Calcareous nannofossil biostratigraphy: historical background and application in Cenozoic chronostratigraphy

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Calcareous nannofossils are considered one of the most powerful biostratigraphical tool in marine carbonate sediments especially in open ocean settings. Their origination goes at least as far back as the Triassic (ca. 220 Ma) when they first biomineralized and produced calcite skeletons. Since then, they have evolved rapidly showing widespread biogeographical distributions. Starting from the 1950s, changes in calcareous nannofossil assemblages have been used to date rocks and sediments and a fundamental step was achieved two decades later with the publication of the first comprehensive biostratigraphical schemes. Standardized quantitative counting methods, unambiguous taxonomy as well as highly resolved data sets provide high-quality biostratigraphical datums which, in turn, result in the precise positioning of calcareous nannofossil biohorizons and in the construction of reliable biostratigraphical frameworks. Here, we use recently published Cenozoic biozonations as a framework to present an overview of the calcareous nannofossil biohorizons which are used in chronostratigraphy to denote Cenozoic Global Standard Stratotype-section and Point. □ *Biostratigraphy, Calcareous nannofossils, Cenozoic, chronostratigraphy, Global Stratotype Standard-section and Point.*

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The first recognition of calcareous nannofossils/coccoliths went back to 1836, when Christian G. Ehrenberg described these peculiar 'morpholites' and/or 'Kalkerdige Crystalldrusen' from a Cretaceous chalk cropping out in the Rügen Island in the Baltic Sea. He did not grasp their organic nature and was convinced that they were rather concretions than microfossils.

After this very important discovery, in 1858, Thomas H. Huxley described the coccolithophores as '…a multitude of very curious rounded bodies, to all appearance consisting of several concentric layers, surrounding a minute clear centre, and looking, at first sight, somewhat like single cells of the plant *Protococcus*; as these bodies, however, are rapidly and completely dissolved by dilute acids, they cannot be organic, and I will, for convenience sake, simply call them coccoliths (Fig. 1)…' Three years later in addition to the coccoliths noted by Huxley, Wallich discovered peculiar spheroidal bodies, which he terms 'coccospheres' and proposed that the coccoliths proceed from the coccospheres (Huxley 1858; Wallich 1861). In the same year, 1861, Henry C. Sorby also published a paper describing the coccoliths he found in the English Chalk. Since then, coccoliths have been considered organic in nature but their importance in rock dating and their role in the biogeochemical cycles were too far away to be understood so that coccoliths/nannoliths would have remained only a scientific curiosity for many years.

After the HMS *Challenger* Expedition (1872–76), Murray & Blackman (1898) published a pivotal study on the biology and biogeography that was followed shortly after by the work of Lohmann (1902), who recognized the presence of flagella and first introduced the term 'nannoplankton'. Once these peculiar organisms started to be widely studied, scientists soon realized that they present very different structures and shapes, which allowed the definition of taxonomic groups based on shared characteristics. However, the complex heteromorphic cycle (polymorphism), the changes forced by external environmental conditions (ecophenotypy) and the diagenetic alterations (preservation) could instead make their classification (parataxonomy) difficult and the species concept somewhat ambiguous.

The next step was the recognition that each taxon has a unique distribution in space and time. Their stratigraphical importance became progressively evident to the geological community starting from the
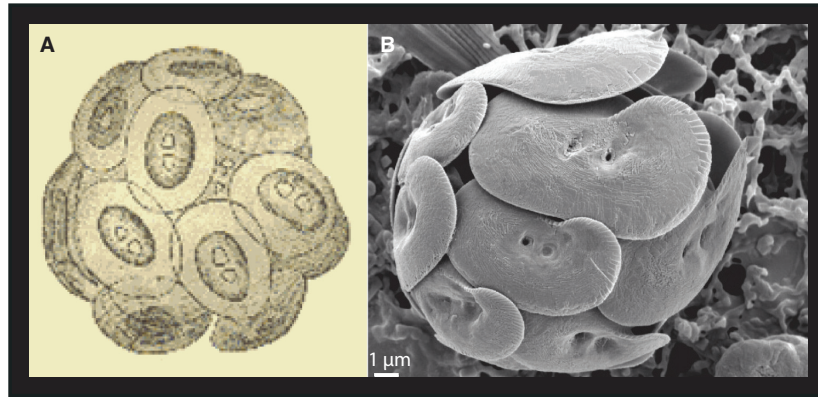
Fig. 1. Coccosphere of *Helicosphaera carteri* (Wallich 1877) Kamptner, 1954. A, Huxley's drawing (1858); B, SEM image (Young *et al.* 2003). [Colour figure can be viewed at wileyonlinelibrary.com]

mid-part of the last century, following a landmark publication that evidenced for the first time their application in biostratigraphy and rock dating (Bramlette & Riedel 1954). This benchmark work, though based on little taxonomic work and data, and possibly biased by technical difficulties, was still able to enlighten that coccoliths are common in some Mesozoic and Cenozoic rocks, especially in deep-sea deposits, and may constitute the main bulk of the chalky sediments. The first stratigraphical distribution of calcareous nannofossil taxa, what is called a range chart, was in fact provided by these authors and represented a fundamental starting point for the development of the calcareous nannofossil biostratigraphy.

Subsequently, availability of deep-sea sediments and cores, provided by ocean research drillings, facilitated the studies on nannoplankton and nannofossils and, in turn, expanded their importance as stratigraphical and chronological tool. Moreover, it became evident that they played also an important role in the bio-geosphere history and therefore they could be used to reconstruct palaeoenvironmental/ oceanographic conditions in the geological past.

The geological history of nannoplankton is intimately related to two other planktonic microfossil groups: dinoflagellates and diatoms. These three new groups entered the fossil record in the Triassic (Falkowski *et al.* 2004; De Vargas *et al.* 2007). The invention of the biomineralization by nannoplankton and their related fossil remains represented an important step in the evolution of the whole marine ecosystem and a significant change in pelagic sedimentation and biogenic carbonate production. Their appearance in the Triassic was a breakthrough in the ocean realm, specifically for the marine carbonate system and the global carbon cycle.

In the modern ocean, calcareous nannoplankton together with dinoflagellates and diatoms play a major role in export flux of organic matter to the ocean interior and sediments but we would emphasize that they are also responsible of the majority of the carbonate production and export in the ocean (Milliman 1993; Falkowski *et al.* 2004). The Mesozoic was the time when this group, together with planktonic foraminifera, acted to shift the primary site of carbonate production from shallow water environments to the open ocean (Brownlee & Taylor 2004; Hay 2004).

Approximately 220 My ago, calcareous nannoplankton started to produce mineralized skeletons and evolved while interacting with changes in climate, ocean structure and chemistry as well as in the geosphere. Increases in biodiversity and rates of evolution occurred through the Jurassic and Cretaceous and continued in the Cenozoic (Fig. 2), and the fairly fast evolutionary changes resulted in several first and last appearance events that represent the basis for detailed biostratigraphical schemes, successfully applied in different intervals of geological time.

Studies published during the last decades have demonstrated that the applications of calcareous nannofossils comprehend the fields of biostratigraphy and biochronology, and expands into palaeoceanography and palaeobiology. Here, we focus on their trait as excellent stratigraphical and chronologic markers in marine sediments and underline their pivotal role as correlation tool involved in chronostratigraphical issues.

Calcareous nannofossil biostratigraphy (and biochronology)

