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PROTISTS FROM CAVES: PRELIMINARY DATA ON POPULATIONS OF THE "COVOLO DELLA GUERRA", BERICI HILLS (VICENZA, ITALY)

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ABSTRACT

Periodic samplings between June 1997 and September 1998 in the karst cave known as the "Covolo della Guerra" (Vicenza, Italy) allowed the monitoring of some chemico-physical parameters, such as illumination, air humidity, air and water temperatures, pH and conductivity of water, and concentrations of heavy metals in water. The initial results on populations of Protists from five different sites of the cave indicated a rich variety of organisms belonging to various taxa, such as Mastigophora, Sarcodina and Ciliophora. Until now about 70 Protist taxa have been detected, 42 belonging to Ciliophora.

Key words: cave, karst, Vicenza, Protists, Ciliophora, Mastigophora, Sarcodina

INTRODUCTION

Increased interest in spelaeology has produced remarkable results for some Italian caves in the last few decades. However, only some sporadic visits with the aim of collecting faunistic material are reported in the literature (Vandel, 1964). Moreover, the collected material concerns only some taxonomic groups, such as Insecta, Chilopoda and Gastropoda (Baccetti, 1966; Minelli, 1974; Pezzoli, 1990).

The "Covolo della Guerra" ("war cave") is one of the most famous caves in the Berici Hills (Vicenza, Italy) (Fig. 1). Its name derives from the fact that it was a shelter for the inhabitants of this area during past wars. An exhaustive description of this cave has been made by Boscolo & Mantovani (1971).

The Berici Hills are situated south of the town of Vicenza, separated by strips of plain from the nearby mountains and hills. The Berici hills, shaped approximately as a lengthened parallelogram, stretch south for 20 kilometres, in the form of a plateau, cut by valleys. These hills are mostly formed of Eocene and Oligocene limestone: consequently, karst morphogenesis is active on their surface (Castiglioni, 1991). In particular, because of the almost flat upper surface, conditions are good for the formation of dolinas, which are very numerous, up to a density of 20 per square kilometre (Mietto & Sauro, 1989).

Owing to the relative ease of exploration of the karst cave, faunistic studies of some invertebrate phyla and Chiroptera have been made (Boscolo 1968; Vernier, 1996).

Since the second part of the 19th century, Protists dwelling in caves in many European countries and in North America have been studied (Landolt *et al.*, 1992; Golemansky & Bonnet, 1994) and in Japan (Sudzuki & Hosoyama, 1991). Studies on Protists have also concerned endoparasites in various cavernicolous invertebrates (Golemansky, 1973, 1980).

As regards the protists living in the Covolo della Guerra, Boscolo (1968) reported only three species of Peritrichida (*Intrastylum steinii, Lagenophrys mono*

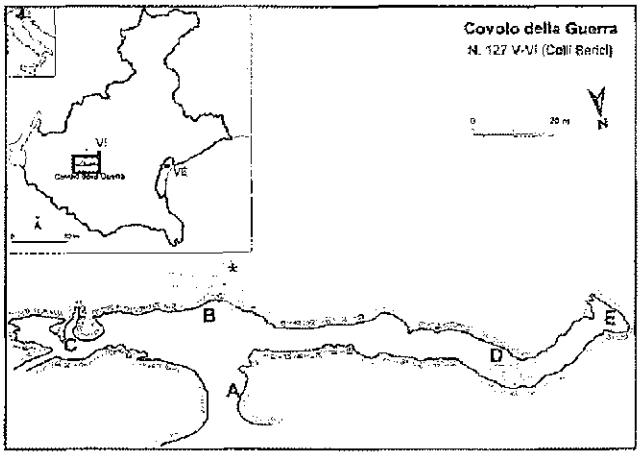
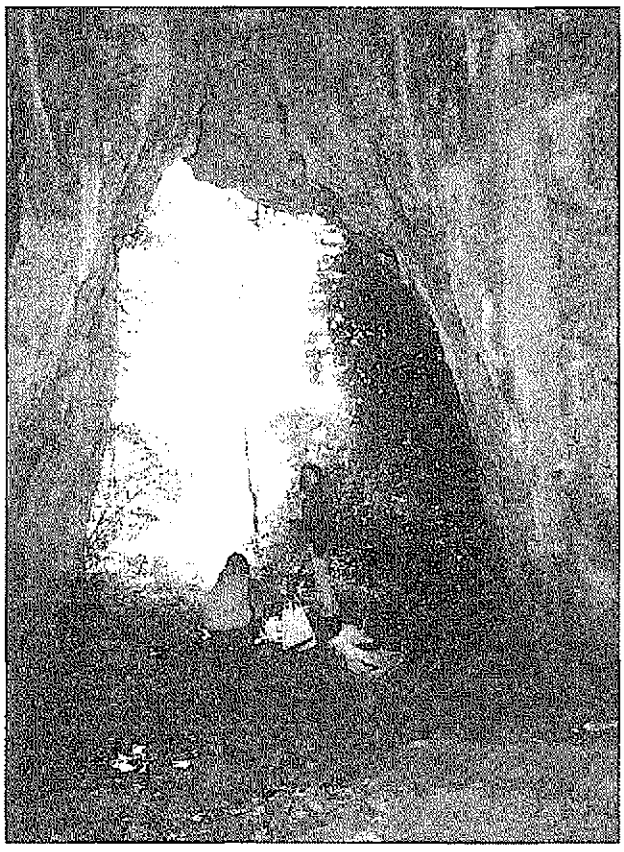


