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FIRST CONTRIBUTION TO THE KNOWLEDGE OF MICROBENTHIC PROTISTS FROM THE VENICE LAGOON

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ABSTRACT

The importance of protists, especially ciliates, in marine communities has been stressed by many authors; since these organisms feed at various trophic levels, e.g., bacteria, algae of various sizes, and other ciliates.

The protists living in the Lagoon of Venice have been poorly studied. It seemed particularly interesting to investigate their populations in this environment, the hydrological dynamics of which have been seriously modified and which is continuously subjected to large-scale anthropic modifications and polluted by waste from several different sources.

This paper presents preliminary data on microbenthic protists in sediment samples collected in May and September 1995 from six stations of the Venice Lagoon. Twenty-one genera of ciliates were found. The highest concentration recorded in station 1, also characterized by the highest concentration of fine sand, was about 100 cells. ml^{-1}, whereas that of flagellates was about 1000 cells. ml^{-1}, with a lower number of taxa in all stations sampled.

Key words: microbenthos, Protists, Ciliates, Flagellates, Venice Lagoon

INTRODUCTION

Since the earlier part of this century it has been recognized that protists form a diversified quota of the biota living within marine benthic habitats. Most work has been concentrated on ciliated protozoa, which appeared to be dominant consumers in some benthic habitats (Kahl, 1930-35; Czapik & Fyda, 1992; Dragesco, 1963a, b; Epstein *et al.*, 1992; Fauré-Fremiet, 1950; Fenchel 1967, 1968, 1969, 1987; Hartwig, 1980a, b) and in recent years also on other protists, such as heterotrophic flagellates, all involved in microbial food webs (Fenchel, 1986; Patterson *et al.*, 1989). Sediment particle size influences the organic content of sediments, water and oxygen penetration, oxygen content, and accessibility by protists or metazoan competitors, and granulometric characteristics have a marked effect on the composition of protist biota. Microbenthic organisms include sessile and vagile unicellular eukaryotic species living on the surface of, or within, sediments; in the former case, the organisms are defined as components of the microbiotecton or epipsammon, in the second as mesopsammon. Many protists cannot be included exclusively in either class (Patterson *et al.*, 1989).

Admittedly, many difficulties are encountered when examining microbenthic commmunities, *e.g.* species determination, collection of qualitative and quantitative data, and the almost complete uselessness of analysing fixed samples (Finlay & Guhl, 1992).

As already mentioned, the protists living in the Lagoon of Venice have been poorly studied: very old data are available in the literature (Kiesselbach, 1936) together with some recent data on a new *Euplotes* morphospecies, *E. margherensis* (Coppellotti & Cisotto, 1996), which has greater resistance to copper than other species of *Euplotes* (Coppellotti & De Gabrieli, 1995).

The Lagoon of Venice is a unique ecosystem which originated nearly 6000 years ago and now consists of a semi-enclosed body of water connected with the Adriatic Sea through three channels (Bendoricchio *et al.*, 1993). The hydrological dynamics of this environment have been modified by various anthropic activities. The Lagoon receives wastes from various sources, all of which are very numerous and complex, such as discharges of treated and untreated domestic sewage, treated industrial effluent, and cooling water from the industrial zone, and also pollutants, such as heavy metals, transported down the waterways flowing into the Lagoon. Particular attention was paid to sediments, because it is well known that heavy metals and organic micropollutants are closely associated with this fraction, from which they may be resuspended (Donazzolo *et al.*, 1984; Martin *et al.*, 1994). For all these reasons, this area may be highly selective. Some other types of organisms, such as macroalgae, have been used as biological indicators for the Lagoon of Venice (Favero *et al.*, 1996).

It seems particularly interesting to investigate protist populations in this peculiar environment, which is continuously subjected to large-scale anthropic modifications. The studied organisms belonged to the taxonomic group of Ciliophora and to the flagellate group, a heterogeneous assemblage of protists equipped with flagella.

MATERIALS AND METHODS

Samples were taken from the sediments of six stations in the Venice Lagoon, chosen for their different environmental characteristics (Fig. 1). Station 1 was located near the hydrobiological station of Chioggia, subject to pollution by urban waste; stations 2 and 3 were located at mussel-farming sites; station 4 was in an area polluted by industrial waste; station 5 lay between the industrial zone of Marghera and the city of Venice, and is polluted by both industrial and city waste; and station 6 was located in a "clean" site in the northern part of the Lagoon.

Samples were collected in May 1995 from all stations and in September 1995 from station 1 only.

