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Size patterns of the coccolith *Watznaueria barnesiae* in the lower Cretaceous: biotic *versus* abiotic forcing

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Abstract

Bodysize is a major trait allowing the assessment of the respective control of evolution *vs.* (palaeo)environment on unicellular micro-organisms. Size patterns can be retraced for fossilisable micro-organisms, especially for coccolithophores, over geological time spans. Here, we present coccolith size patterns of *Watznaueria barnesiae*, which was a dominant and ubiquitous taxon in the Mesozoic. Measurements were conducted at two sites spanning the Upper Berriasian to Lower Hauterivian, namely the Vergol-La Charce section in the epicontinental Vocontian Basin, and the ODP site Leg 185 Hole 1149B located in the Nadezhda Basin, in the Pacific Ocean. The obtained results were treated using multivariate analysis in order to detect significant trends of the measured parameters. Size values of coccoliths from both sites are remarkably similar and display comparable patterns, namely a statistically significant increase in the Lower Valanginian until maximum sizes recorded in the aftermath of the Weissert event (Upper Valanginian). Although *W. barnesiae* coccolith-size fluctuations, in the short term, might be related to environmental changes, the observed long-term size patterns are interpreted as the result of directional selection, in which a single phenotype is favoured through time. Comparison with data available in the literature shows a return to a smaller size after the Turonian.

Keywords: Coccolith; biometry; *Watznaueria barnesiae*; Lower Cretaceous; palaeoenvironment; evolution.

1. Introduction

In modern oceans, the size patterns of marine phytoplankton communities greatly affect food-web structure and organic/inorganic carbon export into the ocean interior (Popp et al., 1998; Hopkins et al., 2015). Body size is a major trait of organism's evolution. Of particular relevance for unicellular organisms, is the fact that diffusion across the cell membrane, especially passive diffusion, is strongly dependent on the surface-area/volume ratio (Lewis, 1976; Aloisi, 2015). Surface-area is proportional to length squared whilst volume is proportional to length cubed, so unless cell-shape changes, larger cells need more resources and produce more waste products, whilst diffusion becomes less efficient. The metabolic rates of unicellular organisms are thus a function of this surface-area/volume ratio (e.g., Atkinson et al., 2003; Fu et al., 2008; Key et al., 2010). In some coccolithophores, such as *Emiliania huxleyi*, culture studies have shown a clear relationship between some physiological parameters and the cell volume (Finkel et al., 2009).

Culture studies under controlled conditions or surface-water sampling in selected oceanic settings allow us to observe the short-term responses of coccolithophore size to change in physical or chemical parameters, such as the pH or the saturation state of ocean waters (e.g., Riebesell et al., 2000; Iglesias-Rodriguez et al., 2008; Beaufort et al., 2011). However, these studies provide data that are limited in terms of time-duration, and do not allow testing of the impact of evolution on organism's size changes.

With coccolithophores we have the chance to measure body size back in deep geological time, to address questions about the relative importance of biotic controls (such as competition, predation, grazing, infection, etc.) *versus* abiotic forcing (such as climate, geography, extreme events, etc.) in shaping the evolution of life on Earth. One complication is that coccolithophore fossils are predominatly isolated coccoliths rather than complete coccospheres. However, Henderiks (2008) has shown that for the dominant Cenozoic placolith genera there is a linear relationship between coccolith and coccosphere sizes, and that coccosphere size is proportional to cell size.

Here we present data on coccolith size changes of the species *Watznaueria barnesiae*, which is the dominant coccolith in Lower Cretceous sediments. This morpho-species is known for its ubiquity in Mesozoic oceans (Mutterlose et al., 2005), and for its longevity (first

68 occurrence at the end of lower Toarcian for *W. fossacincta*, ~181 Ma, or Middle Jurassic,
69 ~168 Ma for *W. barnesiae*; last occurrence at ~64 Ma; Mattioli and Erba, 1999; Lees et al.,
70 2005). Biometry can be safely applied to *Watznaueria barnesiae* because this coccolith is
71 known for its resistance to diagenetic alteration (Roth and Krumbach, 1986). We have
72 predominantly measured isolated coccoliths, but whenever possible we also measured entire
73 coccospheres in order to test if the size relationships observed by Henderiks (2008) apply to
74 *Watznaueria*.
75 For this study, we selected Lower Cretaceous samples (upper Berriasian to basal Hauterivian;
76 ~140-133 Ma) from the Vocontian Basin, SE France (Vergol and La Charce composite
77 section; Fig. 1b). This section has been thoroughly studied for sedimentology (Greselle et al.,
78 2011), ammonite content (Reboulet, 1996; Reboulet and Atrops, 1999; Reboulet et al., 2003;
79 Reboulet and Rard, 2008; Kenjo, 2014; their ammonite zonal schemes are here updated
80 according to the standard zonation established by Reboulet et al., 2014; 2018), nannofossil
81 assemblages and geochemistry (Mattioli et al., 2014), and for biometric studies of
82 nannoconids (Barbarin et al., 2012). In order to discriminate the local *versus* global control on
83 coccolith size, we have used for comparison to the epicontinental Vocontian Basin samples
84 from the ODP Leg 185 Hole 1149B located in the Nadezhda Basin, in the Pacific Ocean (Fig.
85 1c; Plank et al., 2000). The two sites can be precisely correlated by nannofossil
86 biostratigraphy (Lozar and Tremolada, 2003; Mattioli et al., 2014). The Berriasian-
87 Hauterivian interval has been selected because it is characterised by a marked perturbation of
88 the carbon cycle indicated by a ~2‰ positive excursion of $\delta^{13}C_{carb}$, namely the Valanginian
89 Weissert event (Erba et al., 2004). This interval is also characterised by a nannoconid decline
90 (Erba and Tremolada, 2004), and is interpreted as a cool interlude within a global greenhouse
91 climatic period (Lini et al., 1992; Gréselle et al., 2011; Föllmi, 2012). This episode of well-
92 documented environmental change is thus ideal to determine whether such environmental
93 change impacted the morphology of *Watznaueria barnesiae* coccoliths.
94
95 Figure 1 approx. here
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97 **2. Material and methods**
98
99 In this paper, specimens of *W. barnesiae* were measured along with *W. fossacincta*. This latter
100 form, which is rare in the studied samples, presents the same morphology as *W. barnesiae*,
101 but has a slightly open central area. *Watznaueria fossacincta* first occurred at the end of the

lower Toarcian (Mattioli and Erba, 1999), while *W. barnesiae*, which has a closed central area, first occurred in the basal Bathonian (Mattioli and Erba, 1999; Fig. 2). Bornemann and Mutterlose (2006) performed a biometric study on Albian *Watznaueria* and concluded that there is no difference between these two morphotypes other than the central opening size, which is a gradational character. They explicitly reject using them as two separate species. Our observations suggest that this conclusion is valid for the entire Cretaceous. In the rest of the text, we will refer to them as a single entity, *W. barnesiae*, including both morphotypes. The measured parameters are the coccolith length (L) and width (W) in distal view, and the inner cycle length (l) and width (w). In the Vergol section, 38 coccospheres were also measured for their external and inner diameter and, when possible, the coccolith length was also measured (Fig. 2b).

Figure 2 approx. here

A minimum of thirty coccoliths of *Watznaueria barnesiae* per sample were measured in 60 samples of the Vergol-La Charce composite section. Samples were collected from marl-limestone couplets regularly distributed along the Vergol-La Charce composite section (Fig. 3). For the ODP Site 1149B, several samples were prepared but only 11 samples could be analysed from the cores 16R to 27R, where the siliceous component is relatively limited and nannofossils common (Fig. 4). Samples were prepared following the random settling technique of Beaufort (1991) and Geisen et al. (1999), which ensures a homogeneous dispersion of coccoliths on the slide. A small amount, 10 to 20 mg, of powdered rock was diluted in 500 ml of water. After 24 hours of settling on the cover slide, the water was very slowly evacuated, and turbulence was carefully avoided. Once dried, the cover slide was mounted on a microscope slide using Rhodopass. This method ensures that coccoliths are well isolated on the slide, they are not overlapping or tilted.

