

New record of a dinoflagellate species, *Lessardia elongata* in the Black Sea

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Lessardia elongata Saldarriaga & Taylor is identified for the first time in the north-western Black Sea in the present study. This species has probably been recorded with different names in the Black Sea due to lack of detailed investigations with scanning electron microscopy (SEM) or fluorescence microscopy. Our identification is based on observations with an electron microscope in September 2004 and with an epifluorescence and light microscope in June 2006. Cells were 5–10 µm wide, 22–30 µm long in formaldehyde fixed samples, fusiform, transparent, and had a faint broad girdle. Epitheca was slightly larger than the hypotheca and recently ingested prey could often be seen in the antapical half of the cell within very conspicuous vacuoles. Thin and faint thecal plates were hardly visible under epifluorescence microscope after staining the cells with fluorescence brightener 28 in the fixed samples. Cell abundances up to 18,400 cells l⁻¹ and 87,000 cells l⁻¹ were observed in September 2004 and June 2006, respectively.

Keywords: new dinoflagellate, *Lessardia elongata*, Peridinales, Podolampaceae, Black Sea

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Classification of naked dinoflagellates has been arbitrary for a long time (Fensome *et al.*, 1993; Daugbjerg *et al.*, 2000) due to insufficient data and they were generally classified in the order Gymnodiniales (Saldarriaga *et al.*, 2003). Recent studies using ultrastructural and molecular data helped to clarify this classification (Shao *et al.*, 2004). Consequently, several new genera of naked dinoflagellates (e.g. *Akashiwo*, *Karenia*, *Karlodinium* Daugbjerg *et al.*, 2000; *Takayama* De Salas *et al.*, 2003 and *Togula* Flø Jørgensen *et al.*, 2004) have been created and some species, previously thought as naked, were discovered to be thecate (e.g. *Katodinium rotundatum* was reclassified to the peridinialean genus *Heterocapsa*, Hansen, 1995). However, there are still many poorly studied species of dinoflagellates, which appear naked.

Lessardia genus and *Lessardia elongata* Saldarriaga & Taylor species were for the first time described in the samples taken from Georges Bank (north-west Atlantic, off the coast of Massachusetts, USA (Saldarriaga *et al.*, 2003)). It was suggested by Saldarriaga *et al.* (2003) that this species had been identified as '*Gymnodinium elongatum* Hope 1954 or *Gymnodinium* sp.' in the past and observed along Norwegian and Danish coasts (Hansen & Larsen, 1992), in the north-west Atlantic (Shapiro *et al.*, 1989) and the north-east Pacific (Shapiro *et al.*, 1989; Sherr & Sherr, 2002). After revealing their thecal plates, Saldarriaga *et al.* (2003) classified this species in the order Peridinales and the family Podolampaceae, rather than in naked Gymnodiniales. Recently, it was also reported by Throndsen *et al.* (2007)

that this species was sporadically observed in south Norwegian fjord areas in summer.

In the present study *Lessardia elongata*, which was not mentioned previously in the Black Sea (Ivanov, 1965; Bodeanu, 1987/1988; Eker *et al.*, 1999; Velikova *et al.*, 1999; Moncheva *et al.*, 2001; Uysal, 2001; Eker-Develi & Kideys, 2003; Gomez & Boicenco, 2004), was identified for the first time in this area using scanning electron microscope (SEM) in samples from the 2004 cruise and under epifluorescence microscope in samples from the 2006 cruise.

During the present study samples were collected from the western Black Sea on two different cruises. The first cruise was on board of the RV 'Poseidon', along the Romanian and Ukrainian waters (2–7 September 2004); samples were taken from the subsurface chlorophyll-*a* maximum layer at 22 stations. The second cruise was on board of the RV 'Akademik' along Romanian and Bulgarian waters (2–17 June 2006); samples were taken from surface waters at 30 stations. In both sampling periods phytoplankton samples were immediately fixed with buffered formaldehyde to have a final concentration of 2% onboard. For SEM (JEOL 840) examination, phytoplankton samples from the September 2004 cruise were filtered through polycarbonate membrane filters with 0.2 µm pore size. After filtration membranes were placed on a SEM stub and sputter-coated with gold and searched for cells with the SEM.