*Fig. 1: The karst cave Covolo della Guerra and location of sampling sites (A, B, C, D, E); * indicates the third, long, narrow branch of the cave, not easily accessible and not yet studied. Modified from Mietto & Sauro (1989).*

*Sl. 1: Jama Covolo della Guerra in lokacija vzorčišč (A, B, C, D, E); * ponazarja tretji (dolgi in ozki) odsek jame, ki je težko dostopen in tudi še neraziskan. Prirejeno po Miettu in Sauru (1989).*

a

b



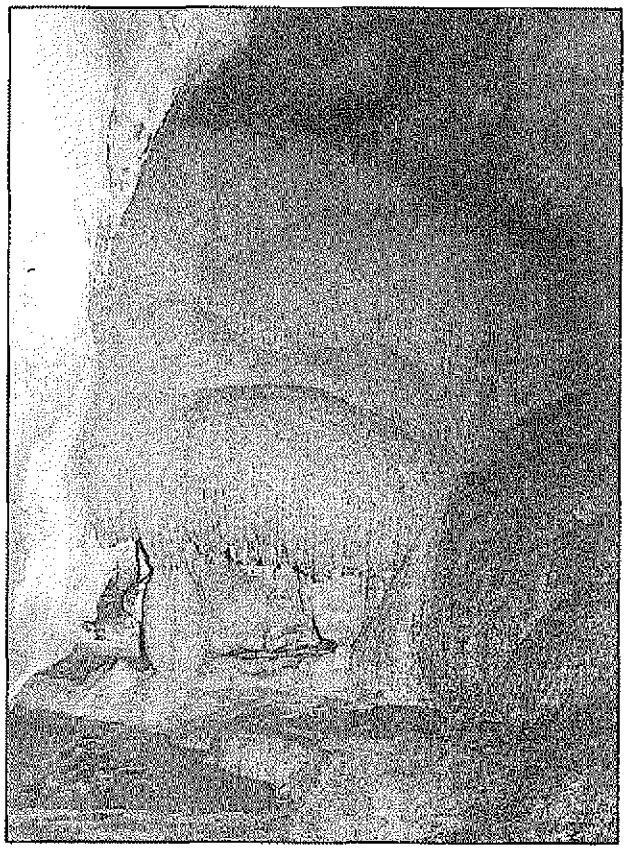


Fig. 2: The cave Covolo della Guerra. a) Entrance of cave; b) Detail of "Grande Colata", a large concretion of stalactites and stalagmites.

Sl. 2: Jama Covolo della Guerra. a) Vhod v jamo; b) "Grande Colata", velik sklop stalaktitov in stalagmitov.

listrae, Lagenophrys sp.) and one belonging to Suctorida (*Acineta* sp.). These ciliates were recovered from the gills or appendages of the amphipod *Niphargus costozzae* or the isopod *Monolistra berica*. It seemed particularly interesting to investigate Protist populations in this peculiar environment, in view of the value of such organisms in constituting simple models for a monitoring system (Boikova, 1990; Coppellotti 1998). Checking the karst environment was necessary, taking into account the presence of a spring, in connection with the hydrological system of this area.

This paper presents initial results from the northern part of the Covolo della Guerra, dealing with the composition of Protist communities and some parameters of chemico-physical conditions, including heavy metal determination.

