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Volume and carbonate production estimates of early Palaeogene calcareous nannofossils

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To assess the role played in the carbonate budget by calcifying phytoplanktonic organisms, it is necessary to provide reliable quantitative estimates of the calcite secreted by calcareous nannoplankton, which certainly have had a vital role in the global carbon cycle since the Jurassic by supplying organic carbon and calcium carbonate to the deep ocean. Reconstructed volume/mass estimates of early Palaeogene calcareous nannofossils based on previous methodologies/techniques applied to extant nannoplankton and/or fossil assemblages suggest that carbonate produced by specific taxa significantly varies both in time and in space. Changes in the calcite within a single taxon and all the more so in the calcium carbonate produced by the entire assemblage are influenced by modifications of palaeoenvironmental conditions and are used as palaeoecological and/or palaeo-oceanographic proxy. ☐ *Calcareous nannofossils, carbonate production, IODP, Paleocene–Eocene, volume estimation.*

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Calcareous nannofossils are a major group of calcifiers in the oceans. The varieties of morphologies, ultrastructures and size of coccoliths/nannoliths imply that quantification of calcite secreted by any individual taxon is necessary to evaluate the contribution of this group to the total carbonate production, which in turn can evidence for increases and decreases of carbonate productivity variations through time. During the past decades, volume calculation of extant calcareous nannoplankton has been performed in order to convert coccolith fluxes data into carbonate export productivity. One of the most efficient methods of estimating the volume of coccoliths was described by Young & Ziveri (2000), and there are several case studies of estimates of recent coccolithophore carbonate contribution (e.g. Paasche 1962; Honjo 1976; Beaufort & Heussner 1999; Broerse *et al.* 2000; Sprengel *et al.* 2000; Bornemann *et al.* 2003; Erba & Tremolada 2004; Beaufort 2005; Ziveri *et al.* 2007). The technique was also used successfully for Mesozoic coccoliths/nannoliths (Williams & Bralower 1995; Mattioli & Pittet 2002; Tremolada & Young 2002). Based on these useful approaches, we tested these methodologies on early Palaeogene sediments from several DSDP/ODP sites.

The study areas are located at different latitudes both in the Atlantic (DSDP 401 and ODP 690, 1051, 1260, 1263 sites) and in Pacific (ODP Site 1209) oceans with the intention of giving a global perspective (Fig. 1). Calcareous nannofossils show prominent intraspecific changes in linear morphometric parameters (e.g. width, length, diameter) ranging by about a factor of 10 – this implies a 1000-fold variation in volume. The large morphometric fluctuations observed in coccoliths should not be disregarded because they likely provide, if duly analysed, a more valuable estimation of coccolith/nannolith volumes and carbonate production (Young & Ziveri 2000; Baumann 2005). The carbonate export productivity and the amount of buried carbon are the main mechanisms to store carbon into sediments/rocks (e.g. Anderson *et al.* 1990; Marinov & Sarmiento 2004). Today, as in the past, variations in productivity ($CaCO_3$ and C_{org}) and preservation result in changes in carbon fluxes among the different reservoirs and, consequently, in the global carbon cycle. An estimation of the carbonate export productivity of calcareous nannoplankton thus represents a robust potential tool for paleoecological and paleoceanographic reconstructions (e.g. Tremolada *et al.* 2008). One of the main goals of this work is to achieve volume and mass estimations of the

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most abundant Paleocene and early Eocene calcareous nannofossil taxa, which include *Coccolithus*, *Toweius*, *Sphenolithus*, *Fasciculithus*, *Discoaster* and *Zygrhablithus*, by means of morphometric measurements and geometrical calculation.

Material and methods

Material

Samples from ODP Legs 198 (Shasky Rise), 208 (Walvis Ridge), 207 (Demerara Rise) and 171B (Blake Nose) and DSDP Leg 48 (Bay of Biscay) were investigated to perform volume estimates of most abundant Paleocene calcareous nannofossils. On this basis, we are able to evaluate quantitatively the contribution of calcareous nannoplankton mass to the total carbonate production and, consequently, evidence for significative modifications. The study material includes samples from different areas taken across the middle-late Paleocene interval (Table 1). This choice is based on the hypothesis that the accuracy of volume estimations can be biased by the assumption that a single mean volume can be unanimously used for each coccolith species. In principle, specimens from samples either recovered in different areas/latitudes or with different ages could be larger or smaller with respect to the estimated average volume value. A significant variation in size or more specifically in a selected morphometric parameter (l) should be thus taken into account, because it strongly affects the volume estimate and consequently the calcite carbonate production. In addition, the K_s value can also vary and generate further change in coccolith volume. Bearing in mind this general assumption, we investigate how variability of K_s and l can actually influence the final evaluation of carbonate production.

Volume estimates

Volumes of calcareous nannofossils presented in Table 2 are calculated using a volume function, $V = K_s \times l^3$, where K_s is a constant depending on the shape and l is a characteristic dimension, this formula is valid for any given shape assuming that this shape is scale invariant (Young & Ziveri 2000). As these authors highlighted, the unique documented relationship of calcareous nannoplankton size to shape is the increase in the number of elements with size. This allometric effect does not modify the cross-sectional profiles of a particular taxon and will not eventually alter the volume estimation. The volume determination of each calcareous nannofossil taxon consists of two steps: the determination of an average size for each considered taxon, which is based on estimates of specific morphological parameter (e.g. length (L), width (W) and/or diameter (D); Fig. 2A), and the determination of the shape factor constant (K_s). Volumes of discoasterids and placoliths were calculated using images of cross-sections (Fig. 2B) taken by means of mobile mounts. A little amount of sediment was put on a glass slide, where Zeiss immersion oil (DIN 58,884; ISO 8036-1; n_e = 1518 (23 °C); ν_e = 42) was added and used as moulting tool with the coverslip. This preparation allows the movement and rotation of nannofossil specimens, whose cross-sectional shapes were photographed using an image analysis system consisting of a CCD camera mounted on a Leitz Laborlux microscope with parallel nicol owing to the low bire-

