

BCLME Project EV/HAB/02/03  
Ref. RAF/00/G32 – Benguela Current Large Marine Ecosystem

# INVESTIGATION INTO THE DIVERSITY AND DISTRIBUTION OF CYSTS OF HARMFUL ALGAL BLOOMS WITHIN THE BENGUELA CURRENT LARGE MARINE ECOSYSTEM REGION

## Final Report



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for

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## GLOSSARY OF TERMS AND ACRONYMS

ASP: amnesic shellfish poisoning
BCLME: Benguela Current Large Marine Ecosystem
DSP: diarrhetic shellfish poisoning
HAB: harmful algal bloom
MCM: Marine and Coastal Management
CMS: Centre for Marine Studies
MFMR: Ministry of Fisheries and Marine Resources (Namibia)
IIM: Instituto de Investigacao Marinha (Angola)
NSP: neurotoxic shellfish poisoning
PSP: paralytic shellfish poisoning
UCT: University of Cape Town
YTX: yessotoxin

## Executive Summary

Sediment samples were collected from 185 stations along the coasts of South Africa, Namibia, and Angola, to determine the composition, diversity, horizontal distribution and abundance of resting cysts in sediments from the BCLME region. At least 47 dinoflagellate cyst types, representing 8 motile-defined genera and 1 cyst-defined genus, were observed. Cysts of heterotrophic dinoflagellates dominated the assemblage for the BCLME region as a whole. Total cyst abundance ranged from 0 – 3975 cysts ml<sup>-1</sup> wet sediment. The majority of cysts occurred along the coasts of Namibia and South Africa, where higher densities were observed. Of particular interest, and importance, is the distribution and abundance of cysts of toxic species. Three toxic cyst species were observed in the sediments of the BCLME region: *Alexandrium catenella*, *Lingulodinium polyedrum*, and *Protoceratium reticulatum*. The presence of these cysts, in particular where higher abundances were found, indicates potential seedbeds for initiation of future vegetative growth (blooms) and subsequent outbreaks of toxic episodes or other harmful events.

Results from this BCLME project, EV/HAB/02/03, provide a good indication of the cyst assemblages present in the coastal upwelling areas of the BCLME region, giving a detailed description of cyst species composition, diversity, distribution and abundance. This data provides information relating to the geographic ranges of cyst-forming HAB species critical for the assessment, planning and prediction of HABs.

## **1. Introduction**

### **1.1. Harmful Algal Blooms**

Proliferations of algae in marine waters can have adverse effects and alter ecosystems in ways that humans perceive as harmful. The scientific community refers to these events with a generic term, "Harmful Algal Blooms (HABs)" (Smayda 1997). HABs are natural phenomena that have occurred throughout recorded history. In recent years, the incidence of HABs appears to be increasing in frequency and intensity worldwide (Hallegraeff 1993), adversely affecting ecosystems and societal interests like fisheries, human health, and tourism. There is no general rule to define harmful concentrations of cells in an algal bloom, but these harmful blooms are usually characterised by the proliferation, and occasional dominance, of a particular species of toxic or otherwise harmful alga, and in many instances these proliferations discolour the water, a phenomenon known as red tide. Conversely, low-density populations can cause severe problems due to the high potency of the toxins produced by some species. Toxic species may cause mass mortalities of fish, shellfish, marine mammals, seabirds and other animals (Adams *et al.* 1968, Anderson 1994, Scholin *et al.* 2000), and human illness and death may result from contaminated seafood. Toxic species are filtered from the water by shellfish, such as clams, mussels, oysters and scallops, the shellfish then act as intermediate vectors, accumulating the algal toxins to high levels, and transmitting the toxins to consumers at higher trophic levels. Several so-called shellfish poisoning syndromes are recognised: Paralytic Shellfish Poisoning (PSP), Amnesic Shellfish Poisoning (ASP), Diarrhetic Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP). Blooms of non-toxic phytoplankton can also cause harm due either to mechanical damage, such as the clogging of fish gill tissue (Boalch 1979), or to the indirect effects of biomass accumulation, such as anoxia (Smayda 1997). Both toxic and non-toxic blooms will in some cases alter the trophic structure of marine systems, thereby causing trophic dysfunction (Smayda 1997, Horner *et al.* 1997, Anderson 1998).

### **1.2. Dinoflagellate cysts**

Dinoflagellates are one of the most important groups of phytoplankton, they are typically microscopic, single-celled, motile organisms, either autotrophic (photosynthetic), heterotrophic (feeding on other phytoplankton cells, e.g. diatoms, small flagellates), or mixotrophic (combination of auto- and heterotrophy). The phylum is mostly marine with less than 10% being freshwater species. Dinoflagellates are

abundant in the coastal and neritic zones, where HABs often cause the most harm. Dinoflagellates are among the most frequent producers of HABs, out of the approximately 100 species of HAB producing phytoplankton, dinoflagellates account for 75% of all HAB species (Smayda 1997). Reproduction in dinoflagellates is primarily asexual, however, many species are also capable of sexual reproduction. Those species capable of sexual reproduction, form gametes that fuse into a swimming cell (planozygote) which, in most cases, transforms into a resting cyst stage (hypnozygote), these cysts are morphologically distinctive and differ from the motile planktonic stage (Pfeister and Anderson 1987, Nehring 1993, Anderson *et al.* 1995). To date, among the approximate 2,000 extant dinoflagellate species, more than 200 are known to produce cysts (Head 1996).

Cysts play an important role in species dispersal (Anderson *et al.* 1995), survival under unfavourable conditions (Dale 1983, Nehring 1993), termination of blooms (Heiskanen 1993), and initiation of future blooms (Anderson *et al.* 1983, Cembella *et al.* 1988, Ishikawa and Taniguchi 1996). Therefore, information on the distribution and abundance of cysts in sediments is essential for understanding the ecology and bloom dynamics of many species. Surveys of cyst assemblages are also useful in that they can provide early warnings of the presence of harmful species in a given area, and can reveal species not observed in the plankton, the motile cells possibly being rare, short-lived, fragile or difficult to identify (Hesse *et al.* 1996). Cysts also have the potential to record their history within sediments. Many species produce cysts which are preservable/preserved in sediments (Head 1996, Dale 2001), The cyst wall in most species is thick, and very resistant, being composed of organic matter (sporopollenin). Organic cyst walls are extremely resistant to natural decay, and can therefore persist in the sediments for a very long time, providing an integrated record over time of the cyst-producing dinoflagellates. Therefore, it is possible to establish the long-term history of cyst deposition, and potentially the history of dinoflagellate blooms, thereby providing an indication of the potential for future blooms within an area.

The long viability of dinoflagellate cysts in sediments indicates that once cyst-forming species are present within an area, their cysts constitute a potential source for the initiation of future blooms. Cysts germinate into vegetative cells after a dormant period, and are thought to provide the inoculum for vegetative growth and bloom initiation in the water column (Anderson and Wall 1978, Walker and Steidinger 1979), assuming environmental conditions are favourable. Knowledge of the distribution of

cysts within marine sediments is useful in that it provides information on potential bloom distribution.

### **1.3. HABS in the BCLME region**

HABs frequently occur within the BCLME region (Pitcher 1998), in both the northern and southern Benguela, they tend to be observed most frequently close inshore, where their visual, and at times, harmful effects are most apparent. In particular HABs are known to occur in coastal waters of South Africa (Pitcher and Calder 2000) and Namibia (Pitcher 1998), and whilst there have been reports of red tides off Angola (Silva 1953, Paredes 1962), there is in general a great disparity in the amount of available information about HABs within the region. The harmful effects of high-biomass, non-toxic blooms in the Benguela include die-offs resulting from anoxia or hypoxia (Hart 1934, Copenhagen 1953, Grindley and Taylor 1962, 1964, Pieterse and Van der Post 1967, Horstman 1981, Matthews and Pitcher 1996, Pitcher and Cockcroft 1998, Cockcroft *et al.* 2000). Other effects of high biomass blooms include those that may cause mechanical or physical damage (Grindley and Nel 1968, Brown *et al.* 1979) or those that may alter the food web (Pitcher *et al.* 1999). Toxic species have caused mass mortalities of fish, shellfish, marine mammals, seabirds and other animals (Brongersma-Sanders 1948, 1957, Copenhagen 1953, Horstman 1981, Horstman *et al.* 1991). Human illness is caused by contaminated seafood when toxic phytoplankton are filtered from the water by shellfish that accumulate toxins to levels that are potentially lethal to humans and other consumers (Sapeika 1948, 1958, Grindley and Sapeika 1969, Popkiss *et al.* 1979, Horstman 1981, Horstman *et al.* 1991, Pitcher *et al.* 1993, Pitcher and Matthews 1996). Of these shellfish poisoning syndromes, PSP and DSP are common in the Benguela.

HABs within the BCLME region are typically attributed to dinoflagellates, however, due to the absence of HAB monitoring programs in Angola and Namibia, the frequency, duration and extent of HABs in these countries is unknown, as are the species involved. Also, the diversity, abundance and distribution of cysts of HAB species, and the history of blooms are poorly established in the majority of the BCLME region. The object of this project was to assess the diversity, distribution and abundance of cyst species, including the spatial limits of specific HAB species, thereby providing a long-term history of blooms and cyst deposition and an indication of seed sources, and the potential for future harmful blooms within the region.

## **2. Methodology**

### **2.1. Sampling areas and sediment collection**

For the analysis of surface cyst assemblages a total of 185 surface sediment samples were collected from within the BCLME region (Fig 1), using a grab sampler. Along the Angolan coast, 17 samples were collected from an area between 5°S and 17° S; along the Namibian coast, 99 samples were collected from an area between 17°S and 26.5°S; along the west coast of South Africa, 69 samples were collected from an area between 30.7°S and 35.5°S. For analysis of the vertical profiles of cyst assemblages and abundances within the sediments, 5 samples were collected by means of a core sampler (Fig 2). The sediment samples from Angola and, the majority of the Namibian samples were collected during the *Alexander v. Humboldt* – Angola-Benguela Expedition (AHAB) Cruise, January to June 2004. Some samples from Namibia were also collected by staff from MFMR. Sediment samples previously collected by L. Joyce and MCM during 2001 - 2003 from along the west coast of South Africa were made available and incorporated into this project.

### **2.2. Sediment analysis**

Sediment samples were stored in the dark at 4°C, to prevent cyst germination, until analysis was undertaken. For cyst identification and enumeration the sediments were further processed and analysed using standard wet sieving methods (Wall and Dale 1968, Matsuoka and Fukuyo 2000). A subsample of the grab sample was removed for processing. The core samples were sectioned every 1cm and each section analysed. A small amount of the sediment was gently sonicated for 1 minute to separate cysts from detrital particles, from this, a 2ml subsample was filtered through a 125µm and a 20µm mesh sieve. The slurry remaining on the 20µm mesh was backwashed onto a watch glass, and a process of panning was performed to separate cysts and lightweight particles from heavier particles. The supernatant water with cysts was transferred into a beaker and the final volume noted. From the final volume, 1ml was removed by pipette and placed on a Sedgewick-rafter chamber and examined under an Olympus BX-60 light microscope. Photographs of cysts were taken using a digital camera attachment. Cyst concentrations are presented as the number of cysts ml<sup>-1</sup> wet sediment.

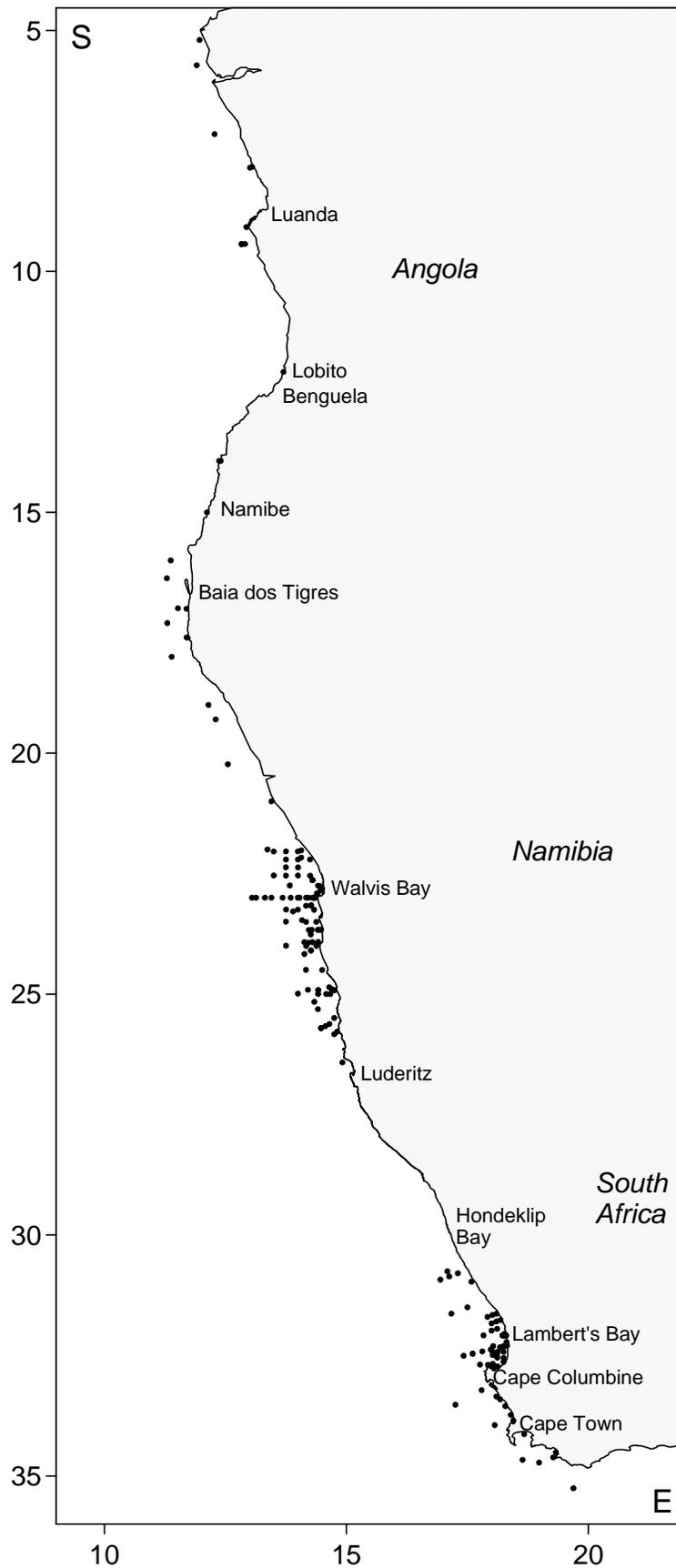


Fig 1. Map of the BCLME region showing the locations of sediment sample collections

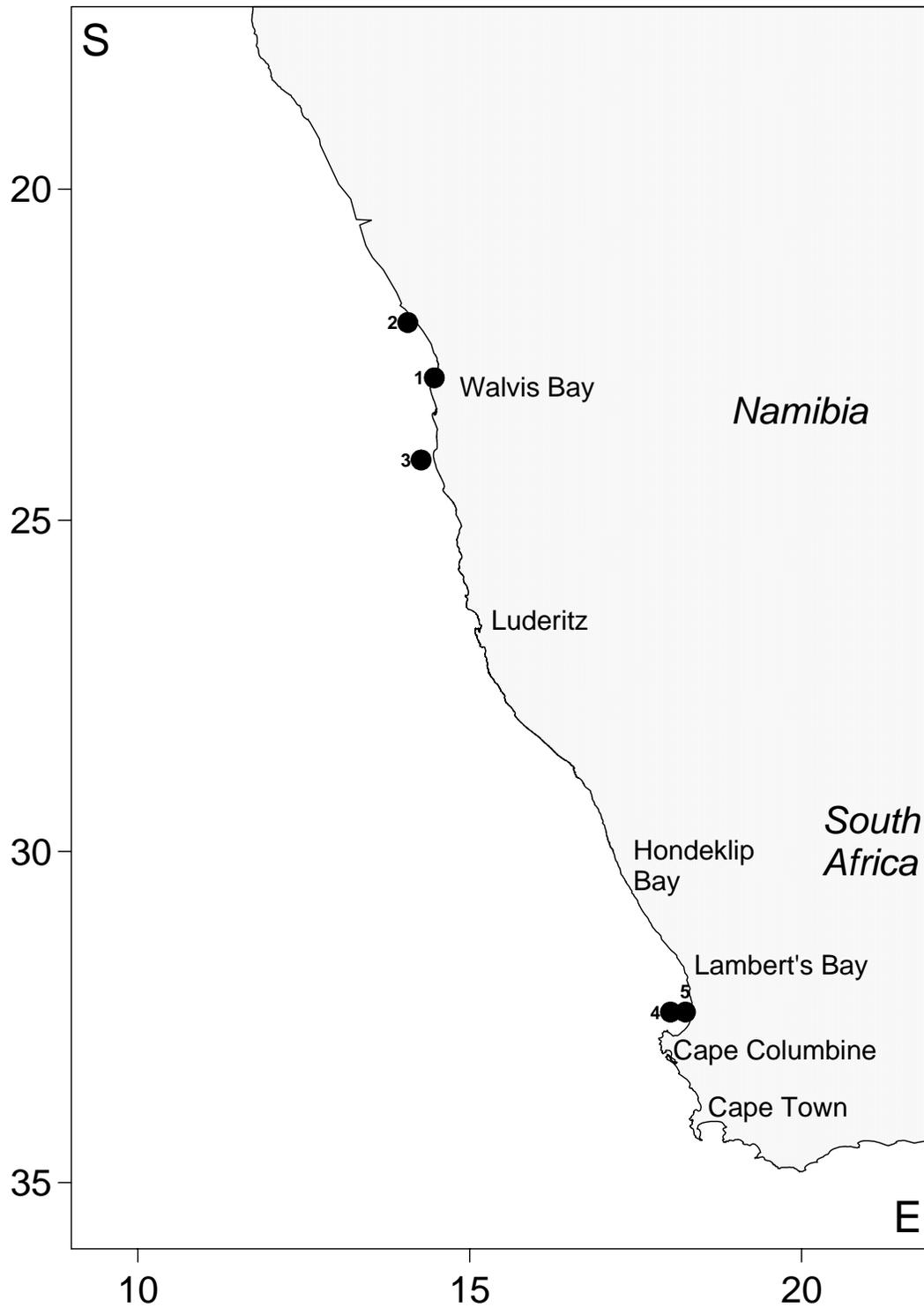


Fig 2: Map of the BCLME region showing the locations of core sample collections

### **3. Results**

#### **3.1. Total cysts in surface sediments**

A detailed species list of cyst types found in the surface sediments of the BCLME region has been established (Table 1). Photographs of these cysts are presented in Plates 1 - 11. Fig. 3 shows the horizontal distribution and abundance of total cysts within the region. Total cyst abundance ranged from 0 – 3975 cysts ml<sup>-1</sup> wet sediment.