MATERIALS AND METHODS

Five sampling sites were chosen in the Covolo della Guerra, Berici Hills, Vicenza (45°27'20" N, 0°52' 27" W), indicated on the land registry map as N. 127 V-VI (Fig. 1). The entrance to the cave (Fig. 2a) is 150 metres above sea level; the cave itself has a total length of 652 metres, and a difference in level of 43 metres. Collection sites are indicated in Fig. 1. Site A is at the entrance, site B on the wall opposite the entrance, site C in the left branch of the cave, site D in the right branch, at the base of a large concretion of stalactites and stalagmites, called the "Grande Colata" ("great flow") (Fig. 2b), and site E is at the spring. Data regarding the third, long (537 m) and narrow branch of the Covolo della Guerra, which is not easily accessible, will be collected in the near future.

Samples and measurements of chemico-physical parameters were taken every two months in the period June 1997 - September 1998. In every site, when possible, the following parameters were measured: water and air temperatures, using a Checktemp 1 Hanna thermometer; relative humidity using a psychrometer, water pH (HI270 Hanna pHmeter), illumination (HD 8366 luxmeter), and conductivity (HI8733 Hanna conductivity meter).

Sampling of sediments was carried out by pressing a plexiglass tube 3 cm in diameter into the sediment; the part from 0 cm to 2.0 cm below the surface was collected. Samples thus consisted of 14 ml each. They were placed in plastic 250-ml bottles and 100 ml of fresh water taken from the environment were added. Water was collected by sucking into a plastic 500-ml bottle. Material from the wall (site B) was collected by scraping with a plastic spoon. Samples were taken to the laboratory in a refrigerated container and observations started after 3 hours.

The material was examined first under a Wild M8 stereomicroscope, and then on a Leitz Diaplan microscope at magnification 310x or 500x. Phase contrast at 500x was often used.

Most Protists were isolated and cultured in the laboratory in distinct strains at 12°C in the dark in mineral water supplemented with bactotryptone, yeast extract or proteose-peptone. Subsequently, 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). Drawings of protargol- or silver nitrate-impregnated specimens were made with the aid of a *camera lucida* under a Diaplan Leitz microscope. Flagellates were identified after staining with acid Lugol's iodine solution (Leakey *et al.*, 1994). The taxonomic scheme of Levine *et al.* (1980) was used. Genus and species descriptions by Kahl (1930-1935), Matthes *et al.* (1988), Foissner & Berger (1996), Foissner *et al.* (1991, 1992, 1994, 1995), Patterson (1996) and Warren (1986) were also used.

Metals (Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd) were determined in waters with an inductively coupled plasma atomic emission spectrometer (ICP-AES, Spectro).

RESULTS AND DISCUSSION

Chemico-physical data

Periodic samplings between June 1997 and September 1998 in the Covolo della Guerra karst cave allowed the monitoring of some chemico-physical parameters. The overall results are shown in Tab. 1.

There is complete darkness in most of the cave, *i.e.* the left and right branches and the site where the spring flows. Illumination values are in the ranges of 240-3300 lux and 10-85 lux, respectively at the entrance (site A) and on the opposite (site B).

Owing to the existence of a small stream which flows from the spring in the right branch of the cave and to the continual dripping of water which percolates from outside, humidity is very high. Indeed, there are puddles both in the two branches and immediately after the entrance. Water temperatures may reach 6.8°C in winter and 14.4°C in summer. The minimum value (6.8°C) is lower than that (11°C) recorded by Boscolo (1968) in the period October 1964 - April 1968, whereas the maximum one (14.4°C) is the same. Moreover, relative humidity in the air is always high and may reach 100%, maintaining a value of 85-90% at the spring site (E).

Air temperatures may reach a maximum of 16.3°C inside the cave, with higher values at the entrance.

pH measurements of water at sites A, C, D and E always indicated basic values between 7.60 and 8.60; the values in the puddles in the two branches were higher than in the spring. It is noteworthy that the pH values recorded by Boscolo (1968) were always acidic and ranged between 6.5 and 6.7. The higher values

Tab. 1: Chemico-physical data in Covolo della Guerra (June 1997-September 1998).
Tab. 1: Kemijsko-fizikalni podatki iz jame Covolo della Guerra (junij 1997 - september 1998).

