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Short communication

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First record of a rare species, *Polyasterias problematica* (Prasinophyceae), in Balsfjord, northern Norway

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Abstract: *Polyasterias problematica* is a poorly investigated prasinophyte species, practically known only from a few, primarily historical studies. Fragmentary information on the species indicates that *P. problematica* is mainly distributed in the euphotic zone of the Canadian and Russian Arctic, Atlantic Canada, the mid-Atlantic and some European seas. Here, we present the first record of this species in the Balsfjord waters, northern Norway. A detailed morphological description, with line drawings and microscopy-based photographs, as well as information on its up-to-date geographical distribution and possible explanations for its extraordinary scarcity are provided.

Keywords: Balsfjord; northern Norway; planktonic protists; *Polyasterias problematica*; Prasinophyceae.

Balsfjord, a 45 km long and 2–3 km wide fjord with a south-south-east orientation (69°N, 19°E; Figure 1), is an example of a well-monitored environment in northern Norway, with a long history of planktonic protist studies (e.g. Gaarder 1938, Eilertsen et al. 1981, Bech 1982, Eilertsen and Taasen 1984, Lutter et al. 1989, Sandberg 1996, Degerlund and Eilertsen 2010). Previous investigations have provided knowledge on the qualitative and quantitative protist community structure of the area, which is represented by taxa belonging to the Arctic and boreal regions (Throndsen et al. 2007).

Through recent plankton studies at the Svartnes station (69°22′N, 19°07′E) in Balsfjord, we found and identified a single specimen of the extremely rare and poorly known prasinophyte *Polyasterias problematica* (Cleve) Meunier (Figure 2). This organism was collected in a short-term sediment trap deployed from 19 to 20 June 2017 in a thermally stratified water column with a temperature of 11°C at the surface and a gradual decrease to approximately 7°C, 6°C and 5°C at depths of 15 m, 30 m and 50 m, respectively. The sediment trap (KC Denmark) consisted of two transparent polyvinyl chloride (PVC) cylinders (opening diameter 72 mm, cylinder height 450 mm) mounted on a steel frame equipped with a steering fin. Five pairs of sediment traps were mounted on a mooring to collect material at depths of 20, 30, 50, 90 and 120 m. The sediment trap mooring was anchored to the seafloor to avoid drifting. The specimen of *P. problematica* was found in the sediment trap deployed at the 30-m depth, a sufficient distance from the seafloor at 170 m to avoid resuspension of sediment material. After a 24-h deployment, the sediment trap mooring was retrieved and the contents of the two cylinders from each depth were gently pooled together. From this material, a 100-ml subsample was taken for phytoplankton identification. Samples for cell identification and enumeration were fixed with glutaraldehyde-Lugol's solution (GA-Lugol; 2% final concentration, Rousseau et al. 1990). Subsamples were qualitatively and quantitatively analysed under an inverted microscope (Nikon Eclipse TE-300) equipped with phase and interference contrast optics, an ocular magnification of 10× and objective magnification of 10× (Nikon Plan Fluor, 0.30 NA) and 40× (Nikon Plan Fluor, 0.60 NA), using protocols by Utermöhl (1958) and modified by Edler (1979), as described in Kubiszyn et al. (2014).

The taxonomic identification of this species was made on the basis of schematic line drawings and morphological descriptions given in Cleve (1900a,b) and Meunier (1910, 1919), as well as a single light microscopy-based photograph of the dorsal view, presented by Brunel (1962) and Bérard-Therriault et al. (1999). In addition to these works, we did not find any other study describing or illustrating the morphological characteristics of this