##### **3.1.1. Angola**

From the 17 sites sampled along the coast of Angola, 21 cyst types representing 7 dinoflagellate genera were recorded. The number of cyst types recorded at any single station ranged between 2 and 8, with an average of 4 cyst types per site. Cyst concentrations were very low, ranging from 38 to 200 cysts ml<sup>-1</sup> wet sediment, and cysts belonging to heterotrophic dinoflagellates dominated the assemblage. Two toxic cyst species were found in the sediments along the Angolan coast: *Lingulodinium polyedrum* and *Protoceratium reticulatum*.

##### **3.1.2. Namibia**

From the 99 sites sampled along the coast of Namibia, 41 cyst types representing 9 dinoflagellate genera were recorded. The number of cyst types recorded at any single station ranged between 0 and 33, with an average of 14 cyst types per site. Cyst concentrations were high, ranging from 0 to 3975 cysts ml<sup>-1</sup> wet sediment, and cysts belonging to heterotrophic dinoflagellates again dominated the assemblage. Three toxic cyst species were found in the sediments along the Namibian coast: *Alexandrium catenella*, *Lingulodinium polyedrum* and *Protoceratium reticulatum*.

##### **3.1.3. South Africa**

From the 69 sites sampled along the west coast of South Africa, 29 cyst types representing 9 dinoflagellate genera were recorded. The number of cyst types recorded at any single station ranged between 0 and 16, with an average of 9 cyst types per site. Cyst concentrations ranged from 0 to 1650 cysts ml<sup>-1</sup> wet sediment and cysts belonging to heterotrophic dinoflagellates again dominated the assemblage. Three toxic cyst species were found in the sediments along the west coast of South Africa: *Alexandrium catenella*, *Lingulodinium polyedrum* and *Protoceratium reticulatum*.

Table 1: Cyst types found in the sediments of the BCLME region (\* Toxic species)

Cyst types		Angola	Namibia	South Africa
Biological name	Palaeontological name			
<b>Gonyaulacales</b>				
<i>Gonyaulax</i> sp.	<i>Spiniferites delicatus</i>		+	+
<i>Gonyaulax spinifera</i> complex	<i>Spiniferites ramosus</i>		+	+
Unidentified <i>Spiniferites</i> spp		+	+	+
<i>Lingulodinium polyedrum</i> *	<i>Lingulodinium machaerophorum</i>	+	+	+
<i>Gonyaulax spinifera</i> complex	<i>Nematosphaeropsis</i> sp.	+	+	
<i>Protoceratium reticulatum</i> *	<i>Operculodinium centrocarpum</i>	+	+	+
<i>Alexandrium catenella</i> *	-		+	+
<b>Gymnodiniales</b>				
<i>Polykrikos kofoidii</i>	-		+	+
<i>Polykrikos schwartzii</i>	-	+	+	+
<b>Calcareous Peridinales</b>				
<i>Scrippsiella lachrymosa</i>	-		+	+
<i>Scrippsiella precaria</i>	-		+	
<i>Scrippsiella ramonii</i>	-		+	
<i>Scrippsiella trifida</i>	-		+	
<i>Scrippsiella trochoidea</i>	-	+	+	+
<i>Scrippsiella</i> sp.	-		+	+
<b>Organic Peridinales</b>				
<i>Protoperidinium americanum</i>	-	+	+	+
<i>Protoperidinium avellanum</i>	<i>Brigantedinium cariacense</i>	+	+	+
<i>Protoperidinium claudicans</i>	<i>Votadinium spinosum</i>		+	+
<i>Protoperidinium compressum</i>	<i>Stelladinium stellatum</i>		+	+
<i>Protoperidinium conicoides</i>	<i>Brigantedinium simplex</i>		+	+
<i>Protoperidinium conicum</i>	<i>Selenopemphix quanta</i>	+	+	+
<i>Protoperidinium denticulatum</i>	<i>Brigantedinium</i> sp.		+	+
<i>Protoperidinium latissimum</i>		+		
<i>Protoperidinium leone</i>	<i>Quinquecuspis concreta</i>	+	+	+
<i>Protoperidinium oblongum</i>	<i>Votadinium calvum</i>	+	+	+
<i>Protoperidinium pentagonum</i>	<i>Trinovantedinium applanatum</i>	+	+	+
<i>Protoperidinium subinermis</i>	<i>Selenopemphix nephroides</i>	+	+	+
Protoperidinium sp 2				+
Protoperidinium sp 3				+
Protoperidinium sp 4			+	+
Unidentified Round Brown	-	+	+	+
<i>Zygabikodinium lenticulatum</i>	<i>Dubridinium caperatum</i>	+	+	+
Unidentified sp 1			+	+
Unidentified sp 4		+	+	
Unidentified sp 5		+	+	
Unidentified sp 7		+	+	
Unidentified sp 8			+	
Unidentified sp 9			+	
Unidentified sp 11			+	+
Unidentified sp 12			+	
Unidentified sp 13			+	
Unidentified sp 14			+	
Unidentified sp 15		+		
Unidentified sp 16		+		
Unidentified sp 17			+	

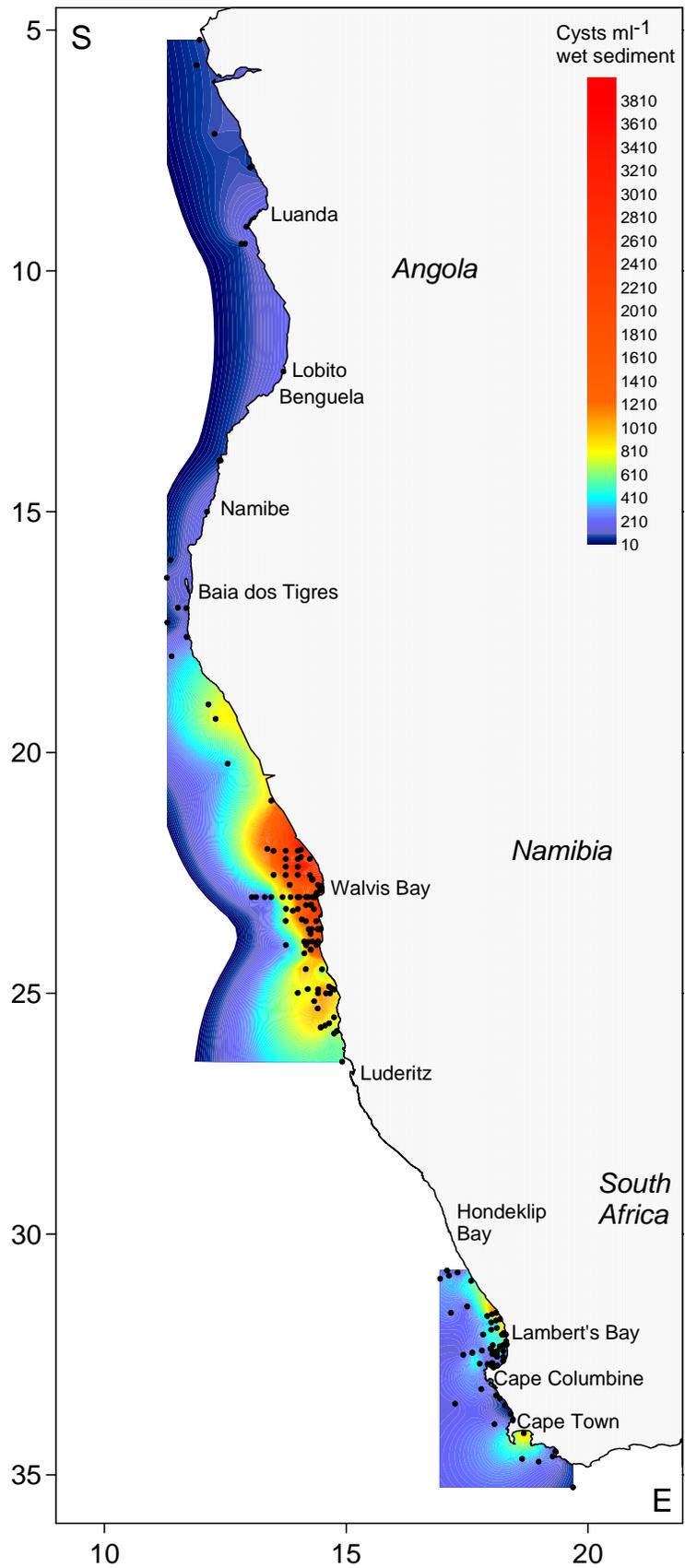


Fig 3. Total cyst distribution and abundance in the sediments of the BCLME region

## **3.2. Toxic species**

### **3.2.1. *Alexandrium catenella***

The toxic dinoflagellate *Alexandrium catenella*, which produces the toxin saxitoxin, and several derivatives of this molecule, is responsible for outbreaks of PSP in the southern Benguela region. The symptoms of PSP include tingling and numbness of the mouth, lips and fingers, accompanied by general muscular weakness and incoordination. Acute doses inhibit respiration, and death may result from respiratory paralysis. Confirmed cases of PSP in the southern Benguela have been known since 1948 (Sapeika 1948, 1958, Popkiss *et al.* 1979, Pitcher and Cockroft 1998). In 1997, sardine (*Sardinops sagax*) mortalities were attributed to the ingestion of *A. catenella* (Pitcher and Calder 2000). There have been no reported cases of PSP from either Angola or Namibia.

Cysts of *A. catenella* were found in the sediments of the southern Benguela and the northern Benguela (abundances ranged from 13-238 cysts ml<sup>-1</sup> wet sediment). Distribution maps of cysts of *A. catenella* have been produced for the BCLME region showing its northern and southern limits of distribution (Figs. 4 and 5). It appears that cyst beds of *A. catenella* do not extend any further north than 22.5°S, its northern limit, or any further south than 33°S, its southern limit. The distribution of cysts of *A. catenella* was very localised, only being found in the vicinity of Walvis Bay and Lüderitz off Namibia, and on the southern Namaqua shelf off South Africa, where maximum concentrations occur downstream of the Cape Columbine upwelling cell.

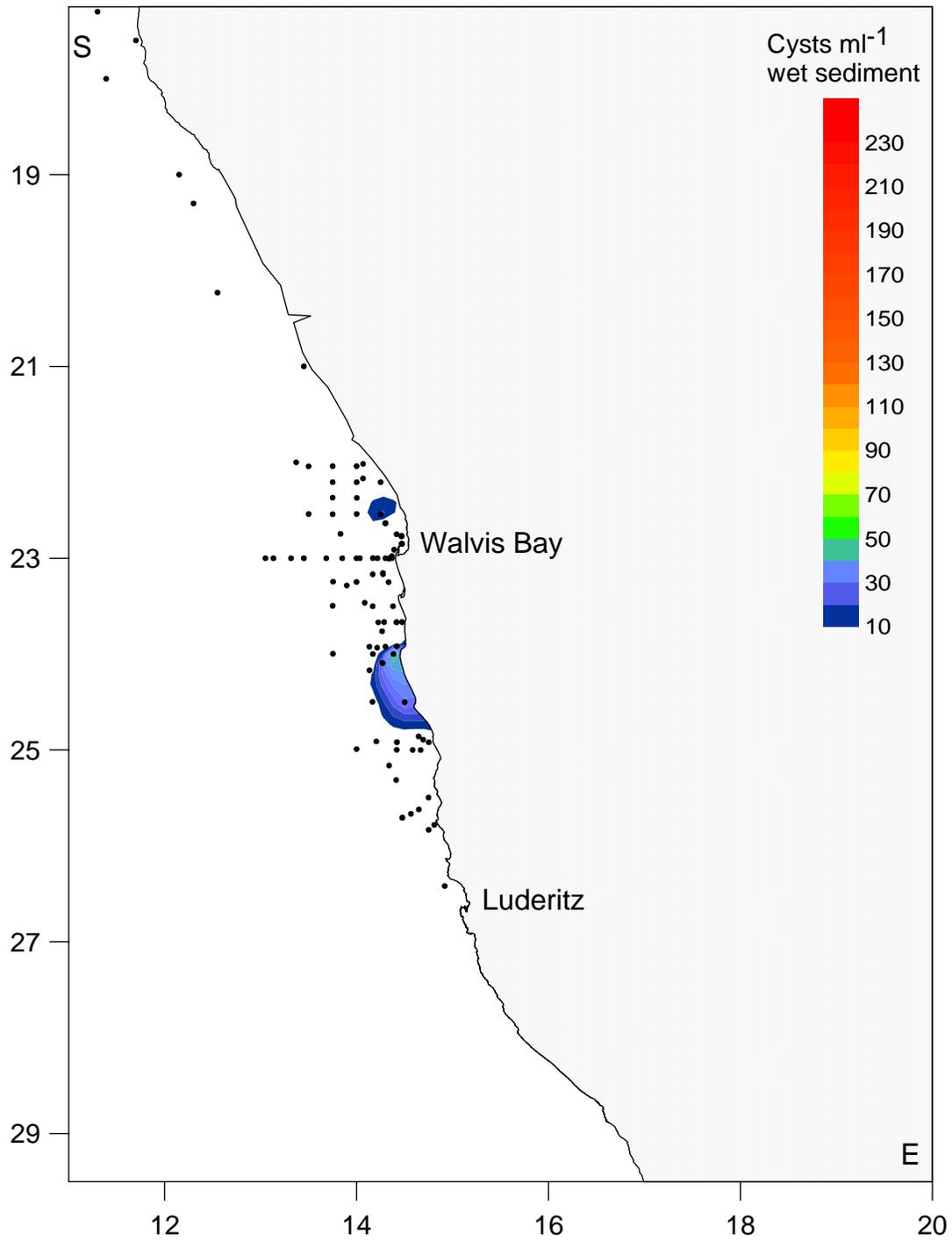


Fig 4: Horizontal distribution map of cysts of *Alexandrium catenella* in Namibia

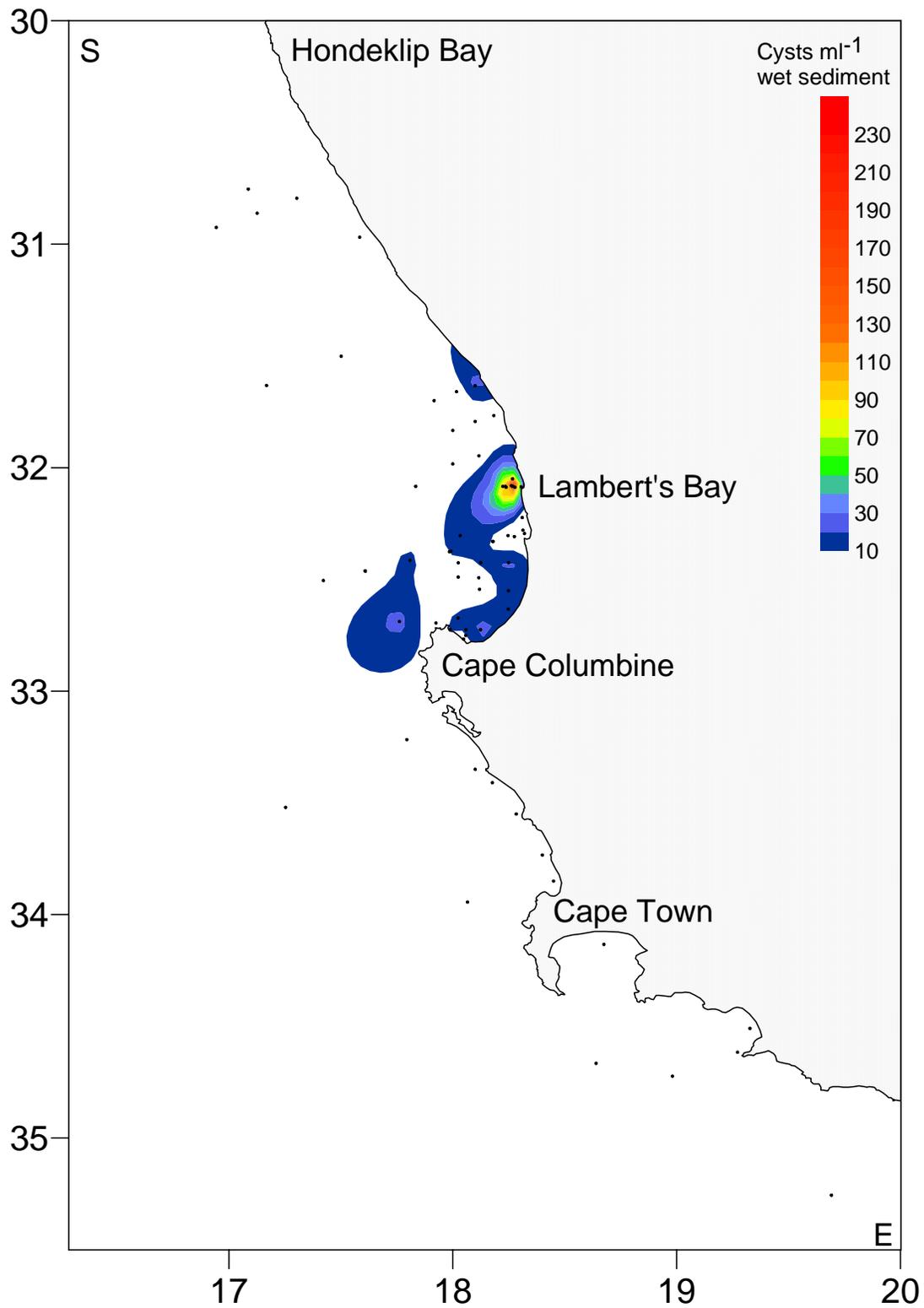


Fig 5: Horizontal distribution map of cysts of *Alexandrium catenella* in South Africa

### 3.2.2. *Lingulodinium polyedrum*

*L. polyedrum* is a toxic dinoflagellate that produces yessotoxins (YTX) (Paz *et al.* 2004). The toxic syndromes of YTX are not well defined in humans, but *L. polyedrum* is often associated with the mortality of fish and benthic organisms. This species has been known to form blooms off the coast of California (Kofoid 1911, Allen 1921, Eppley and Harrison 1975), and commonly forms blooms in the Adriatic Sea (along the Italian and Yugoslavian coastlines) (Marasovic 1989, 1991, Bruno *et al.* 1990) and in Scottish sea-lochs (Lewis *et al.* 1985). The first report of toxicity in this species was made by Schradie and Bliss (1962). They found a toxin profile similar to the toxins of *Alexandrium catenella*, however, most recently more in-depth studies have linked this species with production of YTX (Paz *et al.* 2004). There have been no reports of HABs associated with this species in the Benguela region.

