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FULL LENGTH ARTICLE

Benthic dinoflagellates from Red Sea, Egypt: Early records

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Abstract Dinoflagellates from Red Sea are hardly studied, in particular the benthic forms. Samples collected from shallow intertidal zone, Ain Sokhna, Egypt were microscopically examined. Three genera with seven species were recorded. The most frequently-encountered was *Katodinium* sp., a small mushroom-like with epitheca being consistently larger than hypotheca. Light micrographs revealed the presence of a nucleus in the hyposome and descending cingulum. Scanning electromicrographs (SEM) confirmed this orientation and revealed the presence of apical pore system. Another species showed similarity to the mushroom-like morphology but with large conical episome and small hyposome. Heterotrophic, naked *Gyrodinium* cf *dominans* and *Gyrodinium* sp. were also observed where in the former, there were conspicuous longitudinal striations. A frequently-observed species had naked *Gyrodinium*-like morphology but with much smaller size. One photosynthetic species had a characteristic stigma similar to type B eyespot in "dinotoms" and episome being slightly larger than hyposome. *Gymnodinium* sp. with sulcus extending slightly in the episome but deeply to the end of hyposome was also recorded. This genus is reported to be mostly toxic and its presence should be monitored. Finally, this study presents some early records for benthic dinophytes from rather underexplored locality and raises alerts about genus with reported toxicity.

Introduction

Dinoflagellates are unicellular eukaryotic protists with characteristic morphological features upon which their taxonomic characterization relies (Hoppenrath et al., 2009). The shape and extent of the sulcus and the cingulum displacement are morphological characters that have taxonomic value at the genus and species levels. Similarly, the absence of thecal plates underneath the plasma membrane or their presence and the pattern of tabulation are all morphological characters that are taxonomically informative (Hoppenrath et al., 2009, 2014). Dinoflagellates are mostly marine (nearly 90%) and only 10% are freshwater. The majority of dinoflagellates are planktonic and only small percentage is benthic (Hoppenrath et al., 2014). Benthic dinoflagellates are usually found in the interstitial spaces between sand grains and in the intertidal shallow zone where mixing is observed. Benthic dinoflagellates are found epiphytically on macroalgae and corals in the intertidal and subtidal zones. (Hoppenrath et al., 2014). Benthic naked dinoflagellates and thinly-thecate species are usually

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too delicate to handle, too tiny to observe and collect and too difficult to culture as they usually feed on living preys. In the past, studies focused mainly on basic morphology and fine structure description. Nowadays, many dinoflagellate taxonomists adopt a combined taxonomic approach for more accurate description of taxa (Takano and Horiguchi, 2004; Moestrup and Daugbjerg, 2007; Hoppenrath et al., 2012). This approach overcomes pitfalls resulting from using single aspect of identification. It encompasses general morphology, fine structural studies and molecular and phylogenetic analyses to give a holistic picture of the taxa involved and allow the construction of robust taxonomy (Takano and Horiguchi, 2005). Red Sea is one of the richest water bodies with unique biodiversity that is largely underexplored (Zakaria and Al-Shehri, 2011). There are few studies that focused on certain species of benthic dinoflagellates from Red Sea, but not from Ain Sokhna, Egypt where the study took place, e.g. (Catania, 2012 who studied dinoflagellates from Saudi Arabia; Saburova and Chomérat, 2014 who studied dinoflagellates from Gulf of Aqaba but not from Egypt) but no intensive comprehensive studies on those communities were found. It is noteworthy that there has been a dramatic increase in recent revelations of toxicity of certain benthic dinoflagellates such as *Ostreopsis ovata* (Faimali et al., 1985) and *Gambierdiscus* (Catania, 2012). Moreover, there are some reports on the occurrence of dinoflagellates in the Egyptian Mediterranean waters (Aleem, 1993; Ismael and Halim, 2012) we therefore investigated some of the species present in the Red Sea coastal area located 80 km on Cairo-Suez road.