Images of at least 30 coccoliths per samples were taken under a LEICA DM750P, polarising microscope at 1000X. Images were taken with a LEICA EC3 camera using the software LAS (LEICA Application Suite). Measurements were done using ImageJ. The measurement precision is ± 0.1 µm, estimated by 30 repeated measurements of the calibration micrometre. In the analysed images, 1 µm corresponds to 17.7 pixels. Measurement of 30 specimens per sample is statistically robust when working on a single morphospecies (Suchéras-Marx et al., 2010), because it gives a maximal error of 10% in the estimate of the mean (95% confidence interval) and, with an increasing sample size (e.g., 50, 100 measurements or more), the error

136 percentage of the average value decreases very little. We applied the Suchéras-Marx et al.
137 (2010) test to our dataset, and we obtained similar results. Thus 30 specimens per sample
138 seem to be sufficient to evaluate the mean size of the population.
139 In addition to the length and width parameters, we have calculated the surface areas of the
140 inner and outer cycles of the coccolith, and its ellipticity (Figs. 3 and 4). Statistical analyses
141 have been then applied in order to test the robustness of the observed size trends. A
142 confidence interval at 95% of the average size values has been calculated as follows:

143 $CI_{average\ (\alpha=0.5)} = 1.96 * (\sigma/\sqrt{N})$

144 where σ is the standard deviation of the population, and N is the number of measured
145 specimens in each sample. Most of the statistical tests applied below need the analysed
146 data to be normally distributed. A Shapiro-Wilk test applied to our dataset shows that all the
147 samples have a normal distribution with $-2.7 \times 10^{-19} < p < 0.03$.
148
149 Figures 3 and 4 approx. here
150
151 *2.1. Statistical treatment of biometric parameters*
152
153 In order to test if there is a significant stratigraphical difference for the various parameters of
154 size analysed, an ANOVA (parametric ANalysis Of VAriance) was applied to the coccolith
155 length values of both sections using the software PAST. The null hypothesis is that all the
156 samples have the same average size values. We used the test of Levene, which analyses the
157 homogeneity of the variances and, when Levene was significant with a $p < 0.05$, the test F of
158 Welch was subsequently used. When the result of the test F of Welch was significant, the null
159 hypothesis could be rejected meaning that at least one sample differs significantly and non-
160 randomly from all the others.
161 Eventually, we have applied to the dataset the test of Tukey allowing comparison of samples
162 to each other for a given parameter and assessing the degree of similarity or, conversely, of
163 difference. This test is particularly useful for the Vergol-La Charce composite section, where
164 the number of samples is elevated. The percentage of the difference between groups made of
165 similar samples is then calculated. A MANOVA (Multivariate analysis of variance) was
166 applied, in order to assess the difference between the studied samples on the basis of four
167 parameters, namely L, W, l and w of *W. barnesiae* coccoliths.
168 A Run test was finally applied in order to assess the robustness of the observed trends of the

different size parameters. In this test, each value is compared to the median value with a null hypothesis stating that the number of higher values is equal to the lower ones, thus there is not a preferential trend. If the test is significant ($p < 0.05$) the null hypothesis can be rejected with a risk α, which is calculated, and a significant trend is assessed.

2.2. Detection of modal trends in the analysed dataset

In order to discriminate between a uni- or a pluri-modal distribution of the analysed set of data, and to identify the existence of one or more distinct groups within the totality of coccolith length measurements, mixture analysis was applied to the Vergol-La Charce measurements. This is a robust analysis tool performed with the software PAST, based on a maximum-likelihood method. The minimum values of Akaike Information Criterion (AIC) helped identifying the groups obtained by mixture analysis showing the lowest overfitting (Hammer et al., 2001). The number of bins used for mixture analysis (8 in the present work, 0.5 µm each) depends on the size of the dataset and follows the Sturges' formula, as described in Ferreira et al. (2017).

3. Results

3.1. Coccolith length versus *coccosphere size*

Coccolith length plotted *versus* coccolith width for the Vergol-La Charce composite section and the ODP Site 1149B shows that these two parameters have a positive linear relationship (Fig. 5a). Coccolith length varies at both sites from ~3.1 µm to 7.7 µm, coccolith width varies from 2.6 µm to 6.5 µm. In the following, we show coccolith length trends. Outer and inner diameter of the coccosphere could be measured for 19 specimens (Fig. 5b). Coccospheres are slightly elliptical and there is a very good coefficient of correlation between these two parameters (r=0.83). The external diameter of the 38 coccospheres plotted *versus* the size of their coccoliths shows that coccospheres vary between 6.5 and 12 µm (Fig. 5c), while coccolith lengths are between 3.9 and 6.6 µm. The coefficient of correlation between these two parameters is 0.56 showing that, although coccolith size is dependent on the coccosphere that produced it, there is a considerable degree of size variation.

Figure 5 approx. here

3.2. Coccolith size and shape through time

In the Vergol-La Charce section (Fig. 3), the size and the shape of *W. barnesiae* coccoliths display long term changes. Average coccolith-length (L) fluctuates between 4.8 and 5.5 µm in the upper Berriasian-lower Valanginian interval, until 44 m in the lower part of the NK 3A nannofossil zone. In the same interval, coccolith ellipticity (E) attains some of the highest values while the ratio between the surface of the coccolith and that of the inner cycle (S/s) shows some of the lowest values. Average coccolith-length overall shows higher values with weaker fluctuations in the upper part of lower Valanginian (upper part of the NK 3A zone) and during the Weissert event (upper Valanginian), except for a single sample at 97 m with very low values. Ellipticity is the lowest during the Weissert event with small fluctuations, the S/s ratio is conversely quite high. Coccolith size is the highest in the uppermost Valanginian, along with quite low ellipticity values and high S/s ratio.

This overall trend is confirmed by the Tukey test used for the contrast analysis which is based on the results of the ANOVA. In synthesis, all the similar samples with a *p* value issued from the Tukey test close to 1 have been grouped. Overall, 6 groups were recognized on the basis of this variance analysis (right side of Fig. 3; Fig. 6a and b). The comparison of each of these groups to the others allowed the calculation of the percentage of the difference between them (Fig. 6b). The statistical difference between the samples belonging to these groups has been then tested using a MANOVA (Fig. 6a). The samples are similar when the *p*-value is > 0.05. The smaller the *p*-value, the more different the samples. In synthesis, the samples at the base of the section (groups 1 and 2) are significantly different from the samples corresponding to the Weissert event and the interval above (groups 4 to 6). Group 3 (formed of a few samples) represents a transition between the two other sets of samples.

ODP Site 1149B shows a pattern very similar to that of the Vergol-La Charce composite section. Below the Weissert event *W. barnesiae* coccoliths show average lengths between 5 and 5.5 µm on average, are strongly elliptical and have weak values of S/s ratio. Coccolith ellipticity is the lowest and the S/s ratio is the highest during the Weissert event. Size is the highest with relatively weak ellipticity in the aftermath of the Weissert event.

For the ODP Site 1149B, the limited number of samples allowed a simpler data treatment. When the Tukey test revealed a *p*-value < 0.05, samples are significantly similar on the basis of coccolith length (Fig. 6d). Thus, three groups of samples are identified: from 23R to 27R spanning the lower Valanginian until the base of the Weissert event, from 20R to 22R in the

upper Valanginian, and from 16R to 19R uppermost Valanginian to lower Hauterivian. However, the MANOVA analysis based on all the considered parameters (L, W, l and w of *W. barnesiae* coccoliths) shows that samples 34R and 23R are significantly similar, thus 3 groups of samples are significantly different (Fig. 4 right side; Fig. 6c). So, the samples of ODP Site 1149B corresponding to the Weissert event and just above display very peculiar characters with respect to the other groups of samples located below and above the event interval, and this pattern is very similar to that shown at Vergol-La Charce.

Figure 6 approx. here

3.3. W. barnesiae *coccolith-size modes*

The observed size shifts could be due to a variable mixture of larger and smaller species, this is the reason why mixture analysis was applied. However, the minimum values of Akaike Information Criterion (AIC) are obtained from mixture analysis when a unimodal distribution is considered. A mode of 5.5 µm is observed (Fig. 7). As expected, similar results were obtained when considering the coccolith width (data not shown). This suggests that only one group of *W. barnesiae* occurs in terms of coccolith length, at least for the Vergol-La Charce section in which a significant number of specimens could be measured.

