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Research Article

# NEW DINOFLAGELLATE (*DINOFLAGELLATA*) SPECIES FROM THE ODESSA BAY OF THE BLACK SEA

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## Abstract

The annotation list consists of 28 species and 4 infraspecies taxa of dinoflagellates found in the Odessa Bay of the Black Sea, of which 24 species and 3 infraspecies on the list are new to the Black Sea. The invasive species are described and the possible ways of introduction of these species with ship ballast waters are discussed.

## INTRODUCTION

Knowledge of Black Sea dinoflagellates is presented in two monographs (Kiselev 1950, Black Sea… 1998) and from descriptions of species in several floristic and hydrobiological studies (Kuzmenko 1966; Pitsik 1967; Roukhiyainen 1970, 1971; Senichkina 1973; Georgieva 1993; Krakhmalniy 1994, 2001; Krakhmalniy and Terenko 2002 a, 2002 b). Of the 364 species of dinoflagellates that are described in Kiselev (1950), 155 have been recorded in the Black Sea. Ivanov (1965) also provides descriptions of 164 Black Sea species (173 infraspecies taxa) of dinophytes.

Pitsik (1979) mentions 205 species and subspecies of the number of dinophyte taxa in Black Sea phytoplankton. Using floristic surveys for the coasts of Bulgaria, Romania, Turkey and Georgia, Gomoiu and Skolka (1998) list 210 species (220 infraspecies taxa) of dinophytes. Although the systematic composition of most armored Black Sea dinoflagellates has been well studied, new species are revealed yearly. Recently the dinoflagellates *Alexandrium monilatum* (Howell) Balech, *Oxyphysis oxytoxoides* Kof., *Gymnodinium uberrimum* (Allman) Kof. et Sw. were noted for the Bulgarian coast (Moncheva *et al.* 2000). According to studies of dinoflagellates of the Ukrainian shelf, 99 species (101 infraspecies taxa, including the nomenclature type of the species) were observed (Black Sea… 1998).

The occurrence of “red tides” has also encouraged more dinoflagellate studies, including the morphology and ecology of toxic species. In recent years near the Odessa coast in the Black Sea “red tides” have been caused by *Akaschiwo sanguinea* (Hirasaka) G. Hansen et Moestrup and *G. simplex* (Lohm.) Kof. et Sw. These species were encountered earlier in small numbers in the plankton and later produced high cell concentrations (Terenko and Kurilov 2001, Alexandrov 2001). It has been noted that the mixotrophic species *A. sanguinea*, *G. simplex*, *G. marinum* Sav.-Kent., plus heterotrophic *Gyrodinium cornutum* (Pouch.) Kof. et Sw., *Diplopsalis lenticula* Bergh and *Oblea rotunda* (Lebour) Balech, frequently become highly abundant in Odessa Bay in summer and early autumn.

In the past decade, the problem of invasive species in marine coastal zones introduced with ballast waters from ships coming from other parts of the World Ocean has aroused much interest (Initial risk…1999, Leppäkoski and Olenin 2000, Exotic species… 2001). It is feasible that toxic species could be included among other phytoplankton that have been introduced into the Black Sea with ballast waters and for Mediterranean species to enter naturally through the Dardanelles and Bosphorus straits. Baseline studies were carried out within the framework of the Globallast project in the Odessa port area to reveal invasive species, including dinoflagellates, and also to prevent introduction of exotic organisms with ship ballast waters.

The objective of this study is to update the data on new dinoflagellate species identified in the Black Sea, and to identify the invasive dinoflagellate species that have been introduced with ship ballast waters.

## MATERIAL AND METHODS

Water samples for studying the dinoflagellates in the plankton community were collected annually in the Black Sea coastal zone of Odessa Bay from 1993 to 2002 at seven stations. Joint studies were also provided with water samples in August to November 2001 and in July and August 2002 at fifty stations. Altogether in the Odessa Bay and adjacent area 480 bathymetric samples were taken from a 0.5 m depth below the sea surface, and 62 samples in Odessa port from the sea surface and near bottom depths of 3 to 16.5 m. The 1 – 2 litre samples were concentrated by reverse filtration through nucleoporous (nuclear) filters with 1 μm size pores and observed unfixed. Cell counts of the ultra- and nanoplankton were viewed in a 0.05 ml chamber with a light microscope "Biolam" (with magnification 600 x). Large and rare forms were counted in a 5 ml chamber. These dinoflagellate forms were selected with a thin capillary pipette. They were mounted on a glass slide and studied live. When necessary "a hanging drop" method was used for longer studies. Then separate drops were placed in a large volume of fixing liquid. A quick live fixation with glutaric dialdehyde was made for permanent mounts. Lugol`s solution was for used for fixation and 40% formaldehyde 1:10 solution for additional fixation.

