Assessment of Dwarfing as a Response to Environmental Stress buildup in the end Cretaceous.

A Thesis

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by

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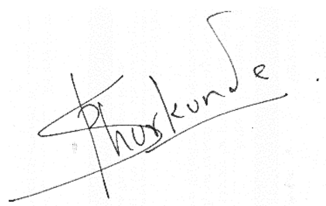
Certificate

This is to certify that this dissertation entitled “Assessment of Dwarfing as a Response to Environmental Stress buildup in the end Cretaceous” towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Ms. Shraddha Sanjay Bhurkunde at Indian Institute of Technology, Bombay under the supervision of Dr. Jahnavi Punekar, Assistant Professor, Department of Earth Science, during the academic year 2018-2019.



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This thesis is dedicated to my father & mother - Sanjay Bhurkunde and Sunita Bhurkunde

Declaration

I hereby declare that the matter embodied in the report entitled Assessment of Dwarfing as a Response to Environmental Stress buildup in the end Cretaceous.are the results of the work carried out by me at the Department of Mathematics, Indian Institute of Science Education and Research, Pune, under the supervision of Dr. Jahnavi Punekar and the same has not been submitted elsewhere for any other degree.

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I thank my family and friends for their support and encouragement.

Abstract

Planktonic foraminifera has been reported as a model organism for studying Biostratigraphy, Evolutionary biology, Heterochrony and Geochemistry. Certain taxa of Planktic foraminifera have been reported to undergo dwarfing as an ecophenotypic variation under stress environment. Previous studies that have reported dwarfing for Cretaceous- Tertiary intervals used Relative species abundances or basic morphometric analysis for a fixed number of individuals in the population. In this study I identified species that appear to dwarf using Relative species abundance and documented dwarfing in the pre K-T boundary interval using absolute values of morphometric analysis. This analysis was performed for the entire population size for the late Maastrichtian *Plummerita hantkeninoides* zone CF1 of Bidart, France. An objectively replicable metric system was established and measurements were performed manually using image analysis software. Most of the selected species seem to exhibit a dwarfing trend in the suspected interval: a low Magnetic Susceptibility zone which forms the top ~60 cm in Bidart, France. Results from this study should be replicated on other prominent sections and intervals in order to make further conclusive statements. These preliminary results propose Dwarfing as a proxy to identify stress intervals along with other known ones such as Magnetic Susceptibility and Fragmentation Index.

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Introduction:

Planktonic foraminifera (foramen/hole - bearers) are a group of single celled marine protozoa characterized by secreting tests (shells) mainly made of calcium carbonate. Their first appearance in the fossil record dates back to the Jurassic period. Ever since they have been extensively studied for their importance in understanding paleo-environment and oceanography, geochemistry, biostratigraphy and evolutionary study. High abundance, wide spread distribution, high diversity in the marine sediment record, sensitivity to environmental changes, short lifespan and rapidly evolving lineages makes them ideal for the aforementioned studies.

The calcareous test is what remains of the forams in the fossil records. It is a record of the paleo-temperature, pH, salinity, etc as Test morphology can be interpreted as a function of the adaptations made to survive in the then ambient water environments. These solid carbonate tests are made of a series of chambers which are added as the organism grows.

Availability of the biogenic carbonate ion for building test can vary according to the dynamics of the surrounding water which can then affect the test morphology (shape and size). These dynamics are greatly influenced during climatic changes and environmental transitions and can cause calcification crisis. Fig (1) highlights the changes in relative species richness of Planktic forams in the past 300 My. The red vertical lines represent the times of potential calcification crisis. Dwarfing i.e. reduction in size has been reported as an adaptation strategy to such environmental stresses in Planktic Foraminifera. (Wade and Olsson, 2009)

It can also be implied from Fig. (1) that the relative species richness of Planktic forams is greatly affected in the end Cretaceous roughly 65.72-65.0 Ma. Before understanding about dwarfing in end Cretaceous let’s have an overview of how dwarfing was identified and manifested in Planktic forams.

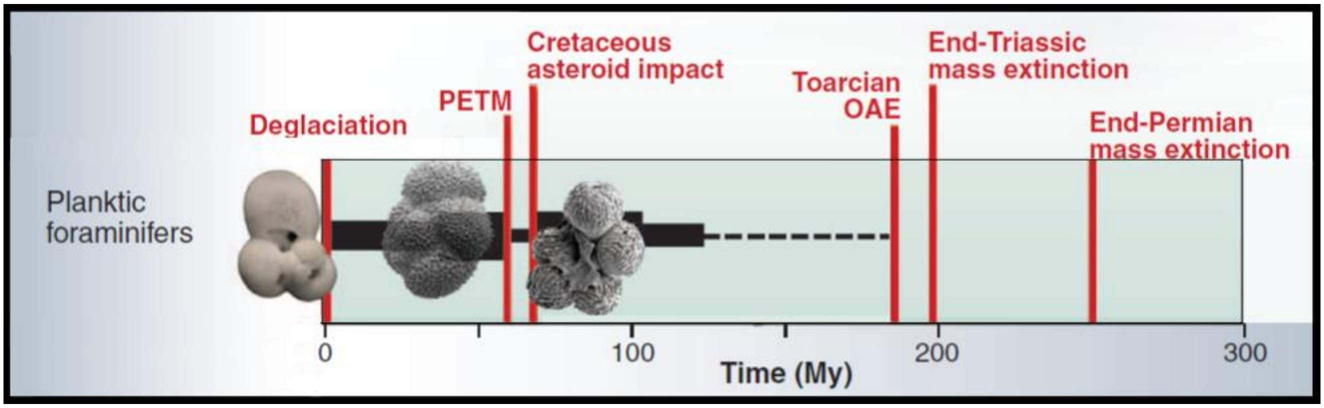

Fig. (1): Adapted from Honisch et al., 2012 : Idealized diversity trajectories of plankton foraminifera: the thickness of the black bar indicates relative and smoothed species richness through potential ocean acidification events.

Reduction in size for Planktic foraminifera has been defined in a variety of ways: "Lilliput effect'' first termed by Urbanek,1993, reports dwarfing as high abundance of smaller species in the descendants of an assemblage post extinction as a result of extinction of the large, complex and more ornate species. Wade et al, 2009 continues this notion of defining Lilliput effect as an aftermath response. Survival of smaller less ornate species because of some selective (unidentified) advantage over large complex forms. This type of reduction in size is discussed in an elaborate study in Abrahmovich and Keller, 2009 where they define Lilliput effect not as only a post event syndrome but as a continuum and umbrella term for morphologic and intraspecies size reductions.

Abrahmovich and Keller, 2009 studies Lilliput effect during Late Maastrichitian interval over a variety of paleo-environments which are associated with stress. The study shows that the morphic size reductions (different species) manifesting Lilliput effect as a function of stress. As the conditions changed from optimum to high stress to catastrophic, the assemblage dwelled high diversity and high relative abundance of K strategists to high relative abundance of r strategist to extinction of K strategists and dominance of disaster opportunists respectively. They used the term dwarfing to refer to intraspecific size reduction i.e. occurring between individuals of the same species rather than morphic size reduction which is interspecific i.e. between different species.

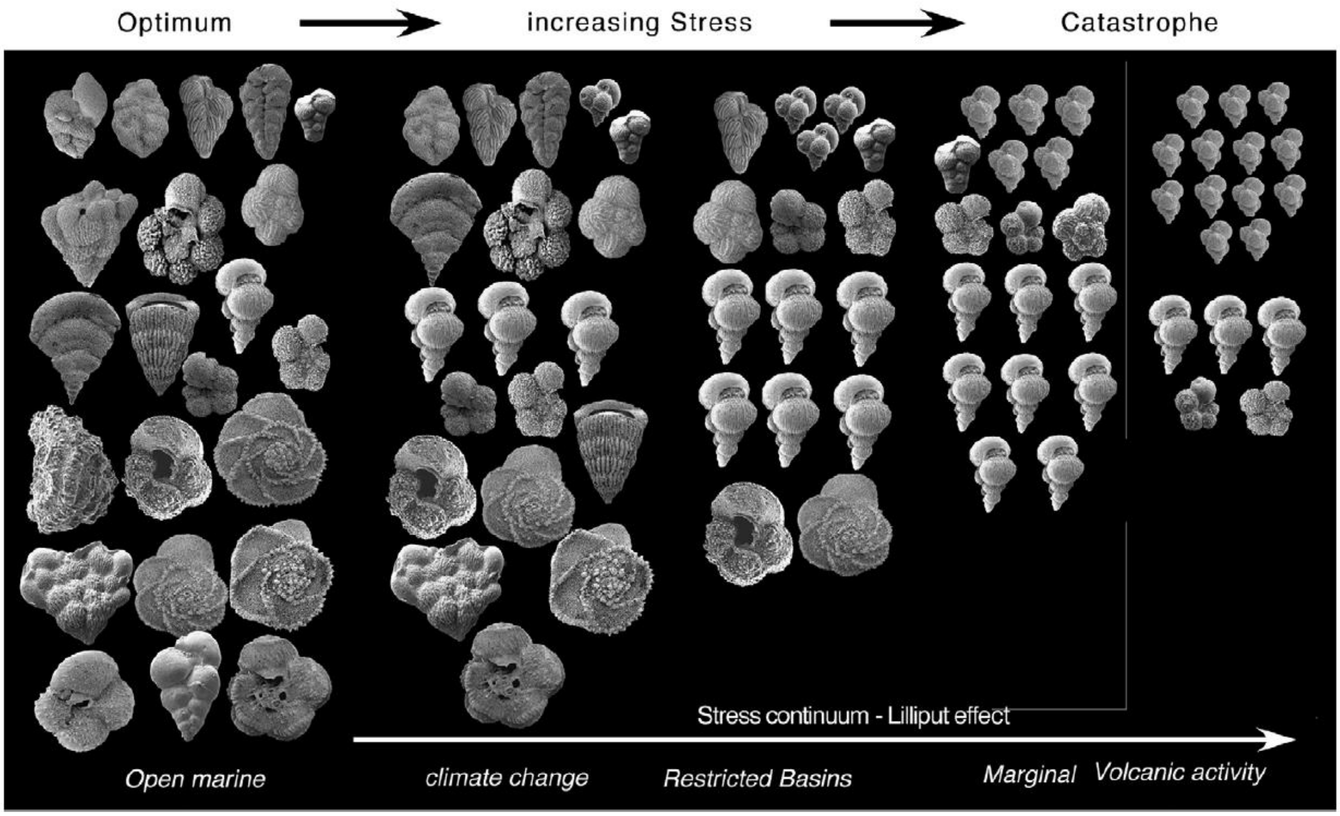

Fig. 2. Reproduced from Abrahmovich and Keller, 2009: Lilliput effect: Progression of Intraspecific and Morphic size reduction as a function of stress.

Dwarfing in Planktic foraminifera has been reported to manifest in non – optimal, unstable and eutrophic environments (Phleger, 1960a). This study aims to document the reduction in the mean test size preceding an extinction event which has been characterized as “Pre- Extinction Dwarfing” in Wade and Olsson, 2009. The causes of terminal dwarfing are unknown but given its ecophenotypic nature, it seems that environmental perturbations somehow interfere with its morphogenesis.

The following table lists some of the important foraminiferal studies and compares its methodology, sites, features and time intervals with the current study.