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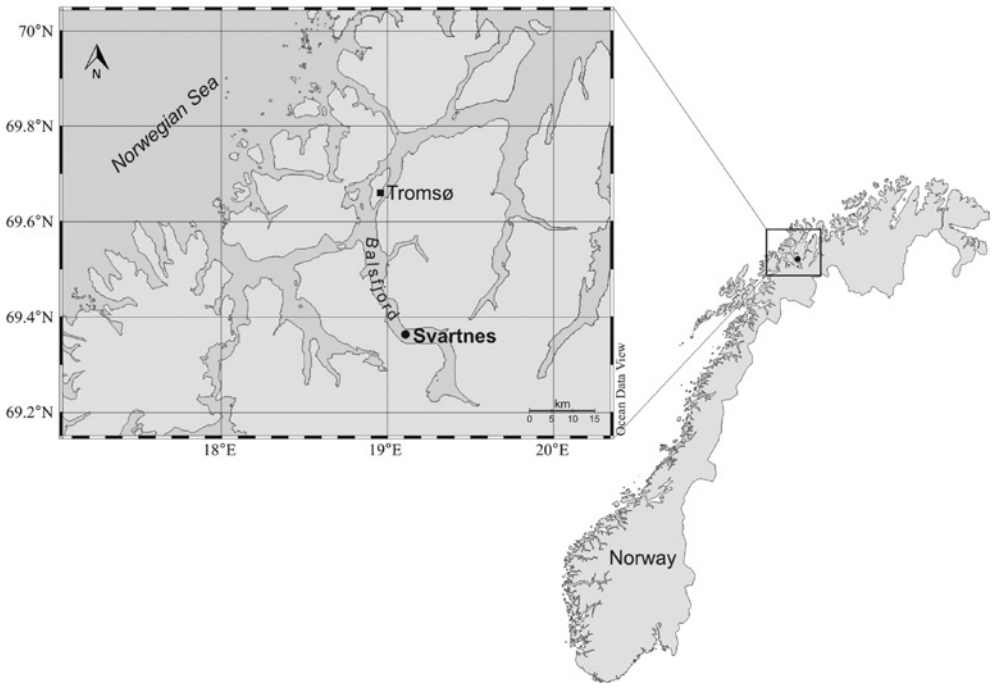

Figure 1: Location of Balsfjord, northern Norway, with the Svartnes sampling station in the Svartnes Basin.

species. The hitherto pelagic records of *P. problematica*, largely referring to papers at least 50 years old, indicate the Arctic-boreal distribution of the species (Figure 3, Table 1). According to these papers, *P. problematica* is mainly distributed in the euphotic zone of the Canadian and Russian Arctic, Atlantic Canada, the mid-Atlantic and some European seas (Figure 3, Table 1). Based on the large range of the species occurrence, it appears to prefer brackish or euryhaline waters. The literature documented a biogeographical distribution that generally corresponds to the raw plankton data integrated in the Ocean Biogeographic Information System [OBIS: http://www.iobis.org; 249 records from 1903 to 2012, of which almost half are up to 1911; 70% of the later data was obtained in the continuous plankton recorder (CPR) survey; Figure 3]. Although, OBIS is considered to be the most complete and comprehensive data repository in existence on the distribution of marine taxa, due to the known issues with regards to taxonomic completeness, geographical biases, and biogeographical accuracy (Webb et al. 2010), the given number and positions of records should, however, be regarded as uncertain and treated with caution. As a palynomorph, *P. problematica* has been observed on the continental shelves of the Canadian and Russian Arctic, the North Atlantic, the north-eastern Pacific, and in a series of marine basins extending from the Mediterranean to the Aral seas (Figure 3, Table 1), where it is generally linked to low saline/brackish cold surface waters in a marine environment with proximity to river inlets and/or meltwater plumes (Kunz-Pirrung 1998, Sorrel et al. 2006, Price and Pospelova 2011, Milzer et al. 2013). Unfortunately, because many of the cited studies, conducted in a relatively large area (*inter alia*, throughout a bay or along multi-kilometre transect), do not provide the exact position of the *P. problematica* record (e.g. Meunier 1910, Bursa 1961, Horner 1984), the location of points in Figure 3 is largely indicative. Most studies are limited to qualitative species information, in some cases supplemented by comments on the rareness of the cells. Scarcely occurring data on abundance range from 1 to 750 cells l^{-1} (Anderson 1979, Pederson 2004, Martin et al. 2006). Here, we present the first record of *P. problematica* in the northern Norway area. We also provide a detailed description of the morphological characteristics and possible explanations for its extraordinary scarcity.

The basionym of *Polyasterias problematica* [synonym: *Ovum hispidum problematicum* (Cleve) Lohmann] is *Hexasterias problematica*, described by Cleve (1900a). This cell was first observed by Hensen (1887; presented as "Röhrenstatoblast") in the western Baltic Sea. Cleve (1900a) established the genus *Hexasterias* (Chlorophyta) on the basis of observations of individuals with six rays, which he considered as conservative. Later studies have shown that the number of rays may vary from four to eight (this difference still remains unclear) and the 6-arm forms were the least common (Meunier 1910). Since Meunier (1910) considered