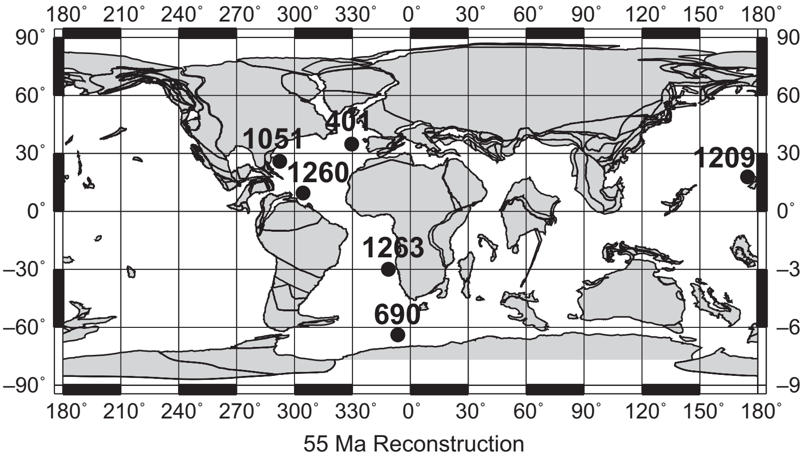

Fig. 1. Location of sites considered in this study (DSDP 401 and ODP 690, 1051, 1209, 1260, 1263) is positioned on a palaeogeographical map (equidistant cylindrical projection) at 55 Ma <http://www.odsn.de/odsn/services/paleomap/paleomap.html>.

Table 1. List of studied samples.

Leg	Site	Hole	Core	Type	Section	Top (cm)	Bottom (cm)	Depth top (mbsf)	NP zone
48	401	–	16	R	2	0	1	219.00	NP7/8
113	690	B	25	H	5	5	6	210.25	NP6
171	1051	A	56	X	4	60	61	520.00	NP9
171	1051	A	58	X	4	140	141	540.00	NP7/8
198	1209	B	22	H	2	97	98	197.57	NP9
198	1209	B	22	H	4	41	42	200.01	NP9
198	1209	B	22	H	3	91	92	199.01	NP9
198	1209	B	23	H	4	1	2	209.11	NP7/8
198	1209	B	23	H	5	149	150	212.09	NP5-NP7
207	1260	A	35	R	2	140	141	320.00	NP7/8
207	1260	A	32	R	2	30	31	290.00	NP9
208	1263	A	39	X	1	2	3	326.42	NP7/8

Table 2. Values of K_s, key morphological parameter, volumes and masses of selected calcareous nannofossil taxa.

				K_s range			Range dimension (μm)			
Species	Volume (μm^3)	Dimension used	K_s adopted	Min	Max	Mean dimension (μm)	Min	Max	Height	Weight (pg)
Discoaster multiradiatus	332.65	Diameter	0.14	0.12	0.21	13.25	8.46	19.59		901.47
Discoaster mohleri	86.89	Diameter	0.14			8.47	4.38	18.90		235.48
Fasciculithus hayi	135.04	Height	0.45	0.30	0.59	6.70	3.55	9.06		365.97
Fasciculithus hayi		Diameter				6.38	3.89	10.39		
Fasciculithus alanii	124.49	Height	0.44	0.26	0.59	6.59	4.51	9.53		337.38
Fasciculithus alanii		Diameter				6.23	3.95	8.20		
Fasciculithus tympaniformis	79.23	Height	0.56	0.36	0.68	5.22	2.21	8.91		214.70
Fasciculithus tympaniformis		Diameter				5.27	2.43	8.41		
‘Fasciculithus tympaniformis’	92.19	Cylinder				5.22			4.31	249.84
Zygrablithus bijugatus	189.60	Height	0.40	0.27	0.55	7.81	5.96	9.47		513.81
Zygrablithus bijugatus		Diameter				5.64	4.48	7.10		
Sphenolithus moriformis	67.55	Height	0.49	0.37	0.60	5.18	7.91	3.31		183.06
Toweius eminens	38.91	Axis >	0.08	0.06	0.09	8.00	6.10	9.29		105.45
Toweius eminens		Axis <				7.03	4.97	8.47		
Ericsonia		Axis >				7.57	5.63	9.51		
Ericsonia		Axis <				7.38	5.42	9.39		
*Coccolithus pelagicus**	34.43	Axis >	0.06			8.31	5.34	12.30		93.31
Coccolithus pelagicus		Axis <				7.01	4.33	10.98		

*K_s value is that recommeded by Young & Ziveri (2000).

fringence at ×1250 magnification and an Apple Macintosh PowerPC with a SCION LG-3 framegrabber card.

In order to calculate K_s constant, an iterative routine implemented in *NIH-Image* software by Young & Ziveri (2000) was used. Software package and macro routines written by J. Young (available at <ina.tmsoc.org/CODENET/CoccoBiom/>) were also used to elaborate raw data. Morphometric investigations were carried out on standard smear slides from unsettled sediment solution (Bown & Young 1998). Size measurements were performed by collecting images of nannofossils and determining the characteristic chosen dimension, on at least 150 specimens for each analysed species (Table S1). An auxiliary analysis at scanned electron microscope (SEM) was performed to check the 3-D structure of each study taxon (e.g. thickness and size; Fig. 2C).