Sampling site	Relative humidity (%)	Illumination (lux)	Air temperature (°C)	Water temperature (°C)	Water pH	Conductivity (µS)
Entrance (A)	70-100	240-3300	9.0-20.0	7.0-14.0	8.47	*
Wall (B)	80-100	10-85	9.1-15.7	*	*	*
Left branch (C)	82-100	0	8.6-16.3	6.8-14.4	8.37-8.60	304-460
Right branch (D)	78-100	0	10.8-15.5	9.8-13.6	8.42-8.49	460-510
Spring (E)	85-90	0	8.0-14.8	11.0-12.5	7.6-7.84	475-496

* Values not determined.

Tab. 2: Protist taxa recovered from Covolo della Guerra (June 1997-September 1998).
Tab. 2: Taksoni enoceličarjev iz jame Covolo della Guerra (junij 1997 - september 1998).

Phylum **SARCOMASTIGOPHORA**
- Subphylum **Mastigophora**
  - *Astasia* sp.
  - *Entosiphon sulcatum*
  - *Peranema trichophorum*
  - *Polytoma uvella*
  - *Monosiga ovata*
  - *Bicosoeca* sp.
  - *Bodo putrinus*
  - *Bodo caudatus*
  - *Rynchomonas nasuta*
- Subphylum **Sarcodina**
  - Class **Lobosea**
    - *Hartmanella vermiformis*
    - *Acanthamoeba* sp.
    - *Naegleria* sp.
    - *Vannella mira*
    - *Vexillifera aurea*
    - *Cryptodifflugia oviformis*
    - *Difflugia sp.*
    - *Thecamoeba* sp.
    - *Arcella hemisphaerica*
  - Class **Filosea**
    - *Cyphoderia grandis*
    - *Euglypha* sp.
    - *Tracheleuglypha* sp.
    - *Thecamoeba* sp.
  - Class **Heliozoea**
    - *Actinophrys sol*
    - *Heterophrys* sp.

Phylum **CILIOPHORA**
- Class **Kinetofragminophorea**
  - *Loxodes rostrum*
  - *Lacrymaria olor*
  - *Amphileptus incurvata*
  - *Litonotus lamella*
  - *Litonotus crystallinus*
  - *Colpoda inflata*
  - *Colpoda steini*
  - *Colpoda cucullus*
  - *Chilodonella uncinata*
  - *Acineta pyriformis*
  - *Acineta tuberosa*
- Class **Oligohymenophorea**
  - *Colpidium colpoda*
  - *Colpidium kleini*
  - *Cinetochilum margaritaceum*
  - *Glaucoma scintillans*
  - *Tetrahymena pyriformis*
  - *Paramecium caudatum*
  - *Cyclidium heptatrichum*
  - *Cyclidium glaucoma*
  - *Philasterides armatus*
  - *Uronema nigricans*
  - *Vorticella astyliformis*
  - *Vorticella aquadulcis*
  - *Vorticella campanula*
  - *Vorticella convallaria*
  - *Vorticella infusionum*
  - *Vorticella picta*
  - *Vorticella pyriforme*
  - *Vorticella turgicula*
- Class **Polyhymenophorea**
  - *Metopus es*
  - *Brachonella spiralis*
  - *Halteria grandinella*
  - *Strobilidium humile*
  - *Holosticha kessleri*
  - *Holosticha pullaster*
  - *Oxytricha fallax*
  - *Oxytricha hymenostoma*
  - *Oxytricha saprobia*
  - *Sterkiella histriomuscorum*
  - *Stylonychia pustulata*
  - *Aspidisca lynceus*
  - *Aspidisca* sp.

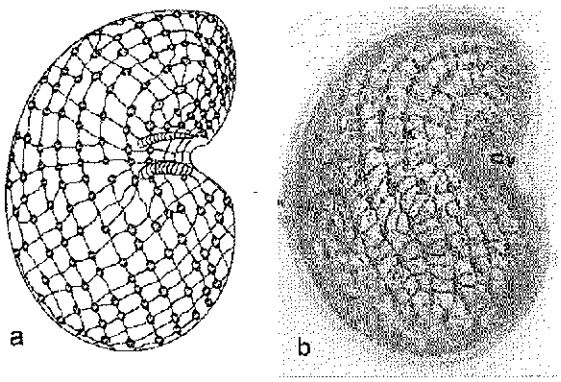


Fig. 3: Colpodid ciliate Colpoda inflata. *a) Camera lucida drawing of silver nitrate impregnated specimen, ventral side; b) Microphotograph of a specimen. Cy=cytostome; K=kineties; k=kinetosome. 1400x.*
Sl. 3: Migetalka Colpoda inflata*r iz reda Colpodida. a) Risba (camera lucida) primerka, impregniranega s srebrovim nitratom, trebušna stran; Mikrofotografija primerka. Cy = citostom; K = kinete; k = kinetosom. 1400-kratna povečava.*

