EMILIANIA HUXLEYI FLUCTUATION AND ASSOCIATED MICROALGAE IN SUPERFICIAL SEDIMENTS OF THE ROMANIAN BLACK SEA SHELF

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Abstract: Several sites from the NW Black Sea, namely, the Romanian Black Sea shelf, situated at water depths ranging from 17 m to 80 m, have been investigated. The superficial sediments, *i.e.*, the water/sediment interface, were analyzed for their calcareous nannoplankton content. Both quantitative and qualitative nannofloral analyses have been performed. The nannofloral assemblages that are *in situ* contain only *Emiliania huxleyi* and *Braarudosphaera bigelowii*. Offshore, in front of the Danube mouth, only resedimented calcareous nannofossils from older Mesozoic-Tertiary deposits are present. Concerning the nannofloras *in situ*, the maximum bloom of *Emiliania huxleyi* in the studied area is placed in the region where the influx in sediments and waters rich in nutrients is significant, due to the input of both the Danube and Dniepr rivers, at a distance exceeding 30-40 km from the coastline. Apart from the coccolithophore algae, other groups of organisms, such as silicoflagellates, diatoms and dinoflagellates, were recorded. Their distribution pattern clearly reflects the marine setting; hence, differences in abundance and diversity between the inner and outer shelves have been observed.

Key words: NW Black Sea; water/sediment interface; inner and outer shelves; calcareous nannoplankton; diatoms; silicoflagellates; dinoflagellates.

1. INTRODUCTION

The calcareous nannofloras represent a major component of oceanic phytoplankton. Nowadays, coccoliths seem to be adapted at very high salinity fluctuations: *Coccolithus pelagicus* has been found in the Dead Sea, at 250 ‰ (Tappan, 1980), while *Emiliania huxleyi* lives in the Black Sea, at salinities below 20‰ (Bukry, 1974; Aksu *et al*., 2002). In general, the oceanic species tolerate narrow fluctuations of salinity, while coastal species are adapted at wider salinity changes (Brand and Guillard, 1981). *Emiliania huxleyi* is dominating the calcareous nannoplankton assemblages of the Black Sea, including the NW region of this marine basin (Giunta *et al*., 2007, Melinte-Dobrinescu and Briceag, 2011).

The first occurrence of *Emiliania huxleyi* was recorded, in the Atlantic Ocean, 289,000 years ago (Bickert *et al*., 1997), while in the Eastern Mediterranean the species firstly occurred 265,000 years ago (Lourens *et al*., 2004). The first common appearance of *Emiliania huxleyi* in the Black Sea, indicating the instauration of a stable normal marine regime, was approximated at 3,500 years BP (Bukry, 1974).

It is well-known that *Emiliania huxleyi* has an unusual behavior in the actual coccolithophore world: in certain environmental conditions it overproduced coccoliths, leading to the well-known blooms (Paasche, 2002). According to Tyrrell and Merico (2004), blooms of *Emiliania huxleyi* should be considered only at cell concentration of at least 1,000,000 per liter. *Emiliania huxleyi* blooms are recorded in many marine regions, including the Black Sea, with a maximum of 10,000,000 cells/liter observed in 1992 (Mankovsky *et al*., 1996).

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The coccolith blooms produced the "bright water" phenomenon (in satellite images), due to the light-scattering of the huge amount of coccoliths, or of "milky sea" (due to the turbid waters), as observed from the sea surface. These blooms have significant environmental impacts, consisting of increasing water albedo, large fluxes of calcium carbonate on the water surfaces, and a decrease in light and heat depth penetration (Tyrrell and Merico, 2004). Blooms of *Emiliania huxleyi* could act as an important source of dimethyl sulfide (DMS) and calcium carbonate, altering the optical properties of the surface mixed layer (Balch *et al*., 1991). No significant bloom was recorded in the Black Sea between the years 1978-1986. Later, satellites captured images of widely distributed blooms of *Emiliania huxleyi* (Cokacar *et al*., 2001; Iglesias-Rodriguez *et al.,* 2002).