Calcareous nannofossils have undeniable qualities when used in biostratigraphy. First, they are usually abundant in marine sediments and this implies that
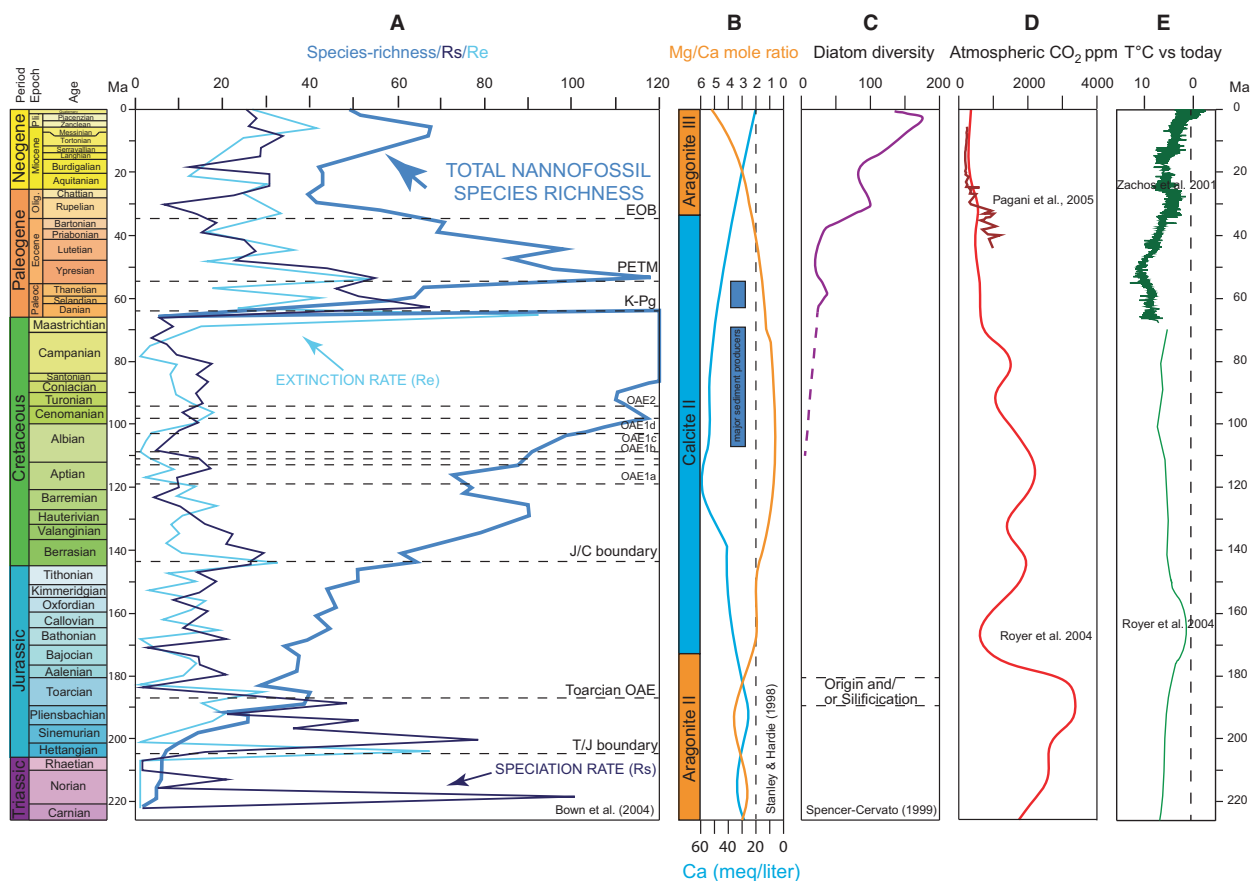
Fig. 2. Calcareous nannofossil evolutionary rates and diatom diversity plotted against selected physical environmental parameters. A, species-richness, speciation rate and extinction rate of calcareous nannofossils through the last 220 Myr (Bown *et al.* 2004); B, aragonite and Calcite seas and major sediment producers (Stanley & Hardie 1998); C, diatom diversity (Spencer-Cervato 1999); D, atmospheric CO_2 (Royer *et al.* 2004; Pagani *et al.* 2005); E, temperature versus today value (Zachos *et al.* 2001; Royer *et al.* 2004). [Colour figure can be viewed at wileyonlinelibrary.com]

they constitute one of the most important and continuous palaeontological record, at least for the last 220 Myr. Secondly, deriving from planktonic organisms, their taxa overall display extensive biogeographical distributions (cosmopolitan), which is an indisputable benefit if one tries to correlate the same stratigraphical level over wide areas. Moreover, they have rapidly evolved, which means that hundreds of appearances and disappearances can in principle be used either to subdivide or date rock strata. Finally yet importantly, the tiny size (from ca. 1 to 30 μm) is sometimes of great usefulness specifically when the amount of the sample to study is limited.

Biohorizons

The biohorizon represents the basis of biostratigraphy and is defined as a stratigraphical boundary or surface recognized in a succession of sedimentary rocks, where a change in the fossil content is observed. According to this definition, a biohorizon is any difference in the palaeontological content, which differentiates two packages of strata.

First (lowest) occurrences, last (highest) occurrences, and the beginning and end of acme/paracme intervals are the most commonly used biohorizons (Fig. 3). An important observation is that the first enter (appearance) or the exit (disappearance) of a taxon in/from the geological record is often preceded or followed by sporadic occurrences prior or after their common and continuous presence (core distribution). This is why the first or last continuous and relatively common occurrence of a taxon may represent a better biohorizon than the absolute first or last occurrence (Backman *et al.* 2012; Agnini *et al.* 2014). This is especially true for calcareous nannofossils for which quantitative abundance patterns have been often produced and allow for very detailed discussions on species stratigraphical distributions/ranges and biohorizon placements. Traditionally, micro-palaeontologists have used First Occurrence (FO) and Last Occurrence (LO) to
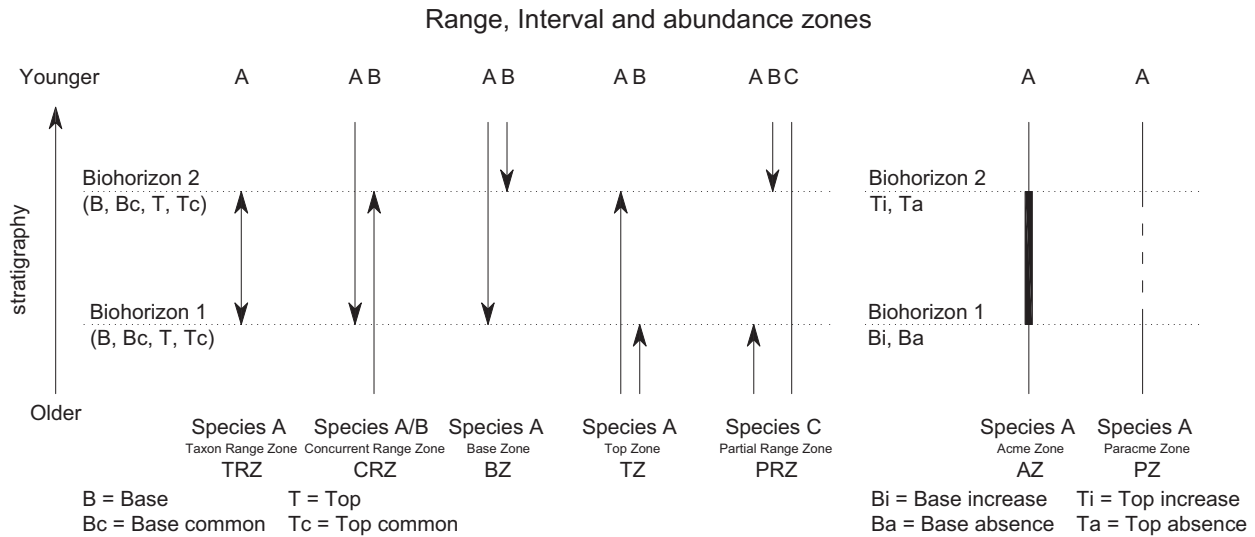
Range, Interval and abundance zones

Fig. 3. Range, Interval and Abundance Zones used in calcareous nannofossil biozonations. Redrawn and implemented after Wade *et al.* (2011) and Backman *et al.* (2012).

describe the appearance and disappearance of a taxon. More recently, other authors have suggested that the use of the terms 'first' and 'last' is incorrect being related to the concept of time, while biostratigraphy does not deal directly with time and better explains the stratigraphical relative relationships existing among rock strata (e.g. Aubry 2014). For this reason, it has been proposed to use Lowest Occurrence (LO) to indicate the lowest documented occurrence of a taxon in a specific section and Highest Occurrence to denote the highest documented occurrence in a specific section (ICS 2017). The presence of an acronym (LO) with two opposite meanings have created ambiguity in the literature because LO may refer to the Last Occurrence or the Lowest Occurrence. To avoid any possible misunderstanding and according to recently published Cenozoic calcareous nannofossil biozonations (Backman *et al.* 2012; Agnini *et al.* 2014), we prefer to use the concept of Base (B) and Top (T) to describe the stratigraphical lowest and highest occurrences of taxa. Additional biohorizons such as the Base common and continuous (Bc) and Top common and continuous (Tc) are proposed to be employed in those cases in which sporadic occurrences were observed before the Base or after the Top of a taxon (Fig. 3).