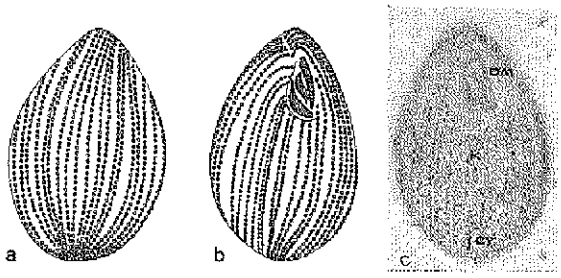


Fig. 4: Hymenostomatid Glaucoma scintillans. *a) Camera lucida drawing of silver nitrate impregnated specimen, ventral side. b) Drawing of dorsal side. c) Microphotograph of same specimen. 2000x. OM=oral membranelles; Cp=cytoproct; Ki=kineties.*
Sl. 4: Glaucoma scintillans *iz reda Hymenostomatida. a) Risba (camera lucida) primerka, impregniranega s srebrovim nitratom, trebušna stran. b) Risba hrbtne strani. c) Mikrofotografija istega primerka. 2000-kratna povečava. OM = ustne membranele; Cy = citoprokt; K = kinete.*

recorded in the present work may be due to the great accumulation of guano from the large colonies of various species of Chiroptera, which live in both branches of the cave.

Analysis of heavy metals in waters indicated no presence of Cr, Mn, Fe, Co or Ni, whereas Cu, Zn and Cd were detected up to a maximum of 33, 38 and 0.54 ppb, respectively. It must be noted that the values of Cu and Zn are in the standard range for freshwaters, whereas that of Cd slightly exceeds the baseline value. The presence of Cd must be carefully considered before using these waters for drinking.

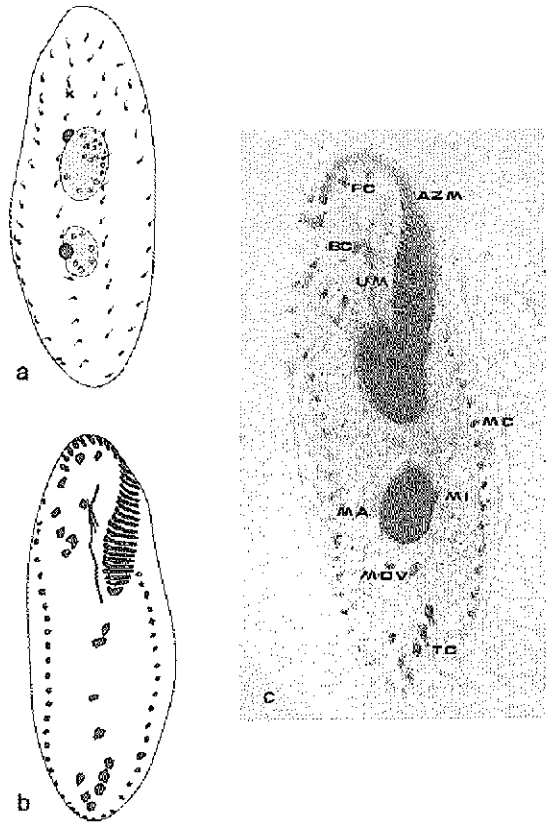


Fig. 5: Morphology of hypotrichid ciliate Sterkiella histriomuscorum *with protargol impregnation. a) Camera lucida drawing of dorsal side of a specimen, 950x; b) drawing of ventral side of same specimen, 950x; c) microphotograph of same specimen. 1400x. AZM=adoral zone of membranelles; BC=buccal cirrus; FC=frontal cirri; k=kinetosome; MC=marginal cirri; MDV=midventral cirri; MA=macronucleus; MI=micronucleus; TC=transverse cirri; UM=undulating membrane.*
Sl. 5: Morfologija migetalkarja Sterkiella histriomuscorum *iz reda Hypotrichida, impregniranega s protargolom. Risba (camera lucida) hrbtne strani primerka. 950-kratna povečava; risba trebušne strani istega primerka, 950-kratna povečava; c) mikrofotografija istega primerka, 1400-kratna povečava. AZM = obustni predel membranel; BC = obustni ciri; FC = prednji ciri; k = kinetosom; MC= marginalni ciri; MDV - srednjetrebušni ciri; MA = makronukleus; MI = mikronukleus; TC = prečni ciri; UM = valovita membrana.*