Cysts of *L. polyedrum* were found in the sediments of the southern Benguela and the northern Benguela regions. Distribution maps of *L. polyedrum* have been produced for the BCLME region showing its northern and southern limits (Figs. 6-8). The distribution of cysts of *L. polyedrum* is slightly more widespread than that of *A. catenella*, but throughout the region the abundances of *L. polyedrum* are very low (ranging from 13-38 cysts ml<sup>-1</sup> wet sediment). The northern distribution limit reaches 5°S, and the southern limit is 32°S.

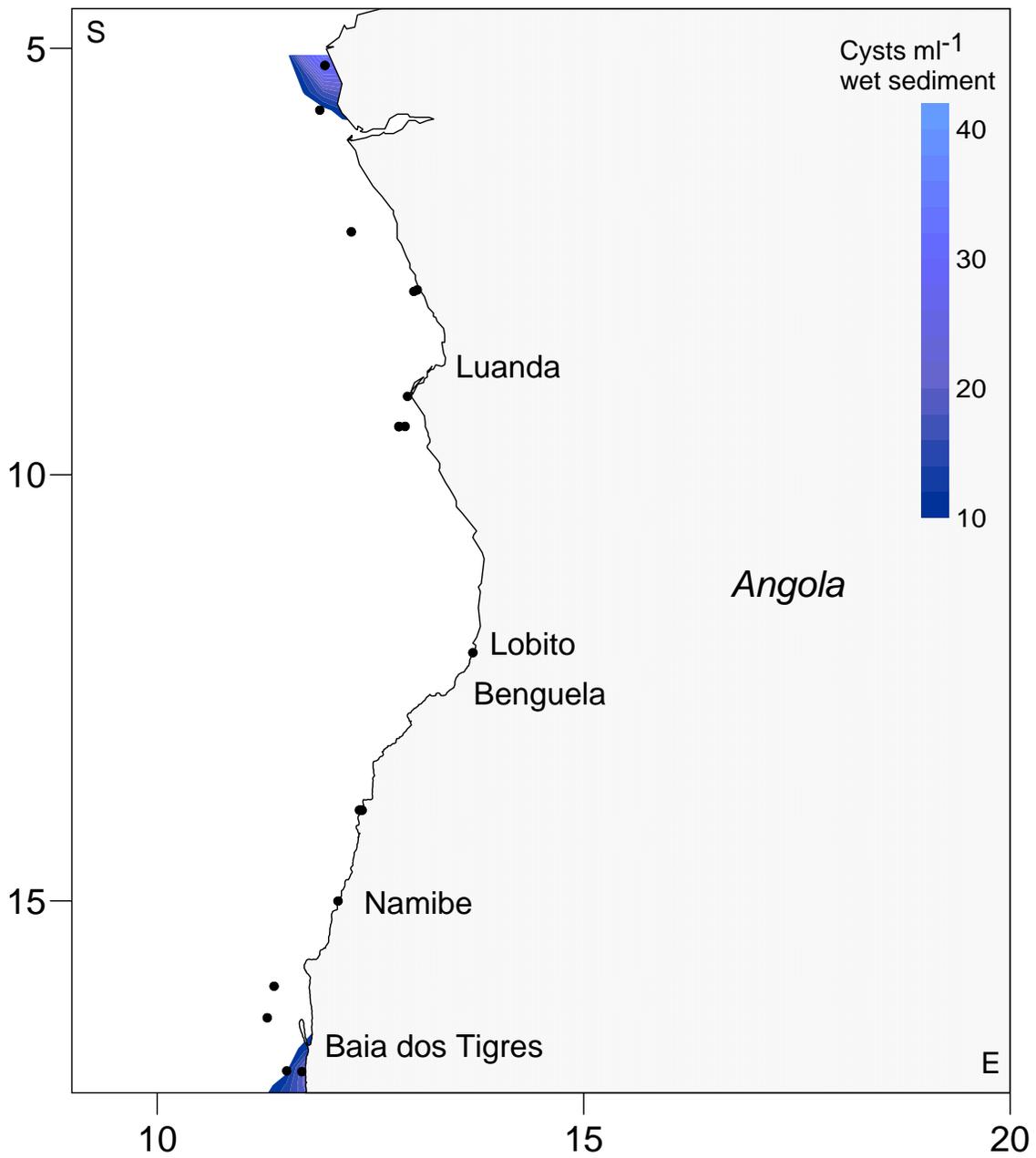


Fig 6: Horizontal distribution map of cysts of *Lingulodinium polyedrum* in Angola

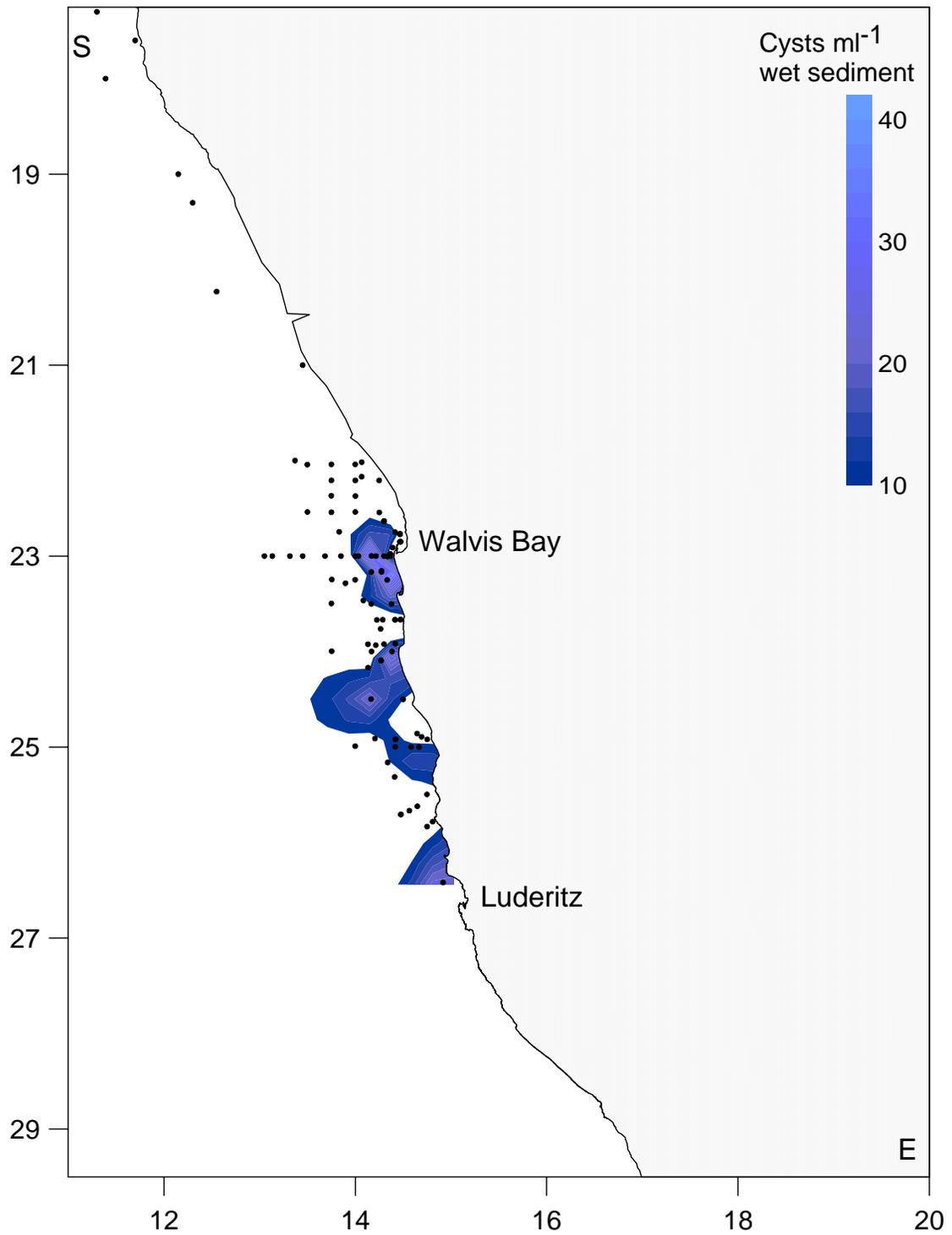


Fig 7: Horizontal distribution map of cysts of *Lingulodinium polyedrum* in Namibia

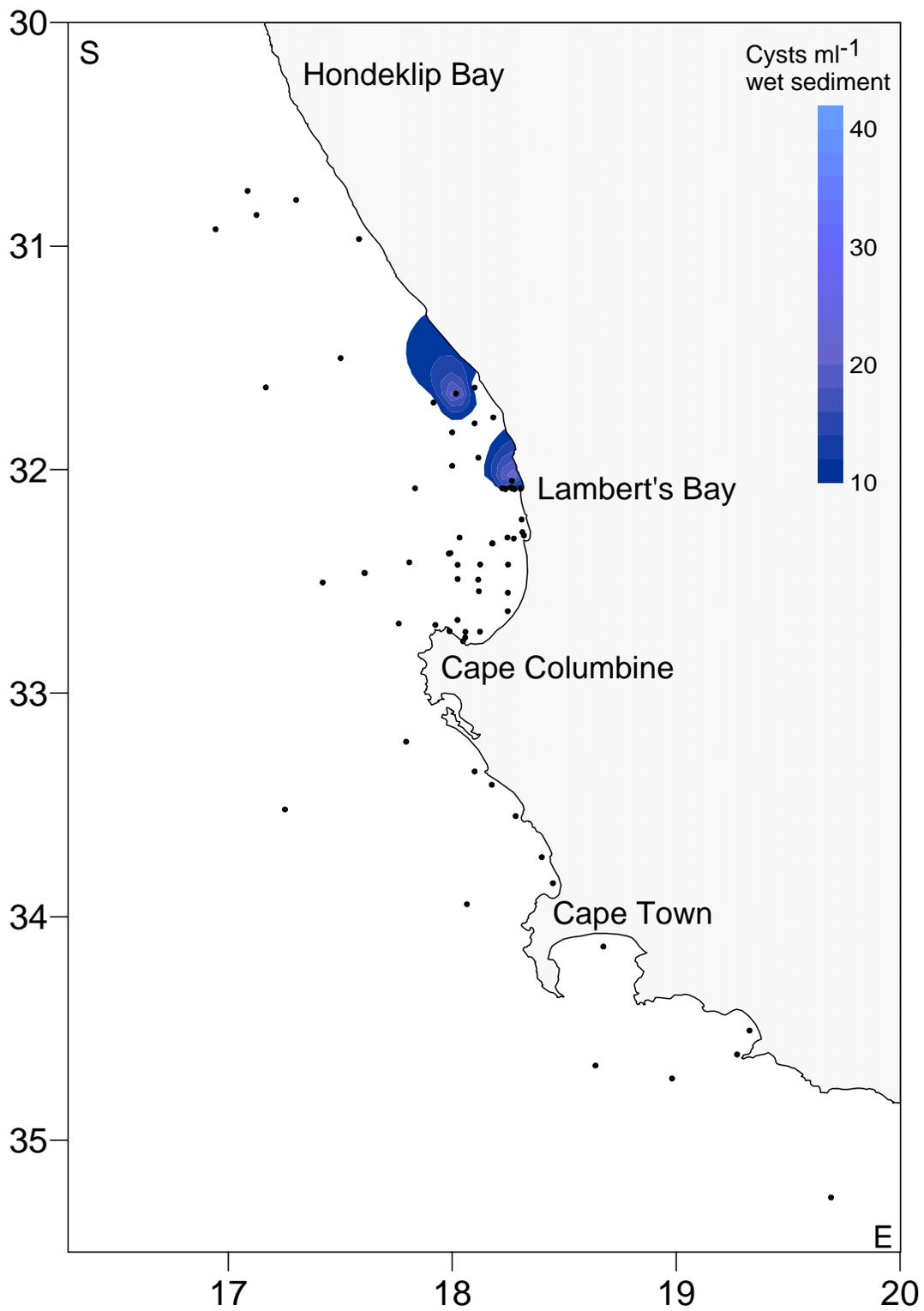


Fig 8: Horizontal distribution map of cysts of *Lingulodinium polyedrum* in South Africa

### 3.2.3. *Protoceratium reticulatum*

*P. reticulatum* is also a toxic dinoflagellate that produces YTX (Satake *et al.* 1997). *P. reticulatum* has been associated with large mortalities of both white (*Donax serra*) and black (*Choromytilus meridionalis*) mussels on the west coast of South Africa (Grindley and Nel, 1968, 1970, Horstman 1981). Initially these events were attributed to blooms of *A. catenella* (Horstman 1981). In 1966, the mass mortality of white mussels was associated with a bloom of an unknown dinoflagellate species (Grindley and Nel 1968, 1970). The responsible dinoflagellate was subsequently described as a new species, *Gonyaulax grindleyi* by Reinecke (1967) (now known as *Protoceratium reticulatum*). However, it was not clear what mechanism was involved in the death of the mussels. Toxicity tests revealed that the mussels were toxic, so the authors concluded that the source of the toxin was *A. catenella*, as it had been observed in offshore samples. In 1974, a similar mussel mortality was again associated with a bloom of *G. grindleyi* and on this occasion mussels remained toxic for 6 months without any trace of *A. catenella*, indicating that *G. grindleyi* was the probable source of the toxin (Horstman 1981). In the late-1990s Japanese scientists confirmed the toxic properties of *G. grindleyi* (Satake *et al.* 1997, Yasumoto and Satake 1998).

Cysts of *P. reticulatum* were found in the sediments of the southern Benguela and the northern Benguela regions (abundances ranged from 13-163 cysts ml<sup>-1</sup> wet sediment). Distribution maps of *P. reticulatum* have been produced for the BCLME region showing its northern and southern limits (Figs. 9-11). The northern distribution limit reaches 5°S, and the southern limit is 35°S. Within the northern Benguela region, maximum concentrations of cysts of *P. reticulatum* occur in sediments along the coast of Namibia, downstream of the Central Namibian upwelling cell. Within the southern Benguela region, maximum concentrations of cysts of *P. reticulatum* occur in sediments south of Cape Town.

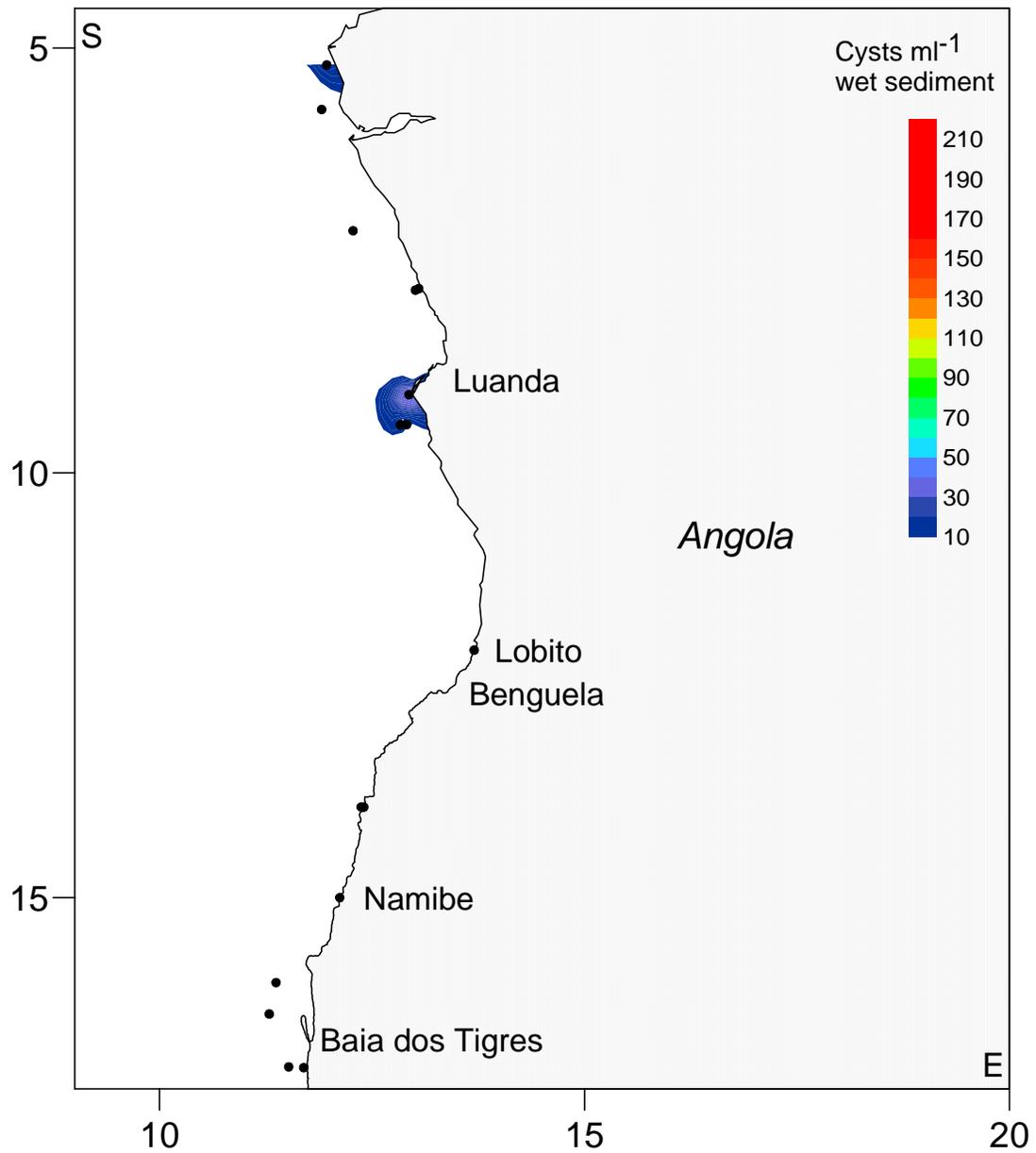


Fig 9: Horizontal distribution map of cysts of *Protoceratium reticulatum* in Angola