K_s values of species belonging to *Sphenolithus*, *Fasciculithus*, *Zyghrablithus* and *Toweius* were determined calculating the width (or long axis for heterococcoliths) of the side view of at least 30 specimens for each species (Fig. 2A). Pictures of cross-sections (side views) were taken and then analysed following the same method described above (Fig. 2B). A special correction was adopted for *Zyghrablithus* that accounts for the ellipticity of its basal side (Fig. 2A). Length and width of each specimen were measured using ImageJ software (Fig. 2A). According to Young *et al.* (1996), the accuracy of these measurements is ±0.1 μm. An exception was made for *Coccolithus pelagicus* (heterococcolith) for which we used K_s values provided by Young & Ziveri (2000).

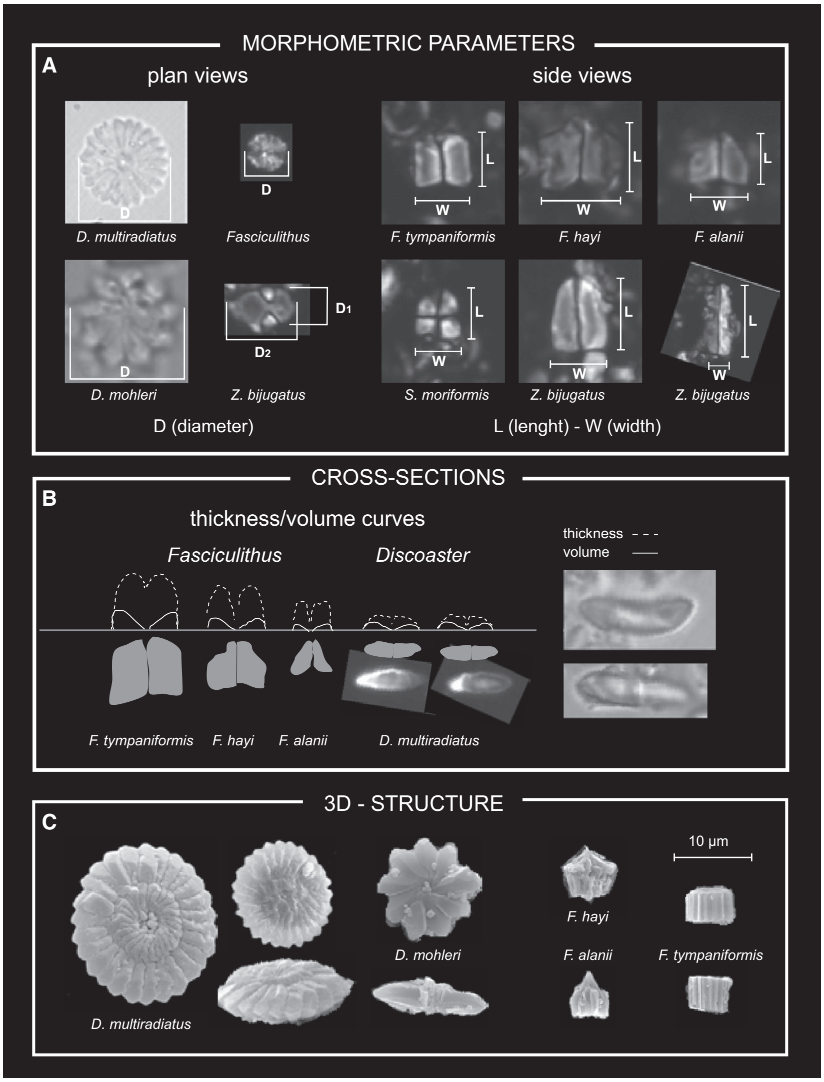

Fig. 2. Transmitted microscope and SEM images and cross-section of selected calcareous nannofossil taxa. A, Images of calcareous nannofossil specimens captured by a CCD camera and measured with ImageJ software. B, Volume of rotation calculated from cross-sectional shapes using with NIH-macro software (Young & Ziveri 2000). C, Images of selected nannofossil species captured by a scanning electron microscope.

From volume estimates to carbonate production

To calculate the carbonate contribution, we have determinate the weights of any given coccolith species converting the estimated mean volume (V) to mass (m) by multiplying the density of calcite ($\rho_{\text{calcite}} = 2.7\ \text{g/cm}^3 = 2.7\ \text{pg}/\mu\text{m}^3$). To this aim, we use this simple formula proposed by Young & Ziveri (2000):

$$m_{\text{coccolith}} = \rho_{\text{calcite}}\ (\text{pg}/\mu\text{m}^3) * V_{\text{coccolith}}$$

Although this method is widely used for the estimation of coccolith volumes, it cumulates a large maximum error of approximately 50% (Young & Ziveri 2000). Nevertheless, it remains the most precise technique available to estimate the carbonate contribution produced by each taxa.

Statistical analysis

The relationship between length (L) and width (W) of selected species belonging to *Fasciculithus*, *Zyghrablithus* and *Sphenolithus* is also investigated. Histograms showing the frequency of two considered morphometric parameters (L and W) are plotted one against the other to eventually estimate the degree of predictability of one morphometric parameter based on the other morphometric parameter. In particular, we report in detail statistical analysis performed on *F. tympaniformis*, *Z. bijugatus*, *S. moriformis* and *C. pelagicus* using PAST

(PAlaeontological STatistics) software package ver. 2.15 (Hammer *et al.* 2001).