Counting methods and their reliability

In the last sixty years, calcareous nannofossil biostratigraphical data have been collected by different authors using different counting methodologies or abundance estimations and this large amount of data have enlightened the importance of the quality of

the biostratigraphical datums itself (Fig. 4). Semi-quantitative and relative abundance counts (Backman & Shackleton 1983; Rio *et al.* 1990) are able to depict very precisely stratigraphical ranges for any given species, and this, in turn, allows for a correct recognition and positioning of a biohorizon, this can improve the reproducibility of datums and provide higher degree of correlability over wide, regional to superregional areas. Range charts, which are based on qualitative abundance estimations of

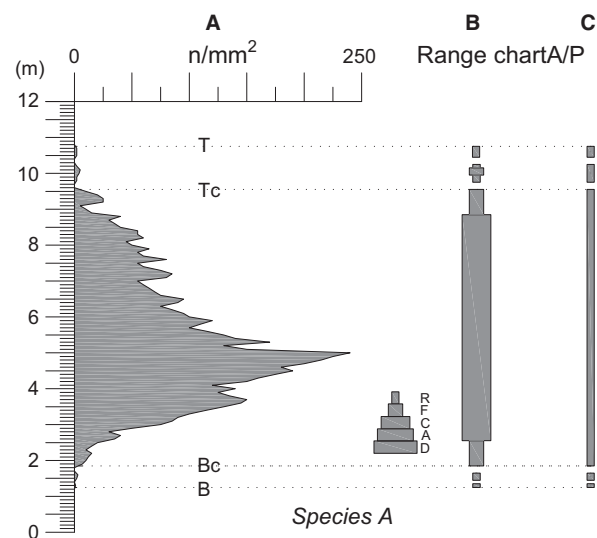*Fig. 4.* Idealized plots of *Species A* using the same initial data set. A, semi-quantitative abundance pattern (number of specimens/mm^2). B, range chart: D = dominant (>100 specimens per field of view – FOV); A = abundant (>10–100 specimens per FOV); C = common (>1–10 specimens per FOV); F = few (1 specimen per 1–10 FOV); R = rare (<1 specimen per 10 FOV). C, abundance/presence (A/P) plot.

taxa, represent a precious data set but the main pitfall is that they do not result in abundance distribution patterns but rather in oversimplified stratigraphical ranges. Finally, absence/presence data, which are still commonly used in applied fields, as for instance in oil companies, are a poor way to gain insight on abundance patterns despite their usefulness for getting quick biostratigraphy.

Last but not least, we would underline one more time the importance of acquiring high-resolution data. Regrettably, low-resolution data could be affected by profound distortions of the real data due to the serious loss of information. An example of the aliasing effect is reported in Figure 5, where low-resolution and high-resolution abundance patterns of an idealized *species A* are compared. The positions of the Base and Top of *species A* show remarkable differences that would eventually result in inconsistent biostratigraphical data. This is why collecting and analysing samples should guarantee the capture of all the necessary details. A good strategy should thus consider and try to find an acceptable compromise between the amount of time spent analysing the sample and the quality of the final biostratigraphical data.

The take-home message is that biostratigraphy is a quite complicate discipline and having a quantitative highly resolved data set is an essential requisite to collect good-quality data and discuss on the reliability and reproducibility of a biohorizon. For this reason, a shared standardization in terms of counting methods and resolution is auspicable.

Taxonomy

Besides the use of poor-quality data, possible inconsistency of biostratigraphical results is linked to ambiguous taxonomy. Biostratigraphy is strictly related to systematic as clear, unambiguous and shared taxonomic concepts for marker species are undoubtedly needed to obtain reliable biostratigraphical data.

As for taxonomy, calcareous nannofossils are *per se* quite a complicated group of microfossils because the planktonic algae producing them are characterized by heteromorphic life cycles, during which the organism secretes different coccoliths or organic scales or naked cells (e.g. Billard & Inouye 2004). Every single species could in fact produce different covers each of which is described as a different independent species when observed in the sediments/assemblages. It also happens that morphologically similar calcite plates do not belong to the same species but rather represents a cryptospecies (De Vargas *et al.* 2004). These evidences have been available from studies on living coccolithophores but likely
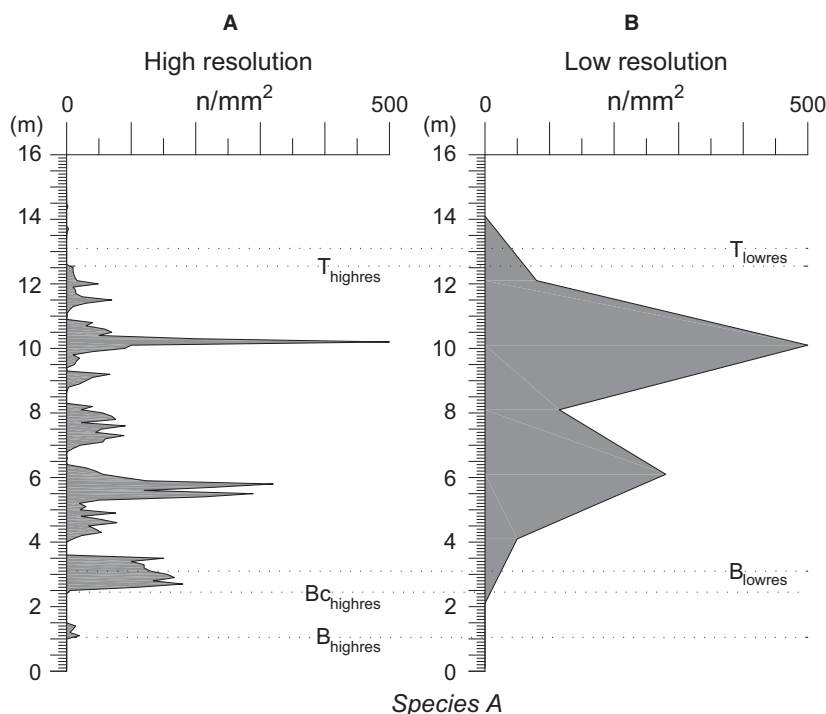

Fig. 5. High-resolution A, and low-resolution B, abundance patterns of *Species A*. The aliasing effect is particularly evident in the low-resolution data set where the signal is completely distorted by the loss of information. The sampling resolution is of 10 cm in A and of 100 cm in B. Biohorizons are positioned at the mid-points.

represent a commonality of fossil species. Parataxonomy rather than a natural classification system could thus describe nannofossils and be used to subdivide this group.

Some taxonomic issues are instead related to the formal description of a species, and this usually happens when the description of a new species is incomplete, missing fundamental morphological characters, or ambiguous. Moreover, biostratigraphers are sometimes rough palaeontologists as they disregard formal species descriptions or tend to use their own taxonomic concept without mentioning it. All these misconducts potentially result in an incorrect recognition of specimens and consequentially of the species range. However, though the innumerable complications, calcareous nannofossil taxonomy is overall in an acceptable state, although attention and caution should always be paid to avoid any possible misinterpretation and/or confusion.

Biozones

The fundamental units in biostratigraphy are the biozones. These are bodies of strata that are characterized on the basis of their contained fossils. The Base and Top of each biozone is defined by biohorizons, which include any change in features related to the content and distribution of fossils in strata (ICS 2017). This is a quite simple definition. Once you have defined the Base and Top biohorizons, you have defined the biozone. Any variation with respect to the formal definition of a specific biozone, in a biostratigraphical scheme, should be officially emended, otherwise you would obtain the undesirable effect of having a biozone represented by different bodies of strata in the same section. In principle, any change in the calcareous nannofossil content could be used to subdivide rock strata. Anyway, the most widely used biohorizons are first occurrences, last occurrences and changes in abundance of taxa. A number of range, interval and abundance zones can be defined using Base (B, Bc, Bi and Ba) and Top (T, Tc, Ti and Ta) of a particular taxon or a combination of two taxa (Fig. 3).

Biozonations

Biohorizons and, hence, biozones are ordered in stratigraphical order and eventually result in a biostratigraphical zonation/scheme (biozonation). Since Bramlette & Riedel (1954) published their pivotal work on the potentiality of calcareous nannofossils as biostratigraphical tools, decades passed before comprehensive syntheses became available to the community (e.g. Martini 1971; Sissingh 1977; Roth 1978; Okada & Bukry 1980). A prodigious effort has been made in collecting an enormous data set based on marine successions cropping out on land or located on the bottom of the seafloor. This large amount of information provides a much-refined biostratigraphical framework, which has been used to produce more reliable biozonations for the Cenozoic and Mesozoic.

Biochronology

Biostratigraphy and biochronology are tightly connected; the former focuses on relative age dating of rock strata/sediments based on biohorizons, and the latter provides age estimations of these biohorizons. The numerical ages estimated for each biohorizon depend on the reliability of the biohorizon *per se* but also on the time-scale adopted to calibrate the bioevent. An astronomically Tuned Neogene Time Scale (ATNTS) has been presented by Lourens *et al.* (2004), and minor changes further refined the ATNTS2012 (Hilgen *et al.* 2012). The Astronomical time-scale has been extended to the Oligocene (Pälike *et al.* 2006) and reconstructed for the Palaeocene–Early Eocene (Westerhold *et al.* 2007, 2008; Hilgen *et al.* 2010; Vandenberghe *et al.* 2012). Recent studies have tried to close the Eocene gap so as to eventually develop a continuous astronomically calibrated geological time-scale for the entire Cenozoic Era and potentially open the way for extending the ATS into the Mesozoic (T. Westerhold, U. Röhl, T. Frederichs, C. Agnini, I. Raffi, J.C. Zachos & R.H. Wilkens, in review). In the next years, the numerical ages estimated for calcareous nannofossil biohorizons will be inevitably subject to change just as a consequence of the instability of the early Palaeogene time-scale.