Figure 7 approx. here

4. Discussion

4.1. Relationships between coccolith and coccosphere size

Fossil coccolithophore cell geometry and size allow detailed assessment of past cell physiology and have important implications for the interpretation of coccolithophore-derived biogeochemical palaeo-proxies that are affected by algal volume-to-surface area ratios (e.g., Henderiks and Pagani, 2007; Plancq et al., 2012; Gibbs et al., 2013). Coccolith size is also a useful proxy for calcite production and carbonate burial by coccolithophores (e.g., Young and Ziveri, 2000; Suchéras-Marx et al., 2014). The relationship between coccolith size and coccosphere size has never been explored in Mesozoic sediments, probably because of the difficulty to recover entire coccospheres in ancient sediments, however this approach

represents a powerful tool to investigate coccolithophore cell geometry and size (Gibbs et al., 2013).

The material we studied and, especially, the Vergol-La Charce samples provided us with the possibility to analyse at the same time the coccosphere size and the coccoliths they hold. Our results allow considerations on the coccosphere and cell dimensions and, when comparing our results (Fig. 7) to the data shown by Henderiks (2008; fig. 3), *Watznaueria* displays a similar relationship to that of typical Cenozoic placoliths, suggesting that a change in mean coccolith size most likely reflects a change in cell size. Unfortunately, the number of analysed specimens is too low to establish a clear relationship between the coccolith and the coccosphere size. In fact, in spite of a general positive correlation, the correlation coefficient we record is relatively weak to establish a coccolith size rule as it was done for Cenozoic genera (Henderiks, 2008). It is possible that besides the limited sampling size, our analyses were hampered by the observations which were made under an optical microscope. Further SEM analyses should be performed in order to better elucidate size rules for lower Cretaceous coccoliths.

4.2. Significance of obtained results

Since the nineties, coccolith biometry has received a strong input and now represents a very powerful proxy for paleoenvironnemental interpretations, as well as for taxonomy and evolution investigations. Most of the papers have shown bivariate graphs or frequency distribution of simple parameters, such as coccolith length and width (e.g., Young, 1990; Bornemann et al., 2003; Bornemann and Mutterlose, 2006; Linnert et al., 2014; Lübke et al., 2015; Lübke and Mutterlose, 2016). Renaud et al. (2002) introduced the use of parametric analysis of variance (ANOVA) in order to study the changes of morphological differences and dynamics of the living coccolithophore *Calcidiscus leptoporus*. Barbarin et al. (2012) used smoothing functions and tested the significance of the observed stratigraphical trends of the nannolith *Nannoconus*. Giraud et al. (2006) have tested the relationships between various biometric variables in the *Watznaueria britannica* pool using Principal Component Analysis. Mixture analysis or Principal Component Analysis have been recently used to discriminate between a uni- or pluri-modal distribution of size parameters (Suchéras-Marx et al., 2010; Barbarin et al., 2012; Ferreira et al., 2017).

In this paper, following the guidelines of the Ferreira et al. (2017) paper, we have tested the significance of the fluctuation of one (L) or more (L, W, l, w) parameters, using ANOVA and

MANOVA respectively. These different tests allowed us to identify groups of samples which are significantly different from the others in the two studied localities. The general trend in both studied sites is a significant size increase in the *W. barnesiae* coccolith length from ~5 µm to ~6.5 µm in the course of Valanginian. Slight differences between the two sites are very likely due to a lower sample resolution in the ODP Site 1149B, making it difficult to precisely assess the exact lower and upper boundary of the Weissert event.

Parallel to the size increase, morphology changed significantly across the Valanginian. In both the studied sites, there is a general trend of decreasing ellipticity and increasing ~~of~~ coccolith-to-inner cycle ratio with the general increase of size. This is a known trend for Cenozoic and extant species *(Pseudoemiliania lacunosa* or *Emiliania huxleyi*; Young, 1989; Henderiks, 2008) and for upper Jurassic coccoliths (*W. britannica*; Giraud et al., 2006). Biometry applied to these forms shows that larger coccoliths are globally less elliptical. This constitutes an allometric trend and seems to indicate that the growth of coccoliths is more effective for the minor axis, thus within a given genus larger coccoliths tend to be less elliptical. However, the *W. barnesiae* specimens measured in this work display some of the largest sizes in the aftermath of the Weissert event, where ellipticity is also significantly high. It is therefore not excluded that besides the allometric trends in coccoliths, with ellipticity decreasing along with size increase, peculiar environmental conditions occurring during the Weissert event may have forced *W. barnesiae* to adopt a peculiar coccolith morphology.

4.3. Environmental versus *evolutionary control on coccolith size and shape*

In the literature, there is conspicuous evidence of micro-organism size changes during major palaeoceanographic events. Significant size decrease is documented for coccoliths and nannoliths during anoxic or hyperthermal events. This is the case for the Toarcian anoxic event (T-OAE) where *Schizosphaerella* and coccoliths turned out to be smaller (Mattioli et al., 2004; Suan et al., 2010), or the OAE1a where some coccolith species display malformation and dwarfism (Erba et al., 2010; Lübke and Mutterlose, 2016). Conversely, during the PETM interval the size of *Discoaster* increased in response to higher temperatures and low nutrient concentrations in surface waters (Tremolada et al., 2012). Size and calcification degree of coccoliths seem also to increase in response to arid conditions, likely acting on the saturation state of oceanic surface-waters. This was the case for the late Oxfordian (Giraud et al., 2006) or the Tithonian (Bornemann et al., 2003), but also for the nannoconid recovery that occurred concomitant with the acme of the Weissert event (Barbarin

et al., 2012). The size of *W. barnesiae*, however, was evidently less sensitive to environmental changes than that of other coccoliths. In fact, *W. barnesiae* size was not significantly affected by environmental shifts during either OAE1a or OAE1d, leading the authors to interpret it as having high environmental tolerance (Bornemann and Mutterlose, 2006; Lübke and Mutterlose, 2016). Also, no clear relationship exists between *W. barnesiae* size patterns and proxies for palaeoenvironmental changes or geochemical cycling (Fig. 8), although the size record is discontinuous in the Lower Cretaceous.

When comparing *W. barnesiae* size distribution data from the two sites considered in this account located respectively in the Tethys (Vergol-La Chance) and in the Pacific (ODP Site 1149B), it is surprising to observe how closely both the bulk size distributions (Fig. 4) and the Early Cretaceous size increase trend (Fig. 8) match in both sites. Analogous co-variation in mean *Watznaueria* pool size were presented by Bornemann et al. (2003), who studied three different oceanic sites of Tithonian to early Valanginian age, and by Erba et al. (2010) who reported trends of *W. barnesiae* size in two sites in Tethys and Pacific Ocean during the Aptian. These observations lead to two main deductions, namely that (i) the genetic flux was not interrupted between populations in the Tethys and Pacific Oceans in the Lower Cretaceous likely related to a circum-global circulation, and (ii) *Watznaueria* size trends are more likely driven by supra-regional or evolutionary changes than by local environmental conditions. We have compiled data on average coccolith length from various papers (Fig. 8), and some peculiar patterns can be observed for the Cretaceous. Bornemann et al. (2003) measured the *Watznaueria* pool, which is composed of *W. barnesiae*, *W. fossacincta*, *W. britannica* and *W. manivitiae*, in three Atlantic sites. The average size per sample for this pool was very high in the Tithonian (7-8 µm), then sharply decreased at the Jurassic/Cretaceous boundary to around 5 µm in the mid-Berriasian. This shift corresponds to a drastic change in the species-specific *Watznaueria* composition, large-sized forms of this genus (*W.* cf. *manivitae*, > 8 µm) were very abundant in the Tithonian, while *W. barnesiae* became dominant within the genus by the Berriasian.