In the Mediterranean Sea, the distribution pattern of living coccolithophores (Knappertsbush, 1993) indicates that the highest frequencies of *Gephyrocapsa oceanica* (species more related to open marine environment) occur in regions of minimum salinity (37‰), with values 1.5-2‰ lower than the normal salinity values of eastern Mediterranean surface waters. Most of the Quaternary sediments of the Black Sea lack *Gephyrocapsa oceanica*, or other present-day typical oceanic species, such as *Calcidiscus leptoporus*, *Helicosphaera carteri* and *Umbilicosphaera tenuis*, according to Aksu *et al*. (2002). This pattern is linked to the low salinities of the Black Sea, below the tolerance of most coccolithophores (Winter *et al*., 1994), but also to unstable environmental conditions and high fluctuation of the nutrient input.

In the NW part of the Black Sea (Romanian Black Sea shelf), in the water column, only one species of calcareous nannoplankton, *i.e*., *Emiliania huxleyi*, occurs. In the modern nannofloral assemblages of superficial sediments, *i.e*., at the water/sediment interface, the above-mentioned species co-occurs with *Braarudosphaera bigelowii* (Giunta *et al*., 2007; Melinte-Dobrinescu and Briceag, 2011; Briceag *et al*., 2012).

This paper presents the fluctuation pattern in abundance of *Emiliania huxleyi* and *Braarudosphaera bigelowii* observed in several locations, situated between 17 and 80 m water depths on the northern part of the Romanian Black Sea shelf. Based on the gathered data on calcareous nannoplankton distribution, and also on other groups of micro-organisms, such as silicoflagellates, diatoms and dinoflagellates, an ecological interpretation is advanced herein.

2. MATERIAL AND METHODS

A total of 16 stations (numbered from 1 to 10 and from 13 to 18), located at various water depths, were sampled for calcareous nannoplankton analysis (Fig. 1). The sampling has been achieved with a multicorer Mark II-400 that is able to take undisturbed cores up to 40 cm length. All the samples were collected in the first decade of May 2012, in the framework of the BS ERA.NET project, WAPCOAST.

For calcareous nannofossil analysis, the studied sample proceed from the interface water/sediment, 0-3 cm bsf (below sea floor) for all studied stations, except for the stations 1, 2 and 3, where the interval 0-5 cm bsf was investigated. Smear-slides were prepared directly from the untreated sediments, *i.e*., mostly muds and silts, in order to preserve the original nannofloral composition. A small amount of sediment was scraped onto a glass coverslip and diluted with distilled water. The suspension was smeared with a flat-sides toothpick along of the coverslip that was fixed by using Norland Optical Adhesive. Qualitative and quantitative studies were performed, by using an Olympus LM (light microscope), with 1600 x magnification.

For the qualitative investigations, counting in a fixed area that corresponds to 75.5 fields of view was performed. The results were converted by using the formula given by Giunta *et al.* (2007) for calcareous nannoplankton of the Black Sea. The counts were converted into population density (number/mm^2) using the following formula:

$$\text{Class} = \text{number of individuals observed/field of view number} * \text{field of view area.}$$

In the same investigated smear-slides, besides calcareous nannoplankton, *i.e*., coccolithophore algae, microplankton, such as silicoflagellates, dinoflagellates and diatoms were also identified. The latter groups of organisms were described, mainly, from a qualitative point of view (generic and specification determination).

3. RESULTS

3.1. Lithology

All the studied stations are characterized, from a lithological point of view, by the deposition of the Shallow Unit (as described by Oaie and Melinte-Dobrinescu, 2012), a shallower correspondent of Units 1 and 2 of Ross and Degens (1974) in the NW inner shelf of the Black Sea. Hence, the cored intervals are composed by muds, interbedded with coquina levels. Alternating sequences of mud, clay and silt were also observed. Sand levels, centimetre-thick, are present, especially in the Stations 9 and 10. Coquina layer in a sandy matrix, containing marine (*Mytilus galloprovincialis*, *Cardium spp*.) and freshwater (*Dreissena* and *Monodacna*) molluscs could be observed.