Overview of Cenozoic calcareous nannofossil biostratigraphy

Cenozoic calcareous nannofossils are considered the most powerful biostratigraphical tool for correlations over wide areas in the marine realm. The main input to the development and refinement of biozonations based on calcareous nannofossil biohorizons has been and is coupled to the tremendous amount of data retrieved and available from sediments recovered by Ocean Drilling Programs (i.e. DSDP, ODP and IODP). However, sedimentary successions cropping out on land are also crucial to create a complete and comprehensive data set, which includes data from different biogeographical domains and depositional settings.

A first Cenozoic biozonation was published by Martini (1971), and defined as 'standard' by the author. This biostratigraphical scheme used an alphanumerical notation: NP (Nannoplankton Palaeogene) for the Palaeogene with a total number of 25 biozones and NN (Nannoplankton Neogene) for the Neogene which consists of 21 biozones. In the same years, D. Bukry was studying calcareous nannofossil assemblages in kilometres of DSDP core sediments which allowed him constructing an alternative Cenozoic zonation (Bukry 1973, 1975), with biozones afterwards formally code-numbered as CP (Coccolith Palaeogene), and CN (Coccolith Neogene), zones by Okada & Bukry (1980). An important synthesis of nannofossil biostratigraphy was published in the 1980s by Perch-Nielsen (1985) and precious contributions came also from Young (1998) and Hine & Weaver (1998), who synthesized the nannofossil biostratigraphy for the Neogene and the Quaternary, respectively.

Regional biozonations have also been played an important role in defining similarities and differences between areas. For the Palaeogene, high latitudes and Mediterranean biozonations have been proposed (Catanzariti *et al.* 1997; Varol 1998; Fornaciari *et al.* 2010) and important improvements have been achieved for Neogene calcareous biostratigraphy for the Mediterranean region (Fornaciari & Rio 1996; Fornaciari *et al.* 1996).

More recently, two new biozonations valid for the low-middle latitudes have been published for the Neogene–Quaternary and the Palaeogene (Backman *et al.* 2012; Agnini *et al.* 2014). The main aim of these works was to re-evaluate the biozonations of Martini (1971) and Okada & Bukry (1980) through the integration of those biohorizons that have proved to be reliable and substituting problematic (unreliable) biohorizons with new ones. The code system used is CN (Calcareous Nannofossils) followed by letters indicating the Epoch (P – Palaeocene, E – Eocene, O – Oligocene; M – Miocene and PL – Pliocene/Pleistocene; Figs 6, 7).

Cenozoic calcareous nannofossil biochronology

From the publication of the first Cenozoic time-scale (Funnell 1964), biostratigraphy has served as a framework to check chronological data (e.g. magnetostratigraphy, cyclostratigraphy and radiometric dating). Subsequently, the biostratigraphical data have been calibrated to chronological data and provide numerical ages for the biohorizons.

Since then, Cenozoic calcareous nannofossil biochronology has amazingly developed (Berggren *et al.* 1985, 1995; Gradstein *et al.* 2012) and reviews of astro-biochronological calibration of Neogene and Quaternary nannofossil datum events have been provided (Raffi *et al.* 2006; Backman *et al.* 2012). Going back through the Palaeogene, the time-scale is to be considered as a work in progress and, even if biostratigraphical biohorizons are sufficiently tested and integrated with magneto- and astro-cyclostratigraphy, the numerical ages now available will be subjected to changes anytime the time-scale would change (see Agnini *et al.* 2014, for detailed discussion).

The role of calcareous nannofossils in Cenozoic chronostratigraphy

To completely understand the all-important role that calcareous nannofossils play in the Cenozoic chronostratigraphy, we will discuss the biohorizons that possibly denote the nine Palaeogene, the eight Neogene and the five Quaternary stages, used as correlation biohorizon and/or complementary criterion for recognition of chronostratigraphical GSSPs. Every Cenozoic chronostratigraphical unit has been or will be defined by a specific boundary level in a reference section, the Global Stratotype Standard-section and Point (GSSP), based on different marker events of optimal correlation potential. Except for very few exceptions (e.g. base of Holocene), calcareous nannofossils provide clear biohorizons that denote or approximate the position of chronostratigraphical boundaries in the Cenozoic. Starting from the base of the Palaeogene, we will give a complete overview of these biohorizons. In this discussion, ages of the chronostratigraphical units as well as ages of the considered biohorizons are those reported in the recently published Cenozoic calcareous nannofossil biozonations or in the Geological Time Scale 2012 (Backman *et al.* 2012; GTS12; Agnini *et al.* 2014). We are aware that this may cause some inconsistencies with some data published in literature, especially for the Palaeogene interval. However, the Palaeogene time-scale is in continuous change and both the numbers reported in this work and the GTS12 could not be valid anymore in the next future. The idea here is just to give a comprehensive summary of the calcareous nannofossil biohorizons in relation to the position and definition of chronostratigraphical units and, even more importantly, their relative ranking and spacing.

The Palaeogene Stages

The GSSP of Danian Stage was ratified in 1991 (Molina *et al.* 2006) at the El Kef section (Tunisia); this boundary coincides with the base of the
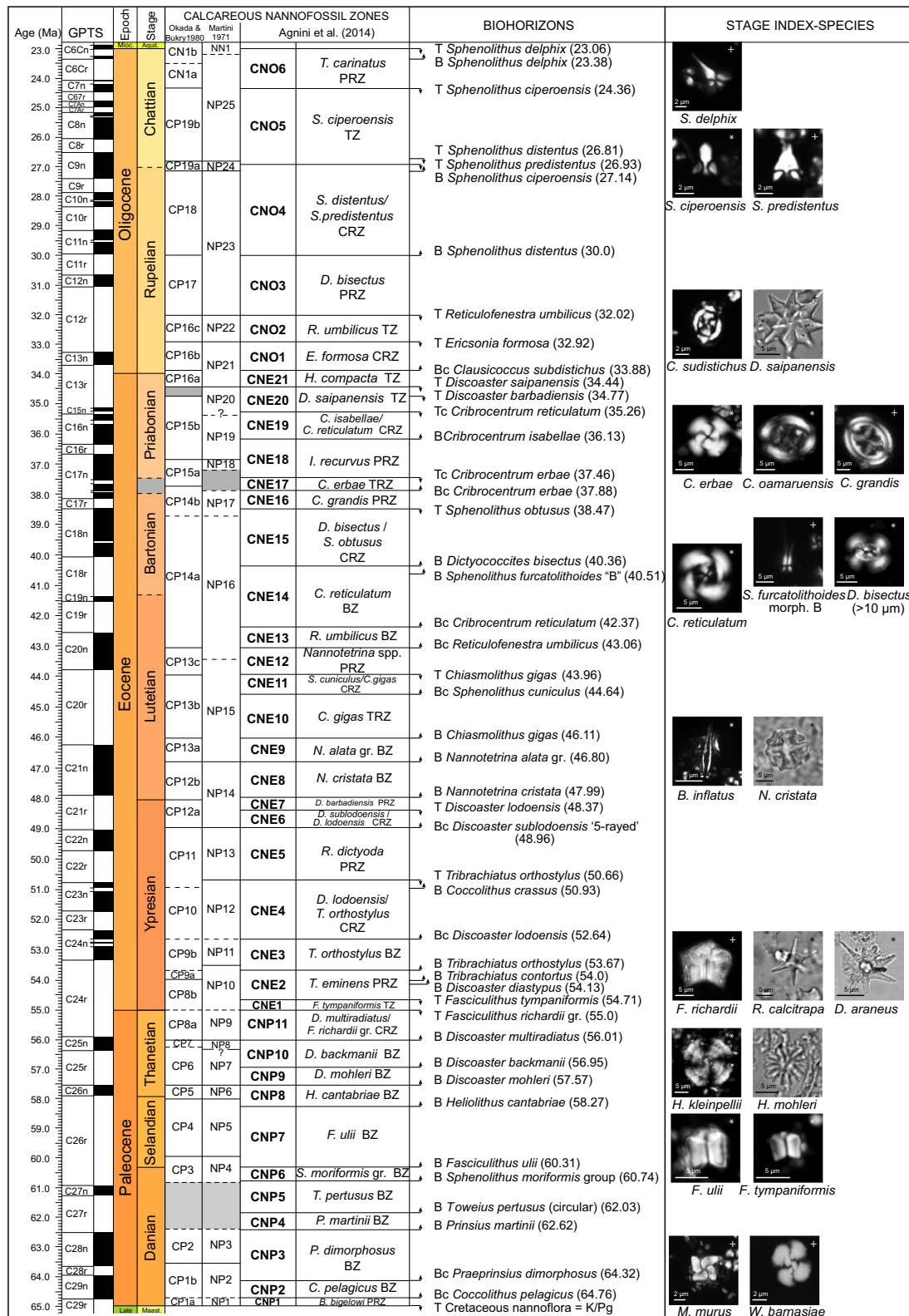
Fig. 6. Palaeogene calcareous nannofossil biozonations (modified from Agnini *et al.* 2014): CP (Okada & Bukry 1980), NP (Martini 1971), CN (Agnini *et al.* 2014). The Geomagnetic Polarity Time Scale (GPTS) is after Pälike *et al.* (2006), from the top of Chron C13r (33.705 Ma), to the base of Chron C19n (41.510 Ma) in the Middle Eocene, and after Cande & Kent (1995; CK95), from the top of Chron C20n (42.356 Ma) downward. Grey boxes and dashed lines show the uncertainty in defining chronostratigraphic and biozone boundaries. CN biochronology from Agnini *et al.* (2014). On the right, images of CN stage index-species are taken from literature (see Appendix S1 for details). * = Base; + = Top; x = crossover. [Colour figure can be viewed at wileyonlinelibrary.com]