Sampling was carried out by pressing a plexiglass tube 3.2 cm in diameter some 10 cm into the sediment and that part of sediment from 0 cm to 2 cm below the surface was collected. Samples thus consisted of 16 ml each, and were placed in plastic 500-ml bottles. A minimum of three samplings was made for each station. 100 ml of seawater, filtered through 0.22 μm Sterivex-GS mesh (Millipore) and having the same salinity as that of the collection site, were added to the sediment samples.

Samples were taken to the laboratory in a refrigerated container and were observed within 6 hours.

Three procedures were adopted for examination of samples:

1) Uhlig's "seawater ice" method (Uhlig, 1964) for extracting interstitial microfauna: sediments were filtered through nylon nets (200, 100, 60 and 45 μm meshes).

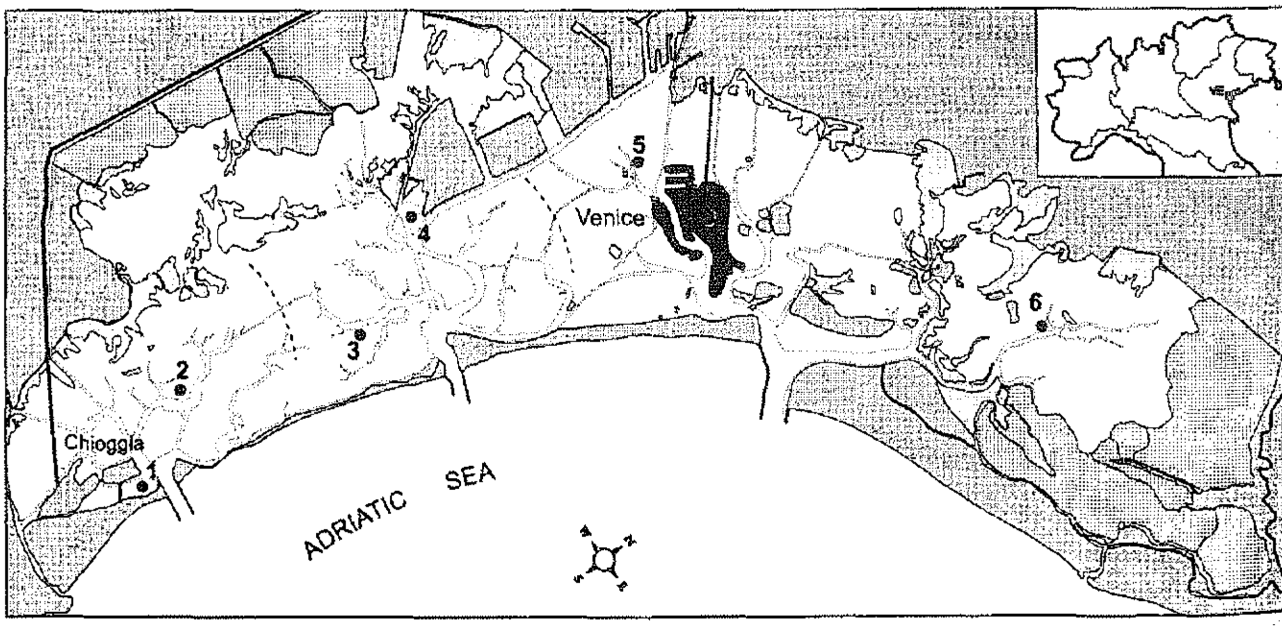

Fig. 1: Lagoon of Venice and location of sampling stations.
Sl. 1: Beneška laguna in lokacija vzorčnih postaj.

This method was only used for sediments composed of relatively large sand particles with low percentages of silt and clay, which are washed through with the protozoa.

2) Three sub-samples were prepared by withdrawing 10 ml of diluted sediment and adding 90 ml of filtered seawater. Ciliates and flagellates were counted in five 100-µl drops for each sub-sample under a Leitz Diaplan microscope at magnifications of 310x or 500x, following the indications of Finlay & Guhl (1992).

3) Six sub-samples were prepared from each bottle by withdrawing 5 ml, to which 45 ml of seawater were added. Quantitative observations and initial identification of ciliates were carried out on every sub-sample under a Wild M8 stereomicroscope. Living ciliates were removed by a Pasteur pipette and directly counted.

The data on protozoan densities reported in this work were obtained using method 2 for flagellates and method 3 for ciliates, after washing of all sediments by Uhlig's "seawater ice" method where possible.

