

First bloom report of *Polykrikos hartmannii* (Dinophyceae) in the coastal waters of İzmir Bay, Aegean Sea (Eastern Mediterranean)

Primer reporte del florecimiento de *Polykrikos hartmannii* (Dinophyceae) en las aguas costeras de la Bahía de İzmir, Mar Egeo (Mediterráneo oriental)

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ABSTRACT

Background. Harmful algal blooms have increased in frequency, intensity and distribution in the last decades around the world. This increase has also been observed for the waters of the Mediterranean Sea. In November 2023, several reddish patches were observed in İzmir Bay (Aegean Sea); the dinoflagellate *Polykrikos hartmannii* was the causative agent. **Goal.** The purpose of this study was to report for the first time a bloom of this species in İzmir Bay. **Methods.** During the bloom, surface samples of seawater were collected using 1 liter plastic bottles at eleven sampling stations and fixed with Lugol for cell counting, which were performed under an OLYMPUS BX-50 microscope, using 1 ml Sedgewick-Rafter chambers. Samples were collected for live analysis and correct identification of the responsible species. **Results.** The moderate bloom occurred in November 2023 was caused by *Polykrikos hartmannii*. During the bloom two-celled chains were the predominant species, with single cells occurring less frequently. The highest cell abundance (4.8×10^4 cells L⁻¹) was observed at station 29 in the inner bay. Cell sizes were 29.7 - 34.6 µm long, and 39.6 - 44.5 µm wide. **Conclusions.** Living samples allowed correct identification of *P. hartmannii*. Cells tend to become round or disrupted when preserved with Lugol's solution. This may be why this species has not been previously reported. The maximum abundance of *P. hartmannii* was recorded in İzmir Bay at a water temperature of 14.84 °C and a salinity of 38.02 ‰, coinciding with highest values of nutrients. The bloom occurred in the shallowest area with limited water circulation and significant riverine inflow.

Keywords: phytoplankton, unarmored dinoflagellates, *Polykrikos hartmannii*, İzmir Bay, eastern Mediterranean.

RESUMEN

Antecedentes. Los florecimientos de algas nocivas se han incrementado en frecuencia, intensidad y distribución en las últimas décadas en todo el mundo. Este aumento también se ha observado en aguas del mar Mediterráneo. En noviembre de 2023 se observaron manchas rojizas en la Bahía de İzmir (Mar Egeo); la especie responsable fue el dinoflagelado *Polykrikos hartmannii*. **Objetivo.** El objetivo del presente estudio fue reportar el primer florecimiento de esta especie en la Bahía de İzmir. **Métodos.** Durante el florecimiento, se recogieron muestras superficiales de agua de mar, utilizando frascos de plástico de 1 litro en once estaciones de muestreo y se fijaron con Lugol para el recuento de células, los cuales se realizaron en un microscopio OLYMPUS BX-50, utilizando cámaras Sedgewick-Rafter de 1 ml. Se colectaron muestras para su análisis in vivo y la correcta identificación de la especie responsable. **Resultados.** El florecimiento moderado ocurrido en noviembre de 2023 fue causado por *Polykrikos hartmannii*. Durante el florecimiento predominaron las cadenas bicelulares, siendo menos frecuente la presencia de células individuales. La mayor abundancia (4.8×10^4 células L⁻¹) se observó en la estación 29 en la bahía interior. El tamaño de las células fue de 29.7 – 34.6 µm de largo y 39.6 – 44.5 µm de ancho. **Conclusiones.** Las muestras vivas permitieron identificar correctamente a *P. hartmannii*, ya que los organismos fijados con Lugol se deforman o se disgregan. Esta puede ser la razón de que esta especie no se haya descrito anteriormente. La máxima abundancia de *P. hartmannii* se registró en la Bahía de İzmir, con una temperatura del agua de 14.84 °C y una salinidad de 38.02 ‰, coincidiendo con valores altos de nutrientes. El florecimiento ocurrió en la zona somera, con circulación limitada de agua y entradas fluviales significativas.

Palabras clave: fitoplancton, dinoflagelados desnudos, *Polykrikos hartmannii*, Bahía de İzmir, Mediterráneo oriental.