Results and data interpretation

A first source of uncertainness in determining coccolith/nannolith volumes is related to interspecific and intraspecific variability of K_s. Our data indicate that between species K_s significantly varies from 0.06 to 0.56 by about one order of magnitude and separate values per species should thus be adopted as suggested in Young & Ziveri (2000). Overall, coccoliths, such as *C. pelagicus* ($K_s = 0.06$), have small K_s values differently from nannoliths, which show greater mean K_s values with a larger range of variability. For instance, the K_s value estimated for discoasterids is of ca. 0.14, while fasciculiths are characterized by K_s values larger than 0.5 (Fig. 3; Table 2). The K_s intraspecific variability is also to be considered. We have analysed data from a specific taxon, that is *F. tympaniformis*, for which we have a large data set acquired from samples recovered in various areas and/or having different ages. Although K_s values of *F. tympaniformis* vary significantly of about 0.2, ranging from ca. 0.4 to ca. 0.6 in all the studied sites, the K_s mean values are very consistent among different sites (Fig. 4A–E; Table 2). Histogram plots representing the frequency distribution of K_s rise rather smoothly to a single peak and then decline. Smoothed histograms, obtained by superimposing a smooth curve on the rectangles, display an unimodal pattern with a very slightly, almost imperceptible, negative skewness, suggesting a good symmetry in the distribution of K_s values.

A second source of uncertainness in determining coccolith/nannolith volumes is related to size variability. Variations in size naturally occur within coccoliths as a result of both genotypic and ecological causes (e.g. Young 1994; Paasche *et al.* 1996; Beaufort *et al.* 2011). Size variations are in fact intimately correlated with changes in key morphometric parameters, which are used to calculate volume and mass for a single taxon. Within a single species, morphometric parameters could vary by an order of magnitude but the volume and consequently the mass are in a ratio of 1–1000 with respect to the morphometric parameter (*l*). Size variation is thus much more effective than K_s variation in controlling the volume estimated for a single species. For this reason, we decided to investigate the frequency distribution of selected morphological parameters in calcareous nannofossil species (Fig. 5). In general, these parameters show a wide range of variability with maximum values ca. 2–3 greater than minimum values (Fig. 5). The frequency distributions of morphological dimensions display unimodal slightly positively skewed patterns with upper tail heavier than lower tail (Fig. 5). As a consequence, mode values are generally slightly lower than mean values, but no large differences exist between the two.

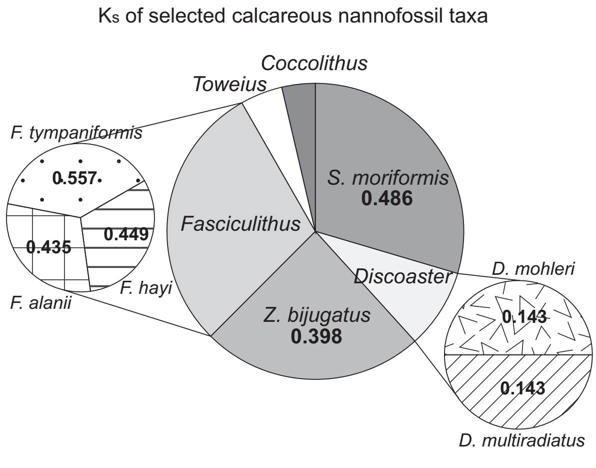

Fig. 3. Pie chart showing K_s values of selected calcareous nannofossil taxa.

Further statistical analyses were also undertaken to see whether selected morphological parameters (length vs. width and short axis vs. long axis) covary and to quantify the strength of the relationship between the observed variables. Almost all the investigated taxa show nice correlation between the two variables. The only exception is represented by *Z. bijugatus*, which shows low values both of correlation ($r = 0.263$) and determination ($r^2 = 0.069$) coefficients (Fig. 6). The ambiguity in the taxonomic concept of *Z. bijugatus* (Deflandre *in* Deflandre & Fert 1954) may be a possible justification for these low correlation coefficients. To date, *Z. bijugatus* is differentiated into forms with moderately tall spines (*Z. bijugatus bijugatus*), short spines (*Z. bijugatus nolfii*, *Z. bijugatus sileensis*) and spines with lateral horns (*Z. bijugatus cornutus*) (Young *et al.* 2012) and other morphotypes likely occurred in calcareous nannofossil assemblages. In fact, *Z. bijugatus* species concept has a wide range of variability, and a formal splitting into different species would be desirable in order to reassess this genus satisfactorily. A more consistent taxonomy could indeed results in higher correlation coefficients between morphological parameters investigated for this taxon.

The high degree of predictability generally observed for these morphometric parameters suggests a kind of linear growth for the most part of investigated taxa, whether we are talking about heterococcoliths and nannoliths and with the only exception of holococcolith *Z. bijugatus*. In principle,