Phytoplankton samples from the June 2006 cruise were settled to concentrate cells to count under the microscope using a Sedgewick-Rafter cell. Samples were kept immobile ~2 weeks in the laboratory, after this time the samples were concentrated from a total volume of ~250 ml to 20–50 ml by siphoning the supernatant using thin curved tubes (Eker *et al.*, 1999). Microscopic visualization and image acquisition of the cells stained with 0.01% fluorescent brightener 28 (Sigma) were performed using an F-View digital

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monochromatic camera and Cell P software coupled to an Olympus IX-81 motorized inverted microscope with an Olympus U-RFL-T epifluorescence attachment. Cell volume, calculated considering a fusiform shape, was $300 \pm 250 \mu\text{m}^3$ on average and ranged from $\sim 200\text{--}800 \mu\text{m}^3$; $N = 400$. The *in situ* temperature and salinity measurements were performed using a Seabird-19 CTD.

Cells were 22–30 μm long, 5–10 μm wide in formaldehyde fixed samples, spindle shaped, transparent and lack chloroplasts (Figures 1, 2a–g). The epitheca was slightly larger than the

hypotheca and had a weakly impressed cingulum (Figure 2c, d). Recently ingested prey could often be seen in the antapical half of the cell within very conspicuous vacuoles (Figure 2a, f, g). Empty vacuoles of cells were sometimes visible (Figure 2d, e). Sulcal plates were also observed (Figure 2h). The nucleus was located in the apical half of the cell (Figure 2i). Faint thecal plates were seen barely on Figure 2j.

Cell abundance of *Lessardia elongata* ranged from 950–18,400 cells l^{-1} in September 2004 and from 400–87,000 cells l^{-1} in June 2006 in the western Black Sea. Abundance of this species was higher at the coastal stations than at the offshore stations.

There was a significant negative correlation between cell abundance of *Lessardia elongata* and salinity ($r^2 = 0.39$, $P < 0.00018$). The temperature and salinity ranges were 21.3–24.4°C and 10.4–17.7 psu during September 2004 sampling and 16.7–20.9°C and 4.2–18.5 psu along the stations during June 2006 sampling.

Lessardia elongata Saldarriaga & Taylor, a thecate dinoflagellate, was previously identified as athecate *Gymnodinium elongatum* Hope 1954 (Saldarriaga *et al.*, 2003) since it may appear like an athecate species under light microscopy and was observed in different regions with this name or as *Gymnodinium* sp. previously (Shapiro *et al.*, 1989; Hansen & Larsen, 1992; Sherr & Sherr, 2002). Although neither *L. elongata* nor *G. elongatum* has been reported in any peer reviewed publication in the Black Sea so far (Ivanov, 1965; Bodeanu, 1997/1988; Eker *et al.*, 1999; Velikova *et al.*, 1999; Moncheva *et al.*, 2001; Uysal, 2001; Eker-Develi & Kideys, 2003; Gomez & Boicenco, 2004), it has been observed and included in total phytoplankton abundance and local protocols since the 1980s with different names; *Gymnodinium* sp., *Cryptaulax* sp., *Gymnodinium elongatum* or *Gymnodinium*

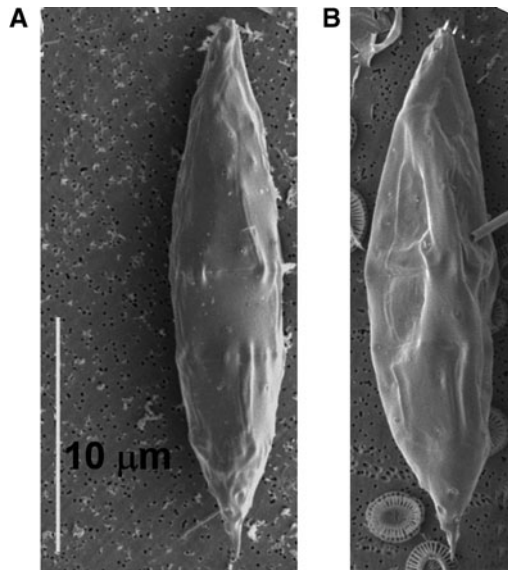


Fig. 1. SEM micrographs of *Lessardia elongata*; both are side view.

