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Note

First record of *Ostreopsis* cfr. *ovata* on macroalgae in the Northern Adriatic Sea

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Abstract

Ostreopsis ovata is an epiphytic potentially toxic dinoflagellate. It has a world-wide distribution, normally associated with other epiphytic or benthic dinoflagellates. In tropical seas *O. ovata* is often associated with the genera *Gambierdiscus*, *Coolia* and *Prorocentrum*, causing cinguatera fish poisoning. Recently, *Ostreopsis* spp. blooms in the Tyrrhenian and southern Adriatic Sea have been related to human health problems, such as breathing and skin irritation. Here we report the first record of *Ostreopsis* cfr. *ovata* in the Northern Adriatic Sea. *O.* cfr. *ovata* was isolated from macroalgae in two areas, the Gulf of Trieste (Italy) and close to Rovinj (Croatia). The microalga was identified by scanning electron microscopy and by fluorescence light microscopy. Size range and thecal pore structure were similar to those described for *O.* cfr. *ovata* in previous studies. *Ostreopsis* cfr. *ovata* was present on all the macroalgae collected, particularly browns and reds. The microalgal association on macroalgae was mostly composed of *Ostreopsis* sp., *Coolia monotis* and *Coscinodiscus* sp.

Keywords: *Ostreopsis* sp.; Epiphytic dinoflagellate; Macroalgae; Northern Adriatic Sea

1. Introduction

The genus *Ostreopsis* Schmidt (1901) belongs to the family Ostreopsidaceae Lindeman (1928). It has a world-wide distribution (Taylor, 1979; Tognetto et al., 1995; Faust et al., 1996; Chang et al., 2000; Vila et al., 2001; Sansoni et al., 2003) and some *Ostreopsis* species can produce palytoxins that can cause harm from ingestion of fish (Sansoni et al., 2003; Taniyama et al., 2003; Usami et al., 1995).

The genus was previously described in 1901 with the species *O. siamensis* Schmidt (1901) and lately re-described by Fukuyo (1981) with two other new species: *O. ovata* Fukuyo and *O. lenticularis* Fukuyo. To date, nine different species of *Ostreopsis* have been described (Faust et al., 1996; Faust, 1999). The taxonomy of the species is based on some morphological characters as thecal plates, shape, size, trichocyst pores and thecal pore size.

Ostreopsis are epiphytic dinoflagellates living on macroalgae, very often in assemblages with other microalgae such as *Coolia monotis*, *Prorocentrum lima* and *Coscinodiscus* sp. (Vila et al., 2001), *C. monotis*, *P. lima* and *P. compressum* (Chang et al., 2000) or *C. monotis*, *Oxyrrhis marina* and *Amphidinium* sp. (Taylor, 1979). In tropical areas *O. ovata* usually forms an assemblage with *P. lima*, *P. concavum*, *O. siamensis*, *O. lenticularis* and *Gambierdiscus toxicus*, species considered responsible for ciguatera (Carlson and Tindall, 1985; Bomber and Aikman, 1989).

During recent years *Ostreopsis* spp. produced blooms in the Tyrrhenian and Southern Adriatic Sea. Blooms have been related to human health problems in tourists attending the beaches and inhaling marine aerosols (Sansoni et al., 2003).

To date in the Gulf of Trieste we have not been aware of any bloom in the water column, but we decided to investigate if the species was present in epiphytic stages on some macroalgae assemblages along the coast, and whether it could be found also in the more oligotrophic environment of the rocky coasts of the Istrian Peninsula. We report for

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the first time the presence of *Ostreopsis* cfr. *ovata* in the Northern Adriatic Sea. From the morphological description our species can be assigned to *O. ovata*, but as this species in previous studies showed variable dimensions, only the molecular characterization will definitely solve the taxonomical problem.

2. Materials and methods

During September and October 2006 macroalgae samples were collected in the Gulf of Trieste (Italy) and close to the town of Rovinj (Croatia). Sampling sites were shallow in protected places, the Italian macroalgae were collected at 2 m depth in a small harbour while the Croatian ones were from 1 m depth on a rocky coast. The macroalgae comprised *Padina pavonica* (Linnaeus) J.V. Lamouroux, *Laurencia obtusa* (Hudson) J.V. Lamouroux, *Corallina officinalis* Linnaeus, *Amphiroa rubra* (Philippi) Woelkerling, *Cystoseira crinita* Duby. *Ulva rigida* C. Agardh was present and collected only at the Italian site.