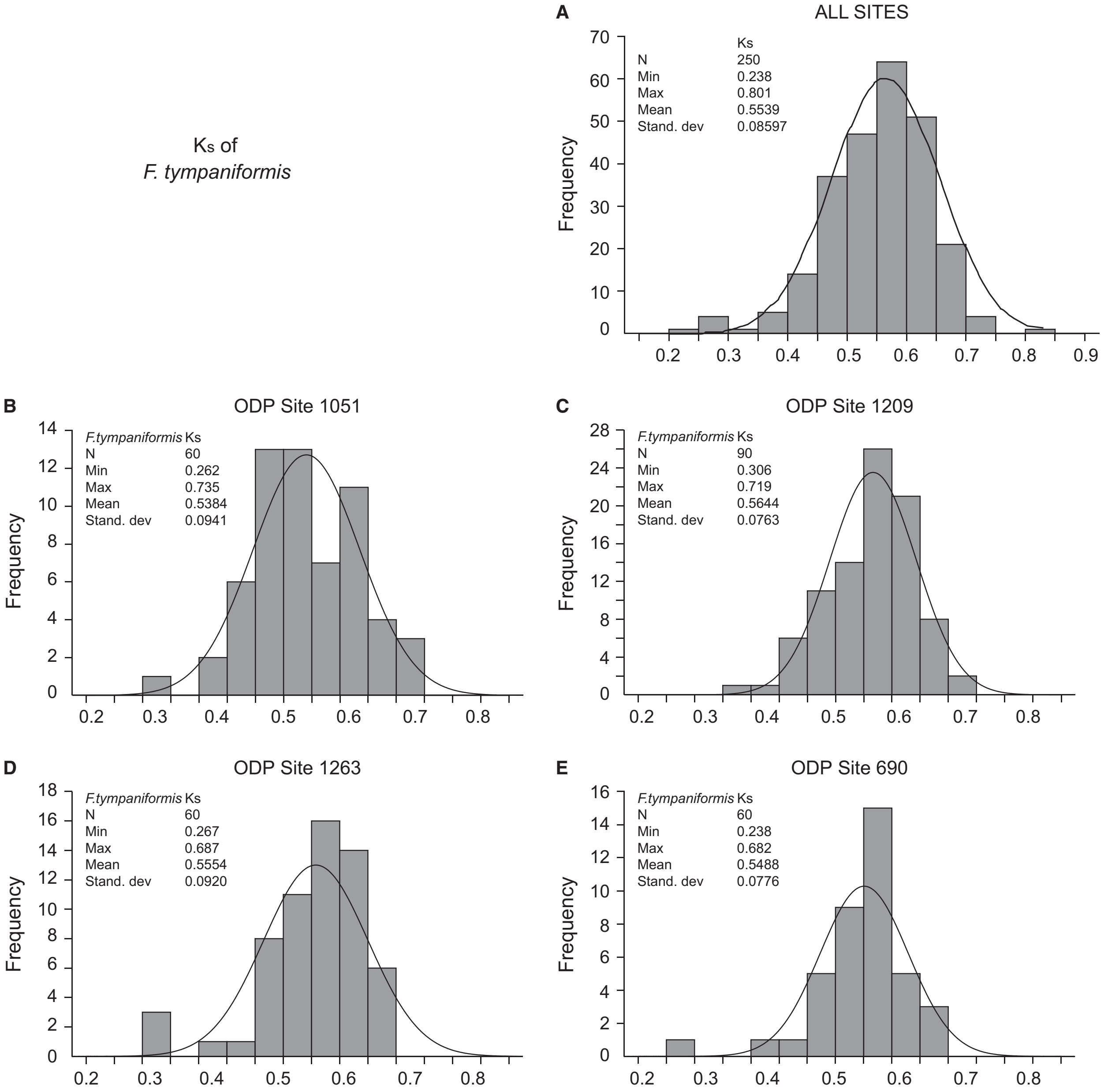

Fig. 4. Histograms representing the frequency distribution of K_s values in *F. tympaniformis*. A, total mean value. B, Mean value estimated for ODP Site 1051A. C, Mean value estimated for ODP Site 1209B. D, Mean value estimated for ODP Site 1263A. E, Mean value estimated for ODP Site 690B.

we cannot assume that coccolith shapes are totally scale invariant; on the contrary, allometric effects have been already documented in a few studies (Young 1989), but data collected in this study indicate a good linear correlation between different variables suggesting a substantial isometric morphological growth. In fact, if this is the case, an increase or decrease in size does not produce any significative shape modification in coccoliths and this implies that the volume estimation calculated is actually a good approximation of the true value.

A final issue on values of morphometric parameters measured in fossil coccoliths/nannoliths should be also taken in account. As a matter of fact, the fossil specimens investigated in this study come from samples which were recovered in different geographical areas and/or have different ages. Geographical location as well as age could indeed produce size variation on coccoliths/nannoliths. In order to start to investigate whether and/or how time and space control size in early Palaeogene nannoplankton taxa, we plot the frequency distribution of key