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INTRODUCTION

Harmful algal blooms (HABs) represent a growing global threat that frequently occurs in bays and exhibits increased frequency, scale and duration. This phenomenon results in severe consequences for human health, economies, marine ecosystems and surrounding creatures (Anderson *et al.*, 2008; Anderson *et al.*, 2012; Grattan *et al.*, 2016; Montes *et al.*, 2018; Brown *et al.*, 2020; Fire *et al.*, 2021; Hallegraeff *et al.*, 2021; Otero & Silva, 2022). The formation of HABs, particularly in coastal environments, occurs in response to changing physicochemical factors, such as tides, winds or available nutrients. These have different timescales of variability, and therefore, blooms can be short-term episodic events, recurrent seasonal phenomena, or rare events associated with exceptional climatic or hydrological conditions (Cloern, 1996). However, among the species causing HABs, there are several that can continue blooming over the long term even if environmental conditions have changed, making the characterization of the mechanisms causing HABs even more complex (Park *et al.*, 2018).

Dinoflagellates, a diverse and ecologically significant group of marine phytoplankton, are the primary causative organisms in approximately 75% of documented harmful algal bloom events (Smayda, 1997) and are responsible for numerous red tide events, characterized by the discoloration of the sea surface (Holmes *et al.*, 1967; Eppley & Harrison, 1975; Franks & Anderson, 1992; Anderson *et al.*, 2002; Imai *et al.*, 2006; Ferrante *et al.*, 2013; Jeong *et al.*, 2013; Park *et al.*, 2013). Many dinoflagellates, including unarmored species, possess the adaptive ability to form resting cysts throughout their life cycles (Head, 1996). This ability not only enables their survival in adverse environmental conditions, but also is associated with various factors such as genetic recombination, continuation and cessation of blooms, recurrence of annual blooms, protection against viral, herbivorous or parasitic attacks, and geographical expansion of populations (Anderson & Wall, 1978; Hallegraeff & Bolch, 1991; Matsuoka & Fukuyo, 2002; Figueroa *et al.*, 2010). This plays a crucial role in HAB dynamics, contributing to both the recurrence and geographic expansion of blooms (Bravo & Figueroa, 2014; Tang & Gobler, 2015; Yang *et al.*, 2018).

The unarmored, gymnodinoid dinoflagellate *Polykrikos hartmannii* W.M. Zimmermann 1930 is a mixotrophic, cyst-producing and ichthyotoxic species that forms single-celled zooids or two-celled pseudocolonies (Hulburt, 1957; Tang *et al.*, 2013; Lee *et al.*, 2015). The species was first described by Zimmermann (1930) as *P. hartmannii* and then was subsequently placed in the genus *Pheopolykrikos* by Matsuoka & Fukuyo (1986) due to shared characteristics with *Pheopolykrikos* spp. Hoppenrath *et al.* (2010) revealed the presence of a nematocyst-taeniocyst complex, a diagnostic feature of the genus *Polykrikos*, leading to the reassignment of the species back to *Polykrikos*. Within current taxonomic nomenclature, the accepted name for this species is *P. hartmannii* (Guiry & Guiry, 2024).

Polykrikos hartmannii exhibits a global distribution pattern, based on its various life stages, including cyst, vegetative and motile stages that have been documented in diverse aquatic habitats, including lagoons, rivers, estuaries and marine environments. This species has been reported from Canada (Pospelova *et al.*, 2010; Price & Pospelova, 2011), the USA (Hulburt, 1957; Steidinger & Tangen, 1997; Pospelova *et al.*, 2004; Badylak & Phillips, 2004; Hoppenrath *et al.*, 2009; Hoppenrath *et al.*, 2010; Tang *et al.*, 2013), Mexico (Morquecho & Lechuga-Devéze, 2003; Peña-Manjarrez *et al.*, 2005; Gárate-Lizárraga *et al.*, 2009, 2016; Gárate-