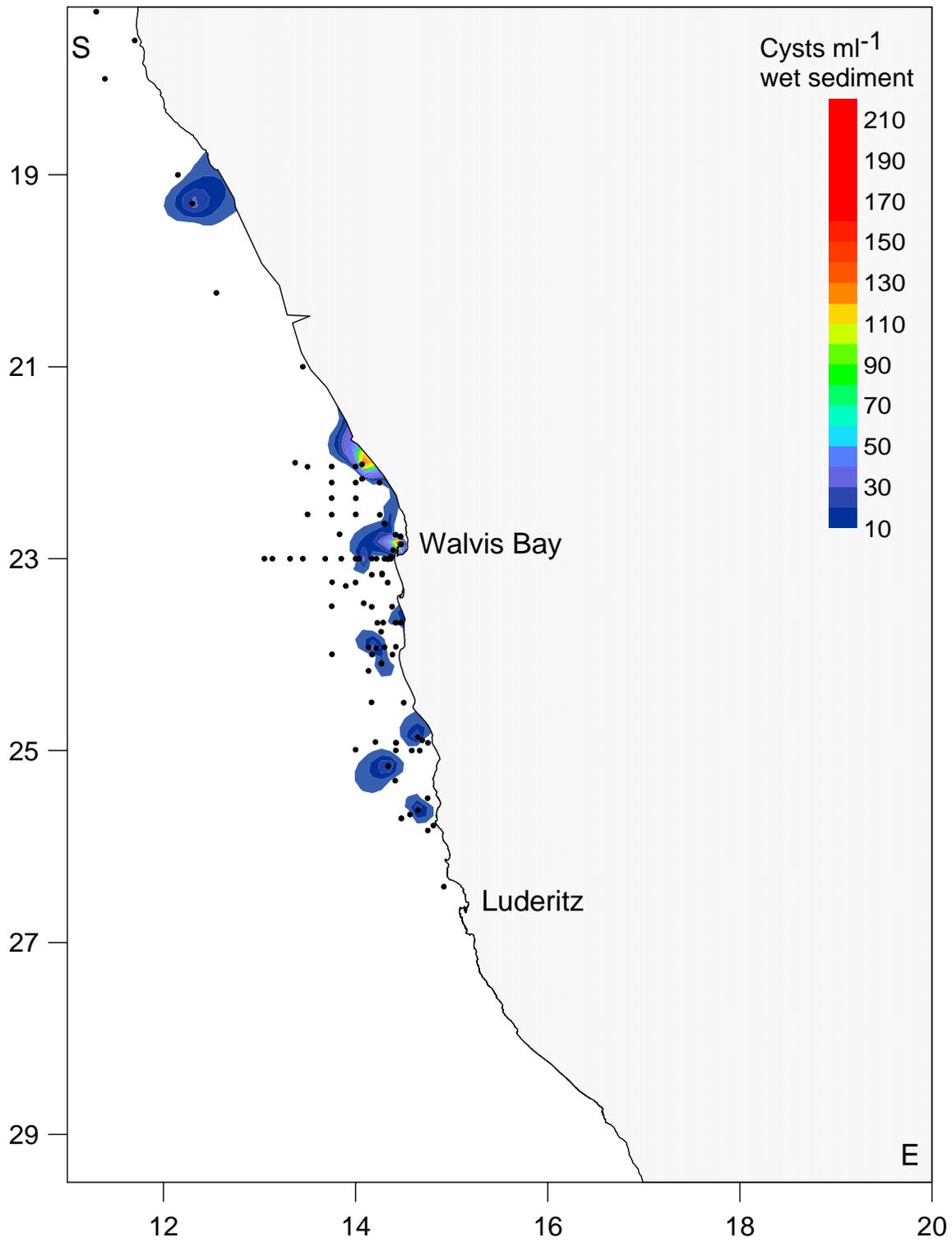


Fig 10: Horizontal distribution map of cysts of *Protoceratium reticulatum* in Namibia

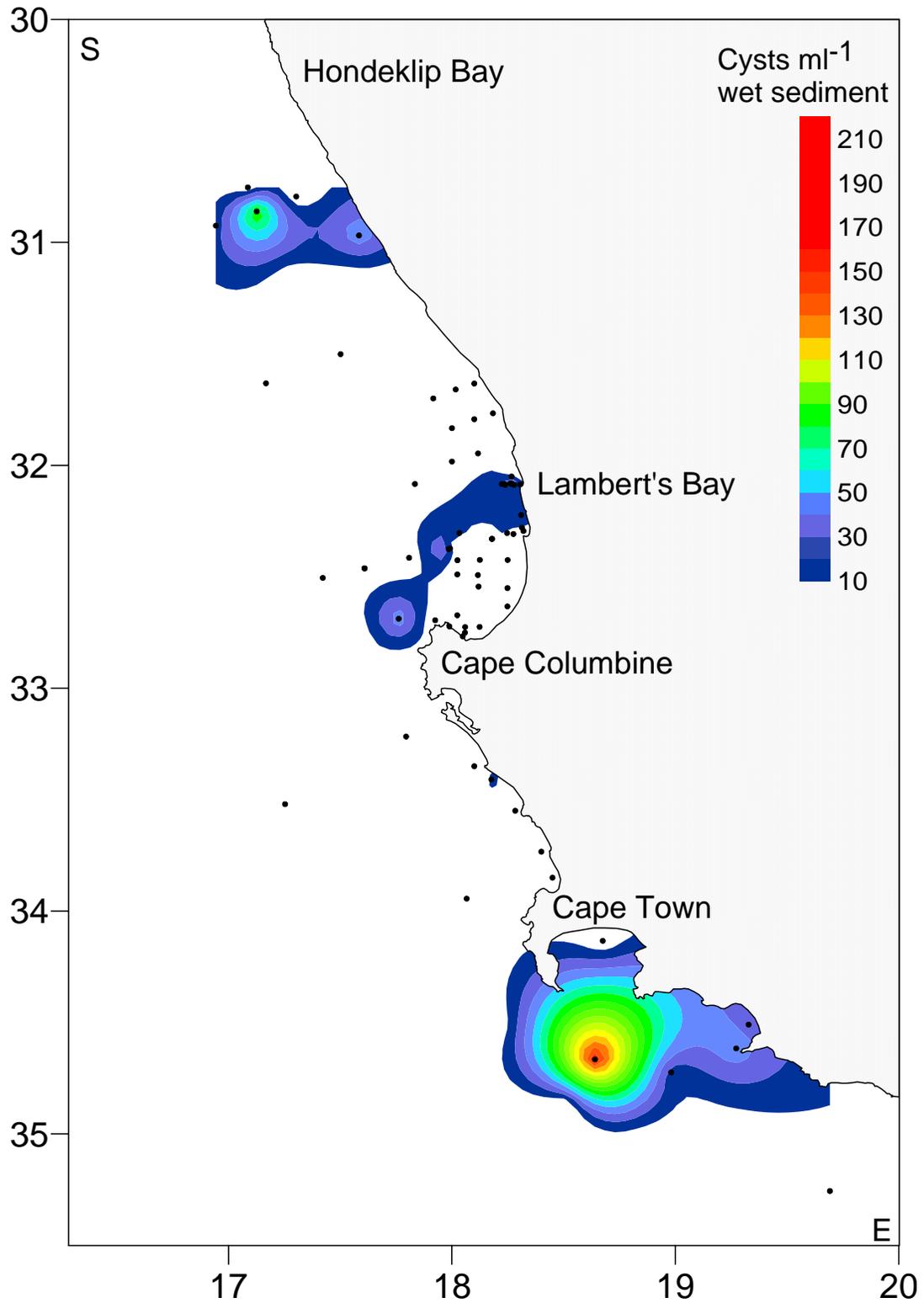


Fig 11: Horizontal distribution map of cysts of *Protoceratium reticulatum* in South Africa

### 3.3. Vertical distributions of cysts

Among the 5 sites sampled, notably different vertical profiles of cysts within the sediments were found (Figs. 12 - 16), some with cysts more numerous at the surface, others with maxima at depth. When looking at the profiles for total cysts, there were 3 noticeable peaks; core 1 at 14cm depth (3525 cysts ml<sup>-1</sup> wet sediment), core 2 at the surface (3950 cysts ml<sup>-1</sup> wet sediment), and core 3 at 7cm depth (2963 cysts ml<sup>-1</sup> wet sediment). These peaks indicate blooms, or periods of high productivity, have taken place, and all these peaks were dominated by heterotrophic protoperidinioid cysts; core 1 peak was dominated by *Zygabikodinium lenticulatum*, core 2 peak was dominated by *Protoperidinium pentagonum*, and core 3 peak was dominated by unidentified round brown cysts. Looking specifically at the vertical profiles of the 3 toxic species, it is clear that they are present down-core, with some obvious peaks associated with *A. catenella*, namely core 5 at 2.5cm depth (100 cysts ml<sup>-1</sup> wet sediment) and *P. reticulatum*, namely core 1 at the surface (213 cysts ml<sup>-1</sup> wet sediment) and core 2 also at the surface (113 cysts ml<sup>-1</sup> wet sediment), although these peaks were not exceptionally high, it is believed they correspond to the occurrence of past blooms or periods of high productivity.

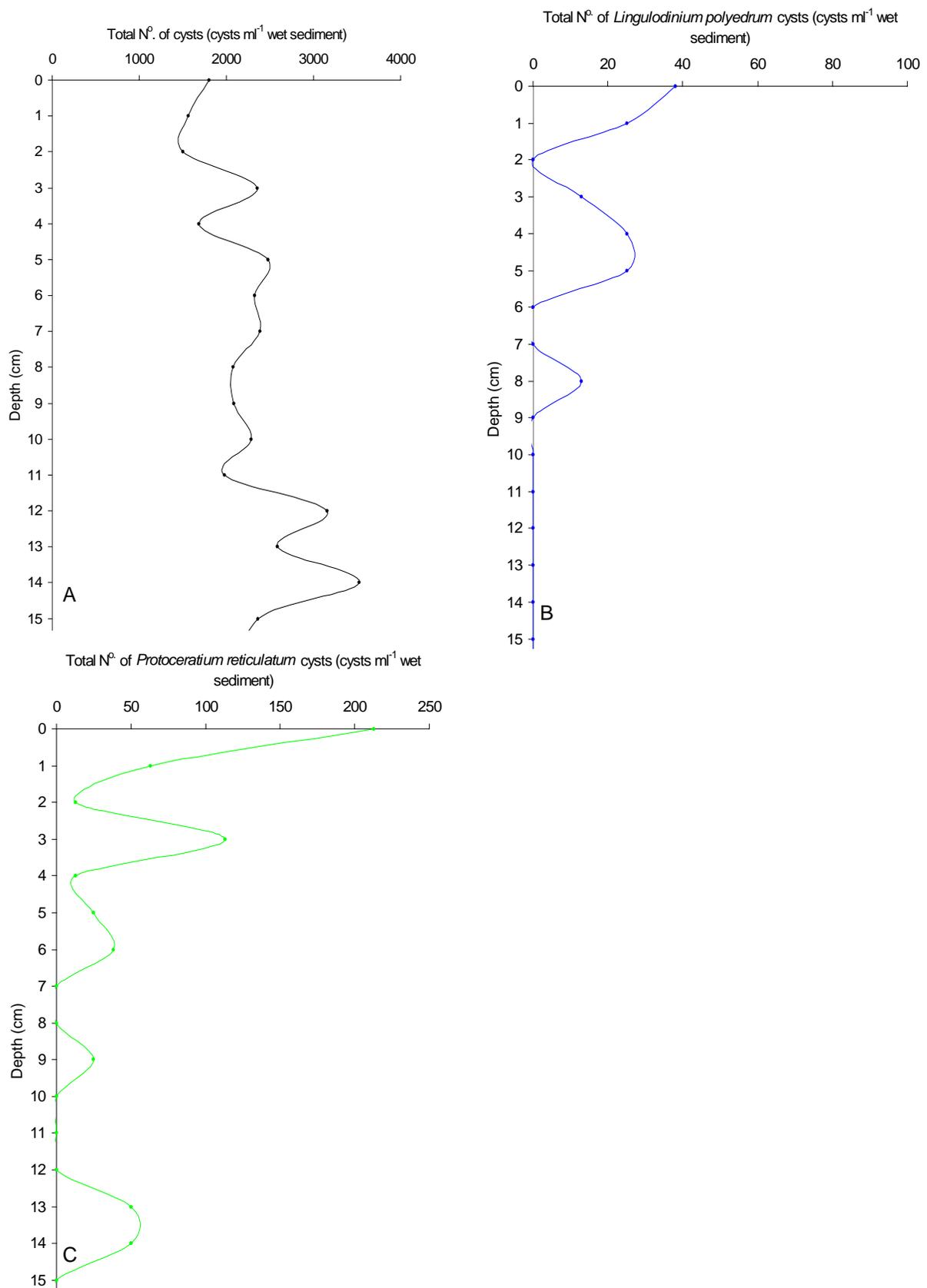


Fig 12: Core 1. Vertical profiles of cyst types in the sediments, (A) total cysts, (B) *Lingulodinium polyedrum* and (C) *Protoceratium reticulatum*

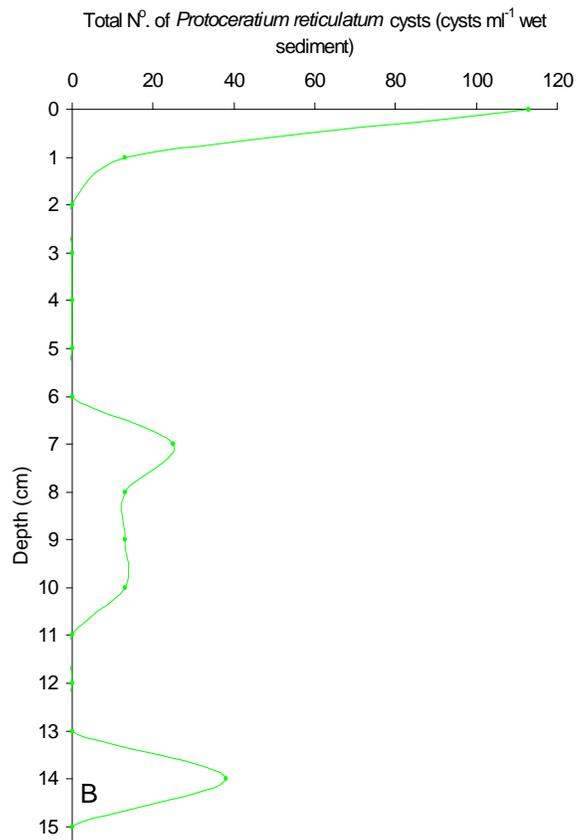
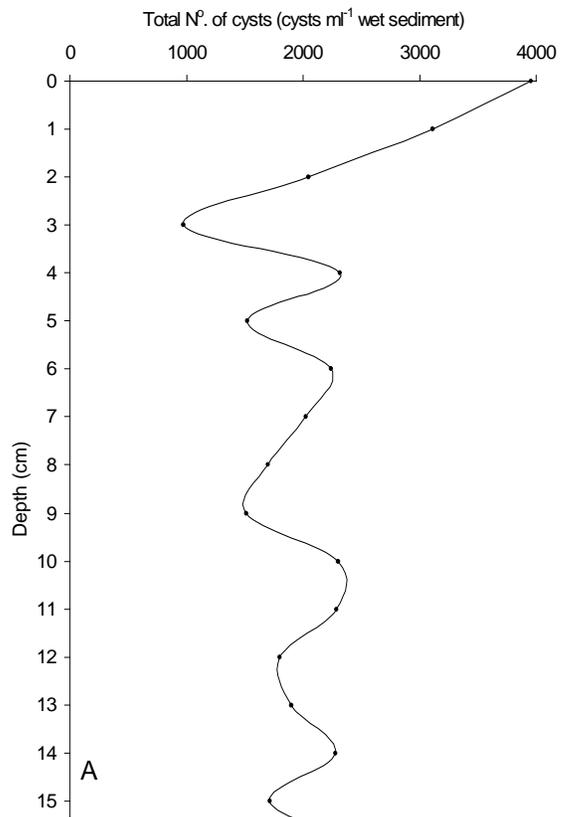


Fig 13: Core 2. Vertical profiles of cyst types in the sediments, (A) total cysts and (B) *Protoceratum reticulatum*