3.2. Calcareous nannoplankton

In several studied stations, at the interface water/sediment, blooms of *Emiliania huxleyi*, consisting of more than 1,500 individuals/mm^2, were recorded (Plate 1). According to the frequency of *Emiliania huxleyi*, expressed in individuals/mm^2, three nannofloral intervals were observed (Table 1):

1. Nannofloral interval 1 is characterized by a very high abundance of *Emiliania huxleyi*, over 1,500 individuals/mm^2. Very high values of *Emiliania huxleyi*, around 1,900

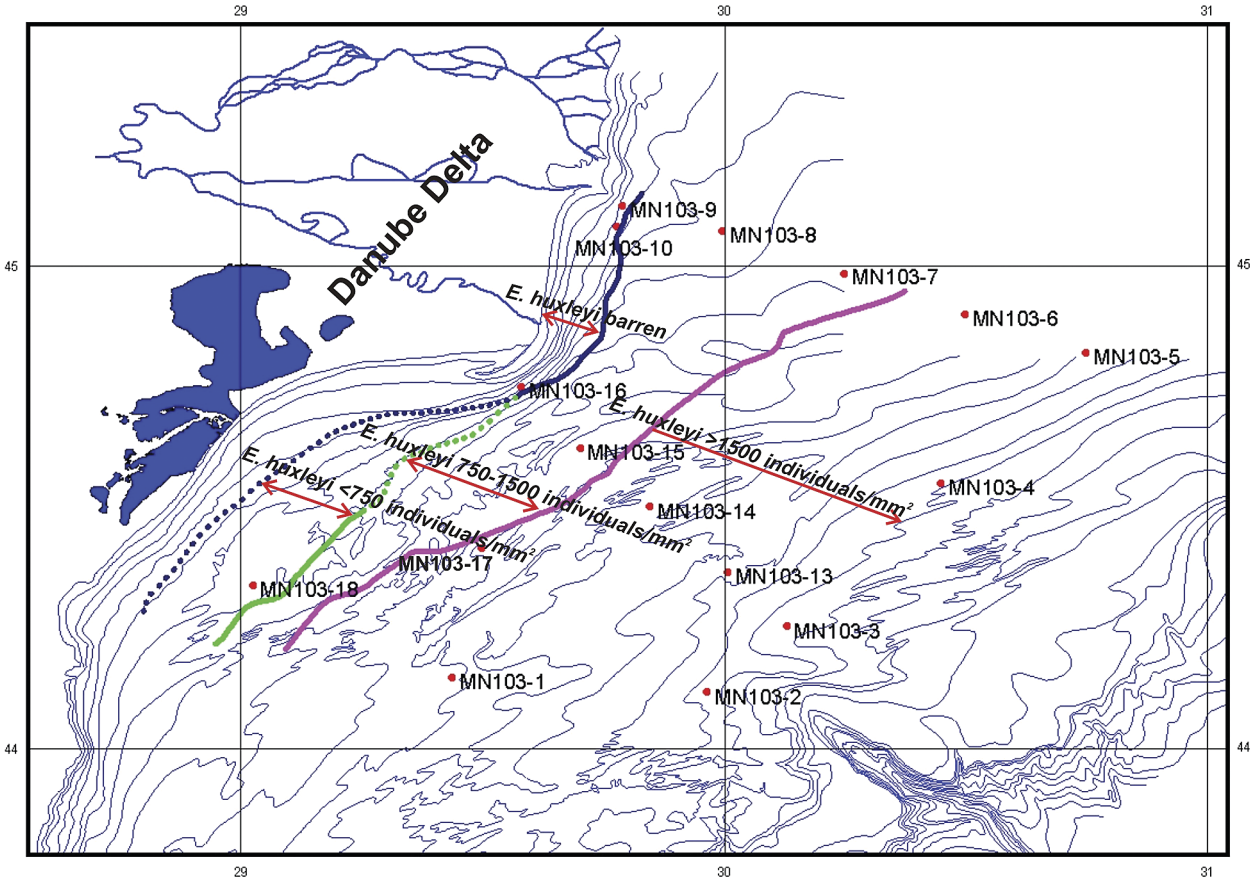

Fig. 1. Location of the studied section on the northern Romanian Black Sea, inner and outer shelves, and distribution of the calcareous nannoplankton species *Emiliania huxleyi* at the interface water/sediment, according to quantitative analysis.