In most cases, quantitative data refer to genera more than to species, due to the difficulty in collecting simultaneously both qualitative and quantitative data on ciliates. Impregnation by protargol or silver nitrate was used for correct identification of most ciliates, following respectively Wilbert or Chatton-Lwoff procedures as modified by Foissner (1991). Some particularly fragile ciliates, such as those belonging to Kinetofragminophorea, were fixed before impregnation with Raikov's (1978) fluid, normally used in electron microscopy. Drawings of protargol-impregnated specimens were made with the aid of a *camera lucida* under a Diaplan Leitz microscope. The taxonomic scheme by Levine *et al.* (1980) was used. Species descriptions by Kahl (1930-1935), Curds (1975), Curds & Wu (1983), Dragesco (1963a, b), and Carey (1992) were also used. Flagellates were identified with the aid of Prescott's (1962) and Schiller's (1933-1937) descriptions.

Salinity values were determined with a refractometer. Samples were analysed for organic matter content and for grain-size composition according to Buchanan (1984).

RESULTS AND DISCUSSION

Abiotic data

In May 1995 salinity was at a minimum value of 28 ‰ at station 1 and peaked at 35 ‰ at station 5. A value of 30 ‰ was measured at all other stations. In September 1995, salinity was 35 ‰ at station 1.

Water temperatures were respectively 16°C and 21°C at all stations.

Table 1 lists data referring to the May samplings. The sediments were characterized by compositions of differing percentages: the highest sand content was recovered at station 1 (97.39%) and the lowest at station 6 (14.92%). The highest contents of fine sand (250-125 µm) were measured at station 1 (54.61%) and of median sand (500-250 µm) at station 4 (49.49%). Very high contents of silt were found at stations 6 (68.39%) and 5 (49.55%). Very little organic matter was present at stations 1 (0.26%) and 4 (0.22%).

	Stations						
Grain-size fractions (µm)	1	2	3	4	5	6	
1000-500	0.38	3.07	1.90	10.73	13.57	0.49	
500-250	34.82	12.11	13.40	49.49	7.24	0.57	S
	97.39*	58.73*	63.72*	94.74*	45.00*	14.92*	A
250-125	54.61	26.58	32.31	28.93	4.95	1.55	N
125-63	7.58	16.96	16.11	5.58	19.24	12.30	D
							S
63-15.6	0.65	14.71	18.81	2.19	27.50	42.54	I
	1.24*	25.28*	26.58*	3.40*	49.55*	68.39*	L
15.6-3.9	0.59	10.57	7.77	1.21	22.05	25.85	T
							C
							L
<3.9	1.37	15.99	9.70	1.86	5.45	16.70	A
							Y
Organic matter	0.26	3.13	0.72	0.22	2.13	1.64	

Tab. 1: Grain-size percentage distribution and organic matter content of sediments examined in may 1995.
** Total percentage values refer to sand and silt, respectively.*
Data kindly supplied by Prof. R. Brunetti.
Tab. 1: Porazdelitev zrnavosti (v odstotkih) in vsebnost organske snovi v sedimentu, raziskanem v maju 1995 (v %).
** Skupne odstotkovne vrednosti se nanašajo na pesek in mulj.*
Podatki s prijaznim dovoljenjem prof. R. Brunettija.

Microfauna

Taxa found in the May samplings in all 6 stations are listed in Table 2. With our counting procedures, flagellates were the most numerous protists (approximately 1000 cells. ml^{-1}), although higher numbers of ciliate taxa were recorded at almost all stations. The highest number was recorded at station 1, where ciliates belonging to Kinetofragminophorea were represented by 9 genera, Polyhymenophorea by 6, and Oligohymenophorea by 4. The most frequent genera were *Remanella, Strombidium* and *Aspidisca*. *Remanella* is common in fine marine sand, its diet comprising diatoms and flagellates. *Strombidium* is generally found in the oxidized zone, where it feeds on diatoms and small phytoflagellates.

	Sampling stations					
Taxa	1	2	3	4	5	6
Flagellates						
Amphidinium sp.	++++					
Gymnodinium sp.	++					
Techadinium sp.	+++					
Oxyrrhis sp.	+++					
Anisonema sp.	++	+++	++	+++		+
Chlorogonium sp.	++	+++	+++	++	+	+
Bodo spp.	++++	++++	++++	++++	+++	+++
Other species	++	++	++	++	++	++
Ciliates						
Kinetofragminophorea						
Lacrymaria sp.	+		+		+	
Mesodinium pulex	+	+			+	
Litonotus sp.	+					+
Trachelocerca schultzei	+					
Trachelocerca sp.	+					
Tracheloraphis sp.	+					
Remanella spp.	+++					
Geleia sp.	+					
Conchostoma longissimum	+					
Colpoda cucullus	+					
Oligohymenophorea						
Frontonia sp.	+	+			+	+
Uronema sp.	+			+		
Pleuronema coronatum	+	+		+	+	+
Cyclidium sp.	++	+				+
Polyhymenophorea						
Condylostoma remanei	+					
Strombidium spp.	+++	+	+	+	+	+
Tintinnopsis sp.		+				
Holosticha spp.	+					
Aspidisca leptaspis	++					
Aspidisca sedigita	++					
Aspidisca spp.	+++		+	+		+
Diophrys appendiculata	+	+				
Euplotes bisulcatus	+					
Euplotes rariseta	+			+		
Euplotes vannus	++	+				++
Uronychia transfuga		+				