Figure 8 approx. here

The average size of ~5 µm measured in the upper Berriasian in three Atlantic sites by Bornemann et al. (2003) corresponds thus to the record of the two sites (one in the Tethys and the second in the Pacific) studied in this account. The most significant size increase of the *W. barnesiae* pool occurred during the Valanginian and across the Weissert event, with average

sizes attaining ~6.5 µm. This size shift has turned out to be statistically significant and is likely an example of Cope's rule, as already proposed for different marine microorganisms (Schmidt et al., 2006) and for Jurassic coccolithophores (*Discorhabdus*, López-Otálvaro et al., 2012; *Lotharingius*, Ferreira et al., 2017). Size evolution of the *W. barnesiae* lineage seems to start near a limiting boundary (~5 µm on average), and then to diffuse away from an originally small-sized ancestor, following the left-wall model (Stanley, 1973). In spite of a discontinuous record of *W. barnesiae* size for the rest of the Cretaceous, it seems that average sizes stayed quite stable in the aftermath of the Weissert event from late Valanginian until Turonian, regularly fluctuating between ~5.5 to ~6.5 µm or even lesser. Apparently, no significant shifts occurred during the OAE 1b or Cenomanian/Turonian events (Bornemann and Mutterlose, 2006; Linnert et al., 2014). During the OAE 1a, Erba et al. (2010) reported a size decrease of *W. barnesiae* of about 1 µm from background values, which might attest for the impact of palaeoceanographic changes, likely linked to increased pCO_2 levels, on coccolith size (Fig. 8). However, size fluctuations of similar amplitude are also observed in the Berriasian-Hauterivian interval, where a clear trend to size increase is observed. *Watznaueria* radiated in times of elevated levels of pCO_2 (Fig. 8). Its resilience to periods of enhanced pCO_2, like during the OAE 1a, might result from an "evolutionary memory" of past atmospheric composition, as described for Cenozoic species by Henderiks and Rickaby (2007).

Watznaueria barnesiae seems therefore to represent an example of directional selection, in which a single extreme phenotype (in terms of size) is favoured (e.g., Kingsolver and Pfennig, 2004). The stationary model proposed by Stenseth and Maynard-Smith (1984), stating that evolution is largely driven by abiotic changes such as temperature, water column stratification or primary productivity does not seem to apply to the *W. barnesiae* size evolution, although some extreme events (like OAE 1a) have produced a temporary size decrease. This model conversely applies to other micro-organisms, as it is shown by the correlation observed between size evolution of Cenozoic foraminifera and palaeoceanographic perturbations (Wei and Kennett, 1983; Schmidt et al., 2004a, 2004b).

Although few measurements are available, *W. barnesiae* size seems to decrease to background values from the Coniacian to Santonian (Fig. 8; *W. barnasiae* represents on average > 86% of the *Watznaueria* pool measured by Linnert et al. (2014). This size decrease started some 20 Myrs before the disappearance of the morpho-species which happened at the end of the Cretaceous (Lees and Bown, 2005), although it sporadically occurred as a survivor in basal Danian sediments (Bown, 2005).

5. Conclusions

Watznaueria barnesiae offers a unique opportunity to monitor the coccolith size and, to a certain extent, the cell size over a very long time span, > 100 Myrs. The present paper focuses on the upper Berriasian to basal Hauterivian interval, but comparisons with previous papers allows considerations to be made for the whole Cretaceous period. This dominant and eurytopic taxon is composed of at least two morphotypes (namely, *W. fossacincta* and *W. barnesiae*) but, because of its longevity (~110 Myrs), it might be suspected to be composed of a pool of cryptic species. If so, these are not distinguishable on the basis of biometric criteria, because, as shown in this paper, the size distribution of *W. barnesiae* coccoliths is unimodal. Whatever the genetic significance of this morpho-species, the coccolith size and shape are remarkably similar in oceanic sites which are far away from each other, and subject to different oceanographic regimes. This pattern seems to indicate that a genetic flux occurred between populations in a context of a circum-global oceanic circulation.

The smallest size of *W. barnesiae* is recorded in the Berriasian with average values at around 5 µm. Then a statistically significant size increase is observed across the lower Valanginian, starting before the palaeoceanographic Weissert event up to average values as high as 6.5 µm in the uppermost Valanginian/basal Hauterivian. Coccolith size stayed then quite steady oscillating between 5.5-6.5 µm from the Hauterivian to Turonian. No significant size changes are observed in the middle Cretaceous apart from a temporary decrease during the OAE 1a, although dramatic oceanic and climatic events occurred, such as the OAE 1a or the Cenomanian/Turonian event. However, a decreasing size trend is observed from the Coniacian.

Thus, the long-term pattern of the *W. barnesiae* coccolith size seems to be more likely related to evolutionary trends than to a body-size response to drastic environmental changes. *Watznaueria barnesiae* appears to have been a very robust taxon, poorly sensitive to environmentally-driven changes, as far as its coccolith size is concerned. Although we do not have any detailed size record data for the Jurassic period, which witnesses the rise to dominance of this taxon within the nannofossil assemblage, *W. barnesiae* lineage seems to follow Cope's rule in the Berriasian-Barremian interval, which is the tendency for lineages to evolve to larger body size. In this regard, *W. barnesiae* displays a certain similarity with the Jurassic genus *Lotharingius* also belonging to the Watznaueriaceae (Ferreira et al., 2017). *Watznaueria barnesiae* size changes seem therefore to illustrate a directional selection, in

which a single extreme phenotype (in terms of size) is favored through time, then returning to smaller size during its late stratigraphic range. If this model held true, then due to the dominance of this taxon in Mesozoic rocks, *W. barnesiae* size could be used in future works along with quantification of its export fluxes for assessing the transfer of particulate inorganic and organic carbon to the sedimentary reservoirs, thus implementing biogeochemical modelling.

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Figure legend

Figure 1. (**a**) Global palaeogeographic map for the lower Cretaceous, redrawn from Zella et al. (2015), where the approximate position of the two studied sites is shown. (**b**) In the lower Cretaceous, the epicontinental Vocontian Basin was surrounded by shallow-carbonate platforms and was opened to the Tethys Ocean to the East (after Gréselle et al., 2011). (**c**) The ODP Site 1149B was located in the western Pacific Ocean, Nadezhda Basin.

Figure 2. (**a**) Micrograph of a *W. barnesiae* coccolith in optical microscope, crossed polars. The measured parameters are shown, namely: coccolith length (L) and width (W), and inner-cycle length (l) and width (w). (**b**) Coccosphere of *W. barnesiae* in optical microscope (parallel polars) with the parameters which were measured, namely the outer and inner diameter of the coccosphere and, when possible the coccoliths forming it.

Figure 3. Vergol-La Charce composite section. Average coccolith length per sample with 95% confidence interval, average coccolith ellipticity and average ratio coccolith/inner cycle surface. The stratigraphic patterns of these biometric parameters are plotted against the stratigraphic column (after Gréselle et al., 2011), the age, and the $\delta^{13}C_{bulk}$

values. The ammonite zonation is built after Reboulet (1996), Reboulet and Atrops (1999), Reboulet and Rard (2008), Kenjo (2014), and updated according to the standard zonation established by Reboulet et al. (2014; 2018). Abbreviated ammonite names are: Inostr. = Inostranzewi; Platy. = Platycostatus; Proneco. = Pronecostatum; Callid. = Callidiscus. The position of organic matter-rich levels, the Barrande layers (Reboulet et al., 2003), and of the Weissert event are shown by shaded areas. On the right side of the figure are shown the groups of samples that are significantly different based on statistical analyses applied to the measured parameters.

Figure 4. ODP Site 1149B. Average coccolith length per sample with 95% confidence interval, average coccolith ellipticity and average ratio coccolith/inner cycle surface. The stratigraphic patterns of these biometric parameters are plotted against cores, lithology, age model, and the $\delta^{13}C_{bulk}$ values (after Plank et al., 2000). The position of the Weissert event is shown by shaded area. On the right side of the figure are shown the groups of samples that are significantly different based on statistical analyses applied to the measured parameters.