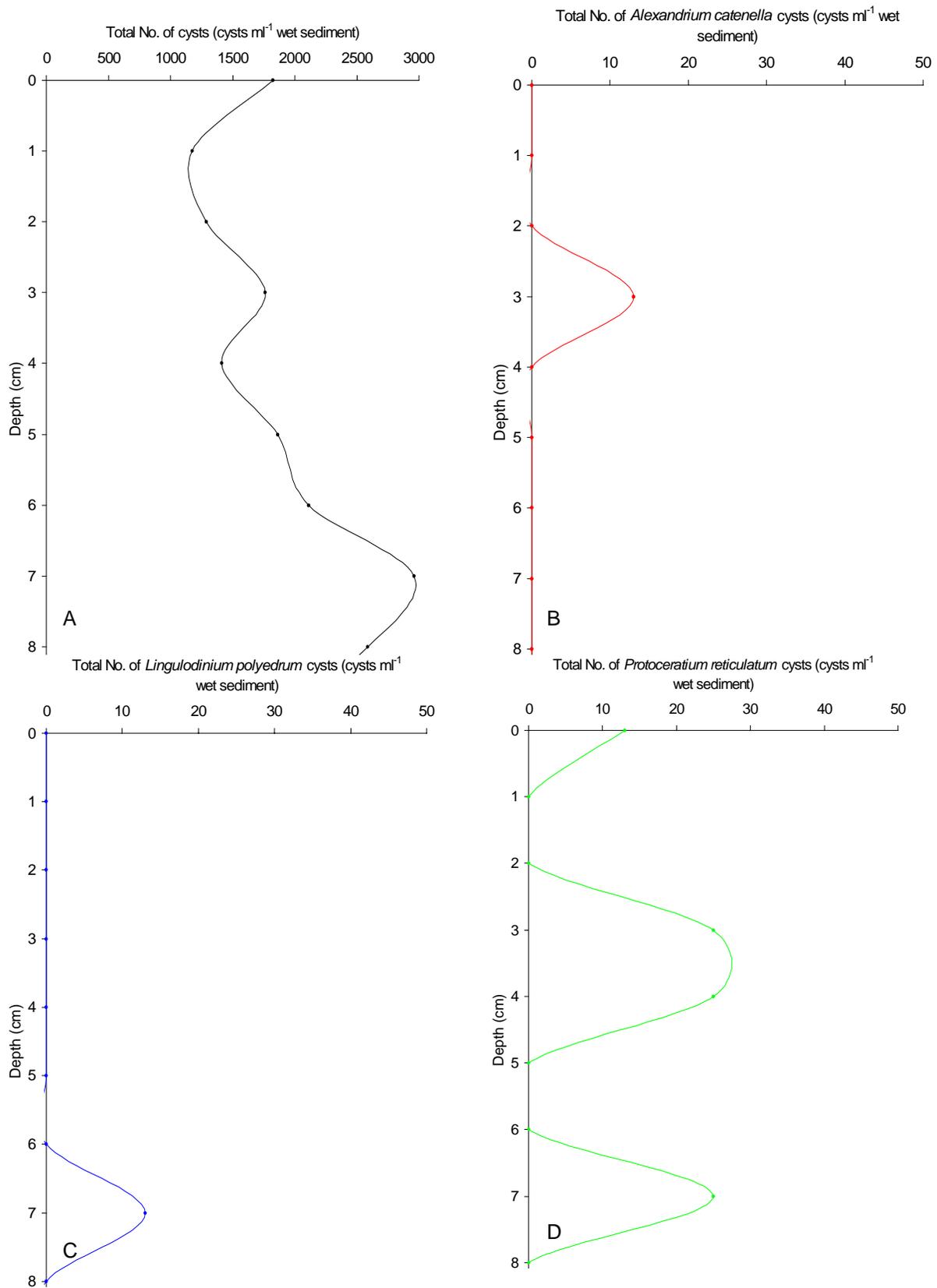


Fig 14: Core 3. Vertical profiles of cyst types in the sediments, (A) total cysts, (B) *Alexandrium catenella*, (C) *Lingulodinium polyedrum* and (D) *Protoceratium reticulatum*

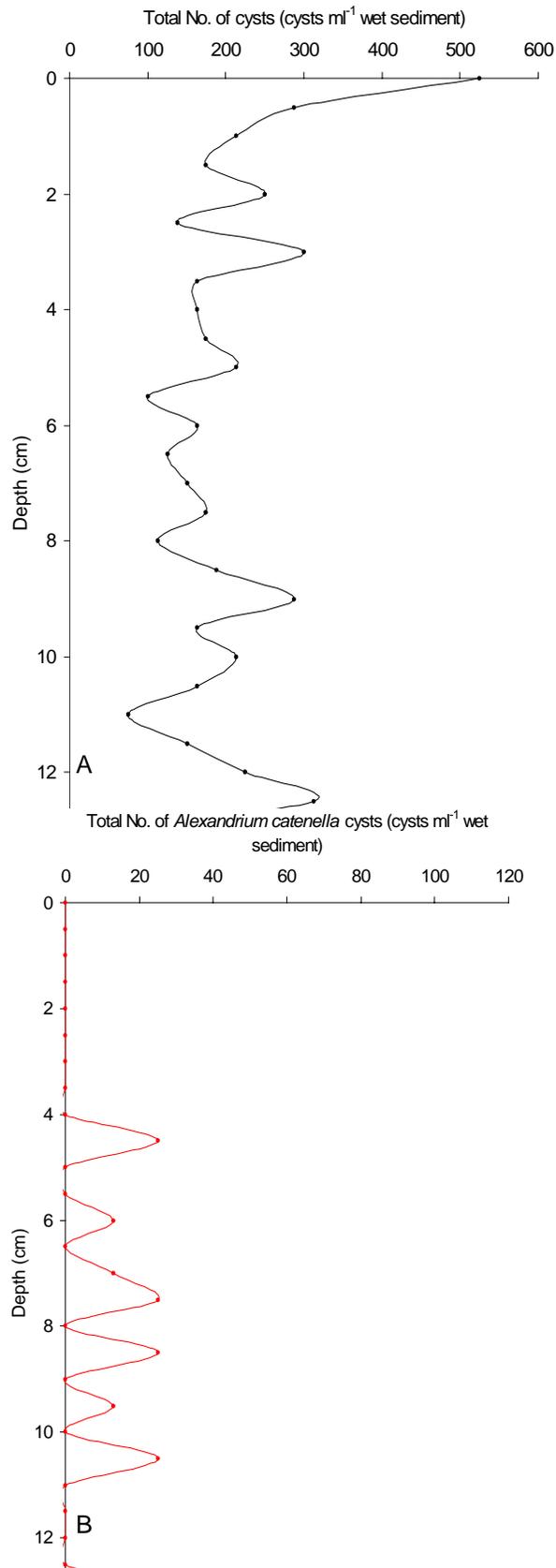


Fig 15: Core 4. Vertical profiles of cyst types in the sediments, (A) total cysts and (B) *Alexandrium catenella*

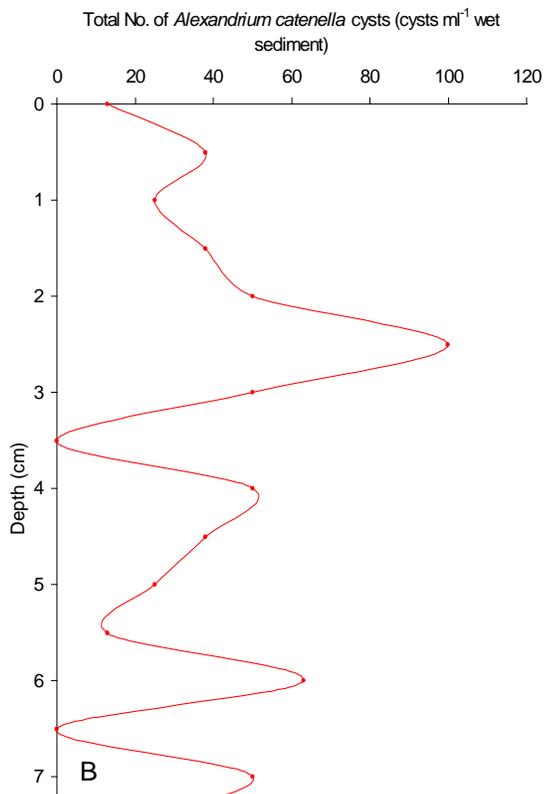
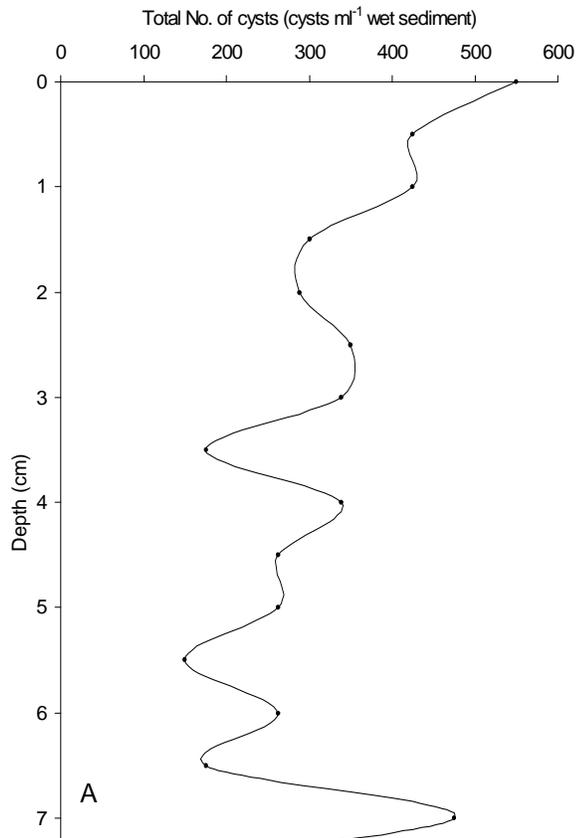


Fig 16: Core 5. Vertical profiles of cyst types in the sediments, (A) total cysts and (B) *Alexandrium catenella*

## 4. Discussion

### 4.1. Total cysts in surface sediments

Cysts of heterotrophic dinoflagellate species dominate the cyst assemblage throughout the whole of the BCLME region, the most abundant cysts being Protoperidinioid types. It has been argued (Dale *et al.* 1999) that strong coastal upwelling usually favours diatoms and other phytoplankton groups rather than dinoflagellates, and that this is reflected in the cyst record by relatively low cyst abundance and by cyst assemblages that are dominated by heterotrophic species. However, the southern Benguela region is well known for the many red tides attributed to high biomass dinoflagellate blooms (Pitcher and Calder 2000). Most of the dinoflagellates that form blooms in this area are non-cyst forming genera, e.g. *Ceratium*, *Prorocentrum*, *Dinophysis*, *Gymnodinium*, *Noctiluca*. Pitcher and Calder (2000) compiled a list of dinoflagellate species reported to form red tides, or to be toxic, from the west coast of South Africa. Of the 22 species on that list only 4 (18%) are cyst-forming species: *Alexandrium catenella*, *Protoceratium reticulatum*, *Scrippsiella trochoidea* and *Gonyaulax spinifera*. All these cyst types were found in the sediments along the west coast of South Africa. For the northern Benguela, records of blooms, and species involved, are not as detailed, but from the few records that are available it is apparent that the majority of dinoflagellate blooms in the region are also attributed to non-cyst forming species. Off the coast of Namibia, the most common genera are *Gymnodinium*, *Prorocentrum*, *Gyrodinium*, *Dinophysis* and *Ceratium* (Pitcher 1998). Off the Angolan coast, common genera are *Ceratium*, *Noctiluca*, and *Prorocentrum* (Silva 1955, Silva 2003). Thus, because the majority of the bloom-forming dinoflagellates in the BCLME region are non-cyst forming species, this subsequently leads to relatively fewer cysts from autotrophic species. As a result, the dominance of heterotrophic cyst types in the sediments can be attributed to the dominance of autotrophic dinoflagellates with no known cyst stage rather than a consequence of the dominance of diatoms and other groups of phytoplankton.

Results of this study clearly show that several more species of cysts have been found along the Namibian coast than that of the South African or Angolan coasts. Higher cyst concentrations were found in the sediments along the coast of Namibia and along the west coast of South Africa. Total cyst numbers were extremely low off Angola. Previous studies have shown that differences in cyst abundance and assemblage composition between areas are primarily caused by two factors: (1) differences in the

abundance of vegetative cells and in their cyst production efficiencies, and/or (2) differences in the sedimentary regime (Balch *et al.* 1983, Blanco 1995).

It is well known that dinoflagellate cysts behave like fine particles (Dale 1983). Therefore it is common to find higher concentrations of dinoflagellate cysts in muddy rather than sandy sediments (Nehring 1993). The continental shelf of the Benguela region is covered by layers of sediments, primarily of biological origin (biogenic). Two significant features of the Benguela shelf are the two extensive mud belts, each about 500km long. The northern mud belt lies over the middle shelf off the coast of Namibia, and the southern belt extends from the Orange River southwards. The existence of these mud belts indicates areas of high accumulation, and the observed distributions of dinoflagellate cysts and abundances coincide with the locations of the mud belts, higher abundances being found in the locale.

The low cyst abundances off Angola could possibly be due to sedimentary processes unfavourable for sedimentation along the coast of Angola, however, it is more likely to be as a result of lower productivity along the coast of Angola. Coastal waters along the majority of the Angolan coast are known to be generally less productive than waters off Namibia or South Africa. Only a few studies of phytoplankton species composition and abundance have been conducted along the Angolan coast (Silva 1953, 1955, 1958, Silva 2003, Paredes 1962), but Silva (1955) found dinoflagellate abundance overall to be low, even though it differed slightly along the coast, with Baía dos Tigres, in the south, having the lowest biomass, while Benguela and Luanda, both in the north, had marginally higher biomass. This coincides with the distribution of low cyst abundances (38-200 cysts ml<sup>-1</sup> wet sediment) found off the coast of Angola (Fig 1). The lower productivity experienced off Angola is more than likely related to the northern extent of the upwelling system. The northern boundary being defined as the Angola-Benguela Front, typically occurring between 14-17°S. Therefore, the majority of the Angolan coast does not technically fall under the direct influence of the upwelling system, which is considered extremely productive.

Higher cyst abundances were found off Namibia and South Africa in the coastal upwelling system. Slightly higher cyst abundances were observed off Namibia (0 to 3975 cysts ml<sup>-1</sup> wet sediment) than off South Africa (0 to 1650 cysts ml<sup>-1</sup> wet sediment), but this is not surprising. A characteristic difference between the northern and southern Benguela is that concentrations of chlorophyll *a* are generally higher off Namibia than off South Africa. Cyst distribution along each of these coasts appears to coincide with the

distribution of phytoplankton biomass. Lower values of chlorophyll *a* are frequently recorded around 27-28°S, the base of the Lüderitz upwelling cell, with higher values downstream of the cell. The regions of highest cyst abundances, and diversity, in sediments along the coast of Namibia are located downstream of the Lüderitz and Central Namibian upwelling cells (Fig 1).

The southern Namaqua shelf area along the west coast of South Africa is characterised by stratified conditions favourable for dinoflagellate growth, and retentive circulation patterns that facilitate the build-up of dense blooms during late summer. Within the coastal upwelling system cyst-forming dinoflagellate species appear to utilise the physical regime, to prevent washout from the coastal environment (Joyce and Pitcher 2004). Here, the dinoflagellate populations appear as surface blooms associated with equatorward flow in the region of the upwelling front, which is displaced from the coast during the active phase of upwelling (Pitcher *et al.* 1998). During these conditions cyst formation and their flux into the predominantly poleward undercurrent serves to maintain populations within their area of origin. Alternately, blooms accumulate inshore following a decline in wind stress causing cross-shelf currents to be directed onshore. Under these conditions the onshore movement of the upwelling front and associated bloom is often accompanied by barotropic poleward flow (Pitcher *et al.* 1998), thereby retaining both vegetative and cyst populations within their area of origin. The region of highest cyst abundances, and diversity, in sediments along the west coast of South Africa is located on the southern Namaqua shelf, downstream of the Cape Columbine upwelling cell (Fig 1).

These regions, off Namibia and South Africa, are especially susceptible to the formation of harmful algal blooms, often attributed to high biomass dinoflagellate blooms. Owing to these blooms and the retentive circulation characteristics of each area, cyst formation and deposition is high.

#### **4.2. Toxic species**

The mapping of dinoflagellate cysts, in particular those of toxic species, in sediments is a useful means to indicate areas where blooms have occurred or where vegetative growth may occur in the future (White and Lewis 1982, Anderson *et al.* 1982, Tyler *et al.* 1982, Anderson and Keafer, 1985). Despite the dominance of *Protoperidinium* cyst types in the sediments of the BCLME region, 3 toxic cyst species were found in the sediments; *Alexandrium catenella*, *Lingulodinium polyedrum* and

*Protoceratium reticulatum*, and it is the occurrence of these cyst types that are of particular importance to the region.

Cysts of *A. catenella* were found off Namibia and the west coast of South Africa, but the distributions are restricted to small areas. Cysts of *A. catenella* were only found at a few sites off Namibia, in the vicinities of Walvis Bay and Lüderitz, and in very low numbers (a maximum of 50 cysts ml<sup>-1</sup> wet sediment). Along the South African coast cysts of *A. catenella* are restricted to the southern Namaqua shelf, but in much higher numbers (a maximum of 238 cysts ml<sup>-1</sup> wet sediment). The high abundance of cysts of *A. catenella* found in the surface sediments of this region can be attributed to a bloom of *A. catenella* during October/November 2002. The fact that cysts of *A. catenella* were found in the sediments along the Namibian coast is interesting. Pieterse and Van der Post (1967) reported that in the northern Benguela, *Gonyaulax tamarensis*, (now *Alexandrium tamarense*), a recognized PSP-producing dinoflagellate, was one of the species regularly responsible for red tide in the Walvis Bay region. Despite frequent blooms of this species, mussel poisoning was never reported on the Namibian coast (Pieterse and Van der Post 1967). In the mid-1990s attempts to market mussels grown in the Lüderitz region failed because the cultured mussels consistently tested positive for PSP. The responsible dinoflagellate has subsequently been identified as *A. catenella*, and not *A. tamarense*. The cysts of *Alexandrium* sp. found in the sediments off Namibia were isolated, and germinated in the laboratory, and confirmed to be cysts of *A. catenella*, no cysts of *A. tamarense* were found. This study has been successful in establishing the previously unknown northern extent of *A. catenella* distribution, and it seems likely that the blooms of *A. tamarense* described by Pieterse and Van der Post (1967) were probably blooms of *A. catenella*.

The discovery of cysts of *L. polyedrum* in the BCLME region is also interesting. This species is known to be toxic and is associated with harmful events in other regions round the world (Paz *et al.* 2004). No known blooms of this species, or harmful events associated with it, have been reported for the BCLME region, and it has not even been listed as a species that occurs in the water column. The fact that cysts of *L. polyedrum* have been found in the sediments reveals that vegetative cells of this species do occur in the plankton, consequently, there is a potential risk for outbreaks of toxicity or harmful effects associated with this species. Cysts of *L. polyedrum* were more widely dispersed throughout the region, occurring along the coasts of Angola, Namibia and South Africa, but only in very low numbers (a maximum of 38 cysts ml<sup>-1</sup> wet sediment).

Cysts of *P. reticulatum* were also more widely dispersed throughout the region, again occurring along the coasts of Angola, Namibia and South Africa. Distinctive areas of higher abundances occurred along the coasts of Namibia (a maximum of 213 cysts ml<sup>-1</sup> wet sediment) and South Africa (a maximum of 163 cysts ml<sup>-1</sup> wet sediment).