Nannofossil biozonation chart (Fig. 7)

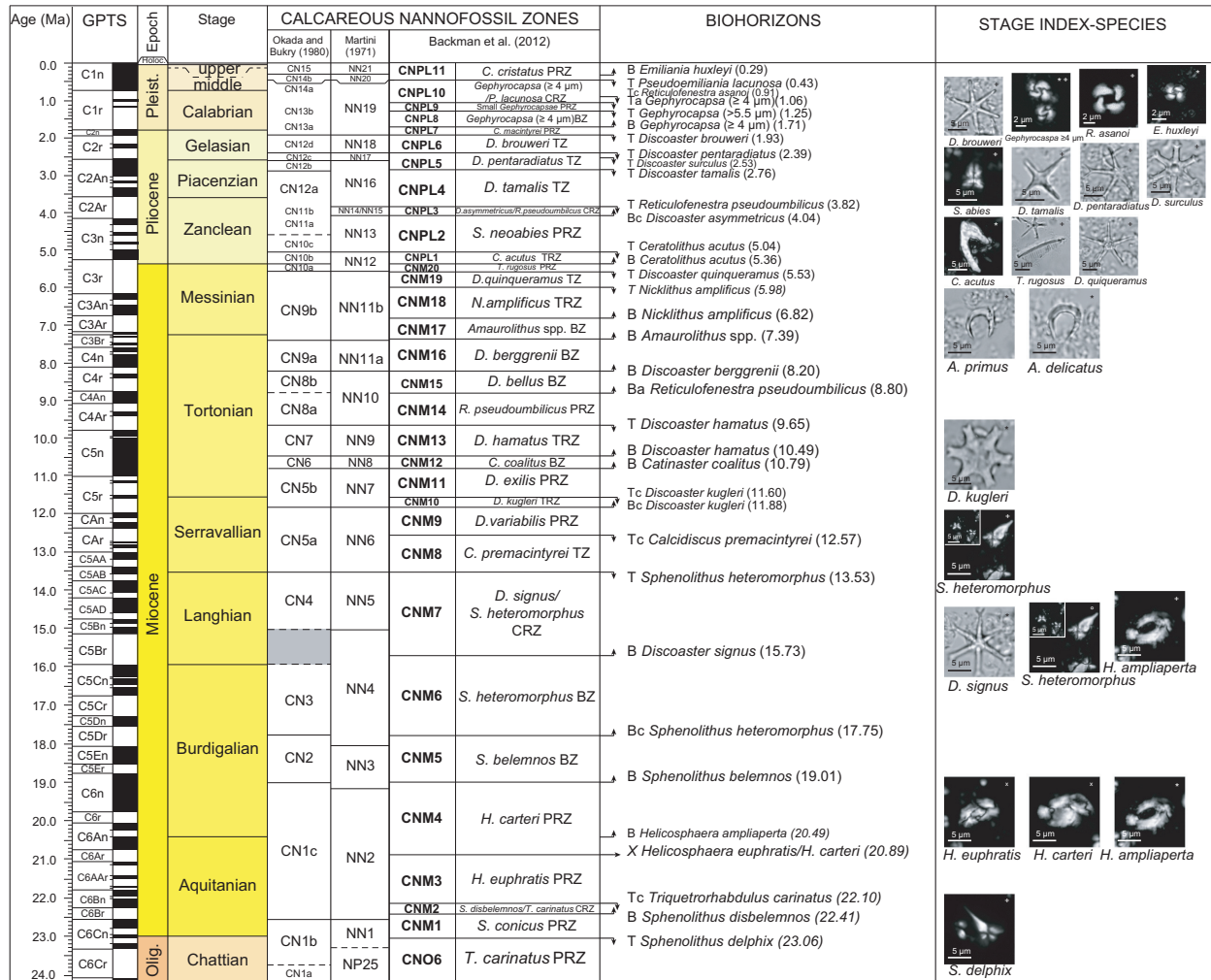| Age (Ma)	GPTS	Epoch	Stage	Okada and Bukry (1980)	Martini (1971)	Backman et al. (2012)	Biohorizons
0.0	C1n	Pleist. / Holoc.	upper / middle	CN15 / CN14b / CN14a	NN21 / NN20	CNPL11 *C. cristatus* PRZ	B *Emiliania huxleyi* (0.29)
1.0	C1r		Calabrian	CN13b / CN13a	NN19	CNPL10 *Gephyrocapsa* (≥4 µm) / *P. lacunosa* CRZ; CNPL9 Small *Gephyrocapsa* PRZ; CNPL8 *Gephyrocapsa* (≥4 µm) BZ; CNPL7 *C. macintyrei* PRZ	T *Pseudoemiliania lacunosa* (0.43); Tc *Reticulofenestra asanoi* (0.91); Ta *Gephyrocapsa* (>4 µm) (1.06); T *Gephyrocapsa* (>5.5 µm) (1.25); B *Gephyrocapsa* (≥4 µm) (1.71)
2.0	C2r	Pliocene	Gelasian	CN12d	NN18	CNPL6 *D. brouweri* TZ	T *Discoaster brouweri* (1.93)
3.0	C2An		Piacenzian	CN12c / CN12b / CN12a	NN17 / NN16	CNPL5 *D. pentaradiatus* TZ; CNPL4 *D. tamalis* TZ	T *Discoaster pentaradiatus* (2.39); T *Discoaster surculus* (2.53); T *Discoaster tamalis* (2.76)
4.0	C2Ar / C3n		Zanclean	CN11b / CN11a / CN10c	NN14/NN15 / NN13	CNPL3 *D. asymmetricus/R. pseudoumbilicus* CRZ; CNPL2 *S. neoabies* PRZ	T *Reticulofenestra pseudoumbilicus* (3.82); Bc *Discoaster asymmetricus* (4.04)
5.0	C3n			CN10b / CN10a	NN12	CNPL1 *C. acutus* TRZ; CNM20 *T. rugosus* PRZ	T *Ceratolithus acutus* (5.04); B *Ceratolithus acutus* (5.36)
6.0	C3r	Miocene	Messinian	CN9b	NN11b	CNM19 *D. quinqueramus* TZ; CNM18 *N. amplificus* TRZ	T *Discoaster quinqueramus* (5.53); T *Nicklithus amplificus* (5.98); B *Nicklithus amplificus* (6.82)
7.0	C3An / C3Ar / C3Br					CNM17 *Amaurolithus* spp. BZ	B *Amaurolithus* spp. (7.39)
8.0	C4n / C4r		Tortonian	CN9a / CN8b / CN8a	NN11a / NN10	CNM16 *D. berggrenii* BZ; CNM15 *D. bellus* BZ; CNM14 *R. pseudoumbilicus* PRZ	B *Discoaster berggrenii* (8.20); Ba *Reticulofenestra pseudoumbilicus* (8.80)
9.0	C4An / C4Ar						
10.0	C5n			CN7	NN9	CNM13 *D. hamatus* TRZ	T *Discoaster hamatus* (9.65); B *Discoaster hamatus* (10.49)
11.0	C5r			CN6 / CN5b	NN8 / NN7	CNM12 *C. coalitus* BZ; CNM11 *D. exilis* PRZ; CNM10 *D. kugleri* TRZ	B *Catinaster coalitus* (10.79); Tc *Discoaster kugleri* (11.60); Bc *Discoaster kugleri* (11.88)
12.0	C5An		Serravallian	CN5a	NN6	CNM9 *D. variabilis* PRZ; CNM8 *C. premacintyrei* TZ	Tc *Calcidiscus premacintyrei* (12.57)
13.0	C5Ar / C5AA / C5AB / C5AC / C5AD						
14.0	C5Bn		Langhian	CN4	NN5	CNM7 *D. signus / S. heteromorphus* CRZ	T *Sphenolithus heteromorphus* (13.53)
15.0	C5Br						B *Discoaster signus* (15.73)
16.0	C5Cn		Burdigalian	CN3	NN4	CNM6 *S. heteromorphus* BZ	Bc *Sphenolithus heteromorphus* (17.75)
17.0	C5Cr / C5Dn / C5Dr						
18.0	C5En / C5Er			CN2	NN3	CNM5 *S. belemnos* BZ	B *Sphenolithus belemnos* (19.01)
19.0	C6n						
20.0	C6r / C6An		Aquitanian	CN1c	NN2	CNM4 *H. carteri* PRZ	B *Helicosphaera ampliaperta* (20.49); X *Helicosphaera euphratis/H. carteri* (20.89)
21.0	C6Ar / C6AAr					CNM3 *H. euphratis* PRZ	Tc *Triquetrorhabdulus carinatus* (22.10)
22.0	C6Bn / C6Br					CNM2 *S. disbelemnos/T. carinatus* CRZ; CNM1 *S. conicus* PRZ	B *Sphenolithus disbelemnos* (22.41)
23.0	C6Cn	Olig.	Chattian	CN1b / CN1a	NN1 / NP25	CNO6 *T. carinatus* PRZ	T *Sphenolithus delphix* (23.06)
24.0	C6Cr						