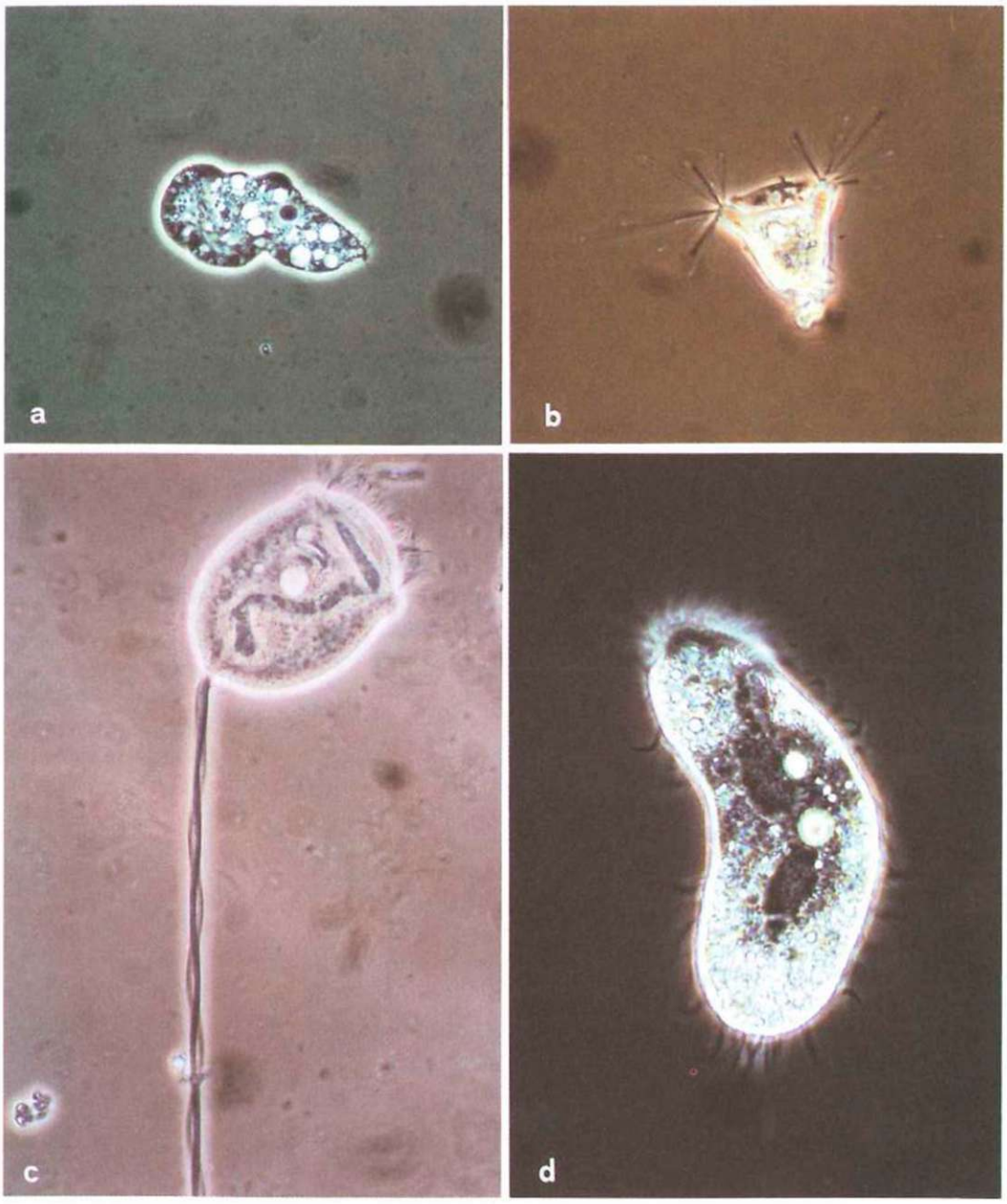


Fig. 6: Phase contrast images of living protists. a) *Naegleria sp.* ***(Sarcodina, Lobosea); b)*** *Acineta tuberosa* ***(Ciliophora, Suctorida); c)*** *Vorticella picta* ***(Ciliophora, Peritrichida); d)*** *Oxytricha fallax* ***(Ciliophora, Hypotrichida). 550x.***