+,(<5 CELLS ml^{-1}); ++, (5-15 CELLS ml^{-1}); +++, 15-100 CELLS ml^{-1}); ++++, (>100 CELLS ml^{-1}).

Tab. 2: List of taxa in samplings of May 1995.
Tab. 2: Seznam taksonov v vzorcih iz maja 1995.

Taxa	May	September
Flagellates		
Amphidinium sp.	++++	
Gymnodinium sp.	++	
Techadinium sp.	+++	
Oxyrrhis sp.	+++	
Anisonema sp.	++	+++
Chlorogonium sp.	++	++
Bodo spp.	++++	++++
Other species	++	++
Ciliates		
Kinetofragminophorea		
Lacrymaria sp.	+	+
Dileptus sp.		+
Mesodinium pulex	+	+
Litonotus sp.	+	+
Trachelocerca binucleata		+++
Trachelocerca schultzei	+	++
Trachelocerca sp.	+	++
Trachelonema minima		++
Tracheloraphis sp.	+	+
Remanella spp.	+++	+
Geleia sp.	+	+
Conchostoma longissimum	+	+
Colpoda cucullus	+	
Oligohymenophorea		
Frontonia sp.	+	+
Uronema sp.	+	+
Pleuronema coronatum	+	+++
Cyclidium sp.	++	+
Polyhymenophorea		
Condylostoma remanei	+	
Strombidium spp.	+++	+
Holosticha spp.	+	+
Aspidisca leptaspis	++	+
Aspidisca sedigita	++	+
Aspidisca spp.	+++	++
Diophrys appendiculata	+	+
Euplotes bisulcatus	+	
Euplotes rariseta	+	
Euplotes vannus	++	++

+,(<5 CELLS ml^{-1}); ++, (5-15 CELLS ml^{-1}); +++, 15-100 CELLS ml^{-1}); ++++, (>100 CELLS ML^{-1}).

Tab. 3: List of taxa in samplings of station 1 in May and September 1995.
Tab. 3: Seznam taksonov v vzorcih s postaje 1 iz maja in septembra 1995.

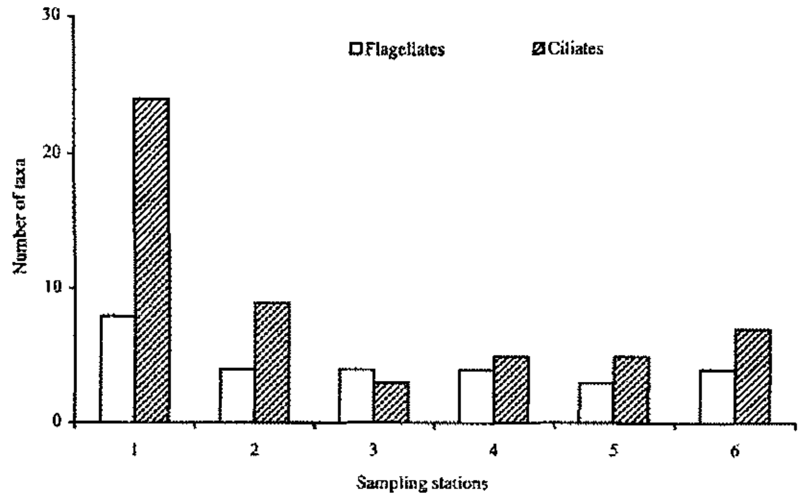

Fig. 2: Number of taxa of Ciliates and Flagellates in six sampling stations of Venice Lagoon in May 1995.
Sl. 2: Število taksonov migetalkarjev in bičkarjev na šestih vzorčnih postajah v Beneški laguni maja 1995.

Ciliate density was very low at the other stations, Oligohymenophorea and Polyhymenophorea being the main groups.The lowest number of genera was found at station 4, *i. e.* 3 genera, whereas 9 were recorded at station 3. *Strombidium* and *Pleuronema* were the most frequently observed genera at all stations.