The macroalgae were collected by diving, carefully cut and placed in plastic bottles under water. In the laboratory, macroalgae samples were gently placed in a petri dish and observed under a Leica Wild M10 microscope at 31.5×. *Ostreopsis* sp. and *C. monotis* were isolated by micropipette as a single cell directly from macroalgae and part of the samples were fixed with formaldehyde solution for counting.

Temperature and salinity of the water were measured during sampling by means of a WTW Microprocessor Conductivity Meter LF 196.

Plate tabulation was examined under epifluorescence microscopy using an Olympus IX 71 inverted microscope at 320× and 400× following the calcofluor method of Fritz and Triemer (1985), using fluorescent brightner 28 (Sigma–Aldrich Co., St. Louis, MO, USA). Dimensions of living cells were determined by measuring the dorsoventral (DV) and the AP (anteroposterior) diameters, and the width by an inverted microscopy Leitz Labovert at 320×. At least 100 cells for every strain were measured. DV/AP ratios were calculated.

For SEM analyses, cells were fixed with 4% formaldehyde and stored at 4 °C for three days. They were then washed in distilled water and dehydrated in an ethanol series (30%, 50%, 70%, 80%, 95% and 100%). The supernatant was removed after the sedimentation of the cells (about 3 h). Samples were CO_2 critical point dried, coated with Au/Pd and examined on a Leica – Cambridge Stereoscan 420i electron microscope.

3. Results and discussion

O. cfr. *ovata* was found at both sites in the Northern Adriatic Sea. *O.* cfr. *ovata* was associated with all species of macroalgae collected, only on *Ulva rigida* it was scarce. It was associated on all the macroalgae with *C. monotis* and *Coscinodiscus* sp.. In the samples observed *Ostreopsis* preferred brown and red macroalgae, especially *C. crinita* and *C. officinalis*.

Under the light microscope the cells appeared lens or tear shaped. The size ranged between 29.6 and 70.8 μm DV and between 18.5 and 53.1 μm width. In particular for the Italian sample the DV diameter was 30–71 μm (average 55 μm) and the width 19–53 μm (average 36 μm), while for the Croatian sample DV was 33–67 μm (average 55 μm) and width 19–44 μm (average 34 μm) (Table 1). AP diameter ranged from 15 to 30 μm (average 23 μm) and the DV/AP ratio value was 1.5–2.8 (average 2.4) (Table 2).

The dimensions were similar to those found by other authors for *O. ovata* (Aligizaki and Nikolaidis, 2006; Chang et al., 2000; Penna et al., 2005). In particular the DV/AP ratio was higher than 4 (Penna et al., 2005) or ≈3 (Aligizaki and Nikolaidis, 2006) in *O.* cfr. *siamensis*, while in *O. ovata* the ratio was lower than 2 (Penna et al., 2005; Aligizaki and Nikolaidis, 2006).

Light microscopy epifluorescence showed a plate pattern Po, 3′, 7′′, 6C, 6S?, 5′′′, 1p, 2′′′′, for both isolates (Figs. 1, 2) as identified for *Ostreopsis* species (Faust and Gulladge, 2002). In the epitheca the plate 1′ was long and hexagonal, in contact with plates 2′, 3′, 1′′, 2′′, 6′′ and 7′′ (Fig. 1). In the hypotheca plate 1p was dorsoventrally elongated long and narrow (Fig. 2) with a *l*/*w* ratio of 2.17 (Table 2).

