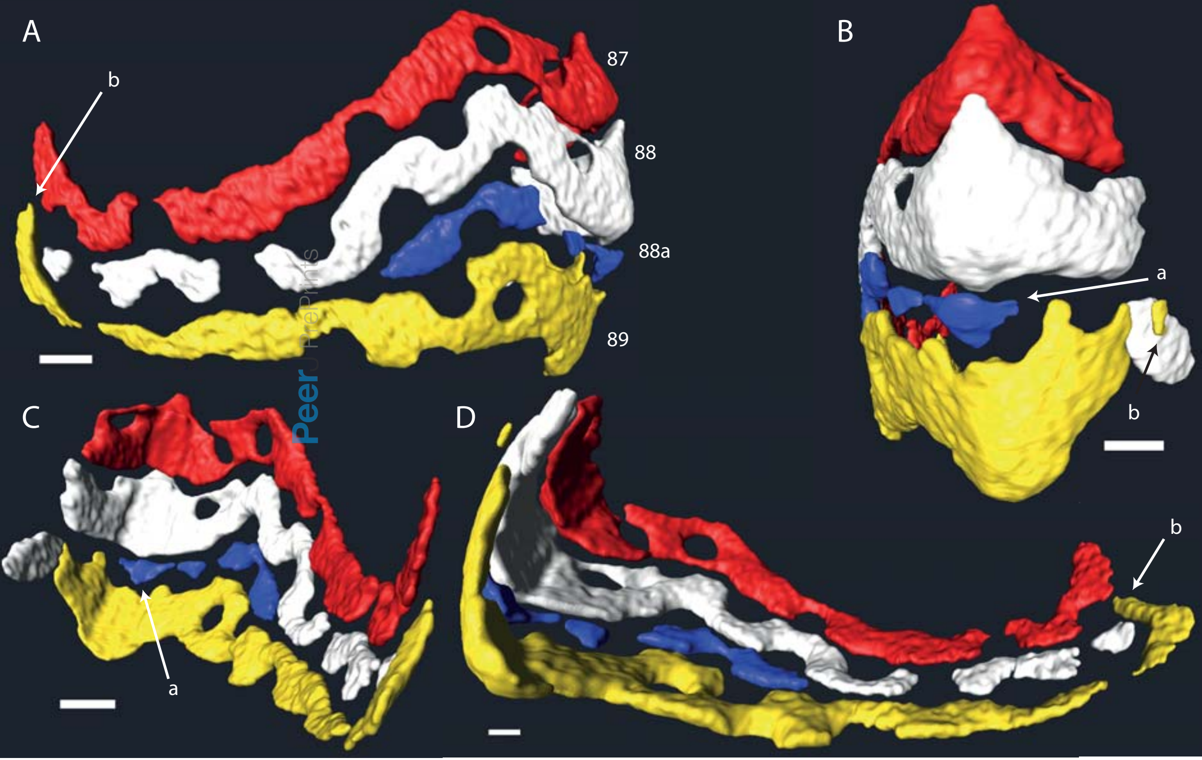
Fig. 3. Chamber shape per whorl in specimen 06KW01_19. Note the difference with specimen 06KW01_05 (Fig. 2). A: whorl 1; B: Whorl 2; C: whorl 3; D: whorl 4; E: whorl 5; G: first half of whorl6

Fig. 4. Average diameter of the whorls in 74 specimens of *Nummulites djokdjokartae.* Group A1 is indicated in black, group A2 in white. Error bars indicate 90% percentile. Numbers above error bars indicate number of specimens.

Fig. 5. Histogram of the proloculus size in *N. djodjokartae*. In black specimens with >= 5 whorls are indicated

Fig. 6. Virtual equatorial (A, B) and axial (C, D) sections of specimen 06KW01_02 (type A2; A, C) and 06KW0110 (type A1; B, D). Scale bars represent 0.5 mm.

Fig. 7 Biometrics of 74 specimens of *N. djokdjokartae*. A: numberof chambers perwhorl; B: chamber length. Group A1 is indicated in black, group A2 in white. Error bars indicate 90% percentile. Numbers above error bars indicate number of specimens.