As regards flagellates, *Anisonema, Chlorogonium* and *Bodo* could be observed at almost all stations, while Dinoflagellates such as *Amphidinium, Gymnodinium, Techadinium* and *Oxyrrhis* were identified only at station 1, where the most numerous genera were the bacterivorous *Bodo* and the phagotrophic *Amphidinium*. It must be noted that diatoms were very abundant in samples from all stations.

The richness of taxa in the May samplings at all stations is reported in Fig. 2. The highest numbers of both flagellate and ciliate taxa are clearly present at station 1. In particular, ciliate taxa numbered 24, and at all stations 26.

On the basis of the data obtained, it was decided to take samples only from station 1 in September 1995. The taxa of this station in May and September are listed in Table 3. Flagellate density was seen to have decreased, mainly because of the disappearance of Dinoflagellates from the September samplings. A remarkable increase in the density of some Karyorelictida, such as the predators *Trachelocerca* and *Tracheloraphis*, was also noted. Indeed, *Trachelocerca* was found to be represented by at least three species. *Trachelonema* also appeared at station 1 in the September sampling.

Direct counts of ciliates in the September samplings yielded the following data: about 70 cells. ml^{-1} of Kinetofragminophorea, especially Karyorelictida; 20 cells. ml^{-1} of Oligohymenophorea, especially *Pleuronema coronatum;* and about 15 cells. ml^{-1} of Polyhymenophorea, without any dominant genus. The morphological traits of some ciliate species after protargol impregnation are shown in Figs. 3, 4 and 5.

These preliminary data indicate that the studied stations of the Lagoon of Venice are characterized by relatively low densities of ciliates.

It is well known that ciliates are most frequent in fine sand (250-125 μm). In finer sand (125-63 μm) their numbers decrease drastically, and in coarser sands (500-250 μm) they also occur in smaller numbers (Fenchel,1969). Larger amounts of organic matter lead to a strongly reducing environment. The richness of station 1 with respect to the other stations may be explained by the composition of its sediments (see Table 1) and by the amount of organic matter. In fact, the highest percentage (54.61%) of fine sand was found at this station. As regards organic matter, a value of 0.26% was recorded at station 1 and a similarly low value (0.22%) at station 4. However, station 4 was located in the industrial zone of the Lagoon, which is known to be highly polluted by heavy metals (Donazzolo *et al.,* 1984; Basu & Molinaroli, 1994) which may thus strongly limit the biological characteristics of the environment and lead to the creation of a highly selective area for microfauna.

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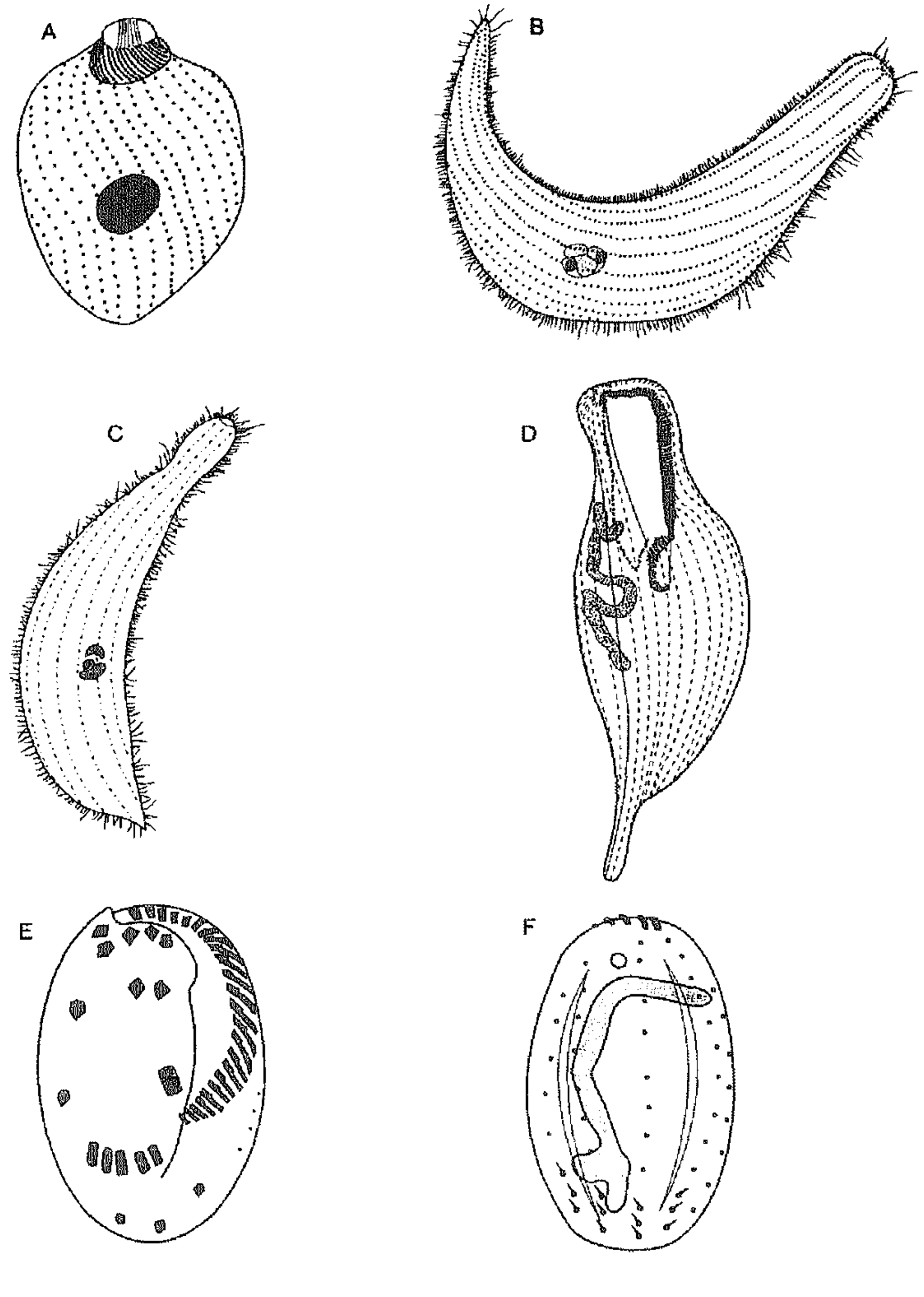