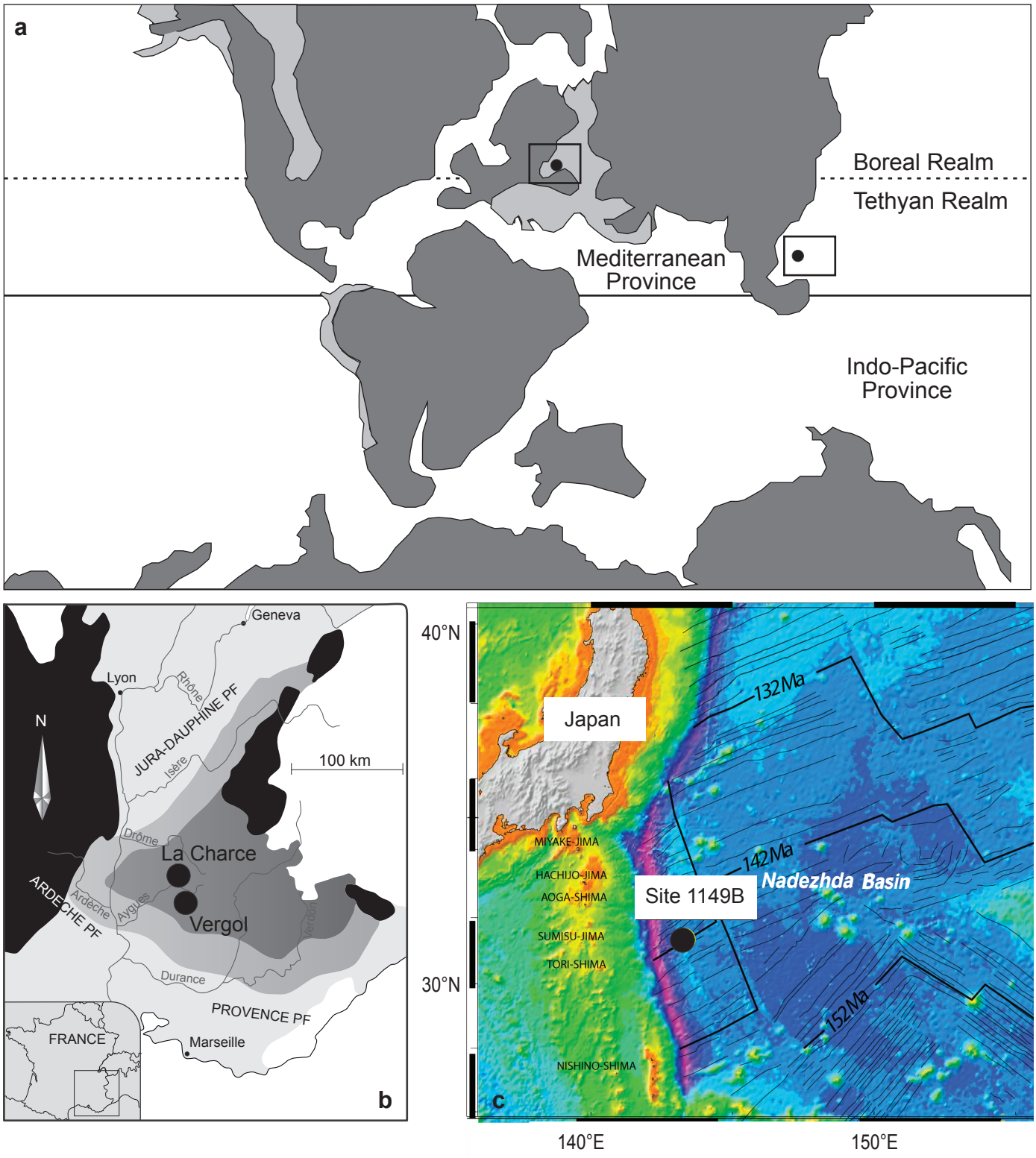
Figure 5. (**a**) Bivariate plot of *W. barnesiae* coccolith length *versus* width for the two studied sites. The two shown parameters have a positive linear relationship. (**b**) The plot of outer *versus* inner diameter for 19 measured coccospheres shows a very significant coefficient of correlation (r = 0.83). (**c**) When plotting coccosphere outer diameter *versus* the coccolith length measured on the coccospheres, the correlation coefficient is positive but data show a certain dispersion.

Figure 6. (**a**) The statistical difference between the samples belonging to the different groups has been tested using a MANOVA. The samples are similar when the *p*-value is > 0.05. The smaller the *p*-value, the more different the samples are. (**b**) Eventually, the percentage of difference between the 6 recorded groups has been tested. See the text for more explanation. (**c**). The samples were directly compared to each other using a MANOVA. Samples are statistically different when *p* is lower than 0.05. The lower the *p*-values, the higher is the difference between samples. (**d**) Solid lines show the groups of samples that are statistically different from each other.

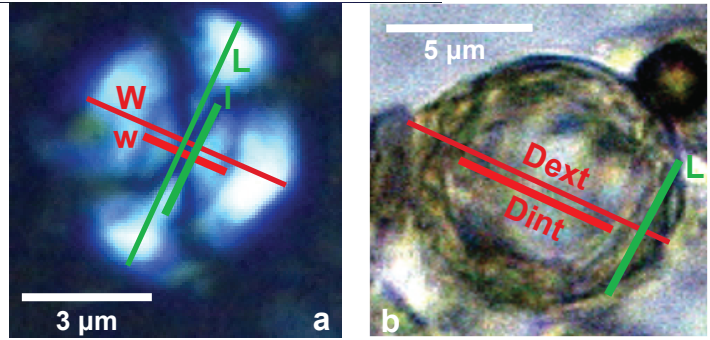
Figure 7. Results of the mixture analysis applied to the 1918 *W. barnesiae* specimens measured in the Vergol-La Charce section (coccolith length). The minimum values of Akaike Information Criterion (AIC) helped to detect the groups obtained by mixture analysis which show the lowest overfitting. For the studied dataset, a uni-modal distribution provided the lowest AIC values. The number of bins used for mixture

analysis (8 in the present work, 0.5 µm each) has been chosen according to the Sturges' formula (Ferreira et al., 2017). The modal value is 5.5 µm.

