First record of the dinoflagellate *Tripos rotundatus* in the Adriatic Sea

Nika Pasković*, Iris Dupčić Radić

University of Dubrovnik, Institute for Marine and Coastal Research, Kneza Damjana Jude 12, P.O. Box 83, HR-20000 Dubrovnik, Croatia

Abstract – This report presents the first record of *Tripos rotundatus* (Jørgensen) Gómez in the Adriatic Sea. The species was found in a net sample in the 50 – 100 m depth layer, taken on July 2021 off the southern coast of the Adriatic Sea. The species *T. rotundatus* was probably previously misidentified as the morphologically similar species *T. digitatus* (Schütt) Gómez.

Keywords: biodiversity, NE Mediterranean, phytoplankton, southern Adriatic, taxonomy

Introduction

Dinoflagellates are an important group of protists with a remarkable diversity of life forms (i.e. free-living, parasites, and mutualistic symbionts), habitats (plankton and benthos), and nutrition modes (heterotrophic, chloroplastcontaining) (Gómez 2012).

In the Mediterranean Sea, 673 taxa have been identified, while 322 taxa have been reported for the Adriatic (Gómez 2003). In general, dinoflagellates are organisms dominantly found in oligotrophic waters (Gómez 2003).

Among dinoflagellates, the genus *Tripos* Bory includes the greatest number of taxa (~800) (Gómez 2021) and is globally widespread in marine waters. Previously, species of the genus *Tripos* were known as the marine section of the genus *Ceratium* Schrank, until morphological and molecular data supported the separation of marine and freshwater species of *Ceratium* at the genus level and restricted *Ceratium* to freshwater species (Gómez et al. 2010). Marine species are consistently large and robust, often with horns (Gómez 2021).

The aim of this study is to report the first record of *Tripos rotundatus* (Jørgensen) Gómez for the Adriatic Sea.

Materials and methods

Sampling was conducted on July 17, 2021 at the Lokrum coastal station, near Dubrovnik (southern Adriatic Sea, 42°37'21" N, 18°06'05"E) (Fig. 1). Vertical profiles of tem-

perature and salinity were measured from the surface to the bottom (90 m depth) at each meter using a multiparametric conductivity-temperature-depth (CTD) probe, and density (sigma-t) was calculated from these data. Water samples were collected using 5-L Niskin bottles for dissolved oxygen, nutrients, chlorophyll a (Chl-a), and phytoplankton on the surface and at depths of 5, 10, 20, 50, 75, and 100 m. Net samples were collected using a Nansen net with 53 µm mesh and 200 µm mesh in two layers: 0-50 m and 50-100 m. Dissolved oxygen was determined by Winkler titration, and oxygen saturation (O_2/O_2) was calculated from the solubility of oxygen in seawater as a function of corresponding temperature and salinity (Weiss 1970, UNESCO 1987). Nutrient samples were analysed in the laboratory using a spectrophotometer according to Strickland and Parsons (1972). To estimate Chl-a, 1 L subsamples were filtered through Whatman GF/F glass microfiber filters and were analysed fluorimetrically (Holm-Hansen et al. 1965). The trophic index (TRIX) was calculated to classify the trophic status of the coastal marine area (Vollenweider et al. 1998). Phytoplankton samples were preserved in neutralized formaldehyde (2.5% final concentration) and observed with an Olympus IX-71 inverted microscope according to the Utermöhl method (Utermöhl 1958). For a detailed description of the method used to analyse nutrients, Chl-a, and phytoplankton (for details see Jasprica et al. 2022). The nomenclature of taxa follows Guiry and Guiry (2023).

^{*} Corresponding author e-mail: nika.paskovic@unidu.hr



Fig. 1. Position of the coastal Lokrum station in the southern Adriatic Sea, where the dinoflagellate species *Tripos rotundatus* (Jørgensen) Gómez was found, 17th July 2021 (derived and adapted from Google earth).

Tab. 1. Physico-chemical parameters, chlorophyll *a* and total phytoplankton abundance in two layers (0-50, 50-100 m) of the water column on the Lokrum station in the southern Adriatic Sea, 17th July 2021 (n = 7).