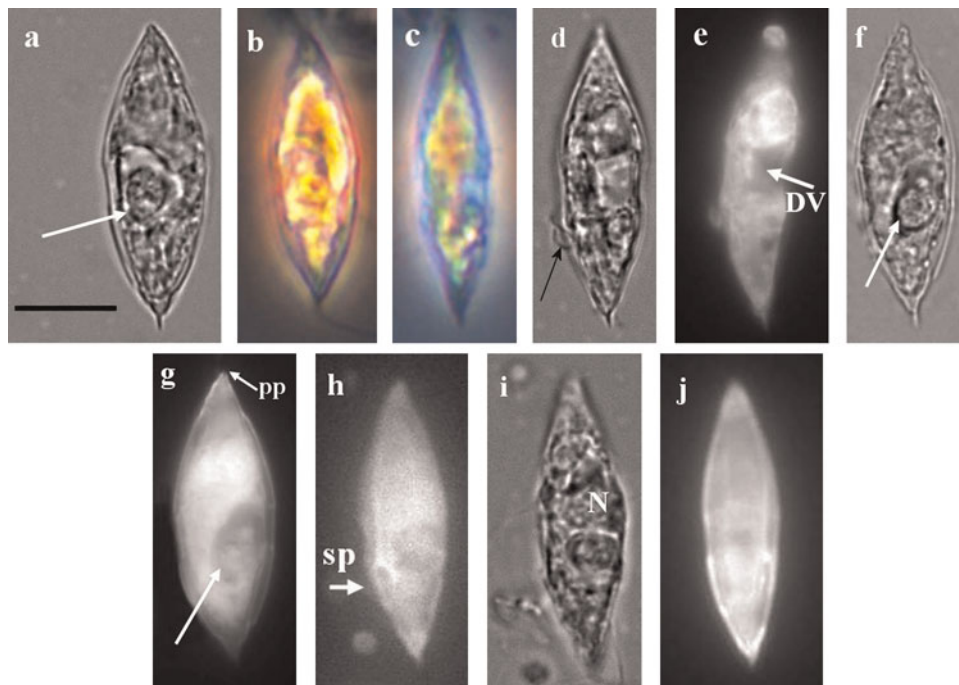


Fig. 2. Morphology of *Lessardia elongata*. (a) Phase contrast light micrograph showing digestive vacuole with ingested prey (arrow); (b) transparent appearance of another cell; (c) transparent cell in which epitheca clearly seems greater than hypotheca; (d) cell with a broad girdle and a flagellum (arrow); (e) the same cell as Figure 2d stained with fluorescent brightener 28, under UV light, empty digestive vacuole (DV); (f) cell with digestive vacuole with ingested prey; (g) same cell as Figure 2f illuminated with UV light, pore plates (pp) located at the apical side; (h) sulcal plates (sp) shown with UV light; (i) the nucleus (N); (j) faint thecal plates observed with UV light. Scale bar: 10 μm .

fuscus (V. Velikova, unpublished; N. Derezyiuk, Odessa University, Ukraine, personal communication). In the present study, based on our fluorescence and SEM observations, we confirm that the spindle shaped dinoflagellate, which was sporadically observed in the Black Sea waters, is *Lessardia elongata*.

Heterotrophic microplankton temporarily plays a major role in the transfer of food and energy to higher trophic levels within the pelagic system (Hansen, 1991; Tillmann & Hesse, 1998). One of the heterotrophic microplankton species, *Lessardia elongata*, was recorded for the first time in the western Black Sea in this study.

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