Studies	Interval	Site/s	Methodology	Features
MacLeod et al., 2000	K-T, P-E, Late Eocene	Brazos for K-T,North Atlantic,Gulf of Mexico. Eastern South Atlantic for late Eocene	Morphometric analysis : Area, Roundness, width	Heterochrony
Abramovich and Keller, 2003	K-T	South Atlantic DSDP Site 525A	Relative population abundances	Dwarfing- general size reduction
Abramovich and Keller, 2009	K-T	Tunisia, Egypt, Texas, Argentina, South Atlantic and Indian Ocean	Relative Species Abundance	Lilliput effect Intraspecific dwarfing termedand Morphic size reduction
Olsson and Wade,2009	PETM	western North Atlantic Ocean, Equatorial Pacific Ocean and Caribbean Sea	Basic analysis: Maximum chamber width and length, No. of chambers	Intraspecific dwarfing termed -pre-extinction dwarfing;
Coccioni et al., 2016	CLIP	Gubbio, Italy	Percentage abundance, Maximum test diameter, Coiling	Morphotype Analysis of R. cushmani

Brombacher et al., 2017	-	-	Calibration of Repeatability of morphometry	Detailed Morphometric analysis
Falzoni et al., 2018	OAE 2	Eastbourne, SE France, Tarfaya, Morocco	Morphometric analysis on ten specimens per species in each sample. (total 2 species)	Planktonic foraminiferal response to OAE2
Current study	K-T	Bidart,France	Relative abundance for Target species; Morphometric analysis of whole population (all specimens of alternate samples of each species (total 8 species)	Documentation of Intraspecific dwarfing

Table 1: Previous foraminiferal studies with their methodology, sites, features and time intervals.

None of the aforementioned studies feature detailed morphometric analysis of entire population for documenting dwarfing pre K-T BOUNDARY interval. Dwarfing in the end cretaceous interval has been studied for wide time brackets - 250-500 ky. Recent discoveries (Punekar et al., 2015) show remarkable changes, such as the low magnetic susceptibility and high foraminiferal test fragmentation in the stratigraphic section preceding the K-T boundary which makes the top ~60 cm of Bidart, France (choice of section of study). Notice the low-MS interval and foraminiferal test fragmentation in Fig.3.

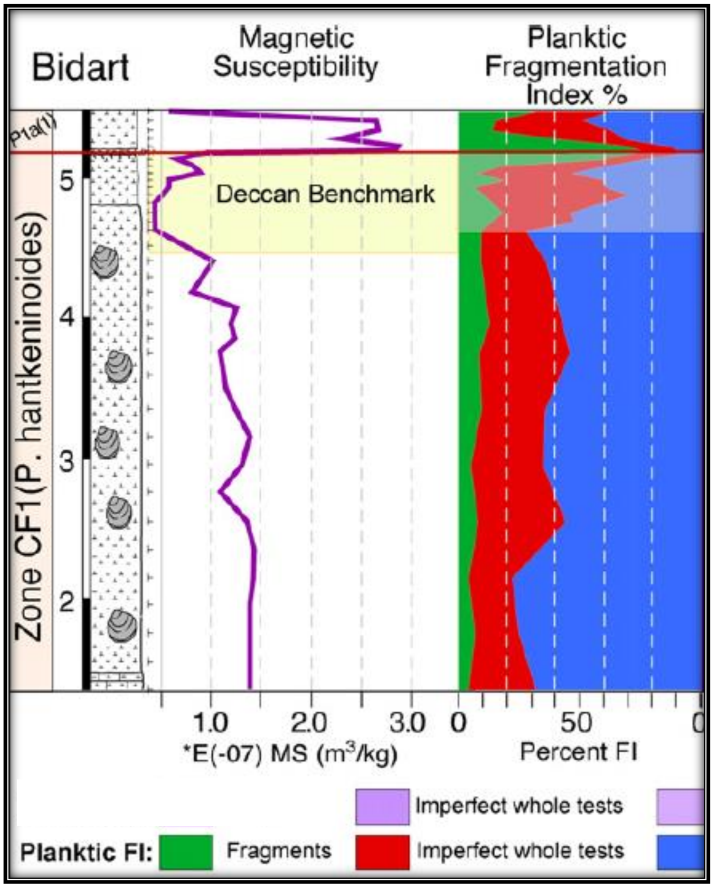

Fig. 3: Adapted from Punekar et al., 2015: Featuring Percentage Fragmentation Index of Planktic foraminifera and Magnetic Susceptibility for Bidart, France. Notice the top 60 cm – 50 ky of CF1 – benchmark.

However this interval just before KTB was not analyzed for dwarfing. Also most of the previous identification of dwarfing depended on anomalous relative abundances of species and not absolute test measurements.

The aim of this study is to: 1) establish a replicable and objective metric system which can be morphotype specific 2) Identify target species for dwarfing using relative species abundance 3) Compare the morphometric variation across the 50 ky of Bidart, France for entire population of each alternate sample of CF1 4) Compare the variations for the different morphometric parameters for the given species.

This study is an extensive documentation of pre-extinction dwarfing with absolute morphometric analysis for entire population size.

Location and Lithology

a) Elles, Tunisia:

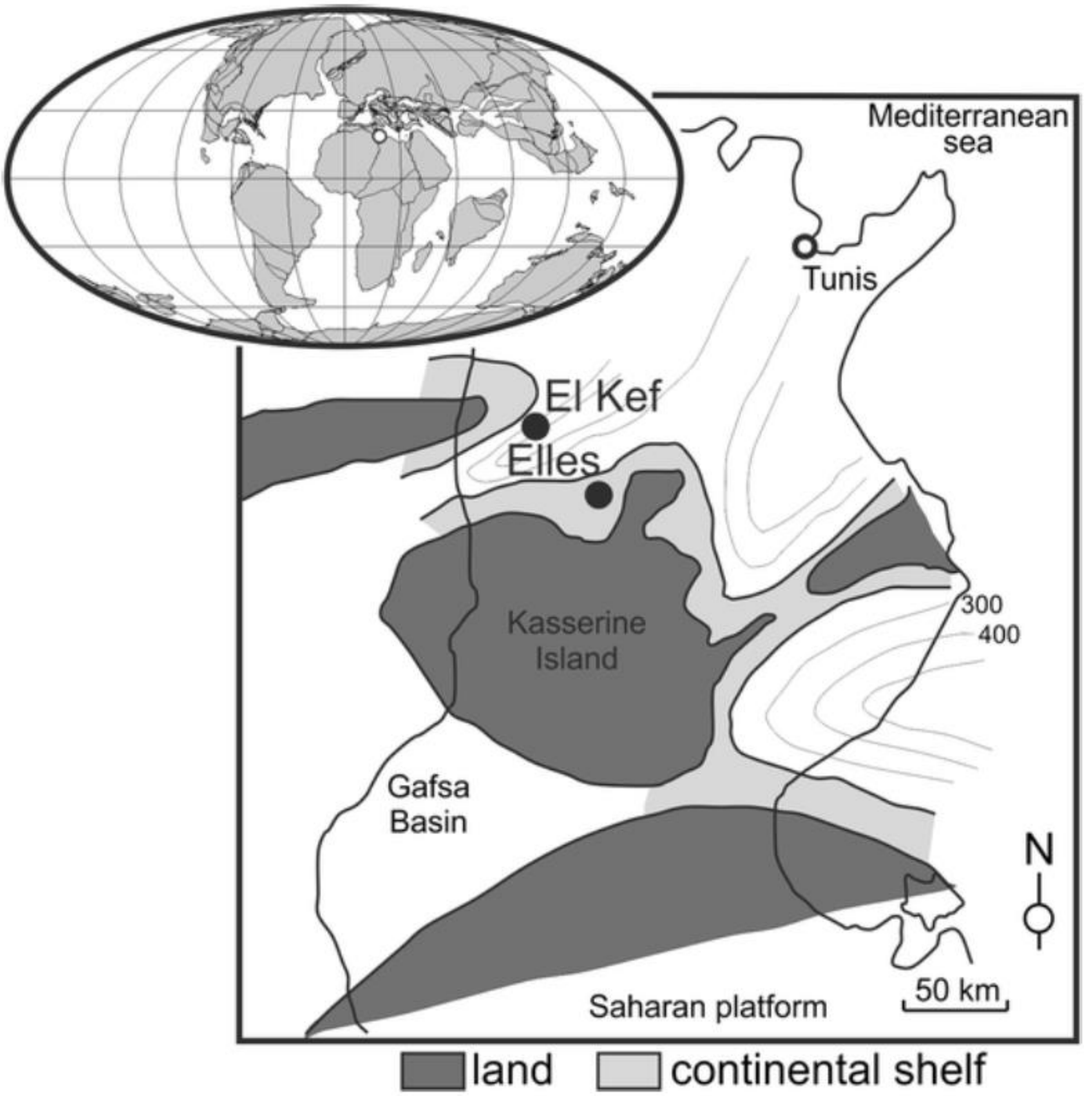

Fig. 4: Reproduced from Thibault et al., 2015*:* Palaeogeographic position of the Tunisian sections: Elles and El Kef :. Modified after Burollet (1967)

Elles,Tunisia is a provisional GSSP for K-T boundary given its elaborate and continuous section with high sedimentation rate. It is located 35 km to south-east of El Kef (GSSP for K-T) in Tunisia. These Tunisian sections are reportedly well suited for end-cretaceous studies given the high abundance of well- preserved high diversity assemblages of upper Maastrichtian. These sections are similar to each other when it comes to their paleogeographic settings. Although both are located on continental shelves, El Kef is slightly farther than Elles. The K- T boundary at Elles is about 20 cm thick. It consists of grey sandy- silty marls. Grey marls make the upper Maastrichtian sediments with some intercalation of siltstones.

b) Bidart, France:

The Bidart KTB section outcrops at W1°35′, N 43°26′; consists of hemipelagic to pelagic deposits of biogenic limestones (Danian), marl and calcareous marl (Maastrichtian), deposited at a rate of 3 to 4 cm per 1000 years. Bidart section consists of only CF1 interval of Maastrichtian identified using *Plummerita hantkeninoides* which makes a high resolution study of this section possible. Below the KTB, sampling spanned for 350 cm where for the bottom 300 cm, sampling was done at intervals of 15 cm whereas for the top 50 cm it was at intervals of 5 cm.