Table 1
Dorsoventral diameters (DV), width, average and standard deviation of two isolates

Italy	DV	Width
Maximum	70.8	53.1
Minimum	29.6	18.5
Average	55.3	36.4
Standard dev.	8.0	6.4
Median	55.5	37.0
Croatia		
Maximum	66.6	44.4
Minimum	33.3	18.5
Average	54.8	34.3
Standard dev.	7.1	4.7
Median	55.5	33.3

Table 2
Anteriorposterior diameter (AP), ratio DV/AP, average, standard deviation of two isolates and *l*/*w* of plate 1p

	DV	Width	AP	DV/AP
Maximum	59.2	40.7	29.6	2.8
Minimum	40.7	25.9	14.8	1.5
Average	52.5	32.9	22.6	2.4
Standard dev.	6.5	4.4	4.4	0.4
Median	53.7	33.3	22.2	2.4
Plate 1p			*l*/*w*	
Maximum			2.47	
Minimum			2.00	
Average			2.17	
Standard dev.			0.12	
Median			2.16	

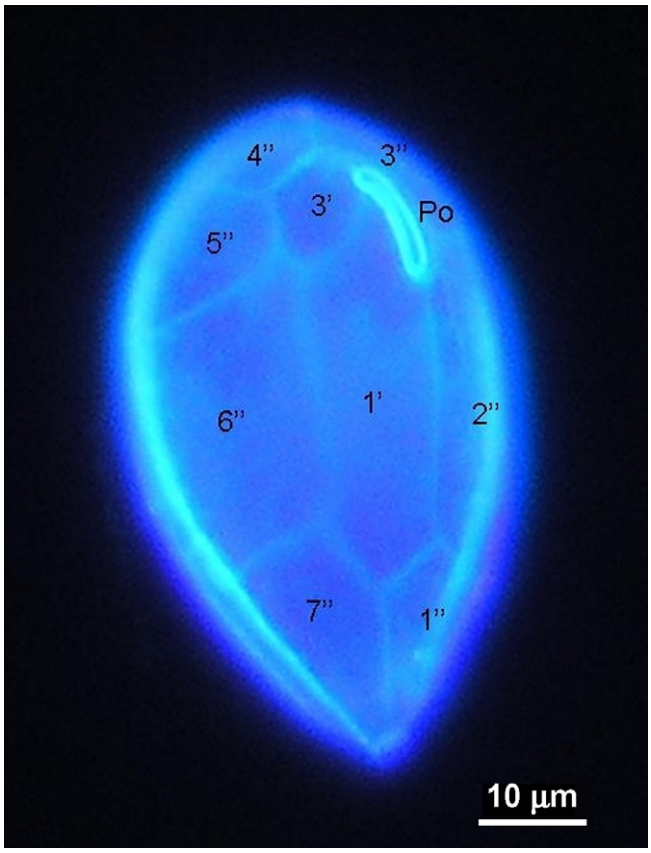

Fig. 1. LM epifluorescence. Epithecal view of *O.* cfr. *ovata*.

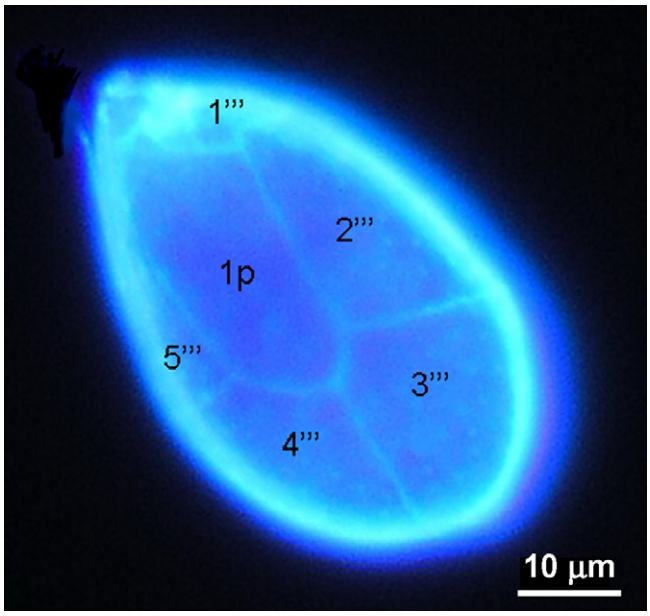

Fig. 2. LM epifluorescence. Hypothecal view of *O.* cfr. *ovata*.

SEM observations confirmed the presence of a smooth thecal surface covered with spaced thecal pores. The average size of thecal pores was 0.25 µm. The ventral pore structure (Vp) for both strains was similar and showed a characteristic structure. In apical view the apical pore plate (Po) generally appeared to be a short, almost straight slit (Fig. 3). The length of the Po ranged between 5.9 and 7.7 µm for the internal part and 6.6–9 µm for external one. This is in the same range as reported by Faust et al. (1996) (8 µm) and Aligizaki and Nikolaidis (2006) (11 µm). In previous studies the thecal pore size, viewed externally, in *O.* cfr. *siamensis* ranged from 0.11 to 0.56 µm and one predominant size class was observed, in *O. ovata* ranged from 0.16 to 0.55 µm and two pore size classes were observed (Penna et al., 2005).