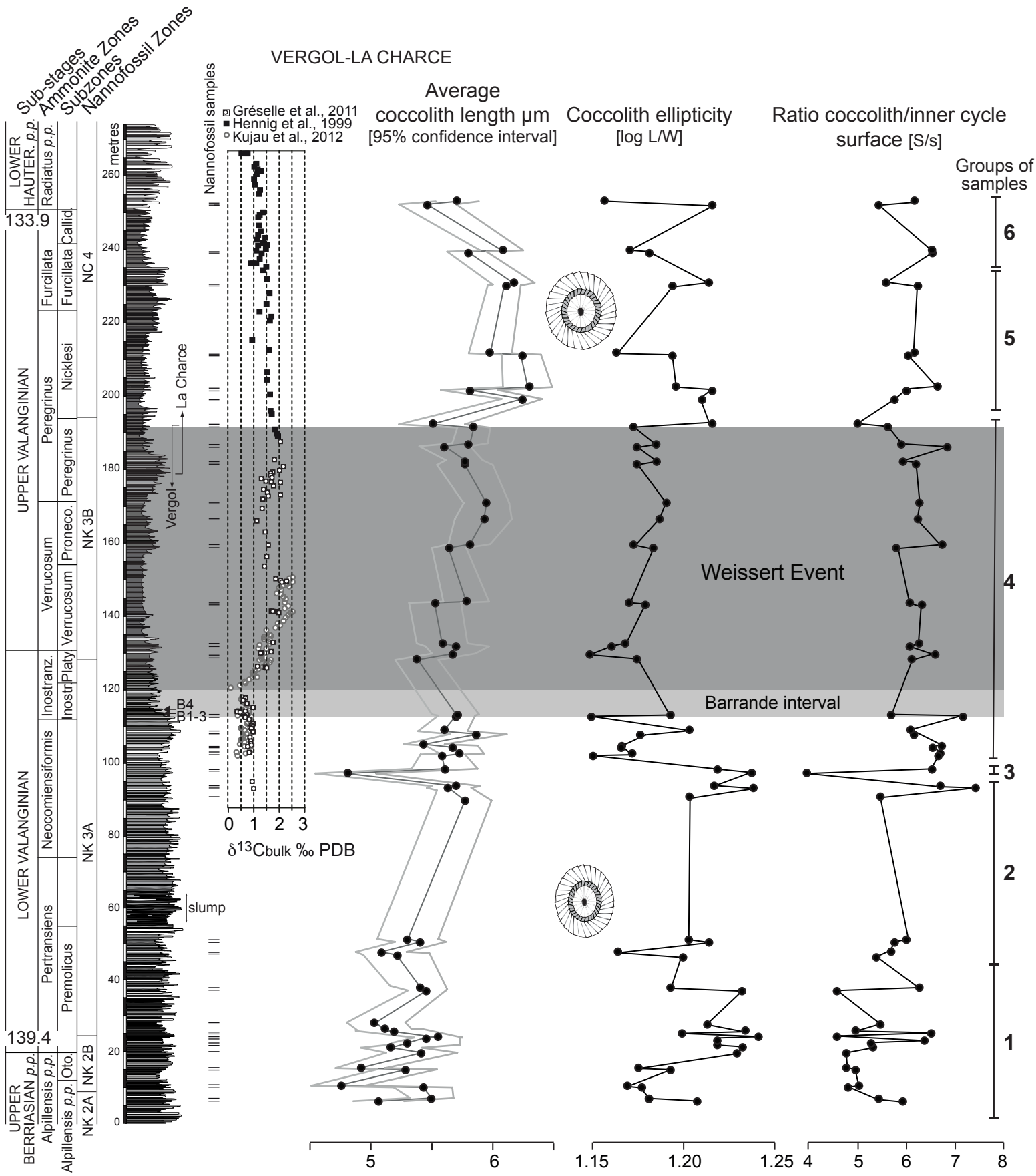
Figure 8. Average values per sample of *W. barnesiae* coccolith-length as deduced from this study (grey lines) and from the literature. First and last occurrence datums of *W. barnesiae* are shown. Also, the stratigraphic position of the most significant palaeoceanographic events of the Mesozoic is displayed (shaded lines). Please, note that the sharp size decrease in size reported by Bornemann et al. (2003) and occurring at the Tithonian-Berriasian is mainly related to a species-specific change. The *Watznaueria* pool contained less than 50% of *W. barnesiae* in the Tithonian, but more than 90% in the Berriasian. The Berriasian sizes documented by Bornemann et al. (2003) are thus mainly related to *W. barnesiae*. Also for the upper Cretaceous sizes, Linnert et al. (2014) measured all the *Watznaueria* pool. However, this is largely dominated (more than 86%) by *W. barnesiae*. The observed size trend is thus mainly related to *W. barnesiae* size fluctuations. The $^{87}Sr/^{86}Sr$ mean LOWESS fitted line through the data is after Gradstein et al. (2012). The $\delta^{13}C_{carb}$ curve is after the compilation of Gradstein et al. (2012). The $\delta^{18}O$ curves resulted from belemnite calcite or from planktonic foraminifers (for the Campanian-Maastrichtian interval in tropical, subtropical regions), according to the compilation of Gradstein et al. (2012). Error bars show the 90% confidence envelope. For the temperature scale, see Gradstein et al. (2012). The reconstruction of the atmospheric CO_2 in the considered time span is redrawn from Berner (2004). Black boxes on the right side show the ages of the Large Igneous Province emplacement (Gradstein et al., 2012): Karoo-Ferrar, at ~183 Ma; Paranà-Etendeka, at ~136-133 Ma; Ontong Java Plateau-Manihiki Plateau, at ~125-123 Ma; Kerguelen Plateau-Rajmahal Traps, at ~118 Ma; Caribbean-Columbian Province, at ~90 Ma; Deccan Traps, at ~66-65 Ma.