individuals/mm², were observed in the Station 4 and Station 13;

2. Nannofloral interval 2 is characterized by a high abundance of *Emiliania huxleyi*, between 700 and 1,500 individuals/mm²;
3. Nannofloral interval 3 occurs in the Station 18. It is characterized by a low abundance of *Emiliania huxleyi*, below 700 individuals/mm²;
4. Barren interval, in which only resedimented taxa from Mesozoic-Tertiary deposits occur, was observed in the stations 9, 10 and 16, that are situated close to the shoreline.

The calcareous nannoplankton species *Braarudosphaera bigelowii* was observed only in the sample 0-5 cm bsf of Station 1, at a water depth of around 55 m. The frequency of this species is extremely low, around 0.4 individuals/mm² (Table 1).

Table 1. Data of quantitative analysis of the calcareous nannoplankton species *Emiliania huxleyi* and *Braarudosphaera bigelowii* in the studied stations from the northern Romanian Black Sea shelf. **E. h.** - *Emiliania huxleyi*; **B. b.** - *Braarudosphaera bigelowii*. Values are expressed in individuals/mm².

1	E.h.	B.b	2	E.h.	B.b	3	E.h.	B.b	4	E.h.	B.b	5	E.h.	B.b	
0-5 cm	1547.4	0,4	0-5 cm	1633.6	0	0-5 cm	1984.5	0	0-3 cm	1936.6	0	0-3 cm	1847.4	0	

6	E.h.	B.b	7	E.h.	B.b	8	E.h.	B.b	9	E.h.	B.b	10	E.h.	B.b	
0-3 cm	1902.3	0	0-3 cm	1127.4	0	0-3 cm	953.2	0	0-3 cm	0	0	0-3 cm	0	0	

13	E.h.	B.b	14	E.h.	B.b	15	E.h.	B.b	16	E.h.	B.b	17	E.h.	B.b	18	E.h.	B.b
0-3 cm	1978.4	0	0-3 cm	1972.4	0	0-3 cm	942.6	0	0-3 cm	0	0	0-3 cm	1620.3	0	0-3 cm	553.8	0

LEGEND

> 1500
700-1500
<700
0

As concerns the taxa proceeding from older deposits, most of them are Cretaceous (dominated by *Watznaueria barnesiae* and *Micula decussata*, two species that are known as some of the most solution-resistant to various diagenetic processes) and Miocene ones. The species proceedings from the Lower and Middle Miocene deposits are more numerous than the Cretaceous ones, and yielded, even if many are delicate-structured taxa, a good conservation degree. The amount of resedimented species is less than 10%, except for the stations 9, 10 and 16, where no nannofloras *in situ* were recorded.

3.3. Other investigated groups of organisms

Diatoms

The analysis of superficial sediments sampled in the studied sites revealed the presence of centric taxa (*Coscinodiscophyceae*), such as *Coscinodiscus*, *Aulacoseira*, *Cyclotella, Chaetoceros, Pseudosolenia*, as well as pennate ones without a raphe (*Fragilariophyceae*) and with a raphe (*Bacillariophyceae*), like *Bacillaria* and *Nitzschia*. To note that in the sites situated offshore the Danube mouth, *i.e.*, 9, 10, 11 and 16, at a water depth up to 40 m, planktonic diatom taxa that characterize a fresh-water environment, such as *Aulacoseira distans*, were identified.

Silicoflagellates

The silicoflagellates are generally rare in the studied samples. The dominant taxonomic group is *Distephanus* genus, mainly represented by the typical hexagonal shaped *Distephanus speculum* and by *Distephanus pulchrus* (Plate 1). Subordinately, species of the genus *Dictyocha* are also present.