The presence of these cyst species in the sediments, and in particular the areas of higher abundances of *A. catenella* and *P. reticulatum*, indicate potential seedbeds for initiation of future vegetative growth (blooms) and subsequent outbreaks of toxic episodes or harmful effects. For dinoflagellates with a distinctive cyst stage, studies of vegetative and cyst cycles, coupled with studies of their physical environment, may reveal strategies of introduction and maintenance of local populations. The ability of dinoflagellate cysts to sink rapidly (Anderson *et al.* 1985, Joyce and Pitcher 2004), and to maintain their viability in bottom sediments under adverse conditions over long periods provides many dinoflagellate species with a means of sustaining local populations. Most dinoflagellate cysts have an endogenously controlled mandatory dormancy period lasting from weeks to months, during which germination is not possible (Anderson *et al.* 1985, Ishikawa and Taniguchi 1996). Following the dormancy period, the germination of cysts is likely to depend on a range of external factors (Nehring 1993), and the number of germinating cysts may be considerably high. Within an upwelling system there are several physical mechanisms that may potentially resuspend cysts, thereby introducing them into the photic zone and so contributing to the development of future vegetative populations.

#### **4.3. Vertical distributions of cysts**

Quantitative reconstruction of the history of dinoflagellate blooms requires a good understanding about the relationship between the magnitude of a motile dinoflagellate bloom population and the abundance of resting cysts produced by that population. Despite the common use of dinoflagellate cysts to reconstruct phytoplankton productivity, few studies have investigated the quantitative relationship between the size of motile dinoflagellate blooms and the abundance of resting cysts. In comparing plankton tow data with surface grab samples from Trondheimsfjord, Dale (1976) found that some cysts appeared over- or under-represented relative to motile cell numbers. Motile populations of *Protoceratium reticulatum* are believed to produce relatively large numbers of cysts (1:2 cyst:theca ratio) while *Gonyaulax digitale* and protoperidinioid species are much less prolific cyst producers (1:500 and 1:120, respectively). Similar

results (Lewis 1988, Anderson and Keafer 1985) suggest that *Lingulodinium polyedrum* and *Alexandrium tamarense* produce relatively few cysts (ca. 4:1000). Nonetheless, Keafer *et al.* (1993) were still able to show that bloom years can usually still be discerned as peaks of cysts, and non-bloom years have much lower cyst abundances.

Results from the 5 cores show that significant numbers of dinoflagellate cysts were found below the sediment surface. There were 3 discernable peaks for total cysts present (core 1, core 2 and core 3) indicating that blooms had taken place. These peaks were dominated by heterotrophic protoperidinioid species. The observed vertical profiles of the 3 toxic cyst types found were somewhat inconclusive, all that was really highlighted was that these toxic species were present down-core, indicating that these species have been present in the BCLME region for a number of years. There were some obvious peaks associated with *A. catenella* and *P. reticulatum*, but not with particularly high abundances, however, it is believed the observed peaks correspond to the occurrence of past blooms or periods of high productivity. These two species have formed blooms in the region previously, but due to the lack of records, especially in the northern Benguela, the size (cells L<sup>-1</sup>) of the majority of these blooms are unknown. The observed vertical profiles of cysts are likely to be as a result of differences in germination, predation, settling characteristics or biological reworking (bioturbation) (Keafer *et al.* 1993, Ishikawa and Taniguchi, 1996).

An important question that needs consideration is, how deep in the sediments can cysts occur yet still pose an excystment threat? Cysts in sediments can remain viable for many years, germination is delayed or inhibited due to anoxic conditions, temperature gradients in the sediments, or other unknown inhibitory mechanisms possibly present in the benthos. Sediment disturbing processes, e.g. bioturbation, scouring by bottom currents, tidal and storm surge and fishing activities, could expose and resuspend buried cysts. Biological reworking can occur down to depths of 20cm (Marcus and Schmidt-Gengenbach 1986), this level of animal activity can create a mixed zone with a significant water content, given the vertical cycling of materials in this layer and the high water content, it seems possible that subsurface cysts could perhaps participate in the bloom seeding process (Anderson *et al.* 1982, Nehring 1996). However, the biological and physical mechanisms underlying surface and subsurface cyst concentrations in the sediments of the BCLME region are not known.

## 5. Conclusion

HABs in the Benguela region pose a threat over an extensive area spanning the coastlines of Angola, Namibia and South Africa. There is a great disparity in the amount of available information on HABs within the majority of the Benguela region, especially the northern Benguela. Results from this project have achieved a good baseline of information on the occurrences of dinoflagellate cysts, with respect to the diversity, distribution and abundance of species. The distribution and abundance patterns of cyst assemblages in the sediments appear to reflect production more than anything else. Lower cyst concentrations were found off Angola as a result of lower productivity related to the northern extent of the upwelling system. Higher concentrations of cysts were found off Namibia and South Africa, as a result of higher productivity related to the coastal upwelling system. The distribution and abundance of cysts also indicate that certain areas are more suitable environments for the accumulation and retention of cysts, e.g. the areas downstream of the Lüderitz and Central Namibian upwelling cells (Namibia) and downstream of the Cape Columbine upwelling cell (South Africa). Of particular importance are the cysts of HAB species within the surface sediments of the BCLME region. 3 toxic species were found, and given the widespread distribution and in particular the areas of higher cyst abundance of these species, which could act as seedbeds, there is a potential risk with respect to initiation of future vegetative growth (blooms) and the associated outbreaks of PSP from *Alexandrium catenella*, or other toxic episodes, namely YTX poisoning from *Lingulodinium polyedrum* and *Protoceratium reticulatum*.

## Acknowledgements

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## EXPLANATION OF PLATES

Scale bars = 20 µm.

### Plate 1

(1a – b) *Spiniferites delicatus*; (2a – b) *Spiniferites ramosus*; (3a – 5c) unidentified *Spiniferites* spp.; (6a – d) *Lingulodinium polyedrum*

### Plate 2

(1a – l) *Nematosphaeropsis* sp.

### Plate 3

(1a – h) *Protoceratium reticulatum*; (2a – h) *Alexandrium catenella*

### Plate 4

(1a – h) *Polykrikos kofoidii*; (2a – h) *Polykrikos schwartzii*

### Plate 5

(1a – e) *Scrippsiella lachrymosa*; (2a – b) *Scrippsiella precaria*; (3a – b) *Scrippsiella ramoni*; (4a – d) *Scrippsiella trifida*; (5a – f) *Scrippsiella trochoidea*; (6a – e) unidentified *Scrippsiella* sp.

### Plate 6

(1a – g) *Protoperidinium americanum*; (2) *Protoperidinium avellanum*; (3a – d) *Protoperidinium claudicans*; (4a – c) *Protoperidinium compressum*; (5) *Protoperidinium conicoides*

### Plate 7

(1a – f) *Protoperidinium conicum*; (2a – d) *Protoperidinium denticulatum*; (3) *Protoperidinium latissimum*; (4a – e) *Protoperidinium leone*

### Plate 8

(1a – f) *Protoperidinium oblongum*; (2a – f) *Protoperidinium pentagonum*

**Plate 9**

(1a – h) *Protoperidinium subinerme*; (2a – b) unidentified *Protoperidinium* sp 2;  
(3a – b) unidentified *Protoperidinium* sp 3; (4a – d) unidentified *Protoperidinium* sp 4; (5)  
unidentified Round Brown cyst; (6a – g) *Zygabikodinium lenticulatum*

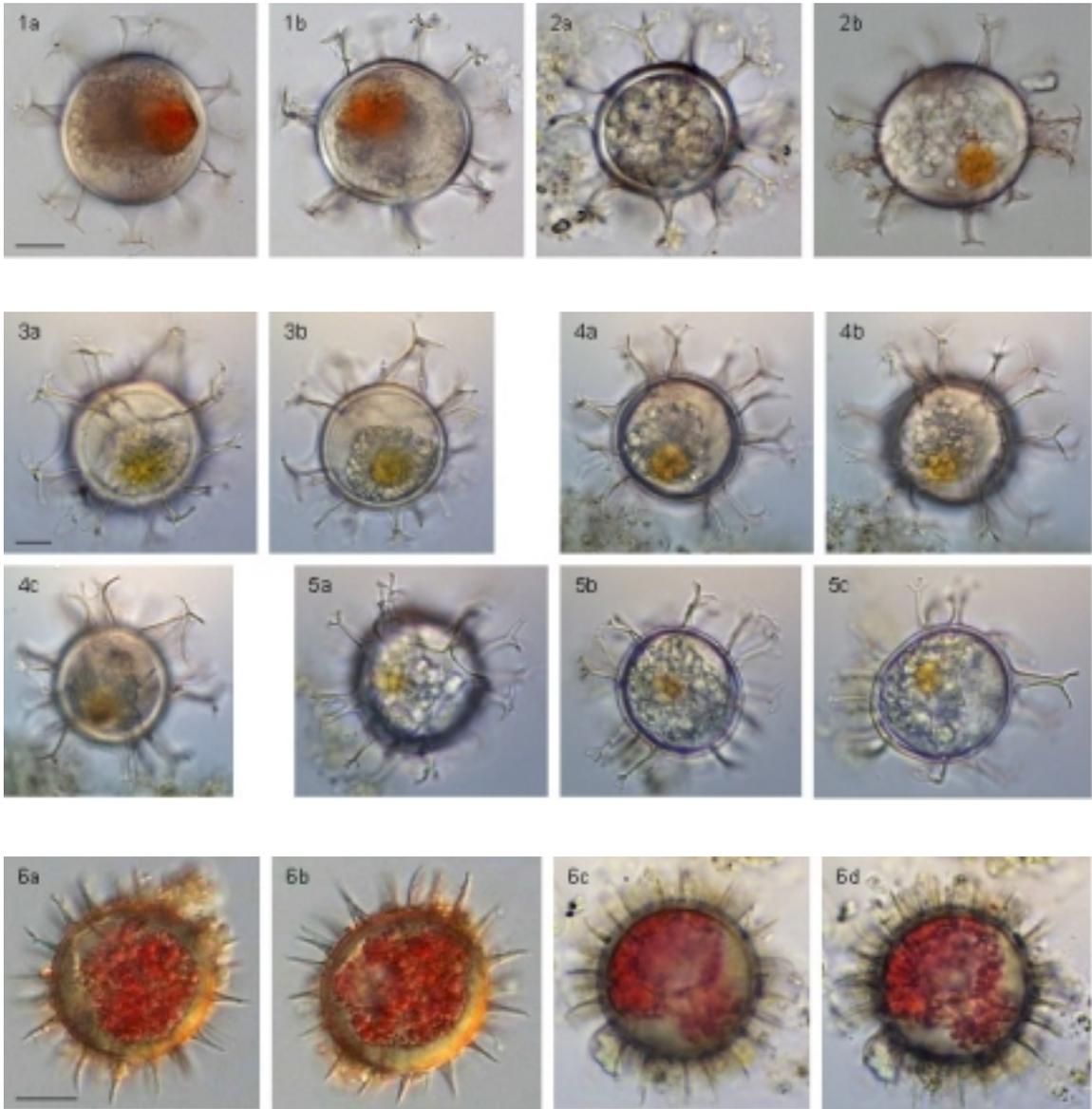
**Plate 10**

(1a – b) unidentified sp 1; (2a – d) unidentified sp 4; (3a – b) unidentified sp 5;  
(4a – b) unidentified sp 7; (5a – b) unidentified sp 8; (6a – h) unidentified sp 9

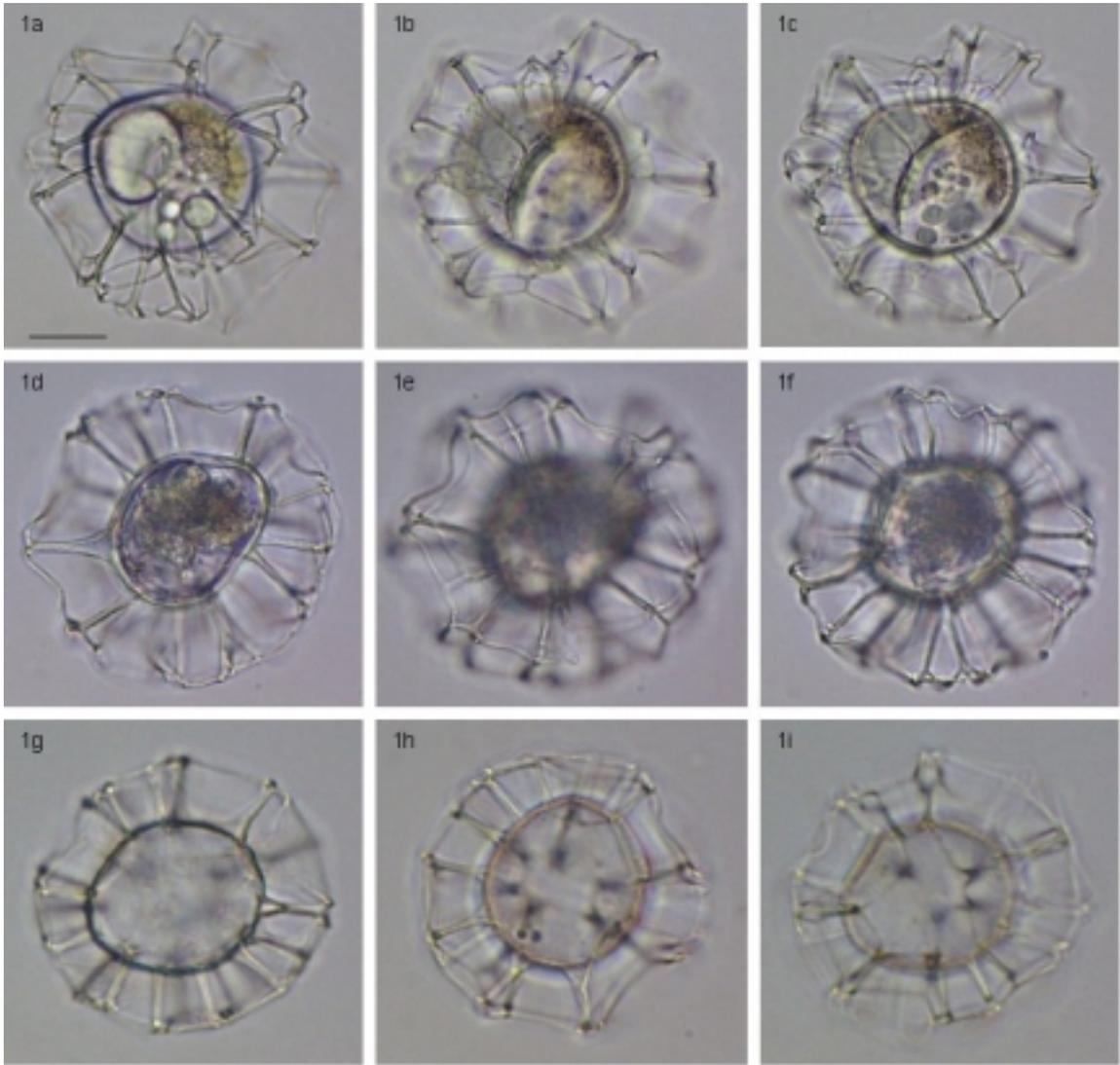
**Plate 11**

(1a – c) unidentified sp 11; (2a – b) unidentified sp 12; (3a – c) unidentified sp 13;  
(4a – b) unidentified sp 14; (5a – c) unidentified sp 15; (6a – b) unidentified sp 16;  
(7a – b) unidentified sp 17

Plate 1



**Plate 2**



**Plate 3**

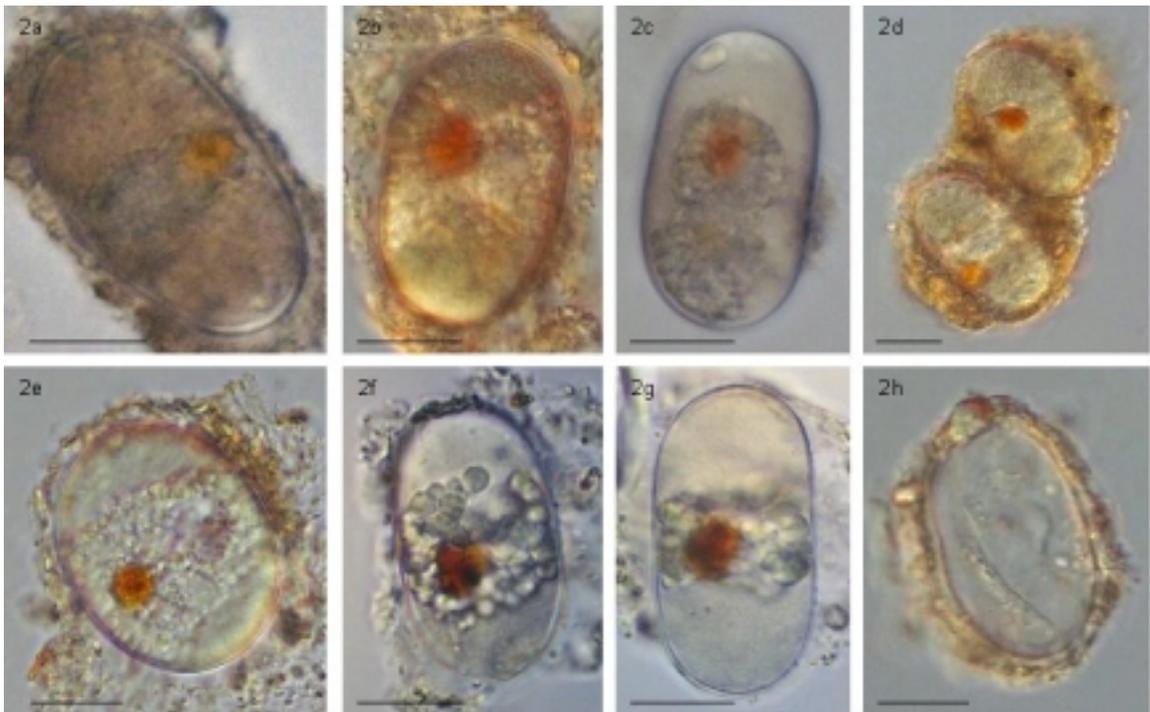
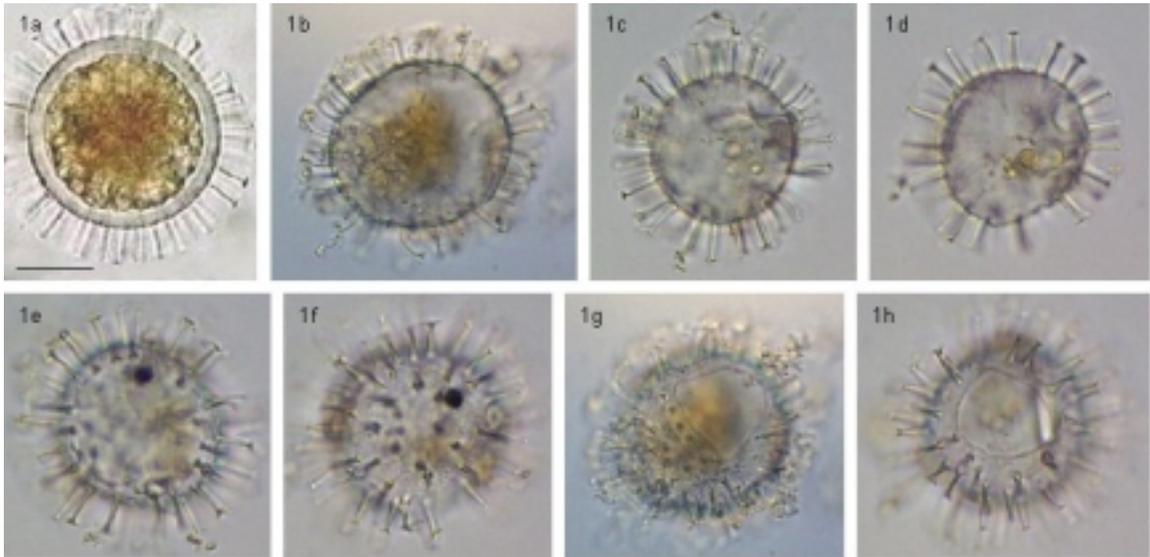


