# First record of the diatom pathogen *Diatomophthora perforans* cf. subsp. *pleurosigmae* (Oomycota) from the Mediterranean microphytobenthos

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**Abstract:** Knowledge about pathogens of diatoms and macroalgae in the Mediterranean is scarce. This paper reports the first record of the oomycete *Diatomophthora perforans* subsp. *pleurosigmae* infecting the Mediterranean microphytobenthic diatom *Pleurosigma* cf. *intermedium*, which was detected in the context of marine environmental surveys of the brine outfalls of two seawater desalination plants.

**Keywords:** Bacillariophyta; *Ectrogella*; Microphytobenthos; Oomycota; *Pleurosigma intermedium*

Diatoms constitute much of the standing stock on soft seabed (sand, mud) of shallow-water marine ecosystems throughout the world. This also applies to the Mediterranean (Álvarez-Blanco and Blanco 2014). As it was highlighted a few years ago, zoosporic pathogens infecting microphytobenthic diatoms were very much understudied until recently (Scholz et al. 2016a). Even though there has been increased research effort since then (Scholz et al. 2016a, Scholz et al. 2014, 2016b, Scholz et al. 2017a, Scholz et al. 2017b), the Mediterranean remains understudied in this regard (Reñé et al. 2023).

The present study into the presence of benthic diatom parasites in shallow waters on the southeast coast of Cyprus became possible because of an intensive sampling program within the framework of the EU project WATER-MINING, exploring the impacts of brine discharge from two seawater desalination plants in Larnaca and Dhekelia (Xevgenos et al. 2021). At the Larnaca site, the sampling protocol was based on a grid established by previous studies (Argyrou 1999, 2000). Sampling stations were positioned at distances of 0 (L0) and 50 (L50) meters from the discharge pipe (33°39.264' E, 34°52.160' N), with a reference station (33°39.113' E, 34°51.393' N) located at 1400 meters (LRef) south from the pipe, adjusted for consistent bathymetry. At Dhekelia, the sampling transect mirrored the Larnaca gradient where feasible, with stations placed at 0 (D0) and 50 (D50) meters from the discharge pipe (33°45.599' E, 34°58.773' N) and a reference point situated at 1245 meters to the east (33°46.414' E, 34°58.726' N; DRef). Overall, four sampling campaigns were conducted: one of each in May, August, and November 2022, as well as in February 2023, to capture temporal variations and sediment samples were collected as recently described (Grammatiki et al. 2025a, Grammatiki et al. 2025b). Benthic diatom identification and further analysis were performed in a specialised microphytoalgal taxonomy laboratory, where light microscopy was carried out (Axiovert and Axiophot Zeiss, Oberkochen, Germany).

Along a monitoring survey of the benthic diatom communities at the locations described above using microscopy and eDNA metabarcoding (Grammatiki et al. 2025b), diatoms were screened for potential infections by zoosporic pathogens, following the protocols described in Scholz and Einarsson (2015) and Scholz et al. (2016b).

During the screening of live samples for the occurrence of infections by zoosporic parasites, the oomycete *Diatomophthora perforans* cf. subsp. *pleurosigmae* (H.E. Petersen) A.T. Buaya *et* Thines infecting the diatom *Pleurosigma* cf. *intermedium* (Figure 1) was found in all three (a, b, c) May samples at station LRef as well as in August at station L50 (b sample) and LRef (b and c samples). No other parasite was detected during the screening. The morphology of the parasite found in the samples agreed well with the species description by Petersen (1905) (as *Ectrogella perforans*), showing usually one to five endobiotic holocarpic thalli, which seem to be naked at the early development stages. Later, the thalli were surrounded by a thin colourless smooth wall and broadly tubular at maturity (around 90–120 μm long by 30–55 μm in diameter). During maturity of the parasite, the host chloroplast was disintegrated, forming thin residual plugs at thallus opposite apices with lipid globules. The 4 to 8 discharge tubes were very short and tubular, with 7–12 μm in diameter and had a very thickened base, slightly protruding outside. The few zoospores which could be observed were pyriform (3–6 μm long by 2.3–4 μm broad), seemly uniflagellate, with an anterior refractive droplet. No resting spores were observed.

One subsample was used for cultivation of sediment-dwelling diatoms. For this, 20 ml of sterile artificial seawater enriched with f/2 + Si was added to each sample in 50 ml centrifuge tubes, which were sealed with cellulose stoppers. The artificial seawater salt (Tropic Marin Classic®, Aquarientechnik GmbH, Wartenberg) dissolved in de-ionised water was used with a salinity of 30 and a pH of 8.3. The tubes were placed in racks and cultivated at 16±0.5 °C, 12:12 h light: dark regime and at an irradiance of 80 µmol photons $m^{-2}$ $s^{-1}$ using Master TL-D 18W/840 light (Phillips, Germany). After four weeks, samples were taken and examined. Infections were only observed in the diatom *Pleurosigma* cf. *intermedium*, and the abundances of *D. perforans* reached $0.107\times10^{2}$ cells $cm^{-3}$. Unfortunately, all attempts to further isolate the parasite were unsuccessful.

The detection of *D. perforans* as described here is based on morphological identification via microscopy only. Confirmation of the presence of this parasite in Cyprus was not feasible via molecular techniques, as no metabarcoding markers that detect oomycotes were used and the metabarcoding dataset was limited (Grammatiki et al. 2025a, Grammatiki et al. 2025b).

This organism was recently taxonomically re-classified as *Diatomophthora perforans* subsp. *pleurosigmae* following its successful isolation in culture, enabling DNA sequencing work (Buaya et al. 2020). It has previously been reported from Denmark, the USA, France and Sweden (Sparrow 1960) and, more recently, Norway (Buaya et al. 2020). For the Mediterranean, no published record was found (e.g., Van den Wyngaert et al. 2025).

To the best of our knowledge, this constitutes the first record ever of this diatom-infecting, zoosporic pathogen from the Mediterranean microphytobenthos. While some knowledge exists regarding similar pathogens infecting Mediterranean macroalgae (e.g. Fletcher et al. 2015, Strittmatter et al. 2013), there is a critical shortage of records of such infections in Mediterranean microalgae, even though they are likely widespread and ecologically very significant (Frenken et al. 2017, Gleason et al. 2011, Gleason et al. 2013, Scholz et al. 2016a). We hope that the present finding will elicit increased research effort into this little-known aspect of Mediterranean biodiversity and also to cover such organisms in future metabarcoding studies.

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**Author contributions:** BS, EA and FCK designed this study. FCK raised the funding and organized the expeditions to Cyprus. FCK, EA, KG, MML, VR and MP performed field work. BS performed lab work, including taxonomic identification of diatoms and associated organisms. BS wrote a report for the project WATER-MINING. FCK and BS wrote the first draft of this paper. All authors participated in the final editing of the paper.

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**Conflict of interest statement:** There is no conflict of interest to declare.

**Compliance with ethical standards:** This work is fully compliant with the ethical standards of the University of Aberdeen, San Diego State University and the University of Cyprus.

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**Figure legend**

**Figure 1:** Differential interference contrast microscopic pictures of *Diatomophthora perforans* cf. subsp. *pleurosigmae* infecting the diatom *Pleurosigma* cf *intermedium* during maturing of the intracellular thallus. **A.** Multiple infections resulting in several thalli. **B.** Single thallus differentiation with visible zoospore initials. Scale bars: 10 µm