Dinoflagellates

The most numerous species observed in the studied stations belong to the genera *Alexandrium*, *Ceratium, Dinophysis, Gymnodinium*, *Gyrodinium*, *Goniaulax, Peridinium, Prorocentrum, Protoperidinium,* and *Scrippsiella*. These taxa are generally known to tolerate high salinity fluctuations and were frequently described from the NW Black Sea, *i.e.*, the Romanian and Bulgarian shelves (Bodeanu, 1992; Velikova *et al*., 199; Gomez and Boicenco, 2004, among others). As in general, dinoflagellates are planktonic species, the identified dinoflagellates in the sediments are most probably cysts or resting stage.

4. DISCUSSION AND CONCLUSIONS

In the NW Black Sea inner shelf, the calcareous nannoplankton species *in situ* are composed by just two species: *Emiliania huxleyi* and *Braarudosphaera bigelowii* (Melinte-Dobrinescu and Briceag, 2011; Briceag *et al*., 2012) that are the only taxa that can survive at salinity variation from this region These two species survive at very high salinity fluctuations, being two of the most cosmopolitan nannofloral living species. For instance, *Emiliania huxleyi* is present, nowadays, in the surface-waters of the Sea of Azov, at 11‰, but also in the Red Sea, up to 41‰ (Fig. 2). *Braarudosphaera bigelowii* is also present at the very high salinity of the Red Sea, but not in the Sea of Azov; the minimum salinity where it could be found is around 17‰, in the Black Sea (Bukry, 1974; Giunta *et al*., 2007).

Fig. 2. Present-day distribution of the calcareous nannofloral taxa *Emiliania huxleyi* and *Braarudosphaera bigelowii* in various marine settings (after Melinte-Dobrinescu and Briceag, 2011; data from Bukry, 1974 and Giunta *et al.,* 2007).

The known seasonal phytoplankton fluctuation in the Black Sea consists of a significant diatom and dinoflagellate-dominated spring production, followed by calcareous nannoplankton bloom of *Emiliania huxleyi*. The latter taxon reaches its maximum in abundance in the second half of May-early June interval, showing a gradual decrease in the months of July and August. Being Blooms of *Emiliania huxleyi* are mainly located in the upper 20 m layer of water, where most favorable light and temperature conditions are accomplished, at a water temperature above 20^{0}C (Mankovsky *et al*., 1996; Moncheva and Krastev, 1997; Eker *et al*., 1999; Cokacar e*t al*., 2004; Oguz and Merico, 2006).

The data obtained, based on calcareous nannoplankton are consistent with the fluctuations in ostracod and foraminiferal assemblages published by Briceag and Ion,(2013). Hence, the above-mentioned authors indicated that, in the inner shelf of the Black Sea, in the Station 9, situated offshore in front of the Sulina branch of the Danube Delta, at a water depth of around 17 m, the character of the ostracod assemblages is a brackish-Caspian one. Our analysis pointed out that this station does not contain any nannofloras *in situ*. By contrast, in the Station 4, placed on the outer shelf, at a water depth of 78 m, the dominant character of the ostracod assemblages is a marine Mediterranean one (Briceag and Ion, 2013). Besides, they reported consistent assemblages of benthic foraminifer assemblages, dominated by *Ammonia* spp. Our calcareous nannoplankton investigations indicated in the Station 4, within the interval 0-3 cm, there is a significant bloom of *Emiliania huxleyi*, of 1936.6 individuals/mm^2, which is the maximum recorded in all stations presented herein.