Renema, Cotton Fig. 1

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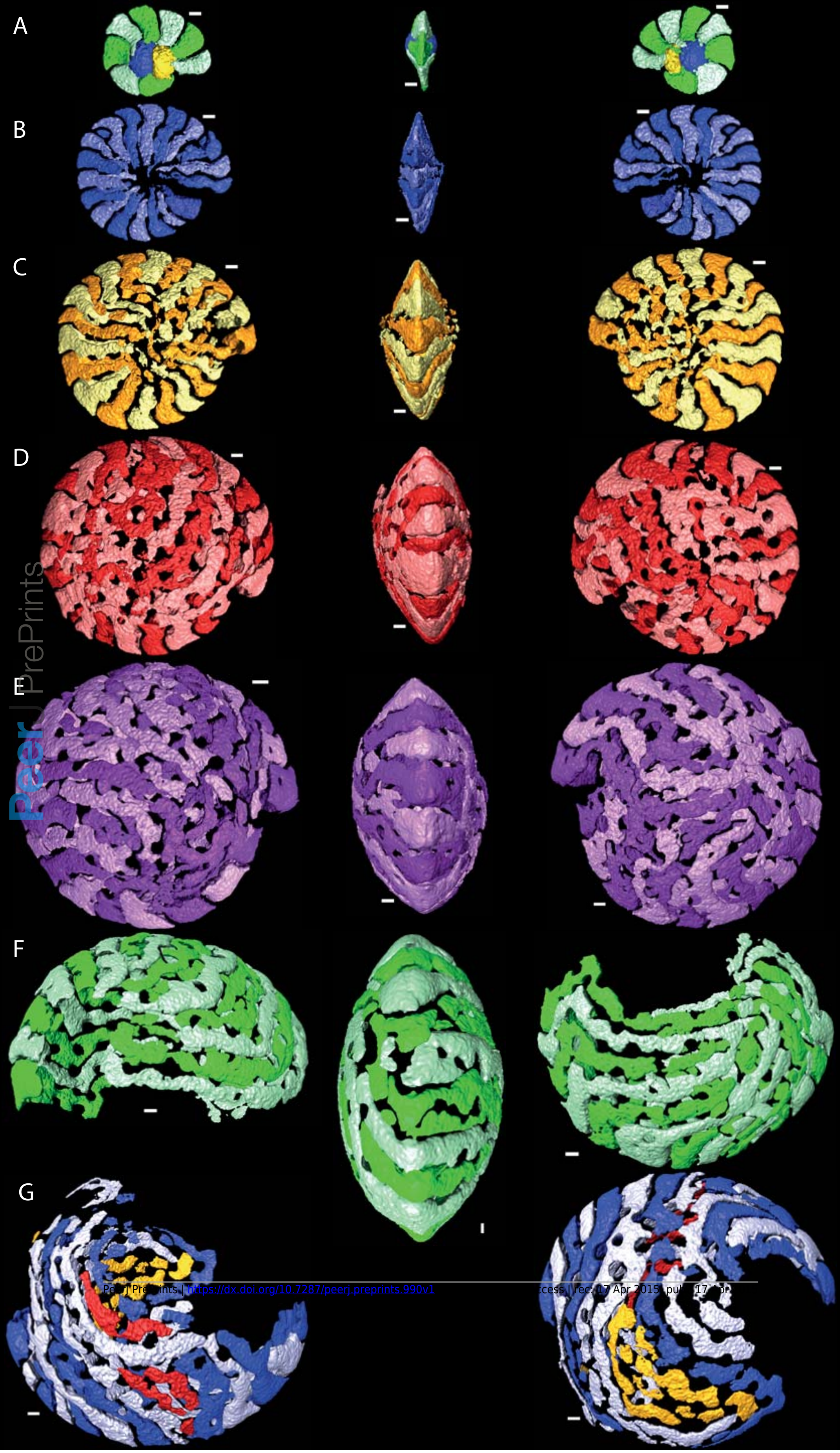

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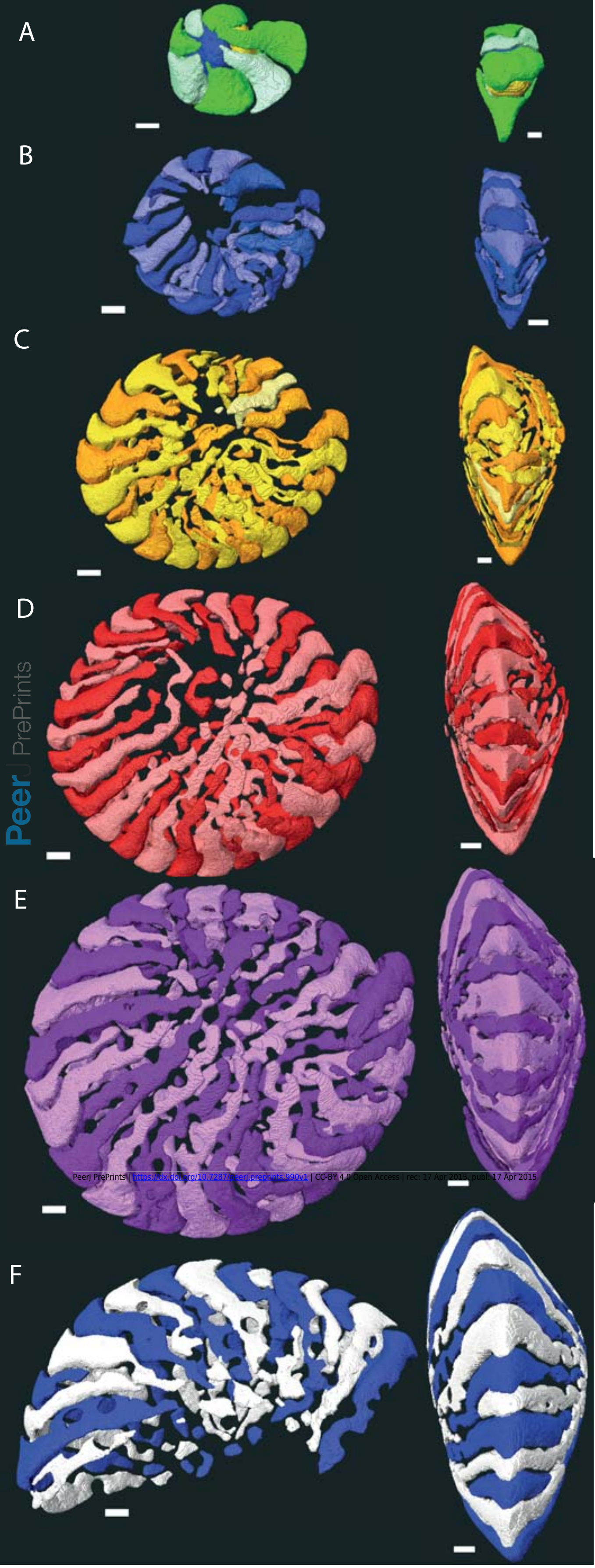

