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SHORT PAPER

Second Habitat Record of *Polykrikos hartmannii* W. Zimm. (Dinophyceae) in the South Aegean Sea, Eastern Mediterranean

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Abstract

Polykrikos hartmannii, a harmful and ichthyotoxic marine dinoflageallate, has been widely distributed in temperate and tropical waters. In this study, *P. hartmannii*, previously recorded from fossil's, is recorded for the first time in the South Aegean Sea, Eastern Mediterranean from living cells. This study provides information on the new distribution areas of the species in the south-eastern Aegean Sea.

Keywords: Dinoflagellates, ichthyotoxicity, Eastern Mediterrannean Sea, aquaculture.

Introduction

Marine dinoflagellates are one of the major components in plankton communities and play an important role as primary producers in marine ecosystems (Shin, Yoon, Kim & Matsuoka, 2011) and they are also useful bio-indicators of environmental changes. Dinoflagellates known as forming cysts and creating blooms in the suitable conditions that can be responsible for red tide, harmful and toxic bloom events, a serious threat to public health and marine fisheries (Ruiz, Carlton, Grosholz & Hines, 1997). The Mediterranean Sea would favour the richness of dinoflagellates which are typical microalgae in the oligotrophic waters (Gomez, 2003). Although the Mediterranean Sea represents only a small percentage of the world's oceans, contains nearly 43 % of the world's marine dinoflagellates species, including c. 88% of the dinoflagellates genera (Gomez, 2006). There are two main groups of dinoflagellates: the thecate (or armoured) forms that have a series of relative rigid thecal plates of different sizes and shapes and the so-called unarmoured (or naked) forms that lack of these structures and are generally more fragile (Escobar-Morales & Hernández-Becerril, 2015).

The species, *Polykrikos hartmannii* is unarmoured, small to medium-sized two-celled pseudo-colony or as single cell gymnodinioid microalgae of the class Dinophyceae. First description of the species was given by Zimmermann (1930) and then, it was re-described by Matsuoka and Fukuyo (1986) as the species, *Pheopolykrikos hartmannii*, which was determined as synonym of *Polykrikos hartmannii* (Escobar-Morales & Hernández-Becerril, 2015). *Polykrikos hartmannii* is currently accepted name, taxonomically (Guiry & Guiry, 2017). The species was recorded is widely distributed in temperate and tropical waters. Nevertheless, there is a little report on its ecology and distribution. Much of the attention in recent years has been focused on the regions of the eastern Pacific including Mexico (Pena-Manjarrez, Helenes, Gaxiola-Castro & Orellana-Cepeda, 2005; Gárate-Lizárraga, Band-Schmidt & Grayebdel Alamo, 2008) and Indian ocean including Thailand (Mizushima, Matsuoka & Fukuyo, 2007; D'Costa, Anil, Patil, Hegde, D'Silva & Chourasia 2008) and the western Pacific including Korea, Japan and China (Wang, Matsuoka, Qi, Chen & Lu, 2004; Shin *et al*., 2010). In order to understand harmful algal bloom ecology, scientists have studied the resting cysts of dinoflagellates as bio-indicators of environmental changes. Only in the western part of Atlantic Ocean, it has been carried evolutionary history and molecular phylogenetic data of polykrikoid species (Pospelova, Chmura & Walker, 2004; Hall *et al*., 2008; Hoppenrath & Leander, 2007; Hoppenrath, Yubuki, Bachvaroff & Leander, 2010). However, detailed information on the presence and distribution of the genus *Polykrikos* in the western Mediterranean Sea is limited; it was recorded from Tyrrenian Sea (Gomez, 2003). The species was also documented as resting cysts accumulated in surface sediment in the western part of the Black Sea by

Moncheva (2015) and the Sea of Marmara (Gemlik Bay) by Balkıs, Balci, Giannakourou, Venetsanopoulou & Mudie (2016), in which upper layer is characterised by the waters coming from the Black Sea (Figure. 1).

This study presents first documentation of the occurrence of *Polykrikos hartmanni* in the Eastern Mediterranean Sea. It contributes to the species richness, and supplies new information about spreading new areas of *P. hartmannii* in the Mediterranean Sea, especially for; Turkish water along the South Aegean Sea.

Material and Methods

Study Area

The sampling area is located in Güllük Bay in the eastern part of the South Aegean Sea (Eastern Mediterranean). The rapid growth of fish farming and tourism has been paralleled by the very rapid urbanization of the coastal zone at the bay located in the province of Muğla, which is the most important marine aquaculture centre of Turkey and contributes to the marine aquaculture production with 35.63%, and contributes economically 47.27%. (Yucel-Gier, Pazi & Küçüksezgin, 2013; Yıldırım & Okumuş, 2004).