When determining the species of armored dinoflagellates, the sheath was separated from the cell contents by squeezing the cells between the glass slide and coverslip in a small volume of fluid. For viewing the empty sheaths with a light microscope the seams between the lamina were dyed with an iodine-containing dye (Konovalova 1998).

For identifying the unarmored dinoflagellates different methods were used for isolating cells, studying them live, and for live fixation. For studying the life cycles of some unarmored dinoflagellates, the method of using mixed and pure cultures was employed. The cells of separate species were selected with a capillary pipette under a binocular MBC-10 microscope. They were placed in Petri dishes with a GPM nutrient medium (Loeblich 1975) prepared earlier using sterile autoclaved Black Sea water of 17 psu salinity. They were cultivated in a thermostat at a temperature of 20 ± 2°C providing a light regime of 12 hrs light: 12 hrs darkness.

When determining species identification and when recording rare dinoflagellate taxa, a light microscope Motic Images 2000 DMWBI – 223 with a digital chamber was used with magnification 1000 x. Permanent preparations were made of new Black Sea species which were kept in the Department of Hydrobiology of Active Sea Zones of the Odessa Branch of the Institute of Biology of Southern Seas, National Academy of Sciences of Ukraine.

## RESULTS AND DISCUSSION

**Table 1**

New dinoflagellate species for the Black Sea and its littoral near Ukraine (original data).

| Taxa | Water Temperature [°C] | Salinity [psu] | Date when first encountered |
|---|---|---|---|
| Division *Dinophyta* | | | |
| Class *Dinophyceae* | | | |
| Order *Gymnodiniales* | | | |
| Family *Gymnodiniaceae* | | | |
| *Amphidinium conradi* (Conrad) Schill. ++ | 5,5 | 8,6 | III, 2001 |
| *A. inflatum* Kof. ++ | 10,0 | 17,8 | V, 2001 |
| *A. lanceolatum* Schröd ++ | 17,0 – 20,0 | 15,0 – 16,0 | VI, 1996 |
| *A. lacustre* Stein ++ | 0,5 – 3,0 | 17,5 – 17,8 | I, 2002 |
| *A. larvale* Lindem. ++ | 2,5 – 5,0 | 9,9 | I, III, 1999 |
| *A. vigrense* Wolosz. + | 8,0 – 10,0 | 14,9 | XI, 1995 |
| *Cochlodinium geminatum* (Schütt) Schütt ++ | 26,0 | 12,1 | VII, 1998 |
| *C. helicoides* Lebour ++ | 0,5 | 17,7 | I, 2002 |
| *C. helix* (Pouch.) Lemm. + | 11,5 | 10,0 | IV, 2001 |
| *C. polykrikoides* Margelef ++ | 25,0 – 26,0 | 14,0 – 15,0 | VIII, IX, 2001 |
| *Gymnodinium aeruginosum* Stein ++ | 6,5 – 22,0 | 10,2 – 14,1 | III, VI, 1998 |
| *G. aureolum* (Hulburt) Hansen ++ | 25,0 – 26,6 | 13,0 – 14,5 | VIII, 2002 |
| *G. blax* Harris.++ | 23,5 | 13,2 | VIII, 1998 |
| *G. heterostriatum* Kof. et Sw. ++ | 6,5 – 19,0 | 13,8 – 14,9 | III, IV, IX, 2001 |
| *G. lacustre* Schill. ++ | 22,0 | 15,9 | V, 1999 |
| *G. pygmaeum* Lebour ++ | 12,0 – 15,0 | 9,9 – 11,7 | V, XI, 2001 |
| *G. stellatum* Hulburt ++ | 0,5 – 6,5 | 13,8 – 17,7 | I, III, XII, 2001 |
| *G. uberrimum* (Allman) Kof. et Sw. + | 12,7 – 18,0 | 9,7 – 14,0 | VIII, IX, 1999 |
| *Gyrodinium ovum* (Schütt) Kof. et Sw. ++ | 5,0 | 15,6 | III, 2001 |
| Family Polykrikaceae | | | |
| *Polykrikos kofoidii* Chatton ++ | 14,5 – 23,5 | 10,2 – 13,2 | V, VI, VIII, IX, X, 1998 |
| Family Warnowiaceae | | | |
| *Warnowia maculata* (Kof. et Sw.) Lind. ++ | 0,5 | 17,5 | I, 2002 |
| *W. schuettii* (Kof. et Sw.) Schill. ++ | 2,0 | 17,0 | XII, 2001 |
| Order Gonyaulacales | | | |
| Family Ceratiaceae | | | |
| *Ceratium furca* var. *eugrammum* (Ehr.) Jörg. + | 8,0 | 16,9 | X, XI, 1999 |
| *C. fusus* var. *schüttii* Lemm. ++ | 4,0 | 15,5 | II, 2001 |
| *C. longirostrum* Gourr. + | 20,8 | 14,8 | X, 1999 |
| *C. pulchellum f. dalmaticum* (Böhm.) Schill. ++ | 18,0 | 13,7 | VI, 2000 |
| *C. strictum* (Okam. et Nishik.) Kof. ++ | 3,5 | 17,9 | XII, 2001 |
| *C. tripos* (O.F. Müll.) Nitzsch. var. *tripos* ++ | 21,0 – 24,2 | 13,0 | VIII, 2000 |
| Family Goniodomaceae | | | |
| *Goniodoma striatum* Mang. ++ | 22,0 – 25,0 | 14,0 – 14,9 | VIII, 2001 |
| *Alexandrium pseudogonyaulax* (Biecheler) Horiguchi ex Yuki et Fukuyo ++ | 25,0 – 26,6 | 13,0 – 14,5 | VIII, 2002 |
| Order Peridiniales | | | |
| Family Oxytoxaceae | | | |
| *Oxytoxum turbo* Kof. ++ | 12,2 | 16,7 | XI, 2000 |
| Class Noctiluciphyceae | | | |
| Order Noctilucales | | | |
| Family Kofoidiniaceae | | | |
| *Spatulodinium pseudonoctiluca* (Pouchet) Cachon et Cachon ++ | 14,9 – 18,5 | 14,0 – 16,1 | VI, 2001 |