The maximum bloom of *Emiliania huxleyi* was found in the studied Stations 2, 3, 4, 5 and 13, at a distance more than 30-40 km of the coastline. This pattern is probably due to the enrichment in nutrients in this area, where both the Danube

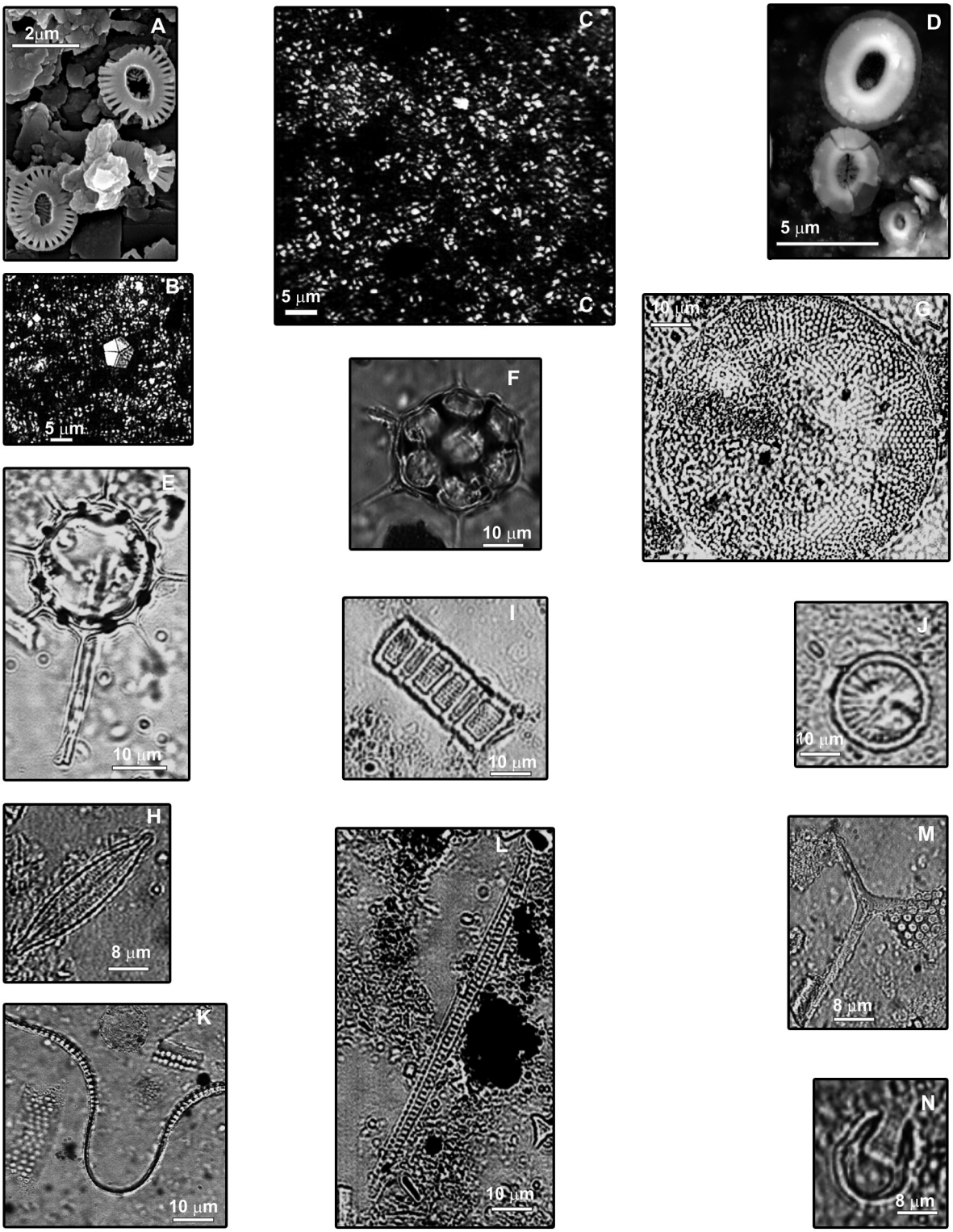

Plate 1. Microphotographs of the calcareous nannoplankton taxa and microfloras encountered in the studied stations. All microphotographs have been taken at LM (light microscope), except Figs. A and D, which have been taken at SEM (Scanning Electron Microscope). Scale bar is given in microns. **A-D: calcareous nannoplankton**; A - Individuals of *Emiliania huxleyi*, Site 4, 0-3 cm bsf (bellow sea-floor); B - Bloom of *Emiliania huxleyi* and a single specimen of *Braarudosphaera bigelowii* (in the central part of the microphotograph), Site 4, 0-3 cm bsf; C - Bloom of *Emiliania huxleyi*, Site 1, 0-3 cm bsf; D - Tertiary calcareous nannofossils resedimented in the superficial sediments, Site 16, 0-3 cm bsf; **E-F: silicoflagellates**; E - *Distephanus pulchrus*, Site 17, 0-3 cm bsf; F - *Distephanus speculum*, Site 17, 0-3 cm bsf; **G-L** and **N: diatoms**; G - *Coscinodiscus* sp., Site 9, 0-3 cm bsf; H - *Navicula* sp., Site 13, 0-3 cm bsf; I - *Aulacoseira distans*, Site 18, 0-3 cm bsf; J - *Cyclotella* sp., Site 10, 0-3 cm bsf; K - *Nitzschia* sp., Site 18, 0-3 cm bsf; L – *Bacillaria paradoxa*, Site 1, 0-3 cm bsf; **M – radiolarian** spicule, Site 17, 0-3 cm bsf; N – *Chaetoceras* sp., Site 1, 0-3 cm bsf.

and Dniepr influences (sediments and waters rich in nutrients) could be assumed. The samples of the Stations 9 and 16, offshore the Danube mouth, contain no calcareous nannoplankton taxa *in situ*. Relatively low concentrations of coccolithophore algae were recorded in the Station 18, placed at around 20 km from the coastline.

The diatoms are present in all the studied samples from the water/sediment interface. In the Black Sea, there are planktonic and benthic diatoms, the latter ones living on different types of substrates in the photic zone. The currents and waves could agitate the bottom sediments and the macroalgae communities, where some of the benthic diatoms are fixed. Therefore, the benthic diatoms may frequently raise and reach the plankton community. Notably, the production-based diatom bloom is preferentially confined to the upper 20 m layer, reflecting strong light limitation. The bloom could partly extend down to the base of the euphotic zone, around 50 m (Oguz and Merico, 2006).