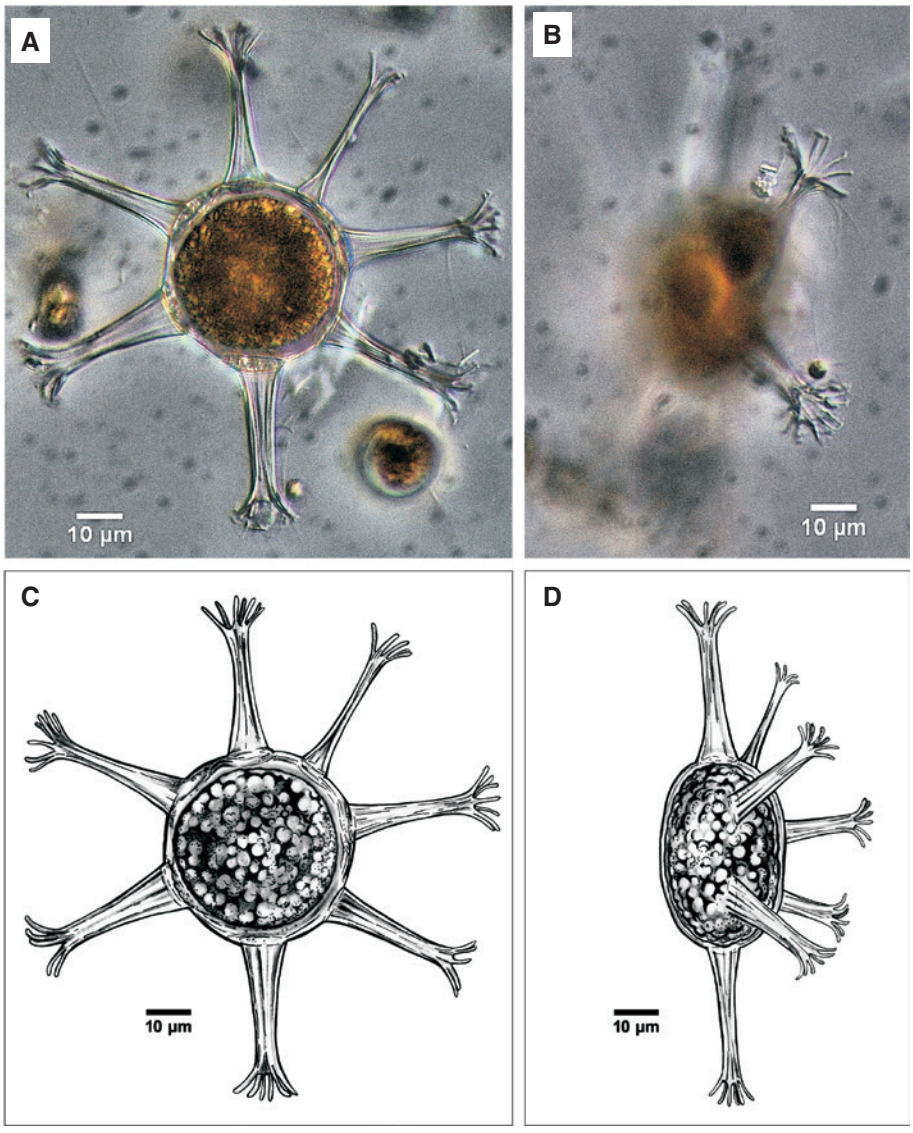

Figure 2: Micrographs (A, B) and line drawings (C, D) of *Polyasterias problematica* taken in light microscopy at 400×.
(A) Stack image of a cell with seven arms in dorsal view. (B) Side view of a cell showing the hook-like structures of the arms. (C) Schematic dorsal view of a cell. (D) Schematic side view of a cell.

that introducing the term *Hexasterias octoradiata*, as an example for an 8-arm form, would be too paradoxical, he decided to replace the genus name with *Polyasterias*, which did not clearly indicate the number of processes. Interestingly, he negated the taxonomic affiliation of the species proposed by Cleve, claiming that the cell does not have the characteristics of a plant and it is probably an egg or a cyst of an unknown animal, with which it will soon be bound. Additionally, Lemmermann (1908) expressed doubts about the taxonomic classification of *Hexasterias*, but these considerations mainly concerned its affiliation with the Pleurococcaceae. Until recently, many authors did not trust the validity of its affiliation in the Prasinophyceae and preferred to present it as an "other" or "unknown" specimen (e.g. Horner 1984, Heiskanen et al. 2005). Additional taxonomic confusion, causing difficulty in merging species data, results from the use of more than one species name in the literature. Generally, in planktonic works, it appears as *P. problematica*, while in sediment studies, it is included as *H. problematica*. In pelagic investigations, the exceptions are Häyrén (1931), Horner (1984) and Heiskanen et al. (2005) (*H. problematica*), as well as Bērziņš (1932) (*Ovum hispidum problematicum*), whereas in sediment approaches, the exceptions are Rubino et al. (2009) and Van Hauwaert (2016) (*P. problematica*).