This paper summarizes a detailed study of dinophyte algae in Odessa Bay of the Black Sea (Table 1). The armored dinoflagellates were best studied stored in fixed material. Most of the identified plankton dinophytes (23 species) were unarmored and have not been sufficiently studied in the Black Sea. The list has been prepared according to the system of dinoflagellates of R. Fensome (1993). The names of the taxa represent recent changes in synonymics and include 32 species (36 infraspecific taxa), first recorded in the Black Sea littoral region (designated as ++ the symbol + in the list) of which 27 species (30 infraspecific taxa) are new to the Black Sea (designated as ++).

Among these taxa were *Cochlodinium polykrikoides*, a cosmopolitan species (Steidinger and Tangen 1996) and *Spatulodinium pseudonoctiluca* which is widely distributed and has been reported in the Mediterranean Sea (Dodge 1985). The most frequently encountered species were *Gymnodinium uberrimum, G. stellatum, G. aureolum, Cochlodinium polykrikoides* and *Polykrikos kofoidii.* The rare species included *Spatulodinium pseudonoctiluca*, *Warnowia maculata*, *W. schuettii* and *Cochlodinium helicoides*.

**Table 2**

Dinoflagellates – introduced species in the Black Sea.

| Species | Date of discovery | Origin | Vector of introduction |
|---|---|---|---|
| *Gymnodinium uberrimum* | 1999 | Europe, freshwater bodies | Shipping or other means (river runoff) |
| *Gymnodinium aureolum* | 2002 | North Atlantic | Shipping, ballast waters |
| *Spatulodinium pseudonoctiluca* | 2001 | North Atlantic, Mediterranean Sea | Shipping, ballast waters |
| *Cochlodinium polykrikoides* | 2001 | North Atlantic or Indian Ocean | Shipping, ballast waters |
| *Alexandrium pseudogonyaulax* | 2002 | Mediterranean Sea or Sea Japan | Shipping, ballast waters |