Plate 4

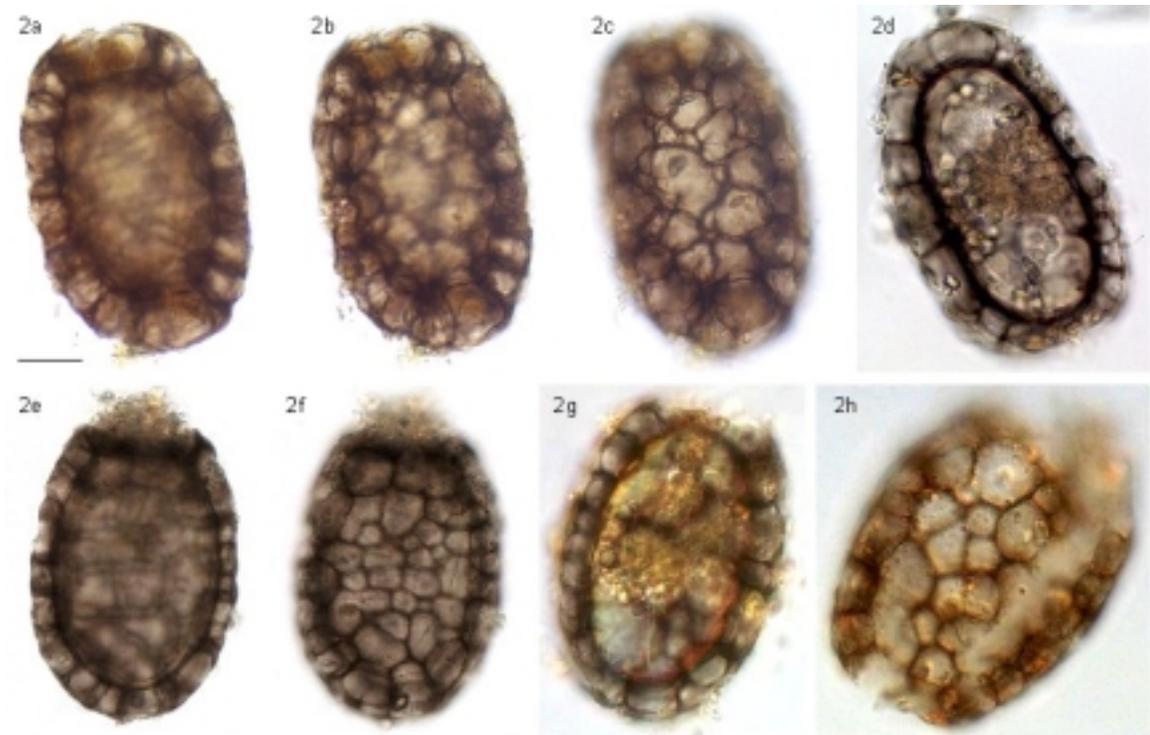
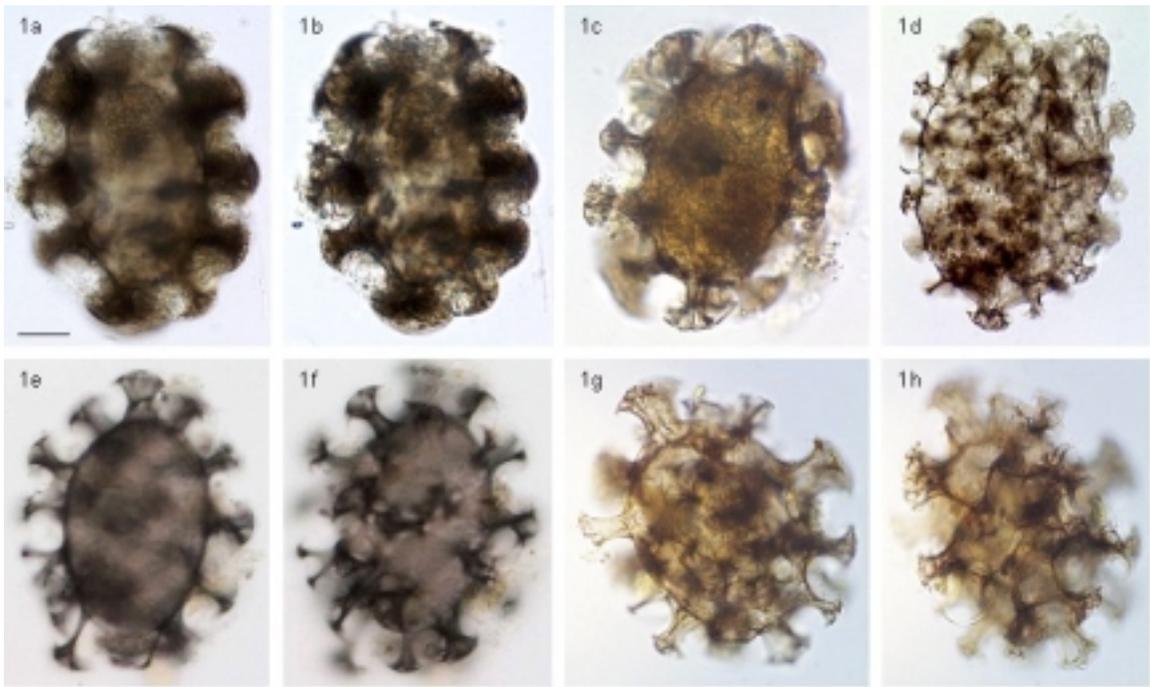


Plate 5

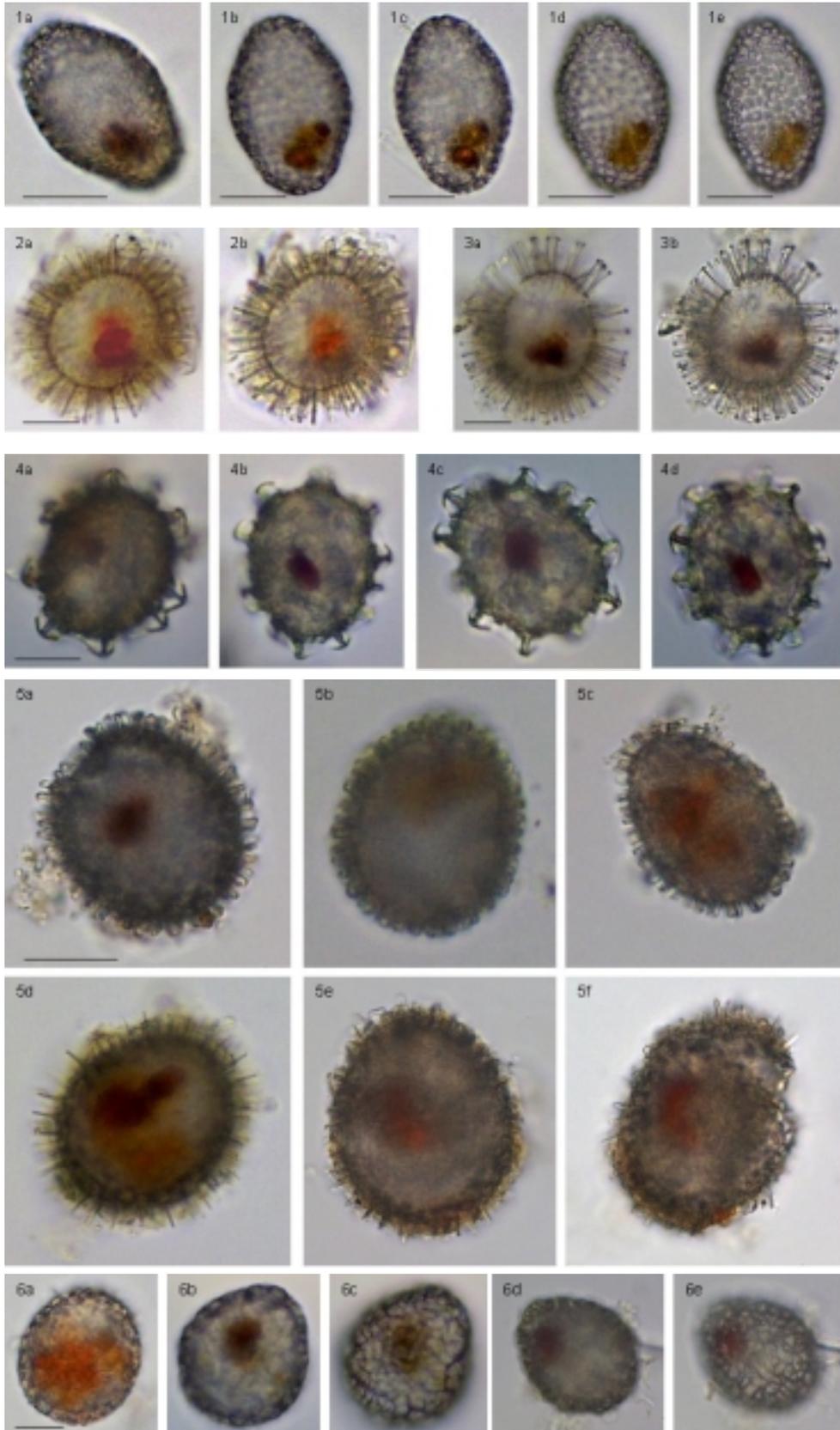


Plate 6

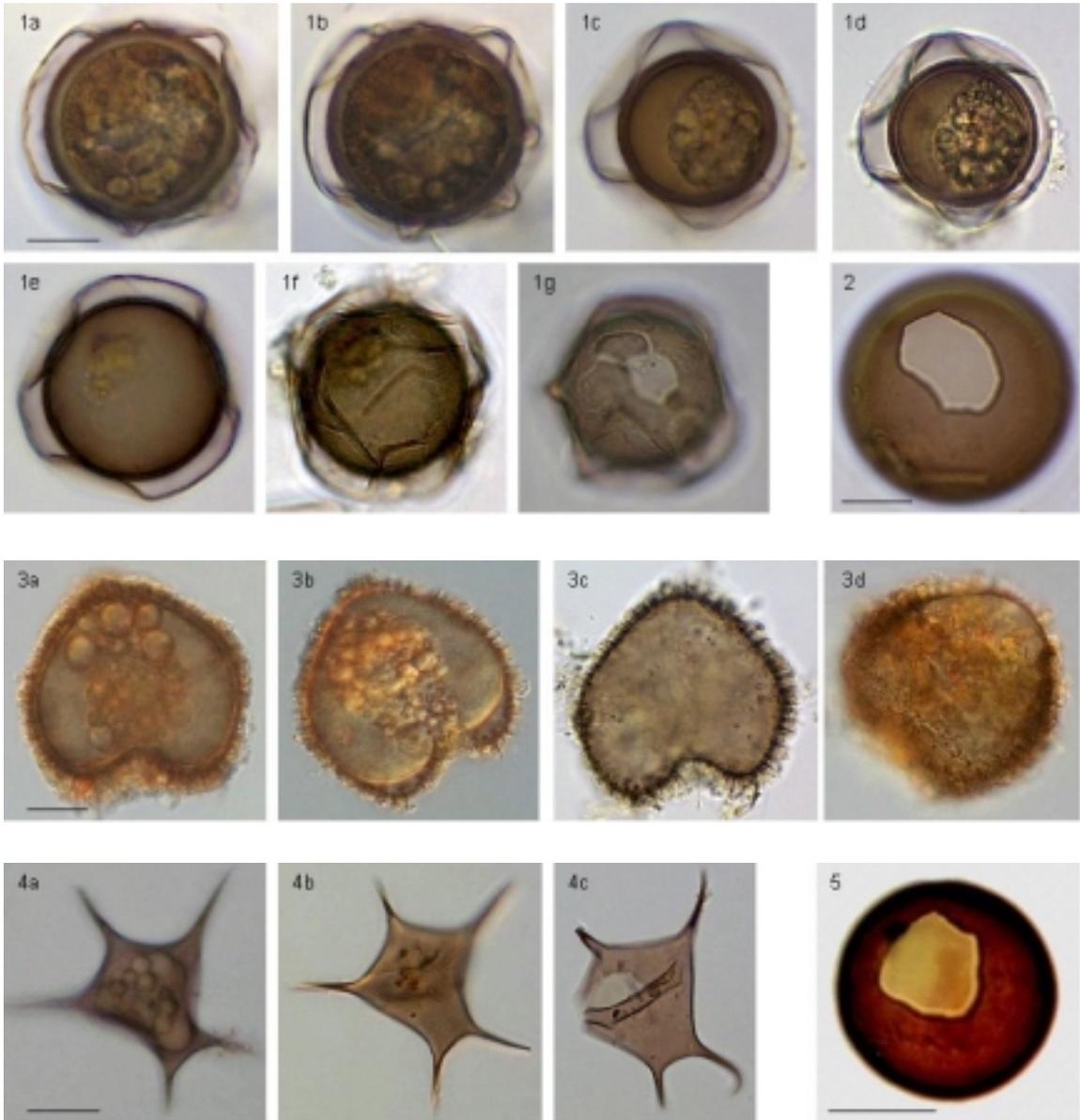
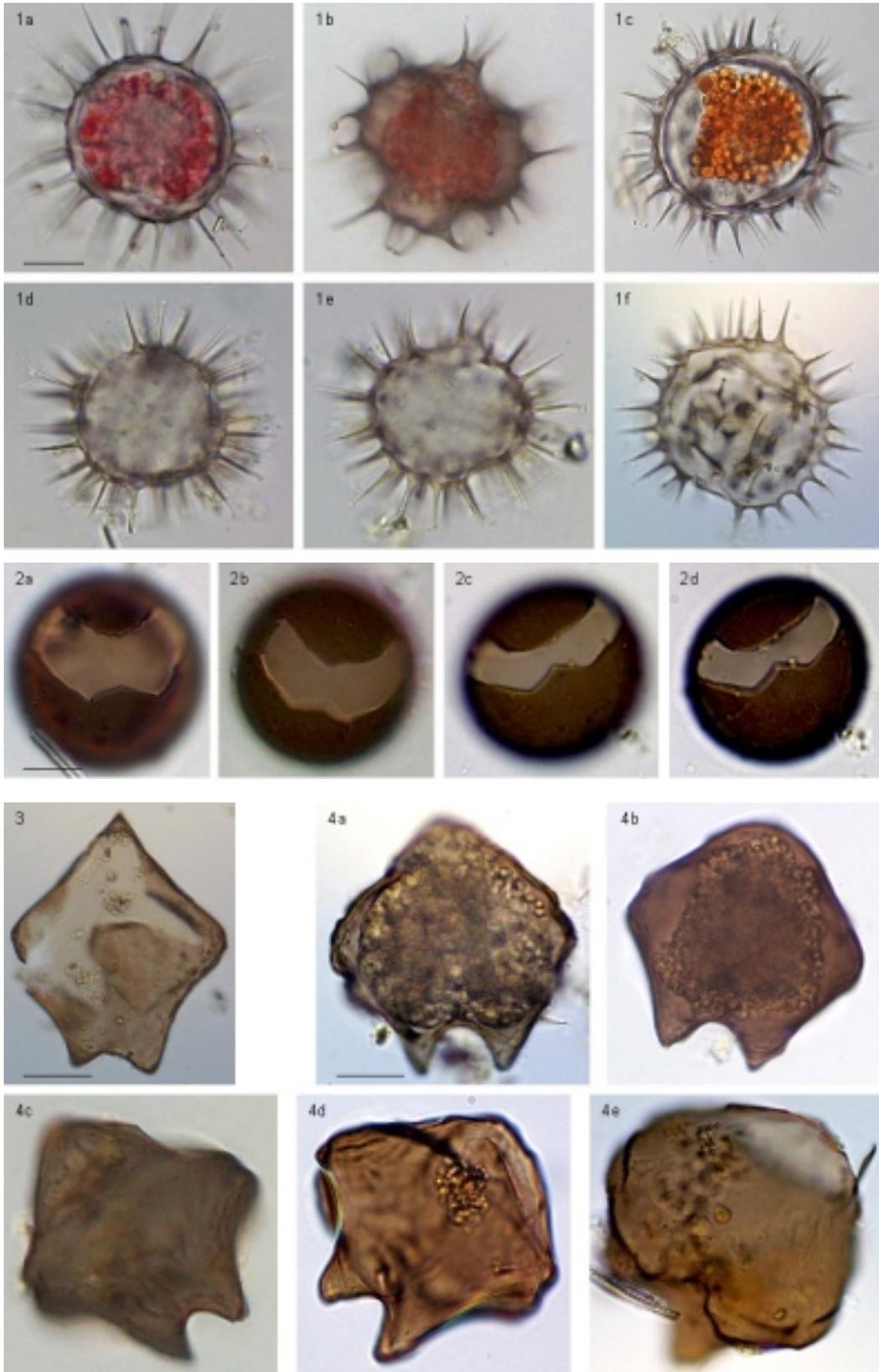