Lizárraga, 2014; Escobar-Morales & Hernández-Becerril, 2015; Escarcega-Bata *et al.*, 2023), Japan (Matsuoka & Fukuyo, 1986; Fuji & Matsuoka, 2006), China (Wang *et al.*, 2004; Chai *et al.*, 2020; Liu *et al.*, 2023), Indonesia (Mizushima *et al.*, 2007; Rukminasari & Tahir, 2021; Rachman *et al.*, 2022), India (Godhe *et al.*, 2000; D'costa *et al.*, 2008), Malasia and Singapore (Hii *et al.*, 2021), Korea (Kim *et al.*, 1990; Kim *et al.*, 2008; Pospelova & Kim, 2010; Shin *et al.*, 2011; Thangaraj *et al.*, 2017; Kwak *et al.*, 2022), Israel (Rubino *et al.*, 2017), Bulgaria and Ukraine (Dzhembekova *et al.*, 2017) and Turkey (Balkis *et al.*, 2016; Aktan & Keskin, 2017). Despite its global distribution, there are few records of *P. hartmannii* causing significant blooms (Kim *et al.*, 1990; Badylak & Phillips, 2004; Gárate-Lizárraga *et al.*, 2009, 2016; Tang *et al.*, 2013; Gárate-Lizárraga, 2014; Thangaraj *et al.*, 2017).

This study provides the first documented bloom of *P. hartmannii* that occurred in the Mediterranean Sea, expanding our understanding of the distribution of this species and contributing to the known biodiversity of the region.

MATERIAL AND METHODS

Phytoplankton samples were collected from 11 sites in İzmir Bay during a bloom event in November 2023 (Fig. 1). The sampling area is located in the Eastern Mediterranean (38° 20' - 38° 40' N, 26° 30' - 27° 10' E). It is a closed bay opening into the Aegean Sea, with a surface area of 500 km², a water capacity of 11.5 million m³, and a total length of 64 km (Kontas *et al.*, 2004). İzmir bay is divided topographically into three sections: inner bay (St. 29), middle bay (St. 22 and 28), and outer bay (St. 6, 15, 17, 20, M2, F1, F3 and F5). The wider and deeper section extending northwest-southeastward between the Karaburun Peninsula and the Gediz Delta is referred to as the outer bay. The width of the outer bay, which has a length of 45 km, reaches 24 km at its mouth. The depth in the outer bay varies between 45 and 70 m. The deepest point reaching 71 m is located at the mouth of the outer bay (between Foça and Karaburun). In the inner and middle bays, the depth increases from East to West. The deepest point (ca. 21 m) of the inner bay is in the central region.

Surface samples of the bloom were obtained using 1-liter plastic flasks. These samples without preservation were examined about one hour after the collection with an Olympus BX-50 (Olympus Optical Co. Ltd., Japan) microscope connected to a digital camera (ToupTek XCAM-1080). Some samples were fixed with Lugol's solution for enumeration, and cell enumerations were conducted using a 1-ml Sedgewick-Rafter counting chamber. Live samples were used to identify *Polykrikos hartmannii* based on their distinctive morphology, including the cell shape, size, motility, and chloroplast color (Hulburt, 1957; Matsuoka & Fukuyo, 1986; Steidinger & Tangen, 1997; Hoppenrath *et al.*, 2009; Hoppenrath *et al.*, 2010; Tang *et al.*, 2013; Gárate-Lizárraga, 2014; Escobar-Morales & Hernández-Becerril, 2015; Kwak *et al.*, 2022).

Surface water samples were collected using 101 Teflon Niskin type bottles attached to a CTD (SeaBird SBE 25Plus/SBE 27 pH Sensor) Rosette System (SBE 32C 12 universal sampling bottle). Surface water was sampled to determine the temperature (°C), salinity (‰), orthophosphate (μmol L⁻¹), nitrite + nitrate nitrogen (μmol L⁻¹), ammonium nitrogen (μmol L⁻¹) and silicate (μmol L⁻¹) at the time of sampling using a CTD and water sampler that makes real-time measurements at each station (MMG, 2017).

RESULTS

Characterization of the environment. An algal bloom and associated water discoloration in November were studied. This phenomenon followed a period of excessive rainfall. The bloom and subsequent color change dissipated during high wind conditions and re-emerged once the winds subsided. The bloom exhibited passive movement consistent with currents. Water discoloration ranged from reddish-brown to rust colored (Fig. 2) and was likely correlated with the cell abundance. In İzmir Bay, apart from the discoloration caused by excessive proliferation, no fish mortality or harmful effects were observed.