**Table 3**

Quantitative parameters of dinoflagellate species introduced into the Black Sea.

| Taxa | Area of discovery | Average cell size, μm | | Average cell volume, $\mu m^3$ | Abundance, cells · $l^{-1}$ | | |
|---|---|---|---|---|---|---|---|
| | | length | width | | Min | Max | Average |
| *Gymnodinium uberrimum* | Odessa port, adjacent area | 43,0 | 35,5 | 28 382 | 20 870 | 36 000 | 28 435 |
| *Gymnodinium aureolum* | Odessa port | 42,0 | 36,25 | 28 906 | 2 600 | 66 600 | 37 800 |
| *Spatulodinium pseudonoctiluca* | Odessa port, Sukhoi liman | 131,3 | 71,5 | 187 105 | 11 | 69 | 41 |
| *Cochlodinium polykrikoides* | Odessa port, Sukhoi liman | 34,8 | 30,45 | 16.900 | 102 | 700 | 487 |
| *Alexandrium pseudogonyaulax* | Odessa port | 45,0 | 40,6 | 38 850 | 231 | 2 721 | 1 476 |

The discovery of these Black Sea dinoflagellates was mainly due to the application of improved research methods and live species identification. However, there is a high probability that the appearance of alien species in the Black Sea is due to accidental human introduction. Five dinoflagellate species (Table 2) are referred to as invasive species believed to be introduced with ship ballast waters. This group includes species which in previous dinoflagellate biodiversity studies were not characteristic of Black Sea plankton, or were not recorded for other seas as introduced species.

The species list includes *Spatulodinium pseudonoctiluca,* which was likely introduced from the Mediterranean Sea and *Cochlodinium polykrikoides*. Both are rare species which dominate in the pelagic community (Table 3). There was a mass development of *Gymnodinium aureolum* and *Alexandrium pseudogonyaulax* in plankton, and their cysts were found in silty port sediments in August 2002.

The following are original descriptions of local populations of the new Black Sea dinoflagellate taxa. Data on the habitat and morphological features of *Gymnodinium uberrimum* have been provided earlier (Exotic species… 2001, Terenko 2002).

***Spatulodinium pseudonoctiluca* (Pouchet) Cachon & Cachon ex Loeblich et Loeblich, 1969 (=*Gymnodinium pseudonoctiluca* Pouchet, 1885)**. The species has a complicated life cycle with several stages of development (Konovalova and Selina 2002). Cells at the trophont stage were observed, as well as an interim phase prior to the mature cell. The cells ranged from 128–133.4 μm in length, and 52–87 μm in width. The episoma is elongated due to a cylindrical tentacle-like protrusion of the apex and the sides are severely concave. The hyposoma is enlarged, approximately three times greater than the episoma with almost straight or slightly convex sides and a thickened antapex. The groove is slightly wider than the girdle, extending from the middle of the episoma almost to the antapex. The membrane is thin, the species belongs to the unarmored dinophytes. Nutrition is heterotrophic with chloroplasts lacking. Benthic diatom inclusions were noted in the cytoplast. The nucleus is round, located in the cell centre and frequently surrounded by large food particles and vacuoles.

**Habitat.** This species was first observed in Odessa Bay and Sukhoi liman in June 2004 at of 14.9 – 18.5°C, salinity 14.0 – 16.1 psu. Population density was low, less than 1000 cells · $l^{-1}$.

**General distribution.** The species is neritic, moderately thermophilic, widely distributed, but rarely encountered. Described in the northeast Atlantic (Pouchet 1885), and observed in the Mediterranean Sea, near the coast of Korea (Sournia 1986) and in the Sea of Japan (An illustrated…1997, Konovalova and

Selina 2002). Kiselev (1950) observed it in the Northern Artic Ocean, and Okolodkov (1998) in the Kara Sea.

***Alexandrium pseudogonyaulax*** **(Biecheler) Horiguchi ex Yuki et Fukuyo, 1992 (=*Goniodoma pseudogonyaulax* Biecheler, 1952)**. The main, most significant and reliable differentiating criterion of the species is its cell shape. *A. pseudogonyaulax* cells are wide, lens-like, and the cell width exceeds its length. The cells in the Black Sea correlate with the detailed description of the species by Yuki *et al.* (1996).