To note that in the vicinity of the coastline, in the northern Romanian Black Sea inner shelf, up to a water depth of 40 m, some fresh-water planktonic diatoms, *i.e.*, *Aulacoseira distans*, have been observed. This taxon was identified especially in the sites placed in front of the Danube Delta, *i.e.*, the mouth of the Sulina branch. We may suppose that, as this species is commonly found in the diatom fresh-water communities of the Danube, it was transported in the Delta front. On the other hand, the salinity of surface-water from some stations, *i.e.*, 9 and 10, where this species is commonly present, is low, up to 7 ‰. So, it is possible that this taxon is part of the *in situ* diatom assemblages that could be found in front of the Danube Delta.

Concerning the dinoflagellates identified in the studied samples, we observed low species richness, and this is probably due to specific environmental conditions in the Danube Delta front, such as high nutrient concentrations, but a seasonal significant changing in nutrient ratios. These factors are known to produce, in NW Black Sea, during last decades, monospecific blooms of some dinoflagellates, *i.e*, *Noctiluca scintillans*, *Prorocentrum cordatum*, *Heterocapsa triquetra*, *Scrippsiella trochoidea*, among others (Bologa *et al*., 1995; Mihnea, 1997; Velikova *et al*., 1999).

The silicoflagellates encountered in the studied stations are, in general, very rare, being mainly present in samples 0-3 cm bsf, below 60 m water depth, *i.e.* the stations 2, 3, 4, 5 and 17. The silicoflagellate assemblages are mainly composed of taxa belonging to the *Distephanus* and *Dictyocha* genera. It is to note that some of the taxa identified in the studied samples, such as *Distephanus speculum*, could produce toxic blooms to fish (Nielsen and Aertebjerg, 1984). So far, no such biotic events were recorded in the NW Black Sea Romanian region; we may suppose the same for the future, as the abundance of the above-mentioned species is generally low in all analyzed settings (the inner shelf and the outer one).

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