Fig. 7. Neogene calcareous nannofossil biozonation (modified from Backman *et al.* 2012): CP (Okada & Bukry 1980), NP (Martini 1971), CN (Backman *et al.* 2012). The Geomagnetic Polarity Time Scale (GPTS) is from Lourens *et al.* (2004). Grey box and dashed lines show the uncertainty in defining biozone boundaries. Biochronology is after Backman *et al.* (2012). On the right, images of CN stage index-species are taken from literature (see Appendix S1 for details). * = Base; + = Top; x = crossover; ° = acme end. [Colour figure can be viewed at wileyonlinelibrary.com]

Palaeogene system (Palaeocene series) and was defined at the base of a dark clay layer that documents the last among the big five mass extinctions. Calcareous nannofossils suffered a profound extinction event followed by a relatively slow recovery. The disappearance of Cretaceous nannoflora (e.g. the genera *Watznaueria* and *Micula*) characterizes the base of the Cenozoic and could serve to denote the base of the Danian Stage (Fig. 6).

The GSSP of Selandian Stage, the second stage in the Palaeocene series, was ratified in 2008 (Schmitz *et al.* 2011) at the Zumaia Section, in coincidence of the base of the Itzurun Formation. Two calcareous nannofossil biohorizons are in fact useful to approximate the base of the Selandian, the Base of *Fasciculithus/Lithoptychius ulii* (Aubry *et al.* 2011), which also coincides with the so-called second radiation of

fasciculiths (*sensu* Steurbaut & Sztrákos 2008; Monechi *et al.* 2013), and the Base of *Fasciculithus tympaniformis* (Fig. 6). The first biohorizon predates the boundary by 21 kyr while the second postdates the base of the Selandian by ca. 80–100 kyr (Bernaola *et al.* 2009).

Similarly to the Selandian GSSP, also the GSSP of the Thanetian Stage was ratified at the Zumaia Section (Schmitz *et al.* 2011). This boundary is positioned at the base of a clay interval within the Itzurun Formation and coincides with the base of Chron C26n (Dinarès-Turell *et al.* 2007). The evolution of the genus *Heliolithus* and specifically, the appearance (Base) of *Heliolithus kleinpelli* was reported to consistently occur in the uppermost part of Chron C26r (Backman 1986; Bralower *et al.* 2002; Agnini *et al.* 2007, 2014), in correspondence with

the Early Late Palaeocene Event (ELPE; Westerhold *et al.* 2008) and thus potentially represents a good approximation of the base of the Thanetian (Fig. 6). However, at Zumaia, this biohorizon is positioned in a significantly lower stratigraphical position with respect to all the other data available from literature. One possible explanation for this inconsistency could be linked to taxonomic uncertainty in *H. kleinpellii* recognition, because of the presence of intermediate forms between *Heliolithus cantabriae* and *H. kleinpelli*. Hence, although this biohorizon is considered reliable thus suggesting its use to approximate the Thanetian GSSP, the alternative biohorizon proposed is the Base of *Discoaster mohleri* (Fig. 6). This biohorizon is found to occur at the very base of Chron C25r, that is ca. 350–400 kyr after the boundary.

The GSSP of the Ypresian Stage was ratified in 2003 (Aubry *et al.* 2007) and is located at the base of 'Bed 1 of the Esna Formation' at the Dababyia Quarry section, in coincidence with the onset of the negative carbon isotope excursion (CIE) marking the base of the Palaeocene–Eocene Thermal Maximum (PETM). Recently, Khozyem *et al.* (2014) have evidenced potentially serious limiting factors of this GSSP and above all the presence of an erosion surface at the base of the Palaeocene–Eocene boundary. Nonetheless, the CIE remains the primary criterion to denote the base of the Ypresian. Related to this extreme warming episode and the perturbation in the carbon cycle are profound modifications in the terrestrial and marine biota (e.g. Sluijs *et al.* 2007). Among these changes, several events are observed in the calcareous nannofossil assemblages such as the lowest occurrence (Base) of *Rhomboaster* spp. and *Discoaster araneus*, and the extinction (Top) of the large *Fasciculithus richardii* group (Fig. 6). These biohorizons all occur in coincidence, or after few kyrs, from this boundary thus allowing for a precise recognition of the base of the Eocene series.

The GSSP of the Lutetian Stage was ratified in 2011 (Molina *et al.* 2011) and placed in a dark marly level (positioned at 167.85 m) in the Gorrondatxe section (Spain) in coincidence with the Base of the calcareous nannofossil *Blackites inflatus* (Fig. 6), ca. 800 kyr above the base of Chron C21r. The final decision on the proposal of using the appearance of *B. inflatus* to identify the base of the Lutetian was eventually taken because this biohorizon is the closest to the position of Lutetian historical stratotype (Molina *et al.* 2011). However, this taxon is sometimes absent in frankly open ocean sections (see T. Westerhold, U. Röhl, T. Frederichs, C. Agnini, I. Raffi, J.C. Zachos & R.H. Wilkens, in review). Moreover, the comparison of multiple ODP-IODP

Sites drilled in the equatorial Atlantic (Site 1258; T. Westerhold, U. Röhl, T. Frederichs, C. Agnini, I. Raffi, J.C. Zachos & R.H. Wilkens, in review), SE Atlantic (1262–1267; T. Westerhold, U. Röhl, T. Frederichs, C. Agnini, I. Raffi, J.C. Zachos & R.H. Wilkens, in review) and NW Atlantic (U1409–U1410, Norris *et al.* 2014), and the Possagno section (Italy; Agnini *et al.* 2006) have evidenced that Base of *B. inflatus* consistently lies at Chron C21r/C21n transition and it is always found to slightly predate the Base of *Nannotetrina cristata* (= Base of *Nannotetrina* spp.) (Agnini *et al.* 2014; Norris *et al.* 2014; T. Westerhold, U. Röhl, T. Frederichs, C. Agnini, I. Raffi, J.C. Zachos & R.H. Wilkens, in review). Although the ranking and spacing observed for these two biohorizons in the aforementioned successions are perfectly in agreement with data from the GSSP section, the position with respect to magnetostratigraphy is instead significantly different, suggesting that some of the data available from the Gorrondatxe section may need a thoughtful revision. Nevertheless, at least at present, this biohorizon is used to define the Lutetian GSSP.