Materials and methods

Sample collection and examination

Sampling was performed in October 2014 (Autumn) from Ain Sokhna area, Suez Governorate approximately 120 km east of Cairo, Egypt (for map see Fig. 1). The latitude and longitude coordinates of the study area are 29.6000° N and 32.3167° E. Sampling was aimed at the intertidal zone from bottom to top in a vertical manner. Samples were taken from a depth of 60 cm. Microscopic examination was performed using Leica inverted microscope and supplemented with Leica suite application software. Epifluorescent microscope was used (oil immersion lens) with differential interface contrast accessories. For morphometric measurements, tables of calibrations of ocular micrometer with slide micrometers were used.

Scanning electron microscopy

Fixation in Lugol's solution (AppliChem, Germany) was performed on dinoflagellate cells, either picked or used directly from raw samples, for a week to ensure sedimentation of all dinoflagellates (Morton, 2001). The fixed sample was then loaded onto 5 μm isopore membrane (TMT PO1300, Milipore) and washed with distilled water twice for 10 min to prevent the formation of salt crystals from remaining sea water. The cells mounted on the filter were then subjected to dehydration with ethanol series (30–50%, 70–85–95%) once at each concentration for 10 min. The final step of dehydration with ethanol using 100% concentration was repeated twice. At each step, the ethanol was loaded into syringe connected to the filtration unit and injected gradually before stopping with some ethanol still in syringe for ten minutes after which the rest of ethanol was injected and the filtrate was discarded (Sampedro et al., 2011). This allows through rinsing of sample. The hexamethyldisilazane (HMDS), previously used by Jung et al. (2010) as dehydrating agent was then used. The HMDS was loaded with a syringe into filtration unit, allowed to stand for an hour, then the rest was injected through and the filtrate was discarded. The filter-containing sample was left to dry at room temperature for twenty minutes. The sample was put in an oven for five minutes at 55 °C. The membrane was then mounted onto electron microscope stub (0.5″ Aluminum specimen stub G301, Agar scientific) using glued double-face sticker (Plano, Germany). The stub was then inserted in gold–palladium sputter for coating with metal under argon saturated atmosphere. When a vacuum was created the sputtering started at 40 mA for 150 s and the sample was ready for electron microscopy. The Scanning electron microscope (Tescan-Vega, Germany) supplied with VEGA3-software was used to visualize cells at 15–20 kV.

Epi-fluorescence test of plate pattern

For epifluorescence microscopy, cells were stained with 1% calcofluor white (Sigma) solution. A drop of stained cell solution was placed on a microscope slide and covered with a cover

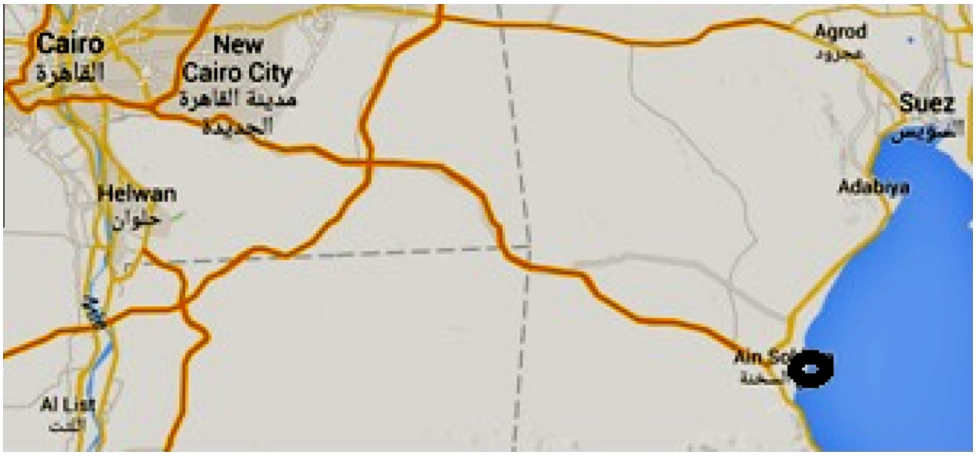

Figure 1 Map showing study area (denoted by a circle). The map was obtained from Google maps.