Fig. 3: ***Camera lucida drawings of protargol-impregnated ciliates. A:*** *Lacrymaria sp.,* ***700x; B:*** *Trachelocerca schultzei,* ***450x; C:*** *Trachelocerca binucleata,* ***450x; D:*** *Condylostoma remanei,* ***290x; E:*** *Euplotes bisulcatus,* ***ventral view, 1100x; F:*** *Euplotes bisulcatus,* ***dorsal view, 1100x.***

Sl. 3: Risbe migetalkarjev (napravljene ob pomoči tako imenovane camera lucida), prepojenih s protargolom. A: *Lacrymaria sp.,* ***700x; B:*** *Trachelocerca schultzei,* ***450x; C:*** *Trachelocerca binucleata,* ***450x; D:*** *Condylostoma remanei,* ***290x; E:*** *Euplotes bisulcatus,* ***pogled s trebušne strani, 1100x; F:*** *Euplotes bisulcatus,* ***pogled s hrbtne strani, 1100x.***

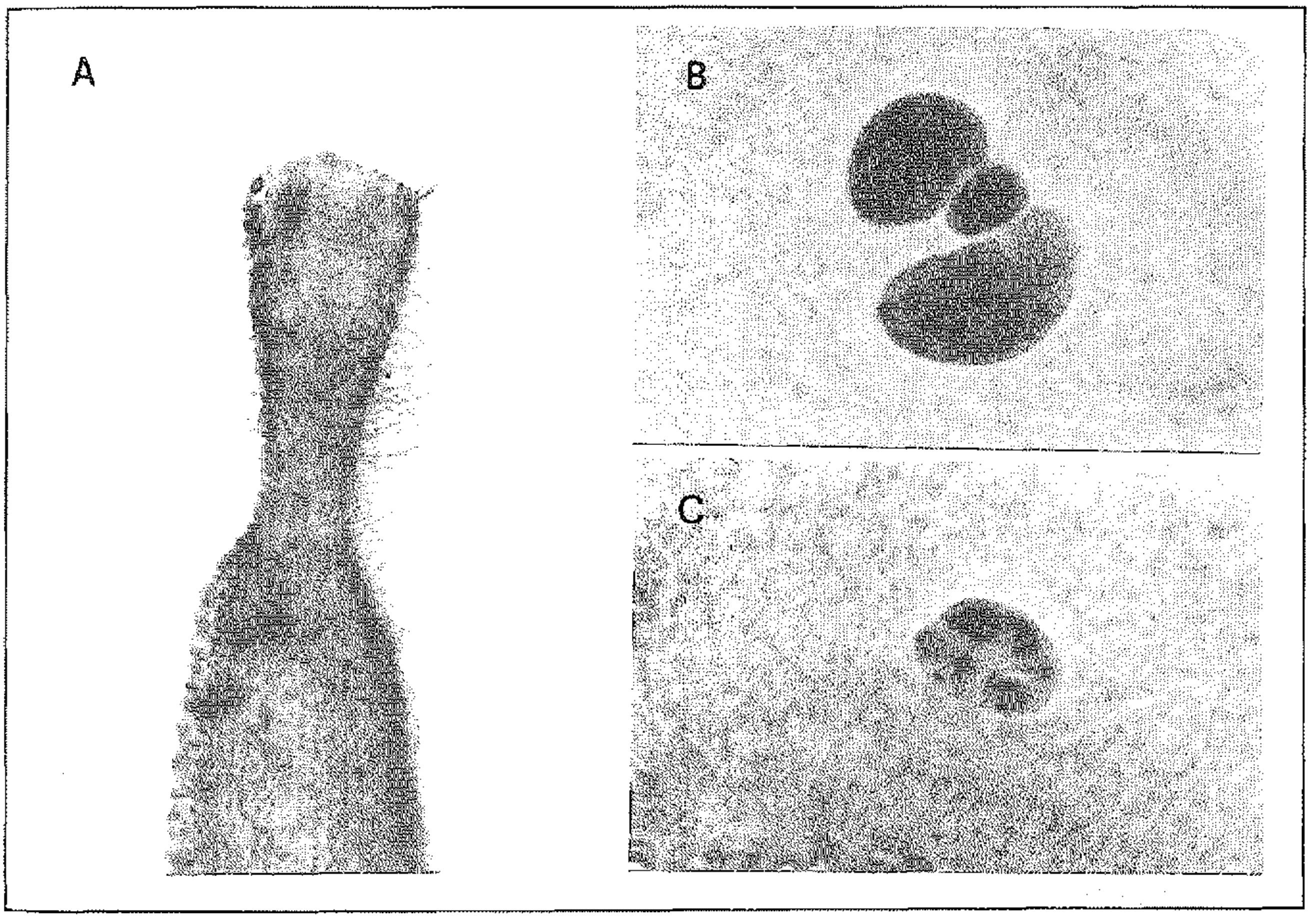

Fig 4: Micrographs of protargol-impregnated specimens. A: *Trachelocerca binucleata,* ***anterior part of cellular body, 960x; B:*** *Trachelocerca binucleata,* ***nuclear apparatus, 1280x; C:*** *Trachelocerca schultzei,* ***nuclear apparatus, 1280x.***

Sl. 4: Mikrografi primerkov, prepojenih s protargolom. A: *Trachelocerca binucleata,* ***prednja stran celičnega telesa, 960x; B:*** *Trachelocerca binucleata,* ***jedrni aparat, 1280x; C*** *Trachelocerca schultzei,* ***jedrni aparat, 1280x.***