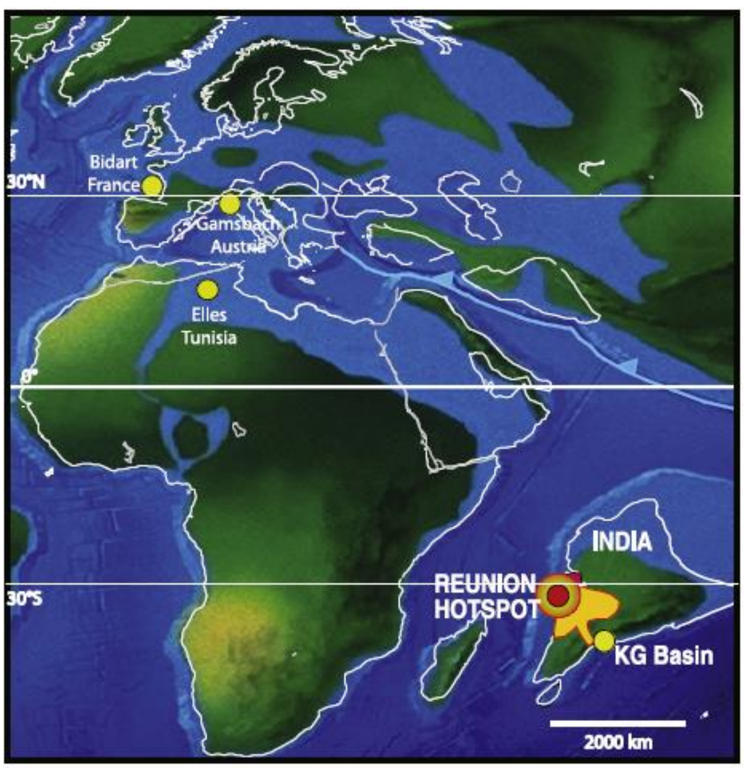

Fig.5: Adapted from Punekar et al. 2015. Palaegeographic map of 66 Ma showing the study sections, Bidart (France) and the preliminary study section, Elles (Tunisia, provisional GSSP)

relative to the location of the focal point of Deccan volcanism

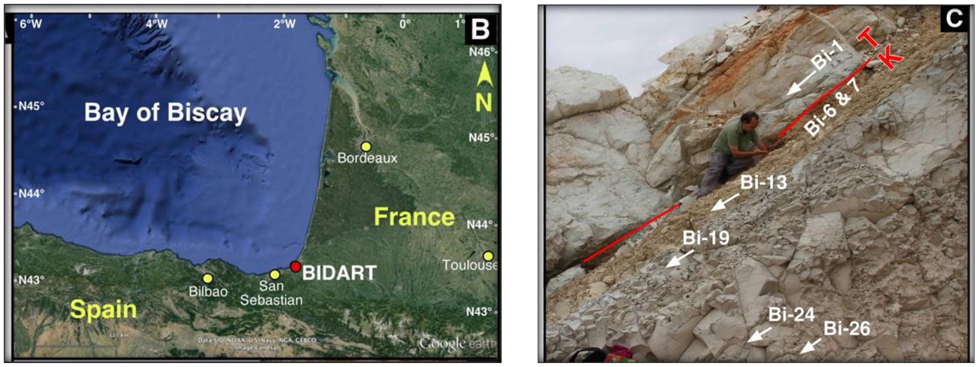

Fig.6: Adapted from Punekar et al., 2015: (B) current day location of Bidart section on Google Earth. (C) KTB highlighted

Material and methods:

a) Metric system:

Morphometric analysis of an entire population size in Planktic foraminifera is very time consuming and analysis of the data gets complicated. Therefore, it is rarely performed on the entire population. To establish an efficient and standardized protocol for morphometric analysis, establishing a metric system was a key element.

Standardization was performed with 8 Planktic foraminiferal species of different morphotypes: *Abathomphalus mayaroensis* (subcircular, trochospiral), *Planoglobulina brazoensis* (subtriangular,multiserial), *Globotruncanita stuarti* (circular, trochospiral), *Guembelina globulosa* (subtriangular, biserial), *Globotruncana fornicate* (circular, coiled with a low trochospire), *Pseudotextularia elegans* (subtriangular, biserial), *Globigerinelloides volutus* (circular, planospiral) and *Gublerina rajagopalani* (subtriangular, biserial). SEM images of these species were used for analysis. Conventions help regiment abstract and complicated systems. Following conventions were made to keep the results objective and replicable:

- Distance measured along the growth axis = length
- Distance measured perpendicular to the growth axis = width
- Area of 2D projection is the area projected of the 3D globular Planktic forams on a 2D image.
- Area of 2D projection is reasonable only when orientation of mounting is same for all specimen of that species.
- For biserials and multiserials, chambers are numbered in ascending order along the growth axis.
- For trochospirally coiled species, only the number of chambers in the outer whorl should be calculated as the identification of the inner whorl chambers is very arbitrary, as there is variability in the overlapping of chambers among specimens.

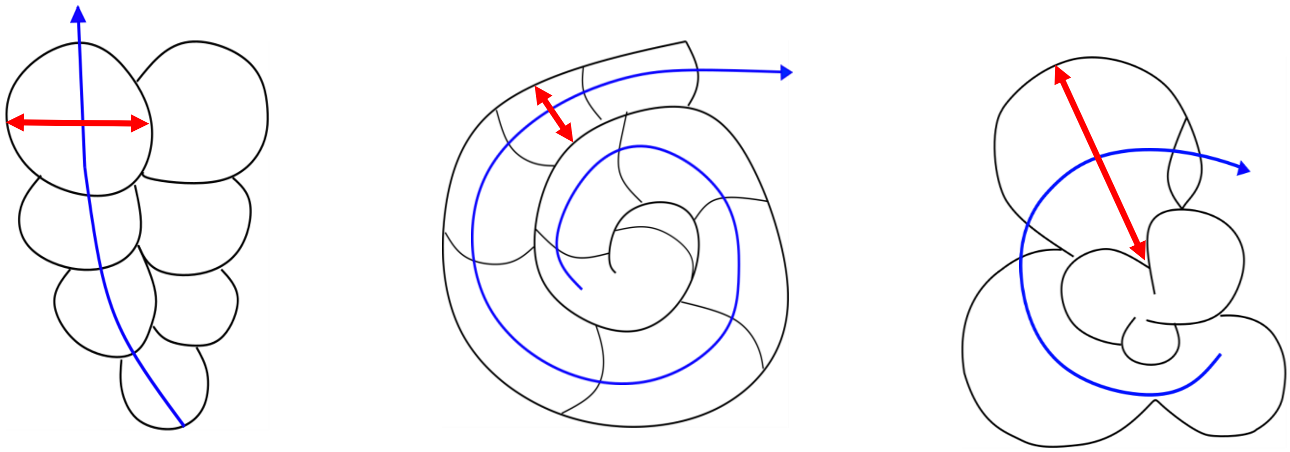

Fig. 7: Schematic of morphotypes of the selected species. (a) is Biserials whereas (b) and (c) is Trochospirals. Blue lines in each schematic is the growth direction. Red lines are perpendicular to the growth direction and is conventionally called the width.

Here we present the metrics selected for the eight planktonic foraminiferal tests:

Species	**Metrics**			
A. mayaroensis	No. of chambers	Thickness(max)	Area of 2D projection	Long & short axis(dorsal side); diagonals
P. brazoensis		Thickness(max)	Area of 2D projection	Max. length and width
G. stuarti	No. of chambers in the outer whorl		Area of 2D projection	Length and wdith
G. globulosa	No. of chambers	Thickness(max)	Area of 2D projection	Chamber dimensions; Max. length and width
G. fornicata	No. of chambers	Thickness(max)	Area of 2D projection	
P. elegans	No. of chambers	Thickness(max)	Area of 2D projection(not reliable)	Max. final chamber length; vertical height
G. rajagopalani		Thickness(max)	Area of 2D projection	Max. length and width
G. volutus	No. of chambers		Area of 2D projection	

Table 2: Metrices for the eight morphotypes used for standardization.

b) Preliminary Study: Elles, Tunisia

Sediment section of Elles Tunisia is widely spread spanning across CF1, CF2 and CF3 zones of Upper Maastrichtian (0 -3200 mbsf). The aim of this preliminary study was to look for a dwarfing trend using crude analysis of *Guembilitria* chamber measurements. *Guembillitria* has been reported as a disaster opportunist which made it a suitable candidate for this study.

The study was based on data made available by Gerta Keller's laboratory at Princeton University. Sample names used are same as in the original study. For this study, 10 well distributed samples : ES5(0 mbsf), ES17(150 mbsf), ES34(320 mbsf), ES36(340 mbsf), ES50(480 mbsf), ES84(820 mbsf), ES185(1830 mbsf), ES220(2180 mbsf), ES237(2350 mbsf), ES250(2480 mbsf) were used. Here the K-T boundary almost coincides with ES5. The data for measured length and width for all chambers (roughly 7-8 chambers) using SEM images of Guembilitria of Elles,Tunisia was already available. Since Guembilitria belong to trochospiral morphology therefore assuming spherical chambers, average chamber diameter was calculated for all the specimens in the selected samples by taking a mean of chamber width and length. However, there was a slight discrepancy due to the presence of juveniles in the sample. For standardizing of this study, the average no. of chambers for each sample was also calculated and plotted.

c) Bidart, France - Archived slides:

For the morphometric analysis we used archived slides prepared in Gerta Keller's laboratory at Princeton University. These were split into two size fractions: 63–150 μm and more than 150 μm. Approximately 300 specimens were picked, mounted and identified based on standard taxonomy for each sample (e.g., Robaszynski et al., 1983–1984; Olsson et al., 1999). These archived slides were then shipped. Transportation of archived slides also led to a bit of clustering and misplacement of specimens within each sample. To ensure efficient data collection: the clustered and misplaced specimens were identified and glued again taxonomically. Mounting on the slide was done using Tragacanth gum which is a water based glue.

d) Identifying target species:

Total 72 species were identified in this CF1 spanning section with suspect interval from BS - 1 to Bi - 37. Dwarfing as reported in Abrahmovich et al. (2009) was identified using comparative study of relative species abundances in smaller and larger size fractions. Relative species abundance is number of specimens of a species in that sample compared to total no. of individuals in that sample. The ideal species to study dwarfism should have either or both characteristics:

- High abundance in 63-150 μm size fraction in suspect interval
- Disappearance in >150 μm size fraction in suspect interval

To identify these, relative species abundance of all 72 species throughout the section in both size fractions was plotted and compared. Six species were identified as target species: *Globotruncana arca, Rugoglobigerina rugosa, Globotruncana mariei, Pseudotextularia elegans, Pseudoguembelina palpebral, Planoglobulina carseyae.*

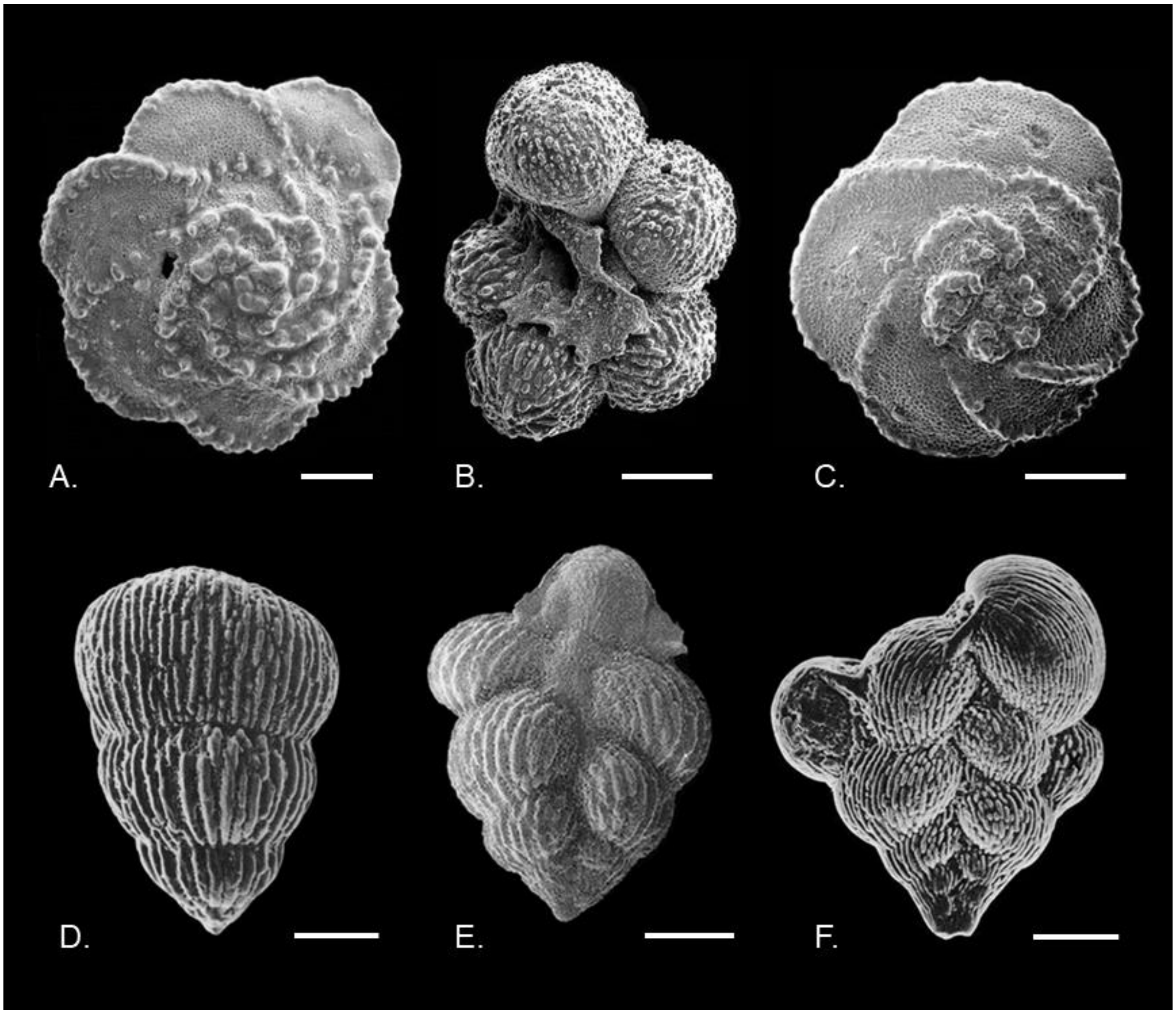

Plate I. Target species from Bidart, France >150 µm size fraction.