The GSSP of the Bartonian Stage has not been formally defined yet, but a magnetostratigraphical study performed in the Barton Clay of Alum Bay (England), where the Bartonian was first described, indicates that magnetochrons C18n, C18r and possibly C19n have been recognized with the base of the Bartonian lying within Chron C18r (ISPS 2017). In 2010, Jovane *et al.* proposed the base of Chron C18r as primary criterion for defining the base of the Bartonian Stage. However, with the exception of magnetostratigraphy, other correlation tools such as calcareous plankton biohorizons are not available in this time interval. The Top of planktonic foraminifer *Gumbelitrioides nuttalli*, which serves to define the base of Zone E11 (Berggren & Pearson 2005) is found, though with an uneven distribution, up to Zone E14, a datum that evidences for the low reliability of this bioevent. The Base of *Cribrocentrum reticulatum* is now used to mark the base of Zone CNE14 (Agnini *et al.* 2014) and, with respect to magnetostratigraphy, is found to occur in the lower mid-part of Chron C19r (Fig. 6), well before the position reported in Jovane *et al.* (2007) that, instead, is rather correlative with an increase in abundance observed in the Tethyan realm (Fornaciari *et al.* 2010; Tori & Monechi 2013; Agnini *et al.* 2014). Although any discussion on reliability of criteria for defining the Bartonian GSSP is beyond the scope of this work, on the basis of previous arguments, we would like to suggest to take into consideration the possibility to define it in correspondence with the Middle Eocene Climatic Optimum (MECO,

at ca. 40.5 Ma; Bohaty *et al.* 2009) during which several biotic and geochemical markers are globally recognized. Specifically, among calcareous nannofossil biohorizons, the Top of *Sphenolithus furcatolithoides* morphotype B and the Base of *Dictyococcites bisectus* (>10 *μ*m in size = *Reticulofenestra stavensis*) could be used to approximate the boundary (Agnini *et al.* 2014).

The GSSP of the Priabonian Stage has not been defined yet, but the leading candidate GSSP section has been proposed in Northern Italy, at Alano di Piave (Veneto). In the Alano section, the 'Tiziano Bed', a crystal tuff layer, has been proposed to define the boundary (Agnini *et al.* 2011). Close to this stratigraphical level, nannofossil biohorizons Base of *Chiasmolithus oamaruensis*, Top of *Chiasmolithus grandis* and Base common (Bc) of *Cribrocentrum erbae* have been detected (Agnini *et al.* 2011). The first two biohorizons have a low degree of reliability, especially the appearance of *C. oamaruensis*, which however has been traditionally used to recognize the base of the Priabonian (e.g. Berggren *et al.* 1985, 1995). Thus, the Bc of *C. erbae* could be used as a primary criterion, although the real strength of this proposal is related to the occurrence of other good stratigraphical markers, as the extinction of planktonic foraminifer *Morozovelloides crassatus* (Wade *et al.* 2012), or the base of Subchrons C17n.3n and C17n.2n (Agnini *et al.* 2011; GTS12), that are in fact observed close to the proposed boundary (Fig. 6).

The GSSP of the Rupelian Stage was ratified in 1992 (Premoli Silva & Jenkins 1993) and defined in the Massignano section (Italy) at the base of a greenish grey marl bed in which both the planktonic foraminifera *Hantkenina* and *Cribrohantkenina* become extinct, at an age estimation of 33.9 Ma. In terms of calcareous nannofossil biostratigraphy, the boundary lies within Zone NP21 and Subzone CP16a (Martini 1971; Okada & Bukry 1980), whose base are marked by the Top of the *Discoaster saipanensis* and *Discoaster barbadiensis*, respectively. These two biohorizons are known to occur tightly spaced, predating the Eocene–Oligocene boundary by ca. 500–600 kyr. Recently, the Bc of *Clausicoccus subdistichus* has been proposed to define the base of Zone CN01 (Agnini *et al.* 2014) with an age estimation of 33.9 Ma. This biohorizon represents an increase in abundance of the nominate taxon and could in fact be used to better approximate the base of the Rupelian. Although the precise recognition of Bc of *C. subdistichus* is possible only if a quantitative counting approach is adopted, nonetheless, this biohorizon could represent a promising criterion, to be further checked (Fig. 6).

The GSSP of the Chattian has been ratified by IUGS in 2016 and defined in the Monte Cagnero section, Central Italy (Coccioni *et al.* 2008, in press). The Chattian GSSP is located at metre level 197 in coincidence with the highest common occurrence (HCO or Tc) of planktonic foraminifer *Chiloguembelina cubensis* (= base of Zone O5; Berggren & Pearson 2005), in the lower part of Chron C9n. This biohorizon has been chosen as the primary marker for defining this chronostratigraphical unit. In terms of calcareous nannofossil biostratigraphy, the Top of *Sphenolithus predistentus*, which marks the base of Zone CNO5 (Agnini *et al.* 2014), lies just below the HCO of *C. cubensis* and thus predates the base of the Chattian by less than 100 kyr if the astrocyclostratigraphical model of Coccioni *et al.* (in press) is adopted.

The Neogene Stages

The GSSP of the Aquitanian Stage was ratified in 1996 in Lemme-Carrioso Section (Italy) (Steininger *et al.* 1997), at 35 m from the top of the section that coincides with the base of Subchron C6cn.2n (23.03 Ma; GTS12). The authors suggested that, in terms of calcareous nannofossils, the best approximation of the Aquitanian can be achieved using the Top of *Sphenolithus ciperoensis*, which marks both the base of Zone NN1 (Martini 1971) and Subzone CN1a (Okada & Bukry 1980). More recently, Shackleton *et al.* (2000) based on data from ODP Sites 926 and 929, and DSDP Site 522 (Raffi 1999; Shackleton *et al.* 1999), stated that the biostratigraphical marker more relevant for correlating the Oligocene–Miocene boundary is the short-range nannofossil *Sphenolithus delphix*. Since then, the Top of *S. delphix* has been successfully used to approximate the base of the Aquitanian Stage in carbonate-rich marine sediments (Fig. 7).

The GSSPs of the Burdigalian and the Langhian stages have not been defined yet. The definition of Burdigalian GSSP is a recurrent problem. The option to have the Burdigalian GSSP defined in an astronomically tuned deep marine section seems to make this problem very difficult to be solved. Till now, no good candidate sections are available, and the option to formally designate this boundary in an ODP core will be seriously considered and discussed within the Working group designated by the Subcommission on Neogene Stratigraphy (SNS), and within the SNS itself. The calcareous nannofossil biohorizons usually considered for approximating the base of Burdigalian are Base of *Helicosphaera ampliaperta* (20.43 Ma; Backman *et al.* 2012) or, secondarily, the crossover in abundance between

Helicosphaera euphratis and *Helicosphaera carteri* (20.89 Ma; Backman *et al.* 2012; Fig. 7).

As regards the Langhian Stage, the Working Group of the SNS have identified three potential GSSP candidates: the Mediterranean La Vedova (Italy) and St. Peter's Pool (Malta) sections and the Atlantic Site DSDP 608. The primary proposed correlation criteria are the Base of planktonic foraminifer *Praeorbulina glomerosa curva*, which falls at the base of Chron C5Bn.2n (15.16 Ma; GTS12; Iaccarino *et al.* 2011), and the top of magnetic polarity chronozone C5Cn.1n (15.97 Ma; GTS12; ICS 2017). Moreover, previous results from the Langhian historical Stratotype section (Fornaciari *et al.* 1997) suggested that the *Preorbulina* datum (i.e. *Praeorbulina sicana* auctorum), the top Chron C5Cn.1n and the Tc of *H. ampliaperta* are closely spaced and could thus serve to approximate the base of the Langhian Stage. However, a detailed review of the *Globigerinoides-Praeorbulina* evolutionary lineage has evidenced that the *Praeorbulina* datum (= Base of *Praeorbulina* spp.) should be moved upward where the first species belonging to genus *Praeorbulina* (namely *P. glomerosa curva sensu* Turco *et al.* 2011) appears. Based on this reasoning, the critical interval where the Langhian Stage should be defined spans from the Top Chron C5Cn.1n and the base of Chron C5Bn.2n (= Base of *P. glomerosa curva*). Depending on the final decision for the position of this chronostratigraphical unit, the nannofossil biohorizons Base of *Discoaster signus* (15.73 Ma; Backman *et al.* 2012) or Top common of *H. ampliaperta* (Di Stefano *et al.* 2011; 14;.86 Ma in Backman *et al.* 2012) could be used to approximate the Langhian GSSP (Fig. 7).

The GSSP of the Serravallian Stage was ratified in 2007 in the Ras il Pellegrin section (Malta) (Hilgen *et al.* 2009) at the base of the Blue Clay Formation, in correspondence with the oxygen isotope event Mi-3b. These authors emphasized the importance of combining geochemical and bio-magnetostratigraphical data to recognize the exact position of the boundary in open ocean settings. In terms of calcareous nannofossil biostratigraphy, the base of the Serravallian Stage is approximated by the Top of *Sphenolithus heteromorphus* and, secondarily, by the Tc of *Cyclicargolithus floridanus* (Fig. 7). However, it is worth noting that the Top of *S. heteromorphus* shows a slight diachrony if Atlantic Ceara Rise and Mediterranean data are compared with younger ages estimated for open ocean settings (13.523 Ma; Backman & Raffi 1997; Backman *et al.* 2012) with respect to the Mediterranean area (13.654 Ma; Abels *et al.* 2005).