The anthropogenic and natural pressures affect the marine ecosystem and thus the regional economy. Eutrophication risk and various pollutants in Güllük Bay are the result of multiple anthropogenic activities; including domestic waste waters, agriculture run off, tourism, aquaculture, port activities, ballast waters, and introducing the exotic species. As a result of these anthropogenic pressures, symptoms of the decline of the water quality and increasing eutrophication were recorded in the coastal areas of the region (Aktan, 2011; Aktan & Topaloğlu, 2011). In recent years, the region has characterised by mass mortality of fish associated with decreases in water temperature in the winter period (Birincioğlu, Aydoğan & Avci, 2013) and it caused serious economic losses in the aquaculture industry.

Sampling and Identification of the Species

Water samples for phytoplankton analysis were collected using plastic flasks in January 2008 during the high mortality events of fish from marine aquaculture area at Güllük Bay in the South Aegean Sea (Figure. 2). Some of the samples are fixed with neutralized formalin (4%). Microscopic studies were made on unfixed-living and fixed material with a Nikon TE2000U inverted microscope at X400 magnification. Identification of *Polykrikos hartmannii* was performed on living samples and based on general characteristics such as shape, size, motility of cells, colour of chloroplasts (Hulburt, 1957; Steidinger & Tangen, 1995; Escobar-Morales & Hernández-Becerril, 2015; Hoppenrath *et al.*, 2010). Identified taxa were controlled with the check-lists (Koray, 2001; Balkıs, 2004) for the distribution in Turkish seas and also with current literature. Preserved samples were also used to search for certain details and relative abundances were determined in subsamples (Utermöhl, 1958). Water temperature was measured with a thermometer. The samples for nutrients were kept frozen (-20°C) until analysis in the laboratory spectrophotometrically according to Parsons, Maita & Lalli (1984).

Results and Discussion

The first observation of *Polykrikos hartmannii* in the South Aegean Sea was recorded at 10°C in January 2008, but it did not play an important role in terms of density and not form a bloom. During the sampling period, in terms of abundance and the number of species, diatoms were the most important group, followed by dinoflagellates. *Cheatoceros* spp., *Leptocylindrus minimus* Gran, and *Skeletonema costatum* (Grev.) Cleve were the main dominant species from diatoms. Dissolved nutrient concentrations were recorded as average 2.15±1.27 μgl^{-1} P for reactive phosphate; 5.38±0.85 μgl^{-1} N for the inorganic nitrogen (addition of nitrate nitrogen, nitrite nitrogen and ammonium nitrogen).

Identification of *P. hartmannii* was performed on living samples. The size of cells was 82±3µm long and 46±2µm wide and it has a characteristics shape of cells with cylindrical body and numerous small circular yellow-brown chromatophores in the pseudocolonial cells (Figure. 3).

P. hartmannii are common dinoflagellates reported from different geographical regions and it forms resting cysts in their life cycle during unfavourable conditions (Figure. 1). When the environmental conditions are favourable, cysts may germinate and may form bloom. Resting cysts of *P. hartmannii* was recorded in surface sediment of the Gulf of Gemlik, the Sea of Marmara (Balkıs *et al.*, 2016). In the present study, first record of the vegetative cells of *P. hartmanii* was given from south-eastern Aegean Sea. Previous studies related to the presence and distribution of this species has been usually focused on their resting cysts in generally. Identification of vegetative cells of unarmoured dinoflagellates is difficult due to their delicate forms and studies performed on fixed samples do not provide adequate accuracy. Systematic studies mostly require living samples and/or particular techniques and protocols such as use of scanning/transmission electron microscopy, molecular sequencing, etc. (Larsen & Nguyen, 2004; Escobar-Morales & Hernández-Becerril, 2015).

Algal blooms in coastal marine area are an increasing problem and significant threat to human health, marine ecosystems and resources (including effects on aquaculture and tourism) all over the world