The cells in the Black Sea had the following dimensions – length 37.7 – 43.5 μm (mean 40.6 μm), width – 43.5 – 46.4 μm (mean 45.0 μm). The length/width ratio is 0.89 – 0.94 μm. In the Black Sea the cells are slightly larger than those in the Sea of Japan – length 32.5 – 42.5 (mean 37.3), width 37.5 – 50.0 (mean 43.5) (Selina and Konovalova 2001).

According to Steidinger and Tangen (1996) the species is toxic. However, recent studies by Hallegraaf (2002) give evidence for a lack of toxicity.

**Habitat**. In the Black Sea the species was first noted in July 2002 in Odessa port (t = 25.8$^{o}$C, S = 14.5 psu) with average population density of 1500 cells · $l^{-1}$.

**General distribution**. Reported in brackish lagoons in the south of France and Portugal, along the coasts of Italy, Norway (Steidinger, Tangen 1997) and Japan (Yuki *et al.* 1996), and also in the eastern seas of Russia – Okhotsk and Japan Seas (Selina, Konovalova 2001).

***Gymnodinium aureolum*** **(Hulburt) Hansen, 2000 (=*Gyrodinium aureolum* Hulburt, 1957)**. Due to its morphological similarity this species was considered to be *Karenia mikimotoi* (=*Gymnodinium mikimotoi*). However, detailed studies of its morphology and pigment composition in recent years (Hansen *et al*. 2000, Hansen 2001) have shown that *Gyrodinium aureolum* is a separate taxon which was transferred to *Gymnodinium*. The main characteristic feature of the species is the horseshoe shape of the apical groove which widens toward the antapex. Fixation of this delicate unarmored dinoflagellate with Lugol`s solution and glutaric aldehyde causes cell deformation. The live cells viewed with a microscope also cease to move and begin to disintegrate. The main problem when identifying the species is an almost immediate disappearance of the horseshoe shaped apical groove.

The cells of the Black Sea species population were 40.6 – 43.5 μm in length, 34.8 – 37.7 μm in width, from an egglike shape to wide ellipsoidal, and slightly concave ventrally. The girdle is moderately deep, the edges are shifted not more than by 1.5 times the length of the cell. The chloroplasts are large, yellow-brown, and multiple.

For many years it was considered this species caused “red tides” that were toxic for fish. In recent years Hallegraff (2002) showed *G. aureolum* lacked toxicity, while *K.* m*ikimotoi* was a toxic species.

**Habitat.** Recorded first in the Black Sea in August 2002 at 26.0 – 26.7$^{o}$C and 13.1 – 14.8 psu salinity. Average density was 37800 cells · $l^{-1}$.

**General distribution**. A euryhaline species, widely dispersed in temperate sea and ocean waters. Present along the coast of the USA and Brazil, in the waters of Norway, Sweden, Scotland, Ireland, Denmark and “blooming” noted along the Tasmanian coast.

***Cochlodinium polykrikoides*** **Margelef, 1961 (=*Cochlodinium heterolobatum* Silva, 1967)**. Cells often form short chains composed of not more than 8 cells. Separate cells are oval, at places of linkage somewhat flattened, compressed. Cells 30 – 40 μm in length, 20 – 30 μm in width (Taylor *et al*. 2003). Conic episome, double blade hyposome. Deep girdle descending making 1.8 – 1.9 revolutions around the cell.

The cells of the Black Sea were 29.0 – 40.6 μm in length, 26.0 – 34.8 μm in width, linked in twos. Cysts encountered in silty sediments of Black Sea ports.

The species is potentially toxic, causes death of fish fry, however, the composition of the toxin is unknown (Steidinger and Tangen 1996). The amount of the toxins in cells of Black Sea populations has not been determined.

**Habitat.** First recorded in Odessa Bay and the Black Sea in August 2001, September 2002 at 25$^{o}$C and 14.0 – 15.0 psu salinity. Average density – 487 cells · $l^{-1}$.

**General distribution**. Cosmopolitan, encountered in tropical waters at high temperatures. Recorded along the USA coast (Seaborn *et al*. 2003), causing toxic “blooming”, along the coasts of Japan, Korea and Puerto Rico.

These new species of dinophyte algae which have been identified in the Black Sea may be the result of their natural introduction into these waters through the Dardanelles and Bosphorus straits in the Mediterranean Sea and from the Atlantic Ocean, in addition to their entry with cargo ship ballast waters.

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