The morphological characteristics and abundance of *P. hartmannii*. Analysis of live samples allows us to identify a monospecific bloom caused by *Polykrikos hartmannii*. The two-celled colonies were the predominant form observed, with single cells occurring less frequently and never dorsoventrally compressed. The cell size was 29.7 - 34.6 μm long and 39.6 - 44.5 μm wide, with the round epicone often smaller than or sometimes equal to the cylindrical hypocone. In the two-celled colonies, the posterior zooid is slightly rounded at the antapex, whereas the anterior zooid was straight at the apex (Fig. 3C). Cells are often heavily pigmented, and numerous small chloroplasts of brown-greenish, yellow color are present (Figs. 3A; 3D). The nucleus of the anterior cell is in the hypocone, while that of the posterior cell is in the center of the epicone (Fig. 3E).

In November 2023, the species exhibited a moderate bloom. The highest cell abundance (4.8×10^4 cells L^{-1}), was observed at station 29 (the inner bay). The lowest cell abundance (11 cells L^{-1}) was observed at station 6 (the outer bay).

Physical and chemical variables. The water temperature varied between 14.84 and 19.21 $^{\circ}\text{C}$, while salinity levels ranged from 37.83 ‰ to 39.23 ‰. Nutrient concentrations during the bloom were 0.02 - 2.23 μM

$\text{PO}_4\text{-P}$, 0.05 - 12.06 μM NO_x , 0.04 - 24.15 μM $\text{NH}_4\text{-N}$ and 0.45 - 22.25 μM Si (Table 1). The maximum abundance of *P. hartmannii* was recorded in İzmir Bay with a water temperature of 14.84 $^{\circ}\text{C}$ and salinity of 38.02 ‰.

DISCUSSION

The cell abundance is consistent with the previous reports of the *P. hartmannii* bloom in the Gulf of California, Mexico (Gárate-Lizárraga *et al.*, 2009: max. 3.5×10^4 cells L^{-1} ; Gárate-Lizárraga, 2014: max. 3.3×10^4 cells L^{-1}), but lower than others reported from Korea (Kim *et al.*, 1990: 1×10^6 cells L^{-1} ; Thangaraj *et al.*, 2017: 1.1×10^6 cells L^{-1}) and the USA (Tang *et al.*, 2013: max. 8.3×10^6 cells L^{-1}). An exceptional bloom of *P. hartmannii* was reported in the northern part of the state of Guerrero, Mexico, which reached densities of 5263×10^3 cells L^{-1} (Gárate-Lizárraga *et al.*, 2016).

During the study period when the maximum cell abundances of *P. hartmannii* were observed, it is notable that the values of orthophosphate, nitrite + nitrate nitrogen, and silicate also reached their maximum levels. The presence of such high abundances in the inner bay is primarily a result of its “closeness”, characterized by limited water exchange with the open water of the İzmir Bay. This restricted exchange contributes to an elevated trophic state in the water. *P. hartmannii* bloom occurs in early fall in İzmir Bay following a heavy rain season. This is consistent with the observations of Gárate-Lizárraga (2014), who noted a similar pattern despite different temperatures, suggesting a potential link between bloom formation and post-rainy season conditions. *P. hartmannii* also blooms in other seasons, including summer (Gárate-Lizárraga *et al.*, 2009; Thangaraj *et al.*, 2017) and fall (Tang *et al.*, 2013; Kim *et al.*, 1990). While *P. hartmannii* blooms are typically associated with warmer waters (22 - 31 $^{\circ}\text{C}$; Badylak & Philips, 2004; Gárate-Lizárraga, 2014,