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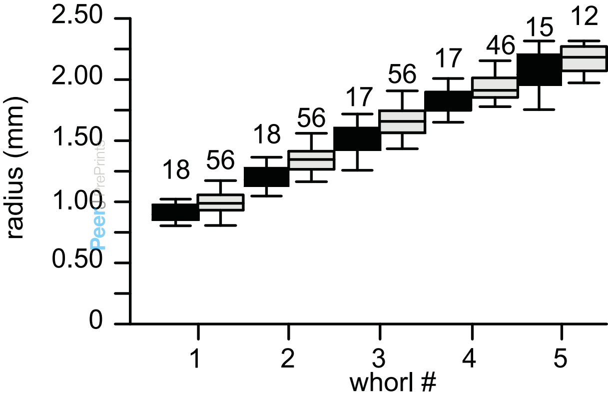

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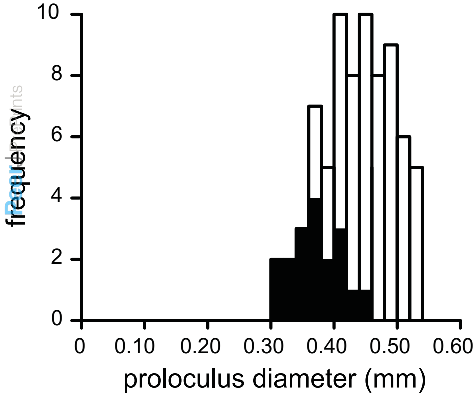
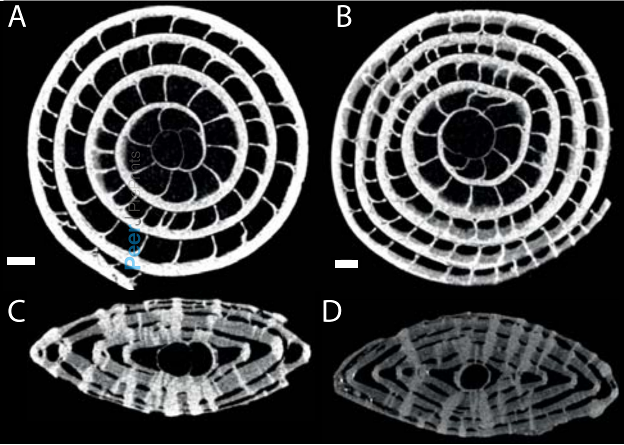


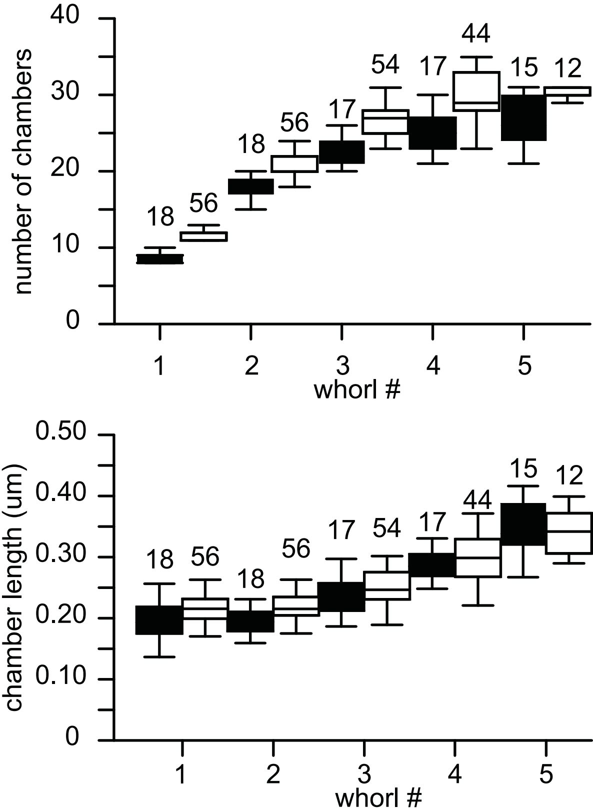
Renema, Cotton Figure 5



Renema, Cotton Fig. 6

A

B



Renema, Cotton, Figure 7

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