Despite the different views on species taxonomic affiliation, both Cleve (1900a,b) and Meunier (1910, 1919) presented very similar species descriptions, to which the

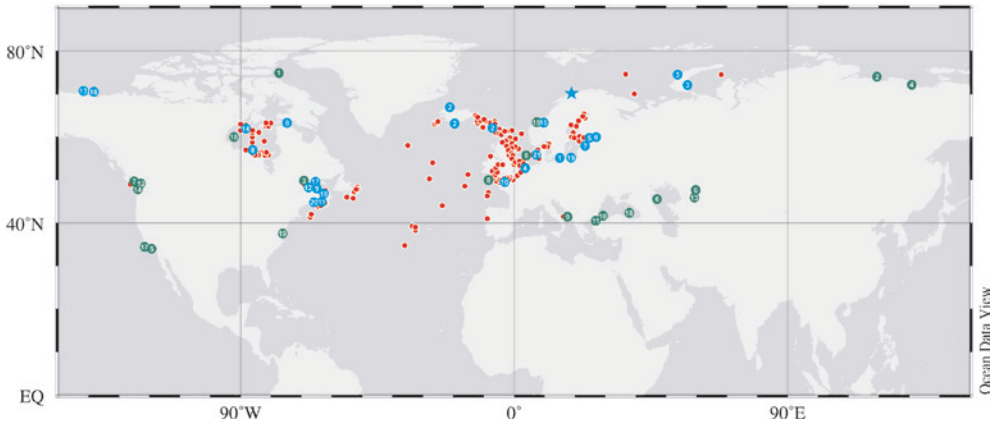

Figure 3: Map showing the distribution of pelagic (numbered blue circles) and sediment (numbered green circles) records of *Polyasterias problematica* from the literature, and the Ocean Biogeographic Information System (red dots); a blue star: this study. References are presented in Table 1.

specimen from Balsfjord clearly corresponds (Figure 2). According to the description, *P. problematica* is discoidal, biconvex with a central spherical body with a diameter of approximately 40 µm. The cell is filled with a dense, granular protoplasm. The cell from Balsfjord had seven cylindrical, empty rays distributed at equal distances along the edge (Figure 2). Rays were straight, nearly as long as the diameter of the disc (approximately 30 µm), and surrounded by a hyaline membrane with distinct parallel wrinkled filaments. The rays were more or less widened at the base, open-ended, with distinct hook-like structures of undetermined function. In side view, most of the rays were inclined in the same direction, but to a much lesser extent compared to the drawings by Cleve (1900a,b) and Meunier (1910, 1919). Due to the inability to turn the cell to the side view without its returning to the dorsal position, we present this arrangement only in the drawing (Figure 2D). Since the cell content was strongly stained with iodine, we were unable to observe the chloroplasts. Unfortunately, our specimen of *P. problematica* was lost during an attempt to discolour the cell with sodium thiosulfate, and the search for another specimen in the remaining material had no success. However, since the glutaraldehyde reacts and damages DNA, an eventual genetic analysis would not be possible anyway. This specimen was found in a sample dominated by *Chaetoceros furcillatus* Bailey, *C. tenuissimus* Meunier, *C. debilis* Cleve, *Pseudo-nitzschia* cf. *delicatissima* (Cleve) Heiden/*pseudodelicatissima* (Hasle) Hasle, *Skeletonema* cf. *marinoi* Sarno *et* Zingone and *Thalassiosira gravida* Cleve/*antarctica* Comber.

The extraordinary scarcity of the species records, in general, may be due to several factors. First, because the species occurrence is very limited in time and space, it can be missed during routine plankton survey; the ephemeral presence is probably also one of the reasons for the rarity of the palynomorph in marine sediments (Mudie et al. 2011). Second, due to low cell abundance (at most, 2–3 cells per sample have been found in previous studies) and the plankton counting methodology (analysis of a small sample volume), the species can be missed at the stage of sample preparation and examination. Taking into consideration the large cell size (approximately 100 µm) and the characteristic morphology, we exclude the possibility of the species being overlooked in the plankton chamber. Third, due to the morphological similarity of *P. problematica* to some dinoflagellate cysts [species classified as a Dinophyceae, *inter alia*, in Loeblich and Loeblich (1970)] and the clear lack of literature and taxonomic references for the species, the possibility of inaccurate taxonomic identification should also be questioned.