The association resembled that found by Vila et al. (2001) along the Catalan coast, with *Ostreopsis* sp.,

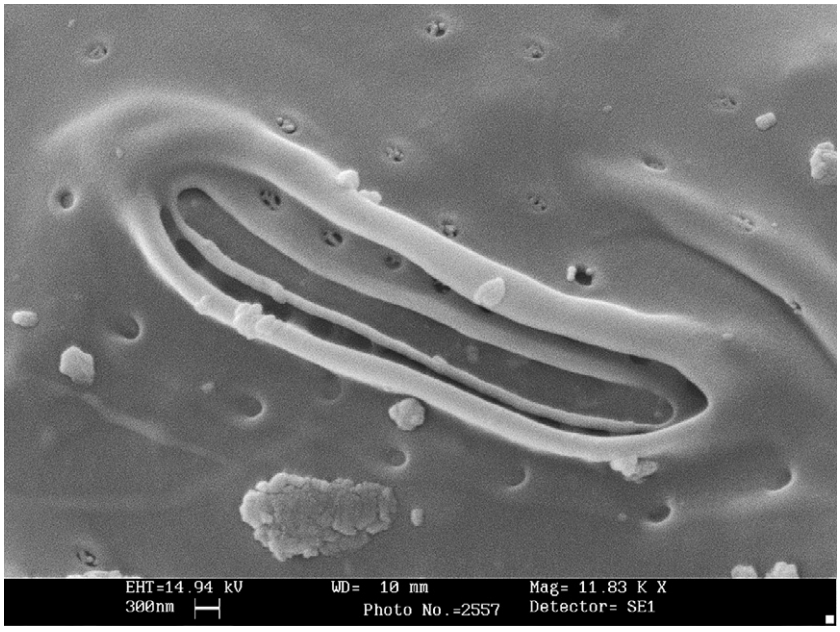

Fig. 3. SEM. Detail of the pore plate (Po) with large multipores structure.

C. monotis, *P. lima* and *Coscinodiscus* sp.. This similarity could suggest a latitudinal gradient for the Mediterranean Sea, with different species compositions within the benthic association.

Temperature at the two sites examined was 20.6 °C (Croatia) and 22.4 °C (Italy), and salinity 37.8 psu (Italy) and 38.6 psu (Croatia). In previous studies *Ostreopsis* sp. was found at temperatures ranging between 11.5 °C and 29.7 °C (Chang et al., 2000; Vila et al., 2001; Aligizaki and Nikolaidis, 2006), and salinity between 37.2 and 38.1 psu (Vila et al., 2001). Temperature does not seem to affect the genus distribution (Carlson and Tindall, 1985). Vila et al. (2001) did not find a clear seasonal pattern in the relative abundance of epiphytic organisms.

In the Gulf of Trieste during the last decade there has been an increase in temperature and salinity values. In particular, summer temperature at a depth of 10 m has a rising trend of 0.22–0.23 °C y^{-1} and salinities showed a most pronounced positive trend of 0.28–0.34 y^{-1} (Malačič et al., 2006). The presence of *Ostreopsis* in the Northern Adriatic could be linked to the increase in temperature and salinity during recent years but it is also possible that this genus was already present in the past but its presence on macroalgae was never tested.

The presence of *Ostreopsis* sp. on macroalgae must be maintained under observation, because it has been demonstrated that a positive correlation exists between its concentrations in the water column and in the sediment with those on macroalgae (Vila et al., 2001; Aligizaki and Nikolaidis, 2006).

Based on these preliminary results, the species in the Northern Adriatic area seems to present an opportunistic behaviour at different trophic situations, and shows a wide distribution on different macroalgae substrates. Further, researches in other areas of the Adriatic Sea are required to define the spatial distribution of the species; molecular characterization and toxicity analyses are also planned to identify the presence and the possible noxious effects of different *Ostreopsis* species.

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