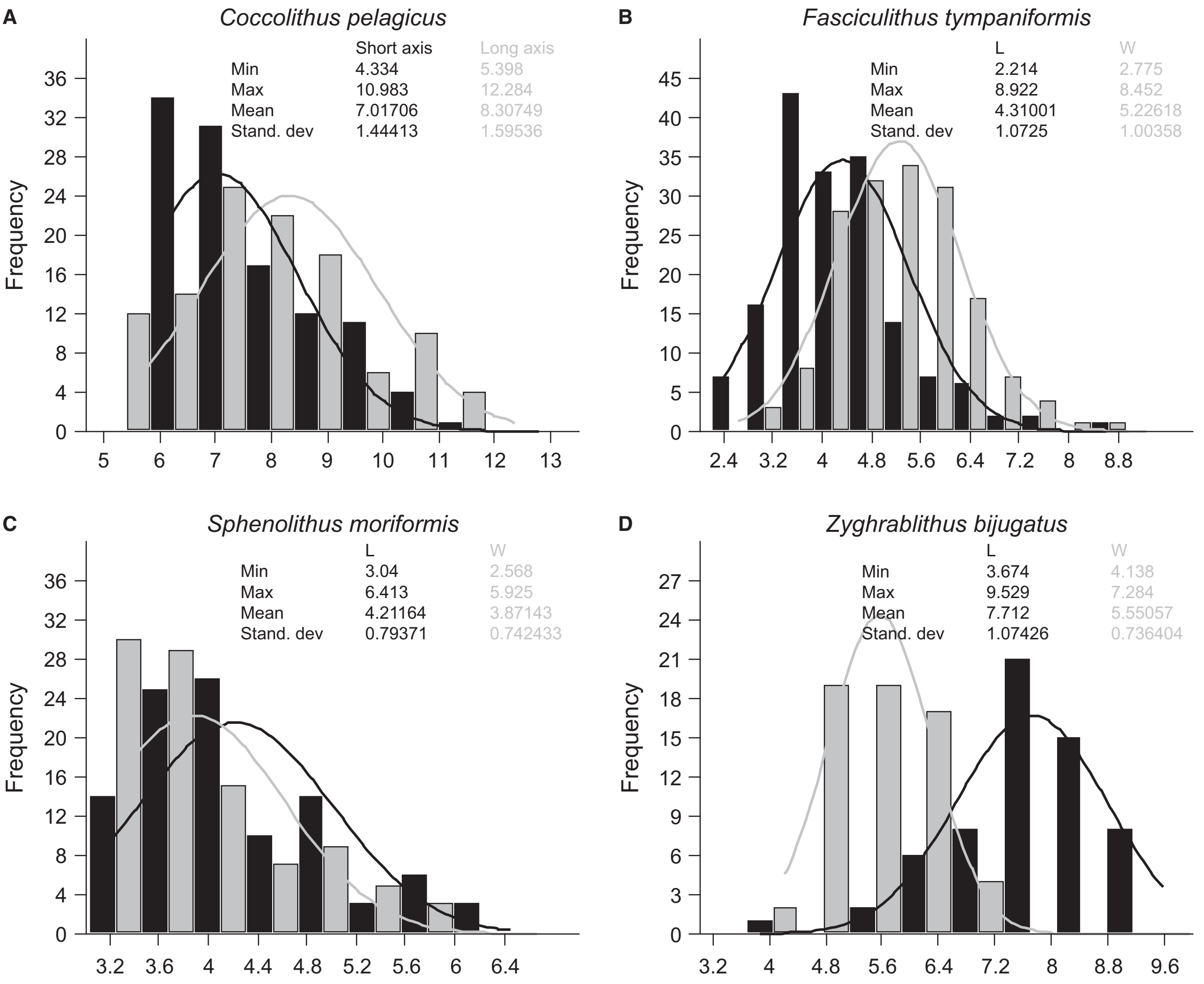

Fig. 5. Frequency distribution of key morphological parameters in selected calcareous nannofossil species. A, *C. pelagicus* (short axis vs. long axis); B, *F. tympaniformis* (*L* vs. *W*); C, *S. moriformis* (*L* vs. *W*); D, *Z. bijugatus* (*L* vs. *W*).

morphometric parameters for which we have a sufficient quantity of data potentially subdividable for two subdatasets. The first one includes the data divided by age (Fig. 7A, B; NP7/8 and NP9), and the second comprehends data having the same age (NP7/8) but divided by provenance geographical areas (Fig. 7C, D; sites 1209, 401, 1260 and 1263).

Looking at the first data set, the two considered taxa, *D. mohleri* and *F. tympaniformis*, display an increase in size over time (Fig. 7A, B). *D. mohleri* shows a pronounced increase in diameter (*D*) size varying from ca. 8.0 μm, observed in Zone NP7/8, to 9.4 μm for Zone NP9 (ca. +17.5%). A similar, although less distinct, increase is also observable in the length (*L*) of *F. tympaniformis* for which we see a variation of ca. +0.1 μm (ca. +2.5%) going forward in time (Fig. 7A, B). This partial data set suggests that, at least these taxa, tend to increase in size trough time well complying with the Cope's rule (Cope 1896). Actually, this law, which theorizes a continuous increase in body size of organisms over time, is still the base of much debate in the scientific community and several examples or counter examples to argument its validity are available in the literature (e.g. Gould 1997; Hone & Benton 2005; Heim *et al.* 2015). A pilot study on size changes in coccolithophorids has been performed by Aubry *et al.* (2005), who claimed for an increase in the average size of coccoliths during the Mesozoic based on a compilation of literature data. By contrast, a recent study by Herrmann & Thierstein (2012) documents a general size decrease of calcareous nannofossil assemblages over the Cenozoic. Bearing in mind the narrowness of our data, we observe an overall increase in size but at a finer taxonomic scale, within the same species, over time, but more data on a larger number of taxa for a longer time interval is needed to test comprehensively this issue.