A - Globotruncana *arca*

B - Rugoglobigerina *rugosa*

C - Globotruncana *mariei*

D - Pseudotextularia *elegans*

E - Pseudoguembelina *palpebra*

F - Planoglobulina *carseyae*

e) Imaging:

On the archived slides for each sample, picked specimens were identified and mounted in same orientation species-wise. In order to obtain comparable results for morphometric analysis, orientation of the mounted individuals of a species was important.

Images were taken using a Leica m1655 MP HD stereozoom microscope with camera m170 and resolution at 1.1µm with standard 0.8x Planachromatic objective. It was also equipped with an inbuilt measurement software, Leica Application Suite (LAS core). Although using a stereo zoom microscope added a little scatter, using a Scanning Electron Microscope (SEM) for these many specimens would have required a lot of specimen preparation and was more time intensive. To avoid the instrumental errors due to the scatter, all the post imaging measurements were done manually.

The morphometric parameters used for each specimen of Trochospirals are as follows 1) Average diameter; 2) Area of 2D projection; 3) the number of chambers in the outer whorl; For Biserials: 1) Maximum length; 2) Maximum width; 3) total number of chambers; 4) Area of 2D projection. These parameters were selected because these might best reflect the change in test size (dwarfing) when subjected to variations in environmental conditions.

Analysis and comparison of results yielded by the entire population (all individual of a given species of that sample) may be ambiguous if the numbers of chambers are not taken into account. As this may lead to comparing a population majorly comprising of juveniles (not fully developed) with a population mostly of adults.

Out of the selected parameters, Area of 2D projection was measured using image analysis program – ImageJ. All other parameters were measured using Leica Application Suite (LAS core) in real-time. Maximum Length and Width were measured using ‘straight’ tool from the Measure panel pn live images. Average diameter for trochospirals was calculated by taking mean of 4 diameters (two diagonals and two perpendiculars). Using this software, a graphical database was created with a scale bar put in each captured image.

f) Post imaging measurements:

Magnification used to capture images varied for each image. Calculations, if made without calibrating the measurement software, would hence bring calculation error. To calibrate the pixels of the image to its value, the scale bar of the image was traced using ‘straight’ tool. Using ‘set scale’ option from the Analyze tab, the traced pixels were set to scale by entering the value in the ‘known distance’ tab of the popped up window. Thus, all the measurements made thereafter were adjusted to the scale. For area measurement, ‘freeform’ tool was used to trace the shape. By selecting ‘measure’ tool from Analyze tab, area, length and perimeter of the traced shape was displayed on the measurement window.

g) Composite parameters:

To detect any altered proportions, composite parameters were used: Roundness for Trochospirals and Aspect ratio for Biserials.

Roundness: It’s a constant that determines how close a figure is to a perfect circle. Mathematically it can be represented as:

$$\text{Roundness (R)} = \pi * \text{Perimeter}^2/\text{Area}$$

For Calculating Perimeter:

we know for a circle with radius ‘r’, $\text{Perimeter (P)} = 2*\pi*r$

$\text{Area (A)} = \pi*r^2$

$\text{Diameter (D)} = 2*r$ ----------------------------------(1)

Therefore, $P = 2*A/r$

$P = 4*A/D$ (from (1))

$R = \pi*16*A/D^2$

The values for Diameter were the Average diameters as mentioned previously, area was Area of 2D projection.

Aspect ratio: A constant which obtained by taking a ratio of Maximum Length to Maximum Width.

Results and discussion

a) Preliminary study in Elles shows consistency in chamber sizes:

Here is a general overview of the lithology: Fig (8) has alternate blue and pink bands running across the litholog which represent the cold and warm periods respectively. The middle blue band marks the onset of Deccan phase – 2, whereas the top blue band is the major pulse of Deccan phase - 2. The delta 18 O trends which are used as a proxy for temperature is in red for Planktic forams - *Rugoglobigerina rugosa* and blue for benthics – *Anonalinoides acuta*.

In the litholog, the values of the average chamber sizes of the selected ten sample is plotted. The preliminary study shows a uniform change in chamber size for each sample. There appears to be a consistent trend in all the chambers of the population. For e.g.: For ES220, we see the chamber diameters of this sample are more on the right side (higher values) compared to other chambers. This is consistent in all its chambers. However a high resolution study (with more samples) will be needed to make the claims certain.

Elles, Tunisia is an elaborate section with high number of samples and spanning three chrons - CF1, CF2 and CF3. It makes morphometric analysis for the entire population of each sample very time intensive. Therefore the choice of section for population level analysis was a slightly less extensive one that could fit in the time frame of the project - Bidart, France. Also, Bidart France has unusually high diversity than Elles for Planktic foraminifera.

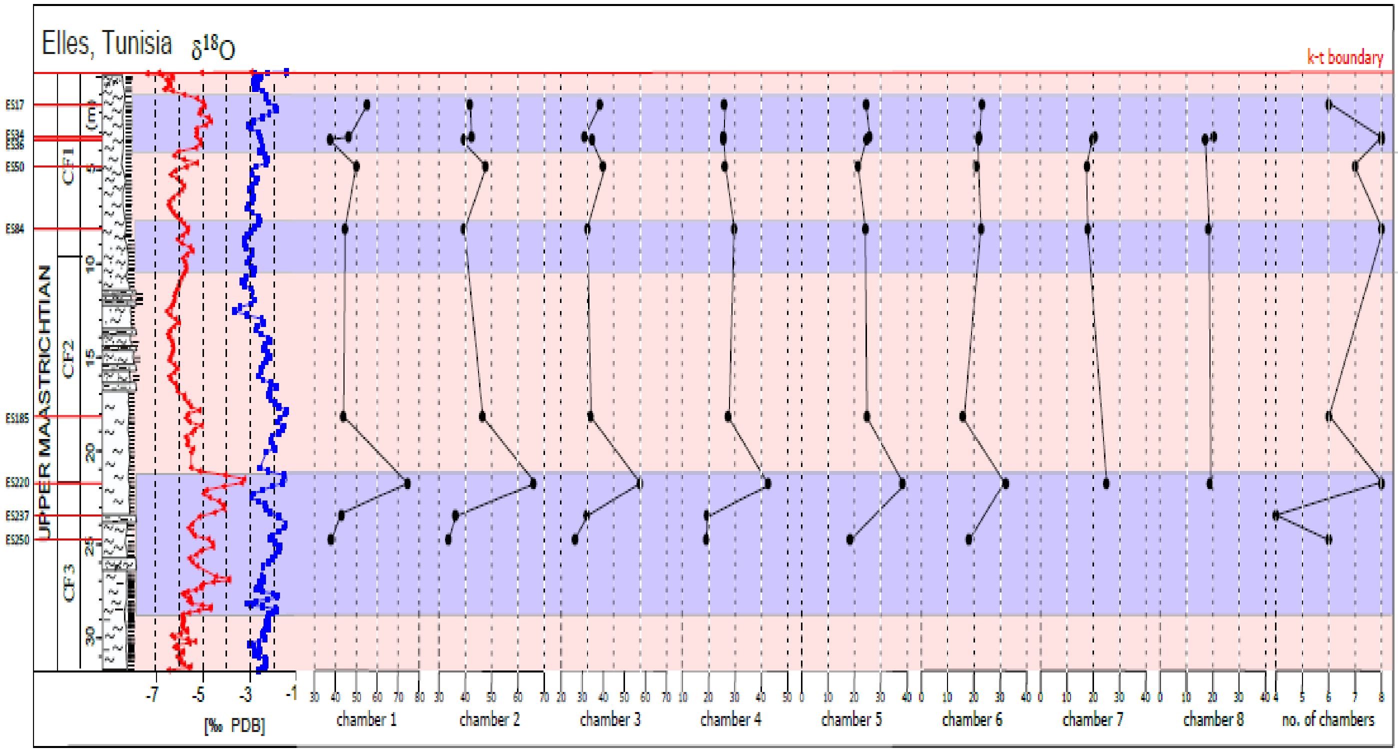

Fig. 8: Results of Preliminary study: Average chamber diameter of selected samples plotted against Elles, Tunisia litholog.

b) Morphometric Analysis at Bidart, France indicates existence of a dwarfing trend:

Here is a general overview of all the lithologs: The total range of the index species *P. hantkeninoides* defines the CF1 zone of Upper Maastrichtian. Right below the Marly Limestone is the KTB - Cretaceous - Tertiary boundary highlighted as a bright red line. Though the sampling was done right from BS- 1 (newest) to Bi – 37(oldest), this study revolved around the idea of comparing the dwarfing trend in suspect interval and the trend in samples of CF1 outside the suspect interval for each species. The suspect interval is defined with the data obtained in Punekar et al., 2015.

As Fig: (9) shows that *Guembilitria* of 38- 63 microns has significant peaks in the top 0.7 – 0.8 m with highest value at KTB. A lot of changes seem to take place in this interval. It is therefore marked as the suspect interval. All the data points outside the suspect interval will be somewhat like controls to compare with the data points in the suspect interval.

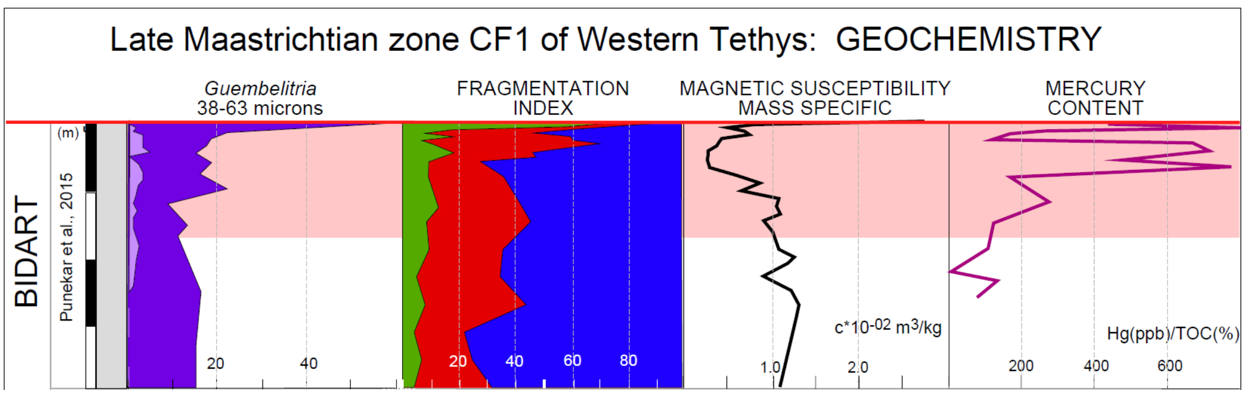

Fig 9: Adapted from Punekar et al. 2015: a)Guembelitira (38- 63 μm) abundance b)Fragmentation Index c)Magnetic Susceptibility d) Mercury content of Bidart, France.