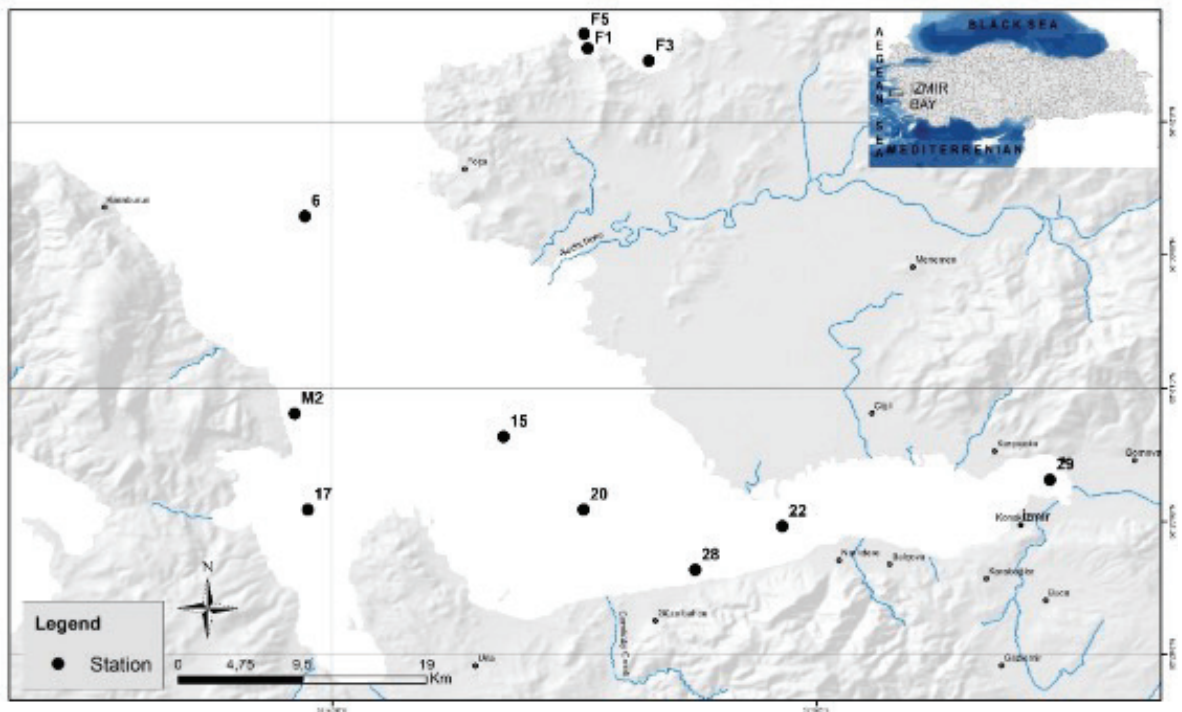


Figure 1. Map of the sampling stations in Izmir Bay, Aegean Sea, in November 2023.

Gárate-Lizárraga *et al.*, 2016; Thangaraj *et al.*, 2017), the maximum abundances in İzmir Bay occurred at a notably lower temperature of 14.84 °C. This was accompanied by a wider temperature range (15 - 28°C) observed throughout the study period by Tang *et al.* (2013). Aktan and Keskin (2017) observed that the vegetative stage can occur at temperatures as low as 10°C, supporting the adaptability of the species to varying thermal conditions. This highlights the potential for *P. hartmannii* to bloom under diverse environmental conditions.

Laboratory studies have shown that *P. hartmannii* possesses several key features that likely contribute to its successful bloom formation. Lee *et al.* (2015) found evidence of a mixotrophic nature, allowing it to utilize both light and organic matter for nutrition. This mixotrophy probably provides a competitive advantage during blooms, potentially explaining the observed monospecific blooms. Chai *et al.* (2020) demonstrated its ability for homothallic reproduction, which simplifies reproduction and potentially aids in population maintenance and growth during blooms. Tang *et al.* (2013) showed acute ichthyotoxicity in a laboratory culture. Kwak *et al.* (2022) demonstrated a wide temperature tolerance for germination (10 - 30°C), with high germination rates exceeding 90 % at 15 - 20 °C in a laboratory setting. This flexibility in germination temperature likely contributes to the bloom-forming potential of this species. Overall, these studies conducted in a laboratory setting highlight several factors that may contribute to the success of *P. hartmannii* blooms.