Plate 7



**Plate 8**

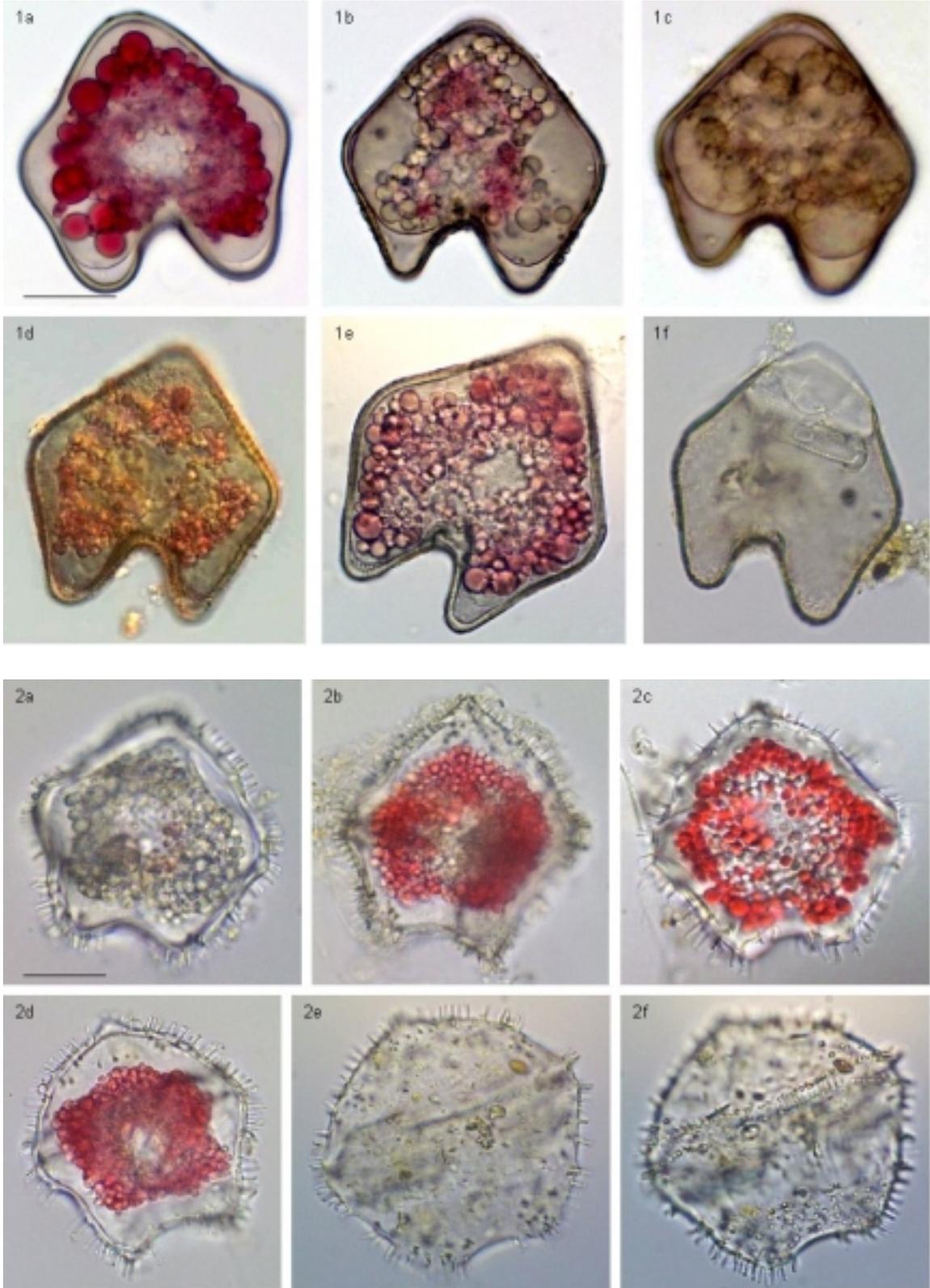
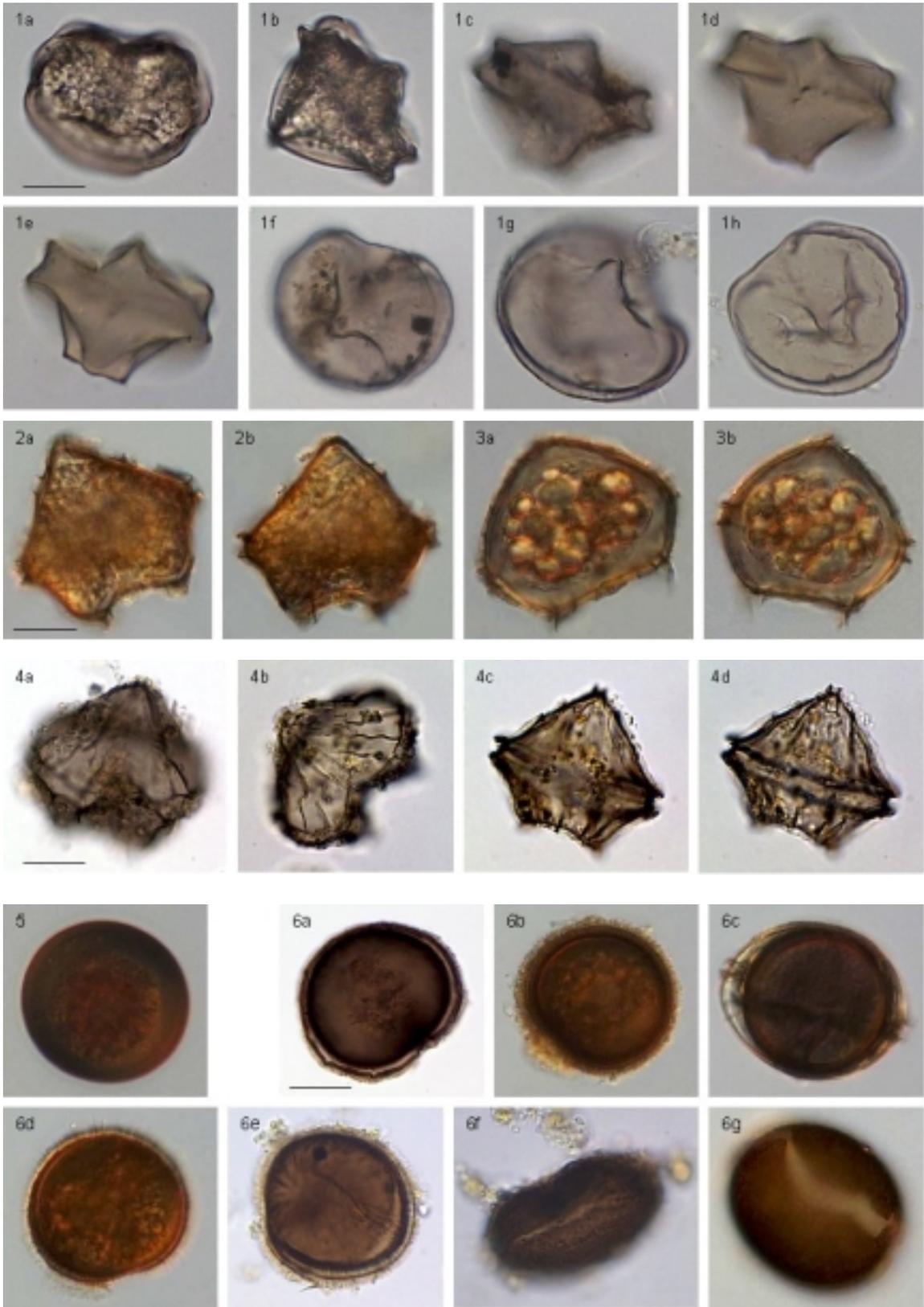


Plate 9



**Plate 10**

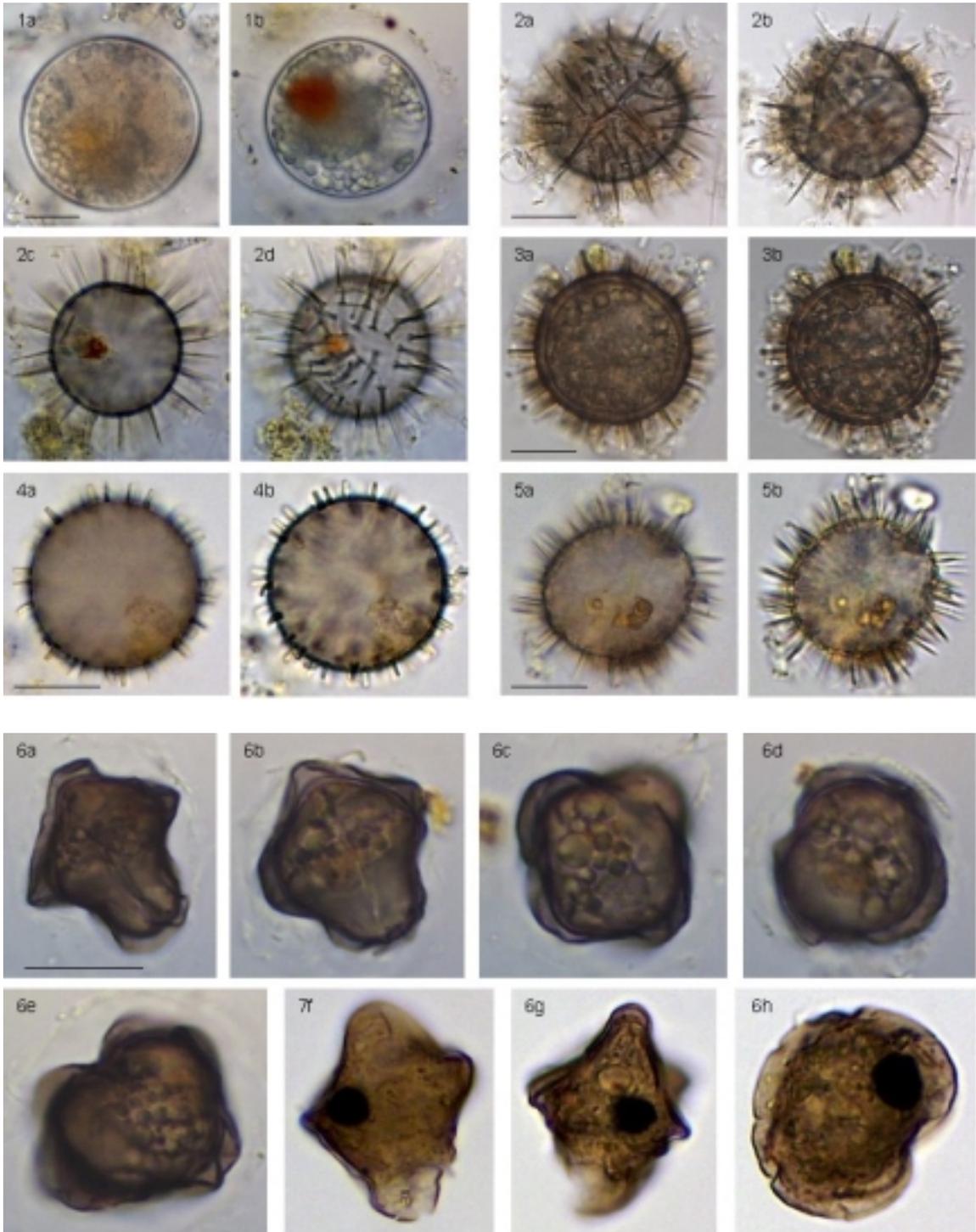
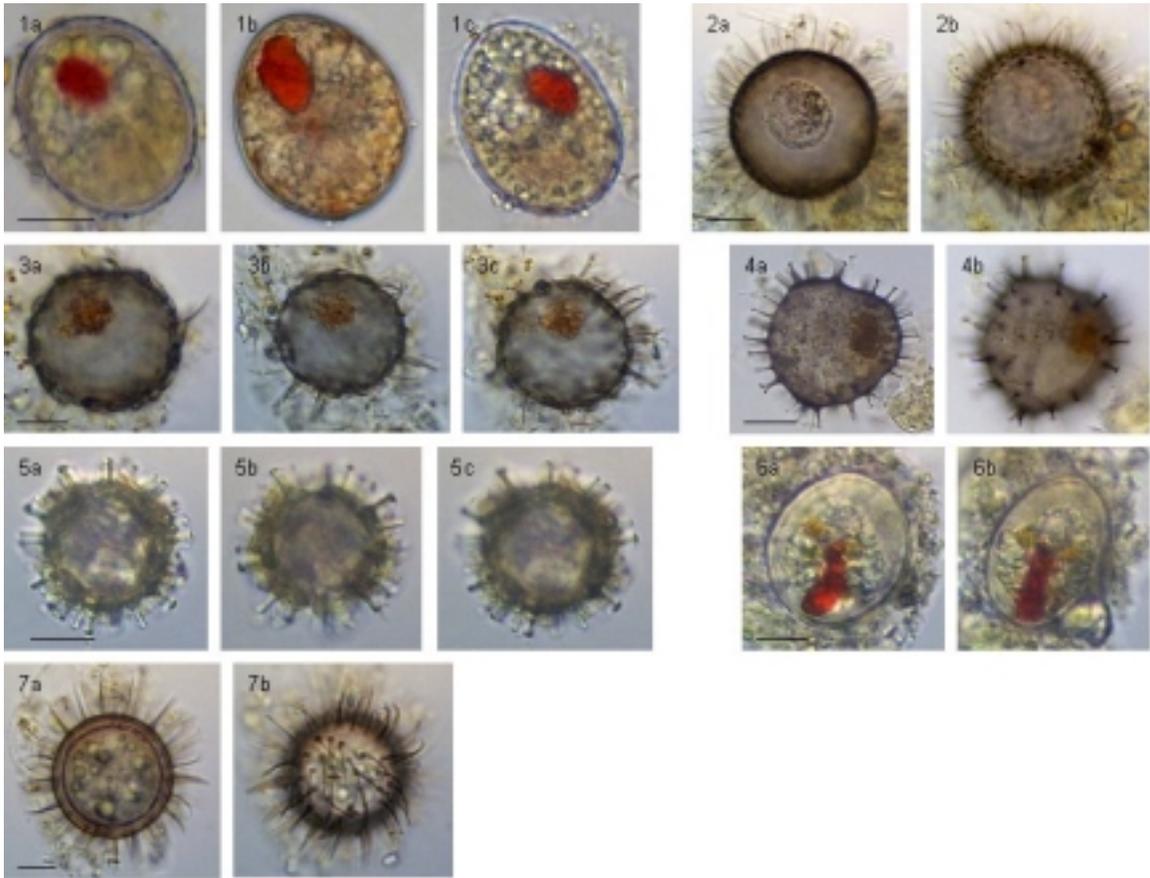


Plate 11



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## APPENDIX 1

### Contributions in kind to the project

#### ***Collection of sediment samples***

All the sediment samples from Angola and, the majority of the Namibian samples were collected using 'ships of opportunity'. The samples were collected during the *Alexander v. Humboldt* – Angola-Benguela Expedition (AHAB) Cruise, January to June 2004, specifically on Legs 3, 4, 5, 7, 8, and 9. A total of 119 samples were collected, equating to 119 hours of ship time (1 hour per sample). Financially, the in-kind contribution is estimated as; ZAR **396,666.00** for ship time (at an average cost of ZAR 80,000.00/24hr); ZAR **47,600.00** for man hours sampling (at an average cost of ZAR 200.00/hr, with 2 people per sample).

Some samples from Namibia were also collected by staff from MFMR, Namibia. A total of 13 samples were collected by MFMR staff, equating to 13 hrs of ship time. Financially, the in-kind contribution is estimated as; ZAR **32,500.00** for ship time (at an average cost of ZAR 60,000.00/24hr); ZAR **3,400.00** for man hours sampling (at an average cost of ZAR 200.00/hr, with one person sampling).

Sediment samples previously collected by L. Joyce and MCM during 2001 - 2003 from along the west coast of South Africa were made available and incorporated into this project. Samples were collected on cruises run by MCM, using the *FRS Africana* and *Sardinops*. A total of 23 samples were collected on the *Africana*, and 28 samples on the *Sardinops*, equating to 23 and 28 hrs ship time respectively. Financially, the in-kind contribution is estimated as; ZAR **146,666.00** for ship time (at an average cost of ZAR 80,000/24hr for the *Africana*, and 60,000/24hr for the *Sardinops*); ZAR **20,400.00** for man hours sampling (at an average cost of ZAR 200.00/hr, with 2 people per sample).

After some samples had been collected they were couriered to Cape Town and the courier service was paid for by MFMR and/or MCM. Financially, this in-kind contribution is estimated at ZAR **3,000.00**.

Assistance from G. Pitcher (direct input and advice) totalled 50 hours, financially, this in-kind contribution is estimated as; ZAR **15,000** (at an average rate of ZAR 300.00/hr for a senior scientist).

The total financial costing of in-kind contributions to this project is estimated at ZAR **665,232.00**.

**Summary of contributions in-kind to the project**

	<b>Cost (ZAR)</b>
<b>Ship time</b>	
- Alexander v. Humboldt	396,666
- MFMR	32,500
- Africana & Sardinops (MCM)	146,666
<b>Man hours sampling</b>	
- Alexander v. Humboldt	47,600
- MFMR	3,400
- Africana & Sardinops (MCM)	20,400
<b>Shipment of samples</b>	3,000
<b>Assistance from Associate Investigator (G. Pitcher – MCM)</b>	15,000
<b>Total financial costing of in-kind contributions</b>	<b>665,232</b>

## APPENDIX 2

### Problems encountered

#### ***Capacity building and training***

It was initially proposed that a training workshop would be held at the University of the Witwatersrand (WITS), Johannesburg, South Africa (to be convened by Dr Stuart Sym), the aim of the workshop being to build capacity in taxonomic skills. The dates for this workshop were never finalised, but it was intended to be held in August/September 2004. As part of this workshop the Lead Investigator of this project was to contribute a series of lectures on cyst sampling techniques, taxonomy and identification. Unfortunately, the capacity building component of this project never happened, largely due to the fact that the group at WITS were not successful in tendering for the project, so the workshop fell through.