Our observation of *P. problematica* in Balsfjord raises a question about the origin of this organism in the studied waters. Because it is the first record of this species in the region (Figure 3), we cannot state whether *P. problematica* was dispersed naturally, by human transportation or both. However, since all associated planktonic protists were typical of marine waters and commonly observed in the North Atlantic and Arctic waters, and because the observed cell was in very good condition, the probability of introducing this species in ballast waters seems to be doubtful. If the distribution range of *P. problematica* actually covers northern Norwegian waters, it will most likely be re-observed in the area sooner or later. Therefore, this paper offers a valuable reference material for the taxonomic identification of *P. problematica* in order that future works will pay more attention to this species. Since nothing is known about living (not preserved) *P. problematica*, the observation of living cells would be particularly valuable

Table 1: References with numbers used for reporting occurrences of *Polyasterias problematica* from pelagic studies (blue circles) and sediment studies (green circles) in Figure 3.

Reference number	Reference	Sampling localities
Plankton studies		
1	Hensen 1887	Baltic Sea
2	Cleve 1900a	Iceland and Faroes, Atlantic Ocean
3	Meunier 1910	Barents and Kara seas
4	Meunier 1919	North Sea
5	Välikangas 1926	Gulf of Finland, Baltic Sea
6	Häyrén 1931	Gulf of Finland, Baltic Sea
7	Bērziņš 1932	Gulf of Riga, Baltic Sea
8	Bursa 1961	Hudson Bay and Strait, Atlantic Ocean
9	Brunel 1962	Baie des Chaleurs, Atlantic Ocean
10	Paulmier 1969	Estuary of Morlaix and Penze River, Atlantic Ocean
11	Saifullah and Steven 1974	Saint Margaret's Bay, Atlantic Ocean
12	Cardinal and Bérard-Therriault 1976	St. Lawrence Estuary, Atlantic Ocean
13	English and Horner 1977	Prudhoe Bay, Beaufort Sea
14	Anderson 1979	Hudson Bay, Atlantic Ocean
15	Hegseth 1982	Trondheimsfjorden, Norwegian Sea
16	Horner 1984	Point Barrow-Demarcation Point area, Beaufort Sea
17	Bérard-Therriault et al. 1999	Gulf of St. Lawrence, Atlantic Ocean
18	Pederson 2004	Atlantic provinces of Canada
19	Heiskanen et al. 2005	Gulf of Gdańsk, Baltic Sea
20	Martin et al. 2006	Bay of Fundy, Atlantic Ocean
21	Rick et al. 2016	North Sea
Sediment studies		
1	Mudie 1992	Baffin Bay, Atlantic Ocean
2	Kunz-Pirrung 1998	Laptev Sea
3	St-Onge et al. 1999	Saguenay Fjord, Atlantic Ocean
4	Polyakova et al. 2005	Laptev Sea
5	Pospelova et al. 2006	Santa Barbara Basin, Pacific Ocean
6	Sorrel et al. 2006	Caspian and Aral seas
7	Esenkulova 2009	Strait of Georgia, Pacific Ocean
8	Mertens et al. 2009	North and Celtic seas
9	Rubino et al. 2009	Gulf of Manfredonia, Adriatic Sea
10	Verleye et al. 2009	Black Sea
11	Mudie et al. 2010	Black Sea
12	Pospelova et al. 2010	Strait of Georgia, Pacific Ocean
13	Mudie et al. 2011	Aral Sea
14	Price and Pospelova 2011	Saanich Inlet, Pacific Ocean
15	Milzer et al. 2013	Trondheimsfjorden, Norwegian Sea
16	Shumilovskikh et al. 2013	Black Sea
17	Bringué 2015	Santa Barbara Basin, Pacific Ocean
18	Heikkilä et al. 2016	Hudson Bay, Atlantic Ocean
19	Van Hauwaert 2016	Chesapeake Bay, Atlantic Ocean

and would allow a better understanding of its morphology-function relationship and phylogenetic affiliation.

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sample analysis and taxonomic identification, iconography preparation, and manuscript writing; CS – sample collection and contribution to the manuscript.

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Bionotes

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