Sl. 6: Kontrastne podobe živih enoceličarjev. a) *Naegleria sp.* ***(Sarcodina, Lobosea); b)*** *Acineta tuberosa* ***(Ciliophora, Suctorida); c)*** *Vorticella picta* ***(Ciliophora, Peritrichida); d)*** *Oxytricha fallax* ***(Ciliophora, Hypotrichida). 550-kratna povečava.***

Protists

Initial results on Protist populations indicated a rich variety of organisms belonging to various taxa, such as Mastigophora, Sarcodina and Ciliophora (see Tab. 2), and about 70 taxa have been identified until now. Particular attention was paid to the last class. Indeed, until now, at least 42 species of ciliates have been detected. Previous work (Boscolo, 1968) reported only three species of Peritrichida and one belonging to Suctorida, living on the gills or appendages of one species of Amphipoda and one of Isopoda. All Protists were found in puddles, walls, mud or guano deposited by the large populations of Chiroptera living in the branches of the cave. The Protists identified belong to taxa living in damp soil, moss or a freshwater environment, according

to previous findings reported by Golemansky & Bonnet (1994) on caverniculous Protists. The extensive presence of bacteria in summer, especially in the bat guano, sustains many bacterivorous Protists, such as *Colpidium colpoda*. However, it must be noted that, according to Foissner (1987), many soil ciliates prey on other ciliates, zooflagellates, and/or naked and shelled amoebae, contrasting with the widely accepted view that most soil ciliates are bacterivorous.

In winter, many ciliates are recovered as temporary and protective cysts, developed in laboratory conditions with culture methods favouring the growth of bacteria. This was the case of many Hypotrichida *(Oxytricha, Sterkiella, Stylonychia)* and Colpodida *(Colpoda)*. Figs. 3-5 show the morphology of some representative Protist taxa, after techniques of impregnation with silver proteinate or nitrate, and Fig. 6 shows four species of Protists observed *in vivo* by phase contrast microscopy.

The high diversity of Vorticellidae, which are represented by at least eight species, is noteworthy. Owing to the complex systematics of this taxon, it is possible that other species, new to science, are present. Studies are in progress to extend taxonomic studies to other groups of ciliates, especially Hypotrichida. The physiological characteristics of ciliate Protists living in the karst environment will also be considered, taking into account the fact that ciliates, essential components of nearly all ecosystems, are simple models for ecotoxicological studies, due to their relative ease of culture, short life-cycle, cosmopolitan distribution, and sensitivity to environmental changes. Future studies will also concern that part of the Covolo della Guerra cave which branches off to the south and which is not easily accessible.

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JAMSKI ENOCELIČARJI: PREDHODNE RAZISKAVE O NJIHOVIH POPULACIJAH V KRAŠKI JAMI "COVOLO DELLA GUERRA" V HRIBOVJU BERICI (VICENZA, ITALIJA)

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POVZETEK

Posledica povečanega zanimanj za speleologijo je nekaj prav presenetljivih odkritij iz obdobja zadnjih nekaj let v italijanskih jamah. Toda na podlagi zbranega favnističnega gradiva so bila do danes posebno raziskane le nekatere taksonomske skupine, na primer Insecta, Chilopoda, Gastropoda in Chiroptera.

Zaradi pomena organizmov, ki živijo v skoraj vseh ekosistemih, se je zdelo še posebno zanimivo raziskati, kot preproste modele za sistem njihovega spremljanja (monitoringa), populacije enoceličarjev (s posebnim poudarkom na migetalkarjih) v italijanski kraški jami "Covolo della Guerra".

Predhodni rezultati, dobljeni v severnem delu jame, zadevajo nekaj kemijsko-fizikalnih parametrov (ki so glede na tamkajšnji izvir vsekakor vredni pozornosti) in govorijo o precejšnji raznoterosti enoceličarjev, ki pripadajo različnim taksonom, kot na primer Mastigophora, Sarcodina in Ciliophora. Do danes je bilo raziskanih 70 taksonov, 42 od katerih so migetalkarji.

Ključne besede: jama, kras, Vicenza, enoceličarji, Ciliophora, Mastigophora, Sarcodina

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