Globally, most records of *P. hartmannii* document its presence in cyst form (Matsuoka & Fukuyo, 1986; Morquecho & Lechuga-Devéze, 2003; Wang *et al.*, 2004; Pospelova *et al.*, 2004; Fuji & Matsuoka, 2006; Mizushima *et al.*, 2007; D'costa *et al.*, 2008; Pospelova & Kim, 2010; Pospelova *et al.*, 2010; Price & Pospelova, 2011; Fertouna-Bellakhal *et al.*, 2014; Balkis *et al.*, 2016; Rubino *et al.*, 2017; Di Poi *et al.*, 2019; Dzhenbekova *et al.*, 2020; Rachman *et al.*, 2022; Kwak *et al.*, 2022; Liu *et al.*, 2023). Records of its motile or vegetative form are less common (Hulburt, 1957; Matsuoka & Fukuyo, 1986; Peña-Manjarrez *et al.*, 2005; Kim *et al.*, 2008; Hoppenrath *et al.*, 2009., 2010; Escobar-Morales & Hernández-Becerril, 2015; Aktan & Keskin, 2017; Chai *et al.*, 2020). Blooms of this dinoflagellate

occur in diverse coastal habitats worldwide, including the Mexican Pacific (Gárate-Lizárraga *et al.*, 2009, 2016; Gárate-Lizárraga, 2014), the South Sea and East Sea in Korea (Kim *et al.*, 1990; Thangaraj *et al.*, 2017), and the USA (Badyalak & Phipps, 2004; Tang *et al.*, 2013).

Previous studies have identified both the cyst (Gómez, 2003; Fertouna-Bellakhal *et al.*, 2014; Rubino *et al.*, 2017) and vegetative forms (Aktan & Keskin, 2017) of *P. hartmannii* in the Mediterranean Sea. This study represents the first recorded bloom of the species in the Aegean Sea. Although the dinoflagellate cyst composition has been analyzed in İzmir Bay (Uzar *et al.*, 2010; Aydin *et al.*, 2011; Aydin & Uzar, 2013; Aydin *et al.*, 2014; 2015), *P. hartmannii* was previously unrecorded in studies of the region's dinoflagellate cyst composition. The inner bay, where the bloom occurred in November 2023, is the shallowest area with limited water circulation and significant riverine inflows. Sediment dredging activities carried out in this area to enhance limited water circulation may have introduced or reintroduced the *P. hartmannii* resting cysts into İzmir Bay. These disturbances, combined with the favorable temperature regime, potentially explain the sudden appearance of *P. hartmannii* in the bay. However, an alternative explanation for the previously undetected presence of *P. hartmannii* in İzmir Bay lies in the inherent fragility of its cells. Their delicate morphology could cause the cells to deform during the standard fixation procedures used for microscopic identification, potentially leading to cell shape changes. When combined with a lack of distinctive characteristics, identification becomes significantly more complex (Gómez *et al.*, 2024). Because of the fragility of *P. hartmannii* cell wall, it is necessary for future studies to address molecular identification techniques (for example, Single-cell PCR), since these morphotypes could present genetic divergence compared to other morphotypes collected in distant regions. Furthermore, the rapid mobility and light sensitivity of dinoflagellates can hinder identification even when analyzing living samples (Gárate-Lizárraga *et al.*, 2009). Microscopic examinations revealed a decrease in motility of live cells exposed to light for 20 minutes, followed by complete cessation of movement, cell swelling, and ultimately, cell lysis (Supplementary Material Video 1 and Video 2).



Figure 2. Different views of the *Polykrikos hartmannii* bloom in İzmir Bay, Aegean Sea, Eastern Mediterranean, in November 2023.

Table 1. Physicochemical variables measured in Izmir Bay in November 2023. Temperature (C°), Salinity (‰), PO₄-P: orthophosphate, NOx: nitrite + nitrate nitrogen, NH₄-N: ammonium, Si: silicate.

Stations	Temperature (C°)	Salinity	PO ₄ -P	NOx	NH ₄ -N	Si
6	19.21	38.56	0.02	0.34	0.47	1.15
15	18.26	38.42	0.07	0.26	1.36	1.78
17	16.78	38.56	0.02	0.05	0.04	0.84
20	18.13	38.34	0.04	1.13	0.65	3.52
22	15.25	37.83	0.15	2.03	3.47	4.14
28	15.87	37.92	0.18	1.82	2.50	4.12
29	14.84	38.02	2.23	12.06	24.15	22.25
F1	17.37	39.23	0.02	0.05	0.19	0.58
F3	17.43	39.23	0.02	0.05	0.70	0.45
F5	17.35	39.23	0.02	0.05	0.72	0.56
M2	17.46	38.71	0.02	0.05	0.04	1.18