The population average of each sample is denoted a black dot marked against its respective sample position. The green vertical (runs along the litholog) represent the mean value of all the population averages. Since the hypothesized dwarfing is an intraspecific dwarfing, an adaptation to environmental stress, the lithologs are made species-wise. It allows comparing trends in all parameters in a single figure.

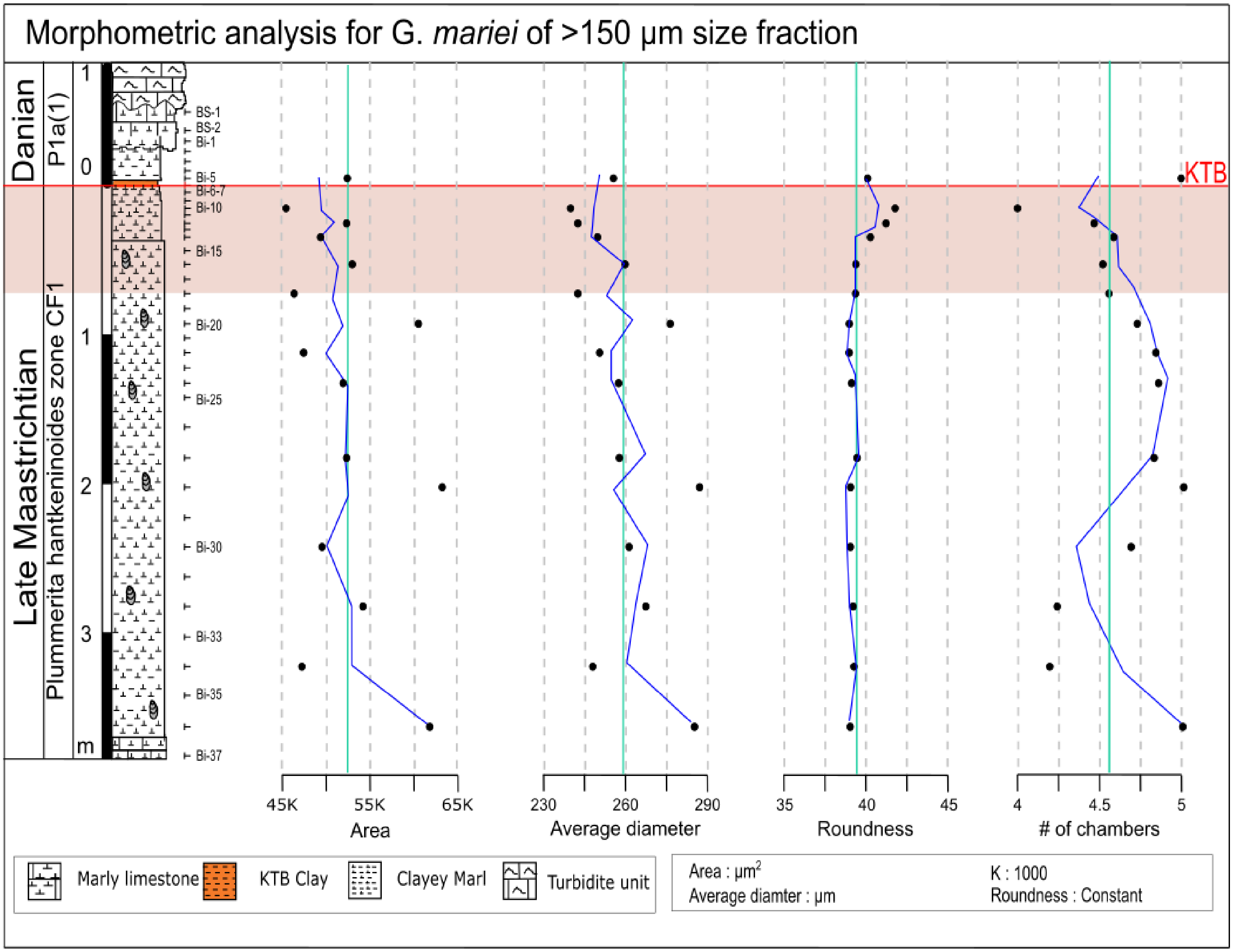

Fig.10 : Morphometric analysis for *G. mariei* of >150 size fraction: Area; Average diameter; Roundness; Number of chambers.

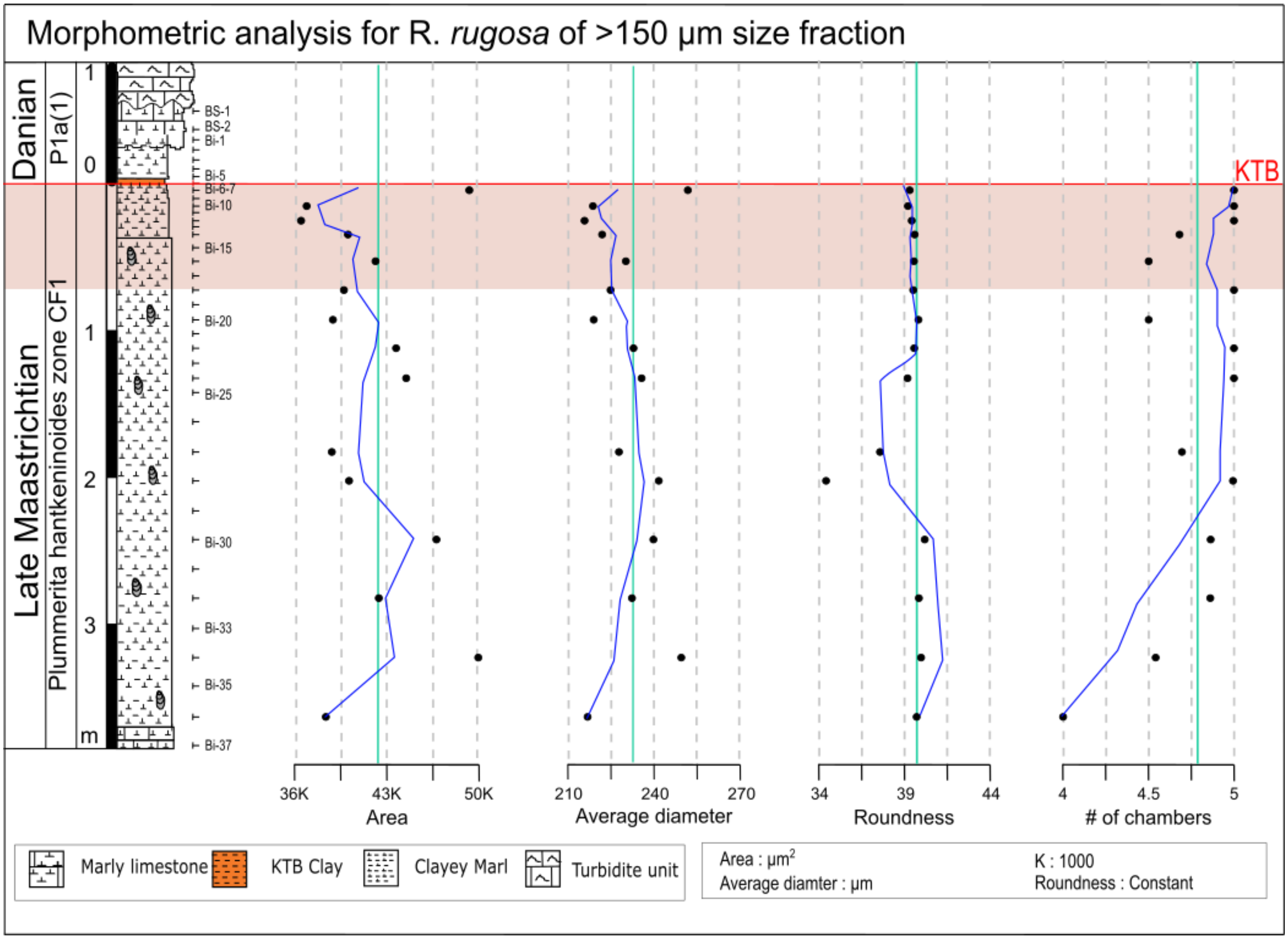

Fig.11: Morphometric analysis for *R. rugosa* of >150 size fraction: Area; Average diameter; Roundness; Number of chambers.

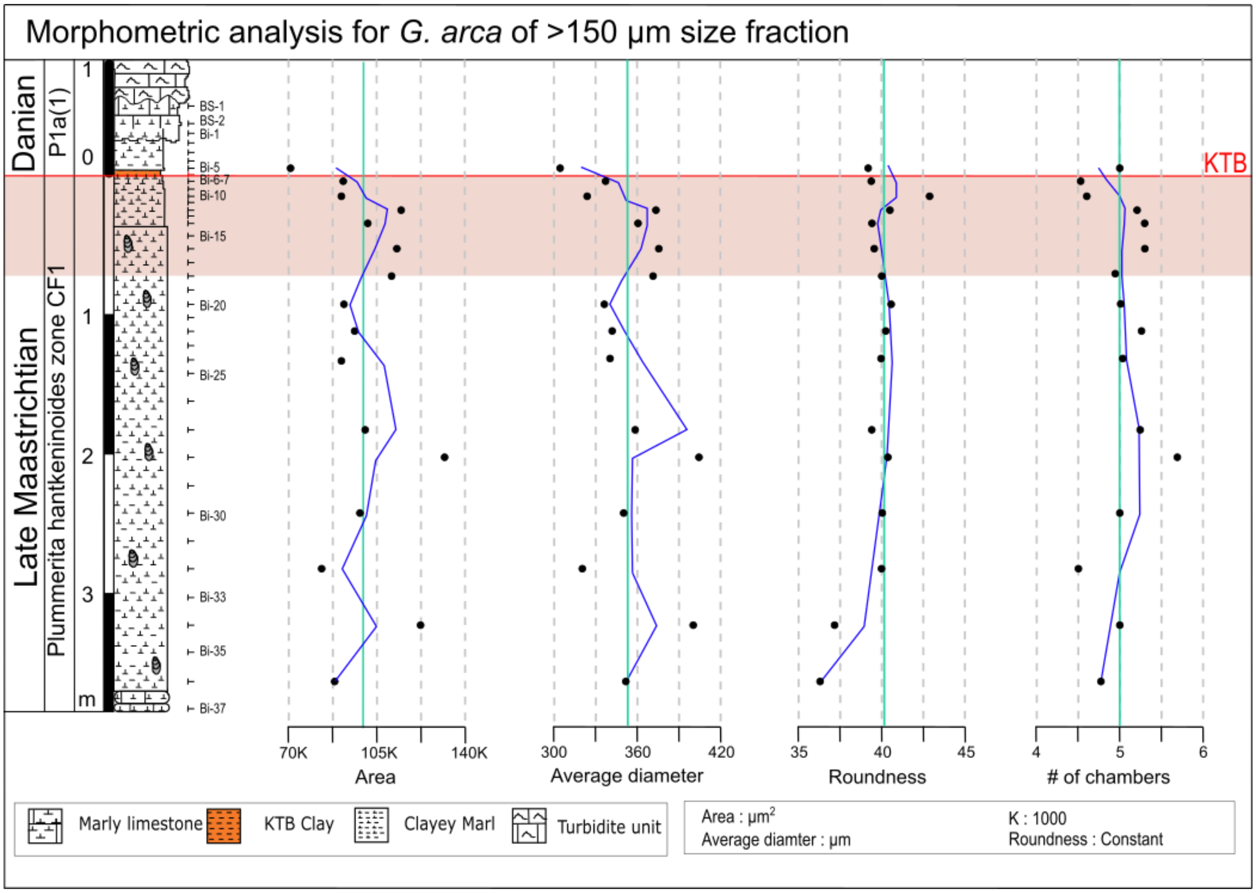

Fig.12: Morphometric analysis for G. *arca* of >150 size fraction: Area; Average diameter; Roundness; Number of chambers.