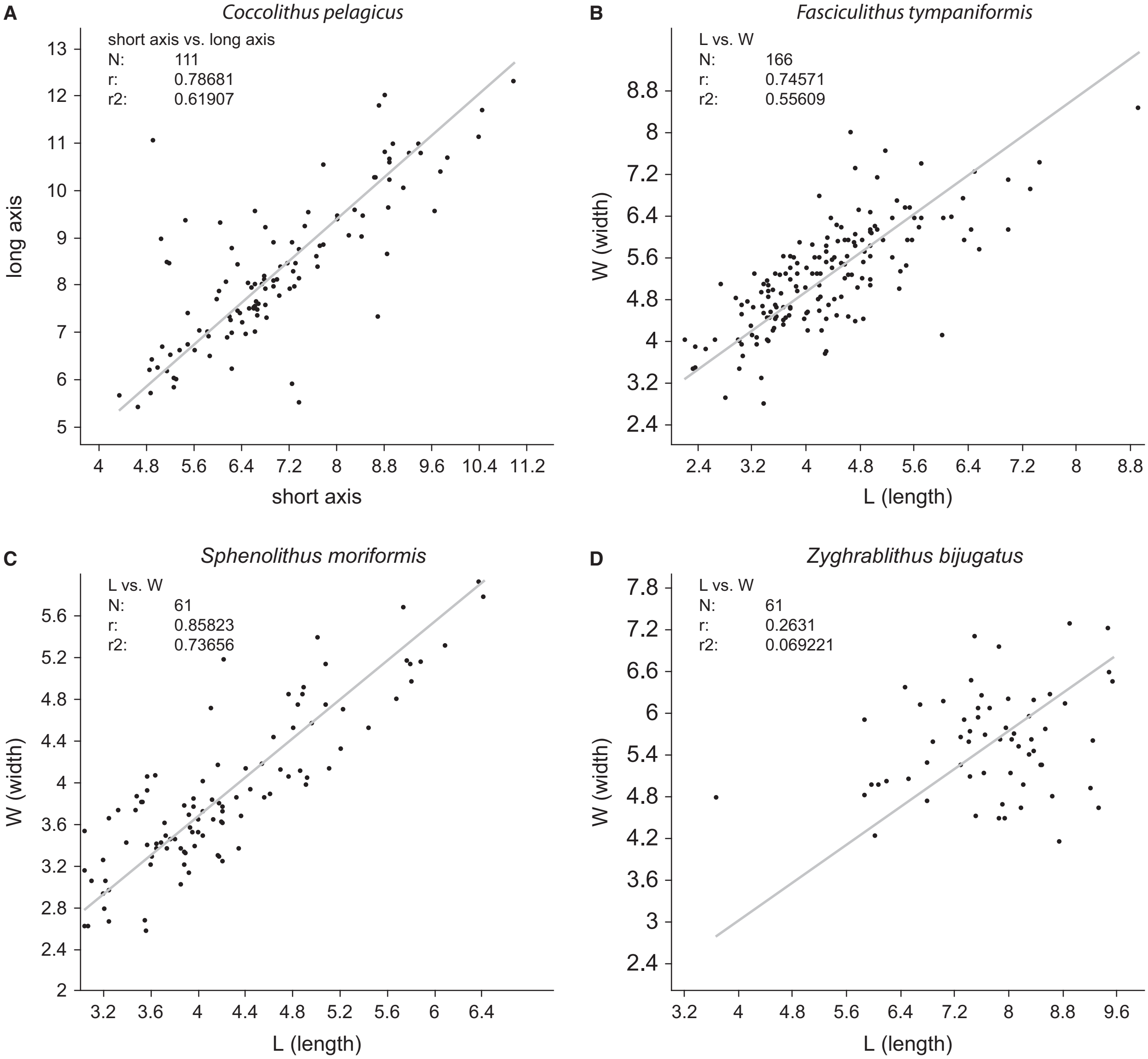

Fig. 6. Linear regression modelling the relationship between key morphological parameters in selected calcareous nannofossil species. A, *C. pelagicus* (short axis vs. long axis); B, *F. tympaniformis* (*L* vs. *W*); C, *S. moriformis* (*L* vs. *W*); D, *Z. bijugatus* (*L* vs. *W*).

However, in order to avoid the time effect just discussed above, we have analysed only data derived from samples having the same age (NP7/8) but different provenance areas. Looking at this second data set, the investigated taxa generally show a rather wide range of size variation (Fig. 7C, D). *Discoaster mohleri* and *F. tympaniformis* both exhibit large geographical variations in average values of investigated morphological parameters, *D* and *L* mean values display differences of ca. 1.0–1.5 μm among different sites; however, this large variability is not controlled by latitude (temperature) as evidenced by the absence of any clear latitudinal gradient in the distribution of morphological parameter values. For instance, the *D* value of *D. mohleri* is virtually indistinguishable if measured at Equatorial Site 1260 rather than at mid-latitude Site 1263 (Fig. 7C, D). Similarly, *L* values of *F. tympaniformis* calculated at low (Site 1209) or mid-high (Site 401) latitudes are almost the same (Fig. 7C, D). A possible explanation for these results could be related to the low latitudinal gradients existing during the late Paleocene time (Zachos *et al.* 2001), such weak difference in climate conditions between low and high latitudes could thus not be enabled to control the *D. mohleri* and *F. tympaniformis* distribution and to some extent their sizes. Other environmental factors, such as nutrient availability, salinity or chemical changes of sea surface waters, could be