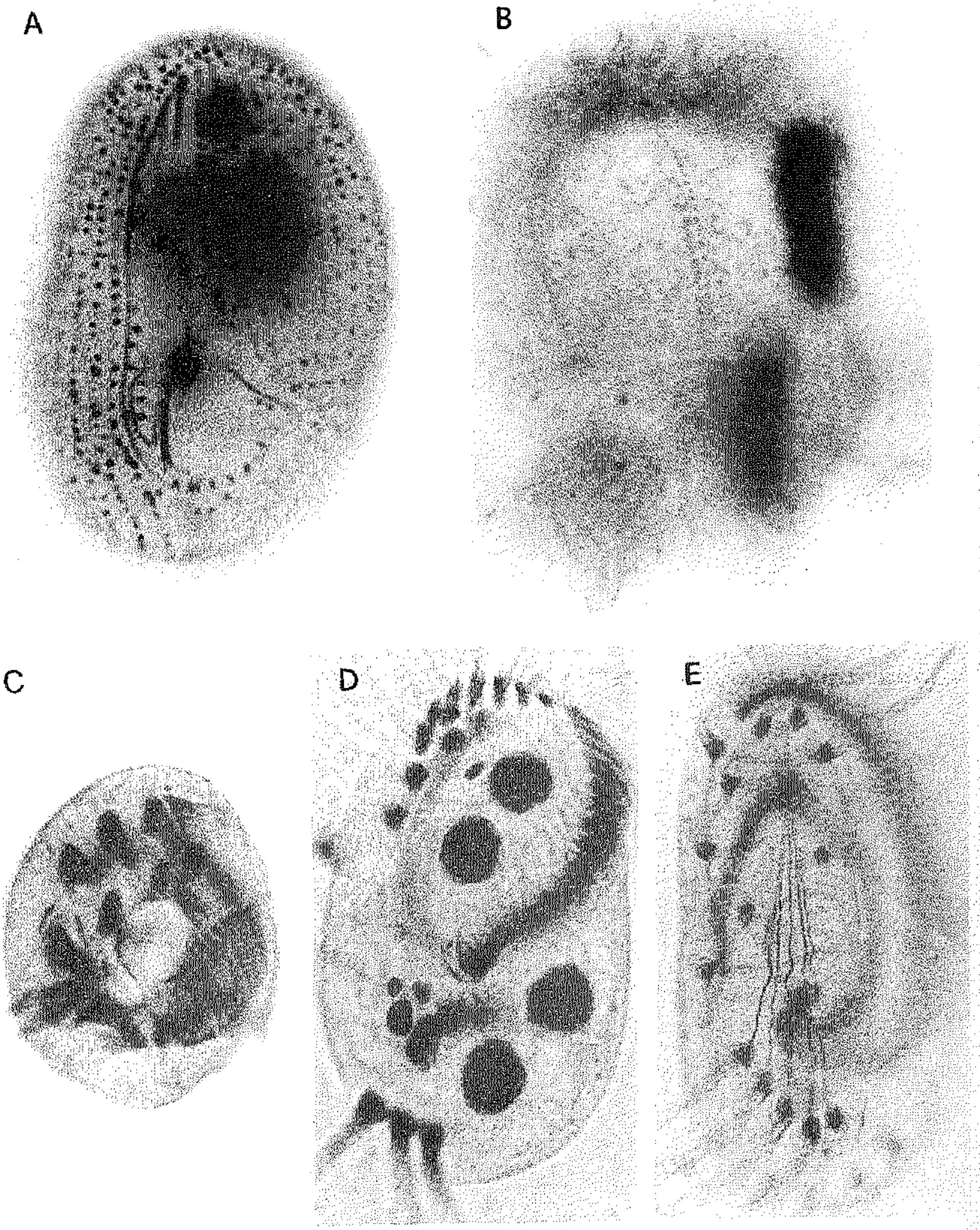

Fig. 5: Micrographs of protargol-impregnated specimens. A: *Pleuronema coronatum,* ***ventral view, 1120x; B:*** *Uronychia transfuga,* ***dorsal view, 1280x; C:*** *Aspidisca sedigita,* ***ventral view, 1280x; D:*** *Diophrys appendiculata,* ***ventral view, 1280x; E:*** *Euplotes vannus,* ***ventral view, 960x.***

Sl. 5: Mikrografi primerkov, prepojenih s protargolom. A: *Pleuronema coronatum,* ***pogled s trebušne strani, 1120x; B:*** *Uronychia transfuga,* ***pogled s hrbtne strani, 1280x; C:*** *Aspidisca sedigita,* ***pogled s trebušne strani, 1280x; D:*** *Diophrys appendiculata,* ***pogled s trebušne strani, 1280x; E:*** *Euplotes vannus,* ***pogled s trebušne strani, 960x.***

PRVI PRISPEVEK K POZNAVANJU MIKROBENTOŠKIH ENOCELIČARJEV V BENEŠKI LAGUNI

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POVZETEK

Na pomen enoceličarjev, posebno migetalkarjev, v morskih združbah so opozorili že mnogi avtorji, saj se ti organizmi hranijo na več trofičnih ravneh, npr. z bakterijami, algami različnih velikosti in drugimi migetalkarji. Enoceličarji, ki živijo v Beneški laguni, so slabo preučeni, zato se nam je zdelo še posebno zanimivo raziskati njihove populacije v tem okolju, katerega hidrološka dinamika se je do danes že temeljito spremenila. Nenehno je namreč izpostavljena velikim antropogenim vplivom in onesnaževanju z odpadki iz več različnih virov.

Pričujoči članek predstavlja preliminarne podatke o mikrobentoških enoceličarjih v vzorcih sedimenta, zbranih v maju in septembru 1995 na šestih postajah v Beneški laguni. Ugotovljenih je bilo 21 rodov migetalkarjev. Njihova največja koncentracija, ki je bila zabeležena na postaji 1 in katere značilnost je tudi največji delež mivke, je bila približno 100 celic. ml^{-1}, največja koncentracija bičkarjev pa okrog 1000 celic. ml^{-1}, z manjšim številom taksonov na vseh vzorčnih postajah.

Ključne besede: mikrobentos, enoceličarji, migetalkarji, bičkarji, Beneška laguna

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