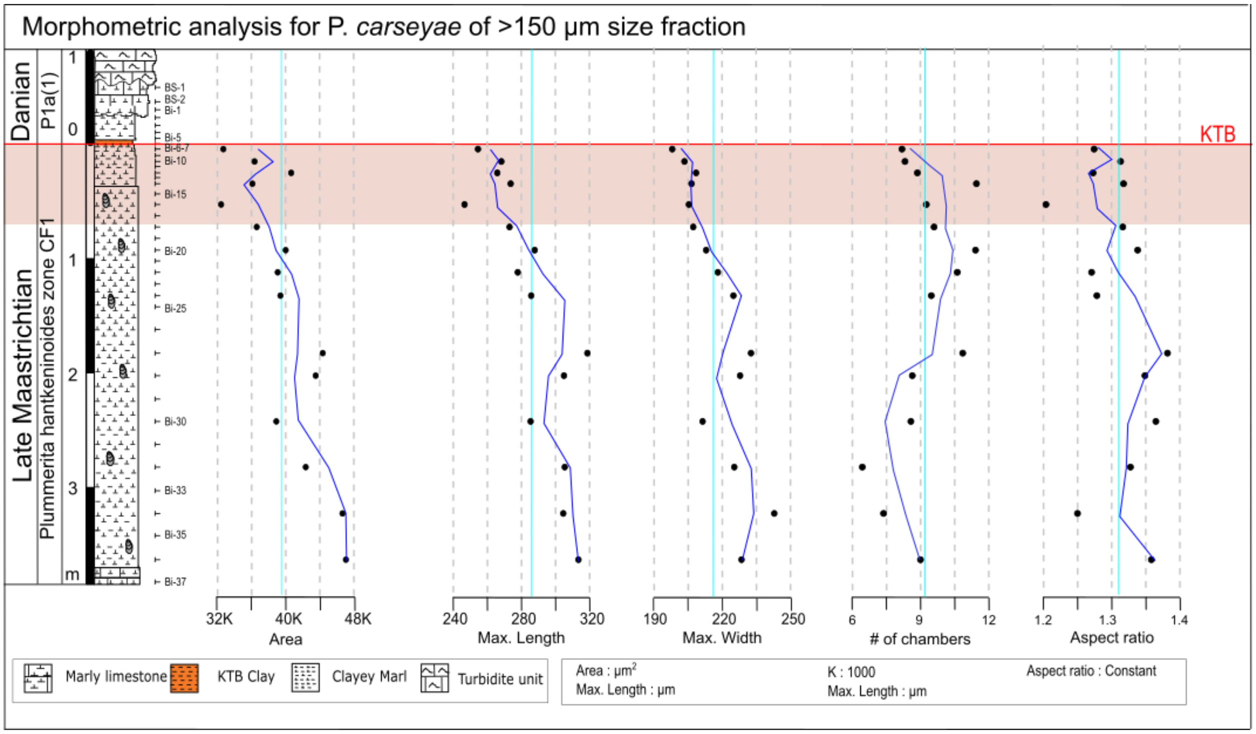

Fig.13: Morphometric analysis for *P. carseyae* of >150 size fraction: Area; Average diameter; Roundness; Number of chambers.

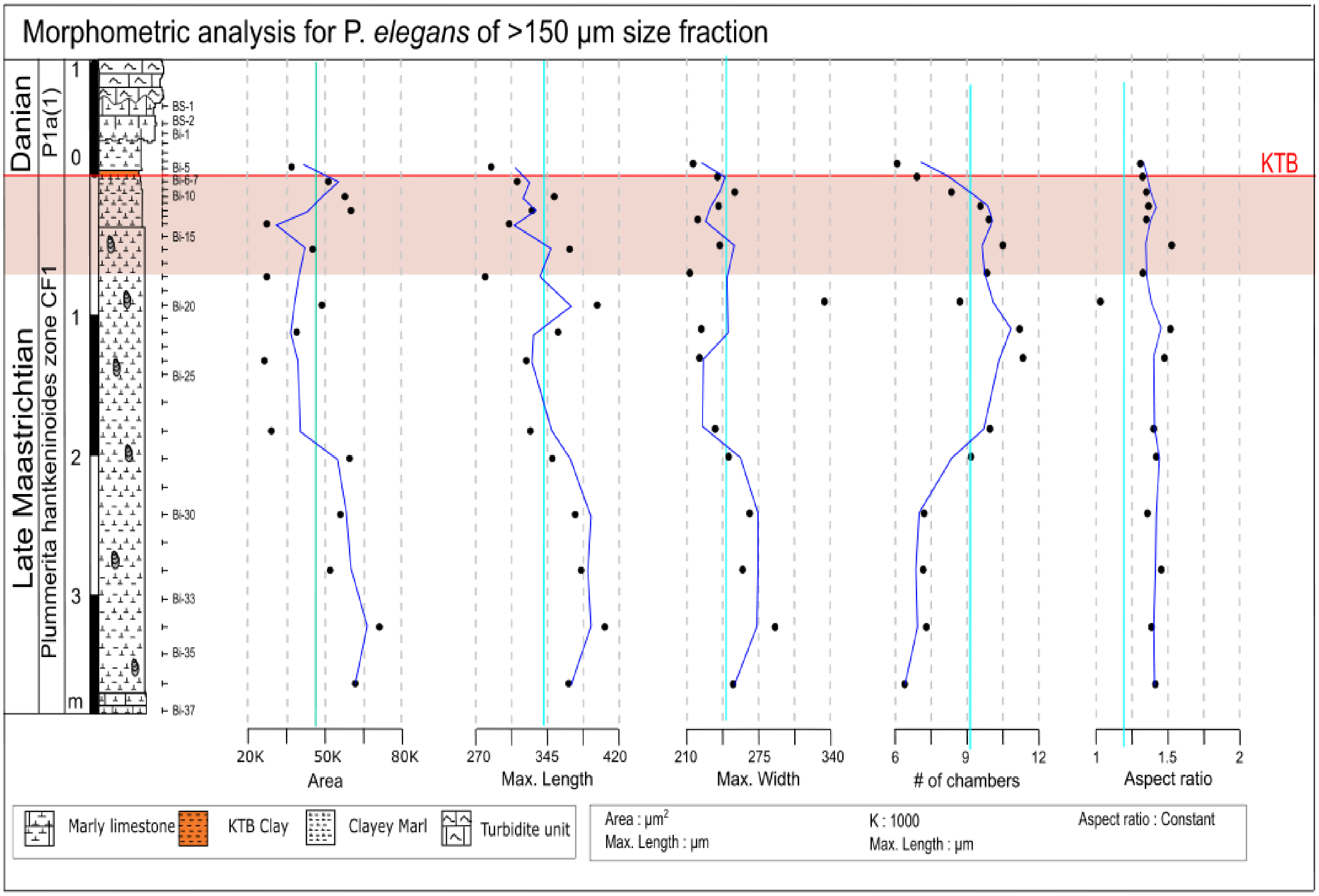

Fig.14: Morphometric analysis for *P. elegans* of >150 size fraction: Area; Average diameter; Roundness; Number of chambers.

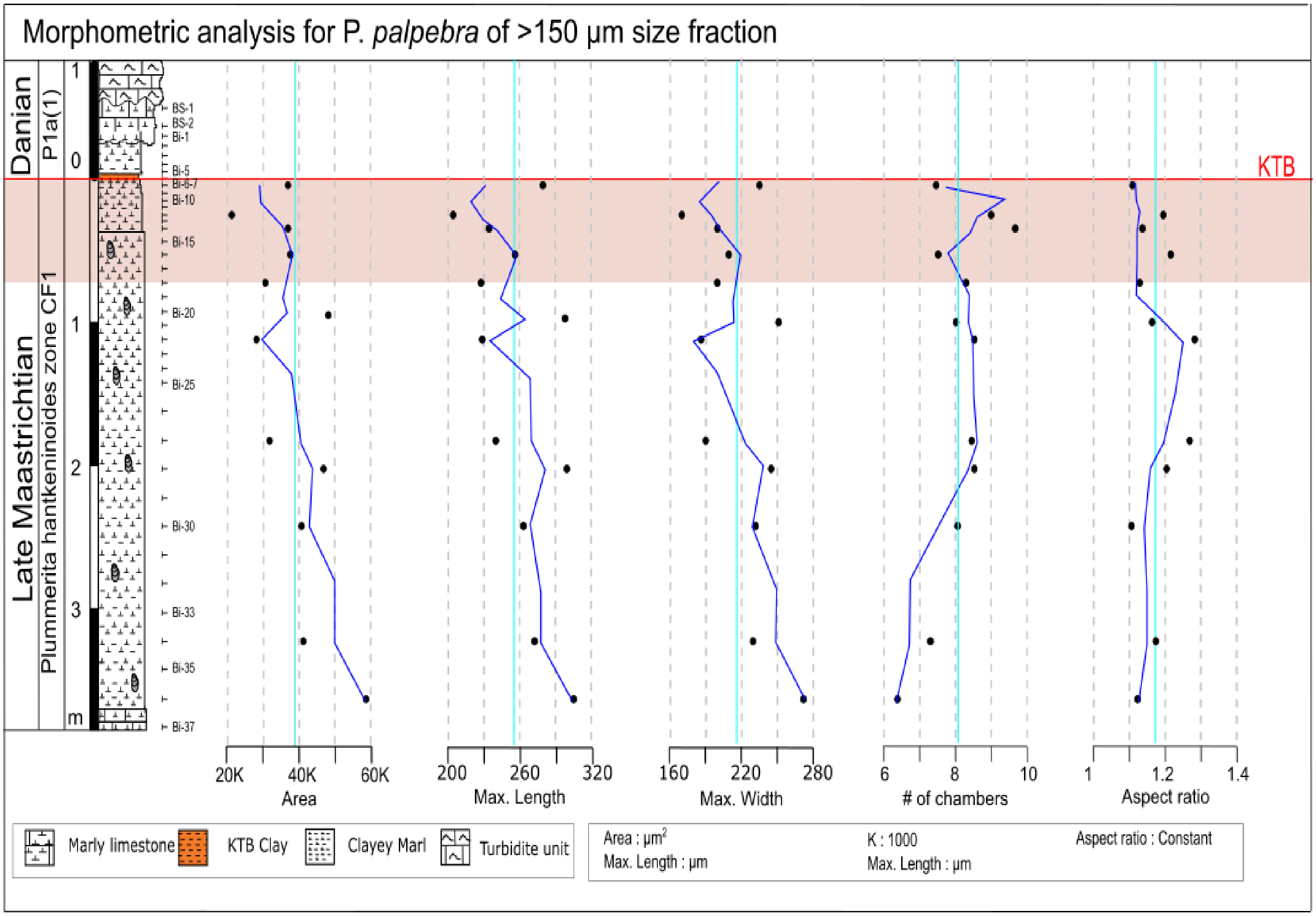

Fig.15: Morphometric analysis for *P. palpebra* of >150 size fraction: Area; Average diameter; Roundness; Number of chambers.