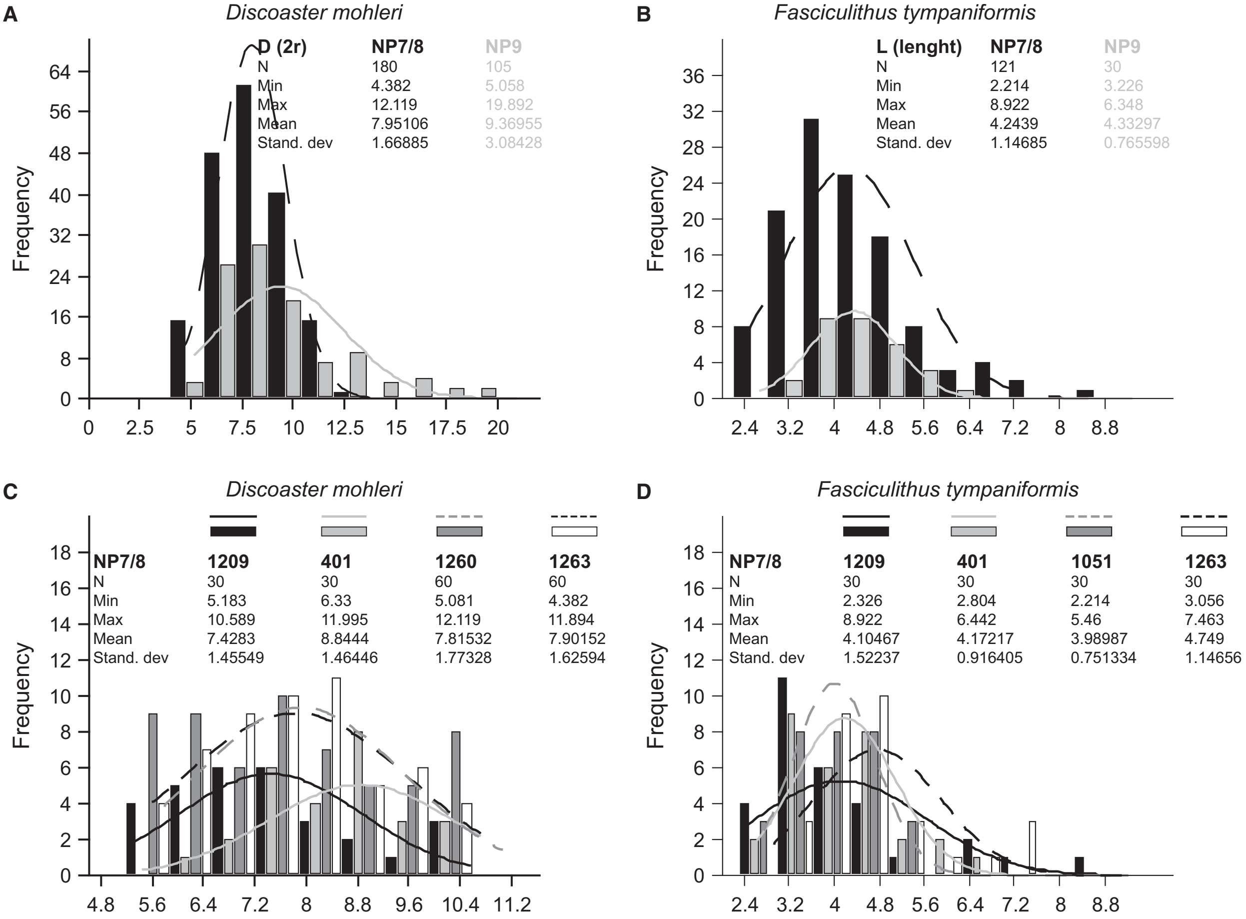

Fig. 7. Examples of variations of key morphometric parameters trough time and space for two species of calcareous nannofossils. A, *D. mohleri* (subdivided by age); B, *F. tympaniformis* (subdivided by age), C, *D. mohleri* (subdivided by space); D, *F. tympaniformis* (subdivided by space).

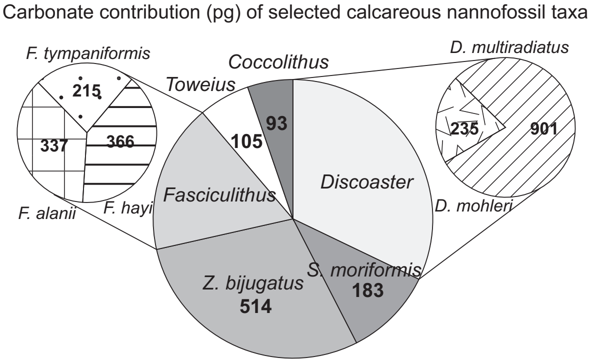

Fig. 8. Cake graph showing carbonate production (pg) of selected calcareous nannofossil taxa.

the real responsible for the observed changes and/or variations of the investigated morphological parameters.

This is not a comprehensive discussion because more data are needed to evidence clear trends of variation in morphological parameters of different calcareous nannofossil species over time and space. In any case, a gain of this study lies in the fact that our data confirm significant size variations in calcareous nannofossils thus suggesting caution when mean values of morphological parameters calculated in a specific region are used to estimate volume/mass of a taxon in a different geographical area, in fact further measurements are desirable in order to fix a local mean value.

Once we have calculated K_s and l values, we can determine the weight for every single taxon. In particular, to obtain a plausible estimation of calcium carbonate produced by calcareous nannofossils, we have investigated taxa that were either common in late Paleocene assemblages or at least heavily calcified. Overall, *Toweius* and *Coccolithus* dominated middle to late Paleocene assemblages (e.g. Haq & Lohmann 1976; Haq *et al.* 1976, 1977a,b; Agnini *et al.* 2007) and this imply they constituted an important part of $CaCO_3$ produced by coccolithophorid communities; on the other hand, discoasters, fasciculiths, sphenolithus and *Z. bijugatus* were definitively less abundant than heterococcoliths within the same assemblages but each of these nannoliths/holococcoliths secreted a larger amount of calcite. Calcite production for early Palaeogene *C. pelagicus* is ca. 90 pg/specimen, consistently with data available for extant *C. pelagicus* (Young & Ziveri 2000), whereas nannoliths/holococcoliths show a wide weight range from ca. 200 to ca. 900 pg (Fig. 8; Table 2), that represents a two- to ten-fold increase in carbonate production if compared with the most common heterococcoliths (*Coccolithus* and *Toweius*).

Conclusion

Morphometric analyses of selected calcareous nannofossil taxa were performed with the aim of providing volume and mass estimations of the most common species present in Palaeogene assemblages. Coccolith volumes were calculated using a volume function, $V = K_s \times l^3$.

K_s values measured for every single taxon generally display a wide range of variation but, at the same time, very constant mean values if measured at different sites, eventually suggesting that K_s values given here represent a quite robust datum that can be used with a good degree of certainty in Paleocene–Eocene assemblages. By contrast, the characteristic dimensions used to calculate volume values (l) show prominent variations both in time and in space that is why we suggest caution in assuming a single mean value to each coccolith species and strongly recommend to perform morphometric analysis in every single-study section before calculating volumes and masses of different coccoliths.

K_s and l values calculated in this study allow us to estimate volumes and masses for several calcareous nannofossil taxa, which represents the first step if we want to reconstruct the history of carbonate production of calcareous nannoplankton over time and possibly use this parameter as a palaeoceanographic proxy of sea surface carbonate productivity.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Number of specimens counted for each analysed species.