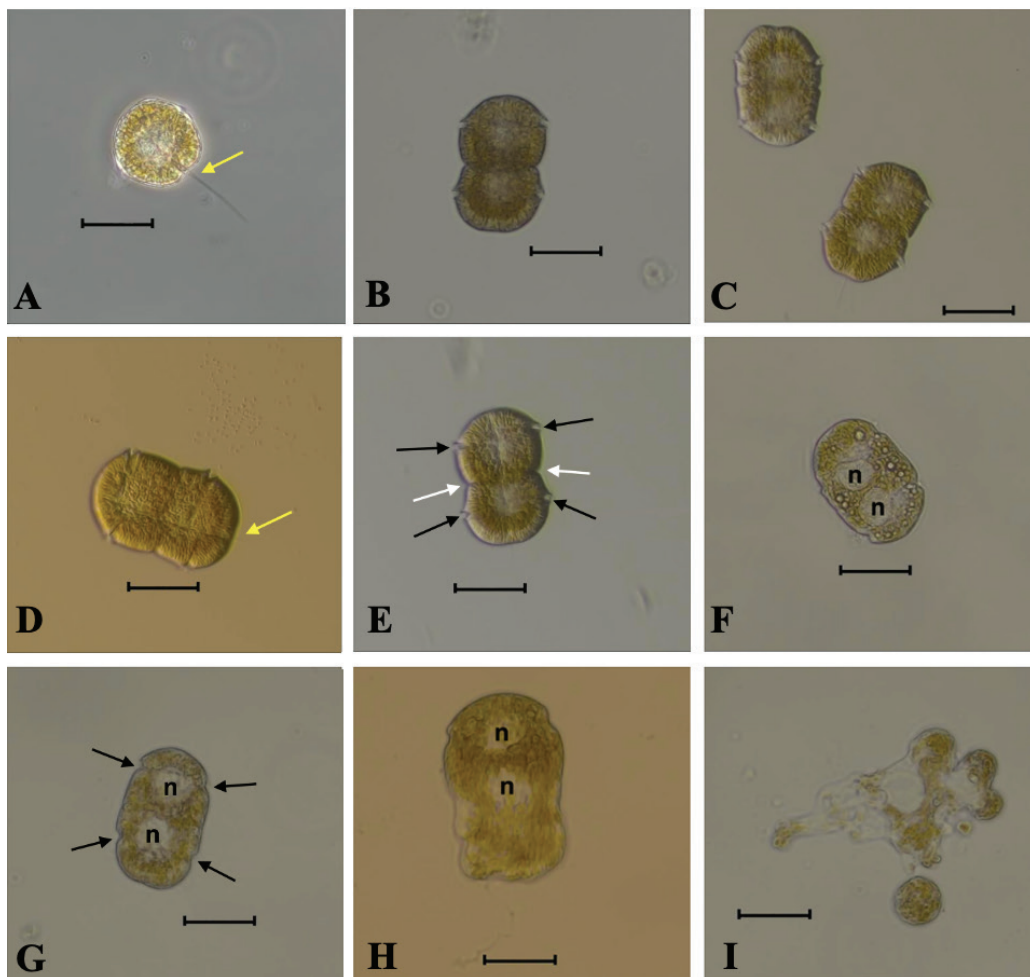


Figure 3. Light microphotographs of *Polykrikos hartmannii* from Izmir Bay (A-I): single cells of *Polykrikos hartmannii* (A); ventral view of a two-celled chain showing general cell shape, cingulum, and the connection between the two zooids (B-E); a colony stressed by longer exposure in deeper focus, showing nucleus (n) (F, G) and lysis of the cell (H, I). Black arrows show two transverse furrows, white arrows show the visible border between the two zooids, and yellow arrows show the longitudinal flagellum. Scale bars: 40 μm

Further monitoring of the unarmored dinoflagellate *P. hartmannii* motile and cyst forms in İzmir Bay, including investigations into the physicochemical and biological factors driving its bloom formation, is crucial for understanding its ecological role and potential impacts. These efforts will yield invaluable insights for future management and mitigation strategies related to this species. This first observed *P. hartmannii* bloom highlights the need to investigate bloom-formation mechanisms and potential environmental impacts specific to the Mediterranean region.

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