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The comparison of the results obtained permits the following general observations. These observations are based on the average values measured for the entire population of each mentioned sample which thus shows fluctuations based on the intra-sample and inter-sample variability.

1) *G. mariei* (Fig 10):
    - Bi-5, Bi-28 and Bi-36 (Bi-5 falls in the suspect interval) have equal values of the population average for number of chambers (highest value for number of chambers) but Area of 2D Projection and Average Diameter values for Bi-5 is roughly equal or less than the mean whereas Bi-28 and Bi-36 takes high values.
    - Although for Bi-12, Bi-14, Bi-16 and Bi-18 (falls in the suspect interval) the population average for the number of chambers is the mean value yet which has low values for Area of 2D Projection and Average Diameter.
    - Bi-10 has the lowest population average for the number of chambers (no. of adults) manifesting into lowest values for Area of 2D Projection and Average Diameter.
    - For Bi-20, the population average for the number of chambers is more than the mean value and their values for Area of 2D Projection and Average Diameter are high compared to the mean value.
    - Bi-22 stands as an outlier: although the population average for the number of chambers is more than the mean value and its values for Area of 2D Projection and Average Diameter are low. This could be a result of having one of the smallest population among all the samples.
    - For Bi-30, the population average for the number of chambers is more than the mean value whereas for Bi-32 it’s lower than the mean but both have their values for Area of 2D Projection and Average Diameter roughly equal to the mean value.

- For Bi-34, low number of chambers manifests into low values of Area and Average Diameter.

2) *R. rugosa* (Fig 11):
   - Bi-8, Bi-10, Bi-12, Bi-18, Bi-22, Bi-24 and Bi-28 (Bi-8, Bi-10, Bi-12, and Bi-18 falls in the suspect interval) have the highest number of chambers (no. of adults).
   - But Bi-10, Bi-12, Bi-18 have low values for Area of 2D Projection and Average Diameter.
   - As Bi- 8 has only one specimen in the sample, reliability of this data is questionable and it also stands as an outlier with high values for Area of 2D Projection and Average Diameter.
   - The Area and Average Diameter for Bi-22 and Bi-24 are more than the mean values.
   - For Bi-28, the Area is slightly less than the mean whereas the Average Diameter is more than the mean value.
   - For Bi-27, the population average for the number of chambers is less than the mean value thus their values for Area and Average Diameter is lower than the mean value.
   - For Bi-30 and Bi-32, the population average for the number of chambers is slightly more than the mean value and their values for Area of 2D Projection and Average Diameter are equal or more than the mean values.
   - Although for Bi-34 the population average for the number of chambers is lower than the mean value, their values for Area of 2D Projection (highest value among all the samples) and Average Diameter is high.
   - Bi-36 has the lowest population average for the numbers of chambers (no. of adults) manifesting into low values for Area of 2D Projection and Average Diameter. It has only two specimens in the population.

3) *G. arca* (Fig. 12):

- For Bi-5 and Bi-8,(falls in the suspect interval) the population average for the number of chambers is roughly equal to the mean value, but the Area of 2D Projection and Average Diameter are low.
- For Bi-12, Bi-14, Bi-16 and Bi-18, (falls in the suspect interval) the population average for the number of chambers is more than the mean which manifested into more than mean values for Area of 2D Projection and Average Diameter.
- For Bi-20, Bi-22 and Bi- 24, the population average for the number of chambers is roughly equal to the mean, their values for Area of 2D Projection and Average Diameter was slightly less than mean.
- For Bi-27 and Bi-30, the population average for the number of chambers is slightly more than the mean value and their values for Area of 2D Projection and Average Diameter are roughly equal to the mean value.
- For Bi-28, the population average for the number of chambers is more than the mean value and so is their value for Area of 2D Projection and Average Diameter.
- For Bi-32 and Bi-36, the values for Area of 2D Projection and Average Diameter are less than the mean value as the population average for the number of chambers (no. of adults) is the lowest among all the samples for Bi-32 and less for Bi-36.
- Although for Bi-34 the population average for the number of chambers is the mean value, yet the values for Area of 2D Projection and Average Diameter are high.

4) *P. carseyae (*Fig. 13):

- For Bi-8 and Bi-10 (falls in the suspect interval) the population average for the number of chambers is slightly less than the mean value, but the values of Area of 2D Projection, Maximum Width and Maximum Length were low.

- For Bi-12, Bi-16 and Bi-18, (falls in the suspect interval) the population average for the number of chambers is almost equal to the mean value but the Area of 2D Projection, Maximum Width and Maximum Length were less than or equal to the mean values.
- For Bi-20, Bi-22 and Bi- 24, the population average for the number of chambers is roughly equal to the mean, but the Area of 2D Projection, Maximum Width and Maximum Length were roughly equal to the mean values.
- For Bi-27, the value of number of chambers is more than the mean value and the values for Area of 2D Projection, Maximum Width are high whereas highest value for Maximum Length.
- For Bi-28, and Bi-36, the average number of chambers is slightly less than the mean value and but it has high values for Area of 2D Projection, Maximum Width and Maximum Length
- Although for Bi-30, Bi-32 and Bi-34, the average number of chambers are less than the mean value (lowest for Bi-32) their values for Area of 2D Projection, Maximum Width and Maximum Length are high.

5) *P. elegans* (Fig.14):

The orientation of some of the specimens was sideways, to avoid this mounting-induced error, these specimens were not considered while calculating the Area of 2D Projection. All the discrepancies in Area of 2D Projection is also mentioned below.

- For Bi-5, (falls in the suspect interval) the population average for the number of chambers is less than the mean value, so the Area of 2D Projection, Maximum Width and Maximum Length are low.
- For Bi-8 and Bi-10(falls in the suspect interval), the population average for the number of chambers is equal or less than the mean value so are their

values for Maximum Width and Maximum Length. But the value for Area of 2D Projection is more than the mean value as 21% for Bi-8 and more than 50% for Bi-10 of the total specimens were not in the right orientation.

- For Bi-12, Bi-14 and Bi-18(falls in the suspect interval), the population average for the number of chambers is more than the mean value and but the value for Maximum Width and Maximum Length is less than the mean for Bi-12, Bi-14, and Bi-18.
- For Bi-20, the population average for the number of chambers is equal to the mean value whereas it has high value for Maximum Length, the highest value for Maximum Width but average value for Area. It had only three specimens out of which a large specimen was not included in the area calculation due to its wrong orientation.
- For Bi-27 and Bi-28, the population average for the number of chambers is slightly more than the mean value and their values for Maximum Width and Maximum Length are roughly equal to the mean value. However, the value of Area of 2D Projection is very low and more than the mean for Bi-27 and Bi-28 respectively. The mounting orientation for 30% in Bi-27 and 59% in Bi-28 was sideways.
- Although Bi-30, Bi-32, Bi-34 and Bi-36 have below mean values for the number of chambers yet their values for Maximum Width, Maximum Length and Area of 2D Projection are more than the mean values
- For Bi-34, the Area of 2D Projection and Maximum Length is highest.

6) *P. palpebra (*Fig.15):

- For Bi-8, (falls in the suspect interval) the population average for the number of chambers is slightly less than the mean value and so are the values for Maximum Width and Maximum Length. But the value for Area of 2D Projection is more than the mean value.
- For Bi-12, Bi-14 and Bi-18, (falls in the suspect interval) the population average for the number of chambers is slightly more than the mean value

but their values for Area of 2D Projection, Maximum Width and Maximum Length are less than the mean values.

- For Bi-16, the population average for the number of chambers is slightly less than the mean value and so are their values for Area of 2D Projection, Maximum Width and Maximum Length.
- For Bi-20, the population average for the number of chambers is equal to the mean value whereas it has high values for Maximum Length, Maximum Width and Area of 2D Projection.
- For Bi-22, the population average for the number of chambers is more than the mean, but the values for Area of 2D Projection, Maximum Width and Maximum Length are low. Bi-22 has only two specimens with contrasting values for all the parameters.
- For Bi-27 and Bi-28, the population average for the number of chambers is slightly more than the mean value but the Area of 2D Projection, Maximum Width and Maximum Length are low for Bi-27 and high for Bi-28. In Bi-27, some of the specimens are missing with some reworked and broken specimens, therefore it is not a reliable data point.
- For Bi-30, the population average for the number of chambers is equal to the mean value and so are its values for Area of 2D Projection, Maximum Width and Maximum Length.
- Although for Bi-34 and Bi-36, the population average for the number of chambers is the lowest among all the samples, the values for Area of 2D Projection, Maximum Width and Maximum Length are high for Bi-34 and highest for Bi-36.

d) Analysis method: 3 point moving average highlights the underlying trend.

Taking a 3 point moving average comes handy to highlight an underlying trend in a series of data which is not clear enough because of the variations within. Calculating a moving average makes it is possible to remove some of these variations.
This method of analysis was used on all the parameters of each species. The analysis was done as follows:

1. A series of data was produced by taking the mean for each sample (represented as dots on the lithologs).
2. Added up the first 3 numbers of the series and divided the value by 3. This obtained value became the first 3 point moving average.
3. Added up the next 3 numbers (2nd, 3rd and 4th) of the series and divided the value by 3, which gave the second 3 point moving average.
4. Repeated the second step until the last 3 numbers.
5. This new series of data was then plotted as a line trend in the same lithologs (blue lines).

An evident dwarfing trend can be defined as lower values in the suspect interval and higher values of biometrics for samples outside the suspect interval with a gentle slope similar to the Magnetic Susceptibility from Fig. 3. And Fig.16 After calculating 3 point moving averages, the following observations are made:

1. *G. mariei:*

   A very prominent dwarfing trend seems to exist for Area of 2D Projection and Average Diameter.
2. *R. rugosa:*

   Dwarfing trend exists with lot of fluctuations and some fluctuations for Area of 2D Projection and Average Diameter respectively.
3. *G. arca:*

   Drawing inferences only from 3 point moving average may not show a dwarfing trend for Area of 2D Projection. No. of chambers in the outer whorl should be compared too. However, it is somewhat visible for Average Diameter.

4. *P. carseyae:*

   Extremely prominent dwarfing trends for Area of 2D Projection, Maximum Length and Maximum Width

5. *P. elegans:*

   A very prominent dwarfing trend seems to exist for Area of 2D Projection and Maximum Length. Dwarfing trend for Maximum width has some outlier peaks.

6. *P. palpebra:*

   There seems to be an evident trend for dwarfing for all its parameters.

c) Composite parameters highlight the outliers:

Along with the other metrics, a composite parameter is also plotted in the Bidart lithologs which is Roundness for Trochospirals and Aspect ratio for Biserials.

Roundness shows very few fluctuations, this constant remains roughly the same for each species in the conducted study: 40.26 for *G. arca*, 38.30 for *G. mariei* and 40.21 for *R. rugosa.* As studied in A. Brombacher et al., 2017, Roundness is mostly a species specific value but it was verified to be true only for three out of the six trochospiral species they studied.

Some of the outliers mentioned in the morphometric analysis have either a very high or very low value for Roundness. As we know, Roundness is proportional to Area/ Diameter^2, if the Area of 2D projection is too high compared to the Diameter square, a high value of Roundness is yielded and vice versa.