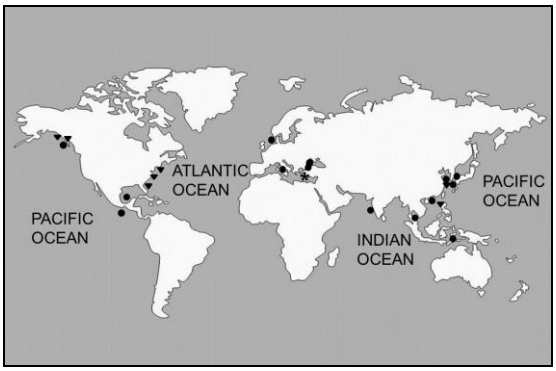

Figure 1. Distribution of *Polykrikos hartmannii* in the world seas: Symbols are indicated vegetative cell (▼), resting cyst (●) and first vegetative cell record from the Aegean Sea (Eastern Mediterranean) in the present study (*). (According to Chatton, 1933; Hulburt, 1957; Matsuoka & Fukuyo, 1986; Nehring, 1997; Lee, Kim, & Lee, 1998; Morquecho *et al*., 2003; Badylak & Phlips, 2004; Wang *et al*., 2004; Pena-Manjarrez *et al*., 2005; Fujii and Matsuoka 2006; Mizushima *et al.*, 2007; D'Costa *et al*., 2008; Shin *et al*., 2010; Hoppenrarth *et al*., 2010; Shin *et al*., 2011; Srivilai, Lirdawitayaprasit & Fukuyo, 2012; Tang *et al*., 2013; Garate - Lizargara, 2014; Moncheva, 2015; Escobar-Morales & Hernández-Becerril, 2015; Balkıs *et al*.,2016).

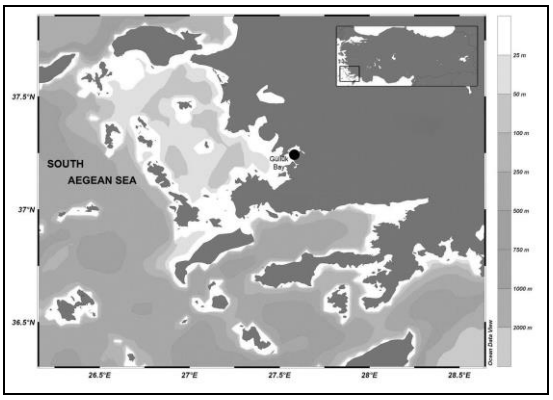

Figure 2. Location of the records of *P. hartmannii* in Güllük Bay in the south Aegean Sea.

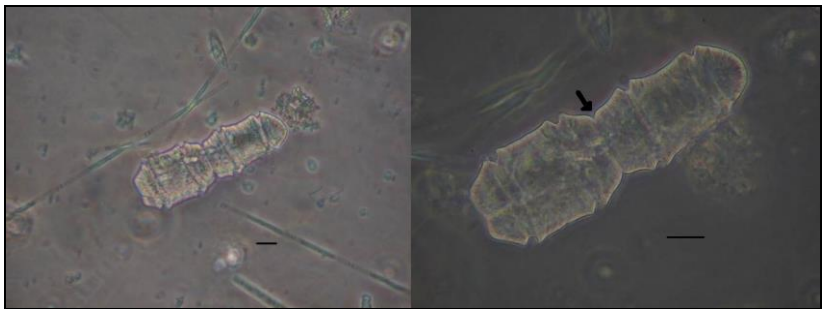

Figure 3. Vegetative pseudocolonial cells of *P. hartmannii* from Güllük Bay in the South Aegean Sea. Arrow indicates the visible border between the cells. Scale bars are 10µm.

(Okaichi, 2003; Giacobbe *et al.*, 2007; Hernandez-Beceril *et al.*, 2007; Ferrante, Sciacca, Fallico, Fiore & Conti, 2013). They occur as a result of complex interactions of environmental factors. They develop in response to favourable conditions such as increasing nutrient inputs, chancing hydrodynamism for cell growth and accumulation (Hall *et al.*, 2008; Basterretxea, Garcés, Jordi, Angles & Masó, 2007).

There are some records on blooms of the *P. hartmannii* and its toxicity from the Atlantic ocean in the warm seasons (when water temperatures generally exceeded 25°C) (Gárate-Lizárraga, 2013; Badylak & Phlips, 2004; Tang, Harke & Gobler, 2013). As for the present study, sampling area has been characterized by high freshwater inputs by the river after severe rainfall in winter period. Low water temperature cannot provide a suitable environment for the growth of this species. Low water temperature (10

°C) may be limited growth of the species.

P. hartmanii has been referred as a harmful and ichthyotoxic species in the literature, but limited toxicity studies are available although it is widely distributed all over the world seas. Acute ichthyotoxicity of *P. hartmannii* to juvenile sheepshead minnows (*Cyprinodon variegates* Lacepède, 1803) was given from the Atlantic Ocean by Tang *et al*. (2013). Considering the natural and anthropogenic pressures on the region, the presence of this species represent a potential risk due to the ichthyotoxicity and potential harmful effect of massive blooms, ecosystem and human health as well as local economy (i.e. tourism and fish farms). The record of this species contributes to the knowledge on the existence and spreading areas of new species, which is important in terms of the ecological and economical management of the resources along the coastal areas in the south-eastern Aegean Sea.

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