Parameters	Layer (m)	
	0 - 50	50 - 100
Temperature (°C)	15.93 - 26.25	15.43 - 15.95
Salinity	36.56 - 38.93	38.93 - 38.96
Density (kg m ⁻³)	24.36 - 28.78	28.79 - 28.93
Oxygen saturation (O_2/O_2')	0.95 - 1.06	0.83 - 0.86
Phosphate (µM)	0.01 - 0.06	0.04 - 0.06
Total inorganic nitrogen (µM)	0.23 - 0.33	0.29 - 0.60
Nitrate (µM)	0.01 - 0.08	0.06 - 0.09
Nitrite (µM)	0.008 - 0.03	0.008 - 0.32
Ammonium (µM)	0.21 - 0.23	0.19 – 0.22
Silicate (µM)	1.49 - 4.84	1.23 – 4.06
Chlorophyll a (mg m ⁻³)	0.06 - 0.16	0.17 - 0.32
Total phytoplankton abundance (cells L ⁻¹)	$9.1 \times 10^4 - 1.4 \times 10^5$	$7.7 \times 10^4 - 2.1 \times 10^5$

Results

Vertical thermal stratification of the water column was found during sampling date. The water column in the upper layer (50 – 0 m) was stratified, with a temperature range (15.93 – 26.25 °C), salinity (36.56 – 38.93) and density (24.36 – 28.78) (Tab. 1). O_2/O_2 ' ranged from 0.83 to 1.06 and was lower (0.83 - 0.86) in the bottom layer.

TIN was calculated as the sum of nitrate (NO₃), nitrite (NO₂), and ammonium (NH₄). NH₄ accounted for the highest proportion of TIN in the entire water column, 59.5%, and NO₃ for the lowest, 16.3%. PO₄ ranged from 0.01 to 0.066 μ M, with the highest value measured at 10 m depth.

 SiO_4 ranged from 1.23 to 4.84 μM , with higher values in the upper 50 m.

The highest Chl-*a* (0.32 mg m⁻³) and phytoplankton abundance (2.1×10^5 cells L⁻¹) were found at a depth of 75 m. TRIX ranged from 1.1 to 2.8, classifying the station as oligotrophic.

Altogether, 48 phytoplankton taxa were identified in seven samples. Thirty-five taxa were dinoflagellates, 10 diatoms and three coccolithophorids. Among larger phytoplankton cells (> 20 μ m cell long) *Thalassionema nitzschioides* (Grunow) Mereschkowsky, *Oxytoxum sphaeroideum* Stein, *Oxytoxum variabile* J. Schiller, *Oxytoxum caudatum* Schiller were the most abundant (> 945 cell L⁻¹). Nanophytoflagellates (2–20 μ m cell long) dominated (96.3%) in total phytoplankton abundance.

Tripos rotundatus was found in the net sample in the 50–100 m depth layer (Fig. 2).

Discussion

Water column stratification is a common occurrence during summer, as is the occurrence of a thermocline and of water column stability (Ninčević Gladan et al. 2015).

The low nutrient and Chl-*a*, and in general the low trophic status, indicated a summer situation common in the oligotrophic coastal area. Moreover, the oligotrophy of the Lokrum station is confirmed by the abundance of phytoplankton dominated by nanophytoplankton, typical of the spring and summer period in the coastal southern Adriatic Sea (Caroppo et al. 1999), and by the highest abundance of dinoflagellates, typical organisms of oligotrophic waters (Gómez 2003).

As *T. rotundatus* was pooled as *T. digitatus* (Schütt) Gómez, and most of the records are not illustrated, we have



Fig. 2. Dinoflagellate species *Tripos digitatus* (Schütt) Gómez (A, B) (Gómez et al. 2021, with permission), *Tripos rotundatus* (Jørgensen) Gómez (C, D) found for the first time in the Adriatic Sea at the Lokrum station on 17^{th} July 2021. Black arrows indicate the morphological differences between the species *T. rotundatus* and *T. digitatus*. Scale bars = 50 µm.

little information on the distribution of the species. *Tripos rotundatus* was previously reported as *T. tasmaniae* (E.J.F.Wood) F.Gómez in Australian Pacific waters (Wood 1963).

Tripos rotundatus also had a different taxonomic status in the literature (Guiry and Guiry 2023). Originally, *T. rotundatus* was named *Ceratium digitatum* Schütt var. *rotundatum* Jørgensen (Jørgensen 1920). The species was found west of the island of Rhodes in the Aegean Sea in August 1910. Our data coincided with Jørgensen's findings, regarding the sampling season (in summer) and the position of the station (coastal).

Tripos digitatus, a morphologically similar species, was found in the Mediterranean Sea (Gómez 2003) and in the northern Adriatic Sea (Revelante 1985). The differences in the morphological characteristics of these two taxa have been recently highlighted (Gómez 2021). There are differences in the epitheca and hypotheca. The epitheca of *T. digitatus* is strongly directed dorsally, the left antapical horn is directed anteriorly, and the apex has a short projection. In *T. rotundatus*, the epitheca is less bent towards the dorsal side, the short projection on the apex is missing, and the left antapical horn is directed laterally (Gómez 2021). The morphological description of the species *T. rotundatus* follows the morphology of the taxa recorded at the Lokrum station. In our case, the cell of *T. rotundatus* was 110 µm long and 30 µm wide.