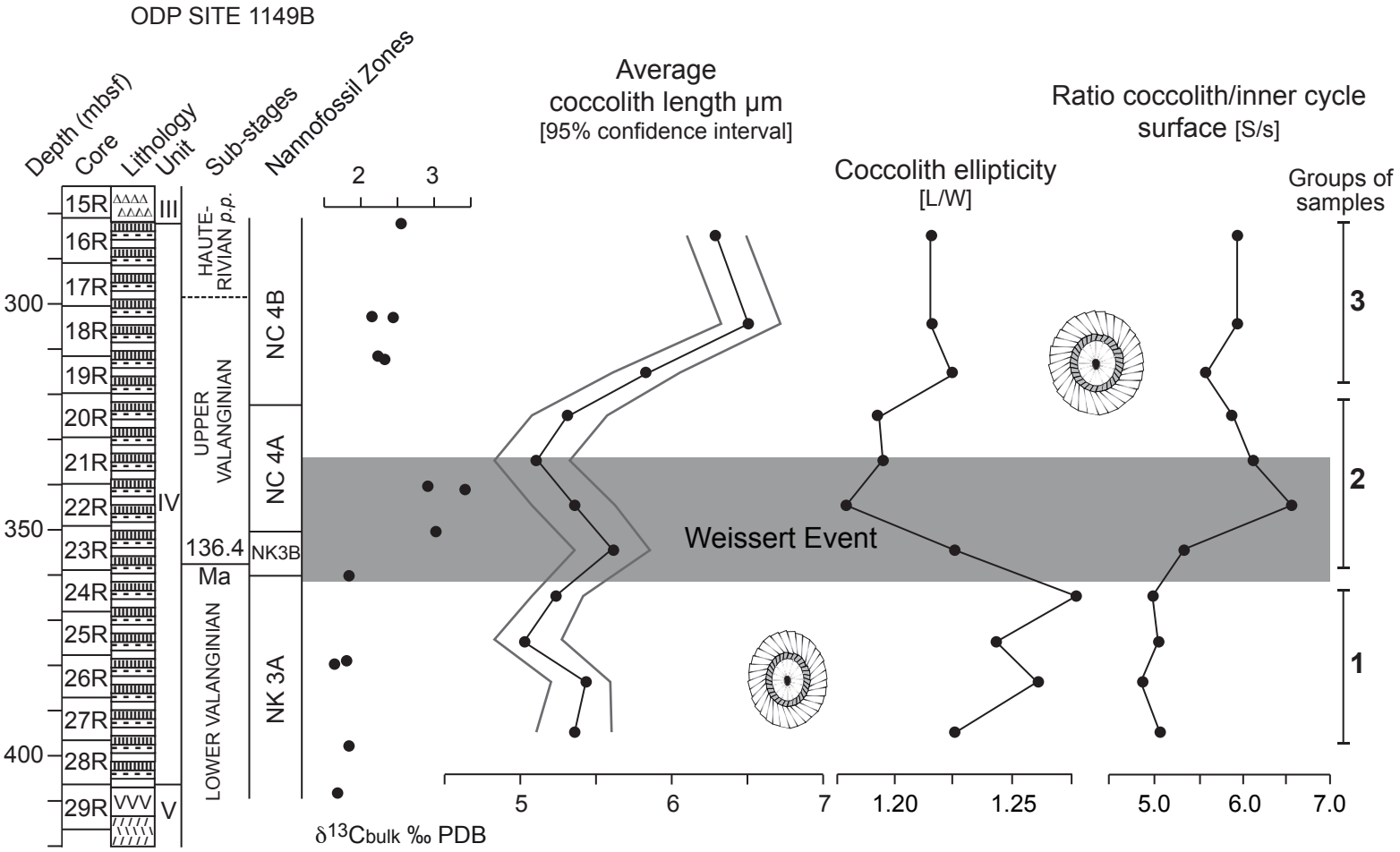
Gollain et al. Figure 1



Gollain et al. Figure 2

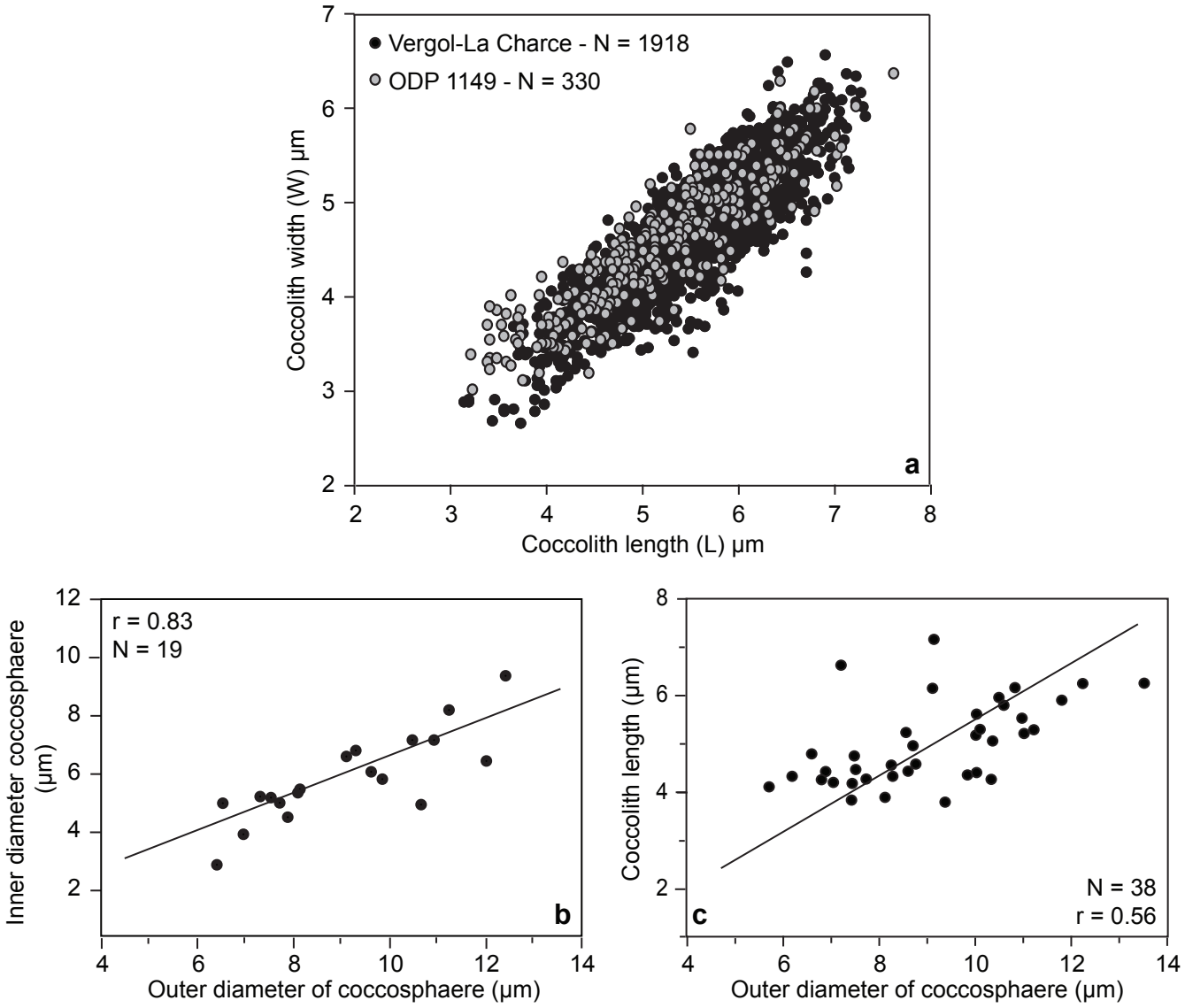


Gollain et al. Figure 3

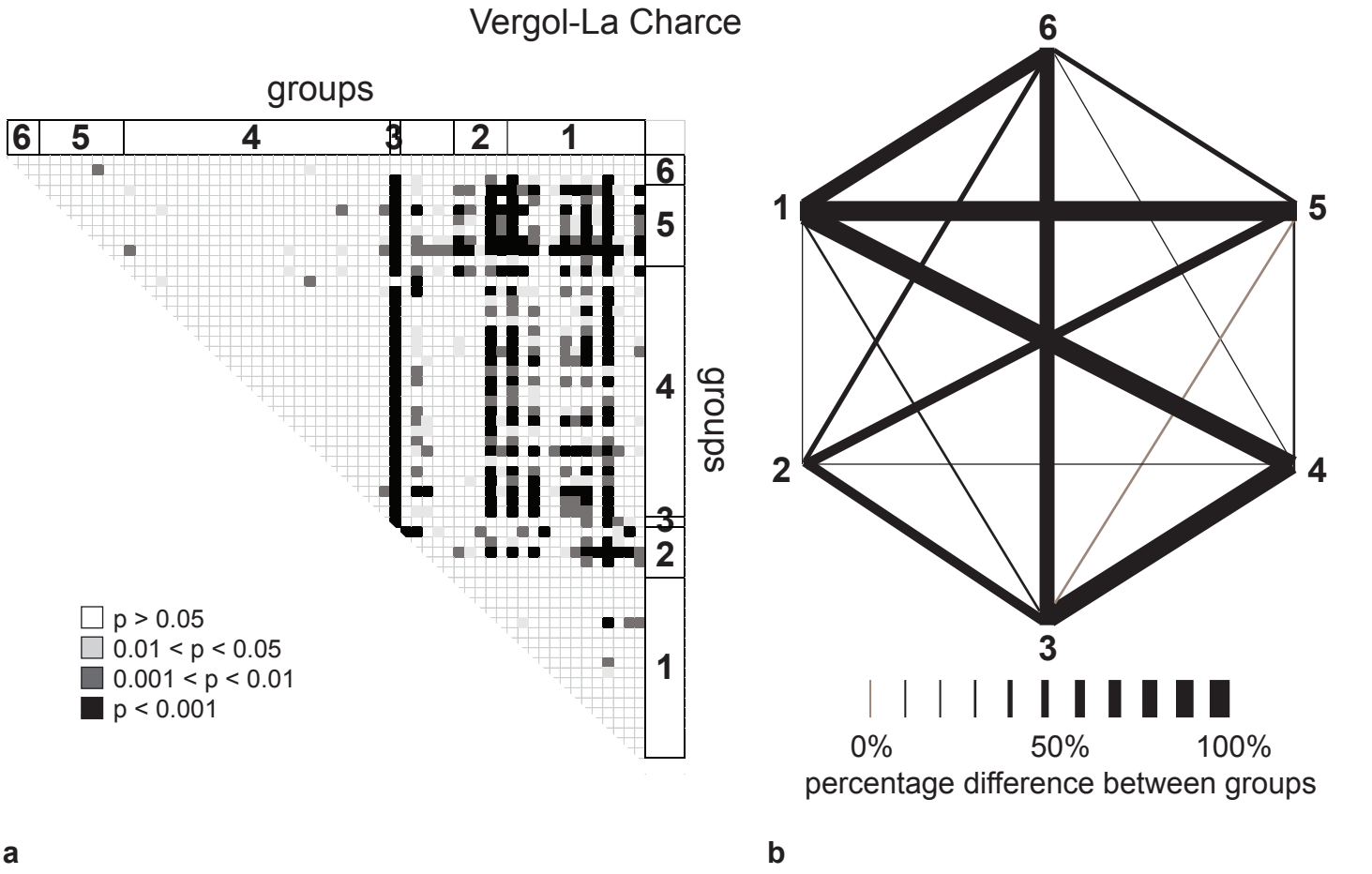


Unit III: interbedded radiolarian chert, porcellanite and siliceous clay
Unit IV: interbedded radiolarian chert and radiolarian/nannofossil chalk/marl
Unit V: basalt