The GSSP of the Tortonian Stage was ratified in 2003 in the Monte dei Corvi Beach section (Italy) (Hilgen *et al.* 2005) at the mid-point of the sapropel of small-scale sedimentary cycle 76 (11.63 Ma; GTS12), close to the Top of common calcareous nannofossil *Discoaster kugleri*, the Top of planktonic foraminifer *Globigerinoides subquadratus* and the base of short normal Subchron C5r.2n (at 11.67 Ma; GST2012). The Tortonian GSSP coincides with oxygen isotope event Mi-5 of Miller *et al.* (1991). This GSSP is clearly defined and we confirm that the best calcareous nannofossil biohorizon to approximate the Serravallian/Tortonian boundary is the Tc of *Discoaster kugleri* (11.60 Ma, Backman *et al.* 2012; Fig. 7).

The GSSP of the Messinian Stage was ratified in 2000 in the Oued Akrech section (Morocco) (Hilgen *et al.* 2000) at the base of the reddish layer number 15. According to the authors, this point is closely coincident with the appearance of planktonic foraminifer *Globorotalia miotumida* group and the calcareous nannofossil *Amaurolithus delicatus*, and is associated with Subchron C3Br.1r (7.25 Ma; GTS12). More recently, Raffi *et al.* (2006) have evidenced that the appearance of *Amaurolithus* spp. (= Base of *Amaurolithus primus*; Raffi *et al.* 1998) is a more reliable datum than the Base of *A. delicatus* that seems to be restricted in the Mediterranean area. For this reason, the appearance of the horseshoe-shaped *Amaurolithus/Nicklithus/Ceratolithus* evolutionary lineage (= Base of *A. primus*), which marks the base of Zone CNM17 (7.39 Ma; Backman *et al.* 2012), is the preferred calcareous nannofossil biohorizon to approximate the Tortonian–Messinian boundary.

The GSSP of the Zanclean Stage, and the Pliocene Series, was ratified in 2000 in the Eraclea Minoa section (Italy) (Van Couvering *et al.* 2000) at the base of the Trubi Formation in correspondence with the insolation cycle 510 (5.33 Ma; GTS 12) and predating by only 96 kyr the onset of the Thvera magnetic event (C3n.4n). Three are the calcareous nannofossil biohorizons that serve to denote the Messinian/Zanclean boundary: the closest to the boundary, and virtually coincident to it, is the Base of *Ceratolithus acutus* (5.36 Ma; Backman *et al.* 2012), followed just above by the Top of *Triquetrorhabdulus rugosus* (5.23 Ma; Backman *et al.* 2012), which shows a very consistent calibration both in the Mediterranean (Di Stefano *et al.* 1996; Castradori 1998) and equatorial Atlantic areas (Backman & Raffi 1997). Below the boundary, the Top of *Discoaster quinqueramus* that occurs in open ocean sections at 5.53 Ma (Backman *et al.* 2012) serves to approximate the Messinian/Zanclean boundary but is useless in the Mediterranean area because of the Salinity Crisis (Fig. 7) interval. The presence of three highly reliable

biohorizons restricted in ca. 300 kyr guarantees for a very precise recognition of the Zanclean GSSP using calcareous nannofossil biostratigraphy.

The GSSP of the Piacenzian Stage was ratified in 1997 in the Punta Piccola section (Sicily, Italy) (Castradori *et al.* 1998) at the base of the beige marl bed of small-scale carbonate cycle 77, in correspondence with the precessional cycle 347 (from the present) at 3.6 Ma (GTS12). The Gilbert/Gauss (C2Ar/C2An.3n) magnetic reversal is documented immediately above the GSSP (at 3.596 Ma; GTS12) and is considered the primary tool to denote the base of the Piacenzian. However, the highest occurrence of nannofossil *Sphenolithus* spp. (= Top *Sphenolithus abies*, at 3.61 Ma; Backman *et al.* 2012) is considered a reliable tool for wide correlations, which allows for a precise positioning of the Piacenzian Stage out of the GSSP section.

The Quaternary Stages

The GSSP of the Gelasian Stage was ratified in 1996 as base of Upper Pliocene stage, and in 2009 as base of Pleistocene and Quaternary (Rio *et al.* 1998; Gibbard & Head 2010). The base of the Gelasian is defined in the Monte San Nicola section (Italy) at 62 m, in coincidence with the base of the marly layer overlying sapropel MPRS 250 and correlative with the isotopic stage 103 (2.588 Ma; GTS12). The primary marker to denote this chronostratigraphical unit is the Gauss/Matuyama magnetic reversal (C2An.1n/C2r.2r), which is located only 1 m below the GSSP. Close to the GSSP, calcareous nannofossil biostratigraphy provides some clear biohorizons occurring in close sequential order that are Top of *Discoaster tamalis* (2.76 Ma; Backman *et al.* 2012), Top of *Discoaster surculus* (2.53 Ma; Backman *et al.* 2012) and Top of *Discoaster pentaradiatus* (2.39 Ma; Backman *et al.* 2012), useful to approximate the Piacenzian/Gelasian Boundary (Fig. 7). In particular, the Tops of *D. tamalis* and *D. pentaradiatus*, which mark the base and top of Zone CNPL5 (Backman *et al.* 2012), precisely bracket the base of the Gelasian Stage.

The GSSP of the Calabrian Stage was firstly ratified in 1985 in the Vrica section (Italy) (Aguirre & Pasini 1985; Cita *et al.* 2012) at the base a claystone overlying the sapropelic marker Bed 'e', correlative with the Mediterranean Precession cycle 176 (at 1.806 Ma; GTS12). The top of the Olduvai magnetic reversal (C2n) is located only ca. 15 kyr below the boundary and is thus considered the best approximation for the base of the Calabrian. Aguirre & Pasini (1985) reported the high correlation potential of some of the palaeontological events observed across the boundary (Lourens *et al.* 1996). Among

them, two calcareous nannofossil biohorizons bracket the Calabrian GSSP, the Top of *Discoaster brouweri* (= Top of *Discoaster* spp.), which predates the base of the Calabrian (at 1.93 Ma; Backman *et al.* 2012) and the base of medium size (4–5.5 μm) *Gephyrocapsa* spp., which post-dates it (1.71 Ma; Backman *et al.* 2012). The use of these paired biohorizons could further strengthen the recognition of the Calabrian GSSP (Fig. 7).

The GSSP of the Middle Pleistocene Stage (i.e. Ionian) has not been formally defined, and three candidate sections are presently under consideration (Head & Gibbard 2015). Although a final decision has not been taken yet, the Brunhes/Matuyama boundary (773 ka) has received the greatest support as primary marker of the Ionian Stage (Head & Gibbard 2015). If this will be the final decision, the main calcareous nannofossil biohorizon that will possibly denote this chronostratigraphical unit is the Top absence of medium size *Gephyrocapsa* spp. (4–5.5 μm), with an estimated age of 1.06 Ma (Backman *et al.* 2012) but the Tc of *Reticulofenestra asanoi* could be possibly used as supplementary biohorizon to approximate the Middle Pleistocene GSSP (Maiorano & Marino 2004; 0.91 Ma; Backman *et al.* 2012).

The GSSP of the Late Pleistocene Stage has not been defined yet but two proposals are under scrutiny: the Eemian (Amsterdam Terminal) proposal (Pillans & Gibbard 2012) and the Tarentian (southern Italy) proposal (Negri *et al.* 2015). Whatever will be the final decision, it is quite evident that this Stage will correlate with the Marine Isotope Stage 5. Unfortunately, calcareous nannofossils are not particularly useful in this time interval, even though the Base of *Emiliania huxleyi* (at 0.29 Ma; Backman *et al.* 2012) might very roughly approximate this chronostratigraphical boundary.

The GSSP of the Holocene Stage was ratified in 2008 (Walker *et al.* 2009) in the NorthGRIP ice core (Greenland). There are no calcareous nannofossil biohorizons, which can approximate this boundary satisfactorily.

Concluding remarks

We briefly introduced the first finding of calcareous nannofossils and the successive intensive studies on this group both on the biological and geological point of view. We discussed some basic concepts of biostratigraphy with special reference to calcareous nannofossils, with some attention dedicated to the different methodologies, that would allow to depict the abundance pattern of any single taxon at best,

and emphasize the importance of highly resolved data sets. A standardization of counting methodologies is highly recommendable in order to identify the most useful and reproducible biohorizons, to construct firm biostratigraphical frameworks and to provide reliable biochronology. Finally, we also gave a general review of the fundamental role played by calcareous nannofossils in the Cenozoic chronostratigraphy describing and discussing on the biohorizons that are used to characterize the Cenozoic chronostratigraphical units.

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Supporting Information

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

Appendix S1. Taxonomic and bibliographic information on Figures 6 and 7.