This finding contributes to a better understanding of the diversity of dinoflagellates in the Adriatic Sea. However, further continuous studies of phytoplankton diversity in the coastal regions of all parts of the Adriatic Sea are required.

Acknowledgments

The authors thank to the two reviewers and editor for their sound advice and helpful remarks on improvements for this article.

References

- Caroppo, C., Fiocca, A., Sammarco, P., Magazzu', G., 1999: Seasonal variations of nutrients and phytoplankton in the coastal SW Adriatic Sea (1995 - 1997). Botanica Marina 42, 389–400. http://dx.doi.org/10.1515/bot.1999.045
- Gómez, F., 2003: Checklist of Mediterranean free-living dinoflagellates. Botanica Marina 46, 215–246. https://doi. org/10.1515/bot.2003.021
- Gómez, F., Moreira, D., López-García, P., 2010: Neoceratium gen. nov., a new genus for all marine species currently assigned to Ceratium (Dinophyceae). Protist 161, 35–54. https://doi. org/10.1016/j.protis.2009.06.004
- Gómez, F., 2012: A quantitative review of the lifestyle, habitat and trophic diversity of dinoflagellates (Dinoflagellata, Alveolata). Systematics and Biodiversity 10, 267–275. https:// doi.org/10.1080/14772000.2012.721021
- Gómez, F., 2021: Speciation and infrageneric classification in the planktonic dinoflagellate *Tripos* (Gonyaulacales, Dinophyceae). Current Chinese Science 1, 346–372. https://doi.org/1 0.2174/2210298101999210101231020
- Guiry, M. D., Guiry, G. M., 2023: AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Retrieved on February 13, 2023 from https://www.algaebase. org.
- Holm-Hansen, O., Lorenzen, C. J., Holmes, R. W., Strickland, J.
 D. H., 1965: Fluorometric determination of chlorophyll. ICES Journal of Marine Science 30, 3–15.
- Jasprica, N., Čalić, M., Kovačević, V., Bensi, M., Dupčić-Radić, I., Garić, R., Batistić, M., 2022: Phytoplankton distribution related to different winter conditions in 2016 and 2017 in the open southern Adriatic Sea (eastern Mediterranean). Journal of Marine Systems 226, 103665. https://doi.org/10.1016/j. jmarsys.2021.103665
- Jørgensen, E., 1920: Mediterranean Ceratia. Report on the Danish oceanographical expeditions 1908-10 to the Mediterranean and adjacent seas. 2. Biology, J. 1, 1–110.
- Ninčević Gladan, Ž., Bužančić, M., Kušpilić, G., Grbec, B., Matijević, S., Skejić, S., Marasović, I., Morović, M., 2015: The response of phytoplankton community to anthropogenic pressure gradient in the coastal waters of the eastern Adri-

atic Sea. Ecological Indicators 56, 106–115. https://doi. org/10.1016/j.ecolind.2015.03.018

- Revelante, N., 1985: A catalogue of phytoplankton reported for the Rovinj area of the northern Adriatic. Thalassia Jugoslavica 21, 139–169.
- Strickland, J. D. H., Parsons, T. R., 1972: A Practical handbook of seawater analysis. 2nd edition. Fisheries Research Board of Canada, Ottawa, Canada. http://dx.doi.org/10.25607/ OBP-1791.
- UNESCO, 1987: International Oceanographic Tables, vol. 4. UNESCO, Paris.
- Utermöhl, H., 1958: Zur Vervollkommnung der quantitativen Phyto plankton-Metodik. SIL Communications, 1953-1996, 9, 1-38. https://doi.org/10.1080/05384680.1958.11904091
- Vollenweider, R. A., Giovanardi, F., Montanari, G., Rinaldi, A., 1998: Characterization of the trophic conditions of marine coastal waters with special reference to the NW Adriatic Sea: proposal for a trophic scale, turbidity and generalized water quality index. Environmetrics 9, 329–357. https://doi. org/10.1002/(sici)1099-095x(199805/06)9:3<329::aidenv308%3E3.0.co;2-9
- Weiss, R. F., 1970: The solubility of nitrogen, oxygen and argon in water and seawater. Deep Sea Research and Oceanographic Abstracts 17, 721–735. https://doi.org/10.1016/0011-7471(70)90037-9
- Wood, E. J. F., 1963: Dinoflagellates in the Australian region. II. Recent collections. Australia, C.S.I.R.O., Divison of Fisheries and Oceanography, Technical Paper 14, 4–51.