Gollain et al. Figure 4

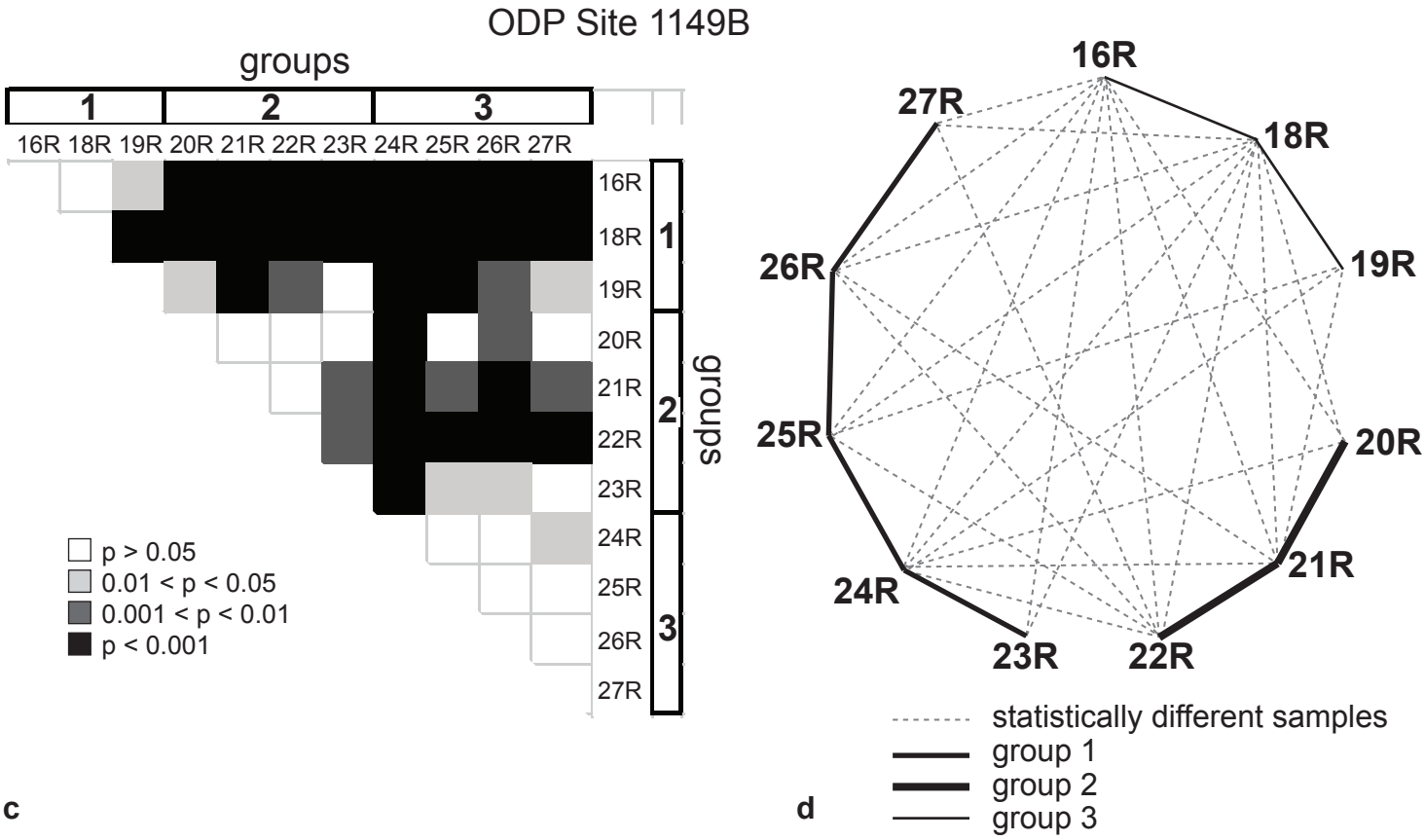


Gollain et al. Figure 5



a

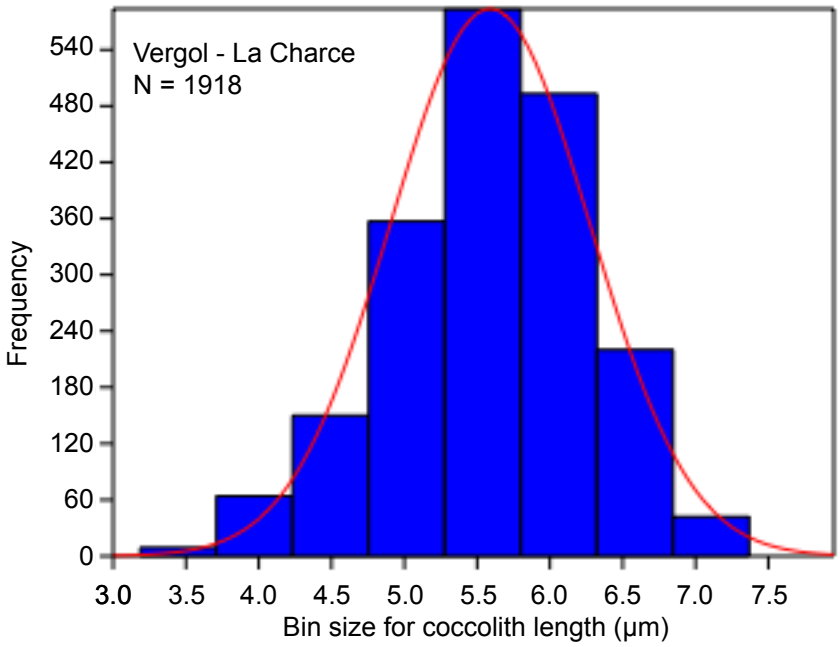
b



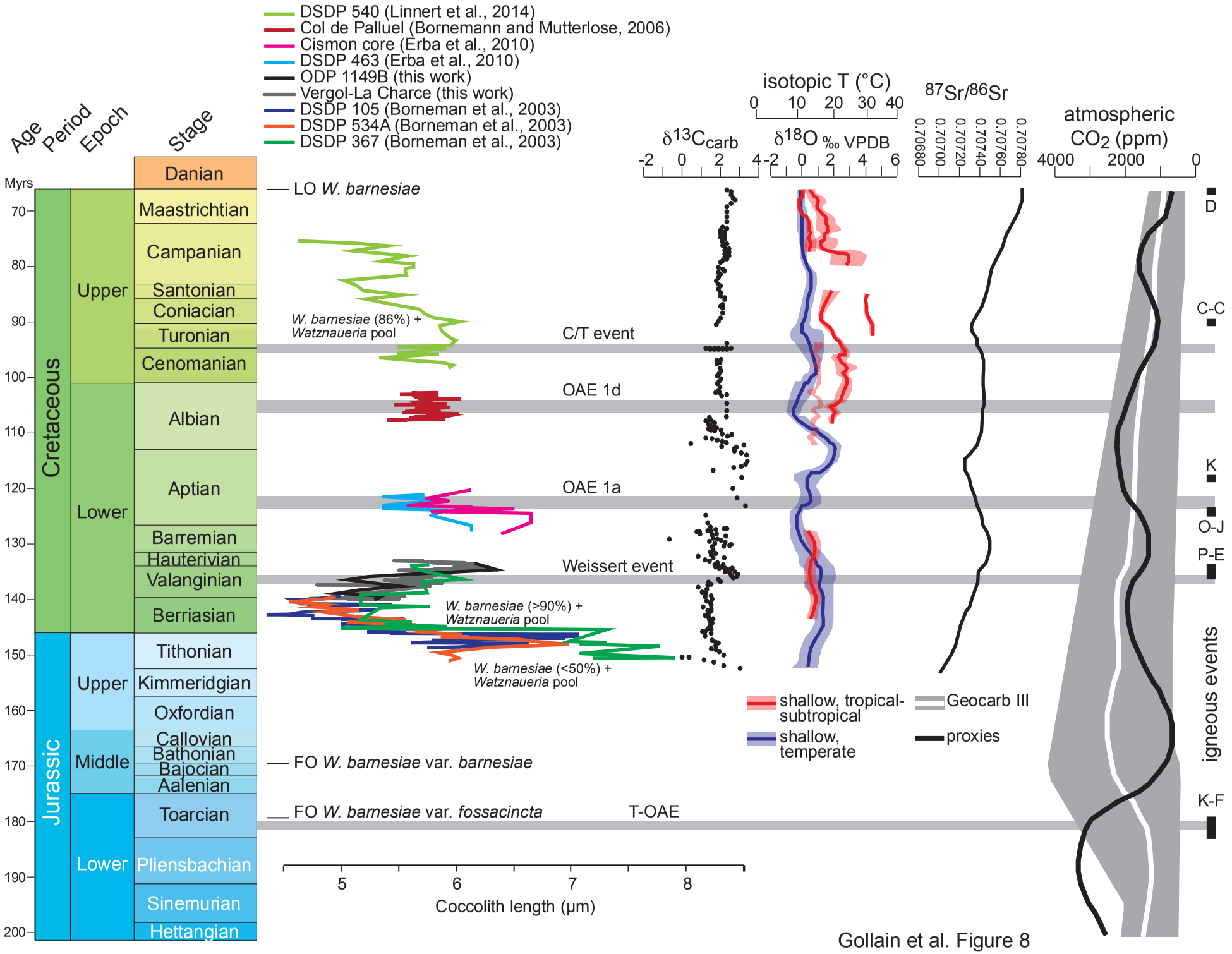
c

d

Gollain et al. Figure 6



Gollain et al. Figure 7



Gollain et al. Figure 8