Aspect ratio is high when the Maximum Length is very high compared to Maximum Width.

Here is a list of the few outliers with anomalous Roundness and Aspect ratio.

- Bi-36 usually has high metric values for most species even when the population average for the number of chambers is equal or less than the mean value. Although for *G. arca*, Bi-36 the value of population average for the number of chambers is slightly less than the mean value, yet the Area of 2D Projection and

Average Diameter are low. This sample stands out with an average roundness of 36.09627.

- In *R. rugosa*, for Bi-28 the value of population average for the number of chambers is more than the mean value but the Area of 2D Projection is slightly less than the mean whereas the Average Diameter is more than the mean value. This discrepancy is highlighted with a very low Roundness value i.e. 34.73196
- The population of *P. carseyae* in Bi-16 (falls under suspect interval) has the least values for Aspect ratio. Although it has average no. of chambers, it has the lowest value for Area and Maximum Length among all the samples.
- Bi-22 (out of suspect interval) among *P. palpebra* has the highest Aspect ratio. This population has average no. of chambers but low values for all its parameters.
- For Bi-27 and Bi-28 (*P. palpebral),* the population average for the number of chambers is slightly more than the mean values but the Area of 2D Projection, Maximum Width and Maximum Length are low for Bi-27 and high for Bi-28. Bi-27 has high Aspect ratio.

Not all outliers have altered Roundness and Aspect Ratio. Also, samples with Altered Aspect Ratio and Roundness do not necessarily stand out of the metric dwarfing trends.

Conclusions & Future Directions:

This study reports following conclusions based on the measurements of the target species of planktonic from the CF1 spanning Bidart section of France.

- A General dwarfing trend exists in most of the identified Target species as postulated. The suspect interval which is prior to the K-T does show population with decreased metric values.
- Most of the identified Target species appear to dwarf pre K-T. Percentage Relative Abundance is thus very useful but a crude method in identification of species which may have dwarfed.
- The dwarfing addressed in this study can be identified as Pre-extinction dwarfing according to Wade and Olsson, 2009 and as Lilliput effect - Intraspecific dwarfing/ Dwarfing according to Abramovich and Keller, 2009.
- Although some of the outliers from the dwarfing trend are also identified as outliers for Roundness and Aspect ratio but the reliability on these Composite parameters for identifying outliers is questionable.

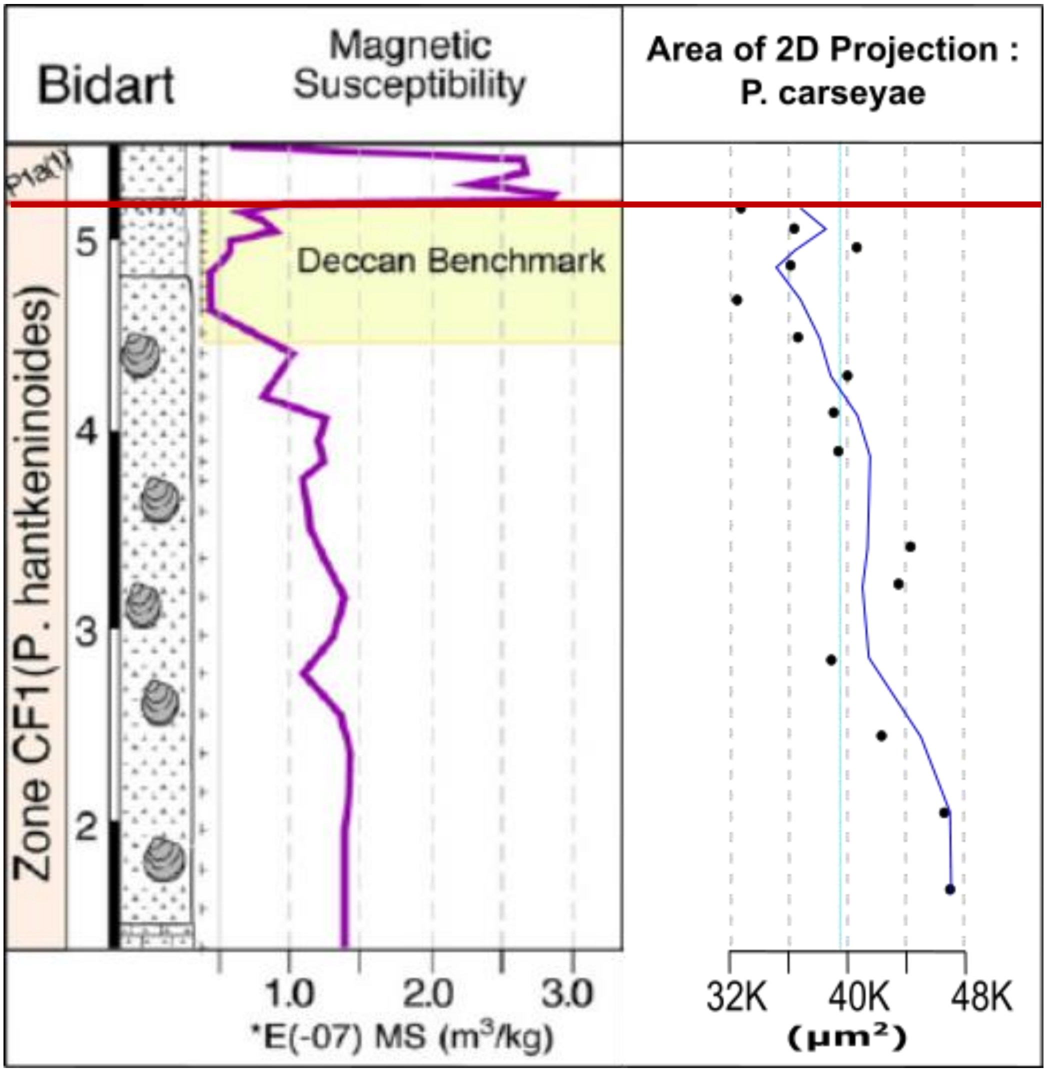

Fig. 16: Comparing the Low MS trend as reported in Punekar et al., 2015 and one the metrics (Area of 2D Projection of *P. carseyae)*

Based on the results from Bidart, France, a directional dwarfing trend exists with dwarfing in the suspect interval. The next step would be to do a high resolution morphometric analysis for Elles, Tunisia with comparative study with pre and post suspect interval –low MS (top 4.5 m) specimens as controls.

R Martínez-Colón *et al*., 2009 documents test deformities and abnormalities induced by anthropogenic stress. Along with quantification of size reduction it would also be interesting to check for any altered test proportions for Elles, Tunisia.

References:

b) Abramovich, S., and Keller, G. (2003). Planktonic foraminiferal response to the latest Maastrichtian abrupt warm event: a case study from South Atlantic DSDP Site 525A. Mar. Micropaleontol. 48, 225–249.
c) Aziz, A., Tantawy, A., Keller, G., and Pardo, A. (2009). Late Maastrichtian Volcanism in the Indian Ocean : Effects on Calcareous Nannofossils and Planktic Foraminifera. Palaeogeogr. Palaeoclimatol. Palaeoecol. 284, 63–87.
d) Brombacher, A., Wilson, P.A., and Ezard, T.H.G. (2017). Marine Micropaleontology Calibration of the repeatability of foraminiferal test size and shape measures with recommendations for future use. Mar. Micropaleontol. 133, 21–27.
e) Coccioni, R., Sideri, M., Frontalini, F., and Montanari, A. (2016). The Rotalipora cushmani extinction at Gubbio (Italy): Planktonic foraminiferal testimonial of the onset of the Caribbean large igneous province emplacement? In The Stratigraphic Record of Gubbio: Integrated Stratigraphy of the Late Cretaceous–Paleogene Umbria-Marche Pelagic Basin, M. Menichetti, R. Coccioni, and A. Montanari, eds. (Geological Society of America), p. 0.
f) Falzoni, F., Petrizzo, M.R., and Valagussa, M. (2018). A morphometric methodology to assess planktonic foraminiferal response to environmental perturbations: The case study of Oceanic Anoxic Event 2, Late Cretaceous.
g) Font, E., Adatte, T., Sial, A.N., Lacerda, L.D. De, Keller, G., and Punekar, J. (2016). Mercury anomaly , Deccan volcanism , and the end-Cretaceous mass extinction. Geology 44, 171–174.
h) Gardin, S. (2002). Late Maastrichtian to early Danian calcareous nannofossils at Elles (Northwest Tunisia). A tale of one million years across the K–T boundary. Palaeogeogr. Palaeoclimatol. Palaeoecol. 178, 211–231.
i) Honisch, B., Ridgwell, A., Schmidt, D.N., Thomas, E., Gibbs, S.J., Sluijs, A., Zeebe, R., Kump, L., Martindale, R.C., Greene, S.E., et al. (2012). The Geological Record of Ocean Acidification. Science (80-.). 335, 1058–1063.
j) Keller, G. (1988). Extinction, survivorship and evolution of planktic foraminifera across the Cretaceous/Tertiary boundary at El Kef, Tunisia. Mar. Micropaleontol. 13, 239–263.
k) Keller, G. (2005). Biotic effects of late Maastrichtian mantle plume volcanism: implications for impacts and mass extinctions. Lithos 79, 317–341.
l) Keller, G., and Abramovich, S. (2009). Lilliput effect in late Maastrichtian planktic foraminifera : Response to environmental stress. Palaeogeogr. Palaeoclimatol. Palaeoecol. 284, 47–62.
m) Li, L., and Keller, G. (1998). Abrupt deep-sea warming at the end of the Cretaceous. Geology 26, 995–998.
n) Macleod, N., Ortiz, N., Fefferman, N., Clyde, W., Schulter, C., and Maclean, J. (2019). Phenotypic response of foraminifera to episodes of global environmental change. In Biotic Response to Global Change, S.J. Culver, and P.F. Rawson, eds. (Cambridge: Cambridge University Press), pp. 51–78.
o) Milner, A., R. Milner, A., and Evans, S. (2000). Amphibians, reptiles and birds: a biogeographical review. In Biotic Response to Global Change: The Last 145 Million Years, pp. 316–332.
p) Olsson, R.K., Hemleben, C., Berggren,W.A., Huber, B.T., 1999. Atlas of Paleocene Planktonic Foraminifera. Smithsonian Contribution to Paleobiology No. 85. Smithsonian Institution Press, Washington D.C., p. 252.

q) Phleger, F.B., 1960a. Ecology and Distribution of Recent Foraminifera. Johns HopkinsPress, Baltimore. 297 pp.
r) Punekar, J., Keller, G., Khozyem, H.M., Adatte, T., Font, E., and Spangenberg, J. (2016). A multi-proxy approach to decode the end-Cretaceous mass extinction. Palaeogeogr. Palaeoclimatol. Palaeoecol. 441, 116–136.
s) Robaszynski, F., Caron, M., Gonzalez Donoso, J.M., Wonders, A.A.H. & The European Working Group on Planktic Foraminifera 1984, Atlas of Late Cretaceous Globotruncanids. Revue de Micropaléontologie, 26: 145-305.
t) Thibault, N., Galbrun, B., Gardin, S., Minoletti, F., and Le Callonnec, L. (2016). The end-Cretaceous in the southwestern Tethys (Elles, Tunisia): orbital calibration of paleoenvironmental events before the mass extinction. Int. J. Earth Sci. 105, 771–795.
u) Wade, B.S., and Olsson, R.K. (2009). Investigation of pre-extinction dwar fi ng in Cenozoic planktonic foraminifera. Palaeogeogr. Palaeoclimatol. Palaeoecol. 284, 39–46.